Sexing of preimplantation mouse embryos by measurement of X-linked gene dosage in a single blastomere

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Summary. Single blastomeres were isolated from zona-free 8-cell mouse embryos and assayed for X-linked hypoxanthine phosphoribosyl transferase (HPRT) activity and autosome-linked adenine phosphoribosyl transferase (APRT) activity. At this stage of development both X chromosomes are active in female embryos. Hence, a bimodal distribution of HPRT:APRT ratios, corresponding to male (XY) and female (XX) biopsied samples, was observed due to the 2-fold difference in gene dosage for HPRT activity. Batches of putative male and female embryos identified in this way were transferred to pseudopregnant recipient females. Development of the seven-eighths embryos was equivalent to that of control zona-free intact embryos. Sex determination by measurement of X-linked gene dosage was accurate and rapid enough to allow transfer of embryos of known sex without the need for cryopreservation.

Keywords: sexing; preimplantation embryo; X-chromosome dosage; mouse

Introduction

The possibility of sexing preimplantation mammalian embryos and the establishment of pregnancy with embryos of either sex has important applications in domestic species for livestock management and breeding. In humans, preimplantation sexing could be of potential benefit in preventive control of X-chromosome-linked genetic diseases which affect only males, such as Duchenne muscular dystrophy, Lesch–Nyhan syndrome and haemophilia. When the determination of sex is destructive to the cells, the approach would be to sex one cell or a few cells biopsied from cleavage-stage embryos or blastocysts, followed by transfer of the identified female or male embryos back to recipient mothers. This approach has been successful with the rabbit in which sex chromatin can be identified histologically (Gardner & Edwards, 1968). A DNA probe specific for the human Y chromosome has been used to identify whole human male preimplantation embryos by in-situ hybridization and autoradiography (West *et al.*, 1987). However, several days are required for autoradiography and this technique, if applied to biopsied embryos, would require their cryopreservation before re-transfer to the mother. However, the use of a biotinylated Y-specific probe has enabled sexing of bovine embryos within 30 h (Leonard *et al.*, 1987). We report here a novel approach to sexing preimplantation embryos by measurement of dosage of the activity of the X-linked enzyme, hypoxanthine phosphoribosyl transferase (HPRT: EC 2.4.2.8) in a single isolated blastomere.

Materials and Methods

Female F1 mice (CBA × C57) were superovulated, mated to males, and 8-cell embryos flushed from the oviducts on the 3rd day of pregnancy in Hepes-buffered M2 medium (Quinn *et al.*, 1982). The zonae pellucidae were removed in acid Tyrode's solution (Nicolson *et al.*, 1975), the embryos washed in Medium M2 and incubated for 10–20 min at

room temperature in Hank's buffered saline without calcium and magnesium and supplemented with 6 mg bovine serum albumin/ml. Single blastomeres were dislodged from each embryo by gentle pipetting. The biopsied embryos and their respective single blastomeres were transferred to paired droplets of Medium M16 (Whittingham, 1971) under oil and incubated at 37°C in 5% CO₂ in air. After incubation for 12 h, each blastomere was washed in phosphate-buffered medium (PB1; Whittingham, 1974) with 4 mg polyvinylpyrrolidone/ml instead of albumin (PB1-PVP; PVP-40, mol. wt 40 000; Sigma, Poole, Dorset, U.K.) and pipetted into a 10 µl capillary tube (Microcap, Drummond Scientific Co., U.S.A.) in 5 µl Medium PB1-PVP. Extracts of the blastomeres were prepared by freeze-thawing the samples in the capillary tubes three times, and the activities of X-coded HPRT and autosome-coded APRT (adenine phosphoribosyl transferase, EC 2.4.2.7) in the supernatants were determined in a single reaction mix as described by Monk (1987). Culture of the embryos in Medium M16 at 37°C in 5% CO₂ in air was continued until the results of the assay of the biopsied samples were known. The embryos were then transferred in batches of putative males and putative females to the uteri of separate pseudopregnant recipients on the 3rd day after mating with vasectomized males. Control zona-free embryos were transferred to the contralateral horns. The resulting fetuses were analysed for gonadal sex on the 15th day of gestation.

