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Cells, cancer, and rare events: Homeostatic metastability in stochastic nonlinear dynamical models of skin cell proliferation

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A recently proposed model for skin cell proliferation [E. Clayton *et al.*, Nature (London) **446**, 185 (2007)] is extended to incorporate mitotic autoregulation, and hence homeostasis as a fixed point of the dynamics. Unlimited cell proliferation in such a model can be viewed as a model for carcinogenesis. One way in which this can arise is homeostatic metastability, in which the cell populations escape from the homeostatic basin of attraction by a large but rare stochastic fluctuation. Such an event can be viewed as the final step in a multistage model of carcinogenesis. Homeostatic metastability offers a possible explanation for the peculiar epidemiology of lung cancer in ex-smokers.

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Cancer represents one of the outstanding health care issues of present times. It is the second leading cause of death in the US after heart disease (2006) [1]. Enormous strides have been made toward understanding the molecular and genetic basis of cancers, particularly since the completion of the Human Genome Project. This has prompted at least one eminent scientist to call for renewed efforts in the "war on cancer" [2]. At the same time, researchers over many decades have collected valuable data on the "dynamics of cancer" [3]. Such epidemiological studies also have the potential to shed light on cancer mechanisms. In particular, it has long been argued that the steepness of the age-incidence curves supports the notion that a cell lineage has to transit through one or more precancerous stages before finally presenting as clinical cancer [3,4]. The transition rates in these so-called multistage models are estimated in the order $10^{-6}-10^{-4}$ per year per cell lineage [3,5].

While the transitions are usually interpreted in terms of somatic mutations, a long-standing puzzle in the epidemiology of lung cancer has been the apparent indifference of the rate of the final transition step to the presence of a carcinogen (tobacco smoke) [6–8]. This has been interpreted as indicating that a nonmutagenic mechanism might be at work. One suggestion is that the final step is epigenetic in nature [7]. Another suggestion is that the final step somehow involves signaling [8]. Here I examine *homeostatic metastability* as a candidate signaling mechanism. Homeostatic metastability focuses on cell population dynamics and makes use of concepts from dynamical systems theory [9]. The idea is that under normal conditions the cell populations fluctuate but remain in the basin of attraction of a homeostatic fixed point. Cumulative somatic mutations in the components of signaling pathways can shrink the size of the basin of attraction until the cell populations can escape by means of a large but rare stochastic fluctuation. If such an escape results in uncontrolled cell proliferation, one has a model for carcinogenesis. Homeostatic metastability fits neatly in the multistage picture since the initial mutations are stochastic rare events, and the final step is also a stochastic rare event, albeit a nonmutagenic one. Homeostatic metastability also fits into the emerging "tissue organization field theory" cancer paradigm [10].

To present a concrete example of homeostatic metastability, I extend a recently developed model for keratinocyte (skin cell) proliferation. The mechanism of epithelial renewal in the epidermis has much in common with that of the lungs although the turnover time may be somewhat shorter [11,12]. Therefore this model may still have relevance to lung cancer. I also deliberately adopt a "physics-oriented" approach in which the extended model is kept as simple as possible to expose the general mechanisms at work (i.e., relying on the generalities of dynamical systems theory). In particular it is not claimed that the biology is necessarily accurately represented.

The epidermis is the outermost part of the skin barrier, comprising 10–20 layers of skin cells which are predominantly keratinocytes [13]. These cells originate in the quasi-two-dimensional basal layer of the epidermis, move up through the middle layers, and are finally shed from the outermost layer at a desquamation rate of the order 10^3 cells h^{-1} mm^{-2} [12]. This means that cells have to proliferate continuously in the basal layer to replenish the suprabasal layers. The recently developed model for keratinocyte proliferation in the basal layer is the single progenitor cell (SPC) model of Clayton *et al.* [14,15]. It is supported by elegant *in vivo* experiments on mouse tail keratinocyte clones with an inducible genetic label. In the SPC model, there are two basal layer cell types or compartments: progenitor cells A and postmitotic cells B. These proliferate according to

$$A \to A + A \text{ rate } \lambda_1, \quad A \to A + B \text{ rate } \lambda_2,$$

$$A \to B + B \text{ rate } \lambda_3, \quad B \to \varnothing \text{ rate } \Gamma. \tag{1}$$

The first three processes represent possible progenitor cell division pathways. The last process represents postmitotic cells leaving the basal layer. The mean-field (or expected-value) population dynamics equations corresponding to these processes are

$$\dot{n}_A = (\lambda_1 - \lambda_3)n_A, \quad \dot{n}_B = (\lambda_2 + 2\lambda_3)n_A - \Gamma n_B, \tag{2}$$

where n_A and n_B are the individual cell densities. Neglecting spatial correlations (i.e., making a "well-mixed" assump-

tion), these equations are exact for the original SPC model since the processes in Eq. (1) are first order with constant rates. They are only approximate for the extended models discussed below where the rates may depend nonlinearly on the cell population densities.

