a model in which band 3 and glycophorin A are not linked in a tight molecular complex, but may be capable of transient lateral association in the membrane.

The third set of implications concerns the generalizability of the molecular mechanism proposed by Knowles et al. (1994). The mechanism, restated, is that receptors with short cytoplasmic tails can activate cells through cooperative actions that induce increased interactions between the cytoskeleton or membrane skeleton and other plasma membrane receptors. Early evidence for this type of mechanism came from studies on lymphocytes, in which locally bound lectins were found to immobilize receptors at locations on the cell surface remote from the site of lectin binding (Edelman, 1976; Henis and Elson, 1981). Contemporary studies on lymphocytes suggest that cell-cell adhesion (mediated by ligand-receptor binding) and cell activation are dynamically interrelated. That is, cells can be activated by ligation of certain adhesion receptors, and cell activation influences the strength of cell-cell adhesion mediated by adhesion molecules at the plasma membrane. Preliminary evidence from a number of laboratories suggests that these effects are likely to involve signal transduction pathways and cytoskeletal rearrangements that affect the lateral mobility and aggregation state of cell surface receptors.

By using the human red cell as a model system, Knowles et al. (1994) have provided clear evidence for bidirectional transmembrane signaling involving the red cell membrane skeleton. "Action at a distance," in which cell activation through one transmembrane receptor affects the structure and function of other receptors in the same membrane, is likely to be the rule rather than the exception in many, if not all, biological membranes.

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Angular Disorder of Weak-Binding Actomyosin Cross-Bridges

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Since a large-scale structural change within the actin-myosin complex (cross-bridge) is predicted to accompany force generation in most models of muscle contraction, a major goal has been the direct electron microscopic (EM) visualization of this complex before and after the power stroke in which force is generated. It is widely accepted that the post-power stroke state is represented well by the tightly associated rigor complex that occurs in the absence of nucleotide or in the presence of MgADP. Under these conditions, EM shows clearly that isolated myosin heads (S1) in solution bind to actin uniformly and stereospecifically at an angle of ~45°. A much more elusive complex is the weak-binding pre-force state that predominates in the presence of ATP.

A report by Walker et al. (1994) in this issue addresses this problem through EM experiments on solutions of actin and S1 during the steady state of the ATP hydrolysis reaction. By performing the experiments at very low (2 mM) ionic strength, which strengthens the binding of S1 to actin, they were able to obtain conditions in which up to 70% of the heads were bound to actin. To capture the short-lived (~1 ms) actin-S1-ATP complex, they froze the samples very rapidly (~10^5 s^{-1}), producing a hydrated complex in vitreous ice, which they then examined directly (without staining) by cryoelectron microscopy. The resulting micrographs show a very *disordered actin-S1 complex in which the S1 molecules appear to bind to actin with a wide range of attachment angles.* In contrast, samples prepared in the absence of ATP, or after waiting long enough that the ATP was converted to ADP, showed the usual uniform attachment of S1 at ~45°.

The observation of angular disorder of weakly attached S1 agrees with most (e.g., Frado and Craig, 1992) but not all (Pollard et al., 1993) previous EM studies of the actin-S1 complex during ATP hydrolysis. The present study by Walker et al. (1994) is important because the techniques used are designed to prevent artifacts that might have affected previous studies. Most previous studies showing disorder did not employ rapid freezing, and the samples were stained before electron microscopy (Frado and Craig, 1992). The present study indicates that the disorder

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previously seen was not an artifact of staining. One previous study showed ordered binding of S1 to actin at an angle indistinguishable from that observed in the absence of ATP (Pollard et al., 1993). That study employed solution conditions and rapid freezing techniques that were similar to those of the present study, but the samples were visualized by making metal-shadowed replicas after freeze-fracture and deep etching. It seems likely that this method is less reliable for preserving native structures than the more direct method employed by Walker et al. (1994). On the other hand, the sharper images produced by Pollard et al. (1993) permitted a more quantitative analysis of S1 orientations.

Questions about possible artifacts due to freezing and fixing cannot be resolved by EM alone, so other structural techniques must aid in the interpretation. EPR spectroscopic measurements, which do not require freezing or fixation, support the conclusion that actin-attached S1 is orientationally disordered during ATP hydrolysis (Berger et al., 1989). Those EPR results went beyond the EM studies to show that the rotational disorder is dynamic on the microsecond time scale; in fact, these myosin head rotations are probably even faster than the freezing rates in the present study. These and other EPR results support a model in which both disordered (as observed by Walker et al., 1994) and ordered (rigor-like, as observed by Pollard et al., 1993) configurations exist, with the force-generating transition involving a transition from disordered to ordered states. This result is supported by structural studies of actin and S1 (Rayment et al., 1993), which suggest that the weak (pre-force, calcium-independent) attachment of S1 to actin may be a nonstereospecific interaction involving a small number of electrostatic interactions, while the stronger (force-bearing, calcium-dependent) attachment that follows involves a much more extensive and stereospecific interaction.

Are the present EM observations relevant to contracting muscle fibers? After all, these experiments were performed at non-physiologically low ionic strength, under conditions where only the very earliest of the intermediates (actin-myosin-ATP) is significantly populated. The answer is probably yes, since spectroscopic (Berger and Thomas, 1993) and EM studies (Hirose et al., 1993) indicate that orientationally disordered heads predominate under physiological conditions in contraction. Further structural studies, both in solution and in muscle fibers, designed to resolve specific intermediates in the actin-myosin ATPase cycle, will be needed to define more clearly the sequence of structural transitions that generates force.

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