

Differential Activation of Core Complex Member FANCM and Downstream Target FANCD2 during the DNA Damage Response

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Objective: According to the current model of the FA pathway, the FA core complex acts upstream of FANCD2 and controls its monoubiquitination (FANCD2-L) in response to DNA damage, a step required for activation of the FA pathway. Meetei *et al.* (*Nat Gen.* 2005) reported that FANCM, member of the FA core complex, is also modified by phosphorylation (FANCM-L) in response to DNA damage. We asked if the induction of FANCD2-L and FANCM-L is stimulated and regulated by the same or by different mechanisms.

Methods: To mimic defined types of DNA damage, we incubated different DNA mini-structures in *Xenopus* egg extracts and determined which types of DNA damage trigger modification (monoubiquitination and phosphorylation, respectively) and DNA recruitment of FANCD2 and FANCM. We used immunodepletion and complementation with recombinant full-length *Xenopus* proteins to determine how FANCD2 and FANCM depend on each other as well as on the ATR/ATRIP complex in response to DNA damage.

Results: We showed recently that FANCD2 is monoubiquitinated (FANCD2-L) and recruited in response to double-stranded linear and forked DNA structures (Sobeck *et al.*, 2007). In contrast, these same DNA structures do not specifically trigger phosphorylation of FANCM (FANCM-L) or recruitment of FANCM to DNA, indicating that FANCM and FANCD2 are activated by different types of DNA damage. However, both FANCD2 and FANCM are activated in the presence of circular dsDNA (plasmid DNA), suggesting that structural aspects of the circular DNA molecule trigger formation of both FANCM-L and FANCD2-L. Interestingly, when egg extracts are depleted of ATR or its binding partner ATRIP, the DNA-induced formation of FANCM-L – but not FANCD2-L – is suppressed, suggesting that DNA-stimulated FANCM-L formation is dependent on the ATR/ATRIP complex. Surprisingly, in a time course following addition of plasmid DNA to egg extracts, we find that activation of FANCD2 precedes the activation of FANCM, suggesting that FANCD2 might act upstream of FANCM-L induction. To test this, we depleted FANCD2 from egg extracts and analyzed the plasmid-DNA induced formation of FANCM-L. We found that FANCM-L formation is inhibited in the absence of FANCD2.

Conclusions: Our study shows that FANCD2 and FANCM are activated by different DNA damage types and suggests that modification of FANCM is part of a feedback mechanism that signals from FANCD2 back to one of the core complex members, FANCM.

Translational Applicability: Understanding how FA proteins are controlled within the DNA damage response by upstream players and by each other is a fundamental requirement for deciphering the FA pathway, which in turn will enable us to specifically screen for drugs that can re-activate crucial FA pathway steps.