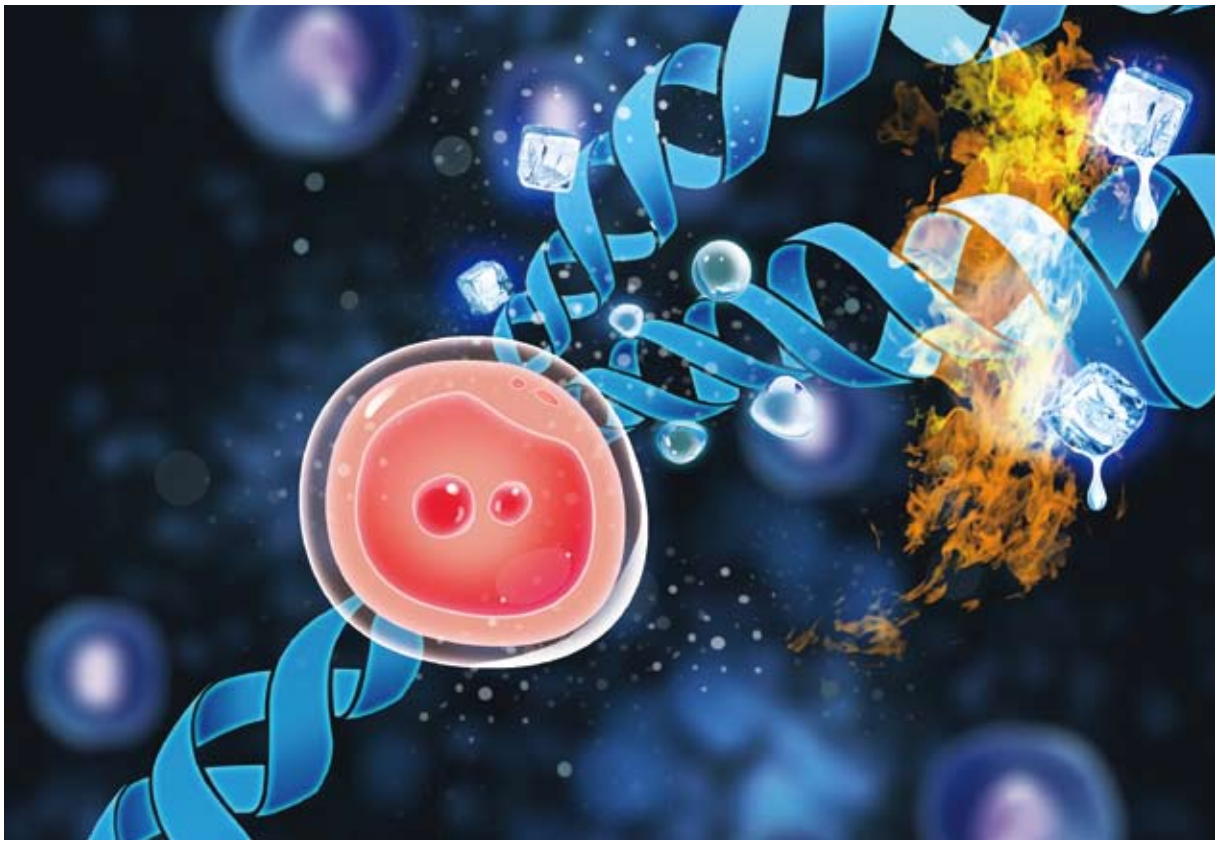
Both Mom and Dad Genomes Actively Reform Themselves: New Scenario Revealed in DNA Demethylation of Mammalian Early Embryos

By SONG Jianlan (Staff Reporter)

DNA demethylation in mammalian zygotes, a biochemical process through which the parental genomes let go the epigenetic marks from their "past lives" to obtain totipotency, has long fascinated biologists: how does it happen? A joint team at the CAS Shanghai Institutes for Biological Sciences tells a surprising story.



