ONCOGENES AND CANCER

6.30.1 Introduction

Key Terms

A **mutator** is a mutation or a mutated gene that increases the basal level of mutation. Such genes often code for proteins that are involved in repairing damaged DNA.

A major feature of all higher eukaryotes is the defined life span of the organism, a property that extends to the individual somatic cells, whose growth and division are highly regulated. A notable exception is provided by cancer cells, which arise as variants that have lost their usual growth control. Their ability to grow in inappropriate locations or to propagate indefinitely may be lethal for the individual organism in which they occur.

Figure 30.1 shows that the incidence of cancer increases exponentially with age in a human population from the age of ~40 to ~80, when it plateaus. Immediately this suggests that cancer is the result of the occurrence of a series of independent events. From the trend of the curve we can estimate that a range of 4-10 stochastic events are required to generate a cancer (for review see 2252).

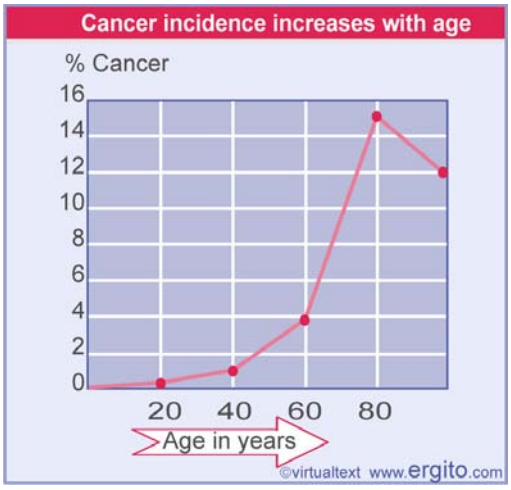

Figure 30.1 The frequency of newly diagnosed cancers increases sharply with age. Each point on the graph gives the incidence over a one year period for the preceding age group (the first point is for 0-20 years of age, etc.). The incidence rate plateaus above age 80.

The basic model for the occurrence of cancer is that cancer is a multistage process in which initiation of a tumor requires several steps, which may then be followed by further changes to strengthen the tumorigenic state. Tumor progression is then driven by selection among the tumor cells for those that can grow more aggressively. Many different types of events contribute to this process at the molecular level (for review

see 2251).

The two major types of change in the genome are the accumulation of somatic mutations and the development of genetic instability. There is still much debate about the relative importance of their contributions to the cancerous state.

Most cancer cells have an increased number of mutations compared to normal cells (2253). As the cancer progresses, the number of mutations increases. However, the rate of somatic mutation is not sufficient to account for the accumulation of mutations that is observed in the cancer cells. **Figure 30.2** illustrates the view that some of the early mutations occur in **mutator** genes (2849). The inactivation of these genes decreases the repair of damaged DNA, and thereby increases the rate at which mutations occur (for review see 2848).

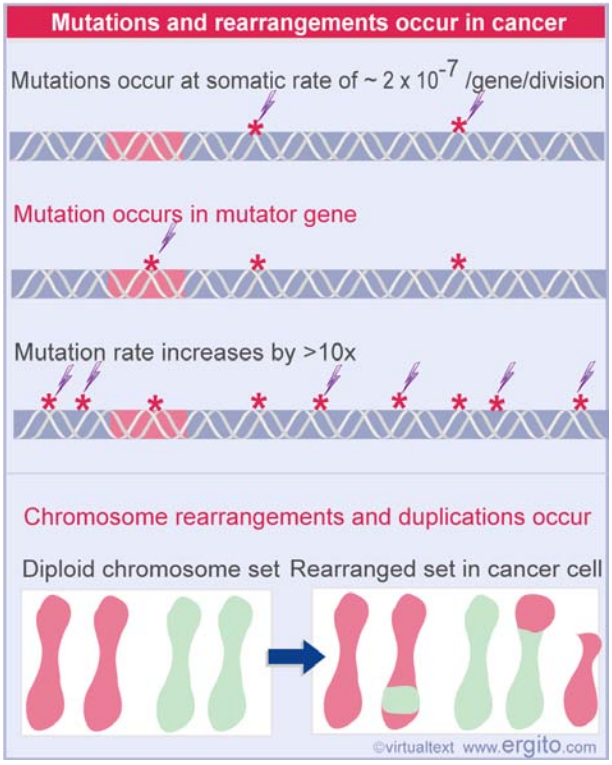

Figure 30.2 Cancer cells accumulate mutations at an increased rate as a result of the mutation of a mutator gene and also undergo changes in the chromosomal constitution.

Genetic instability is reflected in changes in the numbers of genes in cancer cells. This can be the result of small duplications or deletions, translocations of material from one chromosome to another, or even changes that affect entire chromosomes. Instability at the level of chromosome can be caused by systems that act on partitioning at mitosis.

The occurrence of different mutations creates an opportunity to select among the population for cells with particular properties (2850). In the case of cancer, a mutation that increases the growth potential of a cell will give it a selective advantage. **Figure 30.3** illustrates the result that a cell that divides more often,

perhaps because it does not respect the usual constraints on growth, will generate more descendants. At each stage during the progression of a cancer, the cell population is selected for those cells that can grow more aggressively (this meaning initially that they can grow more rapidly and later that they can migrate to start colonies in new locations).

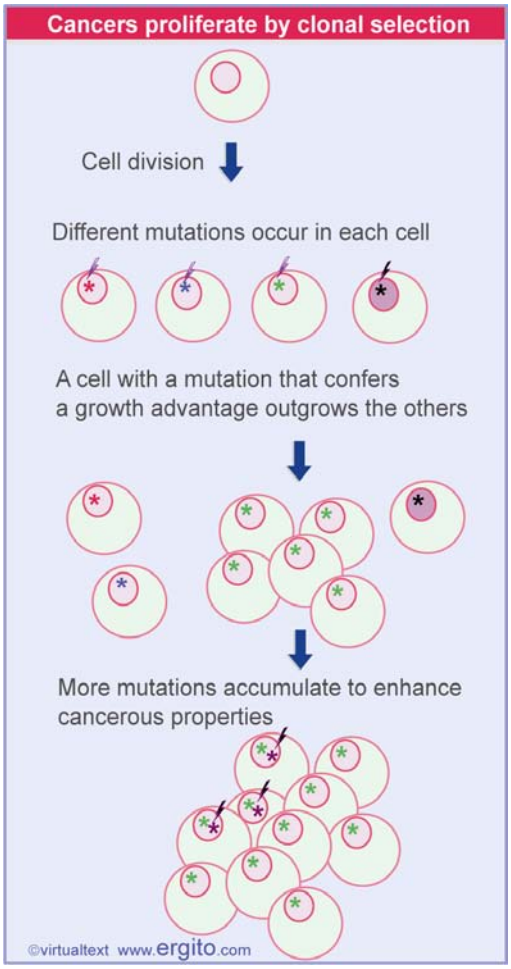

Figure 30.3 When a mutation occurs to confer a growth advantage, the descendent cells will dominate the population. A cancer advances by clonal selection for more malignant cells at each stage.

Our current view of cancer is that it is driven by twin features: an increased rate of mutation is responsible for generating cells with altered growth properties; and the population of cells is then selected for those with an increased rate of proliferation. A cancer progresses by multiple cycles of mutation and selection.

By comparing cancer cells with normal cells, we can identify genes that have been changed by mutation. Those that have direct effects on the generation of a cancer can be divided into oncogenes (where a mutation has activated a gene whose function contributes to the tumorigenic state) and tumor suppressors (where a mutation has inactivated a gene whose function antagonizes the tumorigenic state). In most cases, a cancer arises because a series of mutations have accumulated in a somatic cell,

activating oncogenes and/or inactivating tumor suppressors.

Genetic diversity in the population means that each individual may have a different set of alleles at these loci. Natural selection acts to eliminate alleles in the germline that contribute to cancer formation. However, there are some rare hereditary diseases that are caused by such alleles. In these cases, the affected individuals have a high probability of suffering from a cancer. In addition, susceptibility to cancer is influenced by many other loci, generally known as tumor modifiers. The products of these loci affect the functions of oncogenes or tumor suppressors, either directly or indirectly, but do not themselves have any direct effects (for review see 2404).

Last updated on 8-19-2002

Reviews

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2849. Loeb, L. A., Springgate, C. F., and Battula, N. (1974). *Errors in DNA replication as a basis of malignant changes.* Cancer Res. 34, 2311-2321.

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ONCOGENES AND CANCER

6.30.2 Tumor cells are immortalized and transformed

Key Terms

Immortalization describes the acquisition by a eukaryotic cell line of the ability to grow through an indefinite number of divisions in culture.

Transformation (Oncogenesis) of eukaryotic cells refers to their conversion to a state of unrestrained growth in culture, resembling or identical with the tumorigenic condition.

Metastasis describes the ability of tumor cells to leave their site of origin and migrate to other locations in the body, where a new colony is established.

Crisis is a state reached when primary cells placed into culture are unable to replicate their DNA because their telomeres have become too short. Most cells die, but a few emerge by a process of immortalization that usually involves changes to bypass the limitations of telomeric length.

Primary cells are eukaryotic cells taken into culture directly from the animal.

An **established** cell line consists of cells that can be grown indefinitely in culture (they are said to be immortalized).. The cells usually have had chromosomal changes in order to adapt to culture conditions.

Anchorage dependence describes the need of normal eukaryotic cells for a surface to attach to in order to grow in culture.

Serum dependence describes the need of eukaryotic cells for factors contained in serum in order to grow in culture.

Density-dependent inhibition describes the limitation that eukaryotic cells in culture grow only to a limited density, because growth is inhibited, by processes involving cell-cell contacts.

A **monolayer** describes the growth of eukaryotic cells in culture as a layer only one cell deep.

An **aneuploid** set of chromosomes differs from the usual set by loss or duplication of one or more chromosomes.

Transformed cells are cultured cells that have acquired many of the properties of cancer cells.

Transformed cells grow as a compact mass of rounded-up cells that grows in dense clusters, piled up on one another. They appear as a distinct **focus** on a culture plate, contrasted with normal cells that grow as a spread-out monolayer attached to the substratum.

A **carcinogen** is a chemical that increases the frequency with which cells are converted to a cancerous condition.

Key Concepts

- Immortalization enables cells to overcome a limit on the number of cell divisions.
- Cultured cell lines have been immortalized.

- Transformation consists of a series of changes that release growth constraints on the immortalized cell.

Three types of changes that occur when a cell becomes tumorigenic are summarized in **Figure 30.4**:

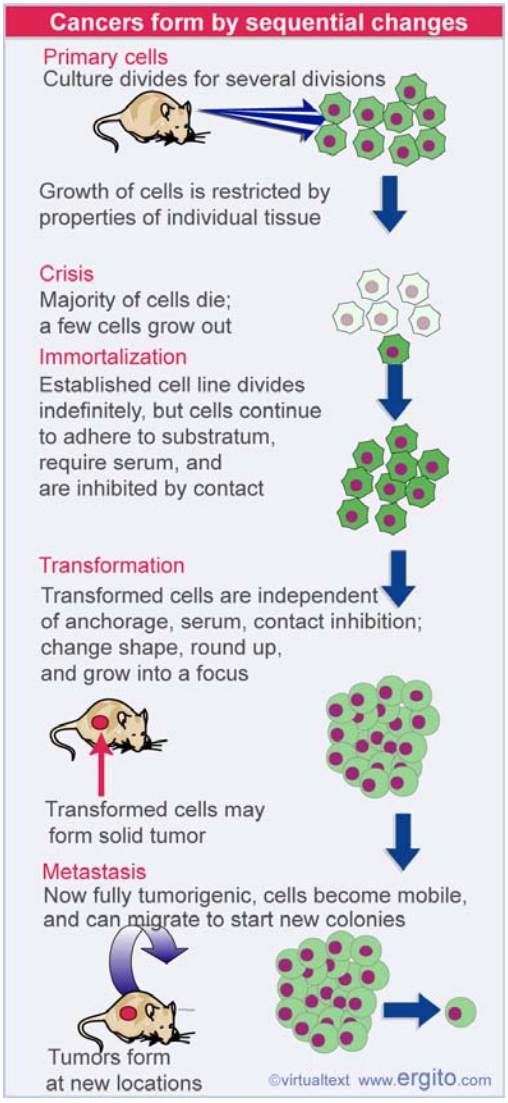

Figure 30.4 Three types of properties distinguish a cancer cell from a normal cell. Sequential changes in cultured cells can be correlated with changes in tumorigenicity.

- **Immortalization** describes the property of indefinite cell growth (without any other changes in the phenotype necessarily occurring).
- **Transformation** describes the failure to observe the normal constraints of

growth; for example, transformed cells become independent of factors usually needed for cell growth and survival.

- **Metastasis** describes the stage at which the cancer cell gains the ability to invade normal tissue, so that it can move away from the tissue of origin and establish a new colony elsewhere in the body.

To characterize the aberrant events that enable cells to bypass normal control and generate tumors, we need to compare the growth characteristics of normal and transformed cells *in vitro.* Transformed cells can be grown readily, but it is much more difficult to grow their normal counterparts.

When cells are taken from a vertebrate organism and placed in culture, they grow for several divisions, but then enter a senescent stage, in which growth ceases. This is followed by a **crisis**, in which most of the cells die. The number of divisions that occur before this happens is sometimes called the Hayflick limit, after the author who discovered the phenomenon (1377).

The survivors that emerge from crisis are capable of dividing indefinitely, but their properties have changed in the act of emerging from crisis. This comprises the process of immortalization. (The features of crisis depend on both the species and tissue. Typically mouse cells pass through crisis at ~12 generations. Human cells enter crisis at ~40 generations, although it is rare for human cells to emerge from it, and only some types of human cells in fact can do so.)

The limitation of the life span of most cells by crisis restricts us to two options in studying nontransformed cells, neither entirely satisfactory:

- **Primary cells** are the immediate descendants of cells taken directly from the organism. They faithfully mimic the *in vivo* phenotype, but in most cases survive for only a relatively short period, because the culture dies out at crisis.
- Cells that have passed through crisis become **established** to form a (nontumorigenic) cell line. They can be perpetuated indefinitely, but their properties have changed in passing through crisis, and may indeed continue to change during adaptation to culture. These changes may partly resemble those involved in tumor formation, which reduces the usefulness of the cells.

An established cell line by definition has become immortalized, but usually is not tumorigenic. Nontumorigenic established cell lines display characteristic features similar to those of primary cultures, often including:

- **Anchorage dependence** – a solid or firm surface is needed for the cells to attach to.
- **Serum dependence** (also known as growth factor dependence) – serum is needed to provide essential growth factors.
- **Density-dependent inhibition** – cells grow only to a limited density, because

growth is inhibited, perhaps by processes involving cell-cell contacts.

- *Cytoskeletal organization* – cells are flat and extended on the surface on which they are growing, and have an elongated network of stress fibers (consisting of actin filaments).

The consequence of these properties is that the cells grow as a **monolayer** (that is, a layer one cell thick) on a substratum.

These properties provide parameters by which the normality of the cell may be judged. Of course, any established cell line provides only an approximation of *in vivo* control. The need for caution in analyzing the genetic basis for growth control in such lines is emphasized by the fact that almost always they suffer changes in the chromosome complement and are not true diploids. A cell whose chromosomal constitution has changed from the true diploid is said to be **aneuploid**.

Cells cultured from tumors instead of from normal tissues show changes in some or all of these properties. They are said to be **transformed**. A transformed cell grows in a much less restricted manner. It has reduced serum-dependence, does not need to attach to a solid surface (so that individual cells "round-up" instead of spreading out) and the cells pile up into a thick mass of cells (called a **focus**) instead of growing as a surface monolayer. Furthermore, the cells may form tumors when injected into appropriate test animals. **Figure 30.5** compares views obtained by conventional microscopy of "normal" fibroblasts growing in culture with "transformed" variants. The difference can be seen more dramatically in the scanning electron microscope views of **Figure 30.6**.

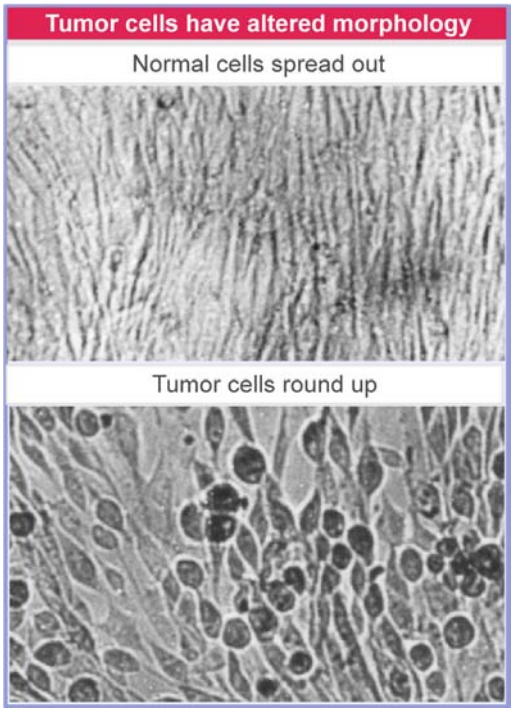

Figure 30.5 Normal fibroblasts grow as a layer of flat, spread-out cells, whereas transformed fibroblasts are rounded up and grow in cell masses. Photographs kindly provided by Hidesaburo Hanafusa.

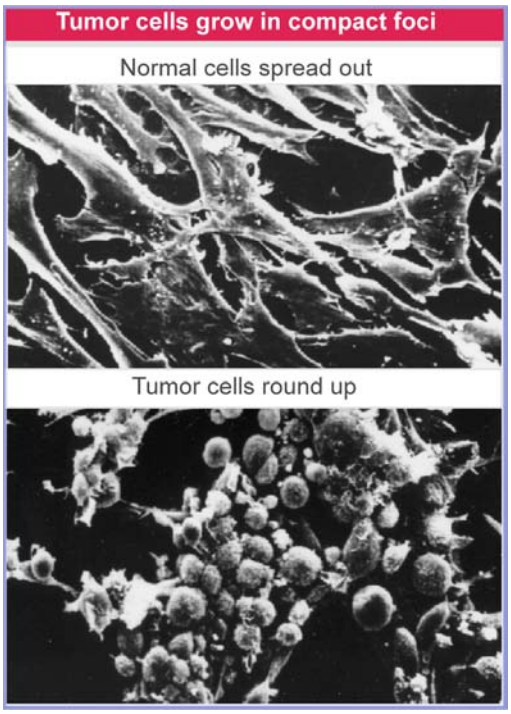

Figure 30.6 Scanning electron microscopy shows that normal cells spread out and form long processes, whereas tumor cells round up into tightly balled masses. Photographs kindly provided by J. Michael Bishop.

The joint changes of immortalization and transformation of cells in culture provide a paradigm for the formation of animal tumors. By comparing transformed cell lines with normal cells, we hope to identify the genetic basis for tumor formation and also to understand the phenotypic processes that are involved in the conversion.

Certain events convert normal cells into transformed cells, and provide models for the processes involved in tumor formation. Usually multiple genetic changes are necessary to create a cancer; and sometimes tumors gain increased virulence as the result of a progressive series of changes.

A variety of agents increase the frequency with which cells (or animals) are converted to the transformed condition; they are said to be *carcinogenic*. Sometimes these **carcinogens** are divided into those that "initiate" and those that "promote" tumor formation, implying the existence of different stages in cancer development. Carcinogens may cause epigenetic changes or (more often) may act, directly or indirectly, to change the genotype of the cell.

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ONCOGENES AND CANCER

6.30.3 Oncogenes and tumor suppressors have opposite effects

Key Terms

Oncogenes are genes whose products have the ability to transform eukaryotic cells so that they grow in a manner analogous to tumor cells. Oncogenes carried by retroviruses have names of the form *v-onc*.

Proto-oncogenes are the normal counterparts in the eukaryotic genome to the (*v-onc*) oncogenes carried by some retroviruses. They are given names of the form *c-onc* .

A **tumor suppressor** is identified by a loss-of-function mutation that contributes to cancer formation. They usually function to prevent cell division or to cause death of abnormal cells. The two most important are p53 and RB.

Key Concepts

- An oncogene results from a gain-of-function mutation of a proto-oncogene that generates a tumorigenic product.
- Mutation of a tumor suppressor causes a loss-of-function in the ability to restrain cell growth.

There are two classes of genes in which mutations cause transformation.

Oncogenes were initially identified as genes carried by viruses that cause transformation of their target cells. A major class of the viral oncogenes have cellular counterparts that are involved in normal cell functions. The cellular genes are called **proto-oncogenes**, and in certain cases their mutation or aberrant activation in the cell to form an oncogene is associated with tumor formation. About 100 oncogenes have been identified. The oncogenes fall into several groups, representing different types of activities ranging from transmembrane proteins to transcription factors, and the definition of these functions may therefore lead to an understanding of the types of changes that are involved in tumor formation.

The generation of an oncogene represents a gain-of-function in which a cellular proto-oncogene is inappropriately activated. This can involve a mutational change in the protein, or constitutive activation, overexpression, or failure to turn off expression at the appropriate time. The simple case of a somatic mutation is illustrated in **Figure 30.7**.

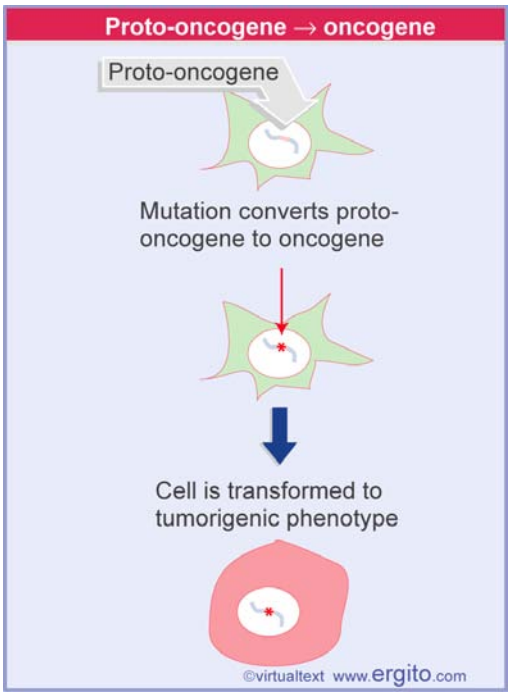

Figure 30.7 Somatic mutation generates a transformed cell by converting a proto-oncogene to an oncogene.

Tumor suppressors are detected by deletions (or other inactivating mutations) that are tumorigenic. The mutations represent loss-of-function in genes that usually impose some constraint on the cell cycle or cell growth; the release of the constraint is tumorigenic. It is necessary for both copies of the gene to be inactivated.

The most compelling evidence for the nature of tumor suppressors is provided by certain hereditary cancers, in which patients with the disease develop tumors that have lost both alleles, and therefore lack an active gene. There is also now evidence that changes in these genes may be associated with the progression of a wide range of cancers. About 10 tumor suppressors are known at present.

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ONCOGENES AND CANCER

6.30.4 Transforming viruses carry oncogenes

Key Terms

A **tumor virus** has the ability to transform an animal cell into a cancerous state.

Key Concepts

- A transforming virus usually carries a specific gene(s) that is responsible for transforming the target cell by changing its growth properties.

Transformation may occur spontaneously, may be caused by certain chemical agents, and, most notably, may result from infection with **tumor viruses**. There are many classes of tumor viruses, including both DNA and RNA viruses, and they occur widely in the avian and animal kingdoms.

The transforming activity of a tumor virus resides in a particular gene or genes carried in the viral genome. Oncogenes were given their name by virtue of their ability to convert cells to a tumorigenic (or oncogenic) state. An oncogene initiates a series of events that is executed by cellular proteins. In effect, the virus throws a regulatory switch that changes the growth properties of its target cell.

Figure 30.8 summarizes the general properties of the major classes of transforming viruses. The oncogenes carried by the DNA viruses specify proteins that inactivate tumor suppressors, so their action in part mimics loss-of-function of the tumor suppressors. The oncogenes carried by retroviruses are derived from cellular genes and therefore may mimic the behavior of gain-of-function mutations in animal proto-oncogenes.

DNA and RNA oncogenic viruses carry different types of oncogenes

Viral Class	Genome	Size	Oncogenes	Origin of Oncogene	Action of Oncogene
Polyoma	dsDNA	5-6 kb	T antigens	Early viral gene	inactivates tumor suppressor
HPV	dsDNA	~8 kb	E6 & E7	Early viral gene	inactivates tumor suppressor
Adeno	dsDNA	~37 kb	E1A & E1B	Early viral gene	inactivates tumor suppressor
Retrovirus (acute)	ssRNA	6-9 kb	Individual	Cellular	activates oncogenic pathway

Figure 30.8 The oncogenes of DNA transforming viruses are early viral functions, whereas the oncogenes of retroviruses are modified from cellular genes.

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ONCOGENES AND CANCER

6.30.5 Early genes of DNA transforming viruses have multifunctional oncogenes

Key Terms

A protein that plays a **permissive** role in development is one that sets up a situation where a certain activity can occur, but does not cause the occurrence itself.

Nonpermissive conditions do not allow conditional lethal mutants to survive.

Key Concepts

- The oncogenes of DNA transforming viruses are early viral functions.
- The oncogene becomes integrated into the host cell genome and is expressed constitutively.
- The oncogenes of polyomaviruses are T antigens, which are expressed by alternative splicing from a single locus.
- Adenoviruses express several E1A and E1B proteins from two genes.

Polyomaviruses and adenoviruses have been isolated from a variety of mammals. Although perpetuated in the wild in a single host species, a virus may be able to grow in culture on a variety of cells from different species. The response of a cell to infection depends on its species and phenotype and falls into one of two classes, as illustrated in **Figure 30.9**:

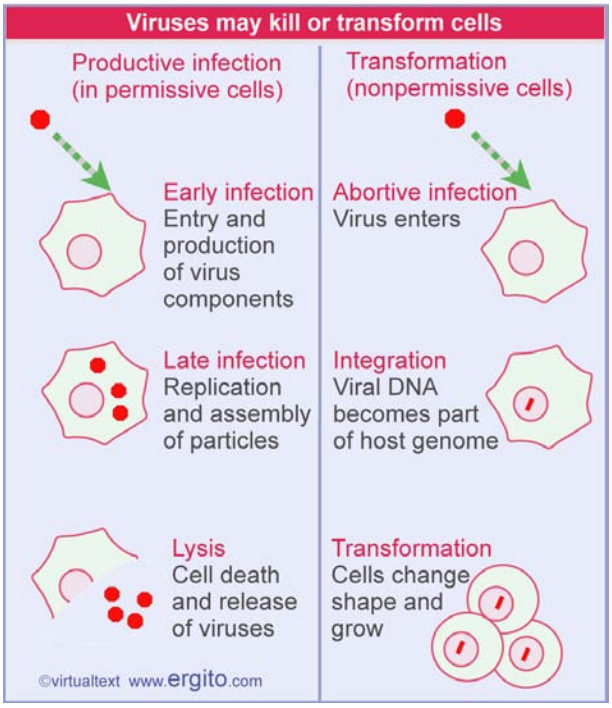

Figure 30.9 Permissive cells are productively infected by a DNA tumor virus that enters the lytic cycle, while nonpermissive cells are transformed to change their phenotype.

