

# Skipping into the E2F1-destruction pathway

J. Wade Harper and Stephen J. Elledge

**F-box proteins act as specificity factors for the ubiquitination, and thus degradation, of target proteins. New results point to the importance of F-box proteins in controlling the transcriptional programs that regulate development and the cell cycle.**

The proper development and successful survival of complex organisms require exquisite spatial and temporal control of gene expression. The levels of certain key transcriptional regulators must be precisely maintained so that they can control gene expression properly. One such regulator is the E2F-1 protein — a cell-cycle-regulated transcription factor that has the unusual property of being both an oncoprotein and a tumour suppressor, depending on its levels (reviewed in ref. 1). The levels and activity of E2F-1 are tightly regulated by multiple means; for example, one mechanism for reducing E2F-1

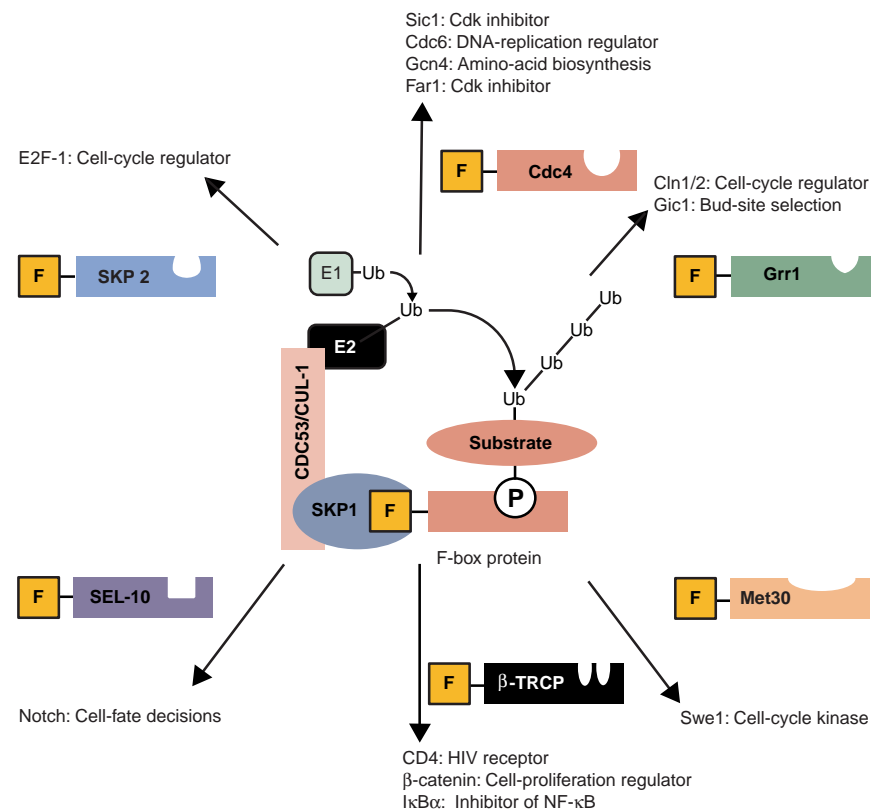
amounts is ubiquitin-mediated proteolysis, although how this process is regulated and the pathways involved have remained elusive. Now, on page 14 of this issue, Marti and colleagues show that the SCF<sup>SKP2</sup> complex, an E3 ubiquitin-protein ligase with no previously known substrate, functions in the cell-cycle-dependent destruction of E2F-1 (ref. 2). These and earlier results suggest a model that explains how both the magnitude and the duration of E2F1-dependent transcription are coupled to cell-cycle control.

Ubiquitin-mediated proteolysis is used to regulate the amounts of a large number

of proteins, but it is particularly useful in the area of cell-cycle control. Here, protein levels must be reduced rapidly to allow for particular cell-cycle transitions and to re-establish a ground state for the next cell cycle. The process of ubiquitin-mediated proteolysis starts with the tagging of substrates with the highly conserved protein ubiquitin. Multiple rounds of ubiquitination produce polyubiquitin chains, which are recognized by the 26S proteasome — the protease that destroys the target protein.

