Cell Proliferation in Normal and Malignant Transformed Cells: Thermodynamic Model of Signal Transduction

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We present a new thermodynamic model for the mechanism of activation and regulation of cell proliferation in the G_1 stage. In this model, the interactions between growth factors and transmembrane proteins play a crucial role in cell growth control for a normal tissue-culture system. We calculate a phase diagram of normal and malignant transformed states of a cell signal transduction system. We propose thermodynamic reasons why cancer cells can continually grow without activation by the growth factors.

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A cell cycle in cell proliferation consists of a series of metabolic stages: the G_1, S, and G_2M periods [1]. In the initial G_1 period, a period between cell division and the beginning of new DNA synthesis, crucial events that regulate cell proliferation take place. In the midstage S period, the genetic information encoded in the cell DNA is duplicated, to be divided between the two daughter cells. Finally, in the G_2M stage, the separation of the two copies of the genome is completed and a cell division occurs.

Phenomenologically, biochemical events are initiated in the G_1 stage through encounters and binding of extracellular molecules of the growth factor (GF) and the specific receptors located in transmembrane proteins (TMPs) on a cell surface. The receptors transmit the binding signal across the membrane to generate appropriate cellular responses. Thus soon after a GF-TMP binding, cell proliferation is activated [2]. This procedure has been well documented for some tissue-culture systems. However, it is known [3] that cancer cells can grow without activation by the GFs. The malignant mutations do not affect the cell-cycle machinery directly, but instead influence the decision of a cell whether to cycle or not. It is now well known that oncogenes, which encode mutated forms of the GF, cell surface receptors and proteins, are involved in the signal transduction [4,5]. Oncogenic mutant receptors produce a signal to grow in the absence of GF or ligands [6].

We present here a thermodynamic model of the regulation of cell proliferation in the G_1 stage, occurring in normal and malignant transformed cells. The key concern of the current approach is to consider the changes in the conformational properties of TMPs. The characterization of TMPs itself remains one of the most challenging biophysical problems, with many unanswered questions (see, for example, Ref. [7]). TMPs are basic channels of signal transduction through cell lipid membranes [8]. Defects in their structures, even small ones, may lead to serious functional (including malignant) distractions in an entire biological system [3–6]. Our goal is to present a phase diagram of states for the TMP-based signal-transduction system in terms of the GF concentration and basic energetic parameters of the system.

TMP model.—Shown in Figs. 1a and 1b is a schematic representation of typical TMP configurations, where the transmembrane blocks, usually in a helix conformation, are drawn as small boxes. In the present paper, we focus our attention on the macroscopic and mesoscopic conformational properties, and ignore the microscopic details of protein structures at an atomic level with length scales shorter than the biophysical structures which interest us here.

The TMP is modeled by a multiblock copolymer which contains quenched sequences of hydrophobic- and hydrophilic-dominated segments. The hydrophobic (usually nonpolar) sequences of TMPs prefer to stay in the interior of a lipid membrane bilayer, where solvent molecules are largely excluded. The hydrophilic (usually polar) sequences tend to stay outside the bilayers. The transmembrane parts of the protein are usually in a helix

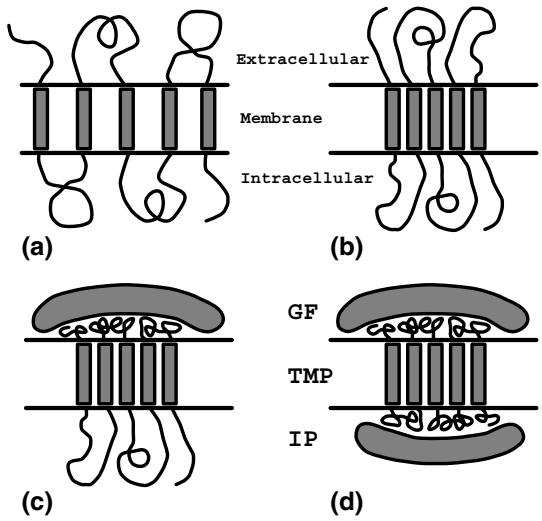

FIG. 1. Sketch of various TMP structures (a) and (b) and TMP binding states with GF only (c) and GF and IP simultaneously (d).

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form, caused mostly by hydrogen bonding. The TMP helices typically have orientation weakly deviating from the bilayer normal, and can form two structures: an aggregated (A) conformation with bundled helices (Fig. 1b), and a nonaggregated (NA) one with separated helices (Fig. 1a).

