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# Completion of Mouse Embryogenesis Requires Both the Maternal and Paternal Genomes

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## Summary

**Transplantation of pronuclei between one-cell-stage embryos was used to construct diploid mouse embryos with two female pronuclei (biparental gynogenones) or two male pronuclei (biparental androgenones). The ability of these embryos to develop to term was compared with control nuclear-transplant embryos in which the male or the female pronucleus was replaced with an isoparental pronucleus from another embryo. The results show that diploid biparental gynogenetic and androgenetic embryos do not complete normal embryogenesis, whereas control nuclear transplant embryos do. We conclude that the maternal and paternal contributions to the embryonic genome in mammals are not equivalent and that a diploid genome derived from only one of the two parental sexes is incapable of supporting complete embryogenesis.**

## Introduction

It is generally believed that both parental sexes contribute equivalent nuclear information to the zygote nucleus since in many animal species viable parthenotes exist. Equivalency of the maternal and paternal contributions to the zygote genome in mammalian species, however, is uncertain. Mammalian parthenotes are inviable, and although experimentally activated mouse eggs have developed as far as the 25-somite stage (Kaufman et al., 1977), most die shortly after implantation.

Possible causes for the death of mammalian parthenotes (Graham, 1974) may be either nuclear or cytoplasmic deficiencies. Nuclear deficiencies would include nonequivalent parental contributions to the zygote nucleus and/or homozygosity for lethal alleles. Alternatively, cytoplasmic deficiencies, such as the lack of an extragenetic contribution by the fertilizing spermatozoon or the inability of the parthenogenetic stimulus to mimic appropriately the stimulus provided by the spermatozoon, could result in the death of mammalian parthenotes. Cells derived from parthenogenetic embryos are viable (Iles et al., 1975), and parthenogenetic and normal embryos can combine to form viable chimeras in which many tissues, including functional germ cells, originated from the parthenogenetic embryo (Stevens, 1978). The full-term development of mouse diploid uniparental embryos (Hoppe and Illmensee, 1977) and of nuclear-transplant embryos that received a nucleus from an inner cell mass cell of a parthogenetic embryo (Hoppe and Illmensee, 1982) argued against a nuclear defect's causing death of the parthenotes. Although these last reports suggest that the lack of development of parthenogenetic embryos is due to the absence of an extragenomic contribution from the spermatozoon, similar attempts by other investigators to produce uniparental mice have not resulted in the birth of live progeny (Modlinski, 1980; Markert, 1982).

More recently, uniparental diploid gynogenetic mouse embryos were derived by the microsurgical removal of the paternal pronucleus following suppression of second polar-body extrusion (Borsuk, 1982; Surani and Barton, 1983). These embryos, however, like parthenotes, died shortly after implantation (Surani and Barton, 1983). Thus uniparental diploid gynogenetic embryos derived by inhibition of the first mitotic division are reportedly viable (Hoppe and Illmenssee, 1977), whereas those derived by inhibition of the second meiotic division are not (Surani and Barton, 1983). The reason(s) for this apparent discrepancy is not known.

To determine whether the maternal and paternal contributions to the zygote are equivalent, we transplanted single pronuclei between one-cell-stage embryos using a recently described nuclear-transplantation technique (McGrath and Solter, 1983). Removal of a single pronucleus, followed by the introduction of a second pronucleus from another embryo, was used to construct biparental diploid gynogenetic and androgenetic and control (one male and one female pronucleus) nuclear-transplant embryos. These embryos were transferred into the oviducts of day 1 pseudopregnant females and permitted to develop to term. On the basis of our results, we conclude that maternal and paternal genomic contributions are not functionally identical and that both are essential to complete embryogenesis.

## Results

From a total of 1528 embryos, 1475 (97%) survived the microsurgical removal of one or two pronuclei. 1452 (98%) of the resulting pronuclear karyoplasts were successfully injected, along with inactivated Sendai virus, into the perivitelline space of the recipient embryos, and 1355 (93%) of the karyoplast:recipient embryo pairs underwent fusion. The overall efficiency of the nuclear-transplantation procedure was 89%.

