

## Models of IP<sub>3</sub> and Ca<sup>2+</sup> Oscillations: Frequency Encoding and Identification of Underlying Feedbacks

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**ABSTRACT** Hormones that act through the calcium-releasing messenger, inositol 1,4,5-trisphosphate (IP<sub>3</sub>), cause intracellular calcium oscillations, which have been ascribed to calcium feedbacks on the IP<sub>3</sub> receptor. Recent studies have shown that IP<sub>3</sub> levels oscillate together with the cytoplasmic calcium concentration. To investigate the functional significance of this phenomenon, we have developed mathematical models of the interaction of both second messengers. The models account for both positive and negative feedbacks of calcium on IP<sub>3</sub> metabolism, mediated by calcium activation of phospholipase C and IP<sub>3</sub> 3-kinase, respectively. The coupled IP<sub>3</sub> and calcium oscillations have a greatly expanded frequency range compared to calcium fluctuations obtained with clamped IP<sub>3</sub>. Therefore the feedbacks can be physiologically important in supporting the efficient frequency encoding of hormone concentration observed in many cell types. This action of the feedbacks depends on the turnover rate of IP<sub>3</sub>. To shape the oscillations, positive feedback requires fast IP<sub>3</sub> turnover, whereas negative feedback requires slow IP<sub>3</sub> turnover. The ectopic expression of an IP<sub>3</sub> binding protein has been used to decrease the rate of IP<sub>3</sub> turnover experimentally, resulting in a dose-dependent slowing and eventual quenching of the Ca<sup>2+</sup> oscillations. These results are consistent with a model based on positive feedback of Ca<sup>2+</sup> on IP<sub>3</sub> production.

### INTRODUCTION

The release of Ca<sup>2+</sup> ions from intracellular stores is a central event in the transduction of hormone and neurotransmitter signals. In a multitude of cell types, the activation of receptors coupled to the phosphoinositide pathway triggers oscillations in the cytoplasmic Ca<sup>2+</sup> concentration ([Ca<sup>2+</sup>]<sub>c</sub>). In many cell types, the strength of the extracellular stimulus is encoded primarily in the frequency of the [Ca<sup>2+</sup>]<sub>c</sub> oscillations, which increases with the degree of stimulation. For example, in rat hepatocytes, the periods of [Ca<sup>2+</sup>]<sub>c</sub> oscillations range over one order of magnitude, from >250 s for low concentrations of hormones, such as vasopressin and noradrenalin, to ~30 s for higher hormone doses (1).

A long-standing question has been whether the oscillations are generated by the cellular Ca<sup>2+</sup> transporters and channels themselves or whether they originate upstream in the signal transduction machinery, between hormone binding to its receptor and the activation of Ca<sup>2+</sup> fluxes. It has been proposed that the periodic release of Ca<sup>2+</sup> ions from the endoplasmic reticulum (ER) can be brought about through the regulatory properties of the IP<sub>3</sub> receptor (IP<sub>3</sub>R), the main type of ER calcium release channel in nonexcitable cells (2–5). Mathematical models have demonstrated how fast activation and delayed inhibition of the IP<sub>3</sub>R by cytoplasmic Ca<sup>2+</sup> can drive repetitive Ca<sup>2+</sup> spiking (6–8). In these models, IP<sub>3</sub> is required to initially open the IP<sub>3</sub>R and sensitize the

channel toward feedback activation by cytoplasmic calcium. Therefore, Ca<sup>2+</sup> oscillations can occur when IP<sub>3</sub> concentration is held at a constant value. However, models based on a simple description of the IP<sub>3</sub>R dynamics generally produce [Ca<sup>2+</sup>]<sub>c</sub> oscillations with short periods (~10–60 s) and thus do not reproduce the long interspike intervals observed experimentally. Long-period oscillations have been obtained when additional mechanisms, such as the regulation of IP<sub>3</sub>R by phosphorylation, stochastic gating phenomena or slow calcium buffers, are included (9,10,11).

Recently, it has become possible to monitor IP<sub>3</sub> changes in intact cells. These experiments have shown that, for some of the agonists used, the IP<sub>3</sub> concentration is highly dynamic and can oscillate together with cytoplasmic calcium (12–15). This raises the intriguing possibility that a coupled IP<sub>3</sub>–Ca<sup>2+</sup> oscillator may generate long-period oscillations and underlie the efficient frequency encoding of the hormone dose.

The existence of both positive and negative feedbacks of Ca<sup>2+</sup> on IP<sub>3</sub> metabolism could mediate fluctuations in cellular IP<sub>3</sub> levels. The production of IP<sub>3</sub> is catalyzed by a diverse family of phosphoinositide-specific phospholipase C (PLC) isoforms (16). All PLC isoforms are activated by Ca<sup>2+</sup> ions, although their sensitivities to [Ca<sup>2+</sup>]<sub>c</sub> vary greatly (17,18). This feedback can have an important role in Ca<sup>2+</sup> wave propagation (19–22). IP<sub>3</sub> is removed by phosphorylation or dephosphorylation through IP<sub>3</sub> 3-kinase (IP<sub>3</sub>K) or IP<sub>3</sub> 5-phosphatase (IP<sub>3</sub>P), respectively. IP<sub>3</sub> removal by IP<sub>3</sub>K is activated by Ca<sup>2+</sup> (23–25). Moreover, it has also been suggested that protein kinase C (PKC), which is activated by receptor-mediated increases in Ca<sup>2+</sup> and diacylglycerol, may inhibit IP<sub>3</sub> production by inactivating agonist receptors (13,26). However, it is presently not clear what

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effects such feedbacks have on  $\text{Ca}^{2+}$  oscillations. Importantly, it is not known whether the involvement of these  $\text{Ca}^{2+}$ -dependent feedback mechanisms serves a physiological role.

Previous models have shown that  $\text{IP}_3$ -mediated  $\text{Ca}^{2+}$  release coupled to  $\text{Ca}^{2+}$ -activated PLC can generate oscillations, without any requirement of  $\text{IP}_3\text{R}$  regulation by  $\text{Ca}^{2+}$  (26,27). These models have been criticized because in some cell types  $\text{Ca}^{2+}$  oscillations can also be elicited by  $\text{IP}_3$  or its nonmetabolizable analogs (3,28,29). The incorporation of  $\text{Ca}^{2+}$  activation of PLC into a  $\text{Ca}^{2+}$  oscillator model based on the above-described  $\text{IP}_3\text{R}$  properties has been reported to modulate  $\text{Ca}^{2+}$  oscillations (30), whereas the inclusion of  $\text{IP}_3\text{K}$  has been found to have practically no effect (31,32).

In this work, we have carried out a systematic modeling study of the interaction between cellular  $\text{Ca}^{2+}$  transports and  $\text{IP}_3$  metabolism. The model includes the dynamics of  $\text{IP}_3$ ,  $\text{Ca}^{2+}$ , and  $\text{IP}_3\text{R}$  and takes into account positive and negative feedback of  $\text{Ca}^{2+}$  on the  $\text{IP}_3$  metabolism. These are mediated by  $\text{Ca}^{2+}$  activation of  $\text{IP}_3$  generation through PLC and  $\text{Ca}^{2+}$  activation of  $\text{IP}_3$  removal by  $\text{IP}_3\text{K}$ , respectively. We have found that each of these  $\text{Ca}^{2+}$  feedbacks strongly modifies the properties of a core oscillator based on  $\text{Ca}^{2+}$  and  $\text{IP}_3\text{R}$  dynamics and, in particular, substantially expands the range of oscillation frequencies. Thus  $\text{IP}_3$  oscillations may underlie efficient frequency encoding of the hormone signal. The model analysis shows that the lifetime of  $\text{IP}_3$  is a critical parameter in the system, the experimental perturbation of which can give information on the feedbacks present. We directly tested this theory in Chinese hamster ovary (CHO) cells by transiently expressing an  $\text{IP}_3$  binding protein composed of the N-terminal ligand-binding domain of the type 1  $\text{IP}_3\text{R}$  fused to green fluorescent protein. The overexpression of this fusion protein exerted a dose-dependent suppression of repetitive agonist-induced  $\text{Ca}^{2+}$  oscillations that is consistent with an oscillator model including positive feedback of  $\text{Ca}^{2+}$  on  $\text{IP}_3$  generation. Taken together, the experimental data and theoretical analysis suggest that  $\text{IP}_3$  oscillations are an essential component of the  $\text{Ca}^{2+}$  oscillator, expanding the richness in the message conveyed by extracellular stimuli.

## MATERIALS AND METHODS

### Mathematical model

The model accounts for the formation and degradation of  $\text{IP}_3$ , the main  $\text{Ca}^{2+}$  fluxes across the ER and plasma membrane, and the  $\text{IP}_3\text{R}$  dynamics (see Fig. 1). It is formulated in terms of four variables: the  $\text{IP}_3$  and  $\text{Ca}^{2+}$  concentrations in the cytoplasm,  $p$  and  $c$ , respectively; the calcium concentration in the ER stores,  $s$ ; and the fraction of  $\text{IP}_3\text{R}$  that have not been inactivated by  $\text{Ca}^{2+}$ ,  $r$ .

#### $\text{IP}_3$ dynamics

$\text{IP}_3$  is produced by PLC, whose activity depends on agonist dose and  $\text{Ca}^{2+}$ . The  $\text{Ca}^{2+}$  sensitivity of PLC can be modeled by (33)

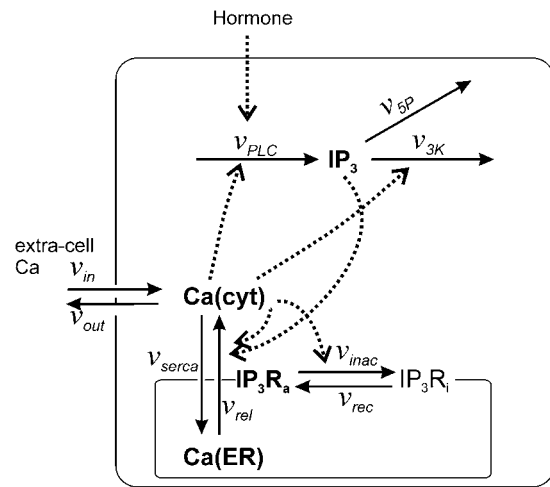


FIGURE 1 Interactions between  $\text{Ca}^{2+}$  transport processes and  $\text{IP}_3$  metabolism included in the model. The solid and dashed arrows indicate transport/reaction steps and activations, respectively. The bold quantities indicate the model variables:  $\text{IP}_3$ , cytoplasmic  $\text{IP}_3$ ;  $\text{Ca}(\text{cyt})$ , free cytoplasmic  $\text{Ca}^{2+}$ ;  $\text{Ca}(\text{ER})$ , free  $\text{Ca}^{2+}$  in the ER;  $\text{IP}_3\text{R}_a$ , active conformation of the  $\text{IP}_3\text{R}$ . The other abbreviations denote:  $\text{IP}_3\text{R}_i$ , inactive conformation of the  $\text{IP}_3\text{R}$ ;  $v_{\text{rel}}$ , rate of  $\text{Ca}^{2+}$  release through the  $\text{IP}_3\text{R}$ ;  $v_{\text{serca}}$ , active  $\text{Ca}^{2+}$  transport into the ER;  $v_{\text{ina}}$  and  $v_{\text{rec}}$  rate of  $\text{Ca}^{2+}$ -induced  $\text{IP}_3\text{R}$  inactivation and recovery rate, respectively;  $v_{\text{PLC}}$ , production rate of  $\text{IP}_3$ ;  $v_{5P}$  and  $v_{3K}$  rates of  $\text{IP}_3$  dephosphorylation and phosphorylation, respectively;  $v_{\text{in}}$  and  $v_{\text{out}}$ , rates of  $\text{Ca}^{2+}$  influx and extrusion across the plasma membrane, respectively.

$$v_{\text{PLC}} = V_{\text{PLC}} \frac{c^2}{K_{\text{PLC}}^2 + c^2}. \quad (1)$$

The maximal rate  $V_{\text{PLC}}$  depends on the agonist concentration, whereas  $K_{\text{PLC}}$  characterizes the sensitivity of PLC to  $\text{Ca}^{2+}$ .  $\text{IP}_3$  is removed through dephosphorylation by  $\text{IP}_3\text{P}$  and phosphorylation by  $\text{IP}_3\text{K}$ , which we model as

$$v_{\text{deg}} = v_{5P} + v_{3K} = \left( k_{5P} + k_{3K} \frac{c^2}{K_{3K}^2 + c^2} \right) p, \quad (2)$$

where  $k_{5P}$  and  $k_{3K}$  are the  $\text{IP}_3$  dephosphorylation and phosphorylation rate constants, respectively. The  $\text{Ca}^{2+}$  dependence of the  $\text{IP}_3\text{K}$  is described by a Hill function with the half-saturation constant  $K_{3K}$  (23). According to Fink et al. (34) and Sims and Allbritton (35), one can assume that the two enzymes are not saturated with  $\text{IP}_3$ , justifying the linear rate law in  $p$ .

