

Ubiquitin signalling: what's in a chain?

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When cellular proteins are attached to a Lys 48-linked polyubiquitin chain, the proteasome will usually degrade them. But attaching such a chain to a yeast transcription factor inhibits its activity without degradation, raising questions about how polyubiquitination regulates transcriptional activation and why the protein is spared destruction.

An emerging theme in eukaryotic transcriptional control is the regulation of gene-specific activation by the ubiquitin–proteasome system^{1,2}. This regulation has some surprising twists. Transcriptional activators can be inhibited in some cases but activated in others by their polyubiquitination. Many of these polyubiquitinated factors are subsequently targeted to the proteasome — a large multi-subunit protease — for degradation; but there are also persistent hints that the proteasome (or a regulatory subcomplex) might participate in transcriptional activation in a way that does not require its proteolytic activity^{3,4}. This has led to the idea of the proteasome functioning as a transcription-complex remodelling factor or sometimes even as a linker between ubiquitinated histones and chromatin-modifying enzymes⁵.

In general, it is difficult to separate effects on transcription-factor activity caused by ubiquitination *per se* as opposed to ubiquitin-dependent proteasomal degradation of the factor. This probably reflects a close and even necessary mechanistic link between the two processes for transcriptional initiation and elongation¹. An exception is the yeast Met4 protein, a bZIP family transcriptional activator that under certain conditions, can be ubiquitinated but remains stable^{6,7}. New results from Flick *et al.*⁸ on p. 634 of this issue now show that the multi-ubiquitinated but long-lived Met4 protein is coupled to a Lys 48-linked polyubiquitin chain at a specific lysine of Met4. This is unexpected because a Lys 48-linked polyubiquitin chain is the classic signal for proteasomal binding and degradation^{9,10}, raising the question of how ubiquitinated Met4 evades degradation.

Ubiquitin is attached to proteins by a series of enzymes (E1, E2 and E3); there are multiple isoforms of E2s and even more E3s, which impart an impressive range of substrate specificities on ubiquitin–protein conjugation¹¹.

For Met4, the E2 is Cdc34 (cell division cycle protein 34) and the E3 is a multi-subunit ubiquitin-protein ligase called SCF^{Met30} (Skp1/Cul1/F-Box (SCF) complex containing the F-box protein Met30). The outcome of a ubiquitination event can depend on whether a single ubiquitin or a ubiquitin polymer is attached to the substrate protein. Mono-ubiquitination serves as a signal for chromatin modifications and for various cellular trafficking events. Polyubiquitin chains bearing different internal linkages between the ubiquitin moieties are also assembled on specific proteins¹². Chains with amide linkages between the carboxyl terminus of one ubiquitin and Lys 48 of the next are the most common, and if they reach a length of at least four ubiquitins, they can bind tightly to the proteasome ($K_D \sim 35$ nM), at least *in vitro*¹⁰. Thus, these chains are thought to target a substrate for degradation. Polyubiquitin chains involving ubiquitin Lys 63 are also generated in cells, but proteins modified with such chains are not rapidly degraded.

Met4 activates the transcription of a battery of methionine biosynthetic (*MET*) genes when methionine levels are low, but it is not active when abundant methionine is available (Fig. 1). High methionine leads to increased intracellular S-adenosylmethionine (SAM), which triggers the inactivation of Met4. In certain media, at high methionine concentration, Met4 seems to be rapidly degraded by the proteasome⁶. However, under other methionine-replete conditions, Met4 is rendered nonfunctional but is relatively stable despite being ubiquitinated^{6,7}. In an earlier paper, Kaiser and colleagues demonstrated a tight correlation between methionine-induced accumulation of multi-ubiquitinated Met4 and loss of Met4-dependent transcription⁷. They found that modified Met4 remains relatively long-lived and still binds to *MET* promoters, but it fails to bring the Cbfl co-factor to these sites⁷. Another group, however, reported that the stable, polyubiquitinated Met4 is no longer recruited to *MET* promoters (Fig. 1). Flick *et al.* demonstrate that a single lysine in Met4 is modified by ubiquitin, despite the presence of 42 other lysine

residues in the protein. Mutation of this lysine, Lys 163, to an arginine blocks Met4 ubiquitination and renders it active irrespective of methionine levels in the medium. However, the mutant Met4 protein can still bind to Met30, the subunit of the SCF^{Met30} E3 ligase that directly recognizes Met4. These and other data make a strong argument that ubiquitination of Met4 on Lys 163 is responsible for its inactivation without causing its degradation.

