J. Anat. (1996) 189, pp. 451–456, with 3 figures Printed in Great Britain

Short Report

Chicken myosin IB mRNA is highly expressed in lymphoid tissues

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(*Accepted 18 June 1996*)

ABSTRACT

Little is known about the functions of members of the myosin I family in vertebrates. Chicken myosin IB is a member of the amoeba-type subclass of myosin I molecules and tissue localisation studies may provide possible clues to the functions of these myosin I molecules. The expression of the mRNA of this unconventional myosin IB was analysed by in situ hybridization and compared with that of the well characterised brush border myosin I on frozen sections of tissues from the adult domestic chicken. High levels of myosin IB mRNA were found in the intestine and spleen, but were not found in other tissues examined such as brain, heart, lung, liver and kidney. In the intestine, myosin IB mRNA was much more abundant in the lamina propria than in the enterocytes, whereas brush border myosin I mRNA was restricted to the enterocytes. In the spleen, myosin IB mRNA expression was abundant in regions of white pulp, namely germinal centres, periellipsoid lymphocyte sheaths and periarteriolar lymphocyte sheaths. Lymphocytes are the major cell type in both the lamina propria and the white pulp of the spleen, which suggests that chicken myosin IB is highly expressed in lymphocytes. Lymphocyte recirculation depends on their migration through the endothelial layer and it is possible that myosin IB may have a role to play in this type of cell motility.

Key words: Myosin I; chicken; in situ hybridization; lamina propria; spleen.

INTRODUCTION

Many new myosin cDNA sequences have been discovered over the past few years. These myosin genes have been placed into families numbered I to IX (Cheney et al. 1993; Bement et al. 1994*a*). Many of these myosin genes are expressed in a single cell line. There are at least 11 myosins present in the human intestinal epithelial line Caco-2 (Bement et al. 1994*a*). In a single cell type, different myosin proteins have distinct but overlapping intracellular distributions (Heintzelman et al. 1994).

Myosin I proteins have a single motor domain (or head region) and a variable number of light-chain binding sites (IQ motifs). Out of all the vertebrate myosin I proteins examined so far, only calmodulin has been found bound to the light-chain binding sites. Myosin I genes have been divided into 4 subclasses based on the homologies of their motor domains (Bähler et al. 1994; Morgan et al. 1994): first is the brush border type with 3–6 IQ motifs; the second subclass is typified by bovine adrenal myosin I with 3–6 IQ motifs; the third by rat myr4 with 2 IQ motifs; and fourth is the amoeba type with 1–2 IQ motifs and a COOH-terminal SH3 domain.

The novel myosin, chicken myosin IB, was identified from a screen of a chick intestinal cDNA library for clones containing a myosin consensus sequence using a PCR based approach (Knight, 1994). It belongs to the amoeba subclass. We have investigated

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its anatomical distribution by in situ hybridization, and have found that in contrast to other myosin I genes it is more abundant in lymphoid tissues than in the other tissue types examined.

MATERIALS AND METHODS

In situ hybridization was carried out essentially as previously described (Snow et al. 1992). Adult chickens were reduced by lethal intraperitoneal injection of pentobarbitone. Organs were rapidly removed. Tissue samples were frozen in OCT embedding compound in a dry ice/70% ethanol mix. Frozen 10 μm sections were cut, fixed in 4% paraformaldehyde, treated with 0.25% acetic anhydride in 100 mM triethanolamine-HCl pH 8.0, 0.9% NaCl, dehydrated in ethanol, defatted in chloroform and dried.

Deoxyoligonucleotide probes complementary to the appropriate mRNA were synthesised by the ABC Unit (Charing Cross Medical School, London). The sequences of the probes were: chicken brush border myosin I TGC ACC TGA TGT AGT TGG GGT TCT TGG AGT bases 1610–1639 (Garcia et al. 1989); chicken myosin IB GCG GAT GTA GTG TGG CGT GCA CTT CAT TAA bases 1767–1796 (EMBL accession number X70400, Knight & Kendrick-Jones, unpublished; Knight, 1994). Each of these probes was complementary to no other sequence in the EMBL database.