Pronuclear DNA demethylation in mammalian zygotes as revealed by the joint team: Active DNA demethylation occurs through oxidation of the methyl group (denoted with melting ice cubes) of 5-methylcytosine by Tet3 dioxygenases (drawn as fire). Whereas passive demethylation due to DNA replication results in a hemimethylated state (denoted by the distribution of two ice cubes only on one DNA strand). (Image by courtesy of Dr. XU)

A recent study by a joint team of scientists at the Shanghai Institutes for Biological Sciences (SIBS), CAS revealed a surprising scenario in early embryonic development of mammals: both paternal and maternal genomes of one-cell mouse embryo (zygote) undergo widespread active and passive DNA demethylation. Their discovery has subverted the earlier belief that only paternal genome undergoes active demethylation while the maternal genome passive demethylation instead through DNA replication, fueling new insights on the role of active and passive DNA demethylation in cell reprogramming and early embryonic development of mammals.

The study, jointly conducted by Profs. XU Guoliang and LI Jinsong's groups at the Institute of Biochemistry and Cell Biology (SIBCB) under SIBS in cooperation with Prof. TANG Fuchou's group from Peking University, also confirmed their earlier discovery that this process of demethylation is mediated by Tet3, a member from the enzyme family of Tet dioxygenases, and somehow independent of another enzyme, the thymine DNA glycosylase (TDG), which was previously revealed as a player in the downstream demethylation.

Published online on Sept 11 and in print on Oct 2 in *Cell Stem Cell*, this finding is the latest one from the intense, continuous efforts by Prof. XU and his colleagues to understand the process and role of DNA methylation/demethylation in mammalian development, which are involved with a mysterious and fascinating epigenetic phenomenon in reproduction: reprogramming.

Back to Pristine: Reprogramming

DNA methylation is a biochemical process where a methyl group is added to the cytosine or adenine of the nucleotides on the DNA chain, and hence decreases the activity of the gene in transcription and subsequently, expression. This, occurring when cells divide or differentiate from embryonic state, can stably silence some genes and shape the cells into certain types of somatic cells. This explains why our organs look so different from each other despite the fact that they share the same sets of genes.

DNA methylation leaves almost permanent, generally unidirectional epigenetic marks on the DNA, making it almost impossible for somatic cells to revert to stem cells. Only in some very special cases, for example when a zygote is formed, can such epigenetic marks be erased by DNA demethylation, a conversion of the methylated bases, say 5-methylcytosine (5mC), back to unmodified cytosines, throwing the pronuclear genome back into a pristine status. The earliest embryo can thus take a fresh start to mark, or "reprogram" how its genes will be expressed. As a result, the zygote is endowed with the privilege called totipotency – the ability to divide and differentiate into all kinds of somatic cells – and hence can develop into an embryo and further an individual organism.

This fascinating process in life activity has puzzled biologists for a long time. How does this reprogramming happen? What and how is it regulated? How do the genes know whether or not they "should" be turned on or off to secure proper development? These questions are pivotal to many important aspects of biomedicine, including the induction of pluripotent stem cells (iPSCs), embryonic development, animal cloning and diseases.

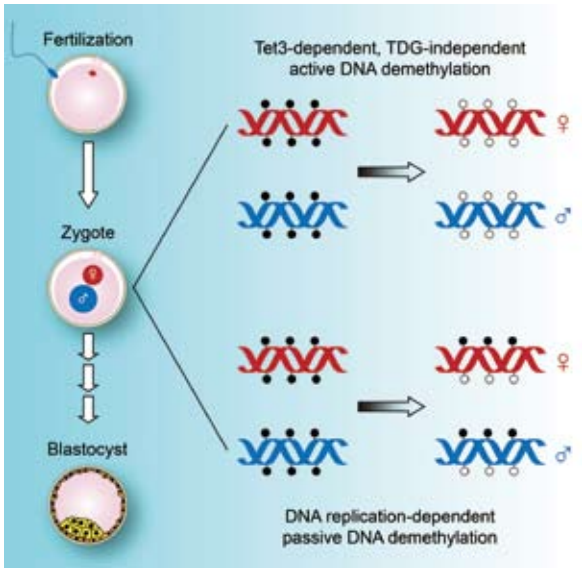
What adds to its glamour is another phenomenon called genomic imprinting, where the alleles on the pronuclear DNA of a zygote are selectively marked along either paternal or maternal line, such that only genes from the paternal or maternal side are expressed. In other words, the paternal and maternal genomes can "remember" part of the epigenetic marks and retain them in the reprogramming.

