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Coordination of Cell Growth and Division by the Ubiquitin-Proteasome System

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Abstract

The coupling of cellular growth and division is crucial for a cell to make an accurate copy of itself. Regulated protein degradation by the ubiquitin-proteasome system (UPS) plays an important role in the coordination of these two processes. Many ubiquitin ligases, in particular the Skp1-Cullin-F-box protein (SCF) family and Anaphase-Promoting Complex (APC), couple growth and division by targeting cell cycle and metabolic regulators for degradation. However, many regulatory proteins are targeted by multiple ubiquitin ligases. As a result, we are only just beginning to understand the complexities of the proteolytic regulatory network that connects cell growth and the cell cycle.

Keywords

Cell cycle; ubiquitin; proteolysis; cyclin; glycolysis

1. Introduction

In order for a cell to make an accurate copy of itself, it must tightly coordinate growth and division. Before dividing, cells must double their complement of macromolecules and organelles, replicate their chromosomes with high fidelity, and then accurately segregate these components into daughter cells. One way that this complex process is coordinated is through the timed expression of key regulatory proteins. Limiting the expression of proteins to the windows of time in which their functions are needed ensures that cell cycle events occur in the proper order and that the cycle proceeds in one direction only.

Protein expression is regulated at several levels. One mechanism utilized by all eukaryotes to establish cyclical patterns of regulatory protein expression is cell cycle-regulated transcription [1]. In budding and fission yeasts, a large fraction of the genomes (12–15% of all genes) are transcribed in a cell cycle-dependent pattern [2–6]. In humans the overall number of cycling genes is similar (~1000), although this represents a smaller fraction of the entire genome [7,8]. While these cyclical transcription profiles suggest that the protein products of these genes also cycle, in order for protein levels to mirror mRNA levels, these proteins must be relatively short-lived and turn over at least once each cell cycle. Thus,

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protein degradation is essential to establish the waves of protein expression that orchestrate cell cycle events.

Most regulated protein degradation occurs through the ubiquitin-proteasome system (UPS) [9] [Please insert a reference to the editorial of this issue]. In the UPS, proteins are first marked for destruction by the covalent attachment of a polyubiquitin chain, which serves as a signal to target proteins to the proteasome for destruction. Ubiquitylation requires an ATP-dependent enzymatic cascade that includes a ubiquitin-activating enzyme (E1), a ubiquitin-conjugating enzyme (E2), and a ubiquitin ligase (E3) [10]. Most regulation of this process is conferred by E3s, which recognize specific protein substrates and catalyze the final step of ubiquitin transfer. Consistent with their key role in determining substrate specificity and regulating ubiquitylation in response to cellular or developmental cues, there are large numbers of E3s in all eukaryotes (~80 in yeast, ~600 in humans) [11]. The majority of these E3s remain poorly characterized *in vivo* and relatively few targets have been identified for most of these enzymes.

The best characterized E3s controlling cell growth and division fall into two classes of multi-subunit complexes, the Skp1-Cullin-F-box (SCF) family and the Anaphase Promoting Complex (APC). Both complexes have well established cell cycle-regulatory roles and are essential for cell division [12–15]. However, the two families utilize different strategies to target substrates in a cell cycle-dependent fashion. SCF ligases are a subgroup of the wider cullin-RING ligases family. They are composed of three core subunits -a structural cullin subunit (Cul1 in vertebrates, Cdc53 in budding yeast), a RING domain protein (Rbx1), and an adaptor protein (Skp1)- as well as one of a large number of modular substrate-specificity subunits called F-box proteins (FBPs) [16–21]. There are large families of FBPs in all eukaryotes and each is thought to recruit a specific set of substrates to the core SCF complex. In almost all instances, F-box proteins interact specifically with epitopes on substrates that have been post-translationally modified, most commonly by phosphorylation. In this way, substrate modification regulates SCF-dependent degradation.

