DNA search and rescue

Sheila S. David

How do DNA-repair enzymes find aberrant nucleotides among the myriad of normal ones? One enzyme has been caught in the act of checking for damage, providing clues to its quality-control process.

DNA-repair enzymes amaze us with their ability to search through vast tracts of DNA to find subtle anomalies in the structure. The human repair enzyme 8-oxoguanine glycosylase (hOGG1) is particularly impressive in this regard because it efficiently removes 8-oxoguanine (oxoG), a damaged guanine (G) base containing an extra oxygen atom, and ignores undamaged bases. Verdine and colleagues previously determined the structure of an inactive hOGG1 variant bound to DNA containing an oxoG-cytosine (C) base pair. Surprisingly, only obvious mechanism by which hOGG1 discriminates between oxoG and G is through a single hydrogen bond to oxoG. It seemed unlikely that this single interaction would be sufficient to allow discrimination between the two bases, particularly given the million-fold excess of G over oxoG within the human genome. In addition, hOGG1 makes extensive contacts with the orphaned cytosine base, which ensures that oxoG is removed only when in the appropriate base-pairing context. Although extensive biophysical and structural studies intimate that there are general features of damaged bases that signal their presence to repair enzymes, the steps involved in finding damaged bases in a sea of normal ones are still unclear. Most mechanisms invoke the enzyme sliding or hopping along the DNA duplex until a damaged site is captured. A particularly intriguing question is whether normal bases are also extruded from the helix during the search process.

Verdine and colleagues reasoned that a structural snapshot of the enzyme encountering a G should provide insight into how the normal base is distinguished from its damaged oxoG counterpart. However, because the enzyme does not specifically recognize G, encountering and rejecting G is a transient process. Indeed, much like a train that stops only at certain locations, hOGG1 probably behaves only when close to oxoG. To capture the enzyme at an undamaged G–C base pair, the authors used an innovative ‘cova lent trapping’ method that they had previously devised to reveal the high-resolution structure of an HIV enzyme bound to DNA. Adding a cysteine thiol moiety in the enzyme and a single thiol-modified base in the DNA promoted the formation of a disulfide bridge — between the two when the enzyme reached the base. Banerjee et al. chose the appropriate site for the crosslink from the structure of inactive hOGG1 bound to an oxoG–C duplex. Using the corresponding duplex containing G rather than oxoG and the trapping trick, they deduced the remarkable structure of hOGG1 bound to the undamaged DNA duplex.

Surprisingly, even though G is forcibly presented to the enzyme and is flipped out from the helix, it does not gain access to the pocket in the enzyme that binds oxoG, but to another site (Fig. 1). Calculations of differences in free energy indicate that both favourable and unfavourable interactions lead to preferential binding of oxoG over G in the oxoG-recognition pocket, and of G over oxoG in the alternative site. This structure captures an intermediate that forms in the process of finding oxoG, and illustrates that the damaged base must pass through a series of ‘gates’, or checkpoints, within the enzyme; only oxoG satisfies the requirements for admission to the damage-specific pocket, where it will be clipped from the DNA. Other bases (C, A and T) may be rejected outright without extrusion from the helix because hOGG1 scrutinizes both bases in each pair, and only bases opposite a C will be examined more closely.

Time-resolved fluorescence studies of the related DNA glycosylase that removes uracil (UDG) have provided evidence for the formation of an uracil intermediate partially removed from the DNA stack that forms
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before the uracil is fully extruded. A uracil analogue that is unable to hydrogen-bond within the uracil–specific pocket may mimic this intermediate. Together, these studies suggest that a multi-step base-recognition process is a common strategy used by BER glycosylases to guarantee accurate base excision. Exploitation of Banerjee and colleagues’ covalent trapping may mimic this intermediate. Together, Banerjee and colleagues’ covalent trapping strategy may result in the detection of other intermediates in this process. In particular, clamping BOGG1 in the proximity of an A–T base pair may give insight into how it is dismissed by the enzyme.

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Techniques

Spectroscopy at a stretch

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Two-dimensional spectroscopy can now be done using visible light. This allows the electronic couplings between energy levels to be measured directly and sheds new light on how molecules function in photosynthesis.

