Stage-specific tyrosine phosphorylation of actin in *Dictyostelium discoideum* cells

ANTON SCHWEIGER, OANA MIHALACHE, MARIA ECKE and GÜNTHER GERISCH
Max-Planck-Institut für Biochemie, 8033 Martinsried bei München, Germany

Summary

A 45 kDa protein in *Dictyostelium discoideum* cells that was recognized by a phosphotyrosine-specific antibody was identified by its binding activity to DNase I and its 2D-electrophoretic behavior as actin. The reactivity of actin with the antibody was transiently enhanced for about 30 minutes shortly after starving cells were reintroduced into nutrient medium. This effect indicates a modification of actin that is regulated under physiological conditions. A similar effect was obtained when growing cells were treated with phenylarsine oxide (PAO), an inhibitor of phosphotyrosine phosphatases. This effect was reversed and the cells fully recovered upon addition of the PAO antagonist 2,3-dimercaptopropanol. Starved cells did not show this enhancement of antibody labelling, which indicates that the response to PAO depends on the developmental stage. Phosphorylated amino acid residues were identified after in vivo labelling with $[^{32}\text{P}]$phosphate in the presence of PAO. Part of the radioactivity in the actin band was recovered as phosphotyrosine, another part as phosphoserine.

PAO caused the cells to form elongated blebs, to round up and finally to become immobilized. Fluorescence labelling with phalloidin of cells that were fixed at different times of PAO treatment revealed a progressive decrease in the staining for actin filaments and showed that these alterations in cytoskeleton organization were readily reversible, in accordance with the reversal of tyrosine phosphorylation at actin.

Key words: actin phosphorylation, cytoskeleton, *Dictyostelium*, protein-tyrosine kinase, tyrosine phosphorylation.

Introduction

Tyrosine phosphorylation of proteins appears to occur ubiquitously in metazoans including sponges (Hunter and Cooper, 1985; Yarden and Ullrich, 1988; Schartl and Barnekow, 1982). Only recently has tyrosine phosphorylation been reported in the lower eukaryote *Dictyostelium discoideum*, which undergoes, during its life cycle, a transition from the unicellular to the multicellular state. The sequences of two cDNA clones from this organism indicate that they encode proteins similar to vertebrate protein-tyrosine kinases (PTKs), and expression of these clones in *Escherichia coli* revealed two different PTK activities (Tan and Spudich, 1990). Three major target proteins of tyrosine kinases have been identified in *D. discoideum* (Schweiger et al., 1990) using an antibody shown to be specific for phosphotyrosine (Fendley et al., 1990). The labelling of two of these proteins has been found to depend on the developmental stage, suggesting the involvement of tyrosine phosphorylation in developmentally controlled processes (Schweiger et al., 1990).

In studies on the interaction of biologically active peptides and proteins with membrane receptors of mammalian cells, phenylarsine oxide (PAO) has been used as an inhibitor of phosphotyrosine phosphatases (Bernier et al., 1988; Fallon, 1990). This dithiol reagent can specifically complex neighboring sulfhydryl groups in proteins (Frost and Lane, 1985). Since PAO does not inhibit PTKs (Garcia-Morales et al., 1990) it leads to high phosphorylation of their substrates. Addition of PAO to 3T3-L1 adipocytes enhances $[^{32}\text{P}]$phosphate labelling of a 15 kDa phosphotyrosine protein that is thought to be involved in the transmission of signals to the glucose transport system (Bernier et al., 1988). By adding PAO to NIH 3T3 cells prior to incubation with insulin, tyrosine phosphorylation of ten or more low-molecular-mass proteins has been observed (Levenson and Blackshear, 1989). The PAO effects can be reversed by the dithiol compound 2,3-dimercaptopropanol (DMP) (Frost and Lane, 1985; Bernier et al., 1987).

