

The Multiple Layers of Ubiquitin-Dependent Cell Cycle Control

Katherine Wickliffe, Adam Williamson, Lingyan Jin, and Michael Rape*

Department of Molecular and Cell Biology, University of California at Berkeley, Berkeley, California 94720

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1. Introduction

Proliferation is driven by an intricate machinery, the smooth operation of which is essential for the development and survival of the organism. The central task of this machinery is to copy the genetic material of a dividing cell and to distribute it evenly between daughter cells. Yet, in higher eukaryotes, the division of every single cell has to be coordinated with the development of the whole organism. Mistakes in this cell cycle program are rarely tolerated: they

give rise to developmental aberrations or cancer and often lead to the organism's demise.

A collaborative effort from many laboratories has transformed our understanding of the cell cycle from a black box to an amazing level of molecular detail. Early groundbreaking studies revealed the crucial role of oscillating kinase activities in driving the embryonic cell cycle.^{1–4} The activation of a kinase, which was first referred to as “maturation-promoting factor” and later identified to be cyclin-dependent kinase Cdk1, was shown to trigger entry of cells into mitosis. Its capability to phosphorylate its substrates is dependent on the periodic synthesis of essential cyclin subunits.^{5–7} Consequently, the removal of cyclins shuts down Cdk1 and leads to exit from mitosis. It was soon realized that cyclins are disposed of by ubiquitin-proteasome-dependent degradation,⁸ which brought ubiquitination into the limelight of cell cycle control.

Subsequent studies extended the role of ubiquitination in regulating proliferation far beyond its part in the tug-of-war between kinase activation and protein degradation. Ubiquitination also regulates checkpoints that ensure the high fidelity of cell division and signaling networks that couple proliferation to differentiation and development. Such a powerful role in cell cycle control comes with its own risk, and many malignancies result from aberrant ubiquitination. In this review, we will discuss key concepts that illustrate how ubiquitination regulates cell cycle progression and how its misregulation can trigger aberrant proliferation and cancer.

2. The Players in Ubiquitin-Dependent Cell Cycle Control

2.1. An Enzymatic Cascade Mediates Ubiquitin-Dependent Cell Cycle Control

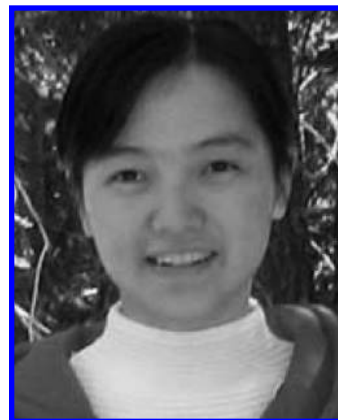
During ubiquitination, a covalent isopeptide bond is formed between the carboxy terminus (C-terminus) of ubiquitin and a nucleophilic side chain in the substrate protein.^{9,10} In the vast majority of cases, ubiquitin is linked to the ϵ -amino group of lysine residues, but modifications also occur at the amino-terminus,¹¹ the hydroxyl group of serine residues,¹² or the thiol group of cysteine residues.^{13,14}

In order to allow the transfer of ubiquitin to its acceptor, it is first activated by a ubiquitin-activating enzyme, E1 (Figure 1). E1 uses ATP to form a phosphodiester bond between the C-terminus of ubiquitin and AMP, before the ubiquitin is transferred to the active-site cysteine of E1 by thioester formation.¹⁵ At least two human E1 enzymes activate ubiquitin,^{16–18} but in most cases, this reaction is carried out by the product of the essential *UBE1* gene. In dividing cells, the Ube1 protein localizes to hotspots of cell cycle control, such as the cytoskeleton, the mitotic spindle,

* Corresponding author. E-mail: mrape@berkeley.edu.



Katherine Wickliffe graduated from Oberlin College with a B.A. in biology. She is currently a graduate student in Molecular and Cell Biology at the University of California, Berkeley, in the laboratory of Michael Rape. Her project focuses on identifying novel proteins that regulate the cell cycle via ubiquitin-dependent degradation.



Lingyan Jin is a graduate student in department of Molecular and Cell Biology at the University of California, Berkeley. Before coming to the U.S. two years ago, she graduated from Fudan University in China with a B.S. degree in Biological Sciences. Lingyan currently works in the laboratory of Michael Rape on identifying new enzymes involved in protein degradation during stem cell differentiation.



Adam Williamson is a graduate student in Molecular and Cellular Biology at the University of California, Berkeley. He graduated from Carleton College in Northfield, MN, with a B.A. degree in Biology. Adam currently studies in Michael Rape's laboratory at Berkeley. His project focuses on identifying new ubiquitin ligases that control mitotic spindle formation.



Michael Rape is an Assistant Professor of Cell and Developmental Biology in the Department of Molecular Biology at the University of California at Berkeley. He received a diploma in biochemistry from Bayreuth University, Germany, in 1999. Michael earned his Ph.D. degree in 2002 with Stefan Jentsch at the Max-Planck Institute of Biochemistry in Martinsried, Germany. From there, he went to the U.S. to pursue postdoctoral research with Marc Kirschner at Harvard Medical School in Boston. Michael accepted his current position at Berkeley in the fall of 2006. His laboratory is interested in dissecting the role of ubiquitination in controlling proliferation and differentiation. Michael has recently received a Pew Scholars Award and an NIH Director's New Innovator Award.

or the spindle midzone.¹⁹ Cell lines carrying thermosensitive mutations in *UBE1* suffer from cell cycle arrest and in fact provided early evidence that ubiquitination is crucial for cell cycle control.²⁰

The charging with ubiquitin triggers conformational changes in E1 that expose a binding site for the recruitment of one of ~60 human ubiquitin-conjugating enzymes, or E2s.²¹ E2s receive the activated ubiquitin on an active-site cysteine by trans-esterification. Most E2s are single domain proteins, some of which have short extensions at the N- or C-terminus. Several of these E2s, including Ubc13, Cdc34, or UbcH10, have central functions in cell cycle control. In few cases, E2 domains are found in large multifunctional proteins, one of which, the 528 kDa BRUCE, localizes to the spindle midzone and functions during the abscission stage of cytokinesis.²²

Once ubiquitin is bound to an E2, its transfer to the substrate relies on ubiquitin-protein ligases, or E3s. The E3s recruit distinct sets of substrates and thereby provide most of the specificity of ubiquitination. They largely come in two different flavors and contain either a catalytic HECT or RING domain. HECT-domains (*H*omologous to *E6AP C*-Terminus) harbor an active-site cysteine, which is charged with ubiquitin by E2s before the ubiquitin is transferred to the substrate. By contrast, RING-E3s (*R*eally *I*nteresting *N*ew *G*ene) do

not have an active site cysteine. Instead, they bind at the same time to the charged E2 and the substrate and activate the E2 to transfer ubiquitin directly to the substrate acceptor.^{23–26}

With ~50 HECT and ~600–1000 RING domain containing proteins, E3s are the most abundant class of ubiquitination enzymes in humans. Many of these enzymes have pivotal roles in cell cycle control. For example, HECT-E3s are named after the E6AP protein, which is hijacked by the human papillomavirus E6 protein to catalyze the ubiquitination and degradation of the cell cycle regulator p53.^{27,28} Other HECT-E3s implicated in cell cycle control include Smurf ubiquitin ligases, which terminate antimetastatic TGF- β signaling,^{29,30} or Itch, which promotes the endocytosis of ErbB4 growth factor receptors.³¹ Among the many RING-E3s controlling proliferation are the anaphase-promoting complex (APC/C),^{32–35} the SCF,³⁶ the tumor suppressor Brca1/Bard1,³⁷ or the oncogene Mdm2.³⁸

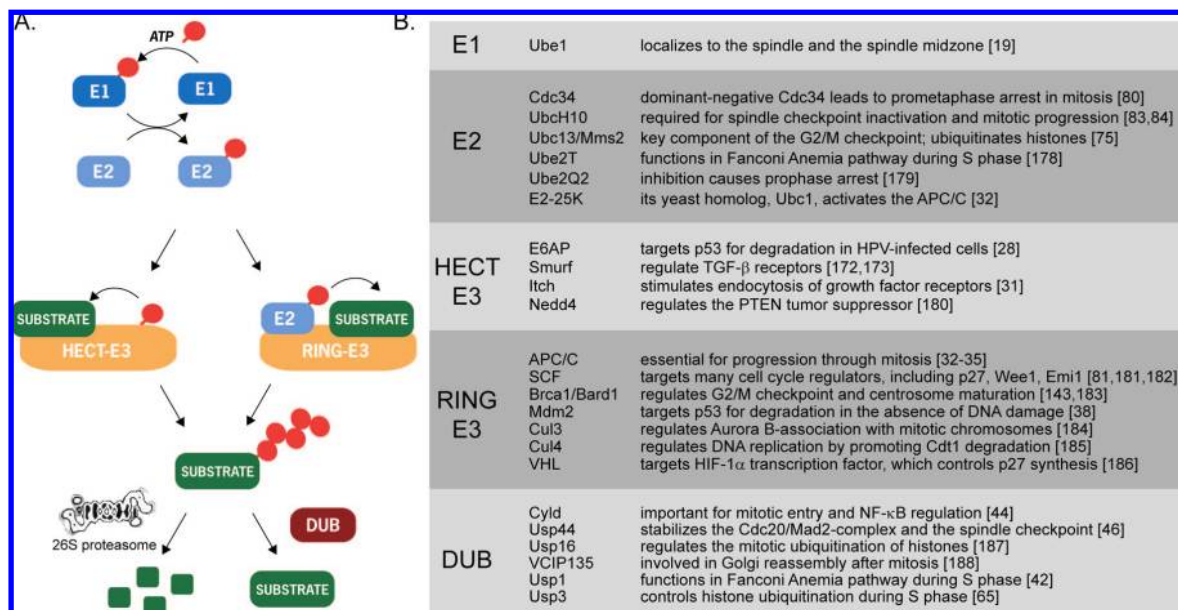


Figure 1. Enzymatic players in ubiquitin-dependent cell cycle control: (A) schematic overview over the enzymatic cascade catalyzing ubiquitination; (B) examples of enzymes at the different steps of ubiquitination that have roles in ubiquitin-dependent cell cycle control.

The modification with ubiquitin can be reversed by ~ 100 human deubiquitinating enzymes (DUBs).^{39,40} DUBs contain a cysteine protease domain or a Zn²⁺-binding JAMM domain,⁴¹ through which they hydrolyze the isopeptide bond between the C-terminus of ubiquitin and the substrate. As regulators of proliferation, DUBs themselves are controlled during cell cycle progression by recruitment of binding partners,⁴² regulation of expression levels,^{43,44} autocatalytic cleavage,⁴⁵ or modifications.⁴⁶ The misregulation of DUBs can result in aberrant proliferation and cancer, which is well understood for the tumor suppressor Cyld.^{44,47}

Proteins decorated with ubiquitin chains containing at least four ubiquitin molecules can be recognized by the 26S proteasome.⁴⁸ The proteasome is a compartmentalized protease, which unfolds ubiquitinated proteins and degrades them in a secluded chamber.⁴⁹ It recognizes ubiquitinated proteins by using dedicated receptors, which either bind the proteasome transiently or are integral components of the proteasomal cap.^{49–51} Before substrates are degraded, ubiquitin is cleaved off by proteasome-resident DUBs so that it can be reused by the cell.⁴⁰ The activities of the proteasome and its associated DUBs are essential for cell cycle progression. Consequently, proteasome inhibition blocks proliferation of cancer cells and has recently been approved for the treatment of multiple myeloma.⁵²

2.2. The APC/C and the SCF: Leading Characters in the Cell Cycle Cast

Two main characters in this impressive cast of enzymes are the anaphase-promoting complex (APC/C) and the SCF.^{32–36} Both enzymes are essential for proliferation in all eukaryotes. In dividing cells, the APC/C orchestrates progression through mitosis and G1, while the SCF operates at all stages of the cell cycle program. Despite differences in their temporal regulation, the APC/C and the SCF are very similar in their basic architecture.

