12 EVOLUTIONARY DYNAMICS OF CANCER

CANCER IS THE CONSEQUENCE of an evolutionary process. Usually we think of evolution as leading to improvement and innovation. But cancer is an evolution that leads backward to selfish cellular proliferation, often destroying the organism in which it occurs. Cancer is the unfortunate byproduct of our design. We are built of individual cells that have their own reproductive machinery and that can sometimes revert to a primitive program of uncontrolled self-replication. Cancer is the "evolution of defection" among our cells.

Computers have viruses, but they do not get cancer. Computers can copy information, and therefore viruses can manipulate this process and reproduce. But computers are not built of smaller self-reproducing units. Therefore silicon-based cancer does not yet exist.

Cancer is a disease of multicellular organisms. For the development of multicellularity, the main obstacle that needed to be overcome was how to establish and maintain cooperation among many individual cells. Cancer is a breakdown of cellular cooperation. Cells must divide whenever needed for the developmental program, but not otherwise. A complicated genetic control network had to evolve to achieve this task. Many of our genes work to ensure that cancer does not happen too early. These genes are involved (i) in

maintaining the integrity of the genome; (ii) in performing error-free cell division; (iii) in determining the developmental program that tells cells when to divide; and (iv) in monitoring the status of the cell and if necessary inducing programmed cell death (apoptosis).

Most cells in the body are continuously listening to reassuring signals from other cells telling them that they are doing all right. If these signals fail to arrive, then the default program for a cell is to commit suicide. Apoptosis is a defense mechanism against cancer. If things go wrong, then cells kill themselves. Cancer cells have escaped from apoptotic control.

The evolutionary process that leads to cancer is different from most other evolutionary processes, because there are so many genes that can be inactivated or modified without any fitness loss for the cell and in many cases even with a fitness gain. Therefore cancer progression can be seen as a "destructive evolution," getting rid of mechanisms that are implemented to protect against cancer.

A relatively large fraction of all possible mutations that can occur in a precancerous cell will increase its somatic fitness (reproductive rate). In contrast, a vanishingly small fraction of all possible mutations that can occur in a well-adapted organism (such as a mouse or a rabbit) will increase its fitness. Hence there should be strong selection pressure on cancer cells to increase their mutation rates. The optimum mutation rate for a cell on the way to cancer is much higher than the normal somatic mutation rate. Throughout this chapter, the term "mutation" is used to include any genetic modification such as point mutations, insertions, deletions, chromosome rearrangements, mitotic recombination, or loss or gain of whole chromosomes or arms of chromosomes.

The idea that cancer is a genetic disease caused by somatic evolution has emerged over the last one hundred years. In 1890 the German physician David von Hansemann noted that cancer cells had abnormal cell division events. In 1914 Theodor Boveri observed that something was wrong with the chromosomes of cancer cells. Today we know that most cancer cells are aneuploid, that is, they do not have the normal number of chromosomes (Figure 12.1). Ernest Tyzzer in 1916 was the first to apply the term "somatic mutation" to cancer. Herman Muller discovered in 1927 that ionizing radiation, which was known to be carcinogenic, was also mutagenic. This observation presented further

evidence for the association between somatic mutation and cancer. In 1951 Muller proposed that for cancer to occur, a single cell had to receive multiple mutations. A few years later, the mathematical modeling of cancer began with a statistical analysis of age-incidence patterns. Work by C. O. Nordling in 1953 as well as Peter Armitage and Richard Doll in 1954 led to the important insight that the emergence of cancer requires multiple probabilistic events.

In 1971 Alfred Knudson discovered that the age incidence of retinoblastoma, a childhood cancer, grows as a linear function of time in the group of children who have multiple cancers in both eyes, but as a slower quadratic function of time in the group of children who have only one cancer. Knudson proposed the concept of a tumor suppressor gene (TSG). Cancer emerges if both alleles are inactivated. In the first group of children, one allele is already inactivated in their germ line, while the second allele is inactivated by a somatic mutation. In the second group of children, both alleles are inactivated by somatic mutations. This is known as Knudson's two-hit hypothesis: it takes two hits to inactivate a TSG (Figure 12.2). On the basis of this discovery, Suresh Moolgavkar and Alfred Knudson developed probabilistic models to describe cancer initiation and progression.

In 1986 the retinoblastoma tumor suppressor gene was identified. In the meantime, about thirty tumor suppressor genes have been found to be associated with human cancers. They have the property that the somatic mutations are recessive: inactivating the first allele is neutral (or nearly neutral), while inactivating the second allele changes the phenotype of the cell and usually increases its net reproductive rate, which constitutes a step toward cancer.

An important TSG is p53, which is mutated in more than half of all human cancers. This gene is located at the center of a control network that monitors genetic damage (including double-strand breaks of the DNA). If there is a certain amount of damage, then cell division will be paused and the cell will be given time to repair itself. If there is too much damage, then the cell will undergo apoptosis. In many cancer cells, the function of p53 is inactivated, which allows these cell to divide in the presence of substantial genetic damage.

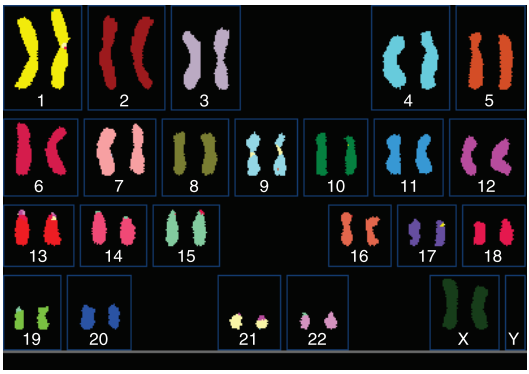
Oncogenes represent another class of genes that are involved in cancer. Oncogenes increase cellular proliferation if one allele is mutated or inappropriately expressed (Figure 12.3). The concept of oncogenes was introduced by Michael Bishop and Harold Varmus in 1976; in 1989 they shared the Nobel

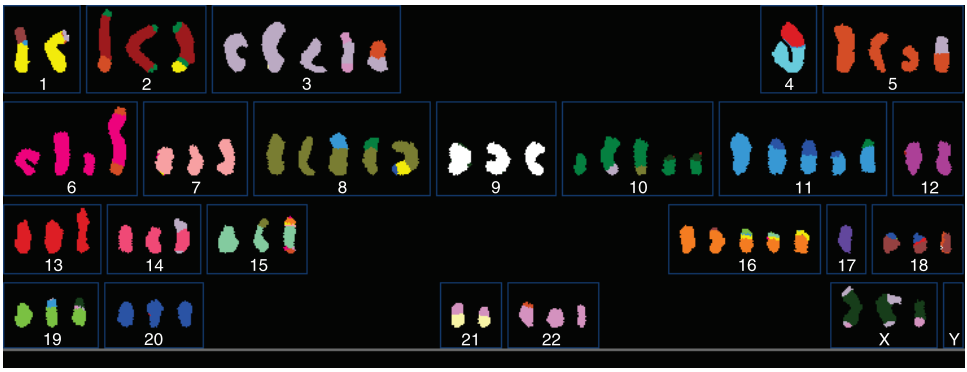
Prize in Medicine for this work. Some viruses carry oncogenes that induce the infected cell to proliferate like a cancer cell. In the last three decades, many oncogenes have been discovered that are involved in various stages of human cancers including tumor initiation, progression, angiogenesis (the process of attracting blood vessels to growing tumors), and metastasis formation.

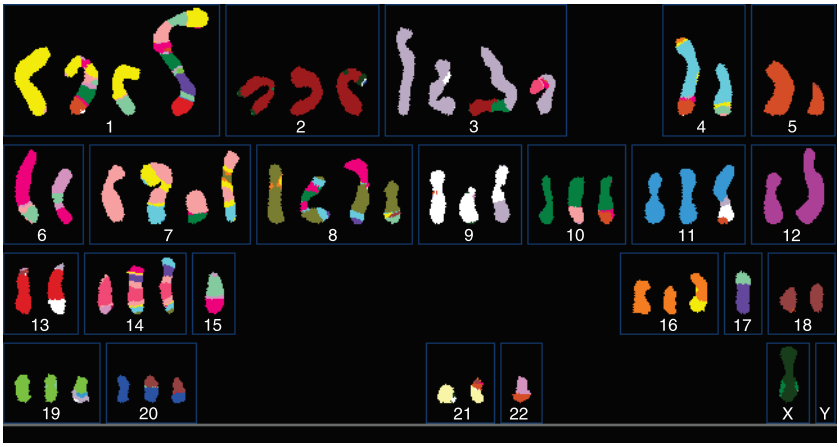
While mutations of tumor suppressor genes and oncogenes tend to increase the net reproductive rate (somatic fitness) of a cell, mutations in genetic instability genes increase the mutation rate. For example, mutations in mismatch repair genes lead to a 50–1,000-fold increase of the point mutation rate, which manifests itself primarily in the accumulation of mutations in the microsatellite regions of the genome. Therefore this type of genetic instability is called microsatellite instability (MIN). About 15% of colon cancers have MIN, but 85% of colon cancers and most other cancers have chromosomal instability (CIN).

CIN is defined as an increased rate of gaining or losing whole chromosomes or large fractions of chromosomes during cell division (Figure 12.4). Often the first allele of a TSG is inactivated by a point mutation, while the second allele is inactivated by loss of heterozygosity (LOH). LOH can be caused by somatic recombination or by loss of (the part of) the chromosome that contains the unmutated allele. In both cases, we obtain a cell that has lost both alleles of the TSG. Christoph Lengauer and Bert Vogelstein have determined that the rate of

Figure 12.1 Normal cells are diploid, with two copies of each autosomal chromosome. The autosomal human chromosomes are labeled 1 to 22 according to decreasing size. In addition, there are two sex chromosomes: in females XX, in males XY. Therefore in a normal human cell there are 46 chromosomes, as shown in the top spectral karyotype. (A karyotype depicts the complete set of chromosomes within a cell.) In most cancer cells, especially in solid tumors, there is aneuploidy, which means the total number of chromosomes is not 46. Some chromosomes exist in more than two copies, others only in one. Moreover, some chromosomes in cancers can be fusions of two or more chromosomes. The middle karyotype is from the breast cancer line HCC1937, which has a BRCA1 mutation. The bottom karyotype shows the chromosomal abnormality in a Capan1 cell (pancreatic cancer) with a BRCA2 mutation. Images courtesy of Joanne M. Staines and Paul Edwards, Cancer Genomics Project, University of Cambridge. See also Davidson et al. (2000).