When examined on denaturing gels, the authors detected multiple ubiquitinated Met4 species in cell extracts (up to ~6 distinct bands) which could represent either the addition of a single ubiquitin to different lysine residues or the addition of ubiquitin polymers. In this case, however, because only one Met4 lysine (Lys 163) is an acceptor site for ubiquitin, the multiple bands imply the assembly of a polyubiquitin chain, with different polyubiquitin–Met4 species bearing different numbers of ubiquitin units on Met4. The authors used multiple approaches to characterize the ubiquitin chains assembled on Met4. First, when a ubiquitin mutant (Ub^{K48R}) that cannot form Lys 48-linked chains was overproduced in cells, a shift toward faster migrating forms of ubiquitinated Met4 was observed. This is predicted if ubiquitin Lys 48-linked chains assemble on the protein: attachment of Ub^{K48R} to Met4 or to the end of a partially assembled polyubiquitin chain on Met4 will prevent further chain elongation. Second, both biochemical analysis of Met4 with Ub^{K48R}-terminated chains and mass spectrometry of purified polyubiquitinated Met4, indicated that a uniform Lys 48-linked polyubiquitin chain is assembled.

In a convincing series of physiological tests, Flick *et al.* show that polyubiquitination of the Met4 activator correlates with a loss of *MET* target gene transcription without significant loss of Met4 protein. For instance, 60 min of methionine deprivation causes a strong induction of *MET* gene expression that is associated with a marked shift of Met4 from a polyubiquitinated to nonubiquitinated form but protein levels of Met4 remain nearly constant. Within 30 min of re-addition of methionine to the medium, *MET*

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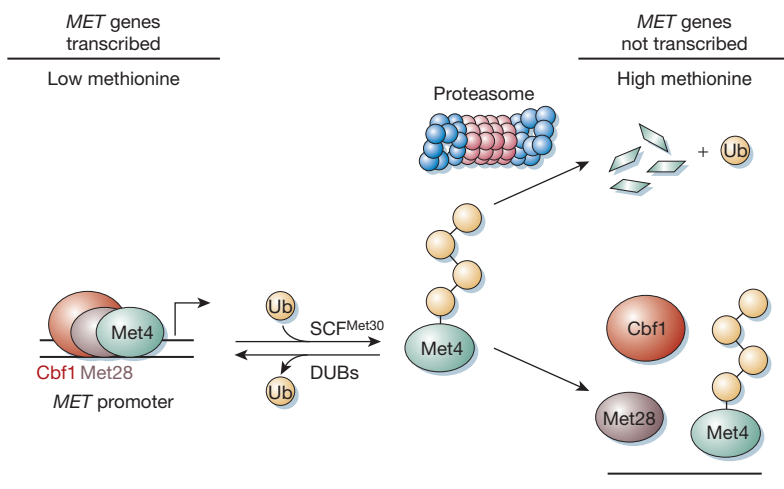


Figure 1 Alternative fates of a polyubiquitinated transcription factor. High levels of methionine inhibit Met4 activity by stimulating its polyubiquitination. It has been reported that under some conditions this leads to Met4 degradation by the proteasome (top pathway)^{6,13}. Under other conditions, the modification still prevents Met4 function, but does not cause it to be degraded (bottom pathway)^{6–8}. Met4, a bZIP protein, binds operator sequences as a complex with Met28, another bZIP factor, and Cbf1, a bHLH protein. DUBs, deubiquitinating enzymes; Ub, ubiquitin.

transcription has largely stopped and almost all of Met4 is ubiquitinated. What regulates these striking switches in ubiquitination state is a central unanswered question.

