the term *bistable-switch*. Thus, there are at least two different but entirely compatible ways of thinking about the switches in fate produced by mutations such as *sevenless* and *cut*, one with strong evolutionary implications and the other with an emphasis on specific gene interactions.

We have considered two genes whose products act at different points in the long and still unknown chain of events that stretches from the determination of a cell in a specific location to become different from its neighbors, to the final differentiation of that cell or its progeny. The *sevenless* gene encodes a membrane-associated protein that probably plays a critical role in cell–cell communication and the initial determination process; the *cut* gene encodes a nuclear, probably DNA-binding protein which seems to be essential in normal differentiation. The switches in cell fate seen when these genes mutate to a loss of function can be interpreted on two levels, one mechanistic and the other evolutionary. As the catalogue of well-analysed genes grows, increasingly substantive interpretations of their role both in neural development and in the evolutionary history of developmental mechanisms can be expected.

Selected references

1 Harris, W. A., Stark, W. S. and Walker, J. A. (1976) *J. Physiol. (London)* 256, 415–439
2 Ready, D. F., Hanson, T. E. and Benzer, S. (1985) *Dev. Biol.* 53, 217–240
3 Tomlinson, A. (1985) *J. Embryol. Exp. Morph.* 89, 313–331
4 Tomlinson, A. and Ready, D. F. (1986) *Science* 231, 400–402
5 Banerjee, U., Renfranz, P. J., Pollock, J. A. and Benzer, S. (1987) *Cell* 49, 281–291
6 Hafen, E., Basler, K., Edstroem, J-E. and Rubin, G. (1987) *Science* 236, 55–63
7 Basler, K. and Hafen, E. (1988) *Cell* 54, 299–311
8 Tomlinson, A., Bowtell, D. D. L., Hafen, E. and Rubin, G. M. (1987) *Cell* 51, 143–150
9 Banerjee, U., Renfranz, P. J., Pollock, J. A. and Benzer, S. (1987) *Cell* 51, 151–158
10 Tomlinson, A. and Ready, D. F. (1987) *Dev. Biol.* 123, 264–275
11 Campos-Ortega, J. A., Jürgens, G. and Hofbauer, A. (1979) *Roux Arch. Ent. Org.* 186, 27–50
12 Reinke, R. and Zipursky, L. *Cell* (in press)
13 Jan, Y. N., Bodmer, R., Jan, L. Y., Ghysen, A. and Dambly-Chaudiere, C. (1986) *UCLA Symp. Molec. Cell Biol.* 49, 45–56
14 Bodmer, R., Barbel, S., Shepherd, S., Jack, J. W. and Jan, Y. N. (1987) *Cell* 51, 293–307
15 Jack, J. W. (1985) *Cell* 42, 869–876
16 Blochlinger, K., Bodmer, R., Jack, J. W., Jan, L. Y. and Jan, Y. N. (1988) *Nature* 333, 629–635
17 Gehring, W. J. (1987) *Science* 236, 1245–1252
18 Scott, M. P. and Carroll, S. B. (1987) *Cell* 51, 689–698
19 Campos-Ortega, J. A. (1988) *Trends Neurosci.* 11, 400–405
20 Ghysen, A. and Dambly-Chaudiere, C. (1988) *Genes & Develop.* 2, 495–501
21 Lewis, E. B. (1963) *Am. Zool.* 3, 33–56
22 Kukalova-Peck, J. (1987) *Can. J. Zool.* 65, 2327–2345
23 Waddington, C. H. (1966) *Principles of Development and Differentiation* Macmillan
24 Edgar, B. A., Odell, G. M. and Schubiger, G. *Dev. Genet.* (in press)
25 Hartenstein, V. (1988) *Development* 102, 869–886

viewpoint

Intracellular Ca^{2+} activates phospholipase C

David A. Eberhard and Ronald W. Holz

It is well established that a receptor-mediated mechanism, perhaps involving a guanine nucleotide binding protein, directly activates polyphosphoinositide-specific phospholipase C. Recent evidence indicates that in excitable tissues a rise in cytosolic Ca^{2+} can also activate the phospholipase C. The activation of phospholipase C by Ca^{2+} can be a direct effect rather than a result of the Ca^{2+}-dependent release of neurotransmitters which activate phospholipase C through a receptor-mediated mechanism. Ca^{2+}-activated phospholipase C may represent a positive feedback system for Ca^{2+}: small increases in cytosolic Ca^{2+} induced by Ca^{2+} influx across the plasma membrane may result in higher cytosolic Ca^{2+} concentrations due to IP_3-induced release of Ca^{2+} from intracellular stores. The activation of phospholipase C by Ca^{2+} may also provide a mechanism for diacylglycerol generation and protein kinase C activation following Ca^{2+} influx. Thus, the regulation of phospholipase C activity by Ca^{2+} may be physiologically important in regulating cytosolic Ca^{2+} and protein kinase C in excitable tissues.

