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REVIEW ARTICLE

Modelling of simple and complex calcium oscillations

From single-cell responses to intercellular signalling

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This review provides a comparative overview of recent developments in the modelling of cellular calcium oscillations. A large variety of mathematical models have been developed for this wide-spread phenomenon in intra- and intercellular signalling. From these, a general model is extracted that involves six types of concentration variables: inositol 1,4,5-trisphosphate (IP_3), cytoplasmic, endoplasmic reticulum and mitochondrial calcium, the occupied binding sites of calcium buffers, and the fraction of active IP_3 receptor calcium release channels. Using this framework, the models of calcium oscillations can be classified into 'minimal' models containing two variables and 'extended' models of three and more variables. Three types of minimal models are identified that are all based on calcium-induced calcium release (CICR), but differ with respect to the mechanisms limiting CICR. Extended models include IP_3–calcium cross-coupling, calcium sequestration by mitochondria, the detailed gating kinetics of the IP_3 receptor, and the dynamics of G-protein activation. In addition to generating regular oscillations, such models can describe bursting and chaotic calcium dynamics. The earlier hypothesis that information in calcium oscillations is encoded mainly by their frequency is nowadays modified in that some effect is attributed to amplitude encoding or temporal encoding. This point is discussed with reference to the analysis of the local and global bifurcations by which calcium oscillations can arise. Moreover, the question of how calcium binding proteins can sense and transform oscillatory signals is addressed. Recently, potential mechanisms leading to the coordination of oscillations in coupled cells have been investigated by mathematical modelling. For this, the general modelling framework is extended to include cytoplasmic and gap-junctional diffusion of IP_3 and calcium, and specific models are compared. Various suggestions concerning the physiological significance of oscillatory behaviour in intra- and intercellular signalling are discussed. The article is concluded with a discussion of obstacles and prospects.

Keywords: bursting; calcium-induced calcium release; calcium oscillations; entrainment; frequency encoding; gap junctions; Hopf bifurcation; homoclinic bifurcation; inositol 1,4,5-trisphosphate; IP_3 receptors.

INTRODUCTION

Many processes in living organisms are oscillatory. Besides quite obvious examples such as the beating of the heart, lung respiration, the sleep-wake rhythm, and the movement of fish tails and bird wings, there are many instances of biological oscillators on a microscopic scale, such as biochemical oscillations, in which glycolytic intermediates, the activities of cell-cycle related enzymes, cAMP or the intracellular concentration of calcium ions exhibit a periodic time behaviour. Calcium oscillations had been known for a long time in periodically contracting muscle cells (e.g. heart cells) and neurons [1], before they were discovered in the mid-1980s in nonexcitable cells, notably in oocytes upon fertilization [2] and in hepatocytes subject to hormone stimulation [3,4]. Later, they have also been found in many other animal cells (cf. [5–10]) as well as in plant cells [11], with many of these cells not having an obvious oscillatory biological function. The oscillation frequency ranges from $\approx 10^{-3}$ to ≈ 1 Hz.

A striking feature of the investigation of calcium oscillations is that almost from its beginning, experiments have been accompanied by mathematical modelling [12–18]. In recent years, much insight has been gained into the processes involved in calcium dynamics at the subcellular, cellular and intercellular levels and, accordingly, the models have become more elaborate and diversified. In particular, bursting oscillations and chaotic behaviour, various types of bifurcations, and the coupling between oscillating cells have been analysed. Moreover, the role of mitochondria as organelles, which are, besides the endoplasmic reticulum (ER), capable of sequestering and releasing calcium, has been studied. These developments are here put into the context of the various simpler models developed previously.

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Abbreviations: IP_3, inositol 1,4,5-trisphosphate; IP_3R, inositol 1,4,5-trisphosphate receptors; PIP_2, phosphatidyl inositol 4,5-bisphosphate; PLC, phospholipase C; RyR, ryanodine receptor; CICR, calcium-induced calcium release; PKC, protein kinase C; SERCA, sarcoplasmic reticulum/ER calcium ATPase; CRAC, Ca^{2+} release-activated current; ICC, IP_3–Ca^{2+} cross-coupling; PTP, permeability transition pore; DAG, diacylglycerol.

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Although focussing on the modelling aspect, we will always aim at relating the model assumptions and theoretical conclusions to experimental results.

A scientific model is a simplified representation of an experimental system. It should meet two criteria often contradicting each other: First, it should describe the features of interest as adequately as possible. Second, it should be simple enough to be tractable and interpretable. We believe that, in model construction, guidance should be sought primarily from the experimental data. For example, the occurrence of self-sustained calcium oscillations can be described by relatively simple, 'minimalist' models (e.g. the two-variable model by Somogyi & Stucki [17], and see Ca_{cyt}/Ca_{er} models, below). However, if, for example, the detailed gating characteristics of the calcium release channel is also to be described, more comprehensive models are needed (e.g. the eight-variable model by De Young & Keizer [18], and see Detailed kinetics of the Ca^{2+} release channels section). Of course, the models should be in accord with physico-chemical laws such as the principle of detailed balance.

This review on calcium dynamics is focussed primarily on deterministic models of the temporal behaviour. Spatio-temporal aspects such as calcium waves (cf. [19]) will be treated in relation to coupled cells (see Coupling of oscillating cells). In the deterministic approach, the mathematical variables are the concentrations of relevant substances and possibly the transmembrane potential; the fluctuations of these variables are neglected. In comparison to stochastic modelling, this approach has the advantage that the mathematical description is simpler. The results derived from deterministic models of calcium oscillations are already in good, and sometimes excellent, agreement with experiment. However, in small volumes, fluctuations may not be negligible. For example, in a cell organelle with a volume of 1 μm^3, a free Ca^{2+} concentration of 200 nM implies the presence of only 120 unbound ions. On the other hand, the binding of Ca^{2+} ions to proteins brings about that a much larger number of ions are present in total. Thus, it is worth investigating whether fluctuations can be assumed to be buffered under these conditions. Stochastic models have been developed for single Ca^{2+} channels [20], intracellular wave propagation [21–25] and intracellular oscillations [26,27].

The deterministic modelling of biological oscillations and rhythms is based on a well-established apparatus to describe self-sustained oscillations in chemistry and physics by nonlinear differential equation systems [28–32]. The same apparatus has been used for the modelling of cell cycle dynamics [33,34], heart contraction and fibrillation [35], glycolytic oscillations [36,37] and cAMP oscillations [5].

The models of calcium oscillations are based on a description of the essential fluxes (Fig. 1). The cytoplasmic compartment is linked with the extracellular medium and several intracellular compartments, most notably the ER and mitochondria, through exchange fluxes. In micro-organisms, special compartments may exist, such as the acidosomal store in *Dictyostelium discoideum* [38]. The cascade of events underlying calcium oscillations has often been described (e.g [5,39]). A central process is the release of Ca^{2+} ions from the ER via channels sensitive to inositol 1,4,5-trisphosphate (IP_3), termed IP_3 receptors (IP_3R) (compare [40–42]). IP_3 and diacylglycerol (DAG) are

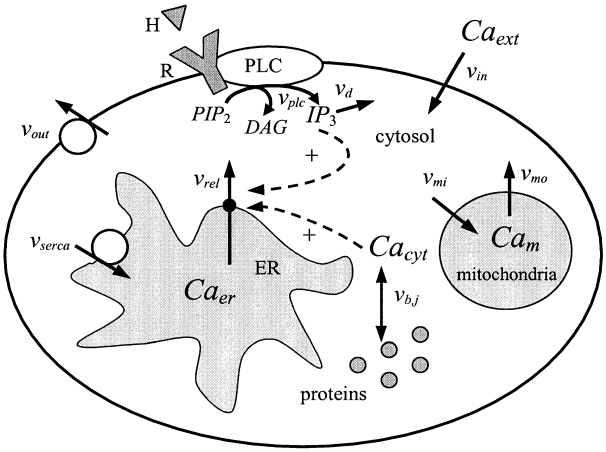

Fig. 1. General scheme of the main processes involved in intracellular calcium oscillations. Meaning of the symbols for reaction rates: $v_{b,j}$, net rate of binding of Ca^{2+} to the j-th class of Ca^{2+} buffer (e.g. protein); v_d, degradation of IP_3 (performed mainly by hydrolysis to inositol-1,4-bisphosphate or phosphorylation to inositol-1,3,4,5-tetrakisphosphate); v_{in}, influx of Ca^{2+} across plasma membrane channels; v_{mi}, Ca^{2+} uptake into mitochondria; v_{mo}, release of Ca^{2+} from mitochondria; v_{out}, transport of Ca^{2+} out of the cell by plasma membrane Ca^{2+} ATPase; v_{plc}, formation of IP_3 and DAG catalyzed by phospholipase C (PLC); v_{rel}, Ca^{2+} release from the ER through channels and leak flux; v_{serca}, transport of Ca^{2+} into the ER by sarco-/endoplasmic reticulum Ca^{2+} ATPase (SERCA).

formed from phosphatidyl inositol 4,5-bisphosphate (PIP_2) by phosphoinositide-specific phospholipase C (1-phosphatidylinositol-4,5-bisphosphate phosphodiesterase, PLC, EC 3.1.4.11). Different isoforms of (phosphoinositide-specific) PLC are activated by hormone-receptor coupled G-proteins (PLCβ), protein kinases (PLCγ) and calcium (PLCδ) [43]. Another ER calcium release channel, particularly prominent in muscle cells, is the ryanodine receptor (RyR), whose physiological activator appears to be cyclic ADP ribose [44]. Opening of the IP_3R, in the presence of IP_3, and of the RyR is also stimulated by calcium binding (calcium-induced calcium release, CICR) [39,41,45,46]. Several isoforms of both receptors have also been shown to be inhibited by high calcium concentrations [41]. (As for oocytes, the signalling pathway via IP_3 is subject to debate [8,47].) Additionally, many other processes may play a role in the signalling cascade in various cell processes, such as activation of protein kinase C (PKC) by DAG and calcium (cf. [41,48]), phosphorylation of the IP_3R by PKC (cf. [41]), 'cross-talk' of the G-protein with this kinase [49,50] and the contribution of the RyR activated by cyclic ADP ribose [44,51].

The steep calcium gradient across the ER membrane is sustained by active pumping through the sarcoplasmic reticulum/ER calcium ATPase (SERCA, EC 3.6.3.8). In hepatocytes, for example, the baseline concentration in the cytosol is about 0.2 μM and rises to about 0.5–1 μM during spikes, while the level in the ER is about 0.5 mM. A similarly high gradient exists across the cell membrane. Various entrance pathways, chiefly calcium store-operated [42,52] and receptor-operated [53], have been described. Ca^{2+} ions are also bound to many substances such as proteins, phospholipids and other phosphate compounds.

For these various reactions and transport processes, flux balance equations can be formulated. Throughout the paper, italic symbols of substances will be used for concentrations while Roman symbols stand for the substances themselves. The general balance equations for the variables of Fig. 1, the concentrations of IP_3 (IP_3), cytoplasmic calcium (Ca_{cyt}), ER calcium (Ca_{er}), mitochondrial calcium (Ca_m), and occupied calcium binding sites of the buffer species j in the cytosol (B_j) are:

$$\frac{\mathrm{d}}{\mathrm{d}t} IP_3 = v_{plc} - v_d \tag{1}$$

$$\frac{\mathrm{d}}{\mathrm{d}t} Ca_{cyt} = v_{in} - v_{out} + v_{rel} - v_{serca} + v_{mo} - v_{mi} - \sum_{j=1}^{n} v_{b,j} \tag{2}$$

$$\frac{\mathrm{d}}{\mathrm{d}t} Ca_{er} = \rho_{er}(v_{serca} - v_{rel}) \tag{3}$$

$$\frac{\mathrm{d}}{\mathrm{d}t} Ca_m = \rho_{mit}(v_{mi} - v_{mo}) \tag{4}$$

$$\frac{\mathrm{d}}{\mathrm{d}t} B_j = v_{b,j} \tag{5}$$

where ρ_{er} and ρ_{mit} are the cytosol/ER and cytosol/mitochondria volume ratios and the rate expressions have the same meaning as in the legend of Fig. 1. Equations similar to Eqn (5) can also be written for the buffers in the ER and mitochondria. Furthermore, the transitions between different states of the IP_3R can play a role in IP_3-evoked calcium oscillations [18,54–57]. Of particular relevance is the desensitization of the IP_3R induced by calcium binding, which can be expressed by the following balance equation

$$\frac{\mathrm{d}}{\mathrm{d}t} R_a = v_{rec} - v_{des} \tag{6}$$

R_a denotes the fraction of receptors in the sensitized state; v_{des} and v_{rec} stand for the rates of receptor desensitization and recovery, respectively.

