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Circadian Gating of the Mammalian Cell Cycle Restriction Point: A Mathematical Analysis

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ABSTRACT A critical decision in the mammalian cell cycle is whether to pass through the restriction point (R-point) or enter the cell cycle. In this letter, we modeled the decision-making system of the mammalian cell cycle entry and the simulated circadian regulation of the R-point driven by external epithelial growth factor (EGF) patterns. Our conceptual model replicated key signaling behaviors observed experimentally, suggesting that the proposed network captured the essential system features. The model revealed the dramatic importance of the EGF dynamics on promoting cell proliferation, showed that the EGF signal duration was more important than the signal strength for driving cells past the R-point, and suggested that the loss of circadian control of the cell cycle entry could be associated with cancer development.

INDEX TERMS Cancer, circadian rhythm, systems biology.

I. INTRODUCTION

CONTROL of the cell cycle entry under circadian humoral epithelial growth factor (EGF) fluctuations plays a critical role in development, stem cell and progenitor renewal, wound recovery, and carcinogenesis. EGF receptor 1 (EGFR1) and human EGF receptor 2 (HER2) are the most studied EGF receptors (EGFRs) that initiate the cell cycle, sustain cell survival, and contribute to various types of cancers when overexpressed, mutated, or improperly regulated. Humoral variations of EGF levels are sensed by the EGFR system though the EGF/EGFR binding. Phosphorylation of EGFR homo and heterodimers occurs within minutes, and the resulting signal rapidly moves forward, toward the restriction point (R-point) control machinery. The R-point is ultimately controlled by the bistable Rb–E2F switch. If the R-point is successfully transitioned, the cell becomes committed to enter the cell cycle, and the retraction of the growth factor no longer affects the cell fate [1].

While the EGF-initiated signaling usually results in a transient extracellular-signal-regulated kinase (ERK) activity in normal fibroblast and epithelial cells, a more sustained ERK signal of sufficient duration is required to pass the R-point [2]. Endocytosis of EGF-bound EGFRs provides immediate attenuation of signals [Fig. 1(a)]. Internalized EGF:EGFR complexes are spatially detached from the signaling system, and the sorting process in endosomes provides fine control of signal strength (through recycling of complexes back to the cell surface) and long-term adaptation (through complex degradation in late endosomes). During the endocytosis process, EGF-bound EGFR-dimers are modified, and show different preferred signaling pathways than the unrecycled EGF:EGFR complexes. Overexpression of EGFR1 or HER2 as well as mutations that undermine the negative feedback regulation of the EGFR-ERK signaling pathway may transform the transient mode of the EGF-induced ERK signaling to the sustained mode, and uncontrollably drive cells past the R-point [3].

Circadian control of the cell cycle entry via EGFRs is crucial for the functional coordination between organs and the suppression of cancer development [4]. Regulated by the suprachiasmatic nucleus (SCN), both global and local EGF levels show clear circadian patterns with moderate oscillation amplitudes [5]. In healthy cells, the temporal distribution of cell cycle phases shows strong, tissue-specific preferences for specific circadian time windows [6]. In contrast, carcinogenesis is often characterized by cell cycle progression gradually losing its synchronization with circadian rhythms. Computational investigations on the circadian gating of the cell cycle checkpoints have attracted increasing attention [7]. However, *in silico* studies focusing on the effects of external growth factor signals on the circadian control of the R-point have not been reported.

II. MODEL DEVELOPMENT

Our study is limited to fibroblastlike and epithelial-like cells. The conceptual model was composed of three distinct modules: 1) a new minimal model of SCN-driven EGF secre-

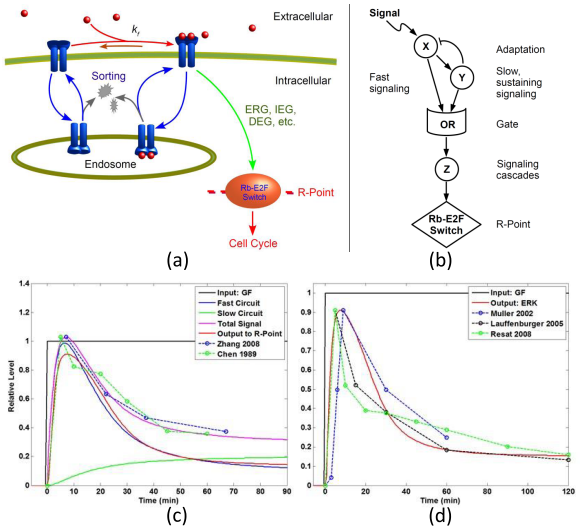

FIGURE 1. Effects of fast and slow EGFR signaling pathways on ERK dynamics. (a) Biological perspective. (b) Model schema. (c) Fitting experimental EGFR data. (d) Fitting experimental ERK data.

