Development 101, 915–923 (1987)
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Nuclear cytoplasmic interactions following nuclear transplantation in mouse embryos

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Summary

We have investigated the development of reconstituted embryos in which enucleated 1- or 2-cell embryos received various advanced nuclei. Enucleated 1-cells developed to the blastocyst stage only when an early 2-cell donor nucleus was transferred but very rarely if the donor nucleus was derived from a late 2-cell, early 4-cell or mid 8-cell embryo. Although an 8-cell nucleus could only support development of an enucleated zygote to the 2-cell stage, it did express the hsp $68/70 \times 10^3 M_r$ proteins that are characteristic of the first embryonic gene activity. These polypeptides were absent in enucleated zygotes that did not receive a donor nucleus. Moreover, an 8-cell nucleus transferred to an enucleated late 2-cell blastomere could also support preimplantation development provided that the nuclear: cytoplasmic ratio was maintained as in intact 2-cell blastomeres. 8-cell nuclei transferred to zygotes that retained at least one pronucleus were able to support development to the blastocyst stage provided that the pronucleus was both fully transcriptionally active and present beyond the late 1-cell stage. This study suggests an active and continued helper role of the resident pronucleus for the participation by an 8-cell nucleus in reconstituted eggs.

Key words: mouse embryo, nuclear transfers, protein synthesis, cytoplasmic interaction, transplantation.

Introduction

Nuclear transplantation studies in the mouse have established that transcriptionally active embryonic nuclei transplanted back into enucleated zygotes are incapable of directing preimplantation development (McGrath & Solter, 1984*a*); development to term also requires the presence of both parental genomes (Barton, Surani & Norris, 1984; McGrath & Solter, 1984*b*). Whereas transfer of both pronuclei into enucleated eggs can result in development to the blastocyst stage (McGrath & Solter, 1983; Surani, Barton & Norris, 1984) only a few (19%) enucleated eggs receiving 2-cell nuclei reached the blastocyst stage and none when receiving nuclei from 4- or 8-cell embryos or inner cell mass cells (McGrath & Solter, 1984*b*). However, advanced nuclei that are transferred into eggs that retain one or both pronuclei can produce triploid or tetraploid blastocysts (Modlinski, 1978). If advanced haploid nuclei are transferred into eggs retaining a single pronucleus, such reconstituted embryos can reach term provided that the two nuclei have opposite parental origins (Surani, Barton & Norris, 1986). Therefore, on its own a nucleus beyond the 2-cell stage cannot be reprogrammed to direct cleavage, but in combination with a resident pronucleus normal preimplantation development is achieved.

Pronuclei are relatively inactive transcriptionally and hence the qualitative pattern of protein synthesis essentially demonstrates utilization of stored maternal mRNA throughout the first cell cycle (reviewed Johnson, McConnell & Van Blerkom, 1984). The first detectable embryonic transcription occurs during the second cell cycle (Pikó & Clegg, 1982; Clegg & Pikó, 1983). Transcriptional activation occurs in two stages: immediately after first division a short burst of transcription during G_1 results in the synthesis of the hsp $68/70 \times 10^3 M_r$ (68/70K) complex a few hours later (Flach *et al.* 1982; Bensaude, Babinet, Morange & Jacob, 1983). The second and major transcriptional activation occurs during G_2 of

the second cell cycle and results in a dramatic switch in the translation pattern concomitant with the destruction of maternal mRNA (Bachvarova & DeLeon, 1980; Flach *et al.* 1982; Bolton, Oades & Johnson, 1984; Howlett & Bolton, 1985). Therefore, taken together with the nuclear transfer data it is suggested that once a nucleus has become active it is incompatible with the preactivation ('inactive') 1-cell cytoplasm except in combination with a resident pronucleus.

We have carried out a systematic study to assess the factors which may influence the fate of reconstituted eggs and embryos following nuclear transfer. Donor nuclei from precisely timed embryos before and after activation of the embryonic genome were transferred to enucleated 1- or 2-cell embryos. The influence of nuclear:cytoplasmic ratio on development and on protein synthesis following nuclear transfer was examined. These studies provide further insight into nuclear:cytoplasmic interactions in early mouse development.

