Spotting the Mistakes, One Molecule at a Time

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adaptor binding site at one end and the ClpX degradation tag at the opposite end effectively stabilizes the interaction between ClpX and PdeA. The authors further suggest that this interaction between the N-terminal domain of PdeA and CpdR enhances the processivity of degradation, supported by the result that the presence of the adaptor CpdR increases the V_{max} of the degradation reaction by 30-fold. Therefore, the interaction between the adaptor and the substrate is the driving force for the degradation originating at a weak degradation tag.

With some substrates, the adaptor binding site is proximal with respect to the position of the ClpX degradation tag (Figure 1Bii), as in the case of the SsrA-tag. As mentioned above, the SsrA-tag supports direct recognition by ClpX. However, the N-terminal half of the tag also supports an interaction with the adaptor protein, SspB (Sauer and Baker, 2011). SspB binds to SsrA-tagged proteins and the N-domain of ClpX, enhancing recognition and thus facilitating unfolding and degradation of SsrA-tagged proteins by ClpXP.

Other substrates utilize a key feature of adaptor-mediated recognition, the ability to tether the substrate to ClpX, but without an external adaptor protein. In this example of self-tethering, the substrate incorporates regions located at positions other than a terminus, which also interact with ClpX. These additional contacts promote engagement of the substrate by ClpX, leading to substrate unfolding (Figure 1C). One example is the phage Mu transposase, MuA, which contains a ClpX degradation tag at its C terminus as well as additional residues that make extended contacts with ClpX to stabilize the association (Abdelhakim et al., 2008).

Regulated proteolysis is essential for development in many organisms. The work presented by Rood et al. (2012) helps clarify the contribution of proteolysis to the *Caulobacter* cell cycle. The incorporation of regulated proteolysis into an already complex regulatory network, such as the transition from G1 to S-phase in *Caulobacter* enables the cell to precisely control the functional activities of cellular components. Rood et al. (2012) present the structural characterization of an adaptor binding domain, thus providing mechanistic insight into how substrates are selected and recognized by cellular proteases.

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Spotting the Mistakes, One Molecule at a Time

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In this issue of *Structure*, Cho and colleagues provide intriguing insight into the first steps of the DNA mismatch repair process. By using single-molecule techniques, they show that the protein MutS undergoes two different types of diffusion on error-containing DNA in an ATP-dependent way.

The DNA mismatch repair (MMR) pathway is a highly conserved process that helps to maintain the integrity of the genome. One of its key functions is the recognition and repair of mismatches that are introduced during DNA replication and that are not repaired by the proof-reading activity of the DNA polymerase. In this issue of *Structure*, Cho et al. (2012) describe an elegant single-molecule approach to directly observe the very first step of the bacterial MMR pathway, the detection of the error by a protein called MutS.

Bacterial MutS is composed of seven domains, including an ATPase, a clamp, and a mismatch-binding domain and can form homodimers and tetramers.

Crystallographic studies have shown that the mismatch-binding domain interacts with the DNA minor groove, which is locally widened when the DNA contains an error (Lamers et al., 2000). Two models have been developed that describe the highly debated role of ATP binding and hydrolysis by MutS in the MMR. One model proposes that ATP hydrolysis by MutS is required to move away from the mismatch by active translocation (Allen et al., 1997). An alternative description is called the "molecular switch model": the recognition of the mismatch by MutS leads to a replacement of ADP by ATP. This exchange induces drastic conformational changes in the protein, which is then released from the mismatch as a sliding clamp (Gradia et al., 1999; Lebbink et al., 2010). Previous single-molecule studies have demonstrated that MutS diffuses one-dimensionally along a homoduplex DNA (not containing a mismatch) and that ATP hydrolysis is not required at this initial step of the MMR (Gorman et al., 2007). By modeling the diffusive movement of a quantum-dot tagged MutS along the DNA, it was inferred that the protein rotates around the DNA while diffusing, a model that is consistent with the tight DNA-protein interactions observed in crystallographic studies.

