cDNA clones coding for α-actinin of *Dictyostelium discoideum*

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cDNA clones coding for *Dictyostelium* α-actinin were isolated from a library in the expression vector λgt11 using a genomic probe that contains α-actinin-specific sequences. The recombinant phages harbored inserts with sizes up to 3.0 kb. They hybridized to a 3.0 kb message in Northern blot analysis and some produced fusion proteins that reacted with monoclonal antibodies directed against different epitopes of *D. discoideum* α-actinin. The sizes of the inserts and the reaction with the monoclonal antibodies indicated that two of the phages carry a nearly complete copy of the α-actinin message.

α-Actinin *Cytoskeleton* (Dictyostelium) *DNA clone*

1. INTRODUCTION

The actin-binding protein α-actinin cross-links actin filaments (review [1]). The molecule is a dimer and has a rod-like structure. α-Actinins have been identified in both muscle and non-muscle cells [2,3]. In *Dictyostelium discoideum* α-actinin consists of two subunits with an apparent molecular mass of 95 kDa [4,5]. It cross-links actin filaments in a Ca^{2+}-dependent manner and thus resembles α-actinin isolated from other non-muscle cells. Previously we have isolated clone λg160 from a genomic library in the expression vector λgt11 [6]. This clone reacted with the α-actinin-specific monoclonal antibody 47-62-17, one of the antibodies used in the screening of the library. It carried an insert of 1.2 kb which recognized a fragment of identical size in *Eco*RI-digested DNA of *D. discoideum* strain AX2 and a 3.0 kb message in a Northern blot. Analysis of the protein sequence derived from the nucleotide sequence of this insert indicated that the cloned DNA was able to code for sequences present in the rod-like tail of α-actinin [6]. Here we present the characterization of α-actinin clones that were isolated from a *D. discoideum* cDNA library using the genomic clone as probe.

2. MATERIALS AND METHODS

2.1. *DNA and RNA isolation from* D. discoideum

DNA was isolated from nuclei of *D. discoideum* strain AX2 after lysing the nuclei in an EDTA-sarcosyl solution (2% sarcosyl, 0.2 M EDTA; pH was adjusted to 8.4 with NaOH) at 65°C [7] and further purified by CsCl-ethidium bromide centrifugation. Total cellular RNA was isolated after lysis of the cells with SDS (1% final concentration) and purified with several phenol-chloroform extractions [8]. For Southern blot analysis DNA was digested with restriction enzymes according to the instructions of the manufacturer (Boehringer, Mannheim), separated on agarose gels in Tris-phosphate buffer, pH 7.8 [9], transferred to nitrocellulose (Schleicher and Schuell, BA85) and hybridized with nick-translated probes in 50% formamide, 2 × SSC, 4 × Denhardt's, 1% sarcosyl, 0.12 M sodium phosphate buffer, pH 6.8, and 0.1% SDS at 37°C for 14–18 h. For Northern blot analysis total RNA (10 μg per lane) was separated

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in 1.2% agarose gels containing 6% formaldehyde [9], transferred to nitrocellulose and hybridized with nick-translated probes as described above.

2.2. *Isolation of cDNA clones*

A cDNA library constructed in λgt11 was kindly provided by Drs R. Kessin and M.-L. Lacombe, Columbia University, NY. For screening with the α-actinin-specific genomic probe the phages were grown on *E. coli* Y1088 [10]. Plaque hybridization, plaque purification and phage DNA isolation were done as described [9]. For production of β-galactosidase fusion proteins the phages were plated out using the *E. coli* host strain Y1090 and grown at 43°C for 3 h. Nitrocellulose filters previously soaked in 10 mM IPTG were laid onto the plates and the plates subsequently transferred to 37°C for an additional period of 10–12 h. The filters were then extensively washed in 10 mM Tris-HCl, pH 8.0, 0.15 M NaCl, 0.05% Tween 20 and incubated with the ^{125}I-labelled antibodies mAb 47-19-2 [11] and 47-62-17 [6] for 2 h.

