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VIEWPOINT

Rashomon at the kinetochore: Function(s) of the Mad1–cyclin B1 complex

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In the film *Rashomon*, four witnesses describe seemingly contradictory views of one event. In a recent analogy, an interaction between the master mitotic regulator cyclin B1 and the spindle checkpoint component Mad1 was independently described by three groups who propose strikingly different functions for this interaction. Here, we summarize their findings and present a perspective on reconciling the different views.

Introduction

The cyclin B1–Cdk1 complex is the master regulator of mitosis. Once cyclin B1 accumulates to a threshold level during G2, full activation of its kinase partner Cdk1 transforms the cell from an interphase to a mitotic state. Activated cyclin B1–Cdk1 phosphorylates a number of cellular targets in order to trigger diverse events such as chromosome condensation, nuclear envelope breakdown, mitotic spindle formation, and chromosome segregation. As mitotic chromosomes form, they assemble kinetochores on their centromere regions in order to dynamically couple to spindle microtubules. Kinetochores that are not yet attached to spindle microtubules act as platforms for the spindle assembly checkpoint, generating a diffusible "wait anaphase" signal that ensures coordinated segregation of all of the chromosomes in a cell (Musacchio, 2015; Corbett, 2017). At unattached kinetochores, spindle checkpoint components catalyze the formation of an inhibitor of the anaphase-promoting complex/cyclosome (APC/C), the ubiquitin ligase that promotes sister chromatid separation and cyclin B degradation to trigger anaphase onset and exit from mitosis (Barford, 2011).

While the kinase activity of cyclin B1–Cdk1 has been shown to promote spindle checkpoint signaling, its precise contributions to the signaling mechanism remain to be clarified (Hayward et al., 2019b; Serpico and Grieco, 2020). Interestingly, analogous to checkpoint proteins, cyclin B1 was shown to localize to unattached kinetochores over a decade ago (Bentley et al., 2007; Chen et al., 2008). However, the mechanism by which this pool of cyclin B1 is recruited to kinetochores and its functional significance remained uncharacterized. Three recent papers (Alfonso-Pérez et al., 2019; Allan et al., 2020; Jackman et al., 2020) describe a direct interaction between cyclin B1 and the spindle checkpoint protein Mad1 and indicate that this interaction contributes to robust checkpoint signaling. However, the studies reach very different conclusions for the mechanistic basis by which the Mad1–cyclin B1 interaction promotes checkpoint signaling. Here, we summarize key findings from the three studies and attempt to reconcile the proposed mechanisms as well as suggest future work that would help address the difference in viewpoints.

A direct Mad1–cyclin B1 interaction

Mad1, along with its binding partner Mad2, makes a heterotetrameric complex that is localized at nuclear pores in interphase and at unattached kinetochores in mitosis (Fig. 1). All three manuscripts report that human Mad1 interacts with cyclin B1. One study (Alfonso-Pérez et al., 2019) narrowed this interaction region to the N-terminal 100 amino acids of Mad1; the other two studies (Allan et al., 2020; Jackman et al., 2020), by characterizing two natural isoforms of Mad1, defined a precise short motif that mediates the interaction with cyclin B1 (Fig. 1 A). Jackman et al. (2020) point out that the cyclin B1 interaction motif in Mad1 has weak homology to a region in the Protein Phosphatase 1 regulator RepoMan that also interacts with cyclin B1–Cdk1 (Qian et al., 2015), suggesting the existence of a new motif class mediating direct cyclin B1 binding.

All three groups show that Mad1 mutants unable to bind cyclin B1 still localize to unattached kinetochores, although distinct effects on recruitment timing relative to nuclear envelope breakdown and on concentration in spatial subdomains of the kinetochore were reported, as detailed below. Mad1 mutants unable to interact with cyclin B1 did not impact mitotic timing in unperturbed mitosis but exhibited faster mitotic exit in the presence of microtubule depolymerizing drugs, suggesting that the Mad1–cyclin B1 interaction contributes to the robustness of checkpoint signaling. While these conclusions are broadly similar and

