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On the encoding and decoding of calcium signals in hepatocytes

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Abstract

Many different agonists use calcium as a second messenger. Despite intensive research in intracellular calcium signalling it is an unsolved riddle how the different types of information represented by the different agonists, is encoded using the universal carrier calcium. It is also still not clear how the information encoded is decoded again into the intracellular specific information at the site of enzymes and genes. After the discovery of calcium oscillations, one likely mechanism is that information is encoded in the frequency, amplitude and waveform of the oscillations. This hypothesis has received some experimental support. However, the mechanism of decoding of oscillatory signals is still not known. Here, we study a mechanistic model of calcium oscillations, which is able to reproduce both spiking and bursting calcium oscillations. We use the model to study the decoding of calcium signals on the basis of co-operativity of calcium binding to various proteins. We show that this co-operativity offers a simple way to decode different calcium dynamics into different enzyme activities.

Keywords: Calcium; Decoding; Hepatocyte; Model; Oscillation; Signalling

1. Introduction

Calcium signalling occurs in many different cell types and carries information from agonists bound at the extracellular side of the cell membrane to targets in the interior of the cell. The information carried serves many different purposes from triggering the developmental program of fertilized mammalian eggs to mobilizing antigenic response of immune cells or mediating cell death [1]. This is why the role of calcium ions as second messengers continues to draw much attention in the field of biochemistry. Calcium signalling in hepatocytes occurs when agonists such as the hormones vasopressin and angiotensin II or nucleotides bind to membrane-spanning receptor proteins on the surface of the cell. In many cases, the calcium concentration in the cytosol displays oscillatory behaviour upon agonist binding. It is the interplay between calcium release and uptake between different compartments that is responsible for this oscillatory dynamics. The oscillations show differ-

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ent frequencies, amplitudes and waveforms. It is generally believed that the information carried by a specific agonist is stored in these patterns [2]. Thus, in order to understand cell signalling mediated by calcium ions, we need to understand how different oscillatory patterns can affect various regulatory proteins in the cell.

Up until now there has been much focus on the encoding of oscillatory calcium signalling, and almost all the attention has been on the mechanisms modulating the signals. Studies of decoding of calcium oscillations have exclusively focussed on how the cell decodes simple frequencies of spiking oscillations, despite the fact that the cell can display signals vastly more complex than periodic spiking [3–12]. The shortage of experimental data is mainly a result of the difficulties in measuring changes in activity of calcium regulated target proteins, both in vitro and in vivo. An example of decoding data obtained only recently is that of calcium oscillation frequency decoding by CaM kinase II [13]. CaM kinase II, which is activated by calmodulin, plays a central role in many cellular processes. CaM kinase II has been the target of many investigations, and discrete activity measurements in vitro have contributed to the understanding of decoding. However, only recently it has been possible to conduct continuous experiments in vivo [4]. Similar to the experimental studies, the theoretical exploration of calcium signal decoding has also been limited to frequency encoded information [14–17], and only one case of decoding in hepatocytes has been studied [7,18].

In the present paper, we present modelling results which may shed some light on how oscillation frequencies, waveforms and the complexity of the signal can affect the activities of regulatory proteins. This subject has received very little attention but some of the ideas presented so far have been reviewed by Schuster et al. 2002 [19] and will be briefly commented later in this paper. In our study, we employ a modification of a model originally proposed by Kummer et al. 2000. This model is a receptor-operated model as opposed to most other calcium models [20–26], which are store operated. This receptor oriented approach was chosen based on experiments showing complex kinetics of the receptor-G-protein, which has been further supported by other experimental results [27,28]. The importance of the receptor specificity both in model and in experiments will be explained in later sections.

It is important to stress that the model used here is qualitative. It deals with levels of calcium in the different compartments, not concentrations. Therefore, we do not take into account the sizes of the different compartments. Although this could easily be done by multiplying the equations of the model with constant volume ratios [29], it does not change the qualitative behaviour, and does not add to the information the model can provide. Therefore, we chose to present our variables without units and only concern ourselves with the qualitative behaviour of the model. The qualitative approach is also used in reducing the number of terms included in the model in order to keep it physiologically plausible, but still simple and comprehensible. Previous work has shown that not all terms (i.e. reactions) involved in calcium signalling have the same impact on the dynamics of the system. If all mechanisms involved in calcium signalling in the cell—that is all transporters, channels, pumps, buffer proteins, membrane potentials, pH changes, up and down regulation of gene expression etc.—were to be included in the model, it would only hamper the understanding of the system. The dynamics of most systems resides in a few core mechanisms, which provide the mathematical backbone of the model. For a review of minimal models, see Schuster et al. 2002 [19]. For the study of the en- and decoding of calcium oscillations we need to create a model, which faithfully reproduces the qualitative dynamics which is experimentally observed, but does not need to respond to every possible physiological event in detail.

We model co-operative calcium binding to proteins and study the different activities of these proteins in response to different types of calcium oscillations. Most proteins, which are influenced by calcium, have several calcium binding sites and bind calcium in a co-operative manner [30]. We show that different waveforms and frequencies can be transformed into very different activation patterns. We also show that coupling the calcium oscillator with a single frequency to a second

calcium-dependent oscillating process can result in oscillations with a broad spectrum of frequencies as well as quasiperiodicity and chaos in the second oscillator. Our results offer the first explanation of how the cell differentiates between bursting and spiking calcium signals, and how the oscillating calcium signal can hold several levels of information in one pattern.

2. Presentation of the model

The model we use for this study is a modification of a recent receptor-operated model [31]. The changes compared to the original model are the inclusion of mitochondria [Eqs. (4) and (5)] and an improvement in modelling the IP_3-receptor of the endoplasmic reticulum (ER) [Eqs. (3) and (5)]. The modifications, which will be discussed in the following sections are introduced in order to increase the model's correspondence with experimental data and thereby rendering it more suited for generating signals for decoding. The model is represented by the following equations:

$$\frac{dG_\alpha}{dt} = k_1 + k_2 \times G_\alpha - \frac{k_3 \times G_\alpha \times PLC}{G_\alpha + K_4} - \frac{k_5 \times Ca_{cyt} \times G_\alpha}{G_\alpha + K_6} \quad (1)$$

$$\frac{dPLC}{dt} = k_7 \times G_\alpha - \frac{k_8 \times PLC}{PLC + K_9} \quad (2)$$

$$\frac{dCa_{ER}}{dt} = -(Ca_{ER} - Ca_{cyt}) \times \frac{k_{10} \times Ca_{cyt} \times PLC^4}{PLC^4 + K_{11}^4} + \frac{k_{16} \times Ca_{cyt}}{Ca_{cyt} + K_{17}} \quad (3)$$

$$\frac{dCa_{mit}}{dt} = \frac{k_{18} \times Ca_{cyt}^8}{K_{19}^8 + Ca_{cyt}^8} - (Ca_{mit} - Ca_{cyt}) \times \frac{k_{20} \times Ca_{cyt}}{Ca_{cyt} + K_{21}} \quad (4)$$

$$\frac{dCa_{cyt}}{dt} = (Ca_{ER} - Ca_{cyt}) \times \frac{k_{10} \times Ca_{cyt} \times PLC^4}{PLC^4 + K_{11}^4} + k_{12} \times PLC + k_{13} \times G_\alpha - \frac{k_{14} \times Ca_{cyt}}{Ca_{cyt} + K_{15}} - \frac{k_{16} \times Ca_{cyt}}{Ca_{cyt} + K_{17}} - \frac{k_{18} \times Ca_{cyt}^8}{K_{19}^8 + Ca_{cyt}^8} + (Ca_{mit} - Ca_{cyt}) \times \frac{k_{20} \times Ca_{cyt}}{Ca_{cyt} + K_{21}} \quad (5)$$

The variables of the model are activated G_α subunit of the receptor complex (G_α), active phospholipase C (PLC), cytosolic calcium concentration (Ca_{cyt}), concentration of free calcium in the ER (Ca_{ER}) and calcium concentration in the mitochondria (Ca_{mit}).

2.1. Active G_α subunit (G_α)

The first variable is the concentration of active G_α subunit of the G protein simulated by Eq. (1). The G-protein is coupled to an agonist specific receptor protein on the cytosolic side of the cell membrane, and exchanges GDP for GTP upon agonist binding. This exchange releases the G_α subunit, which is now active. In the model, it is proposed that depending on the receptor type, the G_α subunit autocatalytically activates other G_α subunits and is able to show a small degree of spontaneous activation. These propositions come from the fact that activation of G_α can show very complicated kinetics, with an acceleration of the GTP/GDP exchange, which could be explained by autocatalysis [27]. The two mechanisms are modelled with the receptor specific terms: (k_1) spontaneous/agonist activation, and ($k_2 \times G_\alpha$) agonist specific autocatalytic activation. The specificity of the receptor terms will be discussed in the end of this section. The inactivation of G_α depends on the active PLC, because the active PLC is a GTPase activating protein, which stimulates hydrolysis of GTP bound to G_α [27]. The inactivation is modelled by: $(k_3 \times PLC \times G_\alpha)/(G_\alpha + K_4)$. This is a Michaelis–Menten term because the inactivation is limited by the amount and turnover number of the enzyme PLC. Another source of inactivation is negative feedback of calcium

dependent kinase (CaDK) on the active receptor complex. This term is modelled by $(k_5 \times \mathrm{Ca_{cyt}} \times G_\alpha)/(G_\alpha + K_6)$.

