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The DDK inbred strain as a model for the study of interactions between parental genomes and egg cytoplasm in mouse preimplantation development

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Summary

The DDK strain of mice has unusual genetic properties. When females of this strain are crossed to males of other strains, they generally exhibit a very low fertility, whereas reciprocal crosses are fully fertile as are the intrastrain crosses. The observed low fertility results from early embryonic lethality, the F_1 embryos dying around the late morula–early blastocyst stage. Nuclear transplantation experiments between hybrid eggs of BALB/c and DDK strains has shown that failure of F_1(DDK♀×BALB/c♂) embryos to develop is not due to the combination *per se* of maternal (DDK) and paternal (BALB/c) genomes but rather to an incompatibility between paternal (BALB/c) genomic contribution and DDK cytoplasm. This incompatibility does not occur between a female BALB/c pronucleus and the DDK cytoplasm, suggesting the involvement of a differential imprinting of parental genomes. Introduction of cytoplasts isolated from DDK 1- to 8-cell embryos into BALB/c♀×BALB/c♂ or BALB/c♀×DDK♂ embryos of the corresponding developmental stage demonstrate that the cytoplasm of DDK embryos prevents the formation of normal blastocysts through a specific interaction with the paternal component of the BALB/c diploid nucleus. Genetic and molecular studies are underway to try and isolate the gene(s) responsible for the failure of (DDK♀×BALB/c♂)F_1 embryos. These experiments should help in our understanding of nucleocytoplasmic interactions and the respective roles of parental genomes in early embryonic development.

Key words: DDK inbred mouse strain, preimplantation development, nuclear transfer, genomic imprinting, nucleocytoplasmic interactions.

Introduction

The development of the preimplantation mouse embryo can be described in apparently simple terms: from fertilization to the third cleavage, blastomeres are generated by successive symmetric divisions resulting in the 8-cell stage in which all the blastomeres seem to retain the same developmental potentialities (Kelly, 1977). At this stage, the first morphological transition, called compaction, takes place (Ducibella and Anderson, 1975; Ducibella *et al.* 1975), profoundly modifying the relationships between blastomeres (Reviewed in Johnson and Maro, 1986; Fleming and Johnson, 1988). Changes in surface properties occur in which the Ca^{2+}-dependent cell–cell adhesion molecule uvomorulin plays a central role (Kemler *et al.* 1977; Hyafil *et al.* 1981; reviewed in Damsky *et al.* 1983; Johnson *et al.* 1988; Takeichi, 1988 and Kemler *et al.* 1988), and gap and tight junctions communication between blastomeres are established (Ducibella and Anderson, 1975; Ducibella *et al.* 1975; Lo and Gilula, 1979; Magnuson *et al.* 1977; Goodall and Johnson, 1984; Goodall, 1986; Fleming *et al.* 1989). At the same time and probably not independently, blastomeres acquire a polarized state resulting in asymmetry which appears crucial for the first differentiation into the two cell lineages present in the blastocyst (Johnson *et al.* 1986; reviewed in Fleming and Johnson, 1988), namely the trophectoderm, which will form the definitive trophoblast of the placenta, and the inner cell mass (ICM), which will generate the other extraembryonic tissues and the embryo proper. Although this phenomenological description appears simple, it covers a complex sequence of molecular and cellular events, which must be tightly controlled both in time and space for a normal blastocyst to be formed.

Several approaches have been used to tackle the problem of cell diversification in the mouse preimplantation embryo. These include (1) an examination of cell potency and cell fate of the blastomeres (reviewed in Pedersen, 1986); (2) a cell biological approach, in which the various cellular components and their evolution are studied in parallel with the development of the embryo (reviewed in Fleming and Johnson, 1988); (3) a biochemical approach, which tends to define markers of

the different preimplantation stages and more specifically of the trophectoderm or ICM (reviewed in Schultz, 1986) and (4) finally, and more recently, a molecular approach has been initiated to try and define the mechanisms that are at work in the early embryo to control DNA replication, as well as transcription and translation, of either the maternal or zygotic messages (Goldman *et al.* 1988; Vassalli *et al.* 1989; Dooley *et al.* 1989; Martinez-Salas *et al.* 1989). Most of these studies have used normal embryos and various ways of interfering with their development. However, there is an alternative possibility, which is to analyse different mutations that perturb in one way or another normal preimplantation development. Several of these have been phenotypically described and studied to various extents (reviewed in Magnuson, 1986).