The rate-limiting step in this protein-degradation pathway is the polyubiquitination process. This involves a cascade of ubiquitin-transfer reactions requiring three components. First a ubiquitin-activating enzyme (E1) activates the ubiquitin, which is then transferred to the second component, a ubiquitin-conjugating enzyme (E2). Specificity in ubiquitination reactions is controlled largely by the third component, ubiquitin-protein ligases (E3s). E3s function as adapters that interact simultaneously with both the substrate and the E2, thereby allowing selectivity in reactions that transfer ubiquitin from the E2 to lysine residues in the substrate<sup>3</sup>. E3s are also often the target of regulation.

There are three major classes of E3s: the HECT-domain proteins, the anaphase-promoting complex, and the largest and most versatile class, the SCF ubiquitin ligases — one of which, SCF<sup>SKP2</sup>, is now shown by



**Figure 1 F-box proteins are interchangeable adaptors that link a core ubiquitin ligase (SKP1–CDC53/CUL-1) with a wide array of substrates. The combination of SKP1, CDC53/CUL-1 and the F-box protein produces an SCF-type E3 complex, shown in the centre of the figure. Substrates destined to be degraded by the 26S proteasome are tagged with polyubiquitin (Ub) chains by the action of E1, E2 and E3 enzymes. F-box proteins link the substrate to the E3 to allow the substrate to be labelled with polyubiquitin. These linkages frequently require that the substrate be phosphorylated. Around the periphery are shown the many different F-box proteins (marked with yellow boxes), and their substrates, that have been identified. The F-box proteins all interact with SKP1 through their F-box and with one or more substrates, such as E2F-1 or Sic1, through other protein-interaction motifs. In SKP2 and Grr1, C-terminal leucine-rich repeats interact with substrates, whereas Cdc4, Met30, β-TrCP and SEL-10 (ref. 14) contain WD40 repeats. Substrates of the SCF are often critical regulatory proteins whose levels need to be altered rapidly for proper cellular function.**

Marti and colleagues<sup>2</sup> to be involved in E2F-1 destruction.

SKP2 was originally identified as a protein associated with complexes of cyclin A and cyclin-dependent kinase 2 (CDK2) in mammalian cells. These cyclin A-CDK2 complexes are involved in the initiation and progression of the S phase of the cell cycle, hence the name SKP2 — for S-phase-kinase-associated protein 2 (ref. 4). SKP2 is required for S phase and its binding to cyclin A allows for association of another protein, SKP1, with cyclin A, but the molecular function of SKP2 has remained unknown.

A link between the SKPs and ubiquitination came with the identification of Skp1 as a suppressor of a temperature-sensitive mutation in one of the cell-division-cycle proteins, Cdc4, in budding yeast<sup>5</sup>. This *cdc4* mutation blocks ubiquitin-mediated proteolysis of the yeast Cdk inhibitor Sic1. In yeast, Cdc4 physically associates with Skp1 through a domain, the F-box, that is conserved in many Skp1-interacting proteins, including cyclin F and Skp2 (Fig. 1)<sup>5</sup>.

We now know that SKP1 and F-box proteins, such as SKP2, are components of the SCF-type E3 ubiquitin-protein ligases that function in the targeted destruction of a great many proteins. SCFs are modular complexes that consist of SKP1, CDC53 (or CUL-1) and one of a variety of F-box proteins (Fig. 1). These F-box proteins function as specificity factors that interact both with particular substrates (through carboxy-terminal protein-protein-interaction domains) and with SKP1-CDC53 (CUL-1) complexes (through an amino-terminal F-box motif)<sup>5-7</sup>. F-box proteins form a large and diverse family, with 15 members in budding yeast and about 100 in *Caenorhabditis elegans*. This means that a wide variety of substrates could be linked to the SKP1-CDC53 (CUL-1) complex, through their specific F-box partner, so pathways involving SCF E3 complexes may be used to regulate many proteins<sup>5,8</sup>.

Genetic and biochemical data have implicated SCF complexes in a wide range of ubiquitination pathways, with a common feature being a requirement for substrate phosphorylation (Fig. 1). This reflects the fact that many F-box proteins interact with substrates in a phosphorylation-dependent manner. Thus the timing of protein ubiquitination and destruction is controlled at the level of the interaction of the substrate with its specific SCF ubiquitin ligase, through the F-box component of this ligase complex.

