—Mini Review—

Oocyte-Specific Linker Histone in Mammalian Species

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Abstract: *The linker histones constitute the major proteins bound to linker DNA, the DNA bridging nucleosome core particles. The composition of the linker histone fraction is tissue- and species-specific, as well as developmentally-regulated. As such, linker histones play a critical role in the higher order packaging of chromatin and thus, inevitably, in an impressive array of regulatory functions. Recently, we uncovered a mammalian oocyte-specific linker histone H1oo homologous to the cs-H1 of sea urchin and to the B4 of the frog in the course of a differential screening project. H1oo protein localized to the intact germinal vesicle (GV) of preovulatory oocytes, to the condensed chromosomes of ovulated oocytes arrested at metaphase (M) II, and to the first polar body. The pronuclei were both positive at the 1-cell stage. Nuclear staining, however, was reduced in 2-cell embryos and was no longer detectable at the 4-cell stage of embryonic development. These findings indicate oocyte-specific linker histones may play some roles in genome-wide reprogramming in vertebrate eggs.*

Key words: *Mouse, Oocyte-specific linker histone, Reprogramming*

Histones and Nucleosomes

Eukaryotic DNA is assembled into chromatin primarily through interaction with small basic proteins described as histones. There are two types of histone: the core histones (H2A, H2B, H3 and H4) and the linker histones such as H1. Two molecules of each of the core histones form an octamer around which 146 bp of DNA is wrapped. The DNA that is in between nucleosome core particles is called linker DNA and is bound by the linker histones (Fig. 1). It has long been believed that the primary function of linker histones is to help create or maintain the compact higher order structure of chromatin fiber. Since the compact chromatin fiber should be refractory to transcription, linker histones have been thought of as non-specific repressors.

Although histone H1 is much less conserved across species than are the individual core histones, it is possible to define a typical structure. Most linker histones have a three-domain structure [1]: a central globular domain, flanked by N- and C-terminal tail domains. The globular domain has long been proposed to bind to linker DNA where it enters and leaves the nucleosome. The C-terminal tail domain is rich in lysine, serine, proline and alanine. This tail domain probably binds to the linker DNA, neutralizing its charge and facilitating chromatin condensation [2]. The most distinctive feature of linker histones is the specific timing of their expression during the life cycle.

To date, eight subtypes of H1 have been identified in mammals [3]. Five of these are collectively termed the somatic subtypes and are called H1a, H1b, H1c, H1d, and H1e. All of these genes are located in the major histone gene cluster together with core histone genes on human chromosome 6 [3] and mouse chromosome 13 [4]. The somatic H1 mRNAs are characterized by a specific 3'-UTR region, lack of polyadenylation signal, and a sequence capable of forming a stem-loop structure, except for the H1c gene [5]. The sixth subtype, termed the replacement variant, H1(0), differs substantially from the somatic subtypes. The gene is located on mouse chromosome 15, the mRNA is polyadenylated, and the protein is smaller and more

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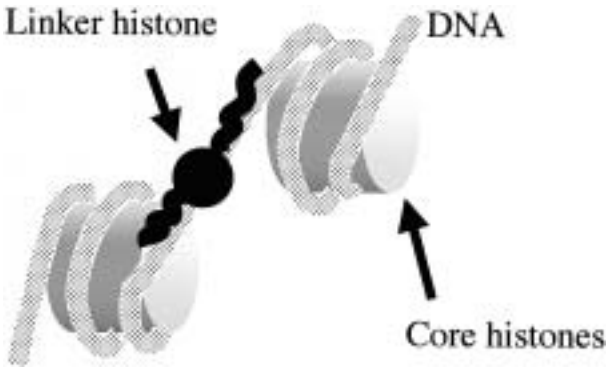

Fig. 1. Schematic diagram of relationship between DNA and histones. Eukaryotic DNA is assembled into chromatin primarily through interaction with small basic proteins described as histones.

lysine-rich than the somatic H1 subtypes [3]. H1(0) is expressed in a variety of terminally differentiated cells. The seventh subtype is H1t, which is expressed only in spermatocytes [5]. The eighth one is oocyte-specific linker histone H1oo described below [6].

Somatic H1 mRNAs are abundant during the S phase but are present only at low levels during the G1 and G2 phases. In contrast, histone H1(0) expression is not tightly linked to DNA replication. It is expressed at a low level throughout the cell cycle. Thus, proliferating cells in culture contain almost exclusively somatic histone H1, with H1c, H1d, and H1e representing up to 95% of the total H1 content [7]. After cells cease proliferation and begin to differentiate, histone H1(0) accumulates as the somatic H1 declines.

