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How molecular motors work in muscle

Much of Howard's Review Article[1] concerns the results of experiments with single myosin molecules and actin filaments. It was a huge surprise when reports of such experiments first appeared (see, for example, ref. 2), and much is being learnt from them that cannot be deduced from experiments on whole muscle fibres, whether intact or after removal of the membrane. But single-molecule experiments do not yet approach the time resolution or the freedom from brownian noise that are easily attainable on larger assemblies of myosin and actin filaments, and their interpretation is subject to many uncertainties — due, for instance, to compliance in the actin filaments and in their attachments to beads or other force-measuring components, and the attachment of myosin molecules or fragments to the base. No doubt the time course of the working stroke of a single myosin head will one day be recorded, but until that is achieved the results of experiments on whole fibres and myofibrils deserve more careful attention than has been given to them by Howard.

Many of Howard's statements can be challenged. Here I will mention only three, of particular relevance to my own work. First, Howard uses a value of 4 nm for the "working distance" of a myosin molecule, defined as the distance over which a myosin head remains attached during a single interaction with an actin filament. But he uses the same value of 4 nm for the "working stroke", the distance over which an attached crossbridge exerts positive force. In rapid shortening, the former is presumably greater than the latter as crossbridges remain attached for a short time after the force they exert has fallen to zero. Howard justifies the use of this value on the grounds that it is the amount of sudden shortening per half-sarcomere needed to bring tension to zero from its value in an isometric tetanus of an isolated frog muscle fibre (Fig. 26 of ref. 3).

However, this quantity is usually interpreted as the average amount by which compliant structures in the half-sarcomere had been strained by the active tension before application of the shortening step. The working stroke is represented in such experiments by the rapid recovery of much of the tension that was present before the shortening step. The slack in a fibre caused by sudden shortening of 13 nm per half-sarcomere is the maximum taken up by this quick recovery process (Fig. 13 of ref. 3). In simulations of the response to a shortening step, this maximum is about equal to the assumed working stroke plus the contribution from linear compliance, whether it is assumed that the working stroke is performed by crossbridges that were already attached before the step (as in my own[4] and many other simulations) or by fresh crossbridges that attach very rapidly after the step. In the latter case, if a stroke of 4 nm is assumed the model cannot account for the 13 nm of shortening observed (Fig. 7 of ref. 5).

Second, Howard points out correctly that "the discovery that the actin filaments contribute about half the compliance of muscle [during isometric contraction] means that the stiffness is not proportional to the number of attached heads" and concludes that "a low duty ratio is therefore not inconsistent with the stiffness measurements" (p. 564 of ref. 1).

The stiffness of an isolated frog fibre contracting under zero load is about one-third of its stiffness during isometric contraction[6], implying 20% of heads attached if it is assumed that all heads are attached in rigor. This is very different from the 1% claimed by Howard.

Last, Howard writes of "a paradox" — that the amount of filament sliding that occurs in the time required by each myosin molecule to hydrolyse one ATP is much larger than the "working distance", referring to two papers (refs 7 and 8 here). He claims to resolve this "paradox" by supposing that much of the sliding takes place while the myosin is detached from actin.

But for many years (for example, ref. 9) it has been supposed that each myosin head acts intermittently and that continuous sliding is brought about by asynchronous action of many myosin molecules, although estimates of the sliding distance per ATP hydrolysed have varied. The controversy raised by refs 7 and 8 was different: those papers report that when a myosin head interacts with an actin filament during rapid shortening, it *remains attached* for a distance of 60 nm (ref. 7) or 40 nm (ref. 8). Staying attached for such large distances remains difficult to explain, and Howard's "resolution" of the paradox is irrelevant because his central postulate is that a myosin head remains attached for only 4 nm, much smaller than 40 or 60 nm.

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Howard replies — Huxley points out that there is a discrepancy between recent mechanical recordings from single myosin molecules *in vitro* and earlier mechanical measurements from muscle fibres *in vivo*: the single-molecule recordings indicate a working distance and maximum duty ratio of 4–6 nm and 10–20%, respectively, only about half the values deduced from the fibre studies. Although it is possible that technical limitations, for example misorientated heads, make the single-molecule recordings suspect, I argued in my Review Article[1] that the discrepancy might be only an apparent one. In particular, the smaller working distance does conform to many of the results from muscle, and the lower duty ratio can even provide a more satisfactory explanation for some of the experiments with muscle fibres. Huxley challenges this view by discussing three additional observations from fibres. But I believe that these too are mainly consistent with the smaller working stroke and duty ratio.

First, Huxley argues that the working distance corresponds to the approximately 13-nm range of displacements over which muscle tension quickly recovers after a sudden shortening[3]. This is true if most of the myosin heads (crossbridges) are bound to the actin filament — that is, if the duty ratio is large.

