Cloning of the cDNA encoding rat myosin heavy chain-A and evidence for the absence of myosin heavy chain-B in cultured rat mast (RBL-2H3) cells

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Summary

The complete amino acid sequence (1961 amino acids) of a vertebrate cellular myosin heavy chain-A was deduced from cDNA clones of a secretory rat mast cell line, the RBL-2H3 cell. The rat, human and chicken cellular myosin heavy chain-A exhibited high similarity in domains that allow binding of ATP and actin. The amino acid sequence of non-muscle myosin heavy chain-A from rat was 96% identical to that in human and 92% identical to that in chicken. Northern blot analysis of mRNA indicated the presence of single message of 7.4 kilobases. Northern blot, reverse-transcriptase polymerase chain reaction, and Western blot with isoform-specific antibodies indicated that RBL-2H3 cells expressed exclusively myosin heavy chain-A. Unlike rat PC12 cells, as well as a wide variety of other cultured cells and tissues, myosin heavy chain-B mRNA and protein were not detectable in RBL-2H3 cells. Because RBL-2H3 cells can be stimulated to release secretory granules as well as newly generated arachidonic acid and cytokines but lack myosin heavy chain-B, this cell line may provide a unique model to study the role of myosin heavy chain-A in cellular responses to antigen and other stimulants.

Introduction

Myosin is a major cytoskeletal protein in all eukaryotic cells. In skeletal, cardiac and smooth muscle cells, the association of myosin and actin filaments permits ATP-dependent contraction. In addition to these sarcomeric muscle myosins, all types of vertebrate cells, including muscle cells, contain other forms of myosin referred to as nonmuscle or cellular myosins (Spudich, 1989; Sellers & Goodson, 1995). All of the functions of nonmuscle myosins are still to be determined, but to date they have been shown to participate in cytokinesis, cell motility, cell shape change (Spudich, 1989), exocytosis of secretory granules (Ludowyke et al., 1989; Choi et al., 1994) and capping (Kerrick & Bourguignon, 1984; Pasternak et al., 1989).

The myosin II molecules are composed of a pair of heavy chains of ~200 kDa and two pairs of light chains of ~15-28 kDa (Spudich, 1989; Hammer, 1991; Sellers & Goodson, 1995). Each myosin heavy chain (MHC) contains two characteristic regions, a globular head-like domain that has ATPase activity and an actin-binding site as well as an intermediate region that forms a double-stranded α-helical coiled-coil when MHCs dimerize. The carboxy-terminal region of about 20 amino acids is thought to form a non-helical tail-like domain.

To date, at least two genetically different isoforms of nonmuscle myosin II heavy chain have been identified in vertebrate cells and referred to as MHC-A and MHC-B. The entire cDNA sequence for...
chicken intestinal epithelial-cell MHC-A (Shohet et al., 1989), human MHC-A (Saez et al., 1990; Simons et al., 1991; Tothaker et al., 1991), chicken brain MHC-B (Takahashi et al., 1992) and human lymphocyte MHC-B (Simons et al., 1991; Phillips et al., 1995) has been determined. Chicken MHC-A and MHC-B have been shown to be the products of two different genes (Katsuragawa et al., 1989; Kawamoto & Adelstein, 1991), and two genes encoding the human nonmuscle MHC isoforms have been localized to different chromosomes (Simons et al., 1991).

A rat mast cell line, the RBL-2H3 cell (Metzger et al., 1986), is widely used as an experimental model for studying antigen-induced signals for release of secretory granules and other stimulatory responses such as the generation of arachidonic acid and cytokines (Beaven & Metzger, 1993). These cells are physiologically triggered through aggregation of membrane receptors for immunoglobulin E (IgE) by multivalent binding of antigen to receptor-bound IgE (Metzger, 1992; Beaven & Metzger, 1993). The aggregation results in rapid Ca\(^{2+}\)-independent tyrosine-phosphorylation of phospholipase C-\(\gamma\)1 (Park et al., 1991) and other proteins (Benhamou & Siragian, 1992; Benhamou et al., 1992; Eiseman & Bolen, 1992), hydrolysis of inositol phospholipids (Park et al., 1991) and, in turn, mobilization of Ca\(^{2+}\) (Beaven & Cunha-Melo, 1988), activation of protein kinase C (Ozawa et al., 1993), and activation of Ca\(^{2+}\)-dependent kinases (Choi et al., 1994). Our previous work has shown that these events are associated with phosphorylation of myosin heavy and light chains; the extent and time course of these phosphorylations correlate with those of release of secretory granules (Ludowyke et al., 1989; Choi et al., 1994).