The APC is similar to the SCF in that it contains a cullin-homology domain subunit (Apc2) and a RING domain protein (Apc11), however the complex is much larger overall, consisting of 13 subunits and estimated at approximately 1MDa [22]. Whereas SCF activity is regulated at the level of substrate modification, APC activity is directly regulated by the cell cycle. The two APC substrate-specific adaptor proteins, Cdc20 and Cdh1, associate in a cell cycle dependent pattern—Cdc20 is expressed and active specifically in mitosis, until it is replaced by Cdh1 in late mitosis and G1 phase [23]. The different strategies employed by SCF and APC ligases set up different windows of activity during the cell cycle. Although many SCF ligases are present throughout the cell cycle and can target substrates at any time, the majority of established SCF cell cycle targets are ubiquitylated during G1 and S phase, while the APC ubiquitylates proteins exclusively during mitosis and G1. In addition, the activity of other cullin-RING ligases (CRLs), such as CRL3 and CRL4^{Cdt2}, overlap with the SCF and APC and also contribute to cell cycle-regulated proteolysis. This review will mainly focus on the established roles of cullin-RING E3s in coordinating growth and the cell cycle, with particular emphasis on how substrates are targeted for degradation during G1 and S phases. For a detailed review of the role of the UPS in protein degradation during mitosis, refer to the related review in this issue by Min and Lindon [Please insert a proper reference to this manuscript].

2. Cell cycle targets of the UPS

Regulated progression through interphase (G1, S and G2 phases) allows a cell time to increase its size and complement of organelles, accurately replicate its chromosomes, and

prepare for entry into mitosis. The UPS coordinates interphase progression by targeting numerous cell cycle regulators for degradation. Two groups of UPS targets, cyclins and cyclin-dependent kinase inhibitors (CKIs), play important roles in establishing cell cycle timing and coordinating cell growth and division. Recent studies demonstrate that many cyclins and CKIs are targeted for degradation by more than one E3, illustrating the complexity of the proteolytic network that regulates cell cycle progression.

2.1. Cyclin degradation controls cell cycle timing

Cell cycle progression is driven by the activity of cyclin dependent kinases (Cdks), which phosphorylate and modulate the activities of hundreds of proteins [24–27]. The activity and specificity of Cdks depend upon their association with one of several activating subunits called cyclins, which are unstable proteins that undergo cell cycle-regulated synthesis and degradation [28,29]. The groups of cyclins whose levels peak in G1 phase or at the G1/S transition play important roles in coordinating extracellular signals with entry into the cell cycle. These cyclins are all targeted for degradation by cullin-RING E3s, primarily by members of the SCF family. Interestingly, most are targeted by multiple E3s, which may help to fine-tune cyclin expression patterns.

In budding yeast, the G1 cyclin Cln3 is the cyclin whose expression is furthest upstream in the cell cycle, and which couples nutrient availability with cell cycle entry. Cells become committed to cell cycle entry upon phosphorylation of the transcriptional repressor Whi5 by Cln3/Cdk1, which inactivates Whi5 and leads to the expression of downstream genes including the G1/S cyclins Cln1 and Cln2 [30,31]. Cells expressing a truncated and stable Cln3 protein have a significantly shortened G1 phase, which results in less time for the cell to grow and a dramatic reduction in cell size [32–34]. Despite the importance of Cln3 degradation for controlling cell cycle entry, the specific E3 that targets Cln3 has remained elusive. Early studies implicated a cullin-RING E3 in Cln3 degradation, since mutations in *CDC34*, the E2 enzyme that functions with SCF family E3s, resulted in Cln3 stabilization [33,35]. However, no single FBP could be identified that regulated Cln3 stability. Recently two FBPs, Cdc4 and Grr1, have been found to redundantly target Cln3 for degradation, and only when both FBPs are inactivated simultaneously is Cln3 stabilized (Figure 1, J. Benanti, *in revision*). It is unclear why this redundancy has evolved, but one possibility is that it may serve to buffer cells against extreme fluctuations in Cln3 synthesis.