In order to identify new tyrosine phosphorylated proteins in *D. discoideum*, we have added PAO to both growing and early developing cells, and have monitored tyrosine phosphorylation using the phosphotyrosine-specific antibody 5E2 (Fendley et al., 1990). With growing cells a pattern of up and down shifts in the labelling of individual proteins upon addition and removal of PAO has been obtained. In this paper we
focus on a 45 kDa protein whose tyrosine phosphorylation is enhanced by PAO in growing but not in developing cells, and provide evidence that this protein is actin. The finding that tyrosine phosphorylation is transiently up-regulated without any PAO treatment when developing cells are transferred to growth conditions, shows that the phosphorylation of actin is regulated under physiological conditions in the absence of any drug.

Materials and methods

Growth and treatments of D. discoideum cells
Cells of D. discoideum strain AX2-214 were cultivated axenically in nutrient medium (Bertholdt et al., 1985) up to densities of not more than 5 x 10^6 cells per ml (Schweiger et al., 1990). Development was induced by washing and resuspending the cells in 17 mM phosphate buffer, pH 6.0 (“non-nutrient buffer”), at a density of 1 x 10^7 per ml. The developing cells were harvested in the preaggregation stage at 4 h of starvation (“t 4-cells”). They were centrifuged, resuspended in nutrient medium at the same density as before and agitated on a rotary shaker at 150 revs/min. PAO (Sigma) was dissolved in dimethylsulfoxide (DMSO) and added to growing cells to a final concentration of 30 μM. Controls showed that the DMSO had no detectable effect on phosphorylation and shape changes of the cells at a final concentration of 0.06%. To remove PAO, cells were washed and resuspended in nutrient medium at an initial cell density of 3 x 10^7 to 5 x 10^7 per ml. 2,3-Dimercaptopropanol (DMP, Sigma) was added to washed cells to a concentration of 200 μM. Cells were cultivated overnight in nutrient medium, with and without PAO and transferred to fresh nutrient medium, or washed and transferred to medium to which 200 μM DMP was added. Phase-contrast micrographs were taken from living cells.

In vivo labeling with [32P]phosphate, extraction of actin and phosphoamino acid analysis
Cells were cultivated overnight in nutrient medium, with phosphate buffer replaced by 10 mM MES, pH 6.5. Subsequently, cell density was adjusted to 1 x 10^6 per ml and 0.2-0.5 mCi/ml of [32P]phosphate (Amersham Buchler, Braunschweig) was added. After 2 h, 30 μM PAO was added and incubation continued for another 2 h. Cells were centrifuged, washed with non-nutrient buffer, freeze/thawed, homogenized in a Dounce homogenizer and extracted with buffer G plus 10% formamide (Zechel, 1980), supplemented with the protease and phosphatase inhibitors aprotonin (200 units/ml, Trasylol, Bayer, Leverkusen), bestatin (0.5 μg/ml), antipain, leupeptin, pepstatin A (each 1 μg/ml, Sigma), orthovanadate (100 μM) and PAO (30 μM).

Immediately after high-speed centrifugation (1 h at 100,000 g) trichloroacetic acid was added to the supernatant to a final concentration of 20%. The precipitate was washed with water and 80% ethanol, and dissolved in 4.5 M urea in SDS sample buffer. Alternatively, the high-speed supernatant was subjected to chromatography on DNase I-Sepharose 4B for actin purification.

For phosphoamino acid analysis, proteins were eluted from dried gels and hydrolyzed as described (Schweiger et al., 1990) and the phosphoamino acids were determined by two-dimensional thin-layer electrophoresis at pH 1.9 for the first and pH 3.5 for the second dimension (Hunter and Selton, 1980).

Purification of D. discoideum actin on DNase I
Cells were treated as for [32P]phosphate labeling in vivo. The high-speed supernatant was passed over a column of DNase I (Type II, Sigma) immobilized on Sepharose 4B (CNBr-activated, Pharmacia, Freiburg), according to Zechel (1980). The bound actin was eluted with buffer G plus 40% formamide.