Results

We have previously shown that both X chromosomes are active in female mouse embryos at the morula stage of preimplantation development (Monk & Harper, 1978; Kratzer & Gartler, 1978). A highly sensitive double microassay, which simultaneously measures the activity of X-coded HPRT and autosome-coded APRT, yielded a bimodal distribution of HPRT activities, and HPRT : APRT ratios, in individual embryos. The 2-fold difference between the modes is consistent with populations of male and female embryos with one or two doses of HPRT activity, respectively (Monk & Harper, 1978). The sensitivity of the assay is such that blastomeres isolated from the 8-cell, or even 16-cell stage, of development and cultured for 12–24 h, can be assayed for HPRT and APRT activities and bimodal distributions are obtained. Such a bimodal distribution of HPRT : APRT ratios in samples derived from single blastomeres isolated from 8-cell embryos of a representative mouse litter is shown in Fig. 1.

Fig. 1. Distribution of the ratios of HPRT activity to APRT activity in samples derived from biopsies of single blastomeres of preimplantation mouse embryos from a single litter. Embryos at the 8-cell stage were flushed from the oviducts on the 3rd day of pregnancy. One blastomere was isolated from each of the 8-cell embryos, cultured for 12 h and then assayed for HPRT and APRT activities (see 'Materials and Methods'). Each symbol represents the HPRT : APRT ratio in a single biopsied sample. Assuming 50% of the blastomeres were derived from female embryos and 50% from male embryos, the average HPRT : APRT ratio values were calculated for each mode of the bimodal distribution to be 2.523 ± 0.210 and 1.224 ± 0.044 (arrows) thus showing a 2-fold difference between the modes.

On the basis of the HPRT : APRT ratios in the isolated blastomeres, putative male and female embryos (now at the blastocyst stage) were transferred to the uteri of separate recipient females. Of 6 recipients used 3 were not pregnant with experimental or control fetuses and therefore were not effectively pseudopregnant at the time of transfer. From the 3 pregnant females the fetuses were dissected out on the 15th day of gestation and gonadal sex was determined (see Fig. 2). These 3 females received 21 experimental (sexed) embryos and 26 control embryos which yielded 15 (71%) and 19 (73%) fetuses respectively. The removal of one blastomere at the 8-cell stage therefore had no apparent effect on post-implantation development. Of the 15 fetuses obtained, 14 were indeed the sex predicted by HPRT dosage (see Table 1), whereas the control fetuses in the contralateral horns of the recipients were randomly females and males.

Fig. 2. Male (a) and female (b) urogenital ridges dissected from mouse fetuses on the 15th day of gestation, showing morphology of the gonads.

Table 1. Biopsy, sexing and transfer of preimplantation mouse embryos

Pregnant recipient	No. of biopsied embryos transferred	Sex (HPRT dose)	No. of fetuses at 14½ days	Sex (gonadal)		No. of control embryos transferred	No. of fetuses at 14½ days	Sex (gonadal)	
				♀	♂			♀	♂
1	4	♂	3	—	3	9	7	3	4
2	9	♀	6	5	1	9	5	4	1
3	8	♀	6	6	—	8	7	2	5

Discussion

For some time now, efforts have been made to pre-determine the sex of an embryo by separating the X- and Y-chromosome bearing spermatozoa followed by in-vitro fertilization. So far this approach has had limited success (Levin, 1987). With the development of techniques of in-vitro fertilization, embryo culture and transfer, the possibility of determining the sex of all or part (biopsy) of a preimplantation embryo becomes a valid approach. In practice, sex could be determined by the presence of a Y chromosome. Cytogenetic techniques have been used to sex one half of bisected bovine embryos (with a 60% efficiency) while the other half may be transferred directly or after freezing and thawing (Picard *et al.*, 1985). However, the reliable production of metaphase chromosomes in a single cell or few cells, and analysis of the presence or absence of a Y chromosome, are likely to be inefficient. DNA probes specific for the Y chromosome enable the identification of Y-chromosome DNA in an interphase nucleus of human or bovine embryos (West *et al.*, 1987; Leonard *et al.*, 1987). Probes for the Y chromosome of other mammalian species may become available in the future. However, it is not yet clear whether the determination of sex by in-situ hybridization can be accurately performed on a biopsy of one cell or a few cells. Also, even with the use of biotinylated probes, there is some doubt that the assay could be carried out quickly enough to avoid the need for cryopreservation of the embryos (West *et al.*, 1987).

Sex determination in the mouse by HPRT activity dosage in single blastomeres biopsied from preimplantation embryos is accurate and rapid enough to allow transfer of embryos of the desired sex without the need for cryopreservation and storage. The procedure may be applicable to other mammalian species. Preliminary experiments have revealed that the developmental profiles of HPRT and APRT activities in human preimplantation embryos are similar to those observed in the mouse (M. Monk, R. Penketh & A. H. Handyside, unpublished).

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