—Mini Review—

Chromatin Remodeling with Oocyte-specific Linker Histones

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Introduction

The term 'epigenetics' defines all meiotically and mitotically heritable changes in gene expression that are not coded in the DNA sequence itself. Epigenetic modification of the genome ensures proper gene activation during development and involves genomic methylation changes, the assembly of histones and histone variants into nucleosomes, and remodeling of other chromatin associated proteins such as linker histones and transcription factors [1]. Additionally, the economic and medical implications of widespread cloning of domestic animals by nuclear transfer have greatly stimulated interest in the basic molecular mechanisms involved in reprogramming the developmental fate of nuclei introduced into eggs and oocytes [2]. An understanding of these mechanisms not only will provide insight into the significance of epigenetic events in establishing a developmental program, but also suggests new approaches towards improving the efficiency of nuclear transfer procedures.

The fundamental structural unit of chromatin is an assemblage, called the nucleosome, composed of five types of histones (designated H1, H2A, H2B, H3, and H4) and DNA. A nucleosome consists of approximately 1.8 turns of DNA wound around a core particle of histone proteins. The core particle is an octamer of 4 types of histones: two each of the H2A, H2B, H3, and H4 proteins. Approximately 166 base pairs are bound to the nucleosome: 146 base pairs are tightly bound to the core particle and the remaining 20 base pairs are associated with the H1 histone [3]. This nucleosome structure is closely similar in all eukaryotes. Although the field of chromatin research has focused on modifications to core histones that signal different gene expression states, it is becoming clear that different subtypes of histones are also important. Recently, Lee *et al.* demonstrate how a linker histone, H1b, can specifically repress the expression of a regulator of skeletal muscle differentiation, the MyoD gene, and thereby restrain the developmental decision to make muscle [4]. They speculate that the complexity of H1 function is attributed, in part, to differential activities of its isoforms.

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Wide Divergence of Linker Histones

Unlike Core histones, which demonstrate remarkable sequence conservation through evolution, linker histones diverge significantly in sequence and structure. Numerous developmentally regulated variants of linker histones exist in different animals, and these variants differ in their globular domains but also, most dramatically, in the length and net charge of the C-terminal tail domains [3]. Within the yeast genome exists a gene encoding a histone H1-like protein with two globular domains that have significant sequence identity with the globular domain of metazoan linker histones (Fig. 1). In contrast, the histone H1 of Tetrahymena lacks a central globular domain entirely with a very similar sequence composition to the C-terminal domain of a metazoan linker histone [3]. In general, mammalian linker histones of the H1 variety possesses a three-domain structure i.e. a central globular domain, flanked by N- and C-terminal tail domains. It is the central globular domain of H1, which is thought to interact with linker DNA and has thus

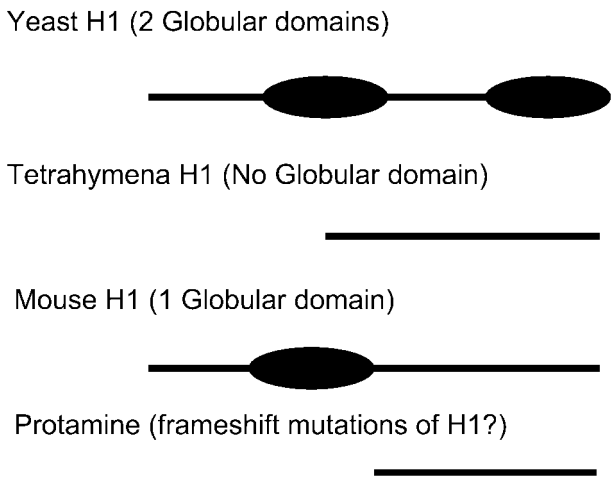

Fig. 1. Schematic drawing of linker histone H1 in different species. The ellipsoids indicate globular domains. The H1 in yeast has two globular domains. In Tetrahymena, H1 lacks a globular domain. In mouse, 8 subtypes of linker histone have been reported, all of which showed three domains structure: a globular domain, flanked by N- and C-terminal domains. In chordates, arginine-rich protamine is converted from the C-terminal domain of lysine-rich H1 as a result from frameshift mutations [5]. Vertebrate protamines could evolve from linker histones.

