UC Berkeley

UC Berkeley Previously Published Works

Title

Ubiquitin-dependent regulation of transcription in development and disease

Permalink

https://escholarship.org/uc/item/2nr5q392

Journal

EMBO Reports, 22(4)

ISSN

1469-221X

Authors

Mark, Kevin G
Rape, Michael

Publication Date

2021-04-07

DOI

10.15252/embr.202051078

Copyright Information

This work is made available under the terms of a Creative Commons Attribution License, availalbe at https://creativecommons.org/licenses/by/4.0/

Peer reviewed

eScholarship.org Powered by the California Digital Library
University of California

Review

EMBO reports

Ubiquitin-dependent regulation of transcription in development and disease

Kevin G Mark[1,*] & Michael Rape[1,2,**]

Abstract

Transcription is an elaborate process that is required to establish and maintain the identity of the more than two hundred cell types of a metazoan organism. Strict regulation of gene expression is therefore vital for tissue formation and homeostasis. An accumulating body of work found that ubiquitylation of histones, transcription factors, or RNA polymerase II is crucial for ensuring that transcription occurs at the right time and place during development. Here, we will review principles of ubiquitin-dependent control of gene expression and discuss how breakdown of these regulatory circuits leads to a wide array of human diseases.

Keywords histone modification; RNA polymerase II; transcription; ubiquitin
Subject Categories Chromatin, Transcription & Genomics; Post-translational Modifications & Proteolysis
DOI 10.15252/embr.202051078 | Received 11 June 2020 | Revised 29 November 2020 | Accepted 1 March 2021 | Published online 29 March 2021
EMBO Reports (2021) 22: e51078

See the Glossary for abbreviations used in this article.

Introduction

Human bodies contain more than two hundred cell types that perform specialized functions and allow us to react to developmental or environmental challenges. Cell identity is established by the expression of tissue-specific subsets of genes, which requires that differentiating cells guide RNA polymerase II (RNA Pol II) to select genomic loci (Djebali *et al,* 2012). While loss of transcription disrupts tissue formation and is incompatible with life, the erroneous execution of gene expression programs can lead to a plethora of developmental abnormalities and is often found at the heart of tumorigenesis (Lee & Young, 2013).

Accurate gene expression relies on transcription factors that bind to specific sequences in gene promoters or enhancers (Zaret & Carroll, 2011). These proteins can collaborate with co-repressors and limit transcription by maintaining chromatin in a compact state (Venkatesh & Workman, 2015). Conversely, when gene expression needs to be stimulated, transcription factors attract co-activators or chromatin remodelers that increase the accessibility of RNA Pol II for the respective genomic loci (Bulynko & O'Malley, 2011). After initiation of RNA synthesis, a distinct set of elongation factors helps RNA Pol II traverse histones within the coding region to allow production of a full-length transcript (Chen *et al,* 2018a). Proteins with roles in transcription termination dissociate RNA Pol II from chromatin, which sets the complex free for another round of gene expression at a distinct locus (Proudfoot, 2016).

Ensuring that this sequence of events occurs at the right time and place, organisms tightly control chromatin architecture and fine-tune the availability and function of transcription factors or RNA Pol II. Central for the underlying regulatory circuits is ubiquitylation, a posttranslational modification that was first documented for a histone with roles in transcriptional control (Goldknopf *et al,* 1975). Several aspects of its biochemistry and biology allow ubiquitylation to exert precise control over gene expression networks: cells can assemble several ubiquitin conjugates of different topologies that elicit distinct responses and hence provide an opportunity to employ similar chemistry for multiple transcriptional outputs (Fig 1A; Yau & Rape, 2016; Haakonsen & Rape, 2019). While, for example, attachment of a single ubiquitin typically changes protein interactions, polymers linked through K11 or K48 of one ubiquitin and the carboxy-terminus of another trigger protein degradation (Chau *et al,* 1989; Jin *et al,* 2008). Cells can further adjust ubiquitin signaling to their needs by synthesizing more complex conjugates, such as branched ubiquitin chains used to dismantle stable protein complexes (Meyer & Rape, 2014; Yau *et al,* 2017; Oh *et al,* 2020). Ubiquitin conjugates can also be edited to modulate their output (Newton *et al,* 2008; Ohtake *et al,* 2018), or disassembled (Komander, 2009; Sahtoe & Sixma, 2015), which creates a cellular language for the dynamic control of gene expression in changing environments.

In humans, the writers of this ubiquitin code are ~ 600 E3 ligases that are counteracted by ~ 100 deubiquitylases (DUBs) (Fig 1B). Multiple effectors with ubiquitin-binding domains decipher the type of ubiquitylation and couple the modification to its intended biological outcome. Many E3 ligases, DUBs, and effectors control gene expression, and their mutation is often deleterious for organismal development. Conversely, hijacking the ubiquitylation machinery with small molecules is beginning to be used to alter gene expression for therapeutic benefit. Here, we will review principles of ubiquitin-dependent control of transcription and discuss how our

1 Department of Molecular Cell Biology, University of California at Berkeley, Berkeley, CA, USA
2 Howard Hughes Medical Institute, University of California at Berkeley, Berkeley, CA, USA
*Corresponding author. Tel: +1 201 2408155; E-mail: markk@berkeley.edu
**Corresponding author. Tel: +1 510 289 4413; E-mail: mrape@berkeley.edu

© 2021 The Authors

Glossary

βTrCP	beta-transducin repeat-containing protein
APC/C	anaphase-promoting complex/cyclosome
ASCL	achaete-scute homolog
AXR	auxin-resistance protein
BAP	BRCA1-associated protein
BCL	B-cell lymphoma
BCOR	BCL6 co-repressor
BRD	bromodomain-containing protein
BRE	brefeldin A sensitivity
BTB	Broad-Complex, Tramtrack and Bric a brac
CBP	CREB-binding protein
CDK	cyclin-dependent kinase
CK	casein kinase
CRBN	protein cereblon
CRY	cryptochrome
CUL	cullin
DDB	DNA damage binding
DCAF	DDB1 and CUL4 associated factor
DDI	DNA damage inducible homolog
DEF	RNA polymerase II degradation factor
DOT	disrupter of telomere silencing protein
dTAG	degradation tag
DUB	deubiquitylase
ER	endoplasmic reticulum
FACT	facilitates chromatin transcription
FBXL	F-box and leucine-rich repeat protein
FBXW	F-box and WD-40 domain-containing protein
GATA	GATA-binding factor
GID	gibberellin-insensitive dwarf protein
Gli	glioma-associated oncogene
GSK	glycogen-synthase kinase
HIF	hypoxia-inducible factor
HUWE	HECT, UBA and WWE domain-containing protein
IAA	indoleacetic acid-induced protein
IκBα	inhibitor of nuclear factor kappa B, alpha
KANSL	KAT8 regulatory NSL complex
KEAP	kelch-like ECH-associated protein
LGE	Large cells
MDM	mouse double minute homolog
MGA	multicopy suppressor of Gam1
MLL	mixed lineage leukemia
MSL	male-specific lethal
MYC	myelocytomatosis
NMYC	v-myc avian myelocytomatosis viral oncogene neuroblastoma-derived homolog
MYSM	Myb-like, SWIRM, and MPN domain-containing protein
NCoR	nuclear receptor co-repressor
NEDD	neural precursor cell expressed developmentally downregulated protein
NF-κB	nuclear factor of kappa light polypeptide gene enhancer in B-cells
NRF	nuclear respiratory factor
PAF	RNA polymerase II-associated factor
PRC	polycomb repressive complex
PROTAC	proteolysis-targeting chimera
RPB1	RNA polymerase II subunit B1
RBX	RING-box protein
RING	really interesting new gene
RNA Pol II	RNA polymerase II
RNF	RING finger protein
RPN	regulatory particle non-ATPase
RSF	remodeling and spacing factor
RSP	reverses SPT-phenotype protein
SALL	Sal-like protein
SCF	Skp, Cullin, F-box containing
SETDB	SET domain bifurcated
SIAH	seven in absentia homolog
SMAD	Mothers against decapentaplegic homolog
SMRT	silencing mediator for retinoid or thyroid-hormone receptors
SMURF	SMAD ubiquitination regulatory factor
SOX	SRY (Sex determining region Y)-box
SPT	suppressor of Ty
STAT	signal transducer and activator of transcription
TBP	TATA-box binding protein
TC-NER	transcription-coupled nucleotide excision repair
TCF/LEF	T-cell factor/lymphoid enhancer-binding factor
TGF-β	transforming growth factor-β
TIR	transport inhibitor response
TLE	transducin-like enhancer
TP	tumor protein
UBR	ubiquitin protein ligase E3 component n-recognin
USP	ubiquitin specific peptidase
VCP	valosin-containing protein
VHL	von Hippel Lindau disease tumor suppressor
WDR	WD repeat-containing protein
WNT	wingless-related MMTV integration site 1
WWP	WW domain-containing protein

increasing understanding of these processes is laying the foundation for therapeutic approaches to modulate gene expression in diseases of aberrant cell specification.

Ubiquitin-dependent control of chromatin architecture

Transcription occurs within the context of chromatin whose basic unit is the nucleosome: an octamer of the core histones H2A, H2B, H3, and H4, wrapped around 147 base pairs of DNA. As nucleosomes can restrict the accessibility of the transcription machinery to promoter or enhancer sequences, their position on chromatin needs to be tightly regulated. While transcription factors can destabilize the interaction of genomic DNA with nucleosomes (Michael *et al,* 2020), they often recruit protein complexes with histone-modifying or chromatin-remodeling activities. As an essential histone modification, ubiquitylation plays a crucial role in the regulatory processes that shape chromatin architecture and, thereby, gene expression.

Histone H2A monoubiquitylation maintains transcriptional silencing

It is befitting to begin a review about ubiquitin-dependent control of gene expression by discussing the modification of histone H2A, the first protein known to be ubiquitylated (Goldknopf *et al,* 1975). Monoubiquitylation of H2A at K119 is catalyzed by polycomb repressive complex 1 (PRC1) (Blackledge *et al,* 2014; Aranda *et al,* 2015) and plays a critical role in restricting gene expression at tissue-specific loci, at the inactive female X chromosome, or at sites of DNA damage (Uckelmann & Sixma, 2017; Brockdorff, 2017). Underscoring the importance of this modification, loss of PRC1 is

 © 2021 The Authors

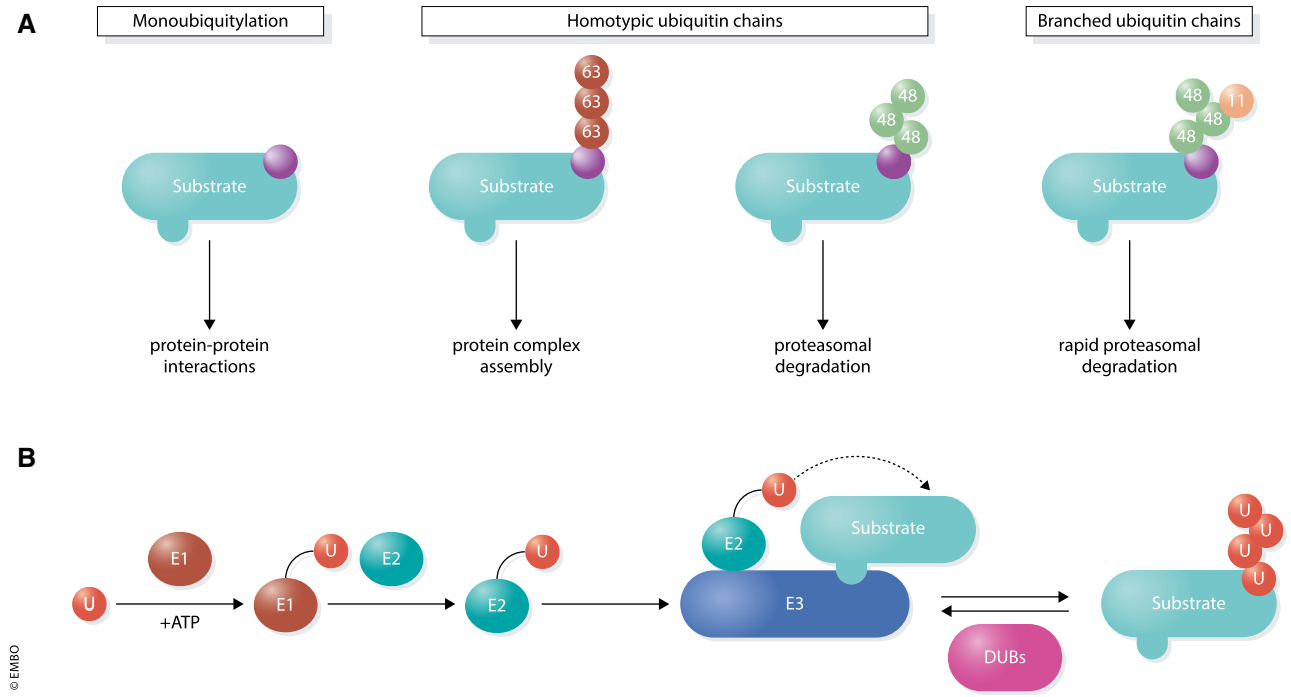

Figure 1. The ubiquitin modification system.

(A) Various structures and functions of different types of ubiquitin conjugates. Monoubiquitylation (left) involves transfer of a single ubiquitin to a substrate. E3 ligases can also connect several ubiquitin molecules together using the C-terminus of one subunit and one of seven lysine (K) residues (K6, K11, K27, K29, K33, K48, K63) or the N-terminal methionine (M1) on the other. Different ubiquitin topologies adopt distinct structural conformations which direct a wide array of substrate outcomes. (B) Ubiquitylation occurs through an enzymatic cascade. E1 enzymes use ATP to form a high-energy thioester bond to ubiquitin through an active site cysteine residue. Charged E1s transfer ubiquitin to one of ~ 40 E2 ubiquitin-conjugating enzymes, which then bind to one of ~ 600 E3 ubiquitin ligases that facilitate the transfer of ubiquitin onto a specific substrate. Approximately 100 DUBs remove ubiquitin from substrates to reverse the ubiquitylation process.

incompatible with life (Voncken *et al,* 2003), and mutations that impede its activity cause microcephaly or learning disabilities (Pierce *et al,* 2018). Aberrant regulation of PRC1 also results in tumors of the brain, liver, colon, breast, lung, prostate or lymphatic system (Grossniklaus & Paro, 2014; Wang *et al,* 2015).

