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Epigenetic modifications during oocyte growth is necessary for development

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Genomic imprinting is the epigenetic mechanism that distinguishes whether the loci that are inherited from maternal or paternal genome lead to parent-specific gene expression. The mechanism also regulates development in mammalian embryos. Genomic imprinting is established after implantation according to the specific markers that are imposed on the genome during gametogenesis; the allele-specific gene expression is then maintained throughout embryogenesis. The genomic imprinting markers are erased and renewed on an own sex basis only in cells that are differentiated into germline cells. Here, I show that the epigenetic modifications that occur during oogenesis perform the crucial function of establishing the allele-specific expression of imprinted genes and development of embryos.

Gene regulated by maternal primary imprinting

In order to directly elucidate the effect of primary imprinting in mice, we firstly developed a procedure for reconstructing eggs that contained one haploid set of genomes derived from a non-growing oocyte and one haploid set of genomes from a fully grown oocytes (ng/fg embryos). This procedure allowed us to examine the developmental ability of oocytes that contain haploid nuclei derived from a non-growing oocyte and as such might not have had the opportunity to undergo complete maternal imprinting. The parthenogenetic embryos (ng/fg PE) developed progressively to day 13.5 of gestation, 3 days further than has been previously observed. Molecular analysis of imprinted genes showed that expression of Pegl/Mest, Peg3, Snrpn, Igf2r and p57 kip2 from maternal (ng) alleles was altered as a result of the disruption of the primary imprinting during oocyte growth. This is the first case that shows that paternally expressed genes can be expressed from the maternal alleles. These findings suggest that the expression of Pegl/Mest, Peg3 and Snrpn is normally regulated by a mechanism of maternal repression that is established during the period of oocyte growth. However, this is not the case for all of the paternally expressed genes; the Igf2 gene was not expressed in the ng/fg PE at either 9.5 and 12.5 dpc, suggesting that these genes are regulated by paternal epigenetic modifications during spermatogenesis.

Production of pups from growing oocyte nuclei

The ability of maternal chromatin to support full term development is attained during oocyte growth. The aim of this study was to identify when during the growth phase the maternal chromatin developed the capacity to support term development. To address this problem mature MII arrested oocytes that contained chromatin from oocytes at different stages of oocyte growth were constructed by micromanipulation. The oocytes were fertilized in vitro, developed to the blastocyst stage in vitro and tranferred to recipients to assay developmental potential. The results demonstrate, firstly, that the origin of the maternal chromatin has no effect on the rate of oocyte maturation, fertilization or development to the blastocyst in vitro. Secondly we demonstrate that maternal chromatin is first competent to support development to term during the latter half of oocyte growth when oocytes are in 60-69 μm in diameter in juvenile mice or 50-59μm in diameter in adult mice. These data show that epigenetic modifications necessary for postimplantation development occur during a specific phase of oocyte growth.

The identification of the stage in the development of the female germ-line that epigenetic changes are completed is important for further studies on the mechanism and regulation of these changes. In addition, the acquisition of epigenetic changes late in the growth phase has implications for development of reproductive strategies for manipulating fertility that involve the growth of mammalian oocytes in vitro.

References

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Table 1. Regulatory expression in the imprinted genes by primary imprinting

Imprinted genes	Mapping	Expressed allele	Mode of regulation
Peg1/Mest	Chr. 6 Prox	paternal	maternal repression
Peg3	Chr. 7 Prox	paternal	maternal repression
Snrpn	Chr. 7 Prox	paternal	maternal repression
Igf2	Chr. 7 Dist	paternal	paternal repression
H19	Chr. 7 Dist	maternal	paternal activation*
$p57^{KIP2}$	Chr. 7 Dist	maternal	paternal repression
Igf2r	Chr.17 Prox	maternal	maternal activation

*through H19 repression by epigenetic modification during spermatogenesis

Table 2. Postimplantation development of blastocysts derived from oocytes constructed with nuclei from growing stage oocytes of adults mice.

Size of oocyte Donors (diameter)	Day of autopsy (dpc)	No pregnants/ recipients	No.of embryos transferrd	No.of implants (%)	No.of live fetuses or pups (%)
20-30 μm	9.5	2/2	14	5 (36)	0 (0)
	10.5	1/1	12	3 (25)	0 (0)
	12.5	2/2	12	4 (33)	0 (0)
40-49 μm	9.5	4/4	36	18 (50)	4 (11)
	11.5	4/4	48	20 (42)	5 (10)
	12.5	2/2	12	5 (42)	0 (0)
	14.5	2/2	11	6 (55)	0 (0)
50-59 μm	19.5	9/12	131	57 (44)	7 (5)**
60-70 μm	19.5	4/7	68	29 (43)	10 (15)
Control (75-80 μm)[a]	19.5	3/3	30	20 (67)	9 (30)

a, Control oocytes were constructed by exchange of GV between fully growm oocytes
**Siginificant ($p<0.01$) from controls