David A. Eberhard and Ronald W. Holz are at the Department of Pharmacology, University of Michigan Medical School, Ann Arbor, MI 48109-0626, USA.

Since the seminal review by Michell in 1975[1] of the relationship between the metabolism of inositol phospholipids and cell receptor activation, considerable effort has been made to link the breakdown of these lipids to specific cell functions. One of the central issues was whether a rise in intracellular Ca^{2+} is a result of or the cause of the hydrolysis of the phosphoinositides. Indeed, one of the suggestions from the Michell review was that phosphoinositide turnover results in the liberation of Ca^{2+} from intracellular stores. Subsequent work has confirmed this idea and we now understand that in many cell types, inositol trisphosphate (IP_3), which is a product of receptor-stimulated hydrolysis of phosphatidylinositol 4,5-bisphosphate (PIP_2) by the enzyme phospholipase C (PLC)[2,3], releases Ca^{2+} from intracellular, non-mitochondrial stores[4].

A rise in intracellular Ca^{2+} can also occur secondary to influx of Ca^{2+} through ligand-gated channels or, in electrically excitable tissue, through voltage-gated ion channels. Micromolar Ca^{2+} activates several different phosphoinositide-specific PLC activities from brain[5,6] and seminal vesicles[7]. In addition, micromolar Ca^{2+} causes production of diglyceride or inositol phosphates from permeabilized platelets[8], prolactin tumor (GH3) cells[9], chromaffin cells[10] and insulinoma (RINm5F) cells[11]. The following discussion presents recent experiments showing that PLC activation can accompany Ca^{2+} influx, and suggests that Ca^{2+} itself is an important physiological activator of PLC in some tissues.

Intracellular PLC activation by Ca^{2+}

Support for direct receptor-mediated activation of PLC comes from demonstrations that, after stimulation of Ca^{2+}-mobilizing receptors, the generation of inositol phosphates precedes or coincides with a rise in intracellular Ca^{2+}, and that IP_3 releases Ca^{2+}

0378 - 5912/88/$02.00

from intracellular stores[4]. However, investigators using preparations derived from excitable tissues have repeatedly described PLC activity that seems to result from an increase in cytosolic Ca^{2+}. Akhtar and Abdel-Latif[12] found that the Ca^{2+} ionophore A23187 caused the breakdown of PIP_2 in iris smooth muscle. Griffin and Hawthorne[13] and Fisher and Agranoff[14] found that A23187 caused the hydrolysis of PIP_2 and PIP in guinea-pig synaptosomes. Kendall and Nahorski observed that the production of inositol phosphates in rat cerebral cortical slices is increased by A23187 (Ref. 15), or by depolarization with elevated K^+(Ref. 16). The effect of the latter involves dihydropyridine-sensitive Ca^{2+} channels. We found that nicotinic receptor-channel stimulation and elevated K^+ increased the formation of inositol phosphates in bovine adrenal chromaffin cells, an effect that could be inhibited by organic Ca^{2+} channel antagonists[10]. All of these effects were either dependent upon medium Ca^{2+}, or were strongly inhibited by EGTA.

Are Ca^{2+} effects direct or indirect?

A major question in the interpretation of these findings is whether PLC activation is a direct consequence of Ca^{2+} influx or is secondary to the Ca^{2+}-dependent release of neurotransmitters, which in turn activates PLC through a receptor-mediated mechanism. For example, in an extension of their study cited above, Akhtar and Abdel-Latif found that the α_1-adrenergic receptor antagonist prazosin blocked the ionophore-induced hydrolysis of PIP_2, indicating that the effect was mediated by catecholamine release from nerve endings in the smooth muscle preparation[17]. Unfortunately, the task of showing conclusively that PLC activation by Ca^{2+} in neural or secretory tissues is *not* caused by a released substance is not so straightforward. In many studies it seems unlikely that the stimulated formation of inositol phosphates results from the release of an endogenous substance, since the activation of PLC requires far less extracellular Ca^{2+} than is generally necessary to support transmitter release. As little as 10 μM Ca^{2+} (the estimated concentration of Ca^{2+} in 'Ca^{2+}-free' media without EGTA) is sufficient for the ionophore-stimulated breakdown of polyphosphoinositides in synaptosomes[13,14] and the ionophore- and depolarization-stimulated formation of inositol phosphates in brain slices[15].