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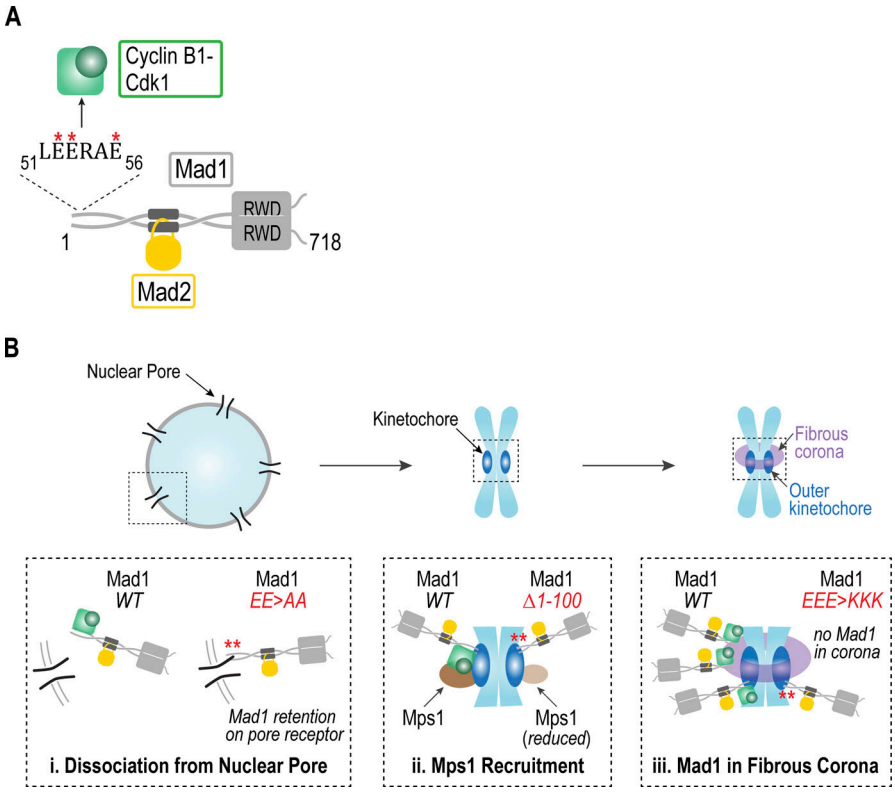

Figure 1. **Mad1 interaction with cyclin B1–Cdk1 and proposed models for the function of this interaction. (A)** Schematic summarizing the direct interaction between Mad1 and cyclin B1–Cdk1 that requires a short motif in the Mad1 N-terminus. The interaction is with cyclin B1 and does not require Cdk1. Red asterisks indicate residues mutated in Jackman et al. (2020) (E52A and E53A) and Allan et al. (2020) (E52K, E53K, and E56K). For simplicity, only one Mad2 molecule is depicted as bound to Mad1; the conserved C-terminal RWD domain of Mad1 is also indicated. **(B)** Three models proposed for the function of the Mad1–cyclin B1 interaction in the discussed papers. **(i)** The first model, proposed by Jackman et al. (2020), is that this interaction promotes Mad1 dissociation from nuclear pores, thereby enhancing its recruitment to unattached kinetochores. **(ii)** The second model, proposed by Alfonso-Pérez et al. (2019), is that this interaction promotes Mps1 recruitment to kinetochores. **(iii)** The third model, proposed by Allan et al. (2020), is that this interaction is important for Mad1 concentration in the fibrous corona, potentially because cyclin B1 scaffolds Mad1 recruitment to this kinetochore subdomain. In the second and third models, wild-type versus mutant Mad1 is depicted schematically on the two sides of a sister chromatid pair. See text for an attempt at reconciling these different models.

generate high confidence with respect to identification of a Mad1–cyclin B1 interaction, the three groups suggest different functions for this interaction. This Rashomon effect is summarized below, before we attempt to reconcile their different viewpoints.