- **Permissive** cells are productively infected. The virus proceeds through a lytic cycle that is divided into the usual early and late stages. The cycle ends with release of progeny viruses and (ultimately) cell death.
- **Nonpermissive** cells cannot be productively infected, and viral replication is abortive. Some of the infected cells are transformed; in this case, the phenotype of the individual cell changes and the culture is perpetuated in an unrestrained manner.

A common mechanism underlies transformation by DNA tumor viruses. *Oncogenic potential resides in a single function or group of related functions that are active early in the viral lytic cycle. When transformation occurs, the relevant gene(s) are integrated into the genomes of transformed cells and expressed constitutively.* This suggests the general model for transformation by these viruses illustrated in **Figure 30.10**, in which the constitutive expression of the oncogene generates transforming protein(s) (oncoproteins).

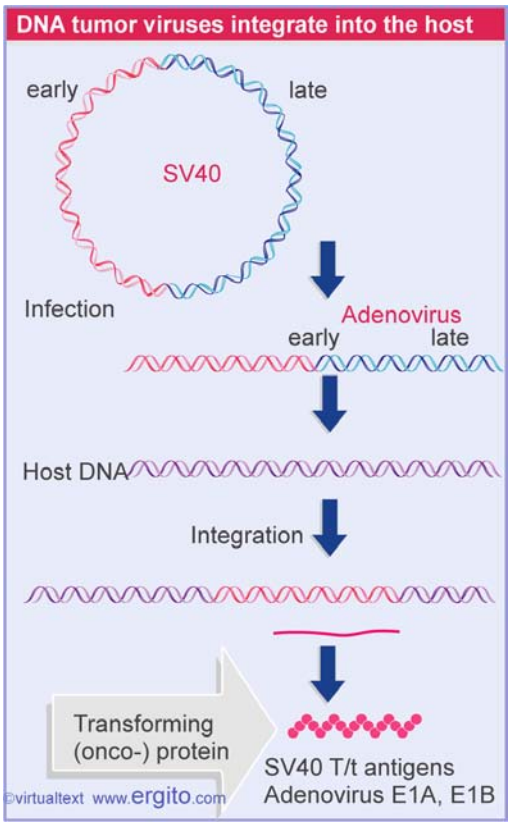

Figure 30.10 Cells transformed by polyomaviruses or adenoviruses have viral sequences that include the early region integrated into the cellular genome. Sites of integration are random.

Polyomaviruses are small. Polyomavirus itself is common in mice, the analogous virus SV40 (simian virus 40) was isolated from rhesus monkey cells, and more recently the human viruses BK and JC have been characterized. All of the polyomaviruses can cause tumors when injected into newborn rodents.

During a productive infection, the early region of each virus uses alternative splicing to synthesize overlapping proteins called T antigens. (The name reflects their isolation originally as the proteins found in *t*umor cells.) The various T antigens have a variety of functions in the lytic cycle. They are required for expression of the late region and for DNA replication of the virus.

Cells transformed by polyomaviruses contain integrated copies of part or all of the viral genome. The integrated sequences always include the early region. The T antigens have transforming activity, which rests upon their ability to interact with cellular proteins. This is independent of their ability to interact directly with the viral genome. SV40 requires "big T" and "little t" antigens, and polyoma requires "T" and "middle T" antigens for transformation.

Papillomaviruses are small DNA viruses that cause epithelial tumors; there are ~75 human papillomaviruses (HPVs); most are associated with benign growths (such as warts), but some are associated with cancers, in particular cervical cancers. Two

virus-associated products are expressed in cervical cancers; these are the E6 and E7 proteins, which can immortalize target cells.

Adenoviruses were originally isolated from human adenoids; similar viruses have since been isolated from other mammals. They comprise a large group of related viruses, with >80 individual members. Human adenoviruses remain the best characterized, and are associated with respiratory diseases. They can infect a range of cells from different species.

Human cells are permissive and are therefore productively infected by adenoviruses, which replicate within the infected cell. But cells of some rodents are nonpermissive. All adenoviruses can transform nonpermissive cultured cells, but the oncogenic potential of the viruses varies; the most effective can cause tumors when they are injected into newborn rodents. The genomes of cells transformed by adenoviruses have gained a part of the early viral region that contains the E1A and E1B genes, which code for several nuclear proteins.

Epstein-Barr is a human herpes virus associated with a variety of diseases, including infectious mononucleosis, nasopharyngeal carcinoma, African Burkitt lymphoma, and other lymphoproliferative disorders. EBV has a limited host range for both species and cell phenotype. Human B lymphocytes that are infected *in vitro* become immortalized, and some rodent cell lines can be transformed. Viral DNA is found in transformed cells, although it has been controversial whether it is integrated. It remains unclear exactly which viral genes are required for transformation.

ONCOGENES AND CANCER

6.30.6 Retroviruses activate or incorporate cellular genes

Key Terms

A **nondefective virus** describes a transforming retrovirus that has all the normal capabilities in replication etc. Its ability to transform depends on its effects on expression of host genes at its site of insertion into the cellular genome.

An **acute transforming virus** carries a gene(s) that originated in a cellular genome. Its transforming capacity is the result of expression of that gene. Because the gene replaced viral sequences, the virus does not have the capacity to replicate independently.

Key Concepts

- Acute transforming retroviruses have oncogenes that are derived from cellular genes.
- Nondefective transforming viruses do not have oncogenes, but activate an equivalent gene(s) in the host genome.

Retroviruses present a different situation from the DNA tumor viruses. They can transfer genetic information both horizontally and vertically, as illustrated in **Figure 30.11**. Horizontal transfer is accomplished by the normal process of viral infection, in which increasing numbers of cells become infected in the same host. Vertical transfer results whenever a virus becomes integrated in the germline of an organism as an endogenous provirus; like a lysogenic bacteriophage, it is inherited as a Mendelian locus by the progeny (see *Molecular Biology 3.12 Phage strategies*).

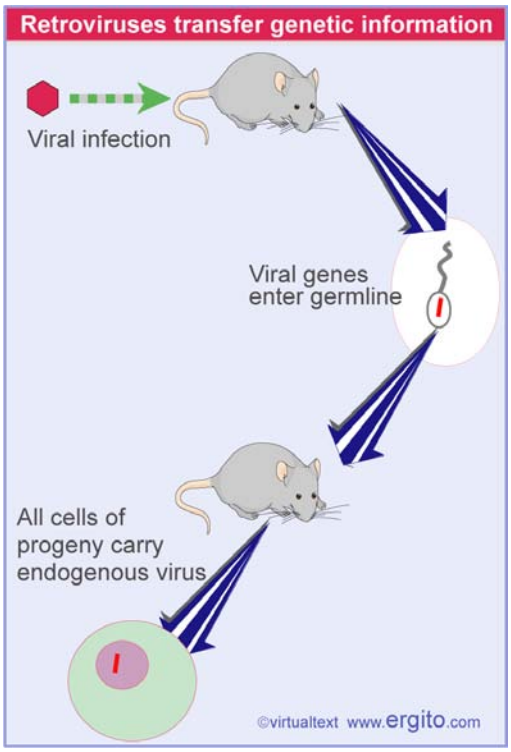

Figure 30.11 Retroviruses transfer genetic information horizontally by infecting new hosts; information is inherited vertically if a virus integrates in the genome of the germline.

The retroviral life cycle propagates genetic information through both RNA and DNA templates. A retroviral infection proceeds through the stages illustrated previously in **Figure 17.2**, in which the RNA is reverse-transcribed into single-stranded DNA, then converted into double-stranded DNA, and finally integrated into the genome, where it may be transcribed again into infectious RNA. Integration into the genome leads to vertical transmission of the provirus. Expression of the provirus may generate retroviral particles that are horizontally transmitted. Integration is a normal part of the life cycle of every retrovirus, whether it is nontransforming or transforming.

The tumor retroviruses fall into two general groups with regard to the origin of their tumorigenicity:

- **Nondefective viruses** follow the usual retroviral life cycle. They provide infectious agents that have a long latent period, and often are associated with the induction of leukemias. Two classic models are FeLV (feline leukemia virus) and MMTV (mouse mammary tumor virus). *Tumorigenicity does not rely upon an individual viral oncogene, but upon the ability of the virus to activate a cellular proto-oncogene(s).*

- **Acute transforming viruses** *have gained new genetic information in the form of an oncogene.* This gene is not present in the ancestral (nontransforming virus); it originated as a cellular gene that was captured by the virus by means of a

transduction event during an infective cycle. These viruses usually induce tumor formation *in vivo* rather rapidly, and they can transform cultured cells *in vitro.* Reflecting the fact that each acute transforming virus has specificity toward a particular type of target cell, these viruses are divided into classes according to the type of tumor that is caused in the animal: leukemia, sarcoma, carcinoma, etc.

When a retrovirus captures a cellular gene by exchanging part of its own sequence for a cellular sequence (see *Molecular Biology 4.17.6 Retroviruses may transduce cellular sequences*), it generates the structure summarized in **Figure 30.12**. Some of the original retroviral sequences (which are usually organized into the genes *gag-pol-env*, coding for coat proteins, reverse transcriptase, and other enzyme activities) are replaced by a sequence derived by reverse transcription of a cellular mRNA. This type of event is rare, but creates a transducing virus that has two important properties:

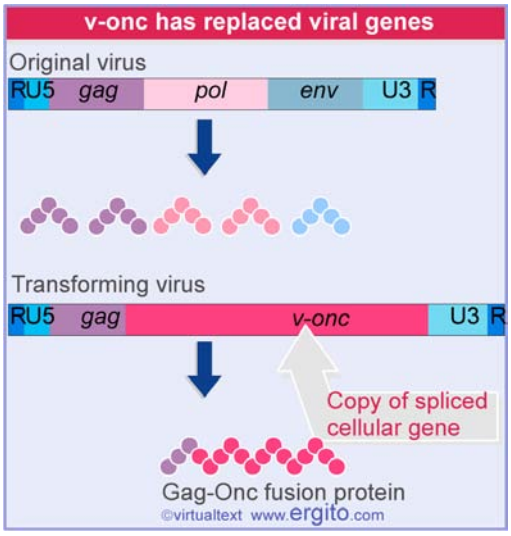

Figure 30.12 A transforming retrovirus carries a copy of a cellular sequence in place of some of its own gene(s).

- Usually it cannot replicate by itself, because viral genes needed for reproduction have been lost by the exchange with cellular sequences. So almost all of these viruses are replication-defective. But they can propagate in a simultaneous infection with a wild-type "helper" virus that provides the functions that were lost in the recombination event. (RSV is an exceptional transducing virus that retains the ability to replicate.)

- During an infection, the transducing virus carries with it the cellular gene(s) that were obtained in the recombination event, and their expression may alter the phenotype of the infected cell. Any transducing virus whose cellular genetic information assists the growth of its target cells could have an advantage in future infective cycles. If a virus gains a gene whose product stimulates cell growth, the acquisition may enable the virus to spread by stimulating the growth of the particular cells that it infects. This is important also because a retrovirus

can replicate only in a proliferating cell. After a virus has incorporated a cellular gene, the gene may gain mutations that enhance its ability to influence cell phenotype.

Of course, transformation is not the only mechanism by which retroviruses affect their hosts. A notable example is the HIV-1 retrovirus, which belongs to the retroviral group of lentiviruses. The virus infects and kills T lymphocytes carrying the CD4 receptor, devastating the immune system of the host, and inducing the disease of AIDS. The virus carries the usual *gag-pol-env* regions, and also has an additional series of reading frames, which overlap with one another, to which its lethal actions are attributed.

ONCOGENES AND CANCER

6.30.7 Retroviral oncogenes have cellular counterparts

Key Terms

Proto-oncogenes are the normal counterparts in the eukaryotic genome to the (*v-onc*) oncogenes carried by some retroviruses. They are given names of the form *c-onc* .

Key Concepts

- A retroviral oncogene is derived by capturing a proto-oncogene from a host genome.

New sequences that are present in an acute transforming retrovirus can be delineated by comparing the sequence of the virus with that of the parental (nontumorigenic) virus. Usually the new region is closely related to a sequence in the cellular genome. The normal cellular sequence itself is not oncogenic – if it were, the organism could scarcely have survived – but it defines a **proto-oncogene**.

The general model for retroviral transformation is illustrated in **Figure 30.13**. The virus gains a copy of a proto-oncogene from a cellular genome. Sometimes the copy is different from the cellular sequence, typically because it has been truncated. In some cases, the difference is sufficient to convert the proto-oncogene into an oncogene. In other cases, mutations occur in the viral sequence that convert the copy into an oncogene.

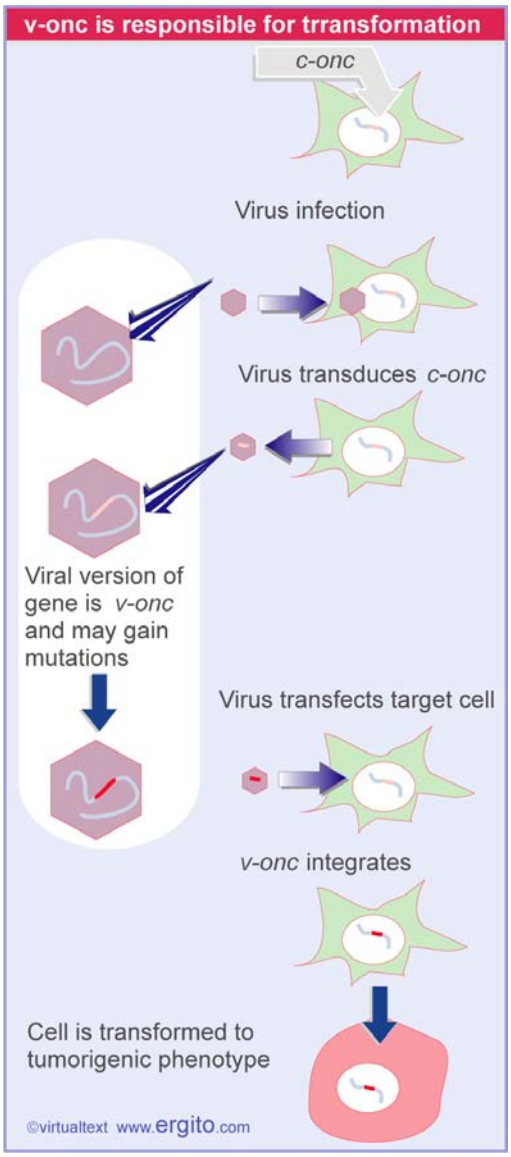

Figure 30.13 A retrovirus may incorporate a cellular proto-oncogene during an infection. Subsequent mutation or other changes in the proto-oncogene make it oncogenic when the virus transfers it to a new cell in another infectious cycle.

The viral oncogenes and their cellular counterparts are described by using prefixes *v* for viral and *c* for cellular. So the oncogene carried by Rous sarcoma virus is called *v-src*, and the proto-oncogene related to it in cellular genomes is called *c-src*. Comparisons between *v-onc* and *c-onc* genes can be used to identify the features that confer oncogenicity (for review see 335; 336).

Oncogenes of some retroviruses are summarized in **Figure 30.14**. The type of tumor results from the combination of the particular oncogene with the time and place in which it is expressed. It is striking that usually the oncogenic activity resides in a single gene. AEV is one of a very few exceptions in which a retrovirus carries more

than one oncogene.

Transforming properties of retroviruses are determined by their v-onc genes

Virus	Name	Species	Tumor	Oncogene
Rous sarcoma	RSV	chicken	sarcoma	*src*
Harvey murine sarcoma	Ha-MuSV	rat	sarcoma & erythroleukemia	*H-ras*
Kirsten murine sarcoma	Ki-MuSV	rat	sarcoma & erythroleukemia	*K-ras*
Moloney murine sarcoma	Mo-MuSV	mouse	sarcoma	*mos*
FBJ murine osteosarcoma	FBJ-MuSV	mouse	chondrosarcoma	*fos*
Simian sarcoma	SSV	monkey	sarcoma	*sis*
Feline sarcoma	PI-FeSV	cat	sarcoma	*sis*
Feline sarcoma	SM-FeSV	cat	fibrosarcoma	*fms*
Feline sarcoma	ST-FeSV	cat	fibrosarcoma	*fes*
Avian sarcoma	ASV-17	chicken	fibrosarcoma	*jun*
Fujinami sarcoma	FuSV	chicken	sarcoma	*fps*
Avian myelocytomatosis	MC29	chicken	carcinoma, sarcoma, & myelocytoma	*myc*
Abelson leukemia	MuLV	mouse	B cell lymphoma	*abl*
Reticuloendotheliosis	REV-T	turkey	lymphatic leukemia	*rel*
Avian erythroblastosis	AEV	chicken	erythroleukemia & fibrosarcoma	*erbB,A*
Avian myeloblastosis	AMV	chicken	myeloblastic leukemia	*myb*

Figure 30.14 Each transforming retrovirus carries an oncogene derived from a cellular gene. Viruses have names and abbreviations reflecting the history of their isolation and the types of tumor they cause. This list shows some representative examples of the retroviral oncogenes.

More than 30 *c-onc* genes have been identified so far by their representation in retroviruses. Sometimes the same *c-onc* gene is represented in different transforming viruses; for example, the monkey virus SSV and the PI strain of the feline virus FeSV both carry a *v-onc* derived from *c-sis*. Some viruses carry related *v-onc* genes, such as in the Harvey and Kirsten strains of MuSV, which carry *v-ras* genes derived from two different members of the cellular *c-ras* gene family. In other cases the *v-onc* genes of related viruses represent unrelated cellular progenitors; for example, three different isolates of FeSV may have been derived from the same original (nontransforming) virus, but have transduced the *sis*, *fms*, and *fes* oncogenes. The events involved in formation of a transducing virus can be complex; some viruses include sequences derived from more than one cellular gene (859).

Given the rarity of the transducing event, it is significant that multiple independent isolates occur representing the same *c-onc* gene. For example, several viruses carry *v-myc* genes. They are all derived from a single *c-myc* gene, but the *v-myc* genes differ in their exact ends and in individual point mutations. The identification of such isolates probably means that we have identified most of the genes of the *c-onc* type that can be activated by viral transduction.

Direct evidence that expression of the *v-onc* sequence accomplishes transformation was first obtained with RSV (see *Great Experiments 10.1 Identification of a retroviral transforming gene*). Temperature-sensitive mutations in *v-src* allow the transformed phenotype to be reverted by increase in temperature, and regained by decrease in temperature. This shows clearly that in this case the *v-src* gene is needed both to initiate and maintain the transformed state.

Reviews

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ONCOGENES AND CANCER

6.30.8 Quantitative or qualitative changes can explain oncogenicity

Key Concepts

- An oncogene usually has qualitatively different (transforming) effects from the proto-oncogene as the result of mutational changes.
- Sometimes the oncogene is transforming because it is expressed at higher levels than the proto-oncogene.

Two general types of theory might explain the difference in properties between *v-onc* genes and *c-onc* genes:

- A quantitative model proposes that viral genes are functionally indistinguishable from the cellular genes, but are oncogenic because they are expressed in much greater amounts or in inappropriate cell types, or because their expression cannot be switched off.
- A qualitative model supposes that the *c-onc* genes intrinsically lack oncogenic properties, but may be converted by mutation into oncogenes whose devastating effects reflect the acquisition of new properties (or loss of old properties).

How closely related are *v-onc* genes to the corresponding *c-onc* genes? In some cases, the only changes are a very small number of point mutations. The *mos*, *sis*, and *myc* genes offer examples in which the entire *c-onc* gene has been gained by the virus; in this case, the small number of amino acid substitutions do not seem to affect function of the protein, and in fact are not required for transforming activity. So the *v-onc* product is likely to fulfill the same enzymatic or other functions as the *c-onc* product, but with some change in its regulation; in these cases, overexpression is responsible for oncogenicity. A good example is *c-myc*, where oncogenicity may be caused by overexpression either by a *v-myc* gene carried by a transforming retrovirus or by changes in the cellular genome that cause overexpression of *c-myc* (for review see 338).

Two cases in which point mutations play a critical role in creating an oncogenic protein are presented by *ras* and *src*.

In the case of *ras*, changes in the regulation of Ras activity that activate the protein can be directly attributed to the individual point mutations that have occurred in the *v-onc* gene. Overexpression of *c-ras* may have weak oncogenic effects, but full oncogenicity requires sequence changes in the protein.

In some cases, a *v-onc* gene is truncated by the loss of sequences from the N-terminus or C-terminus (or both) of the *c-onc* gene, probably as a result of the sites involved in the recombination event that generated it. Loss of these regions may

remove some regulatory constraint that normally limits the activity of the *c-onc* product. Such sequence changes are required for oncogenicity of *src*. *v-src* is oncogenic at low levels of protein, but *c-src* is not oncogenic at high protein levels (>10× normal). The viral and cellular *src* genes are coextensive, but *v-src* has replaced the C-terminal 19 amino acids of *c-src* with a different sequence of 12 amino acids. This has an important regulatory consequence, in activating the Src protein constitutively. In cases where *v-onc* genes are truncations of *c-onc* genes, point mutations may also contribute to the oncogenicity of the *v-onc* product. In the case of Src, changes in two tyrosine residues that are targets for phosphorylation have strong effects on oncogenicity (see *Molecular Biology 6.30.16 Src is the prototype for the proto-oncogenic cytoplasmic tyrosine kinases*).

The characterization of transforming retroviruses played an important role in the definition of oncogenes. However, most events involved in human cancers do not involve viral intermediates, and other mechanisms are responsible for generating oncogenes. But the concept that oncogenes arise by activation of proto-oncogenes is an important paradigm for animal cancers.

Reviews

338. Bishop, J. M. (1985). *Viral oncogenes*. Cell 42, 23-38.

ONCOGENES AND CANCER

6.30.9 Ras oncogenes can be detected in a transfection assay

Key Concepts

- Transformed cells can be distinguished from normal cells by the formation of foci in a culture dish.
- DNA extracted from tumor cells can transform 3T3 target cells.
- A transforming cellular (*c-onc*) gene often has a homologue (*v-onc*) in a transforming retrovirus.
- The *ras* genes are the most common transforming genes identified by this method.

Some oncogenes can be detected by using a direct assay for transformation in which "normal" recipient cells are transfected with DNA obtained from animal tumors (for discussion of the discovery see *Great Experiments 10.3 The discovery of oncogenes in human tumors*). The procedure is illustrated in **Figure 30.15**. The established mouse NIH 3T3 fibroblast line usually is used as recipient. Historically these experiments started by using DNA extracted *en masse*, but now they are usually performed with a purified oncogene. The ability of any individual gene to convert wild-type cells into the transformed state constitutes one form of proof that it is an oncogene. Another assay that can be used is to inject cells into "nude" mice (which lack the ability to reject such transplants immunologically). The ability to form tumors can then be measured directly in the animal.

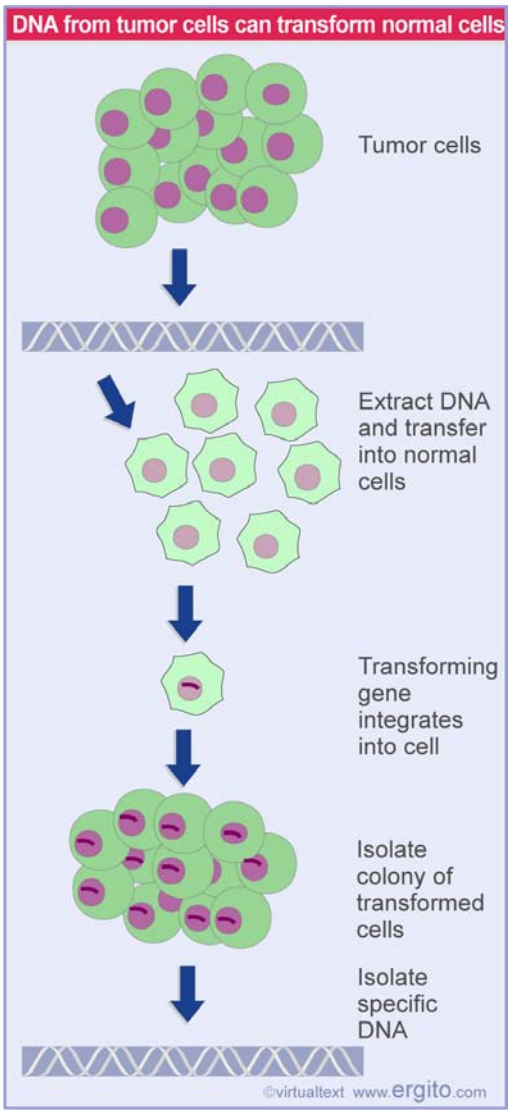

Figure 30.15 The transfection assay allows (some) oncogenes to be isolated directly by assaying DNA of tumor cells for the ability to transform normal cells into tumorigenic cells.

When a cell is transformed in a 3T3 culture (or some other "normal" culture), its descendants pile up into a focus. The appearance of foci is used as a measure of the transforming ability of a DNA preparation. Starting with a preparation of DNA isolated from tumor cells, the efficiency of focus formation is low. However, once the transforming gene has been isolated and cloned, greater efficiencies can be obtained. In fact, the transforming "strength" of a gene can be characterized by the efficiency of focus formation by the cloned sequence.

DNA with transforming activity can be isolated only from tumorigenic cells; it is not present in normal DNA. The transforming genes isolated by this assay have two revealing properties:

- *They have closely related sequences in the DNA of normal cells.* This argues that transformation was caused by mutation of a normal cellular gene (a proto-oncogene) to generate an oncogene. The change may take the form of a point mutation or more extensive reorganization of DNA around the *c-onc* gene.