The free energy difference associated with the helix bundling transition [9] is

$$F_{\mathrm{TMP}} = \phi^{\mathrm{hel}} N^{\mathrm{H}} + Tp\,. \tag{1}$$

Here N^{H} is the total number of hydrophobic monomers inside the bilayer, ϕ^{hel} is the average free energy of each hydrophobic monomer in the conformation of a helix bundle, and T is the temperature in energetic units. This is the mean-field approximation, which is valid for dense-packed helices in a bundle. A TMP sequence parameter [9] is $p = \sum_\kappa \ln N_\kappa^{\mathrm{P}}$, where N_κ^{P} is the length of a TMP coiled loop labeled κ. These loops are formed by hydrophilic (polar) sequences outside the bilayer. The sum $\sum_\kappa$ is taken over all loops on both sides of the membrane bilayer.

Hydrophilic sequences outside the bilayer form Gaussian-like loops in a half space starting from and returning to the membrane surface. In the case of different starting and returning points (when attached NA helices do not interact with each other, Fig. 1a), for a loop of length $N_\kappa^{\mathrm{P}} \gg 1$ the entropy loss [7] is $S_\kappa^{\mathrm{NA}} = -(3/2)\ln N_\kappa^{\mathrm{P}}$. In the case of the same starting and returning point (for A helices, Fig. 1b), the entropy loss is $S_\kappa^{\mathrm{A}} = -(5/2)\ln N_\kappa^{\mathrm{P}}$. Thus the entropy penalty due to the helix bundling transition is $\sum_\kappa (S_\kappa^{\mathrm{A}} - S_\kappa^{\mathrm{NA}}) = -p$.

The transmembrane helices aggregate and form a bundle [10] when $F_{\mathrm{TMP}} < 0$. The transitional value

$$\left(\frac{\phi^{\mathrm{hel}}}{T}\right)_{\mathrm{tr}} = -\frac{p}{N^{\mathrm{H}}} < 0 \tag{2}$$

is the boundary between the regions of aggregated $[(\phi^{\mathrm{hel}}/T) < (\phi^{\mathrm{hel}}/T)_{\mathrm{tr}}]$ and nonaggregated $[(\phi^{\mathrm{hel}}/T) > (\phi^{\mathrm{hel}}/T)_{\mathrm{tr}}]$ helices.

GF-TMP coupling.—The GFs have receptors which prefer to attract hydrophilic amino-acid sequences of TMP loops outside the membrane [11]. It is reasonable [11] to assume that the size of each receptor is much shorter than the unperturbed dimension of a typical hydrophilic loop. A strong attraction between a short GF receptor and links of an external loop leads to the collapse of the loop onto the receptor, which is accompanied by a strong decrease in the distance between loop ends and an aggregation of the corresponding TMP helices connected with the ends. Thus the collapse of the TMP external loops due to GF-TMP binding triggers a bundling transition inside the bilayer.

The free energy of GF-TMP coupling is

$$F_{\mathrm{GF}} = \phi^{\mathrm{GF}} N_{\mathrm{out}}^{\mathrm{P}} + T\,\frac{N_{\mathrm{out}}^{\mathrm{P}} a^2}{R^2} - T\ln\frac{C^{\mathrm{GF}}}{e}\,, \tag{3}$$

where we have considered the interaction energy $\phi^{\mathrm{GF}} N_{\mathrm{out}}^{\mathrm{P}}$ between the GF receptors and all the TMP links belonging to the external loops (the number of the links outside the cell is $N_{\mathrm{out}}^{\mathrm{P}}$ and the energy gain per TMP link when attracted to a GF is ϕ^{GF}). The entropy loss [12] due to the collapse of the external TMP loops is written as $S_{\mathrm{coll}} = -\sum_\kappa^{\mathrm{out}} (N_\kappa^{\mathrm{P}} a^2/R^2) = -(a^2/R^2) N_{\mathrm{out}}^{\mathrm{P}}$, where a is a basic interlink distance, and R is an averaged collapse diameter. The loss of the translational freedom of GF molecules is represented by the last term, where C^{GF} is the GF concentration in the extracellular solution.