Electrophoresis and immunoblotting
SDS-PAGE and immunoblotting techniques were performed as described (Schweiger et al., 1990). Phosphotyrosine proteins were detected with iodinated anti-phosphotyrosine antibody SE2 (kindly supplied by Dr. A. Ullrich, Martinsried; see Fendley et al., 1990). Actin was labelled using monoclonal antibody against D. discoideum actin of the hybridoma line ACT IV (Simpson et al., 1984) purchased from the American Type Culture Collection (Rockville, USA). Goat anti-mouse antibody coupled to alkaline phosphatase was used as second antibody, and 5-bromo-4-chloro-3-indolyl phosphate as substrate.

Two-dimensional gel electrophoresis of actin
The first fraction of the actin peak eluted from the Sepharose/DNase I column was precipitated with 10% trichloroacetic acid. The precipitate was washed several times with water and dissolved in 20 μl of 9.5 M urea, 2% NP-40, 2% Ampholine, 5% β-mercaptoethanol. Proteins were separated by two-dimensional gel electrophoresis according to O’Farrell (1975) and de Robertis et al. (1977). Actin and phosphotyrosine proteins were detected on the same blot. After incubation with the anti-actin antibody, the blot was washed free of the antibody with 0.5 M acetic acid, pH 2.4, for 30 min and several times with blot wash buffer, and was subsequently incubated with the anti-phosphotyrosine antibody.

Phase-contrast and fluorescence microscopy
Growing cells were incubated with 30 μM PAO, washed free of PAO and transferred to fresh nutrient medium, or washed and transferred to medium to which 200 μM DMP was added. Phase-contrast micrographs were taken from living cells.

For labeling F-actin with rhodamine-conjugated phalloidin, samples of 1 x 10^7 cells were sedimented at 1000 revs/min for 3 min, washed once with non-nutrient buffer, resuspended and fixed in 2 ml of 15% picric acid, 2% formaldehyde, 10 mM Pipes buffer, pH 6.0 (B. Humbel, personal communication), and kept for 30 min under repeated agitation.

All these steps were performed at room temperature. The fixed cells were washed twice with Pipes and incubated in 70% ethanol for 10 min. After washing with PBS/glycine and phosphate/BSA/gelatine (PBG), cells were suspended and stained for 1 h with 0.5 μg per ml rhodamine-phalloidin (Sigma) dissolved in PBG (Scheel et al., 1989). Washed cells were suspended in 100 μl PBS and fluorescence photographs taken using a Zeiss Axioplan microscope.

Results

Under certain conditions a 45 kDa protein from D. discoideum cells is strongly labelled with phosphotyrosine-specific antibody
Previously, we identified three major tyrosine-phosphorylated proteins with relative molecular masses of...
developing cells with nutrient medium

Tyrosine phosphorylation of actin

growth-phase cells with PAO

after removal of PAO with DMP

Fig. 1. Immunolabelling with anti-phosphotyrosine antibody of proteins from Dictyostelium discoideum cells. (A) After development for 4 h, starving cells were re-transferred into nutrient medium. Minutes of incubation in nutrient medium are indicated at the bottom. (B) Growing cells in nutrient medium were incubated for 1 h with 30 μM PAO and subsequently divided into three portions: cells of the first sample were left in PAO (left panel), those of the second were washed and resuspended in nutrient medium without PAO (middle panel), and those of the third washed and resuspended in nutrient medium plus 200 μM DMP (right panel). Hours at the bottom indicate times of incubation with PAO (left panel), or times of incubation of PAO-pretreated cells without PAO (middle and right panels). At the times indicated cell samples were sedimented, washed and immediately dissolved and heated in SDS sample buffer. Following SDS-PAGE, proteins were blotted onto nitrocellulose, labelled with 125I-labelled anti-phosphotyrosine antibody, and autoradiographed. Positions of molecular mass markers are shown at the left.