Here we describe one such mutation, carried by the DDK inbred strain. We show how its phenotypic properties, originally described by Wakasugi *et al.* (1967) might be useful in deciphering some aspects of the mechanisms that underlie preimplantation mouse development. In particular, we anticipate that the study of DDK should prove useful in defining the respective roles of the egg cytoplasm and the paternal and maternal genomes (and their interactions) in governing the development of the cleaving embryo. As we will see, the particular features of DDK embryos give support to the notion that imprinting of parental genomes (Surani *et al.* 1984; McGrath and Solter, 1984; reviewed in Solter, 1988 and Surani *et al.* 1988) might already be of functional importance at these early stages of development.

DDK inbred mouse strain as a model of nucleocytoplasmic interactions

(1) The unusual properties of the DDK inbred strain

Wakasugi was the first to describe the unusual properties of a particular inbred strain of mice, DDK. When the females of this strain were crossed with males of other laboratory strains (from now on, genetic constitution of the embryos will be indicated with the female parent coming first followed by the male parent) the fertility, though variable, was generally low. In reciprocal crosses, however, (alien×DDK), the fertility was normal (as is indeed the case in the intracross, DDK×DDK) (Wakasugi *et al.* 1967). It was subsequently shown that low fertility of the (DDK×alien) cross was due to an inherent defect of F_1 embryos, which died around the blastocyst stage, and not to some deleterious effect of the DDK uterine environment towards F_1 embryos, therefore suggesting that an incompatibility existed between the DDK oocyte and the foreign male genetic material (Wakasugi, 1972). Genetic analysis, using various types of crosses between DDK and three other inbred strains (KK, NC and C57BL/6) led Wakasugi (1974) to propose a model that explained the death of (DDK×alien)F_1 embryos. He suggested an incompatibility between a cytoplasmic product made by a DDK gene and the corresponding gene, or gene product, carried by the alien male genome, this in turn leading to the death of the F_1 embryo, probably due to the failure of normal trophoblast differentiation. Finally it should be noted that Buehr *et al.* (1987) have shown that in (DDK×C3H)F_1 embryos (a lethal combination), gap junctional communication is impaired, as compared to embryos of either DDK or other strains of mice. They also showed that aggregation chimeras between normal and the F_1 lethal combination embryos did not permit rescue of the latter.

(2) Nuclear transfers: Nucleocytoplasmic interactions

The advent of an efficient nuclear transfer technique (McGrath and Solter, 1983) made it possible to investigate more directly the respective roles of male and female pronuclei and the egg cytoplasm in the lethality of (DDK×alien)F_1 eggs. The viability of the different reconstituted embryos following nuclear transfer could be easily monitored by culturing them *in vitro* in standard culture medium (Renard and Babinet, 1986). Table 1 shows the results for unmanipulated eggs: (DDK×DDK) and (BALB/c×DDK)F_1 eggs develop normally (91.7% and 88.8% of eggs develop to blastocysts, respectively), whereas the great majority of (DDK×BALB/c)F_1 eggs become grossly abnormal around the 16- to 32- cell–morula stage and die before reaching the expanded blastocyst stage (the death of these F_1 embryos is referred to hereafter as the 'DDK syndrome').

Three main conclusions (Fig. 1) could be reached from different experiments in which various combinations of pronuclei (either male or female from DDK or BALB/c eggs) and egg cytoplasm (DDK or BALB/c) were monitored for their ability to develop into a blastocyst (Renard and Babinet, 1986 and unpublished results).

