significantly contribute to cardiomyocyte proliferation. The mechanism and causal relationship between T3/IGF signaling and this proliferative burst remain unclear, and further studies will be needed to more specifically address these issues. Additional fate mapping studies that conditionally label postmitotic cardiomyocytes would help elucidate this phenomenon and determine the molecular mechanisms responsible for mediating this effect. Indeed, this study takes a step forward in identifying ways to stimulate proliferation in postmitotic cardiomyocytes and may yield valuable insights into therapeutic treatments for inherited and acquired heart diseases.

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Deciphering Functions of Branched Ubiquitin Chains

Jaime López-Mosqueda[1] and Ivan Dikic[1,*]
[1]Institute of Biochemistry II, Goethe University, Theodor-Stern-Kai 7, 60590 Frankfurt am Main, Germany
*Correspondence: dikic@biochem2.uni-frankfurt.de
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The anaphase-promoting complex/cyclosome targets proteins for degradation by catalyzing homotypic ubiquitin chains of different linkage types. In this issue of *Cell*, Meyer and Rape diversify the degradation signals by demonstrating that the APC/C and its cognate E2 conjugating enzymes enhance the rate of substrate degradation by decorating them with branched Lys11 and Lys48 ubiquitin chains.

Ubiquitylation is the most versatile post-translational modification known to date and is involved in the regulation of numerous cellular processes (Grabbe et al., 2011). Ubiquitin (Ub) is predominantly attached to lysine residues of other proteins by a tightly regulated multistep enzymatic process involving activation by E1 enzymes, conjugation by E2 enzymes, and, finally, ligation by E3 ligases. Attachment of Ub may result in monoubiquitylated proteins or modification with Ub chains composed of several Ub moieties. In the latter case, the internal seven lysines or the amino-terminal methionine 1 of Ub are modified in successive rounds of conjugation. Given these multiple linkage sites, the pool of theoretically viable Ub chains with different structures is almost unlimited, underscoring the versatility of the system. However, so far, only a limited number of defined Ub structures were identified in vivo, and research has mainly concentrated on chains of homogenously linked Ub. In this issue of *Cell*, Meyer and Rape (2014) define a physiological role for branched Ub chains in cell-cycle regulation. This may mark the entry into a new era of Ub research and definitely adds a new twist to the deciphering of the Ub code, as well as to the understanding of the enzymatic machinery involved.

In eukaryotes, destruction of cell-cycle regulators is essential for progression through and exit out of mitosis. The anaphase-promoting complex/cyclosome (APC/C) is a large, multisubunit E3 ligase that targets key cell-cycle regulators for ubiquitylation and subsequent degradation by the 26S proteasome (Peters, 2006). The first Ub chains described to relate such signals were Lys48 linked Ub chains, but how the APC/C functioned to catalyze these chain types remained unknown. Mechanistic details emerged from studies in

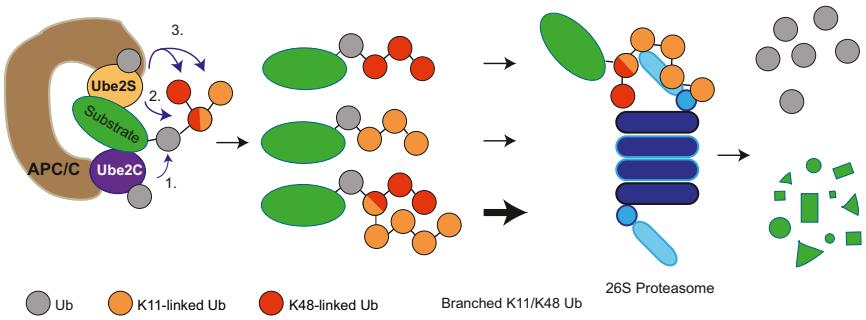

Figure 1. The APC/C and Cognate E2 Conjugating Enzymes Catalyze Branched Lys11 and Lys48 Ubiquitin Chains on Substrates for Proteasomal Degradation

