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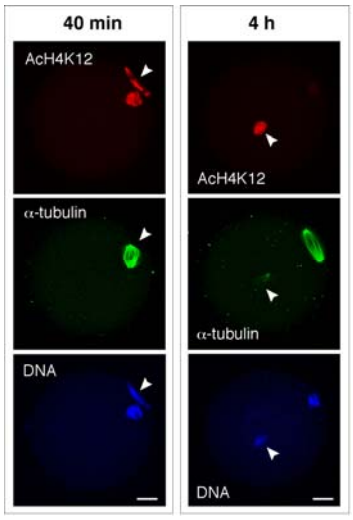
Asymmetric, nucleus-dependent chromatin remodeling in mammalian oocytes

October 19, 2006 – Reprogramming, chromatin remodeling and totipotency are all frequently invoked concepts in the description of developmental processes, but the terms are themselves imperfectly defined and our understanding of the underlying biology is poor. It is agreed, however, that in mammals, totipotency, reprogramming and chromatin remodeling - however you define them - occur immediately after fertilization. Only hours after fusion with a sperm cell, the fertilized egg becomes a totipotent one-cell embryo, able to give rise to an individual. It is known that soon after sperm-egg fusion, the sperm nucleus is stripped of its coat, and its chromatin is erased and reassembled from scratch. But whether this remodeling is accomplished by proteins synthesized after fertilization, or by maternally-supplied factors (or a combination of both) remains unclear.

Now, in an article published in *Developmental Biology*, Naoko Yoshida and colleagues from the Laboratory of Mammalian Molecular Embryology (Tony Perry; Team Leader) address this distinction in the first detailed study of the remodeling of sperm chromatin by metaphase II (mII) oocytes. During natural fertilization, the entry of a sperm induces a cell-cycle change known as meiotic progression, causing the oocyte to start behaving like an embryo. To measure the remodeling effects of mII (maternal), as opposed to post-fertilization (embryonic), cytoplasm on sperm chromatin, the team first eliminated the ability of sperm to induce meiotic progression. They confirmed that this had not otherwise compromised the biological potential of the inactive sperm by injecting them into oocytes and supplying an artificial signal to initiate development; the resulting embryos were able to develop to term, suggesting that the inactivation had not significantly altered the functional potential of the chromatin.



All chromatin remodeling events are equal, but some are more equal than others. Immunofluorescence microscopy of AcH4-K12 (red) in enucleated mII oocytes coinjected with an inactivated sperm head plus a cumulus cell nucleus. Spindles are indicated by α-tubulin staining (green, second row) and genomic DNA is stained blue (third row). Each column represents the same injected oocyte, with times post-injection indicated at the top. Arrowheads show the sperm-derived genome. AcH4-K12 is high in both the sperm and cumulus nucleus after 40 min but markedly declines only in the cumulus nucleus. Scale bars = 20 μm.

Yoshida et al. then analyzed the changes undergone by inactivated sperm chromatin within mII-arrested oocytes. Immunofluorescence microscopy showed that the sperm chromatin decondensed and then recondensed, demonstrating that sperm-derived genomes acquire maternally-derived histones independently of meiotic progression. However, the maternal cytoplasm did exert differential effects on the genomes supplied by each parent. While maternal histone H3 methylation was high, methylation of paternal H3 on lysines 4 and 9 (K4 and K9) remained low.

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Additionally, paternal (sperm) genomic DNA underwent cytosine demethylation, even when the sperm were boiled prior to injection, providing strong evidence that mII cytoplasm is sufficient to mediate the process. This is contrary to previous conclusions, which "it turned out represented something of a *Hineininterpretierung*", according to Perry.

In mitotic cells, the level of histone acetylation influences gene expression, usually in the form of up-regulation, so it could play an important role in very early embryos. In the mII oocyte, paternal, but not maternal, chromatin acquired maternally-derived K12-acetylated H4 (AcH4-K12) independently of microtubule assembly and regardless of whether or not maternal chromatin was present. This state persisted without typical maturation-associated deacetylation, and was unaffected by the level of DNA (cytosine) methylation. In contrast, somatic cell nuclei underwent rapid H4 deacetylation; sperm and somatic chromatin even exhibited unequal AcH4-K12 dynamics when injected together into the same mII oocyte. This implies an extraordinary ability of the mII oocyte to discriminate between two types of exogenous chromatin and modify them in different ways.

But how and why do mII oocytes achieve this discrimination? The inhibition of somatic histone deacetylation showed that histone acetyl transferase (HAT) activity was present in the oocytes together with histone deacetylase (HDAC). From a series of experiments, Yoshida and colleagues inferred that mammalian oocytes are able to specify the histone acetylation status of given nuclei by differentially targeting HAT and HDAC activities. However, the team also found that asymmetric H4 acetylation of the two parental chromatin sets during and immediately after fertilization was dispensable for development when both were hyperacetylated, so the function of this differential targeting remains enigmatic.

These studies delineate non-zygotic chromatin remodeling and suggest a powerful standardized model with which to study de novo genomic reprogramming.

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