Large-scale chromatin morpho-functional changes during mammalian oocyte growth and differentiation

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Abstract

Mammalian oocyte development is characterized by impressive changes in chromatin structure and function within the germinal vesicle (GV). These changes are crucial to confer the oocyte with meiotic and developmental competencies. In cow, oocytes collected from early and middle antral follicles present four patterns of chromatin configuration, from GV0 to GV3, and its progressive condensation has been related to the achievement of developmental potential. During oogenesis, follicular cells are essential for the acquisition of meiotic and developmental competencies and communicate with the oocyte by paracrine and gap junction mediated mechanisms. We recently analyzed the role of gap junction communications (GJC) on chromatin remodeling process during the specific phase of folliculogenesis that coincides with the transcriptional silencing and sequential acquisition of meiotic and developmental capabilities. Our studies demonstrated that GJC between germinal and somatic compartments plays a fundamental role in the regulation of chromatin remodeling and transcription activities during the final oocyte differentiation, throughout cAMP dependent mechanism(s).

Features and significance of large-scale chromatin configuration changes within the germinal vesicle

During follicular development, mammalian oocyte acquires a series of competencies that play critical roles at fertilization and subsequent stages of embryonic development. Recent studies indicate that these competencies involve remodeling of chromatin occurring in the germinal vesicle (GV), when gamete and somatic cells communicate throughout junctional and paracrine mediated mechanisms.[1] Dynamic changes in GV oocyte chromatin structure have been reported in mouse,[2-4] monkey,[5] pig,[6] rat,[7] human,[8,9] horse,[10-12] cattle,[13-16] goat,[17] sheep,[18] rabbit,[19] buffalo,[20] dog,[21-23] ferret,[24] and cat.[25] A direct relationship between oocyte's chromatin configuration and embryonic developmental competence has been proved in mouse[26,27] and cow.[13,28]

In growing mouse oocytes chromatin is initially decondensed in a configuration termed Non-Surrounded Nucleolus (NSN).[2,4,29] With subsequent growth and differentiation, oocyte nuclear organization undergoes a dramatic change in which chromatin becomes progressively condensed, forming a heterochromatin rim in close apposition with the nucleolus, acquiring a configuration termed Surrounded Nucleolus (SN).[2,4,29]

The morphological differences between these two types of oocytes have a biological relevance as NSN and SN morphologies have been correlated with differences in follicle size, oocyte diameter and the age of the mouse.[4,29] Several authors indicate that SN oocytes may represent the more advanced stage of preovulatory oocytes.[4,26,29] In fact, it has been demonstrated that the transition into the SN configuration correlates with the timely progression of meiotic maturation.[2-4] Furthermore, after *in vitro* maturation and fertilization, NSN oocytes are incapable of development beyond the two-cell stage, whereas SN oocytes are capable of development to the blastocyst stage.[26,27] Differences in chromatin morphology have also been correlated with changes in transcriptional activity: NSN oocytes remain transcriptionally active and synthesize all classes of RNA, whereas SN oocytes are associated with global repression of transcriptional activity.[9,30-33]

In cow, oocytes collected from early and middle antral follicles present four patterns of chromatin configuration, from GV0 to GV3 (Figure 1) characterized by the progressive increase in condensation[13] and global DNA methylation.[34] The GV0 stage shows a diffuse filamentous pattern of chromatin in the whole nuclear area; the GV1 and GV2 configurations represent early and intermediate stages, respectively, of chromatin remodeling, a process starting with the appearance of few foci of condensation in GV1 oocytes and proceeding with the formation of distinct clumps of condensed chromatin in GV2 oocytes; the GV3 is the stage where the highest level of condensation is reached with chromatin organized into a single clump. Importantly, oocytes with a GV0 configuration showed a very limited capacity to resume and complete meiosis I after *in vitro* maturation, while virtually all the GV1, GV2 and GV3 oocytes were able to reach MII stage, despite their GV configuration. On the contrary, after *in vitro* fertilization and embryo culture, only a limited percentage of GV1 oocytes reached the blastocyst stage, while GV2 and GV3 oocytes showed a higher embryonic developmental potential.[13]

These results further support the general principle that meiotic and developmental competencies are acquired at sequential stages of oogenesis,[1] alongside with changes in large-scale chromatin structure.[35]

Oocyte growth, chromatin remodeling and key structural modifications in the nuclear and cytoplasmic compartments

