

# Minimal model for signal-induced $\text{Ca}^{2+}$ oscillations and for their frequency encoding through protein phosphorylation

( $\text{Ca}^{2+}$  signaling/inositol 1,4,5-trisphosphate/biochemical oscillations/ $\text{Ca}^{2+}$ -activated protein kinase)

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**ABSTRACT** In a variety of cells, hormonal or neurotransmitter signals elicit a train of intracellular  $\text{Ca}^{2+}$  spikes. The analysis of a minimal model based on  $\text{Ca}^{2+}$ -induced  $\text{Ca}^{2+}$  release from intracellular stores shows how sustained oscillations of cytosolic  $\text{Ca}^{2+}$  may develop as a result of a rise in inositol 1,4,5-trisphosphate ( $\text{InsP}_3$ ) triggered by external stimulation. This rise elicits the release of a certain amount of  $\text{Ca}^{2+}$  from an  $\text{InsP}_3$ -sensitive intracellular store. The subsequent rise in cytosolic  $\text{Ca}^{2+}$  in turn triggers the release of  $\text{Ca}^{2+}$  from a second store insensitive to  $\text{InsP}_3$ . In contrast to the model proposed by Meyer and Stryer [Meyer, T. & Stryer, L. (1988) *Proc. Natl. Acad. Sci. USA* 85, 5051–5055], the present model, which contains only two variables, predicts the occurrence of periodic  $\text{Ca}^{2+}$  spikes in the absence of  $\text{InsP}_3$  oscillations. Such results indicate that repetitive  $\text{Ca}^{2+}$  spikes evoked by external stimuli do not necessarily require the concomitant, periodic variation of  $\text{InsP}_3$ . The model is closely related to that proposed by Kuba and Takeshita [Kuba, K. & Takeshita, S. (1981) *J. Theor. Biol.* 93, 1009–1031] for  $\text{Ca}^{2+}$  oscillations in sympathetic neurones, based on  $\text{Ca}^{2+}$ -induced  $\text{Ca}^{2+}$  release. We extend their results by showing the minimal conditions in which the latter process gives rise to periodic behavior and take into account the role of the rise in  $\text{InsP}_3$  caused by external stimulation. The analysis further shows how signal-induced  $\text{Ca}^{2+}$  oscillations might be effectively encoded in terms of their frequency through the phosphorylation of a cellular substrate by a protein kinase activated by cytosolic  $\text{Ca}^{2+}$ .

Hormonal or neurotransmitter signals evoke repetitive spikes of intracellular  $\text{Ca}^{2+}$  in a variety of cells such as hepatocytes (1, 2), pituitary gonadotropes (3) and somatotropes (4), endothelial cells (5), and fibroblasts (6) as well as in eggs upon fertilization (7, 8). This oscillatory phenomenon is characterized by periods ranging from  $<1$  s to some 30 min, depending on the cell type (9, 10). The ubiquity of signal-induced  $\text{Ca}^{2+}$  oscillations suggests that the effects of second messengers might be encoded primarily by the frequency of their periodic evolution (2–6, 9, 10). Here we present a simple theoretical model for signal-induced  $\text{Ca}^{2+}$  oscillations and show how the latter may be effectively transduced into a frequency-dependent cellular response through protein phosphorylation by a  $\text{Ca}^{2+}$ -activated kinase.

What is the origin of the repetitive  $\text{Ca}^{2+}$  spikes evoked by extracellular stimuli? Inositol 1,4,5-trisphosphate ( $\text{InsP}_3$ ) mediates the action of many external signals in inducing the release of  $\text{Ca}^{2+}$  from intracellular stores (11). It has been suggested (2) that  $\text{Ca}^{2+}$  oscillations originate from a negative feedback exerted by this ion on phosphatidylinositol-specific phospholipase C. An alternative, theoretical model (12), which also relies on  $\text{InsP}_3$  oscillations, predicts the occurrence of  $\text{Ca}^{2+}$  spikes because of cross-catalytic interactions

between the two second messengers. On the other hand, Berridge and colleagues have suggested (9, 10, 13) that the oscillations in  $\text{Ca}^{2+}$  may arise solely from the cycling of this ion between the cytosol and a pool insensitive to  $\text{InsP}_3$ , once a certain amount of  $\text{Ca}^{2+}$  has been released into the cytosol from a second pool, after the rise in  $\text{InsP}_3$  triggered by the external signal. On the basis of their experimental results, Jacob *et al.* (5) considered a variety of possible mechanisms for  $\text{Ca}^{2+}$  spiking and ruled out the cellular membrane potential as a source for the observed oscillations. Likewise, the injection of  $\text{InsP}_3$  into cells has been shown to elicit oscillatory  $\text{Ca}^{2+}$  transients, suggesting that the periodic generation of  $\text{InsP}_3$  can be bypassed as a source of repetitive  $\text{Ca}^{2+}$  spikes (13–16). The model presented below indicates that repetitive spikes of  $\text{Ca}^{2+}$  may arise from a steady increase in  $\text{InsP}_3$  elicited by external stimulation, on the basis of the self-amplified release of  $\text{Ca}^{2+}$  from intracellular stores. That the latter process might lead to  $\text{Ca}^{2+}$  oscillations has been considered for nerve (17) and cardiac cells (18–20). Here we generalize these studies and extend them by taking the role of  $\text{InsP}_3$  explicitly into account.