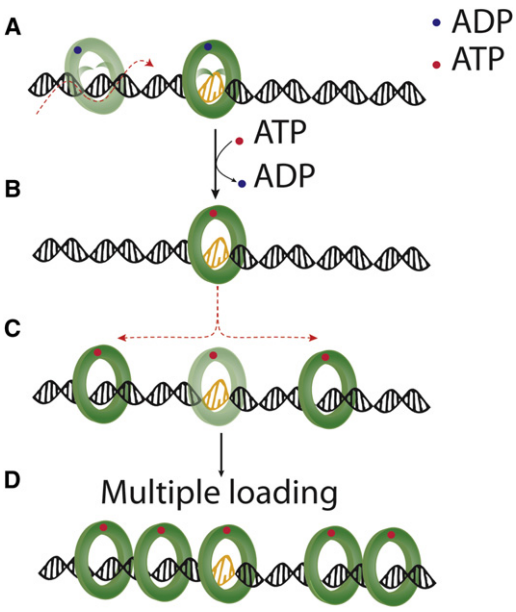

Figure 1. The Molecular Switch Model as a Description of the Initial Step in DNA Mismatch Repair
(A) The ADP-MutS complex diffuses along the DNA by tracking the helical pitch of the DNA.
(B) When MutS detects the mismatch, it undergoes a conformational change accompanying an exchange of ADP for ATP. The resulting ATP-MutS complex is a clamp that is less tightly bound to the DNA.
(C) The ATP-MutS clamp can escape the mismatch in either direction.
(D) By repeating this process, several MutS can be loaded onto the DNA.

Now, Cho et al. (2012) describe single-molecule fluorescence imaging approaches to visualize this coupling between one-dimensional diffusion and rotation. Moreover, they visualize labeled MutS moving along a DNA molecule containing a mismatch, allowing them to observe the interaction between MutS and the error. To achieve this, they image and track in real time the diffusive movement of fluorescently-labeled MutS along a 15 kb long, flow-stretched DNA molecule and simultaneously observe fluorescence resonance energy transfer between the labeled MutS and a fluorophore coupled to the mismatch.

The authors visualized MutS movement along the DNA molecule under several conditions: with and without ATP and under varying ionic strengths and flow rates. Using these protein-motion trajectories and the resulting information on the protein's diffusion, they showed that MutS goes through three phases with each different types of movement: the search of the mismatch, followed by binding to and release from the mismatch.

During the initial search phase, the authors demonstrated that the rate of diffusion of the MutS along DNA is not affected by the ionic strength. This observation is consistent with a model in which MutS is moving along DNA while in continuous contact with the DNA backbone and following its helical pitch (Figure 1A). The continuous electrostatic contact between the protein and DNA renders the binding interface inaccessible to ions from solutions and results in an independence of the diffusion kinetics on the concentration of such ions (Blainey et al., 2006). This conclusion is further confirmed by single-molecule fluorescence polarization measurements the authors performed on different sizes of short duplex DNA that constrain the number of rotations the protein can undergo by scanning the DNA backbone. The shorter the DNA is, the less rotation the protein can make during the time resolution and the broader the polarization distribution of multiple molecules will be.

The next step, binding of MutS to the mismatch, is marked by a decrease of the diffusion coefficient by more than one order of magnitude compared to that in the searching mode, suggesting that MutS is trapped to the mismatch (Figure 1B). Further, the authors showed that the residency time at the mismatch is lower by an order of magnitude compared to that in the absence of ATP. This difference is consistent with the fact that MutS needs ATP binding to be released from the mismatch as proposed by the molecular switch model.

After release from the mismatch, MutS diffuses along DNA but with significantly different diffusional characteristics than the ones displayed in the searching phase (Figure 1C). Most importantly, the diffusion coefficient is dependent on the ionic strength, suggesting that the protein is not in continuous contact with the DNA backbone but instead only undergoes very short-lived electrostatic interactions with the DNA. Whereas the MutS protein can best be seen as a nut circling around a bolt during the search phase, after mismatch release, it is best compared to a washer moving freely along a bolt while still encircling it. The random rotations such a washer-like behavior supports was confirmed by the authors using single-molecule fluorescence polarization measurements.