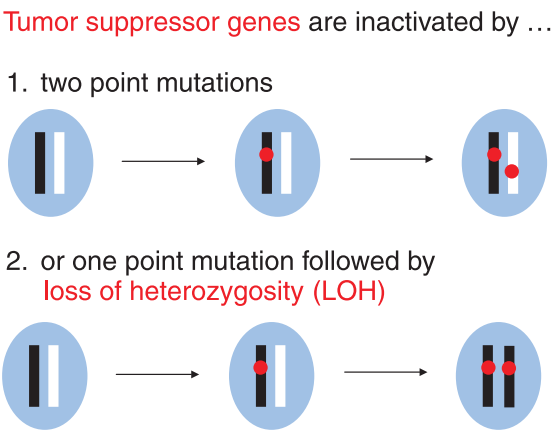


Figure 12.2 A tumor suppressor gene (TSG) is inactivated by two mutational events. The first hit is often caused by a point mutation. The second hit can be another point mutation or a loss of heterozygosity (LOH). There are various mechanisms of LOH, including somatic recombination or loss of a whole chromosome or chromosome arm. If one copy of the chromosome is lost, sometimes the other copy is duplicated. TSGs play central roles in regulatory networks that determine the rate of cell cycling. Inactivation of the function of a TSG modifies the regulatory network and can lead to increased cell proliferation. The basic idea of a TSG is that inactivation of one allele has no (or only a minor) effect, while inactivation of both alleles represents a step toward cancer.

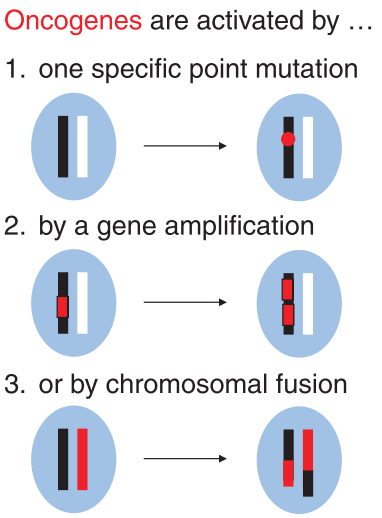


Figure 12.3 Oncogenes must be activated to induce a step toward cancer. It is usually sufficient to mutate one of the two alleles. The activation can occur by a specific point mutation, a gene amplification, or a chromosomal rearrangement. The latter can lead to a fusion gene, where the first half comes from one gene, the second half from another. Activated oncogenes increase cell proliferation.

Chromosomal instability (CIN):
mutations in CIN genes increase the rate of gaining or losing whole chromosomes

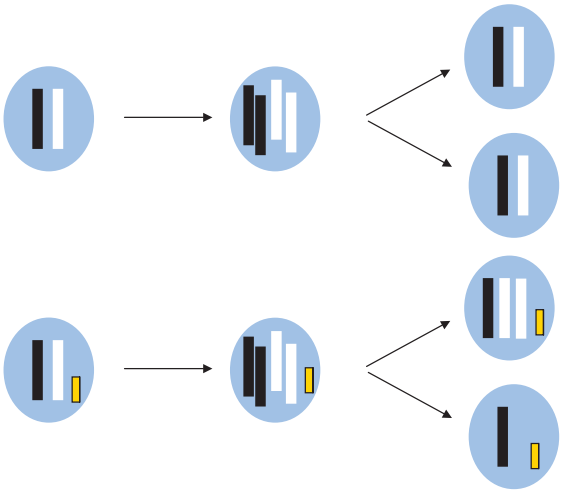


Figure 12.4 Many hundreds of genes work together during cell division to ensure that all chromosomes are properly duplicated and distributed to each of the two daughter cells. Mutations in such genes can lead to chromosomal instability (CIN). The CIN phenotype of a cell is defined by an increased rate of gaining or losing whole chromosomes or arms of chromosomes. The figure shows a normal cell division (top) and one that leads to aneuploidy (bottom). The black and white bars indicate maternal and paternal copies of a chromosome. The yellow bar illustrates a CIN mutation (somewhere in the genome).

losing a chromosome in CIN cancer cells is about 10^{-2} per chromosome per cell division. In contrast, the rate of LOH in non-CIN cells is thought to be on the order of 10^{-7} to 10^{-6}. Therefore CIN leads to a dramatic acceleration in the inactivation of TSGs.

The molecular basis for CIN is just beginning to be understood. A large number of genes that trigger CIN when mutated have been discovered in yeast. These so-called CIN genes are involved in chromosome condensation, sister-chromatid cohesion, kinetochore structure and function, microtubule formation, and cell cycle checkpoints. By comparison with yeast, we expect several hundred human CIN genes, but only a few have been identified so far. These genes include MAD2, hBUB1, BRCA2, and hCDC4.

The classification of CIN genes is based on the mutational events that are required to trigger CIN. Class I CIN genes, such as MAD2, trigger CIN if one allele of the gene is mutated or lost. Class II CIN genes, such as hBUB1, trigger CIN if one allele is mutated in a dominant-negative fashion; this means the

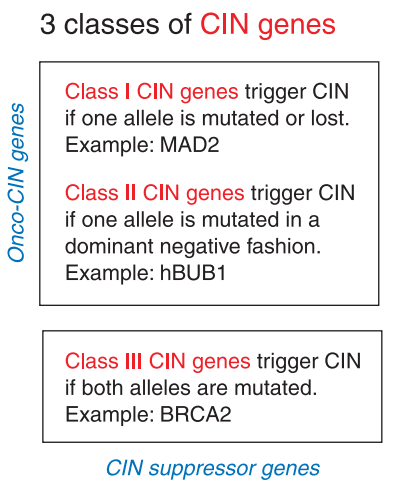


Figure 12.5 CIN is caused by mutations in genes that participate in maintaining genomic integrity during cell division. We can classify CIN genes according to the number and types of mutational events that are needed to activate the CIN phenotype. Class I genes lead to CIN if one allele is mutated or lost. Class II genes lead to CIN if one allele is mutated. Class III genes lead to CIN if both alleles are mutated. There exist examples among human CIN genes for all three classes. Class I and II genes can be called onco-CIN genes, while class III genes are CIN suppressor genes.

mutated allele interferes with the normal function of the unmutated allele. Class III CIN genes, such as BRCA2, trigger CIN if both alleles are mutated. Class I and II CIN genes could be called "onco-CIN genes," while class III genes are "CIN suppressor genes" (Figure 12.5).

Because of the brilliant work of Bert Vogelstein and his friends, colon cancer has one of the best understood evolutionary trajectories. The epithelial layer of the colon has an enormous cellular turnover: a large number of cells are produced and discarded each day. This massive amount of cell division events implies a higher risk of mutations that might lead to cancer. The geometric organization of the colon, however, reduces this risk. The colon is organized into about 10^7 crypts. Each crypt contains thousands of cells. At the bottom of the crypt there is a small number of stem cells that slowly divide to produce differentiated cells. Differentiated cells divide a few times while migrating to the top of the crypt, where they undergo apoptosis. The architecture of the crypt implies that only a small subset of the cells are at risk of acquiring mutations that will become fixed in permanent cellular lineages. Many mutations that arise in the differentiated cells will be washed out. Therefore the number of cells that are at risk of receiving mutations toward cancer is greatly reduced by this architecture (Figure 12.6).

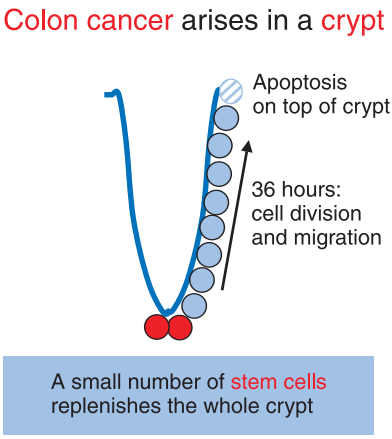


Figure 12.6 The epithelial layers of many tissues seem to require large numbers of rapidly dividing cells. This cell turnover represents a main risk for the emergence of cancer. The colon is organized into roughly 10^7 crypts. Each crypt contains about 1,000 to 4,000 cells. At the bottom of the crypt there are a small number of stem cells (maybe 1 to 4) which divide slowly and differentiate into cells that divide rapidly and migrate to the top of the crypt, where they undergo apoptosis (programmed cell death). This design helps to reduce the risk of cancer, because most cell division events (and therefore most somatic mutations) occur in cells that are short lived. When designing models for the dynamics of colon cancer initiation, we have to take into account that the epithelial layer is subdivided into crypts, and that each crypt has a small effective population size.

Colorectal cancer is thought to be initiated by a mutation that inactivates the adenomatous polyposis coli (APC) tumor suppressor gene pathway. In about 95% of cases, the APC tumor suppressor gene is mutated. In the remaining cases, there are other mutations that affect the same pathway. The crypt in which the APC mutant cell arises becomes dysplastic. The slow accumulation of abnormal cells produces a polyp. The emergence of a large polyp seems to require further mutations, for example, activation of the oncogenes RAS or BRAF. Subsequently 10–20% of these large polyps progress to cancer

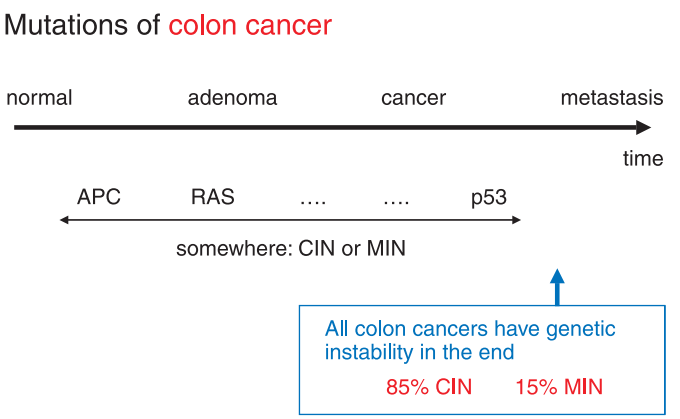


Figure 12.7 The "Vogelgram" illustrates the sequence of mutations that lead to colon cancer. Usually the first step is inactivation of the APC tumor suppressor gene pathway, followed by activation of the RAS or BRAF oncogene. There are one or two additional mutations that are not yet clearly characterized. Eventually there is inactivation of the p53 tumor suppressor gene. While normal colon tissue is genetically stable, all colon cancers have genetic instability in the end. About 85% of sporadic colon cancers have chromosomal instability (CIN). The remaining 15% have microsatellite instability (MIN). At some stage genetic instability must arise. A major question is whether genetic instability is an early event and a driving force of tumorigenesis or a late stage by-product.

by acquiring mutations in genes that are part of the TGF-β pathway, the p53 pathway, or other pathways (Figure 12.7).