Viewpoint 1: Mad1-associated cyclin B1–Cdk1 releases Mad1 from nuclear pore complexes

Mad1 localizes to nuclear pore complexes in interphase through an interaction with the nuclear basket protein Tpr/Megator. Jackman et al. (2020) suggest that cyclin B1 association is critical for the timely release of Mad1 from nuclear pores (Fig. 1 Bi). A mutant in Mad1 that cannot bind cyclin B1 retained its interaction with Tpr in early mitosis and was delayed in its kinetochore targeting. This effect was exacerbated upon inhibition of the checkpoint kinase Mps1, suggesting that cyclin B1–Cdk1 binding to Mad1 may cooperate with Mps1 to release Mad1 from pores and promote a robust checkpoint response. In support of this notion, a recent study highlighted the importance of Mad1 release from nuclear pore complexes for a robust checkpoint response in *Drosophila melanogaster* (Cunha-Silva et al., 2020). Specifically, Mps1 was shown to phosphorylate Megator in early mitosis in order to release Mad1 from pores. A non-phosphorylatable mutant in Megator reduced kinetochore targeting of Mad1 in mitosis and compromised checkpoint signaling, suggesting that delayed release from pores may explain a weakened checkpoint response.

Viewpoint 2: Mad1-associated cyclin B1–Cdk1 promotes Mps1 recruitment to unattached kinetochores

Alfonso-Pérez et al. (2019) contend that the Mad1–cyclin B1 interaction promotes localization of the checkpoint kinase Mps1 to unattached kinetochores. In a mutant lacking the first 100 amino acids of Mad1, the region containing the cyclin B1 association motif, Mps1 recruitment to unattached kinetochores was reduced by half (Fig. 1 Bii). Supporting this idea, recent work has shown that phosphorylation of Mps1 by Cdk1 is required for its recruitment to unattached kinetochores (Hayward et al., 2019a). Of note, Alfonso-Pérez et al.'s localization analysis was conducted using in situ–tagged Mps1, which overcomes limitations imposed by antibody-based detection of this dynamically localized kinase. Unlike Jackman et al. (2020) and Allan et al. (2020), Alfonso-Pérez et al. (2019) did not identify and selectively mutate a specific cyclin B1 interaction motif in Mad1. Thus, a caveat of their analysis is that the deleted region may affect other functions; for example, this region is suggested to be important for Mad1 nuclear localization (Sze et al., 2008). Consequently, whether the reduction in Mps1 recruitment can be attributed exclusively to loss of interaction with cyclin B1–Cdk1 remains to be clarified. Nonetheless, given the central importance of Mps1 in checkpoint signaling, the reduction in Mps1 at kinetochores may account for the weakened checkpoint response observed when Mad1 interaction with cyclin B1 is disrupted.

The results of Alfonso-Pérez et al. (2019) also have implications for the model proposed by Jackman et al. (2020). The N-terminal deletion of Mad1 they analyzed, in addition to removing the cyclin B1 interaction motif, is also predicted to reduce nuclear pore localization (Rodriguez-Bravo et al., 2014). As this deletion exhibited a compromised checkpoint, delayed release from nuclear pores may not explain the checkpoint defect observed when the Mad1–cyclin B1 association was perturbed. Generating and characterizing a precise Mad1 mutation that selectively inhibits nuclear pore association and combining it with

the motif mutation that prevents cyclin B1 binding will be important to assess the extent to which delayed release from pores accounts for the checkpoint defect.

Viewpoint 3: Cyclin B1 scaffolds Mad1 at the kinetochore corona

Allan et al. (2020) identify the same cyclin B1 association motif as Jackman et al. (2020) and employ biochemical reconstitutions with purified components to establish a direct Mad1 interaction with cyclin B1 that is dependent on this motif; notably, Cdk1 is not required for the interaction. The precise binding mechanism involved will be important to elucidate, as it may potentially reveal a new general mode of cyclin binding. Based on functional analysis, Allan et al. (2020) propose that the Mad1–cyclin B1 interaction is critical for recruitment of Mad1–Mad2 to the fibrous corona, the most external subdomain of the kinetochore that expands in the absence of microtubule attachments to form crescent and ring structures (Fig. 1 Biii; Kops and Gassmann, 2020). Checkpoint proteins, as well as the motors CENP-E and dynein, are known to localize to the fibrous corona.

