on neurons in cell culture, raising the question of whether this form of homeostatic axonal plasticity occurs in the intact brain.

Kuba *et al.*[2] neatly answer this question. They had previously found that, in brainstem neurons responsible for encoding sound, the precise location and length of the AIS depend on the characteristic sound frequency that each neuron processes[15]. Now they have tested the effects of hearing loss on the AIS location (Fig. 1b). Removing the cochlea, the auditory part of the inner ear, from one-day-old chicks causes loss of synaptic input to neurons in the nucleus magnocellularis — an essential relay station in the auditory pathway. Kuba and co-workers find that such input deprivation has a dramatic effect on the AIS of these neurons: following hearing loss, the AIS is elongated by roughly 70%. These nucleus magnocellularis neurons lack dendrites, confirming that the excitability changes are indeed restricted to the axon.

Intriguingly, although Kuba *et al.* did not detect changes in the density and subtype composition of sodium channels in the AIS, hearing deprivation increased total sodium currents in the axons, and this could be attributed to the expansion of the AIS. Consequently, smaller current injections were sufficient to trigger action potentials after hearing deprivation, indicating that neuronal excitability had increased to compensate for the reduced synaptic drive.

Together, these papers[1,2] show that the AIS is a powerful target for homeostatic plasticity mechanisms. Notably, the changes in the AIS are bidirectional and reversible — properties crucial for the proposed homeostatic role of such mechanisms.

The two studies also open the door to exploring how AIS plasticity occurs in different types of neuron under various conditions that affect neural circuits. For instance, both teams used relatively crude methods to alter neuronal activity: long-term increases in neuronal activity or sensory deprivation. More subtle and controlled manipulations, over shorter timescales, could reveal whether AIS plasticity is restricted to developmental and pathological conditions, or whether it is a normal physiological mechanism that could dynamically regulate excitability.

The studies identify distinct mechanisms for modulating neuronal excitability — either displacement or extension of the AIS (Fig. 1 a, b). It will therefore be necessary to determine which prevails in different neuronal network states and brain areas. Neither group directly addressed how the changes in the AIS alter the integration of synaptic input by the neurons. This is particularly relevant for inputs mediated by the neurotransmitter γ-aminobutyric acid (GABAergic inputs), which cluster at the AIS[16]. Do these GABAergic synapses move with the AIS, and, if not, how does this affect their ability to influence the generation of action potentials? Finally, if the molecular mechanisms underlying this form of plasticity are identified, they could provide targets for manipulating neuronal excitability in various disease states that involve altered excitability, particularly epilepsy. ■

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DNA REPAIR

How to accurately bypass damage

Suse Broyde and Dinshaw J. Patel

Ultraviolet radiation can cause cancer through DNA damage — specifically, by linking adjacent thymine bases. Crystal structures show how the enzyme DNA polymerase η accurately bypasses such lesions, offering protection.

In this issue, two papers — by Silverstein *et al.*[1] (page 1039) and Biertümpfel *et al.*[2] (page 1044) — describe the crystal structure of the enzyme DNA polymerase η (Polη), which can efficiently and accurately overcome DNA damage caused by ultraviolet radiation. These data are of particular interest because they elucidate how the inactivation of Polη leads to XPV, a variant form of xeroderma pigmentosum — a type of severe skin cancer in humans.

DNA polymerase enzymes mediate DNA replication and repair. In eukaryotes (organisms such as animals, plants and yeast), at least 14 such polymerases, with diverse functions, have been identified[3]. For instance, DNA polymerases of the Y family specialize in DNA-lesion bypass. According to the polymerase-switch model[4], when a high-fidelity replicative polymerase encounters a DNA-distorting lesion, it stalls and is replaced by one or more lesion-bypass polymerases. The bypass polymerases transit the lesion and extend the DNA until the perturbation has been passed. The replicative polymerase then resumes its task of rapidly synthesizing the growing DNA chain.

