Inhibition of Ca^{2+} uptake in *Neurospora crassa* by La^{3+} : a mechanistic study

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Addition of 1 mM- LaCl_3 to *Neurospora crassa* 30 s prior to the initiation of $^{45}\text{Ca}^{2+}$ uptake resulted in a dramatic inhibition of Ca^{2+} influx to 7% of the control value. The lanthanide Gd^{3+} and several other recognized Ca^{2+} channel blockers (ruthenium red, nifedipine, methoxyverapamil) failed to inhibit Ca^{2+} influx. Direct measurement of membrane potential ($\Delta\psi$) with micro-electrodes revealed a La^{3+} -induced depolarization of about 80 mV for 1 mM- La^{3+} in the presence of 1 mM- Ca^{2+} . The depolarization is rapid and partially reversible on La^{3+} washout. The concentration-dependence of the depolarization can be described by a rectangular hyperbola with a $K_{0.5}$ for $\text{La}^{3+} = 0.11$ mM. The La^{3+} -induced depolarization is Ca^{2+} -sensitive, decreasing as external Ca^{2+} increases. The inhibitory effect of Ca^{2+} also exhibits a hyperbolic concentration-dependence, with a $K_{0.5}$ for $\text{Ca}^{2+} = 2.5$ mM for depolarization induced by 1 mM- La^{3+} . While the flux data suggest a direct effect of La^{3+} on Ca^{2+} uptake, the electrophysiological data imply additional effects of La^{3+} on the membrane. Three hypotheses were considered: (1) La^{3+} interacts with K^+ channels; (2) La^{3+} entry into cells carries a large depolarizing current; (3) La^{3+} inhibits the electrogenic H^+ -pump. Hypothesis (1) was eliminated by experiments showing that depolarization occurs regardless of whether the equilibrium potential for K^+ is positive or negative of the resting value of $\Delta\psi$. Hypotheses (2) and (3) remain possible, although La^{3+} influx of the magnitude required to generate the observed depolarization seems very unlikely. We conclude that La^{3+} should be deployed only with considerable caution as a blocker of plasma-membrane Ca^{2+} influx in fungi.

Introduction

Cytosolic free Ca^{2+} ($[\text{Ca}^{2+}]_c$) plays a ubiquitous role as a second messenger in animal and plant cells (Williamson & Monck, 1989; Johannes *et al.*, 1991). A similar function for $[\text{Ca}^{2+}]_c$ in eukaryotic micro-organisms is indicated by the presence of the calcium-binding protein calmodulin (Cox *et al.*, 1982; Davis *et al.*, 1986) and by the capacity of Ca^{2+} ionophores to induce a range of physiological responses (Reissig & Kinney, 1983; Nakashima, 1984; Harold & Harold, 1986).

In resting fungal cells, $[\text{Ca}^{2+}]_c$ resides in the range 100–300 nM (Halachmi & Eilam, 1989; Miller *et al.*, 1990). This low level is sustained by energized Ca^{2+} transport at both the plasma and vacuolar membranes. Efflux of Ca^{2+} to the external medium is probably mediated by an ATPase exchanging Ca^{2+} for (at least two) H^+ (Miller *et*

al., 1990), while Ca^{2+} accumulation in vacuoles is energized by simple $\text{Ca}^{2+}/\text{H}^+$ exchange (Ohsumi & Anraku, 1983). However, by analogy with animal cells, an increase in $[\text{Ca}^{2+}]_c$ during signal transduction is likely to be generated by the opening of discrete Ca^{2+} channels which mediate passive, downhill movement of Ca^{2+} into the cytosol.

Little is known about the nature of Ca^{2+} channels in fungi. Inositol 1,4,5-trisphosphate (InsP_3), which in animal cells is known to be generated by phospholipase-C-mediated hydrolysis of the trace plasma-membrane lipid phosphatidylinositol 4,5-bisphosphate (Berridge & Irvine, 1989), releases Ca^{2+} from vacuolar membrane vesicles of *Neurospora* (Cornelius *et al.*, 1989). In this respect fungi resemble plants rather than animals, where the major site of InsP_3 action is likely to be the endoplasmic reticulum (Berridge & Irvine, 1989). Nevertheless, fungal InsP_3 -elicited Ca^{2+} release is distinguished from that in both animals and plants in being insensitive to heparin (Ghosh *et al.*, 1988; Brosnan & Sanders, 1990). In yeast, Ca^{2+} entry across the plasma membrane is thought to be sensitive to membrane

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Abbreviation: $\Delta\psi$, membrane potential.

voltage ($\Delta\psi$; Eilam & Chernichovsky, 1987), although in the absence of methods for direct determination of $\Delta\psi$ in yeast, the quantitative significance of this finding might be questioned.

