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Development of gynogenetic and parthenogenetic inner cell mass and trophectoderm tissues in reconstituted blastocysts in the mouse

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SUMMARY

The developmental potential of inner cell mass (ICM) and trophectoderm (TE) derived from parthenogenetic or biparental gynogenetic embryos was examined in reconstituted blastocysts with normal TE or ICM, respectively. The results demonstrate that when a normal ICM was introduced inside a trophectoderm vesicle derived from parthenogenetic or gynogenetic blastocysts, postimplantation development was characterized by the almost complete failure of trophoblast proliferation and without compensating cellular contribution from the normal ICM to the outer trophoblast lineage. Consequently, the normal ICMs also failed to develop adequately and only a few retarded embryos were detected on day 11–12 of pregnancy. In most respects, development of these reconstituted blastocysts resembled that obtained with unoperated gynogenetic and a parthenogenetic blastocyst. By contrast, an ICM from a parthenogenetic or gynogenetic embryo introduced inside a normal trophectoderm vesicle induced substantial proliferation of the trophoblast but again without a detectable cellular contribution from the ICM to the outer trophoblast lineage. However, with the improved development of the trophoblast, both the parthenogenetic and gynogenetic ICMs developed substantially better and without a detectable cellular contribution from the TE to the embryo. Almost all the embryos developed at least up to the 25-somite stage and many of them reached the 30- to 40-somite stage. Some of the most advanced day-11 and -12 gynogenones and parthenogenones yet seen have now been obtained in this way. Nevertheless, all the embryos were still smaller than the equivalent control embryos and showed signs of some tissue degeneration. The yolk sac was also suboptimal with poor blood supply and may need to be improved to obtain further improvement in the development of the embryos. The combined results demonstrate that the trophoblast proliferates very poorly even in the presence of a normal ICM, if the TE tissue lacks a paternal genome. However, ICM tissues which lack a paternal genome can develop to an advanced embryonic stage if they are introduced inside a normal trophectoderm vesicle. The results give further insight into the differential roles of maternal and paternal genomes during development of the embryo and extraembryonic tissues in the mouse.

INTRODUCTION

Recent investigations demonstrate that the paternal and maternal genomes have differential roles during embryogenesis and that both are needed for development to term in the mouse (Surani & Barton, 1983; Surani, Barton & Norris, 1984; Barton, Surani & Norris, 1984; McGrath & Solter, 1984*a,b*; Mann

Key words: parthenogenones, gynogenones, inner cell mass, trophectoderm, reconstituted blastocysts, mouse, extraembryonic tissue.

& Lovell-Badge, 1984; Surani, 1985). The maternal genome is relatively more important for preimplantation development up to the blastocyst stage and to some extent for embryogenesis up to the 25-somite stage (Kaufman, Barton & Surani, 1977; Surani & Barton, 1983; Surani *et al.* 1984) but where only the maternal genome is present the extraembryonic tissue, especially trophoblast, is very sparse (Surani & Barton, 1983; Surani *et al.* 1984). By contrast, far fewer eggs with the paternal genome alone reach the blastocyst stage but when they do develop there is extensive proliferation of the extraembryonic tissues, especially the trophoblast (Barton *et al.* 1984). It thus appears that the maternal and paternal genomes exert different influences on the two compartments of the conceptus, the embryo and the extraembryonic tissues: the maternal genome being relatively more important for embryogenesis and the paternal genome for the development of extra-embryonic tissues (Surani, 1985).

Interestingly, other differences between the embryo and extraembryonic tissues with respect to modifications in the DNA and gene expression have been detected. One of the major differences between these two compartments of the conceptus is the preferential inactivation of the paternal X-chromosome in the extraembryonic tissues, while in the embryo either of the two X-chromosomes is inactivated (Takagi & Sasaki, 1975; West, Frels, Chapman & Papaioannou, 1977; Harper, Fosten & Monk, 1982; Lyon & Rastan, 1984). Furthermore, some dispersed and centromeric repetitive DNA sequences in the extraembryonic tissues of the mouse are undermethylated at the CpG sites of CCGG tetramers, while the same sequences remain fully methylated in the embryonic ectoderm which gives rise to the embryo (Chapman, Forrester, Sanford, Hastie & Rossant, 1984). This relative under-methylation may be due both to a contribution by the gametes of under-methylated sequences that remain in that state in the extraembryonic tissues and to demethylation of some specific sequences in these tissues (Chapman *et al.* 1984). Some differences in the activity of the cellular homologues of viral oncogenes are also observed such as the expression of *c-fos* predominantly in the extraembryonic tissues of the day-10 conceptus (Muller, Verma & Adamson, 1983).

One approach to determining developmental potential and interactions between the embryo and extraembryonic components is by reconstitution of blastocysts from inner cell masses (ICMs) and trophectoderm (TE) (Gardner, 1968, 1978) derived from embryos of different genetic constitution. Previous studies have demonstrated that the ICM contributes to all the tissues of the embryo as well as the yolk sac while the TE gives rise mainly to the primary and secondary giant cells of the ectoplacental cone and the extraembryonic ectoderm (Gardner, Papaioannou & Barton, 1973; Gardner & Papaioannou, 1975). Other studies have also shown that trophectoderm vesicles in the absence of ICM can implant but the cells fail to proliferate (Gardner, 1972; Gardner & Papaioannou, 1975; Snow, Aitken & Ansell, 1976; Surani & Barton, 1977) while the ICM alone cannot implant and develop (Gardner, 1972). In this study we specifically investigated the influence of normal TE on the development of ICMs derived from embryos lacking the paternal genomes (gynogenetic and parthenogenetic) since

the trophoblast development in such embryos is especially poor (Surani & Barton, 1983; Surani *et al.* 1984). Reverse experiments were also performed to deduce the developmental fate of normal ICMs placed inside TE derived from gynogenetic and parthenogenetic embryos.

