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MYOSIN FUNCTION IN THE MOTILE BEHAVIOUR OF CELLS

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Summary

Cells undergo a wide variety of movements, such as directed locomotion, extension and retraction of cell surface projections, saltatory movement of intracellular particles and cytoplasmic streaming. These events involve changes in the organization and function of cytoskeletal structures that contain actin and myosin. *Dictyostelium* has proven to be a very useful model system for studying these events. Its actomyosin-based motility resembles that of mammalian cells and has been extensively characterized, from the standpoints both of biochemistry and cell biology. Furthermore, the *Dictyostelium* cytoskeleton can be specifically altered using gene-targeting and other molecular genetic approaches.

Introduction

Myosins are complex multidomain proteins that interact with actin filaments and adenosine triphosphate (ATP) to produce mechanical force and displacement. All myosins contain a globular head domain (approx. 90×10^3 M_r) that contains the binding sites for the myosin light chains and has the catalytic and actin binding properties of the myosin molecule. It has been shown that this globular head fragment, which is also referred to as Subfragment 1 or S-1, is sufficient to cause sliding movement of filaments *in vitro* (Toyoshima *et al.*, 1987; Manstein *et al.*, 1989a) and to produce a force *in vitro* that is comparable with that produced by each head of myosin in muscle during isometric contraction (Kishino and Yanagida, 1988). For historical reasons, members of the myosin superfamily have been divided into two operational categories. The first group consist of the conventional myosins, all of which show the same structural pattern. The COOH-terminal halves of two myosin heavy chains associate with each other in an extended α-helical coiled-coil while the NH_2-terminal half of the polypeptide chains folds to form the globular head domains, each of which binds two light chains. The second group is far more diverse. Unconventional myosins consist of the generic motor domain, that is common to all actin based motorproteïns, attached to a variety of structurally and functionally distinct tail domains and are associated with one or more light chains. Members of this group include single headed as well as double headed myosins (Fig. 1). For a recent review on unconventional myosins see Cheney and Mooseker (1992).

Dictyostelium is an attractive model organism for the study of cytoskeletal proteins and cell motility. This organism resembles higher eukaryotic cells in appearance and motile behaviour and is amenable to classical and molecular genetics. The cell

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physiological function of conventional myosin in *Dictyostelium* has been addressed by applying molecular genetic techniques to alter the expression of the myosin heavy chain gene (*mhc*A) and the gene encoding the essential myosin light chain (EMLC). Overexpression of antisense RNA was used to reduce the expression of the myosin heavy chain (MHC) to less than 1% (Knecht and Loomis, 1987) and the EMLC to less than 0.5% of wild-type levels (Pollenz *et al.*, 1992). The high frequency of homologous recombination events in *Dictyostelium* was exploited in order to generate cell lines that produce a truncated heavy meromyosin fragment (HMM) and less than 0.1% of wild-type levels of intact myosin (De Lozanne and Spudich, 1987). This approach was later extended to eliminate the entire *mhc*A gene, generating cells that are completely devoid of conventional myosin (Manstein *et al.*, 1989b). MHC null cells as well as the other myosin-defective cell lines display the same range of pleiotropic effects on many cellular functions. The fact that three different molecular genetic approaches led to the same phenotype strongly suggested that the observed effects are due solely to the lack of conventional myosin and not to secondary mutations. Definitive genetic proof of this point was achieved by the reintroduction of the cloned gene into the MHC null cells (Egelhoff et al., 1990).

The purpose of this article is to review the work on the cell physiological function of conventional myosin. Special emphasis will be given to recent work using molecular genetics to dissect myosin function in *Dictyostelium discoideum.*

Cleavage furrow formation

The importance of myosin in the division of mitotic cells by cytokinesis has been established by many studies. Microinjection of inhibitory antibodies into starfish blastomeres (Mabuchi and Okuno, 1977) and *Drosophila* embryos (Kiehart et al., 1990) was shown to disrupt cytokinesis. Immunofluorescence studies using *Dictyostelium* (Yumura *et al.*, 1984) and sea urchin blastomeres (Schroeder, 1987) show that myosin concentrates and disperses in the contractile furrow in coincidence with the beginning and end of the furrowing that leads to cell division. In *Dictyostelium*, the myosin that is concentrated in the cortical ectoplasm during interphase becomes dispersed throughout the cytoplasm, in the form of myosin filaments, at late prophase. Most myosin remains localized in the endoplasm during metaphase and anaphase. At the anaphase-telophase transition, myosin accumulates again in the cortical ectoplasm from where it relocates to the equator. Finally, at late telophase, myosin almost exclusively co-localizes with the constricting cleavage furrow (Kitanishi-Yumura and Fukui, 1989). These findings are in good agreement with the contractile ring model which assumes that bipolar myosin filaments pull in both directions on actin filaments that are attached with their barbed ends to the plasma membrane.