- *They may have counterparts in the oncogenes carried by known transforming viruses.* This suggests that the repertoire of proto-oncogenes is limited, and probably the same genes are targets for mutations to generate oncogenes in the cellular genome or to become viral oncogenes.

Oncogenes derived from the *c-ras* family are often detected in the transfection assay. The family consists of several active genes in both man and rat, dispersed in the genome. (There are also some pseudogenes.) The individual genes, *N-ras*, *H-ras*, and *K-ras*, are closely related, and code for protein products ~21 kD and known collectively as $p21^{ras}$.

The *H-ras* and *K-ras* genes have *v-ras* counterparts, carried by the Harvey and Kirsten strains of murine sarcoma virus, respectively (see **Figure 30.14**). Each *v-ras* gene is closely related to the corresponding *c-ras* gene, with only a few individual amino acid substitutions. The Harvey and Kirsten virus strains must have originated in independent recombination events in which a progenitor virus gained the corresponding *c-ras* sequence.

ONCOGENES AND CANCER

6.30.10 Ras proto-oncogenes can be activated by mutation at specific positions

Key Concepts

- *v-ras* genes are derived by point mutations of *c-ras* genes.
- The same mutations occur in the *v-ras* genes of transforming viruses and the mutant *c-ras* genes of tumor cells.
- Almost any mutation at either position 12 or 61 converts a *c-ras* proto-oncogene into a transforming variety.
- The effect of the mutations is to increase Ras activity by inhibiting the hydrolysis of bound GTP to GDP.

Oncogenic variants of the *c-ras* genes are found in transforming DNA preparations obtained from various primary tumors and tumor cell lines. Each of the *c-ras* proto-oncogenes can give rise to a transforming oncogene by a single base mutation. *The mutations in several independent human tumors cause substitution of a single amino acid, most commonly at position 12 or 61, in one of the Ras proteins.*

Position 12 is one of the residues that is mutated in the *v-H-ras* and *v-K-ras* genes. *So mutations occur at the same positions in* v-ras *genes in retroviruses and in mutant* c-ras *genes in multiple rat and human tumors.* This suggests that the normal c-Ras protein can be converted into a tumorigenic form by a mutation in one of a few codons in rat or man (and perhaps any mammal).

The general principle established by this work is that *substitution in the coding sequence can convert a cellular proto-oncogene into an oncogene.* Such an oncogene can be associated with the appearance of a spontaneous tumor in the organism. It may also be carried by a retrovirus, in which case a tumor is induced by viral infection.

The *ras* genes appear to be finely balanced at the edge of oncogenesis. Almost any mutation at either position 12 or 61 can convert a *c-ras* proto-oncogene into an active oncogene:

- All three *c-ras* genes have glycine at position 12. If it is replaced *in vitro* by any other of the 19 amino acids except proline, the mutated *c-ras* gene can transform cultured cells. The particular substitution influences the strength of the transforming ability.
- Position 61 is occupied by glutamine in wild-type *c-ras* genes. Its change to another amino acid usually creates a gene with transforming potential. Some substitutions are less effective than others; proline and glutamic acid are the only

substitutions that have no effect.

When the expression of a normal *c-ras* gene is increased, either by placing it under control of a more active promoter or by introducing multiple copies into transfected cells, recipient cells are transformed. Some mutant *c-ras* genes that have changes in the protein sequence also have a mutation in an intron that increases the level of expression (by increasing processing of mRNA ~10×). Also, some tumor lines have amplified *ras* genes. A 20-fold increase in the level of a nontransforming Ras protein is sufficient to allow the transformation of some cells. The effect has not been fully quantitated, but it suggests the general conclusion that oncogenesis depends on over-activity of Ras protein, and is caused either by increasing the amount of protein or (more efficiently) by mutations that increase the activity of the protein (for review see 340).

Transfection by DNA can be used to transform only certain cell types. Limitations of the assay explain why relatively few oncogenes have been detected by transfection. This system has been most effective with *ras* genes, where there is extensive correlation between mutations that activate *c-ras* genes in transfection and the occurrence of tumors.

Ras is a monomeric guanine nucleotide-binding protein that is active when bound to GTP and inactive when bound to GDP. It has an intrinsic GTPase activity. **Figure 30.16** reviews the discussion of *Molecular Biology 6.28.15 The activation of Ras is controlled by GTP* in which we saw that the conversion between the two forms of Ras is catalyzed by other proteins. GAP proteins stimulate the ability of Ras to hydrolyze GTP, thus converting active Ras into inactive Ras. GEF proteins stimulate the replacement of GDP by GTP, thus reactivating the protein.

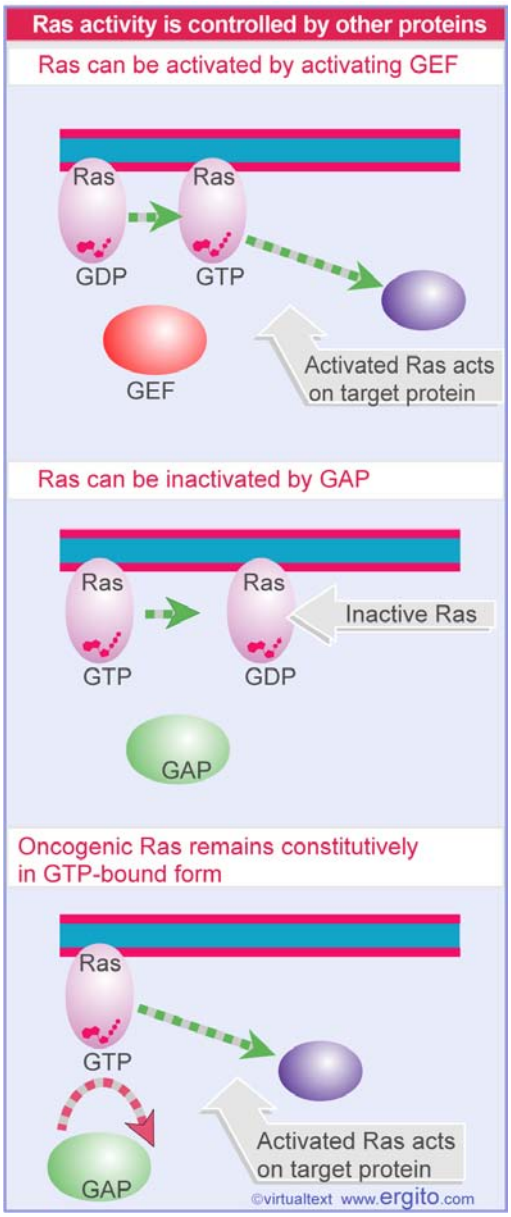

Figure 30.16 Pathways that rely on Ras could function by controlling either GEF or GAP. Oncogenic Ras mutants are refractory to control, because Ras remains in the active form.

Constitutive activation of Ras could be caused by mutations that allow the GDP-bound form of Ras to be active or that prevent hydrolysis of GTP. What are the effects of the mutations that create oncogenic *ras* genes? Many mutations that confer transforming activity inhibit the GTPase activity. GAP cannot increase the GTPase activity of Ras proteins that have been activated by oncogenic mutations. In other words, Ras has become refractory to the interaction with GAP that turns off its activity. Inability to hydrolyze GTP causes Ras to remain in a permanently activated form; its continued action upon its target protein is responsible for its oncogenic activity (for review see 353).

This establishes an important principle: *constitutive activation of a cellular protein may be oncogenic.* In the case of Ras, its effects result from activating the ERK MAP kinase pathway and (possibly) other pathways. The level of expression is finely balanced, since overstimulation of Ras by either increase in expression or mutation of the protein has oncogenic consequences (although mutation is required for a full effect).

Reviews

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ONCOGENES AND CANCER

6.30.11 Nondefective retroviruses activate proto-oncogenes

Key Concepts

- Increased expression of *c-myc* is transforming.
- *c-myc* can be activated by insertion of a nondefective retrovirus near the gene.

A variety of genomic changes can activate proto-oncogenes, sometimes involving a change in the target gene itself, sometimes activating it without changing the protein product. Insertion, translocation, and amplification can be causative events in tumorigenesis.

Many tumor cell lines have visible regions of chromosomal amplification, as shown by homogeneously staining regions (see **Figure 18.32**) or double minute chromosomes (see **Figure 18.33**). The amplified region may include an oncogene. Examples of oncogenes that are amplified in various tumors include *c-myc*, *c-abl*, *c-myb*, *c-erbB*, *c-K-ras*, and *Mdm2*.

Established cell lines are prone to amplify genes (along with other karyotypic changes to which they are susceptible). The presence of known oncogenes in the amplified regions, and the consistent amplification of particular oncogenes in many independent tumors of the same type, strengthens the correlation between increased expression and tumor growth.

Some proto-oncogenes are activated by events that change their expression, but which leave their coding sequence unaltered. The best characterized is *c-myc*, whose expression is elevated by several mechanisms. One common mechanism is the insertion of a nondefective retrovirus in the vicinity of the gene.

The ability of a retrovirus to transform without expressing a *v-onc* sequence was first noted during analysis of the bursal lymphomas caused by the transformation of B lymphocytes with avian leukemia virus. Similar events occur in the induction of T-cell lymphomas by murine leukemia virus. In each case, the transforming potential of the retrovirus is due to the ability of its LTR (the long terminal repeat of the integrated form) to cause expression of cellular gene(s).

In many independent tumors, the virus has integrated into the cellular genome within or close to the *c-myc* gene. **Figure 30.17** summarizes the types of insertions. The retrovirus may be inserted at a variety of locations relative to the *c-myc* gene.

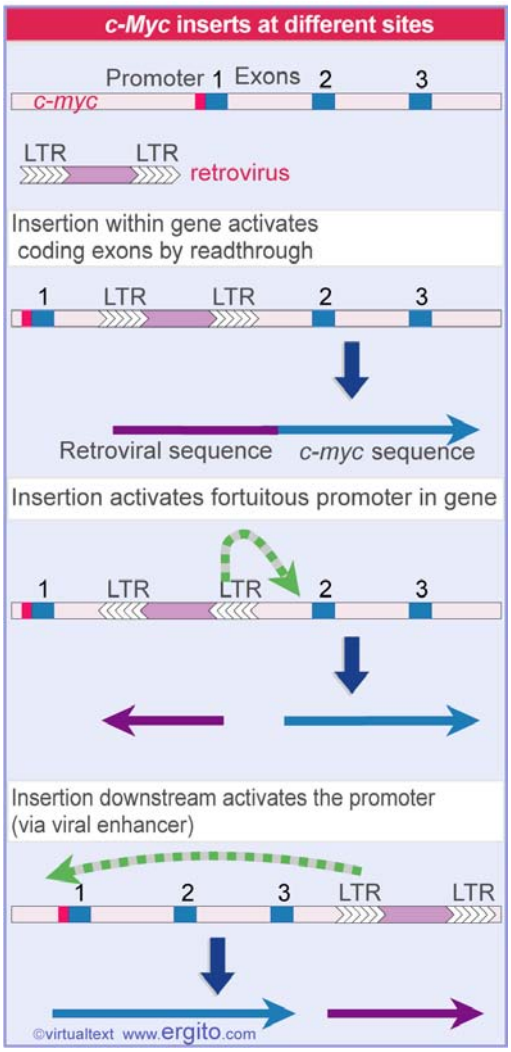

Figure 30.17 Insertions of ALV at the c-myc locus occur at various positions, and activate the gene in different ways.

The gene consists of three exons; the first represents a long nontranslated leader, and the second two code for the c-Myc protein. The simplest insertions to explain are those that occur within the first intron. The LTR provides a promoter, and transcription reads through the two coding exons. Transcription of *c-myc* under viral control differs from its usual control: the level of expression is increased (because the LTR provides an efficient promoter); expression cannot be switched off in B or T cells in response to the usual differentiation signals; and the transcript lacks its usual nontranslated leader (which may usually limit expression). All of these changes add up to increased constitutive expression.

Activation of *c-myc* in the other two classes of insertions reflects different mechanisms. The retroviral genome may be inserted within or upstream of the first intron, but in reverse orientation, so that its promoter points in the wrong direction. The retroviral genome also may be inserted downstream of the *c-myc* gene. In these cases, the enhancer in the viral LTR may be responsible for activating transcription

of c-Myc, either from its normal promoter or from a fortuitous promoter.

In all of these cases, the coding sequence of c-myc *is unchanged, so oncogenicity is attributed to the loss of normal control and increased expression of the gene*.

Other oncogenes that are activated in tumors by the insertion of a retroviral genome include *c-erbB, c-myb, c-mos, c-H-ras,* and *c-raf.* Up to 10 other cellular genes (not previously identified as oncogenes by their presence in transforming viruses) are implicated as potential oncogenes by this criterion. The best characterized among this latter class are *wnt1* and *int2.* The *wnt1* gene codes for a protein involved in early embryogenesis that is related to the *wingless* gene of *Drosophila; int2* codes for an FGF (fibroblast growth factor).

ONCOGENES AND CANCER

6.30.12 Proto-oncogenes can be activated by translocation

Key Terms

A **reciprocal translocation** exchanges part of one chromosome with part of another chromosome.

Key Concepts

- *c-myc* can be activated in lymphocytes by translocations involving the Ig or TCR loci, giving B cell or T cell tumors.

Translocation to a new chromosomal location is another of the mechanisms by which oncogenes are activated. A **reciprocal translocation** occurs when an illegitimate recombination occurs between two chromosomes as illustrated in **Figure 30.18**. The involvement of such events in tumorigenesis was discovered via a connection between the loci coding immunoglobulins and the occurrence of certain tumors. Specific chromosomal translocations are often associated with tumors that arise from undifferentiated B lymphocytes. The common feature is that an oncogene on one chromosome is brought by translocation into the proximity of an Ig locus on another chromosome. Similar events occur in T lymphocytes to bring oncogenes into the proximity of a TCR locus (for review see 341).

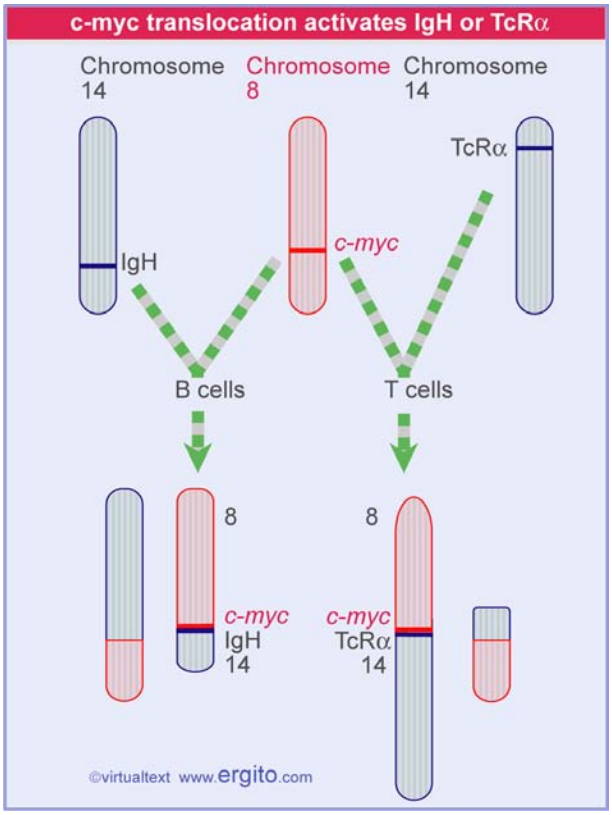

Figure 30.18 A chromosomal translocation is a reciprocal event that exchanges parts of two chromosomes. Translocations that activate the human *c-myc* proto-oncogene involve Ig loci in B cells and TCR loci in T cells.

In both man and mouse, the nonimmune partner is often the *c-myc* locus. In man, the translocations in B-cell tumors usually involve chromosome 8, which carries *c-myc*, and chromosome 14, which carries the IgH locus; ~10% involve chromosome 8 and either chromosome 2 (kappa locus) or chromosome 22 (lambda locus). The translocations in T-cell tumors often involve chromosome 8, and either chromosome 14 (which has the TCR α locus at the other end from the Ig locus) or chromosome 7 (which carries the TCR β locus). Analogous translocations occur in the mouse.

Translocations in B cells fall into two classes, reflecting the two types of recombination that occur in immunoglobulin genes. One type is similar to those involved in the somatic recombination that generates the active genes. These events involve the consensus sequences used for V-D-J recombination. These can occur at all the Ig loci. In the other type, the translocation occurs at a switching site at the IgH locus, presumably reflecting the operation of the system for class switching.

When *c-myc* is translocated to the Ig locus, its level of expression is usually increased. The increase varies considerably among individual tumors, generally being in the range from 2–10× . Why does translocation activate the *c-myc* gene? The event has two consequences: *c-myc* is brought into a new region, one in which an Ig or TCR gene was actively expressed; and the structure of the *c-myc* gene may itself be changed (but usually not involving the coding regions). It seems likely that several different mechanisms can activate the *c-myc* gene in its new location (just as

retroviral insertions activate *c-myc* in a variety of ways).

The correlation between the tumorigenic phenotype and the activation of *c-myc* by either insertion or translocation suggests that continued high expression of c-Myc protein is oncogenic. Expression of *c-myc* must be switched off to enable immature lymphocytes to differentiate into mature B and T cells; failure to turn off *c-myc* maintains the cells in the undifferentiated (dividing) state.

The oncogenic potential of *c-myc* has been demonstrated directly by the creation of transgenic mice. Mice carrying a *c-myc* gene linked to a B lymphocyte-specific enhancer (the IgH enhancer) develop lymphomas. The tumors represent both immature and mature B lymphocytes, suggesting that overexpression of *c-myc* is tumorigenic throughout the B cell lineage. Transgenic mice carrying a *c-myc* gene under the control of the LTR from a mouse mammary tumor virus, however, develop a variety of cancers, including mammary carcinomas. This suggests that increased or continued expression of *c-myc* transforms the type of cell in which it occurs into a corresponding tumor (for review see 345; 347).

c-myc exhibits three means of oncogene activation: retroviral insertion, chromosomal translocation, and gene amplification. The common thread among them is deregulated expression of the oncogene rather than a qualitative change in its coding function, although in at least some cases the transcript has lost the usual (and possibly regulatory) nontranslated leader. *c-myc* provides the paradigm for oncogenes that may be effectively activated by increased (or possibly altered) expression.

Translocations are now known in many types of tumors. Often a specific chromosomal site is commonly involved, creating the supposition that a locus at that site is involved in tumorigenesis. However, every translocation generates reciprocal products; sometimes a known oncogene is activated in one of the products, but in other cases it is not evident which of the reciprocal products has responsibility for oncogenicity. Also, it is not axiomatic that the gene(s) at the breakpoint have responsibility; for example, the translocation could provide an enhancer that activates another gene nearby.

A variety of translocations found in B and T cells have identified new oncogenes. In some cases, the translocation generates a hybrid gene, in which an active transcription unit is broken by the translocation. This has the result that the exons of one gene may be connected to another. In such cases, there are two potential causes of oncogenicity. The proto-oncogene part of the protein may be activated in some way that is independent of the other part, for example, because it is over-expressed under its new management (a situation directly comparable to the example of *c-myc*). Or the other partner in the hybrid gene may have some positive effect that generates a gain-of-function in the part of the protein coded by the proto-oncogene.

Reviews

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ONCOGENES AND CANCER

6.30.13 The Philadelphia translocation generates a new oncogene

Key Concepts

- The Philadelphia translocations create new genes with N-terminal sequences from *bcr* and C-terminal sequences from *c-abl*.
- Both parts of the fusion protein contribute to oncogenicity, which results from activation of the Ras/MAPK pathway.

One of the best characterized cases in which a translocation creates a hybrid oncogene is provided by the *Philadelphia (PH^1)* chromosome present in patients with chronic myelogenous leukemia (CML). This reciprocal translocation is too small to be visible in the karyotype, but links a 5000 kb region from the end of chromosome 9 carrying *c-abl* to the *bcr* gene of chromosome 22. The *bcr* (*b*reakpoint *c*luster *r*egion) was originally named to describe a region of ~5.8 kb within which breakpoints occur on chromosome 22.

The consequences of this translocation are summarized in **Figure 30.19**. The *bcr* region lies within a large (>90 kb) gene, which is now known as the *bcr* gene. The breakpoints in CML usually occur within one of two introns in the middle of the gene. The same gene is also involved in translocations that generate another disease, ALL (acute lymphoblastic leukemia); in this case, the breakpoint in the *bcr* gene occurs in the first intron.

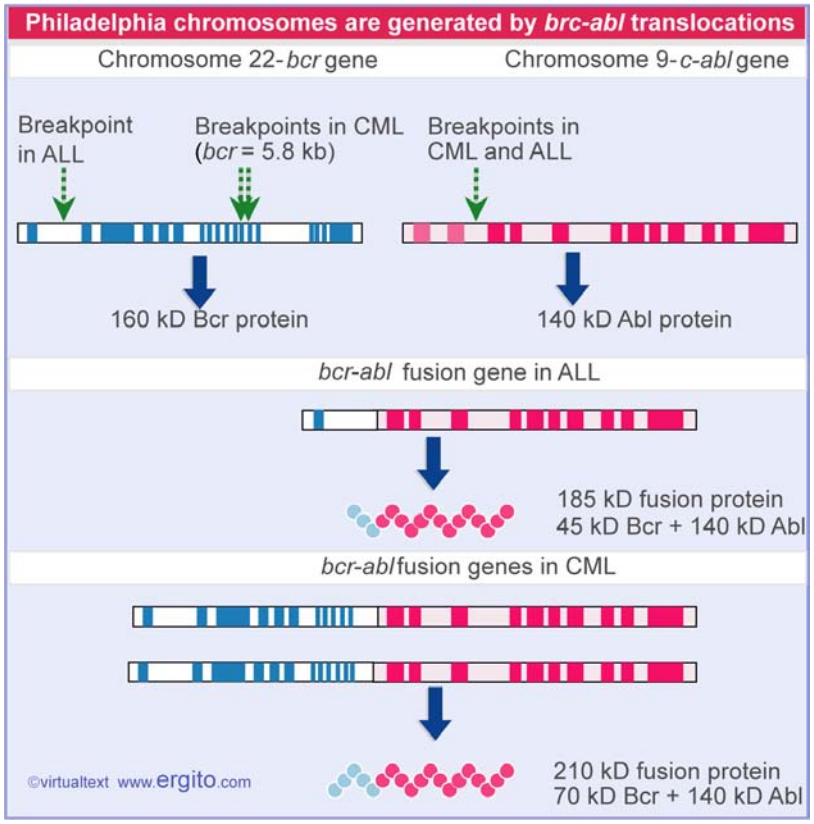

Figure 30.19 Translocations between chromosome 22 and chromosome 9 generate Philadelphia chromosomes that synthesize *bcr-abl* fusion transcripts that are responsible for two types of leukemia.

The *c-abl* gene is expressed by alternative splicing that uses either of the first two exons. The breakpoints in both CML and ALL occur in the intron that precedes the first common exon. Although the exact breakpoints on both chromosomes 9 and 22 vary in individual cases, the common outcome is the production of a transcript coding for a Bcr-Abl fusion protein, in which N-terminal sequences derived from *bcr* are linked to *c-abl* sequences. In ALL, the fusion protein has ~45 kD of the Bcr protein; in CML the fusion protein has ~70 kD of the Bcr protein.

In each case, the fusion protein contains ~140 kD of the usual ~145 kD c-Abl protein, that is, it has lost just a few N-terminal amino acids of the *c-abl* sequence. Changes at the N-terminus are involved in activating the oncogenic activity of *v-abl*, a transforming version of the gene carried in a retrovirus. The *c-abl* gene codes for a tyrosine kinase activity; this activity is essential for transforming potential in oncogenic variants. Deletion (or replacement) of the N-terminal region activates the kinase activity and transforming capacity. So the N-terminus provides a domain that usually regulates kinase activity; its loss may cause inappropriate activation.

Why is the fusion protein oncogenic? The Bcr-Abl protein activates the Ras pathway for transformation. It may have multiple ways of doing so, including activation of the adaptors Grb2 and Shc (see *Molecular Biology 6.28.14 The Ras/MAPK pathway is widely conserved*). Both the Bcr and Abl regions of the joint protein may be important in transforming activity.

ONCOGENES AND CANCER

6.30.14 Oncogenes code for components of signal transduction cascades

Key Concepts

- Oncogenes can be derived from any part of a signal transduction cascade, from the initiating growth factor or receptor to the transcription factor that is the ultimate effector.
- Upstream and downstream components of the Ras pathway are often involved, although not the MAP kinases themselves.

Whether activated by quantitative or qualitative changes, oncogenes may be presumed to influence (directly or indirectly) functions connected with cell growth. Transformed cells lack restrictions imposed on normal cells, such as dependence on serum or inhibition by cell-cell contact. They may acquire new properties, such as the ability to metastasize. Many phenotypic properties are changed when we compare a normal cell with a tumorigenic counterpart, and it is striking indeed that individual genes can be identified that trigger many of the changes associated with this transformation.

We assume that oncogenes, individually or in concert, set in train a series of phenotypic changes that involve the products of many genes. In this description, we see at once a similarity with genes that regulate developmental pathways: they do not themselves necessarily code for the products that characterize the differentiated cells, but they may direct a cell and its progeny to enter a particular pathway. The same analogy suggests itself for oncogenes and developmental regulators: they provide switches responsible for causing transitions between one discrete phenotypic state and another.

Taking this relationship further, we may ask what activities the products of proto-oncogenes play in the normal cell, and how they are changed in the transformed cell. Could some proto-oncogenes be regulators of normal development whose malfunction results in aberrations of growth that are manifested as tumors? There are some examples of such relationships, but do not yet have any systematic understanding of the connection.

Signal transduction pathways are often involved in oncogenesis. The best characterized example is c-Ras, which plays a central role in transmitting the signal from receptor tyrosine kinases (see *Molecular Biology 6.28.14 The Ras/MAPK pathway is widely conserved*). Oncogenic mutations change the regulation of Ras activity.

