Peculiar DNA Marks Widespread in Oocytes

BOR–Papers in Press published 1 May 2013
DOI: 10.1095/biolreprod.113.110460

Driven by new advances in laboratory techniques, researchers are peering ever more closely at the oocyte as it develops from its meiotically quiescent state to the germinal vesicle stage, where it is poised for ovulation.

As the oocyte develops, its nearly naked DNA acquires a heavy load of methyl modifications. This process has long been thought to involve methylation at the cytosine CG sites almost exclusively, but recent studies have shown that non-CG methylation also occurs. Kenjiro Shirane et al. have leveraged advances in “Methylome” analysis to take a close look at non-CG methylation. They first assessed the prevalence of non-CG methylation as oocytes grew, and then pieced together which enzymes were responsible for the modification.

The researchers deployed a modified form of whole-genome bisulfite sequencing, which is a technique for globally assessing methylation that works on nanogram quantities of DNA. In non-growing oocytes taken from neonatal mice at the time of follicle formation, methylation at CG sites was low, at about 2.3%. Methylation levels increased to about 38% in germinal vesicle oocytes, consistent with previous results.

The researchers similarly observed a large increase in methylation at non-CG sites, increasing from apparently no methylation in the non-growing state to a high proportion at the germinal vesicle stage: about 66% of methylation occurred at non-CG sites. The methylation of CG and non-CG sites seemed to occur in a coordinated fashion in the same genomic regions. Moreover, the process required the same regulators, the methyltransferase Dnmt3a and its accessory protein Dnmt3L.

Methylation on non-CG nucleotides occurs de novo, and, unlike CG methylation, is not re-established during cell division. The researchers point out that this may explain why such methylation has also been observed in other non-dividing and slowly dividing cells, such as brain cells and certain pluripotent stem cells. The findings provide a strong basis for future experiments uncovering the biological role of this form of methylation.


Germ Cells Incubated in Nests

BOR–Papers in Press published 8 May 2013
DOI: 10.1095/biolreprod.113.110593

Germ cells proliferate during embryonic development to create the cells that ultimately give rise to sperm and eggs. In many species, this process includes a stage in which germ cells are connected to each other through cytoplasmic bridges, followed by apoptosis of some of the cells. Researchers have now examined this process in mice and found that it may promote optimal germline selection.

Lei Lei and Allan Spradling developed a labeling method that enabled them to activate a fluorescent label, YFP, in embryonic primordial germ cells prior to their mitotic proliferation. The label was activated in only about one primordial germ cell per mouse, enabling the researchers to track the fate of an individual germ cell.

As shown in Figure 1, the researchers found that each primordial germ cell gives rise to a cyst of synchronously dividing, interconnected cells. These cysts typically reach about 30 cells in number before mitotic division ceases, but by then the cysts have begun to fragment, losing some of their cellular connections. As they do so, the fragments associate with fragments from other cysts, although germ cells in the resulting nests of cells are interconnected and synchronized within the original fragment only.

In females, cyst fragmentation and aggregation occurs before and during meiosis I. In both sexes, the nests of cells eventually break apart and, by birth, the germ cells are isolated. The researchers found that apoptosis is uncommon in males, but in females almost 80 percent of these germ cells undergo apoptosis, which is consistent with

![FIG. 1. Image reproduced with permission from Development (DOI 10.1242/dev.093864).](image-url)