The APC/C and Ube2C prime (1) substrates with the attachment of the first ubiquitin moiety. Next, Ube2S extends (2) the ubiquitin chain by attaching a second ubiquitin moiety to Lys11 or Lys48 of the first ubiquitin. By an unknown mechanism (3), Ube2S can subsequently branch the growing ubiquitin chain by attaching ubiquitin moieties on two distinct lysine residues of the same ubiquitin. In cells, any particular APC/C substrate can potentially have either homotypic Lys11 (orange), homotypic Lys48 (red), or branched Lys11/Lys48 (half orange and half red) ubiquitin chains. Substrates marked with branched Lys11/Lys48 ubiquitin chains will be endowed with enhanced binding to and degradation by the 26S proteasome compared to substrates marked with homotypic ubiquitin chains.

budding yeast APC/C (Rodrigo-Brenni and Morgan, 2007), and later in human APC/C (Jin et al., 2008), to reveal that Ub chain formation of defined linkages is catalyzed by the APC/C in cooperation with two cognate E2 conjugating enzymes. In budding yeast, the APC/C first cooperates with Ubc4 to nucleate or prime the first Ub moiety on a substrate. Ubc1 then extends this first Ub moiety on lysine 48 to form Lys48 Ub chains. Analogous to yeast APC/C, human APC/C functions with a set of E2 enzymes, namely Ube2C and Ube2S. Accordingly, Ube2C primes APC/C substrates with the first Ub moiety, whereas Ube2S extends the Ub chain (Figure 1). In this manner, the APC/C is able to catalyze long Lys48 or Lys11-linked Ub chains to target substrates for proteasomal degradation, thus establishing that E2 conjugating enzymes determine APC/C Ub linkage specificity (Garnett et al., 2009; Rodrigo-Brenni et al., 2010; Wickliffe et al., 2011).

Meyer and Rape now demonstrate that the APC/C is able to expand the repertoire of Ub degradation signals by catalyzing the formation of branched Lys11 and Lys48 Ub chains (Meyer and Rape, 2014). Although diverse branched Ub chains can be synthesized in vitro by defined combination of enzymes (Kim et al., 2007) and Lys63/Met1 branched Ub chains have previously been detected in cells (Emmerich et al., 2013), this study moves a step forward by defining a clear function for Lys11/Lys48 branched Ub chains in vivo. Crucial to establishing a functional role for branched Lys11/Lys48 Ub chains was the ability to detect putative Lys11/Lys48 Ub chains in cells. Although conventional mass-spectrometry-based methods have enabled the detection of all eight Ub linkages (Kim et al., 2007), the detection of branched Lys11- and Lys48-linked Ub peptides was not possible with current methods. To overcome this limitation, the authors designed a Ub variant with an internal tandem TEV protease site and a FLAG tag epitope (Ubi-TEV-FLAG). Using this form of Ub, branched Lys11/Lys48 Ub chains could be unambiguously detected in cells for substrates of the APC/C complex, namely Nek2a and Cyclin A. In addition, the repurposing of E2s to build specific Lys11, Lys48, or branched Lys11/Lys48 Ub linkages on prototypical substrates enabled Meyer and Rape to directly compare substrate degradation rates as a function of Ub linkage type. These experiments showed that cell-cycle substrates marked with Lys11/Lys48 branched chains bind with higher affinity to the proteasome than substrates with homotypic Lys11 linked chains. These findings extend previous notions that longer Ub chains enhance binding and degradation by the proteasome (Thrower et al., 2000). Therefore, the rate of APC/C substrate degradation could be dictated by Ub density, rather than by Ub chain length.

Several new questions rise from this work. Previous structural work on Ube2S has shown that Ube2S conjugated to an Ub molecule can interact with a free Ub molecule is such a way that it orients Lys11 of the free Ub toward the catalytic cysteine of Ube2S (Wickliffe et al., 2011). If this is correct, then how does Ube2S now handle a free Ub molecule to link it to a Lys48 of the next Ub? Perhaps branched Ub chains occur mostly on atypical substrates like Nek2a, which bind to the APC/C with higher affinity compared to other known substrates and even before the APC/C is fully active. One can envision a model in which Nek2A is ubiquitylated with a few Lys48 Ub chains during prometaphase but can then re-engage the APC/C and Ube2S for additional rounds of Lys11 Ub modifications. More work will be required to elucidate the mechanism by which the APC/C catalyzes branched Lys11/Lys48 Ub chains.