Each probe was 3′end-labelled with alpha^{35}-SdATP using terminal deoxynucleotidyl transferase. Labelled probe was separated from unincorporated label by NENSORB purification cartridges (Du Pont). Incorporation of radioactive label was about 75%, which would be expected to produce a poly-A tail of 4 nucleotides in length. Hybridization was carried out in 10% dextran sulphate, 4 × SSC, 1 × Denhardt's solution, 1 mM EDTA pH 7.5, 100 μg/ml denatured salmon sperm DNA, 100 μg/ml yeast transfer RNA, 100 μg/ml poly A, 20 mM $NaPO_4$ pH 7.0, 50% deionised formamide, 100 mM DTT. 100 μl of hybridization fluid containing 1,000,000 disintegrations per minute of each probe was added to each section. Hybridization was carried out for 18 h at 28 °C. Stringency washes were in 1 × SSC for 1 h at 52 °C. Sections were exposed to film (Amersham Hyperfilm-beta max) or dipped in Ilford K5 emulsion and counterstained with toluidine blue.

In control experiments, specific hybridization was abolished by adding unlabelled probe at 50 fold excess over labelled probe. No specific hybridization was observed when sections were treated with ribonuclease or with a 30 base probe of random degenerate sequence. As an additional control a probe with identical base composition but specific for chicken myosin V (Espreafico et al. 1992; Sanders et al. 1992) was also used. This probe produced a weak signal relative to that produced by myosin IB. Chicken myosin V mRNA was found at a similar level of abundance in the enterocytes and lamina propria of the intestine. Chicken myosin V mRNA expression was uniform throughout the spleen (Edgar et al. 1996).

RESULTS

We looked initially for the expression of myosin IB mRNA in the intestine because the cDNA clone had been isolated from a chicken intestinal library. In the chicken ileum myosin IB mRNA was most abundant in the lamina propria and lymph nodules, with weaker staining in the enterocytes. Staining was virtually absent in the muscularis mucosae (Fig. 1*a*, *b*). In contrast, brush border myosin I mRNA appeared confined to the enterocytes where it was found to be very abundant (Fig. 1*c*, *d*). This agrees with previous studies showing the localisation of the brush border myosin I protein to the brush border of the enterocytes (Coudrier et al. 1981; Glenney et al. 1982; Bikle et al. 1991; Heintzelman et al. 1994). Figure 1 illustrates the ileum but similar patterns of expression for both myosin I genes were found in the duodenum and jejunum.

Examination of other tissues showed that high levels of myosin IB mRNA were present in the chicken spleen (Figs 2, 3). No specific staining was found in the brain, heart, skeletal muscle, pancreas, lung, liver and kidney.

The expression of myosin IB mRNA in the spleen was not evenly distributed (Fig. 2*a*). It was associated with germinal centres, the periarteriolar lymphocyte sheaths (Fig. 2*b*) and the periellipsoid lymphocyte sheaths that surround the ellipsoid capillaries (Fig. 3). The germinal centres and periellipsoid lymphocyte sheaths contain B lymphocytes and the periarteriolar lymphocyte sheath contains T lymphocytes (White, 1981; Jeurissen, 1991). No hybridization above background levels was observed in red pulp areas and the muscular walls of the artery/arterioles. Brush border myosin I mRNA was absent from the spleen. The findings that myosin IB mRNA is expressed in regions of lymphoid tissue associated with the small intestine and in lymphocyte rich regions of the spleen suggest that myosin IB is abundant in lymphocytes.