In an initial experimental series, biparental gynogenones, androgenones, and control nuclear-transplant embryos were made from embryos obtained from C57BL/6J females mated to BALB/c males. The maternal/paternal origin of the pronuclei was assigned, and one of the pronuclei was removed and replaced with a second pronucleus from another embryo. In a separate experimental series, nuclear transplantation was performed with embryos obtained from BALB/c females mated to C57BL/6J males. In both cases, embryos that survived microsurgery and incorporated the donor pronucleus were introduced into the oviducts of day 1 pseudopregnant females and allowed to develop to term. Since C57BL/6J and BALB/c mice differ in their coat color and glucose phosphate

isomerase-1 (GPI-1) alleles, the maternal/paternal origin of the pronuclei could be verified in progeny by determination of these phenotypic characteristics.

The results (Table 1) show that of 239 putative control embryos, seven agouti male progeny (3%) were born. The agouti phenotype in mice is consistent with their being derived from zygotes possessing one male and one female pronucleus. Of 207 putative gynogenetic and 189 putative androgenetic embryos, one offspring was obtained (Table 1), an agouti male. The agouti phenotype, however, shows that this male possesses a normal maternal/paternal genotype and must have resulted from an incorrect assignment of the parental origin of the pronucleus during microsurgery. Using the proportion of control progeny born, and correcting among the androgenetic embryo population for the expected proportion of lethal YY androgenetic embryos (25%), we should expect the birth of six gynogenetic and four androgenetic offspring. None, however, was observed ($X^2 = 7.93$; $p < 0.01$). Thus nuclear-transplant embryos that possess one male and one female pronucleus developed to term, whereas embryos receiving two male pronuclei or two female pronuclei did not.

Because the gynogenetic and androgenetic embryos produced in this initial experimental series are either C57BL/6J or BALB/c homozygotes while their control counterparts are F1 hybrids, we tested the possible effect of homozygosity on gynogenetic and androgenetic embryo development by removing a pronucleus from an embryo obtained from the C57BL/6J♀ × BALB/c♂ mating and replacing it with a pronucleus obtained from a BALB/c♀ × C57BL/6J♂ mating and vice versa. Maternal/paternal control embryos in this experimental series are therefore C57BL/6J or BALB/c homozygotes, while the gynogenetic and androgenetic embryos are F1 hybrids. The results (Table 2) show that from 109 putative control embryos, 11 progeny (10%) were born (two albino males, two albino females, and seven black males). The coat-color phenotype of all progeny verifies their maternal/paternal origin. No progeny possessing the androgenetic or gynogenetic agouti phenotype were observed. Putative gynogenetic (132) and putative androgenetic (139) embryos were similarly produced, from which four progeny were born. Of the three mice that survived to adulthood, all were albino (two males and one female) and are therefore descended from an embryo possessing one male and one female pronucleus. The fourth mouse, which died within 24 hr of birth, was pigmented, and electrophoretic analysis showed it to be homozygous for the GPI-$1^b$ allele (i.e., C57BL/6J homozygote; data not shown). Thus all four progeny exhibited the control parental phenotype and are instances in which the assigned maternal/paternal origin of the transplanted pronucleus was incorrect. Using the proportion of control progeny born (10%) and correcting for the expected proportion of lethal YY androgenetic embryos, we should expect the birth of 13 gynogenetic and 10 androgenetic offspring. None, however, was observed ($X^2 = 19.4$; $p < 0.001$). Furthermore, the combined results of Tables 1 and 2 show that from a total of 348 control embryos, 18 progeny (5%) were born. From the latter figure, we would anticipate the birth of 19 gynogenones (from 339 embryos) and 14 androgenones (from 328 embryos), for a total of 33 progeny. None, however, was observed ($X^2 = 27.15$; $p < 0.001$). We conclude that gynogenetic and androgenetic embryos cannot complete normal embryogenesis and that their inability to develop is not the result of homozygosity.

