in recent textbooks. But, as mentioned above, DNAs in the entrance-exit region of the nucleosome do not continue the right-angle trajectories defined in the core particle, and bend away from each other before they could cross. This leaves one negative crossing and therefore no paradox in the LH-free nucleosome. The paradox would similarly no longer exist for the LH-containing nucleosome if, as appears likely, the two duplexes in the stem remain parallel and do not wind around each other on going from one nucleosome to the next along the chromatin fiber.

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Smooth and Skeletal Muscle Single-Molecule Mechanical Experiments

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An increasing number of laboratories have now developed techniques for measuring the mechanical properties of single molecules (Finer et al., 1994; Molloy et al., 1995; Saito et al., 1994; Miyata et al., 1994). For studies on actomyosin, the point of making such measurements is that the force, movement, and kinetics of a single cross-bridge power stroke can be measured directly. In this issue, Guilford et al. have used an optical tweezers apparatus to measure the properties of single myosin molecules obtained from different sources. They compare the properties of smooth muscle and skeletal muscle myosins and address the question: How does smooth muscle myosin generate more force than skeletal muscle myosin, both in muscle fibers and in vitro?

The conclusions they draw from their results are straightforward to state, namely that a single molecule of phosphorylated smooth muscle myosin (SMM) develops the same force as that of skeletal muscle myosin (SkM) and has the same working stroke, but has a longer attached lifetime, resulting in a greater fraction of "pulling" cross-bridges. This finding is sufficient to explain both the whole muscle and in vitro motility observations, and suggests another round of experiments to answer more detailed questions about the SMM cross-bridge cycle. Many interesting follow-up questions immediately come to mind. For example, a recent cryoelectron microscopy study has shown that the tail of SMM, in contrast with SkM, undergoes a 35-Å movement upon release of ADP (Whittaker et al., 1995). Can this recently discovered motion be detected with the aid of optical tweezers, and is there a second phase of movement associated with the release of phosphate? A quintessential property of smooth muscle is its ability to maintain tension at a low energy cost: What is the mechanical nature of a cross-bridge in this so-called latch state? SMM is directly regulated by phosphorylation: How, and why, do small amounts of unphosphorylated SMM slow down the motility of other myosins when mixtures of both types are combined and tested in vitro? Further experiments and more detailed analysis of data will be required to answer these more subtle features of SMM cross-bridge interactions.

Optical tweezer techniques are relatively new. The limitations of the apparatus are still being uncovered, and ways of analyzing results are still being developed. Here Guilford et al. present a novel approach to analyzing single molecule mechanical data, Mean-Variance Analysis, which is derived from methods developed for patch-clamp recordings of ion channels in membranes.

Guilford et al. use the "three-bead" arrangement originally developed by Finer et al. (1994) to bring an actin filament into direct contact with a myosin molecule. This is the most commonly used method for making measurements of single actomyosin interactions, during which any individual myosin head remains attached to the actin for a small fraction of the overall time. An actin filament is suspended between two beads, each held

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in an independent optical trap, and the position of one bead is monitored by imaging it onto a 4-quadrant photodiode. This detector can determine the position of the centroid of the image with a resolution of better than 0.5 nm. The bead-actin-bead assembly is then positioned next to a third bead, upon which is deposited a sufficiently low density of myosin that only a single molecule can interact at any one time (see Guilford et al., Fig. 2). Interactions between the myosin molecule and the actin filament can be measured in two principal modes: displacement mode, in which the apparatus is free-running and the traps are maintained at a low stiffness, and force mode, in which a feedback signal is used to hold the beads in fixed positions (ideally, this isometric situation corresponds to infinite stiffness), with the feedback signal being used to monitor the force of interactions between myosin and actin.

