The TRIP from ULF to ARF

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ARF is a key activator of p53, and together they form a critical duo for protection against cancer. Previous evidence had recognized the regulatory potential of ubiquitin-mediated degradation of ARF. The recent identification of TRIP12/ULF as a ubiquitin ligase of ARF adds an important missing piece to the ARF/p53 pathway.

The ARF-mediated activation of tumor suppressor p53 in response to oncogene activation is a major determinant of protection against cancer. In normal cells, p53 activity is dampened by the action of Mdm2, an E3 ubiquitin ligase that targets p53 for proteasomal degradation. ARF binds to Mdm2 and inhibits its action on p53, thus efficiently promoting the accumulation of p53 and the implementation of a defensive response in the form of apoptosis or cell-cycle arrest (Junttila and Evan, 2009). A variety of oncogenes are able to elicit the activation of ARF, most notably Myc overexpression and oncogenic mutant Ras, and absence of ARF in mice severely impairs the tumor suppressive activity of p53 (Junttila and Evan, 2009). A number of transcriptional modulators have been found to mediate the induction of ARF upon oncogene activation (Kim and Sharpless, 2006). The extensively reported role of transcription in determining the levels of ARF has had, however, the adverse effect of overlooking the regulatory potential of ARF protein stability.

ARF and INK4a form a famous odd couple not only for their potent tumor suppressor activity but also for their intricate connections: they are encoded by the same *CDKN2A* genomic locus and share their last two exons, yet the common exons are translated from different reading frames and they have disparate amino acid sequences (Kim and Sharpless, 2006). Still, there is something they share about their amino acid composition that make the couple even more unique: they are 2 of the only about 15 proteins encoded by the human genome that completely lack lysine residues (Ben-Saadon et al., 2004). Interestingly, lysine-less proteins can be ubiquitylated at their N-terminus through what is known as N-terminal ubiquitylation or the *U*biquitin *F*usion *D*egradation (UFD) pathway (Ciechanover and Ben-Saadon, 2004). The UFD pathway, although not exclusive for lysine-less proteins, is the only available choice for ubiquitin-proteasome degradation in the case of lysine-less proteins. This is indeed the case of ARF and INK4a, as respectively reported by the laboratories of Chuck Sherr (Kuo et al., 2004) and Aaron Ciechanover (Ben-Saadon et al., 2004). These observations left in the air the obvious question of identifying the E3 ubiquitin ligase responsible for the N-terminal ubiquitylation of ARF, of INK4a, or, for that matter, of any E3 ubiquitin ligase involved in UFD given that none was known in mammalian cells.

The above gives way to a different chapter of the story that has its roots in yeast, where the UFD pathway had been dissected long ago and UFD4 had been identified as a key E3 enzyme in the pathway (Johnson et al., 1995). It was not until very recently that the team led by Jong-Bok Yoon noticed sequence similarity between yeast UFD4 and a human protein known as TRIP12, and went on to demonstrate that TRIP12 is a bona fide E3 ubiquitin ligase for the mammalian UFD pathway (Park et al., 2009). TRIP12 had been previously identified, but not characterized, among a series of proteins interacting with the thyroid hormone receptor, hence its name *T*hyroid hormone *R*eceptor *I*nteracting *P*rotein 12 (Lee et al., 1995). The identification of TRIP12 as an E3 enzyme of the UFD pathway and the existence of ARF degradation through this pathway set the stage for the next chapter in this fascinating story.