In the end, all colon cancers are genetically unstable. About 15% of colon cancers are diploid and have MIN. The remaining 85% are aneuploid and have CIN. A major question is whether genetic instability arises early or late during the progression to colon cancer.

A quantitative understanding of cancer biology requires a mathematical framework to describe the fundamental principles of population genetics and evolution that govern tumor initiation and progression. Mutation, selection, and tissue organization determine the dynamics of tumorigenesis and should be studied quantitatively in terms of both experiment and theory.

Here I will address the following questions: What are the fundamental principles that determine the dynamics of activating oncogenes and inactivating

tumor suppressor genes? How do mutation, selection, and tissue architecture influence the rate of tumor initiation and progression? And how do quantitative approaches help to investigate the role of genetic instability in tumorigenesis?

12.1 ONCOGENES

Oncogenes contribute to cancer progression if one allele is mutated or inappropriately expressed. Let us explore the basic aspects of the evolutionary dynamics of oncogene activation.

Most tissues of multicellular organisms are subdivided into compartments, which contain populations of cells that proliferate to fulfill organ-specific tasks. Compartments are subject to homeostatic mechanisms that ensure that the cell number remains approximately constant over time. Whenever a cell divides, another cell has to die to keep the total population size constant. Cancer results if the equilibrium between cell birth and death is shifted toward uncontrolled proliferation. Not all cells of a compartment, however, may be at risk of becoming cancerous. Differentiated cells, for example, may not have the capacity to divide often enough to accumulate the necessary number of mutations in cancer susceptibility genes (these are genes that may lead to cancer when mutated, such as tumor suppressor genes, oncogenes, or genetic instability genes). The effective population size of a compartment describes those cells that are at risk of becoming cancer cells. In the following, compartment size will be used synonymously with effective population size within a compartment.

Consider a compartment of replicating cells. During each cell division, there is a small probability that a mistake will be made during DNA replication; in this case, a mutated daughter cell is produced. The mutation might confer a fitness advantage to the cell by ameliorating an existing function or inducing a new function. Then the mutation is "advantageous" in terms of somatic selection. Alternatively, the mutation might impair an important cellular function and confer a fitness disadvantage to the cell. Then the cell proliferates more slowly or dies more quickly than its neighbors. The net reproductive rate is decreased, and the mutation is "deleterious" in terms of

somatic selection. (Somatic selection describes the process of natural selection among cells of the soma of a multicellular organism. Somatic selection leads to cancer.) Finally, the mutation might not change the reproductive rate of the cell. Then the cell proliferates at the same rate as its neighbors, and the mutation is "neutral" in terms of somatic selection. All of these mutations can represent steps toward cancer and are therefore disadvantageous for the organism.

Let us discuss the dynamics of a particular mutation within a compartment. Initially, all cells are unmutated. What is the probability that a single mutated cell has arisen by time t? We measure time, t, in cell cycles. If the relevant cells divide once per day, then the unit of time is one day. Denote by N the number of cells in a compartment, and denote by u the mutation rate per gene per cell division. The probability that at least one mutated cell has arisen by time t is given by

$$P(t) = 1 - e^{-Nut}. \tag{12.1}$$

What is the fate of a single mutated cell? In the simplest scenario, there is a constant probability, q, that this cell will not die, but will initiate a neoplasia. Hence the probability that a compartment has initiated a neoplasia by time t is given by

$$P(t) = 1 - e^{-Nuqt}. \tag{12.2}$$

Alternatively, consider a scenario in which the mutated cell has a relative fitness r compared with a wild-type cell with fitness 1 ("wild type" means unmutated). If $r > 1$, the mutation is advantageous; if $r < 1$, the mutation is disadvantageous; if $r = 1$, the mutation is neutral. Normally, we expect mutations in oncogenes to cause increased net growth rates, $r > 1$; however, a mutation in an oncogene could be kept in check by apoptotic defense mechanisms, and therefore r could be less than one.

What is the probability that such a mutation takes over the compartment? In order to calculate this probability, we consider the Moran process (Chapter 6). The fixation probability of a single mutant with relative fitness r is given by

$$\rho = \frac{1 - 1/r}{1 - 1/r^N}. \tag{12.3}$$

For a neutral mutant, $r = 1$, we have $\rho = 1/N$. An advantageous mutation has a higher fixation probability than a neutral mutation, which has a higher fixation probability than a deleterious mutation. The events in a small compartment, however, are dominated by random drift: if N is small, then even a deleterious mutation can have a fairly high probability of reaching fixation due to chance events.

The probability that a mutation has been fixed by time t is given by

$$P(t) = 1 - e^{-Nu\rho t}. \tag{12.4}$$

Note that any mutation has a higher fixation probability, ρ, in a small compartment than in a large compartment, but $P(t)$ is an increasing function of N for $r > 1$ and a decreasing function of N for $r < 1$. Thus large compartments accelerate the accumulation of advantageous mutations, but slow down the accumulation of deleterious mutations. Conversely, small compartments slow down advantageous mutations, but accelerate deleterious ones. Therefore the compartment size is important in determining the types of mutations that are likely to occur.

We can argue that the most dangerous steps toward cancer are those that lead to cells with increased net reproductive rate, such as mutations in oncogenes or tumor suppressor genes. The best tissue architecture for containing those mutations uses a large number of small compartments. It seems that this is the dominant tissue architecture adopted by human organs that require fast cell division. The mutant cell that carries a fitness advantage is likely to reach fixation in the compartment, but its further spread is at least initially limited by the compartment boundaries. It turns out, however, that this architecture is especially vulnerable to cancer initiation via mutations that lead to genetic instability.

The difference between equations (12.2) and (12.4) is the following: in (12.2) there is a fixed probability q that a mutated cell initiates a neoplasia; in (12.4) the corresponding probability, ρ, depends on the selective advantage of the cell, r, and the effective population size, N, of the compartment. Equation (12.4) describes a situation where the mutated cell has a fitness advantage and must reach fixation in the compartment to initiate cancer progression. Equation (12.2) refers to a situation where a mutated cell might induce a clonal expansion, which is not subject to the constraints of the compartment.

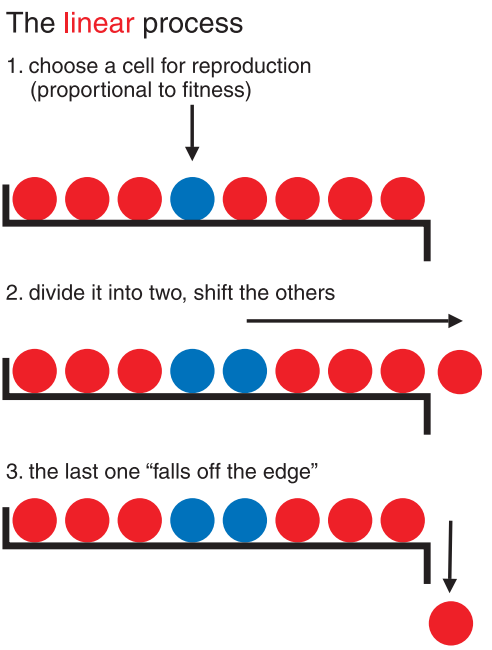


Figure 12.8 The linear process represents the simplest stochastic model that describes the subdivision of somatic tissue into stem cells and differentiated cells. At each time step, a cell is chosen for reproduction proportional to fitness. The cell is replaced by the two daughter cells. All cells to the right are shifted one place. The right-most cell falls off the edge (= undergoes apoptosis). What is the probability of fixation of a mutant cell with relative fitness r that arises in a random position?

12.2 THE LINEAR PROCESS

So far we have considered the evolutionary dynamics of a mutation that arises in a well-mixed compartment. This approach describes a tissue compartment in which all relevant cells are in equivalent positions and in direct reproductive competition with one another—there are no spatial effects. However, we can also envisage theories in which cellular differentiation and spatial structure are explicitly modeled. One simple approach considers N cells in a linear array. At each time step, a cell is chosen at random, but proportional to fitness. The cell is replaced by two daughter cells, and all cells to its right are shifted by one place to the right. The cell at the far right undergoes apoptosis. The cell at the far left acts as a stem cell (Figure 12.8).

Let us now assume that a mutated cell arises that has relative fitness r. The fixation probability of this mutant cell is given by $\rho = 1/N$, irrespective of r, because only a mutation in the left-most cell can reach fixation in the

compartment. A mutation arising in any other cell will eventually be "washed out" of the compartment by the continuous production of cells and their migration from the stem cell to differentiation and apoptosis. The probability that all cells of the compartment are mutated at time t is given by

$$P(t) = 1 - e^{-ut}. \tag{12.5}$$

Here time is measured in units of stem cell divisions. If the stem cell divides more slowly than the other cells, then the accumulation of mutated cells is decelerated.

This "linear process" of cancer initiation has the important feature of balancing out fitness differences between mutations. Advantageous, deleterious, and neutral mutations all have the same fixation probability, $\rho = 1/N$. This is in contrast to a well-mixed compartment, in which the fittest mutation has the highest probability of fixation. In comparison with a well-mixed compartment, a linear compartment delays the development of cancers that are initiated by advantageous mutations, such as mutations in oncogenes and tumor suppressor genes (Figure 12.9).

Incidentally, the linear process prompted the idea of evolutionary graph theory, but its shifting cell population is not covered by the mathematical formalism developed in Chapter 8.

12.3 NUMERICAL EXAMPLES

Three simple numerical examples illustrate how tissue architecture can affect the rate of cancer progression.

(i) Suppose an organ consists of $M = 10^7$ compartments. Each compartment has $N = 10^3$ cells that divide once per day. Let us assume that the rate of activating the oncogene per cell division is $u = 10^{-9}$ and that this activation confers a 10% growth advantage to the cell, which means $r = 1.1$. Then the probability of fixation is $\rho = (1 - 1/r)/(1 - 1/r^N) \approx 0.09$. The probability that a compartment has been taken over by mutated cells after 70 years ($t = 70 \times 365.25$ days) is $P(t) = 1 - \exp(-Nu\rho t) \approx 0.0023$. The expected number of mutated compartments at this age is $M \cdot P(t) \approx 23{,}000$.

