Review

K11-linked ubiquitin chains as novel regulators of cell division

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Modification of proteins with ubiquitin chains is an essential regulatory event in cell cycle control. Differences in the connectivity of ubiquitin chains are believed to result in distinct functional consequences for the modified proteins. Among eight possible homogenous chain types, canonical Lys48-linked ubiquitin chains have long been recognized to drive the proteasomal degradation of cell cycle regulators, and Lys48 is the only essential lysine residue of ubiquitin in yeast. It thus came as a surprise that in higher eukaryotes atypical K11-linked ubiquitin chains regulate the substrates of the anaphase-promoting complex and control progression through mitosis. We discuss recent findings that shed light on the assembly and function of K11-linked chains during cell division.

The ubiquitin code

Information can be transmitted in many ways, be it print media, television, internet, or social networks. Even the shortest notes relayed through these means rely on a code: the symbology of ☺ and ☹ or the more established written word. The more complex this code, the more information can be communicated, but the response remains dependent on the interpretation of the message by the recipient. In eukaryotes, protein ubiquitination follows many of these principles.

Catalyzed by a cascade of E1 ubiquitin-activating, E2 ubiquitin-conjugating, and E3 ubiquitin–protein ligase enzymes, ubiquitin becomes covalently linked to Lys residues in proteins (Box 1) [1–3]. Modification with a single ubiquitin, referred to as monoubiquitination, often alters substrate localization or interactions [4]. This first ubiquitin can also function as the starting point for the synthesis of a polymeric chain, in which ubiquitin molecules are connected through isopeptide bonds between the C-terminus of one ubiquitin and the amino-group at one of seven Lys residues or the N-terminus of another ubiquitin [2]. Depending on the linkage between ubiquitin molecules, these chains can encode distinct information. For example, chains linked through Lys48 of ubiquitin (K48-linked chains) are a targeting device for protein degradation by the 26S proteasome [5,6], whereas K63-linked chains act as molecular scaffolds, bringing together subunits of oligomeric kinase or DNA repair complexes [7,8].

K48- and K63-linked ubiquitin chains were discovered many years ago, and much has been learned about their functions; they are often referred to as 'canonical' ubiquitin chains. By contrast, 'non-canonical' or 'atypical' chains remain incompletely characterized, leaving us with a poor understanding of the breadth of the ubiquitin code. Two atypical chain types, linear and K11-linked ubiquitin chains, were recently identified in cells, where they act in transcription factor activation and cell division, respectively [9,10]. The important roles played by linear and K11-linked chains strongly support the notion that ubiquitination can constitute an elaborate code that cells use to control the activities of key signaling molecules. In the following we discuss insights into this system that have been gained from studying the assembly and function of K11-linked ubiquitin chains.

When are K11-linked ubiquitin chains detected in cells?

In homogenous chains all ubiquitin molecules are connected through the same linkage (Figure 1a). For signaling purposes, chains that contain long stretches of uniform linkage might also be considered homogenous. If multiple linkages are present within a chain these assemblies either have mixed or branched topologies (Figure 1b,c). K11-linkages have been detected in all chain types, and the different topologies might have consequences for their biological functions. For example, homogenous K11-linked chains mediate proteasomal degradation [9,11], whereas mixed K11/K63-linked chains function non-proteolytically during endocytosis or NF-κB signaling [12,13].

The existence of K11-linkages was first suggested by experiments that analyzed the specificity of the E2 Ube2S *in vitro* [14], and proteomics later identified K11-linkages in cells with varying abundance [15–20]. An early analysis found comparable levels of K11- and K48-linkages in yeast [16], whereas a later study reported a lower abundance for K11-linkages in this organism [17]. In asynchronously dividing human cells K11-linkages only represent ~2% of the ubiquitin conjugate pool [19,20]. The differences in the levels of K11-linkages among these studies could be due to technical reasons, such as distinct purification or growth procedures, or they might reveal insight into the regulation of K11-linkage formation. For example, K11-linkages accumulate when cells are stressed by proteasome inhibition, heat shock, and formation of toxic aggregates, or when they passage through a specific cell cycle stage [11,16,19,20].

