number') can be determined using a technique known as Voronoi tessellation[10], which involves the division of the glass's structure into regions centred on individual solute atoms. The basic units of the short-range order that emerges are various polyhedra[11] of around 9 to 13 atoms, with a solute atom in the middle. The precise form of these polyhedral clusters is controlled by the ratio of the effective sizes of solute and solvent atoms, and so changes according to the elemental constituents of the glass. Sheng and colleagues also find a moderate variation in coordination numbers (that is, a range of different, quasi-equivalent clusters) in the same material. This flexibility allows for a more efficient packing of 'soft' atoms without requiring an fcc structure.

Once the three-dimensional positions of the clusters have been mapped out, their topological packing can be determined by a technique known as common neighbour analysis[12]. In three of the metallic glasses investigated by Sheng and colleagues[2] — $Ni_{80}P_{20}$, nickel–boron ($Ni_{81}B_{19}$) and zirconium–platinum ($Zr_{84}Pt_{16}$) — the clusters pack with appreciable icosahedral medium-range order, regardless of the short-range order within the clusters. Each solute-centred cluster shares its solvent atoms with about 12 neighbouring clusters, forming 'super-icosahedra' of 70–80 atoms that are about 1.5 nm wide, or fragments of such structures. As does the fcc-packing model[6], this icosahedral packing of clusters generates cavities similar to those in the random dense packing model[3], into which additional solute species of different sizes may be introduced[13]. This allows bulk metallic glasses to form[14,15].

Sheng and colleagues' *ab initio* calculations indicate that, as the solute content of the metallic glass is increased and solute–solute nearest neighbours become numerically unavoidable, the connection between solute atoms becomes string-like. When the solute strings percolate, as in nickel–niobium glass ($Ni_{63}Nb_{37}$), the solute–solute connection begins to resemble a network, and a spectrum of atomic packing schemes that varies with solute radius and concentration is generated.

The modelling techniques used by the authors do have some inherent limitations. *Ab initio* molecular dynamics simulations can still, for example, only be performed on limited time and length scales because of present limits on computational power. The short time-window also means that the cooling ('quench') rates currently used in simulations are more than 10^{12} K s^{-1}, far above laboratory quench rates of 10^{3}–10^{6} K s^{-1}, restricting the ability of the atoms to sample all possible configurations as they cool.

Nevertheless, the work of Sheng *et al.*[2] will serve to establish a firmer picture of the structure of metallic glasses. This information is fundamental to further applications involving these materials, which are already used commercially because of their exceptional magnetic and mechanical properties. More extensive exploitation of their mechanical properties — in particular their elastic response up to 2% strain — depends on better understanding of their plastic deformation. This deformation, usually leading to failure of the material beyond the 2% elastic limit, is heterogeneous at ambient temperature[16], and occurs in highly localized thin shear bands associated with tiny areas of collective atomic mobility ('shear transformation zones'[17]) that can percolate across the cross-section of the glass. Contrary to the hardening observed in crystalline materials under strain, heterogeneous deformation in metallic glasses has the opposite effect owing to the destruction of medium-range order. Sheng and colleagues' investigations on exactly this scale could thus provide further insight into the effects of deformation, and ways to improve the mechanical properties of metallic glasses. ■

Alain Reza Yavari is at the Laboratoire de Thermodynamique et Physico-Chimie Métallurgiques, Institut National Polytechnique de Grenoble, BP 75, 38402 St-Martin-d'Hères Campus, France, and at the Centre Nationale de la Recherche Scientifique (CNRS) and European Synchrotron Radiation Facilities, Grenoble, France.
e-mail: yavari@ltpcm.inpg.fr

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DNA REPAIR

Tails of histones lost

André Nussenzweig and Tanya Paull

A double-stranded break in DNA can profoundly destabilize a cell's genome. But how does the cell recognize the damage and halt division until it can be fixed? The answer lies in the proteins that package and unravel DNA.

DNA damage induces cell-cycle checkpoints that transiently arrest progression through the cell-division cycle. This delay gives the DNA-repair machinery sufficient time to fix genomic damage before the cell cycle resumes. Two studies, one by Tsukuda *et al.*[1] published at the end of last year and one by Keogh *et al.* in this issue (page 497)[2], demonstrate that modifications to the DNA packaging around the break site help to coordinate DNA repair with cell-cycle checkpoints.