Genetic proof that myosin is indeed required for normal cytokinesis has been established by depletion of the MHC from *Dictyostelium* (De Lozanne and Spudich, 1987; Knecht and Loomis, 1987). Phenotypically, the lack of myosin manifests itself in an inability of the cells to grow in suspension culture. Myosin depleted cells become large and multi-nucleated and massive lysis starts to occur after 4 to 5 days. However, when

Fig. 1. Schematic representation of actin based motor proteins: (A) Conventional myosin has two globular motor domains and a tail that forms the backbone of bipolar filaments. It consists of two identical heavy chains (185 to 230 kD) and two pairs of light chains (14 to 23 kD). Conventional myosins are abundant in muscle tissue but can be found in most eukaryotic cells. (B) Single-headed protozoan myosins containing a basic domain of ~220 amino acids that confers membrane-binding activity and a ATP-independent actin binding site. This second actin binding site is formed by a ~50 amino acids *src* homology domain (SH-3) that is either situated at the tip of the tail, next to a GPA domain, or within a GPA domain. GPA domains are characterized by their unusual concentration of glycine, proline, and alanine residues. *Dictyostelium* myosins IB, ID and *Acanthamoeba* myosins IA, IB, IC are examples of this class of actin based motors. Acanthamoeba myosins IA, IB, IC are associated with one or two specific light chains. (C) Single-headed protozoan myosins that contain a membrane binding site but lack a second actin-binding site. Examples of this group are *Dictyostelium* IA and IE. (D) The *Acanthamoeba* 177 kD myosin has a unique 800 amino acid tail domain except for the SH-3 domain at the tip of the tail. (E) Brush border myosin has similar to the *Dictyostelium* myosins IA, IE a short tail that contains a membrane-binding site. Brush border myosin is associated with 3 to 4 calmodulin molecules. Calmodulin binds also to at least one of the single-headed *Dictyostelium* myosins (Zhu and Clarke, 1992) and the *Dilute* class of actin based motors. (F) The *Dilute* myosins are dimers with two globular motor domains a central rod-like segment and two terminal globular domains. Members of this class are the mouse *Dilute* protein, p190, a protein isolated from chicken brain, and the yeast proteins *MYO*2 and *MYO*4.

Fig. 2. Schematic comparison of the cell cycle of *Dictyostelium* wild-type and myosin null cells. In contrast to the mitotic cell division normally observed with wild-type cells (A), *Dictyostelium* null mutants undergo amitotic cell divisions by "traction mediated cytoplasmic fission" (B).

returned to a surface these cells can fragment into daughter cells by literally crawling away from each other. This amitotic cell division has been termed 'traction mediated cytoplasmic fission' and allows MHC– cells to grow with doubling times of ~12 hours, only slightly slower than the parental cell line (Spudich, 1989). The efficiency of this process is also reflected in the finding that the majority of cells in mid-log phase cultures are small and hardly distinguishable from wild-type cells. Traction mediated cytofission can also occasionally be observed in surface-attached cultures of wild-type cells and may represent cell division at an earlier evolutionary level.

The creation of *Dictyostelium* MHC null mutants in which the entire coding region of the *mhc*A gene was deleted (Manstein *et al.*, 1989b) makes it possible to map the functional domains of the myosin molecule by introducing altered *mhc*A genes into these cells. Egelhoff *et al.* (1991) have shown that complementation of the null mutants with a MHC that lacks the 34 kD carboxy-terminal portion of the molecule partially restores cellular functions like the ability for growth in suspension. However, the removal of phosphorylation sites needed for the proper regulation of myosin filament disassembly, that are contained within the 34 kD fragment, leads to excessive localisation of the truncated myosin to the cell cortex and other cytoskeletal abnormalities. Further extensions of this approach will undoubtedly help to elucidate other structural features of the MHC that are involved in the regulation of contractile activity, correct localisation, assembly and disassembly during cell division.

Intracellular particle movement

In *Dictyostelium* rapid saltatory movements of small intracellular particles and endocytotic vesicles can be readily observed by phase-contrast microscopy. In cells that are actively migrating most of this movement is directed anteriorly, towards the site of cell expansion. Particle movement is normally restricted to the endoplasm and excluded from the ectoplasmic layer and pseudopods. However, in MHC null cells intracellular particles as well as microtubules are found to invade these actin-rich regions. Wessels and Soll (1990) used video enhanced DIC optics in combination with computer-assisted motion analysis systems (Soll, 1988; Wessels *et al.*, 1989) to compare intracellular particle movement in MHC minus cells and wild-type strain AX4. Their results show that while the proportion of intracellular vesicles and mitochondria is similar in AX4 and MHC null cells, the null cells contain far more immobile particles and particle movement lacks directionality. Although in null cells far fewer particles move at rates >1 μm/sec, the maximum velocity of 3 μm/sec observed for AX4 cells exceeds the maximum speed determined for particle movement in MHC null cells only by a factor of two. Another important difference between AX4 and MHC null cells is displayed when cells are pulsed with 1 μM cAMP. While cAMP has no effect on intracellular particle behaviour in MHC null cells, AX4 cells pulsed with cAMP display the same lack of directionality in particle movement and depressed level of particle velocity that is normally observed in null cells.

