A license to kill: Transcriptional activation and enhanced turnover of Myc by the SCF^{Skp2} ubiquitin ligase

Understanding the mechanisms through which the abundance and activity of the Myc oncoprotein is regulated has been a major preoccupation of the cancer and transcription communities. New work, published in the May issue of *Molecular Cell*, reveals that ubiquitination of Myc by the oncogenic SCF^{Skp2} complex not only promotes Myc turnover but is also required for Myc transcriptional activation, suggesting that Myc activity is "licensed" by ubiquitination.

The *c-Myc* gene is a central regulator of proliferation, differentiation, cell survival, and neoplastic transformation. Widespread interest in Myc stems from the fact that it is a powerful oncogene and is found mutated or overexpressed in up to 30% of human cancers (reviewed in Levens, 2003). Myc functions in a transcription factor network that includes the Mad and Max proteins and acts upon E boxes containing genes. The Myc network regulates a bewilderingly large number of genes, approaching 10% of human genes (Levens, 2003).

Given the strength of Myc's oncogenic activity, it is critical that its activity be tightly controlled. Multiple mechanisms ensure proper control in normal cells, including regulated Myc turnover, which occurs through the ubiquitin-proteasome pathway (Salghetti et al., 1999). Indeed, Myc is among the most unstable proteins, with a half-life of ~30 min. Two major mechanisms are responsible for derailing normal Myc regulation: (1) mutations in the structural gene that block its rapid turnover and (2) translocations that cause deregulated high-level expression. However, the molecules that maintain appropriate Myc levels have not been identified. Now two papers (Kim et al., 2003; von der Lehr et al., 2003) in the May issue of *Molecular Cell* have identified one component of the machinery used to control Myc levels through the ubiquitin pathway, the oncoprotein Skp2. Surprisingly, this ubiquitin ligase is also required for induction of Myc-responsive genes, suggesting that ubiquitination not only promotes Myc turnover but "licenses" its transcriptional activity.

To explore the mechanism of Myc turnover, Kim et al. used an elegant yeast genetic system to ask whether Myc turnover occurred via an SCF (Skp1/Cul1/F-box Protein) pathway. When expressed in budding yeast, Myc is quite unstable, with a half-life similar to that seen in mammalian cells. Using temperature-sensitive mutations in SCF components, Kim et al. found that Myc was stabilized under conditions where the mutant SCF subunit was nonfunctional, implicating the SCF pathway in Myc turnover in yeast. F-box proteins serve as substrate-specific receptors for SCF complexes. By looking at Myc stability in cells lacking functional F-box proteins (Grr1, Cdc4, and Met30), Kim et al. found that removal of a single F-box protein—Grr1—caused Myc stabilization. Given that Grr1 is a leucine-rich repeat F-box protein (an Fbl), Kim et al. surveyed a subset of the 18 known mammalian Fbl proteins for those that could induce Myc turnover in Grr1-deficient yeast. Of the genes examined, only one, Skp2/Fbl1, supported Myc destruction.

In an independent study, von der Lehr et al. asked whether Myc can associate with a core component of the SCF ubiquitin ligase, Cul1, and found that it did. By testing three well-characterized F-box proteins, von der Lehr et al. also identified Skp2/Fbl1 as being capable of binding Myc in tissue culture cells. Through a series of domain mapping experiments, both groups demonstrated that Skp2 interacts with Myc via two distinct domains, the N-terminal Myc box II (MbII) domain and the C-terminal bHLHZip domain. If Skp2 is responsible for Myc turnover in vivo, one would expect that loss of Skp2 would lead to Myc stabilization. Both groups demonstrated that this is the case using dominant-negative versions of Skp2 as well as Skp2 siRNA. Taken together, these data are consistent with Skp2 being a component of the Myc turnover machinery, but this is not the end of the story.

Skp2 is, perhaps, the best understood mammalian F-box protein. SCF^{Skp2} is responsible for ubiquitination of the Cdk inhibitor p27 in response to p27 phosphorylation (reviewed in Bashir and Pagano, 2003). Moreover, Skp2 is overexpressed in various tumors and, like Myc, can collaborate with Ras to transform cells. Thus, the finding that a growth-promoting factor is responsible for ubiquitin-dependent destruction of another growth-promoting protein is somewhat counterintuitive.

Previous work from the Tansey laboratory had suggested a possible explanation for this apparently unusual relationship: that ubiquitination of certain transcription factors is required to activate their ability to induce transcription. This idea is based on the fact that many transcriptional activators are unstable, ubiquitinated proteins, and that instability is frequently linked with one or more transcriptional activation domains (TADs) (Salghetti et al., 2000, 2001). For example, in yeast, LexA-VP16 protein fusions cannot activate reporter gene expression under conditions where VP16—the TAD—is not subject to ubiquitination by the SCF^{Met30} ubiquitin ligase (Salghetti et al., 2001). However, artificial attachment of a single ubiquitin to LexA-VP16 allows transcriptional activation in the absence of SCF^{Met30} function. This suggested the possibility that ubiquitination of TADs such as VP16 activates or licenses their transcriptional activity while simultaneously setting up their destruction (Salghetti et al., 2001; Tansey, 2001).