Both the APC/C and the SCF are oligomeric RING-E3s, which have multiple core subunits (4 in the case of SCF; 13 for APC/C).⁵³ In both enzymes, the catalytic activity is provided by RING-finger proteins, Rbx1 or Ro52 in SCF^{36,54}

and Apc11 in APC/C,⁵⁵ which are anchored to the enzyme by a scaffold cullin protein (Cul1 in SCF and Apc2 in APC/C). The various substrates are delivered to the SCF or APC/C by specific adaptors that associate only transiently with the E3s. The SCF, for example, uses ~ 60 F-box proteins, which contain an F-box domain to associate with the SCF-core subunit Skp1, and a substrate binding domain, such as WD40- or leucine-rich repeats.⁵⁶ The equivalent of F-box proteins in the APC/C are the coactivators Cdc20 and Cdh1, which employ WD40-repeats as substrate binding domains.^{57,58}

While the blueprints for SCF and APC/C are similar, differences exist in the regulation of substrate binding to these E3s. The SCF is always eager to promote ubiquitination but only modifies substrates that have been marked by post-translational modifications, such as phosphorylation. Once a substrate is phosphorylated, it is rapidly recognized by the F-box protein and delivered for ubiquitination to the SCF.³⁶ Mutation of phosphorylation sites or kinases stabilizes SCF substrates, which in the case of β -catenin results in aberrant cell cycle control and cancer.⁵⁹ Contrary to SCF, the activity of the APC/C itself is regulated during the cell cycle, and APC/C substrates are usually not modified. The APC/C is activated during mitosis by phosphorylation of core subunits,⁶⁰ and it is shut down during late G1 by degradation of its specific E2 Ubch10,⁶¹ degradation and inhibitory phosphorylation of the substrate targeting factors Cdc20/Cdh1,⁵⁷ and expression of its inhibitor Emi1.^{57,63} Additional inhibitors, such as the spindle checkpoint proteins Mad2 and BubR1, ensure APC/C activation at the proper time and place during the cell cycle, which will be discussed in more detail throughout this review.^{62–64}

2.3. Different Ubiquitin Modifications Have Distinct Functions in Cell Cycle Control

The intimate collaboration of E1, E2s, and E3s results in the transfer of a single ubiquitin (monoubiquitination) or the decoration of substrates with ubiquitin chains (multiubiquitination) (Figures 2). Depending on the modified protein, monoubiquitination recruits different partners that then trigger specific reactions. In eukaryotic cell cycle control, the

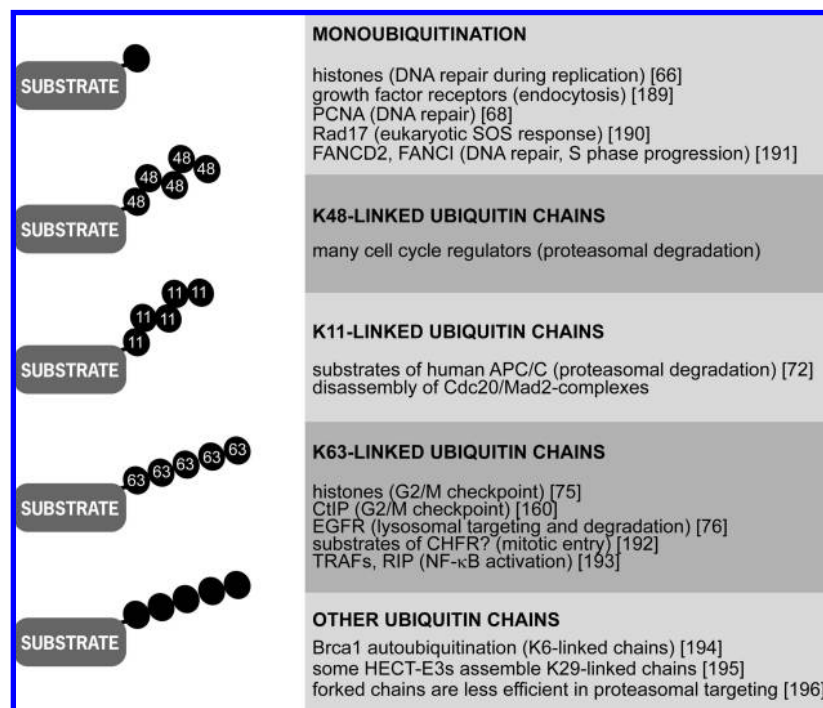


Figure 2. Different ubiquitin modifications have distinct functions in cell cycle control. Substrates can be modified with a single ubiquitin (monoubiquitination) or with ubiquitin chains (multiubiquitination). Ubiquitin chains differ in structure and function, depending on what lysine of ubiquitin is used for chain formation. Examples for different modifications and their substrates in cell cycle control are shown on the right.

monoubiquitination of histones is important for recognition of stalled replication forks and S phase progression,^{65,66} monoubiquitination of growth factor receptors regulates their endocytosis and limits growth factor signaling,⁶⁷ and monoubiquitination of PCNA recruits translesion polymerases to mediate DNA repair, thereby removing road blocks to cell cycle progression.⁶⁸

When chains are assembled, one of the seven lysine residues of ubiquitin serves as acceptor site for the next ubiquitin to be attached. Depending on the lysine residue preferentially employed for chain formation, these chains differ in structure and function.^{69,70} In yeast, all lysine residues of ubiquitin can be modified,⁷¹ but we understand most about the consequences of chains linked through K48, K11, or K63. K48-linked chains are bound by the 26S proteasome; they are the canonical signal for degradation, and essential for proliferation.³² Chains linked through K11 of ubiquitin are assembled by the human cell cycle E3 anaphase-promoting complex (APC/C).^{72,73} These K11-linked chains are recognized by several proteasomal substrate receptors and trigger the degradation of APC/C substrates. Because the APC/C is required for proliferation in all eukaryotes, it is likely that K11-linked chains are essential, too, but this remains to be determined. By contrast, K63-linked ubiquitin chains usually function independently of the 26S proteasome. By recruiting specific binding partners to modified substrates, K63-linked chains activate kinases during inflammation,⁷⁴ orchestrate events in the G2/M checkpoint,⁷⁵ or mediate lysosomal targeting of activated growth factor receptor.⁷⁶

What determines which chain type is formed? In many cases, the ubiquitin chain topology is dependent on the E2. K63-linked chains, for example, are assembled by the heterodimeric E2 Mms2–Ubc13, which functions in cell cycle control due to its role in the G2/M checkpoint. Mms2, a ubiquitin-E2 variant (UEV), has no E2-activity itself.

Instead, it binds the acceptor ubiquitin, which brings K63 of the acceptor ubiquitin into proximity of the active site of the E2 Ubc13.⁷⁷ Since Ubc13 can only function when presented with an acceptor ubiquitin by Mms2, Mms2–Ubc13 is able to elongate ubiquitin chains but may be unable to add the first ubiquitin to a substrate lysine.^{78,79}

In a similar manner, E2s are pivotal for the formation of K48- and K11-linked chains.^{32,72,80} The E2 Cdc34, which is important at multiple cell cycle stages,^{80,81} is responsible for the assembly of K48-linked chains by the cell cycle E3 SCF. This capability of Cdc34 requires a conserved acidic loop in Cdc34, which may directly contact ubiquitin.⁸² Also, the formation of K11-linked chains by the APC/C depends on its E2, UbcH10, which is required for progression of cells beyond metaphase.^{72,73,83,84} The assembly of K11-linked chains by APC/C and UbcH10 relies on short sequence motifs present in substrates and in ubiquitin, which are referred to as TEK boxes.⁷² In addition, UbcH10 cooperates with a novel E2 enzyme, which helps determine the specificity for K11-linked ubiquitin chains (K.E.W., A.W., unpublished).

3. The Different Functions of Ubiquitination in Cell Cycle Control

Ubiquitin is such a crucial regulator of proliferation that discussing every mechanism of ubiquitin-dependent cell cycle control would go far beyond the scope of this review. We will instead focus our discussion on selected examples that illustrate how ubiquitin can take charge over proliferation (Figure 3). We will review how ubiquitin-dependent proteolysis ensures the regulated and unidirectional progression through the cell cycle; how ubiquitination and proteolysis orchestrate the function of cell cycle checkpoints; and how ubiquitination and endocytosis coordinate proliferation with development.

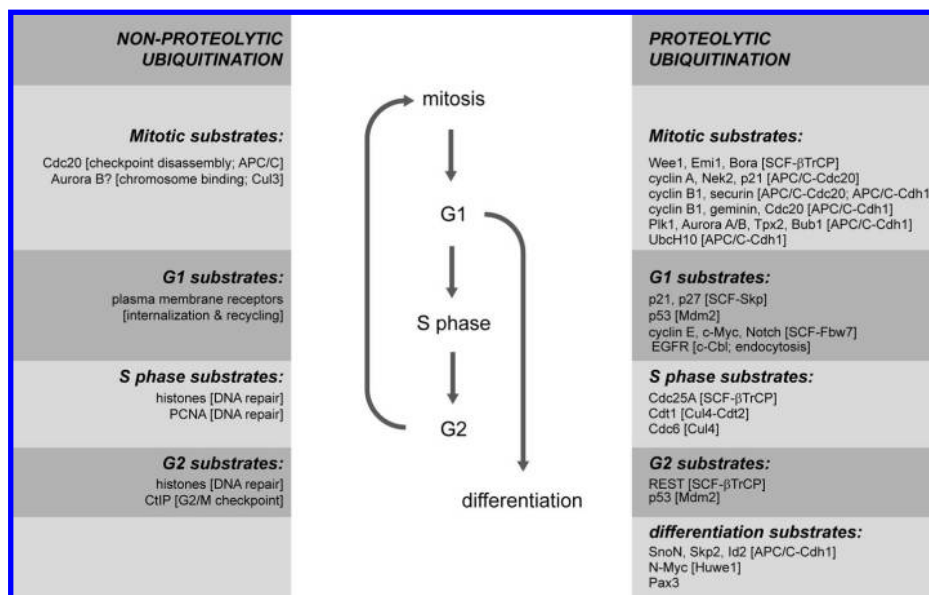


Figure 3. Examples of modified substrates in ubiquitin-dependent cell cycle control.^{178–196} In the left panel are examples of ubiquitinated proteins that are not degraded upon their modification. In parentheses, the function of the relevant modification in cell cycle control is noted. In the right panel are examples of proteins that are degraded after being ubiquitinated at different times in the cell cycle. In parenthesis, the relevant ubiquitin ligases are noted.

