Acetylated and Nonacetylated Actins in Dictyostelium discoideum*

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We have carried out a two-dimensional gel analysis of the actin system of Dictyostelium discoideum. Our results show that on the basis of isoelectric focusing, there is a single major [35S]methionine-labeled species which corresponds both to the actin purified by Uyemura et al. (Uyemura D., Brown, S. S., and Spudich, J. A. (1978) J. Biol. Chem. 253, 9088-9095) and to the Coomassie Blue staining species seen in whole cell lysates of the organism. We also detect a minor labeled actin species, x, which has no corresponding Coomassie Blue staining counterpart. This species turns over much more rapidly than the major actin and has one more positive charge. It is not labeled with [3H]acetate, whereas the major actin is. When D. discoideum RNA is added to an mRNA-dependent rabbit reticulocyte lysate protein translation system, only one major actin is seen, and this species corresponds to the major actin observed in vivo. If endogenous acetyl coenzyme A is removed from the translation system, a second major actin appears corresponding in position to x. These results indicate that in D. discoideum, there is present a single major actin species in addition to a small amount of a rapidly turning over actin which is a nonacetylated form of the major actin. Additional experiments examining these actins through the development cycle of the organism show no consistent differences with the results obtained using vegetative cells.

Actin, long recognized as a major component of the contractile machinery of muscle cells, has been observed in every eukaryotic cell in which it has been sought. In nonmuscle cells, actin is thought to be associated with processes such as cell migration, phagocytosis, cytokinesis, cell shape determination, and mitosis (for review, see Pollard and Weihing, 1974; Clarke and Spudich, 1977). These processes are transient and they involve the rapid formation and disassembly of actin-containing supramolecular assemblies. The formation of these rapidly changing structures might be controlled in part by the selective use of multiple distinct actin species or by the use of ancillary modulating factors operating on the actin, or by both.

Recently, the presence of three distinct actin classes having the same molecular weight but different isoelectric points has been reported in rat, bovine, and chick cells (Whalen et al., 1976; Garrels and Gibson, 1976; Rubenstein and Spudich, 1977, and Storti and Rich, 1976). In chick embryo fibroblasts, all three actin classes occur in approximately the same ratio in total cell extracts, cytoskeleton preparations, and actomyosin preparations (Rubenstein and Spudich, 1977). In cultured skeletal muscle myoblasts, however, selective enhancement of the synthesis of α-actin occurs concomitant with myoblast fusion and myotube growth (Whalen et al., 1976; Rubenstein and Spudich, 1977; Garrels and Gibson, 1976).

The necessity for multiple major actin species apparently does not extend throughout the realm of eukaryotic cells. Only one actin has been observed in Acanthamoeba castellanii (Gordon et al., 1977) and in Physarum polycephalum (Vandekerckhove and Weber, 1978b). Our preliminary observations with actins isolated from the cellular slime mold Dictyostelium discoideum, referred to in a previous report (Uyemura et al., 1978), indicate that there is only one major actin detectable by Coomassie Blue staining of two-dimensional gels of this material. This actin migrated as a single species when the isoelectric focusing (O’Farrell, 1975) was performed in a pH 5 to 7 gradient.

Recently, Kindle and Firtel (1978) reported the isolation of two different actin mRNA species from Dictyostelium. Translation of these messages in a cell-free system gave rise to two species, A1 and A2, on two-dimensional gels which corresponded to two labeled actin species which they saw when cells were labeled in vivo with [35S]methionine.

Since the purification scheme for D. discoideum (Uyemura et al., 1978) results in a 30% yield of the total actin initially present, one can argue that they isolated only one of two actin species initially present. We present here a two-dimensional gel study of the actins made in vivo in both vegetative and developing Dictyostelium cells. In whole cell lysates, we find only one Coomassie Blue stainable species. In cells labeled with [35S]methionine, two labeled species are seen. The major one co-migrates with the Coomassie Blue staining species. The second migrates as if it carried one more positive charge than the first. The major species can be shown to be labeled with [3H]acetate while the second is not. The kinetics of labeling with [35S]methionine shows that the second minor species is turning over more rapidly than the first. Finally, in vitro translation experiments show the same major-minor actin pattern. When protein acetylation is inhibited, what was previously the minor species now becomes the major one.

