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Antagonism of Myc functions by Arf

The Arf-Mdm2-p53 tumor suppressor pathway is activated by sustained hyperproliferative signals emanating from oncoproteins such as Myc. A recent study reveals a novel level of feedback control, whereby induced p19^{Arf} binds to Myc and blocks cell proliferation by selectively impairing its transactivation functions.

When faced with an increased threshold of oncogenic signals that provoke cells to divide at an abnormally accelerated pace, the *Arf* tumor suppressor gene is activated. Its encoded product (p19^{Arf} in mouse or p14^{ARF} in humans) antagonizes the ubiquitin E3 protein ligase activity of the p53 negative regulator Mdm2 (Hdm2 in humans) to trigger a p53-dependent transcriptional response that leads to either cell cycle arrest or apoptosis. These protective responses can be disabled through deletion or silencing of *Arf*, by generation of dominant-negative mutants of p53, or through mechanisms leading to Mdm2 overexpression, so enabling incipient cancer cells to thrive.

Myc was the first oncogene recognized to activate *Arf* gene expression (Zindy et al., 1998), although the mechanism by which it does so remains unclear. When Myc expression is enforced in mouse B-lymphocytes in vivo, its enhancing effects on cell proliferation are inhibited by the Arf-Mdm2-p53 axis, but disabling the pathway cancels Myc-induced apoptosis and allows formation of B cell lymphomas (Eischen et al., 1999). Observations that Myc could trigger a p53 response through the agency of Arf were conceptually satisfying, but additional complexities soon became apparent. First, high and sustained Myc activity is required for *Arf* induction, but the promoter is normally insulated from responding to physiologic Myc signals. Second, cells lacking *p53* or harboring mutant forms of the protein display dramatic upregulation of p19^{Arf}, and reintroduction of p53 represses *Arf* transcription. This feedback control by p53 also extends to c-Myc, again through an unknown mechanism (Figure 1). Most importantly, however, mice engineered to lack both *Arf* and *p53*, or all three genes in the pathway, develop (usually multiple) cancers at a faster rate, and the spectrum of tumor types arising in these mice is much broader than that of animals lacking either *Arf* or *p53*, or both *Mdm2* and *p53*, providing genetic evidence that p19^{Arf} must have p53-independent functions (Weber et al., 2000). Indeed, cells lacking *Arf* and *p53* proliferate faster than those lacking either gene alone, and enforced overexpression of *Arf* can arrest the proliferation of *p53* null mouse embryo fibroblasts (MEFs), albeit inefficiently (Eischen et al., 1999; Weber et al., 2000).

Recent studies now suggest that p19^{Arf} can negatively regulate Myc's transcriptional activity through a direct physical interaction that is seemingly independent of Mdm2 and p53 (Qi et al., 2004). These investigators report some surprising findings that include Myc's ability to bind directly to Arf and to relocalize Arf from its usual storehouse in the nucleolus into the nucleoplasm in both wild-type and p53-deficient MEFs. Most striking, however, are their observations that p19^{Arf} associates with Myc on its tar-

get genes, antagonizing Myc's transactivation functions without impairing its transrepression activities (Figure 1). Specifically, chromatin immunoprecipitation (ChIP) analysis revealed that the Arf protein binds to the promoters of the *eIF4E* and *nucleolin* genes upon Myc activation. Myc binding to these promoters occurs whether or not *Arf* and/or *p53* genes are present, indicating that $p19^{Arf}$ does not affect the recruitment of Myc to its target genes. Myc induced the expression of *eIF4e*, *nucleolin*, *Tert*, *Cdk4*, and *Cul1* in *Arf*/*p53* double null cells, but not in *p53* null MEFs that express very high levels of endogenous $p19^{Arf}$. In contrast, downregulation of *Gadd45* and *Ink4b* by Myc was unaffected by *Arf* status. Together, these results point to a new component of feedback regulation where, following its induction by Myc, $p19^{Arf}$ directly and selectively inhibits Myc's pro-proliferative activities in a p53-independent manner (Figure 1).

