Dispatch

Kinetochore Signalling: The KIss That MELTs Knl1

Mitotic kinetochores coordinate chromosome bi-orientation and anaphase onset by serving as scaffolds for the recruitment of regulatory proteins. Three new studies reveal that multiple interaction motifs of the kinetochore protein Knl1 cooperate to assemble signaling complexes that regulate chromosome segregation.

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Kinetochores are centromeric protein complexes that bind regulators of the spindle assembly checkpoint (SAC) and mediate **(Au : OK ?)** chromosome–spindle interactions in early mitosis [1]. The SAC postpones the initiation of anaphase until all pairs of sister chromatids are connected to microtubules from opposite poles. A host of SAC proteins, including Bub1, Bub3, BubR1, Mad1, Mad2 and Mps1, are recruited to unattached or 'malattached' kinetochores. Kinetochore-associated SAC complexes generate anaphase inhibitors that target Cdc20, an activator of the anaphase-promoting complex. In addition to their essential role in SAC signalling, Bub1 and BubR1 also contribute to chromosome congression during prometaphase by stabilizing correct kinetochore–microtubule interactions and destabilizing erroneous contacts. This regulation is mediated by BubR1-associated protein phosphatase PP2A and the intrinsic kinase activity of Bub1, which do not appear to be required, however, for SAC signaling [2,3].

Key regulators of the SAC and chromosome bi-orientation, including Bub1 and BubR1, are recruited by the kinetochore protein Knl1, also known as Blinkin, Casc5, Spc7 and

Spc105 in various organisms [1,4]. Knl1 is a subunit of the large Knl1–Mis12–Ndc80 network. Its amino-terminal half contains an array of sequence variants of Met–Glu–Leu–Thr (MELT) motifs that turn into high-affinity binding sites for Bub3 after phosphorylation by protein kinase Mps1 (Figure 1, [4–7]). The selectivity for phosphorylated MELT-like motifs (MELTph) is explained by ionic interactions between basic residues of Bub3 and the acidic phosphate group, which are potentiated by Bub3-associated Bub1 [4]. In vertebrates, the amino-terminal region of Knl1 also harbors two short sequence motifs, termed Lys–Ile (KI) 1 and 2, that represent docking sites for the three tetratrico-peptide repeat (TPR) motifs of Bub1 and BubR1, respectively [8,9]. However, neither the KI motifs nor the TPR domains are required for robust targeting of Bub1 and BubR1 to kinetochores. Considerable evidence shows that kinetochore binding of Bub1 is largely mediated by the binding of Bub3 to MELTph [4–7]. Although BubR1 also forms a constitutive heterodimer with Bub3, its recruitment is Bub1-dependent. This indicates that Bub3–BubR1 cannot bind independently to MELTph and is only recruited after the binding of Bub3–Bub1. The further downstream events that lead to the assembly of mature SAC complexes include the recruitment of the Mad1–Mad2 complex and Cdc20, which is facilitated by Bub1 and BubR1, respectively [1] .

Recent papers from Krenn *et al*. [10] (reported in this issue of *Current Biology*), Vleugel *et al*. [11] and Zhang *et al*. [12] shed new light on the cooperative interactions that guide the assembly of signaling complexes on Knl1. A chromosome-arm targeted Knl1 fragment that only comprised the first MELT-like motif and the flanking KI1 + KI2 region (Knl1-M1) was sufficient to recruit Bub1 and BubR1 [11]. Likewise, stably expressed Knl1-M1 co-immunoprecipitated all major SAC components in a Bub1-dependent manner [10]. These included Mps1 and Mad1, which have previously escaped detection in Knl1 complexes. The co-precipitation of SAC components was dramatically increased when Knl1-M1 was fused to the carboxy-terminal kinetochore-targeting domain of Knl1, possibly due to more efficient

phosphorylation by kinetochore-associated Mps1 [10]. Kinetochore-targeted Knl1-M1 elicited a strong SAC response in Knl1-depleted cells, but at best only partially rescued chromosome alignment defects, hinting at an essential contribution of downstream MELT-like motifs to establishing chromosome bi-orientation [10,11]. The relative importance of the Bub binding motifs of Knl1–M1 was further examined by mutagenesis [10,11]. Deletion or mutation of the KI motifs strongly reduced the recruitment of the Bub proteins and dampened the ability to mount a SAC response. Collectively, these data suggest that the KI motifs stabilize the interaction of Bub3–Bub1 with MELT1ph and promote the subsequent recruitment of Bub3–BubR1 (Figure 1). Reconstitution experiments with purified components confirmed this enhancer function of the KI domains [10].