Although the finding that SKP2 is an F-box protein predicts its involvement in ubiquitination, substrates for SCF<sup>SKP2</sup> have remained elusive. SCF<sup>SKP2</sup> can assemble into complexes with cyclin A-CDK2, but there is no evidence that cyclin A is a substrate of this particular E3 (reviewed in ref. 8). A clue to the function of SKP2 came

from the finding that its abundance is itself cell-cycle-regulated, being maximal during S phase. Marti *et al.*<sup>2</sup> noticed that the timing of SKP2 accumulation coincided with the timing of E2F-1 destruction, in S/G2 phase. This led them to investigate whether SKP2 might actually be involved in E2F-1 degradation.

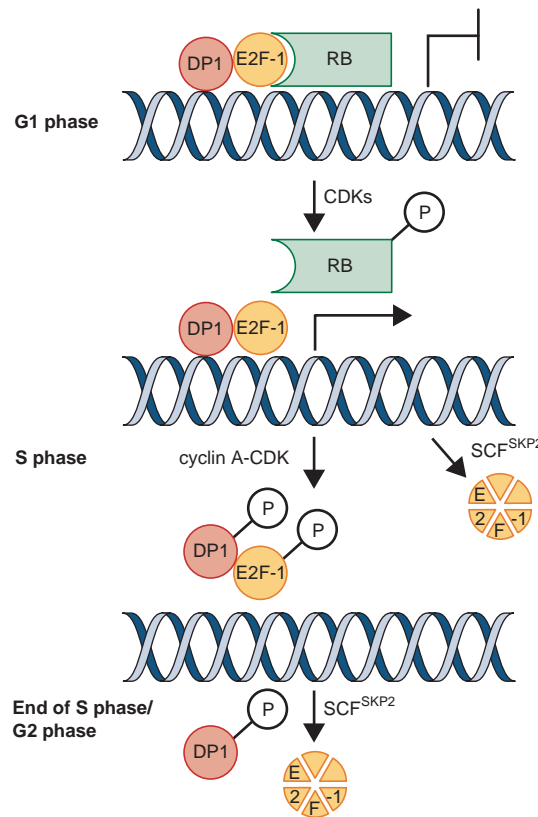
In co-transfection experiments and *in vitro*, SKP2 associated with E2F-1, a property expected of the substrate-receptor component of the E2F-1 E3. In addition, CUL-1 associated with E2F-1 in parallel experiments; the interaction is presumably bridged by endogenous SKP2. Consistent with a role for an SCF in E2F-1 ubiquitination, transfection of CUL-1 into tissue culture cells led to raised amounts of E2F-1-ubiquitin conjugates.

Evidence of SKP2's involvement rests primarily on the finding that mutations in E2F-1 that abolish binding to SKP2 *in vitro* result in stabilization of E2F-1 *in vivo*. Deletion analysis of E2F-1 revealed a SKP2-binding site on the N terminus of E2F-1. An E2F-1 mutant lacking this binding site was stabilized in tissue culture cells and this stabilization correlated with decreased levels of E2F-1-ubiquitin conjugates. A consequence of E2F-1 stabilization through removal of the SKP2-interaction domain was increased activation of transcription from E2F-dependent promoters and increased S-phase entry under conditions of serum deprivation. Thus, the correlation

is strong: SKP2 (and, presumably, SCF<sup>SKP2</sup>) binds to a domain of E2F-1 that, when mutated, leads to increased stability of E2F-1. However, it has not yet been possible to reconstitute E2F-1 ubiquitination by recombinant SCF<sup>SKP2</sup> complexes, and nor has it been possible to remove SKP2 from cells to assess the effects on E2F-1 stability. Such experiments will be important to confirm that SCF<sup>SKP2</sup> is a relevant E2F-1-ubiquitin ligase *in vivo*.