Oncoproteins are organized according to their types of functions in **Figure 30.20**. The left part of the figure groups the oncogenes according to the locations of their products. The boxes on the right give details of the corresponding proto-oncogenes. The functions of many oncogenes remain unknown, and further groups will no doubt

be identified:

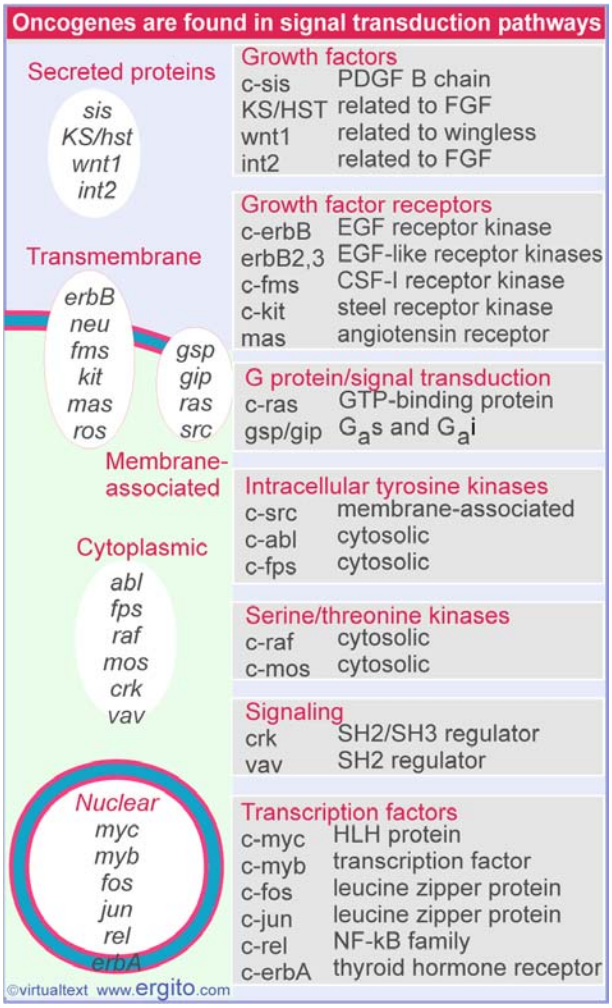

Figure 30.20 Oncogenes may code for secreted proteins, transmembrane proteins, cytoplasmic proteins, or nuclear proteins.

- Growth factors are proteins secreted by one cell that act on another. The oncoprotein counterparts can only transform cells bearing the appropriate receptor. (This is called autocrine transformation; for review see 337; 349.)

- The growth factor receptors are transmembrane proteins that are activated by binding an extracellular ligand (usually a polypeptide). Most often the receptor is a protein tyrosine kinase. Oncogenicity may result from constitutive (that is, ligand-independent) activation of the kinase activity (862). Other early stages in signal transduction are identified by Gsp and Gip, which are mutant forms of the α subunits of the G_s and G_i trimeric G proteins.

- An important group of intracellular protein kinases phosphorylate tyrosine residues in target proteins. c-Src, which associates with the cytoskeleton as well as with the membrane, is the prototype of a family of kinases with similar

catalytic activities (including c-Yes, c-Fgr, Lck, c-Fps, Fyn). We understand the effects of oncogenic mutations on the Src kinase activity in some detail, although we have yet to explain why the altered kinase activity is oncogenic. Other protein tyrosine kinases in the intracellular group are cytosolic; c-Abl is found in both cytosol and nucleus (860; 861; for review see 344).

- A group of cytosolic enzymes are protein serine/threonine kinases, that is, they phosphorylate target proteins on serine or threonine. Little is known about the effects of oncogenic mutations beyond the fact they probably increase or constitutively activate the kinase activities. Mos is an example which can activate ERK MAPK.

- Nuclear proteins include transcription factors of several types. The functions of these proto-oncoproteins are rather well described (see *Molecular Biology 5.22 Activating transcription*). Generally we understand what effects the oncogenic mutations have on the factors, but we cannot yet relate these changes to the activation or repression of a set of target genes that defines the oncogenic state (863; 864; 865).

The common feature is that each type of protein is in a position to trigger general changes in cell phenotypes, either by initiating or responding to changes associated with cell growth, or by changing gene expression directly. Before we consider in detail the potential of each group for initiating a series of events that has an oncogenic outcome, we need to consider how many independent pathways are identified by these factors.

Recall the example of the best characterized mitogenic pathway, the MAPK pathway which consists of the following stages:

growth factor

↓

growth factor receptor (tyrosine kinase)

↓

Ras

↓

kinase cascade (serine/threonine kinases)

↓

transcription factor(s)

When a growth factor interacts with its receptor, it activates the tyrosine kinase activity. The signal is passed (via an adaptor) to Ras. At this point, the pathway

switches to a series of serine/threonine kinases. The targets at the end of the pathway may be controlled directly or indirectly by phosphorylation, and include transcription factors, which are in a position to make widespread changes in the pattern of gene expression.

If a pathway functions in a linear manner, in which the signal passes directly from one component to the next, the same results should be achieved by constitutive activation of any component (so that it no longer needs to be activated by a signal from an earlier component).

A signal transduction pathway, of course, is likely to branch at several stages, so that an initial stimulus may trigger a variety of responses. The activation of components that are downstream will therefore activate a smaller number of end-functions than the activation of components at the start of the pathway. But we can analyze any individual part of the pathway by tracing it back to the beginning as though it were strictly linear.

In the example of the Ras pathway, we know that it is activated by many growth factors to generate a mitogenic response. Mutations in the early part of this pathway, including the *ras* and *raf* genes, may be oncogenic. But oncogenic mutations are not usually found in the following components of the cascade, the MEK and MAP kinases. This suggests that there may be a branch in the pathway at the stage of *ras* or *raf*, and that activation of this branch is also necessary for oncogenicity. Ras activates a cytoskeletal GTPase called Rac, which may identify this branch. However, the ERK MAPK pathway terminates in the activation of several "immediate early" genes, including *fos* and *jun*, which themselves have oncogenic counterparts, suggesting that the targets of the MAPK pathway can be sufficient for oncogenicity.

The central role of this pathway is indicated by the number of its components that are coded by proto-oncogenes. One explanation of the discrepancies between the susceptibilities of MAP kinases and other components to oncogenic mutation may be that the *level* or *duration* of expression is important. It could be the case that mutations in MEK or MAP kinases do not activate the enzymes sufficiently to be oncogenic. Alternatively, the oncogenic mutations (which, after all, represent gain-of-function) may cause new targets to be activated in addition to the usual pathway. The general principle is clear: *that aberrant activation of mitogenic pathways can contribute to oncogenicity*, but we cannot yet explain exactly how the activation of these pathways changes the properties of the cell in terms of immortalization or transformation (for review see 348).

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ONCOGENES AND CANCER

6.30.15 Growth factor receptor kinases can be mutated to oncogenes

Key Concepts

- Oncogenes are generated by mutations that constitutively activate growth factor receptor genes.
- The type of tumor reflects the phenotypes of the cells in which the receptor is expressed.

The protein tyrosine kinases constitute a major class of oncoproteins, and fall into two general groups: transmembrane receptors for growth factors; and cytoplasmic enzymes. We have more understanding about the biological functions of the receptors, because we know the general nature of the signal transduction cascades that they initiate, and we can see how their inappropriate activation may be oncogenic. The normal roles in the cell of the cytoplasmic tyrosine kinases are not so well defined, but in several cases it appears that they provide catalytic functions for receptors that themselves lack kinase activity; that is, the activation of the receptor leads to activation of the cytoplasmic tyrosine kinase. We have a great deal of information about their enzymatic activities and the molecular effects of oncogenic mutations, although it has been more difficult to identify their physiological targets.

Receptors for many growth factors have kinase activity. They tend to be large integral membrane proteins, with domains assembled in modular fashion from a variety of sources. We discussed the general nature of transmembrane receptors and the means by which they are activated to initiate signal transduction cascades in *Molecular Biology 6.28.8 Growth factor receptors are protein kinases*. The EGF receptor is the paradigm for tyrosine kinase receptors. The extracellular N-terminal region binds the ligand that activates the receptor. The intracellular C-terminal region includes a domain that has tyrosine kinase activity. Most of the receptors that are coded by cellular proto-oncogenes have a similar form of organization.

Dimerization of the extracellular domain of a receptor activates the tyrosine kinase activity of the intracellular domain. Various forms of this reaction were summarized previously in **Figure 28.17**. When the cytoplasmic domains of the monomers are brought into contact, they trigger an autophosphorylation reaction, in which each monomer phosphorylates the other (see *Molecular Biology 6.28.9 Receptors are activated by dimerization*).

A (generalized) relationship between a growth factor receptor and an oncogenic variant is illustrated in **Figure 30.21**. The wild-type receptor is regulated by ligand binding. In the absence of ligand, the monomers do not interact. Growth factor binding triggers an interaction, allowing the receptor to form dimers. This in turn activates the receptor, and triggers signal transduction. By contrast, the oncogenic variant spontaneously forms dimers that are constitutively active. Different types of events may be responsible for the constitutive dimerization and activation in different

growth factor receptors (for review see 3648; 3649; 3652).

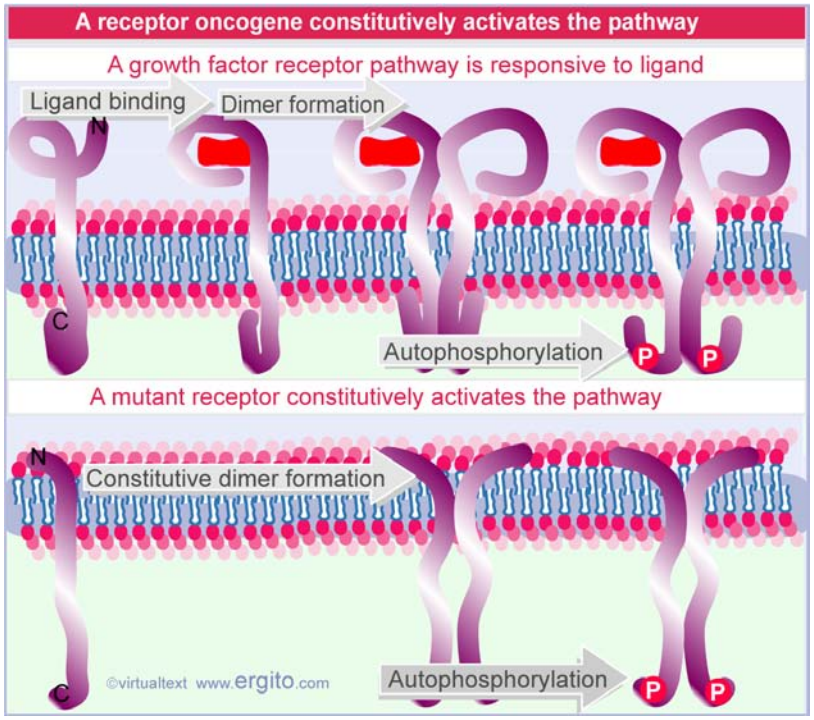

Figure 30.21 Activation of a growth factor receptor involves ligand binding, dimerization, and autophosphorylation. A truncated oncogenic receptor that lacks the ligand-binding region is constitutively active because it is not repressed by the N-terminal domain.

The oncogene *v-erb* is a truncated version of *c-erbB*, the gene coding for the EGF receptor. The oncoprotein retains the tyrosine kinase and transmembrane domains, but lacks the N-terminal part of the protein that binds EGF, and does not have the C-terminus. The deletions at both ends may be needed for oncogenicity. The change in the extracellular N-terminal domain allows the protein to dimerize spontaneously; and the C-terminal deletion removes a cytosolic domain that inhibits transforming activity. There is also an activating mutation in the catalytic domain. So the basis for oncogenicity is the combination of mutations that activate the receptor constitutively (3647).

The general principle that constitutive or altered activity may be responsible for oncogenicity applies to the group of growth factor receptors summarized previously in **Figure 30.20**. Another example of an activation event is provided by *erbB2*, which codes for a receptor closely related to the EGF receptor. An oncogenic form has a key mutation in its transmembrane region; this increases the propensity of the receptor monomers to form dimers (3646).

Some proto-oncogenes code for receptors or factors involved in the development of particular cell types. Mutation of such a receptor (or growth factor) may promote unrestricted growth of cells of the appropriate type. The proto-oncogene *c-fms* codes for the CSF-I receptor, which mediates the action of colony stimulating factor I, a macrophage growth factor that stimulates the growth and maturation of myeloid

precursor cells (3651). *c-fms* can be rendered oncogenic by a mutation in the extracellular domain; this increases dimerization and makes the protein constitutively active in the absence of CSF-I. Oncogenicity is enhanced by C-terminal mutations, which could act by inactivating an inhibitory intracellular domain (for review see 3653).

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ONCOGENES AND CANCER

6.30.16 Src is the prototype for the proto-oncogenic cytoplasmic tyrosine kinases

Key Concepts

- The cytoplasmic tyrosine kinases phosphorylate tyrosine residues in cytosolic proteins.
- Myristoylation of the N-terminus enables Src to associate with the plasma membrane.
- The crucial cellular targets for Src remain unidentified.

The cellular action and basis for oncogenicity of the cytoplasmic group of protein tyrosine kinases is more obscure. The cytoplasmic group is characterized by the viral oncogenes *src, yes, fgr, fps/fes, abl, ros.* (c-Src is actually associated with membranes.) A major stretch of the sequences of all these genes is related, corresponding to residues 80-516 of *c-src.* This includes the SH2 and SH3 domains and the catalytic domain responsible for kinase activity. Presumably the regions outside this domain control the activities of the individual members of the family. In few cases, however, do we know the cellular function of a *c-onc* member of this group.

The paradigm for a cytoplasmic tyrosine kinase in search of a role is presented by the Src proteins. Since its isolation by Rous in 1911, RSV has been perpetuated under a variety of conditions, and there are now several "strains," carrying variants of *v-src.* The common feature in the sequence of *v-src* is that the C-terminal sequence of *c-src* has been replaced. The various strains contain different point mutations within the *src* sequence (3654; 3655; 3656).

Proteins in the Src family were the first oncoproteins of the kinase type to be characterized. Src was also the first example of a kinase whose target is a tyrosine residue in protein. The level of phosphotyrosine is increased about 10× in cells that have been transformed by RSV. In addition to acting on other proteins, Src is able to phosphorylate itself.

Src proteins have several interesting features. **Figure 30.22** summarizes their activities in terms of protein domains.

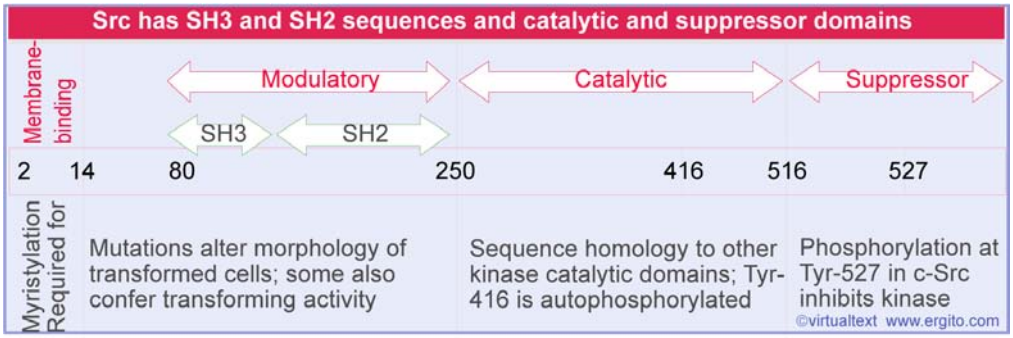

Figure 30.22 A Src protein has an N-terminal domain that associates with the membrane, a modulatory domain that includes SH2 and SH3 motifs, a kinase catalytic domain, and (c-Src only) a suppressor domain.

Both v-Src and c-Src are modified at the N-terminus. The N-terminal amino acid is cleaved, and myristic acid (a rare fatty acid of 14 carbon residues) is covalently added to the N-terminal glycine. Myristoylation enables Src proteins to attach to the cytosolic face of membranes in the cytoplasm. Most of the protein is associated with the cytoplasmic face of the endosomes, and it is enriched in regions where there are cell-to-cell contacts and adhesion plaques.

Myristoylation is essential for oncogenic activity of v-Src, since N-terminal mutants that cannot be myristoylated have reduced tumorigenicity. The simplest explanation for the dependence of transformation on the membrane location of v-Src is that important substrates for Src are located in the membrane.

The biological action of v-Src is qualitatively different from that of c-Src, since increased concentrations of c-Src cannot fully transform cells (3658). The major biochemical difference between v-Src and c-Src lies in their kinase activities. The activity of v-Src is ~20× greater than that of c-Src. The transforming activity of *src* mutants is correlated with the level of kinase activity, and we believe that oncogenicity results from phosphorylation of target protein(s). We do not know whether the increased activity is itself responsible for oncogenicity or whether there is also a change in the specificity with which target proteins are recognized.

Kinase activity plays two roles in Src function (for review see 3657). First, attempts to identify a function for the phosphorylation in cell transformation have concentrated on identifying cellular substrates that may be targets for v-Src (especially those that may not be recognized by c-Src). A variety of substrates has been identified, but none has yet been equated with the cause of transformation. Second, the state of phosphorylation of Src itself controls the transforming activity (see *Molecular Biology 6.30.17 Src activity is controlled by phosphorylation*).

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ONCOGENES AND CANCER

6.30.17 Src activity is controlled by phosphorylation

Key Concepts

- Src autophosphorylates and its activity is controlled by the state of phosphorylation at two Tyr residues.
- Oncogenic variants are derived from c-Src by mutations that cause decreased phosphorylation at Tyr-527 and increased phosphorylation at Tyr-416.
- v-Src lacks Tyr-527 and is constitutively active.
- Src was the protein in which the SH2 and SH3 motifs were originally identified.

Two sites in Src control its kinase activity. It is inactivated by phosphorylation at tyrosine residue 527, which is part of the C-terminal sequence of 19 amino acids that is missing from v-Src. The c-Src protein is phosphorylated *in vivo* at this position by the kinase Csk, which maintains it in an inactive state. Src is activated by phosphorylation at Tyr-416, which is located in the activation loop of the kinase domain.

The importance of these phosphorylations can be tested by mutating the tyrosine residues at 416 and 527 to prevent addition of phosphate groups. The mutations have opposite effects, as summarized in **Figure 30.23**:

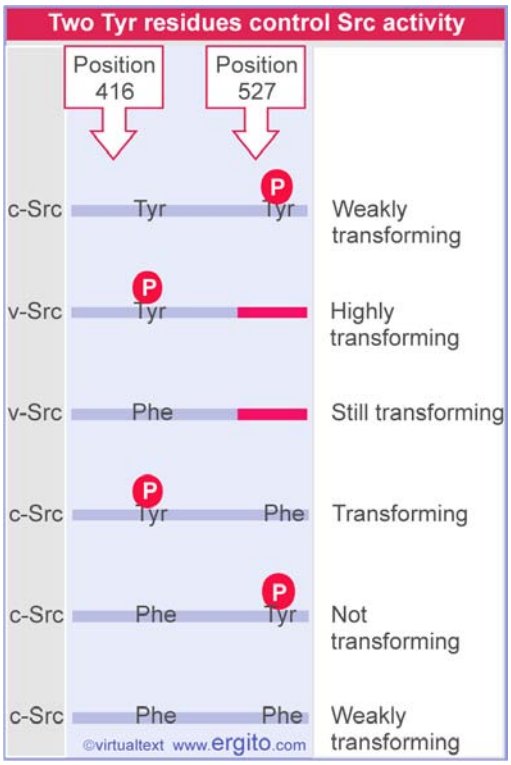

Figure 30.23 Two tyrosine residues are targets for phosphorylation in Src proteins. Phosphorylation at Tyr-527 of c-Src suppresses autophosphorylation at Tyr-416, which is associated with transforming activity. Only Tyr-416 is present in v-Src. Transforming potential of c-Src may be activated by removing Tyr-527 or repressed by removing Tyr-416.

- Mutation of Tyr-527 to the related amino acid phenylalanine activates the transforming potential of c-Src. The protein c-Src$^{Phe\text{-}527}$ becomes phosphorylated on Tyr-416, has its kinase activity increased ~10× , and it transforms target cells, although not as effectively as v-Src. *Phosphorylation of Tyr-527 therefore represses the oncogenicity of c-Src. Removal of this residue when the C-terminal region was lost in generating v-Src contributes significantly to the oncogenic activity of the transforming protein.*

- Mutation of Tyr-416 in c-Src eliminates its residual ability to transform. This mutation also greatly reduces the activity of the c-Src$^{Phe\text{-}527}$ mutant. It also reduces the transforming potential of v-Src, but less effectively. *Phosphorylation at Tyr-416 therefore activates the oncogenicity of Src proteins.*

Point mutations at other positions in c-Src support a correlation in which oncogenicity is associated with decreased phosphorylation at Tyr-527 and increased phosphorylation at Tyr-416. The states of these tyrosines may therefore be a general indicator of the oncogenic potential of c-Src. The reduced phosphorylation at Tyr-527 is responsible for the increased phosphorylation at Tyr-416, which is the crucial event. However, v-Src is less dependent on the state of Tyr-416, and mutants at this position retain transforming activity; presumably *v-src* has accumulated other

mutations that increase transforming potential.

What is the function of c-Src; and how is it related to the oncogenicity of v-Src? The c-Src and v-Src proteins are very similar: they share N-terminal modification, cellular location, and protein tyrosine kinase activity. c-Src is expressed at high levels in several types of terminally differentiated cells, which suggests that it is not involved in regulating cell proliferation. But we have so far been unable to determine the normal function of c-Src. A very large number of proteins have been identified as targets for the Src kinase, most of them identified with signaling pathways, and some with the interactions of the cell with the environment (for review see 3659). c-Src is activated by growth factor receptors, such as the PDGF receptor, suggesting the general view that, like other oncogenes, it is involved in signaling pathways that regulate growth which can be tumorigenic when constitutively activated.

The modulatory region of c-Src contains two motifs that are found in a variety of other cytoplasmic proteins that are involved in signal transduction: these may connect a protein to the components that are upstream and downstream of it in a signaling pathway. The names of these two domains, SH2 and SH3, reflect their original identification as regions of *S*rc *h*omology (see *Molecular Biology 6.28.11 Signaling pathways often involve protein-protein interactions*).

How is c-Src usually activated? Most mutations in the SH2 region reduce transforming activity (suggesting that the SH2 function is required to activate c-Src), and most mutations in SH3 increase transforming activity (suggesting that SH3 has a negative regulatory role). **Figure 30.24** shows a more detailed model for the function of the SH2 domain. The state of phosphorylation at Tyr-527 is critical. In the inactive state, Tyr-527 is phosphorylated, and this enables the C-terminal region of c-Src itself to bind to the N-terminal SH2 domain. When an appropriate receptor tyrosine kinase (such as PDGF receptor) is activated, its autophosphorylation creates an SH2-binding site that displaces Tyr-527. This leads to its dephosphorylation, which triggers a change in conformation allowing Tyr-416 to be phosphorylated.

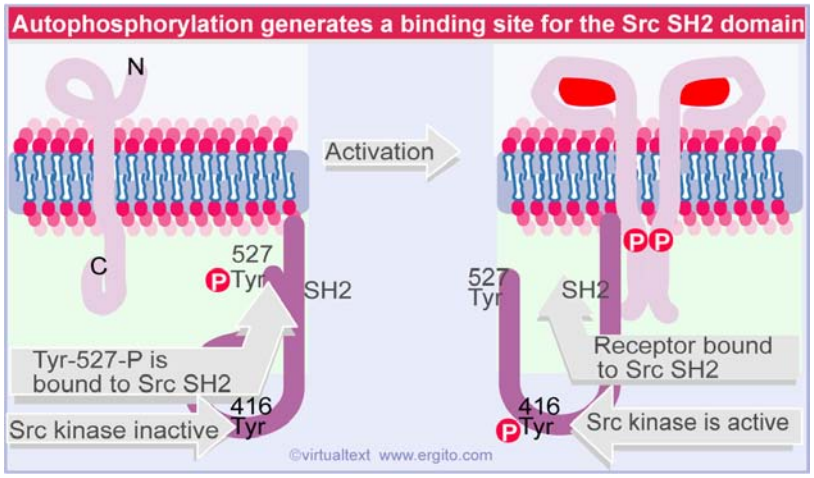

Figure 30.24 When a receptor tyrosine kinase is activated, autophosphorylation generates a binding site for the Src SH2 domain, Tyr-527 is released and dephosphorylated, Tyr-416 becomes phosphorylated, and Src kinase is activated.
This is a static version of an interactive figure; see http://www.ergito.com/main.jsp?bcs=MBIO.6.30.17 to view properly.

Figure 30.25 shows a schematic model based on the crystal structure of Src (3660). The SH2 domain binds to a C-terminal projection of the kinase domain that contains Tyr-527. The SH3 domain binds to a short sequence that connects the SH2 and catalytic domains. The SH2 and SH3 domains are at the back of the kinase domain, so these interactions lock the enzyme in an inactive state. The activation loop in the kinase domain is in a state that does not allow Tyr-416 to be phosphorylated. An activator (such as an activated membrane receptor) binds to both the SH2 and SH3 domains. This causes dephosphorylation of Tyr-527, which triggers an unfolding of the activator loop that allows Tyr-416 to be phosphorylated. The oncogenic v-Src protein is constitutively active because it lacks Tyr-527, so the inactive state cannot be formed.

