Expression of a mutated ras gene in Dictyostelium discoideum alters the binding of cyclic AMP to its chemotactic receptor

Ludérus, M.E.E.; Reymond, C.D.; van Haastert, Petrus; Driel, R. van

Published in:
Default journal

Publication date:
1988

Citation for published version (APA):
Ludérus, M. E. E., Reymond, C. D., Haastert, P. J. M. V., & Driel, R. V. (1988). Expression of a mutated ras gene in Dictyostelium discoideum alters the binding of cyclic AMP to its chemotactic receptor. Default journal.

Expression of a mutated *ras* gene in *Dictyostelium discoideum* alters the binding of cyclic AMP to its chemotactic receptor

M. E. E. LUDÉRUS[1], C. D. REYMOND[2], P. J. M. VAN HAASTERT[3] and R. VAN DRIEL[1]

[1]*Laboratory of Biochemistry, University of Amsterdam, PO Box 20151, 1000 HD Amsterdam, The Netherlands*
[2]*Swiss Institute for Experimental Cancer Research, CH-1066 Epalinges, Switzerland*
[3]*Cell Biology and Morphogenesis Unit, Zoological Laboratory, University of Leiden, 2311 GP Leiden, The Netherlands*

Summary

Dictyostelium discoideum cells contain a _ras_ gene that codes for a polypeptide that is highly homologous to the human _ras_ proteins. Extra copies of the wild-type gene or a gene carrying a missense mutation in codon 12 (_ras_-Gly12 and _ras_-Thr12, respectively) have been introduced into _Dictyostelium_ cells by transformation.

We have investigated the properties of the chemotactic cell surface cyclic AMP receptor in crude membrane preparations of wild-type _Dictyostelium_ cells and _ras_-Gly12 and _ras_-Thr12 transformants. _In vitro_, an ATP- and Ca^{2+}-dependent reduction of the number of cyclic AMP receptors was observed in membranes from all three cell types. The number of available receptors was decreased maximally by about 50%. In the presence of ATP the half-maximal Ca^{2+} concentration required for this process was about 10^{-5} M in wild-type and _ras_-Gly12 membranes, and less than 10^{-7} M in _ras_-Thr12 membranes. Addition of GTP (but not GDP) or the phorbol ester PMA (phorbol-12-myristate-13-acetate) reduced the Ca^{2+} requirement of the process in wild-type and _ras_-Gly12 membranes to the physiological level of less than 10^{-7} M. In membranes derived from _ras_-Thr12 cells addition of GTP or PMA had no effect.

The results indicate that _D. discoideum_ cells contain a cyclic AMP receptor-controlling pathway that can be activated _in vitro_ and involves a GTP-binding protein and a Ca^{2+} plus ATP-dependent activity, possibly protein kinase C. It is concluded that the _ras_ protein specifically interacts with this pathway; the pathway appears to be constitutively activated by the mutated _ras_ gene product.

Key words: *ras* gene, chemotactic cyclic AMP receptor, *D. discoideum*, protein kinase C.

Introduction

Mammalian *ras* genes code for 21 000M_r proteins, which bind GTP and show GTPase activity (Manne *et al.* 1984, 1985; McGrath *et al.* 1984). Mutations at various positions in the *ras* coding region reduce the GTPase activity of the protein, can induce a transformed phenotype and have been associated with tumorigenicity (Taparowsky *et al.* 1982; Balmain & Pragnett, 1983; Fasano *et al.* 1984; Seeburg *et al.* 1984; Der *et al.* 1986). The cellular slime mould *Dictyostelium discoideum* contains a single *ras* gene that codes for a polypeptide highly homologous to mammalian *ras* proteins (Reymond *et al.* 1984). Extra copies of the wild-type *ras* gene (*ras*-Gly12) or of a gene with a missense mutation at codon 12 (*ras*-Thr12) were introduced into *Dictyostelium* cells by transformation (Nellen *et al.* 1984). Expression of the mutated gene has been reported to result in aberrant morphogenesis and enhanced cyclic AMP-induced desensitization of guanylate cyclase (Reymond *et al.* 1986; Van Haastert *et al.* 1987). No effect of overexpression of the wild-type *ras* gene has been observed to date.

We have investigated the binding of cyclic AMP to the chemotactic cell-surface receptor in crude membrane preparations of wild-type *D. discoideum* cells and *ras*-Gly12 and *ras*-Thr12 transformants. Our results indicate that the *ras* protein is involved in a process that reduces the total number of available cyclic AMP receptors *in vitro*. This receptor loss appeared to be the result of an ATP- and Ca^{2+}-dependent activity, possibly protein kinase C.

