24 Is the signal transduction network emanating from the EGF receptor bistable *in vivo*?

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Introduction

Complexity abounds in cell biology. For instance it shows up in the number and the organization of eucaryotic signal transduction pathways. So far as many as eleven MAP kinase family members have been identified in mammalian cells. They constitute different groups of kinases that are involved in different signal transducing pathways [1]. These signal transduction pathways provide the cell with a structured informational processing machinery processing multiple signals at the same time. The result is an integrated adaptive response of the cell towards the signals mediated by activation of a diverse range of transcription factors.

Due to non-linearity prevailing in the kinetics of signal transduction pathways, various emergent properties might arise. One is the ultrasensitivity of the phosphorylated enzyme concentration towards the concentration of the signal, implying the existence of a threshold response below which the system does not respond [2]. The extent of ultrasensitivity can be quantified using the concentration response coefficient of the phosphorylated enzyme with respect to its signal, complying with metabolic control analysis [3,4]. Another interesting emergent property is the possible existence of two steady states a phenomenon known as bistability [5,6,7].

Bistability in signal transduction networks results in two attainable stable steady states characterized by different active regulatory protein concentrations. This may invoke different physiological states or cellular differentiation [7]. The steady state the system will ultimately evolve to depends upon the history of the system, e.g., on its initial conditions. The possibility of bistability in signal transduction has been acknowledged in the literature [5,6,7]. However, to date, this

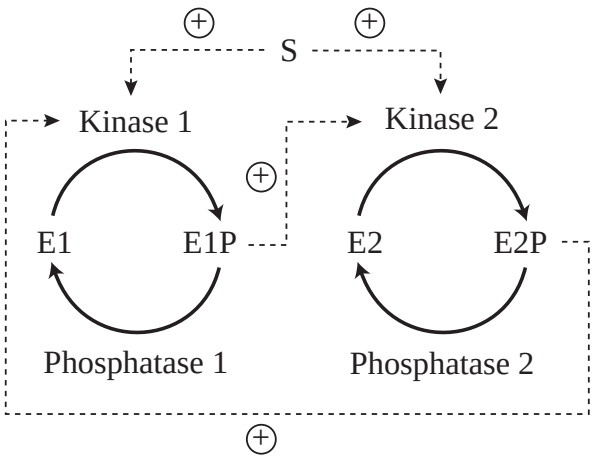

Fig. 24.1 The signal S activates the two kinases which subsequently phosphorylate their substrates. The phosphorylated substrates, being kinases themselves, are capable of phosphorylating the unphosphorylated kinase of the other monocycle.

phenomenon has not been observed in a biological *in vivo* system.

An important signal transduction system in cancer research involves the MAPK pathway downstream of the epidermal growth factor receptor (EGFR) [8]. EGFR is activated upon binding of EGF causing two downstream effects. On the one hand, the sequential activation of GBR2, SOS, Ras and the MAPK pathway (MAPKKK (Raf serine/threonine kinase), MAPKK (MEK1 and MEK2), MAPK (ERK1 and ERK2). On the other hand, activated EGFR causes the activation of phospholipase C-g and protein kinase C (PKC). PKC, in its turn, is capable of activating Raf [9]. Hereby causing cross-talk between activated Ras and PKC. Interestingly ERK is also capable of indirectly activating PKC via phospholipase A-2 [10]. Activated ERK is involved in the initiation of cell cycle related processes.

Recently, Bhalla and Iyengar modelled the signal transduction pathways downstream of the epidermal growth factor receptor (EGFR) [11]. Their model displayed two stable steady-state concentrations of MAPK (ERK) and PKC depending on the concentration of EGF. Interestingly, under certain conditions, MAPK and PKC remained largely in their active phosphorylated form even if EGF was removed. This implies that the transition from steady states with a low degree of activated MAPK and PKC to the steady states with a high degree of activated MAPK and PKC is irreversible. Such irreversible transitions might have a function in the unrestrained growth of cancerous tumors, for it has been shown that many cancers have mutations downstream of the EGF receptor.

Here we present a bistable core model of a signal transduction network which may serve as a guide in the search for bistability in the signal transduction networks emanating from EGFR.