2.2. Phospholipase C activity (*PLC*)

The second variable PLC is activated by G_α, this is modelled by the linear term $(k_7 \times G_\alpha)$. One could argue that this should be a Michaelis–Menten term because there could be saturation of G_α with respect to PLC. However, if it is assumed that the K_M of the MM-term is very small then $(k_7 \times G_\alpha \times \mathrm{PLC})/(K_M + \mathrm{PLC})$ will be essentially equal to $(k_7 \times G_\alpha)$. PLC is enzymatically inactivated, this provides the term $(k_8 \times \mathrm{PLC})/(K_9 + \mathrm{PLC})$. Together, these terms form Eq. (2). Because of the very short half-life of IP_3, its concentration is assumed to be in quasi-steady with PLC. This allows the use of PLC as parameter, where IP_3 should actually have been used and in the following equations whenever IP_3 occurs, it will be replaced by PLC in the model.

2.3. Calcium level of the endoplasmic reticulum (Ca_{ER})

$\mathrm{Ca_{ER}}$, which is the third variable in the model is first released into the cytosol through the IP_3 receptor channels (IP_3R). The term modelling the IP_3R was changed in the new model because like many other models [21,24–26], the original model [31] showed very low levels of calcium in the ER between spikes and bursts. The near depletion of calcium in the ER has been observed in several different cell types, which utilize calcium as second messenger. However, the calcium depletion of the ER is mainly observed in connection with apoptosis or other long-term fundamental cellular processes in these cells [32–35]. In rat hepatocytes, it has been shown that oscillating signals induced by agonist binding to receptor protein does not cause depletion of calcium in the ER [36] This observation renders it important to maintain a certain level of calcium in the ER, not only because experiments support this, but also, because the recurring depletion of $\mathrm{Ca_{ER}}$ in calcium models raises the question, whether the spikes of $\mathrm{Ca_{cyt}}$ are terminated simply because there is hardly any calcium left in the ER to flow into the cytosol. This would imply that a wrong mechanism controls the spike termination.

In order to remedy this deficiency, we improved the description of the inositol 1,4,5-trisphosphate-receptor channel. This receptor is a tetrameric transmembrane protein, which forms gated channels in the endoplasmic reticulum. The opening of the channel is regulated by IP_3, ATP, pH and cytosolic calcium. The increase in IP_3 level upon binding of agonist causes IP_3 to bind to the IP_3R. The binding of IP_3 opens the channel and allows out flux of calcium from the ER, resulting in a rise in $\mathrm{Ca_{cyt}}$. This rise induces further release from the ER trough the same channel, because calcium binds to the cytosolic side of the IP_3R and stabilizes the opening of IP_3R, an effect termed calcium induced calcium release (CICR). The binding of IP_3 is co-operative, and it requires the binding of at least three molecules of IP_3 to open the channel. The IP_3R type dominant in hepatocytes is IP_3R-2, which displays Hill-like saturation kinetics with respect to IP_3 [37–40]. In most models, the Hill coefficient of the IP_3R term is chosen to be 4. Thus, we model the IP_3R with the term:

$$(\mathrm{Ca_{ER}} - \mathrm{Ca_{cyt}}) \times \frac{k_{10} \times \mathrm{Ca_{cyt}} \times \mathrm{PLC}^4}{\mathrm{PLC}^4 + K_{11}^4}$$

This term takes into account that $\mathrm{Ca_{cyt}}$ activates IP_3R and IP_3, which is assumed in quasistationary state with PLC, binds co-operatively to the IP_3R. The Hill coefficient is chosen to be four, but can be varied without losing the capability for showing spiking, bursting and chaotic oscillations. The above term also takes into account that the IP_3R is a channel and hence mediates diffusion in both directions. Calcium is pumped back into the endoplasmic reticulum by an ATP dependent pump modelled by: $(k_{14} \times \mathrm{Ca_{cyt}})/(K_{15} + \mathrm{Ca_{cyt}})$. Together, the two terms form Eq. (3).

2.4. Calcium level of the mitochondria (Ca_{mit})

Addition of a mitochondrial term in the Kummer et al. 2000 model was first presented in Grubelnik et al. 2001 [41]. It was shown that including the mitochondria as a plug-in element in several dif-

ferent models, resulted in amplitude of calcium oscillations in the cytosol, which does not change significantly as the level of agonist is raised. This is a feature, which is seen in virtually all experiments, where a raise in agonist concentration results in increased frequency of the calcium signal. However, this improvement was obtained at the expense of some of the other features of the Kummer et al. 2000 model. In order to make the model and the resulting calcium signals as realistic as possible, we have further modified the equation modelling calcium in the mitochondria (Eq. (4)).

The Rapid Mode of uptake mechanism (RaM) was chosen for modelling the uptake of calcium into the mitochondria [41]. The RaM is a mechanism, which sequesters a significant amount of calcium in the beginning of each cytosolic calcium pulse. This mechanism is two orders of magnitude faster than the uptake mediated by the uniporter.

The driving force of the RaM is most likely the mitochondrial membrane potential. This is experimentally verified by showing that uncouplers, which dissipate the membrane potential inhibits the mechanisms [42]. These experiments provide also evidence that a rapid calcium uptake can be achieved without local calcium puffs and synaptic calcium transmission [43], which is another recently proposed mechanism for calcium uptake [44]. Due to the switch-like uptake conducted by the RaM, its calcium concentration dependence is modelled by a Hill term with a Hill coefficient of eight:

$$\frac{k_{18}\times \mathrm{Ca}_{\mathrm{cyt}}^{8}}{K_{19}^{8}+\mathrm{Ca}_{\mathrm{cyt}}^{8}}$$

Note that the above term does not include any specific contribution from the mitochondrial membrane potential $\Delta\psi_{\mathrm{mit}}$, although this is proposed to drive the uptake of calcium. Sustained calcium oscillations have been shown not to cause significant changes in $\Delta\psi_{\mathrm{mit}}$ [45] in hepatocytes. We, therefore, include the contribution implicitly in k_{18}, assuming that $\Delta\psi_{\mathrm{mit}}$ remains constant [26].

Calcium release from the mitochondria is mediated by the permeability transition pore (PTP), also termed mitochondrial transition pore (MTP) [29]. The term of the PTP mechanism is strongly simplified; the mCICR caused by the PTP is a rather complex process that involves both the matrix pH and the transmembrane potential ($\Delta\psi_{\mathrm{mit}}$) in addition to the increase of the mitochondrial calcium concentration.

During the normal life of the cell, the PTP exists in a closed state and a low conduction state that allows limited diffusion of small ions like Ca^{2+}. It is proposed that the low conductance state has minimal consequence for changing the membrane potential of the mitochondria [26]. During both induced and programmed cell death the PTP irreversibly switches to a stable high conductance state that allows an unselective diffusion of large molecules, which causes large drop in $\Delta\psi_{\mathrm{mit}}$, swelling and release of proapoptotic factors [46–48]. In the model we will only consider the low conductance state, since the high conductance state takes no part in the normal life of the cell. The PTP is modelled by a simple term, which only takes into account that there is an overall mitochondrial calcium-induced-calcium-release effect (mCICR) [26,49] and, when open, the pore allows diffusion of calcium in both directions. Again membrane potential is assumed to be constant and is implicitly included in k_{20}, and k_{20} is held very small compared to k_{18} of the RaM (0.81 and 79, respectively). The term used is:

$$(\mathrm{Ca}_{\mathrm{mit}}-\mathrm{Ca}_{\mathrm{cyt}})\times\frac{k_{20}\times \mathrm{Ca}_{\mathrm{cyt}}}{\mathrm{Ca}_{\mathrm{cyt}}+K_{21}}$$

The physiological validity of implicit inclusion of $\Delta\psi_{\mathrm{mit}}$ can be discussed, and possibly the model would be quantitatively more correct if we included $\Delta\psi_{\mathrm{mit}}$ and pH changes over the different membranes as variables. However, the terms we use to model the mechanisms in the mitochondrial membrane mimic the behaviour observed in experiments, and in order to retain simplicity of the model, we leave these possible variables out.