Equations (2) indicate that the progenitor cell division pathways must be finely tuned ($\lambda_1=\lambda_3$) otherwise the cell populations either grow or vanish exponentially [16]. Thus, writing $\lambda_1=\lambda_3=\lambda r$ and $\lambda_2=\lambda(1-2r)$, Clayton *et al.* find $\lambda\approx 0.16$ day^{-1}, $r\approx 0.08$, and $\Gamma\approx 0.045$ day^{-1}, compatible with the above-mentioned desquamation rate. From Eqs. (2) an additional steady state condition for the cell populations is $\lambda=\Gamma(1-\rho)/\rho$, where $\rho=n_A/n$ is the fraction of progenitor cells and $n=n_A+n_B$ is the total cell density. For the SPC model with parameter values corresponding to mouse tail skin, $\rho\approx 0.22$. The corresponding phase-space portrait and a representative stochastic trajectory are shown in Fig. 1(a). To generate the stochastic trajectory, a patch of area $\mathcal{A}$ initially containing $N_0=200$ cells is simulated, interpreting the processes in Eq. (1) as quasichemical reactions and assuming the system remains well mixed. The standard Gillespie kinetic Monte Carlo algorithm is used [17]. The ordinate in Fig. 1 is n/n_0, where $n_0=N_0/\mathcal{A}$ is the initial cell density. The actual value of $\mathcal{A}$ does not need to be specified.

While providing an eminently satisfactory explanation for the keratinocyte clone data, the original SPC model *lacks* homeostasis in the sense that it possesses a *line* of stable fixed points at $\rho\approx 0.22$, shown in Fig. 1(a). Also it is structurally unstable in the language of dynamical systems theory and not generally robust against perturbations; for example, it cannot accommodate the introduction of a small population of stem cells ($S\rightarrow S+A$ [18]) without making additional fine-tuning assumptions. One obvious solution to this is to extend the model to include mitotic autoregulation [19], representing the fact that cellular fates are governed by integration of autocrine and paracrine signaling factors [20]. Indeed this idea was already suggested by Jones and Simons as an avenue for further investigation [21]. In such an *autoregulating* SPC (ASPC) model, homeostasis would arise as a consequence of the cell population dynamics driving the system to a fixed point of the dynamics. A fixed point in a model of this type represents homeostasis in several ways. First, if the cell populations are perturbed, they will tend to return to the original fixed point. Second, fluctuations in the cell populations will tend to be limited to the vicinity of the fixed point. Third, such a model would be structurally stable from the point of view of dynamical systems theory and able to withstand perturbations (such as the presence of a small population of stem cells) without leading to a qualitative change in behavior. Also it is clear that this behavior should be generic to a wide class of ASPC models since the existence of an isolated stable fixed point is a structurally stable feature of the dynamics [22].

To develop such an ASPC model, in keeping with the physics-oriented approach, I introduce a control parameter $q=q(\rho)$ to describe a bias in the symmetric cell division fates [23]. Thus

$$\lambda_1=\lambda r(1-q),\quad \lambda_2=\lambda(1-2r),\quad \lambda_3=\lambda r(1+q). \tag{3}$$

I additionally suppose that $\lambda=\lambda(n)$ is a decreasing function of the total number density ($\lambda'<0$) representing the fact that the progenitor cell proliferation rate should be reduced if the overall cell density increases. With these assumptions, the fixed points of Eqs. (2) are determined by $q(\rho)=0$ and $\lambda(n)=\Gamma(1-\rho)/\rho$. It is a straightforward exercise to show, in the language of dynamical system theory, that a fixed point is a stable node if $q'>0$, and a saddle if $q'<0$.