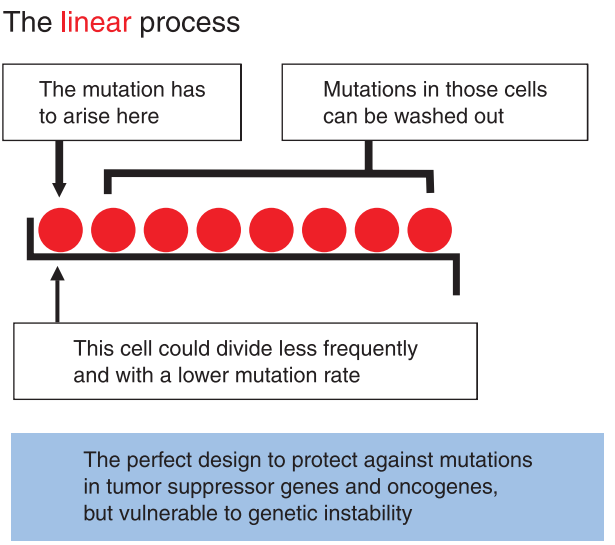


Figure 12.9 The linear process acts as a powerful suppressor of selection. A mutant can only take over the whole compartment if it arises in the left-most cell, which acts like a stem cell. Only the lineage arising from this cell is here to stay. All lineages descending from other cells are transient; mutations that occur in those cells will be washed out. The probability of fixation of a randomly placed mutant is $1/N$, irrespective of its relative fitness. Hence the selective advantage of an activated oncogene or inactivated tumor suppressor gene is negated by the population structure of the linear process. Furthermore, the stem cell can divide more slowly and with a smaller mutation rate than other cells. This effect can further reduce the rate of somatic evolution that might lead to cancer.

(ii) Let us now assume a linear tissue architecture for each compartment. As before, there are $M = 10^7$ compartments consisting of 10^3 cells each. But each compartment is now fed by one stem cell that divides every 10 days. The probability that a compartment has been taken over by mutated cells at time $t = 70$ years is reduced to $P(t) \approx 2.6 \cdot 10^{-6}$. The expected number of mutated compartments at this age is only 26. With these numerical values, the linear architecture reduces the rate of cancer progression about 1,000-fold.

(iii) Finally, consider a population of $N = 10^7$ cells that divide every day. This population size describes, for example, a lesion that has already accumulated mutations in one or a few cancer susceptibility genes. The probability is $P \approx 0.28$ that a mutated cell with relative fitness $r = 1.1$ arises and takes over the whole population within one year. The time until this probability is 1/2 is given by $T_{1/2} = 2.1$ years.

12.4 TUMOR SUPPRESSOR GENES

A normal cell has two alleles of a tumor suppressor gene. The standard idea is that inactivation of the first allele does not lead to a phenotypic change, but inactivation of both alleles increases the net reproductive rate of the cell and therefore represents a step toward cancer.

While an oncogene is activated by one or a few specific mutations, a TSG can typically be inactivated by any mutation that disrupts the function of the gene. Therefore the mutation rate for inactivating an allele of a TSG is much higher than the mutation rate that results in activation of an oncogene. But two events are required to eliminate a TSG, while one event suffices to activate an oncogene.

Consider a TSG, A. Let us introduce the following nomenclature: A "type 0" cell, $A^{+/+}$, is a normal cell with two functioning (wild-type) alleles of the TSG. A "type 1" cell, $A^{+/-}$, has only one functioning allele of the TSG. A "type 2" cell, $A^{-/-}$, has no functioning allele of the TSG.

Let us now ask the most basic question concerning the evolutionary dynamics of TSGs. What is the probability that a single cell with two inactivated TSG alleles has arisen by time t in a population of reproducing cells? The answer, which turns out to be surprisingly difficult, will be presented in subsections 12.4.1–12.4.4. We note that the whole system has only three parameters: the population size, N; the mutation rate for the first hit, u_1; and the mutation rate for the second hit, u_2 (Figure 12.10).

We will assume that u_1 is smaller than u_2, because certain mutational mechanisms, such as mitotic recombination, can only constitute the second hit. In CIN cells, u_1 is much smaller than u_2, because of the dramatically increased rate of losing whole chromosomes.

Evolutionary dynamics of tumor suppressor genes:
given a population of N reproducing cells,
what is the probability that at least one cell
has received two mutations by time t?

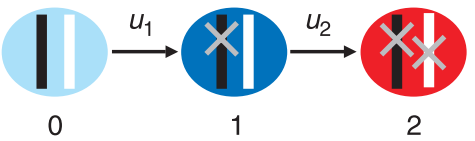


Figure 12.10 How long does it take for a population of reproducing cells to inactivate a tumor suppressor gene? If we assume that the first hit is neutral, then the answer will depend on three parameters: the population size, N; and the mutation rates for the first and second allele, u_1 and u_2.

12.4.1 The Exact Markov Process

The evolutionary dynamics can be described by a Markov process with $N+2$ states. The states $i = 0, \ldots, N$ are transient and indicate the presence of i cells of type 1 and $N-i$ cells of type 0. The state $N+1$ is the only absorbing state and indicates that a cell of type 2 has been produced. The transition probabilities for this Markov process are as follows:

$$
\begin{aligned}
P_{i,i-1} &= \frac{i}{N}\frac{N-i}{N}(1-u_1) \qquad i = 1, \ldots, N \\
P_{i,i} &= \frac{i}{N}\left[\frac{i}{N}(1-u_2) + \frac{N-i}{N}u_1\right] + \left(\frac{N-i}{N}\right)^2(1-u_1) \quad i = 0, \ldots, N \\
P_{i,i+1} &= \frac{N-i}{N}\left[\frac{i}{N}(1-u_2) + \frac{N-i}{N}u_1\right] \qquad i = 0, \ldots, N-1 \qquad (12.6) \\
P_{i,N+1} &= \frac{i}{N}u_2 \qquad i = 0, \ldots, N \\
P_{N+1,i} &= 0 \qquad i = 0, \ldots, N \\
P_{N+1,N+1} &= 1
\end{aligned}
$$

All other entries of the transition matrix are 0.

We are interested in the expected time until absorption into state $N+1$ starting from state $i=0$. Denote by t_i the expected absorption time from state i. We have

$$\begin{aligned} t_0 &= 1 + P_{0,0}t_0 + P_{0,1}t_1 \\ t_i &= 1 + P_{i,i-1}t_{i-1} + P_{i,i}t_i + P_{i,i+1}t_{i+1} \qquad i = 1, \ldots, N \\ t_{N+1} &= 0 \end{aligned} \tag{12.7}$$

This linear system can be solved numerically to get the precise value for t_0. The analytic expressions are complicated. In the following, we will derive excellent approximations for small, intermediate, and large population size.

12.4.2 Small Population Size

In a small population, a type 1 cell reaches fixation before a type 2 cell arises. "Small population" means

$$N \ll 1/\sqrt{u_2}. \tag{12.8}$$

This can be understood as follows. The average fixation time of the first mutation is of order $\tau_1 = N$. This is the expected time it takes to proceed from one mutant cell to N mutant cells in the Moran process, given that fixation does occur. The average waiting time for the second mutation is $\tau_2 = 1/(Nu_2)$. If $\tau_1 \ll \tau_2$, then it is likely that the first mutation reaches fixation before the second mutation arises. From $\tau_1 \ll \tau_2$, we obtain inequality (12.8). Note that each cell divides on average once every time unit. If the population size is N, there are N cell divisions per time unit. Consequently there are N^2 cell divisions in N time units.

In the parameter region given by (12.8), the evolutionary dynamics can be described as transition among three states. State 0 means that all cells are of type 0. State 1 means that all cells are of type 1. State 2 means that at least one type 2 cell has been generated. Denote by $X_0(t)$, $X_1(t)$, and $X_2(t)$, respectively, the probability of being in state 0, 1, or 2 at time t. At time $t=0$, all cells are unmutated. Therefore $X_0(0)=1$, while $X_1(0)=X_2(0)=0$. State 2 is the only absorbing state. For $t \to \infty$, the system converges to $X_0(t)=X_1(t)=0$ and $X_2(t)=1$.

The time derivatives of the three probabilities are given by

$$\begin{aligned}\dot{X}_0 &= -u_1 X_0 \\ \dot{X}_1 &= u_1 X_0 - N u_2 X_1 \\ \dot{X}_2 &= N u_2 X_1\end{aligned} \tag{12.9}$$

In state 0, the rate of producing type 1 cells is Nu_1. The probability that such a cell reaches fixation is $1/N$. Therefore the transition rate from state 0 to state 1 is given by the mutation rate u_1. If the population is in state 1, then the rate of producing type 2 cells is given by Nu_2.

Equation (12.9) is a linear system of ordinary differential equations, which can be solved analytically. The probability that at least one cell of type 2 has been produced by time t is given by

$$P(t) = X_2(t) = 1 - \frac{N u_2 e^{-u_1 t} - u_1 e^{-N u_2 t}}{N u_2 - u_1}. \tag{12.10}$$

For short times, $t \ll 1/(Nu_2)$, we have

$$P(t) \approx N u_1 u_2 t^2/2. \tag{12.11}$$

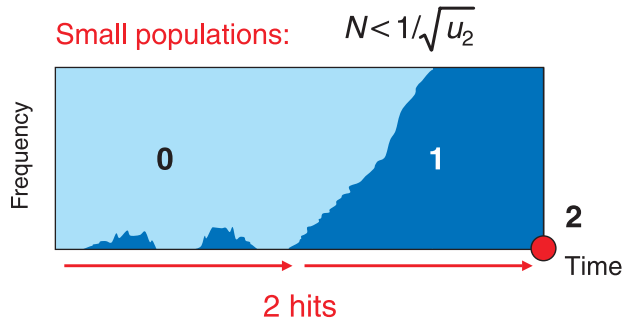
Therefore the probability accumulates as a second order of time. The 2 in the exponent is the same as in Knudson's two-hit hypothesis: it takes two rate-limiting hits to inactivate a TSG in a small population of cells (Figure 12.11).

In contrast, for long times, $t > 1/(Nu_2)$, we have

$$P(t) \approx 1 - e^{-u_1 t}. \tag{12.12}$$

On this time scale, the second hit is fast and can be neglected. Only the first hit is rate limiting.