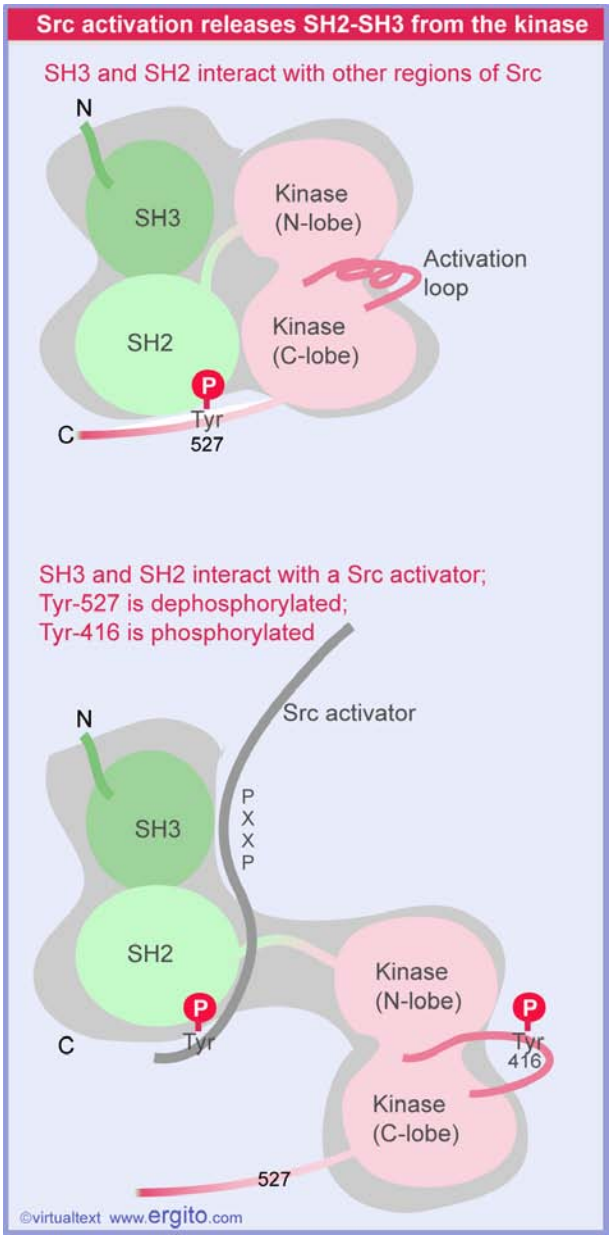

Figure 30.25 Src is inactive when the SH2 domain binds the Tyr-phosphate at position 527, and the SH3 domain binds the connector between kinase and SH2 domains. These interactions are disrupted when a Src activator binds to the SH2 and SH3 domains. This causes Tyr-527 to be dephosphorylated, and changes the conformation of the activation loop of the kinase domain so that Tyr-416 can be phosphorylated.

Alternative ways for activating c-Src may be involved in some oncogenic reactions. For example, the polyoma middle T antigen activates c-Src by binding to the C-terminal region including Tyr-527 and prevents its phosphorylation. Some mutations in the SH2 domain of c-Src can activate the kinase activity (with oncogenic consequences), presumably because they prevent it from sequestering Tyr-527. Mutations in the SH2 and SH3 domains of c-Src can influence its

specificity with regard to transforming different types of target cells, which suggests that these regions provide the connections to other (cell specific) proteins in the pathway.

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ONCOGENES AND CANCER

6.30.18 Oncoproteins may regulate gene expression

Key Concepts

- The *rel* oncogene is a member of the NF κB family.
- The *jun* and *fos* oncogenes code for the subunits of the transcription factor AP1.
- Screening with high density DNA microarrays allows the pattern of gene expression to be compared between tumor cells and normal cells.

It goes almost without saying that it is necessary to make changes in gene expression in order to convert a cell to the transformed phenotype. Many oncogenes act at early stages in pathways that lead ultimately to changes in gene expression. Some act directly at the level of transcription. Retroviral oncogenes include examples derived from the major classes of cellular transcription factors. Several prominent gene families coding for transcription factors are identified by *v-onc* genes: *rel*, *jun*, *fos*, *erbA*, *myc*, and *myb*. In the cases of Rel, Jun, and ErbA proteins, there are differences in transcriptional activity between the c-Onc and v-Onc proteins that may be related to transforming capacity.

The actions of *v-onc* genes may in principle be quantitative or qualitative; and those that affect transcription might either increase or decrease expression of particular genes. By virtue of increased expression or activity they could turn up transcription of genes whose products can be tolerated only in small amounts. Failure to respond to normal regulation of activity by other cellular factors also might lead to increased gene expression. A less likely possibility is the acquisition of specificity for new target promoters. Alternatively, if the oncoproteins are defective in the ability to activate transcription, they might function as dominant negative suppressors of the cellular transcription factors. The first steps towards distinguishing these possibilities lie with determining which functions are altered in v-Onc compared with c-Onc proteins: is DNA-binding altered either quantitatively or quantitatively; is the ability to activate transcription altered? **Figure 30.26** summarizes the properties of some of these oncoproteins.

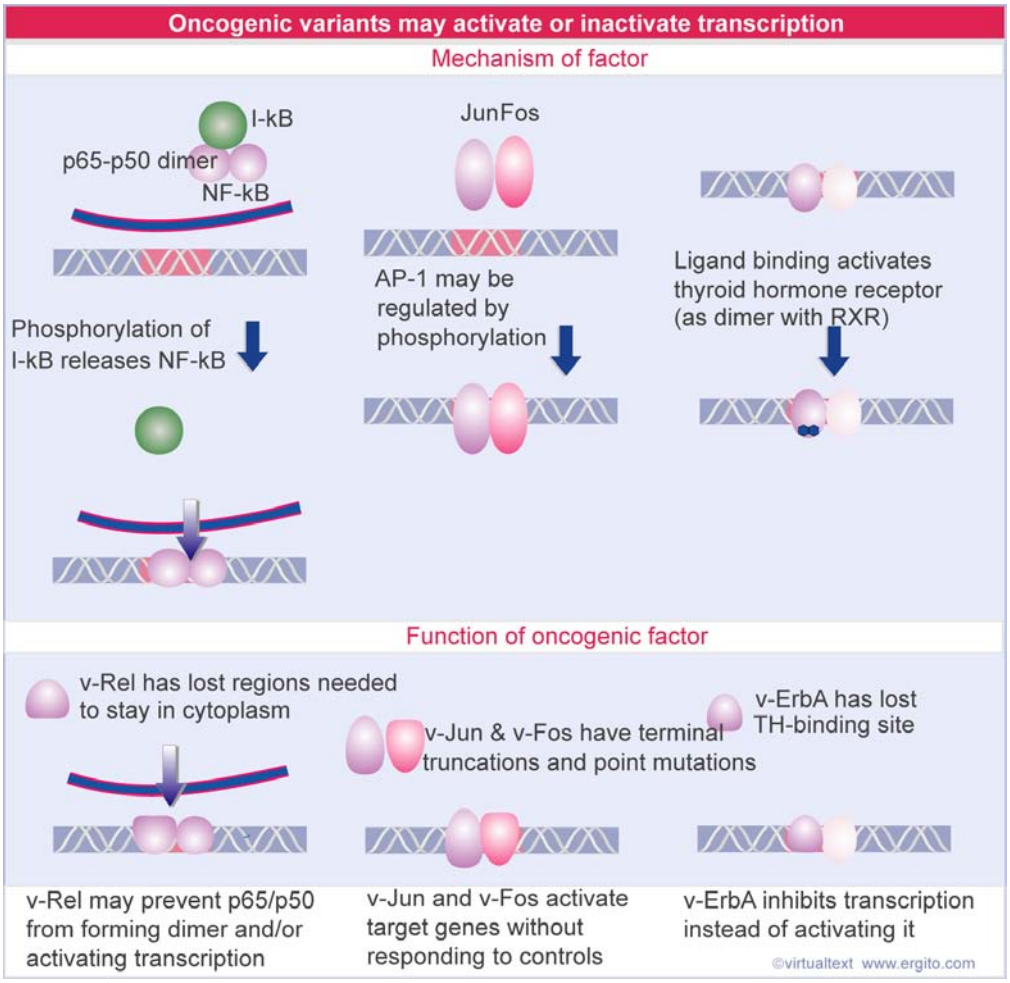

Figure 30.26 Oncogenes that code for transcription factors have mutations that inactivate transcription (v-erbA and possibly v-rel) or that activate transcription (v-jun and v-fos).

The oncogene *v-rel* was identified as the transforming function of the avian (turkey) reticuloendotheliosis virus. The retrovirus is highly oncogenic in chickens, where it causes B-cell lymphomas. *v-rel* is a truncated version of *c-rel*, lacking the ~100 C-terminal amino acids, and has a small number of point mutations in the remaining sequence (3661).

The *rel* gene belongs to a family whose best characterized member is the transcription factor NF- κB. This is a dimer of two subunits, p65 and p50, which is held in the cytoplasm by a regulator, I- κB. (Binding of I- κB masks the nuclear localization sequence in NF- κB). When I- κB is phosphorylated, it is degraded and therefore releases NF- κB, which enters the nucleus and activates transcription of target genes whose promoters or enhancers have the κB motif . This regulatory story is essentially recapitulated in *Drosophila* development, where *dorsal* codes for an NF- κB homologue that is held in the cytoplasm by the *cactus* product, an I κB homologue (see *Molecular Biology 6.31.11 Dorsal protein forms a gradient of nuclear localization*). The two subunits of NF- κB have related sequences, and *c-rel* has 60% similarity with p50 (3662; 3663; 3664).

NF- κB is one of the most pleiotropic transcription factors; indeed, it has been suggested that it may constitute a general second messenger. Many types of stimulus to the cell result in activation of NF- κb and a broad range of genes is activated via the presence of κB binding sites. The members of the NF- κB family form various pairwise combinations that regulate transcription. When v-Rel forms dimers with cellular family members, it may influence their activities either negatively or positively, thus changing the pattern of gene expression (for review see 3665). v-Rel is exclusively nuclear, because it has lost the sequences required for export to the cytoplasm.

The transcription factor AP1 is the nuclear factor required to mediate transcription induced by phorbol ester tumor promoters (such as TPA). An AP1 binding site confers TPA-inducibility upon a target gene. The canonical AP1 factor consists of a dimer of two subunits, coded by the genes *c-jun* and *c-fos*, which activates genes whose promoters or enhancers have an AP1 binding site (863; 865; 3666).

Jun and Fos are transcription factors of the leucine zipper class. Each protein is a member of a family, and a series of pairwise interactions between Jun family members and Fos family members may generate a series of transcription factors related to AP1. Mutations of *v-jun* or *v-fos* that abolish the ability to bind DNA or that damage the transactivation function also render the product non-transforming, providing a direct proof that ability to activate transcription is required for transforming activity (3667).

c-Jun is activated by phosphorylation on two serine residues by the action of the kinase JNK, which is activated by the Ras pathway, and this contributes to the transforming action of Ras. The transforming activity of v-Jun has a more complex basis. v-Jun has a deletion of amino acids 34-60 that includes both these sites of phosphorylation, and so is not regulated by the Ras pathway. Other changes in v-Jun make it constitutively active. However, v-Jun can also interfere with the ability of c-Jun to activate some of its target genes. The transforming activity of v-Jun may therefore depend on both quantitative changes (overexpression or under-expression of particular target genes) or qualitative changes (alteration in the pattern of genes that responds to the factor).

The cellular gene *c-erbA* codes for a thyroid hormone receptor, a member of the general class of steroid hormone receptors (see *Molecular Biology 5.22.10 Steroid receptors are activators*). Upon binding its ligand, a typical steroid receptor activates expression of particular target genes by binding to its specific response element in a promoter or enhancer. The mode of action for thyroid hormone is distinct: it is located permanently in the nucleus, and, indeed, may bind its response element whether or not ligand is present. The effect of hormone binding may therefore be to activate transcription by previously bound receptor.

Ability to bind DNA is required for transforming capacity. *v-erbA* is truncated at both ends and has a small number of substitutions relative to *c-erbA.* Hormone binding is altered; the *c-erbA* product binds triiodothyronine (T_3) with high affinity, but the *v-erbA* product has little or no affinity for the ligand in mammalian cells. This suggests that loss of the ligand-binding capacity (perhaps together with other changes) may create a protein whose function has become independent of the hormone. The consequence of losing the response to ligand is that the factor can no longer be stimulated to activate transcription.

These results place *v-erbA* as a dominant negative oncogene, one that functions by overcoming the action of its normal cellular counterpart. Its action is to prevent transcription of genes that usually are activated by c-ErbA (3668). The implication is that genes activated by c-ErbA act to *suppress* transformation. In this particular case, it seems likely that these genes usually promote differentiation; blocking this action allows the cells to proliferate.

c-jun, *c-fos*, *c-rel*, and also *c-myc* are "immediate early" genes, members of a class of genes that are rapidly induced when resting cells are treated with mitogens, which suggests that they may be involved in a cascade that initiates cycling. So their targets are likely to be concerned with initiating or promoting growth. We should therefore expect an increase in their activities to be associated with oncogenesis, an expectation that may be fulfilled for *v-fos* and *v-myc*, but does not explain the behavior of *v-rel*.

The adenovirus oncogene E1A provides an example of a protein that regulates gene expression indirectly, that is, without itself binding to DNA. The E1A region is expressed as three transcripts, derived by alternative splicing, as indicated in **Figure 30.27**. The 13S and 12S mRNAs code for closely related proteins and are produced early in infection. They possess the ability to immortalize cells, and can cooperate with other oncoproteins (notably Ras) to transform primary cells (see later). No other viral function is needed for this activity (for review see 343).

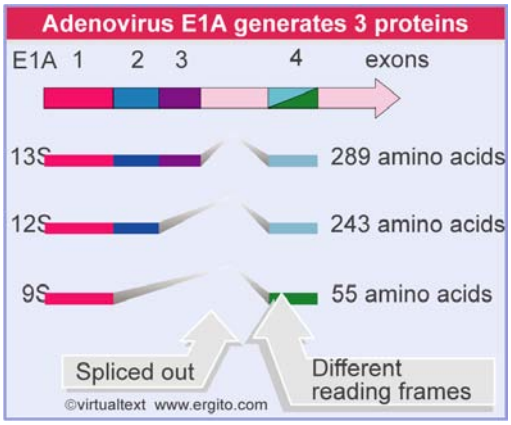

Figure 30.27 The adenovirus E1A region is spliced to form three transcripts that code for overlapping proteins. Domain 1 is present in all proteins, domain 2 in the 289 and 243 residue proteins, and domain 3 is unique to the 289 residue protein. The C-terminal domain of the 55 residue protein is translated in a different reading frame from the common C-terminal domains of the other two proteins.

The E1A proteins exercise a variety of effects on gene expression. They activate the transcription of some genes, but repress others. Mutation of the E1A proteins suggests that transcriptional activation requires only the short region of domain 3, found only in the 289 amino acid protein coded by 13S mRNA. Repression of transcription, induction of DNA synthesis, and morphological transformation all require domains 1 and 2, common to both the 289 and 243 amino acid proteins. This suggests that repression of target genes is required to cause transformation. E1A

proteins act by binding to several cellular proteins that in turn repress or activate transcription of appropriate target genes. Among these targets are the CBP and p300 coactivators, the TBP basal transcription factor, and the cell cycle regulators RB and p27.

A powerful new approach to analyzing the roles of individual genes in cancer has been made possible by the development of techniques to allow simultaneous screening for the expression of many genes. High density DNA microarrays contain probes to the mRNAs of up to 10,000 genes (typically immobilized on a glass slide). The technique is at its most effective for comparing the genes expressed in two related cell types. The technique can be applied to a tumor cell when it is possible to compare it with the original cell type from which it arose, or can be used to compare related tumor cells with different properties. This gives insights into the extent of change in gene expression, and ultimately can be used to identify the particular genes that are involved in stages of cancer development.

Tumor cell lines can be obtained that vary in their ability to metastasize (to spread from the site of origin to colonize new sites in the body). A highly metastatic cell line can be selected from a line that is poorly metastatic without apparently changing the properties of the tumor as such – only the ability to spread appears to be affected. A comparison in two such cases showed that only a small number (<20) genes have a significant change in transcription. This suggests the possibility that metastasis involves relatively minor changes in the pattern of gene expression. Among the affected genes are several whose products act on the actin cytoskeleton, suggesting that changes in cellular motility and/or adhesion to other cells or substratum could be the basis for metastasis (1063).

More widespread changes were detected when benign prostate tumors were compared with metastatic cancers . On average, expression of 55 genes was increased, and expression of 480 genes was decreased (3211). This is interesting in suggesting that cancer progression may have a major contribution from the inhibition of functions that usually suppress cell growth or motility. One of the genes whose expression is increased is *EZH2*, a member of the Polycomb group that forms complex involved in repressing the activity of chromatin (see *Molecular Biology 5.23.16 Polycomb and trithorax are antagonistic repressors and activators*). *EZH2* is responsible for repressing about one third of the genes whose expression is decreased in the metastatic prostate cancer (3212).

Last updated on 1-3-2003

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ONCOGENES AND CANCER

6.30.19 RB is a tumor suppressor that controls the cell cycle

Key Concepts

- Loss of both alleles of *RB* causes retinoblastomas.
- Nonphosphorylated RB prevents cell proliferation.
- In the normal cell cycle, phosphorylation of RB by cdk-cyclin kinases is necessary to proceed into S phase.
- Certain tumor antigens suppress the inhibitory action by sequestering the nonphosphorylated form of RB.
- Several tumor suppressors act by blocking the cdk-kinase complexes that phosphorylate RB.

The common theme in the role of oncogenes in tumorigenesis is that increased or altered activity of the gene product is oncogenic. Whether the oncogene is introduced by a virus or results from a mutation in the genome, it is dominant over its allelic proto-oncogene(s). A mutation that activates a single allele is tumorigenic. Tumorigenesis then results from gain of a function.

Certain tumors are caused by a different mechanism: loss of both alleles at a locus is tumorigenic. Propensity to form such tumors may be inherited through the germline; it also occurs as the result of somatic change in the individual. Such cases identify tumor suppressors: genes whose products are needed for normal cell function, and whose loss of function causes tumors. The two best characterized genes of this class code for the proteins RB and p53.

Retinoblastoma is a human childhood disease, involving a tumor of the retina. It occurs both as a heritable trait and sporadically (by somatic mutation). It is often associated with deletions of band q14 of human chromosome 13. The *RB* gene has been localized to this region by molecular cloning.

Figure 30.28 summarizes the situation. Retinoblastoma arises when both copies of the *RB* gene are inactivated. In the inherited form of the disease, one parental chromosome carries an alteration in this region. A somatic event in retinal cells that causes loss of the other copy of the *RB* gene causes a tumor. In the sporadic form of the disease, the parental chromosomes are normal, and both *RB* alleles are lost by (individual) somatic events (872).

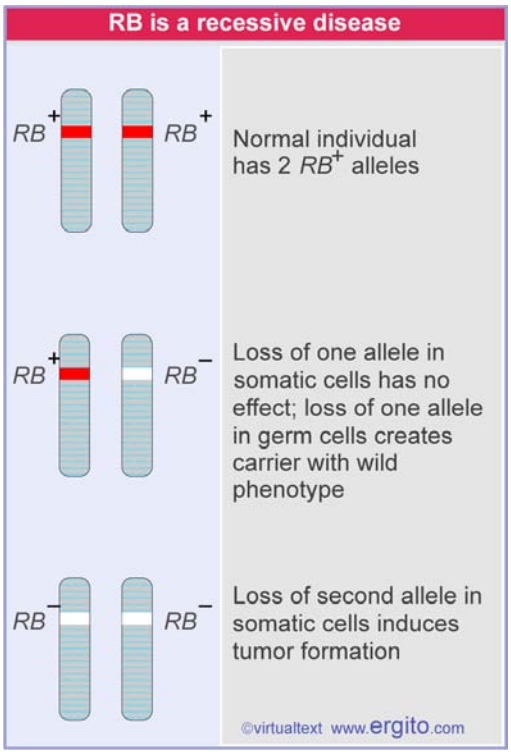

Figure 30.28 Retinoblastoma is caused by loss of both copies of the RB gene in chromosome band 13q14. In the inherited form, one chromosome has a deletion in this region, and the second copy is lost by somatic mutation in the individual. In the sporadic form, both copies are lost by individual somatic events.

The cause of retinoblastoma is therefore loss of protein function, usually resulting from mutations that prevent gene expression (as opposed to point mutations that affect function of the protein product). Loss of *RB* is involved also in other forms of cancer, including osteosarcomas and small cell lung cancers.

RB is a nuclear phosphoprotein that influences the cell cycle (see *Molecular Biology 6.29.17 G0/G1 and G1/S transitions involve cdk inhibitors*). In resting (G0/G1) cells, RB is not phosphorylated. RB is phosphorylated during the cell cycle by cyclin/cdk complexes, most particularly at the end of G1; it is dephosphorylated during mitosis. The nonphosphorylated form of RB specifically binds several proteins, and these interactions therefore occur only during part of the cell cycle (prior to S phase). Phosphorylation releases these proteins.

The target proteins include the E2F group of transcription factors, which activate target genes whose products are essential for S phase. Binding to RB inhibits the ability of E2F to activate transcription, which suggests that RB blocks the expression of genes dependent on E2F. In this way, RB indirectly prevents cells from entering S phase. Also, the RB-E2F complex directly represses some target genes, so its dissociation allows them to be expressed.

Certain viral tumor antigens bind specifically to the nonphosphorylated form of RB.

The best characterized are SV40 T antigen and adenovirus E1A. This suggests the model shown in **Figure 30.29**. Nonphosphorylated RB prevents cell proliferation; this activity must be suppressed in order to pass through the cell cycle, which is accomplished by the cyclic phosphorylation. And it may also be suppressed when a tumor antigen sequesters the nonphosphorylated RB. Because the RB-tumor antigen complex does not bind E2F, the E2F is permanently free to allow entry into S phase (and the RB-E2F complex is not available to repress its target genes) (871).

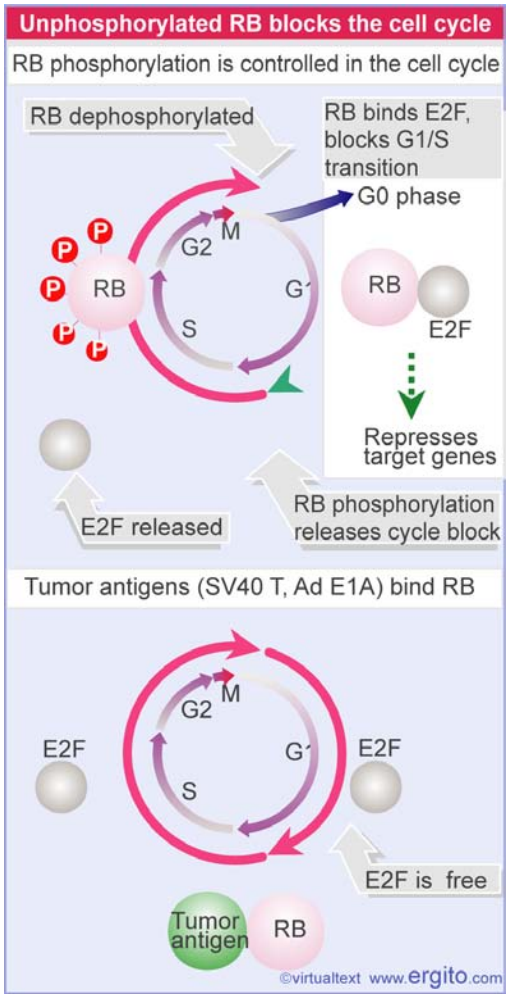

Figure 30.29 A block to the cell cycle is released when RB is phosphorylated (in the normal cycle) or when it is sequestered by a tumor antigen (in a transformed cell).

Overexpression of RB impedes cell growth. An indication of the importance of RB for cell proliferation is given by the properties of an osteosarcoma cell line that lacks RB; when RB is introduced into this cell line, its growth is impeded. However, the inhibition can be overcome by expression of D cyclins, which form cdk-cyclin combinations that phosphorylate RB. RB is not the only protein of its type: proteins with related sequences, called p107 and p130, have similar properties.

The connection between the cell cycle and tumorigenesis is illustrated in **Figure**

30.30. Several regulators are identified as tumor suppressors by the occurrence of inactivating mutations in tumors. As well as occurring in RB itself, mutations are found in the small inhibitory proteins (most notably p16 and possibly p21), and D cyclin(s). Although these proteins (most notably RB) play a role in the cycle of a proliferating cell, the role that is relevant for tumorigenesis is more probably their function in the quiescent (G0) state. In quiescent cells, RB is not phosphorylated, D cyclin levels are low or absent, and p16, p21, and p27 ensure inactivity of cdk-cyclin complexes.

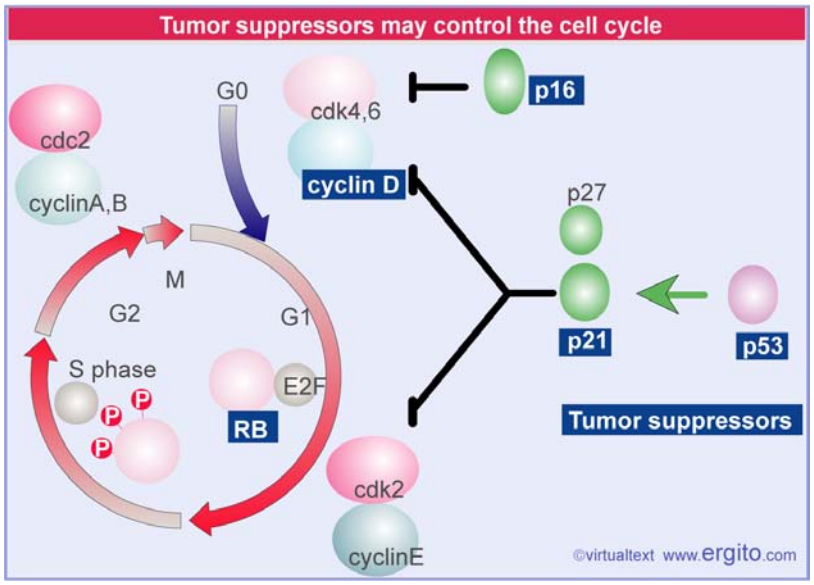

Figure 30.30 Several components concerned with G0/G1 or G1/S cycle control are found as tumor suppressors.

Cyclin D activity is needed for proliferation. Loss of the circuit that suppresses it (p16 or p21) causes unrestrained growth. The control of cyclin D is particularly important in breast cancer. The cyclin D1 gene is amplified in 20% of human breast cancers, and the protein is overexpressed in >50% of human mammary carcinomas. The role of cyclin D1 has been confirmed in mouse models by showing that increased D1 expression causes increased breast cancer (1922), while the deletion of the cyclin D1 gene prevents certain oncogenes from causing breast cancer (1923). Mammary tumors can be caused in cyclin D1-deficient mice by the oncogenes *myc* or *Wnt-1*, but not by the *ras* or *neu* oncogenes. The *neu* oncogene codes for a growth factor receptor that activates Ras, and the Ras pathway leads to activation of the promoter of the cyclin D1 gene, which explains the result. The Myc and Wnt-1 oncogenes must cause breast cancer by a different pathway.