In vertebrates, D-type cyclins (cyclin D1, D2 and D3) play similar roles to Cln3 in yeast. Mitogenic signals promote an increase in cyclin D, which pairs with a G1 specific Cdk (Cdk4 or Cdk6). The cyclin D/Cdk complex then phosphorylates and inactivates the transcriptional repressor Rb, which leads to transcription of genes required for the G1/S transition, including the downstream E-type cyclins [29]. Like Cln3, tight control of cyclin D levels are critical for cells to properly respond to environmental cues. Moreover, cyclin D1 is overexpressed in breast, esophageal, head and neck, and lung carcinomas [36], and cyclin D1 mutations that impair both nuclear export and degradation can transform cells *in vitro* and *in vivo* [37–39]. Elucidating the pathways that regulate cyclin D1 degradation has also been challenging. The APC and several SCF family E3s (including SCF^{Fbx4-αB Crystallin}, SCF^{Fbxw8}, SCF^{βTRCP}, SCF^{Fbxo31}, and SCF^{Skp2}) have been implicated in cyclin D1 degradation (Figure 1) [40–45]. Some of these E3s are likely to only ubiquitylate cyclin D1 in specific conditions. For example, Fbxo31 only targets cyclin D1 after DNA damage [46]. It is not clear how many of these E3s can target cyclin D1 during a normal cell cycle. A recent study deleted and/or knocked down all of these E3s in multiple combinations in mouse fibroblasts and was unable to completely stabilize the protein [47]. Therefore, it seems likely that cyclin D1 is degraded by multiple E3s with overlapping functions.

G1 cyclin/Cdk activity promotes expression of the downstream G1/S cyclins, which are important for triggering entry into S phase and the initiation of DNA replication. In budding yeast, this function is fulfilled by Cln1 and Cln2, both of which are targeted for degradation by SCF^{Grr1} [48]. Interestingly, although Grr1 is the only FBP required for Cln1 and Cln2 destruction *in vivo*, SCF^{Cdc4} can ubiquitylate Cln2 *in vitro* [49], and target it *in vivo* if Cdc4 and Cln2 are co-localized in the cytoplasm (J. Benanti, *in revision*). This demonstrates that two SCF ligases (Grr1 and Cdc4) are capable of targeting all three G1 and G1/S cyclins in yeast.

In metazoan cells, the G1/S cyclin, cyclin E, is targeted by the FBP hCdc4/Fbw7/Ago [50–52]. SCF^{Fbw7} specifically targets phosphorylated Cyclin E that is bound to Cdk2 [53,54]. Interestingly, two additional cullin-RING E3s have been implicated in targeting cyclin E when it is not bound to Cdk2. Mice lacking the FBP Skp2 have elevated levels of cyclin E, and many cells from these mice are polyploid and contain multiple centrosomes, two phenotypes that are associated with cyclin E overexpression [55]. Moreover, SCF^{Skp2} can ubiquitylate free cyclin E *in vitro* [55]. A second cullin-RING E3, CRL3, also interacts with free cyclin E and can ubiquitylate it *in vitro* and *in vivo* [56,57]. Together, these data demonstrate that a complex network of E3s degrade G1 and G1/S cyclins (Figure 1). Future work is necessary to determine whether these multiple cyclin degradation pathways act at the same time and in the same cell type, or if they are active in different cell types or under different physiological conditions, and are therefore are not entirely redundant.