Homogenous K11-linked chains were discovered as the product of the human E3 anaphase-promoting complex

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Box 1. The enzymatics of ubiquitin chain formation

Ubiquitin chain formation starts with ubiquitin being activated in an ATP-dependent manner by one of two E1 ubiquitin-activating enzymes [1]. In this reaction a thioester bond is formed between the carboxy-terminus of ubiquitin and the active-site cysteine of the E1 [84]. This ubiquitin is then transferred to a cysteine in the active site of an E2 ubiquitin-conjugating enzyme [2]. Human cells contain 38 different E2s. Finally, ubiquitin is transferred to the ε-amino group of a substrate lysine with the help of an E3 ubiquitin–protein ligase. Human cells possess >600 different E3s, and these fall into three different classes: (i) HECT-E3s contain an active-site cysteine in their HECT (homologous to E6AP C-terminus) domain [85] and the E2 transfers ubiquitin to this cysteine before the charged HECT modifies the substrate; (ii) RING (really interesting new gene)-E3s bind at the same time to the substrate and the charged E2 and promote transfer of ubiquitin directly from the E2 to the substrate [3]; (iii) finally, the hybrid RBR (RING between RING fingers)-E3s use a RING domain to promote transfer of ubiquitin from the E2 to a cysteine in their second, RING-like domain [86]. The charged RBR-E3 then modifies the substrate. The linkage specificity of ubiquitin chain formation is probably determined by the HECT-E3, the E2 for RING-E3s, and the RBR-E3.

(APC/C), an essential regulator of cell division [9]. *Drosophila* and *Xenopus* APC/C also assemble K11-linked chains [21,22]. When the APC/C is activated during mitosis, K11-linked chains rise dramatically in abundance [11], and blockage of K11-linkage formation in *Xenopus* embryos resulted in cell division defects similar to those observed for APC/C-inhibition [9]. Conversely, when cells exit the cell cycle during differentiation, the levels of K11-linkages appear to decrease [19]. Together with the discovery that most known K11-specific enzymes are linked to mitotic control (see below), these observations raise the possibility that homogenous K11-linked ubiquitin chains are important regulators of cell division in higher eukaryotes.

How are K11-linked ubiquitin chains assembled during mitosis?

The APC/C is the only E3 known to assemble homogenous K11-linked ubiquitin chains. It recognizes its substrates via degenerate degron sequences, referred to as D- and KEN-boxes [23,24], which are sandwiched between a co-factor, Cdc20/Cdh1, and a core APC/C-subunit, APC10 [25]. This mode of binding places substrates close to the RING-subunit APC11, which recruits the E2 Ube2C/UbcH10 to catalyze ubiquitin transfer. Substrate binding to the APC/C can be stabilized by additional means, such as Cks proteins that interact with substrates and phosphorylated APC/C-subunits or a C-terminal IR-appendix that is directly recognized by core subunits of the APC/C [26,27].

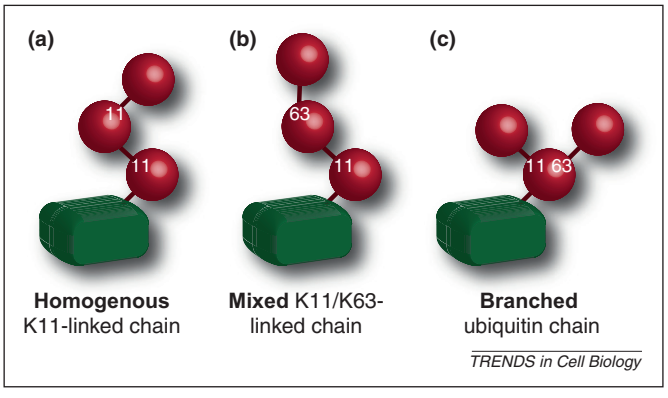

Figure 1. K11-linkages are found in chains of distinct topologies. **(a)** In homogenous K11-linked chains, all ubiquitin molecules are connected through K11-linkages. **(b)** In mixed chains, K11 and other linkages are found, but only one amino-group is modified per ubiquitin molecule. **(c)** In branched ubiquitin chains, a single ubiquitin is connected to at least two other ubiquitin molecules.

Chain initiation

Following binding of substrates to the APC/C, formation of K11-linked chains is initiated by Ube2C (Figure 2) [9,28–31]. Ube2C catalyzes the transfer of ubiquitin to a substrate lysine and also the formation of short, preferentially K11-linked chains [9,32]. *In vitro*, E2s of the Ube2D/UbcH5 family also promote initiation, but several observations argue against a crucial role for these enzymes in K11-linked chain formation in cells. First, depletion of all Ube2D homologs did not affect human cell division [21,33], whereas loss of Ube2C caused mitotic delay in several cell types [30,34–38]. Second, because Ube2D is recognized by most of the >600 human E3 enzymes, only low concentrations of Ube2D are probably available for the APC/C. By contrast, Ube2C only acts with the APC/C, dependent upon an N-terminal APC/C-targeting motif that is absent in Ube2D [39,40]. Third, whereas Ube2C preferentially assembles short K11-linked chains during initiation, Ube2D does not display any linkage specificity [9,32]. Thus, Ube2C is the physiological chain-initiating E2 of mitotic APC/C.