Humans have four Y-family DNA polymerases: Polι, Polκ, REV1 and Polη. The crystal structures of ternary complexes — containing one of these polymerases, together with template and primer DNA, and the deoxyribonucleoside 5′-triphosphate (dNTP) positioned ready for addition to the growing primer chain — have been solved for Polι (ref. 5), Polκ (ref. 6) and REV1 (ref. 7). Together with the structures of the non-human Y-family polymerases Dpo4 (ref. 8) and Dbh (ref. 9), these structures have revealed that some features are unique to a particular Y-family member, whereas others are universal to all members.

For example, like other polymerases, all Y-family polymerases have a hand-like shape, with palm, fingers and thumb domains[10], as well as an active site with evolutionarily conserved carboxylate-containing amino-acid residues (aspartic acid and glutamic acid) and two divalent metal ions, usually magnesium ions (Mg^{2+}). In addition, being generally of low fidelity, Y-family polymerases have a spacious and solvent-accessible active site to facilitate lesion bypass[11]. This feature is in contrast to that of high-fidelity polymerases, the fingers of which close tightly on the nascent base pair to promote accurate replication[10,12]. What's more, Y-family polymerases have a domain termed the little finger, or PAD, which aids in gripping the DNA[11,13]. None of the human Y-family polymerases was found to be highly specialized for the error-free bypass of specific types of DNA damage, although the structures and biochemical evidence provided clues to the nature of the lesions that the enzymes are designed to process[5–7,11,13,14].

The most elusive and intriguing of the human bypass polymerases is Polη. On a functional level, it is essential for error-free bypass of a highly prevalent lesion that results from exposure to ultraviolet radiation, including sunlight[11,13]. The lesion is called a *cis–syn* thymine dimer (Fig. 1a, overleaf): two adjacent thymine bases on the same strand form two covalent bonds to produce an open-book-like

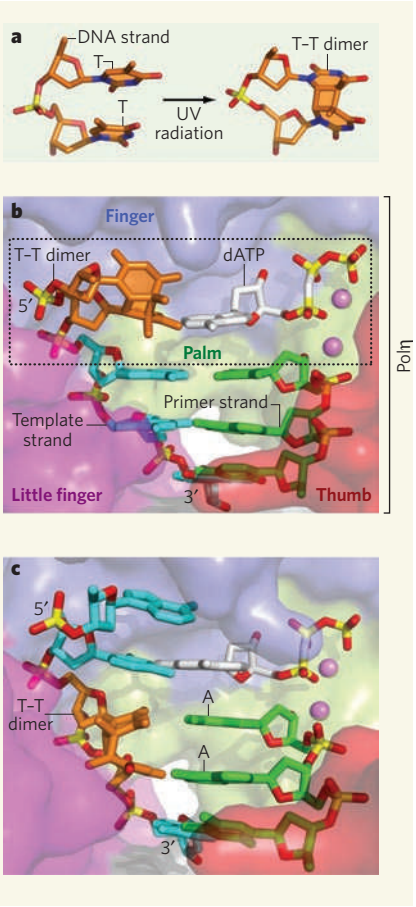

Figure 1 | Accurate lesion bypass by Polη. **a**, Ultraviolet (UV) radiation catalyses covalent linkages between two adjacent thymine (T) bases in a DNA strand so that they form a distorting mutagenic lesion, the T–T dimer. Backbone phosphorus, yellow; oxygen, red; nitrogen, dark blue. **b**, Biertümpfel *et al.*[2] report the structure of human Polη in a ternary complex containing dATP correctly positioned opposite the 3′ base of the thymine dimer in the template strand. This structure reveals an active site (the area within the dashed lines) that is enlarged compared with other Y-family polymerases and that accommodates the two covalently linked thymine bases to permit 'Watson–Crick' hydrogen bonding with the dATP, which is oriented for catalysis mediated by two metal ions (purple spheres). Silverstein and colleagues[1] present a related ternary complex of yeast Polη, uncovering a remarkably similar strategy for thymine-dimer bypass (not shown). Thymine dimer, orange; template bases, blue; primer bases, green; incoming nucleotide, white. **c**, The crystal structure of human Polη is shown in a ternary complex[2] in which the DNA has been replicated past the thymine-dimer lesion by the addition of two nucleotides (A). Unlike other lesion-bypass polymerases, Polη contains a molecular splint — a continuous protein interface between the core (palm and finger) and little finger domains, which cannot be seen in this view — that holds the growing duplex in its normal B-DNA conformation, allowing efficient and accurate extension past the thymine dimer.