In the mammalian ovary, oocytes are naturally arrested at prophase I of meiosis and primordial follicle-enclosed oocytes remain in the resting phase until they are stimulated to grow.[36] Oocyte growth phase includes a series

of modifications in the amount, structure and distribution of the organelles as well as a period of oocyte transcription, which are necessary for the oocyte to achieve meiotic and developmental competence.[36,37] The bovine oocyte and follicle continue to grow in parallel until the follicle reaches a diameter of about 3 mm; thereafter, the oocyte plateaus at about 120-130 μm, while the follicle grows up to 15-20 mm in diameter before ovulation.[36] As the oocyte increases in diameter, key structural modifications and redistribution of the cytoplasmic organelles further occur.[38] Towards the end of the growth phase, the global transcriptional activity decreases and the nucleolus is transformed into an inactive remnant through a mechanism known as nucleolar dismissing.[37,39,40] In cow, the process of chromatin remodeling is timely related with the morphological changes that occur in both the nuclear and cytoplasmic compartment during oocyte growth and differentiation (Table 1 and Figure 2).[13,41] Oocytes with a GV0 configuration are the predominant type of oocytes collected from early antral follicles, between 0.5 and <2 mm in size, with a mean diameter of 108 μm.[13] These oocytes displayed typical structural features of the growth phase with nuclear characteristics and distribution and structure of the cytoplasmic organelles similar to those previously described in oocyte with a diameter <110 μm.[38] Furthermore, at the nuclear level, GV0 chromatin configuration is always associated with high level of RNA synthesis while the transition to condensed state of the chromatin is associated with global repression of transcriptional activity.[28,41] With subsequent growth and differentiation, profound changes in chromatin organization occur and the oocytes gradually achieve the full capability for sustaining embryonic development. As a consequence, oocytes collected from mid-antral follicles represent a heterogeneous population of gametes, characterized by different degrees of chromatin condensation and by different embryonic developmental competence. In fact, GV2 and GV3 oocytes exhibited a higher capability to sustain the preimplantation embryonic development when compared to GV1 oocytes.[13] GV1, GV2 and GV3 stage oocytes, accordingly to their mean diameter (117, 119 and 121 μm, respectively[13]) generally showed the morphological appearance that has been previously described in fully grown oocytes.[36-38,42,43]

Morphological and functional studies in cow described the modifications that typically characterize the bovine oocyte differentiation within the dominant follicle, before the LH surge and during preovulatory development, which is an important step for the attainment of a full developmental competence.[37,44] These changes are generally referred to as *oocyte capacitation*[37] and, at the structural level, include, among others, the reduction of the size of Golgi complex and the convolution of the nuclear envelope.

The overall appearance of GV1 oocytes denotes that they have not completed the changes that normally occur in final differentiation.[41] This could be related with their poor developmental capability. In contrast, both GV2 and GV3 oocytes, accordingly with their higher developmental capability, showed typical signs of pseudo-maturation,[44] with GV3 oocytes in a more advanced stage of differentiation, as indicated by the global repression of transcriptional activity and the appearance of early cellular degeneration, such as the presence of organelle-free areas, degenerative features of cortical granules[41] and reduction of the intercellular coupling between the oocyte and cumulus cells.[13] These observations are in agreement with the hypothesis that GV3 oocytes represent that proportion of gametes which have reached a high developmental capability during follicular growth and that, at the time of collection, are undergoing early events of atresia.[13] This hypothesis also supports the concept that oocyte developmental competence appears to be improved by low levels of atresia.[45-52]

Oocyte development, chromatin remodeling and gap junction mediated interplay with cumulus granulosa cells

Oocyte growth and differentiation depend on the establishment of a patent bidirectional communication mediated by heterologous gap junctions between oocytes and companion granulosa cells during folliculogenesis.[53-55] In mouse, previous studies indicate that the presence of oocyte-associated granulosa cells are required for the progressive repression of transcriptional activity in fully grown oocytes.[32] Moreover, the tight association with companion cumulus cells is required to promote the transition from NSN to SN configuration after gonadotropin stimulation.[32] This hypothesis is supported also by studies where gap junction mediated communications (GJC) between mouse oocyte and cumulus cells were interrupted, due to targeted deletion of the connexin 37 gene (Gja4), and chromatin condensation associated with transcriptional repression failed to occur.[56]