Accurate determinations of the forces developed by the myosin under isometric conditions (no movement of the molecule) require that the stiffness of the apparatus be much greater than that of a cross-bridge (i.e., the cross-bridge is prevented from moving, allowing maximum force to be developed). Although the optical trap itself is quite stiff in feedback mode ($\sim$10 pN nm^{-1}; Simmons et al., 1996), the stiffness corrsponding to the attachment of actin to the two beads at either end is likely to be considerably smaller (Veigel et al., 1997). This allows for some residual (and unwanted) movement of the myosin, thereby reducing the apparent force. For this reason, comparisons that report similarity of forces between different preparations must be treated with caution. This consideration applies to all previously published work in the field. Future technical developments will be to improve the attachment of actin to beads, so that series compliance is reduced, and/or to measure the position of both beads, instead of just one, so that any extension of the bead-filament connections can be measured and appropriate correction factors applied.

To determine the displacement, or working stroke, produced during the attachment of a myosin to an actin filament, the stiffness of the apparatus must be much less than that of the cross-bridge, so that the cross-bridge proceeds through its full working stroke unhindered. At such low stiffnesses, beads held by optical tweezers necessarily exhibit large amounts of Brownian (thermal) motion. Variations in the bead position exhibit a Gaussian distribution, and the motion is heavily damped (see Guilford et al., Eqs. 1 and 3). At the trap stiffnesses used by most investigators ($\sim$0.05 pN/nm), visual inspection of unfiltered data shows peak-to-peak motions of $\sim$50 nm, although on rare occasions beads can diffuse through even greater distances. Thus the movements of the actin filament past the fixed myosin head are typically large compared to the actin monomer spacing. How can displacement events be detected in this background of thermal vibration?

Finer et al. (1994) noticed that attachment of a myosin head to the actin filament increases the stiffness of the link between the actin filament and the "mechanical ground," reflected in a corresponding reduction in thermal movement of the bead-actin-bead assembly. This reduction in thermal noise was subsequently used as the criterion for identifying single myosin attachments (Molloy et al., 1995), and this was achieved in practice by plotting a running estimate of the variance (a measure of the instantaneous stiffness of the system) alongside the position data. The Mean-Variance (M-V) analysis method introduced by Guilford et al. is a sophistication of this idea. M-V analysis was derived from techniques developed by one of the co-authors (Patlak) for analyzing patch-clamp recordings. It involves calculating the mean and variance in a window around each time point and histogramming the number of record coordinates with the same mean and variance. The data are usually presented as a 3-dimensional surface plot. Once the data have been transformed into the mean-variance domain, all time information becomes embedded, but can be extracted in a clever way by varying the number of digital sample points (window size) used for each mean-variance calculation. The number of points accumulated in the "attached pool" then depends upon the ratio of the lifetime of the attached myosin head to the window-size duration (Guilford et al., Fig. 5). Long window sizes detect only long-duration events, because short events are smoothed out; shorter window sizes detect more events, but are increasingly subjected to contamination by background noise, so the choice of proper window size is critical.

Both optical tweezer experiments and patch-clamping recordings permit measurements to be obtained from single molecules. However, there are important differences in the energetics and sources of noise in these two systems. With ion channels, many ions pass through the channel during any single opening, and the overall change in energy of the system is much greater than thermal energy; the signal-to-noise ratio is therefore relatively high and the origin of the noise is mainly instrumental. With single molecule myosin-actin interactions, the energy available is less than (or equal to) the free energy of hydrolysis of one ATP, and this is sufficiently low that signals are never much greater than thermal noise; the signal-to-noise ratio is unavoidably low. Importantly, this noise represents the thermal motion of the actin filament past the myosin head. The noise is therefore in the system studied, rather than in the instrumentation. In fact, the term "noise" may be misleading; thermal motion is better viewed as a "forcing function," albeit a random one. One can take advantage of thermal fluctuations to tell when a cross-bridge attachment occurs, but analysis is hampered because the limited bandwidth of the detectors means that the effective "starting point" for any single power stroke cannot be determined accurately. We cannot think of a direct patch-clamp analogy, but the effect upon the results is as great as varying the clamping voltage randomly by an amount greater than the gating potential.