retained sufficient conservation to allow direct sequence comparison between species and to a degree between somatic H1 histone subtypes. H1 has many subtypes; mammalian somatic cells have six subtypes (H1a, H1b, H1c, H1d, H1e, and H1°) and germ cells have two subtypes (H1t and H1foo). The reason why there are so many subtypes in linker histones remains uncertain, but the expression levels of each subtype seem to be associated with differentiation. According to studies of knocking out a subtype(s) of linker histone, one of the subtypes is not essential but members compensate for each other. The association of histone H1 with DNA may stabilize the interaction of the core histone octamer with DNA and facilitate assembly of the nucleosome array into a higher order structure. It is currently accepted that H1 could have a regulatory role in transcription through modulation of the chromatin higher structure.

Recently, Lewis *et al.* showed an attractive evidence that protamines have been evolved from the C-terminal tail of histone H1 [5]. During the final stages of spermatogenesis, the compaction of DNA in testes is accomplished by the replacement of histones with a class of arginine-rich proteins called protamines. In general, somatic histone H1s typically contain little or no arginine. In contrast, protamines are relaitively small, are composed of >50% arginine, and contain little or no lysine [5]. From the time these nuclear proteins were first characterized, it was suggested that histones of somatic cells and protamines of germ cells were evolutionarily related. Lewis *et al.* clearly showed the evidence that the emergence of protamines in chordates occurred very quickly, as a result of the conversion of a lysine-rich histone H1 to an arginine-rich protamine. They concluded that chordate protamines have been evolved from the tail of sperm specific histone H1 as a result of frameshift mutations. By establishing an evolutionary link between the chromatin-condensing histone H1s of somatic tissues and the chromatin-condensing proteins of the sperm, these results provide unequivocal support to the notion that vertebrate protamines evolved from histones (Fig. 1).

For a long time the role in plant chromatin of H1 histones remained poorly understood. In animals the decrease in any particular variant is immediately compensated by increase in the other variants, without visible effects on major functions. In plants, in which the compensation effect among variants is also strongly manifested, the reversal of the normal proportion of major to minor variants achieved in tobacco using an antisense strategy, led to severe disturbances in chromosome segregation during male meiosis and subsequent male sterility [6]. This result may be explained by the recent demonstration in yeast that the equivalent of H1, Hho1p, suppresses DNA repair by homologous recombination [7, 8]. As homologous recombination is of particular importance during sexual reproduction, it is possible that there is a meiosis-specific mechanism which allows frequent DNA exchange in the presence of H1. It is conceivable that an aberrant proportion of H1 variants in tobacco chromatin during meiosis could have affected such a mechanism and disturbed the normal pairing and segregation of homologous chromosomes. Although little is known about a meiosis-specific mechanism in the presence of linker histone H1 in animals, germ cell specific linker histones H1t and H1foo were clearly shown in mammalian species [9–11]. Thus germ cell specific linker histones may have some roles in homologous recombination during sexual reproduction in mammals.

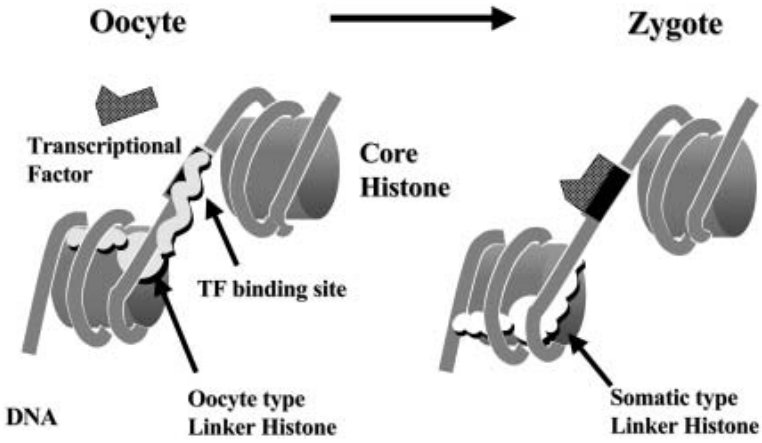

Fig. 2. Control of transcriptional activity with linker histones transition. Transition of oocyte-specific to somatic linker histones controls specific transcription factors (TF) binding to linker DNA. Linker histones function as a developmentally regulated gene selective repressor during Xenopus embryogenesis.