Coupling between oocyte and cumulus cells undergoes dynamic changes during follicle
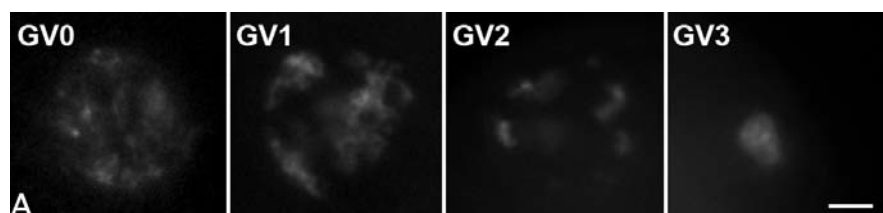
B	GV0	GV1	GV2	GV3	References
Diameter	108.6±0.7	117.1±0.8	119.1±0.7	121.6±0.6	Lodde *et al.*[13]
Transcriptional activity	+	±	-	-	Luciano *et al.*,[28] Lodde *et al.*[41]
Meiotic competence	-	+	+	+	Lodde *et al.*[13] Luciano *et al.*[28]
Developmental competence	-	±	+	+	Lodde *et al.*,[13] Luciano *et al.*[28]
DNA global methylation	±	++	++	++	Lodde *et al.*[34]

Figure 1. A) The four patterns of chromatin configuration, from GV0 to GV3 (scale bar: 10 μm); B) Oocyte size, transcriptional activity, meiotic and developmental competence and DNA global methylation in relation to chromatin configurations.

development and the patency of GJC between the two compartments decreases in parallel with the meiotic resumption of the oocyte.[57-59] However, recent studies performed in cow, horse, dog and cat[60-63] indicated that morphologically healthy oocyte-cumulus cells complexes isolated from antral follicles without evident signs of atresia are a heterogeneous population characterized by different functional degrees of GJC. In cow, in particular, the direct oocyte-granulosa cell communication through gap junction seems a requisite for chromatin remodeling process during the final phase of oocyte growth.[13,28] This is supported by the evidences that, at the time of collection, the pattern of uncondensed chromatin in GV0 oocytes is associated with fully open GJC. On the contrary, the percentage of oocytes with functionally open communications significantly decreases with the increase of chromatin condensation, from GV1 to GV3 oocytes,[13,28] indicating that bovine oocytes that reached the highest level of chromatin condensation have a greater probability in loosing their coupling with follicular cells than oocytes with a lower chromatin condensation.[13] On the other hand, the increase in chromatin condensation may represent a consequence of the premature interruption of communication between oocyte and follicular cells before the final oocyte maturation since the lost of GJC between the germ and somatic compartments has been related with early events of follicular atresia.[64]

The crucial role of GJC in the modulation of chromatin configuration, global transcriptional activity and developmental competence acquisition, has been recently confirmed in bovine oocyte-cumulus cells complexes. The use of culture systems that prolonged GJC, sustained oocyte growth and allowed chromatin to gradually organize from GV0 into the GV1 configuration, thus acquiring the ability to mature and be fertilized *in vitro.*[28] When GJ functionality was experimentally interrupted with the uncoupler 1-heptanol, chromatin rapidly condensed and RNA synthesis suddenly ceased. Interestingly, this effect was nullified by the addition to the culture medium of cilostamide, a specific inhibitor of the oocyte-specific PDE3, an enzyme-degrading cAMP[65-67] indicating that the functional status of GJC may affect both transcriptional activity and remodeling of large-scale chromatin configuration, potentially through cAMP-dependent mechanism(s).[28] Therefore, besides the well-characterized mechanisms of action by which cAMP is known to regulate meiotic resumption,[68,69] these results may suggest that cAMP could be also involved in the control of the activity of factors that modulate transcription and large-scale chromatin remodeling during the final phase of oocyte growth and before the resumption of meiosis.

Interestingly, while in mouse the absence of a

patent bidirectional communication caused the majority of oocytes to remain transcriptionally active with uncondensed chromatin,[32,56] in cow GJC disruption by means of 1-heptanol caused premature chromatin condensation and transcriptional interruption. These experimental models differ substantially in some aspects; thus, it remains to be fully investigated whether this discrepancy might be due to a different physiological status of the animal model or to the growth phase of the follicle from which an oocyte is isolated. Notably, it cannot be excluded

Table 1. Main morphological and structural features of the nuclear and cytoplasmic compartment in bovine oocyte in relation to chromatin configuration (modified from Lodde et al.[41]).