PRC1 installs H2A monoubiquitylation at the proper sites by detecting CpG-rich promoter sequences or chromatin-associated noncoding RNAs (Brockdorff, 2017), which sets in motion a series of events leading to chromatin compaction (Bantignies *et al,* 2011; Denholtz *et al,* 2013; Isono *et al,* 2013) (Fig 2A): The monoubiquitylated H2A is recognized by PRC2 complexes, which methylate K27 on histone H3 (Kalb *et al,* 2014). This recruits SETDB1 to trimethylate K9 in histone H3 (Zhao *et al,* 2016), which serves as a landing platform for chromatin compactors, like HP1 (Nakagawa *et al,* 2008; Machida *et al,* 2018). Trimethylated H3K27 also attracts more PRC1 complexes to seed further H2A modification for stable long-range signaling (Blackledge *et al,* 2014; Tamburri *et al,* 2020; Blackledge *et al,* 2020). Moreover, monoubiquitylated H2A can engage the linker histone H1 to establish repressive higher-order chromatin folding (Jason *et al,* 2005; Fyodorov *et al,* 2018), a reaction that may involve the chromatin remodeler RSF1 (Zhang *et al,* 2017).

To allow for precise gene activation during development, the ubiquitylation of H2A needs to be reversible and multiple deubiquitylases target H2A to counteract PRC1-mediated gene silencing. Most H2A-directed deubiquitylases are subunits of co-activators (Belle & Nijnik, 2014; Chittock *et al,* 2017), such as MYSM1 that associates with the histone acetyltransferase p300/CBP and helps release histone H1 from chromatin (Zhu *et al,* 2007). The H2A deubiquitylases USP16, BAP1 and MYSM1 derepress different sets of lineage-specific genes in stem cells (Scheuermann *et al,* 2010; Yang *et al,* 2014; Gu *et al,* 2016; Campagne *et al,* 2019), while USP21 has a role in liver regeneration (Nakagawa *et al,* 2008). In addition to activating tissue-specific genes, removal of ubiquitin from H2A increases the expression of cell cycle inhibitors to limit stem cell proliferation (Gatzka *et al,* 2015; Wilms *et al,* 2018). Aberrant H2A deubiquitylation can thus deplete stem cell populations, as observed in Down's syndrome and its triplication of *USP16* (Adorno *et al,* 2013). By contrast, inactivation of H2A deubiquitylases can set in place a differentiation block associated with more aggressive cancers and worsened patient outcomes (Carbone *et al,* 2013).

H2B monoubiquitylation facilitates transcription elongation

Contrary to the effects of H2A modification, monoubiquitylation of the neighboring nucleosome subunit, histone H2B, promotes gene expression (Fig 2C; Weake & Workman, 2008; Minsky *et al,* 2008). H2B monoubiquitylation at K120 is carried out by RNF20/RNF40, which is recruited to RNA Pol II as part of the polymerase-associated factor 1 (PAF1) complex. The yeast homolog of RNF20/RNF40,

© 2021 The Authors

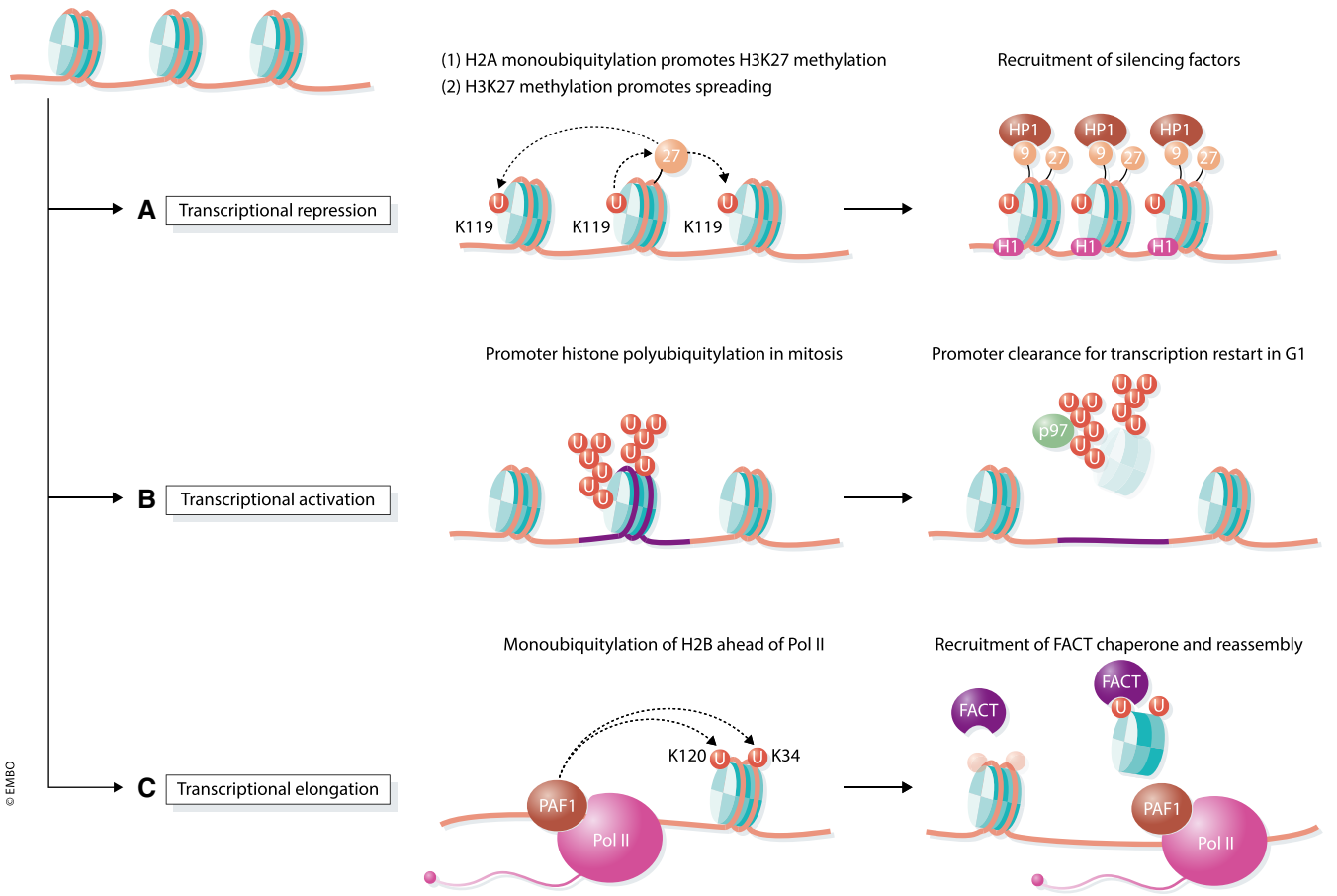

Figure 2. Transcriptional effects of histone ubiquitylation.

(A) Monoubiquitylation of histone H2A at K119 by PRC1 promotes transcriptional silencing. This modification recruits PRC2 to trimethylate histone H3 at K27, a mark which in turn recruits PRC1 to ubiquitylate additional H2A histones, resulting in spreading. H3K27me3 also leads to H3K9 trimethylation, which together with ubiquitylated H2A, recruit factors that compact chromatin and silence transcription. (B) Histone polyubiquitylation promotes transcriptional reactivation following cell division. During mitosis, the APC/C E3 ligase is recruited to specific promoters to polyubiquitylate histones, leading to their extraction by p97/VCP and proteasomal degradation. This action licenses these promoters for rapid transcriptional reactivation when cells enter G1. (C) Monoubiquitylation of histone H2B at K120 and K34 mediates transcriptional elongation. As part of the PAF1 elongation complex, the E3 ligases RNF20/RNF40 and MSL1/MSL2 travel with RNA Pol II and ubiquitylate H2B on nucleosomes obstructing RNA Pol II's path. H2B ubiquitylation promotes nucleosome remodeling, through the FACT histone chaperone, to allow polymerase passage. Ubiquitin is then removed by H2B DUBs to reestablish chromatin behind RNA Pol II.

BRE1, uses its partner LGE1 and liquid-liquid phase separation to increase the local concentration of H2B for efficient modification (Gallego *et al,* 2020). H2B can also be monoubiquitylated at K34 by MSL1/MSL2 (Wu *et al,* 2011; Wu *et al,* 2014), which is similarly recruited to RNA Pol II by the PAF1 complex.

H2B monoubiquitylation stimulates transcription by multiple means. It disrupts higher-order nucleosome assembly by forming a wedge between histone surfaces to prevent their tight packing (Fierz *et al,* 2011; Debelouchina *et al,* 2017). The modification of H2B may also destabilize the nucleosome itself (Krajewski *et al,* 2018), a function that is in part mediated by the FACT histone chaperone that transiently displaces H2A/H2B dimers (Pavri *et al,* 2006; Wang *et al,* 2018; Chen *et al,* 2018b). In addition, monoubiquitylated H2B acts as a platform to attract over ninety effectors, many of which are histone modifiers and chromatin remodelers (Shema-Yaacoby *et al,* 2013). One intriguing effector is the histone H3 methyltransferase, DOT1L, which forms part of a histone crosstalk cascade (Worden *et al,* 2019; Worden *et al,* 2020). Monoubiquitylation of H2B stimulates DOT1L methylation of K79 of histone H3, which in turn promotes H3K4 methylation by MLL to recruit transcriptional activators (Wu *et al,* 2011; Fuchs & Oren, 2014; Levendosky *et al,* 2016; Valencia-Sanchez *et al,* 2019). In this arrangement, H2B ubiquitylation nucleates downstream events that make chromatin more permissive for transcription elongation.

As observed with H2A, the modification of H2B is reversible, and several deubiquitylases remove ubiquitin from H2B to stabilize chromatin in its original state (Zhang *et al,* 2008; Zhao *et al,* 2008; Atanassov *et al,* 2016; Nune *et al,* 2019). For example, the deubiquitylase USP44 inhibits transcript elongation within the NCoR corepressor (Lan *et al,* 2016), and downregulation of USP44 accounts for an increase in H2B ubiquitylation at lineage-specific genes during stem cell differentiation (Fuchs *et al,* 2012; Karpiuk *et al,* 2012; Chen *et al,* 2012). Conversely, USP22 opposes RNF20/RNF40

 © 2021 The Authors

during differentiation of regulatory T cells to limit the immune system's anti-tumor activity (Cortez *et al,* 2020). While defects in H2B monoubiquitylation block cell fate specification, altered levels of ubiquitylated H2B are observed in many cancers (Wang *et al,* 2013; Fuchs & Oren, 2014; Dickson *et al,* 2016; Tarcic *et al,* 2016). Together, these observations underscore that the site of ubiquitylation can be critical for its effects on transcription: Although H2A and H2B are in close proximity within the nucleosome, their modification with ubiquitin has opposite consequences, i.e., inhibition or activation of gene expression.

Histone polyubiquitylation restarts gene expression after mitosis

While monoubiquitylation of histones has long been recognized to control chromatin architecture, histone polyubiquitylation has recently emerged as a means of transcriptional regulation during the cell cycle. As gene expression is downregulated during mitosis (Palozola *et al,* 2017), cells must restart their transcriptional programs every time they enter a new division cycle (Hsiung & Blobel, 2016). This process is facilitated by factors referred to as "mitotic bookmarks" that are deposited at active genes during the G2 phase and persist through the rigors of mitosis into the next G1 (Palozola *et al,* 2019). Despite chromatin condensation during mitosis, bookmarked loci remain accessible to the transcriptional machinery (Martinez-Balbas *et al,* 1995; Michelotti *et al,* 1997; Teves *et al,* 2018).

Early proteins proposed to serve as mitotic bookmarks were transcription factors such as BRD4 (Dey *et al,* 2009), GATA1 (Kadauke *et al,* 2012), or SOX2 (Teves *et al,* 2016; Deluz *et al,* 2016). These included the TATA-box binding protein TBP (Teves *et al,* 2018), which during interphase teams up with WDR5 to recruit RNA Pol II to actively transcribed genes (Tjian, 1996; Wysocka *et al,* 2005; Thomas *et al,* 2015; Guarnaccia & Tansey, 2018). As cells enter mitosis, WDR5 and TBP replace RNA Pol II with the E3 ligase anaphase-promoting complex (APC/C) (Oh *et al,* 2020; Fig 2B). The TBP- and WDR5-bound APC/C modifies histones at transcription start sites with K11/K48-branched ubiquitin chains (Oh *et al,* 2020), a conjugate that recruits the p97/VCP segregase and proteasomes to degrade proteins found in stable complexes (Meyer & Rape, 2014; Yau *et al,* 2017). Accordingly, histone polyubiquitylation clears nucleosomes off promoters for rapid reactivation of transcription in G1. Because the APC/C also triggers mitotic exit (Glotzer *et al,* 1991; Peters *et al,* 1996), this circuit coordinates gene expression with cell division, a role that is particularly important in stem cells (Pilaz *et al,* 2016; Oh *et al,* 2020) (Box 1).

As seen for monoubiquitylation of H2A or H2B, histone polyubiquitylation is likely part of extensive crosstalk between posttranslational marks. In addition to the APC/C, WDR5 binds the histone methyltransferase MLL, which is also required for transcriptional reactivation upon mitotic exit (Blobel *et al,* 2009) and may increase binding of WDR5 to promoters (Wysocka *et al,* 2005). Mitotic WDR5 also interacts with histone acetyl transferases that target residues in histone H3 and H4 (Oh *et al,* 2020). Histone acetyl transferases likely act upstream of MLL, as acetylation of H4K16 is required for H3K4 methylation at a subset of human genes (Katoh *et al,* 2011; Zhou *et al,* 2014), as well as for maintaining a pluripotent state (Spedale *et al,* 2012; Sheikh *et al,* 2019). Revealing another principle of ubiquitin-dependent control of transcription, different types of ubiquitin conjugates collaborate with histone methylation or acetylation to fine-tune gene expression to the needs of cell division and differentiation.

Ubiquitin-dependent control of transcription factor stability and function

Key to regulating access of RNA Pol II to its target sequences are the ~ 1,600 transcription factors that are encoded in the human genome (Juven-Gershon & Kadonaga, 2010; Magnani *et al,* 2011; Lambert *et al,* 2018). A single transcription factor can regulate multiple genes and act in concert with other transcription factors, thereby controlling the expression of specific gene sets during differentiation. Both abundance and activity of transcription factors therefore have to be tightly controlled during development, a task that has often been delegated to ubiquitylation.

Rapid response times through constitutive transcription factor degradation

In its simplest iteration, ubiquitin-dependent degradation eliminates transcription factors that have fulfilled their functions. However, many transcription factors are intrinsically unstable proteins that are constantly synthesized, yet immediately degraded, if they are not needed. While seemingly wasteful, this setup allows cells to stall degradation as a means to translate a developmental signal into rapid transcription factor accumulation. Radial glial cells of the hippocampus, for example, synthesize the transcription factor ASCL1, yet use constitutive ubiquitin-dependent degradation to keep its concentration low. This prevents quiescent precursor cells from premature cell cycle entry, which would deplete the stem cell pool (Blomfield *et al,* 2019). When these stem cells have to undergo self-renewal, however, they simply inhibit ASCL1 degradation, without having to set in motion the machinery for *ASCL1* transcription and translation. Rapid response times are also important for stress responses that detect the many adverse conditions faced by developing organisms. Accordingly, transcription factors responding to hypoxia (HIF1α), oxidative stress (NRF2), or DNA damage (TP53), are constantly turned over under homeostatic conditions, yet instantly stabilized upon stress (Salceda & Caro, 1997; Giaccia & Kastan, 1998; Ohh *et al,* 2000; Ma, 2013).