## Minimal Model for Signal-Induced $\text{Ca}^{2+}$ Oscillations

Following Berridge (9, 10, 13), we assume that the external signal triggers the synthesis of  $\text{InsP}_3$ , whose effect is merely to cause the discharge of an intracellular pool of  $\text{Ca}^{2+}$ , the level of which ion thereby rises in the cytosol (Fig. 1). To determine whether oscillations can occur solely by the cycling of  $\text{Ca}^{2+}$  between the cytosol and the  $\text{InsP}_3$ -insensitive pool, we make the simple hypothesis that as long as the stimulus is present, its effect is to produce a net, constant influx of  $\text{Ca}^{2+}$  from the  $\text{InsP}_3$ -sensitive pool. This pool is considered to remain constant as a result of rapid replenishment by influx of extracellular  $\text{Ca}^{2+}$  through an autoregulatory mechanism controlled by the  $\text{Ca}^{2+}$  content of the  $\text{InsP}_3$ -sensitive pool (21, 22) and by the uptake of cytosolic  $\text{Ca}^{2+}$  after a spike (Fig. 1). Such refilling of the  $\text{InsP}_3$ -sensitive pool may be favored by inositol 1,3,4,5-tetrakisphosphate ( $\text{InsP}_4$ ), a metabolic product of  $\text{InsP}_3$  (23). However, formation of  $\text{InsP}_4$  is not necessary to establish oscillations because these have been observed in pancreatic cells after perfusion with a nonmetabolizable analogue of  $\text{InsP}_3$  (16). The magnitude of the influx,  $v_1\beta$ , from this  $\text{InsP}_3$ -sensitive pool is taken as proportional to the saturation function  $\beta$  of the receptor for  $\text{InsP}_3$  [the cooperative nature of this saturation function (24) is expressed implicitly in  $\beta$ ]; the level of  $\text{InsP}_3$  established upon stimulation increases with the magnitude of the external signal. Thus,  $\text{InsP}_3$  regulates the flow of  $\text{Ca}^{2+}$  into the cytosol, which primes the  $\text{InsP}_3$ -insensitive pool for oscillatory cycles of  $\text{Ca}^{2+}$  release (Fig. 1).

The two variables of the model are the concentration of free  $\text{Ca}^{2+}$  in the cytosol and in the  $\text{InsP}_3$ -insensitive pool

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Abbreviation:  $\text{InsP}_3$ , inositol 1,4,5-trisphosphate.