A concrete model of this type has

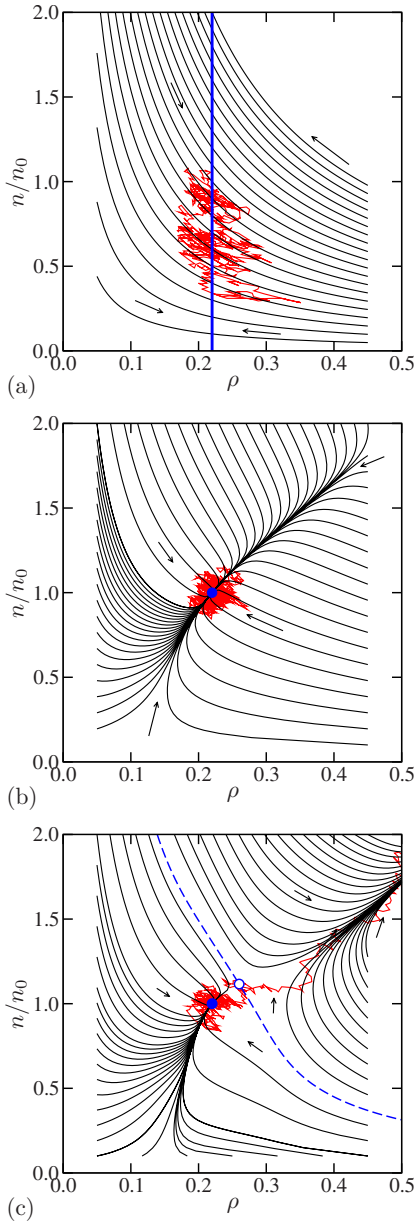

FIG. 1. (Color online) Phase-space portraits for (a) the SPC model, (b) an ASPC model, and (c) an ASPC model exhibiting homeostatic metastability. The axes are the progenitor cell fraction, ρ, and the ratio between the current and initial (and target) total cell densities, n/n_0. Thin lines (black) are phase-space flows, with the direction indicated by the arrows. In (a) the thick line (blue) is the line of fixed points of the SPC model. In (b) and (c) the filled circles (blue) are homeostatic stable fixed points (nodes). In (c) the open circle (blue) is an unstable fixed point (a saddle), lying on the homeostatic basin boundary shown as a dashed line (blue). Jagged lines (red) are representative stochastic trajectories.

$$\lambda = \lambda_0 \left(\frac{n_0}{n} \right)^{\alpha}, \quad q = \tanh \left[\frac{\beta \rho_0 (1-\rho_0)(\rho-\rho_0)}{\rho(1-\rho)} \right], \qquad (4)$$

where $\lambda_0 = \Gamma(1-\rho_0)/\rho_0$. This model has a stable node at a target population density n_0 and progenitor cell fraction ρ_0. These functions have been arbitrarily chosen for illustrative purposes although with care to make sure that they have the appropriate limiting behaviors. For the results presented below I use $\alpha=2$ and $\beta=10$ in Eq. (4), which allows for stochastic fluctuations of moderate amplitude. I have checked that the results are qualitatively insensitive to this choice. Figure 1(b) shows the phase-space portrait and a representative stochastic trajectory for this ASPC model, with a fixed point chosen to lie at $\rho_0=0.22$ and a target population size $n_0=N_0/\mathcal{A}$, where $N_0=200$. Again the actual value of $\mathcal{A}$ does not need to be explicitly specified although it could be interpreted as representing the area of influence of diffusible intercellular signaling factors. Figure 1(b) shows, in contrast to Fig. 1(a), that this ASPC model has an isolated stable fixed point, whose basin of attraction extends to cover the whole plane. The stochastic trajectory is strongly localized to the vicinity of the fixed point.

I turn now to cancer modeling. In this context, carcinogenesis is considered to be unlimited cell proliferation caused either by the simple loss of stability of the homeostatic fixed point or by a large but rare stochastic fluctuation causing the system leaving the homeostatic basin of attraction (homeostatic metastability). My studies of models comprising A, A^*, and B cells, with a process $A \to A^*$ representing a somatic mutation (cf. Klein *et al.* [15]), shows that both phenomena can easily be observed. However the resulting three-dimensional phase-space portraits are tricky to present. To illustrate the mechanism of homeostatic metastability in its simplest form therefore, I return to the original ASPC model but introduce a *re-entrant* bias control function $q(\rho)$. Such a model is a prototypical example of a system which is near a *saddle-node bifurcation*.