3.1. The Role of Ubiquitin-Dependent Proteolysis in Cell Cycle Progression

3.1.1. Do Not Look Back: Generation of Irreversible Cell Cycle Transitions

Cells have to guarantee that their genomic information is replicated only once per cell cycle and that the two identical copies are distributed evenly between the daughter cells. To this end, cells have to ensure that replication always precedes mitosis. The necessary unidirectionality of cell cycle progression depends on irreversible transitions between cell cycle stages, which is achieved by ubiquitin-dependent degradation.

A well characterized example of how ubiquitin-dependent proteolysis generates irreversible cell cycle transitions is exit from mitosis, which depends on the degradation of cyclin proteins.⁸ The A- and B-type cyclins activate the major mitotic kinase, Cdk1, to constitute “maturation promoting factor”, the activity of which is required for establishing and maintaining the mitotic state. Without cyclins, Cdk1 is unable to bind and phosphorylate its many mitotic substrates.⁵ Consequently, the ubiquitination of cyclins A and B by the anaphase-promoting complex (APC/C) and their degradation by the 26S proteasome efficiently shuts down Cdk1 and promotes exit from mitosis. The importance of this proteolytic event is illustrated by cells that fail to degrade cyclins: they arrest in mitosis. Such failure of cyclin degradation can be caused by inhibition of the proteasome,⁸⁵ activation of checkpoints that inhibit the APC/C,⁶² or expression of cyclin mutants not recognized by the APC/C.⁸⁶

The degradation of cyclins is the key mechanism of triggering Cdk1 inactivation and mitotic exit in eukaryotes. To test whether the irreversibility of mitotic exit relies on ubiquitin-dependent proteolysis, mitotic cells can be treated with the Cdk1-inhibitor flavopiridol.⁸⁷ Because active Cdk1 is essential for mitosis, its inhibition by flavopiridol promotes mitotic exit, and this occurs even if the degradation of cyclin B is blocked by proteasome inhibitors.⁸⁷ If flavopiridol is removed from postmitotic cells still containing cyclin B1,

however, the remaining cyclin quickly activates Cdk1 and pushes cells back into mitosis! Upon reverse entry into mitosis, cells break down their nuclear envelopes, condense their chromosomes, and align them at the metaphase plate. If occurring during normal cell cycle progression, such a “reverse transition” would wreak havoc as mitotic checkpoints will not be fully functional and the likelihood of mistakes in chromosome segregation will be high. Since in the absence of drugs, mitotic exit depends on cyclin B1 degradation, such inappropriate reverse transitions cannot occur. Thus, ubiquitin-dependent degradation is essential for the irreversible nature of the M–G1 transition.

Ubiquitin-dependent proteolysis serves a similar role in establishing the irreversible transition from G1 into S, which is pivotal for DNA replication.⁸⁸ During G1, when Cdk activity is low, cells license their origins of replication. However, they initiate DNA replication only after having activated cyclin-dependent kinases at the G1–S transition. Irreversible kinase activation is brought about, at least in part, by the ubiquitin-dependent degradation of a Cdk inhibitor, p27. If degradation of p27 is disturbed, cells can revert back from S into G1 and relicense origins of replication again that have already been used. This allows these cells to amplify genes or chromosomes, an event often observed in tumors.^{89,90}

Consistent with its importance for cell cycle control, the degradation of p27 is tightly regulated. When, during G1, cells commit to another round of cell division, they activate Src and Abl kinases. These kinases phosphorylate p27 on a conserved tyrosine, Y88, which leads to the displacement of p27 from its binding site in the catalytic cleft of Cdk2.^{91,92} This in turn allows cyclin E/Cdk2 to phosphorylate p27 on another conserved threonine, T187, thereby creating a binding site for p27 on the F-box protein Skp2 and its associate Cks1.⁸¹ Together with Cks1, SCF^{Skp2} ubiquitinates p27 and marks the Cdk inhibitor for degradation by the 26S proteasome, which results in a burst of Cdk2 activity, activation of E2F transcription factors, and synthesis of more A- and E-type cyclins.⁹³ The ensuing full activation of Cdk2

commits cells to entry into S. Since no p27 is left, cells cannot revert back into G1 and entry into S is irreversible.

The degradation of p27 provides directionality but also releases a brake in the cell cycle, which is exploited by hyperproliferative tumor cells. For example, amplification of the EGF receptor in breast cancer cells or activation of Abl by the *BCR-ABL* translocation in chronic myelogenous leukemia activates Src or Abl kinases and targets p27 for degradation.^{88,94} Loss of the PTEN tumor suppressor results in increased expression of Skp2 and, conversely, lower levels of p27.⁹⁵ Overexpression of Skp2 is, in fact, observed in many malignancies.⁹⁶ An increased level of p27 degradation is a prognostic factor for disease progression and therapeutic response in cancer, with low levels of p27 correlating with poor prognosis.

3.1.2. Step by Step: Transitions within a Cell Cycle Stage

Not only transitions between cell cycle stages but also the processes within one cell cycle phase have to occur in a defined sequence. This is illustrated during mitosis, which in mammalian cells takes less than one hour. In this short period of time, cells break down their nuclear envelope, condense their chromosomes, attach them to the spindle, distribute the sister chromatids to the two daughter cells, and finally separate the daughters during cytokinesis. Just imagine a cell starting cytokinesis before the chromosomes were even attached to the spindle—big mistake!

The sequence of mitotic events is determined by closely connected processes, including phosphorylation of hundreds of proteins and precisely timed ubiquitin-dependent degradation. The main director of mitotic degradation is the APC/C, which in addition to the cyclins mentioned before, controls the turnover of other mitotic kinases, spindle organizers, centrosomal proteins, kinesins, cytokinesis regulators, and finally its own E2, UbcH10.^{53,57,61,97,98} The APC/C ubiquitinates its substrates in a sequential manner, referred to as “substrate ordering”.^{72,99} As much as we can tell from the analysis of APC/C substrates in multiple species, the sequence of APC/C-dependent ubiquitination reactions is conserved through evolution.^{100,101} Disturbing substrate ordering would be deleterious: for example, the premature degradation of the cytokinesis regulator Plk1 can lead to massive cytokinesis defects and multinucleation.⁹⁷

Consistent with the robustness of cell cycle control, several mechanisms keep up substrate ordering by the APC/C. Anything but an unbiased enzyme, the APC/C discriminates between substrates by catalyzing their ubiquitination with different degrees of processivity.^{99,102} Its “favorites”, such as cyclin B1 or securin, are decorated with long ubiquitin chains within a single binding event. The length of the ubiquitin chains attached to such processive substrates exceeds the four ubiquitin molecules required for recognition by the proteasome.⁴⁸ The most processive substrates of the APC/C are consequently the first proteins to be degraded after the APC/C is switched on to fully active at the metaphase–anaphase transition.

Less preferred substrates, however, require multiple binding events to the APC/C to receive a ubiquitin chain long enough for proteasomal recognition. After dissociation, these distributive, partially ubiquitinated substrates compete for rebinding to the APC/C with all its other substrates. The competitiveness of the mitotic environment is illustrated by a comparison of protein concentrations: each of the rate-limiting APC/C activators Cdc20 and Cdh1 is present at

approximate concentrations of 50 nM, while the combined concentration of all mitotic APC/C substrates exceeds 2–3 μ M (M.R., unpublished). A slight increase in the level of a processive substrate can throw the whole system off balance and bring mitotic progression to a halt.¹⁰³ The dissociated substrates are also recognized by deubiquitinating enzymes, which remove already attached ubiquitin.^{46,99} Together, these mechanisms delay the degradation of distributive substrates to late mitosis or G1. With few exceptions discussed below, the degree of processivity of ubiquitination matches the timing of degradation of APC/C substrates. Substrate ordering by the APC/C, therefore, is the outcome of a fierce competition between ubiquitination and deubiquitination and, thus, similar to a mechanism of kinetic proofreading proposed by Hopfield.¹⁰⁴

The exceptions to the processivity rule are APC/C substrates that are degraded earlier than expected from their processivity of ubiquitination. These substrates are all turned over when the bulk of the APC/C is inhibited by the mitotic spindle checkpoint. They include cyclin A, which activates Cdk1;¹⁰⁵ the mitotic kinase Nek2, which is important for centrosome separation;¹⁰⁶ and the CDK-inhibitor p21.¹⁰⁷ All of these substrates require Cdc20 for degradation, but the mechanisms allowing their degradation despite inhibition of Cdc20 by the spindle checkpoint are only beginning to be understood. It may involve direct binding of substrates to core APC/C subunits, direct competition with spindle checkpoint components for binding to Cdc20, or alternative means of APC/C targeting by Cdk1.^{108,109}

A third mechanism contributing to the efficient degradation of mitotic APC/C substrates is the colocalization of APC/C and substrates at cellular “hotspots”. While much of the APC/C is found in the cytoplasm, it also accumulates on the centrosome and the spindle during mitosis.¹¹⁰ APC/C^{Cdc20} is activated by Cdk1-dependent phosphorylation, and APC/C subunits phosphorylated on Cdk1 consensus sites are first detected on the spindle pole.⁶⁰ The activation of the APC/C on the centrosome or the spindle coincides with the localization of several substrates, including cyclin B1, Tpx2, or Aurora A, to these sites. In *Drosophila*, the APC/C substrate cyclin B1 is first degraded on the centrosome and only later on the spindle and in the cytoplasm.¹¹¹ Losing the connection between the centrosome and the spindle, which occurs in “centrosome-falloff” mutant flies, allows degradation of cyclin B1 at the centrosome but stabilizes it on the spindle.¹¹² Different mechanisms that target inactive APC/C to the spindle or to kinetochores have been proposed,^{113,114} but how active APC/C is brought to microtubules remains to be determined. A recently identified E2-activating enzyme, which can bind both APC/C and microtubules, might provide the missing link (K.E.W., A.W., unpublished).

3.1.3. Road Blocks to Progress: Generating a Regulated Cell Cycle Transition

Especially in higher eukaryotes, it is important not only that cell division occurs with high precision but also that it is integrated into the development of the whole organism. The coordination of proliferation with development depends on signaling by growth and differentiation factors. In order to remain responsive for growth-factor signaling, cells have engineered road blocks against progression through the G1 phase, which depend on ubiquitin-dependent proteolysis brought about by well-studied tumor suppressors.

One of the best understood examples is the human tumor suppressor Fbw7.¹¹⁵ Fbw7 is an F-box protein that recognizes phosphorylated substrates and targets them for ubiquitination by the SCF^{Fbw7} and degradation by the proteasome. Most of the substrates of Fbw7 have important roles in promoting the transition from G1 to S, and their degradation ensures that cells progress into S only after receiving the appropriate signals from their environment. Such Fbw7 substrates include cyclin E, which activates Cdk2 kinase to drive entry into S; the transcription factor c-myc, which promotes entry into S by increasing the synthesis of several cell cycle regulators; and the signaling molecule Notch, which plays a key role in development.^{116–119} The recognition of substrates by Fbw7 requires their phosphorylation on two sites spaced by four amino acids. In most, if not all cases, the kinase responsible for phosphorylation at the –4 site is GSK3, which is regulated by mitogenic signaling.¹¹⁸ If mitogenic signaling is low (i.e., the environment tells the cell not to divide), GSK3-activity is high. Consequently, Fbw7 substrates are fully phosphorylated, ubiquitinated, and degraded, and the cell has to await better conditions to proceed with its cell cycle program.