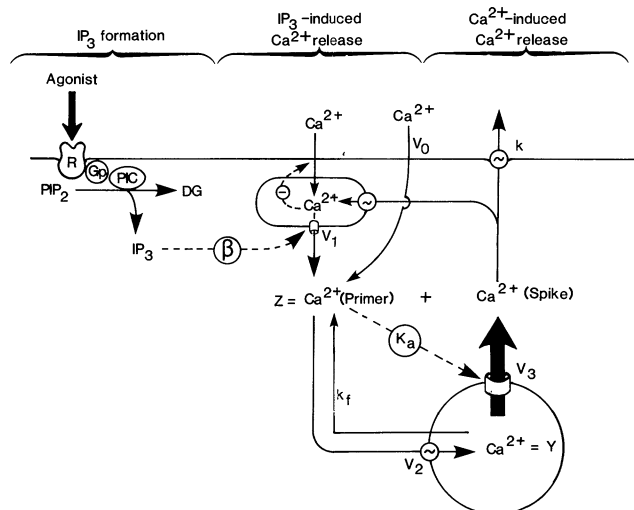


FIG. 1. Schematic representation of the mechanism generating  $\text{Ca}^{2+}$  oscillations, based on the self-amplified release of  $\text{Ca}^{2+}$  from intracellular stores. Agonists act through a transducing mechanism comprising a receptor (R), a G-protein (Gp), and phosphatidylinositol-specific phospholipase C (PIC) to hydrolyze the inositol lipid phosphatidylinositol 1,4-bisphosphate into diacylglycerol (DG) and  $\text{InsP}_3$  ( $\text{IP}_3$ ).  $\text{InsP}_3$  modulates the release of  $\text{Ca}^{2+}$  from an  $\text{InsP}_3$ -sensitive store into the cytosol ( $v_1$ ) and indirectly controls the influx of external  $\text{Ca}^{2+}$  into this store through the capacitative mechanism proposed by Putney (21). In the model, therefore, each level of  $\text{InsP}_3$  controls a constant flow of  $\text{Ca}^{2+}$  into the cytosol, determined by  $v_1\beta$ . The concentration of cytosolic  $\text{Ca}^{2+}$  ( $Z$ ) passes through two phases, one of low concentration, during which primer  $\text{Ca}^{2+}$  is being transferred ( $v_2$ ) into the  $\text{InsP}_3$ -insensitive pool, interspersed with brief  $\text{Ca}^{2+}$  spikes when  $\text{Ca}^{2+}$  stored in that pool ( $Y$ ) is released to the cytosol ( $v_3$ ) in a process activated by cytosolic  $\text{Ca}^{2+}$ . Parameters  $v_0$ ,  $k$ , and  $k_f$  relate respectively to the influx of extracellular  $\text{Ca}^{2+}$  into the cytosol, to the efflux of cytosolic  $\text{Ca}^{2+}$  from the cell, and to a passive leak of  $Y$  into  $Z$ . See text for further details.

(e.g., the endoplasmic or sarcoplasmic reticulum); these variables are denoted by  $Z$  and  $Y$ , respectively. When assuming that buffering is linear with respect to  $\text{Ca}^{2+}$  concentration, the time evolution of the system is governed by the two kinetic Eqs. 1:

$$\begin{aligned} \frac{dZ}{dt} &= v_0 + v_1\beta - v_2 + v_3 + k_f Y - kZ \\ \frac{dY}{dt} &= v_2 - v_3 - k_f Y. \end{aligned} \quad [1]$$

In these equations,  $v_0$  and  $kZ$  relate, respectively, to the influx and efflux of  $\text{Ca}^{2+}$  into and out of the cell, which take place even in the absence of external stimulation; for simplicity, these terms are taken as constant and linear, respectively. The rate of ATP-driven pumping of  $\text{Ca}^{2+}$  from the cytosol into the  $\text{InsP}_3$ -insensitive store is denoted  $v_2$ , while  $v_3$  represents the rate of transport from this pool into the cytosol; the term  $k_f Y$  refers to a nonactivated, leaky transport of  $Y$  into  $Z$  (although not essential for the occurrence of oscillations, this process was found to stabilize the amplitude of  $\text{Ca}^{2+}$  transients at different levels of stimulation). In the above equations, all concentrations and rates are defined with respect to the total cell volume.

When the cell receives an external signal, this triggers an increase in  $\text{InsP}_3$ , which leads to a rise in the saturation function  $\beta$  and, subsequently, to an increase in cytosolic  $\text{Ca}^{2+}$ . We wish to determine the conditions in which this initial rise elicits a train of  $\text{Ca}^{2+}$  spikes. As the system of Eqs. 1 comprises only two variables, it is possible to resolve this

question by resorting to phase plane analysis (25). In particular, the Bendixson criterion indicates that activation of  $v_3$  by  $Z$  is most appropriate for inducing sustained oscillations upon external stimulation (26). This condition corresponds to an activation by cytosolic  $\text{Ca}^{2+}$  of the transport of  $\text{Ca}^{2+}$  from the intracellular store into the cytosol. Evidence in favor of such control has been obtained in skinned cardiac cells (27) and sarcoplasmic reticulum vesicles (28) and could provide the molecular basis for the observed  $\text{Ca}^{2+}$ -induced  $\text{Ca}^{2+}$  release described for muscle (29) and cardiac (30) cells as well as oocytes (31). An alternative view (9, 10, 13, 18) attributes the latter phenomenon to the progressive filling of the intracellular store, which is triggered to release its  $\text{Ca}^{2+}$  when the concentration within the store reaches a threshold for secretion. The present model predicts that at least in the absence of time delays, such a process does not suffice to induce a sustained oscillatory response.

When taking into account the cooperative nature of the pumping process and of the  $\text{Ca}^{2+}$  release from the intracellular store and the positive feedback exerted on the latter transport by cytosolic  $\text{Ca}^{2+}$ , the rates  $v_2$  and  $v_3$  in Eqs. 1 take the form given by Eqs. 2:

$$v_2 = V_{M2} \frac{Z^n}{K_2^n + Z^n}, \quad v_3 = V_{M3} \frac{Y^m}{K_R^m + Y^m} \cdot \frac{Z^p}{K_A^p + Z^p}. \quad [2]$$

In these equations,  $V_{M2}$  and  $V_{M3}$  denote respectively the maximum rates of  $\text{Ca}^{2+}$  pumping into and release from the intracellular store; these processes are described by Hill functions whose cooperativity coefficients are taken as  $n$  and  $m$ ;  $p$  denotes the degree of cooperativity of the activation process;  $K_2$ ,  $K_R$ , and  $K_A$  are threshold constants for pumping, release, and activation.