2.5. Cytosolic calcium level (Ca_{cyt})

Eq. (5), which models the change of $\mathrm{Ca}_{\mathrm{cyt}}$ consists of the terms from Eqs. (3) and (4) but with opposite signs. In addition to the contribution from the ER and the mitochondria calcium flow

is also found between the cytosol and the extracellular space. It has been reported that IP_3 stimulates influx directly from the extracellular space [50], this provides the term $(k_{12} \times \mathrm{PLC})$. Also, a receptor-stimulated influx from the extracellular space has been shown. This influx is due to the fact that some agonist receptors also function as cation channels [51]. The receptor specific influx is modelled by $(k_{13} \times G_\alpha)$. The two influx terms model diffusions and, therefore, should have been represented by a term containing the difference $(\mathrm{Ca_{ext}} - \mathrm{Ca_{cyt}})$. However, the fact that $\mathrm{Ca_{ext}} \gg \mathrm{Ca_{cyt}}$ and, therefore, can be assumed constant and integrated in k_{12} and k_{13}, allows the simplification of both terms. An ATP dependent pump, which is modelled by $(k_{16} \times \mathrm{Ca_{cyt}})/(K_{17} + \mathrm{Ca_{cyt}})$, mediates the pumping of calcium back into the extracellular space.

2.6. The agonist receptor terms

In experiments, different agonist receptors have been shown to employ different dynamics for calcium signal transduction [2,11,31,52,53]. Our modelling results suggest that minor changes in receptor terms have major impact on signal dynamics and as mentioned before the above-presented model is a receptor-operated model. This renders the G_α related terms of the model very important and also allows us to use receptor terms specific to the agonist, we wish to model. We chose to model two receptor types; the nucleotide receptor and the Vasopressin receptor. Therefore, we use receptor-specific parameters for G_α activation and receptor properties. These receptor-specific terms are receptor mediated calcium influx $(k_{13} \times G_\alpha)$ and a receptor-specific autocatalytic G_α activation term $k_2 \times G_\alpha$. Both play a crucial role in determining the possible dynamic states of the model. If the terms are included $(k_2 \neq 0,\ k_{13} \neq 0)$, the model is capable of showing a large variety of behaviours including understimulation (resulting in a low-concentration calcium steady state), simple periodic spiking, periodic bursting, chaotic bursting and overstimulation (resulting in a high-concentration steady state [54]) upon increase in agonist influence modelled by k_2. This corresponds to receptors, which bind, e.g. ATP or UTP. Omitting the two terms $(k_2 = 0,\ k_{13} = 0)$ results in a model, which is able to show understimulation, periodic, spiking with increasing frequency and over stimulation, but no bursting behaviour when varying k_1. This corresponds to the behaviour of receptors binding, e.g. vasopressin [31]. The determination of the specific mathematical receptor and agonist influence terms are based mainly on the calcium response they elicit in the model since experimental data on receptor dynamics is still extremely scarce.

One could argue that that the change in k_1 alone should model the agonist influence on the system, and it is also possible to model most of the described dynamics by varying k_1 instead of k_2, as long as $k_2 \neq 0$ (For example $k_2 = 2.066$), but by doing this the bursting model loses some of its complex behaviour and its ability to show understimulation. Therefore, until more information about the receptor mechanisms is presented, we will model the receptor terms as described above. Therefore, in the following, whenever we discuss the decoding of bursting, we study the model including $k_{13} \times G_\alpha$ and $k_2 \times G_\alpha$ $(k_{13} \neq 0,\ k_2 \neq 0)$ with k_2 modelling agonist influence and whenever discussing decoding of spiking, we refer to the model omitting the terms $k_{13} \times G_\alpha$ and $k_2 \times G_\alpha$ (setting $k_{13} = 0$ and $k_2 = 0$) with k_1 modelling the agonist influence.

The equations were numerically integrated on a computer using either the LSODE integrator or the Rosenbrock method in Berkeley–Madonna (Berkeley–Madonna, USA).

3. Results and discussion

3.1. Dynamic behaviour of the model

Inclusion of mitochondria (Eq. (4)) in the model resulted in a stabilization of the amplitude similar to what was shown previously [29,41]. Fig. 1 shows the changes in the amplitude of calcium oscillations with increasing agonist concentration as simulated by the Kummer et al. 2000 model (Fig. 1a) and by the new bursting model (Fig. 1b). These changes are visualized by bifurcation diagrams in which the amplitudes of the oscillations (both primary and secondary oscillations)

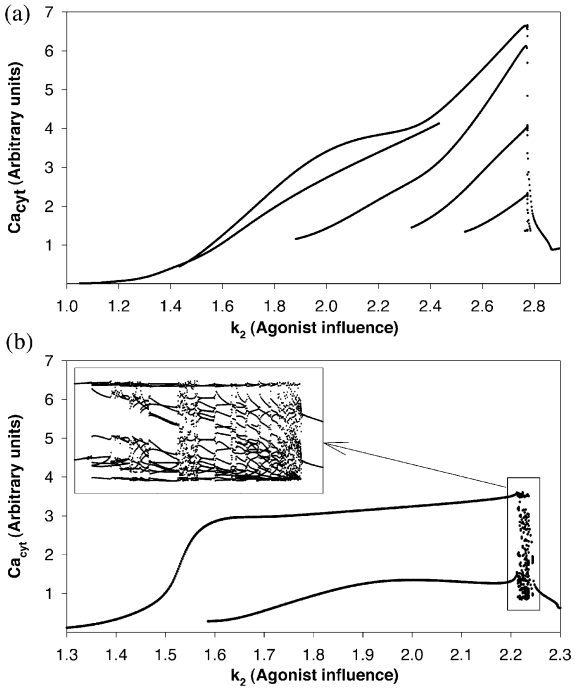

Fig. 1. (a) Bifurcation diagram of the original model, with the equations and parameters from Fig. 2 of Kummer et al. 2000. k_2 runs from 1–3. The amplitude of the calcium oscillations (Both the primary spikes and the subspikes of the bursting pattern) rises throughout the rise in agonist level, which in the model is represented by k_2. (b) New model with inclusion of the mitochondria [Eqs. (1)–(5)]. The initial conditions are: $G_\alpha = 0.01$, PLC = 0.01, $Ca_{cyt} = 0.01$, $Ca_{ER} = 10$ and $Ca_{mit} = 0.001$. These remain the same in all further calculation. The constants of the equations are: $k_1 = 0.01$, $k_3 = 0.64$, $K_4 = 0.09$, $k_5 = 4.88$, $K_6 = 1.18$, $k_7 = 2.08$, $k_8 = 32.24$, $K_9 = 29.09$, $k_{10} = 0.7$, $K_{11} = 3$, $k_{12} = 2.8$, $k_{13} = 13.4$, $k_{14} = 153$, $K_{15} = 0.16$, $k_{16} = 7$, $K_{17} = 0.05$, $k_{18} = 79$, $K_{19} = 3.5$, $k_{20} = 0.81$ and $K_{21} = 4.5$. k_2 runs from 1.2–2.3. In this model the amplitude of the main calcium spikes reaches a plateau, and stays there through the rise in agonist influence. Only the shape and frequency of the signal changes.

are plotted against the level of agonist stimulation, simulated by k_2. In Fig. 1a the amplitude increases with increasing agonist stimulation over almost the complete range of k_2 values (from 1.0 to 2.75). Secondary oscillations emerge as additional lines in the plot at $k_2 = 1.42$.

On the contrary, Fig. 1b shows an amplitude of calcium oscillations, which rises quickly from zero to a plateau at $Ca_{cyt} \approx 3.2$ while the shape of the signal changes from steady state/understimulation ($k_2 < 1.1$) through spiking ($1.1 < k_2 < 1.6$), bursting ($1.6 < k_2 < 2.23$), chaotic bursting ($2.23 < k_2 < 2.24$) and ends with the steady state of overstimulation.

The amplitude stability is due to the fact that the very steep step-like kinetics of the mitochondrial calcium uptake ensures that calcium is very effectively taken up into the mitochondria when the cytoplasmic calcium concentration reaches a certain level [41]. The importance of the step-like kinetics is underlined by the observation that the Hill coefficient of the RaM term has to be larger than 6 to maintain the behaviour of the model.

In the new model, the mean concentration of free calcium remains at least an order of magnitude higher in the endoplasmic reticulum compared to the cytosol. This ratio is still far from the physiological ratio of at least three orders of magnitude (Ratios of up to 1:10 000 have been reported) [36,55]. Nevertheless, it is a considerable improvement of the near depletion of Ca^{2+}_{ER} in most other models. The new model makes it clear that it is not depletion of calcium in the ER, which terminates spikes. For reasons of simplicity we did not include a volume correction factor, which would increase this ratio several orders of magnitude without changing the models dynamic features. Experiments have shown that shortly after calcium ions are released from the ER due to IP_3 binding, mitochondria undergo a transient decrease in $[Ca^{2+}]_{mit}$. This transitory drop in concentration, which is caused by the mCICR effect of the PTP [47], is also seen in the model (data not shown).

The frequency encoding observed in hepatocytes covers a range of periods from 0.3 to 4 min [14]. The frequency encoding abilities of our new model for spiking calcium oscillations (without the terms $k_2 \times G_\alpha$ and $k_{13} \times G_\alpha$) are adequate for our purpose. An increase in agonist stimulation by a factor of 1.75 can result in an increase of the frequency by a factor of 1.7 (Fig. 2). This is still far from the physiological range, but an improvement from the original model.

