Caveat experimentor — is your myosin really inhibited?

Specific cell-permeable inhibitors are useful tools for providing insights into the role of particular classes of proteins or enzymes in a cellular process. For example, the depolymerization of actin filaments by cytochalasins or latrunculin A has highlighted the contribution of actin to functions as diverse as cytokinesis and mRNA localization. The existence of a large superfamily of myosins prompts the obvious question of what this group of motor proteins might be doing in the wide array of cell types in which they are expressed. A low-affinity inhibitor of skeletal muscle myosin, 2,3-butanedione monoxime (BDM), was reported to inactivate several different classes of non-muscle myosin (I, II and V) *in vitro* and myosin-based activity *in vivo* (Cramer, L. P & Mitchison, T. J. *J. Cell Biol.* **131,** 179–189), suggesting that it could function as a general myosin inhibitor. The finding that BDM inhibits various cellular functions, such as growth cone motility, asymmetric protein localization, exocytosis and secretion to name but a few, led investigators to conclude that a myosin(s) are central to each process.

However, a new report (Ostap, E. M. *J. Mus. Res. Cell Mot.* **23,** 305–308 (2002)) raises serious doubts about the use of BDM as a general myosin inhibitor. They find that the actin-activated Mg-ATPase activity (that is, the physiological activity) of baculovirus-expressed myosins that have differing kinetic properties and cellular functions (class I, VI and VI) was unchanged when concentrations of BDM typically used in cellular inhibition studies were included in the assay. Additionally, non-muscle myosin II has also been reported to be unaffected by BDM (Cheung, A. *et. al. Nature Cell Biol.* **4,** 83–88 (2002)). The basis for the discrepancy between these recent reports and the early work is unclear. It should also be noted that not all types of myosin (and there are many!) have been tested and it is possible that one or more could be inhibited by BDM. But, given the relatively high degree of conservation between motor domains, this would seem improbable. Thus, the findings of Ostap raise a red flag about the interpretation of BDM experiments; the true cellular target of this chemical is unknown/unclear and although it is obviously inhibiting interesting processes consistent with myosin function, it is impossible to definitively ascribe a myosin to those activities.

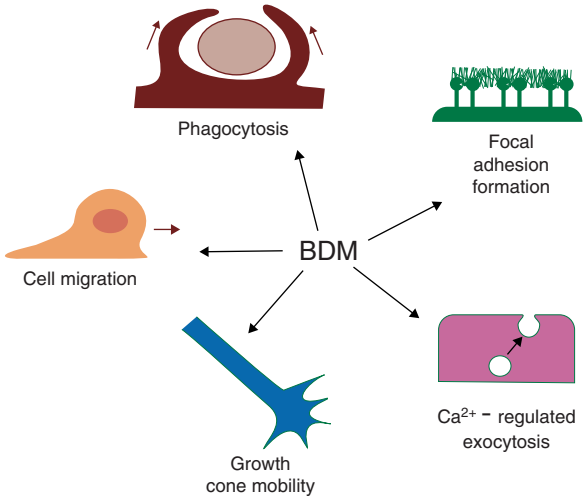

Figure 1 **A subset of the processes in which myosins have been implicated using BDM.**

There can be no doubt that a myosin inhibitor would be a powerful cell biological tool, but candidate inhibitors need to be verified thoroughly before they can be used to draw specific conclusions about the cellular roles of myosins. Hope comes from a new inhibitor of skeletal myosin II, identified by the screening libraries of small molecules (Cheung, A. *et. al. Nature Cell Biol.* **4,** 83–88 (2002)). However, until such time as either a true pan-myosin inhibitor or an inhibitor specific for a particular class of myosin is discovered, researchers will have to resort to the old-fashioned way of inhibiting a myosin — genetics or molecular genetics.

MARGARET A. TITUS

Department of Genetics, Cell Biology and Development, University of Minnesota, Minneapolis, MN 55455, USA.
e-mail: titus@mail.ahc.umn.edu

concentrations than are the early intracellular calcium transients. So, do sperm integrate a number of ionic responses to generate a motor response?

This early phase of signalling saturates at ~25 pM resact, although sperm respond with metabolic and motility changes to doses as high as 1 μM. It is probable that the chemotactic signalling system adapts as sperm move into the resact gradient, permitting them to continue orientating towards the source of the chemical gradient even after saturation of the primary response (Fig. 1). Similarly, sensory systems are known to adapt to permit responses over a wide range of stimulus concentrations. There are, in fact, some hints that sperm signal transduction systems may adapt. For example, components of a sensory adaptation system are present in the sperm flagellum, including G protein-coupled receptor kinase 3 and β-arrestin 2 in the mammalian sperm flagellum[11]. These proteins function sequentially during the adaptation and desensitization of sensory transduction and other signal transduction pathways: phosphorylation of ligand-bound receptors by G protein-coupled receptor kinases causes the subsequent binding of β-arrestin and the steric inhibition of further G protein stimulation. In addition, it is known that the resact guanylyl cyclase-receptor undergoes ligand-induced dephosphorylation that may form part of an adaptation mechanism[12].

In conclusion, Kaupp *et al.* have shown that sperm generate intracellular calcium transients within milliseconds in response to single molecules of an egg-derived peptide, and that these ionic responses result in chemotaxis. Although this study resolves some issues regarding sperm chemotaxis, it raises a number of provocative questions. If, for example, sperm can produce early intracellular calcium transients in response to single resact molecules then does this extend the chemo-activation gradient to which sperm can react to even greater distances? As discussed previously, sperm do not seem to re-orient motility in response to single resact molecules. However, they do generate intracellular calcium transients, suggesting that information can be exchanged between gametes beyond the ~1 mm zone of high react concentrations. A second question regards the mechanisms by which the cGMP that is produced after resact binding activates local calcium channels: is there a direct effect, such as through the cyclic nucleotide-gated channels, known to be present in sperm, or is this indirect, for example through a protein kinase? Third, one wonders about the structural basis of this apparent 5-μm functional unit within the sperm flagellum that resact may activate, and how these early