Perspective

What Myosin Might Do

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HOW CELLS MOVE—HOW THEY DIVIDE, HOW THEY change shape and migrate—is a question that arises in many areas of modern biology. Intensive study of these problems with many different systems and approaches has produced a large amount of information and an appreciation of the complexity of the problem. A task that remains is to organize all the structural, biochemical, and phenomenological observations on cell motility into useful mechanistic models.

Two articles in this issue of *Science*—one by Knecht and Loomis (*1*), the other by De Lozanne and Spudich (*2*)—describe the application of powerful modern genetic techniques to this problem. By different methods, these groups disrupted the expression of a myosin heavy chain in *Dictyostelium* cells. The outcomes of their work begin to make distinctions between what one important protein linked to motility does and does not do, and to demonstrate both the uses and complications of such approaches.

Myosin in muscle and nonmuscle cells. These genetic experiments rest on a long and detailed study of motility, and therefore their contribution is usefully viewed in the perspective of that work. Inevitably, analyses of the mechanisms of movement in nonmuscle cells have been guided and informed by the classical studies of organelles in cells specialized for movement, for example, the cilium, or the sarcomere of skeletal muscle. Decades of work on muscle has produced an extraordinarily high level of understanding, consisting of a detailed correlation between the geometry and function of the structure, and the localization and properties of its component molecules such as actin and myosin. The temptation to transfer that understanding to nonmuscle cells was strengthened by the discovery that many of the proteins known to be essential elements of sarcomere function are present in nonmuscle cells as well. But this parallel does not extend to the phenomenology or even much of the structure of nonmuscle cells. For example, the sarcomere has a restricted motile function-contraction along one axis. Nonmuscle cells display a much larger repertoire of motilities, including elaboration of polarity, movement of parts of the cell with respect to one another, or translocation of the entire cell across a substratum. Too, the sarcomere has a regular geometry, and that geometry changes during contraction in an interpretable manner. No such regular geometric structure occurs in much of the motility apparatus of nonmuscle cells. It seems unlikely, therefore, that the details of biochemistry, localization, and structure will be sufficient to unravel the mechanisms of motility in these cells.

Both the experiments presented here (*1, 2*) focus on the heavy chain of myosin. This major component of the myosin molecule contains the capacity to form characteristic thick filaments, to bind to actin filaments, and to catalyze the hydrolysis of adenosine triphosphate (ATP). These three properties all participate in the structure and function of the sarcomere. In that organelle, thick filaments of myosin chains interdigitate with thin filaments of actin. Their movement with respect to one another, powered by ATP hydrolysis activity localized to the amino-terminal portion of the myosin chain, is the driving force for muscle contraction. The interaction of the myosin with the actin significantly stimulates turnover at the ATP hydrolysis site, producing an order of reaction steps that couples contraction to hydrolysis (*3*).

Mutants in myosin. A gene encoding a *Dictyostelium* myosin heavy chain has been cloned and sequenced (*4*). That gene serves as the starting point for these two strategies to produce mutant cells, deficient in myosin. In the first article, Knecht and Loomis inserted the cloned myosin gene in reverse orientation into a vector, so that it would encode an antisense transcript. Such a transcript can hybridize with the endogenous myosin message and interfere with its proper translation. This technique for producing a phenocopy of a null mutation in diverse organisms was worked out by Izant and Weintraub (*5*). The transformants that contained the antisense gene showed a reduction by at least two orders of magnitude in the level of the corresponding full-length myosin heavy chain protein.

In the second article, sequences encoding only the amino-terminal 140-kilodalton fragment of the myosin heavy chain were inserted into a vector. The normal size of *Dictyostelium* myosin heavy chain is about 240 kD. This amino-terminal domain corresponds to heavy meromyosin, a polypeptide that can be produced by proteolytic cleavage of intact myosin. Heavy meromyosin can interact with actin filaments and hydrolyze ATP, but it cannot form thick filaments. When De Lozanne and Spudich transformed *Dictyostelium* with this vector, they obtained cells in which the myosin sequences of the vector had inserted into the site of the normal myosin heavy chain gene. Such homologous recombination events are frequent and readily exploited in prokaryotes (*6*) and in the fungi (*7*), but have been detected only rarely in other eukaryotic cells. In this case, the transformed cells' normal myosin heavy chain gene was disrupted and not detectably expressed. However, the amino-terminal myosin sequences were expressed, and the corresponding truncated protein was present in roughly the same abundance as is the normal protein in wild-type cells.

Phenotypes. The first result of these two experiments is that they work: two quite different routes to interfering with the expression of this myosin gene produce viable cells. That is, this myosin gene does not behave as if it is essential in this genetic background. A caveat needs to be attached to such experiments, because experience with prokaryotes (*8*) and eukaryotes (*9*) shows that other genetic events, including mutations or duplications of other genes, can compensate rapidly for disruption of essential genes.