tion; 2) a new minimal model of growth factor signaling; and 3) an established model of the Rb–E2F-driven R-point switch [17]. All module equations were formulated in terms of dimensionless state variables. An established mammalian core clock model [8] was used to generate the SCN rhythmic output. Our model was based on the simplification that the SCN regulated global as well as local EGF secretion. The nuclear CLOCK/BMAL1 complex (BN) was chosen as a representative SCN output through which the regulating signals were transmitted to peripheral organs to regulate EGF expression and secretion.

EGF secretion mechanisms were simplified as the early response (1) and delayed response (2) activation machinery, either of which could trigger EGF secretion through an *OR* logic gate (3)

$$\frac{\mathrm{dER}}{dt} = \frac{\beta_{\mathrm{er}}\widehat{\mathrm{ER}}^2}{1+\widehat{\mathrm{ER}}^2} - \alpha_{\mathrm{er}}\mathrm{ER} + \beta_{\mathrm{erbn}}\mathcal{T}(\mathrm{BN}, \tau_{\mathrm{er}}) \quad (1)$$

$$\frac{\mathrm{dDR}}{dt} = \frac{\beta_{\mathrm{dr}}\widehat{\mathrm{DR}}^2}{1+\widehat{\mathrm{DR}}^2} - \alpha_{\mathrm{dr}}\mathrm{DR} + \beta_{\mathrm{drbn}}\mathcal{T}(\mathrm{BN}, \tau_{\mathrm{dr}}) \quad (2)$$

$$\frac{\mathrm{dEGF}}{dt} = \frac{\beta_{\mathrm{egf}}(\widehat{\mathrm{ER}}_e^2 + \widehat{\mathrm{DR}}_e^2)}{1+\widehat{\mathrm{DR}}_e^2 + \widehat{\mathrm{DR}}_e^2} - \alpha_{\mathrm{egf}}\mathrm{EGF} \quad (3)$$

where $\widehat{\mathrm{ER}} = (\mathrm{ER}/K_{\mathrm{er}})$, $\widehat{\mathrm{DR}} = (\mathrm{DR}/K_{\mathrm{dr}})$, $\widehat{\mathrm{ER}_e} = (\mathrm{ER}/K_{\mathrm{er,egf}})$, $\widehat{DR_e} = (\mathrm{DR}/K_{\mathrm{dr,egf}})$, and $\mathcal{T}(\mathrm{BN}, \tau)|_t = \mathrm{BN}|_{t-\tau}$. Rate constants ($h^{-1}$) were obtained from the experimental literature [2], [9], [10]: 1) $\beta_{\mathrm{er}} = 0.05$; 2) $\beta_{\mathrm{erbn}} = 0.69$; 3) $\alpha_{\mathrm{er}} = 1.30$; 4) $\beta_{\mathrm{dr}} = 0.05$; 5) $\beta_{\mathrm{drbn}} = 0.69$; 6) $\alpha_{\mathrm{dr}} = 1.30$; 7) $\beta_{\mathrm{egf}} = 3.75$; and 8) $\alpha_{\mathrm{egf}} = 3.75$. We used the following dimensionless constants: 1) $K_{\mathrm{er}} = 1$; 2) $K_{\mathrm{dr}} = 1$; 3) $K_{\mathrm{er,egf}} = 0.25$; and 4)$K_{\mathrm{dr,egf}} = 0.50$. The EGF secretion module was fit to clinical humoral EGF data [11] by adjusting the time delays (h): 1) $\tau_{\mathrm{er}} = 3.75$ and 2) $\tau_{\mathrm{dr}} = 10$.