As ubiquitylation can be quantitatively tuned, inhibition of constitutive degradation is used to translate a certain amount of a developmental signal into a distinct transcription factor level and gene expression program. This is illustrated by β-catenin, a WNT-responsive transcription factor that determines pluripotency and tissue specification (Fig 3A). In the absence of WNT, β-catenin is phosphorylated by CK1 and GSK3β kinases, which are part of a destruction complex that includes the scaffold proteins Axin and adenomatous polyposis coli. Its phosphorylation marks β-catenin for ubiquitylation by the E3 ligase complex $SCF^{\beta TrCP}$ and proteasomal degradation. Binding of WNT to receptors inactivates the destruction complex, which disrupts β-catenin ubiquitylation and dependent on WNT levels, initiates the synthesis of proteins that govern specific cell fate decisions (Nguyen *et al,* 2009; Vermeulen *et al,* 2010; Yu *et al,* 2012; Nusse & Clevers, 2017; Tammela *et al,* 2017). Cells monitor differences in β-catenin abundance before and after signaling, a mechanism of fold-change detection that helps establish robust gene expression responses despite stochastic

© 2021 The Authors

Box 1. In need of answers

i *How specific are ubiquitylation enzymes and effectors that control gene expression?* Many E3 ligases and DUBs control transcription, yet the whole spectrum of their substrates is typically unknown. As E3 ligases often target multiple proteins, ubiquitylating more than one transcription factor might provide novel ways of coordinating gene expression programs.

ii *How do E3 ligases collaborate to control gene expression?* Multiple E3 ligases can target the same transcription factor in response to distinct inputs or to modify it with complex ubiquitin conjugates containing multiple linkages. How such coordination between ubiquitylation enzymes is established and regulated needs to be addressed.

iii *How does ubiquitin-dependent control of gene expression intersect with other roles of the ubiquitylation machinery?* Addressing this question is of particular importance in understanding the relationship between transcription regulation and ubiquitin-dependent quality control, both of which preserve cell identity and are affected in aging or neurodegenerative disease.

iv *How does ubiquitin-dependent control of protein complex composition affect transcriptional outputs?* As most transcription factors act within dynamic multisubunit complexes, the ability of the ubiquitin system to detect and eliminate aberrant complex subunits is likely crucial for ensuring proper gene expression. Determining transcription factor complex composition on a global scale and discovering new examples of quality control of transcription factor complex composition will be critical.

v *How broadly can small molecules be used to re-wire gene expression in development and disease?* Can molecular glues or PROTACs be used to alter cell fate decisions during development? How important is the proper timing, duration, and efficiency of such therapeutic approaches?

variation in cellular protein expression (Goentoro & Kirschner, 2009; Goentoro *et al,* 2009).

While rapid and quantitative signaling is highly advantageous for cells that need to adapt to changing environments, the circuits described above come at considerable risk. Mutations that prevent transcription factor degradation uncouple developmental inputs from gene expression outputs, with devastating consequences for the organism. Indeed, mutations that prevent E3 ligases from recognizing transcription factors or aberrant activity of E3 ligases often drive tumorigenesis. Inactivation of the destruction complex or the β-catenin phosphodegron is associated with most cases of familial and sporadic colon cancers (Shang *et al,* 2017; Gao *et al,* 2018), whereas mutation or aberrant expression of *VHL*, *KEAP1,* or *MDM2*, the E3 ligases responsible for HIF1α, NRF2, and TP53 degradation, are among the most frequent events across all cancers (Lawrence *et al,* 2014; Gossage *et al,* 2015; Karni-Schmidt *et al,* 2016).

Negative feedback regulation of transcription factor activity

In addition to regulating the speed or amplitude by which developmental signals are transmitted, transcription factor turnover can set the duration of a gene expression response. This function is often built around negative feedback loops, in which a transcription factor induces the expression of its own E3 ligase. In an example of particular importance for disease, TP53 drives the synthesis of the E3 MDM2, which in turn ubiquitylates TP53 to induce its proteasomal degradation (Karni-Schmidt *et al,* 2016). The resulting negative feedback plays a role in establishing the pulsatile nature of DNA repair signaling, when cells take the persistence of DNA damage as basis for their decision to exit the cell cycle (Purvis & Lahav, 2013). The balance between TP53 and MDM2 is skewed in cancer, as *MDM2* is amplified in a large number of hematological and solid tumors (Dembla *et al,* 2018). Elevated levels of MDM2 suppress wild-type TP53 and hamper a cell's response to genotoxic damage, which increases mutagenesis rates to drive tumor evolution (Manfredi, 2010).

While negative feedback regulation is a common principle of ubiquitin-dependent control of gene expression, it comes in several twists. In addition to producing its own E3 ligase, $SCF^{\beta TrCP}$, β-catenin also drives the synthesis of Axin, the rate-limiting component of the destruction complex that marks β-catenin for ubiquitylation and proteasomal degradation (Jho *et al*, 2002; Lee *et al*, 2003). Thus, β-catenin accelerates maturation of its degron to increase rates of ubiquitylation. Conversely, yeast RPN4 promotes expression of proteasomal subunits, in part to provide efficient responses to unfolded proteins accumulating in the cytoplasm (Schmidt *et al,* 2019). Once RPN4 has accumulated and proteasomes have been assembled, the proteolytic machinery degrades the transcription factor (Xie & Varshavsky, 2001).

Signal integration through multiple E3 ligases

Differentiating cells not only need to respond quickly to signals received from their environment, they also have to integrate multiple inputs at a time to make the appropriate fate choice. Ubiquitylation provides a unique opportunity to combine developmental signals into a coherent response, as a single transcription factor can be targeted by several E3 ligases that react to distinct inputs. A striking example is provided by MYC and MYCN, two transcription factors that play crucial roles in relaying anabolic signals to the cell cycle machinery (Fig 3B).

MYC and MYCN release paused RNA Pol II to increase expression of many genes at a time (Baluapuri *et al,* 2020). Ensuring that MYC is activated to the right extent during cell division, its levels are kept in check by phosphorylation of two residues, T58 and S62, and subsequent ubiquitylation by SCF^{FBXW7} (Welcker *et al,* 2004; Welcker & Clurman, 2008). Phosphorylated T58 also mediates binding of MYC by a distinct E3 ligase composed of SCF^{FBXL3} and its co-adaptor CRY2 (Huber *et al,* 2016). Rather than connecting MYC activity to the cell cycle, this regulation establishes proper transcription factor activity in the context of the circadian clock. Mutation of T58 of MYC, which is observed in a high percentage of Burkitt's lymphoma patients (Bahram *et al,* 2000; Gregory & Hann, 2000), thus disrupts control by both the cell cycle and circadian clock. In a similar manner, mutation of the analogous residue in MYCN can lead to neuroblastoma, medulloblastoma, or glioblastoma (Rickman *et al,* 2018).

As the most frequently amplified human oncogene, the levels of MYC must not exceed a threshold that would trigger cell death (McMahon, 2014). MYC and MYCN levels are additionally restricted by the E3 ligase UBR5, which triggers degradation independently of T58 or SCF^{FBXW7} (Qiao *et al,* 2020; Schukur *et al,* 2020). UBR5 thus sets a cap for MYC activity, which is exploited by cancer cells that overexpress UBR5 together with stabilized MYC. As MYC controls the transcription of many ribosomal genes (van Riggelen

 © 2021 The Authors

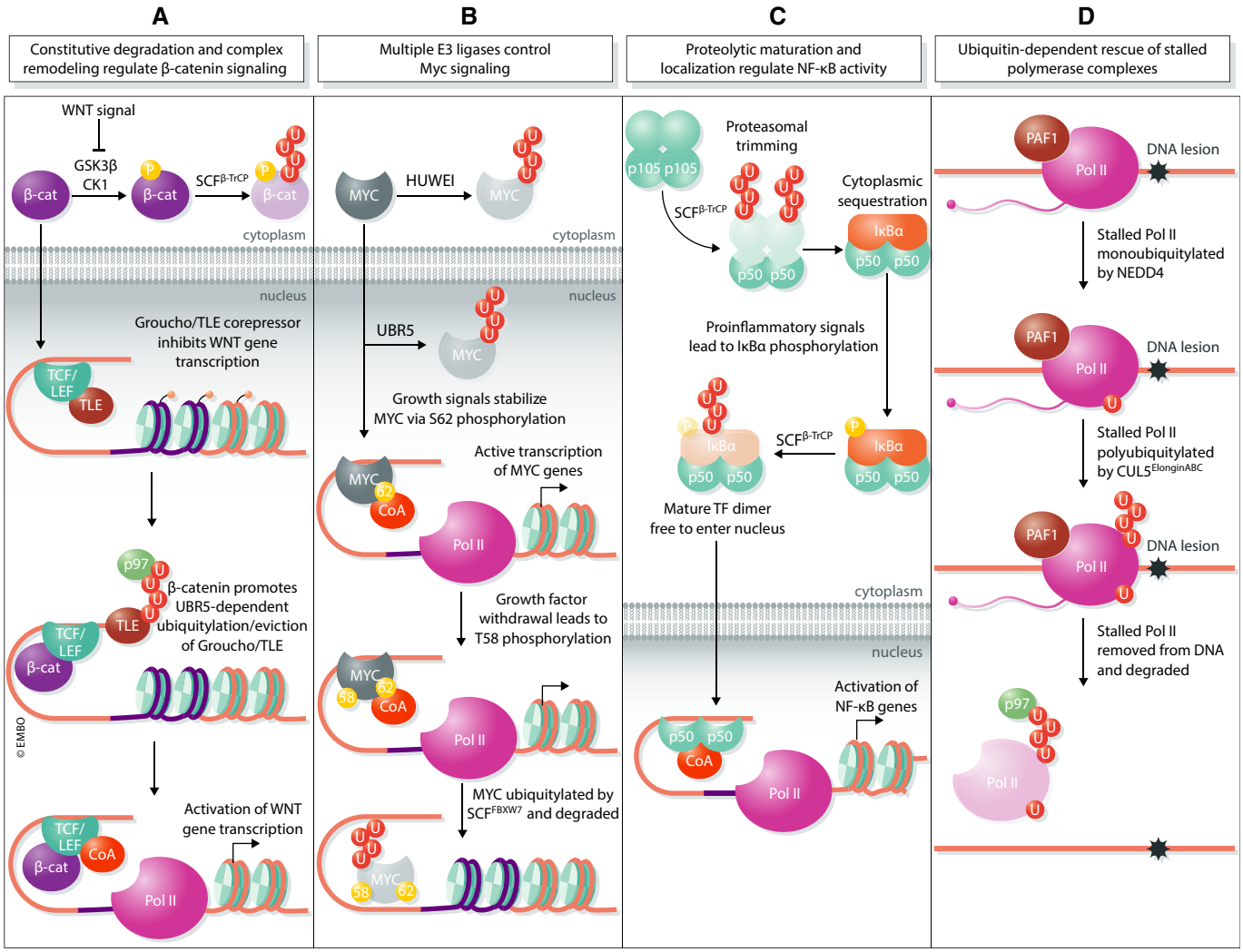

Figure 3. Ubiquitin-dependent regulation of transcriptional machinery.

(A) In the absence of WNT signal, the β-catenin transcription factor (β-cat) is constitutively degraded by a destruction complex consisting of kinases GSK3β and CK1 as well as the ubiquitin ligase $SCF^{\beta TrCP}$. The presence of WNT ligand stabilizes β-catenin by inhibiting its phosphorylation, thus allowing it to translocate into the nucleus. At TCF/LEF transcriptional complexes, β-catenin facilitates the exchange of co-repressors for co-activators (CoA) through UBR5-mediated ubiquitylation and p97/VCP-dependent extraction of the repressive Groucho/TLE subunit. (B) MYC levels are kept low in the cytoplasm and nucleus by the E3 ligases HUWE1 and UBR5, respectively. Upstream growth signals stabilize and activate MYC through phosphorylation of the S62 residue. This primes MYC for subsequent phosphorylation at nearby T58, which occurs upon growth factor withdrawal. T58-phosphorylated MYC is recognized by the SCF^{FBXW7} E3 ligase and degraded to prevent subsequent re-initiation of transcription. (C) The proinflammatory transcription factor NF-κB is synthesized as a 105-kDa precursor (p105) that is ubiquitylated by the $SCF^{\beta TrCP}$ E3 ligase and partially processed by the proteasome to yield a mature 50-kDa form (p50). Mature NF-κB dimers are sequestered in the cytoplasm by an inhibitor, IκBα, until cellular signals lead to degradation of IκBα and liberation of mature NF-κB. Only then can mature NF-κB enter the nucleus and initiate transcription of target genes. (D) RNA Pol II complexes that have stalled, such as at a DNA lesion, are first monoubiquitylated by the E3 ligase NEDD4. A second E3, $CUL5^{ElonginABC}$, adds K48-linked polyubiquitin chains to monoubiquitylated RNA Pol II leading to p97/VCP-dependent removal of the stalled complex from DNA.

et al, 2010), downregulation of MYC by UBR5 decreases protein synthesis by limiting ribosomal biogenesis. Interestingly, UBR5 also eliminates aberrant translation products through quality control of nascent peptides (Yau *et al,* 2017). Another E3 ligase, HUWE1, controls both MYCN levels (Adhikary *et al,* 2005) and excess ribosomal proteins (Sung *et al,* 2016), underscoring the importance of coordinating MYC activity with protein synthesis in dividing cells. Cells therefore implement at least four E3 ligases to establish the appropriate levels of MYC or MYCN under a wide range of conditions.

Transcription factor regulation by multiple E3 ligases is a common theme in development, as also illustrated by members of the SMAD family that control transforming growth factor-β (TGF-β) signaling (Budi *et al,* 2017). Binding of TGF-β growth factors to plasma membrane receptors induces phosphorylation and nuclear translocation of receptor-regulated SMADs, allowing them to promote transcription of genes involved in cell proliferation, migration, and apoptosis (David & Massague, 2018). While nuclear SMADs are ubiquitylated by the E3 NEDD4-L to prevent aberrant re-initiation of transcription (Gao *et al,* 2009), constitutive modification

© 2021 The Authors

of cytoplasmic SMADs by the E3s SMURF1 and SMURF2 sharpens a cell's sensitivity toward changing TGF-β levels and helps establish rapid response times (Zhu *et al,* 1999; Lin *et al,* 2000; Zhang *et al,* 2001). This integrated control of SMADs in multiple compartments is likely pivotal for setting the dynamics of TGFβ-signaling that drive tissue patterning (Sorre *et al,* 2014).