For the purpose of the subsequent analysis we write the balance equation for the  $\text{IP}_3$  concentration in the following form

$$\begin{aligned} \frac{dp}{dt} &= v_{\text{PLC}} - v_{\text{deg}} \\ &= \frac{1}{\tau_p} \left[ \bar{V}_{\text{PLC}} \frac{c^2}{K_{\text{PLC}}^2 + c^2} - \left( \eta \frac{c^2}{K_{3K}^2 + c^2} - (1 - \eta) \right) p \right], \end{aligned} \quad (3)$$

where we introduce the characteristic time of  $\text{IP}_3$  turnover

$$\tau_p = 1/(k_{3K} + k_{5P}), \quad (4)$$

and the ratio of the maximal  $\text{IP}_3\text{K}$  rate to the total maximal degradation rate of  $\text{IP}_3$

$$\eta = k_{3K}/(k_{3K} + k_{5P}). \quad (5)$$

The strength of the positive feedback will be tuned by changing  $K_{\text{PLC}}$  (the  $\text{Ca}^{2+}$  sensitivity of PLC), and the strength of the negative feedback will be

tuned by changing  $\eta$  (the relative expression level of IP<sub>3</sub>K). Although both feedbacks can be present simultaneously, it is useful to first analyze them separately. Therefore, we define the “positive-feedback model” in which PLC is sensitive to Ca<sup>2+</sup> ( $K_{PLC} > 0$ ) and IP<sub>3</sub>K is not expressed ( $\eta = 0$ ), and the “negative-feedback model” in which IP<sub>3</sub>K is present ( $\eta > 0$ ) and PLC is assumed insensitive to physiological Ca<sup>2+</sup> changes ( $K_{PLC} = 0$ ).

Note that the rescaled maximal PLC activity  $\bar{V}_{PLC} = V_{PLC} \tau_p$  equals the steady-state concentration of IP<sub>3</sub> that would be attained in the absence of positive or negative feedbacks.

### Calcium and IP<sub>3</sub>R dynamics

The Ca<sup>2+</sup> release flux through the IP<sub>3</sub>R,  $v_{rel}$ , is modeled according to Li and Rinzel (36). The rate equations for active transport of Ca<sup>2+</sup> across the ER and plasma membranes,  $v_{serca}$  and  $v_{out}$ , respectively, follow Lytton et al. (37) and Camello et al. (38). Calcium influx  $v_{in}$  includes a leak into the cell and a stimulation dependent influx. The balance equation for cytoplasmic Ca<sup>2+</sup> then reads

$$\frac{dc}{dt} = \underbrace{\left( k_1 \left( r \frac{c}{K_a + c} \frac{p}{K_p + p} \right)^3 + k_2 \right) (s - c)}_{v_{rel}} - \underbrace{V_{serca} \frac{c^2}{K_{serca}^2 + c^2}}_{v_{serca}} + \underbrace{\left( \varepsilon \left( v_0 + \phi \bar{V}_{PLC} - V_{pm} \frac{c^2}{K_{pm}^2 + c^2} \right) \right)}_{v_{in} - v_{out}}. \quad (6)$$

The dimensionless parameter  $\varepsilon$  measures the relative strength of the plasma membrane fluxes, which is known to be cell-type specific. We first carry out the model analysis for the simpler case that the plasma membrane fluxes are negligible. Setting  $\varepsilon = 0$  the total Ca<sup>2+</sup> concentration in the cell is conserved and can be expressed as  $c_{tot} = c + \beta s$ , where  $\beta$  is the ratio of effective cytoplasmic volume to effective ER volume (both accounting for Ca<sup>2+</sup> buffering). Therefore, we can insert for the ER calcium in Eq. 6,  $s = (c_{tot} - c)/\beta$ . In the presence of plasma-membrane fluxes ( $\varepsilon > 0$ ) this conservation no longer holds and a kinetic equation for  $s$  must be added:

$$\frac{ds}{dt} = \frac{1}{\beta} \left[ V_{serca} \frac{c^2}{K_{serca}^2 + c^2} - \left( k_1 \left( r \frac{c}{K_a + c} \frac{p}{K_p + p} \right)^3 + k_2 \right) (s - c) \right]. \quad (7)$$

The dynamics of IP<sub>3</sub>R inactivation by cytoplasmic Ca<sup>2+</sup> is described by (35)

$$\frac{dr}{dt} = v_{rec} - v_{inac} = \frac{1}{\tau_r} \left( 1 - r \frac{K_i + c}{K_i} \right). \quad (8)$$

The meaning and numerical values of the kinetic parameters are given in Table 1.

Numerical solutions of the differential equations system, Eqs. 3 and 6–8, were obtained using a fourth-order Runge-Kutta algorithm implemented in XPPAUT (<http://www.math.pitt.edu/~bard/xpp/xpp.html>). Bifurcation analyses were done using the program AUTO2000 (39). Calcium waves with the local kinetics given by Eqs. 3 and 6–8 and diffusion of Ca<sup>2+</sup> and IP<sub>3</sub> were calculated numerically with a finite-difference Crank-Nicholson scheme. Wave speeds for solitary waves were evaluated using AUTO2000.

## Experimental procedures

### Cell culture

CHO cells were cultured in Ham's F-12 media supplemented with 10% fetal bovine serum (FBS) and antibiotics. Cells were seeded onto poly-D-lysine

**TABLE 1** Reference parameter values

	Positive feedback	Negative feedback
<i>IP<sub>3</sub> dynamics parameters</i>		
Half-activation constant of IP <sub>3</sub> K, $K_{3K}$	0.4 $\mu\text{M}$	0.4 $\mu\text{M}$
IP <sub>3</sub> phosphorylation rate constant, $k_{3K}$	0	0.1 $\text{s}^{-1}$
IP <sub>3</sub> dephosphorylation rate constant, $k_{5P}$	0.66 $\text{s}^{-1}$	0
Half-activation constant of PLC, $K_{PLC}$	0.2 $\mu\text{M}$	0 $\mu\text{M}$
<i>Ca<sup>2+</sup> transport and structural parameters</i>		
Ratio of effective volumes ER/cytosol, $\beta$	0.185	0.185
Maximal SERCA pump rate, $V_{serca}$	0.9 $\mu\text{M s}^{-1}$	0.25 $\mu\text{M s}^{-1}$
Half-activation constant, $K_{serca}$	0.1 $\mu\text{M}$	0.1 $\mu\text{M}$
Maximal PMCA pump rate, $V_{pm}$	0.01 $\mu\text{M s}^{-1}$	0.01 $\mu\text{M s}^{-1}$
Half-activation constant, $K_{pm}$	0.12 $\mu\text{M}$	0.12 $\mu\text{M}$
Constant influx, $v_0$	0.0004 $\mu\text{M/s}$	0.0004 $\mu\text{M/s}$
Stimulation-dependent influx, $\phi$	0.0047 $\text{s}^{-1}$	0.045 $\text{s}^{-1}$
Strength of plasma membrane fluxes, $\varepsilon$	0	0
Total Ca <sup>2+</sup> concentration (for $\varepsilon = 0$ ), $c_{tot}$	2 $\mu\text{M}$	2 $\mu\text{M}$
<i>IP<sub>3</sub>R parameters</i>		
Maximal rate of Ca <sup>2+</sup> release, $k_1$	1.11 $\text{s}^{-1}$	7.4 $\text{s}^{-1}$
Ca <sup>2+</sup> leak, $k_2$	0.0203 $\text{s}^{-1}$	0.00148 $\text{s}^{-1}$
Ca <sup>2+</sup> binding to activating site, $K_a$	0.08 $\mu\text{M}$	0.2 $\mu\text{M}$
Ca <sup>2+</sup> binding to inhibiting site, $K_i$	0.4 $\mu\text{M}$	0.3 $\mu\text{M}$
IP <sub>3</sub> binding, $K_p$	0.13 $\mu\text{M}$	0.13 $\mu\text{M}$
Characteristic time IP <sub>3</sub> R inactivation, $\tau_R$	12.5 s	6.6 s

The half-saturation constants for PLC and IP<sub>3</sub>K were taken from Blank et al. (32) and Communi et al. (22), respectively. The IP<sub>3</sub> degradation rate constants were chosen in accordance to Fink et al. (33) and Sims and Allbritton (34). The maximal rate of PLC,  $V_{PLC}$ , is taken as the stimulation-dependent control parameter. In the positive-feedback model (Ca<sup>2+</sup> activation of PLC), the parameters for the Ca<sup>2+</sup> transport processes and the IP<sub>3</sub>R were taken from Li and Rinzel (35). In the negative feedback model (Ca<sup>2+</sup> activation of IP<sub>3</sub>K), we obtained no substantial effect of the IP<sub>3</sub>K on the oscillations with these parameters. However, for different parameters (as given) the IP<sub>3</sub>K effects were pronounced. The differences in the Ca<sup>2+</sup> fluxes between the two models can be accounted for by variations in the expression of the involved proteins. The differences in the Ca<sup>2+</sup> binding properties to the activating site of the IP<sub>3</sub>R can be due to differences in the expression of IP<sub>3</sub>R subtypes, with IP<sub>3</sub>R subtype I having a higher Ca<sup>2+</sup> dissociation constant for the activating site than subtypes II and III (52,53). The table gives the reference parameter set; parameters that are varied in specific simulations are indicated in the respective figure legends.

coated glass coverslips (25 mm) and maintained in culture until 70–80% confluent before experimental protocols.