Another question that arises is how do branched ubiquitin chains enhance proteasomal degradation? It is recognized that general E3 ligase activity, including that of the APC/C, is under the constant opposition of deubiquitinating enzyme activity. Perhaps by branching ubiquitin chains, the APC/C can shift the equilibrium in favor of substrate degradation by facilitating enhanced recognition of ubiquitylated substrates by proteasomal receptors. The findings presented in this manuscript are presumably only the first examples for regulation of an essential cellular process via branched Ub chains and open the black box of a variety of new types of branched Ub signals that need to be carefully analyzed for their in vivo functions.

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Tic-TACs: Refreshing Hair Growth

Demetrios Kalaitzidis[1,2,*] and David T. Scadden[1,2,*]
[1]Center for Regenerative Medicine, Massachusetts General Hospital, 185 Cambridge Street, Boston, MA 02114, USA
[2]Harvard Stem Cell Institute and Harvard University Department of Stem Cell and Regenerative Biology, 7 Divinity Avenue, Cambridge, MA 02138, USA
*Correspondence: dkalaitzidis@partners.org (D.K.), david_scadden@harvard.edu (D.T.S.)
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Although stem cells are subject to niche control, evidence is emerging that they also contribute to generating the niche through their offspring. Using the hair follicle as a model, Hsu at al. demonstrate that the transient-amplifying cells, downstream of stem cells and well-known cell producers, signal back to stem cells to maintain long-term regenerative capacity.

Hair follicles (HFs) actively regenerate in adults and contain a heterogeneous mixture of anatomically defined stem, niche, and differentiated cells. They have proven to be particularly fruitful for discoveries in mammalian stem cell (SC) biology. Whether and how the various stem, progenitor, and niche cells communicate during the progression of the HF growth phases, including intricate coordination between many cell types at lengthening anatomical distances, have not been completely elucidated. Hsu et al. (2014) now provide critical insights into these processes.

HFs contain quiescent SCs located in a structure termed the bulge (Bu-SCs) and more activation-prone SCs anatomically located immediately below the Bu-SCs in the hair germ (HG). SCs in the HF have been demonstrated to be regulated by numerous cellular sources, most prominently by mesenchymal cells located below the HG in the dermal papilla (DP). Other potential niche cells for HF SCs include HF terminally differentiated epithelial cells, adipocytes and nerve fibers, among others (Solanas and Benitah, 2013) (Figure 1A).

HFs cycle between production (anagen), destruction (catagen), and resting (telogen) phases. The multistage anagen phase is initiated when "activated" SCs in the HG (Greco et al., 2009) receive proliferative signals, likely from the DP, and differentiate into transit-amplifying cells (TACs). TACs form a structure termed the matrix and eventually give rise to the differentiated cells that compose the HF (Solanas and Benitah, 2013). As anagen progresses, the HF physically expands relative to its resting state leading to increased distances between Bu-SCs, activated SCs, matrix (TACs), and the DP (Figure 1B).

Previously, HG SCs were demonstrated to proliferate first to initiate HF regeneration, with Bu-SCs lagging behind (Greco et al., 2009). Hsu et al. now show that cells of the HG begin to proliferate in anaphase I (AnaI) and lead to matrix formation in AnaII. Bu-SCs proliferate between AnaII and AnaIII and go quiescent at AnaV. By AnaIII, the HF has doubled in size with the bulge now being 200 μm away from the DP. Given that the DP is required for HF regeneration (Rompolas et al., 2012), this begged the question of how Bu-SCs can be activated by cells from such a far-away place.

The answer lies in the TACs. Using multiple in vivo genetic perturbations, the authors demonstrate that coincident with proliferation and long-term HF regeneration, Sonic Hedgehog (SHH) signaling upregulates Bu-SC activity (Figure 1C). A role for SHH in HF biology was well established (Chiang et al., 1999; Morgan et al., 1998; Brownell et al., 2011), but Hsu et al. provide evidence that TACs uniquely secrete SHH and that this is the critical switch for activating the quiescent Bu-SCs necessary for long-term HF regeneration.

They first show that among the many HF cells (including Bu-SCs), only TACs express high levels of SHH during AnaII–III. They then genetically deleted SHH and show that whereas HG cells proliferate, Bu-SCs do not. They subsequently conditionally deleted the receptor for SHH, Smoothened (SMO), or the downstream transcriptional input of SHH signaling, *Gli2*, and this again led to diminished Bu-SC proliferation, confirming that the effects of SHH on Bu-SCs are direct. Of note, conditional *Gli2* knockout in the