Table 1. Progeny Resulting from Single-Pronucleus Transplantation Using *Either* Embryos from C57BL/6J♀ × BALB/c♂ Matings or BALB/c♀ × C57BL/6J♂ Matings

| | No. of Embryos | Expected Phenotype of Progeny | No. of Progeny | Observed Phenotype of Progeny |
|---|---|---|---|---|
| **C57BL/6J♀ × BALB/c♂** | | | | |
| gynogenones | 140 | black GPI-1B | 0 | — |
| androgenones | 129 | albino GPI-1A | 0 | — |
| maternal/paternal | 154 | agouti GPI-1AB | 2 | agouti males |
| **BALB/c♀ × C57BL/6J♂** | | | | |
| gynogenones | 67 | albino GPI-1A | 1 | agouti male |
| androgenones | 60 | black GPI-1B | 0 | — |
| maternal/paternal | 85 | agouti GPI-1AB | 5 | agouti males |

Table 2. Progeny Resulting from Single-Pronucleus Transplantation *Between* Embryos from C57BL/6J♀ × BALB/c♂ *and* BALB/c♀ × C57BL/6J♂ Matings

| | No. of Embryos | Expected Phenotype of Progeny | No. of Progeny | Observed Phenotype of Progeny |
|---|---|---|---|---|
| gynogenones | 132 | agouti GPI-1AB | 2 | albino (1 male, 1 female) |
| androgenones | 139 | agouti GPI-1AB | 2 | 1 albino male 1 GPI-1B |
| maternal/paternal | 109 | black GPI-1B or albino GPI-1A | 11 | 7 black males<br>4 albino (2 males, 2 females) |

To determine the fate of gynogenetic and androgenetic embryos during the preimplantation and postimplantation stages of development, nuclear transplantation was performed and the embryos were either cultured in vitro or transferred into the oviducts of day 1 pseudopregnant females. Those pseudopregnant females receiving embryos were sacrificed on gestational days 6–11, their uteri excised, and deciduoma subjected to histological analysis. The results of the in vitro culture of embryos (Table 3) show that 100% of the maternal/paternal control nuclear transplant embryos, 88% of putative gynogenetic, and 64% of putative androgenetic embryos developed to the morula-blastocyst stage. Thus diploid embryos possessing exclusively maternal or exclusively paternal pronuclei can successfully develop to the blastocyst stage in vitro. The significantly greater death of androgenetic embryos occurred primarily at the 8-cell stage, presumably the result of YY embryos in this population. Histological analysis of the postimplantation embryos on gestational days 6–11 (Table 4) shows approximately the same number of gynogenetic, control nuclear-transplant, and unmanipulated control embryos implanted. The number of implanted androgenetic embryos was approximately half that observed in other groups, probably due to the presence of YY embryos. Very few biparental gynogenetic and androgenetic embryos survive implantation for very long. The most advanced embryo observed in those groups had 11 somites and was, at the time of sacrifice, approximately 1 day delayed in development. These results indicate that biparental gynogenetic and androgenetic embryos die during the early postimplantation period.

The proportion of control single-pronucleus transplant embryos that successfully developed to term in this study (5%) is less than that obtained in a previous investigation (23%), in which we transplanted both pronuclei (McGrath and Solter, 1984). To determine the basis of this reduction in developmental potential, two additional experiments were performed. Transplantation of both pronuclei from one embryo to another was performed using C57BL/6J and BALB/c mice and these embryos were transferred to the oviducts of pseudopregnant females. From 45 successful double-pronuclei transplants, 16 progeny (36%) were born. This proportion of progeny born from transfer of two pronuclei agrees with our earlier studies and indicates that our results are not a function of strain differences or a technical difficulty peculiar to this investigation. The second experiment was designed to detect any deleterious effect peculiar to the transfer of a single pronucleus. One pronucleus was removed but then returned to the embryo from which it had been obtained. Of 98 such nuclear-transplant embryos, 20 progeny were born (20%). The proportion of progeny born from embryos in which one pronucleus was removed but then returned to the same embryos is not significantly different from the proportion of progeny born from the double pronuclear transfers ($X^2 = 3.34$; $p > 0.05$), but is significantly greater than the proportion of progeny obtained from the control single-pronuclear transplants listed in Tables 1 and 2 (5%) ($X = 16.23$; $p < 0.001$). Although the control embryo population almost certainly contains some inviable gynogenones and androgenones, the calculated decrease of viable embryos (based on the proportion of control progeny among the putative gynogenones and androgenones) does not exceed 1%, and is not sufficient to explain the decreased developmental potential of single-pronuclear-transfer embryos.