In Eq. (3) we kept only leading terms for $N_{\mathrm{out}}^{\mathrm{P}} = \sum_\kappa^{\mathrm{out}} N_\kappa^{\mathrm{P}} \gg 1$ and each $N_\kappa^{\mathrm{P}} \gg 1$. The collapse can also change a GF conformation (for example, make it slightly bend), which could be described by an additional free energy term $F_{\mathrm{conf}}^{\mathrm{GF}} \le O(N_{\mathrm{out}}^{\mathrm{P}})$ in Eq. (3); it was effectively included in the first term through a reduced energy ϕ^{GF}.

One can show (by letting $F_{\mathrm{TMP}} + F_{\mathrm{GF}} = 0$) that for $(\phi^{\mathrm{hel}}/T) > (\phi^{\mathrm{hel}}/T)_{\mathrm{tr}}$ (NA helices), a transitional concentration $C_{\mathrm{A-tr}}^{\mathrm{GF}}$ (to a state with A helices due to the GF-TMP coupling) is given by

$$C_{\mathrm{A-tr}}^{\mathrm{GF}} = \exp\!\left[1 + p + \frac{\phi^{\mathrm{hel}}}{T} N^{\mathrm{H}} + \left(\frac{\phi^{\mathrm{GF}}}{T} + \frac{a^2}{R^2}\right) N_{\mathrm{out}}^{\mathrm{P}}\right]. \tag{4}$$

Therefore, an increase in the GF concentration leads to the start of the bundling transition. Experimentally an increase in the GF concentration leads to the start of cell proliferation [2]. Thus we can conclude that GF-TMP coupling and the corresponding bundling transition eventually lead to cell proliferation. As C^{GF} increases beyond the transitional concentration $C_{\mathrm{A-tr}}^{\mathrm{GF}}$ (this is a command to activate cell proliferation) the TMP-helix bundles are formed. As a direct consequence, the starting and ending points of the intracellular loops have to aggregate within a small region (Fig. 1c). This process of conformational change is the pathway for a transduction of a signal created by a C^{GF} increase. The bundling transition in TMPs merely performs the role of signal relay.

TMP-IP coupling.—Further transduction of the signal relies on the binding of the intracellular proteins (IPs) with the aggregated internal TMP loops. Following the same reasoning as for F_{GF}, we can write

$$F_{\mathrm{IP}} = \phi^{\mathrm{IP}} N_{\mathrm{in}}^{\mathrm{P}} + T\,\frac{N_{\mathrm{in}}^{\mathrm{P}} a^2}{R^2} - T\ln\frac{C^{\mathrm{IP}}}{e}\,, \tag{5}$$

where $\phi^{\mathrm{IP}} N_{\mathrm{in}}^{\mathrm{P}}$ is the interaction energy between an IP and all the TMP links belonging to the internal loops (the number of the links is $N_{\mathrm{in}}^{\mathrm{P}} \gg 1$), and C^{IP} is the IP concentration in the intracellular solution. Again we kept only leading terms with respect to powers of $N_{\mathrm{in}}^{\mathrm{P}}$ for $N_{\mathrm{in}}^{\mathrm{P}} = \sum_\kappa^{\mathrm{in}} N_\kappa^{\mathrm{P}} \gg 1$ and each $N_\kappa^{\mathrm{P}} \gg 1$.

For $(\phi^{\mathrm{hel}}/T) > (\phi^{\mathrm{hel}}/T)_{\mathrm{tr}}$ (NA helices) we find a transitional concentration $C_{\mathrm{A-tr}}^{\mathrm{IP}}$ (to a state with A helices due

to the IP-TMP coupling without the GF involvement for $C^{\mathrm{IP}} > C^{\mathrm{IP}}_{\mathrm{A-tr}}$) from the condition $F_{\mathrm{TMP}} + F_{\mathrm{IP}} = 0$:

$$C^{\mathrm{IP}}_{\mathrm{A-tr}} = \exp\left[1 + p + \frac{\phi^{\mathrm{hel}}}{T} N^{\mathrm{H}} + \left(\frac{\phi^{\mathrm{IP}}}{T} + \frac{a^2}{R^2}\right) N^{\mathrm{P}}_{\mathrm{in}}\right]. \tag{6}$$

