Cytoskeletal accumulation of a specific iso-actin during chemotaxis of *Dictyostelium*

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We have previously reported that stimulation of *Dictyostelium* amoebae with the chemoattractants folate or cAMP elicits a rapid (3–5 s) accumulation of actin in the Triton-insoluble cytoskeleton, a response which may be involved in amoeboid movement (McRobbie and Newell, 1983, 1984). We have now measured the changes in the three major isoactin species A_1, A_2, and A_3 present in the amoebal cytoskeletons and find that it is the most acidic isoactin A_1 that preferentially accumulates in the cytoskeleton in response to these chemotactic pulses. The differing roles of the isoactins are discussed

Actin isoform *Chemotaxis* Dictyostelium *Cytoskeleton*

1. INTRODUCTION

Chemotaxis and cell motility are important throughout the life cycle of the cellular slime mould *Dictyostelium discoideum* [1]. Many intracellular events which may function to control directed cell movement, are now known to result from binding of the developmental stage-specific chemoattractants to their specific receptors [2]. Recently, it was found that such chemoattractants elicit a series of dramatic changes in the actin content of the Triton-insoluble cytoskeleton isolated from several species of cellular slime mould [3,4] and it seems likely that this actin is involved in shape changes and cell translocation during chemotaxis [5,6].

Various isoforms of actin are well characterised in higher eukaryotes and seem to have tissue-specific distributions, suggesting different roles or evolutionary histories for these proteins [7,8]. Despite numerous reports of actin heterogeneity in *Dictyostelium* [3,9–12], there is still some controversy about the genetic or post-translational origins of different isoforms of actin in this organism. Spudich [13] and Uyemura et al. [14] detected only one major actin species following purification, and amino acid sequencing appears to confirm this [15]. However, there are believed to be 17 actin genes in *Dictyostelium* [9] and although the amino acid sequence is compatible with the DNA sequences of at least four genes [15] it does not accommodate others of those described [16]. In vitro translation of actin genes resulted in several isoelectric forms of actin, giving a major species and some minor acidic and basic forms [17]. Others have been unable to repeat this work and suggest that post-translational modification may account for the heterogeneity with acetylation [10,18] and phosphorylation [19,20] being possibilities.

We report a study of the involvement of the three major isoactins we find in *D. discoideum* in the rapid (3–5 s) chemoattractant-mediated cytoskeletal actin accumulation. We show that the increase in actin content is almost totally the result of an increased incorporation of the most acidic isoform and discuss the implications of these results for cell motility.

Abbreviation: cAMP, 3′,5′-cyclic adenosine monophosphate

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2. MATERIALS AND METHODS

L-[^{35}S]Methionine, 1070 Ci · mmol^{-1} in aqueous solution was supplied by Amersham International PLC.

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2.1. *Cell growth and development*

D. discoideum (strain NC4) was grown in the dark in association with *Klebsiella aerogenes* at 22°C on SM nutrient agar [21]. Initiation of development was as described previously [3,4].

2.2. *Incorporation of radiolabel*

To label aggregation-competent cells, the amoebae were allowed to develop by shaking at 22°C and 170 rpm^{-1} in 17 mM phosphate buffer (pH 6.15) at a density of 2×10^7 ml^{-1} in the presence of 5 $\mu Ci \cdot ml^{-1}$ L-[^{35}S]methionine. After 8 h the cells were washed free of label and resuspended at 10^8 ml^{-1} for use in experiments. Preaggregation stage cells were labelled by suspending them at 10^8 ml^{-1} immediately after harvesting, and allowing them to develop for 1 h in the presence of 25 $\mu Ci \cdot ml^{-1}$ L-[^{35}S]methionine before washing out the label and resuspending them at 10^8 ml^{-1} for use in experiments.

2.3. *Isolation of cytoskeletons*

The methods used have been described previously [3,4].

2.4. *Gel electrophoresis*

Cytoskeletons were analysed by 2-dimensional (2D) gel electrophoresis using isoelectric focusing in the first dimension and sodium dodecyl sulphate polyacrylamide electrophoresis in the second dimension according to the method of Garrels and Gibson [7].