There are some limitations to the method. M-V analysis may introduce a bias, because it emphasizes mechanical events that produce a simultaneous change in both variance and mean position, and those events whose duration is greater than the window size used

for the M-V analysis. If the change in mean and/or variance is sufficiently small, or the event duration sufficiently brief, then cross-bridge attachments become difficult to resolve from periods when no actomyosin interaction occurs (this limitation applies equally well to other methods that have been used to distinguish single events in the actomyosin system). Under such conditions, regions of the M-V plot in which no cross-bridge is attached overlap with the "attached" region, and so some form of background subtraction is required. Another problem with M-V analysis, as it is presented, is that it is not clear which particular sequences of the time-series data are included in the attached pool, and which in the detached pool (that is, all time information for points is discarded). This makes some of the potentially exciting analysis of the records difficult, and does not readily allow the method to be cross-checked easily against the raw data to see if sensible results are being obtained. Notwithstanding these restrictions, the method is a real step forward in the automation of analysis.

One significant difference between this report and earlier work concerns the proportion of displacements that occur within the level of the baseline noise. Whereas we reported a majority of such events (Molloy et al., 1995), for Guilford et al. these represent the minority. The distributions of the positions of displacements reported in their paper are generally biphasic, displaying rather tight distributions (SEM $\pm$ 0.7 nm) around displacements of +10 nm and −10 nm (their Table 1). This difference in observations has led to important differences of interpretation. First, to explain the tight distributions, Guilford et al. propose that each attachment occurs very close to the mid-position of the thermal noise (their Fig. 3). Second, the observation of both positive and negative events, occurring at nearly equidistant positions from the baseline, requires that the cross-bridge working stroke be able to go both backward as well as forward. That is, a cross-bridge may push as well as pull, although with a statistical bias for the forward direction. The implication is that Molloy et al. were not able to observe this because, in their work, the two distributions were smeared into one much broader peak. Other groups, however, have also reported seeing only single-peaked distributions (on the positive side), but with a low proportion of displacements occurring within the baseline noise, and consequently computed a much greater working stroke (Finer et al., 1994, ~10 nm; Ishijima et al., 1996, ~23 nm,) than that found by Molloy et al. (1995, ~4 nm).

These relatively new single-molecule studies permit one to ask and answer any number of important questions relating to the detailed mechanism of the cross-bridge cycle. Guilford et al. introduce a new way of analyzing the data, and thereby raise the intriguing possibility that myosin pushes, as well as pulls, and weigh in with their own answer to the controversial question, "Exactly how big is the myosin work stroke?" This line of research seems certain to remain a lively area, at least in the near term.

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Why Do Cyclic Nucleotide-Gated Channels Have the Jitters?

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Cyclic nucleotide-gated (CNG) channels were discovered in retinal rod photoreceptors, where they generate the electrical response to light. Similar channels were found subsequently in cone photoreceptors and in olfactory receptor neurons, where they serve the analogous purpose of generating electrical signals in response to the binding of odorants. CNG channels have recently been identified in a variety of other tissues, both neural and nonneural, but the physiological roles of these channels are uncertain (reviewed in Finn et al., 1996). The retinal rod channel is a heteromultimer consisting of α- and β-subunits (Kaupp et al., 1989; Chen et al., 1993; Körschen et al., 1995), both of which bind cGMP (Brown et al., 1995). A number of functional properties of both native and expressed channels have been elucidated in excised membrane patches containing hundreds to thousands of channels. In terms of gating, for example, channels are known to be activated rapidly by the binding of at least three molecules of cGMP. The channels are therefore exquisitely sensitive to changes in cGMP concentration. In terms of permeation, the pore is similar in several respects to that of voltage-gated Ca^{2+} channels. There is a high-affinity binding site (or sites) for divalent cations formed by a set of pore region glutamate residues. The binding of Ca^{2+} or Mg^{2+} to these residues

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