MATERIALS AND METHODS

Animals

CFLP albino outbred mice (AFRC colony from Banting and Kingman stock) (Gpi-1^a/Gpi-1^a) and non-albino (C57BL/6J×CBA/Ca) F_1 (from OLAC stock, henceforth called F_1) mice (Gpi-1^b/Gpi-1^b) were used in these experiments. Four- to six-week-old females were injected with 5–7·5 i.u. pregnant mare's serum (Intervet Ltd, Cambridge, U.K.) followed 42–48 h later by 5–7·5 i.u. human chorionic gonadotrophin (HCG) (Intervet) to induce superovulation (Fowler & Edwards, 1957).

Preparation of blastocysts

Three types of blastocysts were obtained; fertilized, parthenogenetic and gynogenetic. To obtain normal fertilized blastocysts, CFLP and F_1 females were superovulated and mated with CFLP and F_1 males, respectively. Fertilized F_1×F_1 eggs were obtained on day 1 of pregnancy (day 1 = day of vaginal plug) and 2-cell CFLP×CFLP embryos were flushed on day 2 of pregnancy, to overcome the tendency of eggs from this strain of mice to block at the 2-cell stage when cultured *in vitro* at the 1-cell stage. The F_1×F_1 eggs were cultured for 4 days (96 h) and the CFLP×CFLP embryos for 3 days (72 h), in M16+4 mg ml^{-1} BSA (Whittingham, 1971) to obtain expanded blastocysts. Since approximately 50–60% of the F_1×F_1 blastocysts obtained after culture of either 1-cell or 2-cell eggs can develop to term after transfer to day-3 pseudopregnant recipients (unpublished observations), the 24 h difference in the duration of culture *in vitro* of the F_1×F_1 and CFLP×CFLP blastocysts presumably has no influences on their developmental potential.

To obtain parthenogenetic blastocysts, unfertilized eggs from F_1 mice were obtained at 16·5–17·5 h post HCG. The cumulus cells were removed by incubation with 300 i.u. ml^{-1} hyaluronidase (Ovine testis type V, Sigma) for 3 min and washed twice with M16+BSA. The eggs were then activated in M16+BSA which contained 7% ethanol, for 7 min at room temperature (Cuthbertson, 1983), washed six times with M16+BSA and cultured in M16+BSA containing 5 g ml^{-1} cytochalasin B (in 0·11 dimethylsulfoxide) for 3–3·5 h. The eggs were washed 9 times in M16+BSA and left to culture for a further 2 h. At this time, all diploid eggs containing two pronuclei produced by the suppression of second polar body extrusion by cytochalasin B (Niemierko, 1975) were separated (60–90%) from fragmented and other abnormal eggs and cultured for 4 days (96 h) in M16+BSA.

Biparental gynogenetic eggs were prepared from F_1 ♀×CFLP ♂ eggs. The male pronucleus was removed and a second female pronucleus from a separate group of F_1×CFLP eggs was introduced into the eggs with Sendai-virus-assisted fusion of the karyoplast fragment (Graham, 1971) as described previously (McGrath & Solter, 1983; Surani *et al.* 1984). The reconstituted gynogenetic eggs were also cultured for 4 days (96 h) in M16+BSA.

Reconstitution of blastocysts

Blastocysts were reconstituted using TE and ICM from different embryos. For the preparation of TE all the manipulations were carried out under a Wild M5 dissecting microscope. The zona pellucida was first removed by a brief exposure (5–20 s) to acid Tyrodes medium (Nicolson, Yanagimachi & Yanagimachi, 1975) and the blastocysts were then washed three times in PB1+10% heat-inactivated foetal calf serum (HIFCS) (Whittingham & Wales, 1969). Partial collapse of the blastocysts was then induced by gently sucking the blastocyst into a flame-polished micropipette which was approximately two-thirds to three-quarters the diameter of the expanded blastocyst. The partially collapsed blastocyst with the inner cell mass end still

distinctly visible was cut with a hand-held solid microneedle (approximately 10 μm diameter) while continuously observing under a dissecting microscope. The sawing movement of the needle resulted in bisection of the blastocyst into a larger fragment of trophectoderm alone and a smaller fragment of trophectoderm which contained the inner cell mass. The trophectoderm fragments were cultured in PB1+10% HIFCS for 2–3 h during which time they developed into fully expanded trophectoderm vesicles.

The inner cell masses were obtained by immunosurgery (Solter & Knowles, 1975) as modified by Adams (1985). The blastocysts were placed in 1:5 rabbit anti-mouse antiserum for 30 min at 37 °C. The embryos were washed six times in PB1+10% HIFCS and cultured for a further 15 min in this medium at 37 °C. They were washed six times in PB1+BSA and cultured in this medium containing 1:8 guinea-pig complement (Miles) for 30 min at 37 °C. The outer trophectoderm underwent lysis during this period. The blastocysts were washed six times in PB1+10% HIFCS and cultured in the same medium for a further 30 min at 37 °C. At the end of this period, the inner cell masses were released by repeated pipetting through flame-polished microneedles, approximately 15–20 μm in internal diameter. The ICMs were washed nine times in PB1+10% HIFCS and cultured in this medium. In some instances, especially with gynogenetic and parthenogenetic ICMs, two or three ICMs were aggregated if they appeared smaller than the normal ICMs but larger ICMs were used singly in all cases. Previous studies using aggregated parthenogenetic embryos have shown that development is not improved simply by an increase in the number of cells in blastocysts or inner cell mass (Barton & Surani, unpublished).

The inner cell mass was introduced into the appropriate trophectoderm vesicles with a Leitz micromanipulator using the technique described by Gardner (1968, 1978).

The reconstituted blastocysts were collected in PB1+HIFCS at 37 °C until all the embryos were manipulated. The manipulated embryos were transferred on the same day to the uterine horns of day-3 pseudopregnant CFLP females obtained by mating with vasectomized F_1 males of proven sterility.

