

Formation of germ-line chimaeras from embryo-derived teratocarcinoma cell lines

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The recent availability in culture of embryo-derived pluripotential cells which exhibit both a normal karyotype and a high differentiative ability¹⁻³ has encouraged us to assess the potential of these cells to form functional germ cells following their incorporation into chimaeric mice. We report here the results of blastocyst injection studies using three independently isolated XY embryo-derived cell lines (EK.CP1, EK.CC1.1 and EKCC1.2) which produce a very high proportion (>50%) of live-born animals that are overtly chimaeric. Seven chimaeric male mice, derived from these three lines, have, so far, proved to be functional germ-line chimaeras.

Although teratocarcinoma-derived embryonal carcinoma (EC) cells have been used to construct chimaeras, they have rarely realized the developmental potential demonstrated by embryonic inner cell mass cells used in a similar combination⁴. Indeed, when introduced into the early embryo many EC cell lines show a low rate of colonization and/or a restricted pattern of differentiation⁵, and many chimaeras develop tumours both pre- and postnatally⁴.

We have recently demonstrated that over 15 of our fertilized and parthenogenetically activated⁶ embryo-derived (EK) cell lines of both XX and XY sex chromosome constitutions form normal chimaeras with high efficiency (in preparation). In this study we decided to use fertilized embryo-derived pluripotential XY cell lines, which, unlike most of their tumour-derived EC cell counterparts, readily differentiate *in vitro* and possess a normal euploid karyotype³, an essential prerequisite for the formation of viable gametes. We now present data which clearly demonstrate that such cell lines are capable of forming functional germ-line chimaeras.

Table 1 gives the results of the blastocyst injection experiments and Table 2 the results of the test breeding studies. Figure 1 shows six of the phenotypically male chimaeras which transmitted functional spermatozoa derived from the introduced cells.

It would obviously be desirable to incorporate specific functional genes into the mouse genome. While direct transformation

Table 1 Rates of construction of chimaeras

Cell line	No. injected	No. born (%)	No. chimaeric (%)	Chimaeras			Males		No. of germ-line chimaeras
				Male	Female	ND	Set up	Bred	
EK.CP1	254	167 (66)	74 (44)	40	27	7	23	20	4
EK.CC1.1	69	52 (75)	31 (60)	21	10	0	13	8	1
EK.CC1.2	160	111 (70)	63 (57)	50	13	0	21	7	2
Total:				111	50			35	7 (20%)

The EK.CP1, EK.CC1.1 and EK.CC1.2 cell lines were isolated from delayed blastocysts¹. All of these lines are characterized by the possession of black and agouti coat colour markers. The EK.CP1 cell line was derived from a 129/Sv//Ev strain mouse and is homozygous for the *GPI-1^a* allele at the *GPI-1* (glucose phosphate isomerase-1) locus. EK.CC1.1 and EK.CC1.2 were derived from a substrain of 129/Sv//Ev which is homozygous for the *GPI-1^c* allele at the *GPI-1* locus. All three of these lines possess a normal euploid XY chromosome constitution and have been maintained entirely *in vitro* on feeder layers of inactivated fibroblasts. The breeding data available for some males are incomplete; these might yet prove to have a low-level culture-derived germ-line component. There is a very evident distortion of the sex ratio in the live-born chimaeras—this deviation in favour of males is highly significant (χ^2 , $P < 0.001$) and probably reflects the conversion of a number of 'host' female embryos to male chimaeras. ND, not determined.