(1) The failure of (DDK×BALB/c)F_1 egg development is due to an incompatibility between DDK cytoplasm and BALB/c male pronucleus. This was shown following transfer of both pronuclei from a (DDK×BALB/c)F_1 egg into either an enucleated (DDK×DDK) egg or an enucleated (BALB/c×BALB/c) egg (Fig. 1A). Only the latter were able to develop normally to the blastocyst stage and into living young following transfer to foster mothers while the

Table 1. *Developmental ability of embryos obtained from DDK and BALB/c matings*

Embryo genotype*	Number of compacted morula: Formed at D4 from 1-cell egg†	Number of compacted morula: Developing *in vitro* into blastocysts (%)
DDK-DDK	72/79	66 (91.7)
BALB/c-BALB/c	51/57	44 (86.3)
BALB/c-DDK	116/137	103 (88.8)
DDK-BALB/c	162/196	15 (9.3)‡

*For all the strain combinations, the female genotype is designated first and the male genotype second.

†Embryos were grown *in vivo* from day 2 to day 4 (D4) and subsequently cultured *in vitro*.

‡Significantly different from other groups: $P<0.001$.

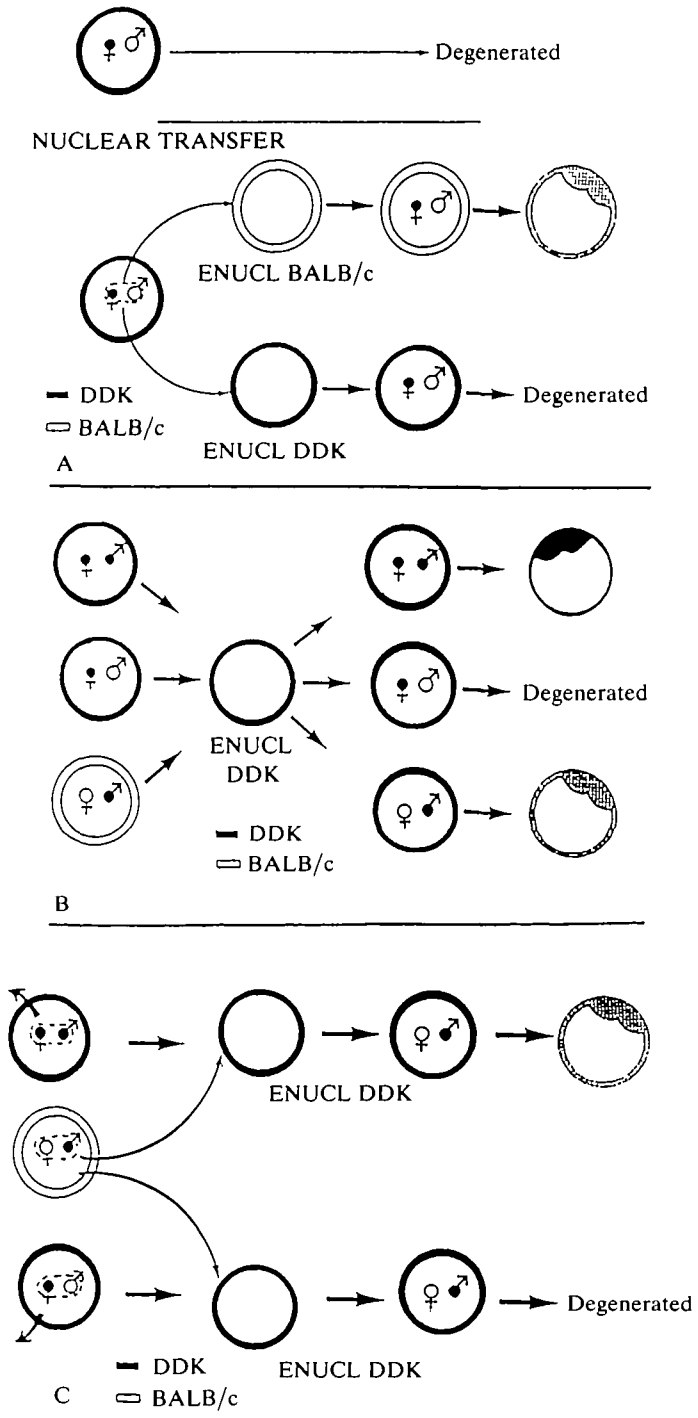

Fig. 1. Effect of different associations of pronuclei and cytoplasm of DDK and BALB/c eggs on the ability of the reconstituted embryos to develop to the blastocyst stage (see text for explanations).

former degenerated around the morula–blastocyst stage. A similar conclusion was reached independently by Mann (1986).

(2) The incompatibility is not manifested between a female alien pronucleus and DDK cytoplasm. This was shown by transferring both pronuclei from a (BALB/c×DDK)F_1 egg into enucleated (DDK×DDK) eggs. These eggs developed normally. Thus, the lethal (DDK×BALB/c)F_1 condition is specifically expressed through an interaction between paternal genomic contribution and DDK cytoplasm (Fig. 1B).

(3) The cytoplasm of (DDK×BALB/c)F_1 eggs is modified at the pronuclear stage, in such a way that it becomes less efficient in its ability to interact with male and female DDK pronuclei to promote normal development. This was demonstrated with enucleated (DDK×BALB/c)F_1 eggs in which female BALB/c and male DDK pronuclei were transferred; these eggs developed poorly compared to control enucleated (DDK×DDK) eggs receiving the same combination of pronuclei (Fig. 1C). This suggests that an aberrant interaction between a cytoplasmic product and a male component has taken place following fertilization before the first cleavage division and this is detrimental for development even when the zygote is reconstructed with otherwise compatible pronuclei.

