# 26 Robustness in a model for calcium signal transduction dynamics

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## Introduction

The task of all communicating systems is to transfer information from one point to another in a reliable manner. In living cells the information transfer involves so-called second messengers which are small molecules or ions that carry information from receptors at the plasma membrane to, e.g., enzymes and genes controlling the biochemical network of the cell. Many different second messengers have been identified and studied. Most studies regarding these molecules concerned the molecular mechanism of their liberation and the nature of their impact. However, up to now, little work has been devoted to the robustness of these signal transduction systems towards internal and external perturbations.

Calcium ions are among the predominant second messengers [1]. Generally, following the binding of an agonist to a receptor at the plasma membrane $G_\alpha$ subunits of a G-protein are activated, bind to phospholipase C (PLC) and thereby activate this enzyme. PLC then catalyzes the release of inositol-1,4,5-trisphosphate ($IP_3$) from the plasma membrane. The newly formed $IP_3$ induces the liberation of calcium ions from internal stores. A massive influx of calcium from extracellular space is also observed following agonist stimulation. Although the general cascade of events is well-known [2], the multiple interactions of all the individual compounds are not fully understood. Using fluorescent dyes, it is possible to follow the concentration of calcium ions *in vivo* and it was shown that it oscillates in response to agonist stimulation [3]. These oscillations show characteristic patterns, if different agonists are used [4], e.g., simple oscillations (spiking) with

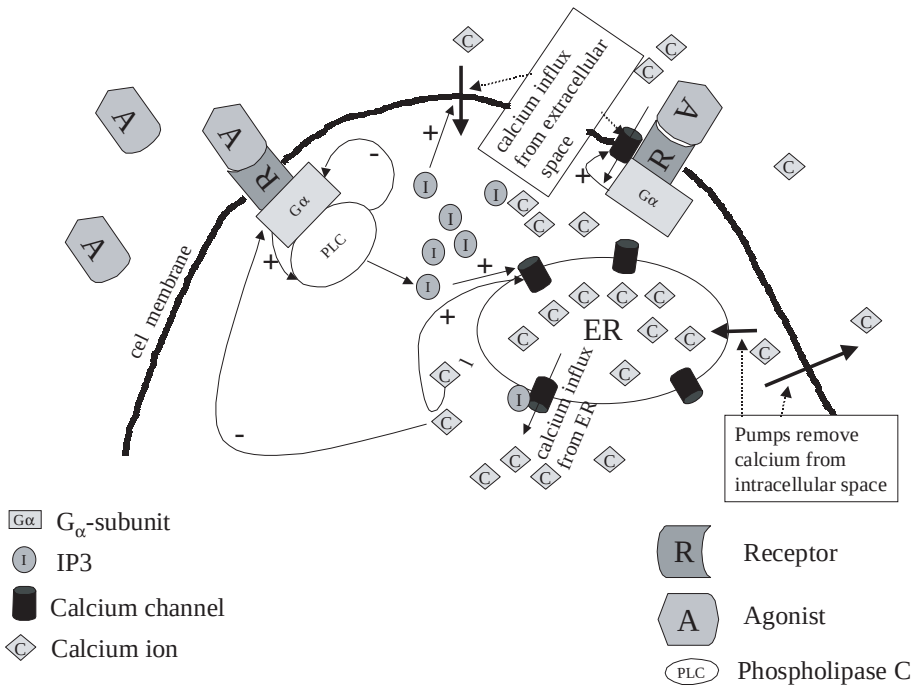


**Fig. 26.1** Scheme for the calcium signal transduction machinery. Upon the activation of the receptor by agonists at the cell membrane, self-enhanced activation of $G_\alpha$ subunits stimulates phospholipase C which produces $IP_3$. $IP_3$ diffuses to and opens calcium channels at the ER which then liberate calcium from the ER. In addition, activated $G_\alpha$ subunits open calcium channels at the plasma membrane and $IP_3$ activates the influx from outside as well. Calcium is pumped out of the intracellular space by calcium pumps at the plasma membrane and at the ER. Furthermore, regulation is supposed to be achieved via the inactivation of the $G_\alpha$ subunits which is stimulated by active PLC and calcium and via the activation of the calcium channels by calcium.

vasopressin and complex irregular oscillations (bursting) with ATP.