Ubiquitin-dependent processing of transcription factors

While degradation is an established consequence of ubiquitylation, this modification can also be used to rapidly activate transcription factors. This surprising role of ubiquitylation has emerged for inactive precursors of transcription factors, which are cleaved, rather than degraded, by the proteasome (Fig 3C). Providing an early example, NF-κB transcription factors are synthesized as cytosolic p100 or p105 precursors that are converted into active p50 or p52 subunits through ubiquitin- and proteasome-dependent cleavage (Palombella *et al,* 1994; Skaug *et al,* 2009; Yilmaz *et al,* 2014). Proteasomal processing occurs in multiple organisms and distinct contexts: proteasomal cleavage of the yeast transcription factors SPT23 and MGA2 modulates lipid composition at the endoplasmic reticulum (ER) membrane (Hoppe *et al,* 2000), yet processing of *Drosophila* Cubitus interruptus controls Hedgehog-dependent transcription during development (Jiang, 2006). Proteasomal processing also regulates gene expression through targets other than transcription factors. In response to transcriptional stress, trimming of the DEF1 protein generates the substrate receptor subunit of an E3 ligase that, in turn, downregulate levels of RNA Pol II (Wilson *et al,* 2013b).

To prevent complete degradation, precursors of proteasomal processing are often tethered to another protein or even an organelle (Hoppe *et al,* 2001; Piwko & Jentsch, 2006; Yilmaz *et al,* 2014). This provides a physical impediment to the proteasome, which in turn can initiate, but not complete, degradation to yield a processed target. Following proteasome action, processed transcription factors need to be extracted from their protective partners, which led to the discovery that the AAA-ATPase p97/VCP is a ubiquitin-selective segregase (Rape *et al,* 2001). Although p97/VCP mostly collaborates with proteasomes, it also allows for the ubiquitin-dependent, but proteasome-independent, activation of the transcription factor NRF1 (Radhakrishnan *et al,* 2014). NRF1 is synthesized as a precursor that is embedded into the ER membrane yet constitutively degraded. In cells experiencing proteasome inhibition, NRF1 evades degradation and is instead cleaved by the ubiquitin-selective rhomboid protease DDI2. P97/VCP then liberates cleaved NRF1 from the ER membrane and allows it to enter the nucleus for renewed synthesis of proteasomal subunits (Radhakrishnan *et al,* 2014; Koizumi *et al,* 2016; Sha & Goldberg, 2016; Vangala *et al,* 2016; Lehrbach & Ruvkun, 2016; Dirac-Svejstrup *et al,* 2020).

Ubiquitin-dependent remodeling of transcription factor complexes

Most transcription factors operate within multisubunit assemblies that define their specificity for particular loci or set the extent of transcriptional activation, and the formation of these complexes needs to be tightly monitored for development. The majority of the ~ 120 transcription factors with BTB domains, for example, form homodimers to engage their proper effector proteins impacting transcription. The transcription repressor BCL6, which is mutated in B-cell lymphomas (Yang & Green, 2019), binds to a SMRT, BCOR, and NCoR co-repressor through each of its BTB subunits (Ahmad *et al,* 2003; Ghetu *et al,* 2008). BCL6, however, also forms aberrant heterodimers that are unable to engage co-repressors and impede gene regulation (Mena *et al,* 2018). A protective pathway referred to as dimerization quality control selectively detects and eliminates the inactive heterodimers to ensure proper BCL6 complex formation (Mena *et al,* 2018; Mena *et al,* 2020).

While dimerization quality control constitutively regulates transcription factor interactions, development or environmental inputs often elicit signal-dependent changes in binding partners to alter gene expression (Mottis *et al,* 2013). This is illustrated by WNT-mediated cell specification, which relies on remodeling of a complex around TCF/LEF family transcription factors (Fig 3A). In the absence of WNT, TCF/LEF is kept in check by the Groucho/TLE co-repressor, but when WNT has been bound by cell membrane receptors, the stabilized β-catenin directs the E3 ligase UBR5 to polyubiquitylate Groucho/TLE (Flack *et al,* 2017). Groucho/TLE ubiquitylation signals p97/VCP-dependent extraction from the TCF/LEF complex to relieve chromatin compaction and initiate efficient transcription.

A frequent consequence of ubiquitin-dependent changes in complex composition is the re-localization of transcription factors in or out of the nucleus. Processed NF-κB, for example, is held back in the cytosol by its inhibitor IκBα, until proinflammatory signals elicit IκBα degradation and nuclear entry of NF-κB (Fig 3C; Winston *et al,* 1999). The NF-κB-dependent resynthesis of IκBα re-exports NF-κB out of the nucleus, thereby establishing pulsatile gene expression (Ashall *et al,* 2009). Such regulation might also occur in the absence of degradation: it has been proposed that monoubiquitylation of TP53 masks its recognition by importin-α3 and thereby prevents it from entering the nucleus (Lohrum *et al,* 2001; Marchenko *et al,* 2010). Conversely, monoubiquitylation may block recognition of export sequences within transcription factors to promote nuclear accumulation (van der Horst *et al,* 2006; Trotman *et al,* 2007).

Together, these examples illustrate the diverse nature of ubiquitin-dependent control of transcription factors. It ranges from fine-tuning protein levels to ensuring that stimulus-induced changes in their activity or location are set in place for the necessary duration. Ubiquitin-dependent control of transcription factor activity and abundance can be established rapidly and with high precision, making it a powerful tool for regulatory circuits that integrate the many inputs received by gene expression networks during development.

Ubiquitin-dependent regulation of transcription elongation

While initiation is often the rate-limiting step in transcription, the entire message must be produced to generate a stable transcript that can be translated into a functional protein. Premature abortion of transcription produces mRNA species that are turned over by nucleases, in part because they would encode truncated and likely misfolded proteins. Despite the dangerous consequences of inefficient transcription, elongation of the message is not a smooth process and RNA Pol II frequently stalls due to topological constraints of chromatin or damaged templates (Selth *et al,* 2010; Wilson *et al,* 2013a). While transcription-coupled nucleotide

 © 2021 The Authors

excision repair (TC-NER) can remove DNA lesions in the path of RNA Pol II (Gaillard & Aguilera, 2013), the polymerase itself is evicted from DNA if repair fails (Woudstra *et al,* 2002; Anindya *et al,* 2007).

Recent studies showed that the largest subunit of RNA Pol II, RPB1, is a central target for ubiquitin-dependent control of transcription elongation. While UV-induced DNA damage broadly downregulates transcription, it is followed by recovery of gene expression once damage has been cleared. During this process, RPB1 is ubiquitylated on a specific Lys residue, K1268, which leads to its rapid proteasomal degradation (Wilson *et al,* 2013a; Tufegdzic Vidakovic *et al,* 2020) (Fig 3D). Proteolytic clearance of stalled RNA Pol II prevents inappropriate re-initiation of transcription for short genes when DNA damage persists. A failure to ubiquitylate RNA Pol II, and its resulting aberrant transcription, yields a Cockayne syndrome-like phenotype characterized by premature aging and neurodegeneration (Anindya *et al,* 2010; Nakazawa *et al,* 2020).

Several E3 ligases act on RNA Pol II to decorate it with distinct ubiquitin conjugates. RPB1 is monoubiquitylated by the E3 ligase NEDD4 (Anindya *et al,* 2007; Harreman *et al,* 2009), while $CUL5^{ElonginABC}$ attaches K48-linked chains for p97/VCP-dependent extraction and proteasomal degradation (Yasukawa *et al,* 2008; Harreman *et al,* 2009). NEDD4 and $CUL5^{ElonginABC}$ likely act on different Lys residues in RPB1 (Somesh *et al,* 2007; Tufegdzic Vidakovic *et al,* 2020). Elongin ABC functions as a transcription elongation factor independent of CUL5 and only recruits the catalytic CUL5-RBX1 module upon stalling (Yasukawa *et al,* 2012; Weems *et al,* 2015). Finally, the E3 ligase WWP2 can ubiquitylate RNA Pol II for extraction at double-strand breaks (Caron *et al,* 2019), which is co-opted by MYCN to alleviate transcriptional stalling in neuroblastoma (Herold *et al,* 2019). Akin to transcription factor regulation by multiple E3 ligases, these findings suggest that RNA Pol II ubiquitylation plays a similar role in coordinating the cellular response to multiple sources of transcriptional stalling.

Regulation of transcription by small molecules targeting ubiquitylation

As highlighted in this review, ubiquitylation plays a pivotal role in controlling gene expression in development and disease. This is dramatically underscored by mutations in the degron motifs of transcription factors, such as TP53, NRF2, β-catenin, or MYC, that drive many cancers. Given the prevalence of aberrant gene expression in disease, it has been a goal of many drug discovery programs to alter the stability of transcription factors for therapeutic benefit. As most E3 ligases and transcription factors lack obvious pockets for small molecules (Gu *et al,* 2018), it only recently became apparent how protein stability can be altered to modulate gene expression. Complementing the physiological regulation of transcription by ubiquitin, our final paragraphs will therefore discuss how the ubiquitylation machinery can be hijacked by small molecules to modulate the transcriptional networks that control development and prevent disease.

E3 ligase inhibition to target unstable transcription factors

A straightforward way to affect gene expression by small molecules are inhibitors of E3 ligases that target transcription factors (Huang & Dixit, 2016), as spearheaded for the tumor suppressor TP53 (Sarek & Ojala, 2007) (Fig 4A). While TP53 is mutated in ~ 50% of cancers, many tumors downregulate TP53 by overexpression of its E3 MDM2. In these cases, inhibition of MDM2 could reactivate TP53-dependent gene expression and help eliminate malignant cells. The first class of MDM2 inhibitors were "Nutlins", which prevent MDM2 from interacting with TP53 (Vassilev *et al,* 2004). Other compounds directly inhibit the E3 ligase activity of MDM2 or destabilize MDM2 by shutting off its protective deubiquitylase USP7 (Turnbull *et al,* 2017; Kategaya *et al,* 2017; Sanz *et al,* 2019) (Fig 4A).

Small molecule inhibitors of E3 ligases are most effective against transcription factors that are constitutively turned over. In addition to TP53, this is illustrated by the antioxidant transcription factor NRF2 (Hayes & Dinkova-Kostova, 2014), whose transient stabilization is of interest for the treatment of neurodegeneration or diabetes (Schmoll *et al,* 2017). NRF2 can be regulated by targeting reactive cysteine residues in its E3, $CUL3^{KEAP1}$ (Zhang *et al,* 2004), using compounds, such as dimethyl fumarate, which has been approved for the treatment of multiple sclerosis (Dodson *et al,* 2019). However, inhibition of NRF2 has to be carefully monitored, as persistent stabilization of this transcription factor induces a gene expression program that triggers reductive stress and prevents muscle stem cell differentiation (Manford *et al,* 2020).

Induced protein degradation to control gene expression

Providing an alternative approach, small molecules can alter gene expression by eliciting the turnover of transcription factors. Such induced protein degradation was first naturally observed in plants, where hormones of the auxin family bridge an E3 ligase, SCF^{TIR1}, with the transcriptional repressors AXR2/IAA7 and AXR3/IAA17 (Sakamoto *et al,* 2001; Dharmasiri *et al,* 2005; Kepinski & Leyser, 2005). In a similar manner, the hormone gibberellin triggers complex formation between the E3 SCF^{GID2} and the transcriptional repressor DELLA (Hauvermale *et al,* 2012). As molecular glues between an E3 ligase and a protein, these hormones induce the ubiquitylation of transcription regulators and control gene expressions programs important for plant development (Fig 4B).

Synthetic molecular glues are now used to modulate transcription factor stability in human cells, as illustrated by the immunomodulatory drug thalidomide that was once prescribed as sleep medication and now is prescribed to combat hematological malignancies (Lasagna, 1960; Vargesson, 2009). Thalidomide and its derivatives direct the E3 $CUL4^{CRBN}$ to degrade zinc finger proteins, many of which are transcription factors (Lu *et al,* 2014; Sievers *et al,* 2018). One transcription factor, SALL4, is essential for limb development, and its induced degradation during early pregnancy likely accounts for the drastic thalidomide-induced birth defects (Donovan *et al,* 2018; Matyskiela *et al,* 2020). While thalidomide was introduced into the clinic without knowledge of its mechanism, the first prospective molecular glue to connect an E3 ligase to a pathological protein was recently reported (Simonetta *et al,* 2019). This compound tethered unphosphorylated β-catenin to the E3 $SCF^{\beta TrCP}$ to induce degradation of this transcription factor in colon cancer cells carrying mutations in the destruction complex. Molecular glues can also elicit transcription factor degradation by means other than directly attracting an E3 ligase: Compounds that bind BCL6, for example, trigger formation of BCL6 fibers that recruit the

© 2021 The Authors

E3 ligase SIAH1 and counteract BCL6-dependent gene repression in lymphoma cells (Kerres *et al,* 2017; Slabicki *et al,* 2020b). Moreover, molecular glues can affect the activity of RNA Pol II itself, by controlling the stability of a cyclin-dependent kinase that regulates transcript elongation (Mayor-Ruiz *et al,* 2020; Lv *et al,* 2020; Slabicki *et al,* 2020a).

Understanding thalidomide's mechanism of action led to the development of bifunctional molecules composed of two warheads, one that binds an E3 ligase and the other that recognizes the target (Winter *et al,* 2015). Having been proposed to elicit degradation almost two decades earlier (Sakamoto *et al,* 2001; Sakamoto *et al,* 2003), such proteolysis-targeting chimeras (PROTACs) induce the recruitment of pathological proteins to E3 ligases to trigger proteasomal degradation of the disease-causing agent. PROTACs have been widely applied to alter gene expression, as they can eliminate transcription factors, such as STAT3 (Bai *et al,* 2019), histone modifiers, such as the PRC2 complex (Potjewyd *et al,* 2020), or readers of histone modifications, such as BRD4 (Winter *et al,* 2015) (Fig 4C). In 2019, the first PROTAC entered clinical trials for the treatment of castration-resistant prostate cancer, targeting the androgen receptor and its transcriptional networks (Wang *et al,* 2020). Degradation tags (dTAGs) (Nabet *et al,* 2018) are PROTACs that target the $FKBP12^{F36V}$ domain which, when fused to a transcriptional regulator, can be a powerful way to acutely change gene expression in the laboratory (Erb *et al,* 2017). Hijacking E3 ligases thus offers multiple opportunities to alter gene expression networks that have been notoriously difficult to target by traditional means.