Recently, we have demonstrated that Ca^{2+} influx into *Neurospora crassa* can be determined if, as in yeast, wall-bound Ca^{2+} is removed by washing cells in ice-cold La^{3+} (Miller *et al.*, 1990). The objectives of this study were to determine (a) whether Ca^{2+} uptake in *Neurospora* is sensitive to any of the pharmacological agents which have been used to define various classes of Ca^{2+} channels in animals (Bean, 1989) and plants (Johannes *et al.*, 1991); (b) whether the inhibitory effects of Ca^{2+} channel antagonists on Ca^{2+} transport are specific; and (c) to what extent such effects can be considered direct, or are mediated indirectly, for example through changes in $\Delta\psi$.

Methods

Fungal culture and flux measurements. *Neurospora crassa* (wild-type strain RL21a) was grown on Vogel's (1956) medium for flux and electrophysiological experiments basically as described by Slayman & Slayman (1979). Briefly, for the flux experiments, liquid cultures (160 ml) were inoculated with about 10^6 conidia ml^{-1} and shaken vigorously for 12 h at 25 °C. Cells were then collected by filtration (Whatman no. 1) and resuspended in 50 ml of Standard Buffer (SB) comprising (concentrations in mM): MES/KOH (25), CaCl_2 (1), KCl (15), glucose (56), pH 5.8. The final K^+ concentration was 25 mM. After a further 15 min incubation with vigorous shaking at 25 °C, unidirectional Ca^{2+} influx was measured by the addition of $^{45}\text{Ca}^{2+}$ (final specific activity, 28 GBq mol^{-1}). At various times thereafter, samples (8 ml) were removed with a syringe, split into two aliquots and filtered separately (Whatman GF/A). Cells were then rapidly washed with 10 ml of ice-cold SB + 10 mM- LaCl_3 to displace Ca^{2+} from binding sites in the cell wall. After drying overnight, cells were weighed and radioactivity determined after addition of 3 ml of xylene-based scintillation fluid. Ca^{2+} uptake is expressed on a cell-water basis using the relationships 2.54 litre cell water per kg dry wt (Slayman & Tatum, 1964).

Electrical measurements. Cells for micro-electrode impalement were grown for 24 h on scratched cellophane placed over agar containing Vogel's medium. Hyphae were continuously perfused with SB solution throughout recordings. Electrical measurements were made with glass capillary micro-electrodes (tip diameter about 0.3 μm) filled with 100 mM-KCl. Micromanipulation was performed with Huxley-Goodfellow micromanipulators (Goodfellow Metals, Cambridge, UK) and the preparation was viewed under bright field illumination with a Nikon Diaphot microscope. Output from the micro-electrode was passed via a high input impedance differential electrometer (FD-223, WPI, New Haven, CT, USA) and recorded on a chart recorder. The circuit was completed with an KCl-agar bridge placed in the external medium.

Results

Addition of 1 mM- La^{3+} 30 s prior to the initiation of $^{45}\text{Ca}^{2+}$ uptake resulted in a dramatic inhibition of Ca^{2+}

Fig. 1. Inhibition of Ca^{2+} influx by 1 mM- LaCl_3 added 30 s before $^{45}\text{Ca}^{2+}$. ■, Control; □, 1 mM- La^{3+} . Data are the means of two replicates from a representative experiment.

Fig. 2. Representative recording showing La^{3+} -induced membrane depolarization by 1 mM- LaCl_3 in *Neurospora crassa*.

influx (Fig. 1). Thus, the absolute value for Ca^{2+} influx declined from 0.32 to only 0.02 mmol (litre intracellular water) $^{-1}$, or 7% of the control value.