In primary cultures of purified bovine chromaffin cells, there is strong evidence that a rise in cytosolic Ca^{2+} directly stimulates PLC[10]. Exocytosis of catecholamine and PLC activation induced by nicotinic receptor-channel stimulation or by elevated K^+ both require extracellular Ca^{2+} and are inhibited by organic Ca^{2+} channel antagonists. Thus, both processes are triggered by an influx of Ca^{2+}. The following indicates that the activation of PLC is not caused by a secreted substance: (1) PLC activation by either agent requires less than one-tenth the extracellular Ca^{2+} concentration than does exocytosis; (2) addition of a nicotinic receptor antagonist after nicotinic agonist-induced secretion is essentially complete (in medium containing 2.2 mM Ca^{2+}) totally blocks subsequent PLC activity; (3) medium from chromaffin cells that had been stimulated to secrete does not increase PLC activity in naive cells; and (4) increasing the incubation volume without increasing cell number does not alter the nicotinic stimulation of PLC. Thus, PLC activation is likely to be a direct result of Ca^{2+} influx rather than an indirect, receptor-mediated effect of a secreted substance. Muscarinic stimulation of bovine chromaffin cells (which does not cause secretion) activates PLC through a receptor-mediated mechanism that does not require Ca^{2+} influx[10]. Hence, in the same cells, the mechanism by which PLC is activated depends upon the type of signal at the plasma membrane.

In summary, in tissues with ligand-gated channels that are permeable to Ca^{2+} or in excitable tissues, two distinct mechanisms for PLC activation exist: direct receptor coupling (probably through a GTP-binding protein[18]) to PLC; or activation induced by a rise in cytosolic Ca^{2+}. This conclusion is shown schematically in Fig. 1.

Ca^{2+} may modulate the response to receptor/G protein activation of PLC

The effects of intracellular Ca^{2+} and receptor/G protein activation on modulation of PLC activity may be interrelated. The ability of muscarinic agonists to stimulate polyphosphoinositide turnover is enhanced by Ca^{2+} ionophore in synaptosomes[14]. The effects of the Ca^{2+} ionophore are probably distal to the muscarinic receptor, since the ionophore does not alter the binding characteristics of the receptor. An interaction between Ca^{2+}- and G protein-dependent mechanisms of PLC regulation is demonstrated by the synergy between Ca^{2+} and the guanine nucleotides in activating PLC in permeabilized cells[9,11]. An increase in cytosolic Ca^{2+} may, therefore, increase the sensitivity of PLC to receptor activation.

Influx of Na^+ may act through Ca^{2+} to activate PLC

Na^+ ions have also been implicated in PLC activation. Gusovsky *et al.*[19] reported that a number of agents that cause Na^+ influx through various mechanisms also increase the accumulation of inositol phosphates in guinea-pig synaptoneurosomes. The effect is blocked by EGTA but does not involve voltage-dependent Ca^{2+} channels[20]. Sodium channel activators also increase inositol phosphate accumulation in chick heart cells[21]. Increased intracellular Na^+ may cause more Ca^{2+} to enter the cytoplasm by Na^+/Ca^{2+} exchange across the inner mitochondrial membrane and across the plasma membrane[22]. Indeed, large elevations of cytosolic Ca^{2+} occur in chick heart cells upon incubation with sodium channel activators[21].