To conclude, accurate transcription is critical for guiding tissue formation and homeostasis. The failure to initiate gene expression at the right time and place or problems in resolving transcriptional stress can lead to cell death and disease. It is clear that cells use ubiquitylation in proteolytic and nonproteolytic ways to control nearly every step of transcription—from modulating chromatin architecture over limiting transcription factor abundance to ensuring transcript elongation. The past years have witnessed groundbreaking improvements in our understanding of ubiquitin-dependent control of gene expression, which led to novel therapeutic strategies that may help patients with cancer and other ailments. It is now possible to mobilize the ubiquitylation machinery to selectively degrade transcription factors that were once deemed "undruggable". As clinical trials are underway, whether these compounds live up to their promise will soon become apparent. If they do, ubiquitin-dependent control of gene expression will be a textbook example of

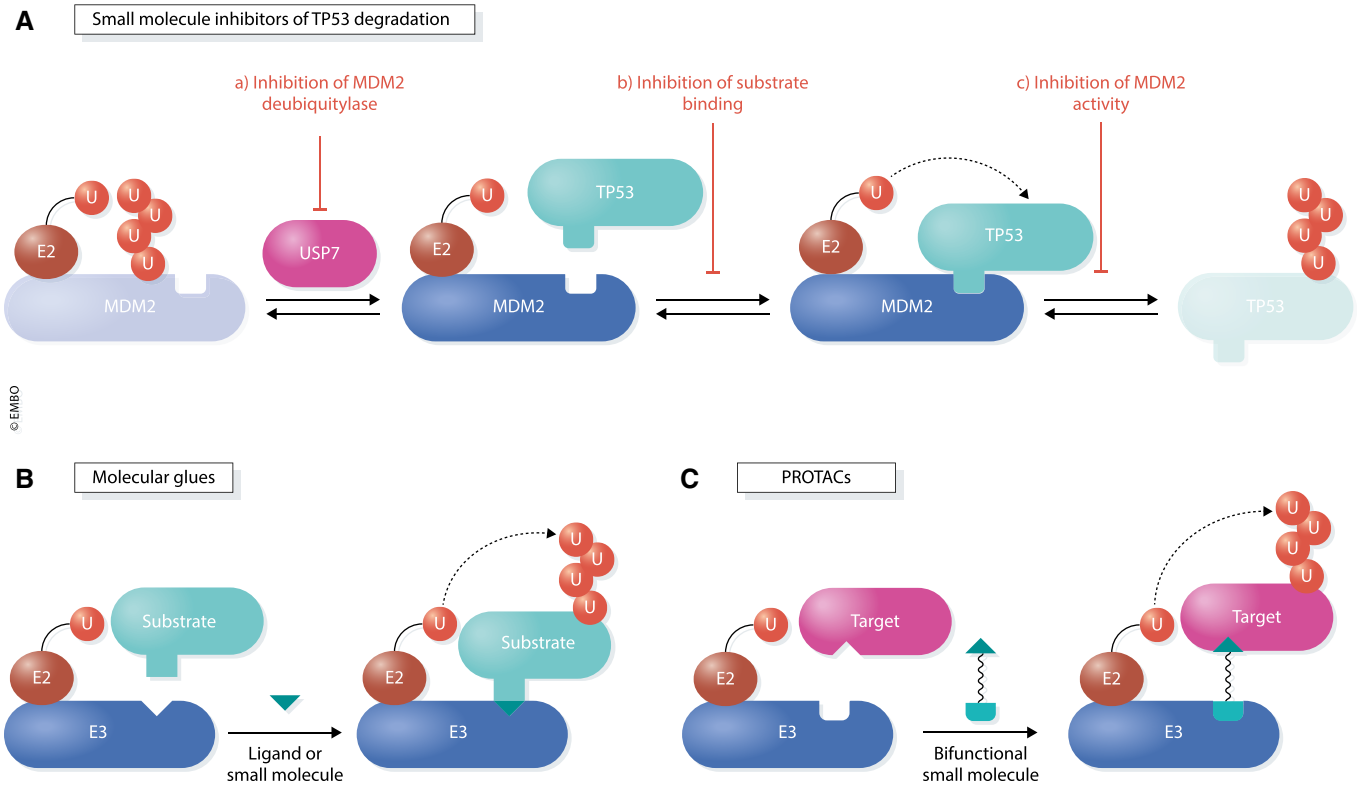

Figure 4. Regulation of transcription by small molecules targeting ubiquitin components.

(A) Mechanisms of small molecules that prevent degradation of the transcription factor and tumor suppressor, TP53, by its E3 ubiquitin ligase, MDM2. (a) Inhibitors of the USP7 deubiquitylase lead to increased autoubiquitylation and destabilization of MDM2. (b) Small molecules, such as the Nutlins, block TP53 degradation by disrupting the substrate-ligase interaction surface. (c) Compounds that block the E3 ligase activity of MDM2, but not substrate binding, also result in accumulation of TP53. (B) Molecular glues are small molecules that alter the surface of proteins, namely an E3 ligase and substrate, to promote their association. These compounds often display low binding affinities for each interactor but simultaneously bind both components to enhance their interaction. (C) PROTACs (proteolysis-targeting chimeras) are heterobifunctional molecules that consist of two small molecules tethered by a linker. One of the small molecules binds a target protein while the other binds an E3 ligase. In this manner, the cell's endogenous ubiquitin–proteasome system can be used to rapidly and selectively eliminate any given protein target-of-interest.

© 2021 The Authors

how fundamental research in understanding principles of signal transduction can have a profound impact on human health.

Acknowledgements

We apologize to our colleagues whose work we were unable to include due to space constraints. We thank D. Haakonsen and E. Oh for critical reading of this manuscript, and we are grateful to all members of our laboratory for their ideas and discussions regarding the role of ubiquitin in transcription. K.G.M. is supported by the National Institute of General Medical Sciences of the National Institutes of Health under award F32GM120956. M.R. is an Investigator of the Howard Hughes Medical Institute.

Conflict of interest

M.R. is a co-founder and consultant to Nurix and scientific advisory board member to Monte Rosa.

References

Adhikary S, Marinoni F, Hock A, Hulleman E, Popov N, Beier R, Bernard S, Quarto M, Capra M, Goettig S *et al* (2005) The ubiquitin ligase HectH9 regulates transcriptional activation by Myc and is essential for tumor cell proliferation. *Cell* 123: 409 – 421

Adorno M, Sikandar S, Mitra SS, Kuo A, Nicolis Di Robilant B, Haro-Acosta V, Ouadah Y, Quarta M, Rodriguez J, Qian D *et al* (2013) Usp16 contributes to somatic stem-cell defects in Down's syndrome. *Nature* 501: 380 – 384

Ahmad KF, Melnick A, Lax S, Bouchard D, Liu J, Kiang CL, Mayer S, Takahashi S, Licht JD, Prive GG (2003) Mechanism of SMRT corepressor recruitment by the BCL6 BTB domain. *Mol Cell* 12: 1551 – 1564

Anindya R, Aygun O, Svejstrup JQ (2007) Damage-induced ubiquitylation of human RNA polymerase II by the ubiquitin ligase Nedd4, but not Cockayne syndrome proteins or BRCA1. *Mol Cell* 28: 386 – 397

Anindya R, Mari PO, Kristensen U, Kool H, Giglia-Mari G, Mullenders LH, Fousteri M, Vermeulen W, Egly JM, Svejstrup JQ (2010) A ubiquitin-binding domain in Cockayne syndrome B required for transcription-coupled nucleotide excision repair. *Mol Cell* 38: 637 – 648

Aranda S, Mas G, di Croce L (2015) Regulation of gene transcription by Polycomb proteins. *Sci Adv* 1: e1500737

Ashall L, Horton CA, Nelson DE, Paszek P, Harper CV, Sillitoe K, Ryan S, Spiller DG, Unitt JF, Broomhead DS *et al* (2009) Pulsatile stimulation determines timing and specificity of NF-kappaB-dependent transcription. *Science* 324: 242 – 246

Atanassov BS, Mohan RD, Lan X, Kuang X, Lu Y, Lin K, McIvor E, Li W, Zhang Y, Florens L *et al* (2016) ATXN7L3 and ENY2 coordinate activity of multiple H2B deubiquitinases important for cellular proliferation and tumor growth. *Mol Cell* 62: 558 – 571

Bahram F, von der Lehr N, Cetinkaya C, Larsson LG (2000) c-Myc hot spot mutations in lymphomas result in inefficient ubiquitination and decreased proteasome-mediated turnover. *Blood* 95: 2104 – 2110

Bai L, Zhou H, Xu R, Zhao Y, Chinnaswamy K, McEachern D, Chen J, Yang CY, Liu Z, Wang M *et al* (2019) A potent and selective small-molecule degrader of STAT3 achieves complete tumor regression *in vivo*. *Cancer Cell* 36: 498 – 511.e17

Baluapuri A, Wolf E, Eilers M (2020) Target gene-independent functions of MYC oncoproteins. *Nat Rev Mol Cell Biol* 21: 255 – 267

Bantignies F, Roure V, Comet I, Leblanc B, Schuettengruber B, Bonnet J, Tixier V, Mas A, Cavalli G (2011) Polycomb-dependent regulatory contacts between distant Hox loci in *Drosophila*. *Cell* 144: 214 – 226

Belle JI, Nijnik A (2014) H2A-DUBbing the mammalian epigenome: expanding frontiers for histone H2A deubiquitinating enzymes in cell biology and physiology. *Int J Biochem Cell Biol* 50: 161 – 174

Blackledge NP, Farcas AM, Kondo T, King HW, McGouran JF, Hanssen LL, Ito S, Cooper S, Kondo K, Koseki Y *et al* (2014) Variant PRC1 complex-dependent H2A ubiquitylation drives PRC2 recruitment and polycomb domain formation. *Cell* 157: 1445 – 1459

Blackledge NP, Fursova NA, Kelley JR, Huseyin MK, Feldmann A, Klose RJ (2020) PRC1 catalytic activity is central to polycomb system function. *Mol Cell* 77: 857 – 874.e9

Blobel GA, Kadauke S, Wang E, Lau AW, Zuber J, Chou MM, Vakoc CR (2009) A reconfigured pattern of MLL occupancy within mitotic chromatin promotes rapid transcriptional reactivation following mitotic exit. *Mol Cell* 36: 970 – 983

Blomfield IM, Rocamonde B, Masdeu MDM, Mulugeta E, Vaga S, van den Berg DL, Huillard E, Guillemot F, Urban N (2019) Id4 promotes the elimination of the pro-activation factor Ascl1 to maintain quiescence of adult hippocampal stem cells. *Elife* 8: e48561

Brockdorff N (2017) Polycomb complexes in X chromosome inactivation. *Philos Trans R Soc Lond B Biol Sci* 372: 20170021

Budi EH, Duan D, Derynck R (2017) Transforming growth factor-beta receptors and smads: regulatory complexity and functional versatility. *Trends Cell Biol* 27: 658 – 672

Bulynko YA, O'Malley BW (2011) Nuclear receptor coactivators: structural and functional biochemistry. *Biochemistry* 50: 313 – 328

Campagne A, Lee MK, Zielinski D, Michaud A, le Corre S, Dingli F, Chen H, Shahidian LZ, Vassilev I, Servant N *et al* (2019) BAP1 complex promotes transcription by opposing PRC1-mediated H2A ubiquitylation. *Nat Commun* 10: 348

Carbone M, Yang H, Pass HI, Krausz T, Testa JR, Gaudino G (2013) BAP1 and cancer. *Nat Rev Cancer* 13: 153 – 159

Caron P, Pankotai T, Wiegant WW, Tollenaere MAX, Furst A, Bonhomme C, Helfricht A, de Groot A, Pastink A, Vertegaal A *et al* (2019) WWP2 ubiquitylates RNA polymerase II for DNA-PK-dependent transcription arrest and repair at DNA breaks. *Genes Dev* 33: 684 – 704

Chau V, Tobias JW, Bachmair A, Marriott D, Ecker DJ, Gonda DK, Varshavsky A (1989) A multiubiquitin chain is confined to specific lysine in a targeted short-lived protein. *Science* 243: 1576 – 1583

Chen S, Li J, Wang DL, Sun FL (2012) Histone H2B lysine 120 monoubiquitination is required for embryonic stem cell differentiation. *Cell Res* 22: 1402 – 1405

Chen FX, Smith ER, Shilatifard A (2018a) Born to run: control of transcription elongation by RNA polymerase II. *Nat Rev Mol Cell Biol* 19: 464 – 478

Chen P, Dong L, Hu M, Wang YZ, Xiao X, Zhao Z, Yan J, Wang PY, Reinberg D, Li M *et al* (2018b) Functions of FACT in breaking the nucleosome and maintaining its integrity at the single-nucleosome level. *Mol Cell* 71: 284 – 293.e4

Chittock EC, Latwiel S, Miller TC, Muller CW (2017) Molecular architecture of polycomb repressive complexes. *Biochem Soc Trans* 45: 193 – 205

Cortez JT, Montauti E, Shifrut E, Gatchalian J, Zhang Y, Shaked O, Xu Y, Roth TL, Simeonov DR, Zhang Y *et al* (2020) CRISPR screen in regulatory T cells reveals modulators of Foxp3. *Nature* 582: 416 – 420

David CJ, Massague J (2018) Contextual determinants of TGFbeta action in development, immunity and cancer. *Nat Rev Mol Cell Biol* 19: 419 – 435

© 2021 The Authors

Debelouchina GT, Gerecht K, Muir TW (2017) Ubiquitin utilizes an acidic surface patch to alter chromatin structure. *Nat Chem Biol* 13: 105 – 110

Deluz C, Friman ET, Strebinger D, Benke A, Raccaud M, Callegari A, Leleu M, Manley S, Suter DM (2016) A role for mitotic bookmarking of SOX2 in pluripotency and differentiation. *Genes Dev* 30: 2538 – 2550

Dembla V, Somaiah N, Barata P, Hess K, Fu S, Janku F, Karp DD, Naing A, Piha-Paul SA, Subbiah V *et al* (2018) Prevalence of MDM2 amplification and coalterations in 523 advanced cancer patients in the MD Anderson phase 1 clinic. *Oncotarget* 9: 33232 – 33243

Denholtz M, Bonora G, Chronis C, Splinter E, de Laat W, Ernst J, Pellegrini M, Plath K (2013) Long-range chromatin contacts in embryonic stem cells reveal a role for pluripotency factors and polycomb proteins in genome organization. *Cell Stem Cell* 13: 602 – 616

Dey A, Nishiyama A, Karpova T, McNally J, Ozato K (2009) Brd4 marks select genes on mitotic chromatin and directs postmitotic transcription. *Mol Biol Cell* 20: 4899 – 4909

Dharmasiri N, Dharmasiri S, Estelle M (2005) The F-box protein TIR1 is an auxin receptor. *Nature* 435: 441 – 445

Dickson KA, Cole AJ, Gill AJ, Clarkson A, Gard GB, Chou A, Kennedy CJ, Henderson BR, Australian Ovarian Cancer Study, Fereday S *et al* (2016) The RING finger domain E3 ubiquitin ligases BRCA1 and the RNF20/RNF40 complex in global loss of the chromatin mark histone H2B monoubiquitination (H2Bub1) in cell line models and primary high-grade serous ovarian cancer. *Hum Mol Genet* 25: 5460 – 5471

Dirac-Svejstrup AB, Walker J, Faull P, Encheva V, Akimov V, Puglia M, Perkins D, Kumper S, Hunjan SS, Blagoev B *et al* (2020) DDI2 Is a ubiquitin-directed endoprotease responsible for cleavage of transcription factor NRF1. *Mol Cell* 79: 332 – 341.e7