Embryos were examined and usually photographed from day 11 onwards to term. Embryos or their remains were dissected and separated from trophoblast and yolk sac. The yolk sac analysed in this study refers to the visceral yolk sac. No attempt was made to separate the parietal endoderm from the trophoblast, which in some instances may not have been possible when the trophoblast failed to proliferate (see later). The tissues were stored at −20 °C until they were analysed for GPI as described previously (Chapman, Whitten & Ruddle, 1971).

One embryo (gynogenetic+normal TE: Table 5, number 12) was fixed in Bouins, wax-embedded, sectioned at 6 μm and stained with haematoxylin and eosin for light microscopy. In this case only the yolk sac and trophoblast were typed for GPI.

RESULTS

Reconstitution of normal blastocysts

Blastocysts were reconstituted using normal ICMs ($F_1 \times F_1$) and TE (CFLP× CFLP) to test if these were capable of normal development. Over 75% of the reconstituted blastocysts which implanted developed normally to day 11–12 (10/13), and a further two normal live young were obtained (Table 1). The embryos were typed for GPI and it was found that in all cases the trophoblast was GPI-1A while the embryo (and yolk sac) was GPI-1B. The live young were non-albino (black and agouti), GPI-1B, and have reached adulthood and bred normally (Table 2).

Reconstitution of blastocysts from normal and parthenogenetic ICMs and TE

In the first instance, ICMs from normal blastocysts (CFLP×CFLP) were introduced into TE from parthenogenetic blastocysts (F_1) (Table 3; Fig. 1). When

Table 1. *Development of reconstituted blastocysts*

Source of		Number transferred to recipients that became pregnant/total transferred	Embryos*			
Inner cell mass	Trophectoderm		Implanted	Normal	Small 25 or more somites	Very retarded†
Fertilized	Fertilized	A 17/23 B 4/9	13	10 2 live born	2	
Fertilized	Parthenogenetic	39/56	29	–	3	10
Fertilized	Gynogenetic	17/22	12	–	3	9
Parthenogenetic	Fertilized	21/39	19	–	14	0
Gynogenetic	Fertilized	26/26	24	–	14	1

* On day 11–12 of pregnancy.
† Conceptuses containing recognizable embryonic tissue.

examined on day 11–12 of gestation we found that from 29 implantation sites, 13 contained some embryonic remains but only 3 of these had recognizable embryos of 20–30 somites. The most advanced 30-somite embryo was small and grossly abnormal and the other two 20- to 25-somite embryos were very small and were dying or dead. The other yolk sacs contained dead or unrecognizable embryonic remains which included two implantation sites on day 12 with embryonic remains. Only in one instance the yolk sac appeared substantial, otherwise it was moderately developed or poor. The trophoblast tissue was very sparse and some remains could only be found with difficulty (see Fig. 1). All embryos and yolk sacs where sufficient tissue was available were typed for GPI and found to be GPI-1A.

Blastocysts were then reconstructed with ICMs from parthenogenetic F_1 embryos and TE from CFLP×CFLP blastocysts. Nineteen implantation sites were detected and 14 of them contained embryos on day 11–12 of gestation (Table 1). All embryos in this instance were well formed and far more advanced and substantial than those described above (Table 4; Fig. 1). Embryos on day 11 contained 25–35 somites and on day 12, 30–40 somites. The largest embryo (Table 4, no. 9) obtained on day 12 was 5 mm long and with at least 40 somites (see Figs 3 and 4). Although this embryo was considerably more advanced and substantial than any unmanipulated parthenogenetic embryo we have yet seen (Barton & Surani, unpublished), it was still smaller than a normal embryo of between 5·8–6·8 mm on day 12 (see Figs 3 and 4). Furthermore, the embryo showed signs of the stunting abnormalities of arrested development as well as degeneration of the tissues, as did the other embryos (1·75–4·0 mm) in this series. The yolk sac in

Table 2. *Development of blastocysts reconstituted from normal ICMs and normal TE**

		Development of conceptuses and GPI analysis				
Embryo no.	Day of gestation	Size: CR length mm	Approx. no. of somites	Embryo	Yolk sac	Trophoblast
1	11	4·5	35	B	B	A
2	11	4·2	35	B	B	A
3	11	4·0	35	B	B	A
4	11	2·5	25	B	B	A
5	12	6·8	45+	B	B	A
6	12	6·5	45+	B	B	A
7	12	6·5	45+	B	B	A
8	12	6·5	45+	B	B	A
9	12	6·3	45+	B	B	A
10	12	5·9	45+	B	B	A
11	12	5·8	45+	B	B	A
12	12	2·8	30	B	B	A
13	TERM	Normal	live born	GPI-1B		
14	TERM	Normal	live born	GPI-1B		

* ICMs were obtained from F_1×F_1 embryos and TE from CFLP×CFLP embryos.

many instances appeared not to have developed as extensively as in the control embryos and the blood supply also appeared to be quite poor. The trophoblast however proliferated extensively and approached trophoblast development in control embryos. All the embryos and their membranes were typed for GPI. In one instance (embryo 13) the embryo and yolk sac contained minor amounts of GPI-1A (GPI-1B expected) which could be attributed to incomplete dissection of the embryo. In another instance (embryo 2) the trophoblast contained some GPI-1B (GPI-1A expected) probably because of incomplete dissection. Otherwise GPI type was as expected.