Journal of Cell Science 90, 701–706 (1988)

Materials and methods

Materials

[5′,8-^{3}H]cyclic AMP (1·55 TBq mmol^{-1}) was purchased from Amersham International (UK), cyclic AMP and dithiothreitol from Serva (Heidelberg, FRG) and 5′-AMP, GDP, GTP and Gpp(NH)p (guanyl-imidodiphosphate) from Boehringer-Mannheim, FRG). PMA (phorbol-12-myristate-13-acetate), 4α-PDD (4α-phorbol-12,13-didecanoate) and geneticin were from Sigma.

Methods

Isolation of crude D. discoideum *membranes.* Transformants of *D. discoideum* strain Ax3 were made and grown as described by Reymond *et al.* (1986). Wild-type Ax3 cells and the transformed cell lines *ras*-Gly12 and *ras*-Thr12 were grown to a density of 5×10^{6} cells ml^{-1}, collected by centrifugation, washed once with 15 mM-potassium/sodium phosphate buffer (pH 6·5) and resuspended in the same buffer at a density of 10^{7} cells ml^{-1}. After starvation for 6 h at 22°C, the cells were washed once in homogenization medium (HM), containing 40 mM-Hepes–NaOH (pH 7·7), 1 mM-dithiothreitol, 0·5 mM-EDTA, 250 mM-sucrose and the following protease inhibitors: 5 mM-benzamidin, 100 μg ml^{-1} aprotinin, 50 μg ml^{-1} trypsin inhibitor, 20 μg ml^{-1} antipain and 0·1 mM-phenylmethylsulphonyl fluoride (PMSF). Subsequently, the cells were resuspended in the same buffer (10^{8} cells ml^{-1}) and lysed by nitrogen cavitation (Janssens *et al.* 1986). The lysate was centrifuged for 10 min at 10 000 ***g*** at 4°C and the pellet was resuspended in HM. The crude membrane preparation was stored in liquid nitrogen.

Cyclic AMP binding assays. Crude membranes (final protein concentration 0·5 mg ml^{-1}) were incubated for 2 min at 22°C in 100 μl of 20 mM-potassium phosphate buffer (pH 7·0). When indicated, ATP, GTP, GDP, PMA or Ca^{2+} was present, in combinations and at concentrations given in the text. A Ca^{2+}-EGTA buffer (1 mM) was used to control the free Ca^{2+} concentration (Bartfai, 1979). Cyclic AMP binding was initiated by adding 10 nM-[^{3}H]cyclic AMP (1·78 TBq mmol^{-1}) in the presence of 20 μM-5′-AMP and 10 mM-dithiothreitol, which inhibits phosphodiesterase activity (Green & Newell, 1975). After equilibration for 5 min at 0°C, the amount of bound [^{3}H]cyclic AMP was determined by measuring the radioactivity in the membrane pellet after 2 min centrifugation at 10 000 ***g***. Non-specific binding was determined by equilibration of [^{3}H]cyclic AMP in the presence of 0·1 mM unlabelled cyclic AMP.

Results and Discussion

Incubation of crude *D. discoideum* membranes in the presence of ATP and increasing Ca^{2+} concentrations reduced equilibrium cyclic AMP binding to the chemotactic receptor (measured at 10 nM-cyclic AMP) by up to about 50% (Fig. 1A). In the absence of ATP no effect of Ca^{2+} was observed. Millimolar Ca^{2+} concentrations were required to reduce cyclic AMP binding in membranes derived from control cells and from *ras*-Gly12 transformants. For membranes from *ras*-Thr12 transformants submicromolar Ca^{2+} concentrations (i.e. in the physiological range) were already sufficient to induce the maximal decrease in cyclic AMP binding.