Loss of Fbw7 function allows cells to proliferate even under adverse conditions, such as those encountered by rapidly dividing tumor cells. Because this endows tumor cells with a growth advantage, Fbw7 function is often impaired in cancer.¹¹⁵ This can occur by deletion of one allele or by mutations in the substrate-binding domain of Fbw7. Conversely, mutations in substrates can also block their phosphorylation or recognition by Fbw7, as observed with c-myc mutations in Burkitt's lymphoma.¹¹⁵ Finally, the viral oncogene SV40 large T antigen (LT) possesses a Fbw7 recognition site, yet binding to Fbw7 does not result in SV40 LT ubiquitination. By functioning as a pseudosubstrate, SV40 LT blocks the substrate-binding site on Fbw7 and stabilizes oncogenic Fbw7 substrates.¹²⁰ The inhibition of E3s by pseudosubstrates has recently emerged as a widespread regulatory mechanism of ubiquitin-dependent cell cycle control, providing a fine example of how studying viral oncogenes can shed light on normal proliferation control.^{63,121}

3.1.4. The Final Transition: Irreversible Exit from the Cell Cycle

A final transition has to be established when cells exit their cell cycle program. This can occur reversibly during periods of starvation or in stem cells that divide rarely and spend most of their time in a quiescent state. However, when cells are instructed to adopt a specific fate during terminal differentiation, they irreversibly cease to proliferate, and this process is dependent on ubiquitin-dependent proteolysis.

The differentiation of dividing stem cells into nondividing, terminally differentiated cells requires tight transcriptional regulation, which is brought about by opposing transcriptional activators and repressors. The degradation of repressors allows cells to rapidly activate genes in response to differentiation cues. A case in point is made by the REST protein, which in stem cells represses the transcription of neuronal genes, such as ion channels or neurotransmitter receptors. Upon differentiation, REST is phosphorylated on C-terminal residues and subsequently recognized by the F-box protein β TrCP.^{122–124} SCF ^{β TrCP} decorates REST with K48-linked ubiquitin chains and targets it for degradation, which coincides with the expression of neuron-specific genes. If this process is blocked, as observed pathologically by

C-terminal frame-shift mutations interfering with REST recognition by β TrCP,¹²⁵ neuronal differentiation is impaired. By maintaining the stem cell character of neuronal precursor cells, stabilized REST behaves like an oncogene, and indeed it is overexpressed in brain cancers.¹²³

In addition to its function during neurogenesis, REST represses the transcription of the essential mitotic spindle checkpoint component Mad2.¹²⁴ In dividing cells, REST is ubiquitinated by SCF ^{β TrCP} and degraded by the 26S proteasome during G2. The degradation of REST is required for achieving expression levels of Mad2 high enough for the functionality of the spindle checkpoint. The stabilization of REST, or its overexpression observed in medullablastoma compromises the spindle checkpoint and harbors the potential of introducing genetic instability. These consequences are reminiscent of deletion of one *MAD2* allele, which predisposes mice to cancer development.¹²⁶ The degradation of REST, therefore, stands out as a spectacular example of the close connections between proliferation and differentiation but also as an Achilles heel of stem cells: if REST is stabilized, stem cells do not efficiently differentiate while at the same time their ongoing proliferation has lost its precision.

Underscoring the importance of coordinating proliferation with development, the other major cell cycle E3, the APC/C, also functions during differentiation. In addition to orchestrating progression of cells through mitosis, APC/C^{Cdh1} regulates synapses in *Drosophila* and *Caenorhabditis elegans*,^{127,128} and controls axonal growth and patterning in the developing nervous system of mice.¹²⁹ Important APC/C^{Cdh1} substrates in differentiating neurons are the SnoN and Id (inhibitors of differentiation) transcriptional repressors.^{130–132} The degradation of these repressors activates the transcription of neuron-specific genes following cell cycle exit. APC/C^{Cdh1} also stabilizes the G1 cell cycle stage, which is the time when cell cycle exit occurs during differentiation. Thus, APC/C^{Cdh1} generates a cell cycle state conducive to differentiation and then contributes to the establishment of a specific cellular fate, thereby elegantly coordinating proliferation with differentiation.

3.2. The Role of Ubiquitination in Cell Cycle Checkpoints

The faithful execution of the cell cycle program is constantly monitored by checkpoint networks, which detect damage or the failure to complete a cell cycle stage. The activation of these checkpoints halts cell cycle progression and provides cells with time to repair the damage. The importance of checkpoints for faithful cell division is underscored by their frequent loss of function in cancer. Ubiquitination plays key roles in checkpoint establishment, maintenance, and inactivation.

3.2.1. To Control and To Be Controlled: Ubiquitin and the Spindle Checkpoint

The complexity of ubiquitin-dependent checkpoint control is revealed during mitosis, when condensed chromosomes have to be attached to the spindle by kinetochore–microtubule interactions.¹³³ It is essential that the kinetochores of the two sister chromatids are connected to microtubules emanating from opposing spindle poles; otherwise both sister chromatids would end up in the same, then aneuploid daughter cell. Since sister chromatids are held together by a ring-shaped protein

complex, cohesin, the correct bipolar spindle attachment of chromosomes generates tension between the sister chromatids. The lack of microtubule attachment at a single kinetochore or lack of tension between sister chromatids activates the spindle checkpoint.⁵⁸ The spindle checkpoint then inhibits the ubiquitin ligase APC/C, the activation of which would trigger sister chromatid separation by targeting inhibitors of the cohesin protease, separase, for proteasomal degradation.¹³⁴ By inhibiting the APC/C, the spindle checkpoint allows cells to correct erroneous chromosome attachment before the sister chromatids are distributed to the daughter cells.

The direct target of the spindle checkpoint is the substrate-recruitment factor and APC/C activator Cdc20. Cdc20 is sequestered in stoichiometric complexes with its inhibitors Mad2 and BubR1.^{62,64} The binding of Mad2 to Cdc20 occurs at unoccupied kinetochores. It involves a template mechanism, where one stably kinetochore-bound Mad2 (through interaction with another checkpoint component, Mad1) changes the conformation in another Mad2 molecule, which is then capable of Cdc20 binding.^{135,136} The resulting Cdc20–Mad2 complex resembles a “seatbelt” conformation, which is very stable.¹³⁷ In addition, the yeast BubR1-homologue Mad3 binds and inhibits Cdc20 as an APC/C pseudosubstrate.¹²¹ The sequestration of Cdc20 in these complexes inactivates the APC/C and arrests cells at metaphase.

After all chromosomes have achieved bipolar attachment to the spindle, sister chromatid separation is rapidly initiated and occurs simultaneously throughout the cell. This indicates that the spindle checkpoint is inactivated in a switch-like manner, which is achieved, at least in part, by ubiquitination. Following the completion of chromosome attachment, the p31^{comet} protein and the E2 UbcH10 catalyze the APC/C-dependent multiubiquitination of Cdc20 itself and potentially other proteins as well.^{84,138} The APC/C-dependent multiubiquitination does not result in Cdc20 degradation but instead triggers the dissociation of Cdc20 from its inhibitor Mad2, activation of APC/C^{Cdc20}, and initiation of sister chromatid separation. It is likely that ubiquitination causes conformational changes in Cdc20 leading to Mad2 release or recruits a ubiquitin-selective segregase, such as p97^{Ufd1/Npl4}, to disassemble the Cdc20/Mad2 complexes in an ATP-dependent manner.^{139,140} Following its activation, the APC/C promotes the degradation of cyclin B1, Mps1, and Bub1, which are all required to maintain spindle checkpoint function.^{141,142} Thus, the APC/C itself initiates events leading to the inactivation of the spindle checkpoint and, consequently, more APC/C activation. Such a mechanism holds great potential for positive-feedback regulation, which could explain the switch-like nature of spindle checkpoint inactivation.

Biological switches solely built on positive-feedback loops are difficult to keep under control; here, spindle checkpoint inactivation could be brought about by local fluctuations in p31^{comet}. The spindle checkpoint, however, employs an additional layer of ubiquitin-dependent regulation to protect cells against unscheduled sister chromatid separation. In early mitosis, when chromosomes attempt to achieve bipolar spindle attachment, the DUB Usp44 reverts the ubiquitination of Cdc20 and stabilizes Cdc20–Mad2 complexes.⁴⁶ Loss of Usp44 abrogates the spindle checkpoint and leads to widespread chromosome missegregation. The phenotype of Usp44 depletion can be rescued by codepletion of UbcH10, providing evidence that Usp44 directly opposes the UbcH10-

dependent ubiquitination underlying spindle checkpoint silencing. The ongoing competition between ubiquitination and deubiquitination results in a very dynamic regulation of the spindle checkpoint and allows cells to rapidly pull the trigger leading to sister chromatid separation.

3.2.2. Ubiquitination Step by Step: The G2/M Checkpoint

A key feature of the spindle checkpoint is its regulation by reversible ubiquitination. A variation on this theme is played by the G2/M checkpoint, which impairs entry of cells into mitosis in response to damaged DNA. An important component of the G2/M checkpoint is the ubiquitin ligase Brca1/Bard1, activation of which blocks mitotic entry through inhibition of cyclin B1/Cdk1.¹⁴³ The loss of ubiquitination by Brca1/Bard1, which occurs in breast cancer cells harboring mutations in the Brca1 RING finger, abrogates the function of the G2/M checkpoint.¹⁴⁴ Such cells can enter mitosis despite DNA damage and accumulate potentially harmful mutations.

Just like the APC/C, Brca1/Bard1 is closely regulated by nonproteolytic ubiquitination. Following DNA damage, cells activate the kinases ATM and ATR, which phosphorylate a histone, H2AX, and a scaffold protein, Mdc1. Together, γ H2AX and Mdc1 form a platform on which DNA repair proteins and G2/M-checkpoint mediators are assembled. One of the earliest proteins to be recruited is the ubiquitin ligase Rnf8, which recognizes phosphorylated Mdc1 through an N-terminal FHA domain.^{145–147} Rnf8 and its E2 Ubc13 decorate histones at the sites of damage with K63-linked ubiquitin chains.⁷⁵ These K63-linked ubiquitin chains are recognized by a ubiquitin-binding protein, Rap80, which together with Ccdc98/Abraxas recruits the Brca1/Bard1 tumor suppressors.^{148–151} Further ubiquitination by Brca1/Bard1 coordinates later events of DNA repair and elicits the checkpoint-dependent G2/M arrest. There are only few substrates of Brca1/Bard1 known at sites of DNA damage, including the CtIP protein mutated in cancer.¹⁵² Rather than promoting CtIP degradation, its Brca1/Bard-dependent ubiquitination stabilizes the interaction of CtIP with damaged DNA and may promote its function in DNA end resection.¹⁵³ Loss of Rnf8, Brca1/Bard1, or Ubc13 all block the function of the G2/M checkpoint.

