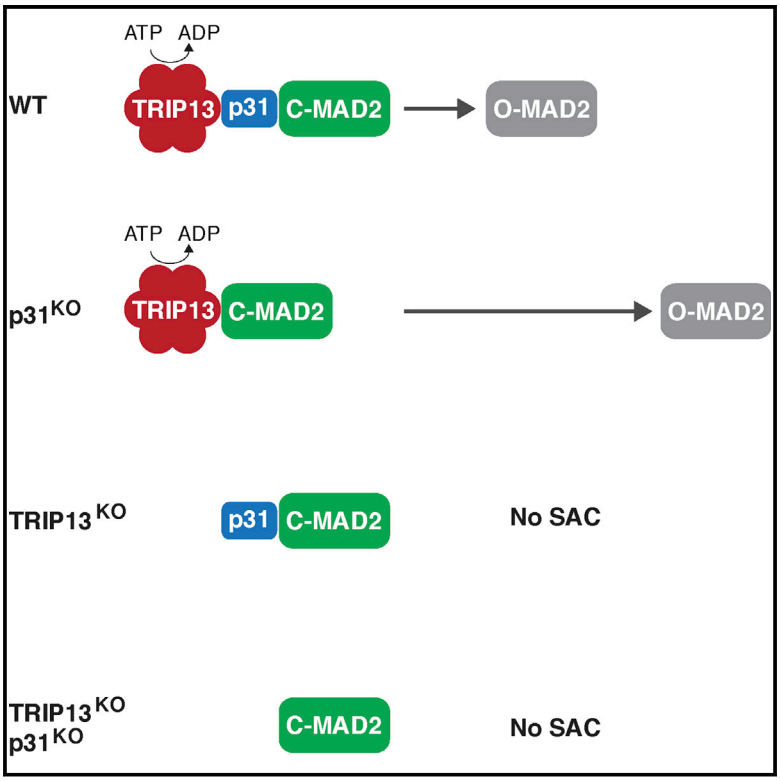
Article

Cell Reports

TRIP13 Regulates Both the Activation and Inactivation of the Spindle-Assembly Checkpoint

Graphical Abstract



Authors

Hoi Tang Ma, Randy Yat Choi Poon

Correspondence

rycpoon@ust.hk

In Brief

Mitotic exit requires the inactivation of MAD2. Ma and Poon show that MAD2 inactivation in mammalian cells requires TRIP13 and is stimulated by p31^{comet}. Moreover, TRIP13-deficient cells containing only C-MAD2 are unable to activate the spindle-assembly checkpoint.

Highlights

- Unperturbed mitosis is relatively normal in p31^{comet}- and TRIP13-deficient human cells
- MAD2 inactivation and mitotic exit are partially impaired without p31^{comet}
- TRIP13-deficient cells contain only C-MAD2
- TRIP13-deficient cells are unable to activate the spindle-assembly checkpoint

Ma & Poon, 2016, Cell Reports *14*, 1086–1099
February 9, 2016
http://dx.doi.org/10.1016/j.celrep.2016.01.001

OPEN ACCESS
CellPress

Cell Reports
Article

TRIP13 Regulates Both the Activation and Inactivation of the Spindle-Assembly Checkpoint

Hoi Tang Ma[1] and Randy Yat Choi Poon[1,*]
[1]Division of Life Science, Center for Cancer Research and State Key Laboratory of Molecular Neuroscience, The Hong Kong University of Science and Technology, Clear Water Bay, Hong Kong
*Correspondence: rycpoon@ust.hk
http://dx.doi.org/10.1016/j.celrep.2016.01.001

SUMMARY

Biochemical studies have indicated that p31^{comet} and TRIP13 are critical for inactivating MAD2. To address unequivocally whether p31^{comet} and TRIP13 are required for mitotic exit at the cellular level, their genes were ablated either individually or together in human cells. Neither p31^{comet} nor TRIP13 were absolutely required for unperturbed mitosis. MAD2 inactivation was only partially impaired in p31^{comet}-deficient cells. In contrast, TRIP13-deficient cells contained MAD2 exclusively in the C-MAD2 conformation. Our results indicate that although p31^{comet} enhanced TRIP13-mediated MAD2 conversion, it was not absolutely necessary for the process. Paradoxically, TRIP13-deficient cells were unable to activate the spindle-assembly checkpoint, revealing that cells lacking the ability to inactivate MAD2 were incapable in mounting a checkpoint response. These results establish a paradigm of the roles of p31^{comet} and TRIP13 in both checkpoint activation and inactivation.