(3) Influence of zygote cytoplasm on paternal genome

The preceding experiments suggest an influence of the paternal genome on egg cytoplasm; however, experimental evidence has suggested a reciprocal and specific effect of the egg cytoplasm on the paternal genomic counterpart during preimplantation development (reviewed in Renard *et al.* 1988). For example, reducing the volume of egg cytoplasm increases the ability of androgenotes to develop to the morula stage (McGrath and Solter, 1986; Howlett *et al.* 1987) and androgenotes tend to compact earlier than parthenotes (Solter, 1988).

It was therefore interesting to see if the DDK cytoplasm can interfere with the activity of the alien (BALB/c) male genome. To address this question, transfer of DDK×DDK cytoplasm was performed into either BALB/c×BALB/c or BALB/c×DDK zygotes at the 2- to 4- and 8-cell stage. In these experiments the only difference between the two kinds of recipient embryos was the origin of the paternal genome. The way of introducing cytoplasm into embryos is illustrated for the 2-cell stage in Fig. 2. Cytoplasts were prepared by gently sucking cytoplasm from a blastomere of a given preimplantation stage. The cytoplast was then inserted under the zona pellucida of the recipient egg (1- and 2-cell stages) or paired with individual blastomeres (4- and 8-cell stages) isolated following exposure of zona-free embryos to calcium-free Whitten's medium (Pratt, 1987). Fusion was then induced by an electric pulse (Barra and Renard, 1988) and development of the treated embryos was monitored. To circumvent the fact that BALB/c embryos are subject to a 2-cell block (Whittingham and Biggers, 1967), experimental 1- and 2-cell embryos had to be reimplanted into a foster mother for 48 h, after which they were flushed and their subsequent development followed *in vitro*. Such a transient *in vivo* culture was not necessary for experimental embryos treated at the 4- and 8-cell stage; these were reconstituted by phytohaemagglutinin-induced aggregation of 4 to 6 blastomeres (Pratt, 1987) and put directly in culture.

