Studies on the Transcription, Translation, and Structure of α-Actinin in *Dictyostelium discoideum*

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Abstract. A clone coding for the F-actin cross-linking protein α-actinin was obtained by screening a genomic library of *Dictyostelium discoideum* DNA in λgt11 with monoclonal antibodies specific for *Dictyostelium* α-actinin. The 1.2-kilobase (kb) genomic clone was confirmed as containing part of the α-actinin gene by comparing its nucleotide sequence with the amino acid sequence of tryptic peptides from purified α-actinin. The clone recognized a 3.0-kb message in a Northern blot. Hybridization to RNA isolated from different developmental stages of several *D. discoideum* strains indicated that the mRNA content increased during early development. A similar result was obtained when the α-actinin content of the cells was followed by Western blot analysis. Hybridization of the clone to DNA from different wild-type strains of *D. discoideum* indicated a polymorphism on the DNA level that coincided with a polymorphism on the protein level. The data suggest (*a*) continuous transcription of the α-actinin gene throughout the development of *D. discoideum*, (*b*) up- and down- regulation of the levels of α-actinin mRNA and protein with maximum levels at the onset of aggregation, and (*c*) a high diversity of α-actinin at the DNA and protein level among different *D. discoideum* strains. The structural data make it conceivable that the highly conserved nature of α-actinin resides only at the functional sites, whereas the helical portions of the α-actinin molecule allow a higher level of diversity throughout evolution.

ACTIN is the major component of the microfilament system. Rearrangements of this system during cell movement are controlled by myosin and its regulatory proteins, by tropomyosin, and by regulatory proteins that bind to G- or F-actin (9, 20, 45). From their function in vitro one can distinguish four classes among the actin-binding proteins: G-actin binding proteins (5, 25, 34), F-actin severing proteins (2, 18), F-actin capping proteins (19, 37), and F-actin cross-linking proteins (1, 4, 7, 8, 10, 13, 16). α-Actinin, a cross-linking protein, can be isolated from both muscle and nonmuscle cells. α-Actinin, as isolated from higher organisms, is composed of two subunits of 100 kD (13) compared with 95 kD per subunit in lower organisms (8, 16). A protein that resembles the nonmuscle α-actinin has been described for *Dictyostelium discoideum* (8, 16).

The primary structures of actin and myosin have been determined (6, 14, 24, 35, 40, 41), the corresponding genes have been isolated and characterized, and their transcription and transcriptional regulation are being investigated (3, 35). In contrast, little is known about the primary structure, genes, and transcriptional regulation of the actin-binding proteins. So far, only cDNA clones coding for erythrocyte spectrin and ankyrin have been described (26).

We have isolated a genomic clone that codes for ~50% of the *D. discoideum* α-actinin molecule. This clone and different monoclonal antibodies against α-actinin enabled us to investigate the transcription of the α-actinin gene in relation to the protein level during the developmental cycle of several *D. discoideum* strains. In addition, we have sequenced the clone and present the first data on the primary structure of an α-actinin.

Materials and Methods

Growth and Development of D. discoideum

D. discoideum strain AX2, an axenically growing derivative of NC4, and various wild-type strains (NC4, V12, WS380B, OHIO, AC4, WS2162, WS7, WS51, WS112, WS269A, WS472, WS526, WS576, WS582, WS583, WS584, WS655, WS656, WS1956, WS2054, ZA3A, K10, MFD, DD44, and DD61) (15) were used. They were grown at 21°C on solid medium (38) with *Klebsiella aerogenes* as food source or in axenic medium (for strain AX2) (43). For development on filters, strains were grown in suspension culture on washed *E. coli* B/r (1×10^{10} cells/ml) in 17 mM phosphate buffer (pH 6.0). Amebas were collected before the bacteria were consumed, centrifuged free of bacteria, and deposited on Millipore filters (Millipore Corp., Bedford, MA) (28). The filters were incubated at 21°C. Cells were harvested from the filters at different stages of development, and aliquots were used for isolation of RNA and sample preparation for immunoblots.

DNA and RNA Isolation

Chromosomal DNA was isolated from partially purified nuclei. To prepare nuclei, whole cells (~5×10^9 cells) were lysed with 100 ml of Nonidet P-40 buffer (10 mM magnesium acetate, 10 mM NaCl, 30 mM Hepes pH

7.5, 10% [wt/vol] sucrose, 2% [wt/vol] Nonidet P-40), and the nuclei pelleted by a low-speed spin.