Reconstitution of the blastocysts from normal and gynogenetic ICMs and TE

Blastocysts were also reconstructed using ICMs and TE from biparental gynogenetic embryos in order to rule out the possibility of any homozygosity of the genotype of parthenogenones influencing the results. In the first instance normal ICMs were introduced into the TE from gynogenetic blastocysts (Table 5; Fig. 2). Twelve implantation sites on day 11 of gestation were detected but only three of them contained recognizable but degenerating embryos, the largest being 3·5 mm

Table 3. *Development of blastocysts reconstituted from normal ICMs and parthenogenetic TE*

		Development of conceptuses and GPI analysis				
Embryo No.	Day of gestation	Size: CR length mm	Approx. no. of somites	Embryo	Yolk sac	Trophoblast
1	11	2·2	30	Head and lower somites cramped – A	Substantial – A	Not retrievable
2	11	–	–	Dead – not separable from yolk sac – A		Very slight (ND)
3	11	–	–	Small, unturned, dead	Small	Slight (ND)
4	11	–	–	Unrecognizable remains	Small	Very slight (ND)
5	11	1·5	25	Dying, beating heart – A	Moderate – A	Slight (ND)
6	11	1·3	20	Dead, half turned, poorly developed head – A	Moderate – A	Slight (ND)
7	11	–	–	Small, contorted, not separable from yolk sac	Small	Very slight (ND)
8	11	–	–	Some membranes and trophoblast remains		
9	11	–	–	Very poor, unturned	Small	Very slight (ND)
10	11	–	–	Poor, not separable from yolk sac	Substantial – A	Very slight (ND)
12	12	–	–	Dead small mass	Small	Very slight (ND)
13	12	–	–	Dead small mass	Small	Very slight (ND)

ND = GPI not determined – degenerate and insufficient tissue.

long, with approximately 25 somites. Another two yolk sacs were substantial, the remainder were small and poor. The trophoblast in all instances was very poor and sparse. Eight embryos or yolk sacs were GPI-1A and developed entirely from normal ICMs. Four embryos or yolk sacs contained traces of GPI-1B probably as a result of incomplete dissection of TE or embryos. One implantation site contained enough trophoblast for GPI typing and was B+A, the A component probably being decidual tissue from the recipient mother from incomplete dissection.

Blastocysts were also reconstructed from gynogenetic ICMs and normal TE (Table 6; Fig. 2). Twenty four of them implanted and fourteen of these had recognizable embryos with 35 to 40 somites on day 11–12 of gestation. In one further site on day 12 only the yolk sac with unrecognizable embryonic remains inside was detected. The embryos were similar to those obtained from parthenogenetic ICM+normal TE reconstituted blastocysts. The embryos were much more substantial than gynogenetic embryos unassisted by normal trophoblast although they were stunted and often degenerating. Of the seven embryos seen on day 11 three had reached the stage comparable to control day 11 in terms of shape and stage of development but they were only one quarter to one third the volume of the normal control. The rest were retarded and even smaller

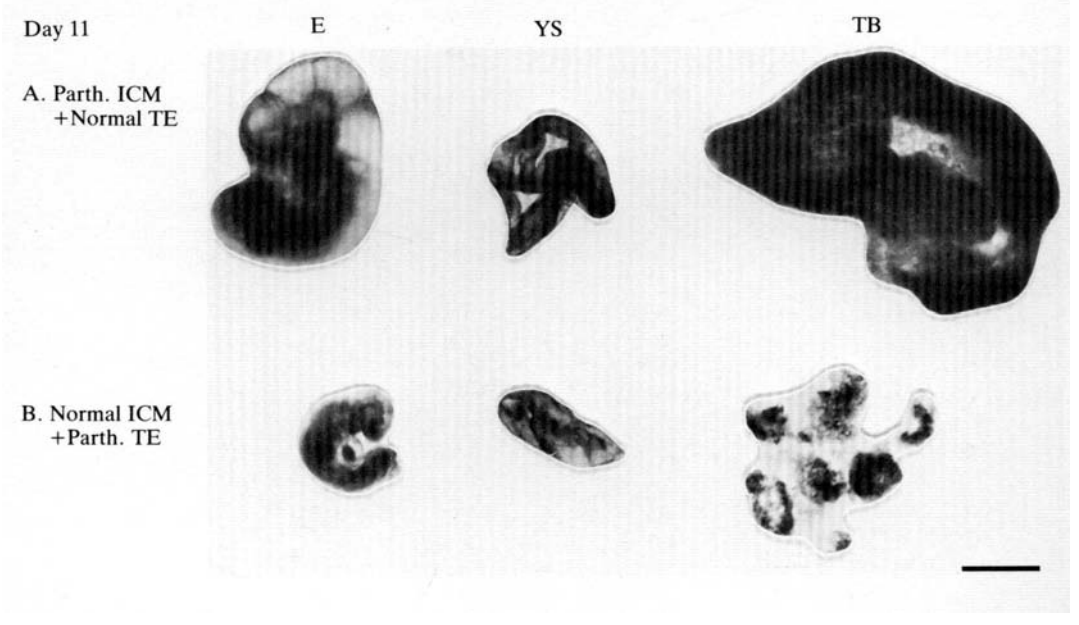

Fig. 1. Embryos and their membranes developed from blastocysts reconstructed from (A) parthenogenetic ICM and normal TE (Table 3, No. 1), and (B) normal ICM and parthenogenetic TE (Table 2, No. 5). E, embryo; YS, yolk sac; TB, Trophoblast and ectoplacental cone. Scale bar equals 1 mm. These are the most substantial embryos obtained on day 11 in each series. The TB in (A) was about normal volume for day 11 but the parthenogenetic embryo, although it had reached an appropriate stage for its age, was stunted and confined tightly within a small yolk sac. In (B) the sparse TB was difficult to dissect from the deciduum, and the yolk sac, though small, was expanded and appropriate to the size of the embryo.

than those described above. Of the day-12 embryos the two largest and most advanced had progressed no further than the best day-11 embryos and they were now about one eighth the volume of a normal day-12 control embryo (4–4·25 mm long compared with 6·8 mm control embryos, see Figs 3 and 4). The 4 mm embryo was processed for light microscopy, its tissues were found to be necrotic and there was a large blood invasion inside the left side of the head (Fig. 5). In all instances the yolk sac was substantial but it was smaller than in control embryos and it had poor blood supply. Apart from two instances of relatively poor trophoblast, the trophoblast was apparently normal in the rest of the implantation sites. Analysis of embryos and yolk sacs by GPI typing revealed that twelve embryos were GPI-1B and were entirely of gynogenetic origin while the other three contained varying amounts of GPI-1A, probably derived as a contaminant of dissected embryos. The trophoblast in all instances was GPI-1A and therefore derived from normal TE.