205 to 220, 107 and 60 × 10³ with the anti-phosphotyrosine antibody 5E2 on immunoblots of cellular proteins from D. discoideum (Schweiger et al., 1990). In a search for other proteins that might be tyrosine-phosphorylated under special conditions, we have tested cells at different stages of development and during its reversal back to the growth phase. A rapid and transient increase in reactivity of a 45 kDa band was found when developing cells that had been starved for 4 h were transferred back to nutrient medium (Fig. 1A). A peak of reactivity with the antibody was reached at 15 to 20 min after transfer. This peak was preceded and accompanied by a peak in reactivity of a 130 kDa protein.

Strong labelling of the 45 kDa band with mAb 5E2 was also observed when growth-phase cells were incubated with 30 μM of the phosphatase inhibitor PAO (Fig. 1B). Labelling of this band strongly increased after 60 min of incubation and persisted until the end of the experiment at 4 h. Under these conditions the 45 kDa protein became the most heavily mAb 5E2-labelled protein that we have found in D. discoideum under any condition tested. The band of the 130 kDa protein, which was already recognizable in cells retransferred to nutrient medium, exhibited a short-lived increase of labelling at about 30 min of incubation with PAO.

The PAO-mediated increase in reactivity of the 45 kDa protein with mAb 5E2 proved to be reversible. When PAO was removed after 60 min by washing, reactivity with the antibody declined slowly within 3 h (Fig. 1B). The PAO antagonist DMP at 200 μM efficiently accelerated the decline of reactivity. Both, with and without DMP, the washed cells survived for at least 20 h, whereas cells continuously incubated with PAO had lysed within that period. Remarkably, the reactivity of a 107 kDa protein, which was already recognizable in untreated cells, was very strong at 20 h after the removal of PAO, long after reactivity of the 45 kDa protein with the antibody had returned to its low basal level.

The PAO effect was only observed in nutrient medium. When 30 μM PAO was added to developing cells at 2 or 4 h of starvation in phosphate or MES buffer, no increase in reactivity of the 45 kDa protein with mAb 5E2 was detected. The effect of PAO on the increase in reactivity of the 45 kDa protein with mAb 5E2 took only place at low Ca²⁺ concentrations. It was suppressed by the addition of 10 mM CaCl₂ to the cells, whereas 10 mM EGTA in the presence of PAO slightly raised the reactivity (data not shown).

Specificity of antibody binding to the 45 kDa protein, and identification of phosphoamino acids

The specificity of the interaction of mAb 5E2 with the 45 kDa protein was examined by adding 1 mM
phosphotyrosine, phosphoserine or phosphothreonine to the incubation mixture during immunolabelling of the blots. Binding of the antibody to the 45 kDa and 130 kDa proteins was substantially and specifically reduced by phosphotyrosine (Fig. 2).

The presence of phosphotyrosine residues in the 45 kDa band was established by phosphoamino acid analysis after incubation of PAO-treated cells with \[^{32}P\]phosphate (Fig. 3). In addition to \[^{32}P\]phosphotyrosine, a high proportion of \[^{32}P\]phosphoserine was obtained by total hydrolysis. Phosphoserine escapes detection by mAb 5E2. Consequently, the data in the present paper that were obtained with that antibody are limited to tyrosine phosphorylation.

**The phosphorylated 45 kDa protein represents a subfraction of actin**

Actin, the predominant cellular protein, runs in SDS-polyacrylamide gel electrophoresis with the same electrophoretic mobility as the phosphorylated 45 kDa protein. The following results show that this protein is, in fact, actin.

