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Laboratory of Neurogenetics and Behavior, The Rockefeller University, Box 63, New York, New York 10021, USA. E-mail: leslie@rockefeller.edu

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SUMO Modification: Wrestling with Protein Conformation

SUMO modification of human thymine-DNA glycosylase facilitates the processing of base excision repair substrates by an unusual mechanism: while leaving the catalytic center unaffected, it induces product release by eliciting a conformational change in the enzyme.

Helle D. Ulrich

Two common strategies to secure a victory are available to a traditional Sumo wrestler: his goal is achieved if he can force his opponent either to step out of the combat arena or to touch the ground with any body part other than his feet. In cell biology, the actions of the small ubiquitin-related modifier SUMO appear to be guided by a similar, but even more flexible set of rules: covalent attachment of SUMO to a protein usually forces the modified target to undergo a change in its localization, its interactions with other cellular components, its stability or its enzymatic activity [1,2]. By affecting the properties of its targets in such ways, SUMO contributes to the regulation of numerous biological processes, ranging from nuclear transport [3] to signal transduction [4], transcription [5] and genome integrity [6].

New SUMO targets are being identified almost by the day, though elucidation of the biological consequences of sumoylation lags far behind the discovery of target proteins. In fact, the mechanisms by which SUMO changes the properties of its targets are rarely well understood on a molecular basis. New work by Steinacher and Schär [7], reported in this issue of *Current Biology*, has now begun to shed light on the mechanism of SUMO function in one particular case.

Human thymine-DNA glycosylase (TDG) promotes DNA base excision repair by recognizing thymine (T) or uracil (U) when mispaired with guanine (G) in double-stranded DNA [8,9]. It cleaves the N-glycosidic bond between the base and the sugar backbone, thus releasing the mismatched base and creating an abasic (AP) site. This structure is then processed by downstream enzymes, which cleave the DNA backbone and initiate restoration

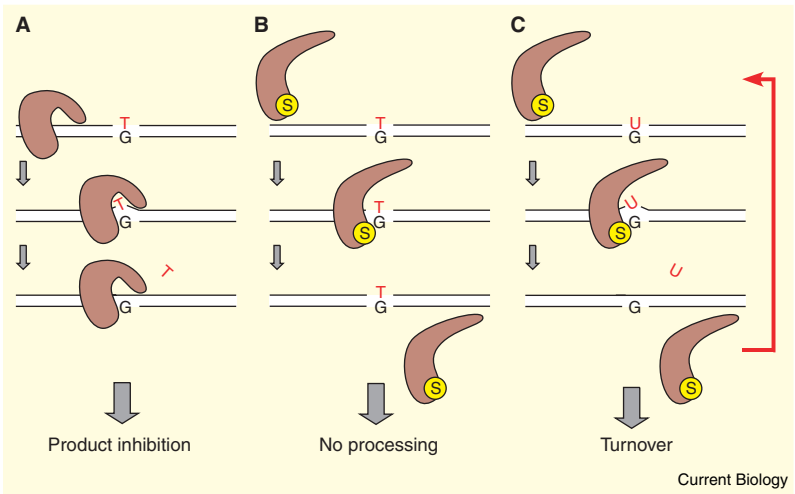

Figure 1. Influence of SUMO modification on the catalytic activity of human TDG *in vitro*.
(A) Unmodified TDG (brown) displays a high affinity for its substrates, including G•T and G•U mismatches in double-stranded DNA, but also for the reaction product, the AP site. Its high affinity is due to the contribution of the amino-terminal domain to non-specific DNA binding and allows the enzyme to process both G•U and G•T mismatches, but also results in a near complete product inhibition due to a failure to release the AP site after excision of the mismatched base. (B) SUMO modification of TDG induces a conformational change in the amino-terminal domain that reduces the overall affinity of the enzyme for DNA. As a consequence, the G•T mismatch, which requires strong DNA binding for recognition, is no longer processed. (C) In contrast to the G•T mismatch, the less demanding G•U mismatch is processed despite a reduced affinity of sumoylated TDG for DNA. Because of the reduction in affinity, however, product inhibition is abolished, and the enzyme is now competent for multiple catalytic turnovers.