These results clearly demonstrate that conventional myosin affects the behaviour of intracellular particles. However, it remains open whether myosin directly interacts with particles or influences their motility by sustaining the general integrity of the cytoskeleton. The observations made after pulsing cells with cAMP suggest a more indirect role of myosin and point at the importance of the molecule in the organisation of the cortical cytoskeleton and in maintaining cell polarity.

Cell motility and chemotaxis

Immunofluorescence studies of *Dictyostelium* amoeba and poly-morphonuclear leukocytes show how myosin may mediate cell polarity in locomoting cells. These cells display a characteristic asymmetric distribution of contractile proteins. Actin and the majority of actin-binding proteins are found throughout the cortex. However, most of the actin is concentrated in the advancing pseudopod, whereas conventional myosin is localized specifically at the posterior end of the cell (Valerius et al., 1981; Stossel et al., 1985; Yumura and Fukui, 1985). For *Dictyostelium*, the correlation between myosin distribution and cell polarity can be further tested by comparing the effects of chemoattractants on wild-type and MHC null cells. Chemotactic cells develop polarity even in the absence of a chemoattractant gradient, however, attractants like cAMP can modulate this inherent polarity. Three different sets of conditions have been used to study the effect of cAMP on *Dictyostelium.* Alternatively, cells were exposed to a non directional pulse of attractant, a stable gradient of attractant, or cAMP was applied locally to individual cells from a micropipette.

It is one particular advantage of *Dictyostelium* as a model system that the response of cells to non-directional pulses of cAMP is so synchronous that it can be followed by

biochemical means in large populations of cells (Berlot *et al.*, 1985). Wild-type cells exposed to uniform solutions of cAMP respond by a large recruitment of actin to the insoluble cytoskeleton within 5 seconds of stimulation, concomitant with rounding up of the cell and the formation of surface blebs. Following this initial 'cringe' reaction, cells proceed to extend pseudopodia and ruffles in all directions, indicating a complete loss of cell polarity (Fukui *et al.*, 1990). When null cells are stimulated in the same way, they form surface blebs and become irregular in shape but, unlike wild-type cells, do not round up, suggesting that myosin is required for an active rounding up response. Additional evidence that conventional myosin contributes as force generator in the rounding up response comes from experiments where *Dictyostelium* cells were depleted of ATP by exposing them to millimolar concentrations of sodium azide (Pasternak *et al.*, 1989). In MHC null cells azide has no effect on the morphology and causes only a small increase in stiffness, while in wild-type cells depletion of ATP causes a rigor-like contraction of the cytoskeleton. Cells that contain conventional myosin retract all surface projections, become spherical, and their stiffness increases five-fold. However, it is important to point out that MHC null cells can round up. Spontaneous rounding up of MHC null cells can frequently be observed with cells grown in suspension and on plastic surfaces and is most likely a passive response to the cytoskeletal changes associated with mitotic events (see Fig. 2).

It is well known that *Dictyostelium* amoeba move by pseudopod extension. Directional stimulation of *Dictyostelium* with the chemo-attractant cAMP results in the almost instantaneous formation of one, rarely more, pseudopods and the progressive movement of the cell content into the enlarging projection. As a consequence the whole cell moves forward in the direction of the source of cAMP. In a stable gradient of cAMP, null cells move with just 20% the efficiency of wild-type towards the source of chemoattractant (Wessels *et al.*, 1988). Furthermore, the initial area of new pseudopods and the maximum area of pseudopods are far smaller than those observed for wild-type cells. This finding could be another manifestation of the lack of directed vesicle movement in the null cells, as vesicle movement towards the anterior of the translocating cell may be involved in membrane insertion for the expanding pseudopod.

Local stimulation of null cells with a micropipette extruding cAMP elicits the fast formation of a pseudopod from the near site of the cell, similar to the initial response observed with wild-type cells. However, the movement of cell content into the newly formed pseudopod is slower and the rate of translocation towards the source of cAMP is only about one third the rate of wild-type cells (Gerisch and Manstein, unpublished observations). This observation confirms that the initial rapid protrusion process does not require myosin and suggests that it is caused by directed actin polymerisation, similar to the formation of an acrosomal process by sea cucumber sperm cells (Tilney and Inoue, 1985).

Conclusion

Conventional myosin has been shown to play a critical role in a number of motility processes. In *Dictyostelium*, myosin is required for the capping of cell surface-receptors

(Pasternak *et al.*, 1989), morphogenetic changes associated with development (Manstein *et al.*, 1989b), the furrowing event of cytokinesis, and the generation of cell polarization. However, even the MHC null cells display many forms of movement, including cell migration, and intracellular vesicle movement, albeit at depressed rates, formation of cell surface extensions, and karyokinesis. These observations are generally in good agreement with studies carried out in other biological systems ranging from yeast to leukocytes and as such endorse the value of a model system like *Dictyostelium* that can be simultaneously studied using biochemical, cell biological and molecular genetic approaches.

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Actin Based Motors