Reminiscent of the regulation of the spindle checkpoint by antagonistic ubiquitination (APC/C, UbcH10) and deubiquitination (Usp44), the Rap80/Brca1/Bard1 complex also contains a deubiquitinating enzyme, Brcc36.¹⁵⁴ Another DUB, Bap1, had also been shown to bind Brca1.¹⁵⁵ The activity of Brcc36 is required for G2/M-checkpoint function, but specific substrates have yet to be identified. Thus, both cell cycle checkpoints are regulated by reversible and nonproteolytic ubiquitination.

3.2.3. Ubiquitin-Dependent Proteolysis in Cell Cycle Checkpoints

Sometimes, cell cycle checkpoints have to resort to more radical means to grant a battered cell enough time to repair the damage. Checkpoints then trigger the degradation of regulators that are required for entry into the next cell cycle stage. A case in point is made by the intra-S checkpoint, which is activated when cells encounter DNA damage during replication. Activation of the intra-S checkpoint promotes the ubiquitination and degradation of the Cdc25A phosphatase,^{156–158} which would otherwise activate Cdk kinases and

promote cell cycle progression. The degradation of Cdc25A therefore stalls cell cycle progression in S phase. The intra-S checkpoint depends on the same ATM, ATR, and Chk kinases as the aforementioned G2/M checkpoint, and Chk1 is required for the ubiquitination of Cdc25A by SCF^{βTrCP}.¹⁵⁷ The failure to degrade Cdc25A, for example by mutation of Chk1 kinase, allows Cdk activation and cell cycle progression despite DNA damage, thus compromising the fidelity of replication. Not surprisingly, high levels of Cdc25A are frequently observed in tumors and correlate with poor prognosis.¹⁵⁹

Cell cycle checkpoints can also turn the tables and inhibit degradation events required for cell cycle progression. The tumor suppressor p53, for example, impairs cell cycle progression in response to DNA damage. To ensure that cells are not inadvertently slowed down, p53 is constantly degraded following its ubiquitination by the E3 Mdm2.³⁸ The detection of DNA damage decreases the activity of Mdm2 toward p53 by several means, including impaired p53 binding, reduced catalytic activity of Mdm2, and deubiquitination.^{38,40} The complete inhibition of Mdm2 leads to stabilization and activation of p53, which can then promote the synthesis of the CDK-inhibitor p21, leading to cell cycle arrest. Mdm2 can also be partially inactivated, which results in p53 monoubiquitination rather than multiubiquitination.¹⁰⁹ As shown with ubiquitin fusion proteins, monoubiquitination of p53 triggers its nuclear export by exposing a nuclear export signal that is recognized by the export factor Crm1.¹⁶⁰ Thus, under those conditions, p53 is only transiently inhibited; the cell remains skeptical enough to retain some p53 just in case.

3.3. Regulation of Growth Factor Signaling by Ubiquitination

The role of ubiquitin in coordinating proliferation with development will serve as a final example to illustrate the intricate mechanisms of ubiquitin-dependent cell cycle control. As already mentioned, mammalian cells decide whether to proliferate or differentiate by recognizing a mixture of extracellular growth and differentiation factors. Cells integrate the signals emerging from multiple receptors before committing to a further round of proliferation. The overexpression of a single receptor disturbs this fine-tuned machinery and results in prolonged signaling and inappropriate cell cycle progression. To limit growth factor signaling, ligand-engaged growth factor receptors are downregulated by ubiquitin-dependent endocytosis. If the activity of the responsible E3 is crippled, cells proliferate without much restraint and the afflicted organism is likely to develop cancer.

3.3.1. EGF Receptors

The importance of endocytosis for appropriate growth-factor signaling and cell cycle regulation is illustrated by the epidermal growth factor receptor, EGFR. Inhibition of EGFR endocytosis or EGFR overexpression is tightly linked to the development of breast cancer. By contrast, the downregulation of EGFR kinase activity or the application of humanized monoclonal antibodies against EGFR has been proven to be of therapeutic benefit.^{161,162}

After engagement with its ligand, the dimerization of EGFR induces its autophosphorylation and activation. This triggers intracellular signaling cascades but, at the same time, also promotes EGFR internalization by endocytosis and

routing to the lysosomal compartment. The complete inactivation of EGFR requires its degradation in the lysosome. Although internalization of EGFR can occur in the absence of ubiquitination, its targeting to the lysosomes depends on K63-linked ubiquitin chains.^{67,76} The lysosomal routing is mediated by the ESCRT pathway, which has several components directly binding to ubiquitin.^{163,164} Thus, ubiquitination is essential for the efficient inactivation of EGFR and constitutes an important negative-feedback loop to limit growth factor signaling.

The activated EGFR is ubiquitinated by the RING-E3 c-Cbl.¹⁶⁵ c-Cbl is paired up with activated EGFR through the adapter molecule Grb, and through recognition of an autophosphorylated tyrosine residue of EGFR by the TKB-domain of c-Cbl.¹⁶⁶ The activity of c-Cbl is absolutely required for EGFR internalization. It is known that c-Cbl, in addition to EGFR, has to ubiquitinate other proteins during endocytosis, which could be substrate adaptors or components of the endocytic machinery.

Mutants of c-Cbl underscore the importance of EGFR endocytosis for cell cycle regulation. A retroviral version, v-Cbl, lacks residues at its C-terminus and is unable to ubiquitinate EGFR.¹⁶⁷ v-Cbl behaves like a dominant negative mutant that protects EGFR from ubiquitination by c-Cbl expressed in the host cell. Prolonged EGFR signaling causes aberrant cell cycle progression, and indeed, v-Cbl expression after retroviral infection leads to increased B cell proliferation, lymphoma, and myelogenous leukemia. In a similar manner, mutations in the RING domain of c-Cbl that block its capability to ubiquitinate and downregulate EGFR lead to prolonged growth factor signaling, aberrant cell cycle control, and cancer.¹⁶⁶

A recurrent theme in ubiquitin-dependent cell cycle regulation is the opposition of E3s by DUBs, which provides the basis for dynamic regulation. So it comes as no surprise that the lysosomal targeting of activated EGFR is subject to DUB regulation. At least two human DUBs regulate EGFR downregulation, AMSH and Usp8/Ubpy.^{168,169} Usp8 is itself a cell cycle regulated protein, which is absent from quiescent cells and is only expressed after these cells have been stimulated to re-enter the cell cycle by growth factor signaling.⁴³ The DUBs function at multiple stages of EGFR endocytosis and control the ubiquitination status of cargo and of components of the endocytic machinery. An oncogenic translocation fusing Usp8 to the PI-3K-subunit p85β lends support to an important function of these enzymes in cell cycle control.¹⁷⁰

3.3.2. TGF-β signaling

TGF-β and related cytokines impair proliferation and promote differentiation in several tissues.¹⁷¹ TGF-β ligands are recognized by heterodimeric receptors (TR-I/TR-II), leading to activation of their kinase activity and trans-phosphorylation. The activated TR-I phosphorylates receptor Smad proteins (R-Smads), allowing them to bind Smad4, travel to the nucleus, and activate the transcription of genes regulating proliferation and differentiation. Loss of TGF-β signaling leads to aberrant cell cycle progression and has been firmly linked to tumorigenesis and metastasis.

A class of inhibitory Smad proteins downregulate TGF-β signaling by stimulating the ubiquitin-dependent degradation of activated receptors. Smad7, for example, teams up with a ubiquitin ligase, Smurf2.¹⁷² The Smad7–Smurf2 complex is transported to cholesterol-rich caveolae, which contain

internalized activated TR-I/TR-II. At this time, Smad7 presents the E2 UbcH7 to the HECT-E3 Smurf2, thereby stimulating Smurf2-dependent ubiquitination reactions.¹⁷³ This leads to modification of both Smad7 and the TRs and targets Smad7 for proteasomal and the TRs for lysosomal degradation.

TGF- β signaling is regulated by ubiquitin-dependent processes at multiple levels and provides a final example for the complexity of ubiquitin-dependent cell cycle control. Key substrates in the ubiquitin-dependent regulation of TGF- β signaling are the Smads. They can be ubiquitinated by a variety of ubiquitin ligases, such as Smurf1, Smurf2, the HECT-E3s Nedd4-2 and Itch, the U-box E3 CHIP, or SCF.^{174–176} This can have distinct consequences, which may be caused by different types of ubiquitination (monoubiquitination versus chains of different topology). For example, whereas Smad3 ubiquitination by Nedd4-2 stimulates its proteasomal degradation, the modification of Smad3 by Itch activates its transcription activator function. The increased degradation of Smads abolishes the capability of cells to respond to antimitogenic TGF- β signaling. This is exemplified by the overexpression of the E3 ectoderm, which targets Smad4 for unscheduled degradation and is found in intestinal tumors.¹⁷⁷

4. Summary: Common Themes in Ubiquitin-Dependent Cell Cycle Control

Ubiquitination is an essential regulator of cell cycle progression in all eukaryotes. It controls a wide variety of reactions important for proliferation and development, such as progression through the cell cycle program, function of cell cycle checkpoints, and coordination of proliferation with development. Ubiquitination can exert specific control over so many processes by changing the abundance or the activity of modified proteins. Ubiquitination itself is tightly regulated and carried out by very specific enzymes. Whether a protein is ubiquitinated or not is often determined by a balance of counteracting ubiquitination and deubiquitination activities. Whenever irreversible transitions have to be accomplished, ubiquitination triggers the proteasomal degradation of crucial regulators. Ubiquitin-dependent proteolysis is also used to dispose of activated growth-factor receptors by targeting them to lysosomes. Finally, nonproteolytic ubiquitination exerts cell cycle control by orchestrating events in cell cycle checkpoints. We believe that the multiple layers of regulation provided by ubiquitin hold great promise for future innovative approaches to arrest the proliferation of cancer cells in more efficient and specific ways than currently available.