Table 3. In Vitro Development of Gynogenetic, Androgenetic, and Control Embryos

| Putative Genotype | No. Degenerate | No. Morula | No. Blastocyst |
|---|---|---|---|
| gynogenones | 3 | 4 | 19 |
| androgenones | 10 | 3 | 15 |
| maternal/paternal | 0 | 0 | 8 |
| unmanipulated control | 0 | 1 | 13 |

Table 4. Postimplantation Development of Gynogenetic, Androgenetic, and Control Embryos

| Putative Genotype | No. of Embryos Transferred | No. of Implantations (%)[a] | No. of Resorptions[b] | No. of Embryos |
|---|---|---|---|---|
| gynogenones | 54 | 19 (35) | 17 | 2[c] |
| androgenones | 42 | 7 (17) | 7 | 0 |
| maternal/paternal | 39 | 13 (33) | 3 | 10[d] |
| unmanipulated controls | 46 | 16 (34) | 2 | 14[d] |

[a] Foster mothers were sacrificed between days 6–11 of gestation.
[b] On histological examination, deciduoma contained massive bleeding and a few giant trophoblastic cells. No remnants of embryos were observed.
[c] One embryo, from a mother sacrificed on day 8 of gestation, was a morphologically normal egg cylinder with three germ layers. The other embryo, from a mother sacrificed on the day 11 of gestation, was morphologically normal, although approximately half the size of corresponding control embryos. It contained 11 somites.
[d] All embryos were morphologically normal and corresponded developmentally to their gestational age.

## Discussion

Shortly after fertilization the haploid maternal and paternal genomes unite to form the zygote nucleus. While both parental sexes contribute equivalent nuclear information to the zygote, this genetic information is not necessarily functionally equivalent. For example, the paternal X chromosome is preferentially inactivated in murine extraembryonic tissues (Takagi and Sasaki, 1975; Harper et al., 1982). It is, however, still unknown if homozygosity for either the maternal or paternal X chromosome results in embryonic lethality. Another example of this phenomenon occurs in embryos with the $T^{hp}$ mutation. Heterozygous embryos that inherit the $T^{hp}$-deleted chromosome 17 from their male parents are viable, whereas embryos that receive the same $T^{hp}$ chromosome from their female parents are inviable (Johnson, 1974). This lethality does not appear to

result from a cytoplasmic defect but from differential functioning of a portion of chromosome 17 during embryogenesis depending on its parental origin (McGrath and Solter, 1984). Normal embryogenesis therefore requires a functional region of maternal (but not paternal) chromosome 17.

The concept of differential functioning of maternal and paternal genomes is further extended and generalized by the present results. After obtaining 18 progeny from 348 control embryos (5%), but no progeny from 339 gynogenetic and 328 androgenetic embryos, we conclude that the maternal and paternal contributions to the embryonic genome are not equivalent and that they differ sufficiently to result in early postimplantation embryonic lethality when either component is absent. Histological analysis reveals that the lethal period of biparental gynogenetic and androgenetic embryos occurs shortly after implantation. This has also been described for uniparental gynogenetic embryos (Markert, 1982; Surani and Barton, 1983) and parthenotes (Graham, 1974). In a recent investigation, Surani and Barton (1983) concluded that the inviability of parthenotes could not be explained by the lack of an extragenomic contribution by the fertilizing spermatozoon since uniparental diploid gynogenones derived from fertilized ova are also inviable. The authors concluded that death may have resulted from lethal homozygosity. The results of the present study also imply a nuclear and not a cytoplasmic defect as a probable cause of the death of mammalian parthenotes. However, we suggest that parthenogenetic embryos die because of a lack of a paternal contribution to the embryonic genome. The comparison of the development of biparental gynogenetic and androgenetic embryos with that of control nuclear-transplant embryos that possess both a male and a female pronucleus permits us to account for any possible deleterious effect from the technical manipulations.

