

Local Regulation of Homeostasis Favors Chromosomal Instability

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Summary

Tissues of long-lived multicellular organisms have to maintain a constant number of functioning cells for many years. This process is called homeostasis. Homeostasis breaks down when cells emerge with mutations in tumor suppressor genes or oncogenes. Such mutated cells can have increased net rates of proliferation, which is increased somatic fitness. We show that the best protection against such mutations is achieved when homeostasis is regulated locally via small compartments. Small compartments, on the other hand, allow the accumulation of cells with reduced somatic fitness. Cells with mutations conferring genetic instability normally have a reduced somatic fitness because they have an increased probability of producing deleterious mutations or triggering apoptosis. Thus, small compartments protect against mutations in tumor suppressor genes or oncogenes but promote the emergence of genetic instability.

Results and Discussion

Cancer susceptibility genes belong to one of two groups: gatekeepers and caretakers [1]. Mutations in gatekeeper genes such as tumor suppressor (TSP) genes and oncogenes alter the growth regulatory or differentiation pathways of the cell [2–6]. These mutations confer a relative selective advantage to the cell, because the increased proliferation rate enables it to outcompete its wild-type neighbors. Such cells have an increased somatic fitness. Mutations in caretaker genes give rise to genetic instability [7–12]. The most common form of genetic instability in human cancers is chromosomal instability (CIN). Cells with CIN have an increased rate of losing or gaining whole chromosomes or large parts of chromosomes. Hence, CIN increases the rate of loss of heterozygosity (LOH), which is important for inactivation of TSP genes. In yeast, it is estimated that mutations in several hundred genes give rise to CIN [13, 14]. The search is currently on for human CIN genes

[15–20]. A major question in cancer genetics is to what extent CIN is an early event and thus a driving force of tumorigenesis or a late-stage consequence [21–25]. CIN by itself should in most circumstances provide a selective cost for the cell: it increases the chance of triggering apoptosis, and it leads to deleterious or lethal mutations. Hence, CIN should normally imply a reduced somatic fitness. The selective cost of CIN, however, can be overcome by an increased probability of generating mutations, such as LOH of TSP genes, that enhance the fitness of the cell. Hence, a costly CIN can hitchhike on mutations that increase the net reproductive rate of the cell.

Tissues are organized into compartments of stem cells and differentiated cells [26]. This architecture works together with somatic selection and apoptosis to protect an organism against neoplastic growth [27, 28]. We will now develop a mathematical model to analyze how compartment size affects the inactivation of a TSP gene with or without CIN.

Consider a tissue of a multicellular organism, and denote by Z the total number of cells at risk of mutating into cancer cells. Let M be the number of compartments and N be the number of cells per compartment. Thus, $Z = MN$. The mutation rate per gene is denoted by u . Typically, we have $u = 10^{-7}$ – 10^{-6} per gene per cell division. Suppose a cell of this tissue can acquire a precancerous state through inactivation of both alleles of a TSP gene. This inactivation can occur before or after mutations that induce CIN (Figure 1A). The transition from a cell with two functioning alleles of the TSP gene, $TSP^{+/+}$, to a cell with one inactivated allele, $TSP^{+/-}$, occurs at a rate of $2u$. This rate is the same in cells with CIN and in cells without CIN. The remaining allele can be lost either through a second mutation or by LOH. The parameters p_0 and p determine, respectively, the rate of LOH in normal and CIN cells. CIN greatly accelerates the rate of LOH; therefore, we have $p \gg p_0$. In vitro studies of colon cancer cell lines suggest $p \approx 10^{-2}$ per cell division [7, 17]. There are no accurate measurements of p_0 , but existing data on inactivation of TSP genes in human cancers suggest that p_0 is of the order of u , perhaps slightly larger. By analogy with yeast, there should be many different genes in human cells that trigger CIN when mutated. We denote by u_c the total mutation rate of acquiring CIN. Suppose there are n_1 genes that induce CIN if one allele is mutated and n_2 genes that induce CIN if one allele is mutated or lost; then, $u_c = 2n_1u + 2n_2(u + p_0)$. We have $n_c = n_1 + n_2$.