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6. References

- Masui, Y.; Markert, C. L. *J. Exp. Zool.* **1971**, *177*, 129.
- Newport, J. W.; Kirschner, M. W. *Cell* **1984**, *37*, 731.
- Cyert, M. S.; Kirschner, M. W. *Cell* **1988**, *53*, 185.
- Gautier, J.; Minshull, J.; Lohka, M.; Glotzer, M.; Hunt, T.; Maller, J. L. *Cell* **1990**, *60*, 487.
- Solomon, M. J.; Glotzer, M.; Lee, T. H.; Philippe, M.; Kirschner, M. W. *Cell* **1991**, *63*, 1013.
- Evans, T.; Rosenthal, E. T.; Youngblom, J.; Distel, D.; Hunt, T. *Cell* **1983**, *33*, 389.
- Murray, A. W.; Solomon, M. J.; Kirschner, M. W. *Nature* **1989**, *339*, 280.
- Glotzer, M.; Murray, A. W.; Kirschner, M. W. *Nature* **1991**, *349*, 132.
- Ciechanover, A.; Heller, H.; Elias, S.; Haas, A. L.; Hershko, A. *Proc. Natl. Acad. Sci. U.S.A.* **1980**, *77*, 1365.
- Hershko, A.; Ciechanover, A.; Rose, I. A. *J. Biol. Chem.* **1981**, *256*, 1525.
- Ciechanover, A.; Ben-Saadon, R. *Trends Cell Biol.* **2004**, *14*, 103–106.
- Wang, X.; Herr, R. A.; Chua, W. J.; Lybarger, L.; Wiertz, E. J.; Hansen, T. H. *J. Cell Biol.* **2007**, *177*, 613.
- Cadwell, K.; Coscoy, L. *Science* **2005**, *309*, 127.
- Ravid, T.; Hochstrasser, M. *Nat. Cell Biol.* **2007**, *9*, 422.
- Dye, B. T.; Schulman, B. A. *Annu. Rev. Biophys. Biomol. Struct.* **2007**, *36*, 131.
- Ciechanover, A.; Elias, S.; Heller, H.; Hershko, A. *J. Biol. Chem.* **1982**, *257*, 2537.
- Ciechanover, A.; Finley, D.; Varshavsky, A. *Cell* **1984**, *37*, 57.
- Jin, J.; Li, X.; Gygi, S. P.; Harper, J. W. *Nature* **2007**, *447*, 1135.
- Grenfell, S. J.; Trausch-Azar, J. S.; Handley-Gearhart, P. M.; Ciechanover, A.; Schwartz, A. L. *Biochem. J.* **1994**, *300*, 701.
- Finley, D.; Ciechanover, A.; Varshavsky, A. *Cell* **1984**, *37*, 43.
- Huang, D. T.; Hunt, H. W.; Zhuang, M.; Ohi, M. D.; Holton, J. M.; Schulman, B. A. *Nature* **2007**, *445*, 394.
- Pohl, C.; Jentsch, S. *Cell* **2008**, *132*, 832.
- Joazeiro, C. A.; Weissman, A. M. *Cell* **2000**, *102*, 549.
- Aravind, L.; Koonin, E. V. *Curr. Biol.* **2000**, *10*, R132.
- Ozkan, E.; Yu, H.; Deisenhofer, J. *Proc. Natl. Acad. Sci. U.S.A.* **2005**, *102*, 18890.
- Reverter, D.; Lima, C. D. *Nature* **2005**, *435*, 687.
- Kee, Y.; Huijbregtse, J. M. *Biochem. Biophys. Res. Commun.* **2007**, *354*, 329.
- Scheffner, M.; Huijbregtse, J. M.; Vierstra, R. D.; Howley, P. M. *Cell* **1993**, *75*, 495.
- Zhu, H.; Kavsak, P.; Abdollah, S.; Wrana, J. L.; Thomsen, G. H. *Nature* **1999**, *400*, 687.
- Di Guglielmo, G. M.; Le Roy, C.; Goodfellow, A. F.; Wrana, J. L. *Nat. Cell Biol.* **2003**, *5*, 410.
- Sundvall, M.; Korhonen, A.; Paatero, I.; Gaudio, E.; Melino, G.; Croce, C. M.; Aqeilan, R. I.; Elenius, K. *Proc. Natl. Acad. Sci. U.S.A.* **2008**, *105*, 4162.
- Rodrigo-Brenni, M. C.; Morgan, D. O. *Cell* **2007**, *130*, 127.
- King, R. W.; Peters, J. M.; Tugendreich, S.; Rolfe, M.; Hieter, P.; Kirschner, M. W. *Cell* **1995**, *81*, 279.
- Sudakin, V.; Ganoth, D.; Dahan, A.; Heller, H.; Hershko, J.; Luca, F. C.; Ruderman, J. V.; Hershko, A. *Mol. Biol. Cell* **1995**, *6*, 185.
- Zachariae, W.; Shin, T. H.; Galova, M.; Obermaier, B.; Nasmyth, K. *Science* **1996**, *274*, 1201.
- Petroski, M. D.; Deshaies, R. J. *Nat. Rev. Mol. Cell Biol.* **2005**, *6*, 9.
- Starita, L. M.; Parvin, J. D. *Curr. Opin. Cell Biol.* **2003**, *15*, 345.
- Brooks, C. L.; Gu, W. *Mol. Cell* **2006**, *21*, 307.
- Nijman, S. M.; Luna-Vargas, M. P.; Velds, A.; Brummelkamp, T. R.; Dirac, A. M.; Sixma, T. K.; Bernards, R. *Cell* **2005**, *123*, 773.
- Song, L.; Rape, M. *Curr. Opin. Cell Biol.* **2008**, *20*, 156.
- Ambroggio, X. I.; Rees, D. C.; Deshaies, R. J. *PLoS Biol.* **2004**, *2*, E2.
- Cohn, M. A.; Kowal, P.; Yang, K.; Haas, W.; Huang, T. T.; Gygi, S. P.; D'Andrea, A. D. *Mol. Cell* **2007**, *28*, 786.
- Naviglio, S.; Matteucci, C.; Matoskova, B.; Nagase, T.; Nomura, N.; DiFiore, P. P.; Draetta, G. F. *EMBO J.* **1998**, *17*, 3241.
- Stegmeier, F.; Sowa, M. E.; Nelapa, G.; Gygi, S. P.; Harper, J. W.; Elledge, S. J. *Proc. Natl. Acad. Sci. U.S.A.* **2007**, *104*, 8869.
- Huang, T. T.; Nijman, S. M. B.; Mirchandani, K. D.; Galardy, P. J.; Cohn, M. A.; Haas, W.; Gygi, S. P.; Ploegh, H. L.; Bernards, R.; D'Andrea, A. D. *Nat. Cell Biol.* **2006**, *8*, 339.
- Stegmeier, F.; Rape, M.; Draviam, V. M.; Nalepa, G.; Sowa, M. E.; Ang, X. L.; McDonald, E. R.; Li, M. Z.; Hannon, G. J.; Sorger, P. K.; Kirschner, M. W.; Harper, J. W.; Elledge, S. J. *Nature* **2007**, *446*, 876.
- Brummelkamp, T. R.; Nijman, S. M. B.; Dirac, A. M. G.; Bernards, R. *Nature* **2003**, *424*, 797.
- Thrower, J. S.; Hoffman, L.; Rechsteiner, M.; Pickart, C. *EMBO J.* **2000**, *19*, 94.
- Elsasser, S.; Finley, D. *Nat. Cell Biol.* **2005**, *7*, 742.