3.2. Signal decoding

By using the improved model, we investigate how the cell can decode different oscillations. For

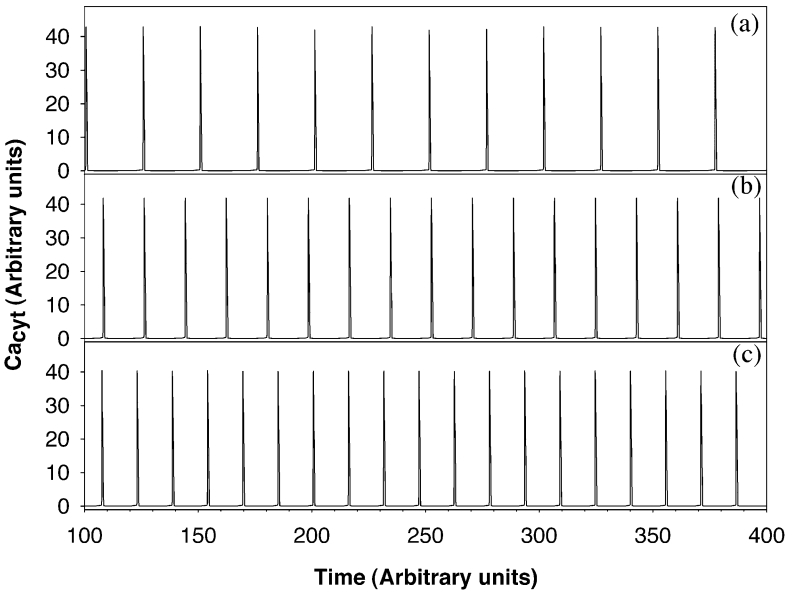

Fig. 2. Frequency encoding by the modified new model [Eqs. (1)–(5)]. The new parameters are; $k_2=0$, $k_3=0.0001$, $K_4=0.783$, $k_5=1.24$, $K_6=0.7$, $k_7=5.82$, $k_8=32.24$, $K_9=29.09$, $k_{10}=0.93$, $K_{11}=2.667$, $k_{12}=0.76$, $k_{13}=0$, $k_{14}=149$, $K_{15}=0.16$, $k_{16}=20.9$, $K_{17}=0.05$, $k_{18}=79$, $K_{19}=2$, $k_{20}=1.5$ and $K_{21}=1.5$. k_1, which now models the level of agonist binding is in (a) $k_1=0.35$, (b) $k_1=0.45$ and (c) $k_1=0.55$.

this purpose, we couple a very simple equation [Eq. (6)], modelling the activity of a hypothetical calcium activated enzyme to the above-described system:

$$\frac{\mathrm{d}Enz}{\mathrm{d}t}=\frac{k_{\mathrm{act}}\times \mathrm{Ca}_{\mathrm{cyt}}^{p}}{K_M^P+\mathrm{Ca}_{\mathrm{cyt}}^{p}}-k_{\mathrm{inact}}\times Enz \qquad (6)$$

We assume that the enzyme has multiple calcium activation sites with co-operative binding. Such enzymes are found in many forms in the cytosol [30]. Calcium activation of the enzyme is modelled by a Hill term with a Hill coefficient p of varying magnitude. The inactivation of the enzyme is modelled as a first order process, the rate of which depends only on the concentration of activated enzyme. A more realistic approach would have been to impose an upper boundary on the value that the variable *Enz* can take. Nevertheless, we found that the results shown in the following section do not change if we introduce an upper value Enz_{tot} for the enzyme activity.

3.2.1. Decoding the frequency of spiking

Frequency decoding has been studied before, and we will, therefore, restrict ourselves to some crucial points. which are closely related to experimental examples. One such example is the control of the Ca^{2+} sensitive intramitochondrial dehydrogenases (Pyruvate dehydrogenase (PDH), 2-oxoglutarate dehydrogenase and NAD^+-isocitrate dehydrogenase) [7]. Upon rise in cytosolic calcium concentration, elevation of the mitochondrial calcium concentration stimulates the dehydrogenases, resulting in an elevation of NAD(P)H concentration in the mitochondria. Experiments have shown that when the frequency of the cytosolic calcium signal rises, so does the frequency of the mitochondrial calcium oscillations. At low calcium oscillatory frequencies, the level of NAD(P)H is also oscillating in the mitochondria. However, if the frequency of calcium oscillations is increased, the NAD(P)H fluctuation decreases, resulting in a constantly elevated NAD(P)H concentration [7,18,56].

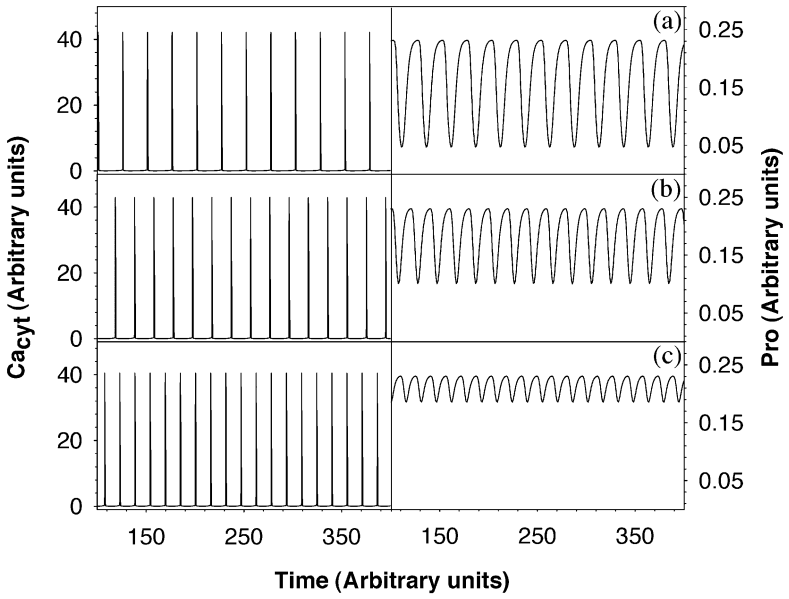

Fig. 3. Three different levels of agonist concentration, mediating three different calcium oscillation frequencies (Ca_{cyt}). These three frequencies are decoded as an increasing mean level of product (Pro). The maximum level of product during oscillation reaching approximately Pro = 0.225 in each time series regardless of the agonist influence. The parameters of Eqs. (6) and (7) are: $k_{act} = 0.1$, $K_M = 0.02$, $k_{inact} = 0.043$, $p = 4$, $k_{enz} = 3$ and $k_{rem} = 3$. The initial conditions are Enz = 0 and Pro = 0 and the parameters of Eqs. (1)–(5) are as in Fig. 2, except for k_1 which is: (a) $k_1 = 0.3$, (b) $k_1 = 0.4$ and (c) $k_1 = 0.55$.

We studied the general mechanism for this finding by using the terms for the enzyme described in Eq. (6) with $p = 4$ coupled to the model system for spiking calcium oscillations (i.e. without the term $k_2 \times G_\alpha$). The enzyme catalyses a reaction with product (Pro). The change in product concentration is modelled with a linear term for product formation catalysed by *Enz* and another linear term modelling the removal of the product from the system. These terms form the following equation:

$$\frac{\mathrm{dPro}}{\mathrm{d}t} = k_{\mathrm{enz}} \times Enz - k_{\mathrm{rem}} \times \mathrm{Pro} \qquad (7)$$

We assume that the concentration of substrate for the reaction remains constant and also that the change in cytosolic calcium concentration due to binding of calcium to the enzyme, is insignificant compared to the amplitude of calcium oscillation. Previous theoretical studies of the decoding mechanism for frequency encoded information used more elaborate models, e.g. for phosphorylation/dephosphorylation cycles [57], since they were attempting to model the CaM kinase II system mentioned above [13].

Fig. 3 shows calcium spiking with increasing frequency following increases in k_1. The increasing oscillation frequency leads to an increase in mean product concentration (Pro). Thus, simple cooperativity of calcium binding to an enzyme is sufficient to qualitatively reproduce the experimentally observed findings of the intramitochondrial dehydrogenase system.

3.2.2. Decoding periodic bursting

In order to study the influence of bursting calcium oscillation on the enzyme activity we studied the enzyme modelled by Eq. (6) coupled to the model system (with inclusion of the terms $k_{13} \times G_\alpha$ and $k_2 \times G_\alpha$) and varied the level of agonist influence (modelled by k_2). Fig. 4 shows how calcium bursts activate the enzyme.