The relative growth rate of CIN cells compared to non-CIN cells is denoted by r . If CIN has a cost, then $r < 1$. If CIN is neutral, then $r = 1$. Inactivation of both alleles of the tumor suppressor gene increases the fitness of the cell to a value of $a > 1$. Without CIN, $TSP^{+/+}$ and $TSP^{+/-}$ cells have a fitness of 1, while $TSP^{-/-}$ cells have a fitness of a . With CIN, $TSP^{+/+}$ and $TSP^{+/-}$ cells have a fitness of r , while $TSP^{-/-}$ cells have a fitness of ar .

The basic structure of the stochastic process is shown in Figure 1B. If the mutation rate is small, $u < 1/N$, then

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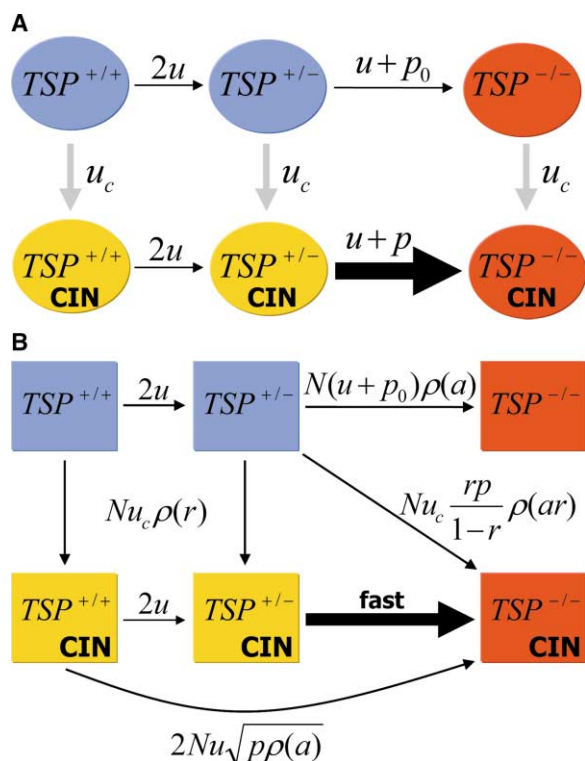


Figure 1. Processes and Rates

(A) Cancer initiation via a tumor suppressor (TSP) gene with or without chromosomal instability (CIN). The mutation rate per gene is given by u . The rate of loss of heterozygosity (LOH) in normal and CIN cells is given by p_0 and p , respectively. We have $p \gg p_0$. CIN mutations are acquired at a rate of u_c .

(B) If the mutation rate is small, $u \ll 1/N$, then compartments of size N are almost always homogeneous, that is they contain only a single type of cell. In this case, the evolutionary dynamics of cancer initiation can be formulated as a stochastic process, which describes transitions among compartments. A wild-type compartment, $TSP^{+/+}$, mutates to a $TSP^{+/-}$ compartment at a rate of $2u$. Without CIN, the $TSP^{-/-}$ compartment is reached at a rate of $N(u + p_0)\rho(a)$. The transition from the $TSP^{+/+}$ and $TSP^{+/-}$ states to the corresponding states with CIN happen at a rate of $Nu_c\rho(r)$. In CIN compartments, the $TSP^{+/-}$ state gives rise to the $TSP^{-/-}$ state at a rate of $N(u + p)\rho(a)$, which is much faster than all other rates. In addition, there are two “tunnels” [23, 30]. The first one originates from the chromosomally stable $TSP^{+/-}$ state and occurs at a rate of $Nu_crp/(1-r)\rho(ar)$. It is important only if $Nrp/(1-r)\rho(ar) \gg 1$. The second one starts from a chromosomally unstable $TSP^{+/+}$ state and takes place at a rate of $2Nu\sqrt{p\rho(a)}$. It is important only if $N\sqrt{p\rho(a)} \gg 1$.