Djebali S, Davis CA, Merkel A, Dobin A, Lassmann T, Mortazavi A, Tanzer A, Lagarde J, Lin W, Schlesinger F *et al* (2012) Landscape of transcription in human cells. *Nature* 489: 101 – 108

Dodson M, de la Vega MR, Cholanians AB, Schmidlin CJ, Chapman E, Zhang DD (2019) Modulating NRF2 in disease: timing is everything. *Annu Rev Pharmacol Toxicol* 59: 555 – 575

Donovan KA, An J, Nowak RP, Yuan JC, Fink EC, Berry BC, Ebert BL, Fischer ES (2018) Thalidomide promotes degradation of SALL4, a transcription factor implicated in Duane Radial Ray syndrome. *Elife* 7: e38430

Erb MA, Scott TG, Li BE, Xie H, Paulk J, Seo HS, Souza A, Roberts JM, Dastjerdi S, Buckley DL *et al* (2017) Transcription control by the ENL YEATS domain in acute leukaemia. *Nature* 543: 270 – 274

Fierz B, Chatterjee C, McGinty RK, Bar-Dagan M, Raleigh DP, Muir TW (2011) Histone H2B ubiquitylation disrupts local and higher-order chromatin compaction. *Nat Chem Biol* 7: 113 – 119

Flack JE, Mieszczanek J, Novcic N, Bienz M (2017) Wnt-Dependent Inactivation of the Groucho/TLE Co-repressor by the HECT E3 Ubiquitin Ligase Hyd/UBR5. *Mol Cell* 67: 181 – 193.e5

Fuchs G, Shema E, Vesterman R, Kotler E, Wolchinsky Z, Wilder S, Golomb L, Pribluda A, Zhang F, Haj-Yahya M *et al* (2012) RNF20 and USP44 regulate stem cell differentiation by modulating H2B monoubiquitylation. *Mol Cell* 46: 662 – 673

Fuchs G, Oren M (2014) Writing and reading H2B monoubiquitylation. *Biochim Biophys Acta* 1839: 694 – 701

Fyodorov DV, Zhou BR, Skoultchi AI, Bai Y (2018) Emerging roles of linker histones in regulating chromatin structure and function. *Nat Rev Mol Cell Biol* 19: 192 – 206

Gaillard H, Aguilera A (2013) Transcription coupled repair at the interface between transcription elongation and mRNP biogenesis. *Biochim Biophys Acta* 1829: 141 – 150

Gallego LD, Schneider M, Mittal C, Romanauska A, Gudino Carrillo RM, Schubert T, Pugh BF, Kohler A (2020) Phase separation directs ubiquitination of gene-body nucleosomes. *Nature* 579: 592 – 597

Gao S, Alarcon C, Sapkota G, Rahman S, Chen PY, Goerner N, Macias MJ, Erdjument-Bromage H, Tempst P, Massague J (2009) Ubiquitin ligase Nedd4L targets activated Smad2/3 to limit TGF-beta signaling. *Mol Cell* 36: 457 – 468

Gao C, Wang Y, Broaddus R, Sun L, Xue F, Zhang W (2018) Exon 3 mutations of CTNNB1 drive tumorigenesis: a review. *Oncotarget* 9: 5492 – 5508

Gatzka M, Tasdogan A, Hainzl A, Allies G, Maity P, Wilms C, Wlaschek M, Scharffetter-Kochanek K (2015) Interplay of H2A deubiquitinase 2A-DUB/Mysm1 and the p19(ARF)/p53 axis in hematopoiesis, early T-cell development and tissue differentiation. *Cell Death Differ* 22: 1451 – 1462

Ghetu AF, Corcoran CM, Cerchietti L, Bardwell VJ, Melnick A, Prive GG (2008) Structure of a BCOR corepressor peptide in complex with the BCL6 BTB domain dimer. *Mol Cell* 29: 384 – 391

Giaccia AJ, Kastan MB (1998) The complexity of p53 modulation: emerging patterns from divergent signals. *Genes Dev* 12: 2973 – 2983

Glotzer M, Murray AW, Kirschner MW (1991) Cyclin is degraded by the ubiquitin pathway. *Nature* 349: 132 – 138

Goentoro L, Kirschner MW (2009) Evidence that fold-change, and not absolute level, of beta-catenin dictates Wnt signaling. *Mol Cell* 36: 872 – 884

Goentoro L, Shoval O, Kirschner MW, Alon U (2009) The incoherent feedforward loop can provide fold-change detection in gene regulation. *Mol Cell* 36: 894 – 899

Goldknopf IL, Taylor CW, Baum RM, Yeoman LC, Olson MO, Prestayko AW, Busch H (1975) Isolation and characterization of protein A24, a "histone-like" non-histone chromosomal protein. *J Biol Chem* 250: 7182 – 7187

Gossage L, Eisen T, Maher ER (2015) VHL, the story of a tumour suppressor gene. *Nat Rev Cancer* 15: 55 – 64

Gregory MA, Hann SR (2000) c-Myc proteolysis by the ubiquitin-proteasome pathway: stabilization of c-Myc in Burkitt's lymphoma cells. *Mol Cell Biol* 20: 2423 – 2435

Grossniklaus U, Paro R (2014) Transcriptional silencing by polycomb-group proteins. *Cold Spring Harb Perspect Biol* 6: a019331

Gu Y, Jones AE, Yang W, Liu S, Dai Q, Liu Y, Swindle CS, Zhou D, Zhang Z, Ryan TM *et al* (2016) The histone H2A deubiquitinase Usp16 regulates hematopoiesis and hematopoietic stem cell function. *Proc Natl Acad Sci USA* 113: E51 – 60

Gu S, Cui D, Chen X, Xiong X, Zhao Y (2018) PROTACs: an emerging targeting technique for protein degradation in drug discovery. *BioEssays* 40: e1700247

Guarnaccia AD, Tansey WP (2018) Moonlighting with WDR5: a cellular multitasker. *J Clin Med* 7: 21

Haakonsen DL, Rape M (2019) Branching out: improved signaling by heterotypic ubiquitin chains. *Trends Cell Biol* 29: 704 – 716

Harreman M, Taschner M, Sigurdsson S, Anindya R, Reid J, Somesh B, Kong SE, Banks CA, Conaway RC, Conaway JW *et al* (2009) Distinct ubiquitin ligases act sequentially for RNA polymerase II polyubiquitylation. *Proc Natl Acad Sci USA* 106: 20705 – 20710

Hauvermale AL, Ariizumi T, Steber CM (2012) Gibberellin signaling: a theme and variations on DELLA repression. *Plant Physiol* 160: 83 – 92

Hayes JD, Dinkova-Kostova AT (2014) The Nrf2 regulatory network provides an interface between redox and intermediary metabolism. *Trends Biochem Sci* 39: 199 – 218

 © 2021 The Authors

Herold S, Kalb J, Buchel G, Ade CP, Baluapuri A, Xu J, Koster J, Solvie D, Carstensen A, Klotz C *et al* (2019) Recruitment of BRCA1 limits MYCN-driven accumulation of stalled RNA polymerase. *Nature* 567: 545 – 549

Hoppe T, Matuschewski K, Rape M, Schlenker S, Ulrich HD, Jentsch S (2000) Activation of a membrane-bound transcription factor by regulated ubiquitin/proteasome-dependent processing. *Cell* 102: 577 – 586

Hoppe T, Rape M, Jentsch S (2001) Membrane-bound transcription factors: regulated release by RIP or RUP. *Curr Opin Cell Biol* 13: 344 – 348

van der Horst A, De Vries-Smits AM, Brenkman AB, van Triest MH, van den Broek N, Colland F, Maurice MM, Burgering BM (2006) FOXO4 transcriptional activity is regulated by monoubiquitination and USP7/HAUSP. *Nat Cell Biol* 8: 1064 – 1073

Hsiung CC, Blobel GA (2016) A new bookmark of the mitotic genome in embryonic stem cells. *Nat Cell Biol* 18: 1124 – 1125

Huang X, Dixit VM (2016) Drugging the undruggables: exploring the ubiquitin system for drug development. *Cell Res* 26: 484 – 498

Huber AL, Papp SJ, Chan AB, Henriksson E, Jordan SD, Kriebs A, Nguyen M, Wallace M, Li Z, Metallo CM *et al* (2016) CRY2 and FBXL3 cooperatively degrade c-MYC. *Mol Cell* 64: 774 – 789

Isono K, Endo TA, Ku M, Yamada D, Suzuki R, Sharif J, Ishikura T, Toyoda T, Bernstein BE, Koseki H (2013) SAM domain polymerization links subnuclear clustering of PRC1 to gene silencing. *Dev Cell* 26: 565 – 577

Jason LJ, Finn RM, Lindsey G, Ausio J (2005) Histone H2A ubiquitination does not preclude histone H1 binding, but it facilitates its association with the nucleosome. *J Biol Chem* 280: 4975 – 4982

Jho E, Zhang T, Domon C, Joo CK, Freund JN, Constantini F (2002) Wnt/beta-catenin/Tcf signaling induces the transcription of Axin2, a negative regulator of the signaling pathway. *Mol Cell Biol* 22: 1172 – 1183

Jiang J (2006) Regulation of Hh/Gli signaling by dual ubiquitin pathways. *Cell Cycle* 5: 2457 – 2463

Jin L, Williamson A, Banerjee S, Philipp I, Rape M (2008) Mechanism of ubiquitin-chain formation by the human anaphase-promoting complex. *Cell* 133: 653 – 665

Juven-Gershon T, Kadonaga JT (2010) Regulation of gene expression via the core promoter and the basal transcriptional machinery. *Dev Biol* 339: 225 – 229

Kadauke S, Udugama MI, Pawlicki JM, Achtman JC, Jain DP, Cheng Y, Hardison RC, Blobel GA (2012) Tissue-specific mitotic bookmarking by hematopoietic transcription factor GATA1. *Cell* 150: 725 – 737

Kalb R, Latwiel S, Baymaz HI, Jansen PW, Muller CW, Vermeulen M, Muller J (2014) Histone H2A monoubiquitination promotes histone H3 methylation in Polycomb repression. *Nat Struct Mol Biol* 21: 569 – 571

Karni-Schmidt O, Lokshin M, Prives C (2016) The roles of MDM2 and MDMX in cancer. *Annu Rev Pathol* 11: 617 – 644

Karpiuk O, Najafova Z, Kramer F, Hennion M, Galonska C, Konig A, Snaidero N, Vogel T, Shchebet A, Begus-Nahrmann Y *et al* (2012) The histone H2B monoubiquitination regulatory pathway is required for differentiation of multipotent stem cells. *Mol Cell* 46: 705 – 713

Kategaya L, di Lello P, Rouge L, Pastor R, Clark KR, Drummond J, Kleinheinz T, Lin E, Upton JP, Prakash S *et al* (2017) USP7 small-molecule inhibitors interfere with ubiquitin binding. *Nature* 550: 534 – 538

Katoh H, Qin ZS, Liu R, Wang L, Li W, Li X, Wu L, Du Z, Lyons R, Liu CG *et al* (2011) FOXP3 orchestrates H4K16 acetylation and H3K4 trimethylation for activation of multiple genes by recruiting MOF and causing displacement of PLU-1. *Mol Cell* 44: 770 – 784

Kepinski S, Leyser O (2005) The Arabidopsis F-box protein TIR1 is an auxin receptor. *Nature* 435: 446 – 451

Kerres N, Steurer S, Schlager S, Bader G, Berger H, Caligiuri M, Dank C, Engen JR, Ettmayer P, Fischerauer B *et al* (2017) Chemically induced degradation of the oncogenic transcription factor BCL6. *Cell Rep* 20: 2860 – 2875

Koizumi S, Irie T, Hirayama S, Sakurai Y, Yashiroda H, Naguro I, Ichijo H, Hamazaki J, Murata S (2016) The aspartyl protease DDI2 activates Nrf1 to compensate for proteasome dysfunction. *Elife* 5: e18357

Komander D (2009) The emerging complexity of protein ubiquitination. *Biochem Soc Trans* 37: 937 – 953

Krajewski WA, Li J, Dou Y (2018) Effects of histone H2B ubiquitylation on the nucleosome structure and dynamics. *Nucleic Acids Res* 46: 7631 – 7642

Lambert SA, Jolma A, Campitelli LF, Das PK, Yin Y, Albu M, Chen X, Taipale J, Hughes TR, Weirauch MT (2018) The human transcription factors. *Cell* 175: 598 – 599

Lan X, Atanassov BS, Li W, Zhang Y, Florens L, Mohan RD, Galardy PJ, Washburn MP, Workman JL, Dent SYR (2016) USP44 Is an integral component of N-CoR that contributes to gene repression by deubiquitinating histone H2B. *Cell Rep* 17: 2382 – 2393

Lasagna L (1960) Thalidomide–a new nonbarbiturate sleep–inducing drug. *J Chronic Dis* 11: 627 – 631

Lawrence MS, Stojanov P, Mermel CH, Robinson JT, Garraway LA, Golub TR, Meyerson M, Gabriel SB, Lander ES, Getz G (2014) Discovery and saturation analysis of cancer genes across 21 tumour types. *Nature* 505: 495 – 501

Lee E, Salic A, Krüger R, Heinrich R, Kirschner MW (2003) The roles of APC and Axin derived from experimental and theoretical analysis of the Wnt pathway. *PLoS Biol* 1: E10

Lee TI, Young RA (2013) Transcriptional regulation and its misregulation in disease. *Cell* 152: 1237 – 1251

Lehrbach NJ, Ruvkun G (2016) Proteasome dysfunction triggers activation of SKN-1A/Nrf1 by the aspartic protease DDI-1. *Elife* 5: e17721

Levendosky RF, Sabantsev A, Deindl S, Bowman GD (2016) The Chd1 chromatin remodeler shifts hexasomes unidirectionally. *Elife* 5: e21356

Lin X, Liang M, Feng XH (2000) Smurf2 is a ubiquitin E3 ligase mediating proteasome-dependent degradation of Smad2 in transforming growth factor-beta signaling. *J Biol Chem* 275: 36818 – 36822

Lohrum MA, Woods DB, Ludwig RL, Balint E, Vousden KH (2001) C-terminal ubiquitination of p53 contributes to nuclear export. *Mol Cell Biol* 21: 8521 – 8532

Lu G, Middleton RE, Sun H, Naniong M, Ott CJ, Mitsiades CS, Wong KK, Bradner JE, Kaelin Jr WG (2014) The myeloma drug lenalidomide promotes the cereblon-dependent destruction of Ikaros proteins. *Science* 343: 305 – 309

Lv L, Chen P, Cao L, Li Y, Zeng Z, Cui Y, Wu Q, Li J, Wang JH, Dong MQ *et al* (2020) Discovery of a molecular glue promoting CDK12-DDB1 interaction to trigger cyclin K degradation. *Elife* 9: e59994

Ma Q (2013) Role of nrf2 in oxidative stress and toxicity. *Annu Rev Pharmacol Toxicol* 53: 401 – 426