3. RESULTS

3.1. *Isolation of α-actinin cDNA clones*

Eight phages were isolated when a *D. discoideum* cDNA library was screened with a 1.2 kb fragment that was previously shown to contain α-actinin-specific sequences. These recombinant phages were tested for the production of fusion proteins by labelling with the two monoclonal antibodies, mAb 47-19-2 and mAb 47-62-17. Six phages showed a positive reaction. After testing with each monoclonal antibody separately the hybrid phages could be divided into two groups (table 1). Two of them, λc33 and λc34, reacted with both monoclonal antibodies; λc21, λc24, λc61 and λc62 reacted with mAb 47-62-17 only. This antibody has led to the isolation of genomic clone λg160 [6]. The results obtained with the cDNA clones indicate that the binding site for mAb 47-62-17 on the α-actinin molecule is located closer to the carboxy-terminus than that for mAb 47-19-2. Electron microscopic data provided evidence that mAb 47-19-2 binds to the ends of α-actinin [12]. The recognition site for this end-binding antibody is thus localized in the amino-terminal end of α-actinin. λc33 and λc34, both reacting with mAbs 47-19-2 and 47-62-17, should therefore contain the complete or nearly complete coding region for α-actinin.

Table 1

Characterization of recombinant λgt11 phages by labelling with α-actinin-specific monoclonal antibodies

Hybrid phage	mAb 47-62-17	mAb 47-19-2
λg160	+	−
λc21	+	−
λc24	+	−
λc33	+	+
λc34	+	+
λc61	+	−
λc62	+	−

3.2. *Characterization of the cDNA clones*

DNA from λc33, λc34, λc21 and λc62 was isolated and characterized further. Upon *Eco*RI digestion five fragments with sizes of approx. 1.2, 0.72, 0.5, 0.3 and 0.22 kb were obtained from λc33 and λc34 in addition to the λgt11 *Eco*RI fragments. The total molecular mass of the inserted DNA of these clones is nearly 3.0 kb, which is identical to the size of α-actinin mRNA. All *Eco*RI fragments of λc33 recognized the same 3.0 kb mRNA in a Northern blot when isolated and nick-translated separately. Fig.1 shows the hybridization of the 1.2

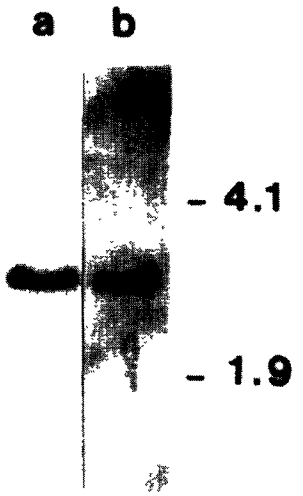

Fig.1. Hybridization of α-actinin-specific cDNA fragments to total cytoplasmic RNA of *D. discoideum* strain AX2. The 1.2 kb (a) and 0.72 kb (b) fragments of λc33 were used as probes. The sizes of the ribosomal RNAs as size markers are given in kb.

and 0.72 kb cDNA fragments to total cytoplasmic RNA of strain AX2. Southern blot analysis with the 1.2 kb fragment of the genomic clone λg160 as probe proved the identity of the 1.2 kb fragments of λc33 and λg160. The incomplete cDNA clone λc21 also harbored this 1.2 kb fragment in addition to two smaller *Eco*RI fragments. In *Eco*RI-digested λc62, which represents the shortest cDNA clone reacting with mAb 47-62-17, a fragment of approx. 0.6 kb hybridized with the 1.2 kb probe. The epitope of mAb 47-62-17 therefore resides in the C-terminal half of the 1.2 kb fragment.

4. DISCUSSION

Several cDNA clones coding for *D. discoideum* α-actinin were isolated from a λgt11 expression library with a genomic DNA probe. All of the described clones reacted with monoclonal antibodies directed against α-actinin. The binding sites of these antibodies on the α-actinin molecule have been shown to be different (Schleicher, M., unpublished). mAb 47-62-17 reacted with all of the fusion proteins produced by the recombinant phages and should therefore recognize an epitope on the α-actinin molecule that is located closer to the carboxy-terminus of the protein than the epitope recognized by mAb 47-19-2. In previous studies [12] the binding site of mAb 47-19-2 to α-actinin was localized to the ends of the α-actinin molecule, whose subunits bind to each other in an antiparallel fashion. Our results with the complete cDNA clones identify this binding site as the amino-terminal end. Considering the blocking function of mAb 47-19-2 [12] we assume that the N-terminal region of the α-actinin subunit is involved in the interaction of α-actinin with F-actin.

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