It might be argued that this effect of La^{3+} relates simply to competition for extracellular Ca^{2+} -binding sites, which are not efficiently depleted of Ca^{2+} during the filtration/wash. However, several lines of evidence suggest that extracellular binding of Ca^{2+} is very small. First, cell-associated Ca^{2+} rises as a linear function of time for at least the first 90 s, with the 'zero-time' intercept comprising less than 0.1 mmol (litre intracellular water) $^{-1}$. Second, preliminary experiments with the lanthanide Gd^{3+} (1 mM) failed to show the inhibition of influx which might have been anticipated if La^{3+} and Ca^{2+} were simply competing for non-specific binding sites in the cell wall. We therefore conclude that La^{3+} inhibits Ca^{2+} transport into the cell. Several other compounds known to be Ca^{2+} channel antagonists in animal cells were tested – ruthenium red (50 μM), nifedipine (50 μM) and methoxyverapamil (80 μM) – but none elicited any inhibited effects on Ca^{2+} influx (data not shown).

Do the effects of La^{3+} on Ca^{2+} influx result from direct interaction of La^{3+} with the Ca^{2+} -permeable pathway, or

Fig. 3

Fig. 4

Fig. 3. La^{3+} -induced depolarization is concentration-dependent in a hyperbolic manner. Experimental data were fitted to the Michaelis-Menten equation to calculate $K_{0.5}$ and maximum depolarization. Each point represents an independent experiment. The total number of experiments was 14.

Fig. 4. Effect of external Ca^{2+} concentration ($[\text{Ca}^{2+}]_0$) on depolarization induced by La^{3+} . Each point is the mean of two independent experiments. The line is fitted according to the relationship $D_{\text{obs}} = D_{\text{Ca}=0} - (I_{\text{max}}[\text{Ca}^{2+}]/(K_i + [\text{Ca}^{2+}]_0))$, where D_{obs} is the observed depolarization, $D_{\text{Ca}=0}$ is the depolarization in the absence of Ca^{2+} , I_{max} is the depolarization at saturating Ca^{2+} and K_i is the inhibition constant for Ca^{2+} .

are the effects indirect? One possible mechanism for inhibition of Ca^{2+} influx might arise, for example, if La^{3+} perturbs $\Delta\psi$. Fig. 2 shows that 1 mM- La^{3+} , applied in identical conditions to those used in the $^{45}\text{Ca}^{2+}$ flux determinations, generates a rapid, extensive and reproducible membrane depolarization amounting to about 80 mV (Fig. 2). This depolarization was succeeded by a spontaneous, but slower partial repolarization which accounted for about 40% of the initial depolarization in Fig. 2, but was more variable between experiments. The effect of La^{3+} on $\Delta\psi$ was only partially reversible, with a sustained 20 mV depolarization remaining after La^{3+} washout.

La^{3+} -induced depolarization is concentration-dependent (Fig. 3). Fitting the data to a rectangular hyperbola yields an estimated maximum depolarization amounting to 87 mV and an apparent $K_{0.5}$ for $\text{La}^{3+} = 110 \mu\text{M}$.

The major portion of the La^{3+} -induced depolarization is Ca^{2+} -sensitive, being reduced as the $[\text{Ca}^{2+}]$ is raised above the control value of 1 mM and increased to as much as 100 mV as the $[\text{Ca}^{2+}]$ is lowered close to zero (Fig. 4). This Ca^{2+} -sensitive inhibition saturates as a function of Ca^{2+} concentration, and has a maximum (I_{max}) = 86 mV and an apparent K_i for $\text{Ca}^{2+} = 2.5 \text{ mM}$.

If the sole action of La^{3+} were to block Ca^{2+} channels, then membrane hyperpolarization might be anticipated because the equilibrium potential for Ca^{2+} is positive of zero. The marked membrane depolarization elicited by La^{3+} demonstrates a more complex action of La^{3+} . In

plant cells, K^+ channels are blocked both by extracellular Ca^{2+} (Tester, 1990) and by La^{3+} (Keifer & Spanswick, 1978; Tester & MacRobbie, 1990). Since the La^{3+} -induced depolarization in *Neurospora* is largely inhibited by extracellular Ca^{2+} , one possible secondary effect of La^{3+} might be interaction with Ca^{2+} -sensitive K^+ channels. This possibility is most simply tested by comparing the polarity and magnitude of $\Delta\psi$ changes in response to La^{3+} for conditions in which the K^+ equilibrium potential (E_K) is first one side, then the other, of the resting $\Delta\psi$ just before La^{3+} addition.