GF or IP coupling with initially bundled TMP.—We further introduce two more transitional concentrations, $C^{\mathrm{GF}}_{\mathrm{NA-tr}}$ and $C^{\mathrm{IP}}_{\mathrm{NA-tr}}$, corresponding to $F_{\mathrm{GF}} = 0$ and $F_{\mathrm{IP}} = 0$, respectively. Then we can write

$$\frac{C^{\mathrm{GF}}_{\mathrm{NA-tr}}}{C^{\mathrm{GF}}_{\mathrm{A-tr}}} = \frac{C^{\mathrm{IP}}_{\mathrm{NA-tr}}}{C^{\mathrm{IP}}_{\mathrm{A-tr}}} = \exp\left(-p - \frac{\phi^{\mathrm{hel}}}{T} N^{\mathrm{H}}\right). \tag{7}$$

The transitional concentrations $C^{\mathrm{GF}}_{\mathrm{A-tr}}$ and $C^{\mathrm{IP}}_{\mathrm{A-tr}}$ in Eqs. (4) and (6) have a physical meaning for initially nonaggregated helices. Let us assume now that the TMPs are already in the helix-aggregated form either because (i) $(\phi^{\mathrm{hel}}/T) < (\phi^{\mathrm{hel}}/T)_{\mathrm{tr}}$ or because of (ii) the GF-TMP initial coupling when $C^{\mathrm{GF}} > C^{\mathrm{GF}}_{\mathrm{A-tr}}$ for $(\phi^{\mathrm{hel}}/T) > (\phi^{\mathrm{hel}}/T)_{\mathrm{tr}}$. Then the subsequent IP-TMP coupling will occur when $F_{\mathrm{IP}} < 0$, with a transitional concentration $C^{\mathrm{IP}}_{\mathrm{NA-tr}}$. Similarly, $C^{\mathrm{GF}}_{\mathrm{NA-tr}}$ describes a minimal GF concentration necessary for GFs to be coupled with helix-aggregated TMPs. Thus the general coupling transitional concentrations are $C^{\mathrm{GF}}_{\mathrm{tr}} = \max\{C^{\mathrm{GF}}_{\mathrm{A-tr}}, C^{\mathrm{GF}}_{\mathrm{NA-tr}}\}$ and $C^{\mathrm{IP}}_{\mathrm{tr}} = \max\{C^{\mathrm{IP}}_{\mathrm{A-tr}}, C^{\mathrm{IP}}_{\mathrm{NA-tr}}\}$.

GF-TMP-IP triple binding.—The GF-TMP and TMP-IP bindings can occur simultaneously. The condition $F_{\mathrm{TMP}} + F_{\mathrm{GF}} + F_{\mathrm{IP}} = 0$ determines a triple transitional concentration $C^{\mathrm{GF}}_{\mathrm{T-tr}}$, which is now a function of C^{IP}:

$$C^{\mathrm{GF}}_{\mathrm{T-tr}} = \frac{C^{\mathrm{GF}}_{\mathrm{A-tr}} C^{\mathrm{IP}}_{\mathrm{NA-tr}}}{C^{\mathrm{IP}}} = \frac{C^{\mathrm{GF}}_{\mathrm{NA-tr}} C^{\mathrm{IP}}_{\mathrm{A-tr}}}{C^{\mathrm{IP}}}. \tag{8}$$

Normal cell proliferation.—We are now ready to examine how a signal is transmitted through a TMP. Under normal conditions, the TMP-IP coupling without activation by GFs is impossible; this is true in normal cells in order to prevent wrong signal transduction. This implies that normally $C^{\mathrm{IP}} < C^{\mathrm{IP}}_{\mathrm{A-tr}}$. On the other hand, in order to have a signal relay after the activation by GFs, the TMP-IP coupling must occur for $C^{\mathrm{IP}} > C^{\mathrm{IP}}_{\mathrm{NA-tr}}$, which implies that normally [i.e., at $(\phi^{\mathrm{hel}}/T) > (\phi^{\mathrm{hel}}/T)_{\mathrm{tr}}$, see Eq. (7)]

$$C^{\mathrm{IP}}_{\mathrm{NA-tr}} < C^{\mathrm{IP}} < C^{\mathrm{IP}}_{\mathrm{A-tr}}. \tag{9}$$

As it follows from Eq. (8), the last inequalities mean that $C^{\mathrm{GF}}_{\mathrm{NA-tr}} < C^{\mathrm{GF}}_{\mathrm{T-tr}} < C^{\mathrm{GF}}_{\mathrm{A-tr}}$. Naturally, under condition (9), very low GF concentrations ($C^{\mathrm{GF}} < C^{\mathrm{GF}}_{\mathrm{T-tr}}$) cannot activate the TMP aggregation and the accompanied signal transduction. Since the triple transitional concentration $C^{\mathrm{GF}}_{\mathrm{T-tr}}$ depends on the IP concentration, the condition $C^{\mathrm{GF}} > C^{\mathrm{GF}}_{\mathrm{T-tr}}$ (start of cell proliferation) can be reached from $C^{\mathrm{GF}}_{\mathrm{NA-tr}} < C^{\mathrm{GF}} < C^{\mathrm{GF}}_{\mathrm{T-tr}}$ by increasing either C^{GF} or C^{IP}.