The nuclei were then lysed at 60°C with EDTA-sarcosyl (2% [wt/vol] sarcosyl, 0.2 M EDTA; the pH was adjusted with NaOH to 8.4), and the lysate subjected to a CsCl-ethidium bromide density gradient centrifugation (0.92 g CsCl per gram solution) (31). Total cellular RNA of *D. discoideum* was isolated after lysis of the cells with SDS (0.5% final concentration) and purified with several phenol-chloroform extractions (30).

Immunoblot Analysis

Cells were pelleted, washed in a small volume of phosphate buffer (17 mM, pH 6.0), immediately lysed by the addition of boiling SDS/EDTA solution (1% [wt/vol] SDS, 2 mM EDTA), and kept in a boiling waterbath for 3 min. Equal amounts of protein (determined by the method of Lowry [22] with bovine albumin as standard) were subjected to SDS PAGE, electrophoretically transferred to nitrocellulose filters (39), and the filters probed with iodinated monoclonal antibodies.

Preparation and Screening of Genomic Libraries

*Eco*RI-digested DNA from *D. discoideum* strain AX2 was used to prepare a genomic DNA library in λgt11 (47). The library was amplified once and screened with iodinated monoclonal antibodies directed against *D. discoideum* α-actinin, as described previously (29). Purification of positive clones, DNA isolation, and subsequent cloning of the genomic DNA insert into a plasmid cloning vector was done by standard methods (23).

Nucleic Acid Analysis

For Northern blots, RNA was separated on 1.2% agarose gels in the presence of 6% formaldehyde; for Southern blots, restriction enzyme–digested DNA of *D. discoideum* was separated on agarose gels in Tris-phosphate buffer (23). Hybridization of the filters was done with nick-translated probes for 16–18 h at 37°C in 2× standard saline citrate (SSC), formamide as indicated, 4 mM EDTA, 1% sarcosyl, 0.1% SDS, 4× Denhardt's solution, and 0.12 M phosphate buffer, pH 6.8. DNA fragments for nick-translation were separated in agarose gels containing Tris-borate buffer (23) and were subjected to electrophoresis onto DE81-paper. Elution from the paper was done as described (12).

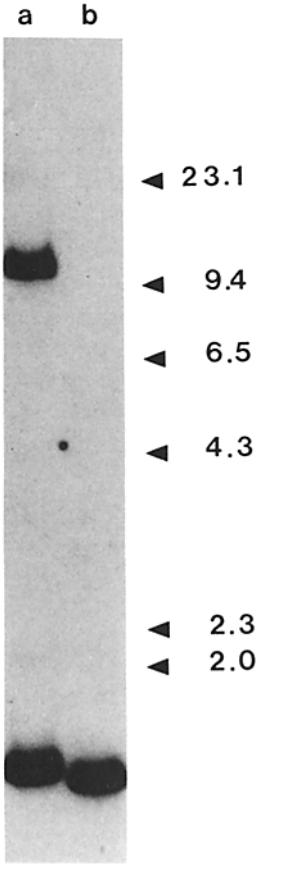


Figure 1. Hybridization of the α-actinin gene probe to DNA from *D. discoideum* strain AX2. Nuclear DNA (10 μg each) was digested with *Hind*III (*a*) or *Eco*RI (*b*), separated on a 0.7% agarose gel, transferred to nitrocellulose, and probed with the nick-translated 1.2-kb fragment. Hybridization was done in the presence of 50% formamide and 2× SSC. The sizes (in kilobases) of the restriction fragments of *Hind*III digested lambda DNA as marker are indicated at the right.

DNA Sequencing

DNA fragments were subcloned in phage M13 mp18 or mp19 (46) using the *E. coli* strain JM101 as host and sequenced by the dideoxy nucleotide chain termination method of Sanger et al. (36). The sequences were analyzed with the program "word-search" of the University of Wisconsin (Genetic Computer Group, J. Devereux).