Last updated on 7-23-2001

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ONCOGENES AND CANCER

6.30.20 Tumor suppressor p53 suppresses growth or triggers apoptosis

Key Terms

A **dominant negative** mutation results in a mutant gene product that prevents the function of the wild-type gene product, causing loss or reduction of gene activity in cells containing both the mutant and wild-type alleles. The effect may result from the titration of another factor that interacts with the gene product or by an inhibiting interaction of the mutant subunit on the multimer.

Key Concepts

- p53 is a tumor suppressor that is lost or inactivated in >50% of all human cancers.
- Wild-type p53 is activated by damage to DNA.
- The response may be to block cell cycle progression or to cause apoptosis depending on the circumstances.

The most important tumor suppressor is p53 (named for its molecular size). More than half of all human cancers either have lost p53 protein or have mutations in the gene, making loss of p53 by far and away the most common alteration in human cancer. Its effects have been demonstrated directly in mice, where loss of p53 alleles causes the occurrence of tumors. **Figure 30.31** shows the survival curves for wild type mice ($p53^{+/+}$), heterozygotes who have lost one allele ($p53^{+/-}$), and homozygotes who have lost both alleles ($p53^{-/-}$). The frequency of tumors is increased from 45% to 80% by loss of the first allele, causing the mice to die sooner; and loss of both alleles shortens the life span dramatically due to the occurrence of tumors in virtually 100% of the mice.

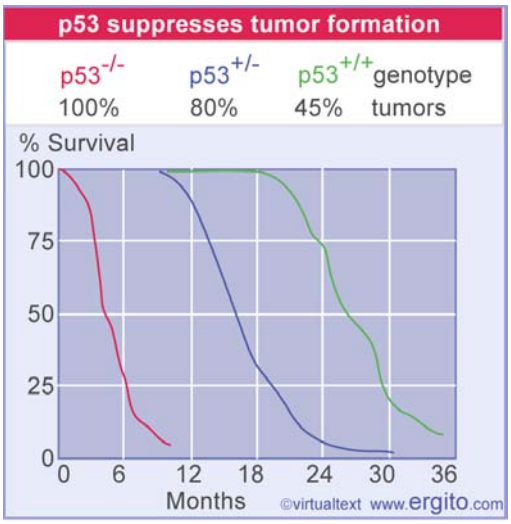

Figure 30.31 Loss of p53 alleles causes mice to die more rapidly because of the accumulation of tumors.

p53 is a nuclear phosphoprotein. It was originally discovered in SV40-transformed cells, where it is associated with the T antigen coded by the virus (see *Great Experiments 10.4 p53 is a tumor suppressor gene*). T antigen is needed to transform cells, and it was thought it might be acting through its effect on p53. A large increase in the amount of p53 protein is found in many transformed cells or lines derived from tumors. In early experiments, the introduction of cloned p53 was found to immortalize cells. These experiments caused p53 to be classified as an oncogene, with the usual trait of dominant gain-of-function (870; 873).

But all the transforming forms of p53 turned out to be mutant forms of the protein! They fall into the category of **dominant negative** mutants, which function by overwhelming the wild-type protein and preventing it from functioning (see *Molecular Biology Supplement 32.9 Complementation*). The most common form of a dominant negative mutant is one that forms a heteromeric protein containing both mutant and wild-type subunits, in which the wild-type subunits are unable to function. p53 exists as a tetramer. When mutant and wild-type subunits of p53 associate, the tetramer takes up the mutant conformation.

Figure 30.32 shows that the same phenotype is produced either by the deletion of both alleles or by a missense point mutation in one allele that produces a dominant negative subunit. Both situations are found in human cancers. Mutations in p53 accumulate in many types of human cancer, probably because loss of p53 provides a growth advantage to cells; that is, wild-type p53 restrains growth. The diversity of these cancers suggests that p53 is not involved in a tissue-specific event, but in some general and rather common control of cell proliferation; and the loss of this control may be a secondary event that occurs to assist the growth of many tumors. p53 is defined as a tumor suppressor also by the fact that wild-type p53 can suppress or inhibit the transformation of cells in culture by various oncogenes (for review see 351; 352). Mutant p53 cells also have an increased propensity to amplify DNA, which is likely to reflect p53's role in the characteristic instability of the genome that is found in cancer cells.

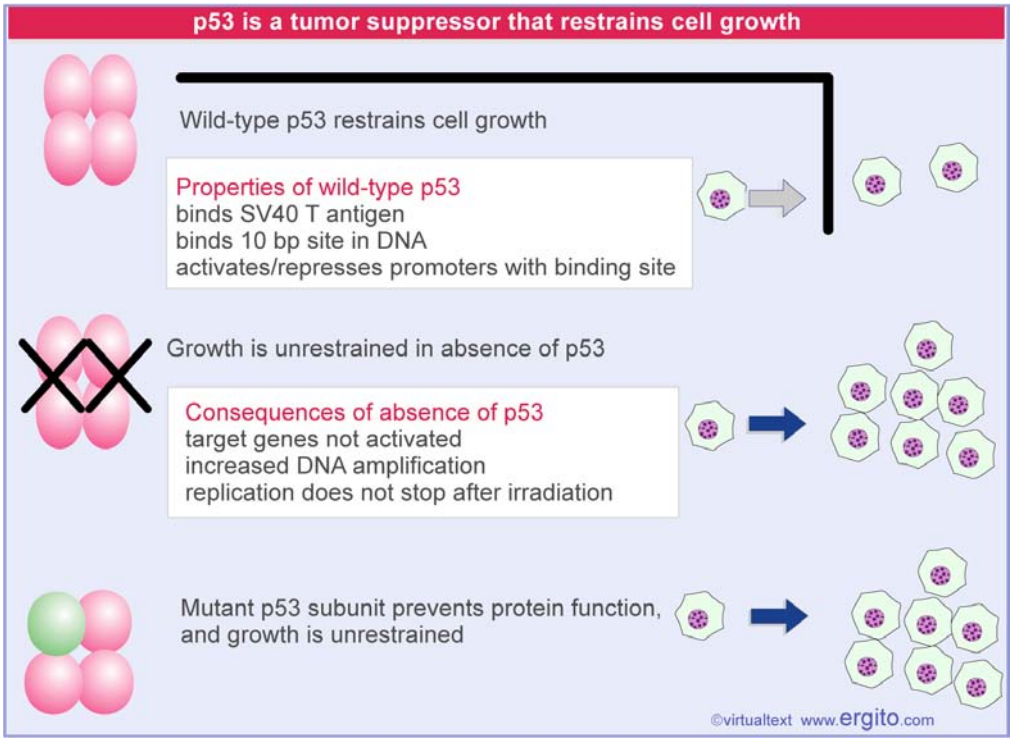

Figure 30.32 Wild-type p53 is required to restrain cell growth. Its activity may be lost by deletion of both wild-type alleles or by a dominant mutation in one allele.

Mutation in p53 is a cause of Li-Fraumeni syndrome, which is a rare form of inherited cancer. Affected individuals display cancers in a variety of tissues. They are heterozygotes that have missense mutations in one allele. These mutations behave as dominant negatives, overwhelming the function of the wild-type allele. This explains the occurrence of the disease as an autosomal dominant (874).

All normal cells have low levels of p53. A paradigm for p53 function is provided by systems in which it becomes activated, the most usual cause being irradiation or other treatments that damage DNA. This results in a large increase in the amount of p53. Two types of event can be triggered by the activation of p53: growth arrest and apoptosis (cell death). The outcome depends in part on which stage of the cell cycle has been reached. **Figure 30.33** shows that in cells early in G1, p53 triggers a checkpoint that blocks further progression through the cell cycle. This allows the damaged DNA to be repaired before the cell tries to enter S phase. But if a cell is committed to division, then p53 triggers a program of cell death. The typical results of this apoptosis are the collapse of the cell into a small heteropycnotic mass and the fragmentation of nuclear DNA (see *Molecular Biology 6.29.25 Apoptosis is a property of many or all cells*). The stage of the cell cycle is not the only determinant of the outcome; for example, some cell types are more prone to show an apoptotic response than others.

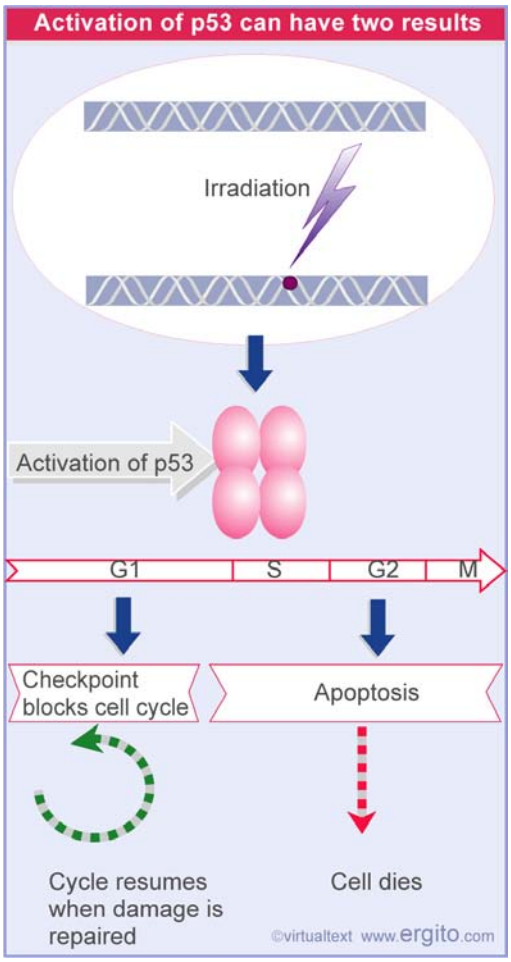

Figure 30.33 Damage to DNA activates p53. The outcome depends on the stage of the cell cycle. Early in the cycle, p53 activates a checkpoint that prevents further progress until the damage has been repaired. If it is too late to exercise the checkpoint, p53 triggers apoptosis.

We may rationalize the existence of these two outcomes by supposing that damage to DNA can activate oncogenic pathways, and that the purpose of p53 is to protect the organism against the consequences. If it is possible, a checkpoint is triggered to allow the damage to be repaired, but if this is not possible, the cell is destroyed. We do not know in molecular terms how p53 triggers one pathway or the other, depending on the conditions, but we have an understanding of individual activities of p53 that may be relevant to these pathways (for review see 354).

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ONCOGENES AND CANCER

6.30.21 p53 is a DNA-binding protein

Key Concepts

- p53 binds to promoters that contain a 10 bp recognition sequence, and may (usually) activate or (less often) inactive transcription.
- p53 also binds to single-stranded regions that are generated in damaged DNA, including those at the telomeres.
- p53 activates the CKI p21, which inhibits the cell cycle in the G1 phase.
- p53 activates the GADD45 repair protein that responds to radiation damage.
- The pathway leading to apoptosis has not been identified.
- p53 is usually present at low levels and has a short half-life.

p53 has a variety of molecular activities. **Figure 30.34** summarizes the responsibilities of individual domains of the protein for these activities:

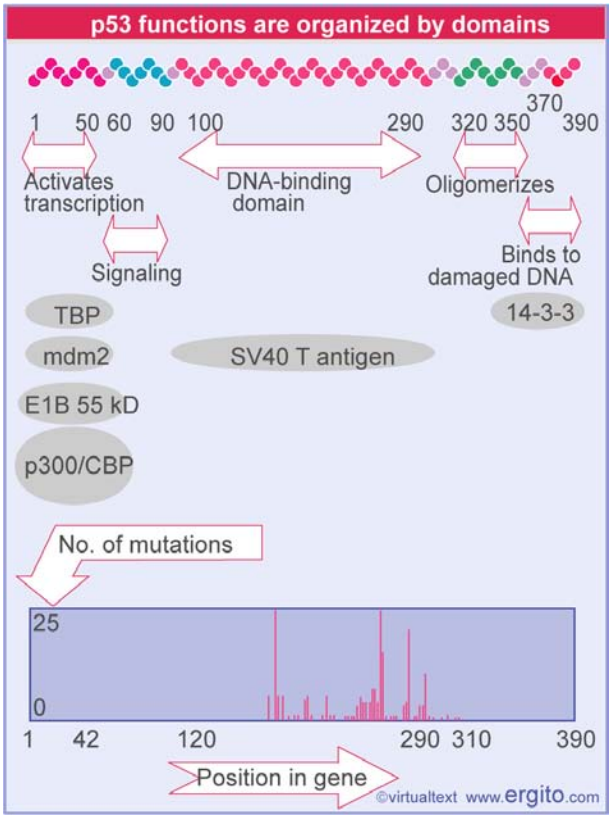

Figure 30.34 Different domains are responsible for each of the activities of p53.

- p53 is a DNA-binding protein that recognizes an interrupted palindromic 10 bp motif. The ability to bind to its specific target sequences is conferred by the central domain.
- p53 activates transcription at promoters that contain multiple copies of this motif. The immediate N-terminal region provides the transactivator domain. p53 may repress other genes; the mechanism is unknown.
- p53 also has the ability to bind to damaged DNA. The C-terminal domain recognizes single-stranded regions in DNA.
- p53 is a tetramer (oligomerization is a prerequisite for mutants to behave in a dominant negative manner). Oligomerization requires the C-terminal region.
- A (putative) signaling domain contains copies of the sequence PXXP, which forms a binding site for SH3 domains.

Mutations in p53 have various effects on its properties, including increasing its half-life from 20 minutes to several hours, causing a change in conformation that can be detected with an antibody, changing its location from the nucleus to the cytoplasm, preventing binding to SV40 T antigen, and preventing DNA-binding. As shown in **Figure 30.34**, the majority of these mutations map in the central DNA-binding domain, suggesting that this is an important activity.

p53 activates various pathways through its role as a transcription factor. The pathways can be divided into the three groups summarized in **Figure 30.35**. The major pathway leading to inhibition of the cell cycle at G1 is mediated via activation of p21, which is a CKI (cell cycle inhibitor) that is involved with preventing cells from proceeding through G1 (see **Figure 29.30** and **Figure 30.30**). Activation of GADD45 identifies the pathway that is involved with maintaining genome stability. GADD45 is a repair protein that is activated also by other pathways that respond to irradiation damage.

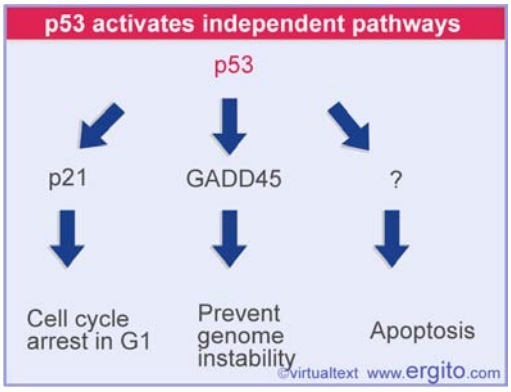

Figure 30.35 53 activates several independent pathways. Activation of cell cycle arrest together with inhibition of genome instability is an alternative to apoptosis.

When it functions as a transcription factor, p53 uses the central domain to bind to its target sequence. The N-terminal transactivation domain interacts directly with TBP (the TATA box-binding protein). This region of p53 is also a target for several other proteins. An interaction with E1B 55 kD enables adenovirus to block p53 action, which is an essential part of its transforming capacity. Other regions of p53 can also be targets for inhibition; the SV40 T antigen binds to the specific DNA-binding region, thereby preventing the recognition of target genes (876).

The stability of p53 is an important parameter. It usually has a short half-life. The response to DNA damage stabilizes the protein and activates p53's transactivation activity.

To function as a transcription factor, p53 requires the coactivators p300/CBP (which are also used by many other transcription factors). The coactivator binds to the transactivation (N-terminal) domain of p53. The interaction between p53 and p300 is also necessary in order for p53 to bind the protein Mdm2, which inhibits its activity (see *Molecular Biology 6.30.22 p53 is controlled by other tumor suppressors and oncogenes*).

The C-terminal domain of p53 binds without sequence-specificity to short (<40 base) single-stranded regions of DNA and to mismatches generated by very short (1-3 base) deletions and insertions of bases. Such targets are generated by DNA damage. One important example of a single-stranded region that activates p53 is the overhanging end that is generated at the telomere of a senescent cell (see *Molecular Biology 6.30.25 Immortalization depends on loss of p53*). The consequence of this interaction is to activate the sequence-specific binding activity of the central domain,

so that p53 stimulates transcription of its target genes. The nature of this connection is not clear, but may be a two-stage process. When p53 binds through its C-terminal domain to a damaged site on DNA, a change occurs in its properties; it then dissociates from the damaged site and binds to a target gene, which it activates.

The ability of p53 to trigger apoptosis is less well understood. It probably depends on the transactivation of a different set of target genes from those involved in activating the G1 checkpoint. The two activities can be separated by the response to adenovirus E1B 19 kD protein, which blocks the apoptotic activity of p53, but does not block its activity to activate target genes. The independence of the effects of p53 on growth arrest and apoptosis is emphasized by the fact that the E1B 55 kD protein blocks transactivation capacity but does not interfere with apoptosis.

p53 can activate apoptosis pathways in two ways. One is to cause the production of proteins that act on the mitochondrion to trigger its apoptotic functions (see *Molecular Biology 6.29.28 Apoptosis involves changes at the mitochondrial envelope*). The other is to produce or activate the cell surface receptors that trigger apoptosis (see *Molecular Biology 6.29.26 The Fas receptor is a major trigger for apoptosis*).

The importance of the connection between tumorigenesis and loss of apoptosis is also shown by the properties of the *bcl2* oncogene. *bcl2* was originally identified as a target that is activated by translocations in certain tumors. It turns out to have the property of inhibiting most pathways for apoptosis (see *Molecular Biology 6.29.28 Apoptosis involves changes at the mitochondrial envelope*). This suggests that apoptosis plays an important role in inhibiting tumorigenesis, probably because it eliminates potentially tumorigenic cells. When apoptosis is prevented because *bcl2* is activated, these cells survive instead of dying (868).

Cells with defective p53 function have a variety of phenotypes; this pleiotropy makes it difficult to determine which (if any) of these effects is directly connected to the tumor suppressor function. Most of our knowledge about p53 action comes from situations in which it has been activated. We assume that the pathways it triggers – growth arrest or apoptosis – are connected to its ability to suppress tumors. Certainly it is clear that the failure of p53 to respond to DNA damage is likely to increase susceptibility to mutational changes that are oncogenic. However, we do not know whether this is the sole role played by p53. $p53^{-}$ mice develop normally, implying that p53's role is not essential for development.

The general definition of their properties shows that both RB and p53 are tumor suppressors that usually control cell proliferation; their absence removes this control, and contributes to tumor formation.

Last updated on 12-11-2001

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ONCOGENES AND CANCER

6.30.22 p53 is controlled by other tumor suppressors and oncogenes

Key Concepts

- Most human cancer cells either have mutations that inactivate p53 directly or have mutations in other loci that lead to loss of p53.
- p53 is destabilized by Mdm2, which targets it for degradation and also directly inhibits its transactivation activity.
- The INK4a-ARF locus codes for $p16^{INK4a}$, which controls RB, and for $p19^{ARF}$, which controls p53 via inactivating Mdm2.
- Loss of INK4a-ARF is a common cause of human cancer.

p53 does not function correctly in most human tumors, but the cause of the problem lies with the gene itself in only about half of the cases. **Figure 30.36** summarizes the causes of p53 deficiency (for review see 1401):

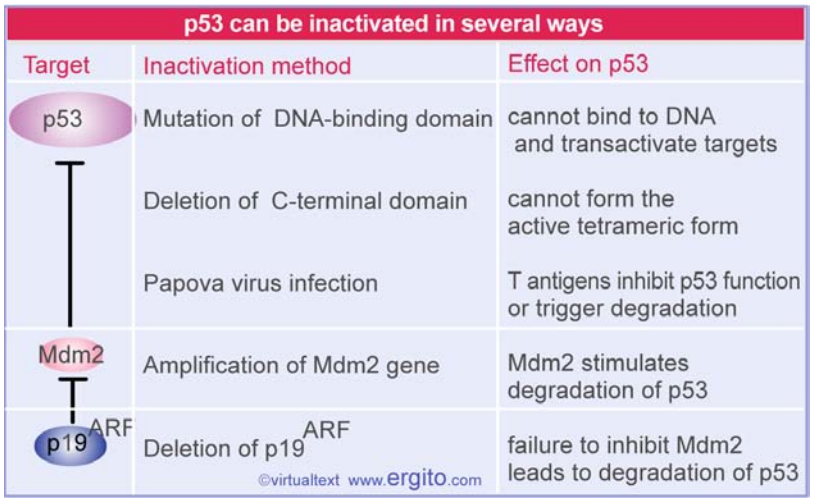

Figure 30.36 p53 is inactivated in human cancers as the result of mutations directly affecting its function or in other genes that affect the level of protein.

- The mutations in p53 itself most often lie in the DNA-binding region, and prevent the protein from binding to promoters to activate the protective response. In some cases, the mutations lie in the C-terminal region that is responsible for forming tetramers, so that active proteins are not produced.
- The major pathway controlling p53 is mediated through the protein Mdm2, which inactivates p53, so the Mdm2 locus behaves as an oncogene. Amplification of the Mdm2 gene causes an increase in expression of the protein,

which reduces p53 function.

- Mdm2 is itself inactivated by the protein $p19^{ARF}$, so deletions of the $p19^{ARF}$ gene lead to increase in Mdm2 and thus to decrease in p53.

Because p53 inhibits growth or triggers apoptosis when it is activated, it is obviously crucial for the cell to restrain the activity unless it is needed. The circuitry that controls p53's activity is illustrated in the upper part of **Figure 30.37**. Proteins that activate p53 behave as tumor suppressors; proteins that inactivate p53 behave as oncogenes (875).

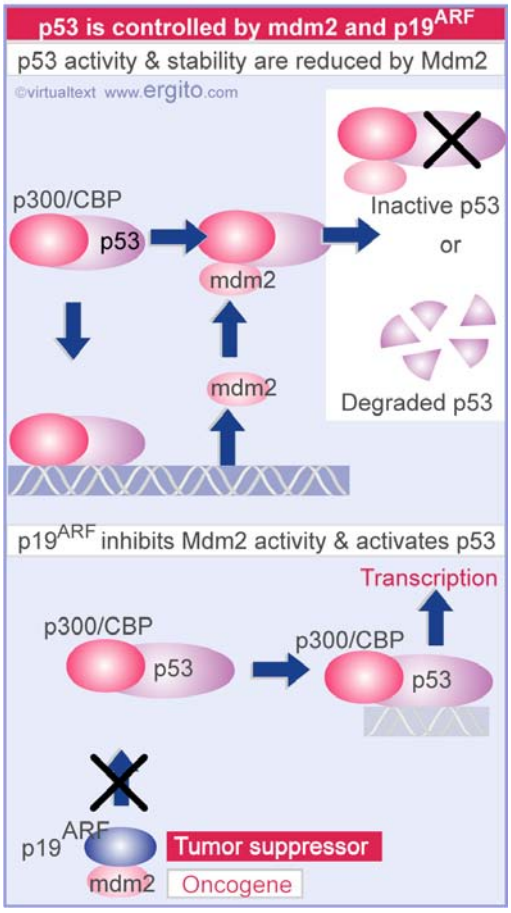

Figure 30.37 p53 activity is antagonized by Mdm2, which is neutralized by $p19^{ARF}$.

A major feature in controlling p53 activity is its interaction with Mdm2 (which was originally identified as the product of an oncogene). Mdm2 inhibits p53 activity in two ways:

- Mdm2 affects p53's stability by acting as an E3 ubiquitin ligase that causes p53 to be targeted by the degradation apparatus.
- Mdm2 also acts directly at the N-terminus to inhibit the transactivation activity

of p53.

In the reverse direction, p53 induces transcription of Mdm2. The consequence of this circuit is that Mdm2 limits p53 activity; and the activation of p53 increases the amount of Mdm2, so the interaction between p53 and Mdm2 forms a negative feedback loop in which the two components limit each other's activities.

INK4A-ARF is an important locus that controls both p53 and RB (for review see 3426). The transcript of the INK4A-ARF gene is alternatively spliced to give two mRNAs that code for proteins with no sequence relationship. $p16^{INK4a}$ is upstream of RB. The second protein is called $p19^{ARF}$ in mouse and $p14^{ARF}$ in man. We will use $p19^{ARF}$ to describe it irrespective of source. As we have just seen, $p19^{ARF}$ is upstream of p53. Deletions of the locus are common in human cancers (almost as common as mutations in p53), and have a highly significant effect, because they eliminate both $p16^{INK4a}$ and $p19^{ARF}$ and therefore lead to loss of both the RB and p53 tumor suppressor pathways.

$p16^{INK4a}$ inhibits the cdk4/6 kinase (see **Figure 29.30**). So it prevents the kinase from phosphorylating RB. In the absence of this phosphorylation, progress through the cell cycle (and therefore growth) is inhibited. $p16^{INK4a}$ is often inhibited by point mutations in human tumors.

$p19^{ARF}$ antagonizes Mdm2, as shown in the lower part of **Figure 30.37**. $p19^{ARF}$ binds to Mdm2 and directly prevents it from ubiquitinating p53. This stabilizes p53 and allows it to accumulate. In effect, therefore, $p19^{ARF}$ functions as a tumor suppressor by inhibiting the inhibitor of the p53 tumor suppressor. Loss of $p19^{ARF}$ or loss of p53 have similar effects on cell growth (and tumors usually lose one or the other but not both), suggesting that they function in the same pathway, that is, $p19^{ARF}$ in effect functions exclusively through p53. The cellular oncogene c-myc, and the adenoviral oncogene E1, both act via $p19^{ARF}$ to activate p53-dependent pathways.

Reviews

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ONCOGENES AND CANCER

6.30.23 p53 is activated by modifications of amino acids

Key Concepts

- p53 is modified by (mostly) phosphorylation or by acetylation in response to treatments that damage DNA.
- Different sensor pathways act through kinases that modify different target sites on p53.

p53 responds to environmental signals that affect cell growth, and many of these signals act by causing specific sites on p53 to be modified. The most common form of modification is the phosphorylation of serine, but acetylation of lysine also occurs. Different pathways lead to the modification of different amino acid residues in p53, as summarized in **Figure 30.38**. There is often overlap between the various residues activated by each pathway. Three principal pathways are identified by the agents that act on p53:

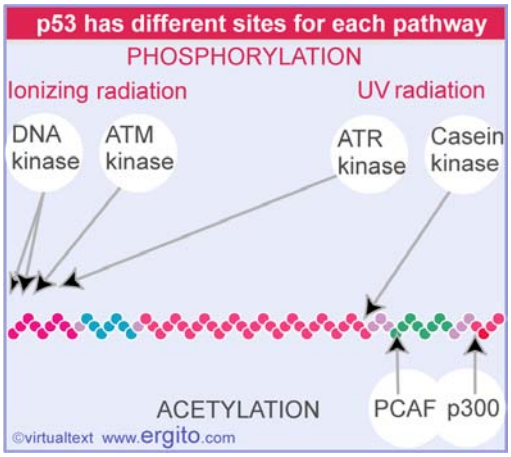

Figure 30.38 Each pathway that activates p53 causes modification of a particular set of residues.