Double-strand breaks in chromosomal DNA are repaired either through direct end-joining or through a process known as recombination in which the broken ends are spliced to the corresponding undamaged DNA on a sister chromosome, and the break is filled in using the undamaged DNA as a template. DNA breaks that are not rapidly rejoined are chewed back by exonuclease enzymes so that a length of single-stranded DNA hangs out from the remaining DNA. These single-stranded intermediates perform at least two crucial functions: the exposed single strands are bound by Rad51 proteins that initiate the search for a complementary template from which to repair them, and they act as a signal to arrest the cell cycle. Resumption of the cell cycle following checkpoint arrest is generally concurrent with repair, suggesting that the elimination of single-stranded intermediates may also control exit from the checkpoint.

In the nucleus, DNA is wrapped about histone proteins to create nucleosomes, and then it is further twisted up into higher levels of packaging. This tight packing probably creates a structural barrier to molecules that recognize and respond to DNA damage. This problem seems to be solved by proteins that control the organization of the chromatin (that is, DNA and its associated proteins). Complexes of such chromatin-remodelling proteins have been found near double-strand breaks; for example, the INO80, SWR1 and NuA4 complexes in budding yeast bind within two kilobases of a break, and are needed for the DNA to be repaired[3–5]. It is proposed that the chromatin-remodelling complexes might unravel the packaging, allowing repair enzymes access to the DNA.

Tsukuda *et al.*[1] show that double-strand breaks do indeed cause a loss of nucleosomes from sequences within a few thousand base pairs of the break, with kinetics that coincide with those of the loading of Rad51 on to single-stranded DNA. The loss of nucleosomes was catalysed by INO80 and facilitated

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by a DNA-repair complex known as Mre11–Rad50–Xrs2, which is rapidly recruited to the break. Furthermore, in yeast strains with mutant Mre11 or INO80 the nucleosomes were not evicted, impairing the subsequent loading of Rad51 on to the DNA. Similarly, in mammalian cells, access of Rad51 to DNA breaks is facilitated by localized chromatin unwinding, which is mediated by the addition of acetyl groups to histones[6].

In addition to chromatin remodelling next to break sites, a histone protein known as H2AX is phosphorylated very rapidly in a large region surrounding the lesion[7]. Phosphorylation occurs at a conserved motif in the carboxy-terminal tail of H2AX. In budding yeast, phosphorylated H2AX (γH2AX) is found at least 25 kilobases to each side of a double-strand break induced in the genome, but is depleted within one to two kilobases of the break[8].

Although phosphorylation of H2AX precedes nucleosome eviction, Tsukuda *et al.*[1] found that it is dispensable for the local displacement of nucleosomes. So, what is the significance of the widespread chromatin modification distal to the break? Previous studies show that γH2AX is required for the large-scale accumulation of checkpoint proteins to the region around a break, and that loss of H2AX results in defective DNA repair and checkpoint signalling[9].

Keogh *et al.*[2] have discovered another function of γH2AX by asking how it reverts to its unphosphorylated state in budding yeast. Near the break, γH2AX is rapidly depleted, but farther away (5–20 kb) the γH2AX is lost only after the binding of the broken strands with the intact donor template. The authors found that a complex (HTP-C) containing the Pph3 phosphatase enzyme specifically regulates the dephosphorylation of γH2AX; and it seems that the mammalian relative of Pph3, protein phosphatase 2A (PP2A), down-regulates γH2AX similarly[10].

Despite having high constitutive levels of γH2AX during normal replication, yeast cells deficient in Pph3 were not growth retarded[2]. Moreover, they could trigger the DNA-damage checkpoint and repair DNA breaks. Nevertheless, the activation of Rad53 and Rad9 — the signalling molecules that initiate the checkpoint — persisted much longer than in normal yeast strains, even after the bulk of DNA damage was repaired. Consequently, checkpoint recovery in Pph3-deficient cells was profoundly retarded. Notably, failure to inactivate the checkpoint was a direct consequence of persistent H2AX phosphorylation because elimination of both γH2AX and Pph3 led to normal recovery kinetics and inactivation of Rad53 and Rad9.

How does the formation of γH2AX over a large chromatin domain keep the DNA-damage checkpoint active? One possibility is that DNA-damage response factors may remain activated and bound to the chromatin as long as γH2AX persists. Consistent with this model, in mice that lack H2AX, checkpoint proteins fail to sustain their interaction with DNA breaks at later time points after DNA damage[11], and cells from these mice exhibit a defect in the checkpoint that acts between the G2 and M phases of the cell cycle[9]. Keogh *et al.* similarly found that H2AX-deficient yeast deactivated Rad53 and escaped the arrest more rapidly than normal cells[2]. Reversal of H2AX phosphorylation during DNA repair might disrupt associations between checkpoint proteins and the chromatin surrounding DNA damage, interrupting the checkpoint-signalling cascade.