However, one of the features of the low-duty-ratio model is that the relative movement of the actin and myosin filaments will bring unattached myosin heads into striking distance of new actin-binding sites. In this way the large range of displacements can be explained by the rapid attachment of fresh crossbridges (and detachment of the initially attached ones)[5]. In this view, the quick-recovery distance is not necessarily limited by the working stroke, and in principle the force might even recover over a substantial fraction of the periodicity of the helical actin repeat (see discussion of Fig. 7 in ref. 5).

Huxley's second two points concern myosin's duty ratio. In the earlier work on muscle fibres, the duty ratio was calculated as the ratio of the stiffness of active muscle to that of rigor muscle (in the latter case it is known that all the myosin heads are attached to the actin filaments[10]). This calculation yields a duty ratio significantly higher than that estimated from the more recent single-molecule, biochemical and other structural data that I summarized[1].

However, a key untested assumption is

239

that all the crossbridges in a rigor muscle contribute to the stiffness. If this is not so — for example if many are bound in 'slack' states — the calculated duty ratio would be lower and the discrepancy might vanish. If the duty ratio is indeed small, the experiments of Higuchi and Goldman[8] are consistent with a small working distance. The experiment of Yanagida et al.[11] remains difficult to explain.

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A new classification for HIV-1

The phenotype of HIV-1 isolates is defined by the cells in which they replicate *in vitro,* but these phenotypes can change *in vivo* with profound implications for viral transmission, pathogenesis and disease progression. Here we propose a new classification system based on co-receptor use, providing a more accurate description of viral phenotype than the present imprecise and often misleading classification schemes.

At present, three classification systems are in use. The first defines primary isolates as macrophage (M)-tropic or T-cell-line (T)-tropic. However, this system disguises the fact that all primary isolates replicate in activated, primary CD4+ T-lymphocytes. The second system categorizes isolates as being either syncytium-inducing or non-syncytium-inducing (NSI) on the basis of whether they form syncytia in MT-2 cells, which express CXCR4 but not CCR5 (ref. 1). However, NSI viruses can readily form syncytia with CCR5-positive cells. The third system defines viruses as either slow/low (SL) or rapid/high (RH) depending on their growth kinetics in culture[2]. These classifications are often used interchangeably, but they are not synonymous.

The identification of some chemokine receptors as having critical roles in the cellular entry of HIV-1 allows us to develop a more precise system for identifying the phenotypic properties of virus strains. A major determinant of HIV-1 tropism (phenotype) lies at the level of virus entry into target cells, which in turn is governed by the expression of co-receptors in conjunction with CD4 (refs 3–5): either CCR5 or CXCR4, or both. CXCR4 use is a defining feature of viruses that form syncytia in T-cell lines; use of CCR5 is a property of NSI, M-tropic viruses; and many T-tropic primary isolates can use both co-receptors[3–5]. A nomenclature based on the co-receptor used would thus provide a precise molecular designation of a given isolate that largely explains its phenotype.

We propose that isolates that use CCR5 but not CXCR4 be termed R5 viruses, that isolates using CXCR4 but not CCR5 be designated X4 viruses, and isolates able to use both co-receptors with comparable efficiency be called R5X4. Whether an X4 or R5X4 virus is a cell-line-adapted isolate should also be specified.

Under this system, R5 viruses are the strains most commonly transmitted sexually, consistent with the high resistance of individuals lacking CCR5 to infection[6,7]. After about five years, viruses evolve in about 50% of patients that are able to use CXCR4, with or without concurrent use of CCR5 (refs 8,9). These viruses would now be called R5X4 and X4 viruses, respectively. Isolates passaged through a permanent T-cell line should be called T-cell line-adapted (TCLA) X4 or R5X4 viruses, and a similar qualifier can be used for viruses adapted to growth on other cells.

This nomenclature takes note of the ability of an isolate to use the major co-receptors, but does not specify whether the isolate can replicate in a particular target cell. The nuances of co-receptor usage in specific contexts are beyond a simple classification system and should be specified by authors if there are perceived ambiguities. That a virus can use a particular co-receptor in transfected cells does not mean that this virus uses the same co-receptor in a more physiological context. The efficiency with which different co-receptors are used by some strains is likely to vary between assay systems; again, authors should clearly explain the limitations of their results, and the significance of extremely low efficiency co-receptor usage should not be over-interpreted. This classification system can also be expanded to take note of other co-receptors if their use by an isolate proves to be a major determinant of tropism; for example, whether isolates use CCR3 and/or CCR5 to enter microglia could define them as R3, R5 or R3-R5 isolates[10].

Our classification system is uncomplicated, is intuitive to those familiar with the usage of the CCR5 and CXCR4 co-receptors by HIV-1, and removes the inaccuracies and confusion associated with the present systems. It is flexible and open to expansion to accommodate emerging knowledge of co-receptor usage, and can encompass HIV-2 and SIV strains, which also use CCR5 and other co-receptors for entry[3–5].

A record of co-receptor use by particular HIV-1, HIV-2 and SIV strains will be maintained by the Los Alamos National Laboratory Sequence Database, together with an expanded version of this article.

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