INTRODUCTION

Mitotic exit is driven by a ubiquitin ligase called anaphase-promoting complex/cyclosome (APC/C) with its targeting subunit CDC20 (Pesin and Orr-Weaver, 2008). Unattached kinetochores or the absence of tension between the paired kinetochores activates the spindle-assembly checkpoint (SAC), which then prevents the activation of APC/C^{CDC20} (Musacchio, 2015). Hence, the SAC ensures that mitotic exit only occurs after all the chromosomes have achieved proper bipolar spindle attachment.

Components of the SAC include a diffusible MCC complex containing MAD2, BUBR1, BUB3, and CDC20, which facilitates the inhibition of APC/C^{CDC20} by MAD2. Binding to APC/C^{CDC20} requires a change in MAD2 from an open conformation (O-MAD2) to a more stable closed conformation (C-MAD2) (Yu, 2006). According to a generally accepted model, the C-MAD2 that binds to MAD1 at the kinetochores serves as a template for converting O-MAD2 into C-MAD2 (De Antoni et al., 2005). The C-MAD2 then forms the MCC, which in turns may serve as a template for converting more O-MAD2 into C-MAD2. This model provides a mechanism for autoamplification and propagation of the SAC signal away from the kinetochores.

While significant progress has been made to unravel the underlying principles of SAC activation, lagging behind is our knowledge of how the SAC is turned off once the checkpoint is satisfied. Specifically, how C-MAD2 is converted back to O-MAD2 is a fundamental but outstanding question. There is compelling evidence that C-MAD2 is neutralized by binding to a protein called p31^{comet} (also called MAD2L1BP) in mammalian cells (Habu et al., 2002). For example, the SAC can be disrupted by p31^{comet} overexpression, and mitotic exit can be delayed by p31^{comet} knockdown (Habu et al., 2002; Xia et al., 2004; Chan et al., 2008a). Crystal structure of p31^{comet} reveals a folding similar to C-MAD2, suggesting that structural mimicry may be involved in checkpoint silencing (Yang et al., 2007).

There is evidence that p31^{comet} is involved in the removal of MAD2 from MCC (Westhorpe et al., 2011). Disassembly of MCC by p31^{comet} is an ATP-dependent process, involving a p31^{comet}-interacting protein called TRIP13 (Eytan et al., 2014; Wang et al., 2014). TRIP13 is an AAA^{+}-ATPase that also functions in meiotic DNA break formation and recombination, checkpoint signaling, and chromosome synapsis (Vader, 2015). Recent structural and in vitro studies indicated that p31^{comet} acts both as an activator and an adaptor for TRIP13 for promoting C-MAD2 to O-MAD2 conversion (Ye et al., 2015). Whether p31^{comet} and TRIP13 are indeed responsible for MAD2 inactivation in the cell remains an important question.

In this study, we investigated the relative role of p31^{comet} and TRIP13 in SAC inactivation. We showed that both p31^{comet} and TRIP13 were not essential for unperturbed mitosis in different cell lines. We also found that C-MAD2 to O-MAD2 conversion was partially impaired in p31^{comet}-deficient cells and completely defective in TRIP13-deficient cells. Paradoxically, cells lacking TRIP13 displayed a relatively short mitosis and were unable to activate the SAC. We further demonstrate that direct binding of p31^{comet} to either MAD2 or TRIP13 was sufficient to promote mitotic exit.

RESULTS

p31^{comet} Is Not Essential for Unperturbed Mitosis

To address unequivocally whether p31^{comet} is required for mitotic exit, p31^{comet} gene (*MAD2L1BP*) was disrupted using TALEN (Figure S1A). Several clones of p31^{comet}-knockout