Molecular Correlates of Primate Nuclear Transfer Failures

Calvin Simerly,[1]* Tanja Dominko,[1]*† Christopher Navara,[1] Christopher Payne,[1] Saverio Capuano,[1] Gabriella Gosman,[1] Kowit-Yu Chong,[1] Diana Takahashi,[1] Crista Chace,[1] Duane Compton,[2] Laura Hewitson,[1] Gerald Schatten[1]‡

Somatic cell nuclear transfer (SCNT) (*1*) in nonhuman primates could accelerate medical research by contributing identical animals for research and clarifying embryonic stem cell potentials (*2*). Although rhesus embryos begin development after embryonic cell nuclear transfer (ECNT) (*3*–*5*), there has only been one report of rhesus births after ECNT (*3*), and that report has not been replicated.

Here, molecular obstacles were identified using 716 rhesus oocytes in four experimental studies: set A, SCNT [rhesus cumulus, umbilical cord blood, epithelial-derived fibroblasts, and inner cell mass–derived precursor embryonic stem cells; 193 oocytes; 62.8% nuclear transfer (NT) success assayed by interphase nucleus formation], and set B, ECNT from dissociated 16- to 32-cell stage embryos (381 oocytes; 97.2% NT success), because ECNT success is greater than SCNT (*1*). Because meiotic spindle removal appeared to be responsible for these NT failures, we performed two additional experiments in which either we did not remove the spindle (set C) or we removed and reinserted it (set D). In set C, NTs into concurrently fertilized oocytes generated tetraploids (55 oocytes; 54.4% success), whereas in set D, fertilization of reconstituted oocytes (that had previously been enucleated and then renucleated) generated diploids (95 oocytes; 67.1% success).

Rhesus NTs (*6*) look superficially normal, yet no pregnancies resulted from 33 embryos transferred into 16 surrogates (compared with seasonably variable 28 to 66% pregnancy rates by assisted reproduction) (*7*). DNA and microtubule imaging showed disarrayed mitotic spindles with misaligned chromosomes (Fig. 1A; all 116 ECNTs and all 30 SCNTs examined displayed aberrant spindles). Despite these defects, cleavages continue, but unequal chromosome segregations produce aneuploid embryos.

NuMA (Nuclear-Mitotic Apparatus), a matrix protein responsible for spindle pole assembly (*8*), concentrates at centrosomes in unfertilized meiotic (Fig. 1B) and fertilized mitotic cells (Fig. 1C). After NT, NuMA is not detected on the abnormal mitotic spindles (Fig. 1D) or in enucleated oocytes. HSET and Eg5 are mitotic kinesin motors (*8*, *9*). HSET, found during meiosis and mitosis, is not detected in NT spindles (Fig. 1E). Eg5 detects centromere pairs at meiosis and mitosis, including misaligned ones on NT spindles (Fig. 1F). Thus, meiotic spindle removal depletes the ooplasm of NuMA and HSET, both vital for mitotic spindle pole formation.

Normal spindles found in tetraploids suggest meiotic spindle removal as the source of NT anomalies. In tetraploids, chromosomes aligned properly on bipolar spindles with centrosomal NuMA (Fig. 1G). NT mitotic spindles could be distinguished from the fertilized spindle by the sperm tail. Similarly, fertilization of reconstituted oocytes resulted in apparently normal divisions. Thus, manipulation of the embryos alone was not the cause of the problem, and proper mitotic spindles can be organized around somatic chromosomes if the meiotic spindle is left intact.

Primate NT appears to be challenged by stricter molecular requirements for mitotic spindle assembly than in other mammals. In cattle, the somatic centrosome is transferred during NT (*10*), whereas mice rely on the oocyte's maternal centrosome (*11*). Also, NuMA and HSET are not exclusively concentrated on the meiotic spindle in mammals other than primates (*8*). With current approaches, NT to produce embryonic stem cells in nonhuman primates may prove difficult—and reproductive cloning unachievable.