Experimental Procedure

Materials—Materials used for isoelectric focusing and gel electrophoresis were those described by O’Farrell (1975). L-[35S]Methionine (>800 Ci/mmol) was purchased from Amer sham. Sodium [3H]acetate (9.4 Ci/mmmole) was purchased from New England Nuclear. mRNA-dependent rabbit reticulocyte lysates supplemented with L-[35S]me-
thionine were prepared by the procedure of Pelham and Jackson (1976). Pure \textit{D. discoideum} actin, prepared by the method of Uyemura \textit{et al.} (1976), was obtained from D. Uyemura and J. Spudich, University of California, San Francisco. All other chemicals were reagent grade quality.

**Growth and Labeling of Cells**—Midlogarithmic phase cells of the axenic strain of \textit{D. discoideum} Ax-3 grown in HL-5 medium were obtained from David Soll, University of Iowa. For labeling vegetative cells with \( ^{14} \text{C} \) methionine, the cells were collected by centrifugation, washed with an aliquot of chemically defined nutrient medium with a 50 mM phosphate solution, pH 6.4, containing 20 mM KCl, 2.5 mM MgCl\( _2 \), and streptomycin sulfate, 0.5 g/liter (LPS), and suspended on a disk of Whatman 50 filter paper soaked in the same solution. The cells were then agitated on a rotary platform for the desired period, harvested by centrifugation, washed with a cold phosphate-buffered saline (0.9% NaCl) solution, and again collected by centrifugation. The cells were immediately lysed in a lysis buffer containing 9.2 M urea, 2% NP-40 nonionic detergent, 0.5 (w/v) β-mercaptoethanol, and 2% (w/v) Ampholines, pH 5 to 7. Labeling with \(^3\text{H}\) Hacetate was performed in a similar fashion except that sodium \(^3\text{H}\)-acetate (9.4 Ci/mmol) was used at a concentration of 1 μC/ml. Furthermore, in this case, the cells were preincubated for 15 min with either 1 mM di-fluorofluorite or 1 mM α-ketoglutarate to prevent conversion of in vivo synthesized \(^3\text{H}\) acetate coenzyme A to amino acids. Label was then introduced into the medium for the desired time.

For labeling with \(^3\text{H}\) methionine during the developmental cycle of the organism, cells were collected by centrifugation, washed with a 50 mM phosphate solution, pH 6.4, containing 20 mM KCl, 2.5 mM MgCl\( _2 \), and streptomycin sulfate, 0.3 g/liter (LPS), and suspended on a disk of Whatman 50 filter paper soaked in the same solution. The cell-covered pad was then placed on two Whatman 3 filter disks of the organism, cells were collected by centrifugation, washed with a cold phosphate-buffered saline (0.9% NaCl) solution, and again collected by centrifugation. The cells were immediately lysed in a lysis buffer containing 9.2 M urea, 2% NP-40 nonionic detergent, 0.5 (w/v) β-mercaptoethanol, and 2% (w/v) Ampholines, pH 5 to 7. Labeling with \[^{35} \text{S}\]methionine was performed in a similar fashion except that sodium \[^{35} \text{S}\]methionine (800 to 1000 Ci/mmol) was used at a concentration of 1 μCi/ml.

**RESULTS**

**In vivo Labeling Studies**—Whole cell lysates of \( ^{14} \text{C} \) methionine-labeled vegetative cells of \textit{D. discoideum} were analyzed by the two-dimensional gel technique using a pH 5 to 7 isoelectric focusing gradient as a first dimension and SDS-PAGE as a second (O’Farrell, 1975). The results are shown in Fig. 1. The top panel shows the Coomassie Blue-stained gel while the bottom panel shows the autoradiogram of this gel. In the stained gel, only a single major actin is present and co-migrates with purified \textit{D. discoideum} actin. However, two labeled putative actin species, A and x, are seen in the autoradiogram. The species labeled A co-migrates with the Coomassie Blue-stained actin while x migrates as if it had the same molecular weight but one more positive charge.

These results indicate that steady state levels of x within the cell are much lower than that of the major actin species. Variation of the labeling time of these cells produced quite different ratios of A to x as shown in Fig. 2. Short labeling times on the order of 10 min produced A/x ratios approximately 1:1 while after a 2-h exposure to label, the ratio of A/x was approximately 12:1. These results indicated that x was turning over at a much faster rate than was A or was possibly acting as a precursor for the major actin.

