Zygote 6 (February), pp 65–73. © 1998 Cambridge University Press Printed in the United Kingdom

Nuclear envelope removal/maintenance determines the structural and functional remodelling of embryonic red blood cell nuclei in activated mouse oocytes

Daniel Szöllösi[1], *Renata Czołowska*[2] *Ewa Borsuk*[2], *Maria S. Szöllösi*[3] *and Pascale Debey*[3,4]
INRA, Jouy-en-Josas, France, University of Warsaw, Poland, IBPC, Paris and Muséum National d'Histoire Naturelle, Paris, France

Date submitted: 9.7.97. Date accepted: 20.11.97

Summary

Nuclei of embryonic red blood cells (e-RBC) from 12-day mouse fetuses are arrested in G_0 phase of the cell cycle and have low transcriptional activity. These nuclei were transferred with help of polyethylene glycol (PEG)-mediated fusion to parthenogenetically activated mouse oocytes and heterokaryons were analysed for nuclear structure and transcriptional activity. If fusion proceeded 25–45 min after oocyte activation, e-RBC nuclei were induced to nuclear envelope breakdown and partial chromatin condensation, followed by formation of nuclei structurally identical with pronuclei. These 'pronuclei', similar to egg (female) pronuclei, remained transcriptionally silent over several hours of *in vitro* culture. If fusion was performed 1 h or later (up to 7 h) after activation, the nuclear envelope of e-RBC nuclei remained intact and nuclear remodelling was less spectacular (slight chromatin decondensation, formation of nucleolus precursor bodies). These nuclei, however, reinforced polymerase-II-dependent transcription within a few hours of *in vitro* culture. Our present experiments, together with our previous work, demonstrate that nuclear envelope breakdown/maintenance are critical events for nuclear remodelling in activated mouse oocytes and that somatic dormant nuclei can be stimulated to renew transcription at a time when the female pronucleus remains transcriptionally silent.

Key words: Heterokaryons, Mouse oocyte, Nuclear remodelling, Red blood cell nuclei, Transcription

Introduction

During the short post-fertilisation period (metaphase II–interphase transition) of mammalian development, initially dormant female and male genomes interact with activated ooplasm and remodel into pronuclei capable of resuming DNA synthesis during the first embryonic cell cycle. These early post-fertilisation changes are also important for programmed gene expression in early development and for completing genomic imprinting.

Recent nuclear transfer experiments in the sheep have shown that nuclei of somatic origin, transferred into activated and enucleated oocytes, can replace the zygotic nuclei and take over the control of embryonic development leading to the birth of healthy offspring (Campbell *et al.*, 1996; Wilmut *et al.*, 1997). Transplantation of somatic nuclei may become the routine method for production of genetically identical animals (genetic cloning) provided that donor nuclei can interact with the ooplasm of the activated egg and repeat all morphological and biochemical changes that condition remodelling of chromatin into a pronucleus. Analysis of early changes in somatic nuclei from different cell types transferred into activated eggs by cell fusion or nuclear transplantation may contribute to our understanding of the respective roles of nucleus and ooplasm in genome remodelling

All correspondence to: Renata Czołowska, Department of Embryology, University of Warsaw, Krakowskie Przedmiescie 26/28, 00-927 Warsaw 64, Poland. Telephone: +48 22 6 20 03 81 (ext. 475). Fax: +48 22 8 26 86 24. e-mail: embrio@plearn.edu.pl.
[1] INRA Unité de Biologie de la Fécondation, 78352 Jouy-en-Josas, France.
[2] Department of Embryology, University of Warsaw, Krakowskie Przedmiescie 26/28, 00-927 Warsaw 64, Poland.
[3] Laboratoire Associé INRA (Station 806)/INSERM (U-310), IBPC, 13 rue P et M Curie, 75005 Paris, France.
[4] Muséum National d'Histoire Naturelle, 57 rue Cuvier, 75005 Paris, France

and in the regulation of gene expression in early development.