The growth factor signaling module [Fig. 1(b)] was described by (4)–(6). The humoral EGF level produced by the SCN-driven secretion module was the input signal to the fast signaling component (X) representing unrecycled EGF:EGFR complexes. The ERK level (Z) was the output of an *OR* gate with X and the slow signaling component (Y) representing recycled EGF:EGFR complexes as the inputs

$$\frac{dX}{dt} = \frac{\beta_x\hat{X}^2}{1+\hat{X}^2} - \alpha_x X + \beta_{xs}\mathrm{EGF} - \alpha_{xy}Y \quad (4)$$

$$\frac{dY}{dt} = \frac{\beta_y\hat{Y}^2}{1+\hat{Y}^2} - \alpha_y Y + \beta_{yx}X \quad (5)$$

$$\frac{dZ}{dt} = \frac{\beta_z(\hat{X}_z^{\,2} + \hat{Y}_z^{\,2})}{1+\hat{X}_z^{\,2} + \hat{Y}_z^{\,2}} - \alpha_z Z \quad (6)$$

where $\hat{X} = (X/K_x)$, $\hat{Y} = (Y/K_y)$, $\hat{X}_z = (X/K_{zx})$, and $\hat{Y}_z = (Y/K_{yz})$. The rate constants (h^{-1}) were specified as [9], [10], [12]–[14]: 1) $\beta_x = 30$; 2) $\beta_{xs} = 30$; 3) $\beta_y = 0.5$; 4) $\beta_{yx} = 0.5$; 5) $\alpha_{xy} = 131.25$; 6) $\beta_z = 100$; 7) $\alpha_x = 40.2$; 8) $\alpha_y = 0.75$; and 9) $\alpha_z = 80$. The dimensionless constants had the values: 1) $K_x = 1$; 2) $K_y = 0.4$; 3) $K_{zx} = 0.6$; and 4) $K_{yz} = 0.6$.

The R-point switch module [Fig. 1(b)] was described by (7), which exhibits a saddle-node bifurcation

$$\frac{dE}{dt} = \frac{\beta_e\hat{E}^2}{1+\hat{E}^2} + \beta_{\mathrm{ez}}Z - \alpha_e E \quad (7)$$

where $\hat{E} = (E/K_e)$ with $K_e = 0.2889$. The module produced hysteresis in the ERK response between the transient and sustained modes with experimentally derived parameters [9], [10], [13], [14]: 1) $\alpha_e = 1.4$; 2) $\beta_e = 1.4$; and 3) $\beta_{ez} = 0.049$. Validation of this module is presented in [17].

III. RESULTS

A. EFFECT OF FAST AND SLOW PATHWAYS ON EGFR SIGNALING

Fig. 1(c) and (d) shows that the conceptual model was able to capture the essential features of the EGFR signaling events. The model reproduced data for the phosphorylated EGFR1 published in two experimental studies (green circles [15] and blue circles [9]) and measured ERK levels from three experimental studies (blue circles [13], black circles [10], and green circles [9]). Although not shown here, the qualitative behavior of our conceptual model was also consistent with other published experimental and computational results [10], [12], [13]. Overexpression of the fast signaling-associated receptor EGFR1 has been shown to increase the EGFR signaling amplitude and extend the effective signaling time, while transient signal attenuation rates due to endocytosis are comparable with normal cells. By contrast, overexpression of the slow signaling-associated receptor HER2 has been shown to cause lengthening of the EGFR signaling. Based on these comparisons, we concluded that the conceptual model possessed sufficient detail to perform *in silico* studies.

B. R-POINT CONTROL UNDER CIRCADIAN EGF LEVELS

The effect of SCN-driven EGF fluctuations on R-point control was investigated and compared with results obtained with a constant, daily average humoral EGF level. For

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this purpose, scaling factors were introduced to investigate the effects of fast, unrecycled EGFR signaling response strength (ω_x) and slow, recycled EGFR signaling response duration (ω_y) on the EGFR and ERK levels such that $\omega_x\beta_{xs}$ and α_y/ω_y. Two additional scaling parameters were introduced to investigate the relative contributions of the fast (κ_x) and slow (κ_y) EGFR signaling pathways to ERK activation and R-point control such that K_{zx}/κ_x and K_{zy}/κ_y. The critical value $\tilde{K}_e$ was defined as the minimum value of the parameter K_e (7) such that a normal cell ($\omega_x = \omega_y = \kappa_x = \kappa_y = 1$) failed to transition the R-point. For $K_e > \tilde{K}_e$, the cell was quiescent, and for $K_e < \tilde{K}_e$, the cell proliferated. Using the circadian EGF signal generated from the secretion module, the model was simulated with different combinations of the EGFR response parameters (ω_x and ω_y) or the ERK activation parameters (κ_x and κ_y) to determine a $\tilde{K}_e$ value for each parameter combination.