Lazarides and Lindberg (1974) showed that pancreatic DNase I forms a tight 1:1 complex with actin and that actin selectively binds to a Sepharose column to which DNase is attached. Furthermore, 3 M guanidine HCl is required to elute the bound actin. We labeled \textit{D. discoideum} with \[^{35} \text{S}\]methionine for 30 min, homogenized the cells in sucrose and, after centrifugation at 10,000 \( \times \) g for 15 min, applied the supernatant solution to a column of DNase I-agarose equilibrated in 0.5 M sodium acetate plus 1 mM CaCl\( _2 \). No actin was seen in the flow-through fraction or in the 0.75 M guanidine-HCl wash. The material eluted with 3 M guanidine-HCl was dialyzed against water, lyophilized, redissolved in lysin buffer and analyzed on two-dimensional gels. The results are shown in Fig. 3. Clearly, both A and x are selectively bound to the resin. This result coupled with the isoelectric points and molecular weights of A and x indicate that they are actin molecules.

It is evident from these gels that a third species of actin, \( n \), is produced by this procedure. This species is observed both by Coomassie Blue staining and by autoradiography but is

\begin{figure}[h]
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\includegraphics[width=\textwidth]{fig1.png}
\caption{Two-dimensional gel analysis of whole cell lysates of \( ^{14} \text{C} \) methionine-labeled \textit{D. discoideum} vegetative cells. First dimension isoelectric focusing was carried out in a pH 5 to 7 gradient. Five micrograms of protein was applied to each gel. A denotes the major actin spot that co-migrates with purified \textit{D. discoideum} actin. X denotes a second minor actin species. A, autoradiogram of the two-dimensional gel; B, actin region of the Coomassie Blue-stained gel. IEF, isoelectric focusing.}
\end{figure}
Dictyostelium discoideum Actins

FIG. 2. Kinetics of variation of A/x in [35S]methionine-labeled D. discoideum vegetative cells. A culture of D. discoideum cells in a defined medium (see "Experimental Procedures") containing 0.01 mM L-methionine and 125 pCi/ml of L-[35S]methionine and shaking on a rotating platform was sampled at various times following addition of the label. Cells were collected by centrifugation, washed with cold LPS, and immediately dissolved in lysis buffer. Focusing was carried out in a pH 4 to 6 gradient with 3 μg of total cell protein containing 0.15 μg of actin loaded on each gel. After second dimension SDS-gel electrophoresis, the gels were dried. Shown are densitometric tracings of the actin region of the autoradiograms of these gels. a, 5 min; b, 15 min; c, 30 min; d, 3 h.

FIG. 3. DNase I-agarose chromatography of [35S]methionine-labeled D. discoideum cell extract. For experimental details, see "Experimental Procedures." Two-dimensional gels were made using a pH 5 to 7 gradient for first dimension isoelectric focusing. Only the actin regions of the gels are shown. a, autoradiogram of gel obtained when cells used in this experiment were dissolved directly in lysis buffer; b, Coomassie Blue-stained gel of the same sample; c, autoradiogram of the material eluted from the DNase column with 3 M guanidine-HCl; d, Coomassie Blue-stained gel of the 3 M guanidine-HCl eluate. Equal amounts of protein were loaded on each gel. A is the major actin species, x is the possible actin precursor, and n is a new species that appears following DNase chromatography. The ratio of A/n and A/x does not vary if lower amounts of protein are loaded on each gel. Identical results were obtained in three separate experiments.

not present when a portion of the cells used in this experiment were dissolved directly in the 9 M urea, 2% detergent-containing lysis buffer which rapidly denatures all proteins in the cell. We thus believe that this new species may arise artifically as a result of deamidation or possibly sulfur oxidation occurring during the experimental manipulations.

All actins sequenced so far (Elzinga et al., 1973; Elzinga and Lu, 1976; Vandekerckhove and Weber, 1978a, b) have been found to possess a blocked NH₂ terminus which has been shown to be an N-acetyl residue in the case of rabbit skeletal muscle actin. To determine whether our actin species were acetylated, we placed vegetative D. discoideum in a medium containing [3H]acetate for 10 min. Over a similar time period as shown in Fig. 4d, the ratio of labeling of A/x by [35S]-methionine was 2:1 as determined by scanning densitometry. Figure 4a shows the results of the acetate label experiment. The ratio of A/x here is 5:1 suggesting that acetate preferentially labels the major actin when compared with the methionine labeling pattern.