Results (see Table 2) show that the effect of DDK

cytoplast on the development of fertilized BALB/c embryos depends strongly on the nature of the fertilizing sperm. Thus, when DDK cytoplast is introduced into embryos developed from a BALB/c ovum fertilized with DDK sperm, development *in vitro* to the blastocyst stage is normal with more than 70% of the morula forming blastocysts. In contrast, in embryos developed from a BALB/c ovum fertilized with BALB/c sperm, fusion with DDK cytoplast considerably reduced their ability to form a blastocyst. This effect could be obtained at least up to the 8-cell stage. Thus, DDK cytoplasts formed from DDK embryos are able to prevent blastocyst formation of BALB/c embryos *via* a specific interaction with the paternal component of their genome.

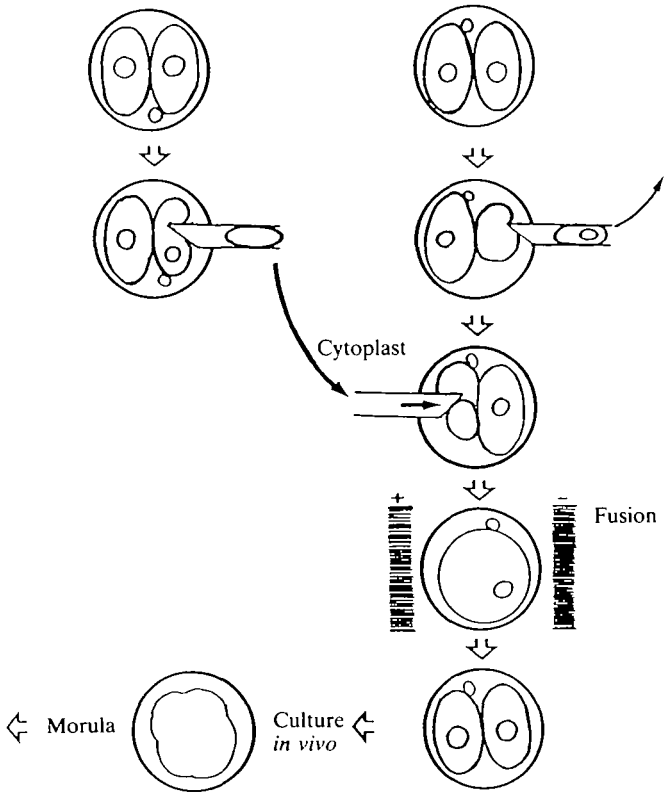

Fig. 2. Introduction of cytoplasm into embryos at the 2-cell stage. A vesicle of cytoplasm (cytoplast) from a donor embryo (left) was gently sucked into a fine pipette according to the original method of McGrath and Solter (1983) and inserted under the zona pellucida of a recipient embryo (right), one blastomere of which had previously been enucleated. Cytoplast/blastomere fusion mediated by electrofusion (Barra and Renard, 1988) resulted in the formation of a single cell, which was first cultured *in vivo* 48 h up to the morula stage then placed *in vitro* to observe subsequent development.

(4) Pattern of protein synthesis in the DDK preimplantation embryo

The results of the reciprocal crosses between DDK and alien strains and the results of the nuclear and cytoplasm transfer experiments suggested that a cytoplasmic product already present in DDK fertilized eggs is involved in the failure of (DDK×alien)F_1 embryos to develop normally. It could be tentatively anticipated that such a product would be either absent or different in eggs from alien strains. The protein synthesis pattern of DDK 1-cell eggs, was therefore examined using two-dimensional gel electrophoresis and compared to that exhibited by BALB/c eggs. A particular polypeptide appeared to be actively synthesized by DDK and to a much lesser extent by BALB/c eggs (see Fig. 3). Although genetic studies (see below) seem to exclude its direct implication in the failure of F_1 embryos to develop, it appears that this polypeptide, called D14, is under strict developmental control and therefore might be of interest in the study of the regulatory mechanisms during very early development (Richoux *et al.* 1991).