Materials and methods

Collection of embryos

Female (C57BL/6J×CBA/Ca)F_1 mice (AFRC colony from Bantin & Kingman stock) were superovulated by injection of 7·5 i.u. pregnant mare's serum (PMS, Intervet) followed 48 h later by an injection of 7·5 i.u. of human chorionic gonadotrophin (hCG, Intervet), and mated with CFLP males (AFRC colony from Bantin & Kingman stock).

Fertilized eggs were recovered from plug-positive females at about 17 h post-hCG. Cumulus cells were removed by incubation in 300 i.u ml^{-1} hyaluronidase (Sigma) in phosphate-buffered medium (PBI; Whittingham & Wales, 1969) plus 4 mg ml^{-1} bovine serum albumin (BSA; Sigma) for 1–2 min at 37°C. Eggs were then washed through six drops of PBI+BSA and cultured under paraffin oil (BDH) in embryo culture medium T6 (modified as below from Quinn, Warnes, Kerin & Kirby, 1984; D. Whittingham – personal communication) plus 4 mg ml^{-1} BSA at 37°C in humidified 5% CO_2 in air. T6 medium: 80·77 mM-NaCl; 25·00 mM-$NaHCO_3$; 5·55 mM-glucose; 1·77 mM-$CaCl_2.2H_2O$; 1·48 mM-KCl; 0·49 mM-$MgCl_2.6H_2O$; 0·39 mM-$NaH_2PO_4.2H_2O$; 0·27 mM-sodium pyruvate; 0·045 mM-sodium lactate; 0·06 mg ml^{-1} penicillin; 0·05 mg ml^{-1} streptomycin.

Haploid activated recipient eggs

Haploid parthenogenetic eggs were obtained by activation of F_1 unfertilized eggs obtained 17 h post-hCG. Activation was carried out using 7% ethanol as previously described (Cuthbertson, 1983; Surani *et al.* 1984). After 4–5 h in culture in T6+BSA eggs containing a single pronucleus and a second polar body were selected for manipulation.

Labelling of embryos

Control recipient and donor embryos and manipulated, reconstituted embryos were labelled for 2 h as 1-, 2-, 4- or 8-cell embryos in T6+BSA containing [^{35}S]methionine (1100 Ci mmol^{-1}, Amersham) at a concentration of 200 μCi ml^{-1}, washed and collected in 10 μl of sample buffer (Laemmli, 1970) and analysed on 10% polyacrylamide gels exactly as described previously (Howlett & Bolton, 1985).

Nuclear transfers

Embryos were placed into PBI+BSA containing 1 μg ml^{-1} cytochalasin D (Sigma) and 0·05 μg ml^{-1} nocodazole (Sigma) each diluted from stocks frozen in dimethyl sulphoxide. After 15–30 min culture at 37°C, eggs and embryos were placed in hanging drops of the same medium for micromanipulation using a Leitz micromanipulator. Donor nuclei were introduced into recipient eggs by fusion of karyoplasts using inactivated Sendai virus as described previously (Barton *et al.* 1984).

Inhibition of DNA or RNA synthesis

Transcriptional inhibition was achieved in two ways: (a) by incubating 1-cells in the RNA polymerase II inhibitor α-amanitin (Boehringer Mannheim) at 11 μg ml^{-1} in T6+BSA (Levey & Brinster, 1978); (b) by irradiating 1-cells in a 5 μl drop of T6+BSA with a mixture of long and short wave (254/356 nm) u.v. light from a Mineralight lamp for 30 s from a distance of 25 cm (method modified from Gurdon, 1960*a*). α-Amanitin treatment allows development to 2-cells (Howlett & Bolton, 1985) whereas u.v.-irradiation blocks eggs as single cells with intact pronuclear membranes (our unpublished observations). The DNA cross-linking agent mitomycin C (Sigma) was used at 2 μg ml^{-1} in T6+BSA to block DNA replication but not transcription (Cozzarelli, 1977). However, blocking the first S phase (with aphidicolin) has been shown to allow the production of hsp 68/70K transcripts but not the later major transcriptional activation (Howlett, 1986).