Eqs. 1 and 2 admit a unique steady-state solution. Linear stability analysis (25) of these equations indicates that the steady state is not always stable. It is well known from studies of physicochemical systems that sustained oscillations of the limit-cycle type develop precisely when the nonequilibrium steady state becomes unstable (32).

We consider a situation in which the system, in the absence of stimulation, is initially in a stable steady state characterized by a low cytosolic  $\text{Ca}^{2+}$  level close to  $0.1 \mu\text{M}$ . Fig. 2 Upper shows how the system responds to an increase in  $\beta$  up to 30% due to a rise in  $\text{InsP}_3$  triggered by the external signal. Repetitive spikes of cytosolic  $\text{Ca}^{2+}$  occur (solid line in Fig. 2 Upper); these oscillations are accompanied by a sawtooth variation of the  $\text{Ca}^{2+}$  content of the intracellular store (dashed line in Fig. 2 Upper). The waveform of the cytosolic  $\text{Ca}^{2+}$  spikes resembles that observed in some experiments; in particular, a progressive rise in  $\text{Ca}^{2+}$  often precedes the  $\text{Ca}^{2+}$  spike (5). The period of the oscillations in Fig. 2 is of the order of 1 s, as in some experimental systems (3, 4, 18). Periods of the order of 1 min or more observed in other cells (1, 2, 5, 6) are readily obtained when the kinetic parameters are divided by a factor of 10–100; such variations of  $\text{Ca}^{2+}$  pumping and transport rates in different cell types are rather common (33) and may result in part from differences in the volumes of various cells and intracellular stores.

A characteristic feature of the oscillations in liver, and perhaps also in other cells, is that the shape of each spike can vary depending on the agonist employed (1, 2). For example, norepinephrine gave narrow symmetrical transients, whereas those induced by vasopressin were much broader (1, 2, 34). Rooney *et al.* (34) have suggested that this broadening of the spike may be explained by the agonist having the additional effect of inhibiting the extrusion of  $\text{Ca}^{2+}$  ( $k$ ). There already is experimental evidence that agonists can inhibit the plasma membrane  $\text{Ca}^{2+}$  pump, with vasopressin being more effective than norepinephrine (35, 36). When we reduced the  $\text{Ca}^{2+}$

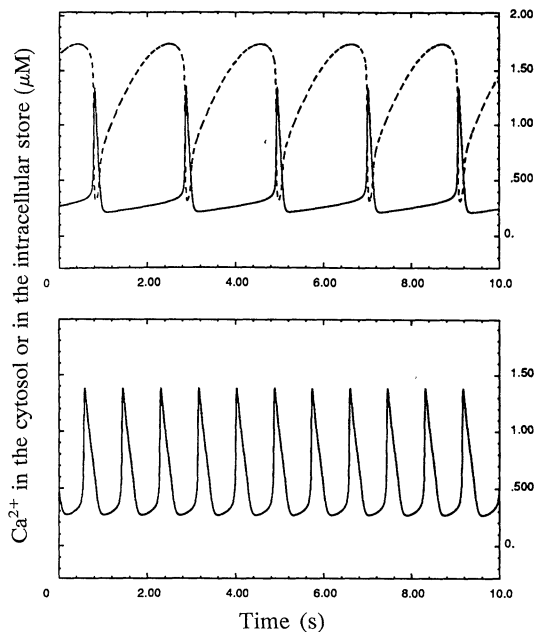


FIG. 2. (Upper) Oscillations in cytosolic  $\text{Ca}^{2+}$  ( $Z$ ; solid line) brought about by an increase in  $\beta$  up to 30.1% triggered by external stimulation. The concentration of  $\text{Ca}^{2+}$  in the  $\text{InsP}_3$ -insensitive intracellular store ( $Y$ ; dashed line) has a concomitant sawtooth variation; for the sake of clarity, the actual level of  $Y$  (defined with respect to the total cell volume) has been decreased by  $0.35 \mu\text{M}$ . The curves are obtained by integration of Eqs. 1 and 2 for the following parameter values, which are in a physiological range (33):  $v_0 = 1 \mu\text{M}\cdot\text{s}^{-1}$ ,  $k = 10 \text{ s}^{-1}$ ,  $k_f = 1 \text{ s}^{-1}$ ,  $v_1 = 7.3 \mu\text{M}\cdot\text{s}^{-1}$ ,  $V_{M2} = 65 \mu\text{M}\cdot\text{s}^{-1}$ ,  $V_{M3} = 500 \mu\text{M}\cdot\text{s}^{-1}$ ,  $K_2 = 1 \mu\text{M}$ ,  $K_R = 2 \mu\text{M}$  (defined with respect to the total cell volume),  $K_A = 0.9 \mu\text{M}$ ,  $m = n = 2$ , and  $p = 4$ . Oscillations also occur, for different parameter values, with  $m = n = p = 1$ . (Lower) Oscillations of cytosolic  $\text{Ca}^{2+}$  obtained for the smaller value of the  $\text{Ca}^{2+}$  extrusion rate constant,  $k = 6 \text{ s}^{-1}$ ; other parameter values are as in Upper.

