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Regulation of Histone Acetylation during First Mitosis in Bovine Clone Embryos

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Histone acetylation as epigenetic marker plays a critical role in gene expression through the interaction of nucleosomes with DNA, modulating the efficiency which RNA-polymerase can interact with promotors to initiate transcription. After fertilization, highly acetylated chromatin takes place and maintain during 1cell stages. The hyperacetylation may lead minor genome activation for survival and cleavage, and then may affect embryonic genome activation and development to blastocyst. In the present study, we examined changes of histone acetylation during mitosis by using immunofluorescence analysis with anti-acetylated histone H4 lysine 5 (anti-AcH4K5) in chromatin of somatic cells and 1cell-embryos fertilized or reconstructed. The degree of H4 hyperacetylation in bESF cells treated with and without trichostatin A (TSA), a specific inhibitor of histone deacetylase, increased at 60h after treatment during cell cycling and the level raised until about twice as much as non-treated cells. Localization of highly acetylated histone through mitosis phase was inquired. As the cells progressed into metaphase in normal bESF cells, the levels histone h4 hyperacetylated chromosomes were dercreased dramatically as chromatin condensed and decreased state of acetylated chromosome was continued from prometaphase to early telophase. Resumption of hyperacetylation was observed at late telophase and the grades of hyperacetylation signals gradually were increased through late telophase/early interphase. However, after TSA treatment, chromosomes in prophase, metaphase, and anaphase were labeled intensely with AcH4K5 antibody, and condensation or decondensation of chromatin was not effected the histone acetylation in TSA treated-cells. In 1cell-embryos during mitosis, acetylation signal of NT embryos disappeared at the first metaphase, whereas IVF and TSA-NT embryos still exhibited acetylated histone H4K5. These differences appears to be due to cell memory of donor cells. Thus, we have found that cloned bovine embryos with primary fibroblasts are abnormally remodeled in terms of histone acetylation as compared to IVF embryos, indicating that epigenetic reprogramming of histone modification aberrantly occurs as early as the pronuclear stage of the reconstructed oocyte.

Key Words : ***Histone acetylation, Trichstatin A, Embryos, Mitosis, Donor cell***