compartments will almost always contain a single type of cell. The resulting system can be solved analytically [23, 29, 30]. Denote by $X(t)$ the probability that a compartment contains only $TSP^{-/-}$ cells without CIN at time t . Denote by $Y(t)$ the probability that a compartment contains only $TSP^{-/-}$ cells with CIN at time t . Time is measured in units corresponding to the average duration of a cell cycle in the particular tissue. For the time scale of a human life, we have $ut \ll 1$, $p_0t \ll 1$, and $pt \gg 1$. In this limit, the probability that a compartment contains only $TSP^{-/-}$ cells without CIN at time t is given by $X(t) = u(u + p_0)N\rho(a)t^2$.

The function $\rho(a) = (1 - 1/a)/(1 - 1/a^m)$ denotes the probability that a single cell with relative fitness a takes

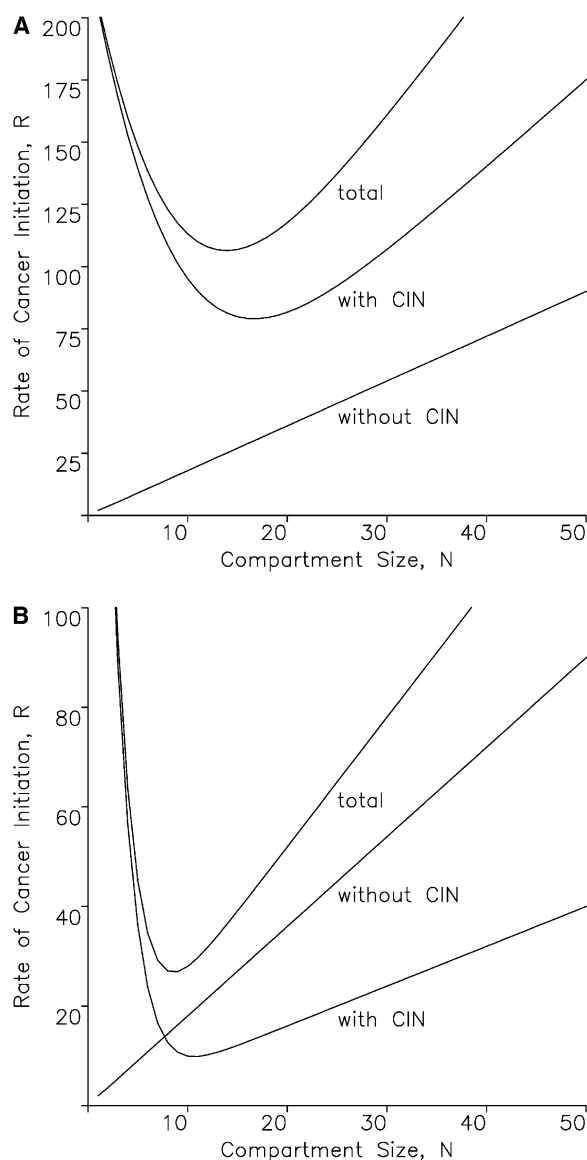


Figure 2. The Effect of Compartment Size on the Frequency of CIN

Small compartments reduce the rate of inactivation of TSP genes without CIN but increase the rate of TSP inactivation via CIN. At the optimum compartment size, there can be a substantial probability that CIN precedes TSP inactivation as a first step toward cancer. Mutation in n_c genes gives rise to CIN. Chromosomally unstable cells have a relative growth rate r and an LOH rate of p . Introduce the rate constants $R_x = X(t)/(u^2t^2)$, $R_y = Y(t)/(u^2t^2)$, and $R = R_x + R_y$. R_x , R_y , and R denote, respectively, the rate constant of cancer initiation without CIN, with CIN, and in total. The probabilities $X(t)$ and $Y(t)$ are given in the text. The parameter values are $n_c = 50$, $u = 10^{-7}$, $p_0 = 10^{-7}$, $p = 10^{-2}$, $a = 10$, and $r = 0.8$ in (A) and $r = 0.5$ in (B).

over the whole compartment. If the mutation has a large fitness advantage, $a \gg 1$, then $\rho(a)$ tends to 1. If the mutation is almost neutral, $a \approx 1$, then $\rho(a)$ tends to $1/N$. The function $\rho(a)$ also works for $a < 1$, where it specifies the probability that a deleterious mutation takes over the compartment. For a $TSP^{-/-}$ cell, we assume $a > 1$.

