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The independence of germ-cell genotype from somatic influence in chimaeric mice

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SUMMARY

Mouse chimaeras made by aggregating embryos homozygous for nine recessive genes with embryos carrying the corresponding dominants were mated with partners of the recessive strain. Progeny were either of the recessive type for all loci examined, or showed the dominant characters at all loci. Tail length in homozygous vestigial-tail progeny was unaffected by whether the fertilizing spermatozoon had undergone maturation in a chimaera or in a control vestigial-tail male. There was thus no indication that the germ cells had been in any way modified by their intimate association with germ cells and somatic tissue of contrasting genotype in the chimaeras.

1. INTRODUCTION

It is a fundamental tenet of Mendelian inheritance that the genetic content of germ cells is contaminated neither by the somatic tissue of the animal or plant in which they develop nor by their fellow germ cells.

Yet germ cells in mammals, as in many other groups, typically develop in very close association with one another and with the somatic cells of the gonad. During much of spermatogenesis, the germ cells are enveloped by the cytoplasm of the testicular Sertoli cells (Brökelmann, 1963; Nicander, 1967), and cytoplasmic connexions between spermatogonia, spermatocytes and spermatids have been demonstrated in several species of mammals (Nicander, 1967; Dym & Fawcett, 1971). In the ovary also the germ cells are linked together in groups by cytoplasmic bridges to form a syncytial organization (Gondos & Zamboni, 1969). Although mammalian oogenesis does not show the massive transfer of cytoplasm and RNA from nurse cells to oocytes characteristic of certain insect groups (e.g. MacGregor & Stebbings, 1970), the mammalian oocyte develops in intimate contact with the surrounding follicle cells, with the cell membranes associated in tight intercellular junctions (e.g. Zamboni, 1972). Mammalian oocytes are pinocytotically active (Anderson, 1972), and incorporate exogenous proteins and perhaps other macromolecules (Glass, 1961, 1970; Mancini *et al.* 1963).

There thus exists ample opportunity for informational macromolecules to enter mammalian germ cells. A low incidence of transformation or 'transgenosis' (a term that makes no assumptions about mechanism, see Doy, Gresshoff & Rolfe, 1973) of germ cells could not be detected in normal matings, since the diploid germ cells are of course identical in

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genetic constitution with each other and with the surrounding somatic tissue, while in the haploid phase any modification would be confounded with other commonly occurring causes of disturbed segregation. Some breeding experiments involving grafted ovaries have been carried out in mice, but in the main these have involved either genetically marked ovaries grafted into F_1 hosts, where a genetic modification in the grafted ovary could not be distinguished from regeneration of host ovarian tissue (e.g. Russell & Hurst, 1945), or grafts into immunologically tolerant hosts, but in strain combinations where a single transformational event would again have gone undetected (e.g. Jones & Krohn, 1960). In any case, the oocytes in an ovarian graft are of the same genetic constitution as the surrounding follicle cells, so that any external influence would require to be mediated by the body fluids.

The embryo aggregation chimaera provides a very different situation. Mouse embryos of genetically contrasting type are aggregated in pairs, usually at the eight-cell stage; provided both components survive embryogenesis, the composite develops into an overtly chimaeric but otherwise normal animal. Primordial germ cells of two genetic types migrate from the yolk-sac wall into the genital ridges, the future gonads, which themselves contain somatic tissue of two genetic types. Since there is no reason to believe that there exists any cell recognition or assortative distribution between germ cell and somatic tissue, female chimaeras will contain some oocytes surrounded by follicles consisting wholly or partly of cells of the other component, and male chimaeras will contain some spermatogonia enveloped by Sertoli cells of contrasting genotype. Some of the consequences for gamete phenotype have already been explored. McLaren, Chandley & Kofman-Alfaro (1972) found that XX germ cells in an XX/XY chimaeric testis entered meiotic prophase before birth, at the usual time for female germ cells, but were unable to continue normal development in the environment of the testis. Burgoyne (1973) reported that head length and breadth and midpiece length of mouse spermatozoa developing in a chimaeric testis behaved autonomously, though there were minor differences between the dimensions of spermatozoa from chimaeras and control males, due perhaps to the different uterine environment to which the chimaeras had been exposed.

The possible consequences of a genetically disparate somatic environment for the genetic (rather than phenotypic) potential of the gametes has received little attention. Published data on the breeding performance of chimaeras is summarized in Table 5 of McLaren (1972). Some of the studies involved aggregations between strains differing at only one easily scorable locus (e.g. C3H↔C57BL); in such cases any modification of germ cells by somatic tissue could not be distinguished from temporal shifts in the proportions of the two types of germ cell due to selection (Mintz, 1968). Some of the strain combinations studied by Mullen & Whitten (1971) differed at two easily scorable loci (e.g. agouti and albino), so that progeny derived from modified germ cells would in principle have been detectable, but no details are given of how many progeny were bred from such chimaeras.

In the present study, breeding tests were carried out on chimaeras derived from aggregation of embryos differing at nine loci, in order to maximize the probability of detecting any modifications of the gametes.

