Under nutrient deprivation conditions, there are three layers of bacterial response. Nutrient deprivation leads to the accumulation of free uncharged tRNAs that bind at the A site of the ribosome and stall translation, thus triggering the first layer of response. The stringent factor RelA recognizes and binds to these blocked ribosomes and catalyzes the synthesis of (p)ppGpp (see ref. 15 and references therein), which then upregulates the transcription of enzymes involved in the biosynthesis of amino acids and downregulates transcription of components of the translational apparatus, such as rRNAs, ribosomal proteins, tRNAs, synthetases and translation factors. The stalling of protein synthesis prevents the production of nascent RelB, and existing RelB populations inside the cell (including those in complex with RelB) are reduced by the ATP-dependent Lon protease. This second layer of response frees the toxin RelE to bind to stalled ribosomes (Fig. 2b)⁸. The blocked ribosome after RelE-mediated cleavage

of the mRNA codon at the A site becomes a substrate for the tmRNA rescue system¹⁶, representing the third layer of response. tmRNA, a hybrid between a tRNA and an mRNA, binds to the A site of the ribosome containing a truncated mRNA and tags the corresponding nascent chains with a degradation signal (Fig. 2b), while allowing translation to terminate normally (reviewed in ref. 17). In this way, aberrant proteins made from truncated mRNAs are degraded while the stalled ribosomes can be recycled. Therefore, the stringent response involving RelBE and the tmRNA system seem to cooperate to increase degradation of nascent chains on stalled ribosomes; the resulting free amino acids may then improve the survival of the cell under harsh conditions.

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The interrogator

Oxidative damage can create 8-oxoguanine (oxoG) lesions on G bases of DNA. If left unrepaired, oxoG is read as a T by the replication machinery, subsequently resulting in a G-C to T-A conversion after a second round of DNA replication. Human 8-oxoguanine DNA glycosylase I (hOGG1) recognizes oxoG-C lesions and initiates base excision repair to remove them. The change from G to oxoG is subtle (addition of O to C8 and protonation of N7), so understanding how hOGG1 discriminates between the two is of interest, particularly because the normal base is so abundant in DNA. A previous structure of oxoG-containing DNA bound to a catalytically inactive form of the enzyme showed that oxoG swings out from the double helix to bind hOGG1 at the recognition pocket (protein and DNA in green, oxoG in red ball-and-stick), leaving its partner cytosine (magenta) 'estranged.' To see whether hOGG1 interacts with nondamaged DNA in a different manner, which may account for the discrimination, Banerjee *et al.* (*Nature* **434** 612–618, 2005) examined complexes of hOGG1 bound to duplex DNA containing a normal G-C base pair.

Crystallization of such a complex is difficult because repair proteins have an inherently weak affinity for DNA that lacks the cognate lesions they recognize. To overcome this problem, the authors engineered a disulfide bond between hOGG1 and the DNA outside the oxoG-binding pocket, between the cytosine of the G-C pair and a newly introduced cysteine on hOGG1. The strategy allowed them to examine the complex crystallographically.

The structure of the complex (protein and DNA in gold, G in blue ball-and-stick) shares an overall similarity with the oxoG-hOGG1 structure, but the G is extruded from the helix and lies at a new site 5 Å outside of the lesion-recognition pocket. Storage of the G-complex over ten days revealed no discernable base excision activity, in contrast to a similar covalent complex containing the oxoG lesion. The authors used free energy simulations to compare binding contributions at the oxoG-binding pocket and at the exosite and found a ~10⁵-fold preference for oxoG over G at the lesion-binding pocket.

Additional calculations revealed changes in a single hydrogen bond and a local dipole moment between oxoG and G that allows stable active site interactions with oxoG but not with G if it were to enter the pocket. These results likely explain why G is not found in the lesion-recognition pocket in the hOGG1-G structure.

Verdine and colleagues suggest that the G-complex represents a late intermediate in the base extrusion for oxoG. When combined with the structure of free hOGG1, the structures of the G- and oxoG-complexes outline the conformational changes that occur as the base is inserted into the lesion-recognition pocket. Although the analysis provides a means for extrahelical oxoG versus G base discrimination, it is unknown whether hOGG1 can discriminate between bases that are not extruded from the double helix. Nonetheless, hOGG1 seems to have a very efficient strategy in place to prevent cleavage of normal, lesion-free DNA.

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