### Plasmid construction and transfection protocols

The cDNA encoding 620 amino acids of the N-terminal rat type 1 IP<sub>3</sub> receptor (40) was ligated in-frame to the C-terminus of the enhanced green fluorescent protein gene in the plasmid pEGFP-C1 (Clontech, Palo Alto, CA) to generate the plasmid pEGFP-LBD. Cell cultures were transiently transfected with either pEGFP-LBD (EGFP-LBD) or pEGFP-C1 (EGFP) using Lipofectamine 2000 (Invitrogen, Carlsbad, CA) according to the manufacturer's protocols. Agonist-evoked [Ca<sup>2+</sup>]<sub>c</sub> responses were recorded in transfected cultures after a 16–48-h incubation period.

### Imaging measurements of [Ca<sup>2+</sup>]<sub>c</sub> and fluorescent proteins

Calcium imaging experiments were performed in a HEPES-buffered physiological saline solution (HBSS) comprising (in mM): 25 HEPES (pH 7.4 at 37°C), 121 NaCl, 5 NaHCO<sub>3</sub>, 4.7 KCl, 1.2 KH<sub>2</sub>PO<sub>4</sub>, 1.2 MgSO<sub>4</sub>,

2.0  $\text{CaCl}_2$ , 10 glucose, 0.1 sulphobromophthalein, and 0.25% (w/v) fatty acid-free BSA. Cell cultures were loaded with fura-2/AM by incubation with 5  $\mu\text{M}$  fura-2/AM plus Pluronic F-127 (0.02% v/v) for 20–40 min in HBSS. The cells were washed and transferred to a thermostatically regulated microscope chamber (37°C). Fura-2 fluorescence images (excitation, 340 and 380 nm, emission 420–600 nm) were acquired at 3–4-s intervals with a cooled charge-coupled device (CCD) camera as previously described (3). Fura-2 fluorescence intensities were corrected for GFP spillover before calculating fluorescence ratio values, by quenching cytosolic fura-2 with  $\text{MnCl}_2$ . Cells expressing recombinant proteins were selected by screening for GFP fluorescence (excitation 488 nm, emission 525 nm).

EGFP-LBD concentration was estimated from a standard curve constructed with known concentrations of six His-tagged EGFP (Clontech; molecular weight of 27,000). Calibration solutions were prepared by diluting the recombinant protein in PBS. An aliquot (5  $\mu\text{l}$ ) was mixed with low-density mineral oil then sandwiched between two glass coverslips. EGFP containing “bubbles” ranging in size from 10 to 50  $\mu\text{m}$  (the approximate range of cell diameters observed in CHO cultures) were imaged with a Nikon 20 $\times$ , 0.75 NA Plan Apo objective on a wide-field microscope. The fluorophore protein concentration was converted into molar value assuming a molecular weight of 27,000 for His-tagged EGFP.

## RESULTS

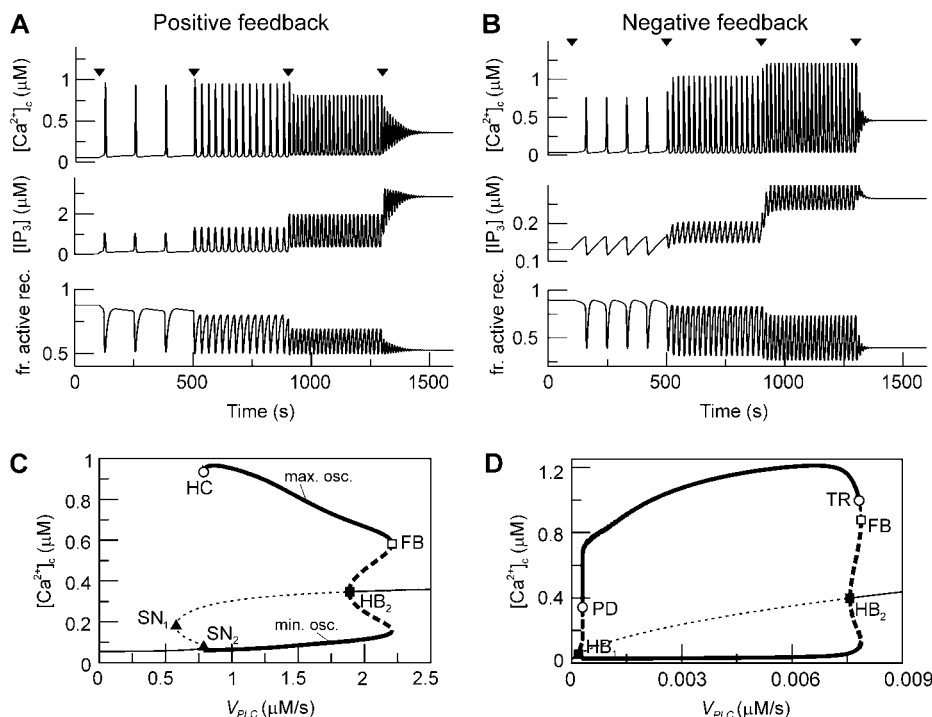
### Positive and negative feedback models exhibit frequency encoding of agonist dose

In many cell types, one observes a strong dependence of the frequency of  $\text{Ca}^{2+}$  oscillations on the dose of the applied receptor agonist, whereas the oscillation amplitude remains nearly constant (1). A stepwise increase of the agonist concentration is accompanied by a prompt rise of the oscillation frequency. In the model, the maximal rate of PLC,  $V_{\text{PLC}}$ , is a

measure of agonist concentration. We subjected the positive-feedback model, with  $\text{Ca}^{2+}$  activation of PLC, and the negative-feedback model, with  $\text{Ca}^{2+}$  activation of  $\text{IP}_3\text{K}$ , to stepwise increases in  $V_{\text{PLC}}$ . The responses are shown in Fig. 2, A and B, respectively, with the time points of  $V_{\text{PLC}}$  increase indicated by arrowheads. Both models exhibit a large range of oscillation frequencies with little change in  $[\text{Ca}^{2+}]_c$  amplitude (Fig. 2, A and B; *top traces*). The pronounced increase in the rate of spiking with increasing stimulus is the hallmark of the experimentally observed frequency encoding. For very large stimuli, a plateau of elevated  $[\text{Ca}^{2+}]_c$  is reached, again in agreement with experimental data.

The  $[\text{Ca}^{2+}]_c$  oscillations in both models consist of a series of sharp spikes with baseline interludes (Fig. 2, A and B). The peak values of  $[\text{Ca}^{2+}]_c$  and  $[\text{IP}_3]$  occur nearly at the same time. The shape of the  $[\text{IP}_3]$  oscillations in the two models is different. In the positive-feedback model,  $[\text{IP}_3]$  exhibits baseline-separated spikes (Fig. 2 A). In contrast, in the negative-feedback model,  $[\text{IP}_3]$  follows a zig-zag pattern: the occurrence of a  $[\text{Ca}^{2+}]_c$  spike leads to an abrupt decrease in  $[\text{IP}_3]$ , after which  $[\text{IP}_3]$  slowly builds up again over the whole oscillation period (Fig. 2 B). The  $\text{IP}_3\text{R}$  activities show similar dynamics in both models.

The behavior of the two model systems for different stimulation strengths can be summarized in bifurcation diagrams (Fig. 2, C and D). We computed the steady states of  $[\text{Ca}^{2+}]_c$  and the maxima and minima of  $[\text{Ca}^{2+}]_c$  oscillations as a function of  $V_{\text{PLC}}$ . For very low PLC activity, both models show a stable steady state of low  $[\text{Ca}^{2+}]_c$ ; similarly, an elevated



**FIGURE 2** Agonist-induced  $\text{IP}_3$  and  $\text{Ca}^{2+}$  oscillations in the positive and negative feedback models. (A) Positive feedback model with  $\text{Ca}^{2+}$  activation of PLC. Changes in  $[\text{Ca}^{2+}]_c$ ,  $[\text{IP}_3]$ , and in the fraction of active receptors  $r$  (*top*, *middle*, and *bottom* panels) after stepwise increases in the agonist concentration (*arrowheads*), modeled by an increase in the maximal rate of PLC ( $V_{\text{PLC}} = 0.3 \mu\text{M/s}$  for  $t < 100$  with successive increases to 0.787, 1, 1.5, and 2.5  $\mu\text{M/s}$ ). (B) Negative feedback model with  $\text{Ca}^{2+}$  activation of  $\text{IP}_3\text{K}$ . The response is shown for a step protocol with  $V_{\text{PLC}} = 0.1 \text{ nM/s}$  for  $t < 100$ , followed by increases to 0.45, 2.5, 5.8, and 10  $\text{nM/s}$ . (C) Bifurcation diagram for positive feedback model; shown are the maxima and minima of the  $[\text{Ca}^{2+}]_c$  oscillations (*thick lines*) and the  $[\text{Ca}^{2+}]_c$  steady states (*thin lines*) as a function of the stimulus ( $V_{\text{PLC}}$ ). Solid and dashed lines indicate stable and unstable states, respectively. HB, Hopf bifurcation; HC, homoclinic bifurcation; SN, saddle-node bifurcation; FB, saddle node of periodics. (D) Bifurcation diagram for negative feedback model. PD, period doubling; TR, torus bifurcation. Between PD and HB<sub>1</sub> and TR and FB there exist complex oscillations (omitted for clarity). The parameter values used are listed in Table 1.

$[\text{Ca}^{2+}]_c$  plateau is reached at relatively high PLC activity (these stable steady states are indicated by *thin solid lines*). For an intermediate range of  $V_{\text{PLC}}$ , the steady states are unstable (*thin dashed lines*). In these regions, both models exhibit oscillations ( $[\text{Ca}^{2+}]_c$  maxima and minima in stable oscillations are depicted by *thick solid lines*). The oscillations arise either via Hopf bifurcations (HB), or, in the case of the positive-feedback model, also by a homoclinic bifurcation (HC).

Further bifurcations are indicated and referred to in the figure legend. In particular, a homoclinic bifurcation is associated with the existence of multiple steady states, which arise through saddle-node bifurcations (Fig. 2 C, SN). Such multistationarity is typical for models that neglect the plasma-membrane fluxes of calcium; this point will be discussed in more detail below. In the negative-feedback model, there are two regions near the Hopf bifurcations  $\text{HB}_1$  and  $\text{HB}_2$  (before the point PD and after the point TR in Fig. 2 D) where irregular and bursting oscillations are observed (results not shown). Because these two regions are extremely narrow, compared to the total stimulation range in the negative feedback model, our focus will be on the regular oscillations.