How light is converted into chemical energy in a photosynthetic system is largely determined by the vibrational and electronic dynamics of the complex biological macromolecules involved. An essential step in the elucidation of these mechanisms is the combined determination of structure and dynamics. The extension of nuclear magnetic resonance (NMR) spectroscopy into multiple dimensions was introduced not long ago as one of the great advances in the study of the structure of proteins in solution. Now, Fleming and co-workers’ have transferred these principles of multidimensional spectroscopy to the electronic spectrum, where the wavelengths are about 10−7 of those in the NMR regime. This has allowed the first direct measurement of electronic couplings combined with the dynamics of excitations transferring between molecular energy levels in photosynthetic antennas. The dimension of a spectroscopy refers to the number of independently variable time intervals between the field pulses that induce the signals. For example, with three femtosecond laser pulses there are three time intervals of interest—that between the first and second pulses (τ, the coherence time); that between the second and third (T, the waiting time); and the interval (t, the detection time) between the third pulse and the detected photon-echo field emitted by the sample in response to all three pulses. The three-dimensional grid of experimental data points obtained by varying these time intervals then defines a response in the time domain that can readily be converted to a three-dimensional spectrum in the frequencies ω0, ω0, and ω0 by Fourier transformation along the three time axes. The unique aspect of such spectra is that they exhibit cross peaks at spectral points (ω0 = ω0, ω0 = ω0) linking two transitions between molecular energy levels at the frequencies ω0 and ω0. These cross peaks are present only because the structural components A and B of the system are able to sense one another’s presence. In other words, A and B are close enough to be coupled and the pulse sequence must be inducing transitions between them. This information is not exposed directly by spectra in one dimension.

Multi-dimensional spectroscopy showing coupling between different structural units has also been demonstrated for electron paramagnetic resonance spectroscopy, for which the wavelengths used are much smaller than in NMR but still in the centimetre range, although the phase is relatively easy to control. More recently, experiments analogous to those using NMR have been performed in the infrared region of the spectrum at a wavelength of 6 μm, enabling the coupling between molecular vibrations to be exposed. Two-dimensional infrared spectroscopy has now become an active field of theoretical and experimental research, with applications in unravelling the dynamic structures of peptides and proteins and liquids.

Fleming and colleagues’ introduce two-dimensional spectra at a wavelength near the visible range (0.5 μm). The spectra are used to spread electronic transitions into two dimensions and expose the pattern of electronic couplings between the seven bacteriochlorophyll groups whose absorption characteristics give these complex molecules their typical colour, and which correspond to the cofactors of a photosynthetic light-harvesting protein. Fleming and colleagues’ spectra of Ω versus Ω over a range of waiting times change with τ because energy is transferring between the cofactors as the system tends to equilibrium in its electronically excited state. The new method allows the energy transfer to be tracked on a cofactor–cofactor basis, providing a picture in space and time of the reorganization of the energy between the bacteriochlorophyll molecules in the light-harvesting complex of the bacterium Chlorobium tepidum.

The experiment clearly reveals which of the cofactors are coupled, and also yields the timescales of the energy flow between them. It is notable that the experimental two-dimensional electronic spectrum can be reproduced by a numerical analysis based on the electronic wavefunctions of the cofactors and a quantum theory of the interaction of cofactors with each other and with the protein environment. Theory and experiment combined have thus generated a molecular-scale map of the energy reorganization occurring within the light harvester, allowing us to visualize how it feeds energy to the reaction centre.

Because of its relatively broad bandwidth, this experimental method will not be restricted to the measurement of equilibrium dynamics. The pulse sequences that generate the photon echo could be applied to a system that has been kicked into a nonequilibrium state by another laser pulse. The new two-dimensional electronic and infrared methods are structural probes of the kinetics of chemical changes over a wide range of timescales dictated only by the ability to introduce spatial or electronic delays between short laser pulses in comparison, the range over which kinetic data can be acquired with NMR is limited at present to tens of milliseconds. Furthermore, with the new techniques, the distance scale of the electronic couplings that can be measured can reach tens of nanometres, much more than is possible with NMR or two-dimensional infrared, both of which operate only at distances up to about 1 nm.

The approach detailed by Fleming and co-workers’ thus opens a new era of electronic spectroscopy—one in which we can expect to visualize combined space–energy relationships between interacting electronic systems at the nanometre scale in a wide variety of applications. Ultimately these approaches will replace conventional ultraviolet, visible and infrared spectroscopy, because not only do they contain the structural information of the conventional spectra but they also tell whether or not the structural elements responsible for the absorption bands are near each other. The