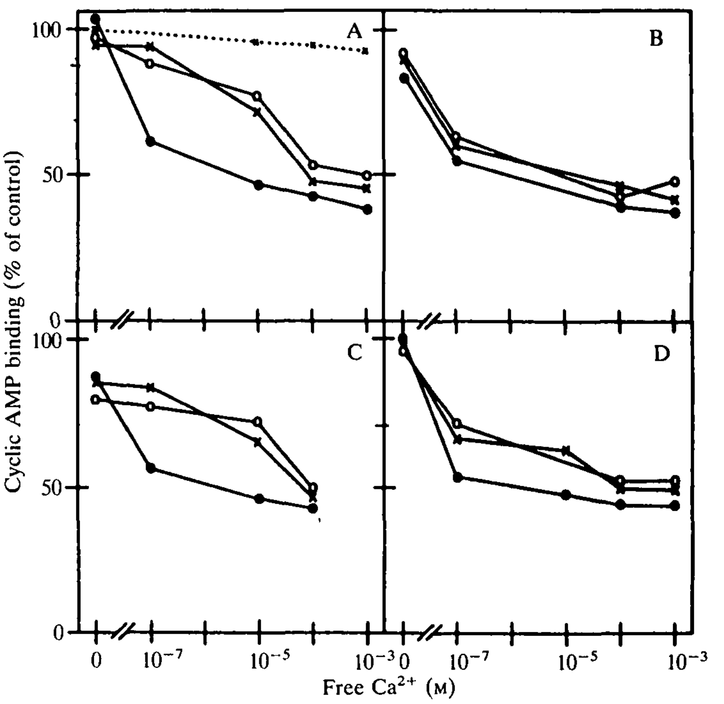

Fig. 1. Effect of ATP, Ca^{2+}, guanine nucleotides and phorbol ester on cyclic AMP binding to the chemotactic receptor. Crude membranes isolated from wild-type Ax3 cells (×) and the transformed cell lines *ras*-Gly12 (○) and *ras*-Thr12 (●) were incubated for 2 min at 22 °C in 100 μl 20 mM-potassium phosphate buffer (pH 7·0) containing free Ca^{2+} concentrations as indicated in the figure, and: A, 1 mM-ATP or no additions (·····); B, 1 mM-ATP and 0·1 mM-GTP; C, 1 mM-ATP and 0·1 mM-GDP; D, 1 mM-ATP and 1 μM-PMA. Subsequently, the membranes were equilibrated with 10 nM-[^{3}H]cyclic AMP for 5 min at 0°C and the amount of specifically bound [^{3}H]cyclic AMP was measured. Each point is the mean of a determination in triplicate of an experiment reproduced four to seven times.

The Ca^{2+} requirement of the process in control membranes and *ras*-Gly12 membranes could be shifted to lower concentrations by the addition of 0·1 mM-GTP (or the non-hydrolysable analogue Gpp(NH)p, result not shown) (Fig. 1B). In *ras*-Thr12 membranes the Ca^{2+} dependency of the process was not affected by GTP (Fig. 1B). GDP did not change the Ca^{2+} sensitivity in any of the membrane preparations (Fig. 1C).

These data indicate that a GTP-binding protein is involved in a Ca^{2+}-dependent process that results in decreased cyclic AMP–receptor binding *in vitro*. Furthermore, the results are consistent with the hypothesis that the mutated, oncogene-like *ras*-Thr12 gene codes for a protein that is irreversibly activated by GTP (Chiarugi *et al.* 1985; Fleischman *et al.* 1986).

