Avian cardiac tropomyosin gene produces tissue-specific isoforms through alternative RNA splicing

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Summary

We have isolated a quail cardiac tropomyosin gene which encodes three distinct isoforms through the use of alternative exon splicing. Characterization of cDNA clones produced by this gene indicate that the gene encodes a unique 284 amino acid cardiac tropomyosin isoform, along with a 248 amino acid cytoskeletal and 284 amino acid smooth muscle isoforms. Northern analyses indicate that the gene is primarily expressed in cardiac muscle, with only minor expression of the cytoskeletal and smooth muscle transcripts.

Introduction

Tropomyosins are a closely related family of proteins present in the contractile apparatus of all cells. In striated muscle, dimeric tropomyosin subunits form head-to-tail polymers which occupy the two F-actin grooves of thin filaments. Through its interaction with the troponin complex, tropomyosin serves to regulate contraction by modulating actin-myosin interactions in response to intracellular calcium concentrations. While tropomyosins are also found in smooth muscle and non-muscle tissue, neither cell type contains a troponin complex; therefore tropomyosin must function in another capacity in these tissues. In non-muscle tissue, tropomyosin is found in association with cytoplasmic filaments, suggesting a role in the regulation of cell shape and movement.

Multiple isoforms of tropomyosin are expressed in a tissue specific manner. In vertebrates, fast-twitch skeletal muscle contains alphafast and betafast subunits of 34 and 36 kDa apparent molecular weight, respectively (Cummins & Perry, 1973, 1974). Slow-twitch skeletal muscle contains alphashow and betashow subunits which are identical in apparent molecular weight to their fast muscle counterparts, but different in their isoelectric points (Bronson & Schachat, 1982; Kardami et al., 1983). Cardiac muscle of small mammals contains only the alphafast skeletal isoform (Lewis & Smillie, 1980), while cardiac muscle of larger mammals contain both alphafast and betafast isoforms (Mak et al., 1975). Smooth muscle contains two additional tropomyosin isoforms, beta and gamma, whose apparent molecular weights are 35 and 43 kDa, respectively (Segura & Saborio, 1982; Sanders & Smillie, 1985). In addition to the muscle-specific isoforms, numerous cytoskeletal isoforms have been isolated from a variety of cell lines and tissues, with apparent molecular weights ranging from 34 to 43 kDa (Schloss & Goldman, 1980; Matsumura et al., 1983; Talbot & MacLeod, 1983; Lin et al., 1985).

The diversity of tropomyosin isoforms is generated from at least four separate tropomyosin genes in higher eukaryotes, each of which is known to encode multiple isoforms through the use of alternate promoters and alternative splicing. Genes which encode alphatropomyosin in fast-twitch skeletal muscle also produce smooth muscle isoforms, several 248 and 284 amino acid fibroblast isoforms, as well as three brain isoforms (Ruiz-Opazo et al., 1985; Flach et al., 1986; Pearson-White & Emerson, 1987; Wieczorek et al., 1988; Lindquester et al., 1989; Lees-Miller et al., 1990b). The tropomyosin gene which encodes alphatropomyosin in slow-twitch skeletal muscle also encodes a 248 amino acid non-muscle isoform (MacLeod et al., 1986; Clayton et al., 1988). The betatropomyosin gene encodes skeletal muscle, smooth muscle and fibroblast isoforms (Helfman et al., 1986; Libri...
et al., 1989; Forry-Schaudies, 1990b). A fourth tropomyosin gene produces 248 amino acid cytoskeletal isoforms (Lewis et al., 1983; MacLeod et al., 1987; Lees-Miller et al., 1990a).

Previously we reported the isolation of a quail tropomyosin gene which encodes at least three distinct protein isoforms: a skeletal muscle alpha-tropomyosin, a smooth muscle gamma-tropomyosin, and a 248 amino acid cytoskeletal isoform (Lindquester et al., 1989). While in mammals the skeletal muscle alpha-tropomyosin is also the predominant cardiac isoform, biochemical and immunological studies have shown the avian alpha-skeletal and cardiac isoforms to be closely related, yet distinct (Hayashi et al., 1977; Montarras et al., 1981). This is consistent with our Northern analyses which indicate that the quail alpha-skeletal gene is not expressed in cardiac muscle, and that only under conditions of reduced stringency will the alpha-skeletal gene hybridize to a cardiac muscle tropomyosin transcript. These data can be explained by the presence of a separate cardiac tropomyosin gene in avians with limited homology to the alpha-skeletal gene.