As an essential step to answer the question of whether cellular myosin is directly involved in the process of secretion in mast cells, we have now isolated and sequenced cDNA clones that encode nonmuscle MHC-A using an mRNA from rat RBL-2H3 cells. The information obtained above enabled the preparation of isoform-specific antibodies against MHC-A and MHC-B and the demonstration that RBL-2H3 cells expressed exclusively MHC-A. Unlike rat PC12 cells, as well as a large number of other cultured cells and tissues, MHC-B mRNA and MHC-B protein were undetectable in RBL-2H3 cells.

**Materials and methods**

**Cell culture**

RBL-2H3 cells were maintained in monolayer culture and grown in Eagle’s minimum essential medium with Earle’s balanced salt solution supplemented with 15% heat-inactivated foetal bovine serum, 100 units ml\(^{-1}\) penicillin and 100 \(\mu\)g ml\(^{-1}\) streptomycin (Gibco Laboratories, Grand Island, NY) in 5% CO\(_2\):95% air at 37°C (Ludowyke et al., 1989). For the purpose of comparison, RBL-2H3 (ml) cells, a stably transfected cell line that contained the gene for the muscarinic ml receptor and that secretes in response to carbachol (Choi et al., 1993), were maintained as described above and used for comparison with parental RBL-2H3 cells. Also, rat pheochromocytoma PC12 cells, a gift from Dr Mari Oshima (National Institute of Child Health and Disease, National Institutes of Health), were grown as monolayers in DMEM supplemented with 7% foetal bovine serum, 7% horse serum, 100 units ml\(^{-1}\) penicillin and 100 \(\mu\)g ml\(^{-1}\) streptomycin (Gibco Laboratories) in 7% CO\(_2\):95% air at 37°C (Oshima et al., 1991).

**RNA isolation and reverse transcriptase–polymerase chain reaction**

Poly (A\(^+\)) RNA was isolated from RBL-2H3 cells by use of a kit (Stratagene, Cambridge, MA). First strand cDNA was synthesized with the cDNA-cycle kit (Invitrogen, San Diego, CA) according to manufacturer’s instructions in 20 \(\mu\)l of reaction mixture that contained 0.5 \(\mu\)g mRNA, 1 \(\mu\)g of random hexamer primer, and 0.2 \(\mu\)g oligo dT primers. Primers were designed from 14 sites of similar sequence (Fig. 1A) in human and chicken cDNA (Shohet et al., 1989). Reverse transcriptase–polymerase chain reaction products were analysed by 0.8% agarose gel electrophoresis. Seven PCR products were isolated and subcloned into the pcRII TA cloning vector (Invitrogen, San Diego, CA). DNA sequences of the cloned PCR products were determined by dideoxynucleotide chain termination using Sequenase Version 2.0 (US Biochemical Corp., Cleveland, OH) and analysed by macDNASIS software.

**Reverse transcriptase–polymerase chain reaction for detection of MHC-B mRNA**

Reagents for the detection of MHC-B mRNA, cDNA and protein in the present studies were based on the unpublished sequence of rat cDNA as deposited in GeneBank (see K. Itoh, Nos U15665 and U15666). Poly (A\(^+\)) RNA and total RNA were isolated from RBL-2H3 cells and PC12 cells, respectively. Primers for PCR-amplification of MHC-B cDNA were 205S/5'-TGGTGTGCTGGCCCTCCTGGA-3' (nt 1587-1608) and 704A/5'-GCTGTCTCAGTCATGCCAGT-3' (nt 1894-1913).

**Northern blot analysis**

For Northern blot analysis of MHC-A and MHC-B, poly (A\(^+\)) RNA (2 \(\mu\)g) was isolated from RBL-2H3 cells and total RNA (12 \(\mu\)g) was isolated from PC12 cells as a control. The RNAs were separated on 1% agarose gels that contained 6% formaldehyde, transferred to nylon membranes (Dupont/NEN, Boston, MA) by capillary transfer and crosslinked by UV irradiation. For hybridization, the filters were incubated for 1 h at 42°C in hybridization buffer I that contained 50% deionized formamide (Oncor, Gaithersburg, MD) and then with either probe MHC-A (a mixture of cDNA fragments, F2, F4 and F6 as shown in Fig. 1) or probe MHC-B (846 bp rat cDNA nt 616-1661) for 16 h at 48°C. The probes were labelled with \(\alpha\)-\[^{32}\text{P}\]-dCTP (3000 Ci mmol\(^{-1}\)) by use of the megalabelling kit (Amer sham, Cleveland, OH). The filters were washed twice.