2.5. *Determination of changes in actin*

Changes in actin isoforms were determined by running 2D gels of cytoskeletons prepared from amoebae prelabelled with L-[^{35}S]methionine. After silver staining [22], the spots representing the isoactins were excised from the gels and radioactivity eluted and determined by liquid scintillation counting [23].

Table 1

Proportion of L-[^{35}S]methionine incorporated into each major isoactin expressed as a percentage of the total incorporated into all three

Preaggregative (1 h) cells	Aggregation-competent (8 h) cells
A_1 = 46.8 ± 2.6	A_1 = 57.3 ± 2.4
A_2 = 24.6 ± 2.3	A_2 = 27.9 ± 3.4
A_3 = 28.6 ± 3.8	A_3 = 14.8 ± 1.0

Each result is the mean ± SEM of 5 separate experiments

3. RESULTS AND DISCUSSION

Isoelectric focusing in the first dimension of 2D gels proved to be an effective means of separating the actin isoforms. Three major isoactins from cytoskeletons were separated on a pH gradient of approx. pH 4–6 with at least two more very minor forms. The three major isoforms were designated A_1, A_2 and A_3 and had isoelectric points of about 5.5, 5.7 and 5.9, respectively. The isoelectric point of the major form of actin has previously been reported as 5.5 [19,24].

Changes in the cytoskeletal content of these three isoactins were found by determination of the amount of ^{35}Slabel present in each spot before or after chemotactic stimulation of amoebae that had been pre-labelled with [^{35}S]methionine. Initially, the proportion of radiolabel that was incorporated into the three actin isoforms in cytoskeletons from unstimulated, pre-labelled amoebae was determined. The results (table 1) indicate that the majority of the label was found in the most acidic (A_1) isoform in both pre-aggregation (1 h) and aggregation competent (8 h) cells. The differences in the amount of label incorporated into each isoform may reflect differences in the size of the pools of these isoforms or differences in the rates of synthesis of each isoform over the period that cells were labelled. Actin is known to be synthesised in large quantities during the pre-aggregation and early aggregation stages of the life cycle [24,25] and simultaneous synthesis of several isoforms has been reported [17].

The changes in cytoskeletal isoactin content following chemotactic stimulation with chemoattractants were then investigated. Vegetative and pre-aggregative stage cells are sensitive to the chemoattractant folate [26], while cells more advanced in development at the stage of aggregation-competence specifically respond to cAMP [27]. The results for both folate (table 2a) and cAMP (table 2b) demonstrate that the major isoactin contributor to the rapid (3–5 s) peak of actin accumulation was the most acidic (A_1) isoform. The others were only minor contributors and in the

Table 2

Changes in cytoskeletal isoactin content following a chemotactic stimulus expressed as percentage change over prestimulus levels

	(a) Preaggregative (1 h) cells, 3 s after a 5×10^{-5} M folate stimulus	(b) Developing (8 h) cells, 5 s after a 5×10^{-8} M cAMP stimulus
Total change	+ 55.8 ± 5.2	+ 74.6 ± 4.5
Change in		
A_1	+106.1 ± 16.1	+166.2 ± 17.1
A_2	+ 20.9 ± 7.0	− 11.7 ± 7.7
A_3	+ 15.0 ± 2.1	− 7.4 ± 7.7

Each result is the mean ± SE of 5 separate experiments

case of cAMP stimulation these isoforms possibly showed slight decreases in their association with the cytoskeleton.

The total extent of the actin changes was comparable to the changes found by scanning densitometry of total cytoskeletal actin samples in one-dimensional gels, indicating that the changes in the three isoforms shown in table 2 probably account for practically all of the actin change previously reported [3,4].

In the light of these results we suggest that whatever the source of actin heterogeneity in *D. discoideum,* the different isoforms may play different roles within the cell. While isoform A_1 participates in the massive cytoskeletal actin accumulation during chemotactic movement, the more basic isoforms A_2 and A_3 may differ in some way that prevents such participation and ensures a more stable, structural role.

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