In addition to the MELT1–KI1–KI2 module, the amino-terminal half of human Knl1 contains an array of 18 MELT-like motifs that are not flanked by KI motifs (Figure 1). These 'naked' MELT-like motifs acted in an additive manner with respect to the recruitment of Bubs and the enhancement of chromosome bi-orientation [11,12]. A tandem array of 4–6 naked MELT-like motifs turned out to be as efficient as native Knl1, indicating that some MELT-like motifs are either not functional or redundant. Nevertheless, 'naked' MELT-like motifs are clearly less efficient than Knl1–M1 in seeding SAC complexes. Indeed, Krenn *et al.* [10] found that kinetochore-targeted MELT1–KI1–KI2 and MELT2–10 were associated with similar amounts of the Bub proteins **(Au: Does this make sense given the previous statement?)**. Intriguingly, a larger Knl1 fragment that combined these motifs (MELT1–10) bound much more of the Bubs than expected from addition, indicating that the MELT1–KI1–KI2 module somehow enhances the recruitment of SAC proteins to the other MELT-like motifs. Possibly, a SAC complex assembled on KNL1-M1 contributes to the recruitment of complexes at downstream MELT-like motifs by positive cooperativity, similar to the recruitment mechanism of oligomerizing gene-silencing and exon-definition complexes [13].

Vleugel *et al*. [11] showed that a conserved TxxF/Y motif that is found amino-terminally to most MELT-like motifs is also essential for efficient recruitment of Bub1 and BubR1. The authors suggested that this TxxF/Y motif may function like a KI motif for naked MELTs. However, both the TxxF/Y and KI motifs were needed for the efficient recruitment of Bubs by Knl1-M1, indicating that they act by distinct mechanisms. At present it is unclear how Bub3–BubR1 can be recruited by a naked MELTph motif since BubR1 hampers the interaction of associated Bub3 with MELTph which, moreover, is already engaged with Bub3–Bub1 (Figure 1). This led Krenn *et al.* [10] to speculate on a direct interaction site between BubR1 and Bub1, consistent with previous findings [2,15]. Using independent approaches, Vleugel *et al.* [11] also concluded that the recruitment of BubR1 is aided by an unidentified kinetochore-localized activity. In any case, mature SAC complexes on naked MELT-like motifs are expected to be rather dynamic unless the Bub1-associated Bub3–BubR1 heterodimer is stabilized by binding to a second, flanking MELTph or to neighbouring SAC complexes. On the other hand, it can be argued that SAC complexes need to be unstable to generate diffusible, BubR1-containing anaphase inhibitors.

The reported findings raise some interesting questions on the coordination of MELT (de)phosphorylation, SAC complex (dis)assembly and microtubule binding by Knl1 [10–12]. Does the (de)phosphorylation of the MELT-like motifs occur in a specific order and, if so, does this organize the (dis)assembly of signaling complexes? The phosphorylation of Knl1 is dynamically regulated during prometaphase [12], and the recruitment of Mps1 to Ndc80 and of PP1 to Knl1 are oppositely regulated by Aurora B **(Au: Reference 14 seems to be missing)** [15,16]. This regulation favours MELT phosphorylation on tensionless kinetochores, but does not immediately suggest a mechanism for an ordered (de)phosphorylation of MELT-like motifs. Possibly, the (de)phosphorylation of MELT-like motifs is guided by resident SAC

complexes that form additional interaction sites with the converting enzymes. Consistent with this notion, immunoprecipitates of Knl1-M1 contained Mps1 [10].

The KI motifs are intrinsically disordered but adopt an α-helical structure upon binding of Bub1 and BubR1 [8,9]. Does this disorder-to-order transition reduce the diffusion range of Knl1-associated PP1 and hamper its ability to dephosphorylate nearby MELT-like motifs? Is the (de)phosphorylation ordered by differences in the affinity of MELT-like motif sequence variants for Mps1 and/or PP1? Similarly, mitotic kinases and phosphatases have been shown to (de)phosphorylate their high-affinity substrates first [17]. Does the (de)phosphorylation of one MELT-like motif affect the (de)phosphorylation rate of a flanking motif? This would be reminiscent of 'sequential' phosphorylation by some protein kinases, which depend on a priming phosphorylation [18]. Does the binding of microtubules to the extreme amino terminus of Knl1 enable the delivery of a phosphatase at the kinetochore that dephosphorylates the nearby bipartite PP1 docking site of Knl1, resulting in PP1 recruitment, MELTph dephosphorylation and SAC silencing [19]?