We recently developed a new qualitative model for calcium oscillations in hepatocytes. This model is able to explain the different oscillatory patterns which are observed in these cells [5]. Here we discuss in detail the robustness of bursting in this system and its implications for the reliability of information transfer via calcium ions.

## Methods

Simulations were performed using the Rosenbrock, LSODE and Adams-routines for the numerical solution of stiff differential equations. The software used was MADONNA (University of Berkeley, Berkeley, Ca, USA) and DYNAMICAL SOFT-

WARE (Dynamical Software Inc., Tuscon, Az, USA).

## Results and discussion

Our model for calcium oscillations in hepatocytes is based on the scheme shown in Fig. 26.1 and consists of the following equations:

$$\frac{da}{dt} = k_1 + k_2 * a - k_3 * a * b/(a + K_4) - k_5 * a * c/(a + K_6) \quad (26.1)$$

$$\frac{db}{dt} = k_7 * a - k_8 * b/(b + K_9) \quad (26.2)$$

$$\frac{dc}{dt} = k_{10} * c * b * d/(d + K_{11}) + k_{12} * b + k_{13} * a - k_{14} * c/(c + K_{15}) - k_{16} * c/(c + K_{17}) \quad (26.3)$$

$$\frac{dd}{dt} = -k_{10} * c * b * d/(d + K_{11}) + k_{16} * c/(c + K_{17}) \quad (26.4)$$

where $a$ represents the concentration of the active $G_\alpha$ subunits of the initial receptor complex, $b$ the concentration of active PLC, $c$ the concentration of cytoplasmic calcium ions and $d$ the concentration of calcium ions in the intracellular stores. $IP_3$ is supposed to be in a quasi-stationary state and follows the concentration of active PLC and therefore is not an independent variable of this model system. We have chosen to present variables and parameters as dimensionless quantities. Since only few of the parameters are known we have estimated the magnitude of all of them.

With this model, it is possible to observe simple oscillations (spiking) as well as complex oscillations (bursting) (Fig. 26.2) in response to different agonists [5]. The crucial terms for the complex oscillations to occur are the autocatalytic term in eq. 1 as well as the feedbacks of calcium and PLC on the inactivation of active $G_\alpha$ subunits in eq. 1. These terms solely depend on the properties of the receptor.

Here we study the robustness of this mechanism by introducing different terms, e.g., for the kinetics of the $IP_3$ receptor at the membrane of the internal stores which has the form $k_{10} * c * b * d/(d + K_{11})$ in our basic model. This is important, because it is known that different types of $IP_3$ receptors exist [6] and if reliability of calcium signal transduction is to be achieved, it should function with different amounts of the receptor (robustness towards parameter variation $k_{10}$) and different types of the receptor (robustness towards different terms).

We substituted $k_{10} * c * b * d/(d + K_{11})$ by $k_{10} * c^4/(c^4 + K_{18}) * b * d^2/(d^2 + K_{11})$ which corresponds to cooperative binding of calcium to the $IP_3$ receptor which was shown to occur for some types of $IP_3$ receptors [2]. We then varied $k_{10}$ between 0.1 and 1000 (leaving all the other parameters as in Fig. 26.2) and observed bursting

