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## New Insight into Control of Parental Gene Expression in Eggs

Researchers have identified a crucial step in a genetic process required for the development of viable eggs. The process, known as imprinting, distinguishes the paternally inherited and the maternally inherited copies of a number of developmentally important genes.

The majority of mammalian genes are present in two copies, both of which are equally expressed and regulated. A small number of mammalian genes, however, are subject to special regulation by a process called gene imprinting. The imprint is a chemical mark, such as methylation, attached to genes during egg or sperm development. Imprinting physically marks genes in such a way that the parental origin of the two copies can be distinguished so that one parent's copy is turned on while the other is silenced. Imprinted genes are the likely reason that maternal and paternal contributions are necessary for normal mammalian development.

Exploring the mechanisms underlying gene imprinting may provide insight into so-called epigenetic control of gene expression, in which the cellular machinery governs the expression of genes in the cell. The function of that machinery, which makes modifications to the genome, remains among the major mysteries in biology.

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- Marisa S. Bartolomei

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Howard Hughes Medical Institute investigator Marisa S. Bartolomei, Andrew Fedoriw, Paula Stein, Petr Svoboda and Richard Schultz at the University of Pennsylvania published their findings in the January 9, 2004, issue of the journal *Science*.

The researchers sought to pinpoint the regulatory role of a protein known as CTCF, which is believed to attach to a control region near imprinted genes.

By binding to this region, called the differentially methylated domain, CTCF blocks the site from the attachment of methyl groups to the DNA -- chemical modifications that the cell's epigenetic machinery uses to silence genes.

Bartolomei and her colleagues focused on the role of CTCF in protecting a gene called *H19*, whose paternal version is silenced in the developing embryo while the maternal version remains active.

To determine whether CTCF might be protecting the maternal copy of *H19* from DNA methylation, the researchers used a technique called RNA interference, in which they genetically engineered mouse eggs to produce RNA molecules that can interfere with a particular messenger RNA (mRNA). In this case, the mRNA for the CTCF protein was targeted. This technique in effect degrades the target mRNA, thereby reducing the level of the protein for which it codes.

The researchers generated and studied a series of mouse eggs that exhibited different levels of RNA interference, resulting in different amounts of the CTCF protein. They found that the lower the level of CTCF in the developing eggs, the higher the methylation of the regulatory domain for *H19*. They also observed that female mice that developed from the eggs with the lowest levels of CTCF protein showed profoundly reduced fertility.

"While this is certainly an indirect experiment -- in that we depleted the CTCF protein and saw that DNA methylation was acquired -- it is nevertheless persuasive evidence of the interaction of CTCF with the differentially methylated domain of *H19*," said Bartolomei. "It's a finding that makes good intuitive sense.

"What's important about this experiment is that it demonstrates that CTCF appears to be actively protecting the *H19* gene," she said. "And importantly, we have demonstrated the utility of this gradation RNA-interference technique that we believe will be invaluable for studying the functions of proteins whose complete ablation by the usual knockout techniques would be lethal."

More broadly, said Bartolomei, such studies investigating the regulation of imprinting are likely to yield new insight into the machinery underlying the epigenetic control of gene expression. "Our findings suggest that there are critical sequences at imprinted genes that are recognized and can be marked by or protected from DNA methylation," she said. "These sequences that regulate imprinted genes are not straightforward, however, but are more complicated ones that we are not able to predict by sequence analysis alone."

An important future direction for the research, she said, is to identify more genes similar to *H19* that are subject to CTCF-dependent imprinting control and to use RNA interference and other techniques to explore the nature of that control.

"Until the availability of this technology, it has been an essentially intractable problem to look at imprinting establishment in the early embryo," said Bartolomei. "But using these newly developed technologies we can now test candidate molecules that might be critical for conferring imprinting.

"We want to identify other paternally methylated and maternally unmethylated regions to see if CTCF might be a general protector against methylation," she said. "Further, we've shown that these embryos produce mice with dramatically reduced litter sizes, and we'd like to understand the nature of the defects as a way to understand the role of CTCF."