Fully-mature antral mouse oocytes are transcriptionally silent but their heterochromatin maintains a transcriptional permissive histone acetylation profile

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Abstract

Purpose The final stages of antral mouse oocytes maturation are characterised by the transition from transcriptionally active NSN to inactive SN oocytes. Here, we studied the profile of histone acetylation changes occurring during the NSN-to-SN transition.

Methods During the NSN-to-SN transition, oocytes were classified based on their chromatin organisation and the immunocytochemical profile of histones H2B, H3 and H4 acetylation was analysed.

Results We described four patterns of immunostaining common to the three acetylated histones, corresponding to

stages of progressive localisation: From a diffused distribution in NSN oocytes to the association with constitutive heterochromatin and then to the nucleolar surface region in SN oocytes.

Conclusions The maintenance of a favourable transcriptional epigenetic context, in the heterochromatin of transcriptionally silent SN oocytes, might be related to the early stages of development when transcripts from these heterochromatic regions are functional to preimplantation progression.

Keywords Mouse · Antral oocytes · Chromatin organisation · Histone acetylation

Capsule In late antral mouse oocytes, chromatin condenses around the nucleolus, becomes transcriptionally silent, but maintains transcriptionally permissive histone acetylation profiles, likely functional to development.

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Introduction

Depending on their chromatin organisation, mouse fully-matured antral oocytes may be classified into two groups: one group comprises oocytes that have a ring of heterochromatin surrounding the nucleolus (named SN oocytes) and the other consists of oocytes that lack a ring of heterochromatin surrounding the nucleolus (NSN oocytes) ([5, 16, 24]; for a review see [26]). Oocytes possessing an NSN or SN chromatin organization have also been described in other mammals, including rat, monkey, pig, bovine and human (for a review see [23]). Antral NSN oocytes are an immature form that in the ovary will acquire the SN-type of chromatin organisation before ovulation [15, 24]. These morphological differences underline biological relevance as they have been correlated with changes in transcription: NSN oocytes are transcriptionally active and synthesise all classes of RNA, whereas SN oocytes become transcriptionally inactive [1, 4, 5, 11, 14, 17, 18]. Furthermore, whilst *in vitro* both types of oocytes are capable of meiosis resumption and reach the metaphase II (MII) stage, MII^{SN} oocytes develop to term, whereas MII^{NSN} oocytes consistently cease development at the 2-cell stage ([9, 25]; for a review see [29]).

Preimplantation development is regulated by the synergic action of cytoplasmic and nuclear determinants inherited by the egg and acquired during the oocyte growth: mRNAs, small interfering RNAs (siRNAs) and transcription factors are all required to regulate the expression of the female genome during these early stages. When the expression of one of the 27 maternal-effect factors identified so far is altered, mouse embryos arrest development, mostly at the 2-cell stage (for a review see [13]). We have recently shown that one of the possible causes of the developmental incompetence of MII^{NSN} oocytes is the down-regulation of two maternal-effect factors, OCT4 and STELLA [27, 28], required for development beyond the 2-cell stage [8, 20].

Besides these maternal-effect factors, the zygote inherits from the egg a genomic epigenetic memory that is also crucial for the regulation of gene expression in early development. The best known epigenetic mechanisms regulating gene expression are DNA methylation (i.e., the acquisition of a methyl group at CpG sites), histone modifications (i.e., histone methylation and acetylation) and chromatin organisation [22]. Our knowledge of the epigenetic signature that regulates gene expression in oocytes and how it contributes to the acquisition of its developmental competence is poor and limited to the major steps that characterise the oocyte growth. The profile of DNA methylation, histone methylation and acetylation increases quantitatively throughout folliculogenesis, reaches a peak in fully-grown SN oocytes [7, 10, 19]. Antral oocytes undergo

deacetylation after GVBD and are acetylated again following fertilisation [6, 12].

Less clear is how and whether the epigenetic profile is modified during the mouse NSN-to-SN transition, when extensive modifications to the chromatin organisation occur.

This transition is marked by specific changes to the localisation of centromeres and of constitutive heterochromatin (CHC) [15]. In both type of oocytes, most of the centromeres co-localise with the A–T rich constitutive heterochromatin [15], for this reason, centromeres and associated pericentromeric chromatin are collectively referred to as chromocentres (CHCs). The antral NSN oocyte is characterised by a number of CHCs (those present in the NOR-bearing chromosomes) already localised near the nucleolus [15] and by CHCs that are interspersed within the nucleus and that will cluster to form a compact ring of heterochromatin around the nucleolus at completion of the NSN-to-SN transition.

To gain a better insight into the epigenetic changes occurring during the NSN-to-SN transition, in the present study we have analysed the profile of histones H2B, H3 and H4 acetylation in mouse fully-grown antral oocytes classified on the basis of the pattern of chromatin organisation observed during this transition.

Materials and methods

Animals and reagents

Female mice (CD-1), purchased from Charles River (Como, Italy) were maintained at 22°C with a light/dark cycle of 14/10 h and fed *ad libitum*. Females were primed with 3.5 I. U. Folligon (Intervet, srl, Milan, Italy) 48 h before the isolation of the ovary. All reagents were purchased from Sigma (Sigma Co., St. Louis, MO) unless otherwise stated.