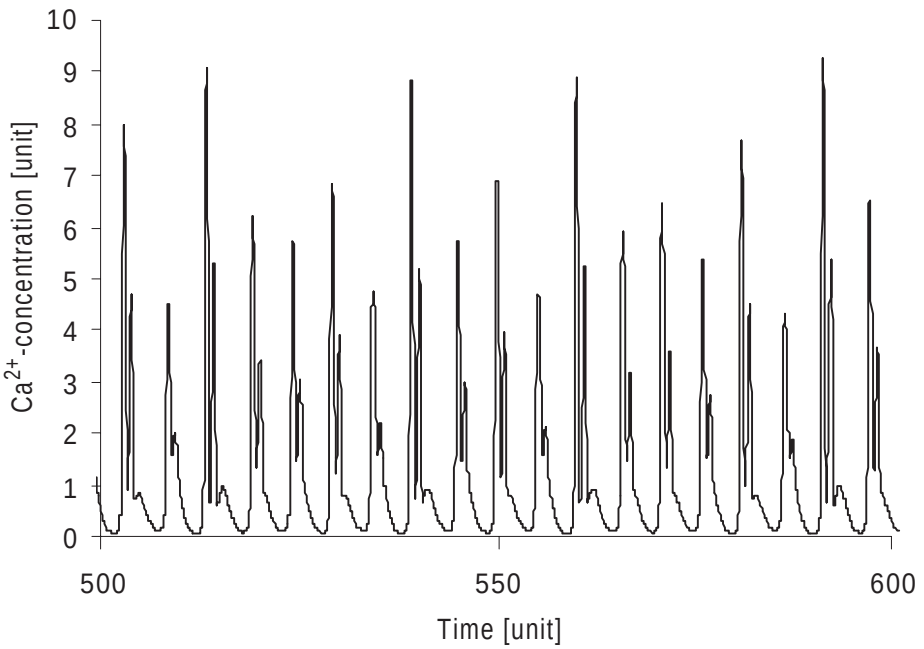


**Fig. 26.2** Bursting of the concentration of free calcium in response to agonist stimulation in eqns. 1-4. Dimensionless parameters: $k_1$=17.85, $k_2$=2.6, $k_3$=2.73, $K_4$=0.21, $k_5$=4.36, $K_6$=3.94, $k_7$=1.24, $k_8$=32.24, $K_9$=29.09, $k_{10}$=2.0, $K_{11}$=2.36, $k_{12}$=0.05, $k_{13}$=13.58, $k_{14}$=153, $K_{15}$=0.16, $k_{16}$=4.85, $K_{17}$=0.05. Initial conditions: a=0.01, b=0.01, c=0.01, d=20.

for the complete parameter range. It was also possible to vary $K_{18}$ and $K_{11}$ between 0.1 and 100 without abolishing the bursting behaviour. Similar robustness was observed for $k_{10} * c^4/(c^4 + K_{18}) * b^4/(b^4 + K_{19}) * d^2/(d^2 + K_{11})$ which corresponds to an additional cooperative binding of $IP_3$ (here represented by active PLC as described above) which was also suggested for certain types of receptors [2].

Apart from the characteristics of the $IP_3$ receptor we also changed the characteristics of the calcium influx term $k_{12} * b$. It has been shown experimentally that calcium influx is influenced in different ways in one cell [7] and the contribution of each way will vary due to different concentrations. $IP_3$ seems to enhance calcium influx [7,8] which was modelled in our basic model as $k_{12} * b$. This term is not crucial for the complex oscillations to occur and it can be substituted by $k_{12}/d$ which models the so-called capacitative influx of calcium. This means that the emptying of the intracellular stores leads itself to an increased influx of calcium from the outside [9]. Therefore the influx term increases with decreasing calcium concentration in the intracellular stores. Varying $k_{12}$ between 0.001 and 0.7 has little effect on the bursting behaviour.

Apart from being reliably transmitted in the presence of different concentrations and kinds of, e.g., $IP_3$ receptors, the transfer of information should also be robust to the presence of noisy conditions in a living cell. In order to examine this latter type of robustness, we studied the effect of noisy perturbations added

to all four variables and saw little sensitivity to perturbations of small amplitude. Enhanced sensitivity was observed in the neighbourhood of bifurcation points. In particular, when the system's unperturbed behaviour was complex periodic, added noise tended to increase the complexity of the solution. Thus, noise added to a period two cycle induced a noisy period four cycle. Due to a similar effect the parameter range within which chaotic behaviour occurs is increased and the observation of complex periodic behaviour appears less likely under noisy experimental conditions. However, even with comparatively high amplitudes of the applied perturbations the qualitative characteristics of the bursting were preserved.

Together these results suggest that bursting in our model provides a mechanism for which reliability in a signal transduction pathway can be achieved. The behaviour is sensitive towards variation of the concentration of agonist and receptor complex, i.e., towards the primary signal. Reliability is then achieved by robustness towards most of the varying conditions within the cell.

## Acknowledgments

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