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SPOTLIGHT

Racing through *C. elegans* mitosis using cyclin B3

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Racecar drivers use left-foot braking, i.e., simultaneously engaging brake and throttle, to carefully balance acceleration and traction when navigating chicanes. In this issue, Lara-Gonzalez et al. (https://doi.org/10.1083/jcb.202308034) show that *C. elegans* embryos employ the molecular equivalent of left-foot braking to faithfully speed through mitosis.

Early embryonic cell cycles are often characterized by rapid rounds of DNA replication and cell division. Only as development progresses, cell cycles lengthen to accommodate transcription, differentiation, cell growth, and cell cycle checkpoints. How organisms achieve the incredibly fast pace of embryonic divisions continues to mystify scientists.

Cell cycle progression is driven by cyclin-dependent kinases (CDKs) in complex with their respective activating cyclins. Both CDKs and cyclins come in different flavors, with different substrate specificities and temporal expression profiles. Mitosis is coordinated by CDK1 associated with A- and B-type cyclins, where cyclin A-CDK1 is thought to be important for mitotic entry and cyclin B-CDK1 for mitotic progression. Timely destruction of A- and B-type cyclins by the anaphase-promoting complex/cyclosome (APC/C) is essential for mitotic exit (1).

Cyclin B3 is the odd one in the family. Cyclin B3s form their own subfamily, sharing higher sequence similarity amongst cyclin B3s from diverse species than with other cyclins from the same organism. Despite being categorized as B-type cyclins, cyclin B3 proteins share several characteristics with A-type cyclins, e.g., their nuclear localization. Yet, whereas A- and B-type cyclins are considered of ancient eukaryotic origin, cyclin B3 is only present in metazoans. In placental mammals, cyclin B3 additionally underwent an unusual expansion of a single exon, resulting in an abrupt gain of a large, intrinsically disordered region that tripled the protein size (2).

Cyclin B3's functional role has been hard to pinpoint. In mice, cyclin B3 seems to have exclusively meiotic functions, with cyclin B3 knockouts resulting in viable adults but sterile females (3, 4). In *D. melanogaster*, cyclin B3 null mutants also cause female sterility, but additional mitotic phenotypes during embryonic divisions have been reported (5, 6). In *C. elegans*, cyclin B3 seems to be involved both in mitosis and meiosis (7, 8), though oocytes depleted of cyclin B3 arrest in metaphase II and not like in vertebrates and flies in metaphase I.

In this study, Lara-Gonzalez et al. (9) first carefully dissected the roles of the different B-type cyclins (cyclin B1, cyclin B2.1 and cyclin B2.2, and cyclin B3) in the *C. elegans* embryo; however, due to the high sequence similarity between cyclin B1 and cyclin B2s, they were not able to knock down these genes individually using double-stranded RNA (dsRNA). To overcome this problem, they elegantly engineered strains that allowed them to express dsRNA-resistant versions of the individual cyclins in trans. Using this approach, they demonstrate that cyclin B1 and cyclin B3, but not cyclin B2, contribute to mitotic progression in a CDK1-dependent manner.

Consistent with previous reports (7, 8), the authors further show that depletion of cyclin B3 in *C. elegans* embryos significantly delays mitotic progression. The observed delay is only partially due to activation of the mitotic checkpoint as abolishing the checkpoint only mildly reduces the delay. Rather, the observed phenotype seems to be the result of cyclin B3 directly impacting and accelerating several mitotic processes including spindle formation, chromosome alignment, and APC/C activation. The latter is in line with cyclin B3's reported role in activating the APC/C in vertebrate metaphase I of meiosis (3) and *D. melanogaster* mitosis (5, 6).

Interestingly, whereas loss of cyclin B3 greatly slows down mitosis, loss of cyclin B1 accelerates mitosis in *C. elegans* embryos. This is surprising because cyclin B1 is usually considered to promote mitotic progression not the least by phosphorylating and activating the APC/C. The observed phenotype is reminiscent of a previously described phosphorylation site mutant in the APC/C activator CDC-20 (10). Genetic experiments by the authors place CDC-20 T32 phosphorylation and cyclin B1 into a shared pathway suggesting that cyclin B1 might decelerate mitosis by phosphorylating CDC-20. However, currently, there is no biochemical evidence that cyclin B1-CDK1 specifically and preferentially phosphorylates CDC-20 T32 directly.