The exact definition for the number of rate-limiting hits is given by the slope of $\log P(t)$ versus $\log t$. If there is one rate-limiting hit, then $P(t)$ is a linear function of time. Two rate-limiting hits mean that $P(t)$ is a quadratic function of time. If $P(t)$ is constant (on a certain time scale), then there are zero rate-limiting hits. The number of rate-limiting hits depends on the time



$$P(t) = 1 - \frac{Nu_2 e^{-u_1 t} - u_1 e^{-Nu_2 t}}{Nu_2 - u_1} \approx Nu_1 u_2 \frac{t^2}{2}$$

Figure 12.11 In a small population of cells, it takes two rate-limiting steps to inactivate a TSG. We have to wait for the first mutation, which will generate a lineage that reaches fixation. Then we have to wait for the second mutation (or loss of heterozygosity, LOH). Thus the first mutation will reach fixation before the second mutation occurs. The probability $P(t)$ grows proportional to t^2, which indicates two rate-limiting hits.

scale. For example, equation (12.10) has two rate-limiting hits on the short time scale, $t \ll 1/(Nu2)$, but only one rate-limiting hit on the longer time scale, $t > 1/(Nu_2)$. On the very long time scale (too long for human life), $t \gg 1/u_1$, equation (12.10) has zero rate-limiting hits.

12.4.3 Intermediate Population Size

In populations of intermediate size, we still have to wait considerable time until the first type 1 cell has been produced. The lineage that arises from such a cell can die out or produce a type 2 cell. In the latter case, the type 2 cell normally arises before the lineage of type 1 cells has reached fixation. "Intermediate population size" means

$$1/\sqrt{u_2} \ll N \ll 1/u_1. \tag{12.13}$$

The average waiting time for a type 1 cell is $1/(Nu_1)$. If $N < 1/u_1$, then this waiting time is longer than the characteristic time scale of cell division. Hence we have to wait for a "long" time until the first type 1 cell is generated. If $N > 1/\sqrt{u_2}$, then a type 2 cell will be generated before the lineage of type 1

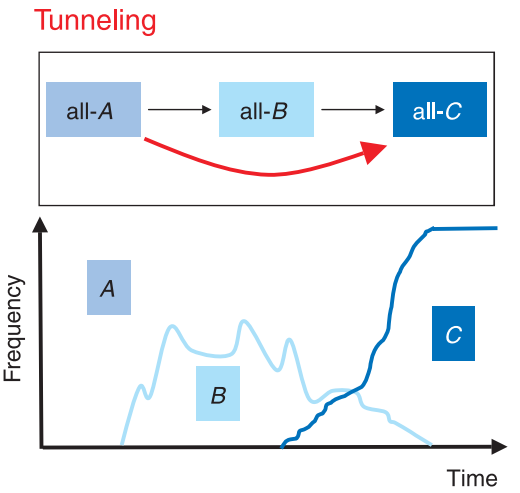


Figure 12.12 If the population size is small compared with the inverse of the mutation rates, then the evolutionary dynamics can be described as a stochastic transition between homogeneous states. Consider two consecutive mutations leading from A cells to B cells and C cells. Initially the population is in state all-A. If a B cell is produced and reaches fixation, the population is in state all-B. Subsequently, if a C cell is produced and reaches fixation, the population is in state all-C. It is possible, however, that a C cell is produced before B cells have reached fixation. In this case, the population moves from all-A to all-C without ever visiting all-B. This phenomenon is called "evolutionary tunneling."

cells has taken over the whole population. We say the population "tunnels" from state 0 to state 2 without ever reaching state 1 (Figure 12.12).

The probability that at least one cell with two hits has arisen before time t is

$$P(t) = 1 - \exp(-Nu_1\sqrt{u_2}t). \tag{12.14}$$

This probability accumulates as a first order of time: it takes only one rate-limiting hit to inactivate a TSG in a population of intermediate size. This one rate-limiting hit is characterized by the waiting time until a type 1 cell emerges, which gives rise to a lineage that will generate a type 2 cell (Figure 12.13). Derivations of equation (12.14) are not trivial and can be found in Komarova et al. (2003) and Iwasa et al. (2005).

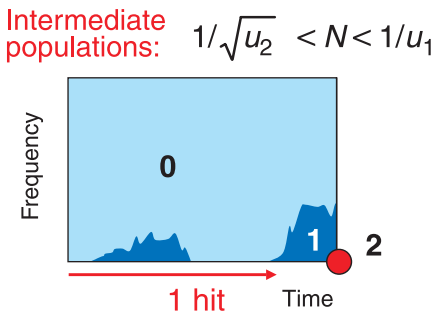


Figure 12.13 For intermediate population size, it takes only one rate-limiting step to inactivate a TSG. We have to wait for the first mutation that gives rise to a lineage that will produce the second mutation. Hence the second mutation occurs before the first mutation has reached fixation. For small t, the probability $P(t)$ grows proportional to t, which indicates one rate-limiting hit.

$$P(t) = 1 - \exp(-Nu_1\sqrt{u_2}t)$$

12.4.4 Large Population Size

In large populations, type 1 cells arise immediately, and their abundance grows as a linear function of time. We simply have to calculate the probability that this growing cell population will generate a type 2 cell. "Large population size" means

$$N \gg 1/u_1. \tag{12.15}$$

In this case, the waiting time for producing type 1 cells, $1/(Nu_1)$, is less than one time unit. Hence type 1 cells arise immediately. The abundance of these cells, x_1, grows according to

$$x_1(t) = Nu_1 t. \tag{12.16}$$

The probability of having produced a type 2 cell at time t is

$$P(t) = 1 - e^{-u_2 \int_0^t x_1(\tau) d\tau}. \tag{12.17}$$

Solving the integral, we obtain

$$P(t) = 1 - \exp(-Nu_1u_2t^2/2). \tag{12.18}$$

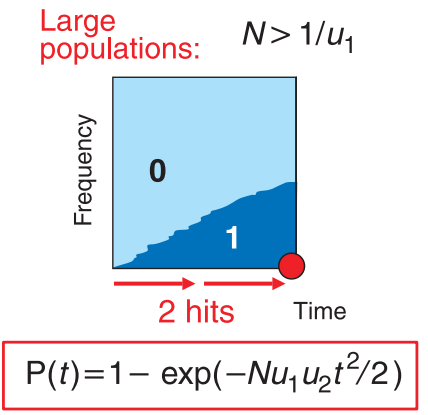


Figure 12.14 In large populations, the first mutation will arise immediately. The abundance of cells with one mutation increases as a linear function of time. We have to wait for these cells to produce the second mutation. For small t, the probability $P(t)$ grows proportional to t^2, which means that it takes two hits to eliminate a TSG in a large population of cells. But neither of these hits will be rate limiting in the overall process of cancer progression, because the events in very large populations of cells occur on a much faster time scale.

This probability again accumulates as a second order of time (Figure 12.14). Eliminating a TSG in a large population of cells is, however, most likely not rate limiting for the overall process of cancer progression. The time it takes to eliminate a TSG in a large population of cells is negligible compared with the time it takes to wait for a mutation in a small population.

12.4.5 Three Dynamic Laws for Tumor Suppressor Gene Inactivation

The three dynamic laws (12.10, 12.14, 12.18) provide a complete description of TSG inactivation. In a normal tissue consisting of small compartments of cells, a TSG is eliminated by two rate-limiting hits. The overall rate of inactivation is proportional to the second order of time. For intermediate population size, only one rate-limiting hit is needed to inactivate a TSG. The rate of inactivation is proportional to the first order of time. In large tumors, it again takes two hits to inactivate a TSG, but neither of them is rate limiting for the overall process of tumorigenesis. Therefore as the population size, N, increases, a TSG is inactivated in two, one, or zero rate-limiting steps (Figures 12.15 and 12.16).

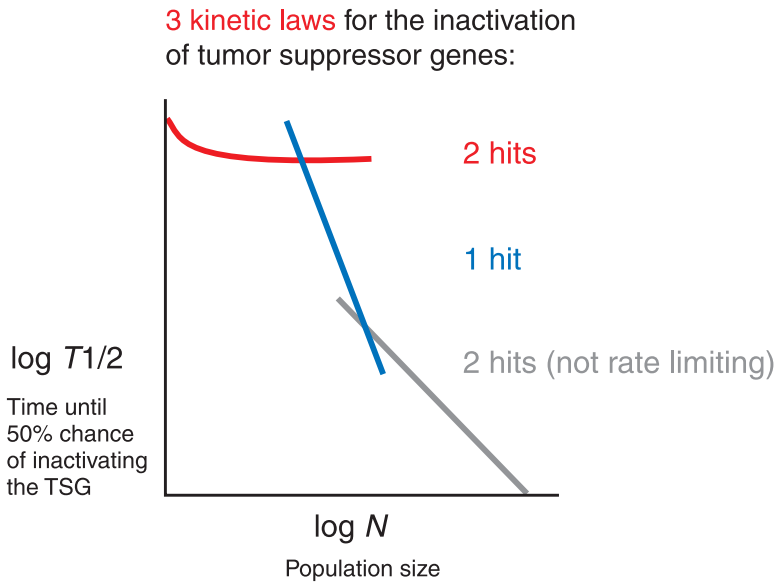


Figure 12.15 There are three kinetic laws for the inactivation of a tumor suppressor gene (TSG). In small, intermediate, and large populations, inactivation takes two, one, and zero rate-limiting hits, respectively. The diagram shows the "half-life of a TSG" versus the population size of cells in a log-log plot. The "half-life of a TSG" is defined by the time until the probability is 1/2 that the TSG has been inactivated.

12.5 GENETIC INSTABILITY

An important question in oncology is to what extent genetic instability is a driving force of cancer progression. Normal cells are genetically stable, but all solid tumors seem to have some form of genetic instability in the end. Therefore genetic instability must arise at some stage of tumorigenesis. One possibility is that genetic instability arises early and accelerates the somatic evolution of cancer via increased mutation rates. Another possibility is that genetic instability is a by-product of the final stages of cancer development.