Linker Histones Regulate Chromatin Remodeling

In Xenopus oocytes and embryos, oocyte-specific linker histone B4 exists alone instead of somatic type H1 during the first divisions after fertilization [12]. B4 is then replaced by somatic H1 at the time of midblastula transition. Accumulation of H1 is a rate-limiting factor for the loss of mesodermal competence. The major difference between H1 and B4 lies in the stability with which these proteins are incorporated into chromatin [13]. In the mouse, histone synthesis is also developmentally regulated. During the course of a differential screening project, we discovered a mammalian oocyte-specific linker histone, H1foo, which is homologous to B4 [11]. The expression pattern of H1foo in preimplantation embryos is developmentally regulated, like Xenopus B4.

Experiments with natural chromosomal templates indicate that the role of linker histones *in vivo* is selective. Histone H1 was selectively released from the chromosomes of a somatic cell leading to the apparently specific activation of the oocyte-type 5S ribosomal RNA genes [14]. Subsequent *in vivo* analysis, using a targeted ribozyme to specifically prevent synthesis of the histone H1 protein, demonstrated that H1 functions as a developmentally regulated gene-selective repressor during Xenopus embryogenesis (Fig. 2) [15]. As embryogenesis proceeds, histone B4 is replaced by the somatic H1 and particular genes, including the oocyte-type 5S ribosomal RNA genes, are repressed [3]. The C-terminal tail domain of linker histones is the site of many regulated phophorylation events. Phosphorylation weakens the interaction of linker histones with DNA *in vitro* and with chromatin *in vivo*. As shown in Fig. 3, phosphorylation stimulates the exchange of linker histones between chromatin fibers *in vitro*. Indeed, Horn *et al.* clearly showed that linker histone phophorylation relieves the repressive effect of linker histones on chromatin remodeling complexes [16]. Several types of studies suggest that histone H1 and HMGN proteins have opposite effects on the structure and activity of the chromatin fiber. Histone H1 stabilizes the high-order chromatin structure, hinders the access of transcriptional coactivators to DNA and acts as general repressor of transcription [3]. In contrast, HMGN proteins are nucleosome binding proteins that reduce the compaction of the chromatin fiber and enhance transcription from chromatin templates (Fig. 3) [17].

Oocyte Specific Linker Histone

We discovered a mammalian oocyte-specific linker histone, H1foo, which is homologous to B4. H1foo is localized to the nucleus of germinal vesicle stage oocytes, M II arrested oocytes, and the first polar body. Early one-cell stage embryos displayed H1foo immunoreactivity in condensed maternal metaphase chromatin, but not in the sperm head. However, following the extrusion of a second polar body, H1foo

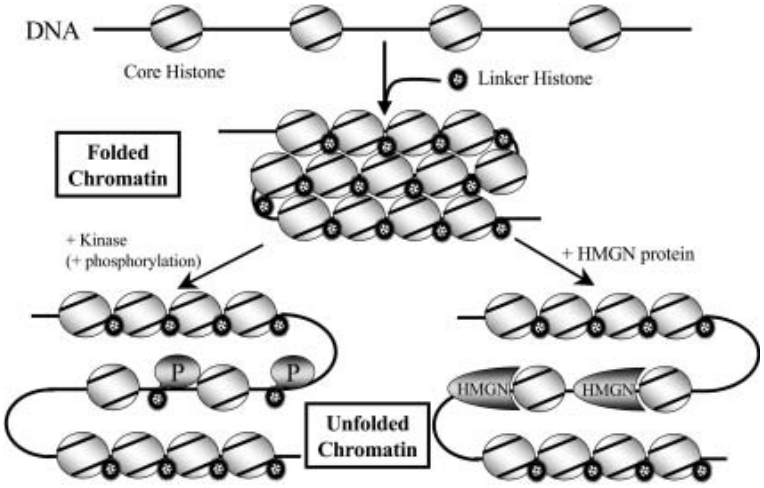

Fig. 3. Chromatin remodeling with linker histones. Linker histones tightly packs DNA with core histones. Phosphorylation of linker histone or replacement of linker histone with HMGN protein relieves the repressive effect of linker histone on chromatin remodeling.