Purification and Proteolytic Digestion of α-Actinin

α-Actinin was purified to homogeneity from aggregation competent cells (AX2) essentially as described (8). The protein was cleaved with *N*-tosyl-L-phenylalanine chloromethyl ketone-treated trypsin (CooperBiomedical, Inc., Malvern, PA) in the presence of 50 mM ammonium bicarbonate for 15 h at 37°C, at a substrate concentration of 1 mg/ml. Protease was added in two portions with a final enzyme/substrate ratio of 1:50 (wt/wt). The resulting peptides were separated and rechromatographed by high performance liquid chromatography on a C18-column using 0.1% trifluoroacetic acid and 0.1% trifluoroacetic acid in acetonitrile as elution solvents.

Protein Sequencing

N-terminal amino acid sequence analyses were performed on a gas phase sequencer 470 A from Applied Biosystems, Inc. (Foster City, CA). The phenylthiohydantoin amino acid derivatives were analyzed by a high performance liquid chromatography system which separates all components isocratically (21).

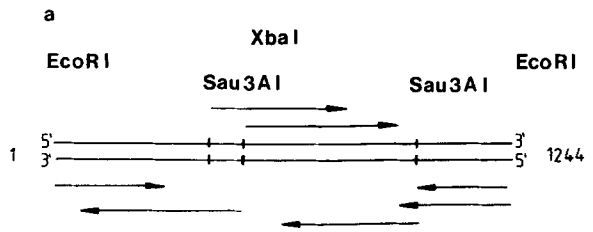
Results

Isolation and Characterization of a Genomic Clone of the α-Actinin Gene of D. discoideum

A genomic library constructed in the expression vector λgt11 was screened with three different monoclonal antibodies directed against *D. discoideum* α-actinin. These antibodies, mAb 47-62-17, mAb 47-19-2, mAb 47-18-9, are highly specific for α-actinin and recognize different epitopes of the molecule (37; Schleicher, M., manuscript in preparation). 12 positive phages were isolated which reacted with mAB 47-62-17 but with none of the other antibodies. All of these recombinant phages harbored an identical insert of ~1.2 kilobases (kb). This 1.2-kb insert recognized a fragment of the same size in a Southern blot containing AX2-DNA cut with *Eco*RI, and two fragments of ~10 and 1.35 kb of AX2-DNA cut with *Hind*III (Fig. 1). The hybridization pattern of the cloned fragment to chromosomal DNA suggests that *D. discoideum* harbors one gene for α-actinin. The result from the hybridization to *Hind*III cut genomic DNA is explained by the presence of a *Hind*III site in the 1.2-kb *Eco*RI fragment. In a Northern blot analysis this probe hybridized to a message of 3.0 kb, a size sufficient to code for a protein of the size of α-actinin (molecular mass of 95 kD per subunit).

Identification of the Clone by DNA and Protein Sequence Analysis

DNA Sequencing. To confirm that the clone codes for the α-actinin of *D. discoideum*, we determined the complete nucleotide sequence of the cloned DNA fragment. A restriction map was established (Fig. 2 *a*), and fragments covering the complete insert were subcloned into suitable M13-cloning vectors. The sequencing strategy is shown in Fig. 2 *a*. Fig. 2 *b* presents the complete sequence of the 1,244-base