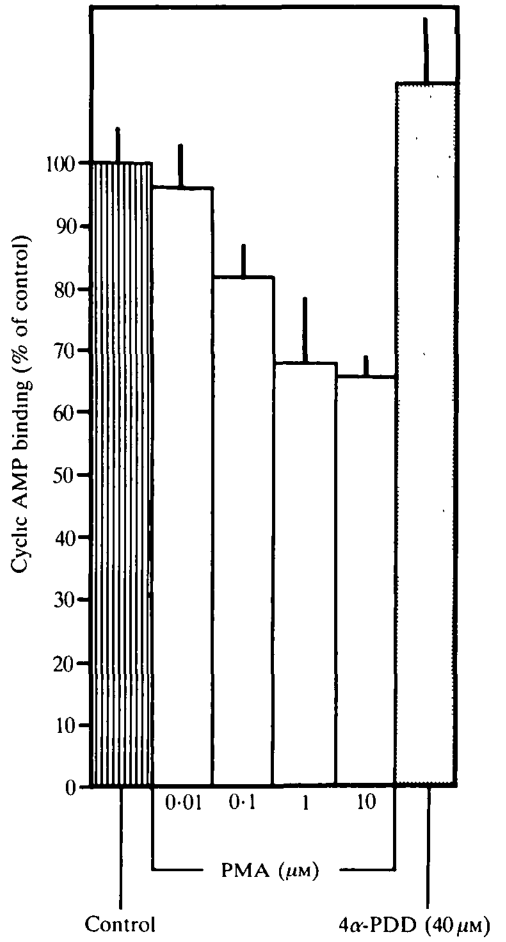

Fig. 2. Effect of phorbol ester PMA and phorbol ester analogue 4α-PDD on cyclic AMP binding to the chemotactic receptor. Crude membranes from wild-type Ax3 cells were incubated for 2 min at 22°C with PMA (concentrations indicated in the figure) or 4α-PDD (40μM), in the presence of 1 mM-ATP and 10^{-7} M-Ca^{2+}. Subsequently, the membranes were equilibrated for 5 min at 0°C with 10 nM-[^{3}H]cyclic AMP in the presence of 20μM-5′-AMP and 10 mM-dithiothreitol. The membranes were then sedimented by centrifugation for 2 min at 10 000 *g* and the amount of specifically bound [^{3}H]cyclic AMP in the membrane pellet was determined. Each value is the mean of the results of a determination in triplicate of an experiment reproduced twice.

Like GTP, the phorbol ester PMA lowered the Ca^{2+} requirement of membranes from wild-type cells and *ras*-Gly12 transformants (Fig. 1D). In the presence of 10^{-7} M-Ca^{2+} and 1 mM-ATP, PMA reduced cyclic AMP binding in these membranes by 30 % and 21 %, respectively, to a level close to that of *ras*-Thr12 membranes in the absence of PMA. Under these conditions a half-maximal effect in wild-type crude membranes was observed at about 100 nM-PMA (Fig. 2). The PMA analogue 4α-PDD, which in contrast to PMA is incapable of activating mammalian protein kinase C (Ashendel, 1985), had no effect on cyclic AMP-receptor binding in crude *D. discoideum* membranes at concentrations up to 40μM (Fig. 2). The requirement for ATP and Ca^{2+}, the stimulatory effect of phorbol ester and the phorbol ester specificity strongly suggest the involvement of protein kinase C in the regulation of cyclic AMP-receptor function (Kishimoto *et al.* 1980; Nishizuka, 1984; Ashendel, 1985; Bell, 1986). If this interpretation is correct, activation of *D. discoideum* protein kinase C requires a significantly higher PMA concentration than has been reported for protein kinase C from mammalian cells (Nishizuka, 1984).

In order to determine the cause of the decrease in cyclic AMP binding after incubation with ATP and Ca^{2+}, equilibrium receptor binding studies were carried out (Fig. 3). In the presence of ATP, but in the absence of Ca^{2+}, the three types of membrane preparations had similar cyclic AMP binding properties. Incubation with ATP and 10^{-7} M-Ca^{2+} dramatically reduced the total number of available cyclic AMP binding sites of *ras*-Thr12 membranes (Fig. 3C). A high Ca^{2+} concentration (10^{-4} M) was required to induce the same effect in membranes from control cells and *ras*-Gly12 transformants (Fig. 3A,B). These data show that the decrease in cyclic AMP binding that was induced by ATP and Ca^{2+} (as seen in Fig. 1) was mainly due to a decrease in available cyclic AMP receptor sites, rather than to a lowered affinity of the receptors. In intact *D. discoideum* cells, a similar effect on cyclic AMP receptors is seen after stimulation of cells with high (micromolar) concentrations of cyclic AMP (Klein & Juliani, 1977; Van Haastert, 1987*a*). This phenomenon is known as receptor-downregulation. It has been associated with desensitization of the receptor-stimulated adenylate cyclase activity (Van Haastert, 1987*a*), but the molecular mechanism underlying the downregulation is not known.

Besides the Ca^{2+} plus ATP-dependent reduction of the number of cyclic AMP binding sites, other conditions *in vitro* have been described that affect cyclic AMP-receptor properties of *D. discoideum*. Addition of GTP (or GDP) to isolated membranes results in a decrease in receptor affinity: the cyclic AMP receptor is converted from a slowly dissociating, high-affinity state to a fast-dissociating, low-affinity one (Janssens *et al.* 1986; Van Haastert *et al.* 1986). The total number of receptors is not affected. GTP and GDP are thought to bind to a guanine nucleotide-binding protein (G-protein), that interacts with the cyclic AMP receptor. As can be seen in Fig. 1C (data points at zero Ca^{2+}), GTP and GDP both reduced equilibrium cyclic AMP binding by about 20 % in all three membrane types. It must be noted that this value can vary considerably from preparation to preparation of membranes (Janssens *et al.* 1986; Van Haastert *et al.* 1986; M.E.E. Ludérus, unpublished). At high Ca^{2+} concentrations

