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Homology Between Actin Coding and Its Adjacent Sequences in Widely Divergent Species

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ABSTRACT Eco RI restriction endonuclease DNA fragments from several representatives of the kingdoms Protista and Animalia were electrophoretically separated and transferred to the nitrocellulose filters. These DNA's were hybridized with [^{32}P]-labelled actin coding sequence from Drosophila melanogaster (Dm). The results indicate that the nucleic acid sequences of the genes coding for actin(s) has been highly conserved throughout evolution. Similar experiments were performed using the sequence derived from the 5' end of Drosophila actin gene as a probe. Cross-hybridization was observed between Drosophila and Acanthamoeba castellanii. This may indicate a functionally important region at the 5' end which has been conserved.

Actin is found in nearly all eukaryotic cells (1,2). Its role in the contractile apparatus of muscles as well as in cell motility, cytokinesis and a number of other processes has been demonstrated (3,1,2). Detection of actin-like protein in Escherichia coli (4) suggests that actin is an ancient molecule having its origin in the prokaryotes. In higher eukaryotes, actin consists of several isoforms (5), which are products of separate genes. In vertebrates at least six different actins exist (6,7), their divergence involving tissue rather than species specificity (6,7). In lower eukaryotes Physarum polycephalum and Dictyostelium discoideum only one isoform has been detected which

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is similar to the mammalian cytoplasmic actins. Analysis of the six actin genes in Drosophila suggests that it synthesizes only the cytoplasmic isoforms (8).

The organization and the number of genes coding for the actins differ extensively among various species, with no apparent direct relationship to the cellular complexity of the organism (9). In organisms where this number exceeds the actual number of variants synthesized, it is not yet clear whether some represent pseudogenes or genes which are used in a selective manner as a function of development and/or tissue specificity.

The high interspecies conservation of actin prompted us to see 1) if the conservation of the molecule at the protein level is reflected at the level of the structural gene(s); and 2) whether some features of the putative control sequences upstream from the 5' end of the gene have also been conserved. Using the Southern blotting hybridization technique (10), we have demonstrated cross-hybridization between Dm actin genes with those of several other highly divergent species. Some cross-hybridization in the sequence adjacent to the 5' end of the Drosophila actin gene was also observed. While this work was in progress, a number of laboratories have independently observed cross-hybridization of the actin genes between certain species (9); none included examination of the 5' adjacent sequences.

MATERIALS AND METHODS

DNA isolation from Drosophila melanogaster pupae, strain Canton S, and from 7 day old chicken embryos was according to Blin and Stafford (11). Calf thymus and salmon sperm DNA were commercially (Sigma) obtained. The remaining DNAs were generous gifts from a number of investigators cited in the Acknowledgements. The Drosophila library used for actin gene screening has been described; a fraction of this library was used in screening with a heterologous actin probe (12). Fig. 1 shows a partial restriction map of the actin containing recombinant phage. The Sal I fragments were subcloned into pBR322 (13). The ^{32}P-labelled probes were synthesized by nick translation (14), using the 0.8 kb Sal I fragment representing the actin coding sequence and the 5.5 kb Sal I fragment including a portion of the intervening sequence, the leader-like sequence and approximately 3

kb of the region upstream from the 5' end of the actin gene. The specific activities obtained were routinely 10^8 cpm/μg. The DNAs from the different species were digested with Eco RI. The transfer of fragments to, and the subsequent hybridizations of the nitrocellulose filters were as described (10,15). The filters were washed and exposed to prefogged Kodak x-ray film at -70°C for 24 hrs to several days using Dupont Lightening Plus Intensifier screens (16). The low stringency hybridization conditions were essentially those of Howley et al., (17). Hybridizations in this procedure were in 30% formamide, 1 M NaCl, 0.01 M Tris (pH 7.4), 10 x Denhardts at 30°-35°C for 48 hrs.