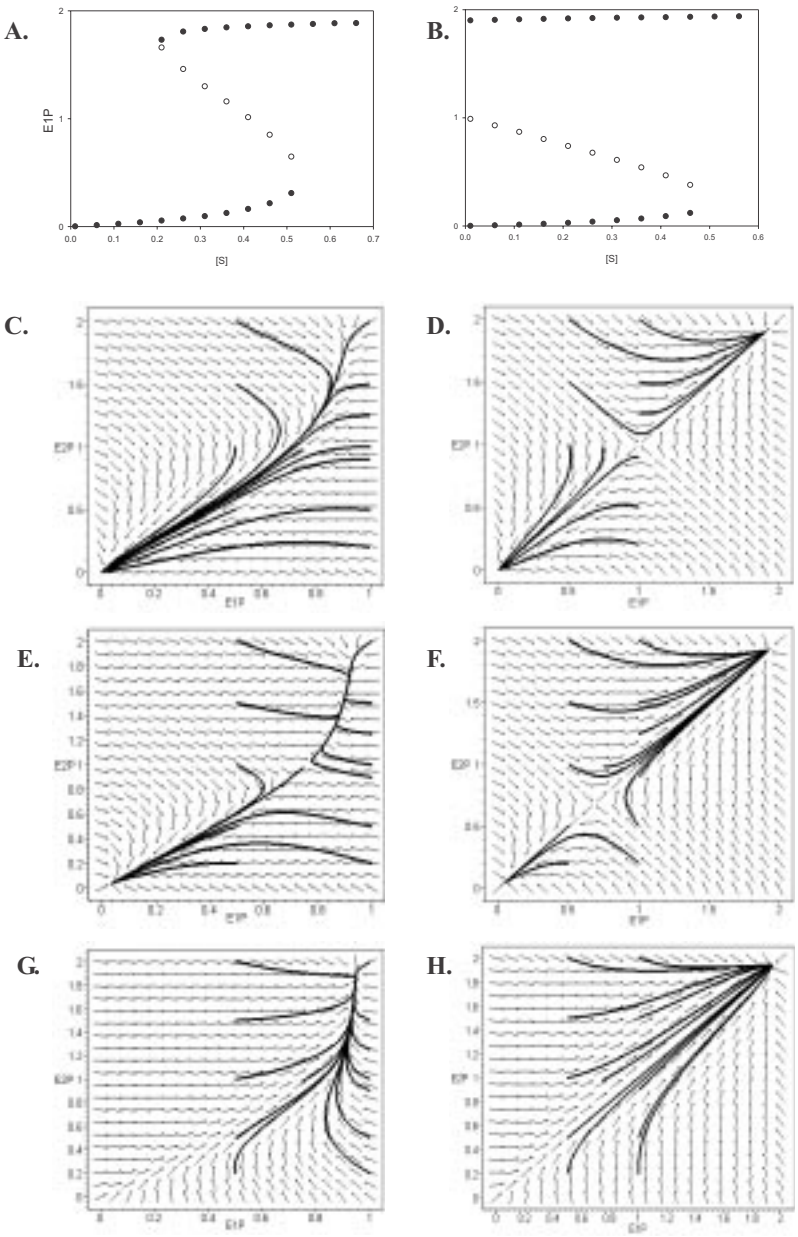

Fig. 24.2 Bifurcation diagrams (open circles: unstable steady states) showing bistability and either A. reversible transitions or B. irreversible transitions. Phase portraits of E1P(t) and E2P(t) at $S = 0$, $S = 0.25$, and $S = 0.6$ for the system with either reversible transitions (C., E., G.) or irreversible transitions (D., F., H.) in its bifurcation diagram. Differential equations used:

$$\frac{dE1P(t)}{dt} = V_{\mathrm{f1}} \times (S + (E2P(t))) \times \frac{(E1t - E1P(t))}{(E1t - E1P(t) + K_{\mathrm{mf1}})} - \frac{V_{\mathrm{1b}} * E1P(t)}{(E1P(t) + K_{\mathrm{m1b}})}$$

$$\frac{dE2P(t)}{dt} = {}_{\mathrm{f2}} \times (S + (E1P(t))) \times \frac{(E2t - E2P(t))}{(E2t - E2P(t) + K_{\mathrm{mf2}})} - \frac{V_{\mathrm{2b}} * E2P(t)}{(E2P(t) + K_{\mathrm{m2b}})}$$

Parameters: $V_{\mathrm{f1}} = 10$, $V_{\mathrm{f2}} = 10$, $V_{\mathrm{1b}} = 10$, $V_{\mathrm{2b}} = 10$, $E2t = 2$, $K_{\mathrm{mf1}} = 0.1$, $K_{\mathrm{mf2}} = 0.1$, $K_{\mathrm{m1b}} = 0.1$, $K_{\mathrm{m2b}} = 0.1$. In the reversible and irreversible case E1t was respectively 1 and 2.

Results

A signal transduction network was modeled (Fig. 24.1). It consists of a signal (S) that stimulates the phosphorylation steps of two kinase/phosphatase monocycles (E1-E1P and E2-E2P). E1P stimulates the kinase of the second monocycle, whereas E2P stimulates the kinase of the first monocycle. Depending upon the parameter values used, the model displays reversible and irreversible transitions from the two stable steady state branches, as can be judged from the bifurcation diagrams (Fig. 24.2A and 24.2B). An irreversible transition is visible in Fig. 24.2B. The system cannot return to the lower branch of steady states once it is located on the upper branch.

Fig. 24.2C to 24.2H display phase portraits of E1P(t) and E2P(t) for both sets of parameters at different concentrations of S. The phase portraits show the evolution of the system's variables from their initial values to their steady-state values. The arrows indicate the direction of time.

Discussion

The model shown in Fig. 24.1 is capable of displaying bistability with reversible and irreversible transitions (Fig. 24.2). The modeled signal transduction network may serve as a core model for the signal transduction pathways emanating from the EGF receptor. A translation of the model into the constituents of the signal transduction pathway emanating from EGFR would be as follows. The first monocycle consists of inactivated (E1) and activated PKC (E1P). PKC is activated by phosphorylated ERK (E2P) and activated EGFR (S). Activated PKC ultimately activates ERK (E2) via subsequent activation of Ras and Raf. Activated EGFR may also activate ERK via the subsequent activation of GBR2, SOS, Ras, and the entire MAP kinase cascade.

Currently, we are investigating whether bistability in the signal transduction pathways emanating from the EGF receptors occurs *in vivo*. The presented model may help us to design appropriate experiments and to understand the emergent properties of this signal transduction network.

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