In conclusion, Cho et al. (2012) present strong evidence in favor of the molecular switch model as a description of the initiation of MMR. The use of single-molecule techniques to study this fundamental process brings a better understanding of the molecular mechanisms and dynamics and allows a direct observation of the different interactions between the key players. As a next step, it will be interesting to study the influence of the nature of the DNA error itself (single-base versus insertion-deletion loop-type mismatches) on the dynamics of MutS activity. Furthermore, crystallographic studies have shown

that MutS locally bends the DNA at the mismatch site. Combining single-molecule fluorescence techniques such as those reported by Cho et al. (2012) with single-molecule force experiments could probe this effect and establish a firm link between the structural work and the mechanics of the mismatch-recognition process. Of course, in order to place the initial steps in the MMR pathway in context and arrive at a full molecular description of the process, an important direction is to visualize the dynamics of downstream events when MutS interacts with other proteins such as MutL. Such studies are underway in other laboratories and undoubtedly will further enrich our understanding of this important process.

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Enzyme or Electrode?

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The process of dissimilatory metal reduction shapes our environment on a global scale by using minerals as terminal acceptors in a biological electron transport chain employed by bacteria under anaerobic conditions. In this issue of *Structure*, Edwards et al. present the structure of an extracellular undecaheme cytochrome involved in the step of electron transfer to metal oxides.

Respiratory metabolism couples transfer of electrons along an electrochemical potential gradient to the generation of proton motive force that serves as an energy source for many physiological processes. We naturally associate the term ''respiration'' with breathing air and the use of molecular oxygen as the terminal electron acceptor, but the general concept of respiratory pathways works for any suitable electron acceptor. One of the best of these, as judged by oxidizing power and abundance, is ferric iron, Fe(III). Consequently, such ''metal respiration'' is widespread in nature, although it faces a substantial logistic problem: in an oxidizing environment, Fe(III) is rapidly precipitated, mainly in the form of ferric hydroxides and hydroperoxides. Iron-respiring bacteria thus have chosen to ''breathe'' on stones, and to do so, they had to develop new ways of delivering electrons to a virtually insoluble substrate.

There are three known strategies metal reducers use to achieve this goal (Newman, 2001). The first is to get Fe(III) into the cell with brute-force, using iron chelators, siderophores, as high-affinity ligands that are secreted into the medium and taken up by specialized receptors for intracellular reduction. The second strategy is fire-and-forget, where respiratory electrons reduce soluble carriers that are then simply excreted. The third, and arguably the most intricate and elegant way, is dissimilatory iron reduction. Here, Gram-negative metal-reducing bacteria construct an electron transfer chain that spans both the inner and outer membrane. It connects the low-potential electron reservoir of the menaquinol pool in the cytoplasmic membrane to a ''metal reductase'' located on the outside of the outer membrane, where it can directly interact with its insoluble substrate (Lovley, 1993). Dissimilatory metal reducers were found to adhere to mineral substrates with a measurable force, and the deletion of an outer-membrane *c*-type cytochrome disrupted this adhesion (Lower et al., 2001, 2007).

Two genera of proteobacteria are established as model systems for dissimilatory metal reduction: *Geobacter*, δ-proteobacteria that couple the oxidation of various aromatic and aliphatic hydrocarbons to the reduction of metal oxides, and *Shewanella*, γ-proteobacteria with similar metabolic capabilities (Weber et al., 2006). The metal-reducing activity of both organisms depends on multiheme cytochromes *c* that reside on the outside of the outer membrane. In *Shewanella oneidensis*, the required proteins form most remarkable complexes around the outer membrane that allow for an ordered translocation of electrons (Beliaev and Saffarini, 1998; Beliaev et al., 2001). A gene cluster of structure *mtrDEF-omcA-mtrCAB* contains two modules consisting of periplasmic decaheme cytochromes