Results

Early 2-cell but not later nuclei can replace pronuclei

Haploid, parthenogenetic F_1 eggs were used as recipients. Donor nuclei from early or late 2-cells, early 4-cells or mid 8-cells were transferred as karyoplasts to enucleated recipient eggs and development *in vitro* of these hybrid embryos was monitored. Fig. 1 demonstrates that early 2-cell (1–3 h postdivision) nuclei can support development to produce expanded blastocysts *in vitro* in the majority of cases (71%). However, this potential is lost rapidly since only 3% expanded blastocysts were achieved on transfer of late 2-cell (12–18 h postdivision) nuclei. In order to rule out the possibility that nuclei early in the cell cycle are intrinsically better at supporting development, early 4-cell (1–3 h postdivision) nuclei were also transferred, but these developed very poorly and only 11% divided more than once. As has been

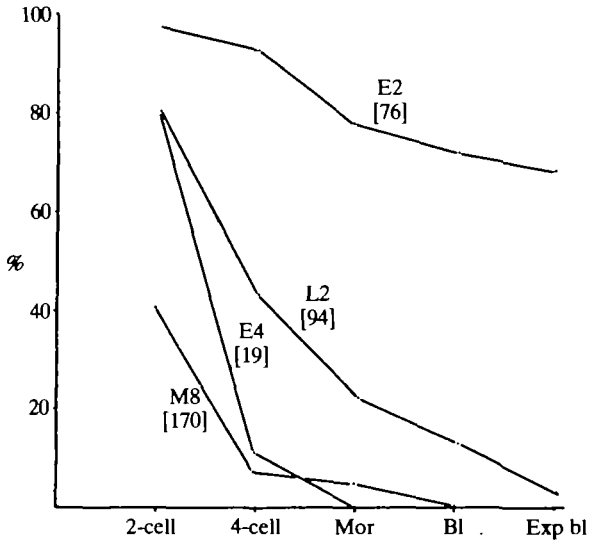

Fig. 1. Development of enucleated zygotes receiving advanced nuclei. Graph showing the proportions of reconstituted embryos reaching the 2-, 4-, morula, blastocyst or expanded blastocyst stages *in vitro*. All embryos were derived from enucleated haploid activated F_1 recipient eggs that received a nucleus from an early (1–3 h postdivision) 2-cell (E2), a late (12–18 h postdivision) 2-cell (L2), an early (1–3 h postdivision) 4-cell (E4) or a mid 8-cell (M8) embryo. The numbers of reconstituted embryos scored for each group is given in brackets.

demonstrated previously, 8-cell nuclei cannot replace pronuclei (McGrath & Solter, 1984*a*); here we found that, on average, 41 % of such hybrids divided once but further development was very poor, since only a single small blastocyst-like structure was achieved from a total of 170 reconstituted embryos.

These results indicate that events occurring during the 2-cell stage prevent nuclei from interacting successfully with 1-cell cytoplasm to direct early development.

Protein synthesis in an egg after transfer of an 8-cell nucleus

We were interested to determine whether there was any alteration to the usual maternally directed pattern of protein synthesis in a zygote after transfer of an advanced nucleus. In particular, we wanted to know whether it was possible to detect any translation products that would indicate that there had been continued transcription from the donor 8-cell nucleus after transfer. Thus, the pattern of protein synthesis in reconstituted embryos was analysed by labelling for 2 h with [^{35}S]methionine 3 h (as 1-cells), 16 h (as blocked 1-cells or early 2-cells) and 30 h (as late 2-cells) after transfer. These patterns were compared with control 1-cells, early and late 2-cells and 8-cells labelled under the same conditions (Fig. 2). The pattern of protein synthesis in the zygote soon after transfer of a donor nucleus (lane B) was identical to that of an unoperated control 1-cell (lane A), that is there was no alteration to the pattern or rate of protein synthesis utilizing stored maternal mRNA nor could any translation products characteristic of 8-cell embryos (lane C) be detected. Although by no means conclusive, we feel that it is possible that if the 8-cell nucleus had continued to produce mRNA at its usual rate then characteristic '8-cell proteins' may have been detected, certainly 8-cell proteins can be seen when a mixture consisting of one labelled 8-cell embryo with seven 1-cell embryos is run on a gel (S.K.H., unpublished observations). At the early 2-cell stage (lane F), and even when blocked as 1-cells (lane E), the reconstituted embryos synthesized the hsp 68/70K proteins as did control early 2-cells (lane D). The hsp 68/70K proteins are encoded by embryonic mRNA which must have been transcribed from the donor nucleus since they were not observed in enucleated eggs that had not received a donor nucleus (lane G). It should be noted that in such enucleated eggs the level of translation of the maternally encoded lower 35K protein (Howlett & Bolton, 1985) is much greater than usually found by this time after fertilization (compare lanes G and H), we have no explanation for this observation. In the late 2-cell reconstituted embryo (lane H), the pattern of protein synthesis remains essentially similar to that of the early 2-cell and there were few, if any, translation products indicative of further transcriptional activation (compare lanes H and I). Presumably on the rare occasions that cleavage beyond the 2-cell stage of such hybrid embryos is achieved the pattern of translation does indeed change to parallel that in control 4-, 8-cells etc.