Fig. 5 shows the results of one such experiment. In order to bring $\Delta\psi$ within the range of accessible values of E_K , the membrane was first depolarized with 1 mM- CN^- . In these conditions, electrogenic H^+ pumping is inhibited considerably (Gradmann *et al.*, 1978). For the control K^+ concentration (25 mM), E_K was -36 mV (assuming cytoplasmic K^+ activity is 100 mM) and a small depolarization from -70 mV to -35 mV resulted on La^{3+} application (Fig. 5a). The mean resting $\Delta\psi$ in the presence of CN^- and La^{3+} was $-43 \pm 7 \text{ mV}$ (six observations). Thus, La^{3+} -induced opening of K^+ channels remains a possible explanation for the depolarization. However, K^+ channel involvement is excluded by the results of experiments in which the external K^+ concentration is reduced to 2.5 mM and E_K therefore shifts to -94 mV . Application of La^{3+} in the presence of CN^- still induces a small depolarization (Fig. 5b) to a mean resting value of $\Delta\psi = -36 \pm 6 \text{ mV}$ (three

Fig. 5. (a) Effect of La^{3+} on $\Delta\psi$ in presence of 1 mM-NaCN at an external K^+ concentration ($[\text{K}^+]_o$) of 25 mM (experiment representative of four performed). (b), as (a) but with $[\text{K}^+]_o = 2.5$ mM (experiment representative of three performed).

observations), despite the considerably more negative value of E_K .

Discussion

Our radiometric studies with $^{45}\text{Ca}^{2+}$ confirm that La^{3+} inhibits Ca^{2+} influx in *Neurospora*, as is the case for other fungi (Borbolla & Peña, 1980; Eilam & Chernichovsky, 1987). Ruthenium red (an inhibitor of endomembrane Ca^{2+} channels), nifedipine (a dihydropyridine) and methoxyverapamil (a phenylalkylamine) – all inhibitors of L-type Ca^{2+} channels in animal cells – were ineffective as inhibitors of Ca^{2+} influx in *Neurospora*.

These results appear to indicate inhibition of a Ca^{2+} channel by La^{3+} . This interpretation is supported by recordings of the effects of La^{3+} on $\Delta\psi$. Previous estimates of Ca^{2+} influx in *Neurospora* (Miller *et al.*, 1990) revealed only a slight stimulation of flux by CN^- , which strongly depolarizes the membrane, and therefore the potent inhibition of Ca^{2+} influx by La^{3+} appears unlikely to have its origins in any decrease in inward driving force on Ca^{2+} associated with La^{3+} -induced depolarization. However, if La^{3+} does indeed interact directly with a plasma-membrane Ca^{2+} channel, then some explanation must be sought for the La^{3+} -induced depolarization itself. Thus, the equilibrium potential for Ca^{2+} is of the order +100 mV, and simple blockage of a

Ca^{2+} channel might be expected to hyperpolarize, rather than depolarize, the membrane.

Three potential alternative modes of action of La^{3+} on membrane electrical properties might be considered. First, by analogy with plant cells (Keifer & Spanswick, 1978; Tester & MacRobbie, 1990), La^{3+} might interact with K^+ channels – perhaps mimicking inhibition by external Ca^{2+} , or possibly antagonizing an inhibitory effect of Ca^{2+} . Clearly, the capacity of Ca^{2+} to antagonize the La^{3+} -induced depolarization (Fig. 4) indicates some form of interaction between the two ions. Nevertheless, an involvement of K^+ -selective channels can be eliminated by the observation that La^{3+} depolarizes the membrane regardless of whether the equilibrium potential for K^+ is positive or negative of the resting $\Delta\psi$.