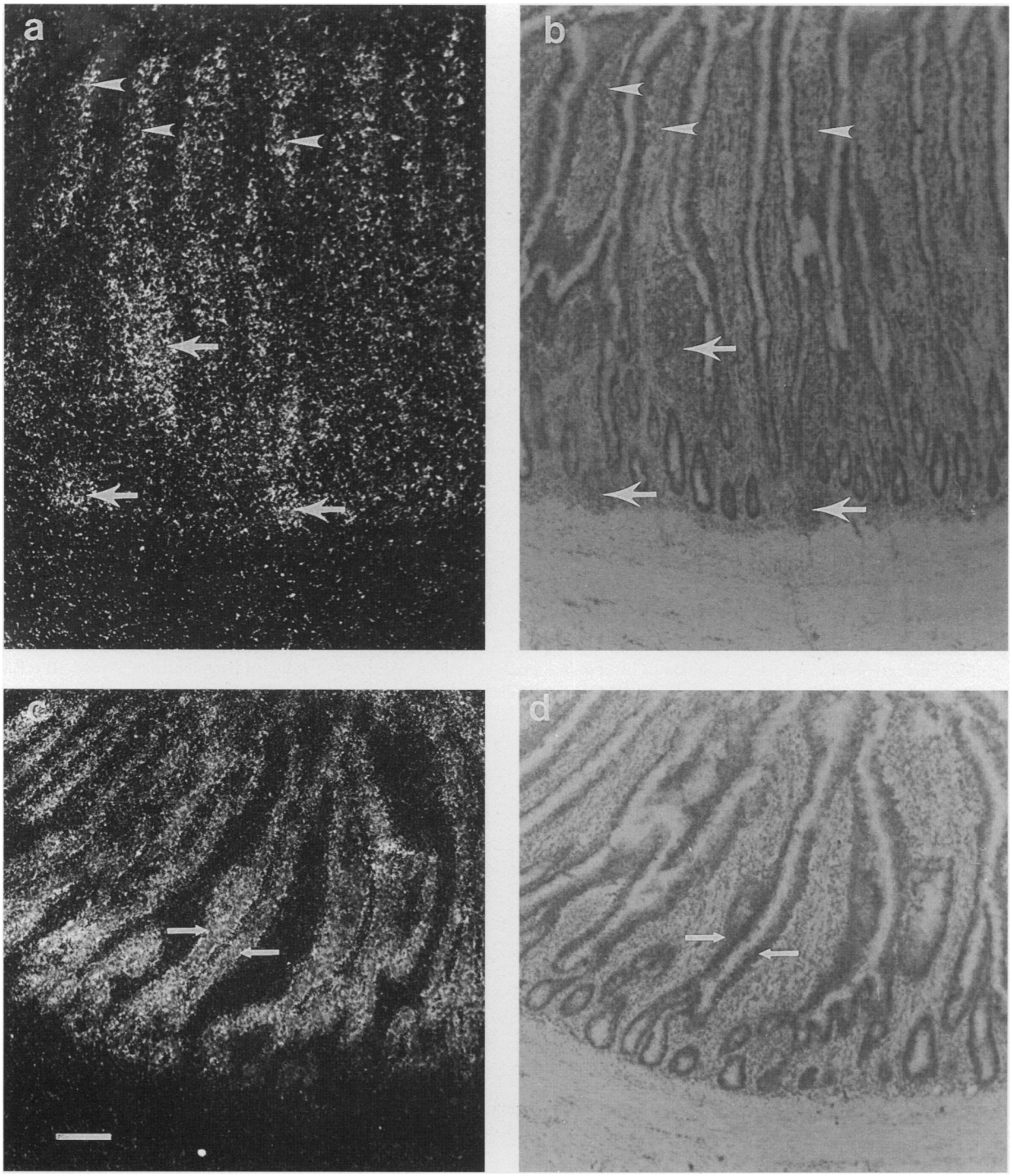

Fig. 1. The distribution of myosin IB and brush border myosin I mRNA in the ileum of an adult chicken. (*a*) was probed for myosin IB and (*c*) for brush border myosin I. (*a*) and (*c*) are darkfield illuminated showing the silver grains from autoradiography and (*b*) and (*d*) are the same fields, counterstained with toluidine blue viewed under brightfield illumination. Myosin IB is highly expressed in lymph nodules (large arrows) and in the lamina propria (arrowheads). Brush border myosin I is located in the enterocytes (small arrows). Neither myosin shows expression in the muscularis mucosae. Bar, 0.1 mm.

DISCUSSION

The discovery of many myosin I genes indicates that their expression is regulated in a tissue specific manner and that each myosin I has a unique role to play in intracellular transport and cell motility. Unlike many myosin I molecules which have multiple light-chain binding sites, the amoeba subclass of myosin I molecules have only one or two IQ motifs. Probably these IQ motifs are the calmodulin light-chain binding