extrusion rate constant  $k$  from 10 to  $6 \text{ s}^{-1}$ , there was a significant enlargement of the  $\text{Ca}^{2+}$  spikes mainly because of slower decay (Fig. 2 Lower), which thus could account for the shape of the transients observed experimentally with some agonists (1, 2, 34). The broadening of the peaks obtained by decreasing the extrusion rate constant at a given value of  $\beta$  was accompanied by an increase in frequency (compare Fig. 2 Upper and Lower), which is a theoretical prediction.

How the period of  $\text{Ca}^{2+}$  oscillations varies with the extent of stimulation, as measured by  $\beta$ , is depicted in Fig. 3. The results agree qualitatively with experimental observations. Thus, the initiation of  $\text{Ca}^{2+}$  spikes and the rise in their frequency upon increasing stimulation are observed in a variety of cells (1–6, 9, 10, 18). The final disappearance of the spikes with the concomitant establishment of a high, constant level of cytosolic  $\text{Ca}^{2+}$  is also observed, for example in endothelial cells stimulated by a sufficiently large amount of histamine (5). The amplitude of  $\text{Ca}^{2+}$  oscillations, measured by the maximum  $\text{Ca}^{2+}$  level, is of the order of 1–1.5  $\mu\text{M}$ ; like the half-width of the  $\text{Ca}^{2+}$  spikes (not shown), it remains practically independent of the extent of stimulation (Fig. 3), in agreement with experimental observations (2, 34).

An important property of the present model is that repetitive  $\text{Ca}^{2+}$  spikes can occur in the absence of  $\text{InsP}_3$  oscillations. Such a situation is supported by a number of experimental observations (13–16), including those on skinned cardiac cells (18), which lack  $\text{InsP}_3$  production. The model further indicates how oscillations may progressively vanish (5) in the absence of external  $\text{Ca}^{2+}$ : bringing the value of  $v_0$  down to zero eventually suppresses the oscillations; the hypothesis of a constant input  $v_1\beta$  from the  $\text{InsP}_3$ -sensitive

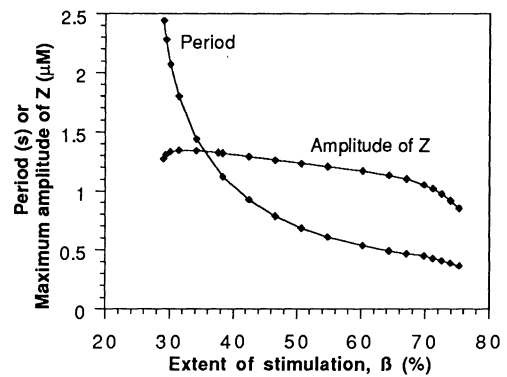


FIG. 3. Period and amplitude of cytosolic  $\text{Ca}^{2+}$  oscillations as a function of the saturation function  $\beta$  of the  $\text{InsP}_3$  receptor, which rises with the external stimulation. For the values of  $v_0$  and  $v_1$  given in Fig. 2, oscillations occur when  $\beta$  ranges from 29.1% to 77.5%. A stable steady state is reached outside this range; this state corresponds to a low value of cytosolic  $\text{Ca}^{2+}$  for  $\beta < 29.1\%$  and to a larger value, close to  $0.7 \mu\text{M}$ , for  $\beta > 77.5\%$ . Similar results are obtained when plotting the amplitude and the period as a function of the influx of extracellular  $\text{Ca}^{2+}$ ,  $v_0$ , for a given value of  $v_1\beta$ . Points denote results obtained by numerical integration of Eqs. 1 and 2 for the parameter values of Fig. 2.

$\text{Ca}^{2+}$  store also ceases to hold in these conditions, as this store can no more be replenished. On the other hand, since the bifurcation parameter whose increase brings about the oscillations is the sum ( $v_0 + v_1\beta$ ), it can readily be seen that oscillations can be triggered by an increase in  $\beta$  due to stimulation by an external signal, or simply by an increase in  $v_0$  originating from an increase in extracellular  $\text{Ca}^{2+}$ . The analysis suggests that for sufficiently large values of  $v_0$  or for sufficiently low values of  $k$ , repetitive  $\text{Ca}^{2+}$  spikes may develop spontaneously (i.e., in the absence of external stimulation). This situation appears to occur, for example, in growth hormone-secreting somatotropes (4) and cardiac cells (37). The results of Fig. 3 also agree with the experimental observation (6, 38, 39) that the frequency of oscillations rises when the level of extracellular  $\text{Ca}^{2+}$  increases.