Variations in ω_x and ω_y produced the 2-D parameter plot in Fig. 2(a), where the green and red isoboles represent the cell mitotic response dividing lines for a constant EGF level of unity magnitude and a circadian fluctuating extracellular EGF stimuli with a mesor of unity, respectively. Under circadian EGF fluctuations, cell proliferation could be activated by substantially smaller increases in the fast pathway response strength ($\omega_x = 2.5$) or slow pathway response duration ($\omega_y = 1.3$) than observed for the constant mesor EGF level. Fig. 2(b) shows the corresponding 2-D parameter isobologram for variations in κ_x and κ_y. Circadian fluctuations had a small effect on the upregulation of the ERK activation required to drive a quiescent cell past the R-point.

The model predicted that a major effect of moderate circadian EGF fluctuations, compared with constant high EGF levels used in most *in vitro* experiments, was to significantly reduce the degree of upregulation of EGFR signaling pathways necessary for an otherwise quiescent cell to become proliferating. This result was consistent with the experimental observations that sustained growth factor receptor signaling is common among cancers, for example, HER2 overexpression in breast cancers [3], [16] and large EGFR1 deletions in brain tumors [18]. According to our model, overexpression of EGFR1 not only undermined receptor-depletion-induced signal attenuation and enhanced strength of the transient fast signaling pathway, but also increased the level of surface signaling EGFR complexes recycled from the endosome. These effects combined to hold slow signaling at a high level.

C. CIRCADIAN TIME OF CELL CYCLE ENTRY

The effects of SCN-driven EGF fluctuations on the circadian time of cell cycle entry were investigated through stochastic simulations designed to capture the effects of intracellular molecular noise. The differential equations of the GFR–R-point signaling module (1)–(7) were modified to form the Langevin equations with 10% white noise. Simulations were performed for three combinations of the EGFR response parameters (points A, B, and C) and three combinations of the ERK activation parameters

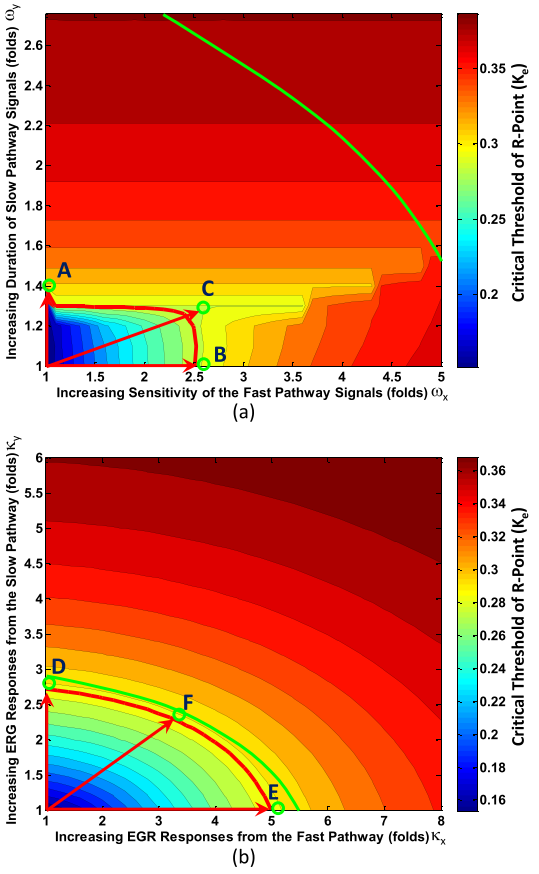

FIGURE 2. Effects of fast and slow pathway modulations on cell mitotic responses for a constant EGF level and circadian EGF fluctuations. Green isoboles: $\tilde{K}_e$ values for constant signals. Red isoboles: $\tilde{K}_e$ values for fluctuating EFG signals. The isoboles divide the parameter spaces into quiescent (bottom left) and proliferating (top right) regions. (a) Effects of ω_x and ω_y. (b) Effects of κ_x and κ_y.

(points D, E, and F) chosen to be just inside the proliferating region for circadian EGF fluctuations (red line) in Fig. 2(a) and (b), respectively.

