

New insights into Cdk2 regulation during meiosis

Michitaka Isoda¹, Petra Mikolcevic¹, Angel R. Nebreda^{1,2}

¹Institute for Research in Biomedicine (IRB Barcelona), Barcelona Institute of Science and Technology, 08028 Barcelona, Spain

²Institució Catalana de Recerca i Estudis Avançats (ICREA), 08010 Barcelona, Spain

Correspondence: michitaka.isoda@irbbarcelona.org, angel.nebreda@irbbarcelona.org

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Cyclin dependent kinases (CDKs) are proline-directed serine/threonine kinases that play a central role in regulating cell cycle progression. Since the activity of CDKs depends on the binding of regulatory subunit named cyclins, the analysis of the CDK functions that have been studied so far has usually gone in parallel to the study of cyclins. However, the proteins referred to as RINGO or Speedy, which have no amino acid sequence homology to cyclins, have been found to function as atypical CDK activators.

The first RINGO protein was identified as a new activator of Cdk1 and Cdk2, which induced the meiotic maturation of frog (*Xenopus*) oocytes. Interestingly, CDKs activated by *Xenopus* RINGO were found to phosphorylate different sites on the kinase Myt1 than those phosphorylated by cyclin-CDK complexes¹. In fact, Myt1 is much more efficiently inhibited by RINGO-CDK than by cyclin-CDK, supporting the idea that RINGO-activated CDKs may have different functions. RINGO proteins are conserved in metazoans and several mammalian RINGO family members have been identified. The best-studied mammalian family member is RingoA, whose mRNA is highly expressed in mouse gonads, suggesting that RingoA might regulate meiotic progression, as reported for *Xenopus* RINGO in oocytes. Genetic studies published during the past decade have provided good evidence that Cdk1 is the only CDK family member that is essential for mouse cell proliferation, and genetic inactivation of Cdk1 has been shown to cause mouse embryonic lethality². In contrast, Cdk2 is not essential for somatic cell proliferation in mouse but it is indispensable for male and female meiosis. Interestingly, the meiotic phenotypes that are observed in Cdk2 knockout spermatocytes and

oocytes have not been reported in any of the mice deficient for particular cyclins. A recent report has shown that the combined deletion of cyclins E1 and E2 produces similar meiotic phenotypes in spermatocytes as the deletion of Cdk2³. However, cyclins E1 and E2 do not co-localize with Cdk2 during prophase I of meiosis, neither at telomeres nor at crossing-over sites, suggesting that they are unlikely to be involved in Cdk2 activation at these specific locations. These findings support the existence of a cyclin-independent mechanism of Cdk2 activation in germ cells.

We have recently reported that RingoA knockout mice are born with the expected Mendelian frequency and do not show any overt differences compared to their wild-type littermates. However, they are sterile and have hypoplastic testes and ovaries, supporting an important role for RingoA in mammalian meiotic progression⁴. Importantly, spermatocytes and oocytes that are deficient in RingoA show identical phenotypes as those deficient in Cdk2⁵, namely they are mostly arrested in a pachytene-like stage of prophase I with non-homologous chromosome pairing, telomere detachment from the inner nuclear membrane (INM) and telomere fusion⁴. In addition, RingoA-deficient spermatocytes and oocytes show strong accumulation of γ H2AX, which is a hallmark of unrepaired double strand breaks. The arrested germ cells then undergo apoptosis probably due to the pachytene checkpoint, which is activated by the accumulation of unrepaired DNA⁴.

RingoA localizes to the telomeric regions in pachytene spermatocytes, as it has been reported for Cdk2⁵, co-localizes with Cdk2 from the leptotene stage and then disappears from telomeres as cells enter the diplotene stage. RingoA and Cdk2 also co-localize along the asynapsed sex chromosome arms in pachytene spermatocytes. Interestingly, RingoA-deficient spermatocytes show impaired localization to telomeres of Sun1, a protein required for telomere tethering to the INM, whereas the telomeric localization of TERB1, another protein required for telomere-INM tethering, is maintained⁴. RingoA-Cdk2 can phosphorylate *in vitro* the N-terminus of Sun1, which is the domain involved in binding to TERB1, so it is possible that RingoA-Cdk2 regulates the tethering of telomeres to the INM through phosphorylation of Sun1, but the detailed physiological mechanism remains unclear. In contrast to the co-localization of RingoA and Cdk2 in telomeres and sex chromosomes, RingoA does not co-localize with Cdk2 at crossing-over sites. Since Cdk2 has been reported to bind the late-recombination marker protein Mlh1⁶, Cdk2 might participate in late-recombination events through the binding to a regulatory subunit other than RingoA.

In addition to the regulation of Sun1 localization, RingoA seems to control chromatin methylation. RingoA-deficient spermatocytes show decreased levels of the histone H3 trimethylated at Lys9 (H3K9-3me), whereas the amount of the histone H3 trimethylated at Lys4 (H3K4-3me) is increased⁴. Reduction of H3K9-me3 is thought to cause telomere shortening, which is actually observed in RingoA-deficient spermatocytes⁴. Moreover, aberrant chromosome methylation has been associated with non-homologous meiotic chromosome pairing⁷, and might be also related to the telomere fusion and non-homologous chromosome pairing that are observed in RingoA-deficient germ cells. How RingoA could modulate histone H3 methylation during meiosis remains unclear, but it is interesting to speculate that histone H3 phosphorylation by RingoA-Cdk2 could be involved.

Our results suggest that RingoA regulates the telomere length, which is critical for meiotic progression⁴. Since the maintenance of telomere length is important for cell immortalization and malignant transformation, further elucidation of RingoA functions may help to understand tumorigenesis mechanisms.

References

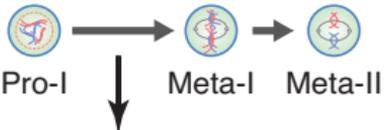
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WT

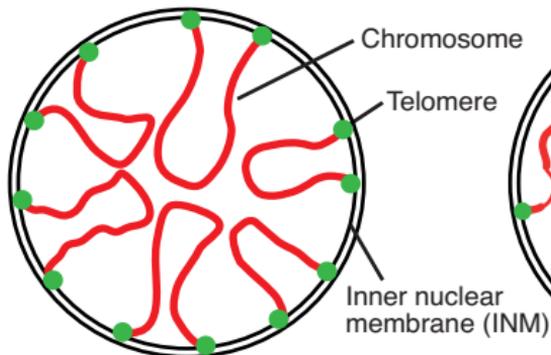
Fertile



Spermatocytes/Oocytes



Pachytene stage



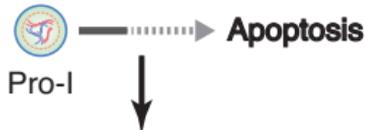
- Attachment of telomeres to INM
- Homologous chromosome synapsis

RingoA KO

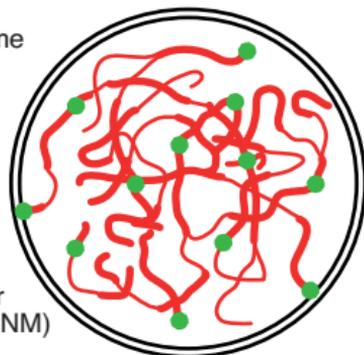
Sterile



Spermatocytes/Oocytes



Pachytene-like stage



- Detachment of telomeres from INM
- Non-homologous chromosome synapsis
- Telomere fusion