TRIMming p53 for ubiquitination

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The function of the p53 tumor suppressor protein is finely tuned through a myriad of interactions with other proteins. These interactions can lead to posttranslational modifications that regulate p53 stability, DNA binding, or promoter-specific transcriptional activation. A number of p53 binding proteins serve as cofactors that participate in the recruitment of p53 to specific promoters and facilitate transcriptional activation by p53. Other p53-interacting proteins regulate transcription-independent activities of p53 and p53 subcellular localization (reviewed in refs. 1 and 2). A new p53 binding partner is identified by Allton et al. (3) in this issue of PNAS, and it turns out to be a member of the tripartite motif protein (TRIM) family, TRIM24.

The TRIM family of proteins is defined by the presence of an N-terminal tripartite motif composed of a RING domain, 1 or 2 B-box motifs, and a coiled-coil region (4). Humans have 60 *TRIM* genes, and these encode proteins that can be further classified on the basis of 1 or 2 additional C-terminal domains. One subgroup, consisting of TRIM24, TRIM28, and TRIM33 [also known as transcription intermediary factor 1 (TIF1) α, TIF1β, and TIF1γ, respectively], contains a PHD domain followed by a BROMO domain at the C terminus. These domains are important for binding to chromatin and are involved in transcriptional repression. TRIM24 protein interacts with retinoic acid receptors in a ligand-dependent fashion to regulate their transcriptional activity. TRIM proteins are conserved in vertebrates and invertebrates (5). *Drosophila* has 7 *TRIM*-related genes, one of which, *bonus*, encodes a PHD and a BROMO domain downstream of the tripartite motif, and is considered an ortholog of *TRIM24/28/33*.

The article by Allton et al. (3) is notable for 2 reasons. First, Allton et al. developed a new knockin mouse and stem cell model based on tandem affinity purification (TAP)-tag fusion with the ORF of the mouse p53 gene. The p53TAP fusion protein allows TAP and analysis of p53 protein partners by mass spectrometry. With extracts prepared from ES cells expressing the p53TAP knockin allele, Trim24 copurified with p53TAP and was identified by mass spectrometry. Allton et al. showed that TRIM24 and p53 interact in various cells, shRNA-mediated repression of *TRIM24* causes endogenous p53 protein levels to rise, and TRIM24 promotes p53 ubiquitination and degradation. They conclude that TRIM24 functions as an E3 ubiquitin ligase for p53. Second, Allton et al. performed a genetic mosaic analysis on *Drosophila* imaginal discs in which they analyzed GFP-marked homozygous *bonus* mutant cells in a heterozygous *bonus* strain. GFP-positive *bonus*$^{-/-}$ cells appeared small and highly apoptotic. Remarkably, RNAi-mediated depletion of *Drosophila* p53 (D-p53) rescued the *bonus* apoptotic phenotype and resulted in *bonus* clones of larger size. This result is reminiscent of the rescue of embryonic lethality in *Mdm2*-null mice conferred by loss of p53, a key finding that established the physiological importance of Mdm2 as a negative regulator of p53 (6, 7). The results by Allton et al. suggest that bonus is a key regulator of D-p53 activity. One expects that D-p53 protein levels will be elevated in homozygous *bonus* mutant cells but that remains to be determined. Extending the relationship between *bonus* and D-p53 to mammals is complicated by the fact that D-p53 is the sole ortholog for p53, p63, and p73, and *bonus* is the sole ortholog for *TRIM24*, *TRIM28*, and *TRIM33*. Further studies are required to determine the physical and functional interactions between these 2 families of proteins.

Unlike *Mdm2*-deficient mice, *Trim24*-deficient mice are viable and fertile (8). *Trim24*-deficient mice also exhibit increased hepatocellular proliferation as a result of deregulated retinoic acid signaling mediated by retinoic acid receptor α. *Trim24*-deficient mice also exhibit a high incidence of hepatocellular carcinoma probably as a consequence of uncontrolled and continuous hepatocyte proliferation. In a *ras*-induced liver carcinoma model, endogenous p53 was shown to block tumor development through the induction of cellular senescence (9). If *Trim24*-deficient hepatocytes express high levels of p53, one would not expect to see increased cell proliferation leading to tumor development. The relationship, if any, between Trim24 and p53 in the liver may be complex and only highlights the need to examine endogenous p53 protein levels not only in the liver but in other tissues of *Trim24*-deficient mice.