Recent data suggest that the SAC is graded rather than switch-like, and that the number of SAC complexes at individual kinetochores decreases with microtubule occupancy [20]. The number of MELT-like motifs are likely to be a key determinant of the amount of SAC complexes that can be assembled on a single kinetochore. This maximum may be achieved in early prometaphase or in the presence of spindle poisons, when most kinetochores are unattached or only attached to a few microtubules. In such conditions, KI motifs are not limiting for SAC complex assembly, as shown by Vleugel *et al.* [11]. However, in late prometaphase, when the SAC response is much weaker, the KI motifs may contribute to setting the threshold for a minimal SAC response. It has been challenging but, gradually, the KInky MELTing-Pot of interactions at Knl1 is revealing its true nature.

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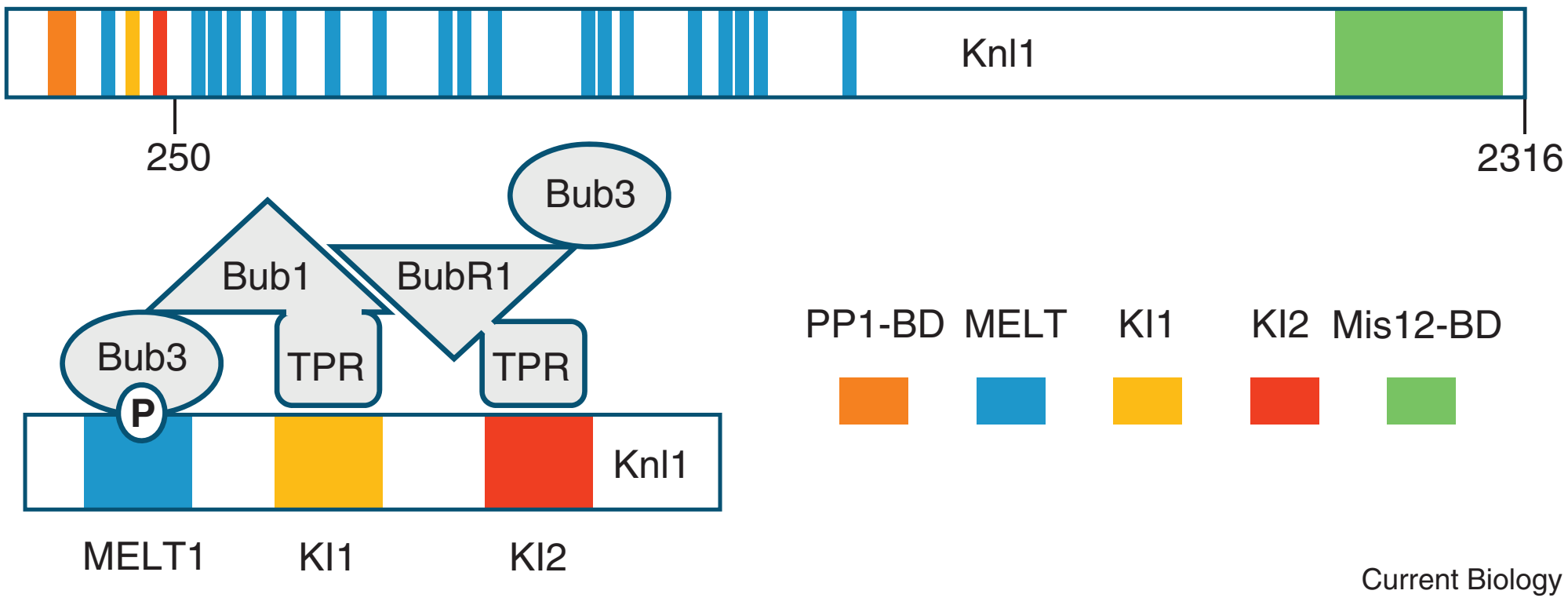
Figure1. Protein interaction motifs of Knl1.

The upper bar shows the localisation of the PP1-binding domain (PP1-BD), MELT-like motifs, KI1 motif, KI2 motif, and the kinetochore binding Mis12-binding domain (Mis12-BD) of human Knl1. The domains are color-coded, as defined in the lower right panel. The lower left panel shows how Bub3–Bub1 and Bub3–BubR1 are proposed to assemble on the MELT–KI1–KI2 module (KNL1-M1).



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