DISCUSSION

This study demonstrates normal development to term of blastocysts reconstituted from normal ICMs and TE. By contrast, when one of the two components of the reconstituted blastocysts was derived from embryos with the maternal genome alone (gynogenetic or parthenogenetic), development was variable, although never normal, depending on whether ICM or TE was used. No detectable differences were however encountered between ICMs or TE derived either from heterozygous biparental gynogenetic or parthenogenetic blastocysts.

Table 4. *Development of blastocysts reconstituted from parthenogenetic ICMs and normal TE*

		Development of conceptuses and GPI analysis				
Embryo no.	Day of gestation	Size: CR length mm	Approx. no. of somites	Embryo	Yolk sac	Trophoblast
1	11	3·1	35	B	B	A
2	11	1·75	25	B	B	A+B 10:1
3	11	2·0	25	B	B	A
4	11	2·0	20+	B	B	A
5	12	2·8	35	B	B	A
6	12	2·6	25	B	B	A
7	12	3·5	40	B	B	A
8	12	2·0	30	B(+A?) v. poor embryo	B	A
9	12	5·0	40	B	B	A
10	12	3·5	35	B	B	A
11	12	3·75	35	B	B	A
12	12	4·0	35	B	B	A
13	12	3·25	35	B+A 5:1	B+A	A
14	12	3·25	35	B	B	A

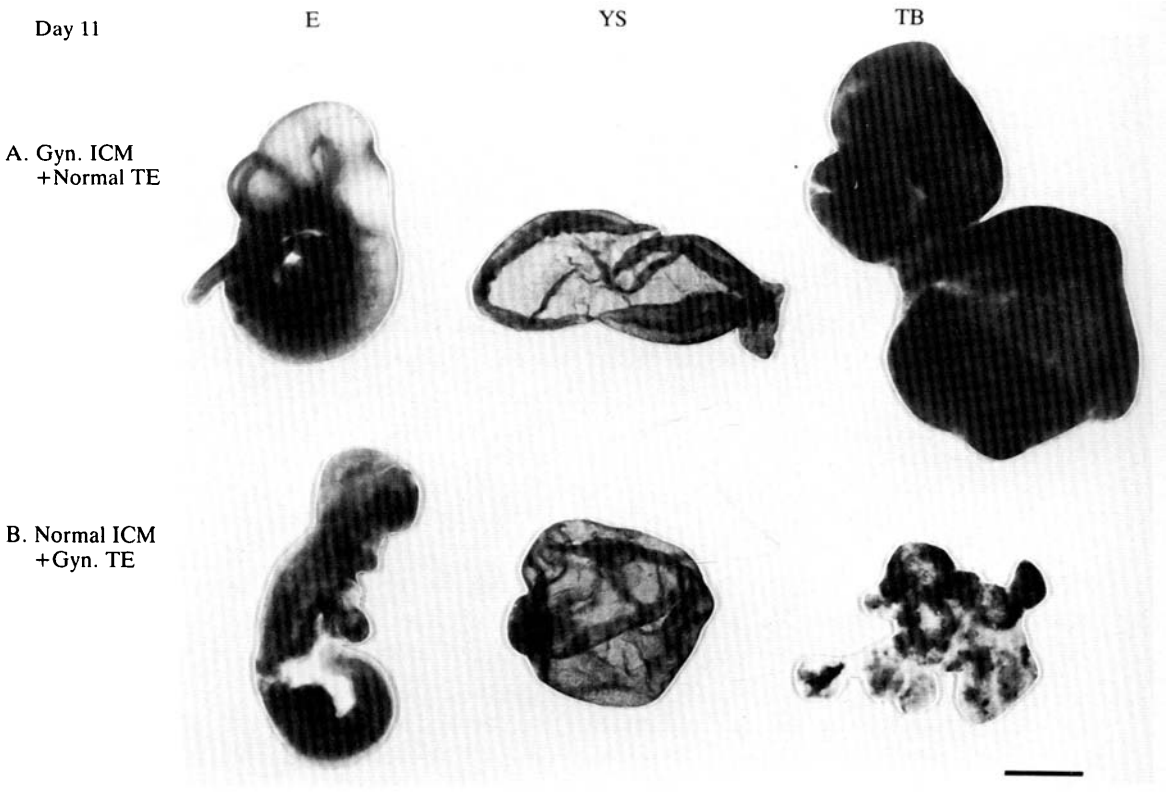

Fig. 2. Day-11 embryos and their membranes developed from blastocysts reconstructed from (A) gynogenetic ICM and normal TE (Table 5, No. 2), and (B) normal ICM and gynogenetic TE (Table 4, No. 4). Scale bar equals 1 mm. The gynogenetic embryo in (A) was well advanced for day 11 but again was small and restricted in a relatively small yolk sac. The embryo from a normal ICM in (B) was already dead and degenerating at the time of dissection.

The results in both cases are therefore attributed to the lack of paternal genome in tissue derived from these blastocysts.