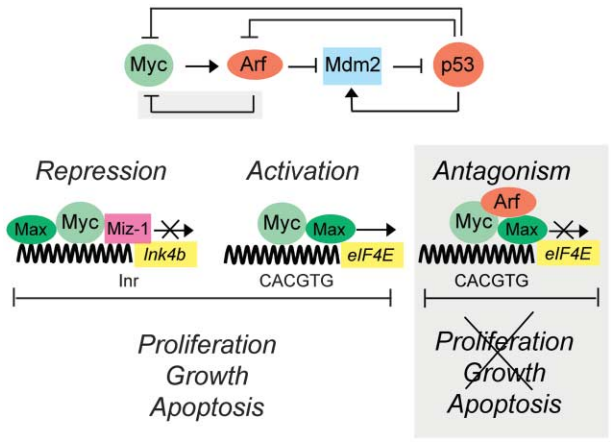

Figure 1. Feedback controls affecting the activities of Myc and Arf

Myc induces *Arf* transcription to trigger a p53 response. p53 activates *Mdm2*, whereas $p19^{Arf}$ inhibits it. p53 also negatively regulates the transcription of both *Arf* and *Myc*. Myc-Max complexes have global effects on chromatin structure, activating numerous target genes by binding to CACGTG consensus sequences and repressing other targets by interacting with Miz-1 on promoter initiator elements (Inr). The $p19^{Arf}$ protein is now revealed to inhibit the activity of Myc on its target promoters (shaded portions of the schematic) (Qi et al., 2004). This antagonism is selective, blocking Myc's ability to activate gene expression, but not affecting gene repression. In turn, Arf cancels Myc's pro-proliferative, but not its apoptotic, functions. We speculate that Arf's ability to antagonize Myc also negatively regulates cell growth (mass).

What are the potential limitations of the analysis? First, $p19^{Arf}$ is a highly basic and "sticky" protein (pI > 12) with a propensity to bind nucleic acids and acidic proteins even under stringent detergent lysis conditions. With its unusual amino acid composition, $p19^{Arf}$ may well interact nonspecifically with other molecules, and it is difficult to devise appropriate experimental controls that deal effectively with this problem. Perhaps not surprisingly, then, Arf has been demonstrated to coimmunoprecipitate with a number of cellular proteins other than Mdm2, including nucleophosmin (at high stoichiometry), E2F1 and DP1, HIF-1α, topoisomerase I, TBP-1, spinophilin, Pex19p, and cyclin G1, although the physiologic significance of these interactions remains uncertain. It has also proven difficult to map Arf binding sites within many of these putative target proteins. Qi et al. (2004) found that Arf could bind to both the N- and C-terminal domains of Myc, which include the transcriptional regulatory and the helix-loop-helix/leucine zipper domains, respectively, although the former segment seems to represent the primary binding site. Second, the authors observed that overexpressed Myc could interact with $p19^{Arf}$ in the nucleoplasm of MEFs to prevent its entry into the nucleolus, but in other cell lines, the two proteins localized to nucleoli, as was also observed by others (Datta et al., 2004). This seems reminiscent of the Arf-Mdm2 interaction in which enforced overexpression of Mdm2 relocalized $p19^{Arf}$ to the nucleoplasm; conversely, overexpression of Arf led to Mdm2 sequestration within the nucleolus (reviewed in Sherr and Weber, 2000). In fact, the results of Qi et al. (2004) also contradict previous results indicating that conditional Myc activation in MEFs provoked accumulation of $p19^{Arf}$, and then Mdm2, within the nucleolus. A caveat for all such studies is that they have generally relied upon diverse cell types and vectors that overexpress vastly different amounts of proteins. This underscores the potential difficulties in determining where within cells Arf antagonism of Myc may occur.

Myc affects not only cell proliferation but also cell growth (mass). At least in part, this may be due to its ability to activate Pol I- and Pol III-dependent transcription (affecting the synthesis of rRNAs, 5S RNA, and tRNAs) and to accelerate rRNA processing (Schlosser et al., 2003; Gomez-Roman et al., 2003). As would be expected, some Myc protein localizes to nucleoli, where its turnover is regulated by an SCF E3 ubiquitin ligase that includes the Fbw7γ F box specificity factor; interference with Myc degradation in this compartment increases cell size (Welker et al., 2004). Intriguingly, Arf inhibits ribosome biogenesis by interfering with rRNA processing (Sugimoto et al., 2003) and, by ChIP, binds to *rDNA* promoters (Ayrault et al., 2004). Therefore, the primordial role of Arf may have been to control cell growth in a p53-independent manner, but in the course of evolution, Arf may have acquired the capacity to efficiently arrest cell proliferation by binding to Mdm2 and activating p53. In light of these new results, this begs the question of whether $p19^{Arf}$ interacts directly with Myc on *rDNA* promoters.

Does inhibition of Myc function by Arf coordinately inhibit both proliferation and growth in incipient cancer cells? For proliferating cells to maintain their size, coordination of these processes is vital. The mechanisms that link the cell division cycle with ribosome production, protein and membrane biosynthesis, nutrient availability, cellular metabolism, and energy production are not clearly understood. Perhaps Myc is a master regulator of these processes. A further understanding of Arf-Myc antagonism will require a more detailed examination of their physical interactions on a broader spectrum of Myc target genes.

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