2.2. Degradation of cyclin dependent kinase inhibitors drives the G1/S transition

Another group of UPS targets that coordinates entry into the cell cycle are the cyclin dependent kinase inhibitors (CKIs). Degradation of CKIs are important for the G1/S transition in both yeast and metazoans. Budding yeast express two CKIs, Sic1 and Far1, both of which are targeted for degradation by SCF^{Cdc4} following their phosphorylation by Cdk (Figure 1) [17,18,58]. Far1 is required to arrest the cell cycle during yeast mating [59], whereas Sic1 is a critical regulator of the G1/S transition during an unperturbed cell cycle. Sic1 is expressed late in the cell cycle when it inhibits Clb/Cdk1 activity and helps potentiate mitotic exit [60–62]. However, the primary function of Sic1 appears to be to inhibit Clb5/Cdk activity that begins to accumulate in G1 phase, thereby delaying the initiation of DNA replication until cells are ready to proceed into S phase. As Cln/Cdk and Clb5/Cdk levels rise during G1 phase, Sic1 becomes hyperphosphorylated, which triggers its sudden destruction by SCF^{Cdc4} [63,64]. Cdc4-mediated Sic1 destruction is required for S phase entry since expression of non-degradable Sic1 arrests cells at the G1/S transition [65].

In vertebrate cells, degradation of the related CIP/KIP family of CKIs, p21, p27 and p57, is also important for cell cycle transitions. All three proteins bind to and inhibit cyclin/Cdk complexes, but also have Cdk-independent functions [66]. One E3 that has been implicated in targeting all three CIP/KIP inhibitors at the G1/S transition is SCF^{Skp2}. Skp2 is itself cell cycle-regulated: it is ubiquitylated by APC^{Cdh1} in G1 phase and only accumulates after Cdh1 activity decreases late in G1 [67]. At the G1/S transition SCF^{Skp2} targets the nuclear pools of p21, p27, and p57 proteins that are bound to cyclin/Cdk2 (Figure 1) [68].

In addition to this common regulation by SCF^{Skp2}, each CIP/KIP inhibitor is also targeted by additional E3s. Of the three family members, p21 degradation has been studied in the most detail. In an unperturbed cell cycle p21 protein peaks at both the G1/S and G2/M transitions [69]. After being degraded at the start of S phase by SCF^{Skp2}, p21 levels re-accumulate until prometaphase, when it is degraded by the APC^{Cdc20} [44,70,71]. Other E3s target pools of p21 that are involved in other functions. For instance, CRL4^{Cdt2} targets PCNA-bound p21 after DNA damage, in order to prevent DNA re-replication [72–74]. Additionally, MDM2/MDMX and CRL2^{Lrr1} target cytoplasmic pools of p21 that are

involved in Cdk-independent functions [66]. Interestingly, some evidence also suggests that proteasomal degradation of p21 may occur independent of its ubiquitylation [75].

Although SCF^{Skp2} targeting of p27 is well established [76–78], other E3s can target it for degradation as well. p27 plays an important role in enforcing cell cycle arrest when cells exit from the cell cycle and enter quiescence (G0 phase). When cells exit quiescence and reenter the cell cycle, p27 is destroyed in a Skp2-independent manner [79]. This degradation depends upon a cytoplasmic E3, KPC (Kip1-promoting ubiquitination complex) [80]. The third CIP/KIP family CKI, p57, is also subject to alternative degradation pathways. In addition to being an SCF^{Skp2} target, p57 is ubiquitylated by SCF^{Fbl12} when degradation is induced by TGFβ1 signaling [81]. Together, these data suggest that unlike cyclin degradation, where E3s may act redundantly by targeting identical pools of cyclin proteins, the multiple E3s that target CKIs appear to act on different pools of protein (Figure 1). This type of regulation allows CKI degradation to be induced in response to a variety of signals.

2.3. Other interphase targets of the UPS

While cyclins and CKIs play primary roles in regulating the timing of G1 phase, and thus couple growth and cell division, there are a number of additional cell cycle regulators that are also targeted by the UPS during interphase. Of particular importance are the degradation of Wee1 and Cdc25, two proteins that regulate Cdk phosphorylation and activity [82,83]. Wee1 adds an inhibitory phosphate to Cdk, thus restraining its activity, delaying the cell cycle, and allowing cells to grow. The Cdc25 family of phosphatases remove Wee1 phosphorylation and thus also impact cell growth. Another important group of UPS targets are proteins required for DNA replication, such as Cdc6 in yeast, and Cdt1 and Orc1 in metazoans [84,85]. Degradation of replication proteins after they fulfill their essential functions helps to prevent re-replication of DNA and maintains the proper order of cell cycle events. Like cyclins and CKIs, almost all of these proteins are ubiquitylated by more than one E3, again emphasizing the complexity of the cell cycle proteolytic regulatory network.