In the first instance we tested the response of TE derived from gynogenetic or parthenogenetic blastocysts by placing normal ICMs inside the trophectoderm vesicles. It was observed that even in the presence of a normal ICM, the trophectoderm failed to proliferate. The result indicates an inherent inability of trophectoderm lacking the paternal genome to proliferate despite the presence of a normal ICM. Furthermore, there was no conclusive evidence of a contribution of cells from the normal ICMs to the outer trophectoderm lineage despite the lack of trophoblast proliferation. Previous studies on reconstituted blastocysts have established an inductive influence of ICM on the proliferation of the trophoblast without the ICM making a direct contribution of cells to the tissue (Gardner *et al.* 1973; Gardner & Papaioannou, 1975). However, when ICMs are isolated from early blastocysts and cultured *in vitro*, they can regenerate trophectoderm cells

(Handyside, 1978; Hogan & Tilly, 1978; Spindle, 1978; Rossant & Tamura-Lis, 1979; Nichols & Gardner, 1984; Adams, 1985). This potential to regenerate trophectoderm cells is lost in ICMs isolated from expanded blastocysts (Gardner, 1975; Rossant, 1975; Adams, 1985). The ICMs used in this study were either too advanced or their transfer inside a trophectoderm vesicle prevented contribution of cells from the ICM to the outer trophectoderm lineage. In any event, the lack of

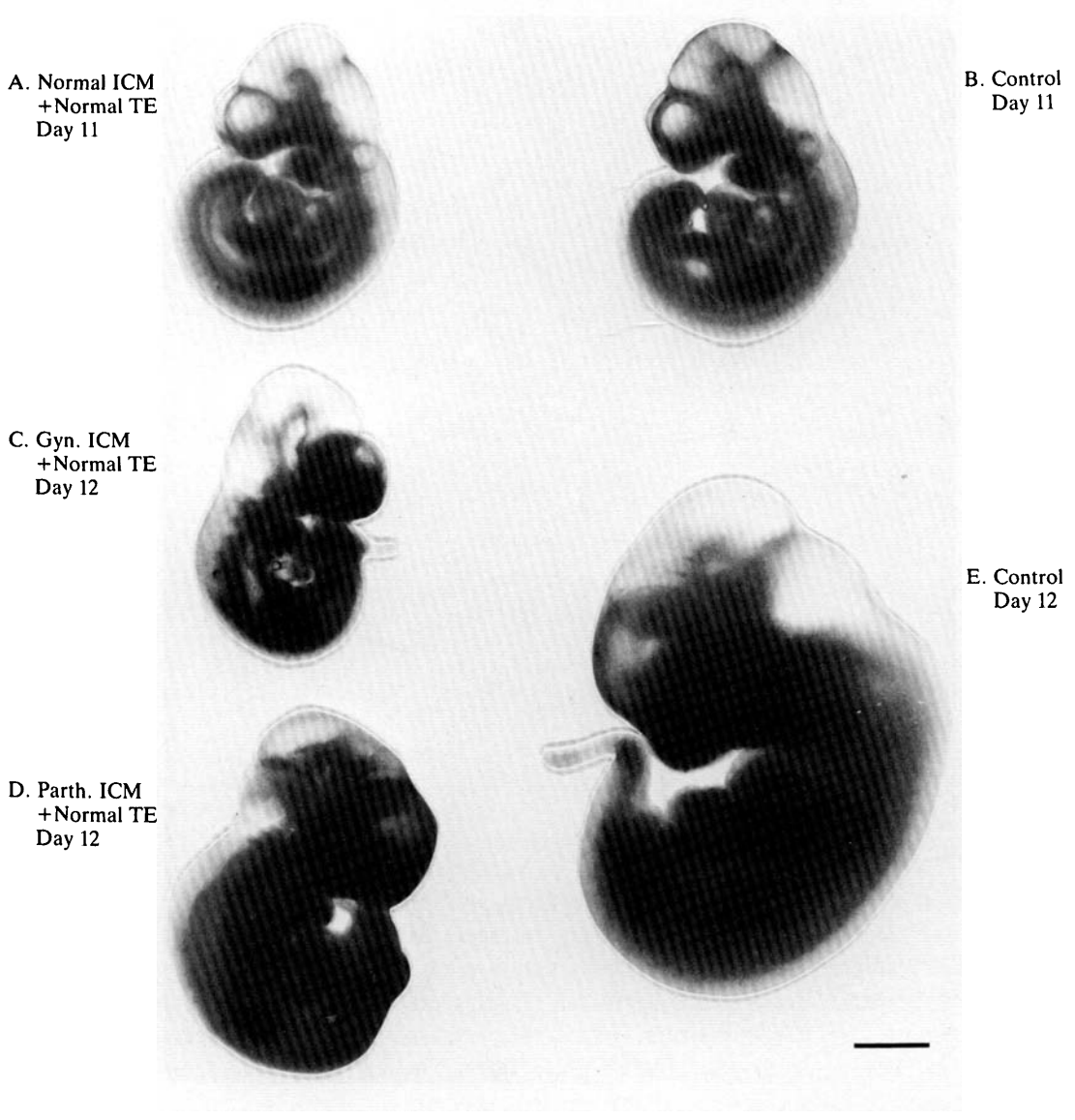

Fig. 3. Control day-11 and day-12 embryos (B and E), day-11 embryo developed from a blastocyst reconstructed from normal ICM and TE of different genetic origin (A), and the most advanced day-12 gynogenetic (C) and parthenogenetic (D) embryos obtained from reconstructed blastocysts using normal TE (Table 5, No. 12 and Table 3, No. 9). Scale bar equals 1 mm. A sagittal section of embryo C is shown in Fig. 5.