This analysis revealed that the 8-cell nucleus had to a certain extent been reprogrammed after transfer, with an apparent switch-off of transcription followed by activation to produce the hsp 68/70K proteins at the normal time. However, this limited reprogramming by the recipient cytoplasm is not sufficient to allow much more than one cleavage division.

Influence of the activity and the amount of cytoplasm

Although it appears that only a nucleus that has not itself undergone transcriptional activation can be returned to the relatively inactive 1-cell cytoplasm and support development, recent results have shown that 4- and 8-cell nuclei can support preimplantation development (Robl, Gilligan, Critser & First, 1986) and even produce live young (Tsunoda *et al.* 1987) when transferred to an enucleated 2-cell blastomere. We repeated these experiments aiming to replace each 2-cell nucleus with an 8-cell nucleus and followed development *in vitro* (Table 1). Indeed, it was

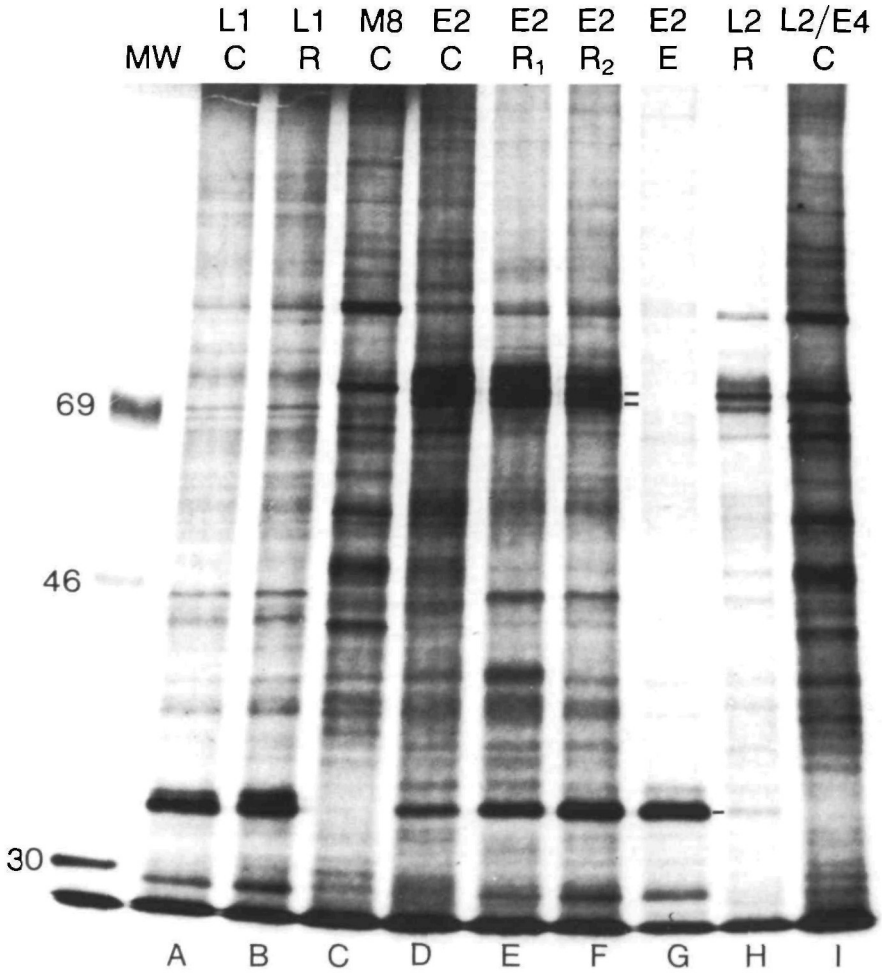

Fig. 2. The pattern of protein synthesis following transfer of an 8-cell nucleus to an enucleated zygote. One-dimensional gel showing the pattern of [^{35}S]methionine-labelled proteins synthesized during 2 h labelling periods in control unmanipulated embryos, reconstituted embryos at various times after nuclear transfer and enucleated eggs not receiving a donor nucleus. Reconstituted embryos were labelled as 1-cells 3 h after transfer (B), as arrested 1-cells (E) or early 2-cells (F) 16 h after transfer or as late 2-cells (H) 30 h after transfer and compared with control unmanipulated 1-cells (A), 8-cells (C; from which donor nuclei were taken), early 2-cell (D), late 2-cell/early 4-cell (I) or enucleated eggs (G) cultured until renucleated embryos were late 2-cells. The hsp 68/70K proteins characteristic of the first embryonic gene activity (Bensaude *et al.* 1983) and the lower band 35K protein that is a characteristic maternally encoded protein (Howlett & Bolton, 1985) are marked. Note the many new proteins characteristic of full embryonic switch-on in the late 2-cell/early 4-cell embryo (I) which continue to be synthesized through to at least the 8-cell stage (C). MW, molecular weight markers.

apparent that an 8-cell nucleus could support preimplantation development when combined with late 2-cell cytoplasm (groups A–C).