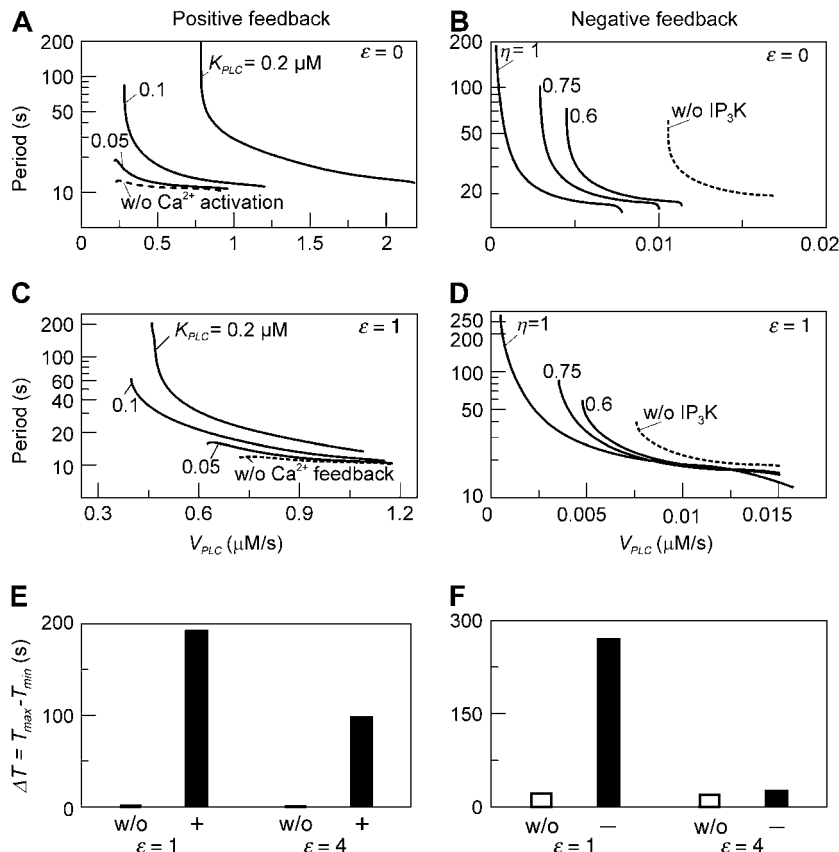
In the two bifurcation diagrams, one notices that the  $V_{\text{PLC}}$  values required for oscillations are considerably smaller in the negative-feedback model than in the positive-feedback model. This is primarily a consequence of the different feedback mechanisms. First, in the positive-feedback model the actual PLC activity is  $\text{Ca}^{2+}$  dependent and, therefore, is

lower than  $V_{\text{PLC}}$  at resting  $[\text{Ca}^{2+}]_c$ . Second, owing to the calcium activation of  $\text{IP}_3\text{K}$  in the negative-feedback model, the  $\text{IP}_3$  degradation rate at resting  $[\text{Ca}^{2+}]_c$  is much smaller than in the positive-feedback model, requiring a smaller rate of  $\text{IP}_3$  production to raise  $[\text{IP}_3]$  and induce oscillations.

### The wide range of oscillation periods is due to interactions of $\text{IP}_3$ and $\text{Ca}^{2+}$ dynamics

To elucidate whether the  $\text{IP}_3$  dynamics participate in frequency encoding, we compared the oscillation periods in models without any  $\text{Ca}^{2+}$  feedback on  $\text{IP}_3$  (resulting in a constant  $[\text{IP}_3]$ ) and in the two feedback models (with  $[\text{IP}_3]$  oscillations).

We begin by discussing the positive-feedback model. In the positive-feedback model, we consider  $\text{Ca}^{2+}$  activation of the agonist-dependent  $\text{PLC}\beta$ . The strength of the positive feedback can be tuned by changing the value of the  $\text{Ca}^{2+}$  activation constant,  $K_{\text{PLC}}$ . For  $K_{\text{PLC}}$  being much lower than the basal  $[\text{Ca}^{2+}]_c$ , PLC is always saturated with  $\text{Ca}^{2+}$  and its activity is independent of variations in  $[\text{Ca}^{2+}]_c$ . In particular, by setting  $K_{\text{PLC}} = 0$  positive feedback will effectively be eliminated. This model with constant  $[\text{IP}_3]$  shows fast calcium oscillations with a period of 10–15 s (Fig. 3 A, *dashed line*). Introducing positive feedback by setting  $K_{\text{PLC}} > 0$  causes oscillations with long periods at low stimulation. The frequency encoding of the stimulus becomes very pronounced when the sensitivity of PLC to changes in  $[\text{Ca}^{2+}]_c$  is



**FIGURE 3** Frequency encoding of agonist stimulus. (A) Positive feedback: oscillation periods observed at different stimulation strengths (varying  $V_{\text{PLC}}$ ). Increasing the half-saturation constant of PLC for  $\text{Ca}^{2+}$ ,  $K_{\text{PLC}}$ , from 0 (no functional positive feedback) to  $0.2 \mu\text{M}$  (functional feedback) greatly enhances frequency encoding. (B) Negative feedback. Increasing the amount of  $\text{IP}_3\text{K}$  relative to  $\text{IP}_3\text{P}$  ( $\eta$ ) enhances frequency encoding. (C, D) The feedback effects shown in panels A and B are preserved when plasma-membrane fluxes of  $\text{Ca}^{2+}$  are included in the models ( $\epsilon = 1$ ). (E) Range of oscillation periods,  $\Delta T = T_{\text{max}} - T_{\text{min}}$ , in the presence (+) and absence (w/o) of positive feedback for two different strengths of the plasma-membrane  $\text{Ca}^{2+}$  fluxes ( $\epsilon = 1, 4$ ). (F) Range of oscillation periods in the presence (–) and absence (w/o) of negative feedback for two different strengths of the plasma-membrane  $\text{Ca}^{2+}$  fluxes ( $\epsilon = 1, 4$ ). We have found that the  $\text{IP}_3\text{K}$  has an impact on the oscillation period only when the  $\text{Ca}^{2+}$  fluxes between ER and cytoplasm are comparatively slow and the  $\text{IP}_3\text{R}$  is less sensitive to  $\text{Ca}^{2+}$  activation. To expose the period effect of the negative feedback, we have chosen different parameter values than in the positive feedback model (see Table 1).

just above basal  $[\text{Ca}^{2+}]_c$  (Fig. 3 A, *solid lines*;  $K_{\text{PLC}} = 0.1$  and  $0.2 \mu\text{M}$ ).

For the negative-feedback model, we recall that there are two removal pathways for  $\text{IP}_3$ , one catalyzed by the  $\text{Ca}^{2+}$ -insensitive  $\text{IP}_3$  5-phosphatase ( $\text{IP}_3\text{P}$ ) and the other by the  $\text{Ca}^{2+}$ -activated  $\text{IP}_3$  3-kinase ( $\text{IP}_3\text{K}$ ). We can modify the strength of the negative feedback by taking different concentration ratios of  $\text{IP}_3\text{P}$  and  $\text{IP}_3\text{K}$ . The feedback strength is expressed by the ratio of the maximal  $\text{IP}_3\text{K}$  rate to the total degradation rate of  $\text{IP}_3$ :  $\eta = k_{3\text{K}}/(k_{3\text{K}} + k_{5\text{P}})$ , where  $k_{3\text{K}} + k_{5\text{P}}$  has been kept constant in the following calculations. The oscillation periods in the absence of negative feedback ( $\eta = 0$ ), and therefore constant  $[\text{IP}_3]$ , are shown in Fig. 3 B (*dashed line*). When  $\eta$  is sufficiently high, the negative feedback has a pronounced effect on the range of oscillations periods; for  $\eta \geq 0.6$  there is an increase in the period range (Fig. 3 B, *solid lines*).

In the positive-feedback model, arbitrarily long periods can be obtained (exceeding the 200 s shown), which are due to the onset of the oscillations via a homoclinic bifurcation (see also Fig. 2 C). The homoclinic bifurcation specifically occurs in the model when the plasma-membrane fluxes of  $\text{Ca}^{2+}$  are neglected, which is a valid simplification for many cell types in which the contribution of these fluxes to  $\text{Ca}^{2+}$  oscillations is small (41). We have also studied the more general case when the plasma-membrane  $\text{Ca}^{2+}$  fluxes are included in the model (see Materials and Methods). Then there is a unique steady state and the homoclinic bifurcation no longer exists. Nevertheless, long-period oscillations are present (Fig. 3 C). Importantly, the dependence of the period range of the oscillations on  $K_{\text{PLC}}$  remains very similar.

We also introduced plasma-membrane fluxes of  $\text{Ca}^{2+}$  into the negative-feedback model (Fig. 3 D). We observed a similar picture as without plasma membrane fluxes, provided that the plasma-membrane fluxes were comparatively moderate. However, when the plasma-membrane  $\text{Ca}^{2+}$  fluxes are large enough, the effect of  $\text{IP}_3\text{K}$  on the oscillation period practically disappears. In contrast, the period behavior in the positive-feedback model is less affected by changes in the magnitude of the plasma-membrane  $\text{Ca}^{2+}$  fluxes. To show this, we evaluated the range of oscillation periods  $\Delta T = T_{\text{max}} - T_{\text{min}}$ , where  $T_{\text{max}}$  and  $T_{\text{min}}$  are the maximal and minimal period that are obtained for low and high stimulation, respectively, for two different strengths of the plasma-membrane  $\text{Ca}^{2+}$  fluxes ( $\varepsilon = 1$  and  $\varepsilon = 4$ ). The positive-feedback model exhibits in both cases a much larger period range than the corresponding model without feedback (Fig. 3 E). In contrast, the increase of the period range through negative feedback is only seen when the plasma-membrane  $\text{Ca}^{2+}$  fluxes are comparatively small,  $\varepsilon = 1$  (Fig. 3 F).

In summary, both positive and negative feedbacks of  $\text{Ca}^{2+}$  on  $\text{IP}_3$  may serve a physiological role by greatly enhancing the range of frequency encoding of the agonist stimulus. The frequency encoding supported by the positive feedback is more robust against variations in the kinetic parameters of the  $\text{Ca}^{2+}$  transport processes.

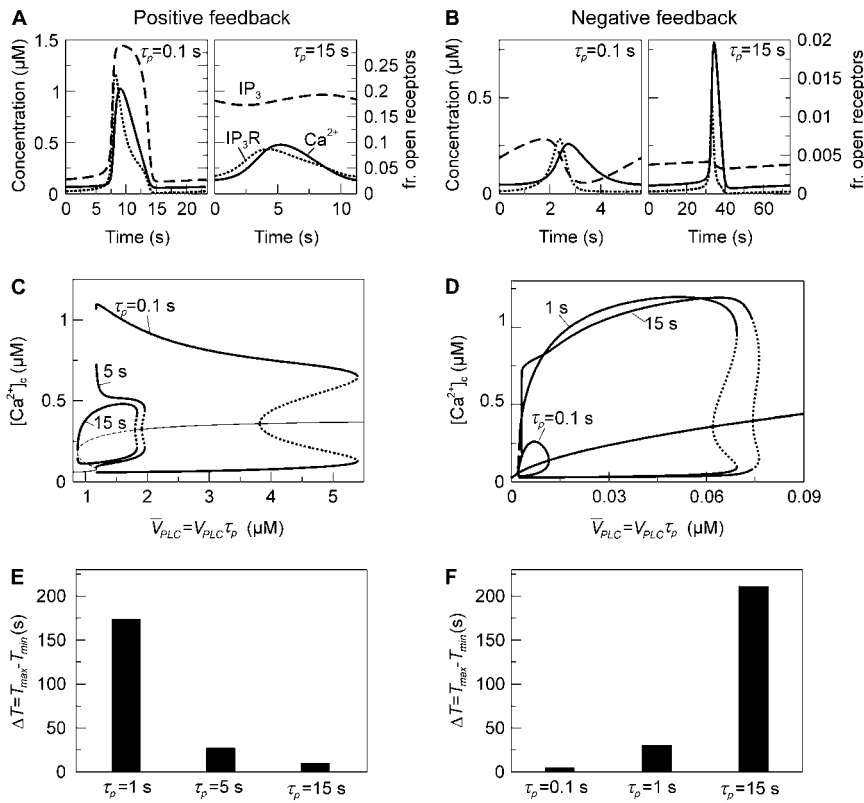
## Positive and negative models respond differently to changes in feedback $\text{IP}_3$ turnover time

In the model simulations, we noticed that the characteristic time of  $\text{IP}_3$  turnover  $\tau_p$  has a decisive impact on the  $\text{Ca}^{2+}$ - $\text{IP}_3$  oscillators. The measured  $\text{IP}_3$  turnover times span a relatively wide range, from 0.1 to  $>10$  s depending on cell type and experimental conditions (34,35). We have found that fast  $\text{IP}_3$  turnover ( $\tau_p \approx 0.1$ –2 s) is associated with long oscillation periods in the positive-feedback model. Conversely, the negative-feedback model exhibits long-period oscillations when the  $\text{IP}_3$  turnover is comparatively slow ( $\tau_p \approx 10$ –15 s).