Actin binds to a DNase I affinity column and can be eluted from the column with formamide. In Fig. 4 binding and elution of the mAb 5E2-recognized protein is compared with that of actin as stained with Coomassie blue. The protein recognized by the antibody efficiently bound to the DNase I column in the presence of 10% formamide and was eluted in 40% formamide together with the first actin containing fractions (Fig. 4A a,b). A similar result was obtained after in vivo labelling with \[^{32}P\]phosphate in the presence of PAO (Fig. 4A c). Quantitative data establish that the antibody-recognized 45 kDa protein co-eluted with the front of the actin peak (Fig. 4B). These results indicate that the phosphorylated protein behaves like a fraction of actin that is less firmly bound to DNase I than the bulk of actin from *D. discoideum* cells. A control with proteins from non-PAO-treated growth-phase cells revealed only a small, nevertheless clearly detectable, amount of protein that was recognized by the anti-phosphotyrosine antibody (Fig. 4B).

Protein eluted from the DNase I column was analyzed by 2-dimensional electrophoresis in order to identify the mAb 5E2-recognized protein with an isoelectric variant of actin that is recognized by an anti-actin antibody (Fig. 5). Among the variants of actin (Vandekerckhove and Weber, 1978a,b), a more acidic fraction was separated by a constriction of the elongated actin spot from less negatively charged actin species. The mAb 5E2 label coincided with the position of the more acidic actin fraction. In summary, the tyrosine phosphorylated protein is characterized as actin on the basis of three independent criteria: its binding to a DNase I column, its relative molecular mass and its isoelectric point.

**The actin cytoskeleton is altered in PAO-treated cells**

The dramatic effect of PAO on tyrosine phosphorylation of actin has prompted us to search for changes that are microscopically visible in PAO-treated cells, even though none of these changes is necessarily caused by tyrosine phosphorylation of actin or any other...
Tyrosine phosphorylation of actin

Fig. 4. Separation of actin on a DNase I column. (A) Proteins extracted as for Fig. 3 were run over a DNase I-Sepharose column. Actin was eluted with 40% formamide. Proteins in the eluate fractions were subjected to SDS-PAGE. Proteins were stained with Coomassie Blue in the gel (a), or labelled with anti-phosphotyrosine antibody on a blot (b). In a parallel experiment, cells were labelled with [32P]phosphate and the dried gel autoradiographed for 32P incorporated into proteins (c). (B) Extracts were prepared from equal amounts of PAO-treated cells or untreated cells and fractionated on DNase I. The amount of actin in eluate fractions 6, 8 and 10 was determined densitometrically on the stained gel and is expressed in arbitrary units of absorbance (open columns). The amount of 125I-labelled anti-phosphotyrosine antibody bound to the blot at the 45 kDa position was determined in a γ-counter and is shown as cts/min (filled columns).

Fig. 5. Two-dimensional gel electrophoresis and immunolabelling of actin. Actin from the DNase I column was analysed by two-dimensional gel electrophoresis. Blotted proteins were first probed with a monoclonal antibody against D. discoideum actin (A) and, after removal of this antibody in dilute acetic acid, with the iodinated anti-phosphotyrosine antibody (B). Numbers at bottom are pH values as determined by elution of gel slices.

cytoskeletal protein. We would also like to point out that substantial serine phosphorylation has been found in the actin band as shown in Fig. 3. PAO has been reported to inhibit phosphotyrosine phosphatases selectively (Garcia-Morales et al., 1990), but in the present paper we have not investigated the control of serine phosphorylation by PAO or retransfer of cells to nutrient medium. PAO altered cell shape and behavior reversibly (Fig. 6). First, the cells lost their tipped pseudopods; instead they extended elongated blebs with rounded ends from their surfaces (Fig. 6A-C). Later, most cells became rounded and lost their ability to move and spread on a glass surface (Fig. 6D). Even then intracellular particle movement was observed. This finding suggests a specific inhibition of actin-based shape changes and motility, and distinguishes the effect of PAO from oxygen depletion or inhibition of ATP synthesis by 2,4-dinitrophenol (Gerisch, 1962). In these cases intracellular particle movement stops after the rounding up of cells, and later when the cells start to lyse Brownian movement is observed. All the effects of PAO could be reversed by 30 min of incubation with DMP (Fig. 6F). Without DMP, only part of the washed cells regained normal shape and motility within 60 min (Fig. 6E).