Chain initiation by Ube2C is strongly promoted by conserved sequence motifs in substrates, referred to as initiation motifs [31]. These initiation motifs are patches of positively charged residues that are located in the proximity of the D-box of the substrate and include the preferentially modified lysines [31,41]. Mutation of all positive charges in initiation motifs to alanine residues interferes with initiation. By contrast, if the positive charge is retained, but all ubiquitin acceptor sites are removed, initiation can still occur at non-optimal lysine residues outside the motif [31]. This suggests that initiation motifs are recognized by an APC/C-component, but whether this is Ube2C, which contains a polar surface next to its active site [42], a core APC/C-subunit, or both, remains to be tested.

Kinetic studies with the SCF (Skp1/Cullin 1/F-box), another E3 required for cell cycle control, pointed to initiation as the rate-limiting step for chain assembly [43], an observation that applies to many substrates decorated with K11-linked chains. Biochemical analyses showed that the rate of initiation by Ube2C is slow compared to the processive chain-elongation step [44,45]. Accordingly, Ube2C levels are limiting for the degradation of many proteins modified with K11-linked chains [30,34,40], and the composition or accessibility of initiation motifs recognized by Ube2C can determine the timing of APC/C-substrate degradation without affecting substrate affinity for the APC/C [31]. As the rate-limiting step, initiation has a profound impact on the processivity of chain formation, explaining why the processivity, and not substrate affinity for the APC/C, correlates with the timing of APC/C-substrate degradation [44].

The initiation of K11-linked chain formation is tightly controlled. Similarly to many cell cycle proteins, the

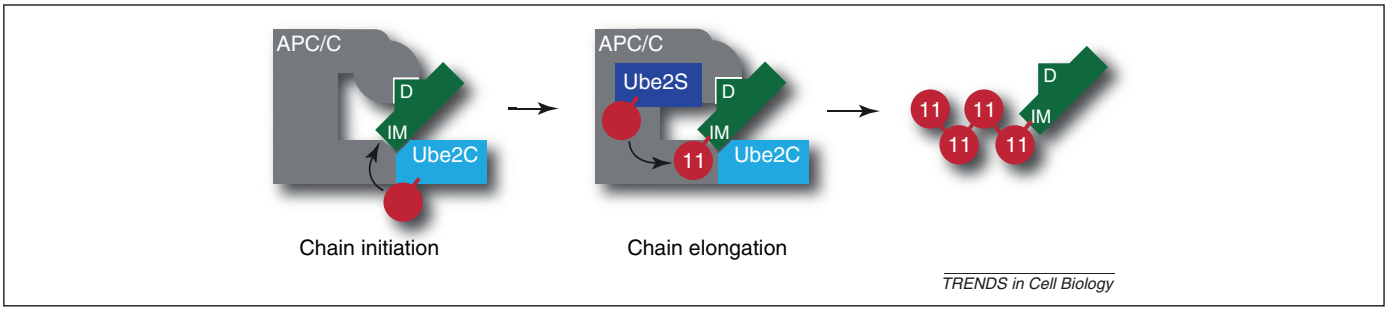

Figure 2. **Mechanism of K11-linked ubiquitin chain formation by the APC/C**. Substrates (green) are bound by the APC/C through degrons referred to as D-boxes (D) or KEN-boxes (not shown). Substrates also contain chain initiation motifs (IM) that are recognized by Ube2C, APC/C, or both, and that promote chain initiation by the E2 Ube2C. Following initiation, chains are extended by the K11-specific E2 Ube2S. Ube2C and Ube2S do not compete for binding to the APC/C.

transcription of Ube2C is regulated and peaks during mitosis [46], and overexpression of Ube2C as the result of an amplification of its genomic locus has been linked to cancer [34]. Higher levels of Ube2C destabilize the spindle checkpoint, a negative regulator of the APC/C that ensures accurate sister chromatid separation [40,47,48]. Accordingly, mice that overexpress Ube2C experience error-prone chromosome segregation, which can lead to tumorigenesis [49]. In addition, the stability of Ube2C is controlled by negative feedback centered on the APC/C [30,37,50]. Following the degradation of most of its substrates, the APC/C promotes autoubiquitination of Ube2C, which results in Ube2C degradation and down-regulation of APC/C-activity. Because the ubiquitination of Ube2C also depends on an initiation motif, APC/C substrates might delay Ube2C degradation by competing for initiation [31]. Nearby sequences in the N-terminus of Ube2C also mediate APC/C binding [40], and N-terminal epitope tags interfere with Ube2C-activity towards the APC/C [31], and this explains previous results with ^{GFP}Ube2C that questioned an important role for Ube2C during mitosis [51]. The mechanism and timing of the APC/C-dependent degradation of Ube2C is conserved in higher eukaryotes [31,37], underscoring the importance of keeping initiation in check.