$\text{Ca}^{2+}$  spiking can also be induced by caffeine (38). It has been argued (10) that this effect is due to a decrease in the threshold constant  $K_R$  for the release of  $\text{Ca}^{2+}$  from the intracellular store. Such a view holds with the observation that a decrease of  $K_R$  in the model can induce oscillations; further decrease in this parameter leads to an increase in frequency and a decrease in the amplitude of the spikes, as observed when increasing the stimulation by caffeine (10).

Useful information has been gained from experiments on the effect of perturbations by cytosolic pulses of  $\text{Ca}^{2+}$  or  $\text{InsP}_3$  in fibroblasts undergoing  $\text{Ca}^{2+}$  oscillations (6). The model accounts for the observation (6) that large pulses of  $\text{Ca}^{2+}$  reset the oscillations at all phases by inducing the cell to behave as if it started at a normal  $\text{Ca}^{2+}$  peak; similar phase advances have been induced by  $\text{Ca}^{2+}$  pulses in sympathetic neurones (40). The model also predicts that instead of the phase advance obtained with larger pulses, delays can be induced after completion of a  $\text{Ca}^{2+}$  spike by subthreshold pulses of  $\text{Ca}^{2+}$ , just below those inducing significant release from the intracellular store; the transient suppression of  $\text{Ca}^{2+}$  oscillations by a critical  $\text{Ca}^{2+}$  pulse has also been achieved. These effects, which can be accounted for by phase plane analysis (G.D. and A.G., unpublished data), have not yet been observed.

### Frequency Encoding Through Protein Phosphorylation

The idea is emerging that  $\text{Ca}^{2+}$  oscillations might be encoded in terms of their frequency (2–6, 9, 10, 12). The advantages

of such encoding have been discussed for other modes of intercellular communication (41–43). How could  $\text{Ca}^{2+}$  oscillations be translated into a frequency-dependent cellular response? One likely way is through protein phosphorylation. Consider a kinase activated in a Michaelian manner by cytosolic  $\text{Ca}^{2+}$  and a phosphatase, which both act on a protein substrate,  $W$ , whose fraction in the phosphorylated form is denoted  $W^*$ . The time variation of  $W^*$  associated with the oscillations of cytosolic  $\text{Ca}^{2+}$  ( $Z$ ) is given by Eq. 3 (see ref. 44 and the legend to Fig. 4):

$$\frac{dW^*}{dt} = (v_P/W_T) \left[ (v_K/v_P) \frac{1 - W^*}{K_1 + 1 - W^*} - \frac{W^*}{K_2 + W^*} \right]$$

with [3]

$$v_K = V_{MK} \frac{Z}{K_a + Z}$$

Owing to the periodic variation in cytosolic  $\text{Ca}^{2+}$  ( $Z$ ) and to the subsequent variation in kinase activity, the value of  $W^*$  periodically rises and decreases with  $Z$  (Fig. 4). A comparison of curves a and b in Fig. 4 shows that the mean level of  $W^*$  increases with the frequency of  $\text{Ca}^{2+}$  oscillations (i.e., with the level of stimulation, measured by  $\beta$ ); this effect is partly due to a change in the mean value of  $Z$ , which is higher at the larger value of  $\beta$  considered. Equally significant, however, is the fact that when the period of oscillations is longer, the protein undergoes significant dephosphorylation from one  $\text{Ca}^{2+}$  peak to the next; much less dephosphorylation occurs between successive  $\text{Ca}^{2+}$  spikes at the higher frequency of oscillations, resulting in the maintenance of a larger fraction of protein phosphorylated.

Efficient frequency encoding through protein phosphorylation only occurs, however, in precise kinetic conditions. The analysis indicates that saturation of the converter enzymes [which favors the occurrence of “zero-order ultrasensitivity” (44)] allows for a better encoding of  $\text{Ca}^{2+}$  oscillations