structure, causing a distortion when incorporated in B-DNA — the most common conformation of DNA. Polη bypasses this lesion in an error-free manner, correctly incorporating an adenine base opposite each of the thymines in the covalently linked dimer[15,16]. How it can do this has remained a structural mystery.

Silverstein *et al.*[1] report two crystal structures of a yeast Polη in ternary complexes: one with incoming dATP and a DNA template strand that contains two normal thymines, and another with dATP and a template strand that contains a *cis–syn* thymine dimer. Biertümpfel *et al.*[2] present a similar structural pair for human Polη (Fig. 1b), together with additional structures showing the thymine dimer after replication has progressed so that two extra nucleotides have been incorporated (Fig. 1c). Together, the structures[1,2] show that key structural elements are conserved across the evolutionary span from yeast to humans. To solve these structures, clever mutational strategies were required to break crystal-packing forces that came into play in an earlier study[17].

The structures provide an elegant explanation for how the dimer is replicated in an error-free manner and for the curious fact that the dimer is as accurately replicated as unmodified thymines. Together with kinetic studies[2], they reveal how extension past the lesion can be efficiently carried out. What is the trick? Compared with other Y-family human polymerases, Polη has a more spacious active site because the polymerase core (palm and fingers) is rotated away from the little finger, allowing two nucleotides to be housed in the active site instead of just one. This is necessary because the two thymines that are linked as a result of ultraviolet-radiation-induced damage cannot be uncoupled. Moreover, van der Waals forces and hydrogen-bonding interactions specifically hold the coupled thymines so that they can be partnered with adenine, hence the higher fidelity for the lesion than for the undamaged thymine.

Another feature that is unique to Polη, accounting for the ready extension past the dimer, is the presence of a stiff spine, or 'molecular splint', that holds the growing duplex chain rigidly in its normal B-DNA conformation, without allowing slippage or other structural anomalies. In this respect, Polη differs from other Y-family polymerases, all of which have a gap between the core and the little finger instead of the continuous protein surface that creates the molecular splint. The open space, the volume of which differs among polymerases, allows greater flexibility and opportunity for error in the growing duplex. Gratifyingly, mutations that are observed in patients with XPV are explained by the structure of the human enzyme. For example, the arginine residue present at position 111 of Polη is part of the molecular splint, and mutation of the bases encoding this amino acid to produce histidine has been observed in patients with XPV and is likely to disrupt the splint.

Every open door leads to another door, and the same holds true for these fascinating Polη structures. Resolving the structures of binary complexes — without the dNTP — is essential to gain insight into conformational changes that may be induced by the binding of the dNTP. So far, all evidence suggests that the large conformational movement, that is, the closing of the fingers on dNTP binding, seen in high-fidelity polymerases does not occur in members of the Y family. But there are indications of more subtle conformational reorganizations, the details of which may depend on the specific bypass polymerase. These early movements need to be understood in order to delineate the mechanistic details of the ensuing chemical reaction of nucleotide addition.

The suggestions that Y-family polymerases in general are engaged in other forms of DNA repair besides lesion bypass are intriguing. For example, Polκ has recently been implicated in nucleotide-excision-repair synthesis[18]. Polη has also been implicated in the repair of DNA strand breaks, specifically in filling in gaps that are created by the displaced loops produced in homologous recombination[19,20]. And Biertümpfel and colleagues' structure suggests that another DNA-binding site on the other side of the enzyme may be used in this process[2].

It will certainly be exciting to determine how Polη is involved in these repair processes and in other cellular processes such as the generation of antibody diversity. Polymerase structure and function remains a research frontier despite the amazing progress that has been achieved in the past decade. ■

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