Chain elongation

Following initiation, K11-linked chains are extended by a dedicated chain-elongating E2, Ube2S/E2-EPF (Figure 2) [21,22,52]. Ube2S interacts with the APC/C cofactor Cdc20 in early mitosis and with Cdh1 in late mitosis and G1 [21]. Additional experiments suggested that Ube2S is also recognized by a core subunit of the APC/C, the identity of which has not been clarified [21]. Apart from the APC/C, Ube2S has only been shown to bind to the cullin-RING E3 subunit VHL [53].

Although Ube2S has negligible activity towards Lys residues in APC/C substrates, it rapidly elongates K11-linked chains [21,22,52]. Ube2S displays an impressive specificity for K11-linkages (>95%), as determined by quantitative mass spectrometry, linkage-specific antibodies, and mutant ubiquitin [11,14,21,22,45,54]. *In vivo*,

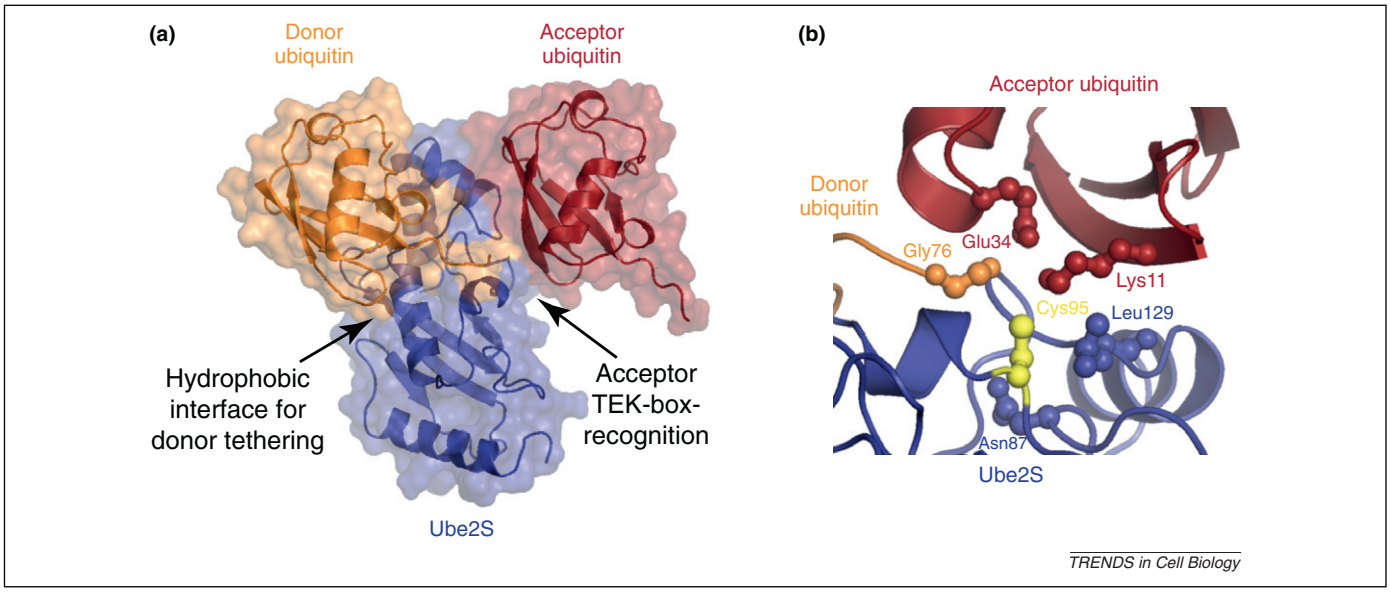

Figure 3. **K11 linkage takes place through substrate-assisted catalysis**. **(a)** Structural model of the ternary complex between Ube2S (blue), donor ubiquitin (orange), and acceptor ubiquitin (red), based on coordinates reported in [45]. The donor ubiquitin is tethered to the E2 by its thioester bond (not shown) and a non-covalent interaction around helix α2 (arrow). The acceptor ubiquitin is recognized through an electrostatic interaction that involves the TEK-box of ubiquitin (arrow). **(b)** The catalytic center of K11-linkage formation consists of residues of the E2 Ube2S (blue) and the substrate acceptor ubiquitin (red). Ube2S contributes the active-site cysteine (yellow), Leu129, and Asn87. The acceptor ubiquitin contributes Glu34 that helps to deprotonate Lys11 and orient it towards the active site of Ube2S. The donor ubiquitin is shown in orange.