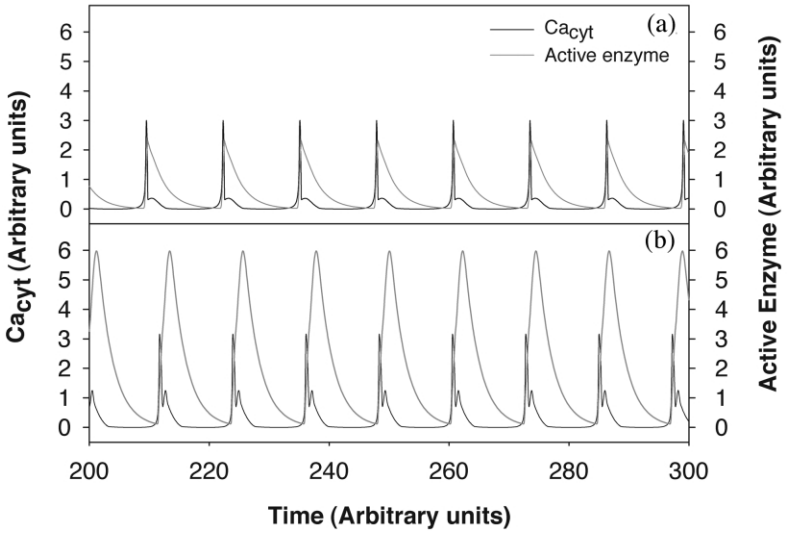

Fig. 4. Two different levels of agonist concentration, resulting in two different but similar levels of bursting, which in turn gives rise to two very different levels of enzyme activity. The parameters of Eqs. (1)–(5) are as in Fig. 1b, except k_2 which is: (a) $k_2 = 1.65$, (b) $k_2 = 1.9$. The parameters of Eq. (6) are $k_{act} = 5$, $K_M = 0.62$, $k_{inact} = 0.4$ and $p = 4$. The initial concentration of activated enzyme is $Enz = 0$.

In Fig. 4a, the bursting is less pronounced compared to Fig. 4b. The frequency and amplitude of the main calcium peaks are almost unaltered; while the levels of active enzyme are increased several fold. In this case, of signal decoding the K_M of the enzyme for Ca_{cyt} is very important. The K_M is 0.62, which is just below the calcium level of the secondary oscillation of the burst in Fig. 4a. This results in low activation of enzyme in Fig. 4a. A rise in agonist influence increases the level of the secondary calcium oscillation without changing the primary peak.

This increases the period of time in which the enzyme is activated during each burst and, therefore, the overall activation of the enzyme is increased. An enzyme with a higher K_M would have been unable to distinguish between the two signals, and its activity would have been unaltered. The point made here is further explored in the end of this section. The above shows that information translatable by enzymes using the very simple kinetics of Eq. (6) can be stored in the shape of the oscillating calcium signal. No other requirement is necessary. This indicates that the cell can vary the activity of calcium-dependent enzymes significantly by varying only the shape of the oscillations, leaving the main amplitude and frequency unchanged. In other words, information is not only encoded in the frequency, but also in the waveform of the oscillations.

Previous model and experimental studies using simple spiking calcium oscillations have shown that an oscillatory calcium signal can decrease the effective calcium threshold for the activation of an enzyme [3,5,14,15]. We verified this also for the bursting calcium oscillations. We calculated the average Ca_{cyt} concentration during periodic bursting, with all parameters as shown in Fig. 4b, to be $Ca_{cyt} = 0.2896$. These oscillations caused an average activity of enzyme of $Enz = 2.0385$ (arbitrary units). By contrast, a steady state level of calcium of $Ca_{cyt} = 0.2896$ only resulted in an average enzyme activity of $Enz = 0.5677$ (arbitrary units). This implies that the oscillatory dynamics of the calcium signal, in this case, increases the resulting enzyme activation more than three times compared to a steady state signal. We conjecture that this is a general property of enzymes that bind calcium co-operatively when decoding an oscillating calcium signal. Although not directly compatible, our

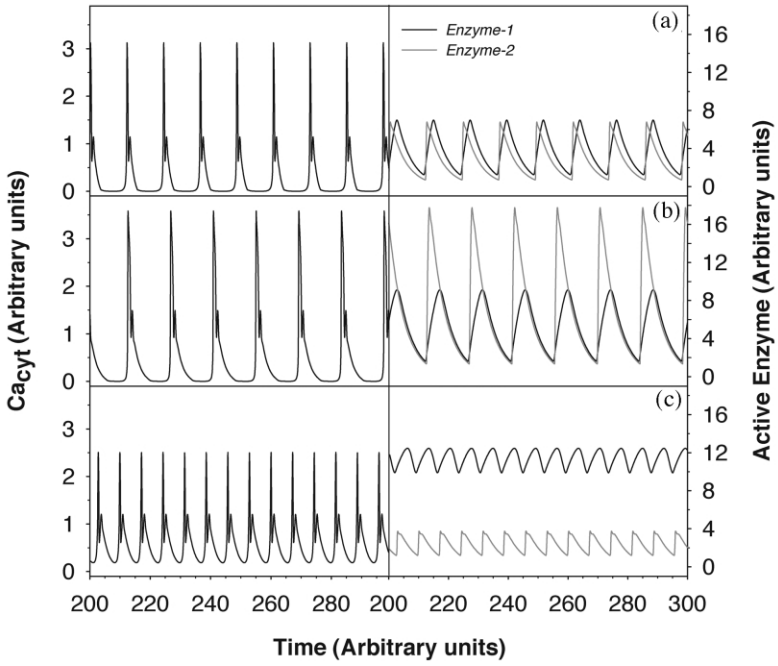

Fig. 5. Decoding by *Enzyme*-1 and *Enzyme*-2 both employing the mechanism of Eq. (6). The enzymes show very different levels of activation upon stimulation by calcium oscillations caused by agonist binding; (b) $k_2=2.21$ and (c) $k_2=2.239$, all though the two enzymes behave similarly at lower agonist concentrations (a) $k_2=1.86$. The enzymes have the following constants; $k_{\text{inact}}=0.2$, $p=4$ and: (*Enzyme*-1) $k_{\text{act}}^{(1)}=3$, $K_M^{(1)}=0.24$, (*Enzyme*-2) $k_{\text{act}}^{(2)}=70$, $K_M^{(2)}=3.7$, the initial concentration of activated enzyme in zero in both cases, and the parameters of Eqs. (1)–(5) are those of Fig. 1.

results obtained with complex periodic oscillations square with previous results obtained with CaM kinase II [13]. Here the activity of CaM kinase II was measured after a series of square pulses of calcium with different frequencies and pulse lengths. Among other things these authors showed that a modest change in pulse length might result in a substantial change in kinase activity.

In order to investigate further the point that a slight change in the shape of the calcium oscillations can result in a dramatic change in coupled enzymatic activity, we employ two different enzymes according to the mechanism of Eq. (6) (*Enzyme*-1 and *Enzyme*-2). They only differ in the K_M and k_{act} of the Hill term. At low values of k_2 (Fig. 5a) the activities of the two enzymes oscillate essentially in parallel. However, at higher values of k_2 the way in which they decode calcium oscillations is very different. We observe that *Enzyme*-1, which has a low k_{act} but also a low K_M, shows high activity compared to *Enzyme*-2 when the periodic bursts show a high amplitude and low frequency (Fig. 5b). *Enzyme*-2, which has a high k_{act} and a high K_M as well, shows a higher activity compared to *Enzyme*-1 when the amplitude of the bursts are low and the frequency high (Fig. 5c). The mean concentrations of calcium in these two time series differ by only 30% (from approx. 0.42 to 0.60) as opposed to the difference in enzyme activation, which is several fold. This is an example of shape modulated signal decoding, where the cell can upregulate the activity of one enzyme and downregulate the activity of another by changing the shape and frequency of the calcium signal slightly.

3.2.3. Decoding complex signals

So far only decoding of simple periodic spiking and bursting has been addressed. However, it was observed experimentally that calcium oscillations

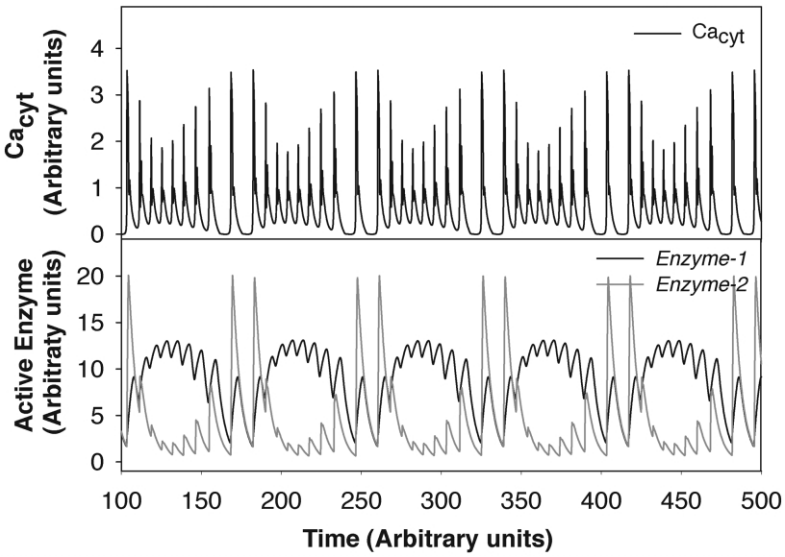

Fig. 6. Decoding of complex calcium oscillations produced by Eqs. (1)–(5) with the constants presented in Fig. 1 and $k_2 = 2.23$. The enzyme activity shows phase locking, and the two enzymes show maximum activity inversely of each other. The enzymes are those presented in Fig. 5.

are often not simple or periodic [8–11,58]. A specific example of reproducible complex behaviour is the calcium signal elicited when a single hepatocyte is exposed to a constant level of ATP. In some cases, the binding of this agonist to P_2-purino receptors induces a signal, which consists of a complex combination of spikes, doublet and bursts. It is believed that the complex signal stems from binding of ATP to two distinct ATP receptors present in the membrane of the hepatocyte. One of the receptors termed P_{2ys} mediates spikes and the other receptor P_{2yl} mediates bursting, the resulting dynamics is generated by a combination the two individual receptor mechanisms [8,59,60]. To our knowledge, no one has so far addressed the questions why ATP in some hepatocytes generate a complex signal, when it generates a periodic bursting signal in others, how this signal is decoded by targets in the cell, and if the targets of the complex signal are the same as those which decode the periodic bursting. We do not attempt to explain this specific example of complex calcium signals, but our model allows us to explore the decoding of a complex signal, which holds some resemblance to the signal induced by ATP.

