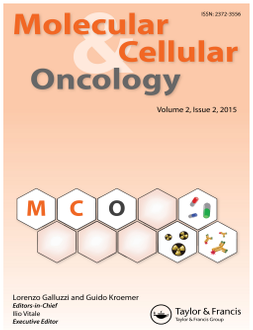

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COMMENTARY

TRAMM, a new player in CENP-E biology

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ABSTRACT
Mitosis is a highly orchestrated process with morphologically defined stages and is subject to checkpoints that ensure the proper distribution of chromosomes. Centromere-associated protein E (CENP-E), a protein expressed during mitosis, is a potential target of cancer therapeutics. Our laboratory has recently implicated a protein called TRAMM (trafficking of membranes and mitosis) in the recruitment of CENP-E to kinetochores.

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Cancer is defined as uncontrolled cell division, often resulting in aneuploid cells. The ability to selectively stop such division has been the subject of cancer research for decades. Intuitively, an ideal therapeutic target would be a protein that is expressed only during the mitotic phase of the cell cycle. Inhibition of the function of such a target could, in theory, prevent cell division. By extension, identifying the full complement of proteins that interact with such a target, and how these other proteins regulate the function of the target, is a necessary requirement for the eventual development of therapeutics.

The mitotic kinesin centromere-associated protein E (CENP-E) integrates several steps within mitosis.[1] As a kinesin motor protein, CENP-E is involved in chromosome congression prior to metaphase by aiding the establishment and maintenance of connections between mitotic chromosomes and spindle microtubules, and by physically moving the chromosomes to the metaphase plate. This motor function resides within the amino-terminal region of the protein. In addition, CENP-E has been reported to bind to a number of different proteins that mediate the spindle assembly checkpoint (SAC), a mechanism that ensures proper chromosome alignment prior to the onset of anaphase. Inhibition of CENP-E activity by either specific antibodies or RNA interference results in arrest of cell division and eventual death of the cell. Since CENP-E plays such a critical function during mitosis and is predominantly expressed during mitosis[2] it has become the focus of cancer therapeutics, with several inhibitors having been designed and undergoing clinical trials.[3]

Following chromosome condensation during prophase, a large protein structure called the kinetochore assembles on the centromeric region of the DNA. The kinetochore is one of the most complex protein assemblies known, with over 100 distinct polypeptides associating with it either stably or transiently.[4,5] How each of these proteins, including CENP-E, is recruited to the kinetochore has been the subject of intense research. Inhibition of several proteins, as well as post-translational modification of CENP-E itself, has been reported to affect recruitment of CENP-E to varying degrees. However, our new study suggests that a previously unknown player in mitosis called TRAMM (trafficking of membranes and mitosis; formerly known as both TTC-15 and TrappC12) affects CENP-E recruitment to an even greater extent.[6]

The revelation that TRAMM functions in mitosis was unexpected. This protein was originally identified as a member of a large complex involved in membrane trafficking called TRAPP (transport protein particle).[7] Indeed, as the twelfth known subunit of this complex, the protein was originally called TrappC12. Inhibition of TRAMM, but not of any other TRAPP subunit, in HeLa cells by RNA interference resulted in a sharp increase in the mitotic index. Analysis of the resulting phenotype revealed a defect in chromosome congression resulting in activation of the SAC.

Biochemical fractionation of cells demonstrated that small amounts of TRAMM fractionated with a nuclear marker. A fraction of this protein associated with mitotic chromosomes and was loosely localized to the kinetochore. The kinetochore localization, combined with the chromosome congression defect, suggested that kinetochore structure may be affected. Indeed, using fluorescence intensity measurements, a number of kinetochore proteins were found to have a reduced presence at the kinetochores of aligned chromosomes in TRAMM-deleted cells. These included proteins that were more distally associated with the centromere but not proteins proposed to be in the inner kinetochore layer.[8] The most profoundly affected protein was CENP-E, whose level at kinetochores was merely 6% of that in control cells. This was notable since the phenotype of a TRAMM knockdown resembled that of CENP-E knockdown. A subsequent recruitment experiment revealed that TRAMM is required for the recruitment of CENP-E to kinetochores.

