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Theriogenology 78 (2012) 1371–1380

Theriogenology

www.theriojournal.com

Replacement of H1 linker histone during bovine somatic cell nuclear transfer

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Received 30 January 2012; received in revised form 6 June 2012; accepted 6 June 2012

Abstract

Linker histone variants are involved in regulation of chromosome organization and gene transcription; several subtypes are expressed in the maturing oocyte and developing embryo. In Xenopus and mice, the transition between linker histone variants occurred following nuclear transfer, and apparently contributed to donor nuclear reprogramming. To determine whether such linker histone replacement occurred after bovine nuclear transfer, red fluorescent protein (RFP) tagged H1e (somatic linker histone H1e) donor cells and Venus tagged H1foo eggs were created, enucleated eggs were injected with donor cells, and embryos were created by fusion. Using fluorescence microscopy, release of H1e in the donor nucleus, acquisition of H1foo by donor chromosomes, and the H1foo-to-H1e transition were observed in live cells. Linker histone replacement occurred more slowly in bovine than murine embryos. Low levels of diffuse red fluorescence (H1e) in the donor nucleus were detected 5 h after fusion, at which time green fluorescence (H1foo) had incorporated into donor chromosomes. However, complete replacement did not occur until 8 h after fusion. We concluded that the linker histone transition was sufficiently conserved among species, which provided further evidence regarding its important role in nuclear reprogramming.

Keywords: Nuclear transfer; Linker histone transition; Nuclear reprogramming; Bovine embryo

1. Introduction

It is well established that H1 Linker histones are involved in various cellular processes, e.g., chromosome organization and gene transcription. A unique subtype of H1 linker histone, "oocyte-specific linker histone" (H1foo), was present in oocytes of several species. The most distinctive feature of H1foo was the specific timing of its expression, which began during oocyte maturation and was maintained during early embryonic development. Based on this expression pattern, linker histones may have important roles during oocyte growth [1] and maturation [2], as well as during fertilization and embryo development [3–7].

In Xenopus, the oocyte-specific linker histone is Histone B4. Following treatment of Xenopus erythrocyte nuclei with egg extracts, somatic linker histone variants (H1 and H1°) were released from chromatin, and the oocyte-specific linker histone B4 was efficiently incorporated into remodeled chromatin. Moreover, this linker histone transition substantially increased transcriptional competence of erythrocyte nuclei [8]. In mammals, H1foo was identified, and its expression pattern was demonstrated in normal preimplantation murine embryos [6]. Similar to the Xenopus model, two

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http://dx.doi.org/10.1016/j.theriogenology.2012.06.004

Table 1
Details of the primers used for gene cloning and mRNA expression detection.

Gene	GenBank Accession no.	Primer sequences	Annealing Temperature (°C)	Product Size (bp)
H1foo[a]	NM001035372	ATCG GCTAGCCTTTCTGCCTGCAAAATGGCTC ATGC AAGCTTACGCCCTCGGCTTCTGCCTTCT	60	1032
H1e[a]	NM001098989	ATCGAAGCTTATGTCTGAAACTGTACCTGCCGC ATCGGGATCCTTGTCCTGCTTTTTGGGGGTC	58	633
β-actin[b]	NM173979	CAAggACCTCTACgCCAACA CTCgATCCAACCgACTgCT	54	444
RFP-H1e[b]	—	AAGCTT ATGTCTGAAACTGTACCTGCCGC CTACAGGAACAGGTGGTGGCG	60	1320

The underlined regions indicate restriction enzyme sites.
[a] Primers used for gene cloning.
[b] Primers used for detection of mRNA expression.

groups have confirmed that somatic linker histones were replaced with H1foo following nuclear transfer in the mouse [9,10]. Human and bovine H1foo cDNA have been identified by direct RT-nested PCR of single oocytes [11]. In addition, the mRNA profile of bovine H1foo was characterized in the oocyte and throughout early embryo development by quantitative reverse transcriptase-polymerase chain reaction (RT-PCR) [12]. However, its function during bovine oocyte maturation and nuclear transfer reprogramming remains unclear.

Recently, two publications showed the important function of oocyte-specific linker histone incorporation into sperm heads or donor nuclei during ICSI and SCNT, respectively. Following human ICSI, there were no decondensed sperm heads without H1foo incorporation, indicating that H1foo incorporation into sperm chromatin was beneficial for sperm nuclei decondensation [13]. In the other study, linker histone transition occurred during cross-species nuclear transfer [14]. Specifically mammalian somatic cell nuclei were transplanted into Xenopus oocytes, and linker histone exchange in transplanted nuclei occurred at an early stage, followed by nuclear reprogramming, associated with reactivation of pluripotency genes (e.g., Oct4, Nanog, and Sox2). It was concluded that oocyte-specific linker histone B4 incorporation into nuclei was required for pluripotency gene reactivation during nuclear reprogramming. Furthermore, the linker histone transition during cross-species nuclear transfer indicated the process was conserved among species. The objective of the present study was to determine whether somatic linker histones were replaced with H1foo during bovine nuclear transfer. By tagging H1foo and H1e with Venus and Red fluorescent protein, respectively, the process was monitored in real-time.

2. Materials and methods

2.1. Construction and identification of pdsred1-H1e and pVenus-H1foo plasmids

Bovine full-length H1e (NM001098989) and H1foo (NM001035372) cDNAs were synthesized by RT-PCR (Fermentas-China, Shenzhen, China) from fibroblast cells and GV-stage oocytes, respectively. Both were inserted into pMD19-T (Takara, Dalian, China) vectors, and the desired recombinants were sent to the Shanghai Sangon Biotechnology, Co (Shanghai, China) for DNA sequencing. All PCR primers are listed (Table 1). The H1e was subcloned into pdsred1-N1 via the HindIII and BamHI sites to generate pdsred1-H1e, and pVenus-H1foo was constructed by inserting H1foo cDNA, obtained by cleaving pMD19-T-H1foo with NheI and HindIII, into pVenus.

2.2. Isolation and culture of bovine ear-derived fibroblast cells

Bovine skin fibroblast cells were obtained from a Holstein cow (3 yrs old) whose genetic background was well known. Briefly, ~1 cm^2 of the cow's ear was isolated using a punch. Under sterile conditions, the samples were washed three times using phosphate buffered saline (PBS) containing penicillin and streptomycin, then cut into small pieces (~1 mm^3). These pieces were cultured in DMEM (Life Technologies Corporation, Beijing, China) containing 20% fetal bovine serum (FBS, Hyclone, Shanghai, China), 100 IU/mL penicillin, 100 μg/mL streptomycin, and 2 mM L-glutamine for ~1 wks at 38.5°C under a humidified atmosphere of 5% CO_2 in air until near confluence. Fibroblasts were subsequently cultured every 6 to 8 days and digested with 0.25% trypsin and 0.04% EDTA (approximately a