Allan et al.'s proposal that cyclin B1 acts as a scaffold for Mad1–Mad2 specifically at the corona is intriguing. The authors establish that disrupting the Mad1–cyclin B1 interaction results in Mad1 loss from the corona in late prometaphase but does not generally disrupt corona formation; in addition, Mad1 localization to the nonexpanding outer kinetochore is maintained, at least partially. Their results also indicate that cyclin B1 does not localize robustly to the corona when its interaction with Mad1 is defective, with cyclin B1 kinetochore localization being significantly reduced in the absence of Mad1. Allan et al. (2020) employ a knockout and replacement strategy to generate HeLa cell lines expressing selectively mutated Mad1 that does not interact with cyclin B1 and show that the mutant protein is significantly reduced in the corona and that the checkpoint is compromised in these mutant lines. However, they did not report the effect of the selectively mutated Mad1 on cyclin B1 localization at kinetochores. Collectively, their results indicate that Mad1 corona localization requires interaction with cyclin B1, but whether this is due to cyclin B1 directly scaffolding Mad1 at the corona, as stated in their title, or is potentially an indirect effect due to loss of Mad1-associated cyclin B1–Cdk1 activity remains to be resolved. Careful analysis of cyclin B1 localization in the mutant Mad1 cell lines would help assess to what extent cyclin B may scaffold Mad1 in the corona. Allan et al.'s results also show that cyclin B1 is not fully removed from kinetochores when its interaction with Mad1 is defective, suggesting the existence of a second kinetochore-based interactor of cyclin B1–Cdk1.

Reconciling the different viewpoints

A potential means of reconciling the different views on the function of the Mad1–cyclin B1 interaction is to focus on the central role of Mps1 kinase in the proposed mechanisms. Mps1 activity is important to release Mad1 from nuclear pores (Cunha-Silva et al., 2020), to phosphorylate the kinetochore scaffold and checkpoint components such as Mad1 in order to initiate checkpoint signaling (London et al., 2012; London and Biggins, 2014; Faesen et al., 2017; Ji et al., 2017), and to assemble the fibrous corona (Sacristan et al., 2018). Thus, a reduction in kinetochore-localized Mps1 caused by loss of Mad1-associated cyclin B1–Cdk1 activity may account for the different observations. An important test for this idea will be to precisely mutate the cyclin B1 interaction motif of Mad1 in the in situ–tagged Mps1 cell line from Alfonso-Pérez et al. (2019) and assess whether reduced Mps1 kinetochore recruitment is indeed due to loss of interaction with cyclin B1–Cdk1. If this turns out to be the case, future efforts would then need to focus on understanding how Mad1-associated cyclin B1–Cdk1 promotes Mps1 recruitment and/or activation. Partial chemical Mps1 inhibition could also be employed to assess whether the different effects described here—delayed pore release, selective loss of Mad1 at the corona, and compromised checkpoint signaling—can be observed following a mild reduction of Mps1 activity. The alternative possibility, if the selective Mad1 mutants do not affect Mps1 kinetochore recruitment, is that locally recruited Mad1–cyclin B1 complexes have distinct functions at the pore and at the kinetochore, acting in parallel to Mps1. Finally, identification of the non-Mad1 kinetochore receptor for cyclin B1, whose existence is suggested by the analysis of Allan et al. (2020), will be important to explore both possibilities in the future.

In addition to extending analysis to reconcile the different views from the three studies, it will be important to assess conservation of the Mad1–cyclin B1 interaction. Mad1–Mad2 localizes to nuclear pore complexes and unattached kinetochores in all organisms analyzed so far, but it is unclear if the cyclin B1 binding motif is conserved beyond vertebrates. Additionally, conservation of cyclin B1 kinetochore localization has also not been assessed.

Conclusion

While an initial reading of the three articles is likely to confuse a reader, the central conclusion that Mad1 recruits a local pool of cyclin B1–Cdk1 activity to ensure robustness of checkpoint signaling is supported by all three studies, establishing a new mechanism by which Mad1 contributes to the spindle checkpoint in human cells. Unraveling precisely how this interaction acts in checkpoint signaling will be stimulated by the different models proposed in these studies, hopefully leading to a resolution of the Rashomon effect in the near future.

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