b

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        10                  30                  50
CATATTGAATCACATGGTGTTAAATTGGTTGGTATTGGTGCTGAAGAGTTAGTTGATAAA
HisIleGluSerHisGlyValLysLeuValGlyIleGlyAlaGluGluLeuValAspLys
        70                  90                  110
AACTTAAAGATGACTTTGGGTATGATTTGGACAATCATTCTTCGTTTTGCCATTCAAGAT
AsnLeuLysMetThrLeuGlyMetIleTrpThrIleIleLeuArgPheAlaIleGlnAsp
        130                 150                 170
ATTTCAATTGAAGAATTGAGTGCCAAAGAAGCCCTTTTACTTTGGTGTCAAAGAAAGACC
IleSerIleGluGluLeuSerAlaLysGluAlaLeuLeuLeuTrpCysGlnArgLysThr
        190                 210                 230
GAAGGTTATGACCGTGTTAAAGTTGGTAATTTCCATACCTCATTCCAAGATGGTCTTGCC
GluGlyTyrAspArgValLysValGlyAsnPheHisThrSerPheGlnAspGlyLeuAla
        250                 270                 290
TTTTGTGCTCTCATCCATAAACATAGACCAGATTTAATCAACTTTGACTCTTTAAACAAA
PheCysAlaLeuIleHisLysHisArgProAspLeuIleAsnPheAspSerLeuAsnLys
        310                 330                 350
GATGATAAAGCTGGTAACTTACAATTGGCTTTTGATATTGCCGAAAAAGAATTGGATATC
AspAspLysAlaGlyAsnLeuGlnLeuAlaPheAspIleAlaGluLysGluLeuAspIle
        370                 390                 410
CCAAAGATGTTGGATGTTTCCGATATGCTCGATGTCGTTCGTCCAGATGAAAGATCAGTC
ProLysMetLeuAspValSerAspMetLeuAspValValArgProAspGluArgSerVal
        430                 450                 470
ATGACCTACGTCGCTCAATACTACCATCACTTTTCTGCCTCTAGAAAAGCTGAAACCGCC
MetThrTyrValAlaGlnTyrTyrHisHisPheSerAlaSerArgLysAlaGluThrAla
        490                 510                 530
GGTAAACAAGTTGGTAAAGTTTTAGATACCTTTATGTTGTTAGAACAAACCAAATCTGAT
GlyLysGlnValGlyLysValLeuAspThrPheMetLeuLeuGluGlnThrLysSerAsp
        550                 570                 590
TATCTTAAAAGAGCCAATGAACTCGTTCAATGGATTAACGATAAACAAGCATCACTTGAA
TyrLeuLysArgAlaAsnGluLeuValGlnTrpIleAsnAspLysGlnAlaSerLeuGlu
        610                 630                 650
TCACGTGATTTTGGTGATTCCATCGAATCTGTTCAAAGTTTCATGAACGCTCATAAAGAA
SerArgAspPheGlyAspSerIleGluSerValGlnSerPheMetAsnAlaHisLysGlu
        670                 690                 710
TATAAAAAAACCGAAAAACCACCAAAGGGTCAAGAAGTCTCTGAATTGGAAGCTATCTAC
TyrLysLysThrGluLysProProLysGlyGlnGluValSerGluLeuGluAlaIleTyr
        730                 750                 770
AATTCATTACAAACTAAATTACGTTTAATTAAACGTGAACCATTTGTTGCACCAGCTGGT
AsnSerLeuGlnThrLysLeuArgLeuIleLysArgGluProPheValAlaProAlaGly
        790                 810                 830
CTCACTCCAAATGAAATCGATTCACCTTGGTCCGCTTTAGAGAAAGCTGAACAAGAACAT
LeuThrProAsnGluIleAspSerProTrpSerAlaLeuGluLysAlaGluGlnGluHis
        850                 870                 890
GCTGAAGCCCTCCGTATTGAACTCAAACGTCAAAAGAAAATTGCAGTTCTCTTACAAAAA
AlaGluAlaLeuArgIleGluLeuLysArgGlnLysLysIleAlaValLeuLeuGlnLys
        910                 930                 950
TACAATCGTATTCTCAAGAAACTCGAAAACTGGGCCACCACCAAATCTGTCTACCTCGGT
TyrAsnArgIleLeuLysLysLeuGluAsnTrpAlaThrThrLysSerValTyrLeuGly
        970                 990                 1010
TCCAATGAAACCGGTGACAGTATCACTGCTGTTCAAGCTAAATTAAAGAATTTAGAAGCT
SerAsnGluThrGlyAspSerIleThrAlaValGlnAlaLysLeuLysAsnLeuGluAla
      1030                1050                1070
TTTGATGGTGAATGTCAATCATTGGAAGGTCAATCAAACTCTGATCTCCTCAGCATTCTT
PheAspGlyGluCysGlnSerLeuGluGlyGlnSerAsnSerAspLeuLeuSerIleLeu
      1090                1110                1130
GCTCAATTAACTGAACTCAACTACAATGGTGTACCAGAACTCACTGAACGTAAAGATACA
AlaGlnLeuThrGluLeuAsnTyrAsnGlyValProGluLeuThrGluArgLysAspThr
      1150                1170                1190
TTCTTTGCTCAACAATGGACTGGTGTTAAATCATCTGCTGAAACCTACAAAAACACTCTT
PhePheAlaGlnGlnTrpThrGlyValLysSerSerAlaGluThrTyrLysAsnThrLeu
      1210                1230
TTAGCTGAACTTGAAAGACTCCAAAAGACTGAAGACTCATTGG*
LeuAlaGluLeuGluArgLeuGlnLysThrGluAspSerLeu
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Figure 2. Restriction enzyme map of the α-actinin genomic clone and its nucleotide sequence. A restriction map of the 1.2-kb *Eco* RI fragment was established using several restriction endonucleases. Appropriate fragments were subcloned into M13mp18 or M13mp19 and sequenced using the dideoxy chain termination method according to Sanger et al. (36). In *a*, a map of restriction endonuclease sites within the genomic clone and the sequencing strategy are shown. The top line in *b* presents the nucleotide sequence and the bottom line, the deduced amino acid sequence. The tryptic peptides, whose amino acid sequences have been determined, are indicated by boxes.