D14, as analysed in DDK embryo has an apparent relative molecular mass of about 36.5×10^3 with a pI of approx. 8. Its synthesis, which is one of the most active in the 1-cell DDK embryo, begins within a few hours following germinal vesicle breakdown, encompasses the 1-cell stage and the first 5 h after the first cleavage division; it then decreases rapidly to finally disappear at 7 h during the 2 cell-stage. However, pulse-chase

Table 2. *Development of BALB/c-BALB/c or BALB/c-DDK embryos following introduction of DDK cytoplasm at different stages of development*

Stage of development*	Embryo genotype†	Number of embryos: Reconstituted	Number of embryos: Developing into morula	Number (%) of blastocysts formed from morula
1-cell	BALB/c-BALB/c	38	19	7 (36.8)
	BALB/c-DDK	23	19	14 (73.7)
2-cell	BALB/c-BALB/c	33	11	0
	BALB/c-DDK	12	4	4 (100)
4-cell	BALB/c-BALB/c	15	10	1 (10)
	BALB/c-DDK	14	14	11 (78.5)
8-cell	BALB/c-BALB/c	7	7	1 (14.3)
	BALB/c-DDK	ND	–	–

* At introduction of cytoplasm from DDK-DDK embryos of the same developmental stage.
† For all the strain combinations the female genotype is designated first and the male genotype second.
ND, not done.

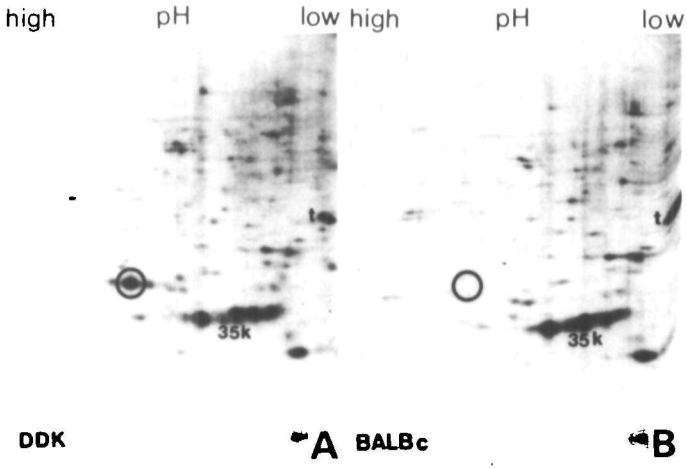

Fig. 3. Comparison of fluorograms showing the patterns of protein synthesis in DDK (A) and BALB/c (B) 1-cell stage embryos: a major synthesis (circle) referred to as D14 in text is found in DDK but not in BALB/c 1-cell stage embryos. Batches of 1-cell stage embryos (n=15) cultured for 3 h (24 to 27 h post HCG) in Whitten's medium containing 1.5 mCi ml^{-1} [^{35}S]methionine were subsequently analysed by two-dimensional electrophoresis (NEPHGE 932 Vh) (Exposure time 30 h). A few known proteins are identified; t: tubulins; 35k: 35×10^3 M_r complex.

experiments indicated that D14 is fairly stable following its synthesis as it can still be found up to the 8-cell stage at least. D14 was not detected in later preimplantation embryonic stages, nor could it be detected in any of the 8 adult tissues examined (liver, kidney, spleen, gut, brain, heart, testis and ovary). Finally, it should be noted that a high level of D14 synthesis is a particular feature of DDK embryos, as none of 7 other laboratory inbred strains of mice exhibited comparable synthesis of the protein. Intriguingly, the only strains found to synthesize a high level of a polypeptide with similar electrophoretic properties to D14, were of feral origin (Richoux *et al.* 1991).