Excitatory amino acid receptors linked to ionic channels may activate PLC through a rise in cytosolic Ca^{2+}

Quisqualate (Q), and *N*-methyl-D-aspartate (NMDA) excitatory amino acid receptors have been linked to PLC activation in the striatum[23] and in cul-

tures of cerebellar granule cells[24]. The ability of NMDA receptor stimulation to activate PLC is profoundly inhibited by physiological concentrations of extracellular Mg^{2+} (Ref. 24); the inhibition by Mg^{2+} is noncompetitive for the NMDA binding site and can be overcome by depolarization. In contrast, Mg^{2+} has little effect on the activation of PLC through Q receptors[24]. The effect of Mg^{2+} on the functional coupling of the NMDA receptor to PLC is remarkably similar to its effect on the NMDA receptor-associated channel[25], which is permeable to Ca^{2+} and Na^{+}. Since Mg^{2+} is thought to inhibit ion influx by directly blocking the channel, it is possible that PLC activation by NMDA receptor agonists occurs as a result of Ca^{2+} entry. The Ca^{2+} dependency of the NMDA activation of PLC has not been demonstrated and the possibility that the activation results from the release of a neurotransmitter that directly activates PLC through another membrane receptor has not been excluded.

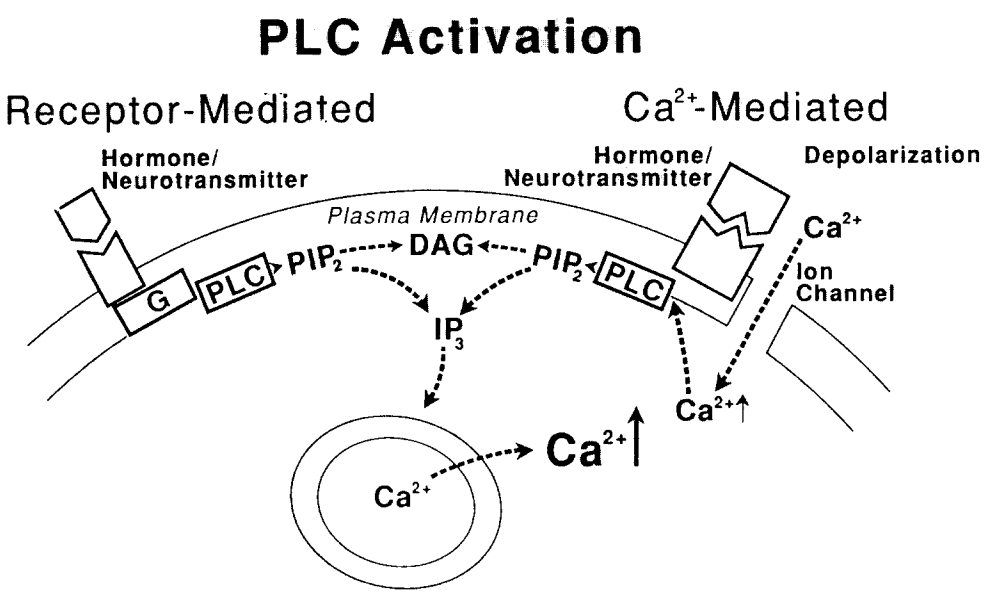

Fig. 1. *Phospholipase C can be activated by a direct receptor-mediated mechanism or by Ca^{2+} influx. On the left a hormone/neurotransmitter receptor is coupled to activation of PLC by a GTP-binding protein (G). This represents a commonly accepted mechanism for activation of PLC. On the right, Ca^{2+} entry either through a hormone/neurotransmitter-gated ion channel or through a voltage-sensitive Ca^{2+} channel causes an increase in cytosolic Ca^{2+}, which in turn activates Ca^{2+}-sensitive PLC. IP_3 generated by either mechanism will release Ca^{2+} from intracellular stores and increase cytosolic Ca^{2+}. For activation of PLC by cytosolic Ca^{2+}, the IP_3-induced release of Ca^{2+} from intracellular stores represents a positive-feedback system for increasing cytosolic Ca^{2+}. The receptor/G protein-activated PLC and the Ca^{2+}-activated PLC may be the same or different proteins. Although it is likely that the receptor/G protein-activated PLC acts at the plasma membrane as depicted, the Ca^{2+}-activated PLC may act at the plasma membrane (as shown) or on membranes of other intracellular organelles, since the activation of PLC by Ca^{2+} need not occur at the plasma membrane.*