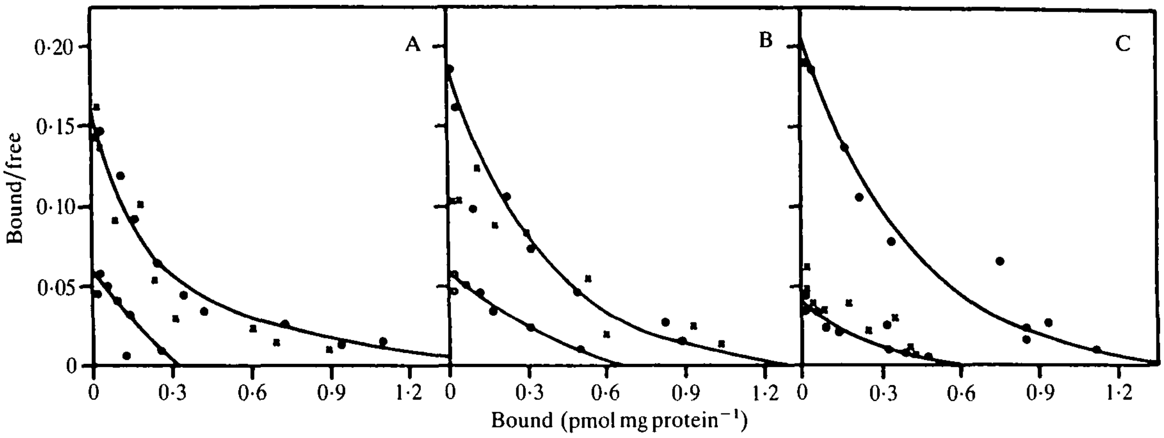

Fig. 3. Equilibrium binding of cyclic AMP to the chemotactic receptor after preincubation with ATP and different free Ca^{2+} concentrations (Scatchard plots). Crude membranes of wild-type Ax3 (A) and the transformed cell lines *ras*-Gly12 (B) and *ras*-Thr12 (C) were incubated for 2 min at 22°C in 20 mM-potassium phosphate buffer (pH 7·0), containing 1 mM-ATP plus 1 mM-EDTA (●), 0·1 μM-free Ca^{2+} (×), or 0·1 mM-free Ca^{2+} (○). Subsequently, the membranes were equilibrated for 5 min at 0°C with [^{3}H]cyclic AMP at concentrations between 0·1 nM and 300 nM in the presence of 20 μM-5′-AMP and 10 mM-dithiothreitol. The membranes were sedimented and the amount of receptor-bound [^{3}H]cyclic AMP was determined. Each point is the mean of the results of a determination in triplicate of an experiment reproduced two to three times.

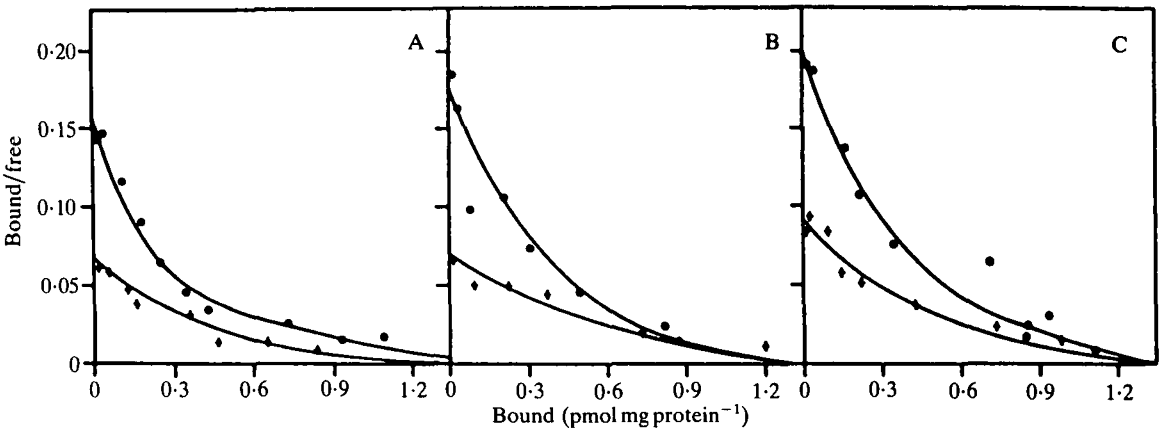

Fig. 4. Equilibrium binding of cyclic AMP to the chemotactic receptor in the presence or absence of Mg^{2+}-ATP (Scatchard plots). Crude membranes of wild-type Ax3 (A) and the transformed cell lines *ras*-Gly12 (B) and *ras*-Thr12 (C) were preincubated in 20 mM-potassium phosphate (pH 7·0), containing 1 mM-ATP and 1 mM-EGTA for 2 min at 22°C in the absence (●) or presence (+) of 1 mM-$MgCl_2$. [^{3}H]cyclic AMP binding curves were determined as described in the legend to Fig. 3. Each point is the mean of results of a determination in triplicate of an experiment reproduced twice.

