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The (Phospho) Needle in the (MELT) Haystack

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The spindle assembly checkpoint promotes chromosome bi-orientation and halts mitotic progression in the presence of improper kinetochore-microtubule attachments. Knl1, a kinetochore protein, acts as a scaffold for SAC signaling. A new study unveils remarkable complexity in the interplay of Knl1 phosphorylation and SAC function (Vleugel et al., 2015).

Cell-cycle checkpoints are crucial for successful cell division, and their inactivation preludes to cell death or transformation. The spindle assembly checkpoint (SAC) facilitates and repairs chromosome attachment to microtubules and delays mitotic exit until chromosomes are bi-oriented (Lara-Gonzalez et al., 2012; London and Biggins, 2014). Like other signal transduction systems, the SAC consists of sensors, transducers, effectors, and—to our pleasure—puzzles that make it hard to understand. A new study in *Molecular Cell* (Vleugel et al., 2015) reports a molecular investigation of one such puzzle, the mechanism that recruits the SAC proteins Bub1 and Bub3 to kinetochores.

Kinetochores connect chromosomes to spindle microtubules (Figure 1A). Two kinetochore subcomplexes, Ndc80-C and Knl1-C, coordinate microtubule attachment with the mitotic cell cycle machinery, forcing the latter into standstill in the presence of unattached chromosomes and again into motion upon chromosome bi-orientation (Lara-Gonzalez et al., 2012; London and Biggins, 2014). Upstream in the pathway, the SAC kinase Mps1 (also known as TTK) binds kinetochores devoid of microtubules to trigger a wave of phosphorylation of downstream targets, including the Knl1-C subunit Knl1 (a.k.a. CASC5, Blinkin, AF15q14, CT29, Spc105, and Spc7) (Figure 1B). Mps1 phosphorylates Knl1 at so-called MELT motifs (from the consensus sequence Met-Glu-Leu-Thr) (Lara-Gonzalez et al., 2012; London and Biggins, 2014). Phosphorylated MELTs bind the SAC protein Bub3, which carries along Bub1, a kinase and a protein interaction scaffold to which Bub3 binds constitutively. Kinetochore Bub1 elicits downstream responses necessary for SAC function and chromosome bi-orientation.

If this scheme appears simple, its details are complex. Knl1 is a large (>2300 residues in humans) unstructured protein carrying six to seven MELT motifs in *S. cerevisiae* and up to 19 in humans (Figure 1C). Furthermore, the repeat unit in humans is more complex than the name MELT suggests, containing TXXΩ (X = any aminoacid, Ω = Phe or Tyr) and SHT (Ser-His-Thr) motifs N- and C-terminal to the MELT sequence, respectively (Krenn et al., 2014; Vleugel et al., 2013). Here, we name this larger repeat "MELT," and the Met-Glu-Leu-Thr sequence "MELT core."

Why so many Bub3-binding motifs? Are they functionally equivalent? Are they redundant? Given the size and complexity of Knl1, these questions pose significant intellectual and technical challenges. Previous analyses suggested that not all MELTs are equal. An array of six copies of human MELT17 functionally replaces Knl1, but an equivalent construct with MELT2 does not (Vleugel et al., 2013). MELT1 was also shown to be more active than its neighbors, a single copy being able to sustain the SAC (Krenn et al., 2014; Vleugel et al., 2013). To approach MELT function systematically, Vleugel and co-workers (2015) engineered a reporter "rescue" construct consisting of 223 residues encompassing MELT12 through MELT14 of human Knl1 (Figure 1C). They pasted this segment between the N-terminal phosphatase-binding motif and the C-terminal kinetochore-targeting motif of Knl1, thus leaving two crucial functionalities of Knl1 intact. They then iteratively modified this scaffold by replacing MELT12 to MELT14 with three identical copies of each of the 19 MELTs. Finally, they tested the 19 resulting MELT constructs in two functional assays probing SAC proficiency and chromosome bi-orientation (Figure 1C). Because all non-repeat sequences in the 223-residue MELT12-MELT14 segment were left intact, a possible concern is that MELTs grafted into this segment from their normal position have altered functionality. Arguing against this, the combined amount of Bub1 recruited to kinetochores by the 19 engineered repeats adds up to the Bub1 amounts normally recruited by full-length Knl1, suggesting that the experimental scheme fairly compares the relative strength of individual MELTs.

When tested in the SAC assay, the 19 MELT arrays behaved in an all-or-none manner. Seven of them restored a robust SAC response, while the remaining 12 failed to respond to spindle poisons, an established SAC trigger. Thus, SAC function requires an activity threshold that only a subset of the 19 MELT motif arrays meets. Conversely, none of the rescue constructs promoted kinetochore-microtubule alignment to levels comparable to those of full-length Knl1, suggesting that chromosome bi-orientation requires a full complement of Bub1:Bub3. Importantly, the same MELT repeats that restored SAC function scored high in the chromosome bi-orientation assay, and the same motifs are also strong Bub1 kinetochore recruiters. This correlation raises considerable interest in the precise sequence determinants of MELT repeats. After showing that the TXXΩ motif is functionally relevant (Vleugel et al., 2013), the

authors now show that phosphorylation of the SHT motif, "primed" by phosphorylation of the MELT core, is also required for kinetochore recruitment of Bub1:Bub3 and SAC proficiency. Specific adaptations at the interface of the human Bub1:Bub3:MELT^{P} complex were identified and probed by mutagenesis thanks to the structure of the equivalent but simpler *S. cerevisiae* Bub1:Bub3:MELT^{P} complex (Primorac et al., 2013).