The idea of a "mutator phenotype" in cancer genetics was first proposed by Larry Loeb in 1974. He argues that somatic selection should favor cells with increased mutation rates, because those cells will more rapidly accumulate other

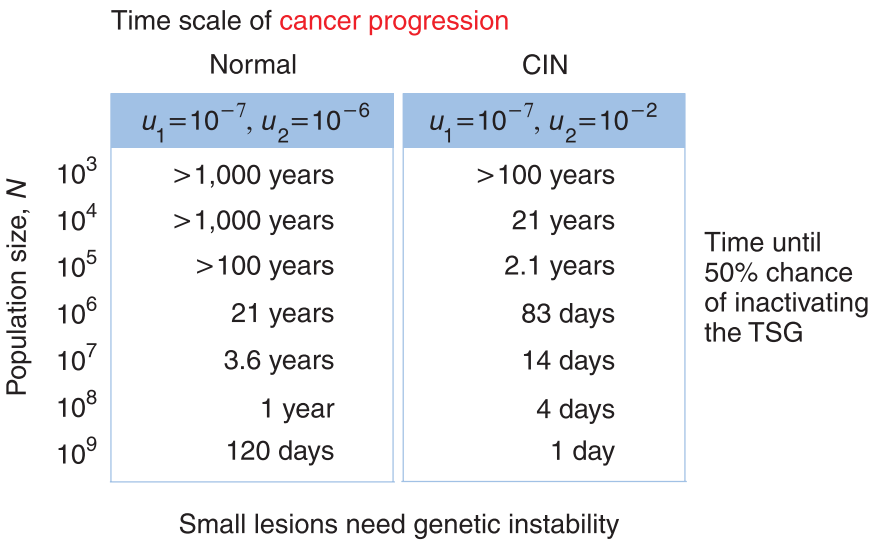


Figure 12.16 Chromosomal instability (CIN) can dramatically accelerate cancer progression. The table shows the elapsed time until there is a 50% chance of inactivating the "next" tumor suppressor gene. We assume that for stable cells the mutation rates for inactivating the first and second allele are $u_1 = 10^{-7}$ and $u_2 = 10^{-6}$, respectively. For CIN cells, the rate of inactivating the second allele (due to loss of heterozygosity, LOH) is $u_2 = 10^{-2}$. For example, a lesion of $N = 10^5$ cells takes 2.1 years with CIN and more than 100 years without CIN to inactivate a TSG. We can also see the dramatic reduction in time scale as the population size (= effective number of cancer cells) increases.

mutations that are necessary for cancer progression. There exists a large literature in evolutionary biology regarding what the optimum mutation rate is in a given situation, but cancer progression represents a rather unique evolutionary scenario. A large number of our genes contribute to the goal that cancer does not happen too early. All these genes are targets for possible advantageous mutations in the somatic evolutionary process that leads to cancer. Since cancer cells have many possibilities for advantageous hits, their optimum mutation rate should be much higher than the normal somatic mutation rate.

In the following, we will explore the most radical possibility of all. We will calculate the probability that chromosomal instability comes before the

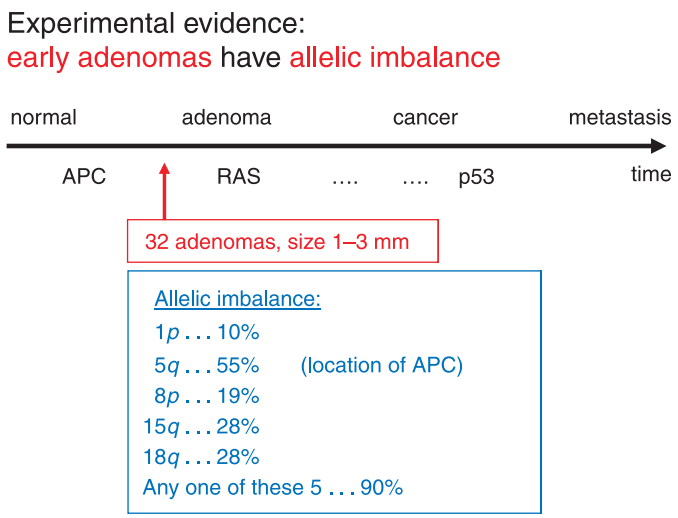


Figure 12.17 Early adenomas have allelic imbalance. Shih et al. (2001) analyzed 32 adenomas 1–3 mm in size. They looked for allelic imbalance (which means either less than two copies or more than two copies) at five different places in the genome: 1*p*, 5*q*, 8*p*, 15*q*, and 18*q*. They found that 90% of the adenomas had allelic imbalance in at least one of those five locations.

inactivation of the first tumor suppressor gene and therefore initiates cancer progression. For colon cancer there is indirect evidence that CIN is an early event: most small adenomas, 1 to 3 mm in size, already have allelic imbalance (Figure 12.17).

12.5.1 Neutral CIN before One TSG

Let us study a case where tumorigenesis starts with the inactivation of a TSG, A, in a small compartment of cells. An appropriate example is the inactivation of the APC gene in a colonic crypt. Initially all cells have two active alleles of the TSG, $A^{+/+}$. One of the two alleles can become inactivated at mutation rate u_1 to generate a cell of type $A^{+/-}$. The second allele can become inactivated at mutation rate u_2 to generate a cell of type $A^{-/-}$. $A^{+/+}$ cells can also receive mutations that trigger the CIN phenotype. This happens at rate u_c and the resulting cell is of type $A^{+/+}CIN$. Such a cell can inactivate the first allele of

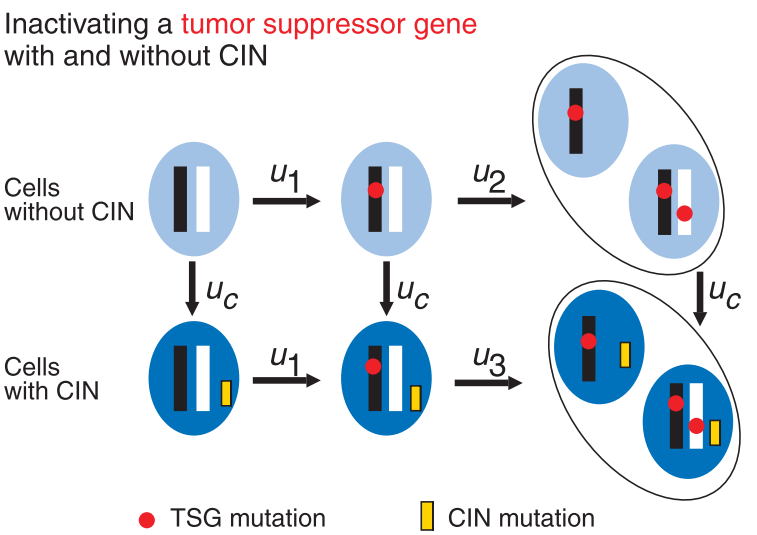


Figure 12.18 There is an ongoing debate as to how early chromosomal instability (CIN) arises during tumor progression. The most radical (and most interesting) suggestion is that CIN precedes the inactivation of the first tumor suppressor gene (TSG) and thus induces the first phenotypic change on the way to cancer. The figure shows the mutational pathway that needs to be analyzed. Inactivation of the first allele is usually caused by a point mutation. Inactivation of the second allele can be caused either by a point mutation or by loss of heterozygosity (LOH). Denote by u_1 and u_2 the inactivation rates of the first and second allele, respectively. Denote by u_c the mutation rate for triggering CIN. If there are many CIN genes, then u_c can be much larger than u_1 or u_2. For a CIN cell, there is rapid loss of heterozygosity (LOH). Hence the rate u_3 is the fastest of all.

the TSG with normal mutation rate u_1 to produce a cell of type $A^{+/-}CIN$. This cell type also arises when an $A^{+/-}$ cell receives a CIN mutation. The $A^{+/-}CIN$ cell rapidly undergoes LOH, at rate u_3, to produce a cell of type $A^{-/-}CIN$ (Figure 12.18).

Therefore, we have the following parameters: u_1 is the mutation rate for inactivating the first allele of the TSG; u_2 is the mutation rate for inactivating the second allele of the TSG in a cell without CIN; u_3 is the mutation rate for inactivating the second allele of the TSG in a cell with CIN; u_c is the mutation

rate for triggering CIN; the effective population size of the compartment is given by N.

The first allele is normally inactivated by a point mutation. The mutation rate per gene per cell division is estimated to be around $u_1 \approx 10^{-7}$. In a normal cell, the second allele can be eliminated by another point mutation or by an LOH event.

If the effective compartment size, N, is much less than the inverse of the mutation rates u_1, u_2, and u_c, then the actual evolutionary dynamics can be approximated by stochastic transitions among homogeneous states. A lineage arising from a mutated cell will usually reach fixation or become extinct before another mutated cell arises. In this case, we can consider a stochastic process with six states (Figure 12.19):

(i) In state X_0, all cells are of type $A^{+/+}$.

(ii) In state X_1, all cells are of type $A^{+/-}$.

(iii) In state X_2, all cells are of type $A^{-/-}$.

(iv) In state Y_0, all cells are of type $A^{+/+}CIN$.

(v) In state Y_1, all cells are of type $A^{+/-}CIN$.

(vi) In state Y_2, all cells are of type $A^{-/-}CIN$.

Let us also denote by $X_0(t)$, $X_1(t)$, $X_2(t)$, $Y_0(t)$, $Y_1(t)$, $Y_2(t)$ the probabilities that the stochastic process is in the corresponding state at time t. The evolutionary dynamics of cancer initiation are given by the following system of linear differential equations:

$$\begin{aligned}
\dot{X}_0 &= -(u_1 + u_c)X_0 \\
\dot{X}_1 &= u_1 X_0 - (u_c + Nu_2)X_1 \\
\dot{X}_2 &= Nu_2 X_1 \\
\dot{Y}_0 &= u_c X_0 - u_1 Y_0 \\
\dot{Y}_1 &= u_c X_1 + u_1 Y_0 - Nu_3 Y_1 \\
\dot{Y}_2 &= Nu_3 Y_1
\end{aligned} \tag{12.19}$$

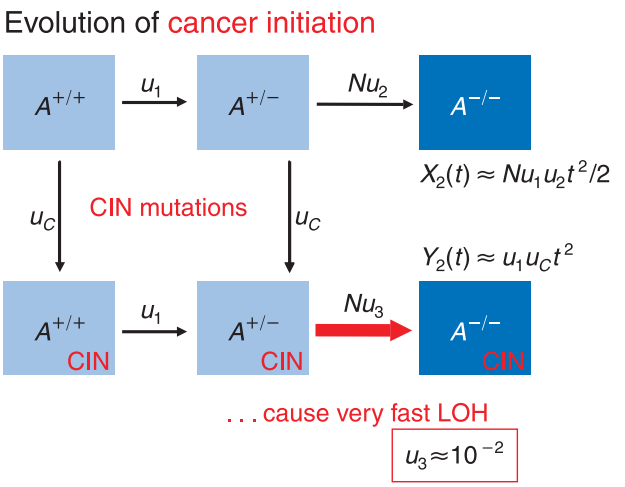


Figure 12.19 Cancer initiation can be studied by a stochastic process that describes transitions between homogeneous compartments. Mutation of the first allele is neutral. Hence the rate of evolution from $A^{+/+}$ to $A^{+/-}$ is given by the mutation rate u_1. Mutation of the second allele leads to a selective advantage. If this advantage is large, then the rate of evolution from $A^{+/-}$ to $A^{-/-}$ is approximately given by Nu_2. If chromosomal instability (CIN) is neutral, its rate of evolution is the mutation rate u_c. CIN has no consequence for inactivating the first allele, but greatly accelerates the loss of heterozygosity (LOH) of the second allele. The red arrow indicates that this transition rate is many orders of magnitude faster than all other transition rates. On the relevant time scale, the probability of inactivating a TSG without CIN is given by $X_2(t) = Nu_1u_2t^2/2$, and with CIN it is given by $Y_2(t) = u_1u_ct^2$. If $Y_2(t) > X_2(t)$, which means $u_c > Nu_2/2$, then it is more likely to initiate cancer via CIN.