Machida S, Takizawa Y, Ishimaru M, Sugita Y, Sekine S, Nakayama JI, Wolf M, Kurumizaka H (2018) Structural basis of heterochromatin formation by human HP1. *Mol Cell* 69: 385 – 397.e8

Magnani L, Eeckhoute J, Lupien M (2011) Pioneer factors: directing transcriptional regulators within the chromatin environment. *Trends Genet* 27: 465 – 474

Manford AG, Rodriguez-Perez F, Shih KY, Shi Z, Berdan CA, Choe M, Titov DV, Nomura DK, Rape M (2020) A cellular mechanism to detect and alleviate reductive stress. *Cell* 183: 46 – 61.e21

Manfredi JJ (2010) The Mdm2-p53 relationship evolves: Mdm2 swings both ways as an oncogene and a tumor suppressor. *Genes Dev* 24: 1580 – 1589

© 2021 The Authors

Marchenko ND, Hanel W, Li D, Becker K, Reich N, Moll UM (2010) Stress-mediated nuclear stabilization of p53 is regulated by ubiquitination and importin-alpha3 binding. *Cell Death Differ* 17: 255 – 267

Martinez-Balbas MA, Dey A, Rabindran SK, Ozato K, Wu C (1995) Displacement of sequence-specific transcription factors from mitotic chromatin. *Cell* 83: 29 – 38

Matyskiela ME, Clayton T, Zheng X, Mayne C, Tran E, Carpenter A, Pagarigan B, McDonald J, Rolfe M, Hamann LG *et al* (2020) Crystal structure of the SALL4-pomalidomide-cereblon-DDB1 complex. *Nat Struct Mol Biol* 27: 319 – 322

Mayor-Ruiz C, Bauer S, Brand M, Kozicka Z, Siklos M, Imrichova H, Kaltheuner IH, Hahn E, Seiler K, Koren A *et al* (2020) Rational discovery of molecular glue degraders via scalable chemical profiling. *Nat Chem Biol* 16: 1199 – 1207

McMahon SB (2014) MYC and the control of apoptosis. *Cold Spring Harb Perspect Med* 4: a014407

Mena EL, Kjolby RAS, Saxton RA, Werner A, Lew BG, Boyle JM, Harland R, Rape M (2018) Dimerization quality control ensures neuronal development and survival. *Science* 586: 452 – 456

Mena EL, Jevtic P, Greber BJ, Gee CL, Lew BG, Akopian D, Nogales E, Kuriyan J, Rape M (2020) Structural basis for dimerization quality control. *Nature* 586: 452 – 456

Meyer HJ, Rape M (2014) Enhanced protein degradation by branched ubiquitin chains. *Cell* 157: 910 – 921

Michael AK, Grand RS, Isbel L, Cavadini S, Kozicka Z, Kempf G, Bunker RD, Schenk AD, Graff-Meyer A, Pathare GR *et al* (2020) Mechanisms of OCT4-SOX2 motif readout on nucleosomes. *Science* 368: 1460 – 1465

Michelotti EF, Sanford S, Levens D (1997) Marking of active genes on mitotic chromosomes. *Nature* 388: 895 – 899

Minsky N, Shema E, Field Y, Schuster M, Segal E, Oren M (2008) Monoubiquitinated H2B is associated with the transcribed region of highly expressed genes in human cells. *Nat Cell Biol* 10: 483 – 488

Mottis A, Mouchiroud L, Auwerx J (2013) Emerging roles of the corepressors NCoR1 and SMRT in homeostasis. *Genes Dev* 27: 819 – 835

Nabet B, Roberts JM, Buckley DL, Paulk J, Dastjerdi S, Yang A, Leggett AL, Erb MA, Lawlor MA, Souza A *et al* (2018) The dTAG system for immediate and target-specific protein degradation. *Nat Chem Biol* 14: 431 – 441

Nakagawa T, Kajitani T, Togo S, Masuko N, Ohdan H, Hishikawa Y, Koji T, Matsuyama T, Ikura T, Muramatsu M *et al* (2008) Deubiquitylation of histone H2A activates transcriptional initiation via trans-histone cross-talk with H3K4 di- and trimethylation. *Genes Dev* 22: 37 – 49

Nakazawa Y, Hara Y, Oka Y, Komine O, van den Heuvel D, Guo C, Daigaku Y, Isono M, He Y, Shimada M *et al* (2020) Ubiquitination of DNA damage-stalled RNAPII promotes transcription-coupled repair. *Cell* 180: 1228 – 1244.e24

Newton K, Matsumoto ML, Wertz IE, Kirkpatrick DS, Lill JR, Tan J, Dugger D, Gordon N, Sidhu SS, Fellouse FA *et al* (2008) Ubiquitin chain editing revealed by polyubiquitin linkage-specific antibodies. *Cell* 134: 668 – 678

Nguyen DX, Chiang AC, Zhang XH, Kim JY, Kris MG, Ladanyi M, Gerald WL, Massague J (2009) WNT/TCF signaling through LEF1 and HOXB9 mediates lung adenocarcinoma metastasis. *Cell* 138: 51 – 62

Nune M, Morgan MT, Connell Z, McCullough L, Jbara M, Sun H, Brik A, Formosa T, Wolberger C (2019) FACT and Ubp10 collaborate to modulate H2B deubiquitination and nucleosome dynamics. *Elife* 8: e40988

Nusse R, Clevers H (2017) Wnt/beta-catenin signaling, disease, and emerging therapeutic modalities. *Cell* 169: 985 – 999

Oh E, Mark KG, Mocciaro A, Watson ER, Prabu JR, Cha DD, Kampmann M, Gamarra N, Zhou CY, Rape M (2020) Gene expression and cell identity controlled by anaphase-promoting complex. *Nature* 579: 136 – 140

Ohh M, Park CW, Ivan M, Hoffman MA, Kim TY, Huang LE, Pavletich N, Chau V, Kaelin WG (2000) Ubiquitination of hypoxia-inducible factor requires direct binding to the beta-domain of the von Hippel-Lindau protein. *Nat Cell Biol* 2: 423 – 427

Ohtake F, Tsuchiya H, Saeki Y, Tanaka K (2018) K63 ubiquitylation triggers proteasomal degradation by seeding branched ubiquitin chains. *Proc Natl Acad Sci USA* 115: E1401 – E1408

Palombella VJ, Rando OJ, Goldberg AL, Maniatis T (1994) The ubiquitin-proteasome pathway is required for processing the NF-kappa B1 precursor protein and the activation of NF-kappa B. *Cell* 78: 773 – 785

Palozola KC, Donahue G, Liu H, Grant GR, Becker JS, Cote A, Yu H, Raj A, Zaret KS (2017) Mitotic transcription and waves of gene reactivation during mitotic exit. *Science* 358: 119 – 122

Palozola KC, Lerner J, Zaret KS (2019) A changing paradigm of transcriptional memory propagation through mitosis. *Nat Rev Mol Cell Biol* 20: 55 – 64

Pavri R, Zhu B, Li G, Trojer P, Mandal S, Shilatifard A, Reinberg D (2006) Histone H2B monoubiquitination functions cooperatively with FACT to regulate elongation by RNA polymerase II. *Cell* 125: 703 – 717

Peters JM, King RW, Hoog C, Kirschner MW (1996) Identification of BIME as a subunit of the anaphase-promoting complex. *Science* 274: 1199 – 1201

Pierce SB, Stewart MD, Gulsuner S, Walsh T, Dhall A, McClellan JM, Klevit RE, King MC (2018) De novo mutation in RING1 with epigenetic effects on neurodevelopment. *Proc Natl Acad Sci USA* 115: 1558 – 1563

Pilaz LJ, McMahon JJ, Miller EE, Lennox AL, Suzuki A, Salmon E, Silver DL (2016) Prolonged mitosis of neural progenitors alters cell fate in the developing brain. *Neuron* 89: 83 – 99

Piwko W, Jentsch S (2006) Proteasome-mediated protein processing by bidirectional degradation initiated from an internal site. *Nat Struct Mol Biol* 13: 691 – 697

Potjewyd F, Turner AW, Beri J, Rectenwald JM, Norris-Drouin JL, Cholensky SH, Margolis DM, Pearce KH, Herring LE, James LI (2020) Degradation of polycomb repressive complex 2 with an EED-targeted bivalent chemical degrader. *Cell Chem Biol* 27: 47 – 56.e15

Proudfoot NJ (2016) Transcriptional termination in mammals: stopping the RNA polymerase II juggernaut. *Science* 352: aad9926

Purvis JE, Lahav G (2013) Encoding and decoding cellular information through signaling dynamics. *Cell* 152: 945 – 956

Qiao X, Liu Y, Prada ML, Mohan AK, Gupta A, Jaiswal A, Sharma M, Merisaari J, Haikala HM, Talvinen K *et al* (2020) UBR5 Is coamplified with MYC in Breast tumors and encodes an ubiquitin ligase that limits MYC-dependent apoptosis. *Cancer Res* 80: 1414 – 1427

Radhakrishnan SK, den Besten W, Deshaies RJ (2014) p97-dependent retrotranslocation and proteolytic processing govern formation of active Nrf1 upon proteasome inhibition. *Elife* 3: e01856

Rape M, Hoppe T, Gorr I, Kalocay M, Richly H, Jentsch S (2001) Mobilization of processed, membrane-tethered SPT23 transcription factor by CDC48 (UFD1/NPL4), a ubiquitin-selective chaperone. *Cell* 107: 667 – 677

Rickman DS, Schulte JH, Eilers M (2018) The expanding world of N-MYC-driven tumors. *Cancer Discov* 8: 150 – 163

van Riggelen J, Yetil A, Felsher DW (2010) MYC as a regulator of ribosome biogenesis and protein synthesis. *Nat Rev Cancer* 10: 301 – 309

Sahtoe DD, Sixma TK (2015) Layers of DUB regulation. *Trends Biochem Sci* 40: 456 – 467

Sakamoto KM, Kim KB, Kumagai A, Mercurio F, Crews CM, Deshaies RJ (2001) Protacs: chimeric molecules that target proteins to the Skp1-Cullin-F box complex for ubiquitination and degradation. *Proc Natl Acad Sci USA* 98: 8554 – 8559

 © 2021 The Authors

Sakamoto KM, Kim KB, Verma R, Ransick A, Stein B, Crews CM, Deshaies RJ (2003) Development of Protacs to target cancer-promoting proteins for ubiquitination and degradation. *Mol Cell Proteomics* 2: 1350 – 1358

Salceda S, Caro J (1997) Hypoxia-inducible factor 1alpha (HIF-1alpha) protein is rapidly degraded by the ubiquitin-proteasome system under normoxic conditions. Its stabilization by hypoxia depends on redox-induced changes. *J Biol Chem* 272: 22642 – 22647

Sanz G, Singh M, Peuget S, Selivanova G (2019) Inhibition of p53 inhibitors: progress, challenges and perspectives. *J Mol Cell Biol* 11: 586 – 599

Sarek G, Ojala PM (2007) p53 reactivation kills KSHV lymphomas efficiently *in vitro* and *in vivo*: new hope for treating aggressive viral lymphomas. *Cell Cycle* 6: 2205 – 2209

Scheuermann JC, De Ayala Alonso AG, Oktaba K, Ly-Hartig N, McGinty RK, Fraterman S, Wilm M, Muir TW, Muller J (2010) Histone H2A deubiquitinase activity of the Polycomb repressive complex PR-DUB. *Nature* 465: 243 – 247

Schmidt RM, Schessner JP, Borner GH, Schuck S (2019) The proteasome biogenesis regulator Rpn4 cooperates with the unfolded protein response to promote ER stress resistance. *Elife* 8: e43244

Schmoll D, Engel CK, Glombik H (2017) The Keap1-Nrf2 protein-protein interaction: a suitable target for small molecules. *Drug Discov Today Technol* 24: 11 – 17

Schukur L, Zimmermann T, Niewoehner O, Kerr G, Gleim S, Bauer-Probst B, Knapp B, Galli GG, Liang X, Mendiola A *et al* (2020) Identification of the HECT E3 ligase UBR5 as a regulator of MYC degradation using a CRISPR/Cas9 screen. *Sci Rep* 10: 20044

Selth LA, Sigurdsson S, Svejstrup JQ (2010) Transcript elongation by RNA polymerase II. *Annu Rev Biochem* 79: 271 – 293

Sha Z, Goldberg AL (2016) Reply to Vangala *et al*: complete inhibition of the proteasome reduces new proteasome production by causing Nrf1 aggregation. *Curr Biol* 26: R836 – R837

Shang S, Hua F, Hu ZW (2017) The regulation of beta-catenin activity and function in cancer: therapeutic opportunities. *Oncotarget* 8: 33972 – 33989

Sheikh BN, Guhathakurta S, Akhtar A (2019) The non-specific lethal (NSL) complex at the crossroads of transcriptional control and cellular homeostasis. *EMBO Rep* 20: e47630

Shema-Yaacoby E, Nikolov M, Haj-Yahya M, Siman P, Allemand E, Yamaguchi Y, Muchardt C, Urlaub H, Brik A, Oren M *et al* (2013) Systematic identification of proteins binding to chromatin-embedded ubiquitylated H2B reveals recruitment of SWI/SNF to regulate transcription. *Cell Rep* 4: 601 – 608

Sievers QL, Petzold G, Bunker RD, Renneville A, Slabicki M, Liddicoat BJ, Abdulrahman W, Mikkelsen T, Ebert BL, Thoma NH (2018) Defining the human C2H2 zinc finger degrome targeted by thalidomide analogs through CRBN. *Science* 362: eaat0572

Simonetta KR, Taygerly J, Boyle K, Basham SE, Padovani C, Lou Y, Cummins TJ, Yung SL, von Soly SK, Kayser F *et al* (2019) Prospective discovery of small molecule enhancers of an E3 ligase-substrate interaction. *Nat Commun* 10: 1402

Skaug B, Jiang X, Chen ZJ (2009) The role of ubiquitin in NF-kappaB regulatory pathways. *Annu Rev Biochem* 78: 769 – 796

Slabicki M, Kozicka Z, Petzold G, Li YD, Manojkumar M, Bunker RD, Donovan KA, Sievers QL, Koeppel J, Suchyta D *et al* (2020a) The CDK inhibitor CR8 acts as a molecular glue degrader that depletes cyclin K. *Nature* 585: 293 – 297

Slabicki M, Yoon H, Koeppel J, Nitsch L, Roy Burman SS, Di Genua C, Donovan KA, Sperling AS, Hunkeler M, Tsai JM *et al* (2020b). Small-molecule-induced polymerization triggers degradation of BCL6. *Nature* 588: 164 – 168

Somesh BP, Sigurdsson S, Saeki H, Erdjument-Bromage H, Tempst P, Svejstrup JQ (2007) Communication between distant sites in RNA polymerase II through ubiquitylation factors and the polymerase CTD. *Cell* 129: 57 – 68