By setting $k_2 = 2.23$ and the rest of the constants as in Fig. 1b, the calcium signal changes to complex periodic bursting which displays a burst pattern that is a combination of the different bursting and spike-like oscillations found in Fig. 5. We have not yet been able to locate or explain the origin of this complex behaviour in the calcium model. This, however, does not hinder that the resulting signal can be used to drive calcium activated enzyme models, such as the one presented by Eq. (6). And this may give us a hint of what the use of a complex signal can be.

The burst patterns are decoded by *Enzyme*-1 and *Enzyme*-2 (from Section 3.2.2) with very pronounced frequency locked oscillations. Thus, oscillations in enzyme activity display large amplitude fluctuations superimposed on small amplitude oscillations. These large amplitude fluctuations lock with the calcium oscillations with a ratio of 6–10 calcium oscillations per large amplitude oscillation in enzyme activity dependent on the agonist level. In Fig. 6 we see how *Enzyme*-1 and *Enzyme*-2 both display this frequency locking of the calcium signal, but in very different ways. When the activity of *Enzyme*-1 is high, the activity

of *Enzyme*-2 is low and vice versa. Again the very simple Hill term of the enzyme equation is responsible for the decoding dynamics of *Enzyme*-1 and *Enzyme*-2. *Enzyme*-1 needs only low levels of calcium, but for a prolonged time, whereas high levels of calcium activate *Enzyme*-2 with an efficiency that allows it to be activated by very short calcium transients. The combination of different bursting patterns in the same timeseries allow the enzymes to be activated not only out of phase with each other, but also with a period dependent on the shape and not the frequency of the driving calcium signal.

The phenomenon of distinct frequency locking and additive behaviour has so far not been seen in any in vivo system. However, it has been observed in vitro [43,61] and in other theoretical studies [62,63]. In addition, the experimental systems explored so far have often shown complex irregular calcium signal when exposed to a single agonist. It would be of great interest to see the calcium responses elicited by two or more natural agonists binding the cell at the same time. So far many modulators of the calcium signal such as Thimerol and Phorbol esters [59,11] have been used in addition to an agonist, but the exploration of multiple agonist binding is extremely scarce. Our guess is that the use of more than one agonists will induce a complex signal, which will hold information about all the agonists used, and that the cell will be able to decode the signal into distinct target responses. The fact that so many agonists use calcium as a second messenger supports this view, but further research has to verify or falsify this.

Another way in which the cell might generate and decode complex oscillatory signals is through coupling of the calcium oscillations to other intracellular oscillators. There are numerous cellular processes, which have been shown to oscillate in vivo [24]. In order to study the general properties of this coupling, and since the simple enzyme activity employed above cannot give rise to oscillations by itself, we coupled our model system to the following equations:

$$\frac{dX}{dt} = j - \alpha \times X \times Y \tag{8}$$

$$\frac{dY}{dt} = \alpha \times X \times Y - \frac{V \times Ca_{cyt} \times y}{K + Y} \tag{9}$$

where X and Y are either other secondary messengers, metabolites or phosphorylated proteins, while j, α, V and K are constants. In this hypothetical oscillatory system, we assume that the variable X is formed at constant rate and later transformed into the variable Y in a simple autocatalytic process. The variable Y is in turn degraded in an enzyme-catalysed process, the rate of which depends linearly on the calcium concentration. For simplicity reasons we assume that transfer of information from calcium to the variables X and Y is one-way, i.e. Eqs. (1)–(5) do not involve these variables. In the absence of the external variable Ca_{cyt}, the solutions of Eqs. (8) and (9) are either a simple steady state or a periodic oscillation. Coupling these equations to Eqs. (1)–(5) results in complex dynamics of the variables X and Y.

In the simplest case X and Y oscillate with the same frequency as the oscillations of Ca_{cyt}. However, we also observe oscillations of X and Y that are subharmonics of the oscillations of Ca_{cyt}. Furthermore, even when the solutions of Eqs. (1)–(5) show simple spiking oscillations of Ca_{cyt}, the corresponding dynamics of X and Y may be quasiperiodic or chaotic. Fig. 7 shows some examples of oscillations of Y that may be obtained when a single parameter, in this case the Michaelis constant K in Eq. (9), is changed. It is worth noting that similar changes may take place if any of the other constants in Eqs. (8) and (9) are changed. The oscillations of Ca_{cyt} show doublet oscillations as in Fig. 4 (Fig. 7a) with the same frequency in all cases. At low values of K, Y does not oscillate at all, but approaches a steady state value of zero. This means that if Y were a second messenger it would not activate its target enzyme(s) under these conditions. If K is between 0.1 and 0.6 we find oscillations of Y that are either subharmonic bursts of Ca_{cyt} (1:4, 1:5, etc. up to 1:10, Fig. 7c–e) or aperiodic (chaotic and quasiperiodic) oscillations (not shown). At $K = 0.6$ the oscillations of Y are 1:1 phase-locked to the oscillations of Ca_{cyt}. This means that with changes in K, the oscillations of Y may show similar behaviour to the oscillations of Ca_{cyt} when we change k_2, i.e. they can be

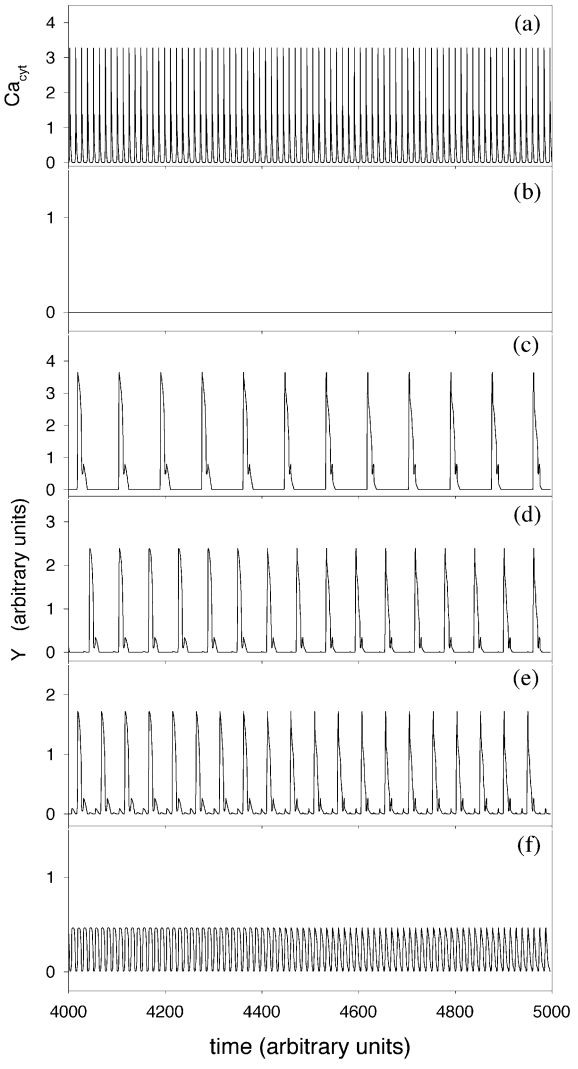

Fig. 7. Coupling of the calcium model defined by Eqs. (1)–(5) to the simple oscillator defined by Eqs. (8) and (9). In (a) we show the oscillations of Ca_{cyt} while in (b) to (f) we show time series of Y for different values of the Michaelis constant K; (b) $K=0.1$; (c) $K=0.3$; (d) $K=0.4$; (e) $K=0.5$ and (f) $K=0.6$. For the calcium oscillator $k_2=2.017$, while the other parameters are as in Fig. 1b. For the simple oscillator $j=0.05$, $\alpha=0.6$, $V=1$ and K is listed above.

decoded in the same way as described above for Ca_{cyt}. Thus, the calcium oscillator may, in addition to controlling one or two enzymes directly, have an indirect effect on many other enzymes through coupling with other signalling systems such as those generating cyclic AMP, NO etc. The result is that signalling systems that operate through both calcium signalling and X and/or Y signalling may be able to regulate the activities of a large number of regulatory proteins and genes independently of each other. These findings once more stress the fact that the cell is able to use diverse properties of calcium oscillations in order to transfer a multitude of information through the cell. It is to be noted that the behaviour shown in Fig. 7 could not be achieved without the coupling of Eqs. (8) and (9) to Eqs. (1)–(5), e.g. by simulating Eqs. (8) and (9) with progressively higher levels of Ca_{cyt}. Eqs. (8) and (9) define a system with only two variables, and, therefore, only simple periodic oscillations can be obtained. The complex oscillations shown in Fig. 7c–e are the result of coupling of two periodic oscillators. Hence, the coupling of Eqs. (8) and (9) to the calcium model (Eqs. (1)–(5)) allows for much more dynamic behaviour and, therefore, also for the transfer of much more information than if the two systems operated independently of each other.