The probability that a compartment contains only $TSP^{-/-}$ cells with CIN at time t is given by

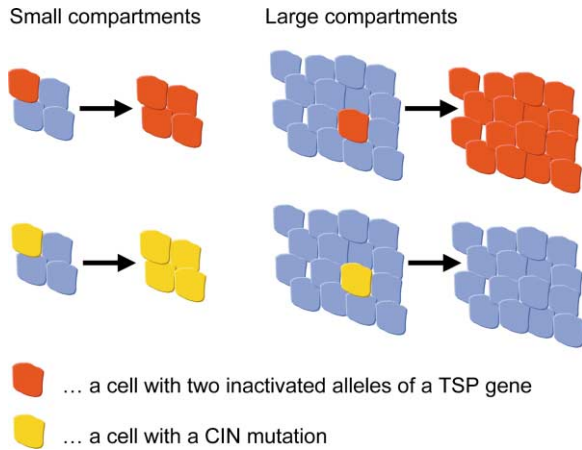


Figure 3. Fixation Probability with and without CIN

Cells with an inactivated TSP gene (red) have a selective advantage. Such cells have a high probability to take over both small and large compartments. After the takeover, there are fewer mutated cells in small compartments than in large compartments, and this reduces the risk of further tumor progression. Here, we suppose the mutation is contained within the boundaries of the compartment. Hence, small compartments are more effective in containing the accumulation of cells with mutations in TSP genes. CIN cells (yellow) normally have a selective disadvantage. Since random drift is important in small compartments, there is still a certain probability that CIN mutations will be fixed. In large compartments, this probability is negligible. Consider, for example, a CIN mutation that reduces fitness by 20%. In a compartment of $N = 4$ cells, its probability of fixation is 0.17. In a compartment of $N = 40$ cells, its probability of fixation is 3×10^{-5} .

$$Y(t) = uu_c t^2 N [2\rho(r) + \frac{rp}{1-r} \rho(ar) + N\rho(r)\sqrt{\rho(a)}].$$

Note that the equations for both $X(t)$ and $Y(t)$ are second order in time. This means that “two hits” eliminate a TSP gene with or without CIN [3, 21]. Figure 2 shows the risk of developing cancer as function of the compartment size, N . We assume that the risk is proportional to the total number of mutated cells in the whole tissue, $Z(X[t] + Y[t])$. The rate of accumulation of non-CIN cells with $TSP^{-/-}$ is minimized by compartments that are as small as possible, that is $N = 1$. The rate of accumulation of CIN cells with $TSP^{-/-}$ is minimized by compartments of intermediate size. Small compartments are best for containing the inactivation of the TSP gene but are vulnerable to CIN mutations. The smallest possible compartment size, $N = 1$, is optimum only if the cost of CIN is very low, which means that r is close to one. In general, the total rate of accumulation of (CIN or non-CIN) cells with $TSP^{-/-}$ is minimized by compartments of intermediate size. At the optimum compartment size, the contribution of CIN cells to the total risk can be substantial. Thus, local regulation of homeostasis via small compartments favors CIN.

Intuitively, small compartments are best for limiting the accumulation of cells with a selective advantage, because the probability is high that such a cell takes over compartments of any size. Small compartments limit the damage (at least initially) by keeping the number of mutated cells small once the takeover has happened.