The effects of PAO on cell shape and motility were paralleled by alterations in the actin filament system (Fig. 7). In normal cells actin filaments are known to be enriched in the cortical region and in cell surface projections, as can be seen in cytoskeletons prepared by combining glutaraldehyde fixation and Triton X-100 extraction (Claviez et al., 1986). The distribution of F-actin in non-PAO-treated control cells, as visualized by fluorescent phalloidin labelling (Fig. 7A), was typical of
A. Schweiger and others

Fig. 6. Alterations of cell shape and motility in PAO-treated cells. Growth-phase cells of D. discoideum exhibited extensive formation of lamellipods and filopods (A). Cells spread on a substratum and treated for 30 (B), 60 (C) or 90 min (D) with 30 μM PAO had lost the capability of spreading and normal pseudopod formation. They often extended elongated blebs from their surface and became rounded. These PAO effects were partially reversed when, after 60 min of incubation, PAO was removed by washing and resuspension of the cells in nutrient medium (E). The effect was almost completely reversed after washing and an incubation for 30 min incubation with 200 μM DMP in nutrient medium (F). Phase-contrast images; bar, 10 μm.

D. discoideum growth-phase cells. The overall result of PAO treatment was a reduced intensity of the phalloidin label, which sometimes became almost undetectable. More often labelling was retained in a thin zone beneath the plasma membrane (Fig. 7B and C). The PAO effect was reversible; normal organization of the actin cytoskeleton was restored upon treatment with DMP (Fig. 7D).

Discussion

Several studies have shown that actin can be phosphorylated at tyrosine and serine residues in vitro. A cell membrane kinase from human placenta uses exogenous actin as a substrate and catalyzes the phosphorylation of serine residues (Machicao and Wieland, 1985). The phosphorylation of tyrosine residues on actin is catalyzed by human insulin-stimulated kinase (Machicao et al., 1983) and also by the pp60c-src kinase (Erikson et al., 1979). Since both these tyrosine kinases also phosphorylate exogenous casein, tubulin, immunoglobulin and histone (Erikson et al., 1979; Petruzelli et al., 1982; Stadtmayer and Rosen, 1983; Donner et al., 1981), the relevance of the in vitro phosphorylation of these proteins to in vivo conditions has been questioned (Stadtmayer and Rosen, 1983), and it has been argued that actin cannot be phosphorylated on tyrosine in vivo (Donner et al., 1981).

The data presented in this paper demonstrate for Dictyostelium that actin phosphorylation occurs in vivo on tyrosine and probably also on serine residues. The tyrosine phosphorylation is reversible and strongly regulated, which means that only under special conditions does it become easily detectable. Under physiological conditions the tyrosine phosphorylation increases transiently in developing cells that are retransferred to nutrient medium. The tyrosine phosphorylation of actin can be artificially strongly enhanced by the phosphatase blocker PAO, but even then the effect depends on the physiological state of the cells. It occurs only in cells growing in the presence of nutrients.

PAO may increase tyrosine phosphorylation in two ways: by directly inhibiting a phosphatase that dephosphorylates actin, or by preventing dephosphorylation of an autophosphorylating PTK, which then remains active and continuously phosphorylates the actin. The earliest effect on protein phosphorylation, observed both in PAO-treated growth-phase cells of D. discoideum and in developing cells transferred to nutrient medium, is an increase in the tyrosine phosphorylation of a 130 kDa protein (Fig. 1). This protein might be an autophosphorylating PTK, but since its phosphorylation decreases in the PAO-treated cells before the strong rise in actin phosphorylation, a direct causal relationship between phosphorylation of this protein and that of actin is unlikely.

The finding that the temporal patterns of tyrosine phosphorylation are specific for individual proteins suggests that PAO interferes in a complex way with different regulatory systems. In immunoblots of total...
Tyrosine phosphorylation of actin cellular proteins we have not seen any other protein whose phosphotyrosine content increases and decreases at exactly the same time as that of actin. This observation might indicate the existence of a specific regulatory mechanism for actin phosphorylation in D. discoideum cells.