During these experiments some of the recipient 2-cell blastomeres fused together before receiving one or two 8-cell nuclei. Whilst development of fused 2-cell cytoplasm receiving two 8-cell nuclei was similar to that of unfused cytoplasm receiving a single 8-cell nucleus (compare group D with B and C), fused cytoplasts receiving only a single 8-cell nucleus developed very poorly (group E). The difference between groups D and E is that the embryos in group E have half the normal nuclear: cytoplasmic (N: C) ratio and of these only 8 % divided more than once. This result suggested that an excess of cytoplasm, i.e. a low N: C ratio was in some way detrimental to development.

Similarly, it has been reported that haploid development, which normally rarely progresses beyond the 2-cell stage (Surani *et al.* 1986), can be improved by reducing the cytoplasmic volume and thereby normalizing the N: C ratio (McGrath & Solter, 1986). We verified this, finding that by reducing the cytoplasmic volume by about one third at the 1-cell stage 5/15 haploid gynogenetic and 2/53 haploid androgenetic blastocysts were produced *in vitro*. No haploid androgenetic blastocysts were achieved in previous studies where the cytoplasmic volume was not reduced (Surani *et al.* 1986). Since increasing the N: C ratio

Table 1. *Development* in vitro *of reconstituted embryos composed of 2-cell cytoplasm and 8-cell nuclei*

Groups		Total	2-cell*	4-cell	Morula	Blastocyst
A	⓪○	2	2	2	2	1 normal
B	⊙⊙	17	17	13	11	5 normal 2 small
C†	⊙○	35	35	28	19	4 small
D	⊙	10	7	7	7	5 disorganized
E	⊙	25	9	1	1	–

* For groups D and E, 2-cell represents division producing two blastomeres which is the second mitotic division for the recipient embryo.

† Category of fusion hybrid produced by Robl *et al.* 1986.

could improve (but not restore) haploid development we wondered whether the development of an 8-cell nucleus transferred to a 1-cell could be improved by reducing the cytoplasmic volume of the recipient. This, however, did not appear to be the case since there was little, if any, improvement when the cytoplasmic volume of the recipient egg was reduced by about one third although the number of embryos dividing once was increased from 41% to 62% (Table 2, compare lines 1 and 2).