the effect of GTP and GDP could not be observed. Van Haastert (1987*b*) has shown that the guanine nucleotide effect can be mimicked by incubating membranes with Mg^{2+}-ATP, thereby activating a putative endogenous protein kinase. We have compared the binding properties of the cyclic AMP receptors of membranes from wild-type cells and the two *ras* transformants after incubation with Mg^{2+}-ATP. Scatchard analysis in Fig. 4 reveals, under these conditions, a similar decrease in receptor affinity and an unaffected receptor number in all three membrane types.

Thus, overexpression of either the wild-type or the mutated *ras* gene did not affect this Mg^{2+} plus ATP-dependent cyclic AMP-receptor regulating mechanism.

The data in Figs 1 and 3 indicate that activating a G-protein or putative protein kinase C had the same effect on cyclic AMP receptors as the presence of the mutated *ras* gene product. In several other receptor systems, receptor properties are controlled by protein kinase C (Shoyab *et al.* 1979; Brown *et al.* 1984; Klausner *et al.* 1984; Serra *et al.* 1986; Dawson *et al.* 1986). The activation pathway of this kinase has been studied extensively in mammalian cells (Nishizuka, 1984; Bell, 1986). In response to ligand binding, various receptors activate a phospholipase C (Berridge & Irvine, 1984), which catalyses the hydrolysis of phosphatidyl inositol 4,5-diphosphate to 1,2-diacylglycerol and inositol

1,4,5-trisphosphate. The latter is thought to release Ca^{2+} from intracellular stores, the former to activate protein kinase C, provided that Ca^{2+} (in micromolar concentrations) is present (Kishimoto *et al.* 1980; Bell, 1986). Several recent reports suggest that a *ras* protein regulates the activity of phospholipase C, possibly by acting as a G-protein-like entity (Blackmore *et al.* 1985; Chiarugi *et al.* 1985; Cockroft & Gomperts, 1985; Fleischman *et al.* 1986).

In *Dictyostelium* cells protein kinase C and phospholipase C have not been identified. However, it was recently shown that inositol 1,4,5-trisphosphate and Ca^{2+} are intracellular messengers for the activation of guanylate cyclase *via* the cyclic AMP receptor (Europe-Finner & Newell, 1986*a*,*b*; Small *et al.* 1986). Moreover, Europe-Finner *et al.* (1988) recently showed that *ras*-Thr12 transformants contain an elevated level of inositol trisphosphate compared to cells carrying only the wild-type *ras* gene. These data suggest that the phosphatidyl inositol signal-transduction pathway is active in this lower eukaryote, and is controlled by a *ras* protein in a direct or indirect way.

Summarizing, our results indicate the involvement of the *D. discoideum ras* protein in a cyclic AMP receptor-controlling pathway *in vitro*, which depends on a protein kinase C-like activity. This pathway appears to be constitutively activated in membranes derived from cells that express the mutated *ras*-Thr12 gene, resulting in the reduction of the number of cyclic AMP receptors. The *D. discoideum ras* transformants that were used in this study as well as in previous work (Reymond *et al.* 1986; Van Haastert *et al.* 1987) have been shown to contain two to four times more *ras* protein than untransformed cells (Reymond *et al.* 1986). Cyclic AMP-receptor binding to intact cells of these *ras* transformants was reported to be indistinguishable from binding to wild-type cells (Reymond *et al.* 1986). Interestingly, we have recently found that further increased expression levels of the mutated *ras* protein (in *ras*-Thr12 transformants) resulted in a dramatic reduction of the number of available cell surface cyclic AMP receptors *in vivo*. A similar increase in the cellular level of wild-type *ras* protein (in *ras*-Gly12 transformants), on the other hand, did not affect the number of available receptors (M. E. E. Ludérus, unpublished). These findings are in agreement with our present observations *in vitro*. Whether the *ras* protein in *D. discoideum* directly interacts with phospholipase C is under investigation.

References

Ashendel, C. L. (1985). The phorbolester receptor: a phospholipid-regulated protein kinase. *Biochim. biophys. Acta* **822**, 219–242.

Balmain, A. & Pragnett, I. B. (1983). Mouse skin carcinomas induced *in vivo* by chemical carcinogen have a transforming Harvey-*ras* oncogene. *Nature, Lond.* **303**, 72–74.