Oocytes isolation

To obtain antral oocytes, ovaries, freed of adnexia, were placed in a pool of fresh M2 medium and antral bulges on their surface were punctured to release oocytes into the medium. Healthy oocytes of 70 µm in diameter with intact germinal vesicles were collected and freed of cumulus cells by a narrow bore micropipette. The oocytes were then incubated in 5 µl droplets of M2 medium containing 0.05 mg/ml Hoechst 33342 overlaid with paraffin oil (BDH, Poole, UK) for 25 min at 37°C in 30 mm petri dishes.

Immunocytochemistry and image acquisition

Oocytes were fixed in 4% formaldehyde in PBS for 30 min, extensively washed in PBS, permeabilised for 15 min in

0.5% Triton X-100 in PBS and incubated in TNT (0.1 M Tris- HCl, pH 7.5, 0.15 M NaCl, 0.05% Tween-20) plus 0.5% blocking reagent (Roche, Boston, MA) for 20 min. Then, oocytes were incubated with antibodies (Santa Cruz Biotechnology) against AcH2B(K5/12/15), AcH3 (K9/14) or AcH4(K5) histones diluted 1:50 in PBS for 1 h at 37°C, washed for 15 min in TNT, incubated with a secondary antibody diluted 1:3000 in TNT for 1 hr at 37°C and then washed for 15 min in TNT. Antibody-reacted oocytes were stained with Hoechst 33342 (0.25 mg/ml in PBS for 5 min), mounted in DABCO, and examined with an Olympus Provis epifluorescence microscope equipped with single-bandpass filters for Hoechst, FITC and PI. A cooled charge-coupled Photometrics CH-350 camera controlled by IP Lab software was used to obtain digital images.

Results and discussion

A total of 123 antral oocytes isolated from the ovaries of four females were analysed by immunocytochemistry, 49 (39.8%) and 74 (60.2%) of them owned an NSN- or SN-type of chromatin organisation, respectively, confirming frequencies previously reported [24, 27]. This analysis, demonstrated the presence of the acetylated form of the three histones in all the oocytes observed (AcH3: 43 oocytes analysed; AcH2B: 37; AcH4: 43). During the NSN-to-SN transition, we detected important changes to the pattern of distribution of the Ab signal within the germinal vesicle (GV). Independently of the Ab used, we observed the same four major patterns of immunostaining, hereafter numbered in succession following the clustering of heterochromatin around the nucleolus during the NSN-to-SN transition. In NSN oocytes we distinguished two main patterns of Ab localisation: pattern 1 (P1), diffused distribution of the Ab-signal, associated with some of the CHCs (Fig. 1e, arrowhead); pattern 2 (P2), Ab-signal diffused both all-over the nucleus and associated to most of the CHCs (Fig. 1f). In SN oocytes also we observed two main patterns of Ab distribution: pattern 3 (P3), a strong Ab-signal associated to the nucleolar region and to those CHCs still distributed within the nucleus (Fig. 1g); pattern 4 (P4), a strong Ab-signal mostly localised around the nucleolus (Fig. 1h). Table 1 summarises the number and frequency of oocytes with a P1 and P2 (NSN) or P3 and P4 (SN) profile of Ab immunofluorescence.

Our data on mouse antral oocytes are in agreement with those described before in pig [2] and show that once established during oocyte growth [10], this epigenetic signature is maintained, although its pattern of localisation changes during the stages of the NSN-to-SN transition: From a diffused distribution in NSN oocytes to the

Fig. 1 A representative immunofluorescence with the Ab for the acetylated form of histone H3. **a–d** staining with Hoechst brings to light the four patterns of chromatin organisation observed during the NSN-to-SN transition. **e–f** immuno localisation of the AcH3-Ab in NSN oocytes; **g–h** immuno localisation of the AcH3-Ab in SN oocytes. Arrow, negative CHCs; arrowhead, positive CHCs; Bar, 5 μ m

association with constitutive heterochromatin and then to the nucleolar surface region in SN oocytes.

Histone acetylation is generally associated with a transcriptionally permissive chromatin and identifies the euchromatic regions of the genome. Furthermore, H3K9 acetylation and H3K4me2 lead to chromatin decondensation [3]. As summarised in Fig. 1 for the AcH3 antibody, we interpret the P1-type of immunostaining (Fig. 1e) as the first step in the transition from the NSN to the SN antral oocyte. At this stage of maturation, NSN oocytes possess a high transcriptional activity [1, 4, 5, 11, 14, 17, 18], their chromatin is positive to the antibodies for the acetylated form of H3, H2B and H4, whilst CHCs are seldom positive to the immunostaining. CHCs become positive during the NSN-to-SN transition. In SN oocytes, the immunostaining signal is localised on CHCs, mostly clustered around the nucleolus [15], at the time when the process of transcription silencing is completed. The maintenance of a favourable transcriptional epigenetic context, in the heterochromatin of transcriptionally silent SN oocytes, might be functional to the early stages of development when transcripts from these

Table 1 Number (%) of NSN or SN oocytes owning a specific pattern of antibody localisation

N. of oocytes analysed	NSN		SN	
	Pattern 1	Pattern 2	Pattern 3	Pattern 4
123	25 (20.3)	24 (19.5)	20 (16.3)	54 (43.9)

heterochromatic regions (i.e., satellite DNA) are functional to preimplantation progression [21].

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