[3H]Acetate, in the form of acetyl-CoA made within the cell, can be converted to amino acids via the Krebs' cycle. This pathway may have been responsible for part of the [3H]acetate seen in A and x and could interfere with detection of specific acetylation. To circumvent this problem, the cells were incubated either with 1 mM α-ketoglutarate or 1 mM fluorocitrate for 15 min prior to the introduction of label. The unlabeled α-ketoglutarate should dilute the specific activity of any [3H]α-ketoglutarate formed, thereby blocking the formation of [3H]glutamic acid, and the fluorocitrate should inhibit aconitase again preventing [3H]acetate from entering the amino acid pool. Under each of these conditions, [3H]acetate labeled only the major actin with an A/x ratio of approximately 30:1 (Fig. 4, b and c). Therefore, the apparent charge difference seen between A and x can be explained by the fact that A is acetylated while x is not. It is of interest that if labeling were carried out with [3H]acetate for 1 h, enough label traversed these metabolic blocks to cause the A/x ratio to approach that seen with [35S]methionine.

FIG. 4. Two-dimensional analysis of D. discoideum labeled with [3H]acetate. For details, see "Experimental Procedures." First dimension isoelectric focusing was carried out in a pH 5 to 7 gradient. Autoradiograms of the actin portions of the gels are shown. a, cells were labeled for 10 min with [3H]acetate; b, cells were pretreated for 15 min with 1 mM α-ketoglutarate and then [3H]acetate was labeled for an additional 10 min; c, cells were pretreated for 15 min with 1 mM α-fluorocitrate and exposed to [3H]acetate for an additional 10 min; d, cells were labeled for 15 min with [3S]methionine. All gels contain approximately 4 μg of total cell protein.
For their gel studies, Kindle and Firtel (1978) used the procedure of Garrels and Gibson (1976) in which SDS was included in the lysis buffer, and 30-cm-long gels were utilized instead of the 12.5-cm gels of O'Farrell (1975). To see whether the resolving power of our gel system was deficient, we lysed cells in our normal lysis buffer to which we added 0.5% (w/v) SDS. Following dissolving of the cells, the lysate was diluted 1:1 with SDS-free lysis buffer and electrofocused in a pH 5 to 7 gradient on 12.5-cm gels. The results shown in Fig. 5 are indistinguishable from those obtained with the normal O'Farrell system: a major actin both Coomassie Blue-stained and labeled with \[^{35}S\]methionine and a second labeled actin without a corresponding stained protein.

Since it has been shown that both the levels of actin and actin mRNA change during the developmental cycle of *Dictyostelium discoideum* (Alton and Lodish, 1977), we questioned whether

![Fig. 5. Two-dimensional gel of \[^{35}S\]methionine-labeled *D. discoideum* using 12.5-cm isoelectric focusing gels and SDS-containing lysis buffer. Focusing was carried out for 12,000 V/h in a pH 5 to 7 gradient. Labeling was for 1 h. a, autoradiogram of the actin region; b, Coomassie Blue-stained gel. If an actin is present in the A_II region (Kindle and Firtel, 1978), it is less than 5% of the material found in the major actin spot by scanning densitometry.](image)

![Table I](image

**Table I**

<table>
<thead>
<tr>
<th>Time after beginning of development</th>
<th>Coomassie Blue-stained gels</th>
<th>15-min labeling time</th>
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<td>2.0:1</td>
<td>6.6:1</td>
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<td>7.2:1</td>
</tr>
<tr>
<td>20</td>
<td>30:1</td>
<td></td>
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</table>

\(^*\) Stained gels were scanned.

\(^*\) Autoradiograms were scanned.