Moreover, several models include, as a variable, the cell membrane potential [58–60]. This may be of importance when calcium oscillations and action potential oscillations interact. However, we restrict this review to the core mechanisms of cytoplasmic calcium oscillations that apply both to electrically nonexcitable and excitable cells.

Most models of calcium oscillations fit into the general system of balance equations (Eqns 1–6). To our knowledge, no model that includes all of the six equations has so far been published, although various combinations of processes have been used. In the Minimal models section, we discuss all classes of minimalist models involving two out of the six variables entering Eqns (1–6) suggested up to now. The section Higher-dimensional models is devoted to more complex models involving three or four out of the six variables mentioned above or additional variables such as the various states of the IP_3R or the concentration of active subunits of the G-protein. The overview of models given in Minimal models and Higher-dimensional models updates and corrects the classification given previously [61].

Different experimental results were obtained concerning the question whether Ca^{2+} outside the cells is necessary for the maintenance of oscillations. Removal of external Ca^{2+} leads to a cessation of oscillations in most cases in endodermal cells [62] and HeLa cells [63]. In other cell types, such as salivary gland cells, external Ca^{2+} is not required [64]. For hepatocytes, Woods *et al.* [65] found that external Ca^{2+} was necessary for oscillations while others found that it was not [66,67] or that inhibition of the plasma membrane Ca^{2+} pump does not prevent oscillations [68]. If oscillations occur in the absence of external Ca^{2+}, they are usually slower and eventually fade away (cf. [69]).

It has often been argued that in calcium oscillations, information is encoded mainly by their frequency [5,12,70–72]. However, a possible role of amplitudes in signal transduction by calcium oscillations has also been discussed [73–75]. Frequency and amplitude encoding will be reviewed in Frequency encoding, based on an analysis of the local and global bifurcations by which calcium oscillations arise (subsections Hopf bifurcations and Global bifurcations). The models addressing the questions of how the oscillatory calcium signal is transformed into a nearly stationary output signal and how the target proteins sense the varying frequency are reviewed in the subsection entitled Modelling of protein phosphorylation driven by calcium oscillations. In the subsection Chaos and bursting, complex temporal phenomena will be discussed. Coupling of oscillating cells allows intercellular communication based on calcium signals, as described in the relevant section below. In the Conclusion, we will review the suggestions concerning the possible physiological significance of oscillatory calcium dynamics in comparison with adjustable stationary levels. Moreover, we will discuss some obstacles and give an outlook on the further development of the field. In particular, we will suggest a possible 'networking' of different modelling approaches in biochemistry. Mathematical fundamentals necessary for the review are outlined in the Appendix.

MINIMAL MODELS

To simulate self-sustained oscillations by a system of kinetic equations, at least two variables are needed (see Appendix). The free cytosolic calcium concentration should be taken as a dynamic variable, because this is the quantity most frequently measured. The only model not including Ca_{cyt} as a dynamic variable published so far is a simplified, two-variable version of a model involving the G-protein [76]. Ca_{cyt} can then be calculated by an algebraic equation (based on quasi-steady-state arguments) from IP_3. In our opinion, this model is not sufficiently supported by experimental data. Experiments show that changes in the activity of the SERCA [77,78] and in receptor-activated calcium influx [79] affect the frequency and spike width of Ca^{2+} oscillations, thus arguing for a participation of Ca^{2+} in the mechanism of oscillations.

Five minimal, two-variable systems including Ca_{cyt} can be conceived from the basic equations (Eqns 1–6), three of which have indeed been studied in the literature (Table 1). Models that include the remaining combinations exist, but are not minimal because they involve also additional

Table 1. Rate laws for three types of minimal models of Ca^{2+} oscillations. In each case, the positive feedback is provided by CICR.

Variables	Cytoplasmic and ER Ca^{2+} (Ca_{cyt}, Ca_{er})	Cytoplasmic Ca^{2+}, active IP_3R (Ca_{cyt}, R_a)	Cytoplasmic Ca^{2+} and Ca^{2+} buffer (Ca_{cyt}, B)
Example	Dupont & Goldbeter [80]	Li & Rinzel [89]	Marhl *et al.* [113] [a]
Limiting process	Ca^{2+} exchange with extracellular medium	IP_3R desensitization	Ca^{2+} binding to proteins
Total cellular Ca^{2+}	Not constant	Constant	Constant
Rate laws			
v_{in}	$v_0 + v_1\beta$	–	–
v_{out}	kCa_{cyt}	–	–
v_{rel}	$k_f Ca_{er} + \beta v_3 \frac{Ca_{cyt}^4}{K_A^4+Ca_{cyt}^4}\frac{Ca_{er}^2}{K_r^2+Ca_{er}^2}$	$\left[k_0 + k_1\left(\frac{R_a Ca_{cyt}}{K_a+Ca_{cyt}}\right)^3\right](Ca_{er}-Ca_{cyt})$	$\left(k_{leak} + k_{ch}\frac{Ca_{cyt}^2}{K_1^2+Ca_{cyt}^2}\right)(Ca_{er}-Ca_{cyt})$
v_{serca}	$v_2\frac{Ca_{cyt}^2}{K_2^2+Ca_{cyt}^2}$	$v_2\frac{Ca_{cyt}^2}{K_2^2+Ca_{cyt}^2}$	$k_{pump}\,Ca_{cyt}$
v_{rec}	–	$k_3\,(1-R_a)$	–
v_{des}	–	$k_{-3}Ca_{cyt}R_a$	–
v_b	–	–	$k_+(B_0-B)\,Ca_{cyt} - k\_B$
Related 2D models	[14,15,17,81,83,84,90]	[97–99,102–104]	[114,120]

[a] In the original model, an effect of the ER membrane potential was included in v_{rel}; however, oscillations are also obtained with the simpler expression given (cf. [115,162]).

variables (see subsections Consideration of the IP_3 dynamics and Inclusion of mitochondria). The following three subsections discuss each class of two-variable models in turn, referred to by the names of the variables involved: Ca_{cyt}/Ca_{er}, Ca_{cyt}/IP_3R, and Ca_{cyt}/protein.

To construct a kinetic model, in the balance equations the dependencies of the flux rates on the model variables must be specified (rate laws). For one representative of each model class, rate laws are given in Table 1, together with references to related models. Although all of these models are minimal in the sense of containing two dynamic variables, there are considerable differences with respect to the complexity of the rate laws. This will be explicitly discussed for the Ca_{cyt}/Ca_{er} models below.

The analysis of two-dimensional models shows that self-sustained oscillations can only occur if one of the model variables exerts an activatory effect on itself (autocatalysis, feedback activation; see Appendix). A prominent feedback loop is CICR exhibited both by RyR and IP_3R Ca^{2+} release channels. Indeed, all three types of minimal models involve CICR. By contrast, a putative activation of Ca^{2+} release by Ca_{er} would not suffice to generate oscillations.

Ca_{cyt}/Ca_{er} models

A model for self-sustained Ca^{2+} oscillations that is not only minimal with respect to the number of variables but also very simple with respect to the rate laws is the 'one-pool model' proposed by Somogyi and Stucki [17]. As shown by Dupont & Goldbeter [80], it can be derived by simplifying a 'two-pool model', in which IP_3-sensitive and IP_3-insensitive stores were considered [14,15,81]. Interestingly, recent findings show that in *Dictyostelium discoideum*, indeed both IP_3-sensitive and IP_3-insensitive stores exist [38].

The following processes are included in the one-pool model (Fig. 1): v_{in}, v_{out}, v_{rel}, and v_{serca}. IP_3 plays the role of a parameter entering the rate expression of v_{rel} and can be set to different values, according to the level of agonist stimulation. We shall discuss the Somogyi–Stucki model here in some detail by way of example, because several interesting features can be seen relatively easily from it. The influx into the cell is assumed to be constant. The transport of Ca^{2+} both out of the cell and into the store is modelled by functions linear in the cytosolic Ca^{2+} concentration, $k_i Ca_{cyt}$. The only nonlinear function is that for the channel flux of Ca^{2+} from the intracellular store. Together with a leak through the ER membrane (or a background conductance of the channel), this reads:

$$v_{rel} = \left[\frac{k_{ch}(Ca_{cyt})^4}{K^4 + (Ca_{cyt})^4} + k_{leak}\right] Ca_{er} \qquad (7)$$

The rate function in Eqn (7) is a simple description of the cooperative behaviour found in CICR (and represents a higher nonlinearity than simple mass action kinetics, $kCa_{er}Ca_{cyt}$; see Appendix). In principle, however, one could simplify the model by using a function quadratic in Ca_{cyt}, in which case the model would coincide with the Brusselator [28]. The system even oscillates if the kinetics of v_{rel} is a product of two Michaelis–Menten terms for Ca_{cyt} and Ca_{er}, and also v_{serca} obeys a Michaelis–Menten kinetics for Ca_{cyt} [82]. In many models [80,83], Ca_{er} enters the rate laws for v_{rel} and v_{serca} through a Hill equation with Hill coefficient two (see Table 1). Friel [84] proposed a model for neurons that is similar to the Somogyi–Stucki model [17], yet with a somewhat more realistic rare law for v_{rel} in that Ca_{er} in Eqn (7) was replaced by (Ca_{er}–Ca_{cyt}) because the release flux is driven by the Ca^{2+} gradient. Moreover, smaller values for the Hill coefficient were used.

For a mathematical analysis of the one-pool model [17,80], it is convenient to sum up the two differential equations, giving

$$\frac{d(Ca_{cyt} + Ca_{er}/\rho_{er})}{dt} = v_{in} - k_{out}Ca_{cyt} \qquad (8)$$

Thus, in any steady state of the system, we have the unique solution:

$$Ca_{cyt} = \frac{v_{in}}{k_{out}} \qquad (9)$$

The stationary value of Ca_{er} in turn is a unique function of Ca_{cyt}. Therefore this model allows exactly one stationary state.

Roughly speaking, the cause for the oscillation is an overshoot phenomenon due to the nonlinearity of CICR. Upon opening of the IP_3R, Ca_{er} is released. However, Ca_{cyt} cannot remain permanently elevated by this flux, cf. Eqn (9). During release, Ca_{er} and therefore also the driving force for the release flux decrease. At some instant, Ca^{2+} extrusion from the cell and Ca^{2+} pumping into the ER overtake release and thus Ca_{cyt} declines. Upon continued stimulation, the process could repeat, giving rise to oscillations. It is an important feature of this model that the total free Ca^{2+} concentration in the cell, $Ca_{cyt} + Ca_{er}/\rho_{er}$, oscillates in the course of Ca_{cyt} oscillations. From this, one can conclude that the essential mechanism counteracting the autocatalytic release is the subsequent depletion of the total Ca^{2+} in the cell. Note that complete depletion of the calcium stores is not required for this mechanism to work (cf. [85]).

To determine the exact requirements for oscillations, intuition is, however, insufficient and we do need modelling. To establish these requirements, a stability analysis is instrumental (see Appendix). A major advantage of the simplicity of the model equations is that the stability calculations can be performed analytically [17,86]. The parameter range in which the steady state is an unstable focus can be determined. In this parameter range, the oscillations can easily be found by numerical integration of the differential equations. The dynamics of Ca_{cyt} exhibits the repetitive spikes found in experiment.

A biologically relevant bifurcation parameter is the rate constant of the channel, k_{ch}, because it increases upon hormone stimulation of the cell mediated by IP_3. For low values of k_{ch}, the steady state is stable. As it increases, a point is reached where stable limit cycles occur. When k_{ch} is increased even further, the oscillations eventually vanish and the steady state becomes stable again. (For a discussion of the bifurcations in this model, see Hopf bifurcations.) From Eqn (9), it can be seen that the steady-state concentration Ca_{cyt} does not depend on the rate constant of the channel. This appears to be in disagreement with experimental observations showing that at very high hormone stimulation, elevated stationary Ca_{cyt} levels occur [17,66,87]. It has been reported for some cell types that hormone stimulation, besides causing IP_3 synthesis, also leads to activation of Ca^{2+} entry into the cell. This can be mediated by store-operated [42,52] and receptor-operated [53] calcium entry. Dupont & Goldbeter [80] modelled the latter effect by including, in the influx rate, a function expressing the occupancy of the cell membrane receptor with hormone, so that the steady-state concentration Ca_{cyt} is indeed increased. This has recently been followed up [83]. The other possible mechanism involves Ca^{2+} entry from the external medium into the cytosol stimulated by emptying of the Ca^{2+} stores [52,88]. However, the mechanism for this phenomenon, called 'capacitative Ca^{2+} entry', via a Ca^{2+} release-activated current (CRAC) is not yet clear [52].

