

Summary: "A Novel Ubiquitin Ligase is deficient in Fanconi Anemia"  
Nature Genetics, Vol 35, 165-170

In this paper the authors are looking for a protein that could act as a ubiquitin ligase in the monoubiquitination of FANCD2. Previous work had never found a component of the Fanconi anemia proteins that contained any sort of ligase activity. In their first figure, they show the isolation of a complex with several 'new' proteins associated. One of these proteins was identified as PHF9. The data in figure one shows that this protein is a stable part of the complex because it not only immunoprecipitates with other proteins in the complex, but other proteins also IP with it. This is true when using antibodies to PHF9 or to a flag when flag-tagged PHF9 was used.

The next figure showed that levels of PHF9 were significantly lower in lymphoblasts taken from people with three Fanconi anemia complementation groups. PHF9 was found in both the cytoplasm and nucleus, but its nuclear localization seemed to depend on FANCA. Since this is similar to what has been shown for other proteins in the Fanconi anemia complex, it seems that PHF9 is also a part of this complex.

**The complex is necessary for PHF9 nuclear localization.**

Additionally, PHF9 has several motifs that are characteristic of E3 ligases and proteins known to carry out protein/protein interactions. The PHD zinc finger motif in PHF9 has two conserved cysteines that are thought to distinguish a ligase from a non-ligase. Ligase activity was also found in GST fusion proteins containing the PHD finger of PHF9. However, when these cysteines were mutated to alanine, ligase activity was lost.

When PHF9 was knocked down using siRNA, it was found that FANCD2 was not monoubiquitinated or distributed to the nucleus

**They found that PHF9 does not colocalize with other proteins involved in DNA repair in specific foci inside the nucleus (see the introduction for the class).**

at levels comparable to wildtype cells. Chromosomal aberrations, a known problem in Fanconi anemia cells, were also seen in the siRNA knockdowns on PHF9.

Finally a screen was performed on individuals with Fanconi anemia but with no known mutations to cause the lack of FANCD2 monoubiquitination.

In fact, they had to show that patients of Fanconi Anemia must exist, because of defects in PHF9. This same disease can happen in people who carry deficient alleles in either one or more of the FA complex-encoding genes. Therefore, if PHF9 is also an important part of the complex, cases of people with FA because of carrying a deficient form of PHF9 should also exist. This is always the strongest evidence for a deficiency in a gene being responsible for a disorder/disease.

One cell line showed a mutation in PHF9 that removed the PHD finger. Since the PHD finger is thought to contain the ligase activity, more evidence shows that PHF9 serves as an E3 ligase for the Fanconi anemia complex. —because it can't monoubiquitinate FANCD2.

Thus the authors conclude that all the members of the Fanconi anemia core complex are necessary for the ubiquitination of FANCD2, but they have now identified a ligase that is part of this complex and is capable of catalyzing the ubiquitination reaction.

Good job!

They also show that FANCD2 monoubiquitination is independent of the other E3 ligase present in the DNA repairing foci, BRCA1 (which by the way is the protein defective in families with hereditary breast adenocarcinoma).