Sorre B, Warmflash A, Brivanlou AH, Siggia ED (2014) Encoding of temporal signals by the TGF-beta pathway and implications for embryonic patterning. *Dev Cell* 30: 334 – 342

Spedale G, Timmers HT, Pijnappel WW (2012) ATAC-king the complexity of SAGA during evolution. *Genes Dev* 26: 527 – 541

Sung MK, Reitsma JM, Sweredoski MJ, Hess S, Deshaies RJ (2016) Ribosomal proteins produced in excess are degraded by the ubiquitin-proteasome system. *Mol Biol Cell* 27: 2642 – 2652

Tamburri S, Lavarone E, Fernandez-Perez D, Conway E, Zanotti M, Manganaro D, Pasini D (2020) Histone H2AK119 mono-ubiquitination is essential for polycomb-mediated transcriptional repression. *Mol Cell* 77: 840 – 856.e5

Tammela T, Sanchez-Rivera FJ, Cetinbas NM, Wu K, Joshi NS, Helenius K, Park Y, Azimi R, Kerper NR, Wesselhoeft RA *et al* (2017) A Wnt-producing niche drives proliferative potential and progression in lung adenocarcinoma. *Nature* 545: 355 – 359

Tarcic O, Pateras IS, Cooks T, Shema E, Kanterman J, Ashkenazi H, Boocholez H, Hubert A, Rotkopf R, Baniyash M *et al* (2016) RNF20 links histone H2B ubiquitylation with inflammation and inflammation-associated cancer. *Cell Rep* 14: 1462 – 1476

Teves SS, An L, Hansen AS, Xie L, Darzacq X, Tjian R (2016) A dynamic mode of mitotic bookmarking by transcription factors. *Elife* 5: e22280

Teves SS, An L, Bhargava-Shah A, Xie L, Darzacq X, Tjian R (2018) A stable mode of bookmarking by TBP recruits RNA polymerase II to mitotic chromosomes. *Elife* 7: e35621

Thomas LR, Wang Q, Grieb BC, Phan J, Foshage AM, Sun Q, Olejniczak ET, Clark T, Dey S, Lorey S *et al* (2015) Interaction with WDR5 promotes target gene recognition and tumorigenesis by MYC. *Mol Cell* 58: 440 – 452

Tjian R (1996) The biochemistry of transcription in eukaryotes: a paradigm for multisubunit regulatory complexes. *Philos Trans R Soc Lond B Biol Sci* 351: 491 – 499

Trotman LC, Wang X, Alimonti A, Chen Z, Teruya-Feldstein J, Yang H, Pavletich NP, Carver BS, Cordon-Cardo C, Erdjument-Bromage H *et al* (2007) Ubiquitination regulates PTEN nuclear import and tumor suppression. *Cell* 128: 141 – 156

Tufegdzic Vidakovic A, Mitter R, Kelly GP, Neumann M, Harreman M, Rodriguez-Martinez M, Herlihy A, Weems JC, Boeing S, Encheva V *et al* (2020) Regulation of the RNAPII pool is integral to the DNA damage response. *Cell* 180: 1245 – 1261.e21

Turnbull AP, Ioannidis S, Krajewski WW, Pinto-Fernandez A, Heride C, Martin ACL, Tonkin LM, Townsend EC, Buker SM, Lancia DR *et al* (2017) Molecular basis of USP7 inhibition by selective small-molecule inhibitors. *Nature* 550: 481 – 486

Uckelmann M, Sixma TK (2017) Histone ubiquitination in the DNA damage response. *DNA Repair (Amst)* 56: 92 – 101

Valencia-Sanchez MI, de Ioannes P, Wang M, Vasilyev N, Chen R, Nudler E, Armache JP, Armache KJ (2019) Structural basis of Dot1L stimulation by histone H2B lysine 120 ubiquitination. *Mol Cell* 74: 1010 – 1019.e6

Vangala JR, Sotzny F, Kruger E, Deshaies RJ, Radhakrishnan SK (2016) Nrf1 can be processed and activated in a proteasome-independent manner. *Curr Biol* 26: R834 – R835

© 2021 The Authors

Vargesson N (2009) Thalidomide-induced limb defects: resolving a 50-year-old puzzle. *BioEssays* 31: 1327 – 1336

Vassilev LT, Vu BT, Graves B, Carvajal D, Podlaski F, Filipovic Z, Kong N, Kammlott U, Lukacs C, Klein C *et al* (2004) *In vivo* activation of the p53 pathway by small-molecule antagonists of MDM2. *Science* 303: 844 – 848

Venkatesh S, Workman JL (2015) Histone exchange, chromatin structure and the regulation of transcription. *Nat Rev Mol Cell Biol* 16: 178 – 189

Vermeulen L, de Sousa EMF, van der Heijden M, Cameron K, de Jong JH, Borovski T, Tuynman JB, Todaro M, Merz C, Rodermond H *et al* (2010) Wnt activity defines colon cancer stem cells and is regulated by the microenvironment. *Nat Cell Biol* 12: 468 – 476

Voncken JW, Roelen BA, Roefs M, de Vries S, Verhoeven E, Marino S, Deschamps J, van Lohuizen M (2003) Rnf2 (Ring1b) deficiency causes gastrulation arrest and cell cycle inhibition. *Proc Natl Acad Sci USA* 100: 2468 – 2473

Wang E, Kawaoka S, Yu M, Shi J, Ni T, Yang W, Zhu J, Roeder RG, Vakoc CR (2013) Histone H2B ubiquitin ligase RNF20 is required for MLL-rearranged leukemia. *Proc Natl Acad Sci USA* 110: 3901 – 3906

Wang MC, Li CL, Cui J, Jiao M, Wu T, Jing LI, Nan KJ (2015) BMI-1, a promising therapeutic target for human cancer. *Oncol Lett* 10: 583 – 588

Wang T, Liu Y, Edwards G, Krzizike D, Scherman H, Luger K (2018) The histone chaperone FACT modulates nucleosome structure by tethering its components. *Life Sci Alliance* 1: e201800107

Wang Y, Jiang X, Feng F, Liu W, Sun H (2020) Degradation of proteins by PROTACs and other strategies. *Acta Pharm Sin B* 10: 207 – 238

Weake VM, Workman JL (2008) Histone ubiquitination: triggering gene activity. *Mol Cell* 29: 653 – 663

Weems JC, Slaughter BD, Unruh JR, Hall SM, McLaird MB, Gilmore JM, Washburn MP, Florens L, Yasukawa T, Aso T *et al* (2015) Assembly of the elongin A ubiquitin ligase is regulated by genotoxic and other stresses. *J Biol Chem* 290: 15030 – 15041

Welcker M, Orian A, Jin J, Grim JE, Harper JW, Eisenman RN, Clurman BE (2004) The Fbw7 tumor suppressor regulates glycogen synthase kinase 3 phosphorylation-dependent c-Myc protein degradation. *Proc Natl Acad Sci USA* 101: 9085 – 9090

Welcker M, Clurman BE (2008) FBW7 ubiquitin ligase: a tumour suppressor at the crossroads of cell division, growth and differentiation. *Nat Rev Cancer* 8: 83 – 93

Wilms C, Krikki I, Hainzl A, Kilo S, Alupei M, Makrantonaki E, Wagner M, Kroeger CM, Brinker TJ, Gatzka M (2018) 2A-DUB/Mysm1 regulates epidermal development in part by suppressing p53-mediated programs. *Int J Mol Sci* 19: 687

Wilson MD, Harreman M, Svejstrup JQ (2013a) Ubiquitylation and degradation of elongating RNA polymerase II: the last resort. *Biochim Biophys Acta* 1829: 151 – 157

Wilson MD, Harreman M, Taschner M, Reid J, Walker J, Erdjument-Bromage H, Tempst P, Svejstrup JQ (2013b) Proteasome-mediated processing of Def1, a critical step in the cellular response to transcription stress. *Cell* 154: 983 – 995

Winston JT, Strack P, Beer-Romero P, Chu CY, Elledge SJ, Harper JW (1999) The SCFbeta-TRCP-ubiquitin ligase complex associates specifically with phosphorylated destruction motifs in IkappaBalpha and beta-catenin and stimulates IkappaBalpha ubiquitination *in vitro*. *Genes Dev* 13: 270 – 283

Winter GE, Buckley DL, Paulk J, Roberts JM, Souza A, Dhe-Paganon S, Bradner JE (2015) DRUG DEVELOPMENT. Phthalimide conjugation as a strategy for *in vivo* target protein degradation. *Science* 348: 1376 – 1381

Worden EJ, Hoffmann NA, Hicks CW, Wolberger C (2019) Mechanism of Cross-talk between H2B Ubiquitination and H3 Methylation by Dot1L. *Cell* 176: 1490 – 1501.e12

Worden EJ, Zhang X, Wolberger C (2020) Structural basis for COMPASS recognition of an H2B-ubiquitinated nucleosome. *Elife* 9: e53199

Woudstra EC, Gilbert C, Fellows J, Jansen L, Brouwer J, Erdjument-Bromage H, Tempst P, Svejstrup JQ (2002) A Rad26-Def1 complex coordinates repair and RNA pol II proteolysis in response to DNA damage. *Nature* 415: 929 – 933

Wu L, Zee BM, Wang Y, Garcia BA, Dou Y (2011) The RING finger protein MSL2 in the MOF complex is an E3 ubiquitin ligase for H2B K34 and is involved in crosstalk with H3 K4 and K79 methylation. *Mol Cell* 43: 132 – 144

Wu L, Li L, Zhou B, Qin Z, Dou Y (2014) H2B ubiquitylation promotes RNA Pol II processivity via PAF1 and pTEFb. *Mol Cell* 54: 920 – 931

Wysocka J, Swigut T, Milne TA, Dou Y, Zhang X, Burlingame AL, Roeder RG, Brivanlou AH, Allis CD (2005) WDR5 associates with histone H3 methylated at K4 and is essential for H3 K4 methylation and vertebrate development. *Cell* 121: 859 – 872

Xie Y, Varshavsky A (2001) RPN4 is a ligand, substrate, and transcriptional regulator of the 26S proteasome: a negative feedback circuit. *Proc Natl Acad Sci USA* 98: 3056 – 3061

Yang W, Lee YH, Jones AE, Woolnough JL, Zhou D, Dai Q, Wu Q, Giles KE, Townes TM, Wang H (2014) The histone H2A deubiquitinase Usp16 regulates embryonic stem cell gene expression and lineage commitment. *Nat Commun* 5: 3818

Yang H, Green MR (2019) Epigenetic programing of B-cell lymphoma by BCL6 and its genetic deregulation. *Front Cell Dev Biol* 7: 272

Yasukawa T, Kamura T, Kitajima S, Conaway RC, Conaway JW, Aso T (2008) Mammalian Elongin A complex mediates DNA-damage-induced ubiquitylation and degradation of Rpb1. *EMBO J* 27: 3256 – 3266

Yasukawa T, Bhatt S, Takeuchi T, Kawauchi J, Takahashi H, Tsutsui A, Muraoka T, Inoue M, Tsuda M, Kitajima S *et al* (2012) Transcriptional elongation factor elongin A regulates retinoic acid-induced gene expression during neuronal differentiation. *Cell Rep* 2: 1129 – 1136

Yau R, Rape M (2016) The increasing complexity of the ubiquitin code. *Nat Cell Biol* 18: 579 – 586

Yau RG, Doerner K, Castellanos ER, Haakonsen DL, Werner A, Wang N, Yang XW, Martinez-Martin N, Matsumoto ML, Dixit VM *et al* (2017) Assembly and function of heterotypic ubiquitin chains in cell-cycle and protein quality control. *Cell* 171: 918 – 933.e20

Yilmaz ZB, Kofahl B, Beaudette P, Baum K, Ipenberg I, Weih F, Wolf J, Dittmar G, Scheidereit C (2014) Quantitative dissection and modeling of the NF-kappaB p100–p105 module reveals interdependent precursor proteolysis. *Cell Rep* 9: 1756 – 1769

Yu M, Ting DT, Stott SL, Wittner BS, Ozsolak F, Paul S, Ciciliano JC, Smas ME, Winokur D, Gilman AJ *et al* (2012) RNA sequencing of pancreatic circulating tumour cells implicates WNT signalling in metastasis. *Nature* 487: 510 – 513

Zaret KS, Carroll JS (2011) Pioneer transcription factors: establishing competence for gene expression. *Genes Dev* 25: 2227 – 2241

Zhang DD, Lo SC, Cross JV, Templeton DJ, Hannink M (2004) Keap1 is a redox-regulated substrate adaptor protein for a Cul3-dependent ubiquitin ligase complex. *Mol Cell Biol* 24: 10941 – 10953

Zhang XY, Varthi M, Sykes SM, Phillips C, Warzecha C, Zhu W, Wyce A, Thorne AW, Berger SL, McMahon SB (2008) The putative cancer stem cell marker USP22 is a subunit of the human SAGA complex required for activated transcription and cell-cycle progression. *Mol Cell* 29: 102 – 111

 © 2021 The Authors

Zhang Y, Chang C, Gehling DJ, Hemmati-Brivanlou A, Derynck R (2001) Regulation of Smad degradation and activity by Smurf2, an E3 ubiquitin ligase. *Proc Natl Acad Sci USA* 98: 974 – 979

Zhang Z, Jones AE, Wu W, Kim J, Kang Y, Bi X, Gu Y, Popov IK, Renfrow MB, Vassylyeva MN *et al* (2017) Role of remodeling and spacing factor 1 in histone H2A ubiquitination-mediated gene silencing. *Proc Natl Acad Sci USA* 114: E7949 – E7958

Zhao Y, Lang G, Ito S, Bonnet J, Metzger E, Sawatsubashi S, Suzuki E, le Guezennec X, Stunnenberg HG, Krasnov A *et al* (2008) A TFTC/STAGA module mediates histone H2A and H2B deubiquitination, coactivates nuclear receptors, and counteracts heterochromatin silencing. *Mol Cell* 29: 92 – 101

Zhao D, Zhang X, Guan H, Xiong X, Shi X, Deng H, Li H (2016) The BAH domain of BAHD1 is a histone H3K27me3 reader. *Protein Cell* 7: 222 – 226

Zhou J, Wu J, Li B, Liu D, Yu J, Yan X, Zheng S, Wang J, Zhang L, Zhang L *et al* (2014) PU.1 is essential for MLL leukemia partially via crosstalk with the MEIS/HOX pathway. *Leukemia* 28: 1436 – 1448

Zhu H, Kavsak P, Abdollah S, Wrana JL, Thomsen GH (1999) A SMAD ubiquitin ligase targets the BMP pathway and affects embryonic pattern formation. *Nature* 400: 687 – 693

Zhu P, Zhou W, Wang J, Puc J, Ohgi KA, Erdjument-Bromage H, Tempst P, Glass CK, Rosenfeld MG (2007) A histone H2A deubiquitinase complex coordinating histone acetylation and H1 dissociation in transcriptional regulation. *Mol Cell* 27: 609 – 621

© 2021 The Authors