Ube2S accounts for the large majority of K11-linked chain formation during cell division [11,45].

The high specificity of Ube2S, combined with established systems for assaying its activity, make it a powerful model for dissecting the mechanism of linkage-specific chain formation [45]. A combination of NMR, computational docking and biochemical analysis revealed that Ube2S engages the thioester-linked donor ubiquitin in an additional non-covalent interaction, which restricts the conformational freedom of the donor and places it in an optimal position for nucleophilic attack by the acceptor Lys11 (Figure 3a) [45]. Tethering the donor ubiquitin increases the processivity of Ube2S, which can add up to 13 ubiquitin molecules to a chain in a single substrate-binding event to the APC/C. The processivity of chain elongation by Ube2S is comparable to that of the E3 SCF and its K48-specific E2 Ube2R1/Cdc34 [43]. Indeed, the surfaces used by human Ube2R1 [45] and yeast Cdc34 [55] to bind to donor ubiquitin are similar to that used by Ube2S, and a small-molecule inhibitor of Ube2R1 interferes with the integrity of the donor-binding site [56]. Interactions with the donor ubiquitin have also been observed with Ubc1 (another K48-specific E2 [57]), the HECT-E3 Nedd4L [58], and the SUMO-E2 Ubc9 [59], suggesting that donor tethering is of general importance for linkage formation.

The acceptor ubiquitin is recognized by Ube2S with very low affinity through an electrostatic interface, which on ubiquitin consists of Lys6, Lys11, Glu34, Lys63, Thr12 and Thr14 [45]. This is the same motif, the 'TEK-box', that was previously found to be required for K11-linkage formation by Ube2C [9]. Nonspecific E2s, such as Ube2D, also require the TEK-box of ubiquitin for formation of K11-linkages [60], revealing a key role for this surface in K11-linkage formation.

The low affinity for the acceptor ubiquitin suggested that the preferred recognition of a specific ubiquitin surface is unlikely to fully account for the high specificity of Ube2S and, indeed, inspection of the ternary complex between Ube2S, donor, and acceptor ubiquitin revealed additional layers of regulation (Figure 3b). It was previously found that the active site of an E2 requires an acidic residue to suppress the pK_a of the acceptor lysine, thereby turning it into a nucleophile ready for attack [61]. Ube2S lacks such an acidic residue and instead depends on a glutamate of the substrate, the acceptor ubiquitin, to activate the target lysine. Indeed, Glu34 of acceptor ubiquitin is positioned to support Lys11 deprotonation and help orient it towards the active site of Ube2S [45]. Because other ubiquitin Lys residues lack an appropriately positioned acidic residue, only Lys11 is used for modification. These studies showed that K11-linkage formation proceeds through a mechanism of substrate-assisted catalysis.

As with initiation, the elongation of K11-linked ubiquitin chains appears to be under tight control. The expression of Ube2S is cell cycle-regulated [46], and increased levels of Ube2S can lead to tumorigenesis [53,62]. Further regulation of Ube2S is achieved by its binding to Cdc20 and Cdh1 at defined cell cycle stages and by its APC/C-dependent ubiquitination and degradation in late G1 [21]. Because the degradation of Ube2S depends on Ube2C activity, and vice versa, the APC/C can compensate to some extent for a reduction in the levels of either E2 enzyme. This might explain why partial depletion of Ube2C has weak effects on mitosis [51], whereas complete depletion of Ube2C or co-depletion of Ube2C and Ube2S cause mitotic arrest [21,38]. Thus, homogenous K11-linked ubiquitin chains are assembled through tightly regulated coordination between an initiating E2, Ube2C, and an elongating E2, Ube2S.

How are K11-linked ubiquitin chains disassembled during the cell cycle?

Deubiquitinating enzymes (DUBs) cleave isopeptide bonds between ubiquitin molecules to oppose signaling through ubiquitin chains. Although some of the ~95 human DUBs display linkage-specificity, the majority of these enzymes act on most linkages with only minor preferences [63]. K11-linkages could, therefore, be disassembled by a K11-specific DUB, or, alternatively, by a nonspecific DUB that is targeted to substrates modified with K11-linked chains through an interaction with the APC/C or the substrate.