If both the conditions

$$C^{\mathrm{GF}}_{\mathrm{NA-tr}} < C^{\mathrm{GF}}_{\mathrm{T-tr}} < C^{\mathrm{GF}} < C^{\mathrm{GF}}_{\mathrm{A-tr}} \tag{10}$$

and (9) are fulfilled, then the C^{GF}-increase signal is transmitted (due to the GF-TMP-IP binding) to the internal proteins (and then it can reach the nucleus) and the proliferation is activated. The daughter cells will continue the process in a similar way unless the conditions (9) and (10) cease to be valid. In the meantime, under the process in the S stage these conditions can be disturbed and thus the GF-TMP-IP system can become disordered. The disordered GFs will move out from the cell-membrane surface and the TMP helices will disaggregate. By this way the signal transduction will be stopped in the normal cells and the biological system will cease to grow.

Malignant cell proliferation.—It is known that oncogenic mutant receptors give a signal to grow in the absence of GF or ligand [6]. To understand this, we postulate that the signal has been transmitted through the cell membrane if TMP-IP coupling has occurred. For GF concentration below the threshold value $C^{\mathrm{GF}}_{\mathrm{T-tr}}$, this coupling can take place, in particular, because of the following malignant transformations.

(i) Spontaneous TMP-helix aggregation. If $(\phi^{\mathrm{hel}}/T) < (\phi^{\mathrm{hel}}/T)_{\mathrm{tr}}$, the TMP continually sends the signal inside the cell without GF-TMP binding. The TMP-IP coupling occurs and an uncontrolled cell proliferation of the population can take place even for normal C^{IP} [Eq. (9)] and $C^{\mathrm{GF}} < C^{\mathrm{GF}}_{\mathrm{T-tr}}$. This is a model of cancer deviations due to malignant activities in TMPs.

(ii) Abnormally high intracellular IP concentration $[C^{\mathrm{IP}} > C^{\mathrm{IP}}_{\mathrm{A-tr}}]$. Here the TMP-IP coupling and "wrong" signal can take place even for $(\phi^{\mathrm{hel}}/T) > (\phi^{\mathrm{hel}}/T)_{\mathrm{tr}}$ and $C^{\mathrm{GF}} < C^{\mathrm{GF}}_{\mathrm{T-tr}}$. This is a model of cancer deviations due to malignant activities in the cell C^{IP}-control system.

Phase diagram.—In Fig. 2 we present a qualitative phase diagram of the signal transduction pathway in terms

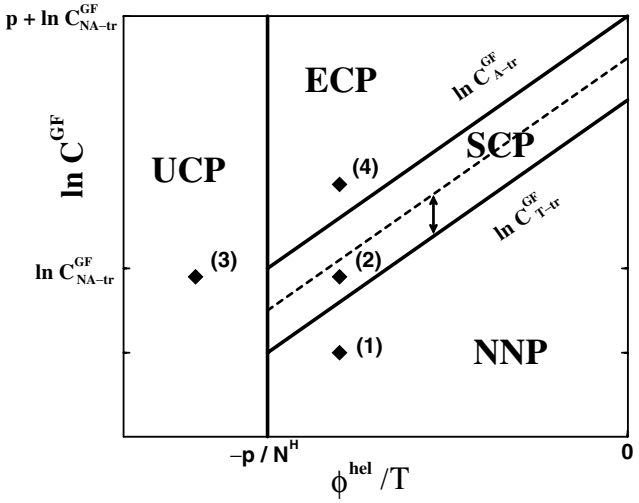

FIG. 2. Phase diagram for the signal transduction pathway, composed of the GF, TMP, and IP, in terms of dependence of the GF concentration on the reduced energy of TMP-helices aggregation. Different regimes are divided by the solid curves.

of the GF concentration vs the reduced energy of the TMP helices bundling (aggregation).