In the light of the reasoning about minimal models given in the Introduction, it is of interest to investigate whether the one-pool model may be simplified further. Neglecting particular fluxes would perturb the Ca^{2+} balance. In particular, neglecting the influx into the cell is interesting in view of experiments where external Ca^{2+} was removed (see Introduction). If both influx and efflux were completely disregarded in the model, the total amount of calcium in the cell would be conserved: $Ca_{er}/\rho_{er} + Ca_{cyt} =$ constant. Thus, the equation system would effectively be one-dimensional, unless additional dynamic variables are included, such as the open probability of the channel [89] or the Ca^{2+} level in an intermediate domain near the mouth of the channel [90].

The flux through the ER membrane channel is pivotal due to its autocatalytic nature. Interestingly, although the leak seems to be negligible in comparison to the CICR flux, it is not. A bifurcation analysis (cf. Frequency and amplitude behaviour) shows that if the leak rate is set equal to zero, the model can indeed give rise to oscillations. However, there is no parameter range with small values of the rate constant of the channel for which a steady state is obtained [91]. This is in disagreement with experiment, because for very low agonist stimulation, no oscillations were found [3,4,17,66]. In conclusion, the one-pool model cannot be simplified any further.

For subtypes I and II of the IP_3R, the dependence of v_{rel} on Ca_{cyt} is more complex than is expressed by Eqn (7) in that at higher values of Ca_{cyt}, this rate decreases [41]. This does not principally alter the behaviour of Ca_{cyt}/Ca_{er} models [83,92].

Ca_{cyt}/IP_3 receptor models

Experimental studies on the IP_3R indicate that the inhibition of this receptor by Ca_{cyt} can play a role in the generation of oscillations if it occurs on a time-scale of seconds compatible with the time-scale of the oscillations while the activation is much faster [55,93,94]. In the Ca_{cyt}/IP_3 receptor models, spikes terminate because the IP_3R is inhibited at high Ca_{cyt} and remains inhibited for some time so that the released Ca^{2+} can be transported back into the ER. Thus, the mechanisms causing the oscillatory behaviour are localized in or near the ER membrane. In contrast to the Ca_{cyt}/Ca_{er} models, the Ca_{cyt}/IP_3R models work without (as well as with) Ca^{2+} exchange across the plasma membrane. Two hypotheses have been put forward (see Detailed kinetics of the Ca^{2+} release channels): (a) transition of the receptor into an inactive conformation upon Ca^{2+} binding [56,93,95,96]; (b) inactivation of the receptor by phosphorylation [94].

The first of these possibilities was studied in two-dimensional models [97–99] with Ca_{cyt}, Eqn (2), and R_a, Eqn (6), being the model variables. As in several other Ca_{cyt}/IP_3R models Eqn (6) was specified to have the form:

$$\frac{\mathrm{d}}{\mathrm{d}t} R_a = k[R_a^{\infty}(IP_3, Ca_{cyt}) - R_a] \qquad (10)$$

motivated by analogy to the Hodgkin-Huxley model of nerve excitation [100,101]. Eqn (10) can be interpreted as a relaxation to the steady state with time constant $1/k$. $R_a^{\infty}(IP_3, Ca_{cyt})$, the steady-state fraction of receptors in the sensitized states, is a decreasing function of Ca_{cyt}. In the models of Poledna [97,98] and Atri *et al.* [99], this function was chosen to be $R_a^{\infty} = K/(Ca_{cyt} + K)$, and $R_a^{\infty} = K^2/(Ca_{cyt}^2 + K^2)$, respectively, where K denotes the equilibrium constant of Ca^{2+} binding. Note that R_a is not the fraction of open receptor subunits *per se* but of the

subunit form that can be in the open state if Ca^{2+} is bound at an activating binding site. The essential positive feedback is again provided by CICR modelled by a Hill equation in the kinetics of Ca_{cyt}.

A more mechanistic, eight-dimensional model was developed by De Young & Keizer [18] (see also Detailed kinetics of the Ca^{2+} release channels). This model was simplified, by using time scale arguments, to two-dimensional models [89,102,103]. For the model by Li & Rinzel [89], the specific form of the rate law entering Eqn (10) as well as the other rate laws are given in Table 1. Also the Ca_{cyt}/IP_3R models obtained by simplification of larger models have a structure reminiscent of the Hodgkin–Huxley models. Accordingly, the Ca^{2+} dynamics can be interpreted as an ER membrane-associated excitability [89,104], so that the term nonexcitable cells often used for hepatocytes, oocytes and other cells exhibiting Ca^{2+} oscillations appears no longer to be appropriate. Moreover, Li & Rinzel [89] also considered a three-dimensional system, in which the Ca^{2+} exchange across the plasma membrane is taken into account.

Ca_{cyt}/protein models

In addition to the sensing of the calcium signal (see Modelling of protein phosphorylation driven by calcium oscillations), Ca^{2+}-binding proteins can exert a feedback on the process of Ca^{2+} oscillations itself. Provided that (a) Ca^{2+} binding to proteins is very fast, and (b) the dissociation constant is well above the prevailing (free) Ca_{cyt}, the overall effect of such buffers is an increase in the effective compartmental volume. In several models, a rapid-equilibrium approximation for Ca^{2+} binding to proteins is used [105–108], which only requires condition (a) to be fulfilled. For example, Wagner & Keizer [105] modified the Ca_{cyt}/IP_3R model of Li & Rinzel [89]. However, the rapid-equilibrium approximation is not always justified [109,110]. Accordingly, several mathematical models [71,106,107,111–115] include the dynamics of Ca^{2+} binding to proteins, showing that the cytosolic proteins can be essential components of the oscillatory mechanism and can play an important role in frequency and amplitude regulation. We have shown earlier by mathematical modelling that, in the presence of Ca^{2+}-binding proteins, Ca^{2+} oscillations can arise even in the absence of an exchange across the plasma membrane and of an intrinsic dynamics of the IP_3R [113]. In Ca_{cyt}/protein models, the role of alternating supply and withdrawal of Ca^{2+} is played by the fluxes of the dissociation and binding of Ca^{2+} to and from binding sites.

Ca^{2+}-binding proteins (as well as Ca^{2+}-binding phospholipids) show a wide range of values of the binding and dissociation rate constants [109,110,116]. Roughly, two types of proteins can be distinguished [116–119]. The first class represents the so-called buffering proteins (also known as 'storage' proteins) such as parvalbumin, calbindin, and also C-terminal domains of calmodulin or troponin C, which bind calcium relatively slowly but with a high affinity [109,116]. The second class, which is referred to as the signalling proteins (also known as 'regulatory' proteins) comprises binding sites that have very high rate constants of binding and dissociation with respect to calcium, but low affinity. Examples are provided by the N-terminal domains of calmodulin or troponin C. Some of these signalling proteins interact with proteins (e.g. CaM kinase II) that transfer the calcium signal by phosphorylating other proteins (see Modelling of protein phosphorylation driven by calcium oscillations). The interplay between buffering and signalling proteins has been examined by modelling studies, using the rapid-equilibrium approximation only for the signalling proteins [71,114,120]. A transfer of Ca^{2+} from the rapid, low affinity, to the slow, high affinity, binding sites, has been mimicked. This is in agreement with observations both in Ca^{2+} oscillations and Ca^{2+} transients, even within one protein molecule as in the case of calmodulin. In skeletal muscle, for example, the Ca^{2+} released into the cytosol first binds to troponin C and, after a brief lag phase, the bound Ca^{2+} population shifts to parvalbumin [116,121]. There, the buffering proteins have the function of terminating the Ca^{2+} transients evoking muscle contraction. Likewise, this mechanism may play a role in the termination of spikes in oscillations.

In the Ca_{cyt}/protein models, the positive feedback necessary for two-dimensional models to generate limit cycles is provided again by CICR. Additional nonlinearities enter the model by the consideration of the transmembrane potential across the ER membrane. While in the model of Jafri *et al.* [111], the transmembrane potential is considered as a dynamic variable, so that the model is three-dimensional (an extended model [112] including the cytosolic counterion concentration is even four-dimensional), the quasi-electroneutrality condition has been used in [71,113,114] to express this variable into the others. The models (directly or indirectly) including the ER transmembrane potential give slightly asymmetric spikes where the upstroke is somewhat faster than the decrease. During the upstroke, the potential is depolarized, which implies that the driving force of the Ca^{2+} efflux from the store is diminished both by the decreasing Ca^{2+} gradient and the decreasing electric gradient.

It should be noted that the magnitude of the ER transmembrane potential is not well known. Because of the high permeability of the ER membrane for monovalent ions it has often been argued that the potential gradient due to Ca^{2+} transport is rapidly dissipated by passive ion fluxes [104,121–123]. An opposing view is that the highly permeant ions directly follow the potential without depleting it, as described by the Nernst equation. An interesting model prediction is that the value of the potential depends on the effective volume of the ER accessible to Ca^{2+} [114].

HIGHER-DIMENSIONAL MODELS

Consideration of the IP_3 dynamics

In the Ca_{cyt}/Ca_{er} models, the IP_3 concentration is considered as a parameter which can be set equal to different, fixed values. This approach is supported by findings showing that IP_3 oscillations are not required for Ca^{2+} oscillations [124]. However, a coupling between oscillations in IP_3 and oscillations in Ca_{cyt} seem to be of importance in some cell types [16,72,76,125–127]. Mechanisms for this coupling are the activating effect of Ca_{cyt} on the δ isoform of PLC [43,63] and on the IP_3 3-kinase (EC 2.7.1.127) [128], and Ca_{cyt} feedback on the agonist receptor [129].

This inspired the idea of the IP_3–Ca^{2+} cross-coupling (ICC) models, in which a stimulatory effect of Ca_{cyt} on the activity of PLC [12,13,18] or on the consumption of IP_3

[130,131] are taken into account, in addition to IP_3 induced Ca^{2+} release. IP_3 is a system variable in these models and oscillates with the same frequency as Ca_{cyt}. Meyer & Stryer [12] first studied a model in which, in addition to IP_3, only two Ca^{2+} pools are considered: Ca_{cyt} and Ca_{er}. As these are then linked by a conservation relation ($Ca_{cyt} + Ca_{er} =$ constant), the model is two-dimensional. It gives rise to bistability rather than oscillations, which is understandable because the cross-coupling between IP_3 and Ca_{cyt} does not fulfil the condition that the trace of the Jacobian be positive (see Appendix). Next, Meyer & Stryer [12] included a Ca^{2+} exchange between cytosol and mitochondria. As the conservation relation now includes Ca_m, the system is three-dimensional, even though Ca_m does not occur explicitly as a variable because the efflux out of the mitochondria is assumed to be constant. In three-dimensional systems, the trace of the Jacobian need not be positive in order to obtain oscillations (in fact, at the Hopf bifurcation, it must be negative, cf. [32]). Thus, violation of the conservation relation $Ca_{cyt} + Ca_{er} =$ constant is not an error, as assumed previously [61], but a prerequisite for the ICC models to generate oscillations. In a later version of the model, Meyer & Stryer [13] proposed to consider, as a third independent variable, a parameter describing the inhibition of the IP_3R by Ca_{cyt} and did not include mitochondria.

Another combination of variables was chosen by De Young & Keizer [18]. The PLC is again assumed to be activated by Ca_{cyt}. A model for Ca^{2+} waves with the same set of variables but a simpler IP_3 dynamics was presented in [99]. The model of Swillens & Mercan [130] involves, as a variable, the level of IP_4 (which is formed from IP_3 by phosphorylation) (see Table 2). In order that this model generates oscillations, these authors included, in addition to the effects mentioned above, an inhibition of v_{rel} by Ca_{er}, an assumption which has not been followed up in later models. In the model of Dupont & Erneux [131], the desensitized receptor is included as a fourth variable. As it involves CICR and receptor desensitization, the IP_3–Ca^{2+} cross-coupling is here not necessary for the generation of Ca^{2+} oscillations.