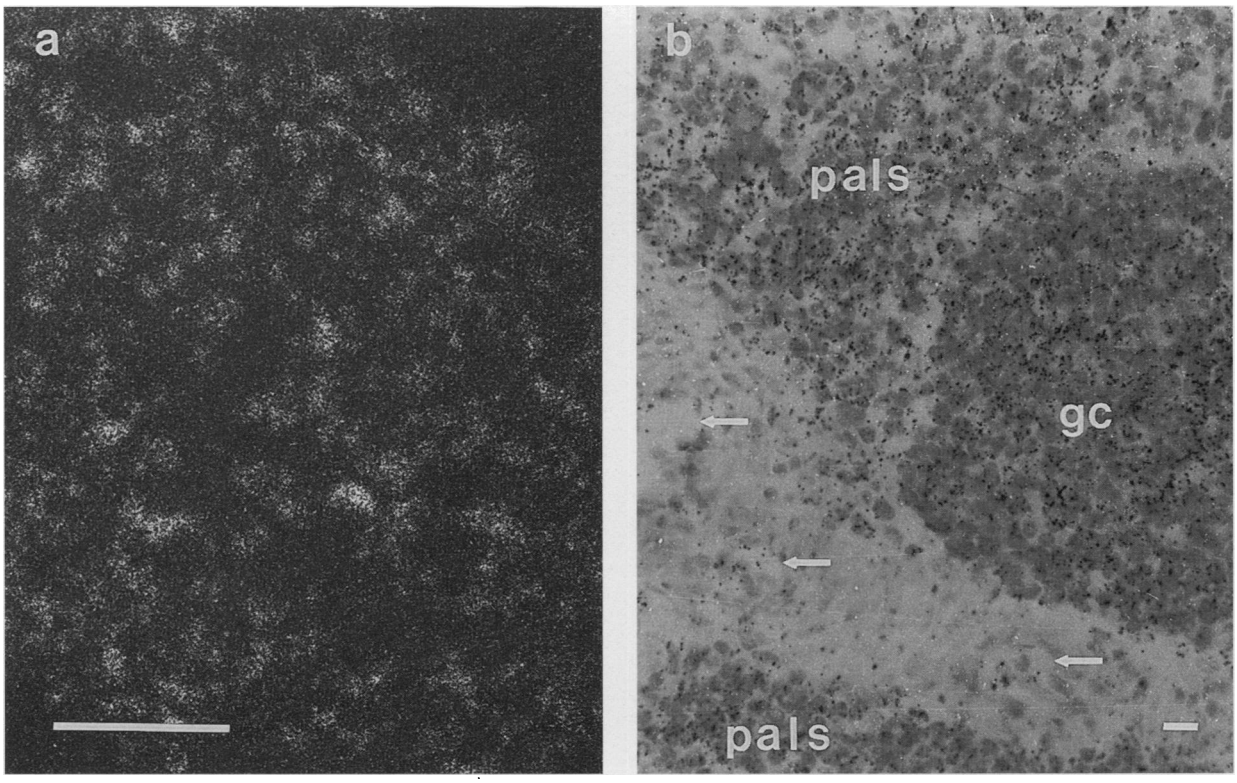

Fig. 2. Distribution of myosin IB mRNA in the spleen of an adult chicken. (*a*) Distribution at low power on Hyperfilm. Bar, 1 mm. (*b*) Brightfield micrograph showing the distribution of silver grains which are visible when viewed at higher magnification. Myosin IB mRNA expression is abundant in the germinal centres (gc) and the periarteriolar lymphocyte sheaths (pals) but is absent in the connective tissue and muscular walls surrounding the arterioles (arrows). Bar, 0.01 mm.

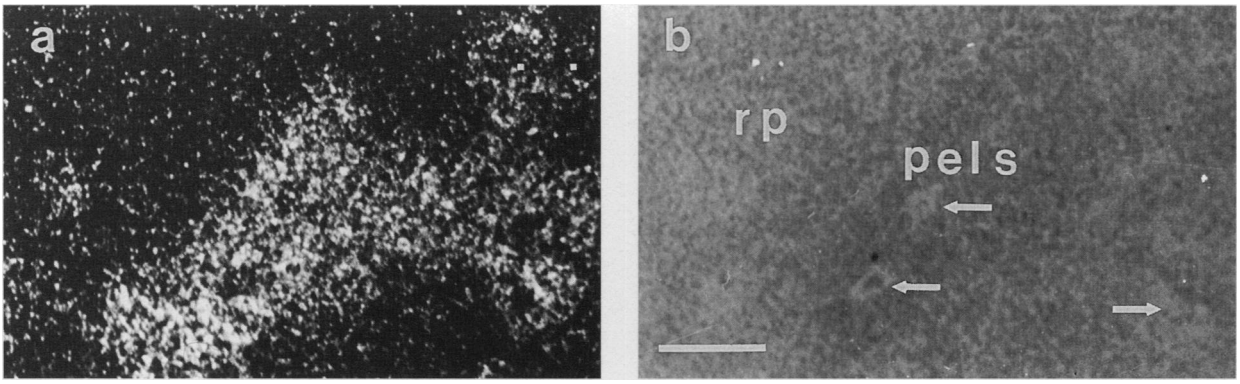

Fig. 3. The distribution of myosin IB mRNA in the spleen of an adult chicken. (*a*) and (*b*) are the same fields viewed under darkfield and brightfield illumination respectively. Myosin IB is abundant in the periellipsoid lymphocyte sheath (pels) but not in the red pulp (rp). The capillaries are indicated with arrows. Bar, 0.1 mm.

sites. The other novel feature is the presence of a Src homology 3 (SH3) domain at the COOH-terminal region. This domain is thought to be a region of protein-protein interaction. The SH3 domain is found in many proteins that bind actin microfilaments or are involved in signalling pathways (Pawson & Schlessinger, 1992).