Recently Forry-Schaudies and colleagues (1990a) reported the isolation of cDNA clones encoding a unique chicken cardiac muscle tropomyosin and a 248 amino acid non-muscle isoform which appear to arise from a common gene. An analysis of the derived amino acid sequence reveals that the chicken cardiac tropomyosin is distinct from chicken skeletal muscle alpha- or beta-tropomyosin. Here we report the characterization of a quail cardiac tropomyosin gene which encodes a unique cardiac isoform, as well as smooth muscle and cytoskeletal isoforms, through the use of alternatively spliced exons. The gene is primarily expressed in cardiac muscle, with only minor expression of the cytoskeletal and smooth muscle isoforms. We also discuss the evolution of the cardiac tropomyosin gene as it pertains to the divergence of the avian and mammalian lines.

Materials and methods

Preparation and screening of cDNA and genomic libraries

Lambda gt10 libraries were prepared as described by Huynh and colleagues (1984) using poly A mRNA isolated from either quail breast muscle, heart muscle or gizzard muscle tissue. Genomic libraries were prepared in EMBL3 from a partial EcoR1 digest of quail liver DNA. A second partial EcoR1 library was kindly provided by Charles Emerson, University of Virginia, USA. The libraries were screened as described by Benton and Davis (1977) using nick-translated tropomyosin cDNA probes described previously (Flach et al., 1986; Lindquester et al., 1989). Filters were hybridized in a solution containing 50% formamide, 4 X SET (600 mM, 80 mM Tris pH 7.8, 4 mM EDTA), 0.2% SDS, 0.1% NaPPI and 50 μg ml⁻¹ heparin at 42° C. Lower stringency hybridizations were performed in a solution containing 35% formamide. Final washes were 2 X SET, 0.1% SDS at 42° C.

DNA sequencing

Restriction fragments to be sequenced were recovered from low melting point agarose gels and subcloned into mp18 or mp19. Dideoxy was performed according to Sanger and colleagues (1977). The sequencing of large restriction fragments was facilitated by the generation of a series of overlapping deletions as described by Dale and colleagues (1985). Sequence analysis was facilitated by the use of IBI Sequence Analysis Software (International Biotechnologies, Inc.)

RNA isolation and Northern analysis

Tissue used for RNA extraction was dissected, rinsed in saline and stored at -80° C until used. The frozen tissue was fragmented in a Waring blender with 20 volumes of 20 mM sodium acetate, pH 5.1, 250 mM NaCl, 10 mM EDTA, 1% SDS, 50% phenol and 500 μg ml⁻¹ heparin. The homogenate was centrifuged at 1200 g for 20 min, and the supernatant was extracted twice with equal volumes of phenol and chloroform. Following ethanol precipitation, the RNA was dissolved in TE and precipitated in 2 M LiCl. Northern blot analysis was done as described previously (Flach et al., 1986). Total RNA (4 μg) was electrophoresed on a 1% agarose gel containing formaldehyde. RNA was transferred to nylon membranes and probed with nick-translated restriction fragments. A 1 kb DNA ladder or RNA ladder (both supplied by BRL) were used as size markers.

Results

Isolation of a cardiac-specific cDNA clone

While the mammalian cardiac tropomyosin isoform is identical to the alpha-skeletal isoform, and presumably encoded by a common gene (Mak et al., 1975; Lewis & Smillie, 1980), studies have shown that in avians the two isoforms are biochemically and immunologically distinct (Hayashi et al., 1977; Montarras et al., 1981). Our work on the quail tropomyosin gene family has shown that the alpha-skeletal gene hybridizes to the predominant cardiac tropomyosin transcript only under conditions of reduced stringency. Forry-Schaudies and colleagues recently reported the isolation of a unique chicken cardiac tropomyosin cDNA clone (1990a). These data suggest the existence of a separate cardiac tropomyosin gene similar but not identical to the alpha-skeletal gene.

To isolate a cDNA clone from such a gene, a lambda gt10 cDNA library prepared from quail cardiac muscle mRNA was probed with a previously described alpha-skeletal cDNA clone (Flach et al., 1986) under stringency conditions which allow the probe to hybridize to both skeletal and cardiac muscle transcripts (35% formamide, four times SET, 42° C). A screen of 200 000 recombinant phage identified 58 cDNA clones, 42 of which were putatively identified as alpha-skeletal tropomyosin clones based on the presence of characteristic restriction sites and their strong homology to the alpha-skeletal probe. Of the remaining 16 clones, cDNA clone C9 was found to contain the largest insert (1.1 kb), and was therefore selected for further examination. The cDNA was subcloned into M13 and sequenced in its entirety utilizing