Insight into the origin of this difference between the two models can be gained by looking at the time courses of the model variables. In the positive feedback model, fast  $\text{IP}_3$  turnover ( $\tau_p = 0.1$  s) yields high-amplitude oscillations in  $[\text{Ca}^{2+}]_c$  and  $[\text{IP}_3]$  (Fig. 4 A, *solid and dashed lines*, respectively).  $[\text{Ca}^{2+}]_c$  and  $[\text{IP}_3]$  rise simultaneously, and  $\text{IP}_3$ -induced  $\text{Ca}^{2+}$  release and  $\text{Ca}^{2+}$ -activated  $\text{IP}_3$  production coincide. After termination of the  $[\text{Ca}^{2+}]_c$  spike,  $[\text{IP}_3]$  returns quickly to a basal level, because  $\text{Ca}^{2+}$ -activated  $\text{IP}_3$  production has ceased and  $\text{IP}_3$  degradation is fast. Also, the  $\text{IP}_3\text{Rs}$  close efficiently after the spike (Fig. 4 A, *dotted line* showing the fraction of open  $\text{IP}_3\text{R}$ ). For slow  $\text{IP}_3$  turnover ( $\tau_p = 15$  s)  $[\text{IP}_3]$  does not sufficiently decline after the  $[\text{Ca}^{2+}]_c$  spike, leading to an increased basal opening of the  $\text{IP}_3\text{R}$ , lower ER  $\text{Ca}^{2+}$  store loading and, consequently, much less pronounced  $[\text{Ca}^{2+}]_c$  spikes (Fig. 4 A).

Not only the spike characteristics but also the oscillation period and the range of agonist stimuli that give oscillations are affected by the  $\text{IP}_3$  half-life. For fast  $\text{IP}_3$  turnover, oscillations in the positive-feedback model are observed over a wide range of stimuli (Fig. 4 C,  $\tau_p = 0.1$  s). Slower  $\text{IP}_3$  turnover leads to reduced oscillation ranges and also much smaller amplitudes (Fig. 4 C,  $\tau_p = 5$  and  $15$  s). Importantly, also, the capacity for frequency encoding of the stimulus, as measured by the period range of oscillations, is high when the  $\text{IP}_3$  turnover is fast (Fig. 4 E).

In the negative-feedback model, changing the timescale of  $\text{IP}_3$  turnover has the opposite effect. When  $\text{IP}_3$  turnover is fast ( $\tau_p = 0.1$  s), a rise in  $[\text{Ca}^{2+}]_c$  triggers, via activation of  $\text{IP}_3\text{K}$ , a pronounced decrease in  $[\text{IP}_3]$ , which in turn limits further  $\text{Ca}^{2+}$  release (Fig. 4 B). Therefore, the  $[\text{Ca}^{2+}]_c$  spikes are relatively small and  $[\text{IP}_3]$  shows strong variations (*solid and dashed lines*, respectively, in Fig. 4 B). When the  $\text{IP}_3$  lifetime is larger ( $\tau_p = 15$  s),  $[\text{IP}_3]$  remains at a relatively high level throughout and the  $[\text{Ca}^{2+}]_c$  spikes are accordingly more pronounced (Fig. 4 B). Moreover, for slow  $\text{IP}_3$  degradation, the range of stimuli where oscillations occur is larger (Fig. 4 D). The capacity for frequency encoding as measured by the range of oscillation periods,  $\Delta T$ , strongly increases with the  $\text{IP}_3$  half-life (Fig. 4 F). This finding agrees with the frequent observation that negative feedback is more prone to oscillate when the controlled variable (here  $\text{IP}_3$ ) responds slowly.



**FIGURE 4** IP<sub>3</sub> turnover time controls feedbacks. (A) Positive-feedback model. Dynamics of [Ca<sup>2+</sup>]<sub>c</sub>, [IP<sub>3</sub>], and the fraction of open IP<sub>3</sub>Rs (solid, dashed, and dot-dashed lines, respectively) during an oscillation period; the fraction of open IP<sub>3</sub>Rs is given by  $[rcp/(c + K_a)/(p + K_p)]^3$  (see Eq. 6). Fast IP<sub>3</sub> turnover yields a pronounced spike (left panel,  $\tau_p = 0.1$  s), whereas slow IP<sub>3</sub> turnover supports only a small-amplitude response (right panel,  $\tau_p = 15$  s). (B) The negative-feedback model shows the opposite behavior, with a small-amplitude response for fast IP<sub>3</sub> turnover (left panel,  $\tau_p = 0.1$  s) and a sharp spike for slow IP<sub>3</sub> turnover (right panel,  $\tau_p = 15$  s). (C) Positive-feedback model. Bifurcation diagram showing the maxima and minima of the [Ca<sup>2+</sup>]<sub>c</sub> oscillations as a function of the stimulus for different values of the IP<sub>3</sub> turnover. The bifurcation diagrams for different values of  $\tau_p$  are compared by plotting them against the product  $\bar{V}_{PLC} = V_{PLC} \tau_p$ ; in this way, the steady-state concentrations of Ca<sup>2+</sup> and IP<sub>3</sub> are identical for a given  $\bar{V}_{PLC}$  (solid and dashed lines indicate stable and unstable states, respectively; the stability of the steady state is shown for  $\tau_p = 15$  s). Both amplitude and range of stimuli leading to oscillations increase with faster IP<sub>3</sub> turnover. (D) The corresponding bifurcation diagrams for the negative-feedback model show the opposite behavior. The amplitude and range of stimuli leading to oscillations increase with slower IP<sub>3</sub> turnover. (E, F) Dependence of frequency encoding on IP<sub>3</sub> turnover in the positive and negative feedback models, respectively. Shown are the differences  $\Delta T$  between the largest (for low stimulation) and smallest (for high stimulation) oscillation period.

To summarize, frequency encoding in the two feedback models poses opposite requirements on IP<sub>3</sub> turnover: positive and negative feedbacks are efficient frequency modulators when the IP<sub>3</sub> turnover is fast and slow, respectively. The critical IP<sub>3</sub> lifetimes estimated in the model indicate that both cases could be realized physiologically.

### Period control is shared by all processes

The calculations have shown that the inclusion of IP<sub>3</sub> dynamics strongly alters the frequency properties of the oscillator and, particularly, leads to long-period oscillations. We have, therefore, quantified the control of the IP<sub>3</sub> dynamics and the other processes present in the model on the oscillation period. To this end, we have used the following sensitivity measure

$$C_i = \frac{\tau_i}{T} \frac{\partial T}{\partial \tau_i}, \quad \text{for } i = er, pm, p, r, \quad (9)$$

which we refer to as period control coefficients (see also Wolf et al. (42)). The  $C_i$  set the change of the oscillation period  $T$  in proportion to the change in the characteristic time  $\tau_i$  of an individual process  $i$ . We analyzed the control of the

following processes: IP<sub>3</sub> metabolism (with  $\tau_p$  as defined in Eqs. 3 and 4), the IP<sub>3</sub>R dynamics (with  $\tau_r$  as defined in Eq. 8), Ca<sup>2+</sup> transport across the ER membrane (achieved by scaling  $v_{rel} - v_{serca}$  with  $1/\tau_{er} = V_{serca}/K_{serca}$  in Eq. 6), and Ca<sup>2+</sup> transport across the plasma membrane (achieved by scaling  $v_{in} - v_{out}$  with  $1/\tau_{pm} = \varepsilon V_{pm}/K_{pm}$  in Eq. 6). A positive period control coefficient implies that a slowing of the respective process (i.e., increase in  $\tau_i$ ) raises the period. At any point, the period control coefficients sum to unity,  $C_p + C_r + C_{er} + C_{pm} = 1$ , so that each coefficient quantifies the relative contribution of a single process to the oscillation period (43).

The control coefficients were calculated for various levels of stimulation in the positive and negative feedback models. Because these levels correspond to different oscillation periods, we can plot the  $C_i$  against the period. Fig. 5, A and B, depict the result for the model without plasma-membrane fluxes of Ca<sup>2+</sup> ( $\varepsilon = 0$ ). Positive and negative feedback models yield a similar picture. The control is distributed between the dynamics of IP<sub>3</sub>, Ca<sup>2+</sup>, and IP<sub>3</sub>R. In long-period oscillations, the IP<sub>3</sub> turnover has the leading control (dot-dashed lines). The IP<sub>3</sub>R dynamics contributes more significantly to setting the period of fast oscillations, especially in the positive-feedback model (dotted lines).

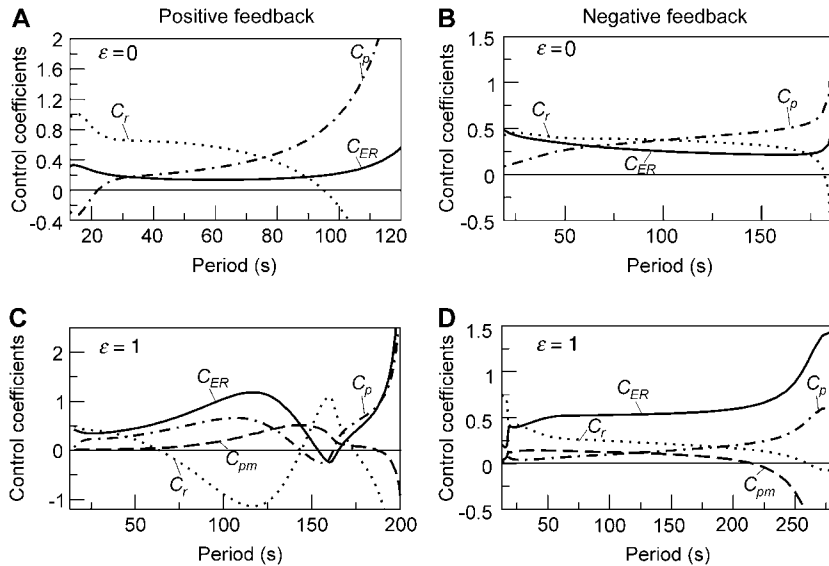


FIGURE 5 Control coefficients for the oscillation period. (A, B) Positive and negative feedback models, respectively, in the absence of  $\text{Ca}^{2+}$  fluxes across the plasma membrane ( $\varepsilon = 0$ ); control coefficients of  $\text{Ca}^{2+}$  exchange across the ER membrane ( $C_{ER}$ , solid line),  $\text{IP}_3$  metabolism ( $C_p$ , dashed line), and  $\text{IP}_3\text{R}$  dynamics ( $C_r$ , dotted line) as function of the period of the oscillations. A positive period control coefficient signifies that a slowing of the corresponding process increases the oscillation period. (C, D) Period control coefficients in the positive and negative feedback models, respectively, in the presence of plasma-membrane fluxes of  $\text{Ca}^{2+}$  ( $\varepsilon = 1$ ). The dash-dotted line indicates the control exerted by  $\text{Ca}^{2+}$  exchange across the plasma membrane ( $C_{pm}$ ).

