p21 stability: Linking chaperones to a cell cycle checkpoint

Progression through the cell cycle is regulated by numerous proteins, one of which is the cyclin-dependent kinase inhibitor, p21. A new study identifies a novel protein complex that stabilizes p21. The stability of this complex is critical in effecting the p53-mediated cell cycle checkpoint.

Transition through the cell cycle involves a series of precise events that ensure order (DNA replication, spindle assembly, nuclear division, and cytokinesis) and directionality. This transition is controlled by numerous proteins whose activities and/or levels oscillate throughout the cell cycle. The first oscillators discovered, the cyclins, increase in level prior to mitosis and fall coincidently with cytokinesis (Murray, 2004). Cyclins interact with specific *c*yclin-*d*ependent *k*inases (cdks) to phosphorylate key regulators of the cell cycle. Inhibitors of the cyclin-cdk complexes were subsequently identified, one of which is p21. Loss of these negative regulators of the cell cycle contributes to increased cell proliferation, which may in turn lead to tumorigenesis. The simultaneous discovery of p21 as the first cdk inhibitor and as a gene transcriptionally regulated by the p53 tumor suppressor was very exciting, and bridged the function of a tumor suppressor with the cell cycle (el-Deiry et al., 1993; Harper et al., 1993).

The cyclin-cdk complexes are also regulated by proteolysis via ubiquitination, which provides exquisite control of protein levels and function. Ubiquitination-mediated protein degradation involves a succession of steps that covalently attach multiple ubiquitin molecules to a substrate, thereby targeting that substrate for degradation. First, ubiquitin is activated by the ubiquitin-activating enzyme E1. Subsequently, a *ubi*quitin-*c*onjugating enzyme (called UBC or E2) transfers the ubiquitin molecule to a ubiquitin protein ligase (E3) that covalently attaches ubiquitin to the targeted substrate at specific lysine residues. The ubiquitin protein ligases provide the target specificity in the system, and key E3 complexes such as the *a*naphase-*p*romoting *c*omplex (APC/C) and *S*kp1/*C*ullin/*F* box protein-related complex (SCF) regulate transition through the cell cycle (Vodermaier, 2004). The first identified, APC/C, controls degradation of securin, the protein that maintains the cohesion of sister chromatids prior to separation, and numerous mitotic kinases. Additionally, the SCF complex is responsible for degrading G1 cyclins, the cdk inhibitors $p27^{Kip1}$ and $p57^{Kip2}$, and potentially p21 in S phase.

Although p21 is degraded by the proteosome, the ubiquitination of p21 has been controversial, since mutation of all lysine amino acids in p21 did not stabilize p21. Recent studies have shown, however, that ubiquitination occurs at the N terminus of p21 by a unique mechanism (Bloom et al., 2003). The discovery of a novel mechanism for regulating p21 protein stability, described in a recent issue of *Molecular Cell*, is highlighted here.

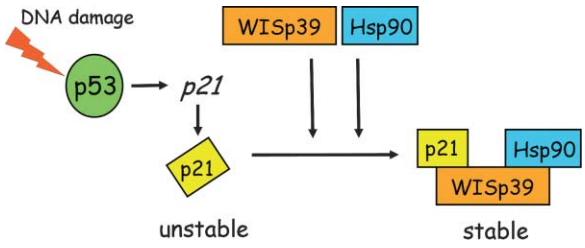

Figure 1. Regulation of p21 at multiple levels

In response to DNA damage, p53 activates transcription of the *p21* gene. An unstable p21 protein is made whose stability is increased by interactions with WISp39 and Hsp90.

Using a two-hybrid screen, Jascur et al. (2005) identified a novel protein, WISp39, that binds p21 and increases p21 stability in cell culture. Several observations indicate that the interaction occurs in vivo. First, other known p21-interacting proteins, PCNA for example, were identified in the screen. Second, endogenous p21 and endogenous WISp39 coimmunoprecipitate, indicating they form a complex. WISp39 binds the N terminus of newly synthesized p21, the region that is ubiquitinated, and may in fact block ubiquitination of p21, although this has not been shown. Moreover, WISp39 contains a TPR (*t*etratrico*p*eptide *r*epeat) domain that allows recruitment of the heat shock response protein Hsp90. Hsp90 is a chaperone responsible for correct folding, function, and stability of various proteins (Chiosis et al., 2004). TPR domains are found in other Hsp90 binding proteins that are called cochaperones. Importantly, mutation of the TPR domain in WISp39 results in loss of interaction with Hsp90, followed by p21 degradation, suggesting that the trimeric complex consisting of p21, WISp39, and Hsp90 is responsible for correct p21 folding and protein stability.

The p53 tumor suppressor is a DNA damage response protein that transcriptionally activates numerous genes (Vogelstein et al., 2000). The protein products of these genes arrest the cell cycle or induce cell death. As such, alterations in *p53* are one of the most common events in tumorigenesis. The first transcriptional target of p53 identified was the cdk inhibitor p21. That p53 transactivation of *p21* does not necessarily yield increased p21 protein levels is another important aspect of the study described by Jascur et al. (2005). DNA damage by ionizing radiation results in stabilization of wild-type p53 and subsequent transcriptional activation of *p21*. However, p21 protein levels are not increased when WISp39 is downmodulated with siRNA or Hsp90 is inhibited with the drug geldanamycin following DNA damage. This exciting observation suggests that p21 levels are increased transcriptionally by p53 and at the level of protein stability by WISp39 and Hsp90 (Figure 1). Thus, two events are required to stabilize p21.

How important is p21 function in tumorigenesis? Mouse embryo fibroblasts from $p21^{Cip1}$ null mice partially bypass the G1/S checkpoint in response to DNA damage, and mice lacking $p21^{Cip1}$ succumb to tumor development (Lozano and Zambetti, 2005). Additionally, onset of spontaneous tumors in mice with a p53 mutant that retains the ability to activate p21 occurs later than in mice with a mutant p53 that is unable to activate p21, suggesting that deletion or inhibition of p21 function may contribute to tumorige-

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-