Bartfai, T. (1979). Preparation of metal–chelate complexes and the design of steady-state kinetic experiments involving metal nucleotide complexes. *Adv. Cyclic Nucleotide Res.* **10**, 219–241.

Bell, R. M. (1986). Protein kinase C activation by diacylglycerol second messengers. *Cell* **45**, 631–632.

Berridge, M. J. & Irvine, R. F. (1984). Inositol trisphosphate, a novel second messenger in cellular signal transduction. *Nature, Lond.* **312**, 315–321.

Blackmore, P. F., Bocckino, S. B., Waynick, L. E. & Exton, J. H. (1985). Role of a guanine nucleotide-binding regulatory protein in the hydrolysis of hepatocyte phosphatidylinositol 4,5-bisphosphate by calcium-mobilizing hormones and the control of cell calcium. *J. biol. Chem.* **260**, 14 477–14 483.

Brown, K. D., Blay, J., Irvine, R. F., Heslop, J. P. & Berridge, M. J. (1984). Reduction of epidermal growth factor receptor affinity by heterologous ligands: evidence for a mechanism involving the breakdown of phosphoinositides and the activation of protein kinase C. *Biochem. biophys. Res. Commun.* **123**, 377–384.

Chiarugi, V., Porciatti, F., Pasquali, F. & Bruni, P. (1985). Transformation of Balb/3T3 cells with EJ/T24/H-*ras* oncogene inhibits adenylate cyclase response to β-adrenergic agonist while increases muscarinic receptor dependent hydrolysis of inositol lipids. *Biochem. biophys. Res. Commun.* **132**, 900–907.

Cockcroft, S. & Gomperts, B. D. (1985). Role of guanine nucleotide binding protein in the activation of polyphosphoinositide phosphodiesterase. *Nature, Lond.* **314**, 534–536.

Dawson, A. P., Comerford, J. G. & Fulton, D. V. (1986). The effect of GTP on inositol 1,4,5-trisphosphate-stimulated Ca^{2+} efflux from a rat liver microsomal fraction. *Biochem. J.* **234**, 311–315.

Der, C. J., Finkel, T. & Cooper, G. M. (1986). Biological and biochemical properties of human *ras* H genes mutated at codon 61. *Cell* **44**, 167–176.

Europe-Finner, G. N., Ludérus, M. E. E., Small, N. V., Van Driel, R., Reymond, C. D., Firtel, R. A. & Newell, P. C. (1988). Mutant *ras* gene induces elevated levels of inositol tris- and hexakisphosphates in *Dictyostelium*. *J. Cell Sci.* **89**, 13–20.

Europe-Finner, G. N. & Newell, P. C. (1986*a*). Inositol 1,4,5-trisphosphate induces calcium release from a nonmitochondrial pool in amoebae of *Dictyostelium*. *Biochim. biophys. Acta* **887**, 335–340.

Europe-Finner, G. N. & Newell, P. C. (1986*b*). Inositol 1,4,5-trisphosphate and calcium stimulate actin polymerization in *Dictyostelium discoideum*. *J. Cell Sci.* **82**, 41–51.

Fasano, O., Aldrich, T., Tamanoi, F., Taparowsky, E., Furth, M. & Wigler, M. (1984). Analysis of the transforming potential of the human H-*ras* gene by random mutagenesis. *Proc. natn. Acad. Sci. U.S.A.* **81**, 4008–4012.

Fleischman, L. F., Chahwala, S. B. & Cantley, L. (1986). *ras* transformed cells: altered levels of phosphatidyl inositol-4,5-bisphosphate and catabolites. *Science* **231**, 407–410.

Green, A. A. & Newell, P. C. (1975). Evidence for the existence of two types of cAMP-binding sites in aggregating cells of *Dictyostelium discoideum*. *Cell* **6**, 129–136.

Janssens, P. M. W., Arents, J. C., Van Haastert, P. J. M. & Van Driel, R. (1986). Forms of the chemotactic adenosine 3′,5′-cyclic phosphate receptor in isolated *Dictyostelium discoideum* membranes and interconversions induced by guanine nucleotides. *Biochemistry* **25**, 1314–1320.

Kishimoto, A., Takai, J., Mori, T., Kikkawa, U. & Nishizuka, Y. (1980). Activation of calcium and phospholipid-dependent protein kinase by diacylglycerol, its possible relation to phosphatidylinositol turnover. *J. biol. Chem.* **255**, 2273–2276.

