nesis (Lozano and Zambetti, 2005). These data are offset by the knowledge that *p21* mutations in human cancers are rare. Besides the possibility of redundant function of other p53 targets, another explanation for the contradictory data is that decreases in p21 stability through deletion or mutation of WISp39 are more common than p21 alterations in tumor development. The regulation of Wisp39 as a function of the cell cycle is also likely.

Counterintuitive to the role of p21 in cell cycle arrest, p21 has also been implicated as a positive regulator of cell survival. For example, loss of *p21* sensitizes cells to undergo uncoordinated DNA replication and death induced by anticancer drugs (Waldman et al., 1996). Because these cells tolerate expression of p21 in the first place, the p21 in these cells may not be functional in its cell cycle-inhibiting capacity. The phosphorylation status of p21 differs in different cell lines and may regulate these activities (Li et al., 2002). Nonetheless, since p21 is overexpressed in some advanced human tumors, downregulation of p21 protein may facilitate more efficient chemotherapy (Seoane et al., 2002). The newfound role of Hsp90 and WISp39 in the stabilization of p21 certainly adds more validity to the belief that targeting Hsp90 could be used to treat cancer, since inhibition of Hsp90 may reduce p21 levels. WISp39 confers specificity on the ability of Hsp90 to promote stability of p21, so we could imagine that an agent that specifically blocks WISp39 interaction with Hsp90 will serve only to target p21 degradation without affecting other Hsp90 targets. This scenario may be useful in some treatment schemes where tumor cells are dependent on p21 for survival.

Many questions remain to be addressed. How does this complex protect p21 from degradation? How does the trimeric complex allow p21 interaction with the cyclin-cdk machinery? Does it affect p21 phosphorylation and interactions with other proteins? This study raises a number of intriguing questions that will need to be addressed in the future.

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# "Dub"bing a tumor suppressor pathway

**The autosomal recessive disease Fanconi anemia (FA) causes bone marrow failure and a hugely increased propensity to develop cancer. Cells from FA patients are prone to chromosome breakage, indicating that FA gene products are required to ensure genomic integrity. Most of the identified FA proteins are components of a nuclear complex whose principal function is to activate FANCD2 by monoubiquitination. Monoubiquitinated FANCD2 accumulates at sites of genome damage, where it probably functions to facilitate DNA repair. A recent paper in *Molecular Cell* (Nijman et al., 2005) reports the identification of an enzyme that is responsible for regulating the FA pathway by deactivating FANCD2.**

Pioneering and painstaking work over the last 15 years has established that mutations in up to 11 different genes could lead to FA (Joenje et al., 1997; Strathdee et al., 1992). The identity of nine of these genes is now known, and recent studies have confirmed the genetic view that they all code for components of a single tumor suppressor pathway. Although the primary sequences of the FA proteins reveal very little about how they function, we know that most of them (FANCA, B, C, E, F, G, and L) interact to form a nuclear complex (FA nuclear complex) (reviewed in Joenje and Patel, 2001; D'Andrea and Grompe, 2003). However, not until the publication of a landmark paper in 2001 did we develop a comprehensive outline of the FA pathway (Garcia-Higuera et al., 2001). This seminal study showed that the FA nuclear complex is essential for the activation of a newly identified key FA protein, FANCD2. The activation step resulted in the conjugation of one ubiquitin polypeptide to a single specific lysine residue (K561) on the FANCD2 protein. The consequences of this modification are to direct FANCD2 to DNA replication or damage-induced nuclear foci. It is very likely that at these sites, FANCD2 directs DNA repair. Despite the evident progress in the FA field over the last few years, there are still many questions that need to be resolved if we wish to gain a complete molecular understanding of the FA pathway. We will need to know more about the precise DNA repair activity in which the FA proteins participate. We will need to understand the functional relevance of the interactions between the "core" FA pathway and other tumor sup-

pressor gene products such as BRCA1, BRCA2 (readers should be aware that biallelic germline mutations in BRCA2 do lead to a phenotype that is akin to FA; Howlett et al., 2002), ATM, NBS1, and the Bloom's syndrome helicase. Finally, we will need to establish how the FA pathway is regulated in cells, what is the precise mechanism for switching it on, and how is it switched off. It is this important latter question that is addressed in a paper published in a recent issue of *Molecular Cell* (Nijman et al., 2005).