4. Summary

The present study demonstrates that oscillating calcium signals are capable of encoding information not only in frequency, but also in shape and complexity of the calcium oscillations. We used a very general property, namely the cooperative binding of calcium to effector enzymes to study the principal possibilities of this information processing. Numerous enzymes in the cell actually show this cooperativity in calcium binding.

We improved the original model of Kummer et al. 2000 to show stable amplitude through spiking, bursting and complex bursting, and to show convincing frequency encoding. With these features the model enabled us to study a variety of possible decoding mechanism.

Since there have been a number of studies on frequency decoding of spiking calcium oscillations, we restricted ourselves in that case to study a phenomenon in calcium signalling which was only recently observed experimentally [7]. In this system the effect of calcium oscillations on intramitochondrial NAD(P)H levels was investigated.

Our theoretical model system decoded the signal in a way very similar to the experimental results. As in the experiment, a low frequency signal resulted in oscillations in the concentration of product, while a rise in frequency caused an elevated concentration of product.

To our knowledge no previous study has attempted to elucidate the mechanism for the decoding of bursting calcium signals. We show that simple cooperativity of calcium binding enables enzymes to react with very different sensitivity to bursting of the signal. This might explain why the cell is using these different patterns of calcium oscillations specifically for different agonists [58,64–66]. Different agonists imply different information and our studies show that calcium sensitive enzymes can easily decode this information.

Finally, we have addressed the issue of decoding complex oscillatory signals. These are often encountered in the literature, but their decoding remains an unsolved problem. We show that the complex oscillations of the model present yet another mean of information processing in the cell. We also show that calcium-sensitive enzymes are able to show frequency locking. Furthermore, by coupling the simple calcium oscillator to a second oscillating signalling system, we show that the combined system is capable of controlling the activity of multiple regulatory proteins, even when the calcium oscillator is operating at a single frequency. Many enzymes in the cell are regulated by more than one signalling molecule. Our studies suggest that this is probably due to the fact that even tiny differences in the properties of one of the signalling pathways will be amplified and lead to highly different signals of the combined system. This provides the cell with an essentially infinity of signals.

We would like to stress again the fact that our study relies on a very simple mechanism known to commonly exist in the cell. Therefore, we conjecture that it is very likely that the proposed decoding mechanisms are actually used by enzymes, even though experimental data in support of this conjecture are still lacking.

Acknowledgments

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References

[1] J.M. Berridge, M.D. Bootman, P. Lipp, Calcium—a life and death signal, Nature 395 (1998) 645–648.
[2] M.S. Nash, K.W. Young, R.A.J. Chaliss, S.R. Nahorski, Receptor-specific messenger oscillations, Nature 413 (2001) 381–382.
[3] R.E. Dolmetsch, E. Xu, R.S. Lewis, Calcium oscillations increase the efficiency and specificity of gene expression, Nature 392 (1998) 933–936.
[4] F. Eshete, R.D. Fields, Spike frequency decoding and autonomous activation of Ca^{2+}-Calmodulin-dependent protein kinase II in dorsal root ganglion neurons, J. Neurosci. 21 (2001) 6694–6705.
[5] W.H. Li, J. Llopis, M. Whitney, G. Zlokarnik, R. Tsien, Cell-permeant caged $InsP_3$ ester shows that Ca^{2+} spike frequency can optimize gene expression, Nature 392 (1998) 933–936.
[6] E. Oancea, T. Meyer, Protein kinase C as a molecular machine for decoding calcium and diacylglycerol signals, Cell 95 (1998) 307–318.
[7] L.D. Robb-Gaspers, G.A. Rutter, P. Burnett, G. Hajnoczky, R.M. Denton, A.P. Thomas, Coupling between cytosolic and mitochondrial calcium oscillations: role in the regulation of hepatic metabolism, BBA-Bioenergetics 1966 (1998) 17–32.
[8] C.J. Dixon, P.H. Cobbold, A.K Green, Adenosine 5′-[αβ-methylene]triphosphate potentiates the oscillatory cytosolic Ca^{2+} responses of hepatocytes to ATP, but not to ADP, Biochem. J. 293 (1993) 757–760.
[9] I. Marrero, A.K. Green, P.H. Cobbold, C.J. Dixon, Bovine growth hormone induces oscillations in cytosolic free Ca^{2+} in single rat hepatocytes, Biochem. J. 313 (1996) 525–528.
[10] T.A. Rooney, S.K. Joseph, C. Queen, A.P. Thomas, Cyclic GMP induces oscillatory calcium signals in rat hepatocytes, J. Biol. Chem. 16 (1996) 19 817–19 825.
[11] A.K. Green, P.H. Cobbold, C.J. Dixon, Thimeosal enhances agonist-specific differences between $[Ca^{2+}]_i$ oscillations induced by phenylephrine and ATP in single rat hepatocytes, Cell Calcium 25 (1999) 173–178.
[12] P. Tompa, R. Toth-Boconadi, P. Friedrich, Frequency decoding of fast calcium oscillations by calpain, Cell Calcium 29 (2001) 161–170.
[13] P. De Koninck, H. Schulman, Sensitivity of CaM Kinase II to the frequency of Ca^{2+} oscillations, Science 279 (1998) 227–230.
[14] D. Gall, E. Baus, G. Dupont, Activation of the liver glycogen phosphorylase by Ca^{2+} oscillations: a theoretical study, J. Theor. Biol. 207 (2000) 445–454.