RESULTS AND DISCUSSION

Since the first actin gene obtained from Drosophila melanogaster (Dm) genomic library was isolated by the use of a heterologous actin probe, (chicken actin mRNA), we were interested to see whether similar conservation existed at the nucleic acid level between other divergent species. Thus, DNA from a number of organisms was analyzed. Acanthamoeba castellanii and Tetrahymena pigmentosa, (Protista), represent primitive unicellular eukaryotes. Sea anemonae, chicken, amphibians, salmon and mouse DNAs represent deuterostomes, while Artemia and Drosophila DNA represent the protostomes. These DNAs were digested with Eco Rl restriction endonuclease, which does not have a recognition site within any of the six Dm actin genes (12). Hence, the Eco Rl digestion of this DNA generates 6 bands which give a positive signal to ^{32}P-labelled actin probe and correspond directly to the number of actin genes in Drosophila (Fig. 2A, lane h). Comparison of the pattern and intensity of hybridization between this and

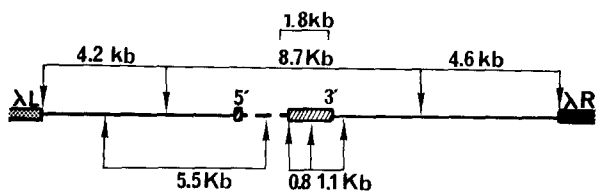

Figure 1 Partial map of λDmA2. The above figure shows restriction endonuclease recognition sites for the enzymes, which were used to generate appropriate fragments for subcloning or direct construction of ^{32}P-labelled probes. The restriction sites shown are: (↑) Eco RI; (↓) Sal I. Several fragments are generated by digestion of DmA2 with Hind III; only the 1.8 kb fragment which contains nearly all of the coding sequence is shown in the figure. Transcriptional orientation of the actin gene is also indicated.

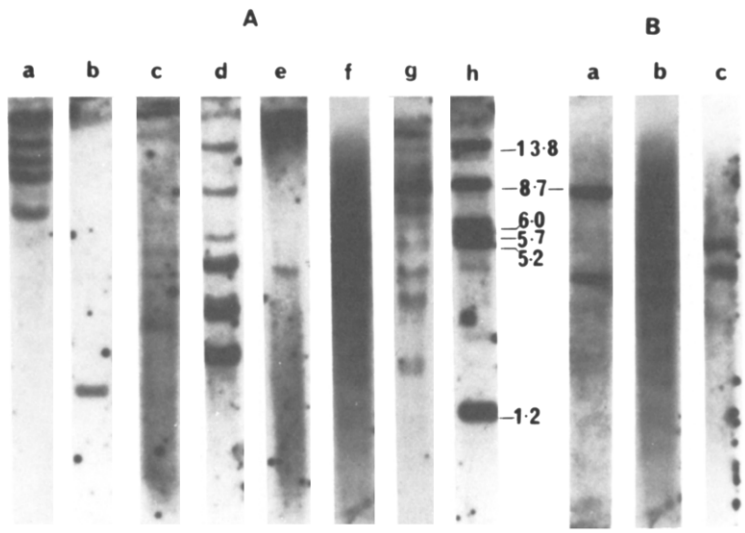

Figure 2 Southern blot analysis of various DNAs with the Drosophila (Dm) actin coding and adjacent sequence. The Eco RI digests of the DNAs used were prepared for hybridization with the ^{32}P-labelled 0.8 kb Sal I fragment as described in Experimental. In Panel A the lanes from left to right indicate the hybridization pattern of this probe with the DNAs from: a) Chicken; b) Tetrahymena pigmentosa; c) Ambystoma maculatum (salamander); d) Metridium dianthus (sea anemonae); e) Notophthalmus viridescence (Notopterus); f) Acanthamoeba castellanii g) Mouse; and h) Drosophila melanogaster. The sizes of the Dm Eco RI fragments, given in kb, which hybridize with actin specific probe are indicated by the numbers on the vertical axis of lane h. The lengths of these fragments were determined using Hind III digested λDNA. Panel B shows a Southern blot analysis of DNAs with the sequence adjacent to the 5' end of Dm actin gene. The experimental conditions were as described for Panel A, except that the 5.5 kb Sal I fragment was used as the probe. The lanes represent hybridization pattern for the DNAs as follows: a) Dm DNA with the 5.5 kb probe; b) Acanthamoeba DNA with 0.8 kb probe; c) Acanthamoeba with the 5.5 kb probe. Fragment lengths were determined as described and are indicated on the vertical axis.