Linkage specificity was observed for various DUBs that contain a catalytic OTU (ovarian tumor) domain [63]. Among these OTU-DUBs, Cezanne preferentially cleaves K11-linkages *in vitro* [54], but its depletion is not associated with proliferation defects [64]. Cezanne could regulate NF-κB activation by deubiquitinating the signaling protein RIP, which is modified with K11/K63-linked mixed chains [12,65]. Moreover, proteomic analysis identified the transcription factor HIF1α as a binding partner of Cezanne [66]. HIF1α is targeted for degradation by an E3 ligase that uses VHL as a substrate adaptor [67], and VHL appears to bind to Ube2S [53]. However, strong candidates for Cezanne-dependent deubiquitination still await discovery.

Based on our current knowledge, it appears more likely that K11-linked chains are disassembled by DUBs that are targeted to their substrates through an interaction with the APC/C. Initial analyses showed that APC/C-dependent ubiquitination is opposed by DUBs present in cell extracts [44]. These and later studies also found that deubiquitination activity co-purified with the APC/C [44,68], and a proteomic survey of the DUB-interactome provided evidence for DUB-binding to the APC/C [66]. Functional interactions with the APC/C are now known for two DUBs, Usp44 and Usp37. Usp44 opposes APC/C-dependent inactivation of the spindle checkpoint in mitosis [69]. One of its substrates is the APC/C activator Cdc20, which is ubiquitinated and degraded by the APC/C during spindle checkpoint inactivation [48,70]; Usp44 also protects Cdc20 from ubiquitination by the APC/C in postmitotic neurons [71], and Usp44-overexpression delays APC/C-activation and chromosome segregation in mouse fibroblasts [72]. Usp37 binds to APC/C^{Cdh1} in G1 and deubiquitinates the APC/C substrate cyclin A [73]. Together with its kinase partner Cdk2, cyclin A inactivates APC/C^{Cdh1} in late G1, a reaction that is facilitated by its Usp37-dependent stabilization. Thus, Usp44 and Usp37 can restrict the activity of the major K11-specific ligase, the APC/C, in addition to deubiquitinating substrates modified with K11-linked chains.

As expected for DUBs with catalytic USP domains, Usp44 and Usp37 display little linkage-specificity *in vitro* ([73]; M.R., unpublished data), and they are primarily

targeted to K11-linked chains through binding to the APC/C. Similar interactions between DUBs and E3s occur with high frequency, which might result in dynamic regulation of ubiquitination [66]. The case of Usp37 shows that the interplay between ubiquitination and deubiquitination can be even more intricate. During exit from mitosis, Usp37 is degraded in an APC/C-dependent manner after being itself modified with K11-linked ubiquitin chains [73]. During G1, several mechanisms turn Usp37 from an APC/C substrate into a stable and abundant APC/C inhibitor, including transcription by E2F, activation by Cdk2, and stabilization as a result of Ube2C- and Ube2S-degradation [21,30,73]. The close connections between the E3 ligase responsible for K11-linked chain formation and DUBs