2. MATERIALS AND METHODS

The chimaeras formed part of a series obtained by embryo aggregation, as described by Bowman & McLaren (1970). Each aggregated pair consisted of one embryo from a multiple recessive stock homozygous for non-agouti, brown, dilute, pink-eye, chinchilla, waved-2, short-ear, vestigial-tail and supernatant NADP IDH type a, and one F_1 embryo carrying the corresponding dominant alleles from crosses of C3H/Bi/McL males with

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Table 1. *The progeny of a series of overtly chimaeric mice of a multiple recessive↔dominant strain combination, 'back-crossed' to the multiple recessive strain.*

Phenotype of young	Germ cell population	Sex of chimaera	No. of chimaeras	Birth* ♀	Birth* ♂	Birth* Dead	Birth* Total	2–3* weeks	Id-1*
Recessive	Single	♀	3	81	94	10	185	136	96
Recessive	Single	♂	10	443	377	39	859	559	287
Recessive	Mixed	♂	1	20	28	0	48	31	0
Total recessive progeny				544	499	49	1092	726	383
Dominant	Single	♀	1	54	54	6	114	90	0
Dominant	Single	♂	6	292	324	10	626	537	0
Dominant	Mixed	♂	1	10	9	0	19	18	0
Total dominant progeny				356	387	16	759	645	0

* At birth the young were classified for pink-eye and vestigial-tail; at 2–3 weeks the survivors were classified for dilute, brown, non-agouti, chinchilla, waved-2 and short-ear; after weaning the Id-1 type of some of the mice was determined.

either C57BL/McL females or CBA/Fa females. Observations on other aspects of the series are described by McLaren & Bowman (1969), McLaren (1972), Grüneberg & McLaren (1972) and McLaren (1975).

The 22 overt chimaeras comprised 4 females and 18 males. The unequal sex ratio reflects the fact that the $XX \leftrightarrow XY$ chimaeras developed as males (McLaren, 1975). At 6 weeks of age, each chimaeric female was mated to one male, and each chimaeric male to two females, from the multiple recessive stock. Control matings were made up in parallel, with multiple recessive animals of approximately the same age as the chimaeras mated to contemporaries (litter-mates where possible) of the animals used in the chimaera matings.

Young were classified at birth for pink-eye and vestigial-tail, and at 2–3 weeks for non-agouti, brown, dilute, chinchilla, waved-2 and short-ear. Liver biopsies were done on some of the 'recessive' young at 3–6 weeks of age, and their Id-1 type determined by starch electrophoresis. Since the F_1 component was heterozygous at the Id-1 locus, Id-1 type of the 'dominant' young was not determined.

At 15 days of age the young of chimaera and control matings had their tails measured to the nearest millimetre. In homozygous vestigial-tail animals, in which the tails are short and curly, an outline of the tail was traced on paper and the length of the midline measured with a 'Curvimetre' recording map measurer. Litter means were used for statistical analysis.

3. RESULTS AND DISCUSSION

With the exception of one male, all the chimaeras bred (Table 1). Seven (1 ♀, 6 ♂) produced progeny of dominant phenotype only, 13 (3 ♀, 10 ♂) of recessive phenotype only, and one male produced both types of progeny, showing that both components of the chimaera had formed functional germ cells. In no case did any of the progeny show segregation at any of the loci examined: young either resembled the multiple recessive strain at all loci or carried all the corresponding dominant alleles.

The expression of the genes in the progeny of chimaeras appeared qualitatively similar to that seen in control matings. A quantitative assessment was made on tail length in homozygous vestigial-tail offspring. The young of female chimaeras were not used, to avoid the complication of possible maternal effects on gene expression during embryonic development. The 10 male chimaeras that produced recessive young were each mated to

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two recessive females, and 10 control recessive males were also each mated to two recessive females. There were thus 20 chimaera/control paired matings available; of these, 14 produced young for paired comparisons. Tail measurements were carried out on 222 young of chimaeric males, from 44 litters, giving a mean tail length of $3{\cdot}93 \pm 1{\cdot}70$ mm, and on 206 young of control males, from 39 litters, giving a mean of $3{\cdot}44 \pm 1{\cdot}52$ mm, calculated from litter means. When the chimaera/control paired matings were weighted according to the number of litters born to each, the (chimaera-control) weighted mean difference in tail length came to $0{\cdot}23 \pm 1{\cdot}83$ mm; that is, there was no significant difference between chimaeras and controls with respect to the tail length of their progeny.

For the nine loci examined there is thus no evidence that any allele was transformed into its homologue under the influence of germ cells or somatic tissue of contrasting genotype, nor is there any indication (for the one locus examined quantitatively, namely vestigial tail) that the expression of the paternal gene was affected by its sojourn in a chimaeric testis. The same loci proved equally refractory to transgenosis when embryos were exposed to DNA of contrasting genotype during cleavage (Snow & McLaren, 1974). Unless the loci selected are very unrepresentative of the genome, we may conclude that the genetic content of mouse germ cells is not subject to contamination by the cellular environment.

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