Physiological implications

The direct receptor-mediated activation of PLC results in production of IP_3 and diacylglycerol (DAG), each of which are second messengers. IP_3 (or metabolites) releases Ca^{2+} from intracellular stores and, thereby, increases cytosolic Ca^{2+} (Fig. 1). DAG activates protein kinase C. When Ca^{2+}, either through depolarization- or ligand-gated channels, enters the cells, the same second messengers are generated. However, in this case the effect of IP_3 to increase cytosolic Ca^{2+} represents a positive feedback system rather than the initial event that increases cytosolic Ca^{2+} (Fig. 1). Because in intact cells PLC activation by Ca^{2+} probably occurs upon small increases in cytosolic Ca^{2+} (see above), IP_3 production may be an important amplification system to increase cytosolic Ca^{2+} when Ca^{2+} influx is small. Large increases in cytosolic Ca^{2+} caused by Ca^{2+} influx are less likely to be significantly increased by IP_3. A positive feedback system whereby cytosolic Ca^{2+} induces increased cytosolic Ca^{2+} through the action of IP_3 involves a number of biochemical steps. It is unlikely to be important where rapid Ca^{2+} changes in response to depolarization are necessary, such as at the neuromuscular junction. However, the pathway may operate in generating slower or more prolonged synaptic or cellular responses. The involvement of PLC and IP_3 in regulating cytosolic Ca^{2+} in the presynaptic terminal could therefore serve an integrating function for incoming signals.

The ability of intracellular Ca^{2+} to regulate PLC activity suggests that a rise in cytosolic Ca^{2+} initially induced by direct receptor-mediated activation of PLC may in turn contribute to the prolonged activation of PLC. The rise in cytosolic Ca^{2+} may be a link in a positive feedback system for PLC. For example, in rat parotid cells, continuous incubation with substance P or a muscarinic agonist causes a large, transient increase in cytosolic Ca^{2+} followed by a lower, sustained increase (which is largely dependent upon extracellular Ca^{2+})[26]. The effects on Ca^{2+} are paralleled by a large, transient and then a smaller, sustained increase in inositol (1,4,5)-trisphosphate and inositol tetrakisphosphate (IP_4). In addition to the inositol phosphates being responsible for the increased cytosolic Ca^{2+}, increased cytosolic Ca^{2+} in the presence of extracellular agonist may contribute to the sustained level of activation of PLC. Thus, PLC once activated may have a tendency to maintain its own activity. A similar positive feedback effect may occur when PLC is activated by Ca^{2+} influx and a small increase in cytosolic Ca^{2+}; a further increase in cytosolic Ca^{2+} induced by inositol phosphates could contribute to sustained PLC activation.

There are a number of homeostatic mechanisms that would keep such a positive feedback mechanism in check. Following termination of the initial

Acknowledgements *We are grateful to S. K. Fisher for his comments about the review and for discussions concerning feedback mechanisms involving PLC. The work was supported by PHS Grants RO 1 DK27959 and PO1 HL18575. RWH is an Established Investigator of the American Heart Association. DAE was supported by PHS Training Grant T32 GM07767 and by a Lutheran Brotherhood Medical Scientist Scholarship.*

stimulus, cytosolic Ca^{2+} would return to resting levels through sequestration in organelles such as mitochondria, and through efflux across the plasma membrane. In addition, elevated intracellular Ca^{2+} may decrease the ability of IP_3 to release sequestered Ca^{2+} (Ref. 27). Furthermore, protein kinase C (PKC) activation by DAG (see below) may inhibit PLC activity through a negative feedback loop[28,29].

DAG and a rise in cytosolic Ca^{2+} will act synergistically to activate PKC[30]. Studies investigating the effects of exogenous activators of PKC indicate that PKC may be involved in increasing the sensitivity of the intracellular exocytotic machinery to Ca^{2+}, in regulating the permeability of ion channels, in the desensitization of receptors, and in synaptic plasticity (see Ref. 31 for review). PKC is translocated and activated in bovine chromaffin cells during nicotinic stimulation[32] or depolarization[33] and in hippocampus following high-frequency electrical stimulation of the perforant pathway *in vivo*[34]. Although it is uncertain whether the activation of PKC in these systems requires activation of PLC, the biochemical machinery exists to link functionally these two enzymes.

Concluding remarks

Recent evidence indicates that a rise in cytosolic Ca^{2+} can be the cause of the activation of polyphosphoinositide-specific phospholipase C as well as the result of its activation. The proposition that Ca^{2+} influx can regulate phospholipase C activity in excitable cells should not be viewed as a challenge to the receptor-activation model of control of phospholipase C. Both modes of regulating phospholipase C occur, even in the same cell. Regulation of phospholipase C activity by Ca^{2+} influx is an extension of some of the known characteristics of phospholipase C and may fulfill the demands of specialized cell functions.