The results presented here are in conflict with those presented by Hoppe and Illmensee (1977). For reasons mentioned above, we do not believe that uniparental gynogenetic embryos can complete development. In addition, the need for a functional maternal portion of the chromosome 17 (McGrath and Solter, 1984) would preclude the development of uniparental androgenetic embryos. One possible explanation for the discrepancy between our results and those reported by Hoppe and Illmensee (1977) might be the incomplete removal of pronuclei because of the small-bore pipette used by those investigators. If a sufficient and appropriate part of the pronucleus remains, it might support the embryos during a critical stage, resulting in a small number of viable progeny. Variations in GPI-1 patterns observed in nuclear-transfer preimplantation embryos (Illmensee and Hoppe, 1981; Hoppe and Illmensee, 1982) are compatible with the possibility of incomplete enucleation.

Two general mechanisms for differential functioning of the maternal and paternal genomes during mammalian embryogenesis may be envisioned. A specific gene(s) may be inherited in a functional form from one parent but in a nonfunctional form from the other. In this model, androgenones and gynogenones should each express a subset of normal gene products, which, when combined, should exactly equal those products found in normal embryos. Alternatively, embryonic gene expression might require interaction between the maternal and paternal genomes (Markert, 1982). In this model both gynogenones and androgenones would again express only a subset of gene products found in normal embryos. However, the combined gene products of gynogenones and androgenones would not equal the spectrum of gene products in normal embryos since the necessary interaction of maternal and paternal genes did not occur. Such a requirement for interaction might also bear on the decreased development of the maternal/paternal control embryos observed in the present study, i.e., the requisite communication and/or synchrony between the resident and newly introduced pronuclei shortly after fertilization might be impaired. However, it is also possible that selective death of female control embryos underlies the decrease in the number of viable controls. Among the 22 control nuclear-transplant progeny that survived to adulthood, 19 were phenotypically male. The significance of this distorted sex ratio and its possible contribution to the decreased development of control embryos is uncertain.

Regardless of the mechanism involved, the differential functioning of parental genomes might be essential for development of embryonic or extraembryonic parts of the conceptus or for both. Recombination of the normal inner cell mass with biparental gynogenetic or androgenetic trophoblast, and vice versa, should address this question. Our model implies that the paternal and/or maternal genome (whole or in part) are somehow conditioned/altered during gametogenesis and that this conditioning is completely reversible (as is the case for the X chromosome). A logical consequence of differential functioning of parental genomes is the unlikelihood of successful cloning of mammals using somatic cell nuclei, since presumably the sex-related conditioning of the paternal and maternal genome has already been reversed in those cells.

## Experimental Procedures

### Embryo Isolation

All embryos were obtained from reciprocal spontaneous matings of C57BL/6J (Jackson Laboratory, Bar Harbor) males and females with BALB/c (Jackson Laboratory, Bar Harbor) males and females. One-cell-stage embryos were isolated by puncturing the ampullae of oviducts from mated females (day 1) in HEPES-buffered Whitten's medium (Whitten, 1971) containing 500 U/ml bovine hyaluronidase (Sigma). Embryos were washed through 4 drops of modified Whitten's medium containing 100 μM Na$_2$EDTA (Abramczuk et al., 1977).

### In Vitro Culture

Embryos were cultured under silicone oil (Dow Corning 2000 Fluid; Kurt Lesker, Pittsburgh, PA) in modified Whitten's medium in an atmosphere of 5% $O_2$, 5% $CO_2$, and 90% $N_2$ at 37°C. Before microsurgery, groups of embryos (five to ten) were cultured for 15–30 min at 37°C in Whitten's medium supplemented with 5 μg/ml cytochalasin B (Hoppe and Illmensee, 1977) and 0.1 μg/ml demecolcine (Sigma) (McGrath and Solter, 1983).

### Microsurgery

Microsurgery was performed on a Leitz Laborlux II fixed-stage microscope using Leitz micromanipulators. Embryos were placed singly in hanging drops of HEPES-buffered Whitten's medium supplemented with 5 μg/ml cytochalasin B and 0.1 μg/ml demecolcine. Nuclear transplantation was performed as previously described (McGrath and Solter, 1983). Determination of the parental sex of the pronuclei was based on their cytoplasmic location, the pronucleus located beneath the second polar body was judged as maternal in origin, while the more eccentrically located pronucleus was judged as paternal. Embryos in which the maternal/paternal origin of the pronuclei could not be decided were discarded. Subsequent to microsurgery, embryos were washed through 4 drops of modified Whitten's medium and returned to the incubator. Embryos that fused with the pronuclear karyoplast were either cultured in vitro in modified Whitten's medium as described above, or transferred to the oviducts of day 1 albino CD-1 (Charles Rivers) pseudopregnant females that had previously mated to vasectomized CD-1 males. Transfer of one-cell-stage embryos into oviducts was performed using a small-bore glass pipette.