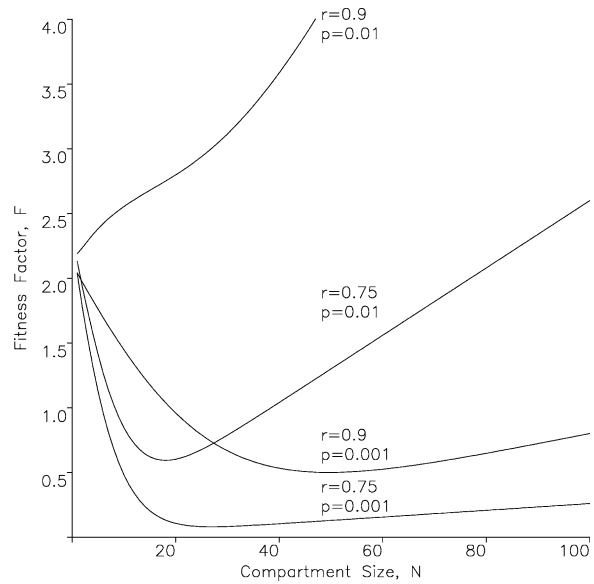


Figure 4. The Risk of Cancer Initiation as a Function of the Compartment Size

CIN mutations can differ in the rate at which they occur, u_i , their selective cost, r_i , and the rate of LOH they induce, p_i . The probability that a compartment contains only $TSP^{-/-}$ cells with CIN at time t is now given by $Y_2(t) = 2ut^2 \sum_{i=1}^n u_i F_i$. The parameter F_i can be interpreted as a “fitness factor” of CIN mutation i . It is given by $F_i = 2N\rho(r_i) + [r_i p_i / (1 - r_i)] N\rho(ar_i) + N^2 \rho(r_i) \sqrt{\rho(a)}$. The figure shows the fitness factor, F_i , for various CIN mutations as a function of the compartment size, N . The parameter values p_i and r_i are given in the figure. Inactivation of the first TSP via CIN mutations with a low selective cost and a high rate of LOH is best contained by the smallest possible compartment size, $N = 1$. For other CIN mutations, there is an intermediate optimum for the compartment size. The other parameter values are $u = 10^{-7}$ and $a = 10$.

Note that this argument applies to mutations that do not immediately break through the boundaries of compartments. Furthermore, in small compartments, random drift is more important than selection. Thus, small selective advantages do not matter in small compartments. In large compartments, on the other hand, selective differences are more important than random drift. Thus, large compartments are more effective in preventing the fixation of disadvantageous mutations such as CIN (Figure 3).

We can extend the model to consider CIN genes with different mutation rates and different effects on the phenotype of a cell. Figure 4 shows the risk of cancer initiation as a function of the compartment size for different CIN mutations. For most CIN mutations, there is an intermediate optimum compartment size that minimizes the rate of cancer initiation. For some CIN mutations with a very small selective cost, which trigger LOH at a high rate, the optimum compartment size is as small as possible, $N = 1$.

In this paper, we have studied how compartment size affects the accumulation of cells with mutations in tumor suppressor (TSP) genes and mutations that give rise to chromosomal instability (CIN). Mutations in TSP genes are best contained by compartments that are as small as possible. If a cell with two inactivated TSP alleles

emerges, it has a high probability to take over the whole compartment. Its further spread, however, is at least initially limited by the boundaries of the compartment. When taking into account CIN mutations, we find that there is an intermediate optimum compartment size. In the absence of TSP mutations, most CIN mutations will have a selective cost: they induce lethal mutations and might trigger apoptosis. Hence, mutations in CIN genes normally reduce the somatic fitness of a cell. In large compartments, cells with a reduced somatic fitness have a small probability of spreading. They are contained by somatic selection. In small compartments, however, somatic selection is dominated by random drift, and, therefore, CIN mutations can spread effectively.

Tissues of multicellular organisms are organized into compartments. This is well known for epithelial tissues [26, 27] but will most likely apply for all tissues. A strong selective pressure that favors the smallest possible compartment size is protection against mutations in TSP genes and oncogenes. Large compartments, on the other hand, are best for containing mutations that lead to genetic instability. Compartments of intermediate size are an evolutionary trade off. If this compromise is reached at small compartment sizes, then CIN mutations are likely candidates for initiating tumorigenesis.

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