3. The UPS couples metabolism and the cell cycle

Another important aspect of cell cycle control is the coupling of the availability of extracellular nutrients and cell cycle entry. As cells exit from mitosis they can either commit to the next cell cycle and pass through a point in late G1 phase termed the restriction point, or they can exit the cell cycle and become quiescent [86]. One of the major determinants of passage through the restriction point is the metabolic activity of the cell. While there are several mechanisms for coupling metabolism and cell division, ubiquitylation by APC and SCF family ubiquitin ligases plays an important role in coordinating glucose metabolism with proliferation, as well as sensing cellular energy status, as described below.

3.1. SCF^{Grr1} coordinates glucose signaling and cell division in yeast

One of the first indications that the UPS plays a role in coordinating metabolism and the cell cycle came from the characterization of the FBP Grr1 in budding yeast. When yeast cells are grown in media containing glucose they repress transcription of genes that are required for the utilization of alternative carbon sources in a process termed glucose repression [87,88]. *GRR1* was originally identified as being required for glucose repression, as well as to activate the transcription of hexose transporter genes in response to extracellular glucose [89–91]. Subsequently, Grr1 was found to target several proteins involved in cell cycle control, including the G1 cyclins Cln1 and Cln2 [48], suggesting that it may function to coordinate glucose-sensing with the cell cycle.

How does Grr1 couple glucose sensing to proliferation? One possibility is that formation of the SCF^{Grr1} complex might be regulated by the presence of glucose. The model is supported

by evidence from yeast-two hybrid assays, which demonstrate that interaction between a truncated Grr1 protein and the SCF adaptor Skp1 is enhanced when cells are grown in glucose as compared to raffinose [92]. However, many Grr1 targets are degraded in the absence of glucose, arguing that glucose is not required for the Grr1-Skp1 interaction. In fact, two glycolysis-promoting factors, the transcription factor Tye7 and the glycolytic activator Pfk27, undergo accelerated Grr1-dependent degradation when glucose is removed from cells [93]. This causes cells to halt glycolysis and turn on gluconeogenesis when glucose levels drop (Figure 2A). Cells expressing non-degradable Pfk27 fail to proliferate in media containing glycerol [93], demonstrating that Grr1 plays a role in coordinating glycolysis and cell division.

Like other FBPs, most regulation of Grr1-dependent ubiquitylation depends upon the regulated phosphorylation of its substrates. Grr1 targets different sets of substrates in the presence and absence of glucose, depending on when substrates become phosphorylated. In the absence of glucose, Pfk27 is phosphorylated by the AMPK homolog Snf1, which promotes its interaction with Grr1 and subsequent degradation [93]. However, when glucose is added to cells, Grr1 targets the signaling proteins Mth1 and Std1, leading to increased transcription of hexose transporter genes [94–96]. Mth1 targeting depends upon phosphorylation by the casein kinases Yck1/2, which only occurs in the presence of glucose [95]. Therefore, while it is possible that glucose has a modest effect on the SCF^{Grr1} complex, most data supports the model that some active SCF^{Grr1} complex is always present and that phosphorylation of specific substrates directs their targeting by Grr1. By ubiquitylating proteins that are phosphorylated in a glucose-dependent manner, Grr1 can couple metabolism and the cell cycle. For example, when diploid cells are grown in glucose, Grr1 blocks cells cycle exit and the initiation of sporulation through the targeting of the kinase Ime2. When glucose is removed Ime2 is stabilized, cells exit the cell cycle and sporulation proceeds [97].