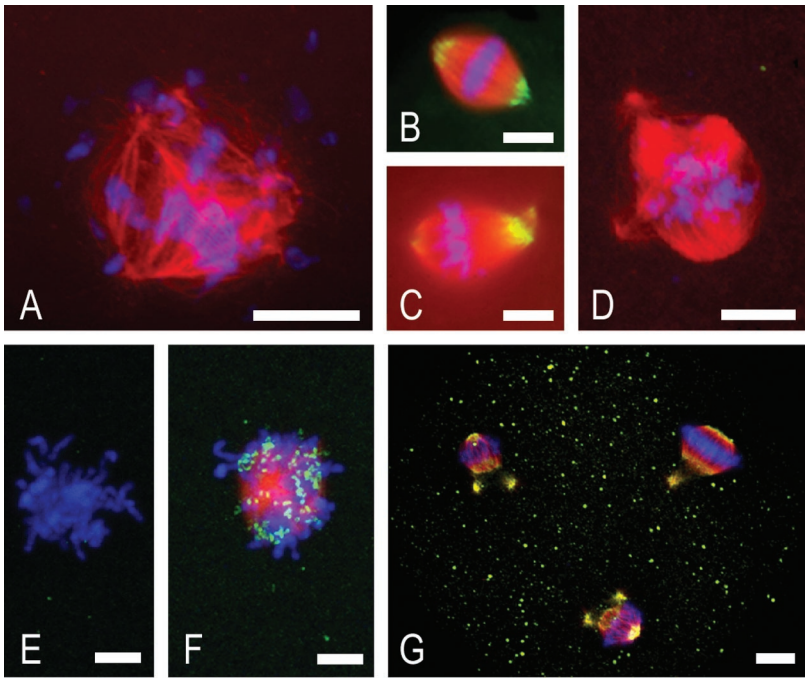

Fig. 1. Faulty mitotic spindles produce aneuploid embryos after primate nuclear transfer. (**A**) Defective NT mitotic spindle with misaligned chromosomes. Centrosomal NuMA at meiosis (**B**) and mitosis (**C**), but not in mitotic spindles after NT (**D**). The centrosomal kinesin HSET is also missing after NT (**E**), but not centromeric Eg5. (**F**). Bipolar mitotic spindles with aligned chromosomes and centrosomal NuMA after NT into fertilized eggs (**G**). DNA, microtubule, NuMA, and kinesin imaging as in (*7*, *8*). Blue, DNA; red, β-tubulin; green, NuMA in (B), (C), (D), and (G); HSET in (E); and Eg5 in (F). Scale bar, 10 μm.

References and Notes

1. I. Wilmut, *Nature Med.* **8**, 215 (2002).
2. J. A. Thomson *et al.*, *Science* **282**, 1145 (1998).
3. L. Meng, J. J. Ely, R. L. Stouffer, D. P. Wolf, *Biol. Reprod.* **57**, 454 (1997).
4. T. Dominko *et al.*, *Cloning* **1**, 143 (1999).
5. S. M. Mitalipov, R. R. Yeoman, K. D. Nusser, D. P. Wolf, *Biol. Reprod.* **66**, 1367 (2002).
6. Materials and methods are available as supporting material on *Science* Online.
7. L. Hewitson *et al.*, *Nature Med.* **5**, 431 (1999).
8. V. Mountain *et al.*, *J. Cell Biol.* **147**, 351 (1999).
9. A. Blangy *et al.*, *Cell* **83**, 1159 (1995).
10. C. S. Navara, N. L. First, G. Schatten, *Dev. Biol.* **162**, 29 (1994).
11. G. Schatten, *Dev. Biol.* **165**, 299 (1994).

12. We thank S. Doxsey, J. Jones, O. Khorram, A. Lewis, V. Mountain, T. Plant, J. St. John, and T. Stearns and Serono Reproductive Biology Institute (Rockland, MA 02370) for hormones and we gratefully acknowledge NIH support.

[1]Pittsburgh Development Center, Magee-Womens Research Institute, Departments of Obstetrics-Gynecology-Reproductive Sciences & Cell Biology-Physiology, University of Pittsburgh School of Medicine, Pittsburgh, PA 15213, USA. [2]Department of Biochemistry, Dartmouth Medical School, Hanover, NH 03755, USA.

*These authors contributed equally to this work
†Present address: CellThera, Worcester, MA 01605.
‡To whom correspondence should be addressed. E-mail: gschatten@magee.edu