Stochastic simulation results for the three cell types with modified ERK activation parameters are shown in Fig. 3(a). The left column contains the results of 100 stochastic simulations for each cell type, while the right column contains histograms of the R-point time distribution where the R-point transition was defined as the time where E2F = 0.5. ERK profiles (magenta curve) of the fast-pathway dominant cells (middle row) were very sensitive to the fluctuations of the external EGF signal and showed substantial stochastic variations between different cells. E2F profiles (red curve) also varied substantially between the three cell types. Slow-pathway dominant cells were activated at roughly the same time during each circadian cycle [Fig. 3(a), top right] as observed experimentally [4]–[6]. This behavior is more clearly represented in Fig. 3(b), where the R-point time distributions for the three cell types are presented in terms of circadian time rather than absolute time. Surprisingly, the fast-slow dual modulated cells produced the widest variability in R-point control [Fig. 3(b), right].

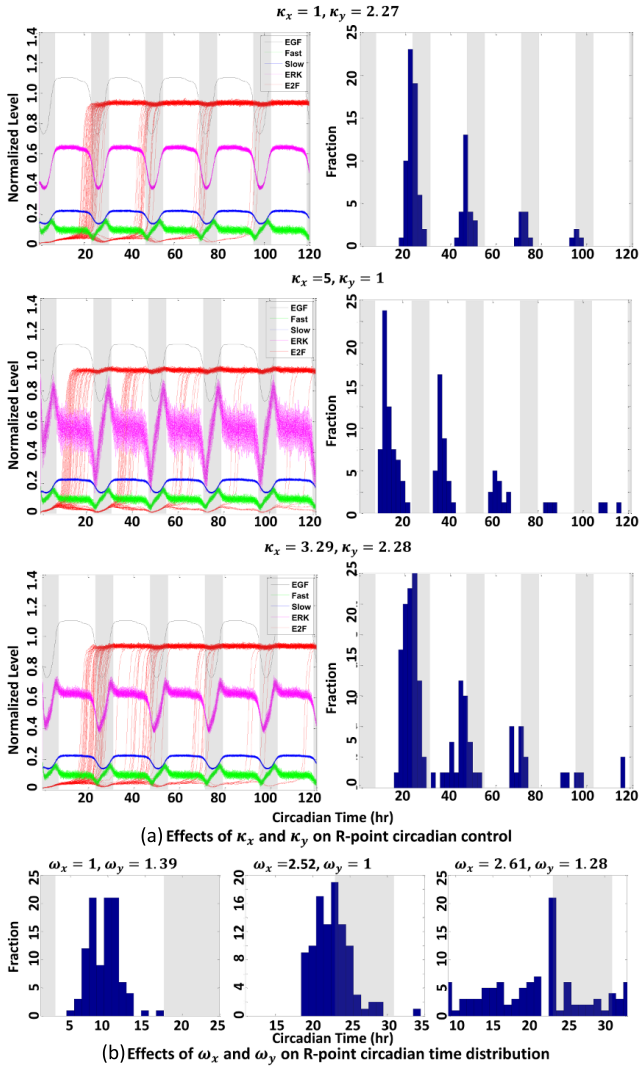

FIGURE 3. Effects of fast and slow pathway modulations on circadian control of cell cycle entry. (a) Left: results of 100 stochastic model simulations for each set of parameters at points D (top), E (middle), and F (bottom) in Fig. 2(b). Right: R-point time distributions for the same three parameter sets. (b) R-point circadian time distributions for points A (left), B (middle), and C (right) in Fig. 2(a). Shaded regions: human physiological night from 10 p.m. to 6 a.m.

Therefore, our model suggested that the combined effects of fast/slow EGFR signaling could have a pivotal role in undermining circadian control of the R-point transition. This prediction could help explain the poor prognosis of EGFR1/HER2 +/+ breast cancer patients [16].

IV. CONCLUSION

This letter represented the first attempt to utilize computational modeling to investigate the effect of circadian growth factor signals on G0/G1 R-point control. Our simulations suggested that: 1) the EGFR pathway associated with slow signaling is more efficient than the fast EGFR pathway for promoting cell proliferation; 2) physiological EGF signals with specific circadian patterns are more likely to trigger proliferation than high concentration EGF pulses of short duration that are commonly used in *in vitro* experiments; and 3) upregulated fast/slow EGFR signaling pathways (e.g., EGFR1/HER2 +/+) may interact to significantly undermine the circadian timing of the R-point transition.

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A MATLAB version of the model is available from the authors upon request.

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