In a three-dimensional model [16], the G-protein is explicitly considered as an important part in the signalling pathway from the agonist to IP_3 formation via PLCβ. The conversion of G-proteins to their active form is described by a separate differential equation, with DAG (which is set equal to IP_3) and Ca_{cyt} being the other variables. (In a follow-up model [76], which was also studied in [132], active PLC was included as a fourth variable.) A direct effect of Ca_{cyt} on PLC is not considered. Rather, the model includes an inactivation of G-protein via PKC, activation of PKC by Ca_{cyt} and a putative positive effect of IP_3 (or DAG) on PLC. In principle, the latter feedback can be used for constructing a two-dimensional model without CICR [76]. However, so far there is no experimental evidence for this mechanism.

Table 2. Overview of some three-dimensional models of Ca^{2+} oscillations.

Model variables	References
Ca_{cyt}, Ca_{er}, IP_3	[12][a] [126,186,189]
Ca_{cyt}, Ca_{er}, Ca in the IP_3-insensitive pool	[186]
Ca_{cyt}, IP_3, inhibition parameter of IP_3R	[12][b]
Ca_{cyt}, IP_3, IP_4	[130]
Ca_{cyt}, DAG (assumed to be equal to IP_3), G_α-GTP	[16]
Ca_{cyt}, PLC, G_α-GTP	[72]
Ca_{cyt}, IP_3, R_a	[99,125][b]
Ca_{cyt}, Ca_{er}, R_a	[89,92,186]
Ca_{cyt}, Ca_m, Ca_{er}	[71,115,162][c]
Ca_{cyt}, B, ER transmembrane potential	[111]

[a] Using the conservation relation $Ca_{cyt} + Ca_{er}/\rho_{er} + Ca_m/\rho_{mit} =$ const. [b] Using the conservation relation $Ca_{cyt} + Ca_{er}/\rho_{er} =$ const. [c] Using the conservation relations $Ca_{cyt} + Ca_{er}/\rho_{er} + Ca_m/\rho_{mit} + B =$ const. and $B +$ free binding sites $=$ const.

Detailed kinetics of the Ca^{2+} release channels

As introduced above, one class of models centre on the dynamics of the IP_3R. Different states of this receptor (e.g. two states [89], five states [54], eight states [18] or 125 states [56]) are distinguished according to the binding of Ca^{2+} and/or IP_3, and the occupancies of the various states are taken as dynamic variables. The transitions between the states are modelled by mass-action kinetics. In most of these models, Ca^{2+} exchange across the plasma membrane is not considered. The models lead to Ca^{2+} oscillations at fixed IP_3 concentration. As a comprehensive overview of these models has been given [103], we will review them here only briefly.

The functional IP_3R consists of four identical subunits [41,133]. Each subunit appears to be endowed with at least one IP_3 binding site and at least one Ca^{2+} binding site. To explain the biphasic effect of Ca_{cyt}, various hypotheses have been put forward. The most commonly shared view is that two Ca^{2+} binding sites exist, with one of these being activating and the other being inhibitory [18,54,99,134]. In the case of independent subunits, this gives rise to seven ($2^3-1 = 7$) independent differential equations for the fractions of the receptor subunit states. The eighth variable is Ca_{cyt}. In the kinetic model of the IP_3R proposed by De Young and Keizer [18], it is assumed that the ligands can bind to any unoccupied site on the receptor irrespective of the binding status of other sites. In the model of Othmer and Tang [134], a sequential binding scheme is proposed: IP_3 has to bind at the IP_3 site before Ca^{2+} can bind to the channel, and Ca^{2+} has to bind to the positive regulatory site before it can bind to the inhibitory site. All of these models reproduce the result that the steady-state fraction of open channels vs. $\log(Ca_{cyt})$ is a bell-shaped curve.

A difficulty in the detailed models of the IP_3R is the uncertainty about the values of the rate constants for the transitions between receptor states. The more different receptor states are considered, the more redundant is of course the parameter identification problem. This is a further motivation, besides the reduction of model dimension, for simplifying the models by the rapid-equilibrium approximation, leading to the models discussed above (cf. [103]). This simplification is feasible if Ca^{2+} binding to the positive regulatory site is a fast process compared with that of binding to the inhibitory site.

The dual effect of Ca_{cyt} and IP_3 on the IP_3R can be considered as an allosteric effect. Along these lines, an alternative approach to describing the kinetics of the IP_3R, based on the Monod model of cooperative, allosteric enzymes was presented [92]. This model is again able to

mimic the bell-shaped curve of the dependence of Ca^{2+} release from the vesicular compartments on Ca_{cyt}, whereas the IP_3 binding process itself is not cooperative. The model is less complicated than the De Young–Keizer model [18] (in which a sort of Hill equation is derived because it is assumed that three subunits have to be in the activated state in order that the channel opens) in that it involves a smaller number of variables (Table 2), but more sophisticated in that a conformational change in the IP_3R is assumed. Further models describing the kinetics of IP_3-sensitive Ca^{2+} channels include those presented in [56,90,135].

The IP_3R can be phosphorylated (with one phosphate per receptor subunit) by protein kinases A and C and Ca^{2+}/calmodulin-dependent protein kinase II (CaM kinase II) [41]. Sneyd and coworkers [94,136] presented models including phosphorylation of subtype III of the IP_3R. The model proposed for pancreatic acinar cells [94] includes four different states of the receptor with one of these being phosphorylated. Moreover, the model includes Ca_{cyt} as a variable. The open probability curve of the IP_3R is calculated to be an increasing function of Ca_{cyt}, as found for type-III IP_3R [137]. The model can explain long-period baseline spiking typical for cholecystokinin stimulation, which is accompanied with receptor phosphorylation, as well as short-period, raised baseline oscillations. It is worth taking into account the existence of three different subtypes of the IP_3R in modelling studies in more detail because experimental work points to a physiological significance of the differential expression of IP_3R subtypes [56,137–139].

Inclusion of mitochondria

It has been known for several decades that mitochondria contribute significantly to Ca^{2+} sequestration [140–143]. Besides the Ca^{2+} uniporter there are several other Ca^{2+} transport processes across the mitochondrial inner membrane, most notably the permeability transition pore (PTP) [144,145] and the Na^+/Ca^{2+} and H^+/Ca^{2+} exchangers [146,147] which appear to function primarily as export pathways. Over a long time, the accumulation of Ca^{2+} was believed to start at Ca^{2+} concentrations of about 5–10 μM (cf. [144]), which is much higher than physiological Ca_{cyt}. Accordingly, except for the model of Meyer & Stryer [12], mitochondria had first been neglected in studying Ca^{2+}-mediated intracellular signalling. Later experiments re-evaluated the role of mitochondria in this context, showing that mitochondria start to take up Ca^{2+} via the Ca^{2+} uniporter at cytosolic concentrations between 0.5 and 1 μM [145,147,148]. This apparent contradiction with the earlier experiments can be resolved by the fact that, in a number of cells, mitochondria are located near the mouths of channels across the ER membrane [149,150]. In these small regions (the so-called microdomains) between the ER and mitochondria the Ca^{2+} concentrations could be 100- to 1000-fold larger than the average concentration in the cytosol [144,151]. It was found that mitochondria indeed sequester Ca^{2+} released from the ER [146,147,152–155]. For example, in chromaffin cells, around 80% of the Ca^{2+} released from the ER is cleared first into mitochondria [156]. In the light of these findings, the role of mitochondria in Ca^{2+} oscillations was studied [148,157–159]. In particular, it was shown that a change in the energy state of mitochondria can lead to modulation of the shape of Ca^{2+} oscillations and waves, which are generated by autocatalytic release of Ca^{2+} from the ER.

These results have stimulated the inclusion of mitochondria in the modelling of Ca^{2+} oscillations [12,71,115,160–162] and Ca^{2+} homoeostasis [163–165]. In the early model of Meyer & Stryer [12], mitochondria are essential for the occurrence of oscillations (see above). The mitochondrial Ca^{2+} efflux is modelled to be constant. However, this assumption is questionable because the efflux must tend to zero as Ca_m tends to zero.

Selivanov *et al.* [161] modelled the so-called mitochondrial CICR (m-CICR) through the PTPs in the inner membrane as observed experimentally [157,158]. They showed that Ca^{2+} oscillations could arise even in the absence of Ca^{2+} stores other than mitochondria. It remains to be seen whether this is physiologically relevant. While PTPs clearly play a role in the Ca^{2+} dynamics in gel suspensions of mitochondria [158] and in apoptosis in intact cells [152], this is less clear for cells under normal physiological conditions [166,167].

In the model presented previously [71], two basic Ca^{2+} fluxes across the inner mitochondrial membrane are taken into account. The Ca^{2+} uptake by mitochondria is, in agreement with experimental data (see above), modelled by Hill kinetics with a large Hill coefficient to describe a step-like threshold function. For the Ca^{2+} release back to the cytosol, the Na^+/Ca^{2+} and H^+/Ca^{2+} exchangers [146,147] but not PTPs are taken into account and described by a linear rate law. The model shows that mitochondria play an important role in modulating the Ca^{2+} signals and, in particular, could regulate the amplitude of Ca^{2+} oscillations [71]. Ca^{2+} sequestration by mitochondria leads to highly constant amplitudes over wide ranges of oscillation frequency, due to clipping the peaks at about the threshold of fast Ca^{2+} uptake (see also [12]). This is in agreement with the idea of frequency-encoded Ca^{2+} signals (see Frequency encoding). Moreover, keeping the global rise of Ca_{cyt} below 1 μM may be of special importance in preventing the cell from apoptosis. Inclusion of mitochondria can also give rise to a dynamics more complex than simple oscillations (see Chaos and bursting).

FREQUENCY AND AMPLITUDE BEHAVIOUR

For a better understanding of biological oscillations, it is of interest to analyse the dependence of frequency and amplitude on certain parameters (e.g. hormone concentration). In particular, this can help elucidate the role of oscillatory dynamics in information transfer. A straightforward method is by numerically integrating the differential equation system for different parameter values [18,80,113]. However, if several parameters are of interest, this method is very time-consuming. A more systematic way, which is, however, restricted to certain parameter ranges, is the analysis of the neighbourhood of the bifurcations from stable steady states leading to oscillations. The behaviour of oscillations near a bifurcation can often be established analytically. For example, so-called scaling laws exist, which give relevant quantities such as frequency and amplitude as functions of a bifurcation parameter.

While extensive bifurcation analysis has been carried out for models of nerve excitation [168–170], this is not the case for models of Ca^{2+} oscillations. (One paper pursuing this aim is [91]). Nevertheless, several papers deal with special aspects of bifurcations in Ca^{2+} oscillations. These will be reviewed below.

Hopf bifurcations

The most frequent transition leading to self-sustained oscillations in the models developed so far is the Hopf bifurcation (see Appendix). Let ε denote some dimensionless parameter measuring the distance from the bifurcation. For Eqn (7), a convenient parameter is $\varepsilon = 1 - k_{ch}/k^*_{ch}$ with k^*_{ch} being the rate constant of the channel flux at the bifurcation. It can be shown analytically that near a supercritical Hopf bifurcation, the frequency remains nearly constant while the amplitude grows proportionally to the square root of ε, $A \propto \sqrt{\varepsilon}$ (Hopf Theorem, cf. [30]). However, it should be acknowledged that Ca^{2+} oscillations often represent so-called relaxation oscillations, which is due to the presence of both slow and fast processes. If the Ca^{2+} channel is open, Ca^{2+} release is much faster than the pump rate or the leak. Intuitively speaking, in relaxation oscillations, the concentration gradient across the ER membrane accumulated during a slow buildup is dissipated during a sudden discharge. The slow build-up is performed during the intermediate phases between spikes, while the discharge occurs during the first part of the spike (upstroke). The second part of the spike is, depending on the system, fast as well or somewhat slower. Changes in oscillation period are mainly due to variation in the duration of the interspike phase.

In relaxation oscillations, the supercritical Hopf bifurcations (as well the subcritical counterparts) have the striking feature that the growth of the oscillation amplitude near the bifurcation occurs in an extremely small parameter range. Numerical calculations for the subcritical Hopf bifurcation in the Somogyi–Stucki model [17] show that this change is confined to less than 10^{-5}% of the value of k_{ch} [91]. As the trajectories occurring in this range have, in the phase plane, the shape of a duck (*canard* in French), they are called *canard* trajectories [31,169]. In fact, for various models, in diagrams depicting the amplitude vs. a bifurcation parameter [80,89,92,107,171], the emergence of periodic orbits is seen as a virtually vertical line (Fig. 2A), irrespective of whether the Hopf bifurcation is subcritical or supercritical. This implies that, practically, Ca^{2+} oscillations often appear to arise with a finite amplitude even at supercritical Hopf bifurcations.