The more detailed phenotypes of the mutants are informative and provocative:

► The mutant cells are defective in cytokinesis. As a result, there is a high frequency of large, multinucleate cells in the cultures. Cells with only one nucleus are present, but there is evidence that such cells can arise by an abnormal pathway, resembling pinching off rather than the usual fission. That myosin participates in normal cell cleavage was suggested by previous work. Myosin has been localized to the contractile ring (*10*). More to the point, injection of antibodies against myosin disrupts cytokinesis but not chromosome separation in some egg cells (*11*).

► The mutant cells show defective aggregation behavior. Normally, *Dictyostelium* cells exhibit ameboid motion, and will walk across a substratum. Under appropriate circumstances, the cells will orient in a chemical gradient and stream toward foci and form aggregates. These aggregates undergo morphogenesis to form a complex structure containing differentiated cells. Both sorts of

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mutants are defective in specific aspects of this process. The mutant cells do aggregate, but only after a very long lag period following the chemotactic stimulus. Moreover, once they do aggregate, they do not undergo normal morphogenesis. However, it is not yet known if this phenotype is a direct consequence of the absence of myosin heavy chain or instead a consequence of the defect in cytokinesis: perhaps multinucleated cells do not aggregate normally even if they have the normal complement of myosin.

► Remarkably, the mutant cells do walk. They may aggregate slowly, and ultimately unsuccessfully, but they do stream together. Individual cells exhibit the features of normal ameboid movement: they ruffle, send out cellular processes such as pseudopodia, and they can locomote across substrata. Most conceptions of how cells walk require just those events that might involve myosin functions as defined by the sarcomere. Moreover, an extensive analysis of myosin form and localization in *Dictyostelium* places much of the molecule in thick filaments at the posterior cortex of the cell (*12*). This localization might be expected if myosin participates in walking.

Functions of myosin. Are we to conclude that this major aspect of motility in these cells is not based on myosin, or an actin-myosin system? There may be other myosin molecules not accounted for by disruption of this gene, although the hybridization data suggest that any other gene encoding a heavy chain must be significantly different from the one in hand (*4*). There is a report of another smaller myosin-like protein in these cells (*13*). The biochemical properties of the mutants allow for other explanations. The mutants produced by the antisense construction still contain somewhat less than 0.5 percent of the normal myosin heavy chain complement, and about 5 percent of a somewhat smaller protein that reacts with antibody to myosin. The mutants produced by the gene disruption contain normal amounts of the truncated molecule, and in vitro studies have shown that a similarly truncated myosin has at least some of the activity of full-length myosin (*14*). Perhaps these proteins are sufficient for at least some normal functions. Experiments to resolve these questions are within the scope of these approaches.

Mutations in cytoskeletal proteins have been studied in other systems. Most pertinent are the results from the budding yeast *Saccharomyces cerevisiae*. This organism contains a single gene encoding an actin that is highly homologous to actins in other eukaryotes. Disruption of that actin gene by homologous recombination is lethal (*15*), unlike the disruption of the myosin gene in *Dictyostelium*. Engineered conditional mutations in that actin gene produce more accessible phenotypes (*16*). At the permissive temperature, at least some of the mutants show an altered pattern of actin staining, especially diminution in the number of actin cables. Shifted to the nonpermissive temperature, these cells eventually die. Before they do, however, interesting phenotypes appear. The deposition of chitin, a yeast cell wall component, is abnormal. In wild-type cells, chitin collars form around the bud neck, and remain associated with the mother cell. But in the actin mutants its deposition is delocalized all over the cell surface. This loss of organization may be associated with a general defect in budding in these mutants. It also may be a reflection of the loss of polarity invoked to explain why the myosin mutants in *Dictyostelium* do not aggregate properly. The yeast actin mutants also show increased sensitivity to high ionic strength. This result is just surprising; nothing that we know of actin biochemistry or its function in muscle predicts such an apparently unrelated phenotype. These results raise the hope that genetics may provide a way to get at unexpected functions and associations of cytoskeletal proteins in the cytoplasm.

Prospects. These two articles succeed in linking modern genetic techniques to a popular and standard system for studying cell motility. That is an advantage because so much is known about the motile apparatus and behavior of *Dictyostelium*. The genetic experiments that are feasible in this and other systems will be extremely valuable. There is a great deal of biochemistry and structural work remaining, and the fundamental events of cell motility are still being studied. The ability of genetic experiments to provide an understanding of in vivo function and interactions, and to identify essential domains of proteins, can help build links between these approaches and help guide choices of what to study in vitro.

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