Oocyte Specific Linker Histones

A shift in the expression of linker histone subtype expression appears to be a notable feature of early embryonic development. There are several examples of the association of a specific linker histone H1 expression with particular transitions during development. In sea urchin, there are at least six different H1 subtypes. A cleavage stage H1 (cs-H1), constitutes the major form of linker histone during the rapid cellular divisions after fertilization [8]. Several hundreds of copies of the alpha subtype are then expressed in early embryogenesis and this expression is dramatically shut down at the blastula stage. The expression of two other H1 subtypes is then turned on at the blastula-gastrula transition. This differential expression of H1 subtypes is also conserved in vertebrates. In frogs, linker histone B4 is the only linker histone present in eggs and during the first divisions after fertilization [9, 10]. In frog, Midblastula transition and the activation of zygotic gene expression are associated with a dramatic decrease in B4 content and a simultaneous accumulation of somatic H1. Based on the sequence similarity of the proteins in question, as well as their expression pattern, it has been proposed that the cs-H1 and B4 constitute trans-species homologues. In mammals, the absence of immunoreactive somatic *H1* histones in fully grown oocytes and early embryos, as indicated by immunofluorescence and immunoblotting, implies that this histone species is absent during oogenesis and early embryogenesis [11, 12]. Indeed, the somatic histone *H1* first becomes detectable in chromatin at the two-cell stage, a time when the embryonic genome becomes transcriptionally active [12]. Clearly then, an oocytic-embryonic switch in the linker histone subtype is likely to occur during mammalian embryogenesis as well. Recently, we uncovered a mammalian oocyte-specific linker histone H1oo homologous to the cs-H1 of sea urchin and to the B4 of the frog in the course of a differential screening [Suppression Subtractive Hybridization (SSH)] project [6]. Amino acid BLAST analysis revealed H1oo displayed the highest sequence homology to the oocyte-specific B4 histone of the frog, the respective central globular domains displaying 54% identity (Fig. 2). Substantial homology to the cs-H1 protein of the sea urchin oocyte was also apparent. While most oocytic mRNAs corresponding to somatic linker histones are not polyadenylated (and remain untranslated), the mRNAs of oocyte-specific linker histones are polyadenylated, a process driven by the consensus signal sequence, AAUAAA, detected in the 3'-untranslated region of the H1oo cDNA.

H1oo Expression and Embryonic Development

In mammals, H1oo mRNA was readily detectable in GV oocytes by RT-PCR (Fig. 3). Following fertilization, however, a substantial decrease was evident in H1oo expression. H1oo transcripts were all but undetectable at the 8-cell stage embryo. Western blot analysis of H1oo protein in GV oocytes disclosed a major 42-*kDa* band as well as a minor 37-*kDa* band (Fig. 3). The minor band could reflect a cross-reaction of the antibody with H1oo-related proteins, or partial cleavage, to mention a few hypothetical possibilities. The H1oo protein also declines rapidly following fertilization and all but disappears by the 2-cell stage. These results