Fig. 4. Amino acid sequence alignment of oocyte-specific linker histones. Identical amino acid residues are shaded. Sequence homology was greatest in the central globular domain, consistent with the observation that linker DNA interacts with the central globular domain of H1 protein.

was detected in the swollen sperm head. Nuclear staining was somewhat reduced in two-cell embryos and was no longer detectable in four-cell embryos [11, 18]. The expression pattern of H1foo in preimplantation embryos is developmentally regulated, like Xenopus oocyte-specific linker histone B4. Additionally, we have identified the human H1foo cDNA in unfertilized oocytes by direct RT-nested PCR of a single cell [10]. So far, oocyte-specific linker histone H1foo has been identified in five species. Amino acid sequence alignment of H1foo was shown in Fig. 4. Sequence homology was greatest in the central globular domain, consistent with the observation that linker DNA interacts with the central globular domain of H1 protein.

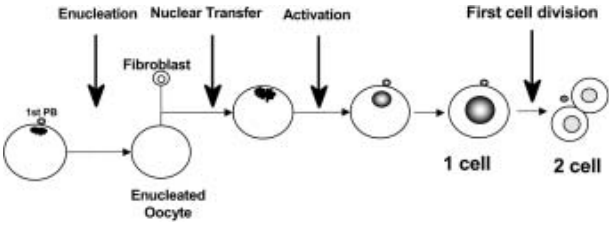

Fig. 5. H1foo rapidly replaces somatic H1 in the course of somatic nuclear transfer. H1foo expression in the nucleus is shaded. H1foo was localized to the metaphase-arrested donor nucleus as soon as 10 min after fusion. Nuclear staining was somewhat less in a two-cell embryo. This H1foo expression pattern in nuclear transfer was the same as that of normal preimplantation embryos.

In an experiment of nuclear transfer in Xenopus, it was shown that somatic type H1 in a donor cell was replaced with oocyte-type B4 soon after transplantation into an oocyte, replacement was mediated by nucleoplasmin, a molecular chaperone which contributes to acquisition of transcriptional competence [19]. In an experiment of somatic nuclear transfer in mouse, we have recently shown that H1foo is rapidly accumulated into the donor nucleus and persists in the nucleus until two-cell stage embryos, then disappears at the four-cell stage. We have previously shown that H1foo is readily detectable in the swollen sperm head shortly after fertilization in normal preimplantation embryos, and nuclear staining of H1foo is somewhat reduced in two-cell embryos and is no longer detectable in four-cell embryos [11]. The developmentally regulated presence of H1foo in a reconstructed embryo is thus similar to that of a normal preimplantation embryo (Fig. 5). Significantly, H1foo was detected in the donor nucleus 10 min after fusion of the donor cell. In Xenopus, the midblastula transition and the activation of zygotic gene expression are associated with a dramatic decrease in B4 content and a simultaneous increase in somatic H1 [2]. In nuclear transfer, the uptake of oocyte-type B4 into donor chromatin and the release of H1 is rapid, taking as little as 15 min from the time of mixing with the egg extract [19]. In the mouse, zygotic gene activation occurs during the two-cell stage, when H1foo begins to decrease. Simultaneous zygotic gene activation and the transition from oocyte-type linker histone to a somatic one strongly suggest that linker histones may play an important role in early development.

To understand the molecular basis of the rapid exchange of linker histone and the differences between H1c and H1foo, we investigated the dynamics of GFP-tagged histone in unperturbed chromatin by fluorescence recovery after photobleaching (FRAP), which can be used to define the mobility of molecules in living cells [20]. Upon bleaching the heterochromatin area, relatively rapid recovery of H1foo-GFP fluorescence reached a plateau after 100 sec. This clearly shows that H1foo-GFP is continuously exchanged in the chromatin regions of the cell nucleus in a similar manner to somatic linker histone H1s [21]. The recovery kinetics of H1foo-GFP were greater than for H1c-GFP. In addition, the immobile fraction of H1c was greater than that of H1foo. These results indicate that H1foo is more mobile than somatic H1c in living cell nuclei. Our FRAP experiments demonstrated that 85% of H1c-GFP was mobile and 15% was immobile. This clearly explains the biphasic reduction pattern of H1 in the course of nuclear transfer. Loss of the majority of H1 is due to the mobile fraction of H1 that can be rapidly replaced with H1foo or diffused into the oocyte cytoplasm, whereas the residue of H1 that remains for several hours is due to the immobile fraction. Interestingly, the time interval between the injection of the somatic cell nucleus into the enucleated oocyte and oocyte activation affects the rate of development [22]. Activation immediately after nucleus injection resulted in significantly less progression to the morulae/blastocyst stage *in vitro* than when activation followed a delay of 1 to 6 h [22]. This interval is consistent with the H1 removal time. Therefore, the remaining somatic H1 may disturb appropriate gene expression and lead to the failure of development. These findings suggest that the rapid replacement of H1 with H1foo may play a significant role in nuclear remodeling.