![Fig. 6. Two-dimensional analysis of actin expression in \[^{35}S\]methionine-labeled *D. discoideum* at different stages in development. First dimension isoelectric focusing was carried out in pH 5 to 7 gradients. Only the actin regions of the two-dimensional gels are shown. a, autoradiogram; sample taken 4 h after start of development and labeled for 15 min. b, Coomassie Blue-stained gel of a 4-h sample. c, Coomassie Blue-stained gel of a 2-h sample. d, autoradiogram; sample taken 12 h after beginning of development and labeled for 15 min. e, autoradiogram; sample taken 12 h after beginning of development and labeled for 2 h. f, Coomassie Blue-stained gel of a 12-h sample. A is the major actin. x is the nonacetylated species.](image)

![Fig. 7. Cell-free synthesis of *D. discoideum* actin in a mRNA-dependent rabbit reticulocyte lysate. Three hundred micrograms of total *D. discoideum* RNA was added to each of two 50-μl rabbit reticulocyte lysate in vitro protein synthesizing assay mixtures supplemented with \[^{35}S\]methionine, 100 μCi/ml, and made mRNA-dependent by prior treatment with micrococcal nuclease (Pelham and Jackson, 1976). Incubations were for 60 min at 37°C. In a control where exogenous RNA was not added, no protein was synthesized. a, only *D. discoideum* RNA was added to the mixture; b, in addition to RNA, oxaloacetate, 1 mM, and pig heart citrate synthetase, 32 units/ml, were added to reduce endogenous acetyl-CoA levels by converting it to citrate (Palmiter, 1977). Two microliters of assay mixture was loaded on each gel. The first dimension isoelectric focusing was carried out in a pH 5 to 7 gradient. Autoradiograms of the actin regions of the gels are shown.](image)
new actin species were produced during this time. At time zero, cells were removed from nutrients and placed on nutrient-free pads to allow development to occur. Every 4 h, \( [^{14}C] \) methionine was fed to a part of the cells and after pulses of 15 min or 2 h, the cells were lysed and analyzed by two-dimensional electrophoresis as described earlier. Representative results are shown in Fig. 6 and Table I. At each stage in the developmental cycle, one can detect a single major labeled species corresponding to the Coomassie Blue-stained actin and a minor species, \( x \). Furthermore, the kinetics of methionine labeling with respect to \( A \) and \( x \) are the same throughout the developmental cycle as seen with vegetative cells.

**Cell-free Synthesis of D. discoideum Actin**—When total \( D. \) discoideum RNA was added to the mRNA-dependent rabbit reticulocyte lysate protein synthesizing system (Pelham and Jackson, 1976) supplemented with \( [^{35}S] \) methionine, the major protein produced was actin. As seen in Fig. 7a, only one major species, \( A \), is seen. This co-migrates with added D. discoideum-unlabeled actin. A minor labeled species, \( x \), can also be seen at a more basic isoelectric point as in the in vivo labeling experiments discussed earlier. When added \( D. \) discoideum RNA was left out of the incubation mixtures, no protein synthesis was detected (data not shown).

When we added oxaloacetate and citrate synthetase to the lysate to prevent protein acetylation by converting endogenous acetyl-CoA to citrate (Palmiter, 1977), we obtained the results seen in Fig. 7b. Now, instead of a single major labeled species \( A \) corresponding to \( D. \) discoideum actin, there are two major species, \( A \) and \( x \), with \( x \) actually more intensely labeled than \( A \). This result confirms the finding of our in vitro experiments that \( A \) is acetylated actin and \( x \) is the nonacetylated precursor form of this protein.

**DISCUSSION**

Analysis of whole cell lysates of \( D. \) discoideum by isoelectric focusing in a pH 5 to 7 gradient indicates that there is only a single major staining actin species and that this species corresponds to the actin purified to homogeneity by Uemura et al. (1978). In vivo labeling of \( D. \) discoideum with \( [^{35}S] \) methionine gave rise to a major labeled actin species which co-migrates with the Coomassie Blue-stained spot plus a minor labeled species with the same molecular weight but one more positive charge. No Coomassie Blue-stained species co-migrated with this labeled species we call \( x \). We believe our major stainable actin (\( A \)) corresponds to \( A_1 \) of Kindle and Firtel (1976). Their \( A_{1H} \) is at a more acidic pH than \( A_1 \), whereas our nonacetylated actin is more basic than \( A \). Furthermore, using 15.5-cm gels and SDS in our lysis buffer (Kindle and Jackson, 1976; Garrels and Gibson, 1976) did not change the gel pattern we see with the usual O’Farrell gel system. Therefore, we have no explanation as to why our results differ with those seen in Fig. 7a.