Upon further increase of the bifurcation parameter, in many models, the oscillations eventually disappear at another Hopf bifurcation with a gradually decreasing amplitude (Fig. 2A). This is because the increase in the parameter reduces time hierarchy. While the bifurcation with a steep increase in amplitude was found more often in experiment [3,4,66] and is certainly physiologically more important because the signal can then be better distinguished from a noisy steady state, also smooth transitions have been observed [17,63]. Some authors have studied situations with parameter values for which time hierarchy is less pronounced at both Hopf bifurcations, so that they both are smoother [18,94,98,125,126].

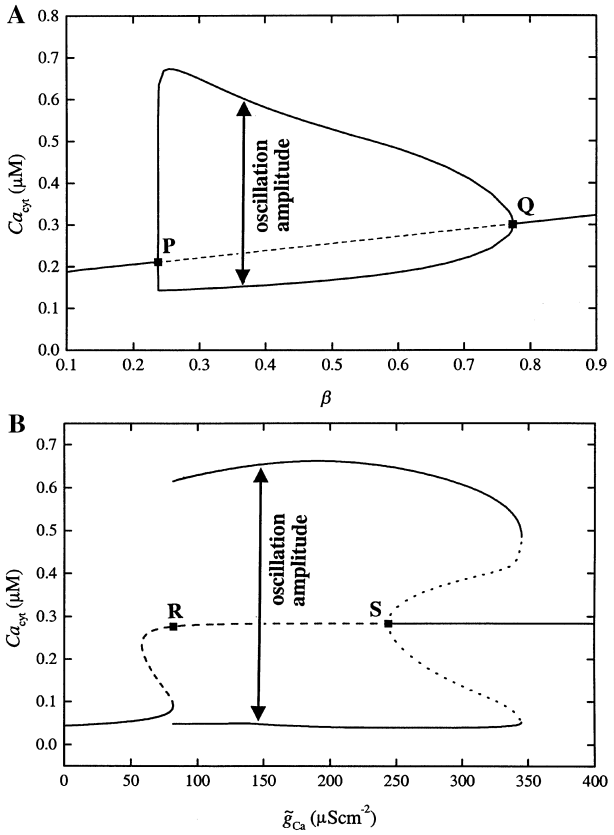

Fig. 2. Bifurcation diagrams for two different models of Ca^{2+} oscillations. Solid lines refer to stable steady states or maximum and minimum values of oscillations. Dashed lines refer to unstable steady states. Dotted lines correspond to maximum and minimum values of unstable limit cycles. (A) One-pool model [80]. β denotes the saturation level of the IP_3R with IP_3. At points P and Q, supercritical Hopf bifurcations with a very steep increase in amplitude and with a gradual decrease in amplitude, respectively, occur. Parameter values are as in Fig. 4 in [80]. (B) Model including Ca^{2+} sequestration by mitochondria [71]. $\tilde{g}_{Ca}$ stands for the maximal ER membrane conductance per unit area. At points R and S, an infinite-period bifurcation and a subcritical Hopf bifurcation with a gradual increase in the amplitude of the unstable limit cycle, respectively, occur.

Global bifurcations

Hopf bifurcations are not the only type of transition by which Ca^{2+} oscillations can arise. For example, in a model including the electric potential difference across the ER membrane and the binding of Ca^{2+} to proteins [113] (see Ca_{cyt}/protein models), a so-called homoclinic bifurcation (see Appendix) was found [91]. For a model of the IP_3R, a homoclinic bifurcation has been discussed briefly in Chapter 5, Exercise 12 in the monograph [101]. A characteristic of the homoclinic bifurcation is that the oscillation period tends to infinity as the bifurcation is approached (see Appendix). In the case of Ca^{2+} oscillations, this is related to a very long duration of the 'resting' phase between spikes, while the shape of spikes remains almost unaltered. It is indeed often found in experiment that spike form is practically independent of frequency. Interestingly, homoclinic bifurcations have also been found for the Hodgkin–Huxley

models of nerve excitation, and are important for the generation of low-frequency oscillations [170].

In a model including the binding of Ca^{2+} to proteins, the ER transmembrane potential and the sequestration of Ca^{2+} by mitochondria [71] (see Inclusion of mitochondria), an infinite-period bifurcation (see Appendix) was found [91]. This bifurcation is also called saddle-node on invariant circle (SNIC) bifurcation [172]. An example is shown in Fig. 2B. As the two newly emerging steady states require an infinite time to be approached or left, the period again diverges to infinity at the bifurcation, while the amplitude remains fairly constant.

Frequency encoding

As mentioned in the Introduction, a widely held hypothesis is that in Ca^{2+} oscillations, information is encoded mainly by their frequency [5,12,70–72,173]. This view is substantiated by the experimental finding that, upon varying hormone stimulation, frequency usually changes more significantly than amplitude. Moreover, Ca^{2+} oscillations usually display a typical spike-like shape with intermediate phases where Ca_{cyt} remains nearly constant. Li *et al.* [174] found in experiments with caged IP_3 that artificially elicited Ca^{2+} oscillations induced gene expression at maximum intensity when oscillation frequency was in the physiological range. On the other hand, the level of activated target protein (see below) is likely to depend also on oscillation amplitude. Accordingly, a possible role of amplitudes in signal transduction by Ca^{2+} oscillations has also been discussed [73–75]. It was shown experimentally that upon pulsatile stimulation of hepatocytes by phenylephrine, not only the frequency but also the amplitude of Ca^{2+} spikes depends on the frequency of stimulation [73]. It was argued that amplitude modulation and frequency modulation regulate distinct targets differentially [175].

For the phenomenon of frequency encoding, it is obviously advantageous if the oscillation frequency can vary over a wide range, while the amplitude remains nearly constant. This is particularly well realized in situations where the period diverges as a bifurcation is approached, while the amplitude remains finite, as it occurs in homoclinic and infinite-period bifurcations. It can be shown that near a homoclinic bifurcation, the period increases proportionally to the negative logarithm of ε, where ε is again some dimensionless distance from the bifurcation, $T \propto (-\log \varepsilon)$ (cf. [30]). In an infinite-period bifurcation, the scaling law reads $T \propto (1/\sqrt{\varepsilon})$. However, it should be checked whether the parameter range in which a significant change in frequency occurs is wide enough to be biologically relevant.

The subcritical Hopf bifurcations in various models do not lead to a diverging period. Nevertheless, time-scale separation in the system and, hence, the relaxation character of the oscillations often become more pronounced near the bifurcation, so that the frequency is indeed lowered drastically (cf. [120]). For the model developed by Somogyi & Stucki [17], for example, an approximation formula for the period, T, as a function of the parameters in the form $T \propto \log(1 + \text{const.}/k_{ch})$ was derived [91]. In general, it may be argued that time hierarchy facilitates frequency encoding. This may be another physiological advantage of such a hierarchy besides the improvement in stability of steady states and the reduction of transition times [86].

It should be acknowledged that in the one-pool models, not only frequency but also amplitude changes significantly depending on agonist stimulation (Fig. 2A). This effect is less pronounced in the two-pool models [80]. As pointed out in Inclusion of mitochondria, the constancy of amplitude is granted particularly well if the height of spikes is limited by sequestration of Ca^{2+} by mitochondria [12,71]. Another mechanism restricting oscillation amplitude is the biphasic dependence of the IP_3R on Ca_{cyt}. Indeed, models including this exhibit fairly constant amplitudes [83,92].

Hopf bifurcations with an extremely steep increase in amplitude share with global bifurcations the abrupt emergence of the limit cycle and the absence of hysteresis. It may be argued that this behaviour is of physiological advantage. A small change in a parameter (e.g. a hormone concentration) can give rise to a distinct oscillation with a sufficiently large amplitude. Thus, misinterpretation of the signal is avoided because, in the presence of fluctuations, a limit cycle with a small amplitude could hardly be distinguished from a steady state. So far, there is no evidence that hysteresis, which would imply that the signal depends on the direction in which the bifurcation is crossed, would be physiologically relevant. Hysteresis occurs, for example, in a subcritical Hopf bifurcation without time-scale separation (Fig. 2B).

Sometimes, it has been argued that the information transmitted by Ca^{2+} oscillations is encoded in the precise pattern of spikes (temporal encoding) rather than in the overall frequency [75]. It is an interesting question whether temporal encoding can be understood as a sequence of frequency changes or whether new concepts are necessary to understand it. In this context, it would be helpful to adopt methods for analysing information in neuronal spike trains (e.g [176]).

Modelling of protein phosphorylation driven by calcium oscillations

Interestingly, the effect caused by the oscillatory Ca^{2+} signal is usually a stationary output, for example, upon fertilizing oocytes, generating a stationary endocrine signal or enhancing the transcription of a gene. In some instances, however, the final cellular output is oscillatory as well, as in the case of secretion in single pituitary cells [177]. The models discussed above provide a sound explanation for the fact that a change in a stationary signal (agonist) can elicit the onset of oscillations. What has been studied much less extensively is how these oscillations can produce an approximately stationary output.

De Koninck & Schulman [178] performed experiments showing that CaM kinase II can indeed decode an oscillatory signal. As this enzyme can phosphorylate a variety of enzymes, the Ca^{2+} signal can be transmitted to different targets. Of particular importance is the autophosphorylation activity of CaM kinase II, because in the phosphorylated form, the enzyme traps calmodulin and keeps being active even after the Ca^{2+} level has decreased. This amounts to a 'molecular memory' [179], by which the oscillatory input is transformed into a nearly stationary output.

It was shown that CaM kinase II activity increased with increasing frequency of Ca^{2+}/calmodulin pulses in a range of high frequencies (1–4 Hz) [178]. However, in electrically

nonexcitable cells, the frequency of Ca^{2+} oscillations is usually below this range. To model the decoding of low-frequency signals, Dupont & Goldbeter [70,180] proposed a model based on an enzyme cycle involving a fast kinase, which is activated by Ca_{cyt}, and a slow phosphatase, which is Ca_{cyt}-independent. Intuitively, it is clear that an integration effect can be achieved in such a system, because the phosphorylation following a Ca^{2+} spike will persist for a while (cf. [69]). The model of Dupont & Goldbeter [70] indeed predicts, with appropriately chosen parameter values, that the mean fraction of phosphorylated protein is an increasing function of frequency. The dependence on frequency is more pronounced if zero-order kinetics for phosphatase and kinase are chosen (cf. the phenomenon of zero-order ultrasensitivity in enzyme cascades [181,182]).

A more detailed model was presented for the liver glycogen phosphorylase [183]. This enzyme includes calmodulin as a subunit. For the Michaelis-type rate law of the phosphorylase kinase, it was assumed that both the maximal activity and Michaelis constant are highly nonlinear functions of Ca_{cyt}. The model shows, both for a sinusoidal input and for oscillations generated by the two-pool model [15], that a given level of active glycogen phosphorylase can be elicited by a lower average Ca_{cyt} level when Ca^{2+} oscillates than when it is stationary.

A mechanism for decoding Ca_{cyt} signals by PKC involving also DAG was proposed by Oancea & Meyer [48] but has not yet been formulated as a mathematical model. A model describing the phosphorylation of CaM kinase and a target protein after cooperative binding of Ca^{2+} to calmodulin as well as the autophosphorylation of CaM kinase was developed by Prank *et al.* [184]. It predicts an increase in activation of target proteins with increasing frequency of the Ca^{2+} signal.

Chaos and bursting

Experimental results very often show more complex forms of Ca^{2+} dynamics than simple, regular oscillations [67,72,185] (for review, see [186]). The most common pattern of such complex oscillations is a periodic succession of quiescent and active phases, known as bursting (Fig. 3). Bursting can be periodic or chaotic. It has been studied intensely in the case of transmembrane potential oscillations in electrically excitable cells [5,60,101,160,172,187]. However, an important difference is worth noting. While often in electric bursting, each active phase comprises several consecutive, large spikes with nearly the same amplitude, in Ca^{2+} bursting, single large spikes are followed by smaller, 'secondary' oscillations.

