Kaguya, the first parthenogenetic mammal – engineering triumph or lottery winner?

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A significant milestone in reproductive science has been achieved with the birth of the mouse 'Kaguya', the first viable parthenogenetic mammal (Kono *et al.* 2004). The work was carried out by Dr Tomohiro Kono and colleagues, and represents a major technical achievement involving the production of many hundreds of reconstructed eggs, from which ten live, and eighteen dead, pups were obtained at day 19.5 of gestation. Of the two surviving pups, one was killed for gene expression studies and the other, Kaguya, was fostered and survived to reproduce successfully by conventional means. This work further expands what is achievable in artificial reproduction and may have important implications for understanding aspects of embryonic development and gene regulation. However, contrary to the views of some commentators in the popular press, it is unlikely to have a major impact on human artificial reproductive technologies.

Genomic imprinting, the differential expression of genes depending on their parental origin, is the main (perhaps the only) barrier to parthenogenetic development in mammals, in which the individual contains no paternal genetic material. In mechanistic terms, genomic imprinting means that the chromatin of certain genetic loci is differentially modified in the parental germlines so that the parental alleles are differentially expressed in the developing embryo. Around fifty genes have been described in mice and humans that exhibit transcriptional silencing of one of the parental alleles during embryonic development (Moore *et al.* 2001, Fig. 1A). Parthenogenetic embryos therefore are deficient in paternally expressed imprinted gene products and exhibit severe growth retardation and intrauterine death.

For nearly a decade, Kono and others have worked to improve the extent to which parthenogenetic embryos can develop *in utero*, thereby revealing important mechanistic details of the imprinting process (Kono *et al.* 1996, 2002, Obata *et al.* 1998, Kato *et al.* 1999, Bao *et al.* 2000, 2003, Sotomaru *et al.* 2002). Principally, their work shows that the imposition of imprints in the maternal germline occurs at a relatively late stage of oogenesis. Therefore, at some imprinted genetic loci, non-growing (ng) oocytes may be 'imprint-neutral' with respect to

maternally imposed imprints, or may retain some paternal imprints that are not removed until later in oogenesis. There is evidence for both of these possibilities (Kono *et al.* 1996, Obata *et al.* 1998, Kato *et al.* 1999, Bao *et al.* 2000, T Kono, unpublished observations). When ng oocytes are used to reconstitute diploidy of unfertilised eggs (Fig. 1B), the result is development far beyond what is normally seen using fully grown (fg) oocytes. However, these improvements notwithstanding, the furthest that such embryos can develop is to day 13.5 of gestation (Kono *et al.* 1996). Molecular genetic analysis of these embryos indicates that, while several paternally expressed imprinted genes are expressed from the ng oocyte genome, the normally maternally expressed *H19* gene is biallelically expressed and the paternally expressed *Igf2* gene is silenced on both ng and fg derived alleles (Obata *et al.* 1998).

Kono's next step was to attempt to correct *H19* and *Igf2* gene dosage in parthenogenetic embryos by introducing chromosomes containing deletions that: (i) abolish *H19* transcription (Kono *et al.* 2002, Fig. 1C), and (ii) abolish *H19* transcription and recover *Igf2* expression (Kono *et al.* 2004, Fig. 1D). The first manipulation extended parthenogenetic development *in utero* to day 17.5 of gestation and the second resulted in the birth of Kaguya. Taken at face value, these results imply that further improvements in the rate of successful parthenogenetic development are possible with deeper knowledge of the imprinting process and more sophisticated manipulations of genotype or epigenotype. Essentially, a type of rational developmental engineering may be achievable.

