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Commentary

Endogenous Folate Accumulation in Oocytes and Preimplantation Embryos and Its Epigenetic Implications

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During mammalian development, chromatin is dynamically reprogrammed first in developing gametes and then after fertilization in the preimplantation embryo [1, 2]. In gametes, following an erasure phase that removes somatic DNA methylation, sex-specific DNA methylation is acquired. For female germ cells, this acquisition of methylation occurs in the growing oocyte, with an increasing progression of DNA methylation coincident with increasing oocyte size [1]. By the time the oocyte is fully grown, acquisition of DNA methylation is complete. During preimplantation development, DNA methylation is maintained primarily at imprinted genes and repetitive elements during global methylation erasure. This is followed by initiation of de novo DNA methylation late in preimplantation development [1]. During these chromatin remodeling periods, dynamic changes in histone methylation are also occurring. Importantly, these methylation patterns are critical to the overall health of gametes and embryos [1–4].

Both DNA and histone methylation are catalyzed by methyltransferases, which transfer a methyl group from the universal donor, S-adenosylmethionine (SAM), to CpG dinucleotides or histone tails [3–5]. Production of SAM is dependent on the 1-carbon folate pathway [3–5]. Thus, folates play a fundamental role in regulating DNA and histone methylation [3, 4]. In keeping with the critical reprogramming events described above, folate accumulation during oocyte and preimplantation development likely is essential to foster correct DNA and histone methylation patterns during gametogenesis and early development. Folates are essential nutrients that are acquired from our diet [3, 4]. Thus, a key question is how the oocyte and early embryo acquire and take up their folate pools. This question was address by Kooistra et al. [5] in their paper ''Folate Transport in Mouse Cumulus-Oocyte Complexes and Preimplantation Embryos'' published in this issue of *Biology of Reproduction*.

Using gene expression studies, Kooistra et al. [5] show that mouse cumulus-oocyte complexes and oocytes harbor transcripts for the reduced folate carrier SLC19A1, an anion exchanger. In preimplantation embryos, *Slc19a1* transcript abundance is very low due to the lack of embryonic gene activation. By comparison, folate receptor *FOLR1* mRNA was present in preimplantation embryos beginning at the 2-cell stage but was lacking in oocytes and zygotes. This ying-yang expression pattern indicates that two distinct mechanisms may be operating in cumulus-oocyte complexes and preimplantation embryos [5].

To functionally characterize SLC19A1 and FOLR1 activity in cumulus-oocyte complexes, germinal vesicle oocytes, and mouse preimplantation embryos, transport experiments were employed to measure uptake of folates and the antifolate methotrexate, whereas biochemical inhibitors were used to distill the uptake mechanisms. The results indicate that folate transport occurs predominately through SLC19A1 in cumulus-oocyte complexes but that FOLR1 is the principal uptake mechanism in embryos from the 2-cell through blastocyst stages. However, most interesting are the final experiments showing that SLC19A1 regulates cumulus cell folate uptake, with little to no uptake in fully grown oocytes. Thus, we are left with an intriguing question: What is the origin of endogenous folates in oocytes and zygotes? A careful look at the methods shows that fully grown oocytes were analyzed by Kooistra et al. Thus, it is reasonable to conclude that oocyte folate accumulation must occur during the early stages of folliculogenesis. Experiments now need to examine growing oocytes to determine when and through what mechanism folate uptake occurs. We are also left with the intriguing paradox that in vitro embryo development often occurs in non-folate supplemented culture medium. Does this mean that the early embryo relies on oocyte folate stores? And if so, why do early embryos have functional FOLR1 folate uptake? Future studies will also need to address whether variation in folate store accumulation is an important determinant of oocyte maturation and eventual embryonic developmental competence.

We must also consider the implication of the Kooistra et al. study on DNA and histone methylation. With regard to DNA methylation in fully grown oocytes, acquisition would be complete. Thus, there may be no further need for folate uptake in fully grown oocytes. However, what would be the ramifications if oocytes failed to store sufficient levels of folates? One repercussion of reduced folate stores during the methylation acquisition phase in growing oocytes may be aberrant establishment of imprinted methylation. Interestingly, we have observed that initiation of methylation acquisition is impaired at the imprinted *Peg1* gene in connexin 37-null oocytes [6]. We postulated that this may be due to reduced stores of a critical metabolite normally transported from granulosa cells to the oocyte via gap junctions. Another

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repercussion of insufficient oocyte folate stores after fertilization may be disruption of imprinted methylation maintenance at the first cell division, when embryonic folate uptake has not yet been initiated through FOLR1. Future studies will need to examine the impact of insufficient oocyte folate pools on DNA and histone methylation patterns during gametogenesis and early development. In summary, as with all good research, the results often generate more questions. The findings of Kooistra et al. [5] certainly make a compelling case for further research to explore these questions.

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