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ORAL PRESENTATION

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HIRA-mediated H3.3 deposition is required for de novo paternal nuclear envelope formation in mouse zygotes

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Paternal genome undergoes dynamic chromatin remodeling after fertilization. The first event is the exchange of protamines with maternally deposited histones including H3.3. Although HIRA has been shown to be responsible for H3.3 deposition in invertebrates, whether such function is conserved in vertebrates is not known. Furthermore, the biological significance of the protamine-histone exchange is also unknown. To address these questions, we depleted the maternal pool of HIRA protein by using an *in vitro* oocyte growth system where siRNA is injected into small growing oocytes with surrounding granulosa cells. In HIRA-depleted zygotes, H3.3 is not incorporated into the paternal genome while protamines are removed, indicating that protamine-removal is uncoupled from H3.3 deposition. Inhibition of H3.3 deposition prevents the de novo nucleosome assembly and the formation of paternal pronucleus. Immunostaining of nuclear envelope components revealed the lack of Lamin B1 and nuclear pore complex assembly and the defect in flattening of endoplasmic reticulum (ER) sheets, the precursor of nuclear envelope, only in the paternal genome of HIRA-depleted zygotes. Furthermore, the HIRA-depletion prevented the localization of Ran, a GTPase required for ER fusion, in the paternal genome. Together, our results not only provide the first direct evidence that HIRA is responsible for H3.3 deposition of the paternal genome in vertebrates, but also suggest that HIRA-mediated nucleosome assembly is a pre-requirement for the recruitment of factors, such as Ran GTPase, involved in ER flattening and nuclear envelope formation during de novo paternal pronucleus formation.

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