3.2. SCF and APC ubiquitin ligases couple glucose metabolism and the cell cycle in mammalian cells

Recent evidence suggests that the UPS also coordinates glycolysis and proliferation in mammalian cells. One protein that has emerged as a central player in this coordination is a homolog of yeast Pfk27, PFKFB3. PFKFB3 is one of several isoforms of the bifunctional 6-phosphofructo-2-kinase/fructose-2,6-bisphosphatases. Unlike other PFKFB isoforms, PFKFB3 has very little phosphatase activity and acts primarily to activate glycolysis by producing the glycolytic activator fructose-2,6-bisphosphate (F-2,6-BP). F-2,6-BP activates 6-phosphofructo-1-kinase (PFK1), which catalyzes a rate-limiting step of glycolysis. Shortly after Pfk27 was identified as the target SCF^{Grr1} ubiquitin ligase in yeast, PFKFB3 was found to be a target of APC^{Cdh1} in neurons [98]. Continuous degradation of PFKFB3 in neurons helps maintain their low glycolytic state. Interestingly, in dividing cells, PFKFB3 is targeted for degradation by both APC^{Cdh1} and SCF^{βTRCP}, which leads to its cell cycle-regulated degradation (Figure 2B) [99,100]. PFKFB3 is transcribed during a discreet window of time during G1 phase. The UPS then fine tunes PFKFB3 protein expression through the coordination of ubiquitin-mediated degradation by APC^{Cdh1} in late mitosis/early G1 and SCF^{βTRCP} in late S phase. PFKFB3 expression in S-phase is critical for cells to proceed into S phase after they have been arrested by glucose depletion, since cells not expressing PFKFB3 remain arrested in G1 [100].

Why might degradation of PFKFB3 be necessary? Some evidence suggests that PFKFB3 may have oncogenic properties and thus its degradation may be important to prevent uncontrolled proliferation. PFKFB3 is expressed at high levels in several types of rapidly dividing cancer cells and may contribute to the altered metabolic state of these cells [101]. Cancer cells exhibit increased glycolytic flux, even in the presence of oxygen, a

phenomenon referred to as the Warburg effect [102]. This phenomenon has been proposed to be necessary so that rapidly dividing cells can meet the increased demand for metabolic intermediates, which are needed for the increased biosynthesis of macromolecules [103,104]. In normal cells oxygen inhibits glycolysis, but this process can be overridden by high levels of F-2,6-BP, suggesting that PFKFB3 overexpression may drive aerobic glycolysis. It is not known whether disruption of proteolytic degradation of PFKFB3 contributes to its increased expression in tumor cells. However, β TRCP mutations that could interfere with substrate targeting have been identified in gastric and prostate cancers [105,106]. In addition, APC^{Cdh1} has some tumor suppressor activity and is downregulated or inactivated in some cancers [82,107]. In future studies it would be interesting to determine if PFKFB3 levels and/or metabolic activity are altered in cells carrying these mutations. All together, these data suggest that PFKFB3 may promote proliferation increasing glycolytic flux to allow cells to have a greater biosynthetic capability. The conserved proteolytic regulation of the related Pfk27 and PFKFB3 enzymes in yeast and humans places the UPS as an important regulator of the coupling between glycolysis and the cell cycle (Figure 2).

3.3. Regulation of the G1/S transition by ATP levels

In addition to providing essential building blocks for the biosynthesis of macromolecules, another important outcome of metabolic processes is the generation of energy in the form of the ATP, which the cell can then use to carry out enzymatic reactions. Since S phase is the major biosynthetic phase of the cell cycle and requires large quantities of ATP, cells also couple ATP availability with passage through the G1/S transition. The mechanism of this coupling has been studied in *Drosophila melanogaster*, where mutations that result in reduced mitochondrial function lead to delayed entry into S phase in the developing eye disk [108]. Two pathways have been implicated in coupling ATP levels to cell cycle progression. The first is a transcriptional mechanism: flies that have elevated levels of reactive oxygen species (ROS) initiate a signaling cascade that leads to the transcriptional upregulation of the Cdk inhibitor dacapo (p27) [109]. The second pathway relies upon the UPS. When the ratio of ATP:AMP in cells is low, this leads to activation of the kinase AMPK. AMPK activation in G1 stimulates p53-dependent transcriptional activation of archipelago (ago), which encodes the F-box protein homologous to mammalian Cdc4. Increased ago promotes ubiquitin-mediated degradation of cyclin E which, in turn, delays the G1/S transition [110].