The distribution of control becomes more complex when the plasma-membrane fluxes of  $\text{Ca}^{2+}$  are included (Fig. 5, C and D;  $\varepsilon = 1$ ), although, interestingly, the plasma-membrane fluxes exert very little period control themselves (dashed lines). There are several notable features. First, the fast oscillations in the positive-feedback model are no longer dominated by the  $\text{IP}_3\text{R}$  dynamics. There is even a rather counterintuitive behavior at intermediate periods where acceleration of the  $\text{IP}_3\text{R}$  dynamics would result in a slowing of the oscillations ( $C_r < 0$ ). Second, the overall tendency that the  $\text{IP}_3$  dynamics are more relevant for slow oscillations is preserved. However, the dynamics of ER  $\text{Ca}^{2+}$  release and refilling now play a more pronounced role in setting the period.

This quantification of period control reveals that no process can be singled out as a unique period controlling factor. Depending on the oscillation mechanism and the reference period, the  $\text{IP}_3$  turnover, the ER  $\text{Ca}^{2+}$  fluxes, and the  $\text{IP}_3\text{R}$  dynamics can all exert strong control.

### How to distinguish $\text{Ca}^{2+}$ feedbacks on $\text{IP}_3$ metabolism experimentally: model predictions

Our analysis has shown that oscillation mechanisms involving  $\text{Ca}^{2+}$ -activated PLC or  $\text{IP}_3\text{K}$  are sensitive to the time-scale of  $\text{IP}_3$ . This offers the possibility to experimentally interfere with the oscillation mechanism by perturbing the  $\text{IP}_3$  turnover.

The overexpression of  $\text{IP}_3$  metabolizing enzymes would accelerate the  $\text{IP}_3$  turnover and also decrease  $[\text{IP}_3]$  (see Eqs. 3–5). Overexpression of either  $\text{IP}_3$  5-phosphatase or  $\text{IP}_3$  3-kinase can abolish  $\text{Ca}^{2+}$  and  $\text{IP}_3$  oscillations. In the case of  $\text{IP}_3\text{P}$  overexpression, this effect can be revoked by an increase in agonist dose, while quenching of oscillations with overexpression of the  $\text{Ca}^{2+}$ -dependent  $\text{IP}_3\text{K}$  is predicted to be irreversible. However, positive- and negative-feedback

models behave in the same way (see Supplementary Material, Fig. S1).

A different result is obtained if the  $\text{IP}_3$  turnover is slowed by introducing  $\text{IP}_3$ -binding proteins ( $\text{IP}_3$  buffer) into the cell. To be specific, we assume a monovalent  $\text{IP}_3$  buffer with on- and off-rate constants  $k_{on}$  and  $k_{off}$ , respectively. The  $\text{IP}_3$  balance equation (Eq. 3) is then modified to

$$\begin{aligned} \frac{dp}{dt} &= \frac{1}{\tau_p} (v_{PLC} - v_{deg}) - k_{on} (B - C)p + k_{off} C, \\ \frac{dC}{dt} &= k_{on} (B - C)p - k_{off} C, \end{aligned} \quad (10a, b)$$

where  $p$  as before stands for  $[\text{IP}_3]$ ,  $B$  denotes the total concentration of the introduced  $\text{IP}_3$  buffer, and  $C$  is the concentration of occupied  $\text{IP}_3$  buffer. If the binding of  $\text{IP}_3$  to the buffer is fast compared to the  $\text{IP}_3$  degradation rate, the amount of occupied buffer will be in equilibrium with  $[\text{IP}_3]$ . This implies  $C = Bp/(K_B + p)$ , where  $K_B = k_{off}/k_{on}$  is the dissociation constant. Summing Eqs. 10a and 10b, and using the equilibrium relation for  $C$ , one obtains for the dynamics of unbound  $\text{IP}_3$

$$\frac{dp}{dt} = \frac{1}{\tau'_p} (v_{PLC} - v_{deg}), \quad (11)$$

with the modified characteristic time of  $\text{IP}_3$  turnover

$$\tau'_p = \tau_p \left( 1 + \frac{BK_B}{(K_B + p)^2} \right). \quad (12)$$

The  $\text{IP}_3$  buffer creates an additional bound pool of  $\text{IP}_3$  that is protected from degradation. Then the buildup of free  $[\text{IP}_3]$  after PLC activation is delayed, because the buffer binding sites also need to be filled. Similarly, the decay of free  $[\text{IP}_3]$  is slowed, because  $\text{IP}_3$  molecules dissociate from the buffer as cellular  $[\text{IP}_3]$  decreases. Precisely these two effects are accounted for by the modified time constant  $\tau'_p$ , which



increases with the buffer concentration (Eq. 12). Note that the balance between the rates of  $\text{IP}_3$  production and degradation is unaffected by the buffer. In particular, the  $\text{IP}_3$  buffer would not change the steady-state concentration of free  $\text{IP}_3$  attained when  $\nu_{\text{PLC}} = \text{const.}$  (The endogenous  $\text{IP}_3$  binding sites have already been accounted for by the characteristic time of  $\text{IP}_3$  turnover,  $\tau_p$ , defined in the absence of the exogenous  $\text{IP}_3$  buffer.)

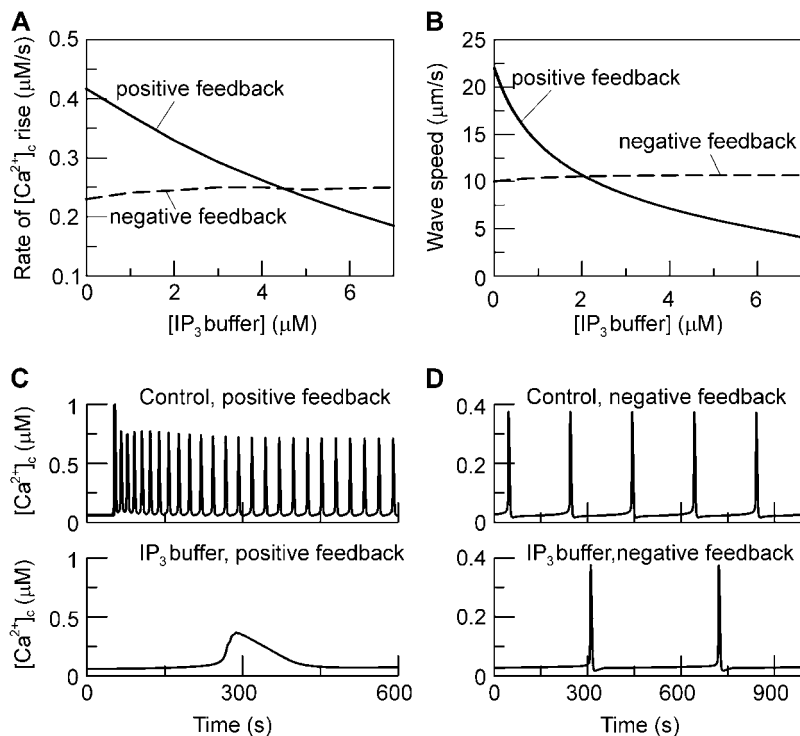
Introducing an exogenous  $\text{IP}_3$  buffer into a core  $\text{Ca}^{2+}$  oscillator model operating with constant PLC activity and  $\text{Ca}^{2+}$ -insensitive degradation (such as the model discussed above in the absence of  $\text{Ca}^{2+}$  feedbacks on  $\text{IP}_3$ ), will delay the rise in  $[\text{IP}_3]$  after PLC activation. However, eventually the same steady-state concentration of free  $[\text{IP}_3]$  would be reached as without buffer. Therefore,  $\text{Ca}^{2+}$  oscillations may set in with an increased latency, whereas spike shape and period would be unaffected by the presence of the  $\text{IP}_3$  buffer.)

When introducing the  $\text{IP}_3$  buffer into the positive- and negative-feedback models, we find that for low concentrations of  $\text{IP}_3$  buffer ( $0 < B < 10 \mu\text{M}$  for  $K_B = 0.13 \mu\text{M}$ ) the oscillations persist in both models. However, depending on which type of  $\text{Ca}^{2+}$  feedback is present,  $\text{IP}_3$  buffer affects the kinetic properties of the  $[\text{Ca}^{2+}]_c$  oscillations in very different ways. In the positive-feedback model, the  $\text{IP}_3$  buffer slows the  $\text{Ca}^{2+}$  responses. The rate of  $[\text{Ca}^{2+}]_c$  rise in an individual spike is predicted to be decreased by the buffer in a dose-dependent manner (Fig. 6 A, *solid line*). Another characteristic property of  $[\text{Ca}^{2+}]_c$  oscillations is the wave speed—the velocity at which a calcium spike propagates through the cell. To evaluate the effect of  $\text{IP}_3$  buffer on wave propagation, we

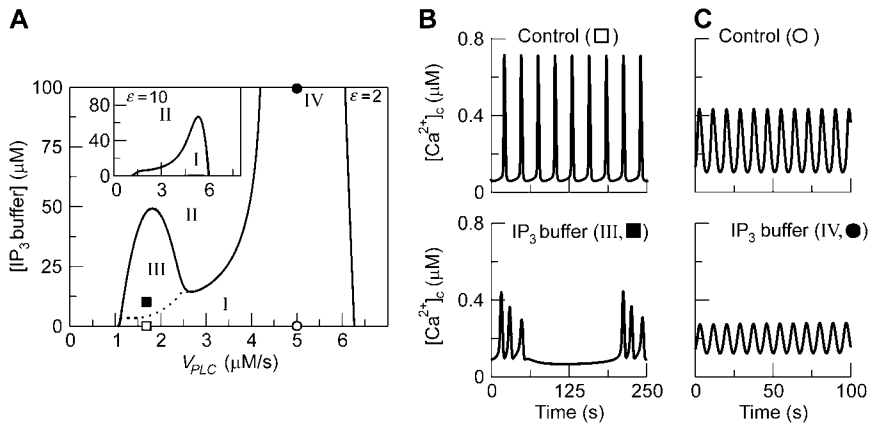
added to the model diffusion of  $\text{Ca}^{2+}$  and  $\text{IP}_3$  in the cytoplasm and solved the resulting reaction-diffusion equations numerically on a one-dimensional domain. The propagation speed of a  $\text{Ca}^{2+}$  spike shows a very similar behavior as the rate of  $[\text{Ca}^{2+}]_c$  rises: it decreases strongly as a function of  $\text{IP}_3$  buffer concentration in the positive-feedback model (Fig. 6 B, *solid line*). In contrast, in the negative-feedback model, the  $\text{IP}_3$  buffer will cause hardly perceptible increases in the rate of  $\text{Ca}^{2+}$  rise (Fig. 6 A, *dashed line*) and the wave speed (Fig. 6 B, *dashed line*). These two properties are well suited for experimental measurements, because they have been found to be remarkably constant in cells not perturbed by  $\text{IP}_3$  buffer (44–46).