An example of a re-entrant $q(\rho)$ is given by inserting an extra factor $(\rho_1-\rho)/(\rho_1-\rho_0)$ in the argument to the tanh function in Eq. (4). This model has a stable node at $\rho=\rho_0$ and a saddle at $\rho=\rho_1$. The saddle-node bifurcation is approached as $\Delta\rho=\rho_1-\rho_0$ vanishes. The phase-space portrait and a representative stochastic trajectory for this ASPC model are shown in Fig. 1(c) for $\Delta\rho=0.04$ (other parameters as for the original ASPC model). The homeostatic basin of attraction is now confined to the lower left region. The saddle lies on the homeostatic basin boundary. The simulations show that the system may escape from the homeostatic basin, typically in the vicinity of the saddle. After this the cell populations grow without limit.

To characterize the escape event, I generate a large set of trajectories and compute an escape rate u from the first passage time distribution (Fig. 2 inset). The main plot in Fig. 2 shows that u decreases approximately exponentially with $N_0\Delta\rho^2$. The dependence of u on α and β is more complex and not necessarily monotonic. The sensitive dependence on the target population size N_0 is typical of system size effects found for other nonequilibrium phase transitions [24] although the scaling collapse onto $N_0\Delta\rho^2$ remains unexplained.

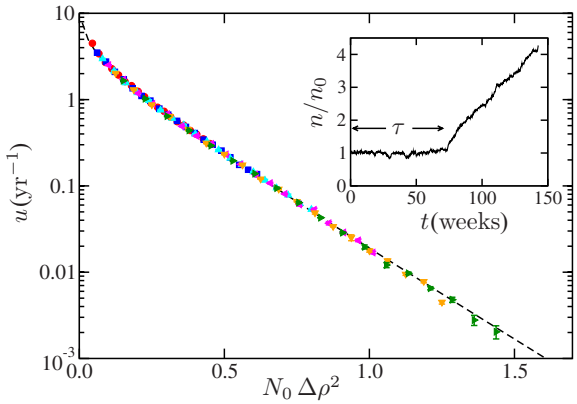

FIG. 2. (Color online) Inset: a typical metastable trajectory from Fig. 1(c); the first passage time τ is found to be distributed as $\sim e^{-u\tau}$ allowing an escape rate u to be defined. Main plot: escape rate u plotted as a function of $x=N_0\Delta\rho^2$. Data were collected for various N_0 and (colored) for $\Delta\rho=0.030(0.005)0.055$ (six values). The line is $u=Ax^{-\gamma}e^{-bx}$, where $A=1.87\pm0.05$ yr^{-1}, $\gamma=0.32\pm0.01$, and $b=4.59\pm0.04$.

The key point is that, extrapolating from Fig. 2, the homeostatic escape rate u can easily be made comparable to multistage transition rates quoted in the introduction [25].

Some general points can be made about directions for future research. First, the present analysis neglects both spatial correlations and, to some extent, number fluctuations. The potentially critical importance of these factors for the behavior of models in two spatial dimensions is well known [26]. The extension of the present ASPC models to fully fledged two-dimensional models is therefore an obvious next step. For example Klein *et al.* were motivated to examine a spatially resolved version of the original SPC model [27]. They found that their original results still hold, albeit with a modified value of $r \approx 0.2$. Another direction in which progress could be made is to improve the representation of the biology, for example, moving to multiscale [28] or agent-based models [29], which can capture the details of the intercellular and intracellular signaling pathways and also the essential stochastic nature of individual cell fates.

A generic conclusion of the present Rapid Communication is that it may not be valid to examine just the *deterministic* consequences of somatic mutations since rare but large fluctuations in cell populations may occur at comparable rates. This makes the task of examining the behavior of more biologically detailed models rather formidable. Brute force methods have been used in the present Rapid Communication since the underlying stochastic processes are rather simple. This may not be possible for more complex models, where it may be necessary to bring to bear more sophisticated techniques such as transition path sampling [30] or forward-flux sampling [31].

Finally, of course, experiments are very desirable. As Frank points out [3], the interpretation of the lung cancer epidemiology data is not unambiguous and should be supported by other experimental evidence. A central feature of the idea of homeostatic metastability is that the cells themselves do not undergo any change if the system escapes from

homeostasis; only the microenvironment changes. Thus, for example, if individual cells from a neoplasm were to be used to seed cancer at sites elsewhere in the organism, one might expect a poor success rate. All such an experiment should do is establish a pool of cell lineages which are in the penultimate precancerous stage, with a concomitant statistical signature in the time-resolved success rate. Recent experiments of this type do indeed show a poor success rate although this is commonly attributed to the hypothesis that only a rare subpopulation of "cancer stem cells" have the potential to develop into new clones [32].

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