It is probable that chicken myosin IB is the chicken homologue of human myosin ID, having 99% identity over the 90 residues available from the partial cDNA (Bement et al. 1994*a*). It is also related to human myosin IC, 72% identity (Bement et al. 1994*b*) and its rat homologue myr3, 73% identity (Stoffler et al. 1995). Tissue distribution of human myosin ID is unknown but the partial human myosin ID cDNA sequence was isolated from a liver cDNA library (Bement et al. 1994*a*). Human myosin IC mRNA expression has been found in the intestinal epithelial cell line Caco-2 and in peripheral blood leucocytes (Bement et al. 1994*a*) and in a wide range of tissues

including intestine, spleen, kidney, prostate, testis, liver and ovary (Bement et al. 1994*b*). Similarly, rat myr3 is also expressed in the spleen and small intestine and is widely distributed in other tissues (Stoffler et al. 1995). Rat myr3 protein is found in the cytoplasm of normal rat kidney cells, being concentrated in elongated structures at regions of cell–cell contact (Stoffler et al. 1995). Con-A treatment of the cells induces an increase in the number and intensity of the myr3 staining at regions of cell–cell contact. These regions contain F-actin and alpha-actinin but lack vinculin. Rat myr3 may be regulated via cell surface signalling pathways and play a role in the formation of cell–cell contacts.

The subclass of myosin I proteins of which chicken myosin IB is a member, is quite ancient because several myosin I genes from *Dictyostelium* and *Acanthamoeba* have a single IQ motif that acts as a light chain-binding site and a COOH-terminal SH3 domain (Jung et al. 1989*a*, *b*). In protozoans, phosphorylated myosin I is active. Phosphorylated myosin IB is concentrated in motile areas of the plasma membrane such as pseudopod and filopod formation and at phagocytic cups and pinocytotic invagination. It is also associated with the membranes of the digestive vacuoles (Baines et al. 1992, 1995).

Of the other myosin I subclasses, brush border myosin I, has limited tissue expression in vertebrates, being found only in intestinal epithelial cells (Bikle et al. 1991; Kawakami et al. 1992). Both bovine and chicken brush border myosin I cDNAs have been cloned (Hoshimaru & Nakanishi, 1987; Garcia et al. 1989). Brush border myosin I is mainly present in the microvilli where it plays a structural role, forming lateral crossbridges, linking the microfilaments to the plasma membrane, in effect zipping the membrane around the microvillus core (Tilney & Mooseker, 1976). However, a number of genes related to brush border myosin I, which includes mouse myosin I alpha (Sherr et al. 1993), mouse MHCI-L (Koslovsky et al. 1993) and the rat myr1 (Ruppert et al. 1993), have widespread tissue distribution, being expressed most strongly in adult lung and embryonic brain but relatively low expression in the spleen. Most other myosin I subclasses have a widespread tissue distribution. Myosin I genes, which belong to the subclass containing rat myr2 (Ruppert et al. 1995), its bovine homologue, adrenal myosin IB (Wagner et al. 1992, Reizes et al. 1994) and a very closely related myosin I (Zhu & Ikebe, 1994), are widely distributed in various tissues. Bovine adrenal myosin IB is enriched in spleen, oesophagus, heart, lung, adrenal gland and stomach but is of low abundance in brain, kidney and small intestine. Rat myr4 belongs to another subclass. Again it has a widespread tissue distribution, being most highly expressed in adult brain tissue (Bähler et al. 1994).

The identity of the cell types found to have a high expression of chicken myosin IB in the present study is not proven, but they are most likely to be lymphocytes. Their identities will become clearer with the development of specific antibodies against myosin IB used in conjunction with cell type markers. At present, the function of chicken myosin IB is unknown but it may play a role in the secretory, phagocytic, and tissue infiltrating activities of lymphocytes. Lymphocyte recirculation depends on their migration through the endothelial layer and it is possible that myosin IB may have a role to play in this type of cell motility.

ACKNOWLEDGEMENT

This work was supported by a grant from the Medical Research Council.

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