Mitotic phosphorylation of TRAMM was documented to occur as the cells entered mitosis and was complete at the onset

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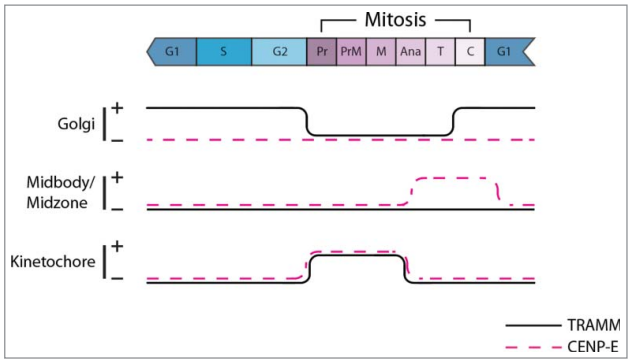

Figure 1. Localization of TRAMM and CENP-E overlaps during early mitosis. During interphase, TRAMM (trafficking of membranes and mitosis) is a component of the TRAPP (transport protein particle) membrane trafficking complex and localizes to Golgi membranes whereas expression of centromere-associated protein E (CENP-E) is suppressed. Mitotically phosphorylated TRAMM, detected from entry into mitosis until anaphase, associates with kinetochores and facilitates the recruitment of CENP-E to these structures. At the onset of anaphase, TRAMM is rapidly dephosphorylated and relocalizes to the emerging Golgi membranes. In contrast, CENP-E localizes to the midzone during anaphase and then to the midbody during cytokinesis. The solid black lines indicate TRAMM and the dashed red lines indicate CENP-E. + and − indicate localization or no localization, respectively, and are not intended to be interpreted quantitatively. Pr: prophase; PrM: prometaphase; M: metaphase; Ana: anaphase; T: telophase; C: cytokinesis.

of anaphase. This temporal phosphorylation correlated with the localization patterns of TRAMM and CENP-E; maximal colocalization was detected during mitotic phosphorylation of TRAMM whereas distinct localization of the 2 proteins was apparent from anaphase onwards. Specifically, after anaphase TRAMM relocalized to the Golgi complex, presumably in preparation for the resumption of membrane trafficking, whereas CENP-E localized to the midzone and ultimately the midbody.

If TRAMM is part of the TRAPP complex during interphase, how is it released from this complex during mitosis to associate with the kinetochore? Size exclusion chromatography revealed that the mitotic form of TRAMM was no longer associated with the TRAPP complex and fractionated at a smaller molecular size. This form of the protein had a slower mobility on SDS-polyacrylamide gels, suggesting that it is mitotically phosphorylated. Thus, it is plausible that mitotic phosphorylation of TRAMM releases it from the TRAPP complex.

Phosphorylation of TRAMM is likely required for more than just release of the protein from the TRAPP complex. Five potential sites of phosphorylation on the TRAMM polypeptide were investigated and a phosphomimetic mutant, in which all 5 of these sites were changed to aspartic acid, was shown to recruit CENP-E more efficiently than the non-phosphorylatable (alanine) mutant. Interestingly, mutation of one of these residues, the invariant Ser184, has been reported in breast cancer cells (http://www.cbioportal.org).

Other membrane trafficking proteins have been reported to have mitotic functions.[9] However, TRAMM appears to be unique in that it cycles between two large complexes to perform its two functions (Fig. 1). Overall, this study identified a new factor important for CENP-E function, raising interesting questions pertaining to the role of CENP-E as a therapeutic target. How exactly does TRAMM recruit CENP-E? What role does phosphorylation of TRAMM play in this recruitment? What other proteins interact with TRAMM? Most importantly, can any of these interactions ultimately be exploited by small molecules as a potential therapeutic? Future studies on this moonlighting protein will begin to reveal answers to these intriguing questions.

Disclosure of potential conflicts of interest

No potential conflicts of interest were disclosed.

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