[15] L.T. Izu, R.A. Spangler, A class of parametrically excited calcium oscillation detectors, Biophys. J. 68 (1995) 1621–1629.
[16] Y. Kubota, J.M. Bower, Decoding time-varying calcium signals by the postsynaptic biochemical network: computer simulations of molecular kinetics, Neurocomputing 26–27 (1999) 29–38.
[17] K. Prank, L. Läer, A. von zur Mühlen, G. Brabrant, C. Schfl, Decoding intracellular spike trains, Europhys. Lett. 42 (1998) 143–147.
[18] G. Hajnoczky, L.D. Robb-Gaspers, M.B. Seitz, A.P. Thomas, Decoding of cytosolic calcium oscillations in the mitochondria, Cell 82 (1995) 415–424.
[19] S. Schuster, M. Marhl, T. Höfer, Modelling of simple and complex calcium oscillations, Eur. J. Biochem. 269 (2002) 1333–1355.
[20] J. Keizer, G. De Young, Simplification of a realistic model of IP_3-induced Ca^{2+} oscillations, J. Theor. Biol. 166 (1994) 431–442.
[21] J.A.M. Borghans, G. Dupont, A. Goldbeter, Complex intracellular calcium oscillations: a theoretical exploration of possible mechanisms, Biophys. Chem. 66 (1997) 25–41.
[22] J.Y. Chatton, Y.M. Cao, J.W. Stucki, Pertubation of myo-inositol-1,4,5-trisphosphate levels during agonist-induced Ca^{2+} oscillations, Biophys. J. 74 (1998) 523–531.
[23] J. Sneyd, M. Wilkins, A. Strahonja, M.J. Sanderson, Calcium waves and oscillations driven by an intercellular gradient of inositol (1,4,5)-trisphosphate, Biophys. Chem. 72 (1998) 101–109.
[24] A. Goldbeter, Biochemical Oscillations and Cellular Rythms, Cambridge University Press, Cambridge, 1996.
[25] G. Houart, G. Dupont, A. Goldbeter, Bursting, chaos and birhythmicity originating from self-modulation of the inositol-1,4,5-trisphosphate signal in a model for intracellular Ca^{2+} oscillations, Bull. Math. Biol. 61 (1999) 507–530.
[26] M. Marhl, T. Haberichter, M. Brumen, R. Heinrich, Complex calcium oscillations and the role of mitochondria and cytosolic proteins, Biosystems 57 (2000) 75–86.
[27] G.H. Biddlecome, G. Bernstein, E.M. Ross, Regulation of Phospholipase C-β_1 by G_q and m1 muscarinic cholinergic receptor, J. Biol. Chem. 271 (1996) 7999–8007.
[28] C.W. Taylor, P. Thorn, Calcium signalling: IP_3 rises again… and again, Curr. Biol. 11 (2001) R352–R355.
[29] M. Marhl, S. Schuster, M. Brumen, Mitochondria as an important factor in the maintenance of constant amplitudes of cytosolic calcium oscillations, Biophys. Chem. 71 (1998) 125–132.
[30] E. Carafoli, L. Santella, D. Brance, M. Brini, Generation, control, and processing of cellular calcium signals, Crit. Rev. Biochem. Mol. Biol. 36 (2001) 107–260.
[31] U. Kummer, L.F. Olsen, C.J Dixon, A.K. Green, V. Bornberg-Bauer, G. Baier, Switching from simple to complex oscillations in calcium signalling, Biophys. J. 79 (2000) 1188–1195.
[32] S.L. Waters, J.K. Wong, R.G. Schnellmann, Depletion of endoplasmic reticulum calcium stores protects against hypoxia- and mitochondrial inhibitor-induced cellular injury and death, Biochem. Biophys. Res. Commun. 240 (1997) 57–60.
[33] R. Foyouzi-Youssefi, S. Arnaudeau, C. Borner, W.L. Kelley, J. Tschopp, D.P. Lew, et al., Bcl-2 decreases the free Ca^{2+} concentration within the endoplasmic reticulum', PNAS 97 (2000) 5723–5728.
[34] J.W. Putney, C.M. Pedrosa Ribeiro, Signalling pathways beween the plasma membrane and endoplasmic reticulum calcium stores, Cell Mol. Life Sci. 57 (2000) 1272–1286.
[35] H.N. Nguyen, C. Wang, D.C. Perry, Depletion of intracellular calcium stores is toxic to SH-SY5Y neuronal cells, Brain Res. 924 (2002) 159–166.
[36] J. Chatton, H. Liu, J.W. Stucki, Simultaneous measurements of Ca^{2+} in the intracellular stores and the cytosol of hepatocytes during hormone-induced Ca^{2+} oscillations, FEBS Lett. 368 (1995) 165–168.
[37] F. Ichas, L.S. Jouaville, S.S. Sidash, J.P. Mazat, E.L. Holmuhamedov, Mitochondrial calcium spiking-a transduction mechanism-based on calcium-induced permeability transition involved in cell calcium signalling, FEBS Lett. 348 (1994) 211–215.
[38] M. Iino, Molecular basis of spatiotemporal dynamics in inositol 1,4,5 trisphosphat-mediated Ca^{2+} signalling, Jpn. J. Pharmacol. 82 (2000) 15–20.
[39] S.K. Joseph, C. Lin, S. Pierson, A.P. Thomas, A.R. Maranto, Heteroligomers of Type-I and Type-III inositol trisphosphate receptors in WB rat liver epithelial cells, J. Biol. Chem. 270 (1995) 23 310–23 316.
[40] T. Miyakawa, A. Maeda, T. Yamazawa, K. Hirose, T. Kurosaki, M. Iino, Encoding of Ca^{2+} signals by differential expression of IP_3 receptor subtypes, EMBO J. 18 (1999) 1303–1308.
[41] V. Grubelnik, A.Z. Larsen, U. Kummer, L.F. Olsen, M. Marhl, Mitochondria regulate the amplitude of simple and complex calcium oscillations, Biophys. Chem. 94 (2001) 59–74.
[42] T.E. Gunter, L. Butinas, G.C. Sparagna, R. Eliseev, K. Gunter, Mitochondrial calcium transport: mechanisms and functions, Cell Calcium 28 (2000) 285–296.
[43] G.C. Sparagna, K. Gunter, S. Sheu, T.E. Gunter, Mitochondrial calcium uptake from physiological-type pulses of calcium, J. Biol. Chem. 270 (1995) 27 510–27 515.
[44] G.A. Rutter, R. Rizzutto, Regulation of mitochondrial metabolism by ER Ca^{2+} release: an intimate connection, TIBS 25 (2000) 215–221.
[45] J.J. Ubl, J.Y. Chatton, S. Chen, J.W. Stucki, A critical evaluation of in situ measurements of mitochondrial electrical potentials in single hepatocytes, Biochim. Biophys. Acta 1276 (1996) 124–132.
[46] J.B. Hoek, E. Walajtys-Rode, X. Wang, Hormonal stimulation, mitochondrial Ca^{2+} accumulation, and the con-

trol of the mitochondrial permeability transition in intact hepatocytes, Mol. Cell. Biochem. 174 (1997) 173–179.
[47] F. Ichas, J. Mazat, From calcium signalling to cell death: two conformations for the mitochondrial permeability transition pore. Switching from low- to high-conductance state, BBA-Bioenergetics 1366 (1998) 33–50.
[48] M. Zoratti, I. Szabo, The mitochondrial permeability transition, BBA-Rev. Biomembranes 1241 (1995) 139–176.
[49] V.A. Selivanov, F. Ichas, E.L. Holmuhamedov, L.S. Jouaville, Y.V. Evtodienko, J. Mazat, A Model of Mitochondrial Ca^{2+} induced Ca^{2+} release simulating the Ca^{2+} oscillations and spikes generated by mitochondria, Biophys. Chem. 72 (1998) 111–121.
[50] C.A. Hansen, L. Yang, J.R. Williamson, Mechanisms of receptor-mediated Ca^{2+} signalling in rat hepatocytes, J. Biol. Chem. 266 (1991) 18573–18579.
[51] M. Klapperstuck, G. Schmalzing, F. Markwardt, Characteristics of binding sites for ATP^4 at the human $P2X_7$ receptor, Drug Dev. Res. 53 (2001) 77–82.
[52] J.Y. Chatton, Y.M. Cao, J.W. Stucki, Agonist specific behaviour of the intracellular Ca^{2+} response in rat hepatocytes, Biochem. J. 328 (2) (1997) 573–579.
[53] C.J. Dixon, N.M. Wods, T.E. Webb, A.K. Green, Evidence that rat hepatocytes co-express funtional $P2Y_1$ and $P2Y_2$ receptors, Br. J. Pharmacol. 129 (2000) 764–770.
[54] T.A. Rooney, D.C. Renard, E.J. Sass, A.P. Thomas, Oscillatory cytosolic calcium waves independent of stimulated 1,4,5-trisphosphate formation in the hepatocyte, J. Biol. Chem. 19 (1991) 12 272–12 282.
[55] J. Meldolesi, T. Pozzan, The endoplasmatic reticulum Ca^{2+} store: a view from the lumen, Trends Biochem. Sci. 23 (1998) 10–14.
[56] G. Hajnoczky, G. Csordas, R. Krishnarmurthy, G. Szalai, Mitochondrial calcium signalling driven by the IP_3 receptor, J. Bioener. Biomembranes 32 (2000) 15–25.
[57] G. Dupont, A. Goldbeter, CaM kinase II as frequency decoder of Ca^{2+} oscillations, BioEssays 20 (1998) 607–610.
[58] T.A. Rooney, E.J. Sass, A.P. Thomas, Characterisation of cytosolic calcium oscillations induced by phenylephrine and vasopressin in single fure-2-loaded hepatocytes, J. Biol. Chem. 264 (1989) 17 313–17 141.
[59] C.J. Dixon, P.H. Cobbold, A.K Green, Oscillations in cytosolic free Ca^{2+} induced by ADP and ATP in single rat hepatocytes display differential sensitivity to application of phorbol ester, Biochem. J. 309 (1995) 145–149.
[60] A.K. Green, C.J. Dixon, P.H. Cobbold, Cytosolic free Ca^{2+} oscillations induced by diadenosine 5,5-P^1,P^3-triphosphate and diadenosine 5,5-P^1,P^4-tetraphosphate in single rat hepatocytes are indistinguishable from those induced by ADP and ATP, respectively, Biochem. J. 310 (1995) 629–635.
[61] T.E. Gunter, L. Butinas, G.C. Sparagna, K. Gunter, The Ca^{2+} transport mechanisms of mitochondria and Ca^{2+} uptake from physiological-type Ca^{2+} transients, BBA-Bioenergetics 1366 (1998) 5–15.
[62] T.R. Chay, Y.S. Lee, Y.S. Fan, Appearance of phase-locked Wenckebach-like rhythms, devil's staicase and universality in intracellular calcium spikes in non-excitable cell models, J. Theor. Biol. 174 (1995) 21–44.
[63] Y.S. Fan, A.V. Holden, An asymmetrical phase locking structure for a non-excitable cell model, Chaos Soliton. Fract. 9 (1998) 1637–1650.
[64] A. Sanchez-Bueno, C.J. Dixon, N.M. Woods, K.S.R. Cuthbertson, P.H. Cobbold, Inhibitors of protein kinase C prolong the falling phase of each free-calcium transient in a hormone-stimulated hepatocyte, Biochem. J. 268 (1990) 627–632.
[65] I. Marrero, A. Sanchez-Bueno, P.H. Cobbold, C.J. Dixon, Taurolithocholate and taurolithocholate 3-sulfate exert different effects on cytosolic free Ca^{2+} oscillations in rat hepatocytes, Biochem. J. 300 (1994) 383–386.
[66] A.P. Thomas, D.C. Renard, T.A. Rooney, Spatial and temporal organization of calcium signalling in hepatocytes, Cell Calcium 12 (1991) 111–126.