Complex Ca^{2+} oscillations may arise by the interplay between two oscillatory mechanisms; this is not, however, the only possibility [188]. The underlying molecular mechanisms as well as the biological significance for intracellular signalling are not yet understood in detail (cf. Conclusions). Different agonists may induce different types of dynamics in the same cell type. For example, while hepatocytes exhibit regular Ca^{2+} oscillations when stimulated with phenylephrine, stimulation of the same cells with ATP or UTP elicits regular or bursting oscillations depending on agonist concentration [67,72,185].

Several combinations of three equations out of the system (Eqns 1–6) have been suggested to explain bursting in Ca^{2+} oscillations. Shen & Larter [189] demonstrated regular bursting and transition to chaos in a model involving Ca_{cyt}, Ca_{er} and IP_3. Both the activatory and inhibitory effects of Ca_{cyt} on v_{rel} are included. Moreover, Ca_{cyt} is assumed to activate IP_3 production. Three combinations of variables giving rise to bursting have been studied by Borghans *et al.* [186]. The first model

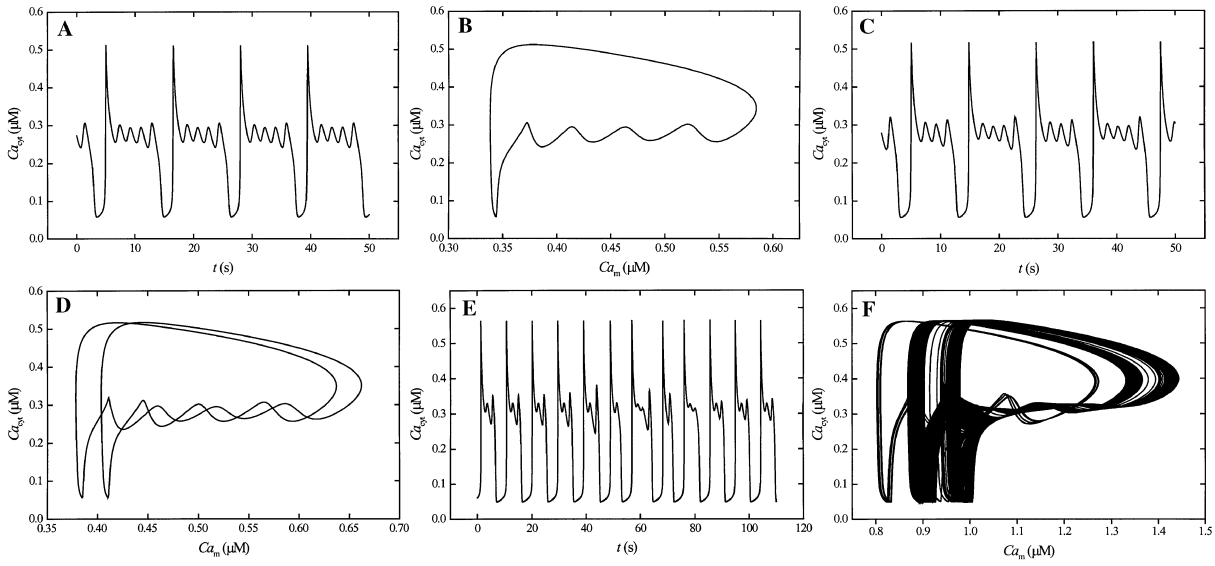

Fig. 3. Dynamic behaviour of the model presented in [115,162] represented as a plot of Ca_{cyt} vs. time (A, C, E) and as a plot in the (Ca_m, Ca_{cyt}) phase plane (B, D, F). (A,B) Simple limit cycle showing periodic bursting. (C,D) Folded limit cycle showing periodic bursting. In the time course, spikes are followed alternately by three or four small-amplitude oscillations. (E,F) Chaotic bursting. Parameter values are as in Table 1 in [115] except for the rate constant of the ER Ca^{2+} channel, k_{ch}, which is 4100 s^{-1} (A, B), 4000 s^{-1} (C, D), or 2950 s^{-1} (E, F).

extends the one-pool model based on Eqn (2) and Eqn (3) by considering the fraction of sensitized IP_3R as a third variable and, accordingly, including Eqn (6). The second model extends an ICC model [130] by including the CICR mechanism. This model can generate not only bursting but also chaotic behaviour. It was further analysed mathematically [126] and shown to admit birhythmicity (i.e. the coexistence of two stable limit cycles, cf. [5]). The third model is based on the two-pool model [14,15] with the Ca^{2+} level in the IP_3-insensitive pool being the third variable. For the first two proposed models, the cause for the transitions between active and quiescent phases can be studied by considering the difference in time scales between the fast, spike-generating subsystem made up of Ca_{cyt} and R_a, or Ca_{cyt} and IP_3, and the slow dynamics of Ca_{er} [186].

Another explanation of complex intracellular Ca^{2+} oscillations has been proposed recently [115,162]. In addition to the ER, also Ca^{2+} sequestration by mitochondria and the Ca^{2+} binding to cytosolic proteins is taken into account. These studies extend earlier work [71] on modelling the possible mitochondrial modulation of Ca^{2+} signals. As the Ca^{2+} exchange across the plasma membrane is neglected, there is a conservation relation involving Ca_{cyt}, Ca_{er}, Ca_m, and B, so that the model is three-dimensional. Simple Ca^{2+} oscillations, periodic and aperiodic bursting and chaos can be obtained with appropriate parameter values (Fig. 3). In all of these regimes, single large-amplitude spikes are followed by small oscillations of nearly constant amplitude. Such small-amplitude oscillations during the quiescent phase are indeed found in experiment, although it is difficult to distinguish them from noise. The transition from a limit cycle to chaos via a folded limit cycle (Fig. 3D) and repeatedly folded limit cycles is known as the period-doubling route to chaos (and was also found in [189]). Interestingly, in other parameter ranges, the succession of behaviours follows the intermittency route to chaos [162]. Besides complex dynamics also birhythmicity and even trirhythmicity can be found [162]. The model predicts that spike amplitudes in the active phases of bursting are remarkably insensitive to changes in the level of agonist. This is due to the fact that mitochondria clip the peaks in Ca_{cyt}, as observed already in the earlier models generating simple oscillations [12,71].

A model proposed by Kummer *et al.* [72] involves the variables Ca_{cyt}, Ca_{er}, and the concentrations of active G_α subunits of the G-protein and active PLC. IP_3 is assumed to be proportional to the latter variable due to quasi-steady-state arguments. The model assumes the presence of two different receptors, for phenylephrine and ATP, both of which activate PLC through the G_α subunit. The rate of G_α activation is modelled as $k_1 + k_2{*}G_\alpha$, with k_1 and k_2 being proportional to the concentrations of phenylephrine and ATP, respectively. (The term $k_2{*}G_\alpha$ describing an autocatalytic activation can be regarded as a linear approximation of $k'_2{*}G_\alpha{*}(GTP_{total} - G_\alpha)$, with the latter complying with the conservation relation for total G-protein.) The model is in particularly good agreement with experimental observations in two respects [72]. First, each oscillation period starts with a large, steep spike followed by a number of pulses of decreasing amplitude around an elevated mean value. Second, varying the parameters k_1 and k_2 independently, one finds that stimulation by ATP can induce (periodic or aperiodic) bursting, while stimulation by phenylephrine can only elicit regular oscillations. From a more theoretical point of view, it is interesting that Kummer *et al.* [72] were able to reduce this model to three dimensions by just excluding Ca_{er} and the fluxes v_{serca} and v_{rel}. The reduced model can still generate chaotic behaviour although the nonlinearities involved are simple Michaelis–Menten rate laws, so that it represents one of the simplest models generating chaos.

In the three-dimensional model of Chay [60,160] (the variables are Ca_{cyt}, Ca_{er} and the cell membrane potential), the essential nonlinearities reside in the ion fluxes across the cell membrane. The model establishes a link between the electrical bursting and calcium bursting in excitable cells. However, experiments indicate that Ca_{cyt} is not likely to be the slow variable underlying electrical bursting in pancreatic β-cells [101].

COUPLING OF OSCILLATING CELLS

Experimental observations

The models discussed so far focus on the temporal evolution of the Ca^{2+} concentration. However, cellular Ca^{2+} transients also have a spatial dimension. In the cytoplasm of single cells, Ca^{2+} gradients can be observed when Ca^{2+} release from the ER is excited at particular subcellular locations [6,42,130]. The local excitation can spread through the cell as a concentration wave, which appears to be propagated by Ca^{2+} diffusion and CICR. In hepatocytes, periodic Ca^{2+} waves are seen that originate from a particular region within a cell [190]. Moreover, in the intact liver and in hepatocyte multiplets, Ca^{2+} waves can spread from cell to cell [191–194]. In contrast to isolated hepatocytes, which exhibit substantial variations of Ca^{2+} oscillation periods between cells when stimulated by hormone, coupled hepatocytes oscillate with the same period [195], or nearly the same period [196]. There are fixed phase relations in that the cells oscillating faster in isolation peak before the slower cells. Thus the intercellular coupling leads to a (near) 1 : 1 entrainment, or synchrony, of the oscillations in adjacent cells. On the larger scale of the liver, periodic Ca^{2+} waves propagate from the periportal to the pericentral region of each liver lobulus independent of the direction of perfusion [193]. The direction of wave propagation may correlate with a gradient in hormone receptor density [197]. Intercellular entrainment of Ca^{2+} oscillations has also been observed in other cell types, such as pancreatic acinar cells [198], articular chondrocytes [199,200], kidney cells [201], and in the blowfly salivary gland [64]. This phenomenon can be viewed a particular instance of the intercellular propagation of Ca^{2+} waves observed in many systems [202–204].

Two pathways have been implicated so far in intercellular Ca^{2+} signalling: (a) the diffusion of cytoplasmic messenger molecules through gap junctions [205–208] and (b) the release of paracrine messengers into the extracellular space and their diffusion to neighbouring cells [209,210]. In the systems in which intercellular entrainment has been observed so far, cells have also been shown to be coupled by gap junctions. In hepatocytes, entrainment is disrupted by gap-junctional uncouplers but not by exclusion of paracrine signalling [195,211].

Modelling approach

To capture the spatial propagation of Ca^{2+} signals, diffusion fluxes of Ca^{2+} and IP_3 must be included in the general balance equations (Eqns 1–6). For Ca_{cyt}, the balance equation then reads

$$\frac{\partial}{\partial t} Ca_{cyt} = \sum_i v_i + D_c \frac{\partial^2}{\partial x^2} Ca_{cyt} \qquad (11)$$

where the v_i denote the Ca^{2+} exchange fluxes with the various compartments, cf. Eqn (2), and D_c is the cytoplasmic Ca^{2+} diffusion coefficient. The Ca^{2+} concentration is now a function of time and spatial position, $Ca_{cyt} = Ca_{cyt}(x, t)$. Likewise the kinetic terms v_i depend on spatial location as functions of $Ca_{cyt}(x, t)$. The spatial dependence of the v_i can also explicitly reflect the subcellular organization of the Ca^{2+} transport processes. A similar balance equation holds for the IP_3 concentration in place of Eqn (11):

$$\frac{\partial}{\partial t} IP_3 = v_{plc} - v_d + D_p \frac{\partial^2}{\partial x^2} IP_3 \qquad (12)$$

where D_p denotes the diffusion coefficient of IP_3. In Eqn (11) and Eqn (12), $\partial^2/\partial x^2$ is the Laplace or diffusion operator. For simplicity, we have given a spatially one-dimensional formulation of the diffusion terms (that can be generalized to two and three dimensions). The x-axis is considered to lie along the direction of Ca^{2+} wave propagation.

As in the case of Eqns (2–4), Eqn (11) can be understood to implicitly contain the effect of fast Ca^{2+} buffering. In addition to the definition of effective rate constants, one can now also define, under certain conditions, an effective diffusion coefficient for Ca^{2+} that includes the effect of Ca^{2+} buffering [105]. In Eqn (11), D_c is understood as such an effective diffusion coefficient. It is generally about an order of magnitude lower than the Ca^{2+} diffusivity in water and also the cytoplasmic IP_3 diffusivity [212]. Moreover, the value of D_c is influenced by the diffusivities and concentrations of Ca^{2+} buffers, which can thus have a decisive impact on the spatial propagation of Ca^{2+} signals [105,108,110].