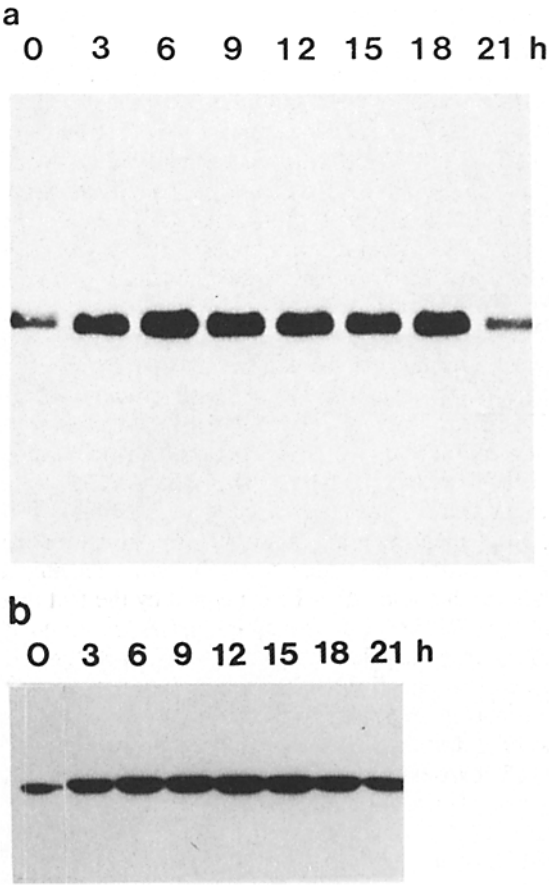

Figure 3. Expression of α-actinin RNA and protein during the development of *D. discoideum* strain AX2. AX2 was grown on *E. coli* B/r, harvested, washed free of bacteria, and developed on filters. The cells started to aggregate between 6 (t6) and 9 (t9) h after deposition on the filters and had reached the tight aggregate stage at t15 and the culmination stage at about t18. Most of the aggregates were in the late culmination stage at t21 and a few had formed fruiting bodies already. At the indicated time points (given in hours after the begin of starvation) cells were harvested and used for RNA isolation. Samples for analysis of the proteins were taken in parallel. Total RNA from the different developmental stages (10 μg per lane) was separated on a 1.2% agarose gel containing 6% formaldehyde, blotted onto nitrocellulose, and hybridized with the 1.2-kb α-actinin-specific probe (*a*). The RNA size (in kilobases) was determined relative to ribosomal RNA. In *b*, an immunoblot of total cellular proteins (20 μg per lane) from cells harvested at the same time as in *a* is shown. After transfer to nitrocellulose the blot was incubated with labeled α-actinin-specific monoclonal antibody 47-19-2.

pair (bp) fragment. This stretch of DNA contains one uninterrupted open reading frame coding for 413 amino acids.

Protein Sequencing. Because only one out of a series of different monoclonal antibodies recognized the polypeptide encoded by the 1.2-kb fragment, we could not rule out the isolation of a "false positive" phage. To prove that the cloned fragment is part of the α-actinin gene, we purified the protein from *D. discoideum* strain AX2, cleaved the protein with trypsin, separated the resulting peptides by high performance liquid chromatography, and determined the amino acid sequences of several peptides. The boxes in Fig. 2 *b* show the localization of six tryptic peptides whose primary structures matched completely the corresponding amino acid sequences derived from the nucleotide sequence. These matches at different positions of the nucleotide sequence proved unequivocally that the 1.2-kb DNA fragment is part of the *D. discoideum* α-actinin gene.