A twist in the tale is that in yeast lacking Pph3, H2AX remains phosphorylated and yet is still displaced from the entire 50-kilobase region around the break as repair occurs. Remarkably, the checkpoint arrest signal is maintained in these yeast even once the break is repaired and the γH2AX molecules are redistributed within the nucleus. If the displaced γH2AX primarily remains soluble, this would mean that DNA-damage signalling can occur 'off' the chromatin. So, at least in budding yeast, dephosphorylation of γH2AX might be happening after its removal from the chromatin (Fig. 1).

In mammals, it is possible that the attenuation of H2AX may be mechanistically distinct, because the dephosphorylating enzyme PP2A seems to bind to γH2AX on damaged chromatin[10], and γH2AX is normally protected from dephosphorylation through interactions with its binding partner MDC1 (ref. 12), a protein that has no direct counterpart in yeast. There may also be other mechanisms of removing γH2AX, for instance the Tip60 complex in the fruitfly *Drosophila* acetylates phospho-H2Av (the *Drosophila* relative of H2AX), leading to its exchange with unphosphorylated H2Av[13].

The phosphorylation of H2AX is among the earliest responses to DNA damage, and controls the widespread accumulation of checkpoint response proteins to large chromatin regions surrounding break sites. Nevertheless, it is not required for the rapid recruitment of DNA-repair factors and chromatin remodelling complexes to the actual site of DNA damage[11]. Tsukuda *et al.*[1] and Keogh *et al.*[2] demonstrate that dephosphorylation of γH2AX and its ejection from chromatin regions distal to the break site are crucial in restarting the cell cycle. In this way, the addition and removal of the phosphate group on the tail of H2AX creates a molecular switch that helps to maintain genomic stability. ■

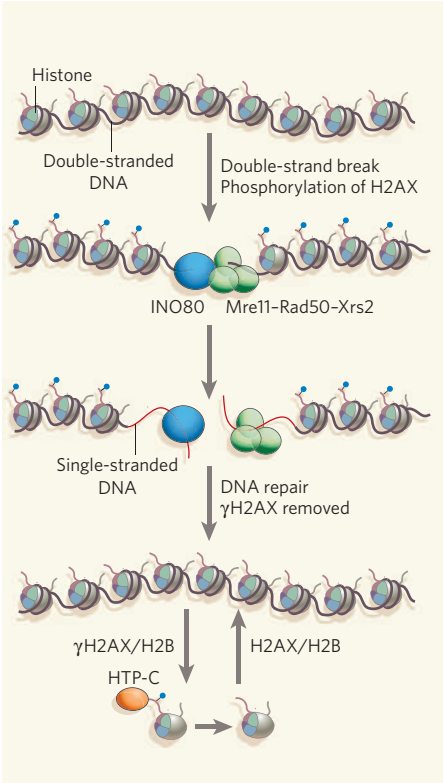

Figure 1 | Chromatin remodelling around double-stranded DNA breaks. Tsukuda *et al.*[1] find that the Mre11–Rad50–Xrs2 DNA-repair complex and the INO80 chromatin remodelling complex are rapidly recruited to breaks and evict nucleosomes in the immediate vicinity of the break. Meanwhile, histone H2AX in a large region around the break is phosphorylated (forming γH2AX, blue dots). Exonucleases chew back one strand of the DNA, forming a single-stranded overhang (red). Before completion of repair by recombination, an unknown remodelling complex mediates the removal of γH2AX from 50 kilobases of chromatin; Keogh *et al.*[2] show that subsequently γH2AX is dephosphorylated by a protein complex containing the enzyme Pph3, an event that is crucial for release from the damage checkpoint. It is unclear whether entire nucleosomes (shown) or just certain histones (γH2AX/H2B dimers) are displaced.

André Nussenzweig is in the Experimental Immunology Branch, National Institutes of Health, Bethesda, Maryland 20892, USA.
e-mail: andre_nussenzweig@nih.gov
Tanya Paull is in the Department of Molecular Genetics and Microbiology, University of Texas at Austin, 1 University Station A5000, Austin, Texas 78712, USA.
e-mail: tpaull@icmb.utexas.edu

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