Rapid communications

Expression of specific genes in early mouse embryos blocked by cytochalasin

Reinald Fundele, Karl Illmensee, Eva-Maria Jägerbauer, Monika Fehlau, and Wolfgang K.G. Krietsch

Summary. Mouse embryos at the two cell stage derived from C57BL/6 x C3H/Aa F₁-females heterozygous at the X-linked phosphoglycerate kinase locus (Pgk-1) were cultured continuously in the presence of cytochalasin B or D. Further cleavage of the two cell embryos was thus prevented and the embryos became polyploid during culture. The onset of expression of the maternally inherited Pgk-1 gene and of the paternally inherited glucosephosphate isomerase (Gpi-1) gene was determined in these polyploid embryos by cellulose acetate gel electrophoresis of single embryos. In contrast to euploid preimplantation embryos developing normally in utero or in culture without cytochalasins, expression of maternal Pgk-1 was never observed at days 4 and 5 of gestation in polyploid two cell embryos, showing that the Pgk-1 allele on the maternally inherited X chromosome is not activated independently of cytokinesis and morphogenesis. Expression of paternally derived Gpi-1, however, occurred in cleavage blocked embryos von day 5 of development. This may indicate that the activation of two genes which are both expressed during preimplantation development and which both code for glycolytic enzymes, is initiated by different signals.

Key words: Mouse embryogenesis - Cytochalasin - Phosphoglycerate kinase - Glucosephosphate isomerase

Introduction

During early embryonic development of the mouse biochemical processes take place which are closely related to morphogenetic processes like cleavage, compaction and blastocyst formation. Stage specificity of molecular changes has been observed in extensive studies of protein synthesis in preimplantation embryos (Levinson et al. 1978; Braude et al. 1979; Chen et al. 1980; Petzoldt and Hoppe 1980). In addition, activation of specific autosomal (West and Green 1983; Gilbert und Solter 1985; Duboule and Bürkí 1985) and X-chromosomal (Krietsch et al. 1982; Krietsch et al. 1986) loci at specific developmental stages has been described. It is of interest to determine, whether these molecular differentiation events are triggered by morphogenetic processes or whether molecular differentiation proceeds independently of morphogenesis (Waksmundzka et al. 1984).

To some extent, morphogenesis and temporally related molecular differentiation may be uncoupled by treatment of early embryos with Cytochalasins B and D (Surani et al. 1980). Both inhibit cytokinesis but not karyokinesis in mammalian cells (Carter 1967). It was demonstrated (Pratt et al. 1981), that embryos whose cytokinesis was blocked by treatment with Cytochalasin D (CytD) produced proteins that were specific to the developmental stage of untreated control embryos. The molecular markers measured were amino acid transport, synthesis of membrane lipids and activity and distribution of alkaline phosphatase. No significant differences were observed between control embryos and embryos cultured in the presence of CytD. Also, when total protein synthesis was used to monitor molecular differentiation of embryos by two dimensional electrophoresis, striking differences were again not observed (Pratt et al. 1981). In a similar experiment, Petzoldt and coworkers (1983) studied the polypeptide profiles of fertilized oocytes that were cultured continuously in the presence of Cytochalasin B (CytB). They observed that these one cell embryos produced an identical protein pattern, when compared to control embryos whose morphological differentiation proceeded normally. Also it was shown, that paternally inherited Gpi-1 and two genes coding for cell surface antigens are expressed in cleavage blocked polyploid one cell mouse embryos (Petzoldt 1986). These and other results suggest that the timing of protein synthesis during preimplantation development of the mouse is correlated with nuclear divisions rather than cellular divisions. To further test this possibility, we have investigated the expression of the X chromosome linked gene Pgk-1 in embryos which were cleavage arrested during the two cell stage by continuous culture in the presence of CytB or CytD.

In contrast to Gpi-1, whose maternally and paternally inherited alleles are activated at the same time during preimplantation development (West and Green 1983; Gilbert and Solter 1985; Duboule and Bürkí 1985; West et al. 1986), the Pgk-1 alleles are activated at different times, dependent on whether the allele is inherited from the mother or the father (Krietsch et al. 1982; Krietsch et al. 1986). Maternal Pgk-1 is already active at day 4 after fertilization, e.g., during preimplantation development, whereas paternal Pgk-1 is first activated on day 6, after implantation has occurred in utero. It is therefore possible to differentiate between maternal and paternal Pgk-1 expression experimentally, without using more sophisticated methods, as described for instance for the analysis of maternal and paternal Gpi-1
activation (Gilbert and Solter 1985). Pgk-1 is the only gene coding for a housekeeping enzyme, for which a parental imprinting has so far been described (Krietsch et al. 1982; Krietsch et al. 1986; Surani et al. 1986).