Quantitative Changes of α-Actinin and Its Message during Development

D. discoideum undergoes a developmental cycle in which, upon starvation, the amebas start to aggregate and form fruiting bodies. *D. discoideum* strain AX2 was harvested at various time points during development, RNA was isolated, and equal amounts per time point separated on a denaturing gel. Hybridization of the 1.2-kb fragment to the RNA showed an increase in the amount of α-actinin RNA during the first 6 h of development. The RNA level remained nearly the same for several hours, but at later stages of development the amount of α-actinin-specific message dropped (Fig. 3 *a*). Samples were taken in parallel for analysis of α-actinin on the protein level. They showed a similar rise and fall in the amount of α-actinin protein present in the cells (Fig. 3 *b*).

α-Actinins of Different Wild-type Strains Show Polymorphisms on Both the Protein and DNA Level

While probing a Western blot that contained cell lysates of different *D. discoideum* wild isolates, we noted that out of 25

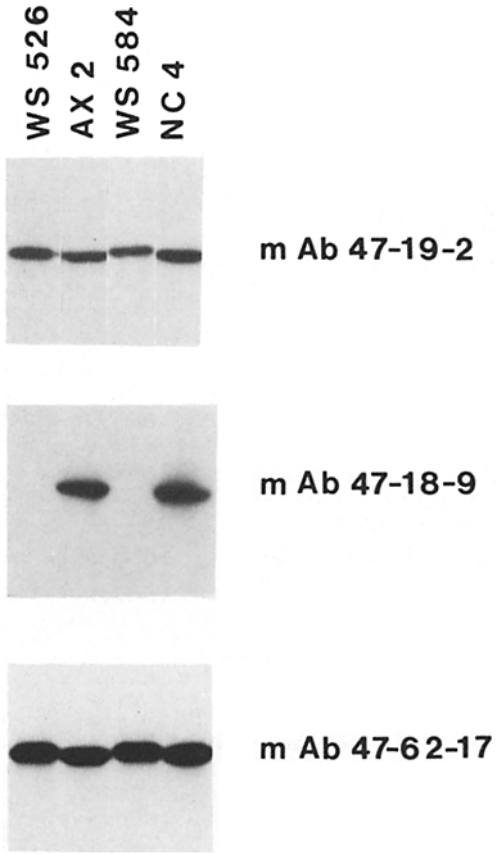

Figure 4. Immunoblots of total cellular protein from *D. discoideum* strains. Equal amounts of protein from growth phase cells of strains WS526, AX2, WS584, and NC4 (the parent strain of AX2) were separated in a 10% SDS polyacrylamide gel and transferred to nitrocellulose. The blots were incubated with different monoclonal antibodies directed against the α-actinin of strain AX2. No protein was detected by mAb 47-18-9 in either WS526 or WS584.