A collaborative study between Bernard's and D'Andrea's groups describes a novel approach to identify a modulator of the FA pathway. As mentioned above, the activation of FANCD2 by monoubiquitination is a key step; loss of this modification disables the whole pathway. Like phosphorylation, monoubiqutination is a dynamic and reversible process whereby an enzyme cascade conjugates ubiquitin to a target protein, and a family of enzymes, the *de ub*iquitinating enzymes, or DUBs for short, are potent at removing this modification. A gene family-specific siRNA library was used to screen for siRNA that results in an accumulation of activated FANCD2. These screens lead to the identification of siRNA that resulted in the persistence of monoubiquitinated FANCD2. The siRNA corresponded to the USP1 gene, and further studies with additional siRNA confirmed that they specifically led to the knockdown of this DUB enzyme. The USP1 gene and protein is under cell cycle regulation, accumulating throughout the S phase and diminishing near the point at which FANCD2 is deubiquitinated. Following DNA damage, both USP1 and FANCD2 are bound to chromatin, where they interact. Some functional work indicates that USP1 knockdown reduces chromosome breakage induced by mitomycin C treatment, perhaps because of an attenuated inactivation of FA pathway. Collectively, the data supports a key role for USP1 in shutting down the FA pathway, acting directly on ubiquitinated FANCD2 on chromatin (Figure 1).

However, this study raises a raft of new questions. Why isn't all of the FANCD2 that is associated with USP1 deubiquitinated—is there another level of regulation that impacts on the activity of the enzyme? Is FANCD2 the only substrate for this enzyme, or are other monoubiquitinated proteins such as PCNA also its substrates? Recent studies have indicated that the FA proteins facilitate some forms of homologous recombination and error-prone repair (Niedzwiedz et al., 2004; Yamamoto et al., 2005); does inactivation of USP1 lead to an enhancement of these repair processes? Although FA is a rare condition, somatic inactivation of the FA pathway occurs in sporadic cancers (Taniguchi et al., 2003; Tischkowitz et al., 2003). Could induction of USP1 reduce the efficacy of the FA DNA damage response in some instances? Finally, modulating the activity of the FA pathway has obvious therapeutic implications. Its inactivation leads to marked sensitivity to certain chemotherapeutic agents such as cisplatin. Getting a better molecular understanding of how the pathway is switched on or off would provide potential therapeutic targets. The current work now sets the stage nicely for studies to unravel the regulation of this conserved and fundamental tumor suppressor pathway.

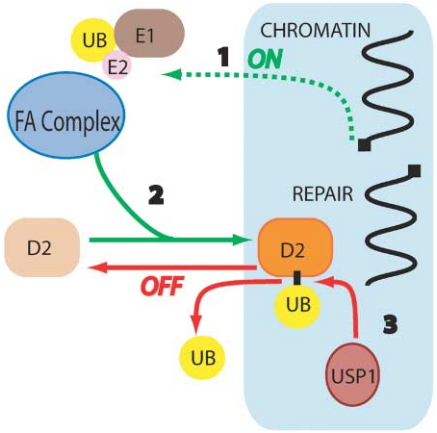


**Figure 1.** Current outline of the FA pathway

(1) DNA damage results in the activation of the FA pathway; this step is not well understood. (2) The FA nuclear complex consisting of most of the known FA proteins activates FANCD2 by the conjugation of a single ubiquitin residue. Modified FANCD2 binds to chromatin surrounding damaged DNA, where it may function to direct DNA repair. (3) At this site, USP1 deubiquitinates FANCD2, resulting in its inactivation and release from chromatin.

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