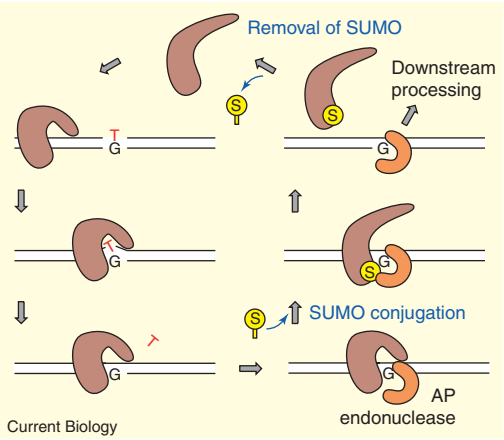

Figure 2. Model for the processing of a G•T mismatch by TDG *in vivo*.

Catalytic turnover on a G•T mismatch could be achieved by repeated cycles of sumoylation and desumoylation. This would entail successive steps of high-affinity substrate binding and base excision by the unmodified form, sumoylation of the DNA-bound form and subsequent release from the product AP site. Once released from the DNA, TDG can be desumoylated by an isopeptidase, and the next reaction cycle can be initiated. Coordination of the sumoylation step with the binding of the downstream enzyme, AP endonuclease, would prevent the AP site from being exposed and incorrectly processed.

of the nucleotide. The reaction intermediate, the AP site, is a dangerous structure if left unprotected, because its uncontrolled processing could have devastating effects on genome integrity [10]. This notion has sparked the hypothesis that the strong affinity for the reaction product displayed by many DNA glycosylases may shield the AP site until a displacement by the downstream AP endonuclease is guaranteed [11,12]. But while strong product inhibition can indeed provide the necessary protection for the AP site, it also invokes the need for a controlled dissociation mechanism.

A role for SUMO in regulating substrate release was first proposed by Schär and coworkers [13] when they identified human TDG as an *in vivo* sumoylation target. Attachment of SUMO to a lysine residue in the carboxy-terminal domain of TDG was found to reduce its affinity for the product AP site *in vitro*, thus allowing turnover on a G•U mismatch. At the same time, processing of a G•T substrate, which is bound with lower affinity by unmodified TDG, was completely abolished in the sumoylated form of the enzyme, again consistent with a reduction in overall DNA binding affinity.

Careful mechanistic analysis has now provided evidence that the basis for the observed change in affinity and catalytic activity of TDG is a direct effect of SUMO on the conformation of the enzyme (Figure 1). Using truncated versions of TDG, Steinacher and Schär [7] showed that the amino-terminal domain contributes to high-affinity, non-specific interaction with double-stranded DNA and is responsible for product inhibition and lack of turnover in the full-length protein. Consistent with this notion, processing of a G•T mismatch is poor in the bacterial uracil-DNA glycosylase MUG, which lacks the regulatory amino- and carboxy-terminal domains [14].

Intriguingly, sumoylated TDG resembled an amino-terminally truncated version with respect to DNA binding and catalytic properties, indicating that attachment of SUMO to the carboxy terminus of TDG modifies the properties of the enzyme's amino-terminal domain. A direct effect of sumoylation on the structure of TDG's amino terminus was supported by an analysis of protein conformation using partial protease digests: while the addition of DNA sensitized unmodified TDG toward proteolysis at a number of sites, no DNA-dependent change in the digestion pattern was observed for the sumoylated protein.

These observations suggest that unmodified, full-length TDG adopts a different conformation when bound to DNA, whereas a similar change does not occur in the SUMO-modified form. Moreover, even in the absence of DNA, protease digestion of sumoylated TDG produced a fragment of the amino-terminal domain that was absent in digests of the unmodified form, implying that sumoylation of TDG directly affects the conformation of the amino terminus.

Without changing the properties of the catalytic domain, modulation of TDG's amino-terminal region by sumoylation within the carboxyl terminus thus provides for an effective product-release mechanism, which allows catalytic turnover on more demanding substrates, such as the G•T mismatch (Figure 2). Given that sumoylation has these effects in an *in vitro* system, modification of TDG is one of the rare cases where SUMO directly controls the properties of its target, independently of its interaction with other cellular components or its subcellular localization. This mode of action stands in contrast to other well-known examples of regulation by sumoylation that mostly appear to involve the creation of or interference with protein–protein interaction surfaces on the modified target [2,4,5].