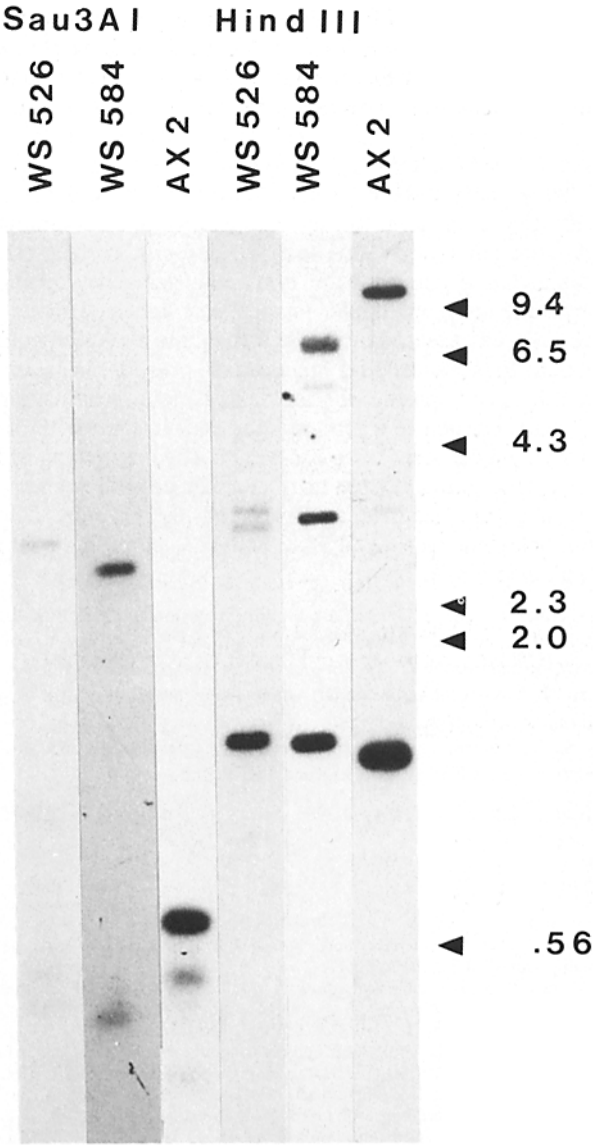

Figure 5. Restriction enzyme polymorphisms in strains WS526 and WS584 detected with the α-actinin gene probe. Total nuclear DNA was isolated from strains AX2, WS526, and WS584, purified in a CsCl-ethidium bromide gradient, and digested with *Hind*III or *Sau* 3AI. The DNA fragments (10 μg) were separated in a 1% agarose gel, transferred to nitrocellulose, and hybridized with the nick-translated cloned 1.2-kb fragment. The sizes (in kilobases) of the *Hind*III-generated lambda DNA fragments as molecular weight markers are indicated at the right.

strains, the two strains WS526 and WS584 showed binding of the α-actinin-specific monoclonal antibody 47-19-2 to a protein that was slightly larger in size than the α-actinin of strain AX2 and the 23 other wild isolates. The monoclonal antibody 47-62-17 also recognized this protein, whereas mAb 47-18-9 did not react with any protein in strains WS526 and WS584 (Fig. 4). This result indicated that the α-actinin molecules of strains WS526 and WS584 are altered in size compared with the α-actinin of strain AX2 and, in addition, lack the epitope that was recognized by mAb 47-18-9. The observed differences on the protein level were accompanied by differences on the DNA level. Southern blots containing different restriction enzyme digests of nuclear DNA from strains WS584, WS526, and, for comparison, AX2, were probed with the 1.2-kb *Eco*RI-fragment. A different hybridization pattern was observed with *Hind*III-cut DNA, where the α-actinin-specific probe recognized two fragments of ~10 and 1.35 kb in AX2 DNA, whereas in similarly digested WS526 and WS584 DNA, a fragment of 1.4 kb was seen. Instead of the 10-kb fragment two new bands appeared. The polymorphism was even more pronounced with *Sau* 3A I-digested DNA, yielding fragments of differing sizes (Fig. 5). No difference was seen when the size of the α-actinin-specific mRNA of WS526 and WS584 was compared with that of the AX2 strain. Looking at α-actinin on the transcriptional level, WS526 and WS584 were comparable to strain AX2 and its parent NC4 with regard to the pattern of expression during the development. However, the hybridization signals in blots containing WS526 and WS584 RNA were much weaker than the ones obtained with AX2 and NC4 RNA, although identical amounts of total RNA were loaded and the hybridizations were done in parallel with an identical probe. This result could either be explained by the fact that in WS526 and WS584 less α-actinin-specific message is present or by assuming that the genes in AX2 and the two strains in question had diverged from each other sufficiently that the normal hybridization conditions did not allow the formation of a stable hybrid between the AX2 probe and their RNA. The latter explanation is supported by results that were obtained when the hybridization and the following wash were done at a reduced stringency (hybridization in the presence of 30% formamide instead of 50% formamide) (Fig. 6).

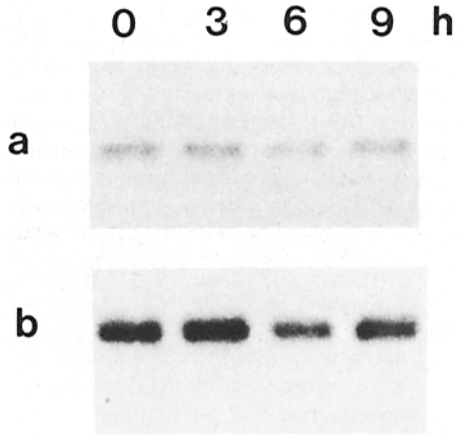

Figure 6. Northern blot analysis of the α-actinin transcript in wild-type strain WS584. Total RNA (10 μg per lane) isolated from several stages of development of strain WS584 was separated on a 1.2% agarose gel in the presence of 6% formaldehyde, transferred to nitrocellulose, and probed with the α-actinin-specific 1.2-kb fragment in the presence of 50% (*a*) or 30% (*b*) formamide. After the hybridization with the probe in the presence of 50% formamide, the counts were melted off and the blot was rehybridized with an identical probe (same specific activity) in the presence of 30% formamide. The salt concentration and the hybridization temperature were kept constant in both experiments. The numbers on top indicate the time (in hours) of harvest of the cells after the beginning of starvation. The size of the RNA was determined relative to ribosomal RNA.