How does the involvement of SKP2 in E2F-1 function fit into the overall regulation of E2F-1? E2F-1 is expressed when the cell cycle enters G1 phase, and is bound both to promoter DNA (through the DNA-binding protein DP1) and to the retinoblastoma protein, RB (Fig. 2). RB acts as a transcriptional inhibitor at such promoters. As CDKs are activated, RB becomes phosphorylated and dissociates from E2F-1, leaving E2F-1-DP1 bound to DNA where it can act in a positive manner to promote transcription. E2F-1 activity is downregulated by phosphorylation mediated by cyclin A-CDK2 during S phase; phosphorylation of DP1 blocks its ability to bind DNA<sup>9</sup>. Thus, the duration of E2F-1 action, as well as its initial activation after phosphorylation of RB, is regulated by the cell-cycle clock.

Marti and colleagues' results<sup>2</sup> indicate that ubiquitin-mediated proteolysis may regulate E2F-1 activity in two more ways. First, SCF<sup>SKP2</sup> may maintain appropriate E2F-1 levels during S phase, thereby con-



**Figure 2** The amounts and activity of E2F-1 are controlled in a cell-cycle-dependent way. During G1 phase, E2F-1 is expressed and binds to both promoter DNA (through the DNA-binding subunit DP1) and the retinoblastoma protein, RB, which inhibits E2F-1-activated transcription from such promoters. When activated cyclin-dependent kinases (CDKs) phosphorylate RB, it dissociates from E2F, thus allowing E2F-1 to activate transcription. As cells enter S phase, E2F-1 is inhibited in two ways: it and the DP1 subunit are phosphorylated by cyclin A-CDK complexes, and so cannot bind to promoter DNA, and it is destroyed by SCF<sup>SKP2</sup>-mediated ubiquitination and destruction. During the transition from S to G2 phase, SCF<sup>SKP2</sup> may be responsible for the removal of E2F-1. SCF<sup>SKP2</sup> is itself regulated in a cell-cycle-dependent way: its abundance is maximal in S phase. Perhaps E2F-1 controls its own fate by regulating transcription of SKP2 during S phase.

trolling the magnitude of the transcriptional response. Second, SCF<sup>SKP2</sup> may be responsible for the removal of E2F-1 after completion of S phase, thereby resetting the balance for the next cell cycle.

At present, there is no evidence that association of E2F-1 with SKP2 requires phosphorylation of E2F-1, unlike other interactions between substrates and F-box proteins. Instead, the timing of E2F-1 ubiquitination seems to depend on the timing of expression of SKP2. If so, this is the first example of an SCF-dependent process that is phosphorylation independent. However, it is conceivable that SKP2 can interact with E2F-1 only after E2F-1 has been displaced from DNA, an event mediated by cyclin A–CDK2. Thus, the timing of ubiquitination could still be phosphorylation dependent, albeit indirectly so.

SKP2 is itself cell-cycle-regulated at the transcriptional level, so perhaps E2F-1 might have a hand in its own destruction by regulating transcription of SKP2, much as mitotic cyclins set up their own destruction by activating the anaphase-promoting complex. This idea remains to be tested. Note that although one of SKP2's substrates

is E2F-1, it must have other substrates too because its role in E2F-1 regulation cannot explain its requirement in the G1-to-S-phase transition.

F-box proteins are a versatile set of tools that can be used by the cell to perform its many tasks. Transcriptional control is particularly well suited to the form of regulation offered by the diversity of F-box proteins. These proteins may play a part in controlling multiple transcriptional pathways in addition to that involving E2F-1. For example, the  $\beta$ -TrCP/slimb protein has been implicated in activation of the transcription factor NF- $\kappa$ B/Dorsal through destruction of the regulator I $\kappa$ B $\alpha$ <sup>10–12</sup>, in inactivation of the  $\beta$ -catenin pathways through constitutive turnover of  $\beta$ -catenin in the absence of signalling through Wnt<sup>10,13</sup>, and in control of the Hedgehog pathway through an unknown mechanism (Fig. 1)<sup>13</sup>. In addition, three F-box proteins in budding yeast (Cdc4, Grr1 and Met30) may be involved in controlling metabolic transcriptional programs as well as cell-cycle functions (Fig. 1)<sup>8</sup>.