The new data provide useful fodder for constructing models to explain the ubiquitin-dependent shutdown of the Met4 activator, but we are also left with a number of puzzles. First, there are the lingering discrepancies between results from different research groups. Rouillon *et al.*¹³ and Kuras *et al.*⁶ have compelling data showing that high levels of methionine can, under certain conditions, cause Cdc34–SCF^{Met30}-dependent ubiquitination and proteasome-dependent degradation of Met4. Flick *et al.* have gone so far as to use the same media formulations as these other groups, but they do not see ubiquitin-dependent Met4 proteolysis. Small variations in conditions might be sufficient to tip the mechanism from a nonproteolytic to a proteolytic one (for example, assembly of slightly longer ubiquitin chains might allow proteasome binding and degradation). Nevertheless, there is general agreement that under some conditions, Met4 can be inactivated by ubiquitination without being degraded.

What, then, prevents the degradation of polyubiquitinated Met4 by the proteasome? Flick *et al.* present preliminary data which suggest that despite its attachment to a Lys 48-linked ubiquitin chain, Met4 cannot bind tightly to the proteasome. There are caveats to

this particular set of experiments but, if taken at face value, they imply that there is something unusual about polyubiquitinated Met4 that impairs proteasome binding. Perhaps the most likely possibility is that the chain is too short for such binding. The precise number of ubiquitins in the chains attached to Met4 is not yet certain, but it does seem that at least some of them can reach or exceed the minimal tetrameric length required for tight *in vitro* association with the proteasome. Another possibility is that a polyubiquitin chain in the structural context of Met4 Lys 163 cannot bind the proteasome. However, it is difficult to imagine how a flexibly tethered polyubiquitin chain is completely blocked from proteasome binding as a result of its particular lysine attachment site. In two other examples where Lys 48-linked polyubiquitin chain attachment fails to cause degradation, binding of the polyubiquitinated substrate to the proteasome is still detected^{10,14,15}. In these examples, the failure of the Lys 48-linked ubiquitin chain to elicit degradation was observed *in vitro*, and the physiological significance of the observations is not yet clear. Perhaps other factors bind to polyubiquitinated Met4 and shield it from the proteasome. This is certainly a topic that will need further investigation. However, if short chain length is the key determinant for preventing degradation, then an important issue to address is what limits chain elongation.

Another fundamental question that remains unresolved is why the ubiquitin-modified Met4 is no longer active as a transcription factor. Here too, discrepant results between laboratories hamper speculation. Kaiser and colleagues⁷ detected little change in Met4 occupancy of *MET* promoters with or without methionine in the medium, but saw a methionine-induced loss of its cofactor Cbf1 there. In contrast, Kuras *et al.*⁶ reported clear evidence for loss of Met4 at *MET* genes in rich medium (containing methionine), a condition in which Met4 was ubiquitinated but not degraded. Interestingly, Met4 continued to bind to and activate certain *SAM* gene promoters under these conditions. This suggests that a subset of promoters still can recruit Met4 when it is polyubiquitinated, whereas others cannot. Presumably, different general transcription or chromatin-remodelling factors are required at these different promoters and are differentially sensitive to Met4 polyubiquitination. A relevant analogy might be the regulation of the proliferating cell nuclear antigen (PCNA) DNA-repair factor; in this case, attachment of a single ubiquitin or a chain of ubiquitins (Lys 63-linked) seems to control the binding or activity of distinct DNA polymerases during DNA repair¹⁶. It was noted earlier that the proteasome itself, or some proteasomal regulatory subcomplex, might function as a general transcription factor. Perhaps differences in proteasome-dependent 'remodelling activity' at these various promoters underlie the observed differences in polyubiquitinated Met4 recruitment. As these conjectures make clear, there is much to the connection between ubiquitin and transcription that remains to be explored. □

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