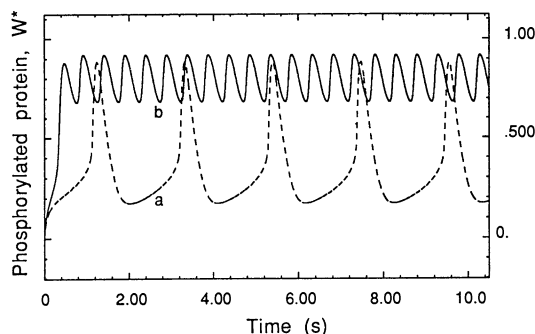


FIG. 4. Oscillations in the level of a protein phosphorylated by a kinase activated by cytosolic  $\text{Ca}^{2+}$ . Curve a is the time evolution of the fraction of phosphorylated protein in the course of the  $\text{Ca}^{2+}$  oscillations shown in Fig. 2 Upper;  $\beta = 30.1\%$ , and the mean fraction of phosphorylated protein over a period,  $\langle W^* \rangle$ , is equal to 0.32. Curve b is the time evolution of protein phosphorylation at a higher level of stimulation;  $\beta = 64.4\%$  and  $\langle W^* \rangle = 0.80$ . The curves are obtained by integration of Eqs. 1–3 with  $W^* = 0$  at time zero for  $v_P = 5 \mu\text{M}\cdot\text{s}^{-1}$ ,  $V_{MK} = 40 \mu\text{M}\cdot\text{s}^{-1}$ ,  $K_a = 2.5 \mu\text{M}$ ,  $W_T = 1 \mu\text{M}$ , and  $K_1 = K_2 = 0.1$ ; these values are in the physiological range for protein phosphorylation systems (45). For these parameter values, the phosphorylation curve for  $W^*$  possesses a sharp threshold, where  $W^* = 0.5$ , in  $v_K/v_P = 1$  (see ref. 44). In Eq. 3,  $v_K$  and  $v_P$  denote the maximum rates of kinase and phosphatase at a given value of  $Z$ ;  $V_{MK}$  is the maximum rate of the kinase at saturation by  $Z$ ;  $K_a$  denotes the constant of activation of the kinase by cytosolic  $\text{Ca}^{2+}$ ;  $K_1 = K_{m1}/W_T$  and  $K_2 = K_{m2}/W_T$ , where  $K_{m1}$  and  $K_{m2}$  denote the Michaelis constants of kinase and phosphatase, while  $W_T$  is the total amount of protein substrate.

as the mean value of the fraction of phosphorylated protein varies over a much larger range (0.2–0.95) with the frequency of  $\text{Ca}^{2+}$  oscillations than it does when the kinase and the phosphatase are not saturated by their substrate (compare curves a and b obtained for the same oscillations in Fig. 5).

The analysis further shows that to obtain a significant variation in phosphorylated protein, cytosolic  $\text{Ca}^{2+}$  would have to oscillate in such a manner that the ratio  $v_K/v_P$  goes above a threshold (here equal to unity; see ref. 44) when  $Z$  reaches its maximum, and below it when  $Z$  decreases to its minimum. Such fine tuning clearly depends on the absolute and relative values of the maximum rates of the kinase and phosphatase, of their Michaelis constants, and on the value of the activation constant  $K_a$ , which corresponds to the  $\text{Ca}^{2+}$  concentration that yields the half-maximum kinase activity.

## Discussion

The above analysis of a simple two-variable model shows that repetitive  $\text{Ca}^{2+}$  spikes may be triggered by external stimulation solely on the basis of the self-amplified release of  $\text{Ca}^{2+}$  from intracellular stores. For oscillations to occur, the latter process should involve the activation of the release by cytosolic  $\text{Ca}^{2+}$  rather than the filling up of intracellular stores beyond a threshold level. Oscillations are brought about by the rise in cytosolic  $\text{Ca}^{2+}$  that is itself induced by the increase in  $\text{InsP}_3$  that follows stimulation. Some stimulations are too weak to produce the rise in  $\beta$  up to the level required for oscillations. This might explain, for example, why in fibroblasts a depolarization by gramicidin and a stimulation by vasopressin are required for inducing oscillations, regardless of the order of the two stimulations; none of the stimuli by themselves can induce repetitive spikes (6). The model suggests that only the combined stimuli raise cytosolic  $\text{Ca}^{2+}$  sufficiently, so that the value of  $(v_0 + v_1\beta)$  increases above the critical threshold required for oscillatory behavior. The single  $\text{Ca}^{2+}$  spike evoked by each stimulus (6) could represent an excitable response.

Besides accounting for a large body of experimental observations, a number of testable predictions were made pertaining to the effect of the parameters on the shape and period of  $\text{Ca}^{2+}$  transients. In the model, an increase in  $(v_0 + v_1\beta)$  will accelerate the oscillations by shortening the phase of cytosolic  $\text{Ca}^{2+}$  accumulation up to the threshold level at which self-amplified release begins, while an increase in  $k$  or  $V_{M2}$  will slow down the production of spikes by increasing the time interval required for triggering  $\text{Ca}^{2+}$  release from intracellular stores. Another prediction concerns the existence of critical pulses of  $\text{Ca}^{2+}$  producing phase delays similar to those