A second possibility is that the depolarization is generated by an in-going La^{3+} current. However, simple calculations based on the near-linear current-voltage characteristic of *Neurospora* do not support this notion either. Thus, the membrane conductance of *Neurospora* lies between 100 and 500 $\mu\text{S cm}^{-2}$ (Gradmann *et al.*, 1978), so a membrane depolarization of 100 mV (Fig. 4) indicates the presence of an inward current of 10 to 50 $\mu\text{A cm}^{-2}$. This value converts to a charge flow of between 25 and 120 meq (litre intracellular water) $^{-1}$ min $^{-1}$ (Sanders *et al.*, 1983), which for a trivalent ion is 8 to 40 mmol (litre intracellular water) $^{-1}$ min $^{-1}$. These values are one or two orders of magnitude higher than the

measured influx of Ca^{2+} [$0.32 \text{ mmol (litre intracellular water)}^{-1} \text{ min}^{-1}$; Fig. 1]. For the depolarization to result solely from an inward La^{3+} current, the membrane would therefore have to be considerably more permeable to La^{3+} than to Ca^{2+} , which is unlikely. For example, semi-quantitative studies on a number of plant tissues have indicated that lanthanides enter cells only slowly, or not at all (Campbell *et al.*, 1974; Campbell & Thomson, 1977; Thomson *et al.*, 1973; Quiquampoix *et al.*, 1990).

A third possible mechanism for La^{3+} -induced depolarization invokes inhibition of the primary plasma membrane H^+ -ATPase. Previous work with charophyte algae (Tsutsui *et al.*, 1987) has suggested that La^{3+} is a potent inhibitor of the plasma-membrane electrogenic pump of plants, and a similar effect appears, by default, to comprise the most likely explanation for the present results. Our data give no indication concerning the location (extracellular or intracellular) at which La^{3+} interacts with transport. However, the clear antagonism by Ca^{2+} of the La^{3+} -induced depolarization suggest that, whatever the cause of the depolarization, La^{3+} interferes with some Ca^{2+} -dependent event.

We conclude that although La^{3+} is likely to interact directly with Ca^{2+} -permeable pathway(s) in the plasma membrane of *Neurospora*, the ion also has other effects on the membrane, which result in a marked membrane depolarization. Considerable caution should therefore be exercised in the interpretation of investigations which rely on La^{3+} to block plasma-membrane Ca^{2+} influx in fungi (for example, during signal transduction).