For higher concentrations of  $\text{IP}_3$  buffer ( $B > 20 \mu\text{M}$ ), the differences between the positive- and negative-feedback models are even clearer. In the positive-feedback model, the  $\text{IP}_3$  buffer completely abolishes the  $[\text{Ca}^{2+}]_c$  oscillations, and instead a single slow  $[\text{Ca}^{2+}]_c$  transient is observed (Fig. 6 C). In the negative feedback model, the  $[\text{Ca}^{2+}]_c$  oscillations persist even for very high concentrations of  $\text{IP}_3$  buffer, although the oscillation period is increased (Fig. 6 D).

As  $\text{IP}_3$  buffering causes the strongest effects in the positive-feedback model, we have analyzed this case in more detail. Fig. 7 A summarizes the results by showing the regions of oscillatory behavior as a function of the stimulus ( $V_{\text{PLC}}$ ) and the concentration of exogenous  $\text{IP}_3$  buffer. Four different regions can be distinguished. In region I, the  $\text{IP}_3$  buffer slows the oscillations with respect to rise time and propagation speed (see *solid lines* in Fig. 6, A and B). In region II, high enough  $\text{IP}_3$  buffer concentrations abolish the



**FIGURE 6** Slowing of the  $\text{IP}_3$  turnover with an  $\text{IP}_3$  buffer. (A) The maximal rate of  $[\text{Ca}^{2+}]_c$  rise during a  $[\text{Ca}^{2+}]_c$  spike decreases as a function of  $\text{IP}_3$  buffer concentration in the positive-feedback model (*solid line*), whereas it is barely affected in the negative-feedback model (*dashed line*). The results are shown for  $V_{\text{PLC}} = 3 \mu\text{M/s}$  (positive feedback) and  $V_{\text{PLC}} = 0.4 \text{ nM/s}$  (negative feedback); similar results are obtained for other values. (B) The intracellular wave speed decreases as a function of  $\text{IP}_3$  buffer concentration in the positive-feedback model (*solid line*), whereas it is barely affected in the negative-feedback model (*dashed line*). A solitary  $\text{Ca}^{2+}$  wave is initiated by a local increase in  $[\text{IP}_3]$ ;  $[\text{Ca}^{2+}]_c$  and  $[\text{IP}_3]$  diffuse with diffusion constants of 20 and  $280 \mu\text{m}^2/\text{s}$ , respectively;  $V_{\text{PLC}} = 0.976 \mu\text{M/s}$  (positive feedback) and  $0.217 \text{ nM/s}$  (negative feedback). (C) High  $\text{IP}_3$  buffer concentration abolishes oscillations in the positive-feedback model ( $B = 50 \mu\text{M}$ ,  $V_{\text{PLC}} = 1.6 \mu\text{M/s}$ ). (D) Oscillations persist in the presence of  $\text{IP}_3$  buffer in the negative feedback model ( $B = 50 \mu\text{M}$ ,  $V_{\text{PLC}} = 0.6 \text{ nM/s}$ ). In all panels the  $\text{IP}_3$  buffer dissociation constant is  $K_B = K_p = 0.13 \mu\text{M}$ . For the positive-feedback calculations:  $\varepsilon = 2$ ,  $\tau_p = 1 \text{ s}$ ,  $\tau_r = 6 \text{ s}$ ,  $v_0 = 0.002 \mu\text{M/s}$ ,  $\phi = 0.001/\text{s}$ . For the negative-feedback model  $\varepsilon = 0.1$ . Other parameters are as listed in Table 1.



with  $\varepsilon = 2$ . When the strength of the  $\text{Ca}^{2+}$  plasma-membrane fluxes is increased ( $\varepsilon = 10$ ), regions III and IV disappear (inset). (B) Example of bursting oscillations observed in region III (top panel, control without  $\text{IP}_3$  buffer; bottom panel,  $[\text{IP}_3 \text{ buffer}] = 10 \mu\text{M}$ ;  $V_{\text{PLC}} = 1.6 \mu\text{M/s}$ ). (C) Example of oscillations in region IV ( $V_{\text{PLC}} = 5 \mu\text{M/s}$ ;  $[\text{IP}_3 \text{ buffer}] = 100 \mu\text{M}$ ), which are characterized by high frequency and low amplitude.

oscillations (shown in Fig. 6 C). In region III,  $\text{IP}_3$  buffer can cause more complex oscillations, such as the bursting oscillations shown in Fig. 7 B. For a large range of stimuli, the oscillations disappear when sufficiently high amounts of  $\text{IP}_3$  buffer are added (transition into region II). However for strong stimulation, there can be an additional domain, region IV (Fig. 7 A). Here, the oscillations persist even at very high  $\text{IP}_3$  buffer concentration but have strongly diminished amplitude (Fig. 7 C). Note that in the presence of sufficiently high  $\text{IP}_3$  buffer, only fast oscillations (at high stimulation) can be retained. The long-period oscillations, which depend on  $\text{Ca}^{2+}$  feedback on PLC are invariably abolished by the  $\text{IP}_3$  buffer.

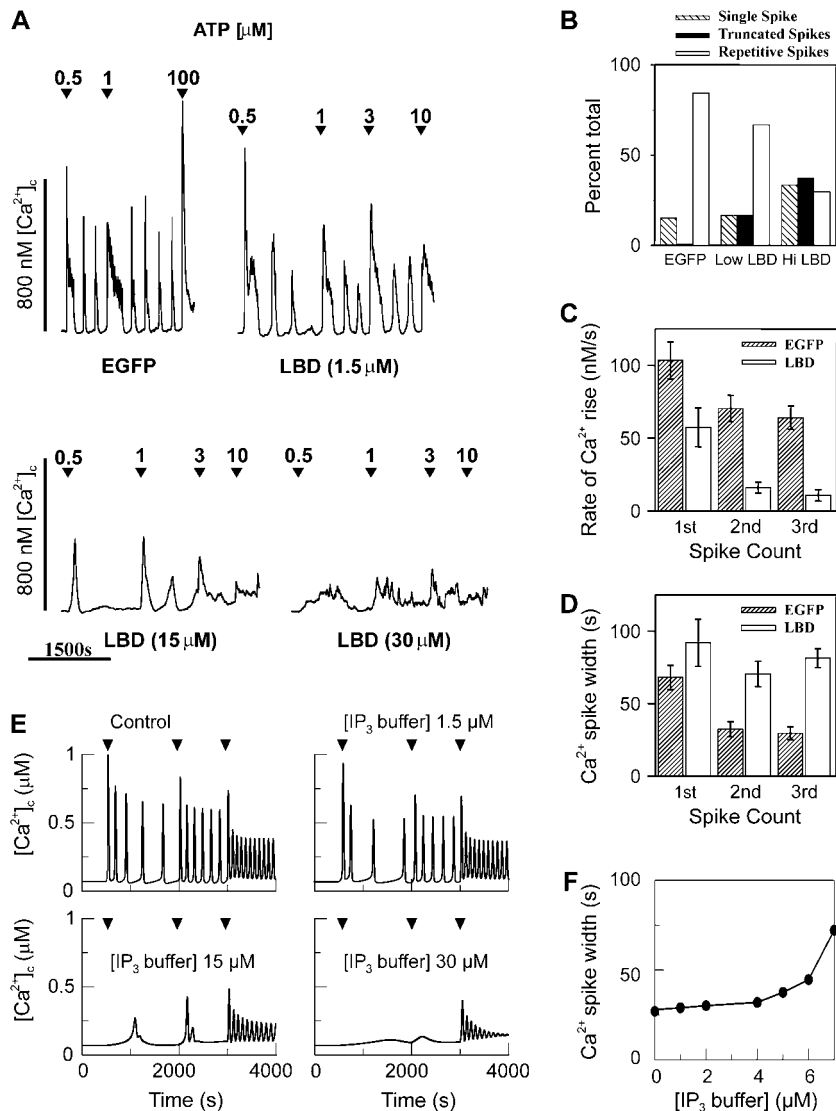
Whether regions III and IV exist, depends on the kinetic parameters of the  $\text{Ca}^{2+}$  fluxes. The inset in Fig. 7 A shows a situation where the  $\text{Ca}^{2+}$  fluxes across the plasma membrane were increased fivefold. Then only regions I and II remain, and sufficiently high buffering of  $\text{IP}_3$  always suppresses oscillations. Closer analysis revealed that if the system can oscillate for constant  $[\text{IP}_3]$  then  $\text{IP}_3$  buffer never completely abolish the oscillations and a region IV exists (A. Politi, unpublished data).

### Expression of an $\text{IP}_3$ buffer suppresses $\text{Ca}^{2+}$ oscillations

The most significant difference in the responses of the positive-feedback and negative-feedback models to the  $\text{IP}_3$  buffer is that agonist-induced oscillations always persist in the latter model whereas they can be abolished in the former. To examine the model predictions experimentally, we took advantage of a molecular  $\text{IP}_3$  buffer developed in our laboratory, which consists of the N-terminal ligand binding domain of rat type 1  $\text{IP}_3\text{R}$  linked to enhanced green fluorescent protein (EGFP-LBD; (47); L. Gaspers, P. Burnett, J. Johnston, A. Politi, T. Höfer, S. Joseph and A. Thomas, unpublished data). CHO cells were transiently transfected with EGFP or EGFP-LBD then challenged with submaximal

and maximal ATP concentrations. The subsequent  $[\text{Ca}^{2+}]_c$  responses were monitored via changes in the fura-2 fluorescence ratio. EGFP fluorescence was utilized to distinguish transfected from nontransfected cells in a given field of view and to estimate the intracellular concentration of the transgene (see Materials and Methods).

The addition of low ATP concentrations elicited periodic  $[\text{Ca}^{2+}]_c$  spikes in >85% of the CHO cells expressing EGFP (Fig. 8, A and B) or nonexpressing cells from cultures transfected with EGFP-LBD (not shown). Agonist-evoked  $[\text{Ca}^{2+}]_c$  oscillations required functional ER  $\text{Ca}^{2+}$  stores (i.e., they were thapsigargin-sensitive), but ceased abruptly upon removing extracellular  $\text{Ca}^{2+}$  suggesting that plasma membrane  $\text{Ca}^{2+}$  fluxes are relatively strong in this cell type (not shown). In both GFP-expressing and nonexpressing cells, the  $[\text{Ca}^{2+}]_c$  increase immediately after agonist challenge was more prolonged and the rate of  $\text{Ca}^{2+}$  rise faster than subsequent  $[\text{Ca}^{2+}]_c$  oscillations (Fig. 8, A and C). No systematic differences were evident in agonist sensitivity or the pattern of the  $[\text{Ca}^{2+}]_c$  spiking between EGFP expressing and non-expressing cells suggesting that neither EGFP nor the transfection reagents per se had significant effects on  $\text{Ca}^{2+}$  signaling. By contrast, the presence of EGFP-LBD had a dose-dependent effect on the agonist-dependent  $\text{Ca}^{2+}$  oscillations in CHO cells (Fig. 8 A). High levels of EGFP-LBD expression correlated with a loss of repetitive  $[\text{Ca}^{2+}]_c$  spiking and the appearance of low amplitude  $[\text{Ca}^{2+}]_c$  increases (Fig. 8, A and B). Moreover, EGFP-LBD expression significantly slowed the rate of  $[\text{Ca}^{2+}]_c$  rise (Fig. 8 C;  $p < 0.01$ ) and significantly broadened the width of the  $[\text{Ca}^{2+}]_c$  spike (Fig. 8 D;  $p < 0.05$ ) compared to EGFP expressing cells. For these data, we only analyzed EGFP-LBD expressing cells where low ATP challenge (0.5 or 1  $\mu\text{M}$ ) evoked at least three sequential baseline-separated  $\text{Ca}^{2+}$  spikes. This was observed predominately in cells expressing low levels of EGFP-LBD and, thus we probably underestimated the actions of  $\text{IP}_3$  buffering on the kinetics of  $[\text{Ca}^{2+}]_c$  oscillations.