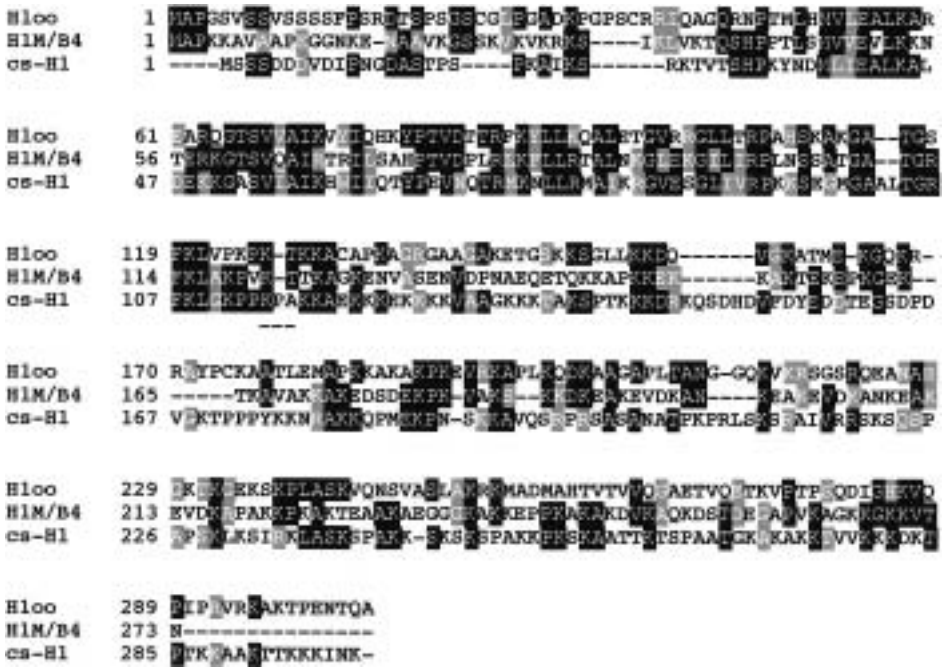

Fig. 2. Amino acid sequence alignment of H1oo with B4 (Xenopus) and cs-H1 (sea urchin). Identical amino acid residues are darkly shaded; similar amino acids are lightly shaded; unrelated residues display a white background. (Redrawn with permission from ref. [6] The company of Biologists LTD.)

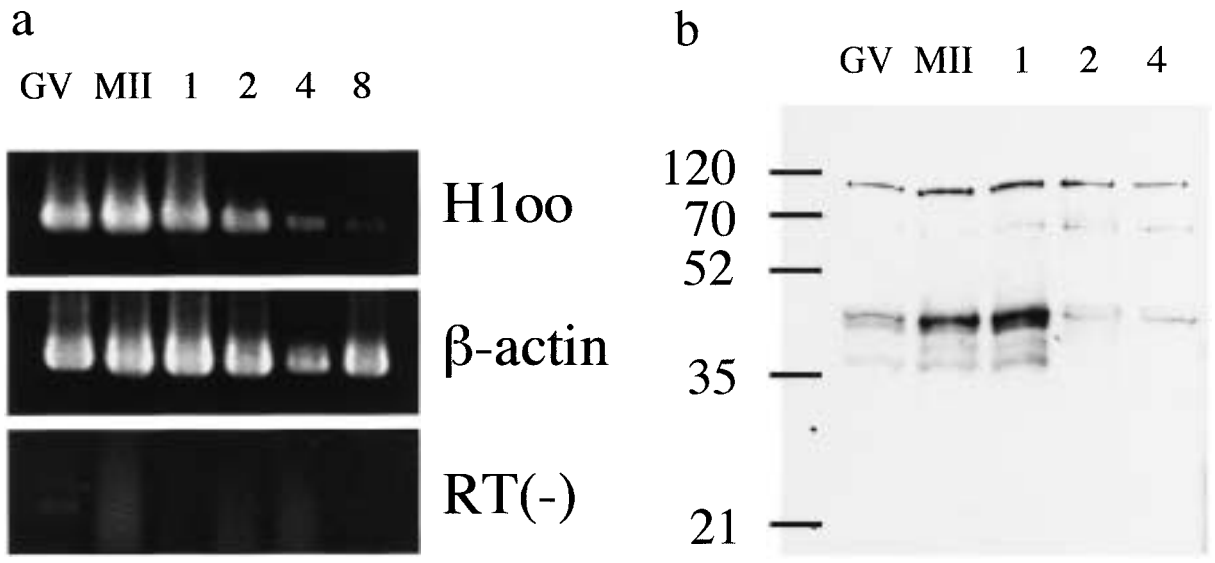

Fig. 3. RT-PCR and immunoblot analysis of H1oo during preimplantation development. a. RT-PCR: A substantial decrease in *H1oo* expression was evident after fertilization. *β*-actin primers were used as a positive control. b. Immunoblotting: H1oo protein declines rapidly and almost disappears at the 2-cell stage. (Redrawn with permission from ref. [6] The company of Biologists LTD.)

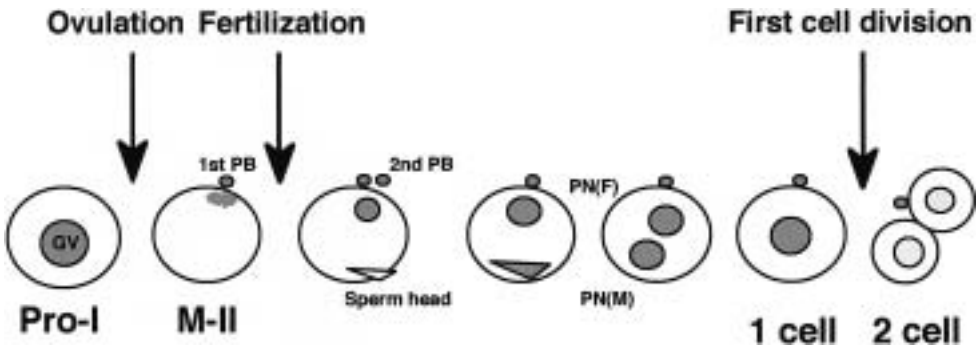

Fig. 4. Schematic diagram of H1oo expression in oocytes and preimplantation embryos. H1oo localized to the nucleus of oocytes and to the first polar body. After fertilization, H1oo was detected in the sperm head. H1oo nuclear staining was reduced in the 2-cell embryo.

suggest that the H1oo transcript is of maternal origin and that it and its encoded protein are promptly degraded following fertilization.