- (50) Schreiner, P.; Chen, X.; Husnjak, K.; Randles, L.; Zhang, N.; Elsasser, S.; Finley, D.; Dikic, I.; Walters, K. J.; Groll, M. *Nature* **2008**, *453*, 548.
- (51) Husnjak, K.; Elsasser, S.; Zhang, N.; Chen, X.; Randles, L.; Shi, Y.; Hofmann, K.; Walters, K. J.; Finley, D.; Dikic, I. *Nature* **2008**, *453*, 481.
- (52) Guédat, P.; Colland, F. *BMC Biochem.* **2007**, *8*, S14.
- (53) Thornton, B. R.; Toczyski, D. P. *Genes Dev.* **2006**, *20*, 3069.
- (54) Sabile, A.; Meyer, A. M.; Wirbelauer, C.; Hess, D.; Kogel, U.; Scheffner, M.; Krek, W. *Mol. Cell. Biol.* **2006**, *26*, 5994.
- (55) Tang, Z.; Li, B.; Bharadwaj, R.; Zhu, H.; Ozkan, E.; Hakala, K.; Deisenhofer, J.; Yu, H. *Mol. Biol. Cell* **2001**, *12*, 3839.
- (56) Jin, J.; Cardozo, T.; Lovering, R. C.; Elledge, S. J.; Pagano, M.; Harper, J. W. *Genes Dev.* **2004**, *18*, 2573.
- (57) Peters, J. M. *Nat. Rev. Mol. Cell Biol.* **2006**, *7*, 644.
- (58) Yu, H. *Mol. Cell* **2007**, *27*, 3.
- (59) Clevers, H. *Cell* **2006**, *127*, 469.
- (60) Kraft, C.; Herzog, F.; Gieffers, C.; Mechtler, K.; Hagting, A.; Pines, J.; Peters, J. M. *EMBO J.* **2003**, *22*, 6598.
- (61) Rape, M.; Kirschner, M. W. *Nature* **2004**, *432*, 588.
- (62) Fang, G.; Yu, H.; Kirschner, M. W. *Genes Dev.* **1998**, *12*, 1871.
- (63) Miller, J. J.; Summers, M. K.; Hansen, D. V.; Nachury, M. V.; Lehman, N. L.; Loktev, A.; Jackson, P. K. *Genes Dev.* **2005**, *20*, 2410.
- (64) Tang, Z.; Bharadwaj, R.; Li, B.; Yu, H. *Dev. Cell* **2001**, *1*, 227.
- (65) Wu, R. S.; Kohn, K. W.; Bonner, W. M. *J. Biol. Chem.* **1981**, *256*, 5916.
- (66) Nicassio, F.; Corrado, N.; Vissers, J. H.; Arecas, L. B.; Bergink, S.; Martijn, J. A.; Geverts, B.; Houtsmuller, A. B.; Vermeulen, W.; Di Fiore, P. P.; Citterio, E. *Curr. Biol.* **2007**, *17*, 1972.
- (67) Sigismund, S.; Woelk, T.; Puri, C.; Maspero, E.; Tacchetti, C.; Transidico, P.; Di Fiore, P. P.; Polo, S. *Proc. Natl. Acad. Sci. U.S.A.* **2005**, *102*, 2760.
- (68) Hooge, C.; Pfander, B.; Moldovan, G. L.; Pyrowolakis, G.; Jentsch, S. *Nature* **2002**, *419*, 135.
- (69) Eddins, M. J.; Varadan, R.; Fushman, D.; Pickart, C. M.; Wolberger, C. *J. Mol. Biol.* **2007**, *367*, 204.
- (70) Varadan, R.; Assfalg, M.; Haririnia, A.; Raasi, S.; Pickart, C.; Fushman, D. *J. Biol. Chem.* **2004**, *279*, 7055.
- (71) Peng, J.; Schwartz, D.; Elias, J. E.; Thoreen, C. C.; Cheng, D.; Marsischky, G.; Roelofs, J.; Finley, D.; Gygi, S. P. *Nat. Biotechnol.* **2003**, *21*, 921.
- (72) Jin, L.; Williamson, A.; Banerjee, S.; Philipp, I.; Rape, M. *Cell* **2008**, *133*, 653.
- (73) Kirkpatrick, D. S.; Hathaway, N. A.; Hanna, J.; Elsasser, S.; Rush, J.; Finley, D.; King, R. W.; Gygi, S. P. *Nat. Cell Biol.* **2006**, *8*, 700.
- (74) Deng, L.; Wang, C.; Spencer, E.; Yang, L.; Braun, A.; You, J.; Slaughter, C.; Pickart, C.; Chen, Z. *J. Cell* **2000**, *103*, 351.
- (75) Wang, B.; Elledge, S. J. *Proc. Natl. Acad. Sci. U.S.A.* **2007**, *104*, 20759.
- (76) Huang, F.; Kirkpatrick, D.; Jiang, X.; Gygi, S.; Sorkin, A. *Mol. Cell* **2006**, *21*, 737.
- (77) Eddins, M. J.; Carlile, C. M.; Gomez, K. M.; Pickart, C. M.; Wolberger, C. *Nat. Struct. Mol. Biol.* **2006**, *13*, 915.
- (78) Petroski, M. D.; Zhou, X.; Dong, G.; Daniellssakani, S.; Payan, D. G.; Huang, J. *J. Biol. Chem.* **2007**, *282*, 29936.
- (79) Windheim, M.; Pegg, M.; Cohen, P. *Biochem. J.* **2008**, *409*, 723.
- (80) Topper, L. M.; Bastians, H.; Ruderman, J. V.; Gorbosky, G. J. *J. Cell Biol.* **2001**, *154*, 707.
- (81) Carrano, A. C.; Eytan, E.; Hershko, A.; Pagano, M. *Nat. Cell Biol.* **1999**, *1*, 193.
- (82) Petroski, M. D.; Deshaies, R. J. *Cell* **2005**, *123*, 1107.
- (83) Townsley, F. M.; Aristarkhov, A.; Beck, S.; Hershko, A.; Ruderman, J. V. *Proc. Natl. Acad. Sci. U.S.A.* **1997**, *94*, 2362.
- (84) Reddy, S. K.; Rape, M.; Margansky, W. A.; Kirschner, M. W. *Nature* **2007**, *446*, 921.
- (85) Skoufias, D. A.; Indorato, R. L.; Lacroix, F.; Panopoulos, A.; Margolis, R. L. *J. Cell Biol.* **2007**, *179*, 671.
- (86) Wolf, F.; Wandke, C.; Isenberg, N.; Geley, S. *EMBO J.* **2006**, *25*, 2802.
- (87) Potapova, T. A.; Daum, J. R.; Pittman, B. D.; Hudson, J. R.; Jones, T. N.; Satinover, D. L.; Stukenberg, P. T.; Gorbosky, G. J. *Nature* **2006**, *440*, 954.
- (88) Chu, I. M.; Hengst, L.; Slingerland, J. M. *Nat. Rev. Cancer* **2008**, *8*, 253.
- (89) Sclafani, R. A.; Holzen, T. M. *Annu. Rev. Genet.* **2007**, *41*, 237.
- (90) Nakayama, K.; Nagahama, H.; Minamishima, Y. A.; Miyake, S.; Ishida, N.; Hatakeyama, S.; Kitagawa, M.; Iemura, S.; Natsume, T.; Nakayama, K. I. *Dev. Cell* **2004**, *6*, 661.
- (91) Chu, I.; Sun, J.; Arnaout, A.; Kahn, H.; Hanna, W.; Narod, S.; Sun, P.; Tan, C. K.; Hengst, L.; Slingerland, J. *Cell* **2007**, *128*, 281.
- (92) Grimmmer, M.; Wang, Y.; Mund, T.; Cilensek, Z.; Keidel, E. M.; Waddell, M. B.; Jäkel, H.; Kullmann, M.; Kriwacki, R. W.; Hengst, L. *Cell* **2007**, *128*, 269.
- (93) Hao, B.; Zheng, N.; Schulman, B. A.; Wu, G.; Miller, J. J.; Pagano, M.; Pavletich, N. P. *Mol. Cell* **2005**, *20*, 9.
- (94) Yang, H. Y.; Zhou, B. P.; Hung, M. C.; Lee, M. H. *J. Biol. Chem.* **2000**, *275*, 24735.
- (95) Mamillapalli, R.; Gavrilo, N.; Mihaylova, V. T.; Tsvetkov, L. M.; Wu, H.; Zhang, H.; Sun, H. *Curr. Biol.* **2001**, *11*, 263.
- (96) Frescas, D.; Pagano, M. *Nat. Rev. Cancer* **2008**, *8*, 438.
- (97) Sullivan, M.; Morgan, D. O. *Nat. Rev. Mol. Cell Biol.* **2007**, *8*, 894.
- (98) Stewart, E.; Kobayashi, H.; Harrison, D.; Hunt, T. *EMBO J.* **1994**, *13*, 584.
- (99) Rape, M.; Reddy, S. K.; Kirschner, M. W. *Cell* **2006**, *124*, 89.
- (100) Mathe, E.; Kraft, C.; Giet, R.; Daek, P.; Peters, J. M.; Glover, D. M. *Curr. Biol.* **2004**, *14*, 1723.
- (101) Parry, D. H.; O'Farrell, P. H. *Curr. Biol.* **2001**, *11*, 671.
- (102) Carroll, C. W.; Morgan, D. O. *Nat. Cell Biol.* **2002**, *4*, 880.
- (103) Marangos, P.; Carroll, J. *Nat. Cell Biol.* **2008**, *10*, 445.
- (104) Hopfield, J. J. *Proc. Natl. Acad. Sci. U.S.A.* **1974**, *71*, 4135.
- (105) Geley, S.; Kramer, E.; Gieffers, C.; Gannon, J.; Peters, J. M.; Hunt, T. *J. Cell Biol.* **2001**, *153*, 137.
- (106) Hayes, M. J.; Kimata, Y.; Wattam, S. L.; Lindon, C.; Mao, G.; Yamano, H.; Fry, A. M. *Nat. Cell Biol.* **2006**, *8*, 607.
- (107) Amador, V.; Ge, S.; Santamaria, P. G.; Guardavaccaro, D.; Pagano, M. *Mol. Cell* **2007**, *27*, 462.
- (108) Wolthuis, R.; Clay-Farrace, L.; van Zon, W.; Yekezare, M.; Koop, L.; Ogink, J.; Medema, R.; Pines, J. *Mol. Cell* **2008**, *30*, 290.
- (109) Li, M.; Brooks, C. L.; Wu-Baer, F.; Chen, D.; Baer, R.; Gu, W. *Science* **2003**, *302*, 1972.
- (110) Tugendreich, S.; Tomkiel, J.; Earnshaw, W.; Hieter, P. *Cell* **1995**, *81*, 261.
- (111) Raff, J. W.; Jeffers, K.; Huang, J. Y. *J. Cell Biol.* **2002**, *157*, 1139.
- (112) Wakefield, J. G.; Huang, J. Y.; Raff, J. W. *Curr. Biol.* **2000**, *10*, 1367.
- (113) Acquaviva, C.; Herzog, F.; Kraft, C.; Pines, J. *Nat. Cell Biol.* **2004**, *6*, 892.
- (114) Ban, K. H.; Torres, J. Z.; Miller, J. J.; Mikhailov, A.; Nachury, M. V.; Tung, J. J.; Rieder, C. L.; Jackson, P. K. *Dev. Cell* **2007**, *13*, 29.
- (115) Welcker, M.; Clurman, B. E. *Nat. Rev. Cancer* **2008**, *8*, 83.
- (116) Koepf, D. M.; Schaefer, L. K.; Ye, X.; Keyomarsi, K.; Chu, C.; Harper, J. W.; Elledge, S. J. *Science* **2001**, *294*, 173.
- (117) Moberg, K. H.; Bell, D. W.; Wahrer, D. C.; Haber, D. A.; Hariharan, I. K. *Nature* **2001**, *413*, 311.
- (118) Welcker, M.; Orian, A.; Jin, J.; Grim, J. E.; Harper, J. W.; Eisenman, R. N.; Clurman, B. E. *Proc. Natl. Acad. Sci. U.S.A.* **2004**, *101*, 9085.
- (119) Tetzlaff, M. T.; Yu, W.; Li, M.; Zhang, P.; Finegold, M.; Mahon, K.; Harper, J. W.; Schwartz, R. J.; Elledge, S. J. *Proc. Natl. Acad. Sci. U.S.A.* **2004**, *101*, 3338.
- (120) Welcker, M.; Clurman, B. E. *J. Biol. Chem.* **2005**, *280*, 7654.
- (121) Burton, J. L.; Solomon, M. J. *Genes Dev.* **2007**, *21*, 655.
- (122) Ballas, N.; Grunseich, C.; Lu, D. D.; Spoh, J. C.; Mandel, G. *Cell* **2005**, *121*, 645.
- (123) Westbrook, T. F.; Hu, G.; Ang, X. L.; Mulligan, P.; Pavlova, N. N.; Liang, A.; Leng, Y.; Maehr, R.; Shi, Y.; Harper, J. W.; Elledge, S. J. *Nature* **2008**, *452*, 370.
- (124) Guardavaccaro, D.; Frescas, D.; Dorrello, N. V.; Peschiaroli, A.; Multani, A. S.; Cardozo, T.; Lasorella, A.; Iavarone, A.; Chang, S.; Hernandez, E.; Pagano, M. *Nature* **2008**, *452*, 365.
- (125) Westbrook, T. F.; Martin, E. S.; Schlabach, M. R.; Leng, Y.; Liang, A. C.; Feng, B.; Zhao, J. J.; Roberts, T. M.; Mandel, G.; Hannon, G. J.; Depinho, R. A.; Chin, L.; Elledge, S. J. *Cell* **2005**, *121*, 837.
- (126) Michel, L. S.; Liberal, V.; Chatterjee, A.; Kirchwegger, K.; Pasche, B.; Gerald, W.; Dobles, M.; Sorger, P. K.; Murty, V. V.; Benzra, R. *Nature* **2001**, *409*, 355.
- (127) van Roessel, P.; Elliott, D. A.; Robinson, I. M.; Prokop, A.; Brand, A. H. *Cell* **2004**, *119*, 707.
- (128) Juo, P.; Kaplan, J. M. *Curr. Biol.* **2004**, *14*, 2057.
- (129) Konishi, Y.; Stegmüller, J.; Matsuda, T.; Bonni, S.; Bonni, A. *Science* **2004**, *303*, 1026.
- (130) Stegmüller, J.; Konishi, Y.; Huynh, M. A.; Yuan, Z.; Dibacco, S.; Bonni, A. *Neuron* **2006**, *50*, 389.
- (131) Wan, Y.; Liu, X.; Kirschner, M. W. *Mol. Cell* **2001**, *8*, 1027–1039.
- (132) Lasorella, A.; Stegmüller, J.; Guardavaccaro, D.; Liu, G.; Carro, M. S.; Rothschild, G.; de la Torre-Ubieta, L.; Pagano, M.; Bonni, A.; Iavarone, A. *Nature* **2006**, *442*, 471.
- (133) Burke, D. J.; Stukenberg, P. T. *Dev. Cell* **2008**, *14*, 474.
- (134) Cohen-Fix, O. *Cell* **2001**, *106*, 137.
- (135) Luo, X.; Tang, Z.; Rizo, J.; Yu, H. *Mol. Cell* **2002**, *9*, 59.
- (136) Mapelli, M.; Massimiliano, L.; Santaguida, S.; Musacchio, A. *Cell* **2007**, *131*, 730.
- (137) Luo, X.; Tang, Z.; Xia, G.; Wassmann, K.; Matsumoto, T.; Rizo, J.; Yu, H. *Nat. Struct. Mol. Biol.* **2004**, *11*, 338.