**FIGURE 8** The effects of a molecular  $\text{IP}_3$  buffer on ATP-evoked  $[\text{Ca}^{2+}]_i$  oscillations in CHO cells. CHO cells ( $n = 5$  independent cultures) were transiently transfected with pEGFP-LBD (EGFP-LBD) or pEGFP-C1 (EGFP). The cells were loaded with fura-2/AM 16–48 h posttransfection and challenged with the indicated ATP concentrations. The traces (A) show typical ATP-evoked  $[\text{Ca}^{2+}]_i$  spikes in CHO cells transiently expressing EGFP or different levels of EGFP-LBD. The intracellular EGFP-LBD concentration was estimated as described in Materials and Methods. (B) Data show the effects of increasing EGFP-LBD expression on ATP-evoked  $\text{Ca}^{2+}$  signals. CHO cells expressing EGFP-LBD were arbitrarily divided into low ( $489 \pm 66$  units;  $n = 18$  cells) or high ( $3170 \pm 480$  units;  $n = 27$  cells) categories using a cutoff of 1000 fluorescence intensity units. The estimated mean EGFP-LBD concentration was  $6 \pm 0.8$  or  $38 \pm 6 \mu\text{M}$ , respectively. The mean EGFP fluorescence intensity was  $7400 \pm 615$  units ( $89 \pm 7 \mu\text{M}$ ;  $n = 198$  cells). Truncated spikes are defined as low amplitude  $[\text{Ca}^{2+}]_i$  oscillations similar to those shown in the bottom traces of panel A. The initial rates of  $[\text{Ca}^{2+}]_i$  rise (C) and the widths of the  $[\text{Ca}^{2+}]_i$  spike (D) were calculated in cells expressing EGFP ( $n = 52$  cells) or EGFP-LBD ( $n = 20$  cells) where ATP challenge (0.5 or  $1 \mu\text{M}$ ) evoked at least three sequential baseline-separated  $\text{Ca}^{2+}$  spikes. The width of the  $[\text{Ca}^{2+}]_i$  spike were determined at half-peak height. (E) The positive feedback model,  $\text{Ca}^{2+}$  activation of PLC, with different  $\text{IP}_3$  buffer concentrations (as indicated) shows a good agreement with the EGFP-LBD experimental data shown in panel A. An increase in ATP is simulated by an increase in the maximal activity of PLC (arrowheads). (F) In the positive feedback model we also observe a significant increase in spike width (calculated at half-peak height). To match the  $\text{Ca}^{2+}$  oscillations in CHO cells all variables have been slowed by a factor 10, reference parameter set as in Fig. 6  $\varepsilon = 10$ . In panel E,  $V_{\text{PLC}} = 0.125, 0.2, 0.4 \mu\text{M/s}$ . Initial condition at  $V_{\text{PLC}} = 0.05 \mu\text{M/s}$ . In panel F,  $V_{\text{PLC}} = 0.2 \mu\text{M/s}$ .

According to the theoretical results, the disappearance of the oscillations and a slowing of the  $\text{Ca}^{2+}$  rise suggests that  $\text{IP}_3$  oscillations driven by positive feedback of  $\text{Ca}^{2+}$  on  $\text{IP}_3$  production are involved in this system. We have simulated the positive-feedback model with relatively strong plasma-membrane  $\text{Ca}^{2+}$  fluxes as observed in CHO cells. At high concentrations of  $\text{IP}_3$  buffer (Fig. 8 E), the model exhibits single transients (for lower agonist dose), and repetitive truncated spikes (for high agonist dose). Both responses closely resemble the experimentally observed patterns in cells expressing high amounts of EGFP-LBD. The  $\text{Ca}^{2+}$  oscillations at lower concentrations of  $\text{IP}_3$  buffer (Fig. 8 F) exhibit a broadening of the individual spikes, which is very similar to the experimental observation in cells expressing low amounts of EGFP-LBD. Also the observed decrease of the rate of  $\text{Ca}^{2+}$  rise is reproduced by the model (data similar to Fig. 6 A). A model with negative feedback

could account for none of the experimental findings (see Fig. 6).

## DISCUSSION

There has been increasing evidence that hormone-evoked periodic  $\text{Ca}^{2+}$  spiking can be accompanied by oscillations of the  $\text{Ca}^{2+}$ -releasing second messenger  $\text{IP}_3$  (12–15). The experimental findings raise the questions of i), the underlying mechanisms of  $\text{IP}_3$  oscillations and ii), their potential functional role. The theoretical analysis and experiments presented here provide insight into both issues.

Several processes could be involved in the generation of  $\text{IP}_3$  oscillations. Feedbacks of  $\text{IP}_3$  and the second product of the PLC reaction, diacylglycerol, on PLC and upstream agonist receptor/G-protein could produce  $\text{IP}_3$  oscillations without involvement of  $\text{Ca}^{2+}$  (26,48). Alternatively, feedbacks

on  $\text{IP}_3$  metabolism may be mediated by  $\text{Ca}^{2+}$ , resulting in coupled  $\text{IP}_3$ - $\text{Ca}^{2+}$  oscillators (27,30,32,49). In this work, we have focused on the latter type of feedback oscillators because they can naturally account for the experimental observations of i),  $\text{Ca}^{2+}$  oscillations at clamped  $\text{IP}_3$  concentration and ii), coupled  $\text{IP}_3$  and  $\text{Ca}^{2+}$  oscillations. We considered prototypical positive and negative feedbacks of  $\text{Ca}^{2+}$  ions on  $\text{IP}_3$  metabolism:  $\text{Ca}^{2+}$  activation of PLC and  $\text{Ca}^{2+}$  activation of  $\text{IP}_3$  3-kinase, respectively.

The incorporation of such feedbacks into a core  $\text{Ca}^{2+}$  oscillator model based on the regulatory properties of the  $\text{IP}_3$  receptor can greatly expand the sensitivity of signal transduction to the hormonal stimulus. The presence of either feedback increases the range of agonist concentrations where one observes  $\text{Ca}^{2+}$  oscillations and enhances the ability to frequency-encode the agonist dose. Thus  $\text{Ca}^{2+}$  feedbacks on  $\text{IP}_3$  metabolism represent a possible mechanism for the generation of robust long-period oscillations. This is likely to be an important component of frequency-modulated  $\text{Ca}^{2+}$  signals, because physiological responses are controlled in this lower frequency range (50,51). Thus our model points to a physiological role of  $\text{IP}_3$  oscillations.

For the positive feedback to modulate the oscillation properties, the  $\text{Ca}^{2+}$  sensitivity of PLC needs to be only somewhat above basal  $[\text{Ca}^{2+}]_c$  ( $K_{\text{PLC}} = 0.1\text{--}0.2\ \mu\text{M}$ ). Such values are in agreement with experimental data (33). This feedback delays the onset of the  $\text{Ca}^{2+}$  spike, because both  $[\text{IP}_3]$  and  $[\text{Ca}^{2+}]_c$  must rise to a certain level for triggering explosive opening of the  $\text{IP}_3\text{R}$ . In this way, long oscillation periods arise for low levels of stimulation, whereas for strong stimuli the high  $\text{IP}_3$  level obviates the need for additional  $\text{Ca}^{2+}$  activation of PLC. We have here specifically assumed that  $\text{Ca}^{2+}$  and agonist act on the same isoform of PLC (e.g.,  $\text{PLC}\beta$ ). However, similar results were obtained in a model variant when the isoform  $\text{PLC}\delta$  is also included in the model, which is a  $\text{Ca}^{2+}$ -sensitive but agonist-insensitive PLC isoform (results not shown).

Negative feedback exerts control on the  $\text{Ca}^{2+}$  oscillations when  $\text{IP}_3$  removal takes place predominantly via  $\text{IP}_3\text{K}$  rather than by the  $\text{IP}_3\text{P}$  (>60% of the removal flux at high  $[\text{Ca}^{2+}]_c$  carried by  $\text{IP}_3\text{K}$ ). Long oscillation periods are generated when  $[\text{IP}_3]$  drops in the wake of a  $\text{Ca}^{2+}$  spike due to  $\text{IP}_3\text{K}$  activation and subsequently recovers slowly to the level needed to activate the  $\text{IP}_3\text{R}$ . We have found that this mechanism requires a finely tuned interplay between  $\text{IP}_3$  metabolism and  $\text{Ca}^{2+}$  fluxes. This sensitivity may explain the discrepancy to the work of Dupont and Erneux (32), who reported only small effects of  $\text{IP}_3\text{K}$  on  $[\text{Ca}^{2+}]_c$  oscillation periods. In contrast, the effects of the positive feedback were found to be robust with respect to the properties of the core  $\text{Ca}^{2+}$  oscillator.

The different modes of action of positive and negative feedbacks are reflected by opposing requirements on the lifetime of  $\text{IP}_3$ . In the case of positive feedback,  $\text{IP}_3$  turnover must be fast to support long-period oscillations, allowing for

i), coincidence of  $\text{Ca}^{2+}$  and  $\text{IP}_3$  spikes and ii), for the rapid removal of  $\text{IP}_3$  in the wake of a spike. In the case of negative feedback, slow  $\text{IP}_3$  turnover is required for the slow recovery of  $\text{IP}_3$  levels in between spikes. In different cellular systems, either one of the  $\text{IP}_3$  feedbacks could play a significant role in controlling  $\text{Ca}^{2+}$  oscillations, depending primarily on the underlying turnover rate of  $\text{IP}_3$ . However, they cannot be expected to act synergistically.

A critical question is the experimental identification of the mechanisms that drive  $\text{IP}_3$  oscillations. The theoretical analysis showed that slowing the  $\text{IP}_3$  turnover by means of an  $\text{IP}_3$  buffer can be used to distinguish between the two feedback mechanisms. An  $\text{IP}_3$  buffer can quench the oscillations generated by an  $\text{IP}_3$ - $\text{Ca}^{2+}$  oscillator based on positive feedback of  $\text{Ca}^{2+}$  on  $\text{IP}_3$ , but not by one based on negative feedback. Our preliminary modeling results indicate that the weak response of the negative-feedback oscillator to  $\text{IP}_3$  buffering could be a general property not limited to a mechanism operating through  $\text{IP}_3$  3-kinase. We obtained very similar results with an alternative mechanism acting through PKC-dependent inactivation of the agonist receptors.

To test this theoretical prediction, we overexpressed the ligand binding domain of the type 1  $\text{IP}_3\text{R}$  in a mammalian cell line, which acts as an  $\text{IP}_3$  buffer. The observed dose-dependent suppression of  $\text{Ca}^{2+}$  oscillations demonstrates that the  $\text{IP}_3$  dynamics play a critical role in the oscillator mechanism. Moreover, the detailed agreement between the experimental data and the simulations of the positive-feedback model is consistent with a coupled  $\text{IP}_3$ - $\text{Ca}^{2+}$  oscillator based on  $\text{Ca}^{2+}$  activation of PLC.

## SUPPLEMENTARY MATERIAL

An online supplement to this article can be found by visiting BJ Online at <http://www.biophysj.org>.

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