One of the few instances in which ARF protein stability has been carefully studied is in relation to nucleophosmin (NPM). NPM is a major component of the nucleolus with important functions regulating cell growth, proliferation, and transformation, plus additional important effect of stabilizing ARF protein levels (Grisendi et al., 2006). Based on this, Wei Gu and coworkers analyzed, by mass spectrometry, the protein complexes bound to NPM. From a simplistic point of view, the initial expectation would be to find proteins that contribute to stabilize ARF. Instead, the authors found TRIP12 and demonstrated that TRIP12 served as an E3 ubiquitin ligase for the degradation of ARF. On this basis, they renamed this protein ULF, for *U*biquitin *L*igase for AR*F* (Chen et al., 2010). To test the functional consequences of TRIP12/ULF, the authors downregulated the protein with RNAi and examined the effect on p53 and cell proliferation. In support of a relevant role of TRIP12/ULF in ARF function, knockdown of TRIP12/ULF resulted in increased stability of ARF, accompanied by accumulation of p53 and cell-cycle arrest. All these functional consequences were dependent on the presence of ARF, since concomitant RNAi knockdown of ARF abolished the increased levels of p53 and allowed the cells to proliferate normally (Chen et al., 2010). Of note, no effects were observed on INK4a levels upon inhibition of TRIP12/ULF, suggesting that INK4a may have a different E3 ubiquitin ligase for its degradation.

To further extend the above observations, the authors examined the interplay between TRIP12/ULF and two known positive regulators of ARF, namely, NPM and Myc. In the case of NPM, the authors conclude that NPM promotes the stabilization of ARF by retaining ARF in nucleoli, thus away from the action of TRIP12/ULF, which resides mainly in the nucleoplasm (Figure 1). In the case of Myc, the study of TRIP12/ULF also brings new and

unexpected light. It had been shown that Myc activates ARF through transcriptional upregulation of its mRNA levels (Zindy et al., 1998). However, this does not seem to be the whole story to Myc and ARF. Wei Gu and coworkers found that Myc inhibits TRIP12/ULF ubiquitin ligase activity toward ARF (Chen et al., 2010). This effect was shown to be dependent on direct Myc-TRIP12/ULF interaction and does not require the transcriptional activity of Myc (Figure 1). Consequently, a transcriptionally deficient Myc protein can increase the half-life of ARF and thus increase the levels of p53 and its target p21 (Chen et al., 2010).

These data raise the possibility that TRIP12/ULF could be a novel sensor of oncogenic stress upstream of ARF. In this regard, it will be interesting to know how TRIP12/ULF activity is regulated when normal cells are challenged by oncogenic stress. Also, TRIP12/ULF might have pro-oncogenic activity if deregulated, since unchecked TRIP12/ULF activity will promote degradation of ARF, rendering cells unprotected from oncogenic stress. If this were the case, targeting TRIP12/ULF activity with small drug inhibitors might offer a novel strategy for therapeutic intervention of cancer, thus following the footsteps of agents such as Nutlin3a that targets Mdm2.

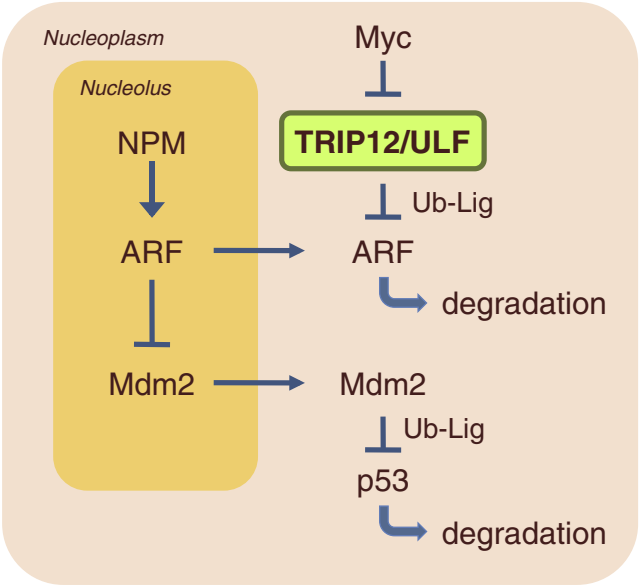

Figure 1. A New Addition to the ARF/p53 Pathway
A recent report (Chen et al., 2010) has identified TRIP12/ULF as a key E3 ubiquitin ligase that adds ubiquitin to ARF (Ub-Lig) and induces its degradation. ARF is protected from TRIP12/ULF when bound to NPM and localized in the nucleolus. Moreover, in the nucleolus, ARF binds and inhibits Mdm2, which, in turn, is the main E3 ubiquitin ligase of p53.

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