We have assumed that the probability that a lineage arising from a single $A^{+/-}$ cell takes over the compartment is $1/N$. Therefore inactivation of the first allele of the TSG is neutral. We have also assumed that the CIN mutation is neutral. Finally, we have assumed that the probability for an $A^{-/-}$ cell to take over the compartment is close to 1, which means this mutation has a strong selective advantage.

Initially, at time $t = 0$, we have $X_0 = 1$ and all other probabilities are 0. The time-explicit solutions of this system can be easily obtained with standard techniques. But on the relevant time scale of human life (about one hundred

years), these solutions take an even simpler form. If the cells divide once per day, then our time, t, is measured in units of days. If the cells divide once per week, then our time, t, is measured in units of weeks. In both cases, we find that $u_1 t$, $N u_2 t$, and $u_c t$ are all much less than 1.

On the relevant time scale, the approximate solutions of system (12.19) take the form

$$\begin{aligned} X_0(t) &\approx 1 \\ X_1(t) &\approx u_1 t \\ X_2(t) &\approx N u_1 u_2 t^2/2 \\ Y_0(t) &\approx u_c t \\ Y_1(t) &\approx u_1 u_c t^2 \\ Y_2(t) &\approx u_1 u_c t^2 \end{aligned} \tag{12.20}$$

For the lifetime of a person, almost all compartments remain wild type, which means that $X_0(t) \approx 1$. Compartments in state X_1 arise as a linear function of time. Compartments in state X_2 arise as a quadratic function of time. Similarly, Y_0 compartments arise as a linear function of time, and Y_1 compartments as a quadratic function. Surprisingly, however, $Y_2(t)$ is equivalent to $Y_1(t)$, and therefore Y_2 compartments also accumulate as a quadratic function of time. The reason is that $N u_3 t$ is much larger than one on the relevant time scale, and the corresponding step is not rate limiting. As soon as the system has reached state Y_1, it will proceed to Y_2. The waiting time for the LOH event in CIN cells is negligible compared with the waiting time for all other mutations in the system.

Therefore we make the interesting observation that it takes two rate-limiting hits to eliminate a TSG both with CIN and without CIN. The additional CIN mutation adds another rate-limiting step, but the subsequent LOH event is no longer rate limiting (Figure 12.20).

We can now estimate the ratio of cancers that are initiated with CIN versus without CIN. We have

$$Y_2(t) : X_2(t) = 2u_c : N u_2. \tag{12.21}$$

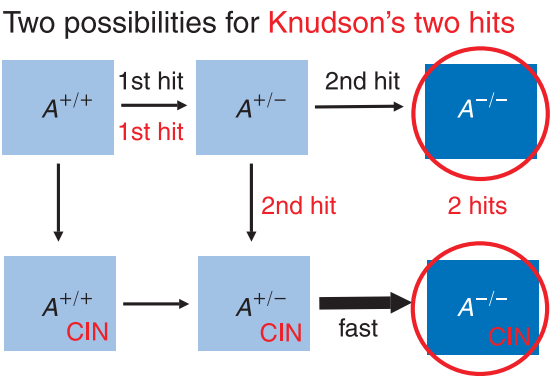


Figure 12.20 Knudson's two-hit hypothesis gave rise to the concept of a tumor suppressor gene (TSG). It takes two rate-limiting hits to inactivate a TSG: one for the first allele and one for the second. These rate-limiting hits were first observed in cancer-incidence data of retinoblastoma. Our analysis shows that Knudson's two hits are entirely compatible with chromosomal instability (CIN). It also takes only two hits to inactivate a TSG with an additional CIN mutation: one hit for the first allele of the TSG and one hit for the CIN mutation. Losing the second allele of the TSG in CIN cells is very fast and therefore not rate limiting.

The ratio is independent of time.

The mutation rate for inactivating the second allele of the TSG in a normal (non-CIN) cell can be written as the sum of the point mutation rate per gene, u, and the rate of LOH, p_0. We have

$$u_2 = u + p_0. \tag{12.22}$$

If there are n_1 class 1 CIN genes and n_2 class 2 CIN genes in the human genome that can be mutated in this particular scenario of cancer initiation, then the mutation rate u_c that triggers the CIN phenotype is given by

$$u_c = 2n_1(u + p_0) + 2n_2u. \tag{12.23}$$

Hence the majority of cancers are initiated by CIN if

$$4n_1(u + p_0) + 4n_2u > N(u + p_0). \tag{12.24}$$

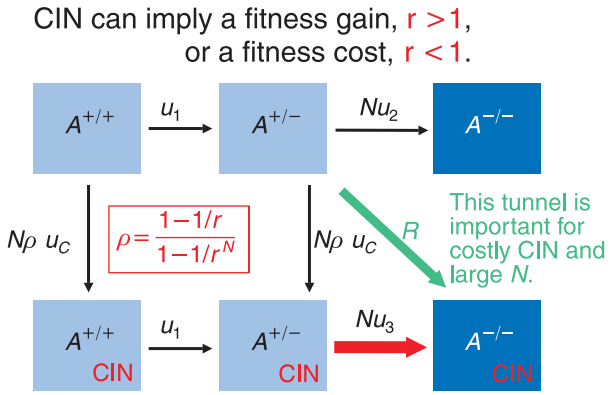


Figure 12.21 If chromosomal instability (CIN) implies a fitness loss or gain, then the transitions from non-CIN to CIN states occur at the rate $Nu\rho$, where ρ is the probability that a newly produced CIN cell takes over the population. But for costly CIN and large N, the tunnel from $A^{+/-}$ to $A^{-/-}$CIN is important. This tunnel has a transition rate $R = Nu_c u_3 r/(1-r)$.

If, for example, $N = 4$ and $u \approx p_0$, then the presence of one class 1 and one class 2 CIN gene suffices to ensure that CIN initiates more than half of all cancers. If there are two class 1 genes and two class 2 genes, then 75% of cancers are initiated by CIN. The calculation so far does not include a possible cost of CIN. Let us do this now.

12.5.2 Costly CIN in Small Compartments

Let us assume that the relative fitness of a CIN cell is $r < 1$; the cost of CIN is $1 - r$. It could be that the CIN phenotype is recognized by apoptotic defense mechanisms, which lead to higher death rates and consequently lower r. Moreover, CIN cells might have a lower fitness, because they accumulate deleterious mutations (Figure 12.21).

In a Moran process with population size N, the probability that the lineage arising from a CIN mutant takes over the population is given by

$$\rho = \frac{1 - 1/r}{1 - 1/r^N}. \tag{12.25}$$

The transition rate from a non-CIN state to a CIN state of our stochastic process is now given by $N\rho u_c$. We obtain the same equations as before, but u_c is replaced by $N\rho u_c$:

$$\begin{aligned}
\dot{X}_0 &= -(u_1 + N\rho u_c)X_0 \\
\dot{X}_1 &= u_1 X_0 - N(\rho u_c + u_2)X_1 \\
\dot{X}_2 &= N u_2 X_1 \\
\dot{Y}_0 &= N\rho u_c X_0 - u_1 Y_0 \\
\dot{Y}_1 &= N\rho u_c X_1 + u_1 Y_0 - N u_3 Y_1 \\
\dot{Y}_2 &= N u_3 Y_1
\end{aligned} \tag{12.26}$$

On the relevant time scale, the solutions are given by

$$\begin{aligned}
X_0(t) &\approx 1 \\
X_1(t) &\approx u_1 t \\
X_2(t) &\approx N u_1 u_2 t^2/2 \\
Y_0(t) &\approx N\rho u_c t \\
Y_1(t) &\approx N\rho u_1 u_c t^2 \\
Y_2(t) &\approx N\rho u_1 u_c t^2
\end{aligned} \tag{12.27}$$

The ratio of cancers that are initiated with CIN versus without CIN is given by

$$Y_2 : X_2 = 2\rho u_c : u_2. \tag{12.28}$$

As before, we assume

$$u_2 = u + p_0 \tag{12.29}$$

and

$$u_c = 2n_1(u + p_0) + 2n_2 u. \tag{12.30}$$

If, for example, $N = 4$, $r = 0.8$, $u \approx p_0$, $n_1 = 2$, and $n_2 = 2$, then about 68% of all cancers are initiated by CIN. If the effective compartment size is small, then even substantial costs of CIN can be tolerated, because the evolutionary dynamics in small compartments are dominated by random drift rather than by selection.

12.5.3 Costly CIN in Large Compartments

For larger compartment sizes, however, the ratio $N\rho$ could become vanishingly small for $r < 1$. If, for example, $N = 100$ and $r = 0.7$, then $N\rho \approx 10^{-16}$; a CIN mutation with this cost could never reach fixation in a population with effective size 100.