Although it is generally more difficult to obtain and analyse solutions for the reaction-diffusion Eqns (11,12) than for systems of ordinary differential equations, such as Eqns (1–6), a number of numerical and analytical tools exist [213,214]. In particular, models based on equations of this type can describe the propagation of intracellular Ca^{2+} waves [101,215,216].

If cells are coupled by gap-junctions, in addition to Eqns (11,12) the junctional fluxes must be included in a model. In the absence of membrane potential differences between the cells, these can be assumed proportional to the concentration differences across the junctions for each substance. For example, for a pair of coupled cells the junctional fluxes from cell 1 to cell 2 of Ca^{2+}, j_c, and of IP_3, j_p, can be written as:

$$j_c = P_c[Ca_{cyt}^{(1)}(\text{right end}, t) - Ca_{cyt}^{(2)}(\text{left end}, t)],$$
$$j_p = [IP_3^{(1)}(\text{right end}, t) - IP_3^{(2)}(\text{left end}, t)] \qquad (13)$$

P_c and P_p are the gap-junctional permeabilities for Ca^{2+} and IP_3, respectively. Both Ca^{2+} and IP_3 have been shown to permeate gap junctions in various cells [217,218]. The effect of fast Ca^{2+} buffering on the gap-junctional fluxes can be accounted for in a similar fashion as for the kinetic terms, and P_c can accordingly be defined as an effective gap-junctional permeability for Ca^{2+} [108]. Ca^{2+} buffering reduces the effective intercellular Ca^{2+} permeability. Direct measurements of Ca^{2+} and IP_3 permeabilities are not available in the literature; however, permeability coefficients for various other molecules have been determined in some systems [219,220]. Gap-junctional conductivities (determining the electrical current through the junctions) are also available for many cell types, yet their relation to permeabilities for particular ionic species is not straightforward [83,221].

Comparison of models and experiments

Synchronization and, more generally, entrainment are common phenomena in systems of coupled oscillators. In the case of the intercellular entrainment of Ca^{2+} oscillations, the participating mechanisms and, specifically, the messenger molecules exchanged between cells have been a focus of the experimental work [217,218,222]. Recently, two models relating to experiments in hepatocytes were proposed. They study two specific entrainment mechanisms: intercellular coupling mediated by diffusion of IP_3 [196] and Ca^{2+} [83], cf. Fig. 4.

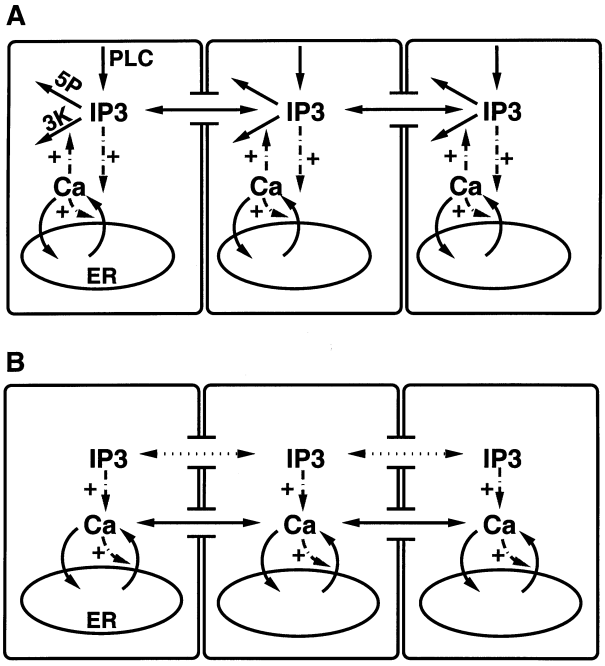

Fig. 4. Two schemes of intercellular coordination of Ca^{2+} oscillations in hepatocytes. (A) Intercellular coupling mediated by gap-junctional IP_3 diffusion; IP_3 oscillates superimposed on Ca_{cyt} because of the assumed Ca^{2+} activation of IP_3 3-kinase (3K) [196]. (B) Intercellular entrainment mediated by diffusion of Ca_{cyt} [83]. No Ca^{2+} feedback on IP_3 consumption has been assumed so that IP_3 is considered to reach a constant level in each cell. Therefore the gap-junctional diffusion of IP_3 (dotted arrows) does not contribute to the dynamic behaviour. 3K, IP_3 3-kinase (EC 2.7.1.127); 5P, IP_3 5-phosphatase (EC 3.1.3.56).

Two extreme possibilities for intercellular coordination can be envisaged. (a) Transient, agonist-induced coordination: intercellular coordination of Ca^{2+} oscillations is a transient phenomenon that is caused by the initial application of hormone and afterwards slowly decays until cells become uncoordinated again. Such a mechanism can in principle work also without any intercellular coupling, though coordination is enhanced by coupling. (b) Active entrainment through coupling: the coordination is inherently caused by the intercellular coupling. As a consequence, cells being uncoordinated in the absence of gap-junctional coupling may become coordinated when coupling is restored, under otherwise constant conditions (e.g. the hormonal stimulus is not changed). In this case, the putative coupling messenger must clearly be sensitive to phase differences of the oscillations in adjacent cells. In the model proposed by Dupont and coworkers [196], Ca^{2+} activation of one of the IP_3-degrading enzymes, IP_3 3-kinase, causes IP_3 oscillations to occur sumperimposed on the Ca^{2+} oscillations (cf. subsection Consideration of the IP_3 dynamics). IP_3 diffusion across gap junctions coordinates the Ca^{2+} oscillations in adjacent cells, but does not lead to stable 1 : 1 entrainment. Immediately after agonist application, there is a transient 1 : 1 coordination which subsequently disappears. The model predictions compare well with a number of experimental results. If there is no Ca^{2+} feedback on IP_3 dynamics, entrainment cannot be brought about by IP_3 diffusion. As shown previously [83], gap-junctional Ca^{2+} fluxes can lead to active 1 : 1 entrainment. Such an autonomous entrainment has been found in experiments with application and subsequent washing out of gap-junctional uncouplers, or transient block of ER Ca^{2+} release [195].

Whether active 1 : 1 entrainment is obtained depends crucially on the gap-junctional permeability. For the hypothesized Ca^{2+} coupling, it was shown that the permeability must lie within certain bounds to obtain correspondence of model simulations and experimental results [83] (a related study was made on intercellular Ca^{2+} waves [223]). If the permeability falls below the critical value for 1 : 1 entrainment (synchronization), entrainment of heterogeneous cells will still occur, but with ratios of the oscillation periods different from 1 : 1. If the frequency ratio of coupled oscillators equals a rational number, this phenomenon is also called phase locking.

Recently, the influence of a number of other processes on intercellular Ca^{2+} wave propagation has been studied for a simple, nonoscillatory model system, including cytoplasmic Ca^{2+} buffering and level of agonist stimulation [108].

CONCLUSIONS

What is the point in oscillations?

A question immediately arising in the context of calcium signalling is why the signal is transmitted by oscillations rather than by adjustable stationary calcium concentrations. This question has often been discussed [12,69,70,86, 113,179,183,224] but surprisingly little work on modelling has been presented so far [70,183,184]. First, it is worth mentioning that not every biological phenomenon necessarily needs to have a reason in terms of evolutionary advantage. It may well be that oscillations just arise because it is hard to avoid them under certain circumstances due to the nonlinearities involved. Moreover, it has even been argued that in certain cells, e.g. cochlear Hensen cells, oscillations are closely related to pathophysiological conditions such as noise-induced hearing loss [225]. The nonlinearities, in turn, are likely to be necessary for a high amplification of signals. The phenomenon of frequency encoding could then be explained by the fact that the mean Ca_{cyt} level increases with increasing frequency due to the special form of the oscillations characterized by spikes and interspike phases of varying length.

Nevertheless, it is of course interesting to speculate about the physiological advantages of oscillatory behaviour. Already the switch between stationary and pulsatile regimes may serve as a (digital) signal, while changes in oscillation frequency may serve as analogue signals. The latter, in turn, may be manifold: they may be encoded by frequency, amplitude, or spike form. It has also been argued that frequency encoded signals could prevent long-lasting receptor desensitization [69] and are more robust to noise [183,226]. Discrete events (spikes) can be recognized as signals better than potentially spurious wanderings of the steady-state concentration [88,207]. Moreover, oscillations are a suitable means for switching on different processes with one and the same second messenger. For example, Dolmetsch *et al.* [224] were able to show that the expression of three different transcription factors in T-lymphocytes was specifically triggered depending on the frequency of Ca^{2+} oscillations.

A special property of Ca^{2+} ion is that concentrations elevated over a longer period are lethal to the cell due to formation of unsoluble Ca^{2+} salts. This harmful effect can be avoided by an oscillatory behaviour. As corroborated by a recent model [183], an oscillatory regime can increase the sensitivity of the Ca^{2+} sensing enzymes to this second messenger because Ca^{2+} can periodically exceed the threshold for enzyme activation even if the average Ca^{2+} level remains below the threshold. Moreover, a very wide range of signal strengths (notably several orders of magnitude) may be achieved. A comparable variation in steady-state levels would imply severe problems with respect to osmotic balance and solvent capacity.

Another advantage arises from the spatial aspect: Coupled oscillators are able to exhibit a wide range of possible behaviours such as synchronization with or without phase shift, phase locking, quasiperiodicity and chaotic regimes. Thus, many more types of different signals could be transmitted from cell to cell than by stationary states.

A further point is the binding to proteins. If the Ca^{2+} level were constant (at different adjustable values), this binding would be in equilibrium, so that the fraction of bound Ca^{2+} were only be determined by the equilibrium constants. In an oscillatory regime, however, also the on and off rate constants are relevant so that the system has more degrees of freedom for fine-tuning regulation.

In view of the models describing bursting and chaos (see earlier), it is interesting to speculate about the physiological role of these phenomena. Again, they might be hard to avoid due to the underlying nonlinearities, as soon as more than two variables are involved. The three-dimensional models showing bursting with small secondary oscillations [72,115,186] show that the effect of one variable can approximately be neglected. Its effect is just a small

fluctuation around a regular oscillation. On the other hand, dynamics with two superimposed oscillatory patterns (Fig. 3) clearly provides more possibilities to encode information. It is interesting to investigate whether this has a physiological significance. For some other biological systems it has been proposed that a possible role of complex (chaotic) oscillations could be the detection of weak signals within cells because of the extreme sensitivity of a chaotic state to periodic forcing [227]. The physiological relevance of chaotic behaviour has been intensely discussed in the case of cardiac chaos [228–230].

Obstacles and prospects

The modelling of Ca^{2+} oscillations is complicated by the wide diversity of the nature of this phenomenon. Their generation in different cell types may not be due to one and the same mechanism (cf. [69]). However, the extensive experimental and theoretical studies on this subject point to a central role of the CICR. Other mechanisms such as the effect of Ca^{2+} on IP_3 turnover or the sequestration of Ca^{2+} by mitochondria play a modulatory role and may be cell-type specific. Accordingly, if not only the occurrence of spike-shaped oscillations in general but more specific phenomena are to be described, specific models must be developed for different cell types, as exemplified by the work on hepatocytes [231], pancreatic acinar cells [94] or pituitary gonadotropes [104]. Relatively little work has been carried out so far on discriminating different models on the basis of experimental data, for example with respect to the mechanisms of spike termination (see Minimal models). Interestingly, even in a given cell, the form and width of spikes may vary depending on the type of agonist used [72,185]. Moreover, the spike form and frequency may vary between different single hepatocytes although being reproducible on the same cell [66], indicating heterogeneity of cellular parameters.

Several problems that arose in the beginning of the work in this field are still unsolved. For example, it is still not clear under what circumstances IP_3 follows a significantly oscillatory regime and whether this is important for modelling Ca^{2+} oscillations. This might depend on oscillation frequency because it was found that PLC is activated by Ca_{cyt} with a saturation at frequencies below the maximum [63]. Moreover, it is still a matter of debate under which conditions frequency encoding or amplitude encoding play the most important role, or whether a more complex mechanism (temporal encoding) is relevant that may have developed during biological evolution.

Future efforts might be spent on a more detailed study of the phosphoinositide pathway, of which the hydrolysis of PIP_2 into IP_3 and DAG is but a tiny part. A number of phosphoinositides linked by kinases and phosphatases have been found to be second messengers [232]. Moreover, it is promising to analyse Ca^{2+} sequestration by the nucleus. As only a very limited number of models describing this have been developed so far [27] (for a review on experimental data, see [233]), we have not included the nucleus in Fig. 1.