### Histological Analysis of Embryos

Pseudopregnant females receiving nuclear-transplant embryos were sacrificed on successive days, their uteri excised, and the deciduoma dissected free. Some deciduoma were examined under the dissecting microscope for the presence of embryos, but the majority were fixed in formalin, embedded, serially sectioned, and hematoxylin/eosin stained. Sections were examined under a light microscope.

### Statistical Analysis

The number of progeny resulting from gynogenetic and androgenetic embryos was compared to the number of progeny developed from control embryos using the $X^2$ contingency test. In all statistical comparisons involving androgenones, 25% of the androgenetic embryos were excluded in order to compensate for the expected production of lethal YY embryos.

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### References

Abramczuk, J., Solter, D., and Koprowski, H. (1977). The beneficial effect of EDTA on development of mouse one-cell embryos in chemically defined medium. Dev. Biol. *61*, 378–383.

Borsuk, E. (1982). Preimplantation development of gynogenetic diploid mouse embryos. J. Embryol. Exp. Morphol. *69*, 215–222.

Graham, C. F. (1974). The production of parthenogenetic mammalian embryos and their use in biological research. Biol. Rev. *49*, 399–422.

Hoppe, P. C., and Illmensee, K. (1977). Microsurgically produced homozygous-diploid uniparental mice. Proc. Nat. Acad. Sci. USA *74*, 5657–5661.

Hoppe, P. C., and Illmensee, K. (1982). Full-term development after transplantation of parthenogenetic embryonic nuclei into fertilized mouse eggs. Proc. Nat. Acad. Sci. USA *79*, 1912–1916.

Harper, M. I., Fosten, M., and Monk, M. (1982). Preferential paternal X inactivation in extraembryonic tissues of early mouse embryos. J. Embryol. Exp. Morphol. *67*, 127–135.

Iles, S. A., McBurney, M. W., Bramwell, S. R., Deusen, Z. A., and Graham, C. F. (1975). Development of parthenogenetic mouse embryos in the uterus and in extrauterine sites. J. Embryol. Exp. Morphol. *34*, 387–405.

Illmensee, K., and Hoppe, P. C. (1981). Nuclear transplantation in Mus musculus: developmental potential of nuclei from preimplantation embryos. Cell *23*, 9–18.

Johnson, D. R. (1974). Hairpin-tail: a case of post-reductional gene action in the mouse egg? Genetics *76*, 795–805.

Kaufman, M. H., Barton, S. C., and Surani, M. A. H. (1977). Normal postimplantation development of mouse parthenogenetic embryos to the forelimb bud stage. Nature *265*, 53–55.

Markert, C. L. (1982). Parthenogenesis, homozygosity, and cloning in mammals. J. Hered. *73*, 390–397.

McGrath, J., and Solter, D. (1983). Nuclear transplantation in the mouse embryo by microsurgery and cell fusion. Science *220*, 1300–1302.

McGrath, J., and Solter, D. (1984). Maternal $T^{hp}$ lethality in the mouse is a nuclear, not cytoplasmic, defect. Nature, in press.

Modlinski, J. A. (1980). Preimplantation development of microsurgically obtained haploid and homozygous diploid mouse embryos and effect of pretreatment with cytochalasin B on enucleated eggs. J. Embryol. Exp. Morphol. *60*, 153–161.

Stevens, L. C. (1978). Totipotent cells of parthenogenetic origin in a chimaeric mouse. Nature *276*, 266–267.

Surani, M. A. H., and Barton, S. C. (1983). Development of gynogenetic eggs in the mouse: implications for parthenogenetic embryos. Science *222*, 1034–1036.

Takagi, N., and Sasaki, M. (1975). Preferential inactivation of the paternally derived X chromosome in the extraembryonic membranes of the mouse. Nature *256*, 640–642.

Whitten, W. K. (1971). Nutrient requirements for the culture of preimplantation embryos *in vitro*. Adv. Biosci. *6*, 129–139.