A theme that has emerged is that F-box proteins can target multiple proteins that

frequently bear no obvious relationship to one another. Matching targets and pathways with an ever-growing number of F-box proteins will be a challenge for the future. □

J. Wade Harper is at the Verna and Marrs McLean Department of Biochemistry, and Stephen J. Elledge is at the Howard Hughes Medical Institute and Department of Biochemistry, Baylor College of Medicine, Houston, Texas 77030, USA.  
e-mail: jharper@bcm.tmc.edu

- Weinberg, R. A. *Cell* **85**, 457–459 (1996).
- Marti, A., Wirbelauer, C., Scheffner, M. & Krek, W. *Nature Cell Biol.* **1**, 14–19 (1999).
- Hershko, A., Heller, H., Elias, S. & Ciechanover, A. *J. Biol. Chem.* **258**, 8206–8214 (1983).
- Zhang, H., Kobayashi, R., Galaktionov, K. & Beach, D. *Cell* **82**, 915–925 (1995).
- Bai, C. *et al. Cell* **86**, 263–274 (1996).
- Skowrya, D., Craig, K. L., Tyers, M., Elledge, S. J. & Harper, J. W. *Cell* **91**, 209–219 (1997).
- Feldman, R. M., Correll, C. C., Kaplan, K. B. & Deshaies, R. J. *Cell* **91**, 221–230 (1997).
- Patton, E. E., Willems, A. R. & Tyers, M. *Trends Genet.* **14**, 236–243 (1998).
- Krek, W., Xu, G. & Livingston, D. M. *Cell* **83**, 1149–1158 (1995).
- Winston, J. T. *et al. Genes Dev.* **12**, 270–283 (1999).
- Yaron, A. *et al. Nature* **396**, 590–594 (1998).
- Spencer, E., Jiang, J. & Chen, Z. J. *Genes Dev.* **13**, 284–294 (1999).
- Jiang, J. & Struhl, G. *Nature* **391**, 493–496 (1998).
- Hubbard, E. J. A., Wu, G., Kitajewski, J. & Greenwald, I. *Genes Dev.* **11**, 3182–3193 (1997).

## The strain of copper on the brain

Transmissible spongiform encephalopathies (TSEs), such as scrapie in sheep and Creutzfeldt–Jakob disease (CJD) in humans, occur as numerous different strains in each species. The existence of these stable variants of the disease is one of the main unresolved issues for Stanley Prusiner's prion hypothesis, or 'protein-only hypothesis', of TSEs. This hypothesis proposes that the infectious agent in TSEs is a rogue form (Prp<sup>Sc</sup>) of a cell's normal prion protein (Prp<sup>C</sup>), possibly along with a putative non-nucleic-acid cofactor.

Strains are a problem for Prusiner's hypothesis because, assuming that each strain represents a different conformational version of the prion protein, it is unclear how the protein can adopt, under physiological conditions, as many different conformations as there are strains of TSEs. Elsewhere in this issue (*Nature Cell Biology* **1**, 55–59; 1999), John Wadsworth and colleagues report an intriguing link between metal-ion occupancy of the prion protein and its biochemical strain characteristics.

Recent research has focused on the copper-binding capabilities of the amino-

terminal domain of Prp<sup>C</sup> as a clue to understanding the protein's normal cellular function. Wadsworth and colleagues look instead at metal-ion occupancy in Prp<sup>Sc</sup>



isolated from two biochemically distinct strains of sporadic CJD. They use metal-chelating agents to disrupt binding of Prp<sup>Sc</sup> to Cu<sup>2+</sup> and Zn<sup>2+</sup>; this treatment slightly alters the electrophoretic mobility of the cleavage products of Prp<sup>Sc</sup> after limited proteolysis of the prion protein using proteinase K — one of the biochemical indicators of strain in TSEs.

It would be tempting to claim that this observation points to a molecular explanation of TSE strains, with metal-ion occupancy determining strain specificity, but this would be premature. For a start, Wadsworth and colleagues found that the effect on Prp<sup>Sc</sup> biochemistry of altering metal-ion occupancy extends to only two of the four biochemically defined strains of CJD, and so does not seem to offer a general explanation for strain variation. It also remains to be shown whether or not these altered strain characteristics can be transmitted experimentally to animals in inocula pretreated with chelators. But this provocative link between the copper-binding capabilities of prion protein and strain variations in Prp<sup>Sc</sup> points research in a new direction.

HARRIET COLES