H1oo is localized to the intact germinal vesicle (GV) of preovulatory oocytes, to the condensed chromosomes of ovulated oocytes arrested at metaphase (M) II, and to the first polar body. Early 1-cell stage embryos (prior to extrusion of the second polar body), featured H1oo immunoreactivity in condensed maternal metaphase chromatin but not in the sperm head. However, following second polar body extrusion, H1oo immunoreactivity was detected in the swollen sperm head as well as in the second polar body. The second polar body remained brightly fluorescent throughout early embryogenesis. Nuclear staining, however, was somewhat reduced in 2-cell embryos, as compared with the 1-cell stage. At the 4-cell stage of embryonic development, nuclear staining was no longer detectable, although as stated earlier, a strong signal persisted in the second polar body. Ontogenetic studies of H1oo are summarized in Fig. 4.

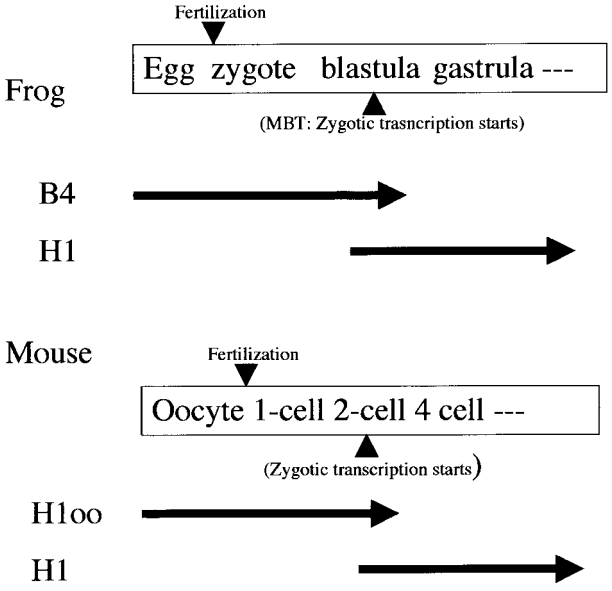

Fig. 5. Developmental regulation of linker histone in frog and mouse.

What is the Function of the Oocyte-specific Linker Histone?

The nucleus of a fertilized egg is totipotent in that all of the differentiated cell types can be derived from it. The success of frog and mammalian cloning through the transfer of somatic nuclei into eggs or oocytes has stimulated interest in the basic molecular mechanisms involved in reprogramming nuclei [13]. However, the efficiency of cloning using somatic nuclei in the adult animal is still low. Although the DNA sequence content of nuclei remains unchanged as development proceeds, the repertoire of genes that are expressed in a given cell type becomes limited. An understanding of these mechanisms will potentially provide insight into the significance of epigenetic events in establishing a developmental program [13]. In Xenopus laevis, nuclei from the blastula stage, when zygotic transcription starts, support development after transfer to enucleated eggs. The genomic potential of more advanced embryonic nuclei declined dramatically [14]. Oocyte-specific linker histone B4 disappear and somatic linker histones appear at this mid-blastula transition phase (Fig. 5) [10]. In the mouse, transcriptional activation of the zygotic gene starts at the 1- to 2-cell stage. Interestingly, the oocyte-specific linker histone H1oo to

somatic linker histone H1s transition happens at the same time as zygotic gene activation starts (Fig. 5) [6]. Additionally, systematic analysis shows a major decline in the developmental potential of donor nuclei (as assayed by blastocyst formation) between the one-cell (90% success) and two-cell stages (12.6% success) [15]. It should be noted that, in these experiments, the transfer of nuclei into oocytes or zygotes could have a different outcome. McGrath and Solter used zygotes as recipients [15], giving poorer results compared with when oocytes were used as recipients [16]. The progressive restriction in the developmental capacity of nuclei correlates with aspects of nuclear function. The time at which transcription begins to occur parallels a rapid decline in the efficiency of successful nuclear transfer. These findings indicate oocyte-specific linker histones may play some roles in genome-wide reprogramming in vertebrate eggs. The loss of oocyte-specific linker histone should facilitate the loss of pluripotency and perhaps totipotency. Further investigation on the function of the oocyte-specific linker histone may improve our understanding of the molecular mechanisms in chromatin remodeling.

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