It is interesting to discuss the interrelations between bistability and oscillations. The Somogyi–Stucki model [17] as well as a simple chemical model [32,234] are examples of oscillating systems that do not exhibit bistability (unless some parameters are set equal to zero). In these models, the oscillations arise via Hopf bifurcations. By contrast, in order that a homoclinic bifurcation or an infinite-period bifurcation can be observed, the model must admit at least two stationary states. This explains why in all models allowing only for one steady state [15,17,80], neither homoclinic nor infinite-period bifurcations can occur. In contrast, more complex models [71,101,113,172] exhibit bistability and global bifurcations.

Moreover, there is another interesting relationship between oscillatory behaviour and bistability. When the Ca_{cyt}/Ca_{er} models are modified in that the exchange fluxes via the plasma membrane are neglected, they cannot give rise to oscillations anymore because the arising conservation relation causes the systems to be one-dimensional. However, the models reduced in this way exhibit bistability. Analogously, the Meyer–Stryer model [12] exhibits bistability (but no oscillations) when the exchange with mitochondria is neglected. The complex interplay between bistability and oscillations deserves further general studies.

In the modelling of coupled oscillating cells, the phenomenon of entrainment, that is the phase locking between a fast pacemaker oscillator and slower, entrained, oscillators has been studied (see Coupling of oscillating salts). Cells having different intrinsic oscillation frequencies attain, upon coupling, fixed frequency ratios which are quotients of small integers. Up to now, only 1 : 1 entrainment has been studied in some detail. However, the results in [196] point to the possible relevance of ratios different from 1 : 1. Similar phenomena can be observed when a cell capable of Ca^{2+} oscillations is stimulated with an oscillating hormone input [73]. Theoretical studies of this type of entrainment are rare [75,76] and worth being extended. Mathematical modelling should further be exploited in conjunction with experimental work to elucidate the control exerted by the various intracellular mechanisms of Ca^{2+} signalling on the one hand, and the gap-junctional diffusion of Ca^{2+} and IP_3 on the other, on the intercellular coordination of Ca^{2+} oscillations.

In biochemistry, the theoretical analyses of stationary states and the modelling of oscillations have surprisingly developed as relatively separate strands over the last decades. A number of well-established theoretical tools such as metabolic control analysis [86,235,236], metabolic flux analysis [237] and structural analysis of metabolic networks [237,238] have been developed to analyse stationary states. Some of these tools are applicable also to oscillatory systems as long as average fluxes are considered, because for these, the stationary balance equations hold true as well. It is certainly of interest to extend Metabolic Control Analysis to oscillatory processes, to answer questions such as: how are frequency and oscillation controlled by the activity of a given enzyme or the permeability of a channel? Although there are a few attempts (cf. [86]), this extension is far from being complete [239]. Moreover, it is worthwile extending structural analysis, which does not require the knowledge of kinetic parameters, to signal transduction systems. This could help answer questions such as: What structure (topology) of such a system is favourable for a high amplification of signals [182,240] or a signal transmission that is robust to noise?

Information is always linked with a high amplification of some quantity [181,240]. For example, the replacement of one nucleotide in the DNA can have a large effect, or a few hormone molecules may elicit dramatic changes. In view of

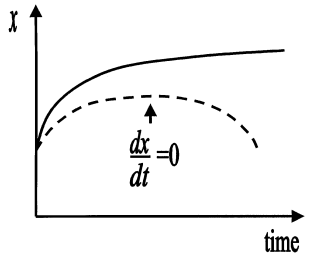

Fig. 5. Scheme illustrating that autonomous oscillations cannot occur in a one-dimensional system. If for a one-dimensional equation, $dx/dt = f(x)$, the curve $x(t)$ were to have a monotonic increasing part and a monotonic decreasing part (dashed curve), it would need to pass a point where the time derivative dx/dt equals zero. At this point, however, $f(x)$ is zero, so that x remains constant and cannot, hence, decrease. Therefore, the dashed trajectory is impossible and oscillations are excluded. In fact, as $f(x)$ tends to zero, the slope of the curve gets smaller and smaller, so that a point where $dx/dt = 0$ can only be reached asymptotically (solid curve).

the small values of the cytosolic Ca^{2+} concentration and the large-scale effects that may be induced by Ca^{2+} oscillations, these oscillations fit into the amplification paradigm. It is worth studying in the future the energetic requirements for amplification in relation to information transfer by Ca^{2+} oscillations.

A general problem in the analysis of chaotic time-series is the difficulty to distinguish deterministic chaos from oscillations superimposed by stochastic noise [241]. The distinction between regular oscillations and bursting is clearly much simpler. Further work could also concern the question whether stochastic resonance (i.e. the amplification of weak signals by noise, cf. [242]) plays a role in Ca^{2+} signalling. First results in this direction have been obtained [132].

Experimentalists sometimes criticize models by saying that these just reproduce what was found earlier in experiment. However, we believe that the quantitative description constitutes a necessary step in the understanding of a cellular system. Mathematical models in cell biology can be very helpful because they explain why a certain phenomenon occurs and may lead to new or deeper insight (such as by distinguishing molecular mechanisms that can give rise to oscillations from those which can not). The molecular interactions involved in Ca^{2+} oscillations (e.g. the activation and inhibition of the IP_3 receptor by its agonists) are so complex that they cannot be understood intuitively. Thus, Ca^{2+} dynamics constitutes an excellent example demonstrating the use of mathematical models. Hopefully, the interaction between experiment and theory will lead to further progress so that modelling increasingly gains predictive power.

APPENDIX: MATHEMATICAL FUNDAMENTALS

For dynamical systems described by autonomous ordinary differential equations, a system of at least two equations is required to describe oscillations (see Fig. 5). Moreover, it can be shown that, with autonomous ordinary differential equations, the system should at least be three-dimensional to describe chaos. This follows from the Theorem of Poincaré & Bendixson for two-dimensional systems (cf. [243]). This theorem says that if, and only if, a trajectory remains for all times, starting with a certain time point, within a finite region of the phase plane without approaching a stationary state, this trajectory is periodic or tends to a periodic trajectory as $t \rightarrow \infty$. To understand this, it is helpful to realize (although this is not a mathematical proof) that a trajectory cannot cross itself because the differential equations,

$$dx_1/dt = f_1(x_1, x_2) \quad \text{and}$$

$$dx_2/dt = f_2(x_1, x_2)$$

determine, for each point x_1,x_2, the direction of the trajectory uniquely. If, in a two-dimensional system, a chaotic trajectory arose, it would have to avoid to tend to a stationary point and to spiral to a limit cycle (Fig. 6). To avoid the latter, it would have to move in opposite directions in increasingly closer positions. This is impossible because, as long as the functions f_1 and f_2 are smooth enough (which is, in biochemical kinetics, always the case), this direction cannot change dramatically for points lying close together.

To analyse the oscillation models mathematically, it is helpful to begin with an investigation of the potential steady states in the system. This is because they can be found more easily than limit cycles and because the stability analysis of steady states can be instrumental in the detection of oscillations. From the Theorem of Poincaré & Bendixson, it follows that, in two-dimensional systems, the existence of

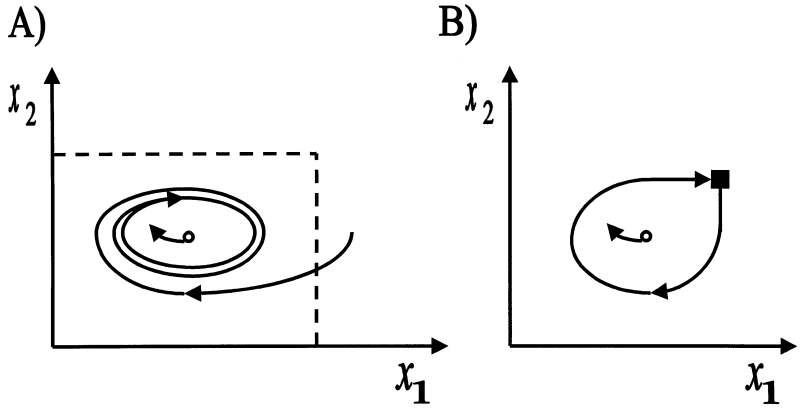

Fig. 6. Schematic illustrations of the Poincaré-Bendixson theorem and a homoclinic orbit. (A) Scheme illustrating the Poincaré–Bendixson theorem. If the trajectory cannot leave the region bounded by the dashed lines and does not tend to a steady state, it must tend to a limit cycle. The empty circle refers to an unstable steady state (unstable focus). (B) Schematic picture of a homoclinic orbit. Full square, steady state that is stable in one direction an unstable in another (saddle point); empty circle, unstable focus.

a finite region as described above and of an unstable focus (i.e. a point from which the trajectory spirals away) lying in this region implies the existence of a stable limit cycle in this region. Moreover, the number of steady states is interrelated with the type of potential transitions to limit cycles (see below).

Positive feedback is a potential mechanism for the generation of self-sustained oscillations ('back-activation oscillator') [29,86,244]. Mathematically, this can be shown by analysing the Jacobian matrix,

$$\mathbf{J} = \begin{pmatrix} \partial f_1/\partial x_1 & \partial f_1/\partial x_2 \\ \partial f_2/\partial x_1 & \partial f_2/\partial x_2 \end{pmatrix}$$

The trace of matrix **J**, that is, the expression $(\partial f_1/\partial x_1) + (\partial f_2/\partial x_2)$ as well as its determinant can be positive in the presence of positive feedback. This leads to an unstable focus. It is worth noting that on the basis of a simple mass-action kinetics, reaction systems with only two variables cannot exhibit limit cycles if only monomolecular and bimolecular reactions are involved [245], while systems with three variables can [32]. Note that the oscillations arising in the well-known Lotka–Volterra model in population dynamics (cf. [246]), which is two-dimensional and involves, from a chemical point of view, only monomolecular and bimolecular reactions, are not limit cycles because they do not attract neighbouring trajectories. The (two-dimensional) Brusselator model [28], which gives rise to limit-cycle oscillations, involves a trimolecular reaction.

When a parameter of the system (e.g. a rate constant) is changed, a point may be reached where the dynamic behaviour changes qualitatively (for example, the number of steady states may change). Such a point is called a bifurcation. An example is provided by a transition from a stable focus (i.e. a steady state for which all trajectories starting in its neighbourhood spiral towards it; the trace of the Jacobian matrix is then negative while the determinant is still positive) to an unstable focus with the emergence of a limit cycle. This bifurcation was called after the mathematician E. Hopf (cf. [5,30,86]. In two-dimensional systems, Hopf bifurcations arise when the trace of the Jacobian matrix equals zero.

Hopf bifurcations are supercritical or subcritical according to whether the limit cycle bifurcating from the steady state is stable (points P and Q in Fig. 2A) or unstable (point S in Fig. 2B), respectively (cf. [30]). At a supercritical Hopf bifurcation, crossing the bifurcation point leads to a smooth transition from a steady state to a limit cycle, if the growth in amplitude is not too steep. Then it is often called soft excitation. At a subcritical bifurcation, a jump from the steady state to infinity or to a coexisting domain of attraction occurs. Very frequently the attractor is a stable limit cycle. Accordingly, the amplitude jumps from zero to a finite value at the bifurcation (hard excitation).

Hopf bifurcations, as well as the transition from monostability to bistability, are called local bifurcations because qualitative changes occur, in the phase space spanned by the system's variables, only in a neighbourhood of the steady state. At global bifurcations, by contrast, a qualitative change occurs in a larger region in phase space. An example is provided by the homoclinic bifurcation (cf. [30]), at which a limit cycle coalesces with an unstable steady state (more specifically, a saddle point) to form a homoclinic orbit (see Fig. 6B) and disappears beyond the bifurcation. The velocity of the trajectory tends to zero as it approaches this steady state (exactly at this point, the velocity is zero). Therefore, the period of the limit cycle tends to infinity as the homoclinic bifurcation is approached. When the bifurcation is crossed in the opposite direction, the limit cycle emerges all of a sudden with a finite amplitude. Another global bifurcation leading to a diverging period is the infinite-period bifurcation (cf. [30]). When a limit cycle disappears in an infinite-period bifurcation, a new steady state appears exactly on the limit cycle and starts dividing into two steady states, with one of them being stable and the other being unstable. The trajectory then runs towards the stable steady state, so that a cyclic orbit can no longer be observed.

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