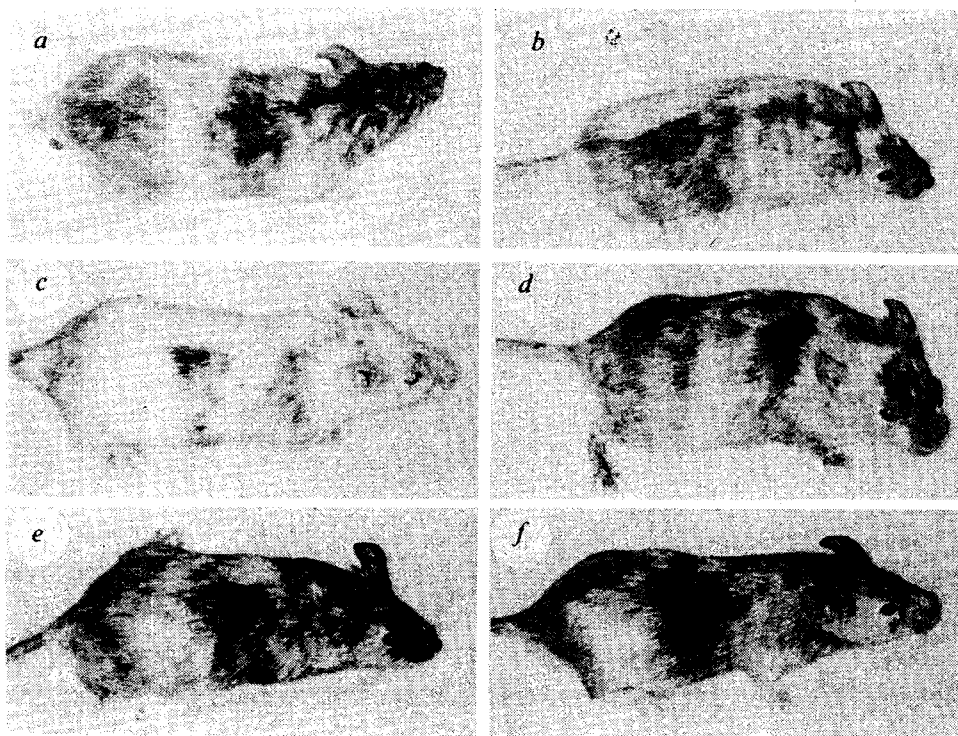


Fig. 1 Six of the seven germ-line chimaeras described in Tables 1 and 2. *a*, CPI.3; *b*, CPI.5; *c*, CPI.11; *d*, CCI.1.3; *e*, CCI.2.6; *f*, CCI.2.8. Between 8 and 12 embryo-derived cells were introduced into the blastocoelic cavity of host-fertilized blastocysts homozygous for the recessive albino locus. The blastocysts were then allowed to re-expand and were subsequently transferred to the uterine lumen of recipients on the third day of pseudopregnancy. All the conceptuses were allowed to develop to term, and live-born animals were scored for the presence of eye and coat pigmentation at or shortly after birth.

of the zygote has recently shown much promise⁷⁻¹³, an alternative approach is the construction of individuals between 'modified' pluripotential cells and normal embryos. This second approach has the advantage that cultured cells are accessible for genetic manipulation, characterization and selection before their incorporation *in vivo*. Several EC cell lines have been selected *in vitro* to carry specific mutations or additional intact 'foreign' chromosomes, and have subsequently been incorporated into chimaeric individuals¹⁴⁻¹⁷. It is, however, a necessary prerequisite to demonstrate that germ-line chimaerism can be reliably achieved. Published rates of construction of germ-line chimaeras using EC cell lines have been disappointingly poor^{18,19}. There has been a report of a single XX cell line, maintained completely *in vitro*, from which two germ-line females have been obtained. In addition to a low rate of chimaera formation (13%), these females produced only four progeny having an EC-derived component^{20,21}.

Clearly, embryo-derived stem cells seem to be particularly efficient at recolonizing the early embryo. This feature, together with the availability of XY lines such as those described here, now allows the routine construction of chimaeric males which are capable of transmitting culture-derived genomes to a potentially limitless number of offspring, and confirms our previous contention of the normality of the genome in these stem cell lines²².

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Note added in proof: A further four males described in Table 1 have proved to be germ-line chimaeras (three from the CCI.2 and one from the CCI.1 cell lines).

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Table 2 Breeding data from the seven functional germ-line chimaeras

Male chimaera	No. of litters	No. of offspring	No. albino	No. black agouti
CPI.3	10	78	0	78
CPI.5	16	170	163	7
CPI.11	2	23	22	1
CPI.34	1	5	0	5
CCI.1.3	1	12	0	12
CCI.2.6	1	13	0	13
CCI.2.8	1	11	0	11

Phenotypically male chimaeras were caged with successive virgin CFLP females homozygous for the *GPI-1^b* allele and the albino locus. Resulting litters were scored for the dominant black and agouti phenotypes. GPI analysis of blood samples taken from all the black agouti offspring has shown the predicted *GPI-1^a1^b* (EK.CPI) or *GPI-1^b1^c* (EK.CCI.1 and EK.CCI.2) genotype, thus verifying the inheritance of these culture-maintained lines. The chimaeras which appear to be breeding from their XY culture-derived component alone are probably sexual mosaics, although phenotypically they are normal males, as any XX germ cells would not be capable of forming functional spermatozoa.

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