Influence of a resident pronucleus

Tetraploid embryos produced by introduction of 8-cell nuclei and even inner cell mass nuclei into non-enucleated, fertilized eggs can give rise to blastocysts (Modlinski, 1978, 1981). Similarly, a haploid 8-cell nucleus introduced into a haploid recipient egg of the opposite parental origin can produce blastocysts and live young (Surani *et al.* 1986). We investigated the nature of the interaction between the resident pronucleus and a transferred 8-cell nucleus that allowed for development.

Diploid 8-cell nuclei were transferred into recipient zygotes that retained either the female (Table 2, line 3) or the male (line 4) pronucleus or cotransferred along with a male pronucleus into an enucleated zygote (line 5) and in all cases blastocysts were produced. Therefore, we reasoned that leaving resident pronuclei with the transferred donor 8-cell nucleus within the recipient for as long as possible before removal of the pronuclei may enable the reconstituted embryos to develop. Thus, 8-cell nuclei were first transferred to intact zygotes from which the resident pronuclei were subsequently removed after 5 h. This allowed a prolonged period of time for the resident and donor nuclei to interact extending up to about 3 h prior to the first cleavage division. However, this manipulation did not improve development beyond the 2-cell stage (line 6).

To investigate if it was necessary for a resident pronucleus to be transcriptionally active in order to allow the transferred 8-cell nucleus to be able to function, the endogenous pronucleus was treated with various agents and its ability to aid development of an 8-cell nucleus was assessed. Hence, two transcriptional inhibitors and a DNA synthesis inhibitor that would block embryonic gene transcription beyond that of the hsp 68/70 genes (see Materials and methods and Howlett, 1986) were tested. U.v. irradiation irreversibly arrests 1-cell embryos (S.K.H. & M.A.S., unpublished observation) and prevents transcription (Gurdon, 1960*a*; Masui & Pedersen, 1975), whilst 11 $\mu g\,ml^{-1}$ α-amanitin for 2 h (or more) blocks transcription irreversibly, allowing first but not second mitosis to occur (Howlett & Bolton, 1985).

Table 2. *Development* in vitro *of reconstituted zygotes receiving an 8-cell nucleus*

Recipient zygote	Donor nucleus	Total	2-cell	4-cell	Morula	Blastocyst
1. Enuc 1-cell	8-cell	170	69	11	9	1
2. Enuc red. 1-cell	8-cell	53	33	2	–	–
3. Hap ♀ 1-cell	8-cell	16	10	6	5	4
4. Hap ♂ 1-cell	8-cell	19	17	14	11	10
5. Enuc 1-cell	8-cell +♂ PN	6	2	–	–	4
6. Late enuc 1-cell	8-cell	75	49*			
7. Irrad hap ♀ 1-cell	8-cell	12	5	–		
8. Enuc 1-cell	8-cell +irrad ♀ PN	10	6	–		
9. αAM hap ♀ 1-cell	8-cell	4	4	–		
10. Enuc 1-cell	8-cell +αAM ♀ PN	3	3	–		
11. Enuc 1-cell	8-cell +MMC ♀ PN	14	3	–		

* 23 of these embryos were transferred to pseudopregnant recipients and examined on day 10 and there were no implants.

Key: Enuc, enucleated; red., reduced; Irrad, irradiated; αAM, α-amanitin; PN, pronucleus; MMC, mitomycin C.

Mitomycin C crosslinks DNA and blocks DNA replication irreversibly but does not apparently affect transcription (Cozzarelli, 1977) and at $2\,\mu g\,ml^{-1}$ blocks first division.