Proper reprogramming is a great wonder to behold given the complexity of the epigenome and the supreme importance of the subsequent embryonic development for a healthy individual organism – wherever disorders occur, serious diseases will follow. What is behind this marvellous process? Where is that "invisible hand"? All hinged on DNA methylation and demethylation, this breathe-taking moment in life has attracted longstanding, intensive attention from researchers.

Get to Know the Players

Over the past years, XU and his colleagues have put most of their efforts into understanding DNA methylation/demethylation and its role in reprogramming and mammalian development. Due to his important contributions to this field, XU was awarded with the Tan Kah Kee Science Award in Life Sciences of 2014.

Accumulated efforts by them and others in this field have revealed that sperms and eggs differ greatly in their epigenetic makeup: sperms show very high level of DNA methylation (represented by 5-methylcytosine, or 5mC) while in oocytes the 5mC level is lower. In wake of fertilization, the pronuclear DNA of the zygote will undergo a series of reprogramming to establish totipotency for the early embryo. A traditional belief is that the paternal genome will undergo large-scale active DNA demethylation before the first mitosis, while the maternal genome will undergo passive demethylation via DNA replication later on during blastomere cleavage. In cooperation with his SIBCB colleague Prof. LI Jinsong, XU revealed in his 2011 research published in *Nature* that this active demethylation



In the one-cell mouse embryo (zygote), the maternal and paternal genomes both undergo Tet3-dependent active demethylation and replication-mediated passive demethylation. (Image by courtesy of Dr. XU)

is dependent on the oxidation of 5mC with the aid of Tet dioxygenases. The products from the oxidation, namely the 5fC and 5caC, are recognized and excised by TDG. The role of this pathway for demethylation mediated by Tet and TDG in zygotes, however, remained unclear.

Now with joining forces from Prof. TANG Fuchou's group at Peking University, XU and LI's labs set out to systematically investigate the molecular mechanism of DNA demethylation occurring in paternal and maternal genomes of mouse zygotes.

Both Parents Active in Reprogramming

With the aid from high-accuracy analytic techniques for epigenetic modification, including an improved reduced representation bisulfite sequencing approach recently developed by XU and colleagues that enabled them to determine the methylomes at base resolution on a genome-wide scale, they investigated Tet3- and TDG-knockout mice and directly looked at the pronuclear DNA before the first mitosis in the zygote, testing the role of active and passive demethylation. At last they demonstrated that aside from passive demethylation via DNA replication, both paternal and maternal genomes undergo global active demethylation in the zygote before the first mitotic division.

Before them, another group reported earlier this year evidence for active demethylation of both parental methylomes, but instead of examining the methylation status in one-cell zygotes directly, they observed this in mouse gametes and early embryos, without validating the role of Tet3 or DNA replication via loss-of-function approaches.

This demethylation, as reported by the team, is dependent on Tet3. At the loci where active demethylation occurred, the 5mCs were replaced by unmodified cytosines, leaving very little 5fC/5caC, residual products from the Tet-mediated oxidation. Surprisingly, the demethylation process is independent of TDG, suggesting existence of other player(s) in the downstream demethylation responsible for eliminating the residuals of 5mC. "This indicates that there may be another demethylation pathway to be identified, in which Tet3, but not TDG is involved," comments Dr. GU Tianpeng, co-author of the paper.

Further Studies Required

Active demethylation soon after fertilization, the team suggested, might benefit the early embryonic development in some possible ways. For example, this might free the embryo from subsequent developmental disorders by preventing transmission of erroneous DNA methylation from the parents, and could also exempt the blastomeres from mistaken remethylation, which could also lead to developmental disorders.

Notably, recent studies indicate a similarity between the global methylation change in human zygotes and that in mouse, suggesting some conservation of the demethylation mechanism as revealed by XU, LI and TANG's research. However, the authors cautioned, further studies are required to understand the functional significance and molecular processes of embryonic DNA demethylation, particularly when inheritance of epigenetic modification and establishment of zygotic totipotency are involved.

For more information please refer to:

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