- (138) Xia, G.; Luo, X.; Habu, T.; Rizo, J.; Matsumoto, T.; Yu, H. *EMBO J.* **2004**, *23*, 3133.
- (139) Rape, M.; Hoppe, T.; Gorr, I.; Kalocay, M.; Richly, H.; Jentsch, S. *Cell* **2001**, *107*, 667.
- (140) Braunstein, I.; Miniowitz, S.; Moshe, Y.; Hershko, A. *Proc. Natl. Acad. Sci. U.S.A.* **2007**, *104*, 4870.
- (141) Palframan, W. J.; Meehl, J. B.; Jaspersen, S. L.; Winey, M.; Murray, A. W. *Science* **2007**, *313*, 680.
- (142) Qi, W.; Yu, H. *J. Biol. Chem.* **2007**, *282*, 3672.
- (143) Yarden, R. I.; Pardo-Reoyo, S.; Sgagias, M.; Cowan, K. H.; Brody, L. C. *Nat. Genet.* **2002**, *30*, 285.
- (144) Ruffner, H.; Joazeiro, C. A.; Hemmati, D.; Hunter, T.; Verma, I. M. *Proc. Natl. Acad. Sci. U.S.A.* **2001**, *98*, 5134.
- (145) Mailland, N.; Bekker-Jensen, S.; Faustrup, H.; Melander, F.; Bartek, J.; Lukas, C.; Lukas, J. *Cell* **2007**, *131*, 887.
- (146) Huen, M. S.; Grant, R.; Manke, I.; Minn, K.; Yu, X.; Yaffe, M. B.; Chen, J. *Cell* **2007**, *131*, 901.
- (147) Kolas, N. K.; Chapman, J. R.; Nakada, S.; Ylanko, J.; Chahwan, R.; Sweeney, F. D.; Panier, S.; Mendez, M.; Wildenhain, J.; Thomson, T. M.; Pelletier, L.; Jackson, S. P.; Durocher, D. *Science* **2007**, *318*, 1637.
- (148) Wang, B.; Matsuoka, S.; Ballif, B. A.; Zhang, D.; Smogorzewska, A.; Gygi, S. P.; Elledge, S. J. *Science* **2007**, *316*, 1194.
- (149) Sobhian, B.; Shao, G.; Lilli, D. R.; Culhane, A. C.; Moreau, L. A.; Xia, B.; Livingston, D. M.; Greenberg, R. A. *Science* **2007**, *316*, 1198.
- (150) Kim, H.; Chen, J.; Yu, X. *Science* **2007**, *316*, 1202.
- (151) Kim, H.; Huang, J.; Chen, J. *Nat. Struct. Mol. Biol.* **2007**, *14*, 710.
- (152) Yu, X.; Fu, S.; Lai, M.; Baer, R.; Chen, J. *Genes Dev.* **2006**, *20*, 1721.
- (153) Sartori, A. A.; Lukas, C.; Coates, J.; Mistrik, M.; Fu, S.; Bartek, J.; Baer, R.; Lukas, J.; Jackson, S. P. *Nature* **2007**, *450*, 509.
- (154) Dong, Y.; Hakimi, M. A.; Chen, X.; Kumaraswamy, E.; Cooch, N. S.; Godwin, A. K.; Shiekhattar, R. *Mol. Cell* **2003**, *12*, 1087.
- (155) Jensen, D. E.; Proctor, M.; Marquis, S. T.; Gardner, H. P.; Ha, S. I.; Chodosh, L. A.; Ishov, A. M.; Tommerup, N.; Vissing, H.; Sekido, Y.; Minna, J.; Borodovsky, A.; Schultz, D. C.; Wilkinson, K. D.; Maul, G. G.; Barlev, N.; Berger, S. L.; Prendergast, G. C.; Rauscher, F. J. *Oncogene* **1999**, *16*, 1097.
- (156) Busino, L.; Donzelli, M.; Chiesa, M.; Guardavaccaro, D.; Ganoth, D.; Dorrello, N. V.; Hershko, A.; Pagano, M.; Draetta, G. F. *Nature* **2003**, *426*, 87.
- (157) Jin, J.; Shirogane, T.; Xu, L.; Nalepa, G.; Qin, J.; Elledge, S. J.; Harper, J. W. *Genes Dev.* **2003**, *17*, 3062.
- (158) Kanemori, Y.; Uto, K.; Sagata, N. *Proc. Natl. Acad. Sci. U.S.A.* **2005**, *102*, 6279.
- (159) Boutros, R.; Lobjois, V.; Ducommun, B. *Nat. Rev. Cancer* **2007**, *7*, 495.
- (160) Carter, S.; Bischof, O.; Dejean, A.; Vousden, K. H. *Nat. Cell Biol.* **2007**, *9*, 428.
- (161) Leahy, D. J. *Cancer Cell* **2008**, *13*, 291.
- (162) Polo, S.; Pece, S.; Di Fiore, P. P. *Curr. Opin. Cell Biol.* **2004**, *16*, 156.
- (163) Malerød, L.; Stuffers, S.; Brech, A.; Stenmark, H. *Traffic* **2007**, *8*, 1617.
- (164) Saksena, S.; Sun, J.; Chu, T.; Emr, S. D. *Trends Biochem. Sci.* **2007**, *32*, 561.
- (165) Levkowitz, G.; Waterman, H.; Ettenberg, S. A.; Katz, M.; Tsygankov, A. Y.; Alroy, I.; Lavi, S.; Iwai, K.; Reiss, Y.; Ciechanover, A.; Lipkowitz, S.; Yarden, Y. *Mol. Cell* **1999**, *4*, 1029.
- (166) Schmidt, M. H.; Dikic, I. *Nat. Rev. Mol. Cell Biol.* **2005**, *6*, 907.
- (167) Robertson, H.; Hime, G. R.; Lada, H.; Bowtell, D. D. *Oncogene* **2000**, *19*, 299.
- (168) McCullough, J.; Row, P. E.; Lorenzo, O.; Doherty, M.; Beynon, R.; Clague, M. J.; Urbé, S. *Curr. Biol.* **2006**, *16*, 160.
- (169) Niendorf, S.; Oksche, A.; Kisser, A.; Loehler, J.; Prinz, M.; Schorle, H.; Feller, S.; Lewitzky, M.; Horak, I.; Knobloch, K. P. *Mol. Cell Biol.* **2007**, *27*, 5029.
- (170) Janssen, J. W. G.; Schleithoff, L.; Bartram, C. R.; Schulz, A. S. *Oncogene* **1998**, *16*, 1767.
- (171) Schmierer, B.; Hill, C. S. *Nat. Rev. Mol. Cell Biol.* **2007**, *8*, 970.
- (172) Kavvak, P.; Rasmussen, R. K.; Causing, C. G.; Bonni, S.; Zhu, H.; Thomsen, G. H.; Wrana, J. L. *Mol. Cell* **2000**, *6*, 1365.
- (173) Ogunjimi, A. A.; Briant, D. J.; Pece-Barbara, N.; Le Roy, C.; Di Guglielmo, G. M.; Kavvak, P.; Rasmussen, R. K.; Seet, B. T.; Sicheri, F.; Wrana, J. L. *Mol. Cell* **2005**, *19*, 297.
- (174) Morén, A.; Imamura, T.; Miyazono, K.; Heldin, C. H.; Moustakas, A. *J. Biol. Chem.* **2005**, *280*, 22115.
- (175) Li, L.; Xin, H.; Xu, X.; Huang, M.; Zhang, X.; Chen, Y.; Zhang, S.; Fu, X. Y.; Chang, Z. *Mol. Cell Biol.* **2004**, *24*, 856.
- (176) Wan, M.; Tang, Y.; Tytler, E. M.; Lu, C.; Jin, B.; Vickers, S. M.; Yang, L.; Shi, X.; Cao, X. *J. Biol. Chem.* **2004**, *279*, 14484.
- (177) Dupont, S.; Zacchigna, L.; Cordenonsi, M.; Soligo, S.; Adorno, M.; Rugge, M.; Piccolo, S. *Cell* **2005**, *121*, 87.
- (178) Machida, Y. J.; Machida, Y.; Chen, Y.; Gurtan, A. M.; Kupfer, G. M.; D'Andrea, A. D.; Dutta, A. *Mol. Cell* **2006**, *23*, 589.
- (179) Banerjee, S.; Brooks, W. S.; Crawford, D. F. *Oncogene* **2007**, *26*, 6509.
- (180) Wang, X.; Trotman, L. C.; Koppie, T.; Alimonti, A.; Chen, Z.; Gao, Z.; Wang, J.; Erdjument-Bromage, H.; Tempst, P.; Cordon-Cardo, C.; Pandolfi, P. P.; Jiang, X. *Cell* **2007**, *128*, 129.
- (181) Watanabe, N.; Arai, H.; Nishihara, Y.; Taniguchi, M.; Watanabe, N.; Hunter, T.; Osada, H. *Proc. Natl. Acad. Sci. U.S.A.* **2004**, *101*, 4419.
- (182) Margottin-Goguet, F.; Hsu, J. Y.; Loktev, A.; Hsieh, H. M.; Reimann, J. D.; Jackson, P. K. *Dev. Cell* **2003**, *4*, 813.
- (183) Joukov, V.; Groen, A. C.; Prokhorova, T.; Gerson, R.; White, E.; Rodriguez, A.; Walter, J. C.; Livingston, D. M. *Cell* **2006**, *127*, 539.
- (184) Sumara, I.; Quadroni, M.; Frei, C.; Olma, M. H.; Sumara, G.; Ricci, R.; Peter, M. *Dev. Cell* **2007**, *12*, 887.
- (185) Jin, J.; Arias, E. E.; Chen, J.; Harper, J. W.; Walter, J. C. *Mol. Cell* **2006**, *23*, 709.
- (186) Mack, F. A.; Patel, J. H.; Biju, M. P.; Haase, V. H.; Simon, M. C. *Mol. Cell Biol.* **2005**, *25*, 4565.
- (187) Joo, H. Y.; Zhai, L.; Yang, C.; Nie, S.; Erdjument-Bromage, H.; Tempst, P.; Chang, C.; Wang, H. *Nature* **2007**, *449*, 1068.
- (188) Wang, Y.; Satoh, A.; Warren, G.; Meyer, H. H. J. *Cell Biol.* **2004**, *164*, 973.
- (189) Haglund, K.; Di Fiore, P. P.; Dikic, I. *Trends Biochem. Sci.* **2003**, *28*, 598.
- (190) Fu, Y.; Zhu, Y.; Zhang, K.; Yeung, M.; Durocher, D.; Xiao, W. *Cell* **2008**, *133*, 601.
- (191) Smogorzewska, A.; Matsuoka, S.; Vinciguerra, P.; McDonald, E. R.; Hurov, K. E.; Luo, J.; Ballif, B. A.; Gygi, S. P.; Hofmann, K.; D'Andrea, A. D.; Elledge, S. J. *Cell* **2007**, *129*, 289.
- (192) Loring, G. L.; Christensen, K. C.; Gerber, S. A.; Brenner, C. *Cell Cycle* **2008**, *7*, 96.
- (193) Adhikari, A.; Xu, M.; Chen, Z. J. *Oncogene* **2007**, *26*, 3214.
- (194) Nishikawa, H.; Ooka, S.; Sato, K.; Arima, K.; Okamoto, J.; Klevit, R. E.; Fukuda, M.; Ohta, T. *J. Biol. Chem.* **2004**, *279*, 3916.
- (195) Wang, M.; Cheng, D.; Peng, J.; Pickart, C. M. *EMBO J.* **2006**, *25*, 1710.
- (196) Kim, H. T.; Kim, K. P.; Lledias, F.; Kisselev, A. F.; Scaglione, K. M.; Skowyra, D.; Gygi, S. P.; Goldberg, A. L. *J. Biol. Chem.* **2007**, *282*, 17375.

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