Discussion

α-Actinin is a rod-shaped protein molecule that can cross-link actin filaments (8, 16). The functional molecule is a dimer. Electron microscopic data indicate that the subunits bind to each other in an antiparallel fashion and that the interaction with the actin filaments is confined to the head part only (42). Biochemical data from *D. discoideum*, i.e., the purification of only one protein subunit, suggest that α-actinin is a homo- rather than a heterodimer (8, 16). The data of the Northern and Southern blots indicate the presence of a single gene for α-actinin in the genome of *D. discoideum* and support the presence of a homodimer. The amino acid sequence derived from the cloned fragment suggests a helical structure for this part of the protein. A comparison of the amino acid sequence to a data bank (11) indicates a mild homology with myosin from *Caenorhabditis elegans* (24), another rod-shaped molecule. The homology resides in the tail region of the myosin molecule. This region also has a helical structure and it is conceivable that the formation of a rod-like secondary structure leads to homologies among different, possibly unrelated molecules. Hybridization and sequencing data of the α-actinin-specific fragment did not reveal any intron/exon structures as is often observed with genes coding for cytoskeletal proteins (3). The relatively small number of introns in *Dictyostelium* genes (32) enhances the efficiency of cloning long, uninterrupted reading frames. This fact may have facilitated the isolation of the α-actinin clone from a genomic expression library.

During the development of *D. discoideum* from the amebal stage to the formation of fruiting bodies, different regulatory mechanisms of gene expression take place. The appearance of the contact site A molecule is strictly regulated and the mRNA as well as the corresponding protein are present at a very distinct time during the development (27, 29). In contrast, the gene coding for the lectin-like molecule discoidin is transcribed only at the onset of development, whereas the protein itself is present at high concentrations until the formation of fruiting bodies (33). The expression of α-actinin is clearly distinguishable from these two examples. The message and protein are present throughout development. A slight increase in amount on both the RNA and protein level is consistently found in aggregating cells. However, these changes are too small to consider the expression of the α-actinin gene as being developmentally regulated. The exact function of α-actinin in vivo is not clarified yet (42), but, assuming a certain role in regulation of the cytoskeleton, the constitutive expression of α-actinin during the developmental cycle may be the consequence of these regulatory functions.

Polymorphisms on the DNA as well as on the protein level have been previously described in *D. discoideum* (17, 44). During our studies we detected in two out of 25 different wild-type strains polymorphic α-actinin using monoclonal antibodies. Compared with AX2 and its parent strain NC4, these two strains, WS526 and WS584, contain α-actinin that differs in size and lacks an epitope recognized by one of the monoclonal antibodies used. The missing epitope is not due to a deletion as judged by the apparent molecular weight in SDS PAGE but is rather the result of a different primary structure in some parts of the molecule. Consistent with this interpretation, there exists in WS526 and WS584 a polymorphism on the DNA level as well. The strongly reduced hybridization signals with RNA from these strains suggest that translated sequences of the α-actinin gene have diverged among these strains. Other actin-binding proteins (severin and capping protein) were apparently unchanged in these strains (37; Schleicher, M., unpublished observations).

In preliminary experiments we have used the α-actinin-specific DNA fragment to probe Southern blots containing DNA from other organisms such as *Xenopus*, mouse, rat, and man. Using conditions of fairly low stringency during the hybridization, we could not detect a signal. Although α-actinin is a conserved molecule throughout evolution with respect to its structure and function, this result can be explained by assuming that the molecule functions by having a rod-shaped structure where only the ends interact with the actin filament and need to be conserved. The intervening rod that keeps the actin filaments apart can then be built from any amino acid sequence that can form a rod-like structure. The results obtained with the WS526 and WS584 strains could be explained similarly.

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