Knl1 varies rapidly in time, and its mutation has been even implicated in human evolution (Prüfer et al., 2014). Previous studies on SAC evolution (Suijkerbuijk et al., 2012; Vleugel et al., 2012) show that a Bub1-ancestor gene duplicated to form Bub1 and BubR1 (Mad3 in some species) paralogs in at least nine independent occasions. Bub1 and BubR1 invariably underwent subfunctionalization, with BubR1 being always required downstream of Bub1 in the SAC (Suijkerbuijk et al., 2012). Unlike Bub1, BubR1 does not contribute to the high-affinity interaction of Bub3 with MELT repeats, and its recruitment to kinetochores requires dimerization with Bub1 (Overlack et al., 2015) (Figure 1B). Thus, it is unlikely that the "weak" MELTs that fail to bind Bub1:Bub3 bind BubR1:Bub3 instead, although they may interact with additional, currently unknown partners. Alternatively, MELT inactivation may reflect a requirement to maintain Bub1:Bub3 at fixed levels vis-a-vis a remarkable size expansion of Knl1 whose functional significance is however presently unclear. The study of Vleugel et al. (2015) suggests that chromosome bi-orientation, not the SAC response, demands high levels of kinetochore Bub1:Bub3 that single MELTs cannot provide. The secret of MELT expansion and inactivation may lie in the interplay of kinase and phosphatase activity required for kinetochore-microtubule attachment.

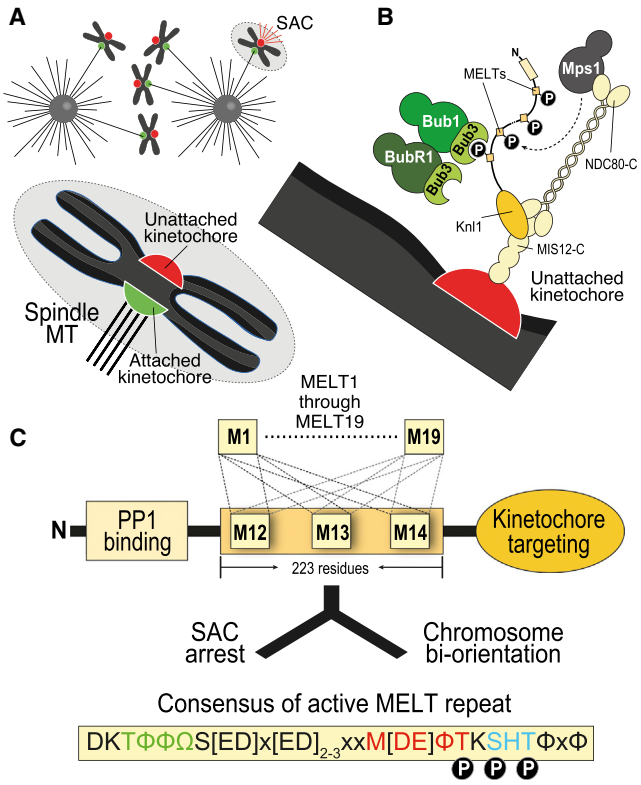

Figure 1. Schematic Depiction of the SAC Response

(A) Chromosomes attach to microtubules via kinetochores (green). Kinetochores not yet bound to microtubules (red) emit the SAC signal.

(B) Molecular components of kinetochores discussed in the main text.

(C) Experimental strategy for testing strength of MELT repeats. Three copies of each of the 19 MELTs of human Knl1 were used to replace MELT12 through MELT14 inserted between functionally relevant domains. The resulting constructs were tested in cells depleted of endogenous Knl1. The consensus of an "active" MELT is shown (x, any amino acid; Φ, hydrophobic; Ω, aromatic).

REFERENCES

Krenn, V., Overlack, K., Primorac, I., van Gerwen, S., and Musacchio, A. (2014). Curr. Biol. *24*, 29–39.

Lara-Gonzalez, P., Westhorpe, F.G., and Taylor, S.S. (2012). Curr. Biol. *22*, R966–R980.

London, N., and Biggins, S. (2014). Nat. Rev. Mol. Cell Biol. *15*, 736–747.

Overlack, K., Primorac, I., Vleugel, M., Krenn, V., Maffini, S., Hoffmann, I., Kops, G.J., and Musacchio, A. (2015). eLife *4*, e05269.

Primorac, I., Weir, J.R., Chiroli, E., Gross, F., Hoffmann, I., van Gerwen, S., Ciliberto, A., and Musacchio, A. (2013). eLife *2*, e01030.

Prüfer, K., Racimo, F., Patterson, N., Jay, F., Sankararaman, S., Sawyer, S., Heinze, A., Renaud, G., Sudmant, P.H., de Filippo, C., et al. (2014). Nature *505*, 43–49.

Suijkerbuijk, S.J., van Dam, T.J., Karagöz, G.E., von Castelmur, E., Hubner, N.C., Duarte, A.M., Vleugel, M., Perrakis, A., Rüdiger, S.G., Snel, B., and Kops, G.J. (2012). Dev. Cell *22*, 1321–1329.

Vleugel, M., Hoogendoorn, E., Snel, B., and Kops, G.J. (2012). Dev. Cell *23*, 239–250.

Vleugel, M., Omerzu, M., Groenewold, V., Hadders, M.A., Lens, S.M.A., and Kops, G.J.P.L. (2015). Mol. Cell *57*, this issue, 824–835.

Vleugel, M., Tromer, E., Omerzu, M., Groenewold, V., Nijenhuis, W., Snel, B., and Kops, G.J. (2013). J. Cell Biol. *203*, 943–955.