Epigenetic Reprogramming and Linker Histones

The two parental genomes are formatted during gametogenesis to respond to the oocyte environment and proceed through development. The zygote biochemically remodels the paternal genome shortly after fertilization and before zygotic gene activation occurs. To successfully recapitulate these processes, the somatic nuclei transferred into an oocyte must be quickly reprogrammed to express genes required for early development. Epigenetic reprogramming after fertilization and nuclear transfer has been studied in frogs and several mammalian species [1]. Differentiated somatic nuclei have the flexibility to

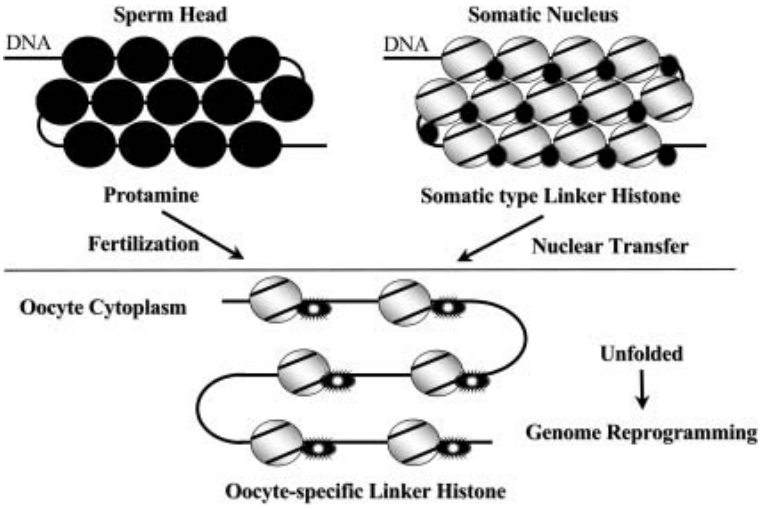

Fig. 6. Epigenetic reprogramming of sperm and somatic chromatin in the oocyte cytoplasm. Tightly packed DNA with protamines in the sperm head and with somatic linker histones in the somatic cells are replaced with oocyte-specific linker histone H1foo in the oocyte cytoplasm. H1foo leads to less stable chromatin that is more favorable for active transcription and DNA replication in early embryos.

dedifferentiate in oocyte cytoplasm and redifferentiate into other multiple lineages during the subsequent embryogenesis. These results indicate that highly efficient reprogramming mechanisms exist in oocyte cytoplasm. Our data have clearly shown that H1foo abundantly exists in MII stage oocyte cytoplasm. Indeed, following the extrusion of a second polar body, H1foo was detected in the swollen sperm head. As shown in Fig. 6, the compaction of sperm DNA is released by the replacement of protamine, which might be evolved from sperm specific linker histone H1, with the oocyte-specific linker histone H1foo. During somatic cell nuclear transfer, the compaction of somatic cell nucleus DNA is also released by the replacement of somatic cell H1 with the H1foo. H1foo has the greater mobility compared with H1 using FRAP in living cells [21]. These findings suggest that the rapid replacement of H1 with H1foo may relax chromatin structure and play a significant role in nuclear remodeling. Recently, Lee *et al.* provide the first evidence that a linker histone subtype operates in a gene-specific fashion to regulate tissue differentiation [4]. The next decade of research on linker histone variants will yield as much insight into genome reprogramming as the past decade has into histone modification.

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