An unsteady scaffold for Myc

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Ubiquitination and degradation of the Myc onco-protein are highly regulated events that have an important function in tumourigenesis. A new study by Rosalie Sears and colleagues now identifies Axin, a tumour suppressor protein best known for its role in the Wnt pathway, as a central co-ordinator of this Myc degradation. Loss-of-function mutations in Axin, therefore, directly enhance Myc levels in human tumours.

Ever since the discovery that enhanced expression of a wild-type *MYC* gene is sufficient to uncover its oncogenic properties in a mouse model of B-cell lymphomagenesis (Adams *et al.*, 1985), it has been accepted in the field that the levels of Myc are an important determinant of normal and pathological cell behaviour. Consequently, the molecular mechanisms that control Myc protein levels have been studied in extensive detail, and we now know that both transcriptional and a plethora of post-transcriptional mechanisms regulate Myc levels in normal and in tumour cells.

For example, Myc is a highly unstable protein that is rapidly turned over by the ubiquitin-proteasome system in normal cells; the rate of turnover is attenuated in many tumour cells. Although several ubiquitin ligases have been shown to associate with Myc, the best understood pathway is the degradation by Fbw7 (Welcker

et al., 2004; Yada *et al.*, 2004). Degradation through this pathway is initiated by phosphorylation of Myc at serine 62, which serves as a priming site for binding of Gsk3. Gsk3 then phosphorylates Myc at threonine 58 and T58-phosphorylated Myc is—after a series of intermediate steps—recognized by the Fbw7 ubiquitin ligase, leading to degradation of ubiquitinated Myc through the proteasome. This pathway offers a prime example of how degradation of Myc can be disrupted during tumourigenesis: many Burkitt lymphomas harbour point mutations in Myc at either T58 or S62, leading to reduced ubiquitination and enhanced stability (Bahram *et al.*, 2000). One would expect, therefore, that similar mutations could be found in at least some of the other tumours in which enhanced levels of Myc have an important function. But this does not appear to be the case: despite intensive efforts, no point mutations of *MYC* have been reported from solid tumours.

So, if point mutants of Myc offer an easy way to enhance protein levels of this protein during tumourigenesis, then why are they never found in solid tumours, despite extensive searches? One answer to this riddle, albeit a partial one, is that other mutations and changes in gene expression occur that can stabilize Myc and that may, therefore, alleviate the necessity for further mutations in Myc itself. Several of these changes affect the kinase cascades that

Figure 1 The figure illustrates the role of Axin in degradation of Myc. Axin facilitates phosphorylation of T58 by Gsk3 and the subsequent de-phosphorylation of S62 by PP2A. Mutations in Axin are found in several tumours and affect different scaffolding roles of Axin, all resulting in enhanced levels of Myc.

control phosphorylation of Myc. For example, the PI3-Kinase pathway is constitutively activated in many tumours by mutations in receptor tyrosine kinases or by loss of the PTEN phosphatase; activation of PI3-Kinase leads to inhibition of Gsk3 activity and subsequently T58 phosphorylation. Similarly, the *FBW7* gene is a tumour suppressor gene that is lost in many, most notably colon, carcinomas (Rajagopalan *et al*, 2004). Finally, a de-ubiquitinating enzyme, Usp28, that antagonizes the function of Fbw7 and stabilizes Myc, is expressed at very high levels in breast and colon carcinoma (Popov *et al*, 2007). Potentially, therefore, many of the mutations found in solid tumours affect Myc turnover, and it is therefore only the genomically relatively stable lymphomas, which resort to mutating Myc itself.

In the current issue of the *EMBO Journal*, Rosalie Sears and colleagues add an important further piece of evidence to support this concept. In this paper, Arnold *et al* show that complex formation between Myc and Gsk3 (as well as PP2A and Pin1 two other proteins involved in degradation) is facilitated by the scaffolding protein, Axin1. Axin1 has a similar role in several signaling pathways: it is best known for its role in the Wnt pathway, where it acts as a platform for Gsk3-mediated phosphorylation of β -catenin, leading to its degradation by the proteasome pathway. Although the Wnt pathway is intimately linked to Myc function, it appears that the function of Axin1 in Myc degradation is mediated by a complex that is unrelated to that mediating catenin turnover, as a key component of the latter complex, the APC tumour suppressor protein, has no role in Myc turnover. Importantly, Axin1 is a tumour suppressor protein and Arnold *et al* go on to show that stabilization of Myc in several tumour cell lines correlates with the presence of

Axin1 mutations that disrupt its ability to act as a scaffold for degradation of Myc (Figure 1).

These data suggest that an additional class of mutations, which may be largely undetected, contribute to the stabilization of Myc by inhibiting Fbw7-mediated degradation of the protein. By further highlighting this pathway, they also point to what appears to be the biggest puzzle with regard to our understanding of how this pathway controls Myc function: the T58A and S62A mutations are not only more stable but also show a strongly reduced pro-apoptotic potential; for example, lymphomas that are generated with these mutants in transgenic mice can arise in the presence of wild-type p53 and Arf and thus escape the otherwise obligatory mutations in the p53/Arf pathway, which mediates apoptosis in response to deregulation of wild-type Myc (Hemann *et al*, 2005). Furthermore, these mutations have altered gene-regulatory properties. How does this fit together? First, it is possible that the mutations that are selected for during lymphomagenesis affect a hitherto unknown protein interaction of Myc that just happens to bind in a region of Myc that also affects turnover. This idea is supported by the observation that not all mutations of Myc that are found in Burkitt lymphomas stabilize the protein. Alternatively, ubiquitination of Myc by Fbw7 not only affects protein turnover but also alters the functional properties of Myc. Indeed, some recent evidence suggests that ubiquitination of Myc affects its transcriptional properties and that not all ubiquitination events target Myc to the proteasome (Kim *et al*, 2003; von der Lehr *et al*, 2003; Adhikary *et al*, 2005; Otto *et al*, 2009). Whatever the exact answer is: the analysis of Myc ubiquitination and turnover is a key to our understanding of the protein and is likely to offer many more surprises.

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