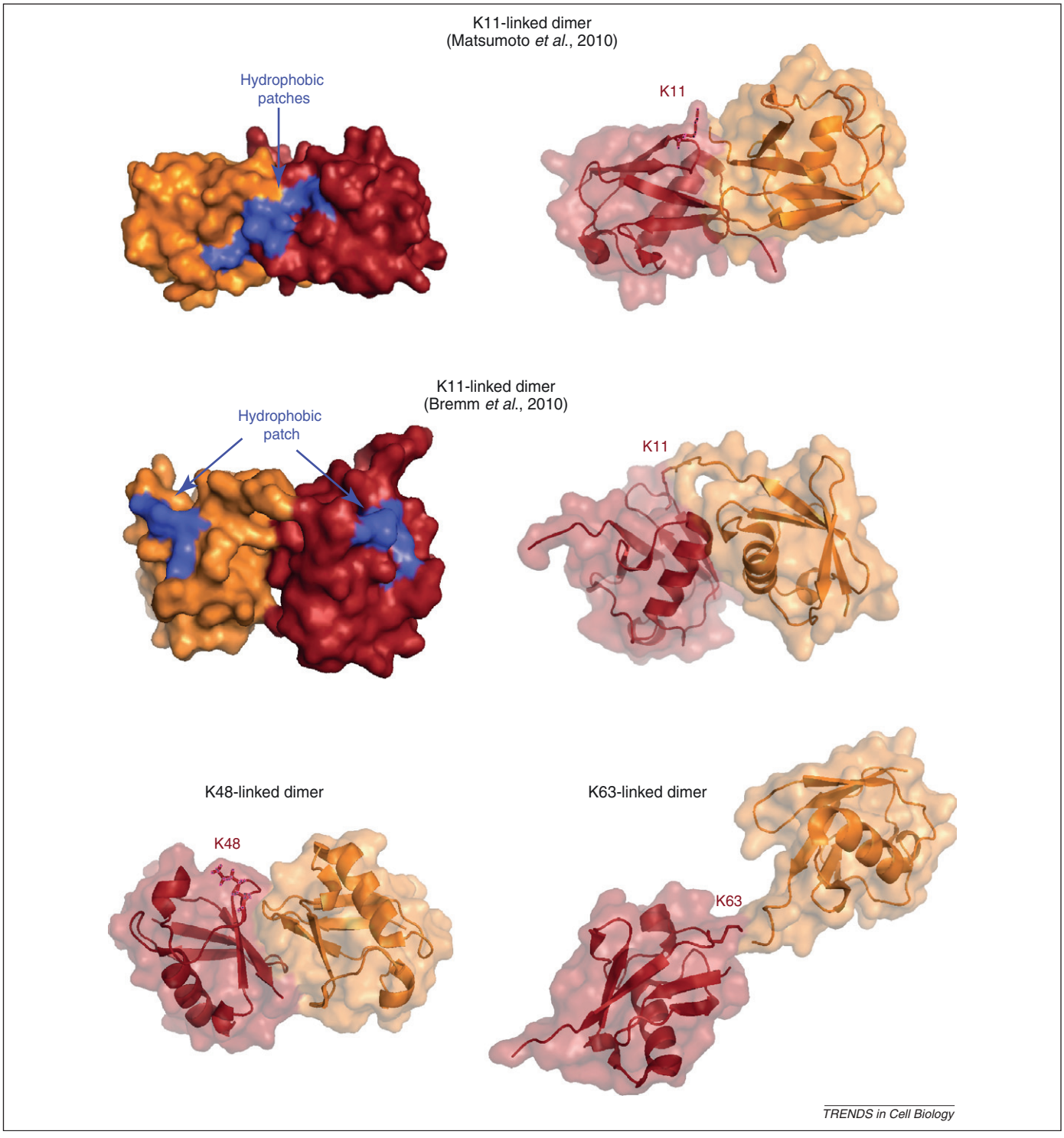

Figure 4. K11-linked ubiquitin dimers have a unique structure. Two structures of K11-linked ubiquitin dimers are displayed, as well as comparable structures of K48- and K63-linked dimers (based on coordinates reported in PDB files 3NOB, 2XEW, 2PEA, 2JF5). The acceptor ubiquitin containing the modified lysine residue is shown in red, the donor ubiquitin is depicted in orange. Both K11-linked ubiquitin dimers show compact structures with the hydrophobic patch of ubiquitin being exposed for potential interactions.

underscore that the assembly of K11-linked chains is a tightly regulated and highly dynamic process, as expected for a key component of cell cycle control.

How are K11-linked ubiquitin chains recognized?

Proteins that specifically interact with K11-linked ubiquitin chains and not with chains of other topologies are likely to exploit structural features that are unique to K11-linkages, a hypothesis that was in principle validated with the development of K11-linkage specific antibodies [11]. Indeed, K11-linked ubiquitin dimers adopt compact conformations that are distinct from K48- or K63-linked dimers, and the differences between the two reported structures of K11-linked ubiquitin dimers, together with NMR-analysis, indicate that K11-linked chains can populate at least two conformations (Figure 4) [11,54]. Importantly, in both conformations the hydrophobic patch of ubiquitin, the major surface recognized by ubiquitin-binding proteins, is exposed and ready to engage in interactions.

Although the unique structures of K11-linked ubiquitin dimers suggest that there could be proteins that specifically interact with chains of this topology, the nature of such binding partners remains elusive. K11-linked chains are recognized by proteasomal receptors for substrate degradation, but the same receptors can also interact with K48-linked chains [9,11,14]. K11-linkages can also be bound by ubiquitin-binding domains in proteins, such as NEMO, which were previously shown to interact with ubiquitin chains of different topologies [12]. However, it is interesting to note that an analysis of substrate adaptors of p97/Cdc48, a segregase that mobilizes ubiquitinated proteins from complexes [74,75], found some enrichment for K11-linkages [75]. Whether this is caused by specific binding to K11-linked chains, and whether it is biologically meaningful, needs to be tested in future experiments. How K11-linked ubiquitin chains are decoded remains, unfortunately, mostly elusive.