- Ionizing radiation induces DNA breaks that activate the kinase ATM (named for the disease ataxia telangiectasia in which its gene is mutated). ATM phosphorylates S^{15}. DNA breaks also activate a DNA-dependent kinase that acts on other sites in the N-terminal region. (Through unknown pathways, ionizing radiation also causes phosphorylation of S^{33}, dephosphorylation of S^{376}, and acetylation of L^{382}.)
- UV radiation and other types of stress activate the kinases ATR (ataxia telangiectasia related) and casein kinase II, which phosphorylate S^{15} and S^{33}, and also cause phosphorylation of S^{392}.

- Some aberrant growth signals, such as those produced by the oncogenes Ras or Myc, may activate $p19^{ARF}$. This inactivates Mdm2 and thus activates p53.

The target sites for these various pathways are located in the terminal regulatory domains of the protein. The modifications may affect stability of the protein, oligomerization, DNA-binding, and binding to other proteins. So p53 acts as a sensor that integrates information from many pathways that affect the cell's ability to divide. *The important point is that each pathway leads to modification of specific residues in p53 that activate its response.*

Phosphorylation can change the properties of a protein by altering the structure in the immediate vicinity, creating or abolishing sites that interact with other proteins. At some of the sites in p53, the phosphorylation has a more widespread effect, and changes the conformation of the polypeptide backbone of the protein. The usual conformation of a protein chain is a series of amino acids arranged in *trans* conformation about the peptide bonds that connect them. This means that the adjacent amino acids lie on opposite sides of the peptide bond. However, as shown in **Figure 30.39**, the ring structure of proline allows a rearrangement in which it lies in *cis* configuration relative to the preceding amino acid. This has a major effect on protein conformation. The reaction is catalyzed by a class of proteins called peptidyl-prolyl-isomerases.

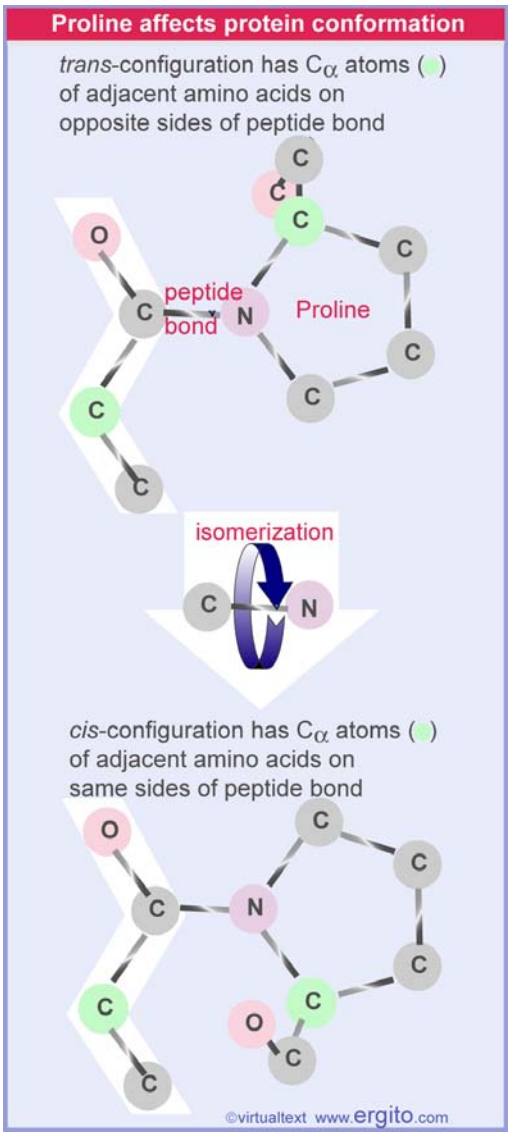

Figure 30.39 Amino acids usually lie in *trans* on either side of the peptide bond connecting them in a protein chain. The ring structure of proline allows isomerization around the bond connecting it to the preceding amino acid, generating the *cis* configuration in which both amino acids lie on the same side of the peptide bond.

Some of the target sites for phosphorylation in p53 are serine or threonine residues followed by a proline. Phosphorylation changes the energetics to favor the *trans-cis* isomerization. The reaction is assisted by a particular peptidyl-prolyl-isomerase, Pin1, which binds to the dipeptide sequence only when the first amino acid is phosphorylated. The importance of the reaction is demonstrated by the fact that mutations in Pin1 impair the ability of p53 to respond to damage in DNA (3213; 3214). Although Pin1 is essentially part of the mechanism by which p53 is activated, in formal terms it behaves like a tumor suppressor.

There are two aspects to the activation of p53. The amount of p53 protein in the cell is determined principally by its degradation, which is a reflection of the activity of Mdm2. Stabilization of the protein is a prerequisite for the response, but is not sufficient. Conformational changes must be triggered by one or more of the modification events that respond to the various sensor systems.

Last updated on 1-3-2003

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ONCOGENES AND CANCER

6.30.24 Telomere shortening causes cell senescence

Key Terms

Senescent cells show visible changes in the appearance of a culture as the result of limitations posed by telomere shortening on the number of chromosomal replications that can occur.

Key Concepts

- Somatic cells usually lack telomerase activity, which means that telomeres shorten with each cell division.
- Cultured cells may go into crisis as the result of reaching zero telomere length.
- Reactivation of telomerase enables cells to survive crisis and to become immortal.

A key event in the limited divisions of growth in cell culture is the shortening of telomeres. A special ribonucleoprotein enzyme, called telomerase, is responsible for extending telomeres (see *Molecular Biology 5.19.18 Telomeres are synthesized by a ribonucleoprotein enzyme*). The function of telomerase is to compensate for the shortening of telomeres that occurs at each replication cycle. Telomerase is turned off in many somatic cells, typically when differentiation occurs.

Continued division in cells that lack telomerase activity (for example, when primary cells are placed into culture) will cause the telomeres to shorten in each generation. The cells become unable to propagate properly when the telomeres become too short to ensure stability at the ends of the chromosomes (see *Molecular Biology 5.19.19 Telomeres are essential for survival*). The consequences are visible as changes in the appearance of the culture, and the cells are said to be **senescent**. Crisis occurs when the cells cannot divide any longer, and the culture dies out. **Figure 30.40** summarizes the events involved in approaching crisis and passing through it.

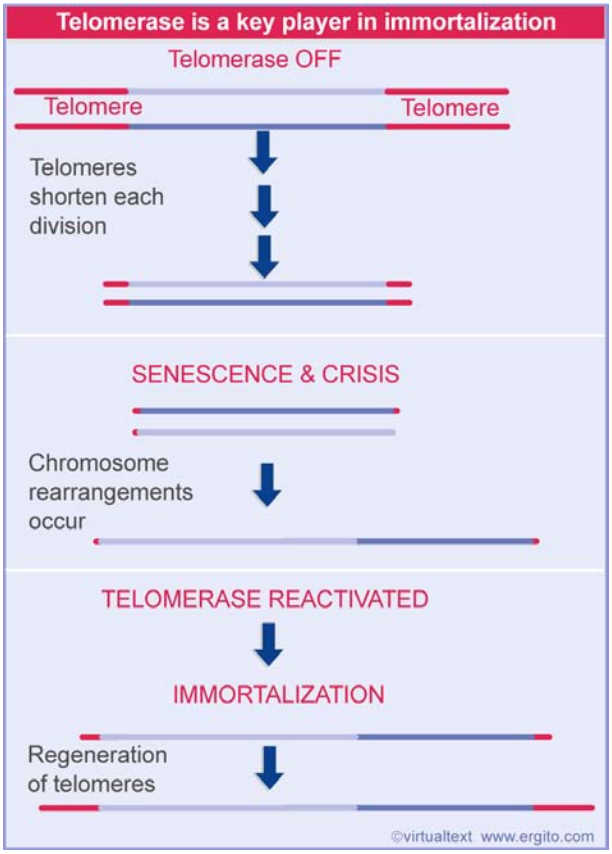

Figure 30.40 In the absence of telomerase, telomeres shorten each cell generation until crisis occurs. Unstable ends cause chromosome rearrangements in senescent cells. The most common means of immortalization is the reactivation of telomerase.

The rare cells that survive crisis pass through a stage at which the ends of their chromosomes are unstable. These ends interact with one another or with other chromosomal regions, and this is the probable cause of the frequent occurrence of chromosomal abnormalities in cultured cells. The mutations that are caused by these abnormalities can contribute to the tumorigenic state.

To continue to divide after passing through crisis, a cell must regain the capacity to replicate its telomeres. This suggests that a critical parameter for immortalization might be the reactivation of telomerase. Telomerase activity can be restored by transfecting the gene for the catalytic subunit into target cells, and this allows them to be perpetuated in culture without passing through crisis (see *Great Experiments 9.2 Immortalizing human cells with telomerase*; 1380; 1390). We might view the finite replicative capacity of primary human cells, in general, or their inability to continue propagation once telomere lengths have become too short, in particular, as a tumor suppression mechanism that in effect prevents cells from undertaking the indefinite replication that is needed to make a tumor (878).

Immortalization is required for cells to be perpetuated indefinitely in tissue culture, and we have to ask how the relevant events relate to the formation of a tumor *in vivo*. The behavior of telomerase shows a parallel between the immortalization of a cell in

culture and the generation of a tumor *in vivo*. The limiting step in production of telomerase is the transcription of the catalytic subunit, which is repressed in differentiated somatic cells and restored in tumor cells. This resembles the reactivation of telomerase activity in cultured cells that have emerged from crisis.

Can we test the role of telomerase in intact animals? Mice in which the gene coding for the telomerase RNA has been inactivated can survive for >6 generations. Mouse telomeres are exceptionally long, and range from 10-60 kb. In the absence of telomerase, telomeres shorten at 50-100 bp per cell division. There are ~60 divisions in sperm cell production, and ~25 divisions in oocyte production, which fits with the observed rate of shortening of ~4.8 kb per male mouse generation. This gives an expectation that after about 7 generations, a telomerase-negative mouse will have run down its telomeres to around zero length.

By the 6th generation, chromosomal abnormalities become more frequent, and the mice become infertile (due to the inability to produce sperm). The effects of lack of telomerase are first seen in tissues consisting of highly proliferative cells (as might be expected). All of these observations demonstrate the importance of telomerase for continued cell division. However, cells from the telomerase-negative mice can pass through crisis and can be transformed to give tumorigenic cells, so the presence of telomerase is not essential, or at least is not the only means, of supporting an immortal state (although reactivation of telomerase is by far the most common mechanism) (see *Molecular Biology 5.19.19 Telomeres are essential for survival*) (879).

Telomerase-negative mice can develop tumors, but do so at a rate lower than wild-type mice (2852). The effect of telomere loss on formation of a cancer cell is therefore confined to its role in provoking a genetic instability that stimulates tumor initiation. After that, it is in fact inhibitory to cancer formation.

There is a curious inconsistency between the results obtained with cultured cells and the survival of telomerase-negative mice. Crisis of mouse cells occurs typically after 10-20 divisions in culture, but we would not expect the telomeres to have reached a limiting length at this point. Mice of the first telomerase-negative generation have passed a greater number of cell divisions without telomerase, and without suffering any ill effects. Mice of the third telomerase-negative generation are to all intents and purposes normal, although their cells have gone through more divisions than would have triggered crisis in culture.

Lack of telomerase is clearly associated with inability to continue growth, and reactivation of telomerase is one means by which cells can behave as immortal. It is not clear whether telomerase is the only relevant factor in driving cells into crisis and to what extent other mechanisms might be able to compensate for lack of telomerase. We do not know what pathway is responsible for controlling telomerase production *in vivo*, and how it is connected to pathways that control cell growth.

Last updated on 8-21-2002

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ONCOGENES AND CANCER

6.30.25 Immortalization depends on loss of p53

Key Concepts

- Loss of p53 is the crucial step in immortalization.

When cells enter senescence as the result of telomere shortening, p53 is activated, leading to growth arrest or apoptosis. **Figure 30.41** shows that the trigger that activates p53 is the loss of the telomere-binding protein TRF2 from the chromosome ends. In effect, TRF2 protects the end of the DNA, but when it is lost, the free 3′ overhanging end activates p53 (1998; 3669).

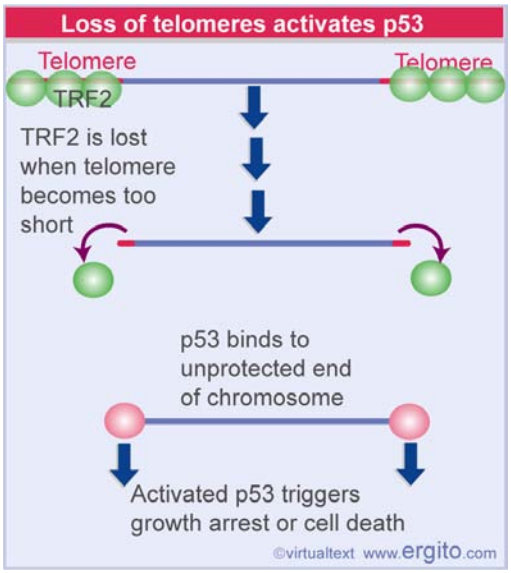

Figure 30.41 TRF2 protects telomeres, but when it is lost, the exposed ends can bind and activate p53.

The loss of p53 is the crucial event that allows the cells to survive and divide. A variety of events can be associated with immortalization, but they converge upon causing either the loss or inactivation of p53 protein. Remember that p53 was discovered as the protein of host cells that binds the T antigen (the transforming protein) of polyomaviruses. A major part of the activity of T antigen is its ability to inactivate p53. The T antigens of different viruses work in different ways, but the consequences of the interaction are especially clear in the case of HPV E6 (the equivalent of T antigen), which targets p53 for degradation. In effect, HPV converts a target cell into a $p53^{-}$ state.

p53 provides an important function in immortalization, but may not be sufficient by itself. Established cell lines have usually lost p53 function, which suggests that the role of p53 is connected with the acquisition of ability to support prolonged growth. However, loss of the known functions of p53 is not enough by itself to explain

immortalization, since, for example, a $p53^-$ mouse is viable, and therefore is able to undergo the usual pattern of cell cycle arrest and differentiation. Primary cells from a $p53^-$ mouse can pass into the established state more readily than cells that have p53 function, which suggests that loss of p53 activity facilitates or is required for immortalization (877).

An interesting convergence is seen in the properties of the tumor antigens from different DNA tumor viruses, . The antigens always bind to both the cellular tumor suppressor products RB and p53. The two cellular proteins are recognized independently. Either different T antigens of the virus bind separately to RB and to p53, or different domains of the same antigen do so. So adenovirus E1A binds RB, while E1B binds p53; HPV E7 binds RB, while E6 binds p53. SV40 T antigen can bind both RB and p53. The loss of p53 (and/or RB) is a major step in the transforming action of DNA tumor viruses, and explains some significant part of the action of the T antigens. The critical events are inhibition of p53's ability to activate transcription, and loss of RB's ability to bind substrates such as E2F. Loss of the tumor suppressors (especially p53) is the major route in the immortalization pathway.

Inability to trigger either growth arrest or apoptosis could lead to continued growth. We do not know whether only one or both of these activities are required for immortalization *in vitro*. We know that more than the growth arrest pathway is needed for p53's contribution to tumorigenesis, because a $p21^-$ mouse shows deficiencies in the G1 checkpoint (as would be expected) but does not develop tumors. The contrast with the increased susceptibility of a $p53^-$ mouse to tumors shows that other functions of p53 are involved besides its control of p21.

We are now in a position to put together the various events involved in immortalization (for review see 2853). **Figure 30.42** summarizes the order in which they occur. Checkpoints normally stop a cell from dividing when its telomeres become too short. Cells then enter replicative senescence and stop growing. If they manage to bypass the checkpoints to enter early crisis, the loss of TRF2 from the telomeres activates p53, which causes growth arrest and/or apoptosis. In the absence of p53 activity, they pass into late crisis, where the dysfunction of the telomeres causes genetic instability as seen in large scale chromosomal rearrangements. To survive this stage, they must activate telomerase or find an alternative means of maintaining the telomeres. A cell that survives through all of these stages will be immortal, but almost certainly will have an altered genetic constitution.

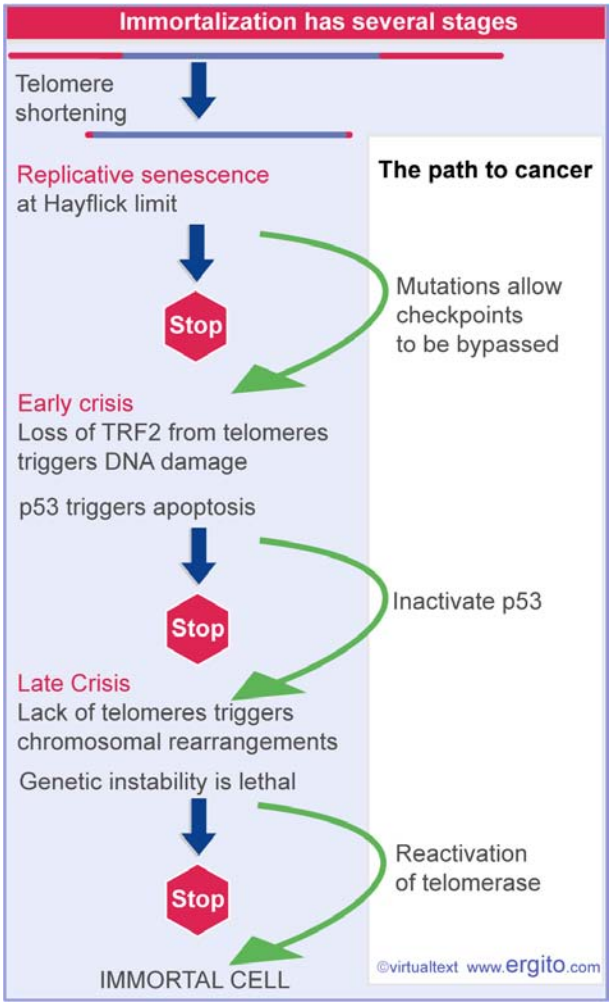

Figure 30.42 Several changes are required to allow a cell to pass the replicative limit and to become immortalized, including bypassing the checkpoints that respond to short telomeres, losing or preventing the ability of p53 to trigger apoptosis, and reactivating telomerase or finding other means to stabilize telomeres.

Last updated on 8-21-2002

Reviews

2853. Maser, R. S. and DePinho, R. A. (2002). *Connecting chromosomes, crisis, and cancer.* Science 297, 565-569.

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ONCOGENES AND CANCER

6.30.26 Different oncogenes are associated with immortalization and transformation

Key Terms

Cooperativity in protein binding describes an effect in which binding of the first protein enhances binding of a second protein (or another copy of the same protein).

Key Concepts

- A tumor cell has independently acquired changes necessary to immortalize it and to transform it.
- Established cell lines grown in culture usually have been immortalized and need to acquire only transforming properties.
- Primary cells require the actions of different oncogenes to be immortalized and to be transformed.

Most tumors arise as the result of multiple events. Some of these events involve the activation of oncogenes, while others take the form of inactivation of tumor suppressors. The requirement for multiple events reflects the fact that normal cells have multiple mechanisms to regulate their growth and differentiation, and several separate changes may be required to bypass these controls. Indeed, the existence of single genes in which mutations were tumorigenic would no doubt be deleterious to the organism, and has been selected against. Nonetheless, oncogenes and tumor suppressors define genes in which mutations create a predisposition to tumors, that is, they represent one of the necessary events. It is an open question as to whether the oncogenes and tumor suppressor genes identified in available assays are together sufficient to account entirely for the occurrence of cancers, but it is clear that their properties explain at least many of the relevant events.

Figure 30.43 gives an overview of the stages of tumor formation. There are two discrete stages, which may loosely be viewed as being concerned with immortalization or with transformation (for review see 346; 350).

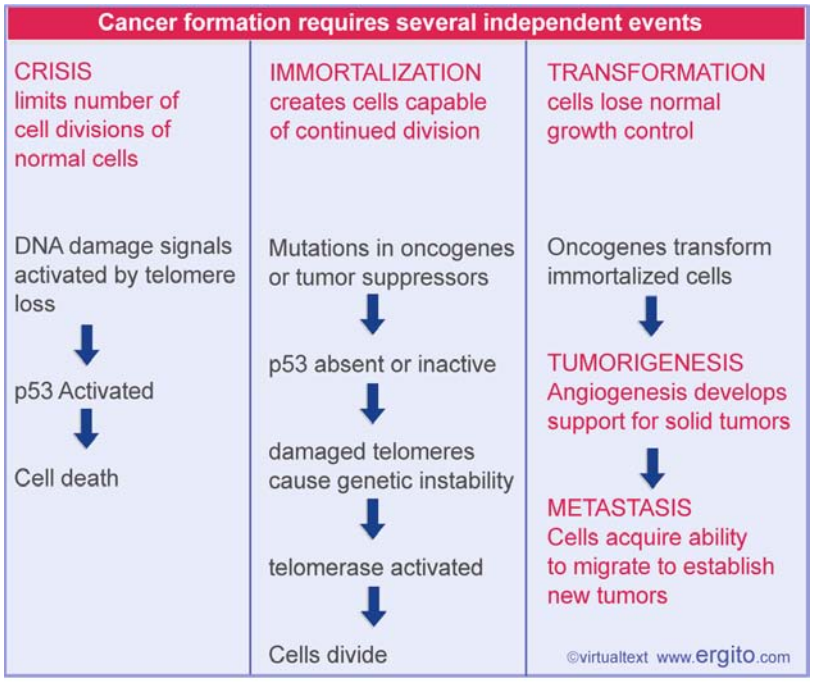

Figure 30.43 Crisis is induced by attempts to divide in the absence of telomerase. Immortalization occurs when p53 activity is lost, as the result of mutation in p53 or in a pathway that acts on it. Transformation requires oncogenes to induce further changes in the growth properties of the cells. For cells that develop solid tumors, angiogenic development is required for provision of nutrients. Metastasis is the result of further changes that allow a cell to migrate to form a colony in a new location.

The immortalization step is to bypass crisis (or its equivalent in the *in vivo* situation). Crisis is provoked when cells continue to divide in the absence of telomerase (see *Molecular Biology 6.30.24 Telomere shortening causes cell senescence*). When the telomeres become too short, damage to DNA is caused by attempts at replication, and this triggers the activation of p53. The role of p53 is to cause cell death. If p53 is absent, a cell may survive, although at the expense of a genetic catastrophe in which telomere malfunction leads to chromosome fusions and other rearrangements.

Immortalized cells can pass through an unlimited number of cell divisions, but they do not have other tumorigenic properties, such as independence of factors required for growth. The second step, transformation, converts immortalized cells into tumorigenic cells. Whether further changes are involved in creating a cancerous state depends on the nature of the tumor cell. A leukemia cell can multiply freely in the blood. However, a cell type that forms a solid tumor needs to develop a blood supply for the tumor (requiring angiogenic development), and may later pass to the stage of metastasis, when cells are detached from the tumor and migrate to form new tumors at other locations.

The minimum requirement to enter the tumorigenic state is therefore the occurrence of successive, independent events that involve different tumor suppressors and/or oncogenes. The need for multiple functions of different types is sometimes described as the requirement for **cooperativity**.

The involvement of multiple functions fits with the pattern established by some DNA tumor viruses, in which (at least) two types of functions are needed to transform the usual target cells:

- Adenovirus carries the *E1A* region, which allows primary cells to grow indefinitely in culture, and the *E1B* region, which causes the morphological changes characteristic of the transformed state.
- Polyoma produces three T antigens; large T elicits indefinite growth, middle T is responsible for morphological transformation, and small T is without known function. Large T and middle T together can transform primary cells.
- Consistent with the classification of oncogenic functions, adenovirus E1A together with polyoma middle T can transform primary cells. This suggests that one function of each type is needed.

In the same way, expression of two or more oncogenes in the cellular transfection assay is usually needed to convert a primary cell (one taken directly from the organism) into a tumor cell.

Several cellular oncogenes have been identified by transforming ability in the 3T3 transfection assay; 10-20% of spontaneous human tumors have DNA with detectable transforming activity in this assay. Of course, 3T3 cells have been adapted to indefinite growth in culture over many years, and have passed through some of the changes characteristic of tumor cells (see *Great Experiments 9.1 The story of 3T3 cells: A voyage of discovery without an itinerary*). The exact nature of these changes is not clear, but generally they can be classified as involving functions concerned with immortalization. Oncogenic activity in this assay therefore depends on the ability to induce further changes in an established cell line.

The principal products of 3T3 transfection assays are mutated *c-ras* genes. They do not have the ability to transform primary cells *in vitro,* and this supports the implication that their functions are concerned with the act of transforming cells that have previously been immortalized. *ras* oncogenes clearly provide one major pathway for transforming immortalized cells; we do not know how many other transforming pathways may exist that are independent of *ras*.

Although *ras* oncogenes alone cannot transform primary fibroblasts, dual transfection with *ras* and another oncogene can do so. The ability to transform primary cells in conjunction with *ras* provides a general assay for oncogenes that have an immortalization-like function. This group includes several retroviral oncogenes, *v-myc*, *v-jun*, and *v-fos*. It also includes adenovirus E1A and polyoma large T. Mutant p53 genes have the same effect. In fact, the action of the immortalizing oncogenes is most likely to cause inactivation or loss of p53. However, note that the distinction between immortalizing and transforming proteins is not crystal clear. For example, although E1A is classified as having an immortalizing function, it has (some) of the functions usually attributed to transforming proteins, and loss of p53 confers some properties that are usually considered transforming.