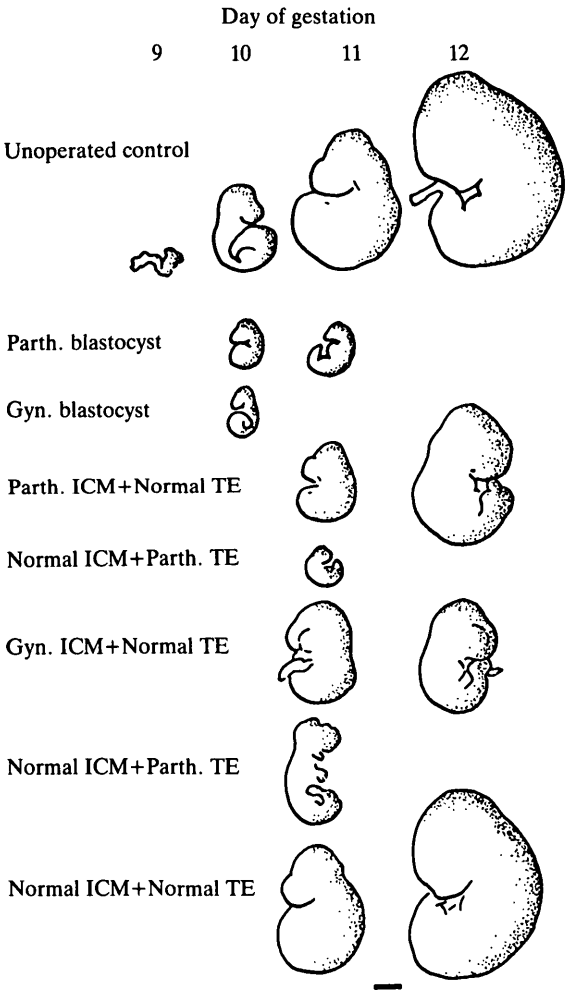

Fig. 4. Schematic diagram of normal control mouse embryos between days 9 and 12 of development and the most advanced experimental embryos obtained in this study. Scale bar equals 1 mm.

trophoblast proliferation had a significant influence on the number and extent of development of normal ICMs in the reconstituted blastocysts. A very few small 25-somite embryos were obtained and they resembled the development of unoperated gynogenones (Surani & Barton, 1983) and parthenogenones (Kaufman *et al.* 1977) in which the trophoblast also fails to proliferate (Surani & Barton, 1983; Surani *et al.* 1984). The yolk sac which is derived from the ICM (Gardner & Papaioannou, 1975) was also poorly developed with meagre blood supply. This study clearly demonstrates that the failure of trophoblast proliferation severely retards development of embryos from normal ICMs.

We also tested the developmental capacity of ICMs derived from gynogenetic and parthenogenetic blastocysts by introducing them into trophectoderm vesicles

Table 5. *Development of blastocysts reconstituted from normal ICMs and gynogenetic TE*

Embryo no.	Day of gestation	Development of conceptuses and GPI analysis: Size: CR length mm	Approx. no. of somites	Embryo	Yolk sac	Trophoblast
1	11	–	–	Unrecognizable remains – A	Extensive – A	Very poor – B+A 1:1
2	11	–	–	Not detectable	Small – A	Very poor (ND)
3	11	–	–	Not detectable	Small – A	Very poor (ND)
4	11	3·5	25	Dying tissue – A+B 20:1	Moderate – A+B 20:1	Poor (ND)
5	11	–	25	Only part found – A	Moderate – A	Very poor (ND)
6	11	–	–	Some remains (ND)	Small – A+B	Very poor (ND)
7	11	–	–	Empty small yolk sac vesicle only – A		Few giant cells (ND)
8	11	–	–	Small yolk sac vesicle only – A		None found
9	11	–	25	Very decrepit – A	Small	Very poor (ND)
10	11	–	–	Empty substantial yolk sac – A		Very poor (ND)
11	11	–	–	Small yolk sac vesicle only – A		Very poor (ND)
12	11	–	–	Small disintegrated embryo	Small-A	Very poor (ND)

ND = GPI not determined – degenerate and insufficient tissue.

from normal embryos. The trophoblast in this instance proliferated normally but without a cellular contribution from ICMs. This clearly indicates that the ICMs can interact with the TE and induce proliferation provided the TE cells contain a paternally inherited genome. With the improved development of the trophoblast, almost all the embryos reached at least the 25-somite stage and indeed some of the most advanced 30- to 40-somite gynogenones and parthenogenones seen to date were obtained. However, these embryos are still smaller than the controls and show various abnormalities already described. Hence, poor development of extraembryonic tissue partly but not wholly accounts for the inability of parthenogenones and gynogenones to reach term. The improved development of gynogenones and parthenogenones may represent almost the maximum development attainable in the absence of a paternal genome. However, this inference about the improved development assumes that the normal trophoblast does not contribute a variety of factors postulated in the rescue of non-viable genotypes by normal cells in chimaeras (McLaren, 1976). Nevertheless, this study concurs with our previous observations (Surani *et al.* 1984; Barton *et al.* 1984) and shows that the maternal genome alone can direct preimplantation and early postimplantation development but the proliferation of extraembryonic tissues probably requires paternal inheritance of specific genes.

Table 6. *Development of blastocysts reconstituted from gynogenetic ICMs and normal TE*

		Development of conceptuses and GPI analysis				
Embryo no.	Day of gestation	Size: CR length mm	Approx. no. of somites	Embryo	Yolk sac	Trophoblast
1	11	2·8	35	B+A 10:1	Substantial – B+A	Poor – A
2	11	3·6	35	B	Substantial – B	A
3	11	1·9	25	B	B	A
4	11	2·9	30	B	B	A
5	11	2·6	30	B	B	A
6	11	3·5	35	B	B	A
7	11	3·2	35	B	B	A
8	12	4·25	35	B	B	A
9	12	3·75	35	B	B	A
10	12	2·5	30	B	B+A 4:1	A
11	12	2·5	30	B	B	A
12	12	4·0	35	ND (sectioned)	B	A
13	12	2·75	35	B	B	A
14	12	–	–	Yolk sac vesicle only (1·2 mm)	B+A 20:1	Poor – A
15	14	3·0	40	Very poor	B	A

ND = GPI not determined.