(5) Genetic studies

The results of a first series of experiments confirmed that genomic and not cytoplasmic (mitochondrial) inheritance (Yonekawa *et al.* 1982; Ferris *et al.* 1982; Gyllensten *et al.* 1985) is responsible for the DDK defect. (BALB/c×DDK)F_1 females were backcrossed to DDK males (backcross 1). Conversely (DDK×BALB/c)F_1 females were backcrossed to BALB/c males (backcross 2). After 20 generations, backcross 1 gave rise to progenies having a DDK nuclear constitution with mitochondrial DNA of BALB/c origin, and backcross 2 a BALB/c nuclear constitution with mitochondrial DNA from DDK. Females from backcross 1 when crossed to BALB/c males were almost sterile, giving birth only occasionally to progeny but appeared fully fertile when crossed with DDK males, thus behaving as the females of the DDK strain. Females from backcross 2, however, appeared normally fertile whether crossed to BALB/c or to DDK males, and therefore behaved as normal BALB/c females. These results show that the DDK syndrome is not dependent on cytoplasmic (mitochondrial) inheritance but rather is purely a genomic (nuclear) property of the DDK strain.

In another experiment, we derived 23 recombinant inbred (RI) strains from the parental BALB/c and DDK strains. 18 of these RI strains were of the DDK type and five of the BALB/c type, as revealed by an *in vitro* test (rate of blastocyst formation among (RI×BALB/c)F_1 embryos). Most of the lines were also monitored by an *in vivo* test (fertility of RI females crossed to BALB/c males). Although several selections and epistatic interactions may have been operating during the inbreeding of these RI strains such a result is consistent with the idea that a very small number of genes are responsible for the DDK syndrome.

These RI strains, which should prove useful for the mapping of the DDK alleles involved in DDK syndrome, also allowed us to check for a possible relationship between D14 synthesis and the DDK phenotype (Richoux *et al.* 1991). For each of the 23 RI strains, the level of D14 synthesis was monitored, using two-dimensional gel electrophoresis of proteins extracted from RI embryos cultured in the presence of [^{35}S]methionine. Seven RI strains showed a level of D14 synthesis comparable to that observed in DDK and of these six exhibited the lethal syndrome. In the 16 remaining strains no synthesis of D14 was observed but of these 12 manifested the lethal syndrome. These results show that a high level of D14 synthesis does not correlate with, and therefore is not directly involved in, the DDK syndrome.

Concluding remarks

There are several mutations or genetic abnormalities known to affect the development of preimplantation mouse embryos (Magnuson, 1986). The DDK strain whose properties were originally described by Wakasugi *et al.* (1967), represents a unique situation in the sense that the expression of the abnormal phenotype is dependent on particular combinations of genotypes and on the direction of the crosses. Using nuclear and cytoplasmic transfer experiments, we have been able to characterize more precisely the lethal interactions between the DDK cytoplasm and the alien male genome. It appears from these experiments that the lethality of (DDK×alien)F_1 embryos results from an interaction between a cytoplasmic component already found in the fertilized DDK egg and encoded in the nuclear genome and the alien male genome or male genome products. Experiments in progress indicate that the cytoplasmic component is of maternal origin (Renard *et al.* in preparation). Thus, the DDK lethal syndrome could be viewed as both a maternal and/or a paternal effect. A differential expression between paternal and maternal alien genomes revealed by the cytoplasm of DDK embryos does in fact occur because the lethal effect which is manifested in the presence of alien paternal genome is not observed with an alien maternal one, a situation that we created by nuclear or

cytoplasmic transfer (see Fig. 1B and Table 2). This result strongly suggests that a differential imprinting of parental genomes is involved and is a prerequisite for normal development of blastocysts. This differential imprinting and its role could be disclosed by the particular genetic constitution of DDK mice. The lethal effect may be initiated as early as the 1 cell stage (see Table 2); however, it is observed as a phenotypic effect only around the late morula to blastocyst stage, when the embryo degenerates. Since transfer of DDK cytoplasm into (BALB/c×BALB/c) embryos could impair their development even when performed at the late 8-cell stage, the initiation of the lethal effect may be later. We are now trying to tackle its analysis at the molecular level. This is a particularly difficult challenge due to the very limited amount of material available; however, it is worth attempting because it should give an insight into some of the pivotal nucleocytoplasmic interactions and the differential activities of the parental genomes in preimplantation mouse embryos.

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