Why does the APC/C assemble K11-linked ubiquitin chains?

Without knowing the specific binding partners for K11-linkages or highly K11-specific DUBs, we can only speculate about the properties of K11-linked chains that serve their function as essential mitotic regulators. One potential clue to understanding the role of K11-linked chains in mitosis comes from studying the conservation of the E2s Ube2C and Ube2S [76]. Both of the K11-specific enzymes are absent from budding yeast, and in this organism the APC/C assembles K48-linked chains [77]. It is an attractive hypothesis that K11-linked, but not K48-linked, ubiquitin chains are able to control reactions that are required for the more complex mitotic regulation of higher eukaryotes. For example, higher eukaryotes have many more APC/C-substrates than do yeast, and modification with K11-linked chains might be a more efficient means of sending proteins for degradation [11]. Moreover, spindle assembly, a process that is very tightly connected to APC/C-regulation, is more complex in higher eukaryotes than in yeast because the former undergo nuclear-envelope breakdown, utilize the Ran-GTPase for microtubule nucleation, and attach multiple microtubules to each kinetochore [78]. K11-linked chains might be more suited to allow the coordinated assembly of mitotic spindles.

Given these observations, it is interesting to note that cells unable to assemble K11-linked chains accumulate in mitosis with defective spindles [21]. Although the aberrant spindle structures could be a consequence of aborted or dysfunctional spindle formation, an alternative explanation is provided by cohesion fatigue [79]. Cohesion fatigue (i.e. the loss of sister chromatid cohesion during a prolonged metaphase arrest) was observed by live imaging of cells that could not assemble K11-linked chains owing to depletion of the APC/C-activator Cdc20, or that did not turn over K11-ubiquitin modified substrates as a result of sustained proteasome inhibition [79]. Events reminiscent of cohesion fatigue were also seen in studies using siRNAs or small molecules to interfere with APC/C activity [38,80]. Because cohesion fatigue depends on continuous kinetochore attachment to the spindle, K11-linked chains might indeed control spindle dynamics or stability.

The connections between K11-linked chains and the spindle were strengthened by the discovery that the APC/C controls Ran-dependent spindle assembly [81]. GTP-charged Ran activates spindle-assembly factors by releasing them from inhibitors of the importin family [82,83]. For two spindle-assembly factors, HURP and NuSAP, activation by Ran also results in exposure of their APC/C-binding sites, which leads to their modification with K11-linked chains and their degradation by the proteasome [81]. However, because the mechanisms that coordinate spindle-assembly factor activation and degradation remain poorly understood, it is not known whether other chain types would also achieve the proper regulation of Ran-dependent spindle assembly.

There is, of course, the possibility that K11-linked ubiquitin chains are not better suited than other chain types to fulfill the many tasks of the APC/C. Instead, it is possible that the properties of the enzymes that catalyze K11-linked chain assembly, rather than chain topology *per se*, have been conserved during evolution. For example, the similarity in the composition of initiation motifs in substrates and the TEK-box in ubiquitin might increase the efficiency of K11-linked chain formation by allowing Ube2C to assemble short chains during initiation [9]. In addition, because Ube2S does not appear to interact with the RING-domain of APC11 [45], Ube2S does not compete with Ube2C for APC/C-binding [21,45], and initiating and elongating E2s can be present on the same APC/C-molecule; such a close collaboration might also be advantageous for targeting many substrates during the short time-span of mitosis. Future work is needed to distinguish between these possibilities and to find a molecular explanation for the role of K11-linked chains in cell cycle regulation.

Concluding remarks

Recent studies have taught us much about the function and assembly of K11-linked chains. The striking accumulation of K11-linked chains in mitosis, and the strong mitotic defects in cells lacking the K11-specific enzymes, have firmly linked this modification to cell cycle control. We know less about the factors that specifically bind to and

read K11-linked chains, either in mitosis or at other cell cycle stages. Moreover, we lack strong evidence that homogenous K11-linked chains function outside of mitosis, possibly because we have yet to identify mechanisms that lead to their postmitotic induction. Hence, whereas it is established that K11-linked chains are able to trigger degradation, it remains unclear whether they also provide more idiosyncratic, non-proteolytic functions that set them apart from canonical K48-linked chains. The discovery of specific functions of K11-linked chains would be exciting and important because it would deepen our insight into the breadth of the ubiquitin code – cracking a code that is an elaborate device for the tight and complex regulation of cell division.

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