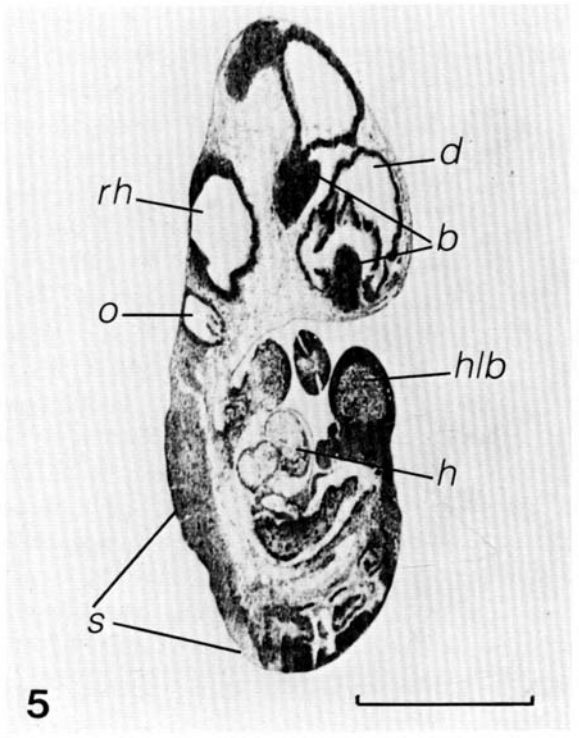

Fig. 5. Sagittal section of day-12 gynogenetic ICM+normal TE (Table 5, No. 12) (see Fig. 3C). Since all the tissues in this embryo were more or less necrotic and the embryo also contracted somewhat during processing, many of the features are obscured to some extent. *b*, blood mass made up of necrotic nucleated blood cells; *d*, diencephalon; *h*, heart; *o*, otic vesicle; *rh*, rhombencephalon; *s*, somites; *hlb*, hindlimb bud (the thick epithelium is probably seen because of the plane of section). Scale bar equals 1 mm.

Embryonic development in the absence of a paternal genome may be further advanced if the yolk sac as well as the trophectoderm is improved. Despite substantial proliferation of the trophoblast, the yolk-sac development was relatively poor with meagre blood supply in the reconstituted embryos. It has been well established that parthenogenetic cells lacking a paternal genome do not die of autonomous cell lethals as they display extensive capacity for proliferation and differentiation in ectopic sites (Iles, McBurney, Bramwell, Deussen & Graham, 1975) and when combined with normal embryos as chimaeras can develop to term (Surani, Barton & Kaufman, 1977; Stevens, Varnum & Eicher, 1977; Stevens, 1978). However, parthenogenetic embryos combined with similar embryos from another strain of mice or indeed androgenetic embryos with a paternal genome have so far failed to reach term (Surani, Barton & Norris, unpublished). Hence although both a maternal and a paternal genome are needed for development to term, it does not appear sufficient to have them present in separate cells of a conceptus. The results indicate that the two genomes are needed together in at

least some of the embryonic cells of a developing conceptus, probably because the maternal and the paternal genomes interact in specific ways inside individual cells.

The results of our study do not agree with the reported birth of homozygous uniparental gynogenetic and androgenetic as well as parthenogenetic mice (Hoppe & Illmensee, 1977, 1982). The main conclusions from the previous studies suggest that the cytoplasm of fertilized eggs but not that of activated eggs, is the most crucial factor in development to term of various genotypes (Hoppe & Illmensee, 1977, 1982). However, in a number of other studies similar eggs fail to develop to term (Modlinski, 1980; Markert, 1982; Surani & Barton, 1983; Surani *et al.* 1984; Barton *et al.* 1984; McGrath & Solter, 1984; Mann & Lovell-Badge, 1984) and display characteristic developmental arrest depending on the genotype (Surani *et al.* 1984; Barton *et al.* 1984). In these studies normal development occurs provided both maternal and paternal genomes are present (Surani *et al.* 1984; McGrath & Solter, 1984), regardless of whether the cytoplasm is from fertilized or activated eggs (Surani & Barton, 1983; Surani *et al.* 1984; Mann & Lovell-Badge, 1984). The differences in the reported results requires further investigation.

In conclusion, the apparent functional differences between maternal and paternal genomes may arise when the homologous chromosomes are spatially separated and exposed to the different cytoplasmic environment of maturing gametes, enabling specific modifications to the DNA. The nature of the specific information introduced is unknown but the differences are apparently inherited and can account for the differences in the behaviour of the homologues in many instances (Sager & Kitchin, 1975). In this respect, the methylation of some dispersed repetitive DNA sequences in the mouse spermatozoa but not the oocytes (Sandford, Forrester, Chapman, Chandley & Hastie, 1984) is of interest because this and other kinds of differences may explain preferential inactivation by heterochromatization of the paternal X-chromosome in the extraembryonic tissue (Chandra & Brown, 1975; Lyon & Rastan, 1984). Such differences may also account for the apparent overall differences of functions of maternal and paternal genes during preimplantation development (Surani, Barton & Norris, in preparation) and embryogenesis as well as the influence of certain mutations in mice being dependent on the parental origin of inheritance (Wakasugi, 1974; Johnson, 1974; McGrath & Solter, 1984*b*). A number of investigations have demonstrated non-complementation of maternal duplication/paternal deficiency and its reciprocal for regions of chromosomes 2, 8, 11 and 17 (Searle & Beechey, 1978; Lyon & Glenister, 1977; Johnston, 1974; Cattanach & Kirk, 1985). For example, mice disomic for chromosome 11 of maternal origin are significantly smaller, but not if their chromosome 11 are of paternal origin (Cattanach & Kirk, 1985). It appears most likely that during the formation of germ cells, the previous modifications of the DNA are first entirely removed prior to or at the beginning of meiosis, as judged by substantial demethylation of both the major and minor satellite DNA (Ponzetto-Zimmerman & Wolgemuth, 1984; Sanford *et al.* 1984) and reaction of the inactive X-chromosome (Johnston, 1981; Monk, 1981), and that during meiosis the cycle is repeated and new modifications introduced.

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