Fanconi proteins get histones moving

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FANCD2 is a central player in the DNA damage response, particularly in the repair of spontaneous and chemotherapy-induced DNA interstrand crosslinks. New work by Sato *et al* (2012a) in *The EMBO Journal* reveals an unexpected novel function of the FANCD2 protein as a histone chaperone, which appears to be essential for this DNA repair role.

Mutation in *FANCD2* or 1 of the 14 other *FANC* genes causes the recessive leukaemia-prone disorder Fanconi anaemia (FA). The disease can be completely recapitulated in *Fancd2*-knockout mice, but only when elevated levels of endogenous DNA crosslinks are induced by simultaneous deletion of aldehyde dehydrogenase 2 (Langevin and Crossan, 2011). The phenotypes of FA are thus the product of accumulated, unrepaired DNA interstrand crosslinks (ICLs) that prevent replication by covalently linking DNA strands. How the FANC proteins interact and respond to environmental or chemotherapy-induced DNA crosslinks has been central to understanding this disorder.

FANCD2 sits at the centre of the puzzle, as it is a substrate for monoubiquitination by the upstream FANC proteins (FANCA, B, C, E, F, G, L, and M) and a landing pad and activator for the downstream DNA repair-associated FANC proteins (FANCD1, J, N, O, and P) (Deans and West, 2011). *In-vitro* studies in *Xenopus laevis* egg extracts show that FANCD2 accumulates in regions adjacent to a site-specific ICL in a replication-dependent manner (Knipscheer *et al*, 2009). In cells, the monoubiquitinated form of FANCD2 localizes to DNA-damage induced nuclear foci, and specifically interacts with the FAN1 nuclease that may be involved in cutting DNA adjacent to the crosslinked region (MacKay *et al*, 2010). Furthermore, chromatin retention of FANCD2 is required for correct localization of the BRCA1 and BRCA2 breast cancer tumour suppressors that also play a key role in the repair of ICLs (Wang *et al*, 2004).

FANCD2 heterodimerizes with the evolutionarily related partner protein FANCI. The recently solved crystal structures of FANCD2 and FANCI revealed an overall saxophone-like shape, single- and double-strand DNA binding regions, and the monoubiquitination site buried within the FANCI-heterodimerization interface (Joo *et al*, 2011). Sato *et al* (2012a) noticed that a region within one of the C-terminal α helical-structured solenoids of FANCD2 also contains sequences homologous to those found in a class of proteins termed as histone chaperones (Figure 1). Using a combination

of biochemical and cell-based assays, they could subsequently show that this region binds histones H3/H4 to promote the mobilization of histones within chromatin (Sato *et al* 2012a).

Recombinant histones H3:H4 form a tetramer that is stable even in the absence of DNA, and this property was used to show that human FANCD2 within cellular extracts, or chicken FANCD2 from a recombinant source is able to interact with H3:H4 independently of DNA. The authors went on to show that this interaction could promote the loading of core histones onto naked plasmid DNA. Recombinant FANCD2 could also promote the formation of a minimal nucleosome complex around a 195-bp fragment at a rate similar to that observed for the canonical histone chaperone Nap1.

Protease mapping was used to confirm the requirement for the C-terminal α -solenoid region to histone interaction and

Figure 1 Functions of FANCD2 at a DNA ICL. The 'saxophone' crystal structure of the FANCD2:FANCI heterodimer is hypothesized to enclose a region of crosslinked DNA that is approached on either side by a replication fork (leading strand is identified by an arrow head). The ubiquitination site of each protein is at the interface of the heterodimer. Interaction of FANCD2-Ubiquitin with the UBZ domain of FAN1 nuclease may guide the specific cutting of DNA 5' to the ICL. The C-terminus of FANCD2 contains a histone chaperone activity that promotes the eviction, exchange or assembly of histones in regions adjacent to the ICL.

nucleosome formation. In fact, the C-terminal third of FANCD2 (residues 953–1439) was sufficient for histone mobilization *in vitro*. This fragment does not contain the monoubiquitination site at K561 and it appears that ubiquitination was not required for the histone chaperone activity *in vitro* when using full-length protein.

To investigate the consequences of loss of FANCD2 or FANCD2 ubiquitination on histone mobility in the nucleus, GFP-H3 movement was followed using fluorescence recovery after photobleaching (FRAP). Intriguingly, movement of nuclear histone H3 content was reduced in the absence of FANCD2 only when cells were saturated with mitomycin c-induced DNA ICLs (however with the caveat that it remains unclear if the change in histone mobility is limited to sites of damage). Contrary to what was seen *in vitro*, ubiquitination of FANCD2 is required for changes to histone localization in cells, perhaps to promote the increased chromatin concentration of the protein required for histone mobilization activity. As deletion of the H3:H4-binding region of FANCD2 not only affected histone mobilization but also led to defects in FANCD2 ubiquitination and chromatin targeting, the authors screened several FA-associated mutations in FANCD2 for their ability to rescue histone mobilization. Surprisingly, FANCD2 with a mutation in the N-terminal half of the protein (R302W) could be both ubiquitinated and chromatin recruited after mitomycin c treatment, but was unable to restore the mobility of GFP-H3. Despite R302 being located a long way from the putative H3:H4 interacting region at residues 1268–1389 in the primary amino-acid sequence, the crystallography data confirm that protein folding brings R302 into close proximity of this region in the tertiary structure (Joo *et al*, 2011; Figure 1). *In-vitro* studies confirmed that FANCD2-R302W had reduced H3:H4 interaction, but normal DNA binding activity. Because this protein is correctly localized in DNA damage-induced nuclear foci, the misfolding presumably only affects the function of FANCD2 in histone mobilization. Thus, if R302W indeed represents a true separation-of-function mutation, then the newly described histone chaperone functions of FANCD2 are downstream of ubiquitination and potentially required for subsequent steps of DNA repair.

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This study raises important questions about the role of chromatin dynamics in the repair of DNA ICLs. Histones around DNA damage sites are extensively modified by phosphorylation, ubiquitination, SUMOylation, and acetylation. These histone modifications act as checkpoint activation signals, binding sites for DNA repair proteins, and suppressors of aberrant transcription (van Attikum and Gasser, 2009). Can such modifications change the histone's ability to be 'chaperoned' by FANCD2 or other proteins? Do histones also have to be moved out of the way so that DNA repair proteins can do their job? Or, are histones reloaded or replaced around DNA damage by FANCD2 to reestablish chromatin integrity? The binding of replication and recombination intermediates by FANCD2 places it in a position of influence, with direct control over local chromatin dynamics during DNA repair. Furthermore, FANCD2 has the ability to decide who replaces any displaced histones—with the ability to directly recruit the FAN1 and SLX4 nucleases and the BRCA1 and BRCA2 recombination controllers (Wang *et al*, 2004; MacKay *et al*, 2010).

Finally, this study examines the histone chaperone activity of FANCD2 on double-stranded, undamaged DNA. Future work should look at its ability to load and shuffle histones in chromatin around structures containing replication intermediates or DNA crosslink damage. Also still missing is a definitive explanation of what monoubiquitination is actually doing to FANCD2 and FANCI, including in its newfound property as a histone chaperone. As Sato *et al* (2012b) have also recently given researchers a new method for producing recombinant ubiquitinated FANCD2, the final pieces in this Fanconi puzzle appear tantalizingly close.

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Conflict of interest

The author declares that he has no conflict of interest.

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