The UCP (uncontrolled proliferation) area corresponds to the condition $(\phi^{\rm hel}/T) < (\phi^{\rm hel}/T)_{\rm tr}$ of GF- and IP-independent TMP helices bundling, i.e., the permanent signal on the internal side of TMP. The next area ECP [externally (IP-independent) controlled proliferation] corresponds to the conditions $(\phi^{\rm hel}/T) > (\phi^{\rm hel}/T)_{\rm tr}$ and $C^{\rm GF} > C^{\rm GF}_{\rm A-tr}$, for which the signal reaches the TMP internal side because of IP-independent GF-TMP coupling. In both UCP and ECP areas the signal can reach the cell nucleus if $C^{\rm IP} > C^{\rm IP}_{\rm NA-tr}$ (in the UCP area this means a permanent malignant proliferation). It does not reach the nucleus in the opposite case.

In two other areas the signal is normally controlled. The NNP (normal nonproliferation) phase exists for $(\phi^{\rm hel}/T) > (\phi^{\rm hel}/T)_{\rm tr}$, $C^{\rm GF} < \min\{C^{\rm GF}_{\rm T-tr}, C^{\rm GF}_{\rm A-tr}\}$, and $C^{\rm IP} < C^{\rm IP}_{\rm A-tr}$. The last area, SCP [self-controlled (IP-dependent) proliferation], exists for $(\phi^{\rm hel}/T) > (\phi^{\rm hel}/T)_{\rm tr}$ only if $C^{\rm GF}_{\rm T-tr} < C^{\rm GF}_{\rm A-tr}$ [i.e., for the normal IP concentration in Eq. (9)], and the area disappears for $C^{\rm IP} \le C^{\rm IP}_{\rm NA-tr}$. (However, for $C^{\rm IP} > C^{\rm IP}_{\rm A-tr}$ an abnormal proliferation can occur in both NNP and SCP areas.)

Let us now consider some scenarios of system development. Suppose that the initial state of a system is represented by the point (1) in Fig. 2, where there is no possibility of cell proliferation for $C^{\rm IP} < C^{\rm IP}_{\rm A-tr}$. An original signal is sent and results in an increase of $C^{\rm GF}$ outside the cells; this causes the system to move to point (2) in the SCP area. Under the proliferation development the new daughter cells will be created while the system is in the SCP area. However, during the proliferation some parameters (which determine the transitional concentrations above) can change; this change can be due to either (i) a normal back signal to decrease or to stop the proliferation, or (ii) a cancer deviation in one or a few parts of the cell system.

(i) Under the normal development the curve $C^{\rm GF} = C^{\rm GF}_{\rm T-tr}$ can move up on the phase diagram (for instance, due to a decrease of $C^{\rm IP}$ inside the daughter cells) and at a certain stage this curve (dashed line in Fig. 2) goes beyond point (2). This is a situation where the GF concentration becomes smaller than that which is necessary to continue the proliferation. The GF-TMP couples will be destroyed. The system can return to the vicinity of point (1) and the transitional curve $C^{\rm GF}_{\rm T-tr}$ can return to the vicinity of the original solid line.

(ii) Under the malignant development in the daughter cells, some malfunction can occur in TMPs and/or IPs. For instance, the malfunction can be associated with irreversible changes in the interaction energies $\phi^{\rm hel}$, $\phi^{\rm GF}$, or $\phi^{\rm IP}$, or the IP concentration inside the transformed cells. If $\phi^{\rm hel}/T$ is decreased, the system can reach the area UCP [point (3) in Fig. 2] with an abnormal permanent $C^{\rm GF}$-uncontrolled proliferation. In another scenario, a sharp increase in $C^{\rm GF}$ can lead to point (4) in the ECP area, where the GF-TMP coupling signal cannot be stopped by a decrease of $C^{\rm IP}$. The signal can be stopped by a $C^{\rm GF}$ decrease only, for instance, as a result of a long-term proliferation, when a majority of the GFs will be caught by a large number of the daughter cells.

In summary, we have presented and discussed a new thermodynamic model for activation and regulation of cell proliferation in the G_1 period for both normal and malignant transformed cells. We have studied the signal transduction pathway between GF and IP (in the cytoplasm inside the cells) through TMP on cell-membrane bilayers. We have calculated a phase diagram of the cell signal transduction system, which includes areas of both normal and malignant proliferation.

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