In *C. elegans* embryos, it seems that cyclin B3 is stepping on the gas while cyclin B1 is pumping the brakes (Fig. 1). How can the opposing behaviors of these two B-type

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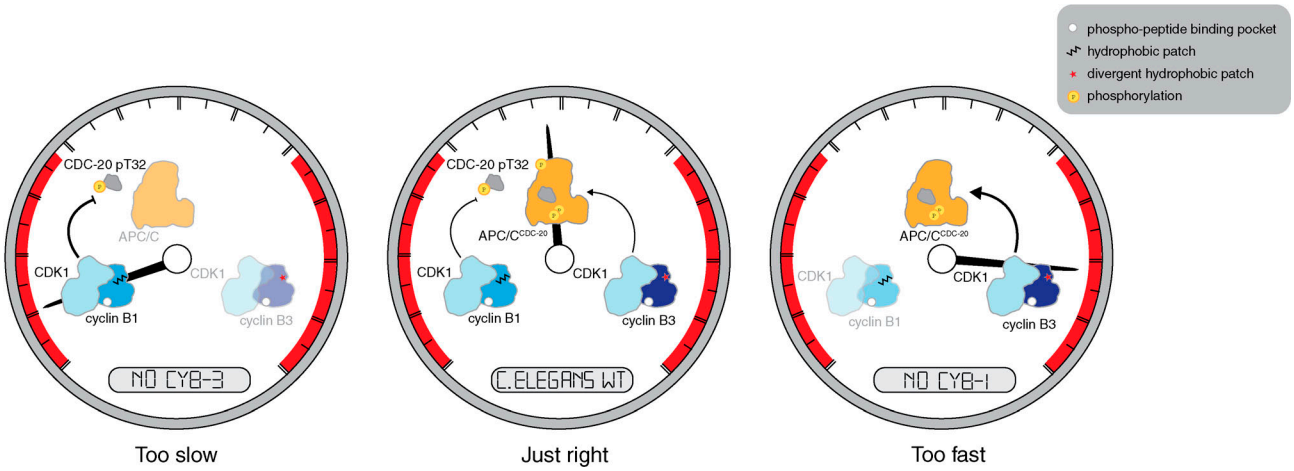

Figure 1. **Cyclin B1 and cyclin B3 fine-tune the speed of mitotic progression during the early embryonic divisions in *C. elegans*.** Cyclin B1 slows down mitosis potentially via directly or indirectly increasing CDC-20 T32 phosphorylation, which prevents CDC-20 from binding and activating the APC/C. Cyclin B3 promotes mitotic progression by positively influencing APC/C activation (e.g., by phosphorylation) and other mitotic processes. Several motifs in cyclin B1 and cyclin B3 including the phospho-peptide binding pocket and the hydrophobic patch, which has diverged in *C. elegans* cyclin B3, could contribute to the observed distinct substrate specificities.

cyclins be explained? Strikingly, when the authors isolated cyclin B1 and cyclin B3 from embryo extracts and measured the associated CDK1 activity toward a standard substrate, histone H1, they found a 25-fold higher substrate phosphorylation for cyclin B3-CDK1 compared with cyclin B1-CDK1. Whereas this difference has been noted before (7, 11), here the difference is convincingly quantified under standardized conditions. The finding suggests that cyclin B3 accelerates mitosis by supercharging CDK1 activity. The significantly increased activity of cyclin B3-CDK1 toward histone H1 could either be explained by an increased substrate affinity, e.g., mediated by specific docking motifs in cyclin B3, or—more intriguingly—by cyclin B3 inducing a structural change in CDK1 that increases the catalytic rate.

Lara-Gonzalez and colleagues tried to address this question by swapping the cyclin box domains of cyclin B1 and B3. Unfortunately, none of the chimeras retained the ability to bind CDK1, which leaves the question unanswered for now. Notably, in *C. elegans* cyclin B3, two critical residues of a conserved motif involved in substrate docking, the hydrophobic patch, are substituted with hydrophilic residues (Fig. 1). It will be interesting to investigate whether reverting these residues to the canonical amino acids (M and L, respectively) impacts substrate specificity or mitotic progression. *Xenopus laevis* cyclin B3-CDK1 preferentially phosphorylates the APC/C inhibitor XErp1/Emi2 but not histone H1 (12) compared with cyclin B1-CDK1. This preference has recently been linked to a phospho-peptide binding pocket in cyclin B3 and surrounding acidic residues (13, *Preprint*). Whereas Emi2 is absent in *C. elegans*, it remains to be explored, whether the phospho-peptide binding pocket of *C. elegans* cyclin B3 plays a distinct functional role in determining CDK1 substrate specificity in mitosis or meiosis.

Clearly, understanding how cyclin B3 makes the embryonic cell cycle engine run at full throttle will require further biochemical and structural investigations into how cyclin B3, and cyclins in general, influence CDK activity toward different substrates and shape the dynamic landscape of substrate phosphorylation.

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