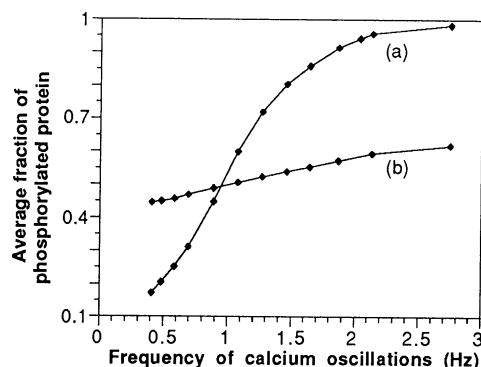


FIG. 5. Frequency encoding of  $\text{Ca}^{2+}$  oscillations through protein phosphorylation. The curves show the average value of the fraction of phosphorylated protein,  $\langle W^* \rangle$ , over a period in the course of the  $\text{Ca}^{2+}$  oscillations in the range given in Fig. 3. Data (points) are obtained as in Fig. 4 for the same parameter values, with  $K_1 = K_2 = 0.01$  (curve a) or 10 (curve b).

observed (6) with  $\text{InsP}_3$ . Finally, although this minimal model cannot account for all variations of  $\text{Ca}^{2+}$  oscillations in response to different ligands, it indicates, however, how each agonist might possess its own "signature" by acting at the same time, in a specific manner, on several parameters such as  $\beta$  and  $k$ .

The present model for  $\text{Ca}^{2+}$  oscillations differs from that proposed by Meyer and Stryer (12) in that it comprises only two variables (instead of three), does not require  $\text{InsP}_3$  oscillations for  $\text{Ca}^{2+}$  spiking or  $\text{Ca}^{2+}$  pumping into mitochondria, and incorporates the effect of extracellular  $\text{Ca}^{2+}$ . On the other hand, the above model is closely related to that proposed by Kuba and Takeshita (17) for membrane potential oscillations linked to  $\text{Ca}^{2+}$  spikes in sympathetic neurones treated with caffeine. One of the models proposed by these authors contains two variables and also relies on the  $\text{Ca}^{2+}$ -induced  $\text{Ca}^{2+}$  release mechanism to produce oscillations. Here, the effect of the external stimulation and of  $\text{InsP}_3$  is taken explicitly into account, and the source of the instability leading to oscillations is made clear by the analysis of the minimal conditions that produce the phenomenon. While Kuba and Takeshita considered activation of the release by both cytosolic  $\text{Ca}^{2+}$  and  $\text{Ca}^{2+}$  in the intracellular store, we showed that the former control suffices for producing oscillations. In fact, the suggestion that oscillations may arise from  $\text{Ca}^{2+}$ -induced  $\text{Ca}^{2+}$  release was first made for cardiac cells in which this mechanism may operate as one of those generating pacemaker behavior (18–20).

That signal-induced  $\text{Ca}^{2+}$  spikes are observed in a large variety of cells suggests the robustness of the mechanism underlying these oscillations. The activation by cytosolic  $\text{Ca}^{2+}$  of the release of  $\text{Ca}^{2+}$  from intracellular stores would provide a simple, reliable, and ubiquitous mechanism capable of inducing  $\text{Ca}^{2+}$  spikes in many different conditions. Although such a mechanism has been demonstrated in muscle and nerve cells as well as in oocytes, its occurrence in other cells in which  $\text{Ca}^{2+}$  oscillations are found needs to be established. The possibility remains, however, that  $\text{Ca}^{2+}$  oscillations have not the same origin in all cell types. The present, minimal mechanism for  $\text{Ca}^{2+}$  oscillations might also operate in conjunction with that proposed by Meyer and Stryer (12) or with a mechanism based on a negative feedback exerted on  $\text{InsP}_3$  production (2, 9), which both rely on concomitant oscillations of  $\text{InsP}_3$ . In either case, such coupling could be the source of the complex  $\text{Ca}^{2+}$  transients that are sometimes evoked by external stimulation (2, 9, 10).

As to the physiological role of  $\text{Ca}^{2+}$  oscillations, the progressive increase in the frequency of  $\text{Ca}^{2+}$  spikes upon increasing the external stimulation could translate into the rising level of some phosphorylated protein that would be the substrate of a  $\text{Ca}^{2+}$ -activated kinase. We showed that this encoding process is effective only when appropriate conditions hold for the kinetics of the kinase and phosphatase involved in the covalent modification. Encoding signals in terms of the frequency of  $\text{Ca}^{2+}$  oscillations provides an alternative to the encoding based on varying the amplitude of a steady level of cytosolic  $\text{Ca}^{2+}$ , whose precise modulation by external signals might prove more difficult to achieve. Moreover,  $\text{Ca}^{2+}$  oscillations allow for selective differential stimulation of cellular processes (2), since the occurrence of a particular response will depend on the rates of the phosphorylation and dephosphorylation reactions controlled by  $\text{Ca}^{2+}$  transients.

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