The site of phosphorylated tyrosine residues in the actin polypeptide chain has not yet been identified. Among the 15 tyrosine residues present in D. discoideum actin, the most carboxy-terminally located residue, Tyr363, is flanked by acidic amino acid residues (Vandekerckhove and Weber, 1980), as they are typical of substrate sites for viral or cellular PTKs (Hunter and Cooper, 1985; De et al., 1986) and also characteristic of the region in metavinculin surrounding Tyr949, which is presumably phosphorylated (Gimona et al., 1988). In the three-dimensional structure of muscle actin, the carboxy-terminal end of the polypeptide chain extends out of the globular molecule as a flexible tail (Kabsch et al., 1990). Tyr363 is only 14 amino acids away from the carboxy-terminal end of the D. discoideum actin chain. It should, therefore, be accessible to kinases and phosphatases.

One impact of in vitro phosphorylation of serine residues on rabbit skeletal muscle actin is the reduction of its affinity for DNase I (Machicao and Wieland, 1985). D. discoideum actin in vivo phosphorylated at tyrosine residues is eluted first from a DNase I column (Fig. 4). This finding might be interpreted in analogy to the in vitro result. However, since D. discoideum produces several actin species that differ slightly in their sequence (Romans and Firtel, 1985), it is also possible that certain actin species that already in their unphosphorylated state bind less strongly to DNase I than others are selectively phosphorylated under the in vivo conditions used.

The in vivo data obtained with D. discoideum show that the PAO-elicited increase in the tyrosine phosphorylation of actin depends on growth conditions. More importantly, the increase in tyrosine phosphorylation that is observed under physiological conditions, i.e. in the absence of PAO, is limited to a transitory state during the recommencement of growth in previously starved cells. The question is, therefore, whether tyrosine phosphorylation is related to an actin function involved in growth. D. discoideum cells feed upon bacteria by phagocytosis, and laboratory strains like the AX2 strain used in the present study take up...
Endocytotic processes that are related to growth and cytosis and internalization of cell-surface proteins are liquid nutrients by pinocytosis. Phagocytosis, pinocytosis and internalization of cell-surface proteins is correlated with the tyrosine phosphorylation of actin, is erasure of developmental traits (Soll and Waddell, 1975; Waddell and Soll, 1977). During erasure the capacity of developing cells to rapidly reaggregate after disaggregation is lost. This loss is accompanied by the disappearance of two cell surface proteins characteristic of developing cells, cAMP receptors and the contact site A cell-adhesion protein. These proteins may be removed by internalization followed by lysosomal breakdown, and the cytoskeleton may be involved in this process.

In antigen-receptor-mediated activation of human B lymphocytes, actin polymerization has been reported to depend on tyrosine phosphorylation of unknown substrate proteins (Melamed et al., 1991). The in vivo effect of PAO on structure and function of the cytoskeleton in D. discoideum points into the opposite direction. Reversible rounding up of PAO-treated cells is paralleled by the decline in phalloidin labelling of actin filaments within these cells. In order to relate the action of PAO on living cells to changes in tyrosine phosphorylation of actin or other cytoskeletal proteins, it will be necessary to establish experimental conditions for the defined in vitro phosphorylation of these proteins, and to compare polymerization and other activities of purified phosphorylated and non-phosphorylated actin under defined conditions.

We thank Dr. Michael Schleicher for discussions, and Christina Heizer for providing antibody. This work was supported by grants of the Deutsche Forschungsgemeinschaft to A. Sch. and of the Fonds der Chemischen Industrie to G.G.

References


mould Dictyostelium discoideum. The accumulation and erasure of 'morphogenetic information'. Develop. Biol. 47, 292-302.


(Received 28 February 1992 - Accepted 7 April 1992)