One way to investigate the oncogenic potential of individual oncogenes

independently of the constraints that usually are involved in their expression is to create transgenic animals in which the oncogene is placed under control of a tissue-specific promoter. A general pattern is that increased proliferation often occurs in the tissue in which the oncogene is expressed. Oncogenes whose expression have this effect with a variety of tissues include SV40 T antigen, *v-ras*, and *c-myc* (588).

Increased proliferation (hyperplasia) is often damaging and sometimes fatal to the animal (usually because the proportion of one cell type is increased at the expense of another). However, the expression of a single oncogene does not usually cause malignant transformation (neoplasia), with the production of tumors that kill the animal. Tumors resulting from the introduction of an oncogene (for example, in transgenic mice) are probably due to the occurrence of a second event.

The need for two types of event in malignancy is indicated by the difference between transgenic mice that carry either the *v-ras* or activated *c-myc* oncogene, and mice that carry both oncogenes. Mice carrying either oncogene develop malignancies at rates of 10% for *c-myc* and 40% for *v-ras;* mice carrying both oncogenes develop 100% malignancies over the same period. These results with transgenic mice are even more striking than the comparable results on cooperation between oncogenes in cultured cells (866; 867).

In some systems, immortalization may be connected with an inability of the cells to differentiate. Growth and differentiation are often mutually exclusive, because a cell must stop dividing in order to differentiate. An oncoprotein that blocks differentiation may allow a cell to continue proliferating (in a sense resembling the immortalization of cultured cells); continued proliferation in turn may provide an opportunity for other oncogenic mutations to occur. This may explain the occurrence among the oncoproteins of products that usually regulate differentiation.

A connection between differentiation and tumorigenesis is shown by avian erythroblastosis virus (AEV). The AEV-H strain carries only *v-erbB*, but the AEV-E54 strain carries two oncogenes, *v-erbB* and *v-erbA.* The major transforming activity of AEV is associated with *v-erbB*, a truncated form of the EGF receptor, which is equivalent to the single oncogene carried by other tumor retroviruses: it can transform erythroblasts and fibroblasts. The other gene, *v-erbA*, cannot transform target cells alone, but it increases the transforming efficiency of *v-erbB.* Expression of *v-erbA* itself has two phenotypic effects upon target cells: it prevents the spontaneous differentiation (into erythrocytes) of erythroblasts that have been transformed by *v-erbB;* and it expands the range of conditions under which transformed erythroblasts can propagate. *v-erbA* may therefore contribute to tumorigenicity by a combination of inhibiting differentiation and stimulating proliferation. In fact, *v-erbA* has a similar effect in extending the efficacy of transformation by other oncogenes that induce sarcomas, notably *v-src*, *v-fps*, and *v-ras.*

Correlations between the activation of oncogenes and the successful growth of tumors are strong in some cases, but by and large the nature of the initiating event remains open. It seems clear that oncogene activity assists tumor growth, but activation could occur (and be selected for) after the initiation event and during early growth of the tumor. We hope that the functions of *c-onc* genes will provide insights into the regulation of cell growth in normal as well as aberrant cells, so that it will become possible to define the events needed to initiate and establish tumors.

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ONCOGENES AND CANCER

6.30.27 p53 may affect ageing

Key Concepts

- Shortening of telomeres below a critical length is associated with reduced longevity.
- Increase of p53 above wild-type levels can decrease tumor formation, but also decreases longevity.

We have very little idea what is responsible for ageing of an animal. The general drift of evolutionary theories of ageing is that natural selection operates only via reproduction, and therefore there is little advantage to the survival of the organism past the stage when it is reproductively active. In other words, there is no selection for longevity beyond the reproductive state. .

We do not know how ageing of the organism relates to changes in individual cells, but one possibility is that ageing results from the accumulation of damage at the cellular level. Within this model, one contribution could be inappropriate expression of genes resulting from failure of regulation.

It is an open question whether aging of the organism is connected with the senescence of individual cells. Cessation of telomerase activity in adult lineages causes telomeres to shorten as cells divide. When telomere lengths reach zero, cells enter the senescent state. Shortened telomeres can reduce lifespan. **Figure 30.44** shows that mice from the fourth or fifth telomerase-negative generations have a slightly reduced lifespan, and mice from the sixth generation have a much reduced lifespan (2248). Whereas normal mice have a 50% survival rate at ~25 months, the figure for the sixth generation mice is ~17 months. Increased cancer incidence accounts for only half of the accelerated deaths. The other mice die from unknown causes (this is typical of ageing), and they prematurely show several of the characteristics of ageing (such as reduced wound healing).

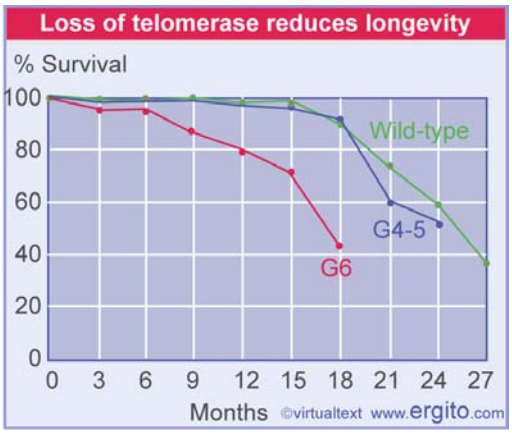

Figure 30.44 Telomerase-negative mice of the sixth generation (G6) have sharply reduced longevity, and mice of the fourth to fifth generations (G4-5) have slightly reduced longevity.

We know that loss of p53 is one of the mechanisms allowing cultured cells to pass through the crisis provoked by loss of telomeres (see *Molecular Biology 6.30.24 Telomere shortening causes cell senescence*). What role might p53 loss play in telomerase-negative mice? A major role is seen in the pattern of inheritance. An increased proportion of the progeny are $p53^{-}$. This is because loss of p53 reduces the apoptosis (death) of germ line cells that is caused by loss of telomerase (2249).

Direct attempts to see whether p53 might have any effect on ageing have been unsuccessful because $p53^{-}$ mice die early as the result of accumulating tumors, and mice that over-express p53 cannot be made, probably because of deleterious effects of excess p53 during embryonic development. However, striking results have been obtained from the serendipitous production of a mouse that has a mutant form of p53 (2250). The mutant gene, called the *m* allele, has lost its first 6 exons, and makes a truncated protein. Heterozygous $p53^{+/m}$ mice have a reduced frequency of tumor formation. Comparison with wild-type and hemizygous p53 mice shows:
p53 +/– (one active allele):>80% tumors
p53 +/+ (two active alleles): > 45% tumors
p53 +/m: 6% tumors.
Mice with two active alleles form tumors at about half the frequency of mice with only one active allele, which corresponds to the relative rate at which one allele is likely to be spontaneously inactivated compared to two alleles (see **Figure 30.31**). The much reduced rate of tumor formation in the $p53^{+/m}$ mice suggests that the *m* allele has the unexpected effect of increasing p53 activity. This is confirmed by directly measuring some of the known responses to p53 in cells from the $p53^{+/m}$ mice.

We might expect the reduction in tumor formation in $p53^{+/m}$ mice to be associated with an increase in longevity, but exactly the reverse is found when they are compared with wild-type mice. Although the $p53^{+/m}$ mice have only 6% tumors and the wild-type mice develop 45% tumors, half of the $p53^{+/m}$ mice have died by 22 months, whereas the wild-type mice survive on average to 27 months. (Mice that are $p53^{+/-}$ or $p53^{-/-}$ die more quickly because of the high accumulation of tumors.) The effect seems to result from the interaction of the *m* mutant protein with the wild-type

protein in the heterozygote, because $p53^{-/m}$ mice have just as many tumors and die just as quickly as $p53^{-/-}$ mice. So the *m* allele does not have any protective effect on its own.

The $p53^{+/m}$ mice show no differences from wild-type mice for the first 12 months, but by 18 months show signs of premature ageing. This suggests that increased activity of p53 has a direct effect in promoting ageing. This raises the possibility that the very same activities of p53 that are needed for protection against cancer also have the effect of causing ageing! This clearly makes the level of p53 activity something that must be very tightly controlled.

Last updated on 1-15-2001

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ONCOGENES AND CANCER

6.30.28 Genetic instability is a key event in cancer

Key Terms

Genetic instability (Genome instability) refers to a state in which there is large increase (×100-fold) in the frequency of changes in the genome as seen by chromosomal rearrangements or other events that affect the genetic content. This is a key occurrence in the generation of cancer cells.

Key Concepts

- Tumor cells have rates of genetic change that are increased above the usual rate of somatic mutation.
- Gross chromosomal changes are observed in most types of colorectal cancer.
- Chromosome rearrangements can be generated by mutations of checkpoint pathways and other pathways that act on the genome in a yeast model system.

The inactivation of tumor suppressors and the activation of oncogenes are key events in creating a tumor, but several such events (typically 4-10 in the case of human cancers) are required to generate a fully tumorigenic state. This number of events would not be predicted to occur during the life of a cell or organism if the individual changes occurred at the normal rate of spontaneous mutation. Many cancers are associated with **genetic instability** that significantly increases the number of events.

Genetic instability is revealed by increases in the frequency of genomic changes. These range from reorganizations at the level of the chromosome to individual point mutations. We can get a sense of their relative importance from their occurrence in colon cancers, where the genetic changes have been well characterized. The majority of colorectal tumors show high rates of gross alteration in chromosomes, often involving changes in the number of copies of a gene. This is the most common way to generate the basic changes that fuel tumorigenesis. In the minority of cases (~15%), there are no gross changes, but there are many individual mutations, resulting from a highly increased rate of mutation (see *Molecular Biology 6.30.29 Defects in repair systems cause mutations to accumulate in tumors*). Either of these types of change in the cell can propagate a tumor; it is rare for both to occur together.

Gross chromosomal alterations involve deletion, duplication, or translocation. They may result in changes in the number of copies of a gene. **Figure 30.45** illustrates two major causes:

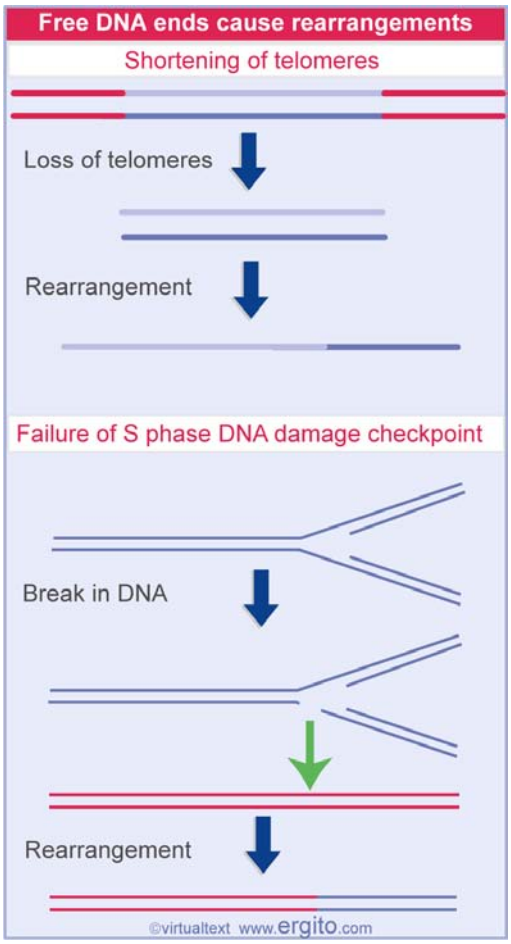

Figure 30.45 DNA ends induce genome rearrangements. This may happen because of the failure of either telomere maintenance or DNA damage checkpoints.

- loss of telomeres because cells continue to divide in the absence of telomerase;
- creation of free double-strand ends that result from an unrepaired break in DNA.

The events that occur when a cell passes through crisis are a paradigm for the generation of chromosomal alterations (for review see 2853). Loss of telomeres induces DNA rearrangements (see *Molecular Biology 6.30.24 Telomere shortening causes cell senescence*). Failure of the normal protective mechanisms allows the damaged cells to survive.

Gross rearrangements can also be provoked by failure of protective mechanisms during a normal cell cycle (for review see 2854). Checkpoint pathways respond to DNA damage by halting the cell cycle in its current phase (see **Figure 29.21** in *Molecular Biology 6.29.13 DNA damage triggers a checkpoint*). The checkpoint triggers an effector pathway that repairs the damage, after which the cell cycle is allowed to proceed. Mutations in the S phase checkpoint pathway in *S. cerevisiae* result in an increase of more than 100× in the rate of genome rearrangements

(2856; 2857). This happens because the cell cycle is allowed to proceed in spite of the presence of breaks in DNA. **Figure 30.46** shows that similar effects are produced by mutations of some recombination-repair pathways or pathways for telomere maintenance. Analogous events could be involved in creating genetic instabilities that lead to cancer.

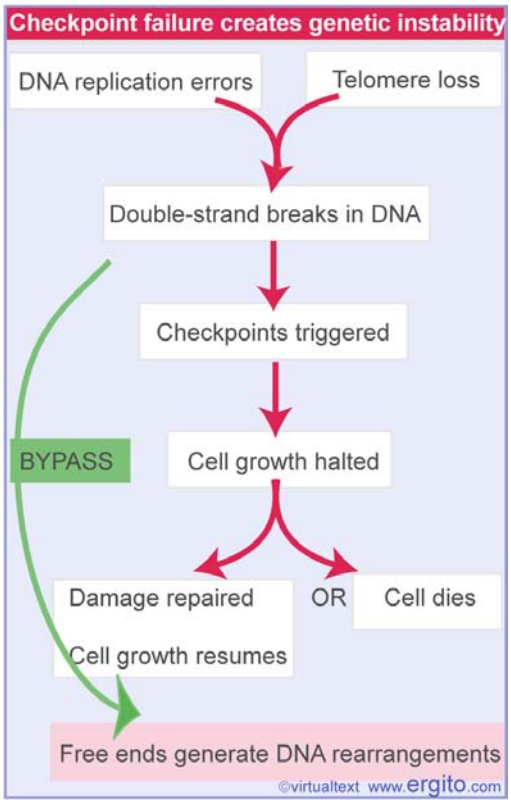

Figure 30.46 DNA replication errors or loss of telomeres may generate double-strand breaks in DNA. Checkpoints detect the breaks and protect the cell from perpetuating errors. If the checkpoints fail, DNA rearrangements occur.

Last updated on 8-21-2002

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ONCOGENES AND CANCER

6.30.29 Defects in repair systems cause mutations to accumulate in tumors

Key Concepts

- Loss of mismatch-repair systems generates a high mutation rate in HNPCC.

All cells have systems to protect themselves against damage from the environment or errors that may occur during replication (see *Molecular Biology 4.15 Recombination and repair*). The overall mutation rate is the result of the balance between the introduction of mutations and their removal by these systems. One means by which cancer cells increase the rate of mutation is to inactivate some of their repair systems, so that spontaneous mutations accumulate instead of being removed. In effect, a mutation that occurs in a mutator gene causes mutations to accumulate in other genes. (A mutator gene can be any type of gene – such as a DNA polymerase or a repair enzyme – whose function affects the integrity of DNA sequences.)

The MutSL system is a particularly important target. This system is responsible for removing mismatches in newly replicated bacterial DNA. Its homologues perform similar functions in eukaryotic cells. During replication of a microsatellite DNA, DNA polymerase may slip backward by one or more of the short repeating units. The additional unit(s) are extruded as a single-stranded region from the duplex. If not removed, they result in an increase in the length of the microsatellite in the next replication cycle (see **Figure 4.28**). This is averted when homologues of the MutSL system recognize the single-stranded extrusion and replace the newly-synthesized material with a nucleotide sequence that properly matches the template (see **Figure 15.47**).

In the human disease of HNPCC (hereditary nonpolyposis colorectal cancer), new microsatellite sequences are found at a high frequency in tumor cells when their DNA sequences are compared with somatic cells of the same patient (2256; 2257). **Figure 30.47** shows an example. This microsatellite has a repeat sequence of AC (reading just one strand of DNA) . The length of the repeat varies from 14-27 copies in the population. Any particular individual shows two repeat lengths, one corresponding to each allele in the diploid cell. In many patients, the repeat length is changed. Most often it is reduced at both alleles, as shown in the example in the figure.

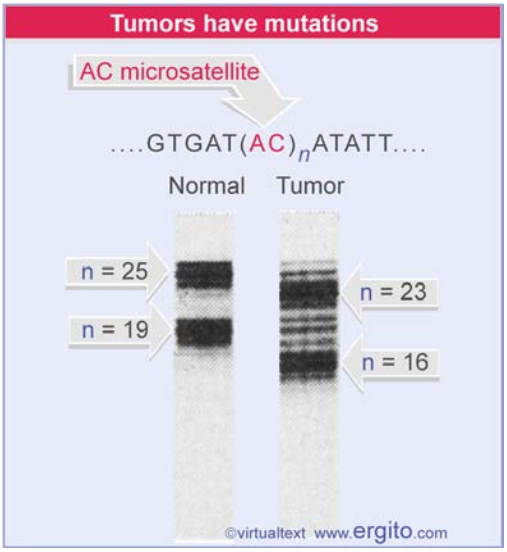

Figure 30.47 The normal tissue of a patient has two alleles for a microsatellite, each with a different number of repeats of the dinucleotide (AC). In tumor cells, both of these alleles have suffered deletions, one reducing the repeat number from 25 to 23, the other reducing it from 19 to 16. The repeat number of each allele in each situation is in fact probably unique, but some additional bands are generated as an artefact during the amplification procedure used to generate the samples. Data kindly provided by Bert Vogelstein. The bands remaining at the normal position in the tumor samples are due to contamination of the tumor sample with normal tissue. (From 2256).

The idea that this type of change might be the result of loss of the mismatch-repair system was confirmed by showing that *mutS* and *mutL* homologues (*hMSH2*, *hMLH1*) are mutated in the tumors (2258; 2259). As expected, the tumor cells are deficient in mismatch-repair. Change in the microsatellite sequences is of course only one of the types of mutation that result from the loss of the mismatch-repair system (it is especially easy to diagnose).

The case of HNPCC illustrates both the role of multiple mutations in malignancy and the contribution that is made by mutator genes. At least 7 independent genetic events are required to form a fully tumorigenic colorectal cancer. More than 90% of cases have mutations in the mismatch-repair system, and the tumor cells have mutation rates that are elevated by 2-3 orders of magnitude from normal somatic cells (for review see 2253). The high mutation rate is responsible for creating new variants in the tumor that provide the raw material from which cells with more aggressive growth properties will arise.

Several human diseases are caused by mutations in the systems that execute checkpoints, including Ataxia telangiectasia (see *Molecular Biology 6.29.13 DNA damage triggers a checkpoint*), Nijmegan breakage syndrome, and Bloom's syndrome, all of which are characterized by chromosomal rearrangements that are triggered by breaks in DNA.

Last updated on 8-21-2002

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ONCOGENES AND CANCER

6.30.30 Summary

A tumor cell is distinguished from a normal cell by its immortality, morphological transformation, and (sometimes) ability to metastasize. Oncogenes are identified by genetic changes that represent gain-of-functions associated with the acquisition of these properties. An oncogene may be derived from a proto-oncogene by mutations that affect its function or level of expression. Tumor suppressors are identified by loss-of-function mutations that allow increased cell proliferation. The mutations may either eliminate function of the tumor repressor or create a dominant negative version.

DNA tumor viruses carry oncogenes without cellular counterparts. Their oncogenes may work by inhibiting the activities of cellular tumor suppressors. RNA tumor viruses carry *v-onc* genes that are derived from the mRNA transcripts of cellular (*c-onc*) genes. Some *v-onc* oncogenes represent the full length of the *c-onc* proto-oncogene, but others are truncated at one or both ends. Most are expressed as fusion proteins with a retroviral product. Src is an exception in which the retrovirus (RSV) is replication-competent, and the protein is expressed as an independent entity.

Some *v-onc* genes are qualitatively different from their *c-onc* counterparts, since the *v-onc* gene is oncogenic at low levels of protein, while the *c-onc* gene is not active even at high levels. In such cases, proto-oncogenes are activated efficiently only by changes in the protein coding sequence. Other proto-oncogenes can be activated by large (>10×) increases in the level of expression; *c-myc* is an example that can be activated quantitatively by a variety of means, including translocations with the Ig or TCR loci or insertion of retroviruses.

c-onc genes may have counterpart *v-onc* genes in retroviruses, but some proto-oncogenes have been identified only by their association with cellular tumors. The transfection assay detects some activated *c-onc* sequences by their ability to transform rodent fibroblasts. *ras* genes are the predominant type identified by this assay. The creation of transgenic mice directly demonstrates the transforming potential of certain oncogenes.

Cellular oncoproteins may be derived from several types of genes. The common feature is that each type of gene product is likely to be involved in pathways that regulate growth, and the oncoprotein has lack of regulation or increased activity.

Growth factor receptors located in the plasma membrane are represented by truncated versions in *v-onc* genes. The protein tyrosine kinase activity of the cellular receptor is activated only when ligand binds, but the oncogenic versions have constitutive activity or altered regulation. In the same way, mutation of genes for polypeptide growth factors gives rise to oncogenes, because a receptor becomes inappropriately activated.

Some oncoproteins are cytoplasmic tyrosine kinases; their targets are largely

unknown. They may be activated in response to the autophosphorylation of tyrosine kinase receptors. The molecular basis for the difference between c-Src and v-Src lies in the phosphorylation states of two tyrosines. Phosphorylation of Tyr-527 in the C-terminal tail of c-Src suppresses phosphorylation of Tyr-416. The phosphorylated Tyr-527 binds to the SH2 domain of Src. However, when the SH2 domain recognizes the phosphopeptide sequence created by autophosphorylation of PDGF receptor; the PDGF receptor displaces the C-terminal region of Src, thus allowing dephosphorylation of Tyr-527, with the consequent phosphorylation of Tyr-416 and activation of the kinase activity. v-Src has lost the repressive C-terminus that includes Tyr-527, and therefore has permanently phosphorylated Tyr-416, and is constitutively active.

Ras proteins can bind GTP and are related to the α subunits of G proteins involved in signal transduction across the cell membrane. Oncogenic variants have reduced GTPase activity, and therefore are constitutively active. Activation of Ras is an obligatory step in a signal transduction cascade that is initiated by activation of a tyrosine kinase receptor such as the EGF receptor; the cascade passes to the ERK MAP kinase, which is a serine/threonine kinase, and terminates with the nuclear phosphorylation of transcription factors including Fos.

Nuclear oncoproteins may be involved directly in regulating gene expression, and include Jun and Fos, which are part of the AP1 transcription factor. v-ErbA is derived from another transcription factor, the thyroid hormone receptor, and is a dominant negative mutant that prevents the cellular factor from functioning. v-Rel is related to the common factor NF- κB, and influences the set of genes that are activated by transcription factors in this family.

Retinoblastoma (RB) arises when both copies of the *RB* gene are deleted or inactivated. The *RB* product is a nuclear phosphoprotein whose state of phosphorylation controls entry into S phase. Nonphosphorylated RB sequesters the transcription factor E2F. The RB-E2F complex represses certain target genes. E2F is released when RB is phosphorylated by cyclin/cdk complexes; E2F can then activate genes whose products are needed for S phase. Loss of RB prevents repression by RB-E2F, and means that E2F is constitutively available. The cell cannot be restrained from proceeding through the cycle. Adenovirus E1A and papova virus T antigens bind to nonphosphorylated RB, and thus prevent it from binding to E2F.

p53 was originally classified as an oncogene because missense mutations in it are oncogenic. It is now classified as a tumor suppressor because the missense mutants in fact function by inhibiting the activity of wild-type p53. The same phenotype is produced by loss of both wild-type alleles. The level of p53 is usually low, but in response to damage to DNA, p53 activity increases, and triggers either of two pathways, depending upon the stage of the cell cycle and the cell phenotype. Early in the cycle, it provides a checkpoint that prevents further progress; this allows damaged DNA to be repaired before replication. Later in the cycle, it causes apoptosis, so that the cell with damaged DNA dies instead of perpetuating itself. Loss of p53 function is common in established cell lines and may be important in immortalization *in vitro*. Absence of p53 is common in human tumors and may contribute to the progression of a wide variety of tumors, without specificity for cell type.

p53 is activated by binding to damaged DNA, for which it uses a (non

sequence-specific) DNA-binding domain. One important target that activates p53 is the single-stranded overhanging end that is generated at a shortened telomere. When it is activated, p53 uses another DNA-binding domain to recognize a palindromic ~10 bp sequence. Genes whose promoters have this sequence and which are activated by p53 include the cdk inhibitor p21 and the protein GADD45 (which is activated by several pathways for response to DNA damage). Activation of these and other genes (involving a transactivation domain that interacts directly with TBP) is probably the means by which p53 causes cell cycle arrest. p53 has a less well characterized ability to repress some genes. Mutant p53 lacks these activities, and therefore allows the perpetuation of cells with damaged DNA. Loss of p53 may be associated with increased amplification of DNA sequences.

p53 is bound by viral oncogenes such as SV40 T antigen, whose oncogenic properties result, at least in part, from the ability to block p53 function. It is also bound by the cellular proto-oncogene, Mdm2, which inhibits its activity. p53 and Mdm2 are mutual antagonists.

The locus INK4A contains two tumor suppressors that together control both major tumor suppressor pathways. $p19^{ARF}$ inhibits Mdm2, so that p19 in effect turns on p53. $p16^{INK4A}$ inhibits the cdk4/6 kinase, which phosphorylates RB. Deletion of INK4A therefore blocks both tumor suppressor pathways by leading to activation of Mdm2 (inhibiting p53) and activation of cdk4/6 (inhibiting RB).

Loss of p53 may be necessary for immortalization, because both the G1 checkpoint and the trigger for apoptosis are inactivated. Telomerase is usually turned off in differentiating cells, which provides a mechanism of tumor suppression by preventing indefinite growth. Reactivation of telomerase is usually necessary to allow continued proliferation of tumor cells. The crisis that is encountered by cultured cells results from shortening of telomeres to the point at which genetic instability is created by the chromosome ends. Loss of p53 is important in passing through crisis, because otherwise p53 is activated by the ends generated by telomere loss.

Several independent events are required to convert a normal cell into a cancer cell, typically involving both immortalizing and transforming functions. The required number of events is in the range of 4-10, and would not normally be expected to occur during the life span of a cell. Early events may increase the rate of occurrence of mutational change by damaging the repair or other systems that limit mutational damage. One important target is the MutSL system that is responsible for removing mismatches in replicated DNA.