"Stochastic tunneling" can still allow a significant fraction of cancers to be initiated by CIN. The stochastic process will never reach states Y_0 or Y_1. From state X_1, however, the process can tunnel to Y_2 without ever reaching Y_1. In state X_1, all cells are of type $A^{+/-}$. Cells of type $A^{+/-}CIN$ are being produced at rate Nu_c. These cells do not reach fixation, but remain near a mutation selection balance with average abundance $Nu_c/(1-r)$. From there they produce $A^{-/-}CIN$ cells at rate ru_3. Therefore the rate of the tunnel from state X_1 to Y_2 is given by

$$R = \frac{Nu_c r u_3}{1-r}. \tag{12.31}$$

The stochastic evolution is described by the system

$$\begin{aligned} \dot{X}_0 &= -u_1 X_0 \\ \dot{X}_1 &= u_1 X_0 - (R + Nu_2) X_1 \\ \dot{X}_2 &= Nu_2 X_1 \\ \dot{Y}_2 &= R X_1 \end{aligned} \tag{12.32}$$

The approximate solutions on the relevant time scale are

$$\begin{aligned} X_0(t) &\approx 1 \\ X_1(t) &\approx u_1 t \\ X_2(t) &\approx N u_1 u_2 t^2/2 \\ Y_0(t) &\approx 0 \\ Y_1(t) &\approx 0 \\ Y_2(t) &\approx R u_1 t^2/2 \end{aligned} \tag{12.33}$$

Remember that the system will never reach states Y_0 and Y_1. Therefore both $Y_0(t)$ and $Y_1(t)$ are zero.

The ratio of cancers that are initiated with CIN versus without CIN is given by

$$Y_2 : X_2 = R : N u_2 = \frac{u_c r u_3}{1-r} : u_2. \tag{12.34}$$

The population size has canceled out of this comparison. If, for example, $r = 0.8$, $u \approx p_0$, $u_3 = 0.01$, $n_1 = 5$, and $n_2 = 5$, then about 38% of all cancers are initiated by CIN.

12.5.4 CIN before Two TSGs

Consider a path to cancer in which two TSGs, A and B, have to be eliminated (Figure 12.22). Initially the compartment consists of N_0 wild type cells, $A^{+/+}B^{+/+}$. Suppose gene A has to be inactivated first. The evolutionary pathway proceeds from $A^{+/+}B^{+/+}$ via $A^{+/-}B^{+/+}$ to $A^{-/-}B^{+/+}$, and subsequently to $A^{-/-}B^{+/-}$ and $A^{-/-}B^{-/-}$. CIN can emerge at any stage of this pathway; once arisen, CIN accelerates the transitions from $A^{+/-}$ to $A^{-/-}$ and from $B^{+/-}$ to $B^{-/-}$. Inactivation of the first TSG induces neoplastic growth. We assume that the $A^{-/-}$ compartment gives rise to a small lesion of N_1 cells. In this lesion, the second TSG has to be inactivated for further tumor progression. Because of the increased compartment size, the evolutionary trajectory will tunnel from $A^{-/-}B^{+/+}$ directly to $A^{-/-}B^{-/-}$. The condition for this tunnel is $1/u_1 > N_1 > 1/\sqrt{u_2}$.

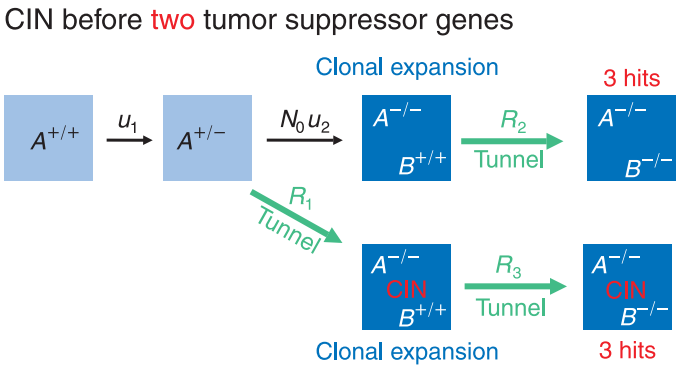


Figure 12.22 Inactivation of the first tumor suppressor gene (TSG) might lead to a moderate clonal expansion. In this example, a second TSG needs to be inactivated for further cancer progression. We can calculate the probability that chromosomal instability (CIN) arises before the inactivation of the first TSG. Even moderate clonal expansion will lead to a population of intermediate size and, therefore, inactivation of the second TSG will occur via a tunnel. Thus it takes three hits to inactivate two TSGs with and without CIN. CIN, however, can accelerate both loss of heterozygosity (LOH) events. We find that one (or a few) costly CIN genes are enough to ensure that CIN comes before the first TSG in a pathway of cancer progression where two TSGs have to be inactivated in rate-limiting situations.

Since we already know that CIN is likely to initiate cancer progression if it has no cost (section 12.5.1) or if inactivation of the first TSG happens in a very small compartment (section 12.5.2), we will only investigate the case where CIN has a substantial cost and the compartment size, N_0, is so large that fixation of a CIN cell is nearly impossible. In this case, evolutionary dynamics can be described as a stochastic process on the following six states:

(i) In state X_0, all cells are of type $A^{+/+}B^{+/+}$.

(ii) In state X_1, all cells are of type $A^{+/-}B^{+/+}$.

(iii) In state X_2, all cells are of type $A^{-/-}B^{+/+}$.

(iv) In state X_3, all cells are of type $A^{-/-}B^{-/-}$.

(v) In state Y_2, all cells are of type $A^{-/-}B^{+/+}CIN$.

(vi) In state Y_3, all cells are of type $A^{-/-}B^{-/-}CIN$.

As before, $X_i(t)$ and $Y_i(t)$ denote the probabilities to be in the corresponding states at time t. Initially, at time $t = 0$, we have $X_0(0) = 1$ and all other states have zero probability. The evolutionary dynamics are given by the following system of linear differential equations:

$$\begin{aligned}
\dot{X}_0 &= -u_1 X_0 \\
\dot{X}_1 &= u_1 X_0 - (R_1 + N_0 u_2) X_1 \\
\dot{X}_2 &= N_0 u_2 X_1 - R_2 X_2 \\
\dot{X}_3 &= R_2 X_2 \\
\dot{Y}_2 &= R_1 X_1 - R_3 Y_2 \\
\dot{Y}_3 &= R_3 Y_2
\end{aligned} \tag{12.35}$$

The rates of the tunnels are given by

$$\begin{aligned}
R_1 &= \frac{N_0 u_c r u_3}{1 - r} \\
R_2 &= N_1 u_1 \sqrt{u_2} \\
R_3 &= N_1 u_1 \sqrt{u_3}
\end{aligned} \tag{12.36}$$

Without CIN, inactivation of two TSGs requires three rate-limiting hits. It takes two hits to inactivate the first TSG. If this leads to a moderate clonal expansion, then the second TSG can be inactivated in one rate-limiting step. With CIN, inactivation of two TSGs also requires three rate-limiting hits. It takes one rate-limiting hit to inactivate one allele of the first TSG, one rate-limiting hit to trigger CIN, and one rate-limiting hit to inactivate both alleles of the second TSG (Figure 12.22).

A numerical analysis of system (12.35) shows that even under the assumption of a substantial cost for CIN and a large compartment size, N_0, only a small number of Class I or II CIN genes are needed to ensure that cancer progression is initiated by a CIN mutation.

The cost of CIN is compensated by an acceleration of every successive TSG inactivation. It is possible that the first TSG, A, is predominantly inactivated in cells without CIN. Thus most lesions that are caused by inactivation of TSG A do not have CIN, but the small fraction of lesions with CIN will

eliminate TSG *B* within the time scale of a human life. In such a situation all (or almost all) cancers will derive from lesions in which a CIN mutation preceded inactivation of the first TSG.

If the inactivation of the first TSG leads to a vast clonal expansion, then the inactivation of a further TSG is not rate limiting. In this case, the analysis of the system leads back to the question of whether CIN arises before inactivation of only one TSG.

There is also the possibility that the clonal expansion caused by inactivation of the first TSG is so small that the non-CIN trajectory requires two hits to inactivate the second TSG. In this case, it takes four hits to inactivate *A* and *B* without CIN, but only three hits to inactivate them with CIN. Thus CIN is at an enormous advantage in this situation.

Throughout this section, we have assumed that the clonal expansion caused by inactivation of *A* occurs with certainty once such a cell has been produced. A correction term for a reduced probability can easily be incorporated. We have also made the plausible assumption that the time needed for clonal expansion can be neglected compared with the other transition rates.

We conclude as follows. In a pathway of cancer progression where one TSG is inactivated in a rate-limiting situation, one or a few neutral CIN genes are enough to ensure that CIN occurs before inactivation of the TSG. In a pathway of cancer progression where two TSGs must be inactivated in rate-limiting situations, one or a few costly CIN genes are enough to ensure that CIN occurs before inactivation of the first TSG. By analogy with yeast, there should be hundreds of CIN genes in the human genome (although in any particular tissue only a subset of them might give rise to a CIN phenotype when mutated). Therefore not only does CIN accelerate cancer progression, but there are also many mutations that lead to CIN. The combination of these two effects must imply that CIN is an early event and an important driving force in cancer progression.

SUMMARY

- Cancer is an evolutionary process.
- A quantitative understanding of cancer progression requires a mathematical analysis of the underlying evolutionary dynamics.

- We have calculated the rate of evolution of activating oncogenes and inactivating tumor suppressor genes. The key parameters include the population size of reproducing cells, the mutation rates, and fitness values.
- Tissue architecture can affect the rate of cancer initiation and the types of mutations that are likely to occur. Small compartments confer protection against mutations in oncogenes and tumor suppressor genes, but are vulnerable to genetic instability.
- The "linear process" is an effective tissue design to delay the onset of cancer.
- In small, intermediate, and large populations of reproducing cells, a tumor suppressor gene is eliminated in 2, 1, and 0 rate-limiting steps, respectively.
- In a small compartment (such as a colonic crypt), it takes two rate-limiting hits to eliminate a TSG with and without CIN. Therefore Knudson's two-hit hypothesis is compatible with the idea that the second hit occurs in a CIN gene.
- For a wide range of plausible parameter values, CIN can precede inactivation of the second allele of a TSG. In this case, the CIN mutation leads to the first phenotypic change on the way to cancer.
- The more CIN genes in the human genome, the more possibilities there are to commit CIN, and the more likely it is that CIN occurs early.
- One (or a few) neutral CIN genes are enough to ensure that CIN precedes inactivation of a TSG in an evolutionary pathway to cancer where one TSG must be inactivated in a rate limiting situation.
- One (or a few) costly CIN genes are enough to ensure that CIN precedes inactivation of the first TSG in an evolutionary pathway to cancer where at least two TSGs must be inactivated in rate-limiting situations.