Fertilized eggs (17–19 h post-hCG) were treated in one of three ways: (a) incubated in $11\,\mu g\,ml^{-1}$ α-amanitin for 2 h followed by extensive washing in control medium; (b) irradiated with u.v. light (see Materials and methods) or (c) incubated in $2\,\mu g\,ml^{-1}$ mitomycin C for 2 h followed by extensive washing. In each case, treated eggs were incubated for a further 2–4 h prior to manipulation in order to ensure as far as possible that no residual drug remained in the cytoplasm or pronuclei. Then two different nuclear transfer protocols were followed. Either a treated egg from which the male pronucleus had been removed was used as the recipient for an 8-cell nucleus (Table 2, lines 7 and 9), or the female pronucleus from a treated egg was cotransferred along with an 8-cell nucleus to a fresh untreated, enucleated egg (lines 8, 10 and 11). This second series of transfers was performed in order to eliminate the possibility that the recipient egg cytoplasm retained residual drug that subsequently contaminated the 8-cell nucleus. We have tested that a mitomycin or u.v.-treated pronucleus could be transferred to a non-enucleated, untreated egg without affecting development (S.C.B. and M.A.S., unpublished observations). Hence, although transcription from the treated pronucleus remains blocked, transcription from other nuclei within the same cytoplasm is unaltered. Thus, either an 8-cell nucleus was (a) transferred to a u.v.-irradiated (line 7) or an α-amanitin-treated (line 9) zygote that had had the male pronucleus removed or (b) cotransferred along with a treated female pronucleus that had been u.v.-irradiated (line 8) or treated with α-amanitin (line 10) or mitomycin C (line 11) into an untreated, enucleated recipient zygote. In no instance did we see cleavage beyond the 2-cell stage. Thus, in all the reconstructed embryos in which an 8-cell nucleus coexisted in cytoplasm (that had or had not been previously exposed to inhibitors) with a treated pronucleus, development resembled that of a lone 8-cell nucleus returned to 1-cell cytoplasm. These results suggest that it is necessary for the pronucleus to be fully transcriptionally active and probably to be capable itself of full transcriptional activation in order for the development of reconstituted eggs to continue.

Discussion

The ability of embryonic mouse nuclei to be reprogrammed sufficiently to allow them to support development when transferred to enucleated 1-cells is severely limited (McGrath & Solter, 1984*a*). We demonstrate here that such a change in nuclear potential occurs during the second cell cycle since most early 2-cell, but virtually no late 2-cell and fewer 4- or 8-cell, nuclei can give rise to blastocysts when returned to enucleated zygotes. Transcriptional activation of the embryonic genome occurs during the second cell cycle (Flach *et al.* 1982) and it seems probable that the process of transcriptional activation irreversibly restricts nuclei and so precludes full reprogramming. Nevertheless, a limited degree of reprogramming of an 8-cell nucleus is observed since the translation pattern following transfer to an enucleated recipient egg suggests that transcription is initially switched off. Previous studies have also indicated cytoplasmic control of transcription in the early mouse embryo (Bernstein & Mukherjee, 1972) and in *Xenopus* eggs (Gurdon & Woodland, 1969). Subsequently, transcription is switched on again to produce the hsp 68/70K proteins at the time which is temporally appropriate for the recipient egg. However, there appears to be little if any further transcriptional reactivation. This implies that the presence of the hsp 68/70K proteins is not sufficient, although it may be necessary, for the major transcriptional switch that follows (as has been inferred from other experiments; Howlett, 1986). It is presumed that in the very rare cases where an 8-cell nucleus was able to promote development to the 4- to 8-cell stage there must have been further transcriptional activation.

When an 8-cell nucleus is transplanted into eggs that retain a resident pronucleus, endogenous nuclear activity is presumably sufficient to carry the embryo through to a point beyond the block normally encountered when a lone 8-cell donor nucleus is present. The endogenous pronucleus must apparently be transcriptionally active beyond the time of hsp 68/70K production since treatments with α-amanitin, u.v.-irradiation and mitomycin C all abolish any 'beneficial' effect on the development of reconstituted eggs containing an 8-cell nucleus. Since in this study a distinguishable genetic marker was not present, it is not possible to determine unequivocally whether or not the 8-cell nucleus contributes any transcripts (other than those encoding the hsp 68/70K proteins) until after full activation of the endogenous nucleus. It will be interesting to address this point directly with appropriate markers. The 8-cell nucleus must subsequently become fully transcriptionally active once again since development consistently proceeds beyond that expected for the haploid pronucleus (of either parental origin) alone and even to term if the donor 8-cell was haploid and of the opposite parental origin to that of the pronucleus (Surani *et al.* 1986).