Table 1. A comparison of the E3 ubiquitin ligases that target p53 for degradation

E3 ligase	Type	p53-responsive	Degradation of p53 after DNA damage	Phosphorylation	Ref.
Mdm2	RING	Yes	No	Ser-166, Ser-188, Ser-395; Tyr-276, Tyr-394*	1, 2
Pirh2	RING	Yes		Thr-154; Ser-155†	10,18
Cop1	RING	Yes	No	Ser-387‡	11,17
ARF-BP1	HECT				12
CARP1/2§	RING		Yes		14
TOPORS	RING				13
Synoviolin	RING				15
TRIM24	RING		Yes		3

*ATM-mediated phosphorylation of Mdm2 on Ser-395 decreases the ability of Mdm2 to degrade p53. Wip1 phosphatase dephosphorylates Ser-395 on Mdm2 to increase the degradation of p53 by Mdm2. Akt/PKB-mediated phosphorylation of Mdm2 on Ser-166 and Ser-188 stabilizes Mdm2. These phosphorylation sites also appear to be necessary for translocation of Mdm2 from the cytoplasm into the nucleus. c-Abl-mediated phosphorylation of Mdm2 on Tyr-276 and Tyr-394 after DNA damage decreases the ability of Mdm2 to degrade p53.

†Pirh2 interacts with calmodulin and is phosphorylated by calmodulin-dependent kinase II on Thr-154 and Ser-155, resulting in a decrease in Pirh2 stability and decreased degradation of p53 (18).

‡ATM-mediated phosphorylation of Cop1 on Ser-387 after DNA damage results in the dissociation of Cop1 from p53 (17).

§CARP1 and CARP2 can ubiquitinate Ser-20-phosphorylated p53 after DNA damage.

Author contributions: E.T. and S.B. wrote the paper.

The authors declare no conflict of interest.

See companion paper on page 11612.

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Trim24 joins several other ubiquitin ligases (Table 1) that promote ubiquitin-mediated degradation of p53 including Mdm2, Pirh2, Cop1, ARF-BP1, TOPORS, Synoviolin, CARP1, and CARP2 (1, 2, 10–15). Interference with the expression of these E3 ubiquitin ligases leads to an increase in p53 stability and an increase in p53-dependent apoptosis or G_1 arrest. With the exception of Mdm2, the physiological role of these ubiquitin ligases in regulating p53 function remains unclear, and one of the great challenges is to determine why p53 is targeted for degradation through so many pathways. Are the E3 ligases that target p53 functionally redundant? It will be important to evaluate the expression patterns of these E3 ligases during different stages of development, in different tissues, and under different stress conditions. It is also important to determine whether these E3 ligases target different isoforms of p53, different oligomeric forms of p53, different post-translationally modified forms of p53, or other p53 family members. A recent study (16), for example, reported that Pirh2 preferentially targets tetrameric p53, and it will be important to extend this finding to the other E3 ligases.

The results by Allton et al. suggest that bonus is a key regulator of D-p53 activity.

Mdm2 binds p53 in unstressed cells and promotes ubiquitination on conserved C-terminal lysine residues (residues 370, 372, 373, 381, 382, and 386) (2). Importantly, Mdm2 binds to various cofactors that influence its ability to ubiquitinate p53 (2). Both p53 and Mdm2 undergo ataxia telangiectasia mutated (ATM)-dependent phosphorylation in response to DNA damage, and these changes result in the dissociation of Mdm2 from p53 resulting in p53 protein stabilization. ATM-mediated phosphorylation of Cop1 after DNA damage similarly results in its dissociation from p53 (17), and Pirh2 stability is decreased upon its phosphorylation by calmodulin-dependent kinase II (18). It will be important to determine the lysine residues on p53 that are ubiquitinated by TRIM24 and the other E3 ligases, identify E3 ligase regulatory cofactors, and learn how p53 ubiquitination is modulated through stress signals.

ACKNOWLEDGMENTS. This work was supported by Canadian Cancer Society Grant 017258.

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