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References

- BEAN, B. P. (1989). Classes of calcium channels in vertebrate cells. *Annual Review of Physiology* **51**, 367–384.
- BERRIDGE, M. J. & IRVINE, R. F. (1989). Inositol phosphates and cell signalling. *Nature, London* **341**, 197–205.
- BORBOLLA, M. & PEÑA, A. (1980). Some characteristics of Ca^{2+} uptake by yeast cells. *Journal of Membrane Biology* **54**, 149–156.
- BROSNAN, J. M. & SANDERS, D. (1990). Inositol trisphosphate-mediated Ca^{2+} release in beet microsomes is inhibited by heparin. *FEBS Letters* **260**, 70–72.
- CAMPBELL, N. A. & THOMSON, W. W. (1977). Effects of lanthanum and ethylenediaminetetraacetate on leaf movements of *Mimosa*. *Plant Physiology* **60**, 635–639.
- CAMPBELL, N., THOMSON, W. W. & PLATT, K. (1974). The apoplastic pathway of transport to salt glands. *Journal of Experimental Botany* **25**, 61–69.
- CORNELIUS, G., GEBAUER, G. & TECHEL, D. (1989). Inositol trisphosphate induces calcium release from *Neurospora crassa* vacuoles. *Biochemical and Biophysical Research Communications* **162**, 852–856.
- COX, J. A., FERRAZ, C., DEMAILLE, J. G., PEREZ, R. O., VAN TUINEN, D. & MARME, D. (1982). Calmodulin from *Neurospora crassa*. General properties and conformational changes. *Journal of Biological Chemistry* **257**, 10694–10700.
- DAVIS, N. N., URDEA, M. S., MASIAZ, F. R. & THORNER, J. (1986). Isolation of yeast calmodulin gene: calmodulin is an essential protein. *Cell* **47**, 423–431.
- EILAM, Y. & CHERNICHOVSKY, D. (1987). Uptake of Ca^{2+} driven by the membrane potential in energy-depleted yeast cells. *Journal of General Microbiology* **133**, 1641–1649.
- GHOSH, T. K., EIS, P. S., MULLANEY, J. M., EBAT, C. L. & GILL, D. L. (1988). Competitive, reversible, and potent antagonism of inositol 1,4,5-trisphosphate-activated calcium release by heparin. *Journal of Biological Chemistry* **263**, 11075–11079.
- GRADMANN, D., HANSEN, U. P., LONG, W. S., SLAYMAN, C. L. & WARNCKE, J. (1978). Current voltage relationships for the plasma membrane and its principal electrogenic pump in *Neurospora crassa*. I. Steady-state conditions. *Journal of Membrane Biology* **39**, 333–367.
- HALACHMI, D. & EILAM, Y. (1989). Cytosolic and vacuolar calcium concentrations in yeast cells measured with the calcium-sensitive fluorescence dye Indo-1. *FEBS Letters* **256**, 55–61.
- HAROLD, R. L. & HAROLD, F. M. (1986). Ionophores and cytochalasins modulate branching in *Achlya bisexualis*. *Journal of General Microbiology* **132**, 213–219.
- JOHANNES, E., BROSNAN, J. M. & SANDERS, D. (1991). Calcium channels and signal transduction in plant cells. *BioEssays* **13**, 331–336.
- KEIFER, D. W. & SPANSWICK, R. M. (1978). Activity of the electrogenic pump in *Chara corallina* as inferred from measurements of the membrane potential, conductance, and potassium permeability. *Plant Physiology* **62**, 653–661.
- MILLER, A. J., VOGG, G. & SANDERS, D. (1990). Cytosolic calcium homeostasis in fungi: roles of plasma membrane transport and intracellular sequestration of calcium. *Proceedings of the National Academy of Sciences of the United States of America* **87**, 9348–9352.
- NAKASHIMA, H. (1984). Calcium inhibits phase shifting of the circadian conidiation rhythm of *Neurospora crassa* by the calcium ionophore A23187. *Plant Physiology* **74**, 268–271.
- OHSUMI, Y. & ANRAKU, Y. (1983). Calcium transport driven by a proton motive force in vacuolar membrane vesicles of *Saccharomyces cerevisiae*. *Journal of Biological Chemistry* **258**, 5614–5617.
- QUIQUAMPOIX, H., RATCLIFFE, R. G., RATKOVIC, S. & VUCINIC, Z. (1990). ^1H and ^{32}P NMR investigation of gadolinium uptake in maize roots. *Journal of Inorganic Biochemistry* **38**, 265–275.
- REISSIG, J. L. & KINNEY, S. G. (1983). Calcium as a branching signal in *Neurospora crassa*. *Journal of Bacteriology* **154**, 1397–1402.
- SANDERS, D., SLAYMAN, C. L. & PALL, M. L. (1983). Stoichiometry of H^+ /amino acid cotransport in *Neurospora crassa* revealed by current-voltage and analysis. *Biochimica et Biophysica Acta* **735**, 67–76.
- SLAYMAN, C. W. & TATUM, E. L. (1964). Potassium transport in *Neurospora*. I. Intracellular sodium and potassium concentrations, and cation requirement for growth. *Biochimica et Biophysica Acta* **88**, 578–592.
- SLAYMAN, C. L. & SLAYMAN, C. W. (1979). Whole cells for the study of transport linked to membrane potential. *Neurospora crassa. Methods in Enzymology* **55**, 656–666.
- TESTER, M. (1990). Plant ion channels: whole-cell and single-channel studies. *New Phytologist* **114**, 305–340.
- TESTER, M. & MACROBBIE, E. A. C. (1990). Cytoplasmic calcium affects the gating of potassium channels in the plasma membrane of *Chara corallina*: a whole-cell study using calcium-channel effectors. *Planta* **180**, 569–581.
- THOMSON, W. W., PLATT, K. A. & CAMPBELL, N. (1973). The use of lanthanum to delineate the apoplastic continuum in plants. *Cytobios* **8**, 57–62.
- TSUTSUI, I., OHKAWA, T., NAGAI, R. & KISHIMOTO, U. (1987). Role of calcium in the excitability and electrogenic pump activity of the *Chara corallina* membrane. I. Effects of La^{3+} , verapamil, EGTA, W-7, and TFP on the action potential. *Journal of Membrane Biology* **96**, 65–73.
- VOGEL, H. J. (1956). A convenient growth medium for *Neurospora* (Medium N). *Microbial Genetics Bulletin* **13**, 42.
- WILLIAMSON, J. R. & MONCK, J. R. (1989). Hormone effects on cellular Ca^{2+} fluxes. *Annual Review of Physiology* **51**, 107–124.