The model put forth here, however, also raises a number of important issues that need to be resolved in order to understand the function of TDG sumoylation in its biological context. For one thing, it postulates a cycle of SUMO conjugation and deconjugation for each catalytic turnover on a G•T mismatch (Figure 2), a plausible hypothesis that is consistent with the reversible and often transient nature of SUMO modification [2,5] and could in principle be tested *in vitro* with the use of the appropriate modifying and deconjugating enzymes.

Yet more important is the question of whether the control of product release by SUMO actually contributes to the shielding of the AP site for a hand-over to downstream enzymes. If this notion is valid, it will be critical to determine the factors that regulate the modification reaction itself: if sumoylation causes an immediate dissociation of TDG from the AP

site, the modification must not occur prior to the arrival of an AP endonuclease at the site of action. If sumoylation takes place without the need for downstream enzymes, dissociation of TDG from the DNA *in vivo* must be slowed down until protection of the AP site is guaranteed.

Takahashi *et al.* [15] recently demonstrated that TDG is also capable of interacting non-covalently with SUMO, a property shared by many other SUMO targets, which is believed to serve as a 'molecular glue' to facilitate the assembly of multi-protein complexes [2,4–6]. As the SUMO interaction motif on TDG is situated adjacent to the modified lysine in the carboxy-terminal domain [15], it is unlikely to contribute to the observed conformational changes in an intramolecular fashion. Instead, non-covalent interaction with SUMO was shown to control the protein's subcellular localization to PML nuclear bodies, thus suggesting one of the more conventional strategies available to SUMO to exert its regulatory power over its targets. Nevertheless, even its influence on TDG's conformation is somewhat reminiscent of a true Sumo wrestler: according to a less frequently applied rule of the game, a bout is won when the opponent's mawashi, the traditional belt of the Sumo wrestler, becomes completely unravelled.

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Cancer Research UK, Clare Hall Laboratories, Blanche Lane, South Mimms EN6 3LD, UK.
E-mail: helle.ulrich@cancer.org.uk

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Bacterial Mitosis: Actin in a New Role at the Origin

MreB is a prokaryotic homolog of actin involved in cellular organization and chromosome segregation. Recent results suggest that MreB is part of a kinetochore-like complex that specifically segregates the replication origin region of the bacterial chromosome.

William Margolin

Mitosis is the process of chromosome separation that precedes cytokinesis. During mitosis in a eukaryotic cell, the mitotic spindle microtubules are connected to chromosomes via a kinetochore, a protein complex that bridges the centromeric sequences on the DNA with the spindle. Prokaryotes also need to segregate their chromosomes in order to produce viable progeny cells; however, no mitotic spindle equivalent has been found in bacteria, perhaps because bacterial cells often have just two daughter chromosomes to separate, and the separation distance is much shorter. No evidence for a bacterial kinetochore has been found either — until now.

In the original model for bacterial chromosome segregation, chromosomes were thought to be attached to the cell membrane and to be segregated passively, as a result of cell elongation [1]. Two breakthroughs led to revision of this model. The first was the discovery that chromosomal origin (*oriC*) regions move toward the cell poles much more rapidly than one would expect were their separation driven just by cell growth, implicating an active partitioning machine [2]. The second was the discovery of prokaryotic homologs of tubulin and actin. In a curious reversal of functions, the tubulin homolog is required for bacterial cytokinesis but not mitosis, while one of several actin homologs has been implicated in chromosome segregation. This actin homolog, MreB, is strikingly similar to actin both in molecular structure and in its ability to assemble into ATP-dependent filaments [3,4].

In the bacterial cell, MreB forms a membrane-associated coiled structure that often extends along much of the cell length [5]. Depletion of MreB in rod-shaped bacilli such as *Escherichia coli* and *Bacillus subtilis*, or crescent-shaped *Caulobacter crescentus*, causes the cells to lose their characteristic shape. Additional