Klausner, R. D., Harford, J. & Van Renswoude, J. (1984). Rapid internalization of the transferrin receptor in K562 cells is triggered by ligand binding or treatment with a phorbolester. *Proc. natn. Acad. Sci. U.S.A.* **81**, 3005–3009.

Klein, C. & Juliani, M. H. (1977). cAMP-induced changes in cAMP-binding sites on *D. discoideum* amebae. *Cell* **10**, 329–335.

Manne, V., Bekesi, E. & Kung, H.-F. (1985). Ha-*ras* proteins exhibit GTPase activity: point mutations that activate Ha-*ras* gene products result in decreased GTPase activity. *Proc. natn. Acad. Sci. U.S.A.* **82**, 376–380.

Manne, V., Yamazaki, S. & Kung, H.-F. (1984). Guanosine nucleotide binding by purified Ha-*ras* encoded p21 protein. *Proc. natn. Acad. Sci. U.S.A.* **81**, 6953–6957.

McGrath, J. P., Capon, D. J., Goeddel, D. V. & Levinson, A. D. (1984). Comparative biochemical properties of normal and activated human *ras* p21 protein. *Nature, Lond.* **310**, 644–649.

Nellen, W., Silan, C. & Firtel, R. A. (1984). DNA-mediated transformation in *Dictyostelium discoideum*: regulated expression of an actin gene fusion. *Molec. cell. Biol.* **4**, 2890–2898.

Nishizuka, Y. (1984). The role of protein kinase C in cell surface signal transduction and tumor promotion. *Nature, Lond.* **308**, 693–697.

Reymond, C. D., Gomer, R. H., Mehdy, M. C. & Firtel, R. A. (1984). Developmental regulation of a *Dictyostelium* gene encoding a protein homologous to mammalian *ras* protein. *Cell* **39**, 141–148.

Reymond, C. D., Gomer, R. H., Nellen, W., Theibert, A., Devreotes, P. & Firtel, R. A. (1986). Phenotypic changes induced by a mutated *ras* gene during the development of *Dictyostelium* transformants. *Nature, Lond.* **323**, 340–343.

Seeburg, P. H., Colby, W. W., Capon, D. J., Goeddel, D. V. & Levinson, A. D. (1984). Biological properties of human c-Ha-*ras* 1 genes mutated at codon 12. *Nature, Lond.* **312**, 71–75.

Serra, M., Smith, T. L. & Yamamura, H. I. (1986). Phorbolesters alter muscarinic receptor binding and inhibit polyphosphoinositide breakdown in human neuroblastoma (SH-SY5Y) cells. *Biochem. biophys. Res. Commun.* **140**, 160–166.

Shoyab, M., DeLarco, J. E. & Todaro, G. J. (1979). Biologically active phorbolesters specifically alter affinity of epidermal growth factor membrane receptors. *Nature, Lond.* **279**, 387–391.

Small, N. V., Europe-Finner, G. N. & Newell, P. C. (1986). Calcium induces cyclic GMP formation in *Dictyostelium*. *FEBS Lett.* **203**, 11–14.

Taparowsky, E., Suard, Y., Fasano, O., Shimizu, K., Goldfarb, M. & Wigler, M. (1982). Activation of the T24 bladder carcinoma transforming gene is linked to a single amino acid change. *Nature, Lond.* **300**, 762–765.

Van Haastert, P. J. M. (1987*a*). Down-regulation of cell surface cAMP-receptors and desensitization of cAMP-stimulated adenylate cyclase by cAMP in *Dictyostelium discoideum*. *J. biol. Chem.* **262**, 7700–7704.

Van Haastert, P. J. M. (1987*b*). Alteration of receptor/G-protein interaction by putative endogenous protein kinase activity in *Dictyostelium discoideum* membranes. *J. biol. Chem.* **262**, 3239–3243.

Van Haastert, P. J. M., Kesbeke, F., Reymond, C. D., Firtel, R. A., Ludérus, M. E. E. & Van Driel, R. (1987). Aberrant transmembrane signal transduction in *Dictyostelium* cells expressing a mutated *ras* gene *Proc. natn. Acad. Sci. U.S.A.* **84**, 4905–4909.

Van Haastert, P. J. M., De Wit, R. J. W., Janssens, P. M. W., Kesbeke, F. & De Goede, J. (1986). G-protein-mediated interconversions of cell-surface cAMP-receptors and their involvement in excitation and desensitization of guanylate cyclase in *Dictyostelium discoideum*. *J. biol Chem.* **261**, 6904–6911.

(*Received 15 February 1988 – Accepted 30 March 1988*)