myosin VI has a rather small lever arm and they cannot be accounted for considering the structural transitions that occur within the myosin motor of other classes. Secondly, this motor produces force towards the minus end of actin filaments, which is the opposite direction of all other characterized myosins. In order to understand the molecular basis of these features, we would like to describe the structure of this myosin in different states of its ATPase cycle. During the ATPase cycle, myosin goes through states of strong and weak affinity for the actin filament. To this day, we solved the structure of three states of the cycle, one state that mimics the state of strong affinity for actin (at the end of the movement on the filament) and two states before the force production. One of them represents the starting point for movement on actin, the pre powerstroke state. The analysis of the structures from the beginning and the end of the powerstroke allows us to understand how myosin VI moves in the opposite direction (toward the minus-end of actin filaments) due to a unique insertion between the motor domain and the lever arm. These structures also allowed us to understand the origin of the large size of the myosin VI lever arm swing. Unexpectedly, we found that a conformational change occurs in the converter which allows an optimized movement of the lever arm during the stroke.

Keywords: myosin, molecular motors, mechanisms enzyme

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How programmed cell death is regulated: Insights from structures of Bcl-2 family protein complexes

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Programmed cell death, or apoptosis, is controlled by the Bcl-2 family of proteins. The family consists of two main groups: the pro-survival group, which includes Mcl-1 and Bcl-xL, and the pro-death group, which includes the essential effector molecules Bax and Bak together with the more distantly related “BH3-only proteins” that are upregulated in response to various death stimuli. Interactions between members of these groups determine the fate of the cell and their deregulation underlies many disease conditions. Indeed, dysregulated apoptosis is a hallmark of many, if not all cancers. Thus, Bcl-2 family members are promising targets for anti-cancer therapeutics. Bax and Bak are thought to be able to adopt at least two distinct structural folds. One of these, the inactive fold, is well characterised and typical of the pro-survival Bcl-2 family proteins. Upon receipt of an apoptotic stimulus Bax and Bak are able to undergo a conformation change leading to an as yet undescribed activated form. One structural consequence is that a domain common to all Bcl-2 family members, the BH3 domain, becomes exposed. The pro-survival Bcl-2 family proteins are able to bind activated Bax and Bak via this BH3 domain and thus inhibit their activity. This provides an extra level of control over this important biological process. We have solved crystal structures for two such complexes, the Mcl-1:Bax BH3 (using a 34-mer BH3 peptide) complex and the Bcl-xL:Bak BH3 (34-mer) complex. In both cases significant structural changes must occur in the published structures of inactive Bax and Bak in order for these complexes to form.

Keywords: apoptosis, cancer, protein complexes

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Structural basis of spindle checkpoint activation and inactivation by Mad2 and p31comet

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In eukaryotes, the spindle checkpoint is a cell-cycle surveillance mechanism that ensures the fidelity of chromosome segregation in mitosis and thus prevents chromosome missegregation and aneuploidy. The status of spindle checkpoint signaling depends on the balance of two opposing dynamic processes that regulate the highly unusual two-state behavior of Mad2. In mitosis, a Mad1-Mad2 core complex recruits additional cytosolic Mad2 to kinetochores through Mad2 dimerization. Mad2 is then converted to a conformer amenable to Cdc20 binding, which facilitates checkpoint activation, halting mitosis. We report the crystal structure of an active Mad2 dimer. Combined with NMR and biochemical studies, we describe the features, kinetics and energetics of the dramatic conformational changes that initiate Mad2 structural activation. The opposing mechanism, spindle checkpoint inactivation, can be initiated by p31comet. This protein binds to Mad1- or Cdc20-bound Mad2 and thereby prevents Mad2 activation and promotes the dissociation of the Mad2-Cdc20 complex. We report the crystal structure of the Mad2/p31comet complex. Surprisingly, the sequentially unrelated p31comet adopts a fold strikingly similar to that of Mad2. It binds at the Mad2 dimerization interface and, by acting as an anti-Mad2 through structural mimicry, exploits the two-state behavior of Mad2 to block its activation. Through a combination of crystallographic and NMR studies, together with biochemical and cell biological studies, we provide a picture of the complex activation and inactivation processes during Mad2-dependent spindle checkpoint signaling.

Keywords: spindle assembly checkpoint, mitosis, structural mimicry

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Structural basis for the effects of PI3Kalpha oncogenic mutations

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PIK3CA, one of the two most frequently mutated oncogenes in human tumors, codes for p110alpha, the catalytic subunit of a heterodimeric PI3Kalpha kinase (p110alpha/p85) that plays a central role in signal transduction pathways. These somatic mutations increase PI3K kinase activity, leading to increased cell survival, cell motility, cell metabolism, and cell cycle progression. The 3.0 Å resolution structure of the complex between the catalytic subunit of PI3Kalpha, p110alpha, and two domains of its regulatory subunit, p85alpha reveals that the majority of the oncogenic mutations occur at the interfaces between p110 domains and between p110 and p85 domains. At these positions, the gain-of-function-mutations disrupt interactions resulting in changes in the kinase domain that may