4. Conclusion

In conclusion, the UPS plays essential roles in controlling cell cycle progression, and in linking the cell cycle with the metabolic activity of the cell. To fully understand how the UPS regulates cell division, one goal in the field has been to catalogue the network of E3-substrate interactions that occur during the cell cycle. However, as the number of E3s that regulate the degradation of cell cycle and metabolic proteins continues to grow, it has become increasingly clear that multiple degradation pathways can control the degradation of most key regulators (Figure 1). This has several important implications for the field moving forward. First, as exemplified by the G1 cyclins Cln3 and cyclin D1, it can be extremely difficult to identify specific E3s involved in regulating a protein's degradation. In the case of the cyclins, this is because mutation of a single relevant E3 does not necessarily have an effect on that target's steady-state protein levels or half-life. Therefore, it may be necessary to use other approaches to first identify E3s that have overlapping functions *in vivo*, and then try to identify the proteins that are redundantly ubiquitinated by these E3s. Given the large number of proteins that are predicted to be cell cycle regulated (as discussed above), elucidating the network of E3s that target these proteins is a formidable challenge.

The network of E3s that regulate the destruction of CIP/KIP family CKIs in metazoans also has implications for future work. A common theme in CKI targeting is that different pools of these regulators are targeted by different E3s, which localize to different compartments, or bind the targets in response to different cues. This suggests that the proteolytic regulatory network controlling the cell cycle is rewired in different conditions. Not only may a specific protein be targeted by a different E3s in a different condition, but a given E3 may target a different array of substrates in that alternate condition, as has been shown for SCF^{Grr1} in yeast.

Finally, the role of the UPS in regulating metabolic switches is just beginning to be explored. As more metabolic proteins are found to be UPS targets, it will be of considerable interest to determine how the E3 network that controls metabolism is interwoven with the E3 network that controls cell division. By regulating both the cell cycle and metabolism, the UPS is poised to play an instrumental role in the coordination of cellular growth and proliferation.

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Highlights

- Coupling of growth and the cell cycle is important for accurate cell proliferation.
- The UPS targets cell cycle and metabolic regulatory proteins for degradation.
- Multiple ubiquitin ligases target most key regulators.
- The UPS plays a key role in coupling growth and cell division.

Figure 1. The E3 network that ubiquitylates G1 and G1/S cyclins and CKIs

Diagram of G1 and G1/S phase cyclins and CKIs, and the E3s that have been implicated in their degradation. Yeast proteins are diagrammed in the top half of the figure, vertebrate proteins are diagrammed in the bottom half. SCF ligases are indicated by their, specific FBPs. Related FBPs are colored similarly (yeast Cdc4 and vertebrate Fbw7 in red, yeast Grr1 and vertebrate Skp2 in blue).

Figure 2. Glycolysis is downregulated through the targeting of 6-phosphofructo-2-kinase by the UPS

One rate-limiting step of glycolysis is the conversion of fructose-6-phosphate (F-6-P) to fructose-1,6-bisphosphate (F-1,6-BP), which is catalyzed by phosphofructokinase (PFK1). The 6-phosphofructo-2-kinase enzymes (Pfk27 in yeast and PFKFB3 in mammals) generate the second messenger fructose-2,6-bisphosphate (F-2,6-BP) which is an essential activator of PFK1. By degrading these enzymes that generate F-2,6-BP, the UPS can downregulate glycolysis in response to specific cues. **(A)** In yeast, low extracellular glucose promotes the ubiquitin-mediated degradation of Pfk27 by SCF^{Grr1}. **(B)** In mammals, glycolysis is restricted to S phase through the combination of cell cycle-regulated transcription and degradation of PFKFB3 in G1 by APC^{Cdh1} and in S phase by SCF^{βTRCP}.