Selected references

1 Michell, R. H. (1975) *Biochim. Biophys. Acta* 415, 81–147
2 Berridge, M. J. and Irvine, M. J. (1984) *Nature* 312, 315–321
3 Fisher, S. K. and Agranoff, B. W. (1987) *J. Neurochem.* 48, 999–1017
4 Berridge, M. J. (1987) *Annu. Rev. Biochem.* 56, 615–649
5 Rye, S. H., Cho, K. S., Lee, K-H., Suh, P. G. and Rhee, S. G. (1987) *J. Biol. Chem.* 262, 12511–12518
6 Rebecchi, M. J. and Rosen, O. M. (1987) *J. Biol. Chem.* 262, 12526–12532
7 Wilson, D. B., Bross, T. E., Hofmann, S. L. and Majerus, P. W. (1984) *J. Biol. Chem.* 259, 11718–11724
8 Haslam, R. J. and Davidson, M. M. L. (1984) *Biochem. J.* 222, 351–361
9 Martin, T. J. F., Lucas, D. O., Bajjalieh, S. M. and Kowalchyck, J. A. (1986) *J. Biol. Chem.* 261, 2918–2927
10 Eberhard, D. A. and Holz, R. W. (1987) *J. Neurochem.* 49, 1634–1643
11 Vallar, L., Biden, R. J. and Wollheim, C. B. (1987) *J. Biol. Chem.* 262, 5049–5056
12 Akhtar, R. A. and Abdel-Latif, A. A. (1978) *J. Pharmacol. Exp. Ther.* 204, 655–668
13 Griffin, H. D. and Hawthorne, J. N. (1978) *Biochem. J.* 176, 541–552
14 Fisher, S. K. and Agranoff, B. W. (1981) *J. Neurochem.* 37, 968–977
15 Kendall, D. A. and Nahorski, S. R. (1984) *J. Neurochem.* 42, 1388–1394
16 Kendall, D. A. and Nahorski, S. R. (1985) *Eur. J. Pharmacol.* 115, 31–36
17 Akhtar, R. A. and Abdel-Latif, A. A. (1984) *Biochem. J.* 224, 291–300
18 Gilman, A. G. (1987) *Annu. Rev. Biochem.* 56, 615–649
19 Gusovsky, F., Hollingsworth, E. B. and Daly, J. W. (1986) *Proc. Natl Acad. Sci. USA* 83, 3003–3007
20 Gusovsky, F., McNeal, E. T. and Daly, J. W. (1987) *Mol. Pharmacol.* 32, 479–487
21 McDonough, P. M., Goldstein, D. and Brown, J. H. (1988) *Mol. Pharmacol.* 33, 315–331
22 Carafoli, E. (1987) *Annu. Rev. Biochem.* 56, 395–433
23 Sladaczek, F., Pin, J-P., Recasens, M., Bockaert, J. and Weiss, S. (1985) *Nature* 317, 717–719
24 Nicoletti, F., Wroblewski, J. T. and Costa, E. (1987) *J. Neurochem.* 48, 967–973
25 Nowak, L., Bregestovski, H., Ascher, P., Herbet, A. and Prochiantz, A. (1983) *Nature* 307, 492–495
26 Merritt, J. E. and Rink, T. J. (1987) *J. Biol. Chem.* 262, 14912–14916
27 Chueh, S-H., and Gill, D. L. (1986) *J. Biol. Chem.* 261, 13883–13886
28 Orellana, S. A., Solski, P. A. and Brown, J. H. (1985) *J. Biol. Chem.* 260, 5236–5239
29 Brock, T. A. *et al.* (1985) *J. Biol. Chem.* 260, 14158–14162
30 Nishizuka, Y. (1984) *Science* 225, 1365–1370
31 Miller, R. J. (1986) *Trends Neurosci.* 9, 538–541
32 TerBush, D. R., and Holz, R. W. (1986) *J. Biol. Chem.* 261, 17099–17106
33 TerBush, D. R., Bittner, M. A. and Holz, R. W. *J. Biol. Chem.* (in press)
34 Akers, R. F., Lovinger, D. M., Colley, P. A., Linden, D. J. and Routtenberg, A. (1986) *Science* 231, 587–589