Bioresource Engineering Laboratory
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(0) Research field

CPR Subcommittee**:** Biology

Keywords:

Developmental biology, embryo, epigenetics, fertilization, mouse

(1) Long-term goal of laboratory and research background

To understand how new life is established at fertilization, how the germline genomes are established during development, and how the resultant embryonic genomes are programmed to contribute to two developmental lineages (the embryonic and placental lineages) in the mammalian life cycle. These are the key questions of developmental epigenetics. Clues should exist within the genomes of normally developing germ cells and embryos, but the information obtained from conventional analyses with these "normal" materials may be limited. Therefore, we reconstruct special embryos using reproductive engineering techniques including microinsemination (sperm or spermatid injection), nuclear transfer cloning and establishment of new stem cell lines, and undertake systematic and comparable analyses. Most studies are performed using laboratory mice (*Mus musculus*) because a variety of reproductive engineering techniques as well as a large amount of genomic information are available for this species. In some studies where mice models are not suitable due to the lack of phenotypes in knockout mice, we produce genetic knockouts of golden (Syrian) hamsters (*Mesocricetus auratus*) Our strong point is that we can make the best use of the mouse reproductive engineering techniques and combine them with cutting-edge genomic analyses. This strategy may provide us with the opportunity to solve the important questions above, which cannot be achieved by the conventional experimental methods.

(2) Current research activities (FY2021) and plan (until Mar. 2025)

(A) Establishment of embryonic stem cells (ESCs) from wild-derived mice by somatic cell nuclear transfer (SCNT)

Laboratory mice are widely used in biological research, but they have a disadvantage of low genetic diversity. To overcome this problem, wild-derived mice with diverse genetic backgrounds have been introduced into research laboratories. The RIKEN BRC has a world-class collection of wild-derived mice, but their major drawback is the difficulty in adapting conventional reproductive engineering techniques and the unavailability of reliable ESCs. To ensure a stable supply and maintenance of wild mouse strains and to maximize the use of their genetic characteristics, we attempted to establish nuclear transfer ESCs (ntESCs) using nuclear transfer cloning technology. Mature oocytes of B6D2F1 (*M. m. domesticus*), a representative F1 crossbred line of lab mice, were used as recipient oocytes, and monocytes and granulocytes recovered from the peripheral blood of wild-derived CASP/1Nga and CAST/Ei (both *M. m. castaneus*) mice were used as nuclear donors. NtESCs were established from cloned blastocyst stage embryos by the so-called 2i method, in which LIF and two differentiation inhibitors were added. The established ntESC lines were subjected to microsatellite analysis and mitochondrial (mt) DNA confirmation for their nuclear and cytoplasmic genomic origins, confirmation of undifferentiated markers, the chromosome number, and teratoma formation analysis. Thirteen lines (5 males and 8 females) from CASP/1Nga and 11 lines (8 males and 3 females) from CAST/ Ei were established. The microsatellite analysis revealed that their nuclear genomes were all derived from the donor wild strains. On the other hand, the mtDNAs were derived from *M. m. domesticus*, which was used as the recipient oocytes, as expected. Injection of high-quality ntESCs (with undifferentiated markers and normal chromosome constitution) into 8-cell stage or blastocyst stage embryos resulted in production of chimeric mice in both strains. Furthermore, germline transmission was confirmed in the male chimeric mice of CAST/Ei by mating tests. Thus, we succeeded in establishing ntESCs with a full pluripotency from wild mice.

Future Plan The wild mice from which ntESCs were established in this study are subspecies of laboratory mice. In the future, we plan to conduct experiments using wild mice of different species of laboratory mice.

(B) Production of offspring from azoospermic mutant mice with a spermatocyte developmental defect

In many mammals including humans, mature spermatozoa as well as immature sperm (spermatids) can normally fertilize oocytes following direct injection. However, younger male germ cells, i.e. primary spermatocytes, have never been considered for fertilizing oocytes owing to their premeiotic state, despite frequent spermatocyte arrest among human azoospermia cases. Here, we propose that primary spermatocytes can be used as potential substitute gametes, provided they are injected into immature oocytes with a reduced cytoplasm. We confirmed that mouse spermatocytes injected into intact immature oocytes could start meiosis together with oocyte chromosomes. However, after transfer of the resultant embryos into recipient females, only 1% of them developed into offspring. Further, high-resolution live-imaging analysis revealed that spermatocyte chromosomes underwent erroneous segregations at meiosis I in most oocytes. However, when we reduced the cytoplasm of the recipient oocytes, the normality of the meiotic chromosomes significantly improved, leading to a remarkably higher birth rate (19%, $P < 0.0001$ vs. control). Moreover, this technique enabled the production of offspring from azoospermic male mice with spermatocyte arrest caused by the deficiency of STX2, an azoospermia factor also found in humans. Thus, female meiotic apparatuses could guide meiotic divisions of male chromosomes with more exactness than was assumed. Furthermore, our study suggests that spermatocyte injection might be a potential treatment for azoospermia patients with spermatocyte arrest.

Future Plan Because of the differences in male and female meiosis, it was unexpected that the chromosomes of the primary spermatocytes would proceed to meiosis in the oocyte, leading to a birth rate of nearly 20%. There are many mutant mice with spermatocyte arrest in the world. We plan to investigate which mutations can be rescued by this method in the future.

(3) Members as of March, 2021

(Chief Scientist)
Atsuo Ogura

(Assitant)
Ayano Tsukahara

(4) Representative research achievements

1. “Highly rigid H3.1/H3.2–H3K9me3 domains set a barrier for cell fate reprogramming in trophoblast stem cells”, Hada M, Miura H, Tanigawa A, Matoba S, Inoue K, Ogonuki N, Hirose M, Watanabe N, Nakato R, Fujiki K, Hasegawa A, Sakashita A, Okae H, Miura K, Shikata D, Arima T, Shirahige K, Hiratani I, Ogura A., **Genes Dev** 36, 84-102 (2022).
2. “Formation of spermatogonia and fertile oocytes in hamsters requires piRNAs.” Loubalova Z, Fulka H, Horvat F, Pasulka J, Malik R, Hirose M, *Ogura A, *Svoboda P., **Nat Cell Biol** 23, 992-1001 (2021).
3. “Easy and quick (EQ) sperm freezing method for urgent preservation of mouse strains.” Mochida K, Hasegawa A, Shikata D, Itami N, Hada M, Watanabe N, Tomishima T, Ogura A., **Sci Rep** 11**,** 14149 (2021).
4. “Improved development of mouse SCNT embryos by chlamydocin analogues, class I and IIa histone deacetylase inhibitors.” Kamimura S, Inoue K, Mizutani E, Kim J-M, Inoue H, Ogonuki N, Miyamoto K, Ihashi S, Itami N, Wakayama T, Ito A, Nishino N, Yoshida M, Ogura A., **Biol Reprod** 105, 543-553 (2021).
5. “Tsga8 is required for spermatid morphogenesis and male fertility in mice.” Kobayashi Y, Tomizawa SI, Ono M, Kuroha K, Minamizawa K, Natsume K, Dizdarević S, Dočkal I, Tanaka H, Kawagoe T, Seki M, Suzuki Y, Ogonuki N, Inoue K, Matoba S, Anastassiadis K, Mizuki N, Ogura A, Ohbo K., **Development** 148, dev196212 (2021).

Laboratory Homepage
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