other DNAs may give a rough estimate about the number of actin genes in the other organisms. The results of a Southern hybridization with the actin probe is shown in Fig 2A. In chicken, (lane a), five intense bands ranging in size from about 8-20 kb are observed. The intensities between these bands and those from Drosophila are comparable, thereby suggesting that in chicken the number of bands may reflect the number of genes, assuming that with respect to the Eco Rl sites, the sequence has been conserved. In case of mouse, (lane g), at least 10 bands of varying intensity and ranging in size from about 2-20 kb are

visualized, indicating that there probably exist multiple copies of actin genes. The unequal intensity of several bands suggests the presence of Eco Rl sites in some of the actin genes or pseudogenes. The most consistent pattern obtained with the amphibian DNAs examined was that of Notopterus where a single band of 4.5 kb was routinely observed. In case of salamander,lane c, three bands of 4-6 kb were visualized. Sea anemonae DNA (lane d) shows 5 intense bands of hybridization suggesting multiple copies of actin genes; two additional faint bands may be a result of an Eco Rl site within some of these. In Tetrahymena (lane b), a single band of approximately 1.5 kb is observed. Lastly, Acanthamoeba (lane f) displays 5 bands of hybridization, 3 of which are intense, again suggesting multiple copies of the gene. At least three different actin proteins have been reported for this organism (18). No hybridization was observed between Dm and Artemia or salmon sperm. Current literature suggest that actin genes are highly conserved, hence we were surprised to find weak hybridization between Dm and the amphibian DNAs. However, no additional bands were detected with lower stringency conditions. The visualization of actin bands in salamander and Notopterus genome is, to the best of our knowledge, the first case where genes which are probably present in a few copies have been detected. Since in most eukaryotes, actin genes comprise multigene families (9), it is likely that a similar situation exists in amphibians. Thus, the weak hybridization observed in amphibians may be due to their greater complexity and/or a greater sequence divergence. Our inability to detect stable hybrids between Dm actin probe and either salmon sperm or Artemia DNA may be due to similar reasons. The availability of the appropriate clones and direct sequencing of the actin coding regions would resolve this question.

The above observations prompted us to see if similar conservation existed in putative control regions 5' to the Dm actin gene. Eco Rl fragments of the different DNAs were hybridized in a southern experiment with the ^{32}P-labelled 5.5 kb Sal I fragment (Fig. 1). The results are shown in Fig. 2B. Our inability to obtain a positive response with this probe in all of the DNAs does not necessarily rule out short sequence homologies, which may be too short to form stable hybrids, especially in genomes of greater complexity.

In Drosophila the 5' sequence used in this study also hybridizes to a 4.2 kb non-actin containing fragment. This is due to the fact that the 5.5 kb Sal I fragment contains part of the 4.2 kb Eco Rl fragment (Fig. 1), which is present only once on the Drosophila genome (12). It is unlikely, that this identical sequence would be present in other species. We also feel that the observed cross-hybridization is not due to the intervening sequence portion of the probe, since to date the intervening sequences in the actin genes of different species do not share sequence homologies (9). In Acanthamoeba two out of five actin bands visualized cross-react with the Drosophila 5' end probe thus indicating homology at or near their 5' end. This conservation may reflect regions important in regulation of the expression of these particular actin genes. Direct nucleic acid analyses of these regions between Drosophila and Acanthamoeba would help resolve this interesting observation.

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