Nucleus	GV0 Eccentric	GV1 Peripheral	GV2 Peripheral	GV3 Peripheral
Undulation of the nuclear envelope	Nearly absent	Slight	Profound	Profound
Nucleolus	Fibrillo granular	Dense fibrillar	Dense fibrillar	Dense fibrillar
Cytoplasmic organelles distribution	Sparse in the cytosol	Homogeneous in the oocyte cortex	Homogeneous in the oocyte cortex	Clustered in the oocyte cortex
Ooplasmic vesicle	Few	Abundant	Abundant	Plentiful
Perivitelline space	Absent	Present	Present	Present
Microvilli	Erected	Bent	Bent	Bent
Mitochondria (location)	Round (small clusters in the cytoplasm)	Hooded (deep cortical)	Hooded (deep cortical)	Hooded (peripheral)
Golgi complex	Present	Reduced	Almost absent	Almost absent
Cortical granules	Singular, all over the cytoplasm	Clustered, deep cortical	Clustered, deep cortical	Clustered, peripheral (some sign of degeneration)
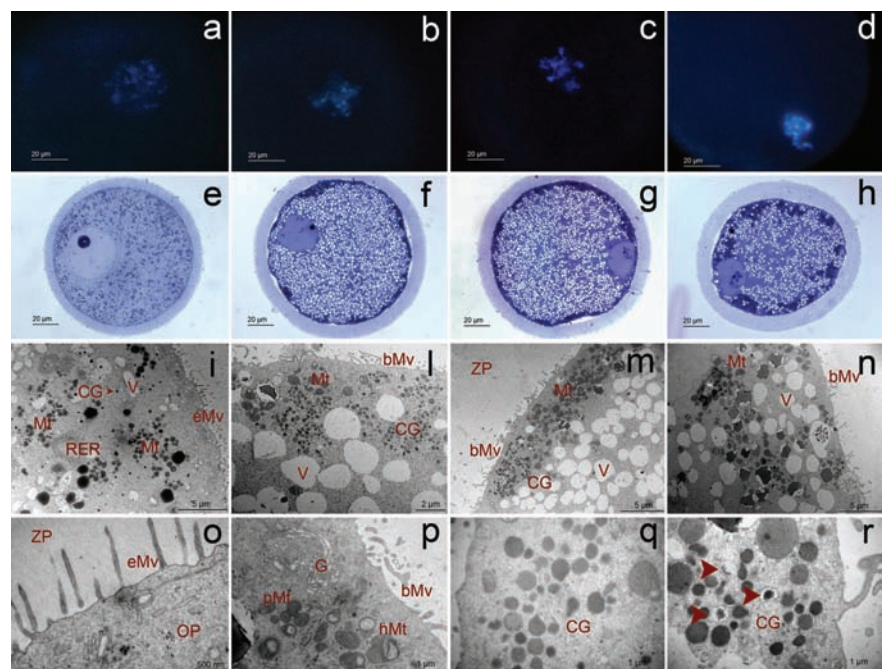
Figure 2. Fluorescence (a, b, c, d), light (e, f, g, h) and transmission (i, l, m, n, o, p, q, r) micrographs representative of GV0 (a, e, i, o), GV1 (b, f, l, p), GV2 (c, g, m, q) and GV3 (d, h, n, r) oocytes. Mt, mitochondria; V, vacuoles; RER, rough endoplasmic reticulum; CG, cortical granules; eMV, erected microvilli; bMv, blent microvilli; ZP, zona pellucida; OP, ooplasm; pMt, pleomorphic mitochondria; hMt, hooded mitochondria; G, Golgi complex (from Lodde et al.[41]).

that just the timing when the functional coupling between oocytes and cumulus cells is interrupted could determine the effect on chromatin structural and functional changes.[28]

Conclusions

The mammalian oocyte nucleus exhibits characteristic chromatin configurations, which are subject to dynamic modifications during oogenesis.

The experimental manipulation of large-scale chromatin structure[25,28,70] can provide a tool to determine the key cellular pathways and factors involved in genome-wide chromatin modifications. Analysis of the functional differentiation of chromatin structure in the oocyte genome in fact have wide-ranging implications for understanding the role of nuclear organization in meiosis, the events of nuclear reprogramming and the spatio-temporal regulation of gene expression during development and differentiation.

Finally, this can provide experimental models to analyze the possible implication of gametes and embryos manipulation in epigenetic disturbances since the process of chromatin remodeling accompanies the epigenetic maturation of the female gamete, which enables an oocyte to develop into a viable embryo after fertilization.

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