# Conscious uncoupling between FANCI and FANCD2 in DNA repair

November 15, 2015

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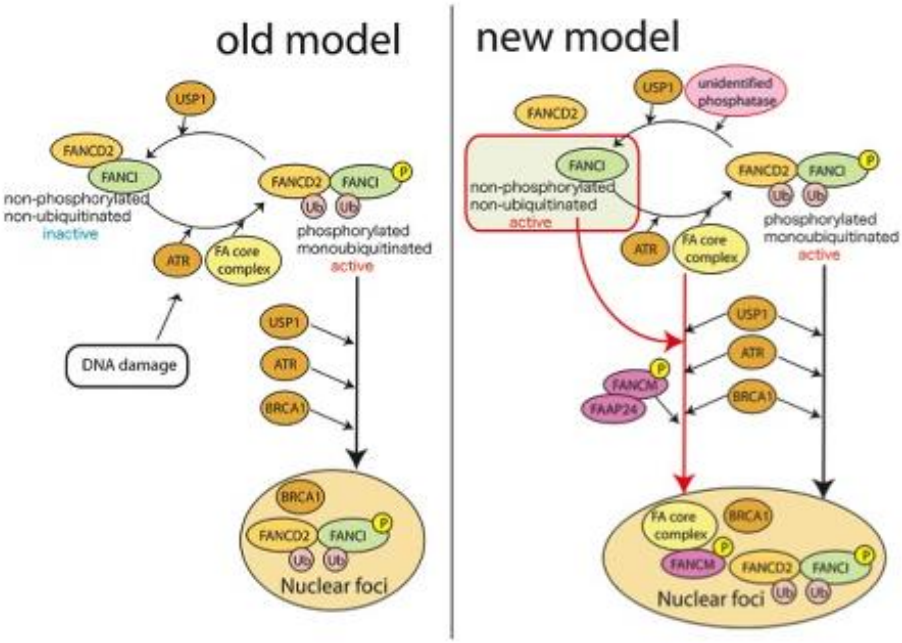


New model of the FA pathway activation. Non-phosphorylated, non-ubiquitinated FANCI plays a critical role in recruitment of the FA core complex at sites of DNA damage. ATR phosphorylates substrates including FANCI and FANCM. The FA core complex monoubiquitinates FANCD2 and FANCI following FANCI phosphorylation, and USP1 deubiquitinates FANCD2 and FANCI. FANCI, FANCM, USP1, and BRCA1 positively regulate recruitment of FA core complex and FANCD2 at sites of interstrand crosslinks (ICLs). Monoubiuqitination of FANCD2 is required for retention of FANCD2–FANCI at sites of ICLs. Monoubiquitinated FANCD2 is also required for downstream events, such as recruitment of various nucleases (not depicted). For the regulation of FANCI phosphorylation/dephosphorylation, ATR and unidentified phosphatase are involved. In this new model, the FANCI ubiquitination/deubiquitination and phosphorylation/dephosphorylation cycle is important for proper activation of the FA pathway. In the old model, only monoubiquitinated and phosphorylated FANCI is considered an active form.

*Image provided Dr. Toshiyasu Taniguchi.*

The study of the Fanconi anemia (FA) pathway has helped elucidate the molecular basis for several biological processes, including DNA repair, protein modification and cancer susceptibility. FA is a clinically heterogeneous genetic disease that results in bone marrow failure, congenital skeletal defects as well as early onset of cancer. The importance of DNA repair was recently highlighted by the award of this year's Nobel Prize in chemistry for mechanistic studies of DNA repair. Several FA genes have been identified and some of these are also breast and ovarian cancer susceptibility genes, such as *BRCA1* and *BRCA2*. Eight FA proteins form a protein complex known as the FA core

complex that is required to both detect and respond to DNA lesions. However, how the FA core complex is recruited to DNA lesions is not well understood. A new Fred Hutch study by the Taniguchi Laboratory (Human Biology and Public Health Sciences Divisions), led by former post-doctoral fellow Dr. Maria Castella and published in *PLOS Genetics*, developed an improved method to visualize the FA core complex, which enabled a detailed study of its regulation.

"The Fanconi anemia pathway regulates DNA repair and plays a critical role in determining sensitivity of cancer cells to commonly used chemotherapeutics, such as cisplatin", said Dr. Toshiyasu Taniguchi. The investigators first refined immunostaining methods to detect nuclear foci of FA core complex subunits revealing that the FA core complex foci formed during the S and G2 phases of the cell cycle and relied on the whole FA complex. The FA core complex is an ubiquitin ligase that monoubiquitinates FANCD2 and FANCI. Because FANCD2 and FANCI form a complex and their monoubiquitination is required for both their DNA localization and efficient DNA repair, the investigators were surprised to find that FANCI, but not FANCD2, was required for FA core complex foci formation. This novel FANCI function required neither its monoubiquitination nor its phosphorylation. Next, the authors searched for additional factors required for the formation of FA core complex foci. To this end, they assayed FA foci in cells lacking one of several proteins known to be involved in the FA pathway. These experiments revealed that depletion of either BRCA1 or USP1 resulted in marked decrease in the formation of FA core complex foci. USP1 is a deubiquitinase, so the authors asked whether FANCI was the relevant USP1 substrate. Consistent with this notion, they found that a non-ubiquitinatable version of FANCI (K523R mutant) partially rescued the formation of FA foci in USP1-deficient cells. In summary, a careful analysis of FA core complex formation revealed new regulatory mechanisms for FA core complex recruitment, including novel roles for several proteins (BRCA1, USP1 FANCI, ATR) that were previously thought to act in a more restricted manner.

Said Dr. Taniguchi "It is believed that activation of this pathway requires monoubiquination and phosphorylation of FANCI. Unexpectedly, we found that non-phosphorylated, non-ubiquitinated FANCI plays a critical role in recruitment of the FA core complex at sites of DNA damage, suggesting that deubiquitination and dephosphorylation of FANCI protein is also critical for the function of the pathway. This finding changes our current view of the Fanconi anemia pathway."

Funding for this study was provided by the National Institutes of Health and the Howard Hughes Medical Institute.

Castella M, Jacquemont C, Thompson EL, Yeo JE, Cheung RS, Huang J-W, Sobeck A, Hendrickson EA, Taniguchi T. 2015. FANCI regulates recruitment of the FA Core complex at sites of DNA damage Independently of FANCD2. PLoS Genet. 11(10):e1005563. doi:10.1371/journal.pgen.1005563.