When cells were labeled at times from 5 min to 2 h, the ratio of the label in the minor \( x \) species to the major \( A \) species changed rapidly, indicating that \( x \) was turning over much more rapidly than \( A \). Either \( x \) was being degraded faster or it was being converted to \( A \). A similar phenomenon was observed in Drosophila melanogaster (Storti et al., 1978) when a minor rapidly metabolized actin was also detected. This Drosophila species was not further characterized.

Since all actins so far studied have blocked NH\(_2\)-terminals (Elzinga and Lu, 1976; Vandekerckhove and Weber, 1978a, b) and since \( x \) has apparently one more positive charge than \( A \), we considered the possibility that \( x \) was a nonacylated form of the major actin in the cell. First, using the DNase I-agarose technique of Lazarides and Lindberg (1974) we showed that both \( x \) and \( A \) were specifically adsorbed to the resin and could be removed only with 3 M guanidine-\( \text{HCl} \) in 0.5 M sodium acetate. This behavior is highly characteristic of all actins since the binding constant of actin to pancreatic DNase I is approximately \( 10^5 \).

Second, when we labeled cells with \( [^{14}C] \) acetyl-CoA under conditions where the \( [^{14}C] \) acetyl-CoA formed was largely prevented from being converted to amino acids, only the major \( A \) species was labeled with an \( A/x \) ratio of 30:1. Under similar labeling times, the labeling ratio of \( A/x \) obtained with \( [^{35}S] \) methionine was 2:1. These results support our hypothesis that \( x \) is a nonacylated form of \( A \) and indicate that the N-blocking residue in mature \( D. \) discoideum actin is probably an acetyl group as is found in rabbit skeletal muscle actin (Alving and Lakl, 1966).

Kinetic labeling experiments with \( [^{35}S] \) methionine during the developmental stage of these cells gives an actin profile identical with that which we observe during vegetative growth of the Ax-3 cells. Therefore, although actin mRNA levels fluctuate during the developmental cycle of the cell, this modulation does not result in the synthesis of a new major structural gene product that we can detect by isoelectric focusing. It is important to note that the two-dimensional gel system will not pick up conservative amino acid substitutions or the insertion or deletion of a small number of uncharged amino acid residues. Thus, a definitive answer as to the number of different structural genes for actin in these cells must await sequencing of the actin genes or of the translation products of these genes.

Experiments utilizing total RNA isolated from \( D. \) discoideum in a cell-free synthesizing system have again shown that there is only one major actin and a minor species, \( x \). In this experiment, a large portion of \( A \) could be converted to \( x \) simply by including oxaloacetate and citrate synthetase to diminish protein acetylation. This result further indicates that \( D. \) discoideum actin is, in fact, acetylated and that a small amount of a nonacylated form of the protein is produced in the cell. Isoelectric focusing shows that \( A \) differs in charge from \( x \) by a single acetyl residue. Since all mature actins so far examined have a blocked NH\(_2\) terminus, our results suggest, but do not prove, that the acetylation we observe probably occurs at the NH\(_2\) terminus.

The labeling kinetics of \( A \) and \( x \) suggest that \( x \) is either being degraded faster than \( A \) or converted to it in a precursor-product relationship. It has been previously reported (Pestana and Pitot, 1975a, b) that \( N \)-acetylation occurs mainly during translation for most proteins, so conversion of \( x \) to \( A \) might be considered unlikely. The pulse-chase experiments needed to test this possibility in vitro are not feasible due to the slow uptake of free amino acids by \( D. \) discoideum cells. As for the possibility that \( x \) is degraded faster than \( A \), Brown (1979) has recently reported that, on the whole, \( N \)-acylated proteins do not have a measurably longer half-life than do nonacylated proteins in mouse L-cells. He correctly points out that he has not examined relative half-lives of acetylated and nonacylated forms of the same protein. In any case, it is certain that the \( N \)-acylase present in \( S. \) discoideum does not \( N \)-acylate every actin polypeptide while it is being synthesized. In future work, we will examine in greater detail the relationship of \( A \) and \( x \) and the reasons for their temporally dissimilar labeling patterns. Little is known about the role of \( N \)-acylation in actin function. The Dictyostelium system, because it has only a single major actin, should provide a convenient model for addressing this problem.
**Acknowledgments**—We thank Barbara Varnum and David Soll for providing us with the *Dictyostelium* and for their helpful discussions. *D. discoideum* actin was kindly provided by Dennis Uyemura and James Spudich.

**REFERENCES**


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