However, Rudolf Jaenisch, quoted recently in *The Scientist*, argues that Kaguya is simply a stochastic event, in which a major component of the epigenetic basis of her viability is unpredictable (Holding *et al.* 2004). He essentially relegates Kono's rationale of using *H19/Igf2* transgenics to a minor role. Implicitly, he argues that if a large number of embryo reconstitution experiments are performed the birth of viable progeny may occur due to random sampling of 'epigenotype space'. His arguments parallel the suggestion that viable cloned animals produced by somatic cell reprogramming are just unique, random events (Surani 2003). However, in Kono's

Figure 1 Schematic of epigenotype at *H19/Igf2* gene locus associated with various manipulations of mouse germ cells. (A) Normal fertilisation. (B) Embryo reconstitution with fully grown oocyte and non-growing oocyte (Kono *et al.* 1996). (C) Embryo reconstitution with fully grown oocyte and non-growing oocyte carrying deletion of the *H19* transcription unit (Kono *et al.* 2002). (D) Embryo reconstitution with fully grown oocyte and non-growing oocyte carrying extensive deletion of *H19* gene region including upstream differentially methylated region/boundary element (Kono *et al.* 2004). Vertical bars: Red bars, *Igf2* gene transcription unit; green bars, differentially methylated region/boundary element upstream of *H19* gene promoter; blue bars, *H19* gene transcription unit. Values refer to day of gestation (developmental stage) reached following each type of manipulation.

experiments, in contrast to somatic cell cloning, the ng oocyte nucleus probably does not undergo extensive reprogramming of chromatin, being already committed to a germline stem cell fate. Also, such oocytes are explanted at a defined developmental stage and are therefore expected to be relatively homogeneous with respect to epigenotype. A more instructive comparison, in embryo reconstitution experiments, may be with the use of haploid spermatid nuclei from the testis or diploid blastomeric nuclei from preimplantation embryos, which undergo relatively high rates of development.

What then, underlies the low rates of parthenogenetic viability in Kono's experiments? One possibility is that the origin of ng oocyte epigenotype variability is due to the random sampling of different combinations of maternally and paternally derived imprinted chromosomal regions at meiosis. Recall that the diploid ng oocyte nucleus contains maternally and paternally derived homologues that may differ systematically (rather than stochastically) at imprinted loci due to incomplete removal of residual maternal and paternal imprints at this stage of oocyte development. At each imprinted locus, maternally and paternally derived homologues are shuffled and randomly segregated at meiosis. Therefore, in Kono's experiments, each resultant haploid ng oocyte nucleus represents one of 2^n combinations of maternal and paternal imprints, where n is the number of imprinted chromosomal regions that remain differentially modified in ng oocytes. For example, if the diploid ng oocyte genome contains eight

imprinted chromosomal regions that systematically exhibit residual differences between maternal and paternal homologues, it follows that there are 2^8 (256) possible epigenotypes, of which perhaps only a small number permit embryo viability. To extend the example: perhaps only the 1 in 256 of ng oocytes that inherit a full set of eight formerly paternal homologues is capable of supporting good embryo development due to the retention of some paternal imprints at these loci. The validity of this hypothesis could be tested using ng oocytes from an F₁ hybrid to identify the distribution of grandmaternal and grandpaternal homologues at imprinted loci in reconstituted embryos exhibiting exceptional development.

Jaenisch also notes that the rescue of parthenogenetic embryo viability by the enhancement of *Igf2* expression (viz Kaguya) is unexpected because *Igf2* is dispensable for viability in normal biparental embryos. However, the contribution of *Igf2* to embryo viability has been tested only in a very limited number of genetic backgrounds. It is quite conceivable that some of Kono's parthenogenetic embryos, having a different epigenotype and gene expression pattern to biparental embryos, benefit from complementation with *Igf2*. However, Kono's 'fascinating riddle' of how *H19/Igf2* normalisation 'caused the modification of a wide range of genes' (Kono *et al.* 2004) may be a red herring because the epigenotype of a parthenogenetic embryo that responds to *H19/Igf2* normalisation may differ from one that does not. The perceived change in gene expression associated with addition of the *H19*^{Δ13}

mutation may therefore reflect the selection of a pre-existing epigenotype that facilitates *Igf2*-mediated enhancement of parthenogenetic development, rather than being a direct result of *Igf2* expression *per se*.

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