It has been shown that haploid gynogenetic or androgenetic embryos undergo the major transcriptional activation during the 2-cell stage (Surani *et al.* 1986). This suggested (and has been confirmed recently; Petzoldt & Muggleton-Harris, 1987) that the N:C ratio does not dramatically affect the timing of the switch-on. Nevertheless, a decreased N:C ratio is detrimental thereafter, as haploid development rarely exceeds the 2-cell stage. Reducing the cytoplasm to produce a normal or near-normal N:C ratio considerably improves their development. This rules out the argument that expression of recessive lethals is responsible for poor haploid development and is probably the explanation of the better development of immediate cleavage parthenogenotes (Kaufman, 1983) which are haploid but have a normalized N:C ratio. These observations suggest that either insufficient transcripts are produced by a haploid genome for the cytoplasmic volume or that a haploid genome cannot overcome the quantity of any putative repressors present in the egg cytoplasm.

An 8-cell nucleus returned to late 2-cell cytoplasm probably does not have to undergo acute reprogramming in terms of gene activity, indeed, the translation pattern remains remarkably constant from the late 2-cell stage through to the 8- to 16-cell stage (see Fig. 2, lanes D and I; and Levinson, Goodfellow, Vadeboncoeur & McDevitt, 1978). In this context, it is interesting that we have observed premature compaction in embryos derived from an 8-cell nucleus transferred back into either an egg that retained a single pronucleus (Surani *et al.* 1986) or an enucleated late 2-cell (seen during the course of these studies). These observations suggest that under both conditions the 8-cell nucleus has some influence over the recipient cytoplasm.

Nuclear transfer studies in other species have shown that embryonic amphibian nuclei progressively lose their totipotency after the blastula stage, although earlier in endoderm of *Rana* compared with *Xenopus* (King & Briggs, 1955; Gurdon, 1960*b*), with occasional production of tadpoles from differentiated *Xenopus* nuclei (Gurdon & Laskey, 1970). It should be noted that in order to obtain successful development, serial transfers of blastula nuclei back into eggs must be performed, such serial transfers have not yet been performed in the mouse. We are testing the possibility of passaging an 8-cell nucleus through an enucleated egg which is then allowed to divide before transferring a resulting '2-cell' nucleus back to a second recipient egg to evaluate whether this could restore or improve developmental potential. In the sheep, 8-cell blastomeres have been combined with anucleate halves of bisected eggs to produce viable offspring (Willadsen, 1986). However, whereas single 8-cell sheep blastomeres can produce lambs (Willadsen, 1981), neither single 4- nor 8-cell mouse blastomeres can produce mice (Tarkowski & Wroblewska, 1967; Rossant, 1976). Furthermore, activation of the embryonic genome of the sheep occurs at the 8- to 16-cell stage (Crosby, Gandolfi & Moor, 1988) and so 8-cell sheep nuclei are probably equivalent to early 2-cell mouse nuclei. Therefore, there may not be such a fundamental difference in the totipotency of nuclei between these two species as appears at first sight, rather a difference in the timing of activation of the embryonic genome.

It is of considerable interest to know what is lost or gained by activated embryonic mouse nuclei that prevents their being fully reprogrammed when returned to 1-cell cytoplasm. It is possible that even during early mammalian development certain information, perhaps even 'imprinted' information that reflects parental origin, is used sequentially such that an 8-cell nucleus cannot go through early mitoses for a second time. In this respect, it is interesting to note that, while diploid androgenetic embryos develop poorly, they cleave and compact earlier than parthenogenetic embryos whose preimplantation development is similar to that of control, fertilized eggs (our unpublished observations). Development may be accompanied by a series of subtle but significant structural modifications of DNA that can be erased (reprogrammed) only during gametogenesis and not simply by transfer of nuclei to egg cytoplasm. Evidence for a suitable molecular mechanism for the process of imprinting in the germline has recently been obtained (Reik *et al.* 1987; Sapienza, Peterson, Rossant & Balling, 1987).

We would like to thank Linda Notton for typing the manuscript. SKH is in receipt of an MRC Training Fellowship.

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(*Accepted 4 September 1987*)