The finding that Skp2 associates with TADs in Myc led Kim et al. and von der Lehr et al. to test whether Skp2 is involved in licensing Myc's transcriptional activity. Several lines of evidence are consistent with this possibility. First, using a Gal4-MbII fusion protein, Kim et al. showed that overexpression of Skp2 stimulates both the ability of the Gal4-MbII protein to be ubiquitinated and to activate reporter constructs, while Skp2 knockdown by RNAi reduced the activity of Gal4-MbII. Thus, Skp2 appears to be important for induction of Myc target genes by MbII. Second, Skp2 expression has the unexpected property of being able to induce the expression of several endogenous Myc-dependent genes in quiescent Rat1 cells, and the extent of activation was similar to that seen with Myc induction. Critically, Skp2 was

unable to activate Myc-responsive gene expression in *c-Myc*$^{-/-}$ Rat1 cells. Moreover, and perhaps most surprisingly, Skp2 was unable to drive *c-Myc*$^{-/-}$ cells into S phase from quiescence, despite being able to destroy p27. Thus, Myc is required for Skp2-driven S phase entry in this setting.

Also consistent with the direct involvement of Skp2 in Myc-dependent transcription, von der Lehr et al. detected Skp2 and Cul1 on the Myc-responsive cyclin D2 promoter using chromatin immunoprecipitation in wild-type but not in *c-Myc*$^{-/-}$ cells. This suggests that SCF^{Skp2} is brought to the cyclin D2 promoter through an interaction with Myc. Several subunits of the 19S regulatory particle of the proteasome, as well as α2 subunit of the 20S proteasome core, were also found associated with the cyclin D2 promoter. Although AAA ATPase subunits of the 19S particle have been previously linked with transcriptional activities (Ferdous et al., 2001), the presence of proteasome core subunits is unusual. This result raises several questions: Is the entire proteasome actually present and therefore able to rapidly destroy ubiquitinated proteins as they are generated at the promoter? If this is the case, how is the balance between ubiquitin-mediated transcriptional activation and ubiquitin-mediated destruction established? Presumably, licensing via ubiquitination sets up a permissive state for transcription that is rapidly eliminated once transcription has initiated via destruction of the transcriptional activator, but the mechanism that controls the licensing window will need to be elucidated if we are to understand the dynamics of this process.

Until now, the major role for Skp2 in promoting proliferation was ascribed to its ability to induce p27 destruction, in keeping with the finding that Skp2 cannot induce S phase entry in cells expressing nonphosphorylatable forms of p27 (Sutterluty et al., 1999). However, while Skp2 can collaborate with Ras to transform cells, Ras alone is unable to transform cells lacking p27, suggesting the existence of additional Skp2 targets (Bashir and Pagano, 2003). Moreover, Skp2 can promote S phase entry from quiescence in mouse fibroblasts lacking p27 (Y. Wei and J.W.H., unpublished data). Therefore, the connection between Skp2 and Myc suggests a new view of the Skp2 pathway: Skp2 expression simultaneously leads to activation of an oncogene (Myc) and destruction of a Cdk inhibitor (p27) that normally serves to impose a barrier against Cdk-driven S phase entry. The unique proliferative advantage these effects would have on the cell provides a simple explanation for why Skp2 is overexpressed in a wide array of epithelial cancers (Bashir and Pagano, 2003). What is not exactly clear from this work is how Skp2$^{-/-}$ mice undergo something approaching normal development, yet presumably lack Myc function, while mice lacking c-Myc display embryonic lethality. One would expect that if Myc activity in vivo uniquely required Skp2, then loss of Skp2 would result in a more severe phenotype. Clearly, further studies are needed to determine whether only a subset of Myc-responsive genes require Skp2 for Myc responsiveness or whether alternative pathways exist for Myc activation in particular cell lineages.

Although Kim et al. (2003) and von der Lehr et al. (2003) demonstrated the Skp2-dependent ubiquitination of Myc in vivo, neither group was able to demonstrate Myc ubiquitination by SCF^{Skp2} in vitro, nor were they successful in demonstrating a direct interaction between Myc and Skp2. Cks1 is an essential accessory factor for p27 ubiquitination by SCF^{Skp2} (Bashir and Pagano, 2003). Might it be the case that an accessory factor for Myc is missing? Finding the ever elusive accessory protein will be critical for elucidating the mechanism by which Skp2 is licensed to kill Myc.

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Selected reading

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