Materials and methods

Substrates and auxiliary enzymes for GPI and PGK staining were obtained from Boehringer Mannheim, FRG. 6-Phosphogluconate dehydrogenase, bovine serum albumin (BSA, No A-7888) and cytochalasins were bought from Sigma, St. Louis, USA. Buffers and inorganic salts were obtained from Merck, Darmstadt, FRG; gelatine and electrophoresis equipment were purchased from Chemetron, Milan, Italy; plastic ware for embryo culture was from Becton, Dickinson and Co., Oxnard, USA. Chemicals for the preparation of Whitten's medium (Hoppe and Pitts 1973) were purchased from BDH Chemicals, Poole, GB. A 0.5% phenol red solution was obtained from Gibco, Paisley, GB.

The mouse strains used in the experiment were bred in our own colonies (Geneva and Munich). The C3H/AA strain carrying the variant allele Pgk-1p (Nielsen and Chapman 1977) was established with mice kindly given to us by J.T. Nielsen and V.M. Chapman, C57BL/6 and BALB/cByJ were originally obtained from the Kleintierfarm in Dödrin AG, Füllinsdorf, Switzerland and from the Zentralinstitut für Versuchstierzucht Hannover, Germany. To obtain female mice heterozygous at the Pgk-1 locus, C57BL/6 females were used. Superovulation was induced by injecting 5 I.U. of pregnant mare serum gonadotropin (both from Laboratorio Nobilis, Boxmeer, Netherlands) 12 hours before mating. The resulting female offspring designated B6C3F1 were used in the experiment. Either spontaneously ovulating or superovulated B6C3F1 females were used. Superovulation was induced by injecting 5 I.U. of pregnant mare serum gonadotropin 60 hours and 5 I.U. of human chorion gonadotropin (both from Laboratorio Nobilis, Boxmeer, Netherlands) 12 hours before mating. The B6C3F1 females were mated with C3H/AA or BALB/cByJ males. Females showing a vaginal plug were killed by cervical dislocation on day 2 of gestation, the day of the vaginal plug being designated as day 1. Two cell embryos were flushed from the oviducts in PB1 (Whittingham and Wales 1969) and cultured in modified Whitten's medium containing 0.5 mg/ml bovine serum albumin, 1.0 mg/ml saponin, and 2 mM dithioerytritol in PB1. The capillaries were then frozen at -22°C for at least 20 min, and after thawing, the contents were blown into black walled cuvettes (2 × 10 mm) containing 140 μl of assay mixture.

Karyotype analysis was performed according to standard methods (Dyban 1983).

Results

When two cell embryos were cultured continuously in the presence of CytoB or CytoD, they became increasingly polyplloid without any further cleavage (Fig. 1). Since the nuclei replicate once, the cleavage blocked embryos are binucleate (Fig. 1) (Surani et al. 1980).

Both X chromosomes are active during oogenesis and accordingly both Pgk-1 loci are expressed (Epstein 1969). In oocytes and zygotes derived from females heterozygous at the Pgk-1 locus this results in a two banded PGK-1 allozyme pattern, although the mature oocyte carries only one Pgk-1 allele. It was shown (Krietsch et al. 1982; Krietsch et al. 1986), that in vivo the relative activities of PGK-1A and PGK-1B remain constant in oocytes and in cleavage stages up to day 4 of gestation, although the absolute PGK activity decreases during this period. Beginning at day 4 and concomitant with an increase in absolute PGK activity, a distinct change in the PGK-1A to PGK-1B ratio takes place, due to activation of a Pgk-1 locus. Since the Pgk-1 allele on the paternally inherited X chromosome is first activated on day 6, this change is due to activation of the maternally inherited Pgk-1 allele. As seen in Fig. 2, activation of maternal Pgk-1 occurs normally in cultured day 4 and 5 blastocysts. In cleavage blocked day 4 and 5 two cell embryo however, there is no indication of maternal Pgk-1 activation, as the activity ratio PGK-1A to PGK-1B remains stable (Fig. 2).