We have previously shown that the nuclei of somatic cells (thymocytes and murine erythroleukemia (MEL) cells from *in vitro* culture), as well as the nuclei of embryonic cells (8-cell stage blastomeres), remodel when transferred to parthenogenetically activated mouse oocytes, and that the extent of remodelling depends on whether the nuclear envelope of the donor nucleus is removed or preserved (Szöllösi *et al.*, 1986*b*, 1988; Czołowska *et al.*, 1992; Borsuk *et al.*, 1996). Two different patterns of remodelling were observed depending on the length of time separating parthenogenetic activation and cell fusion. When the interval was short (25–60 min), nuclear envelope breakdown could occur and the donor cell nuclei were remodelled into pronucleus-like nuclei. When the time period between activation and fusion was greater than 1 h, the nuclear envelope did not break down, and nuclear remodelling was less extensive or absent. These structural changes coincided well with changes in the activity of donor nuclei: remodelling into a pronucleus led to the switching-off of global transcription, whereas preservation of a nucleus with an intact nuclear envelope resulted in the maintenance (MEL and blastomere nuclei) or activation (thymocyte nuclei) of transcription (Borsuk *et al.*, 1996, and unpublished observations).

Primitive erythrocytes of mammals (embryonic red blood cells, e-RBC) originate from yolk sac blood islands. In the mouse on the 12th day of gestation, they constitute a homogeneous population of terminally differentiated cells specialised as oxygen carriers (Kovach *et al.*,1967; Rifkind *et al.*, 1974; Harris, 1986). e-RBC nuclei are in G_0 phase of the cell cycle: they are highly condensed and show only a low level of transcription. In the present work we provide evidence that e-RBC nuclei transferred to activated mouse oocytes change their structure and function, and that the extent of these changes depends on whether the nuclei are introduced shortly or late after activation.Together with previously published studies this result demonstrates that the capacity of nuclei to remodel depends mostly on the activity of cytoplasmic factors operating at the time of fusion, and to a lesser extent on the type of nucleus introduced.

Materials and methods

Primitive erythrocytes

Primitive (nucleated) erythrocytes (embryonal red blood cells, e-RBCs) were obtained from embryos removed from uteri of female mice on the 12th day of gestation. Pregnant females were killed by cervical dislocation or following chloroform anaesthesia. Each fetus was removed within the intact amniotic sac and transferred to phosphate-buffered saline (PBS, Dulbecco A completed with $CaCl_2$ and $MgCl_2$). The amniotic sac was then opened, the fetus was isolated and the maternal blood was quickly removed by washing in a few changes of PBS. After decapitation, the fetus was bled in PBS solution. In every experiment the erythrocyte suspension was prepared freshly before fusion.

Collection and activation of oocytes

Ovulated metaphase II (MII) oocytes were obtained from F_1 (C57BL/10 × CBA/H) female mice induced to ovulate with PMSG (pregnant mare's serum gonadotrophin) and hCG (human chorionic gonadotrophin) (doses: 5–10 IU of each given 48–54 h apart). Oocytes were harvested 15–16 h after hCG injection and treated with hyaluronidase (150 IU/ml) for removal of cumulus cells. For zona pellucida digestion the oocytes were incubated in pronase (0.5% in Ringer solution). Short-term culture of oocytes in drops of M2 medium (Fulton & Whittingham, 1978) under paraffin oil, at 37 °C, was followed by activation and fusion (see below). Oocytes were activated by exposure to 8% ethanol for between 5 min and 5 min 30 s (Cuthbertson, 1983) in drops of the activating medium under paraffin oil (age of oocytes: 16 h to 18 h 30 min after hCG) and cultured in M2 until they were treated for fusion.

Fusion of hybrid partners

Activated oocytes were preincubated for a few minutes in phytohaemagglutinin (PHA, Sigma; 200–500 μg/ml in BSA-free M2). They were then placed on an agar-coated embryological watch glass in BSA-free M2 medium and rolled over a layer of erythrocytes (prepared by dilution of erythrocyte suspension) until erythrocytes were firmly attached to the oocyte surface. The oocytes with attached erythrocytes were treated for 1 min with polyethylene glycol (PEG, Fluka; *Mr* 2000; 45–50% in BSA-free M2), thoroughly washed thereafter in M2, placed in separate drops of M2 under paraffin oil and cultured under standard conditions (37 °C, 5% CO_2 in air). Under this protocol the number of fusions is unpredictable and heterokaryons may contain singular or multiple (over 10) e-RBC nuclei.

Light and electron microscopy

Oocyte–erythrocyte heterokaryons were produced according to the following variants: group 1, e-RBC were fused with activated oocytes shortly (25–45 min) after activation, then cultured for 1–5 h; group 2, fusions were performed between 1 h and 7 h after activation

https://doi.org/10.1017/S0967199400005098 Published online by Cambridge University Press

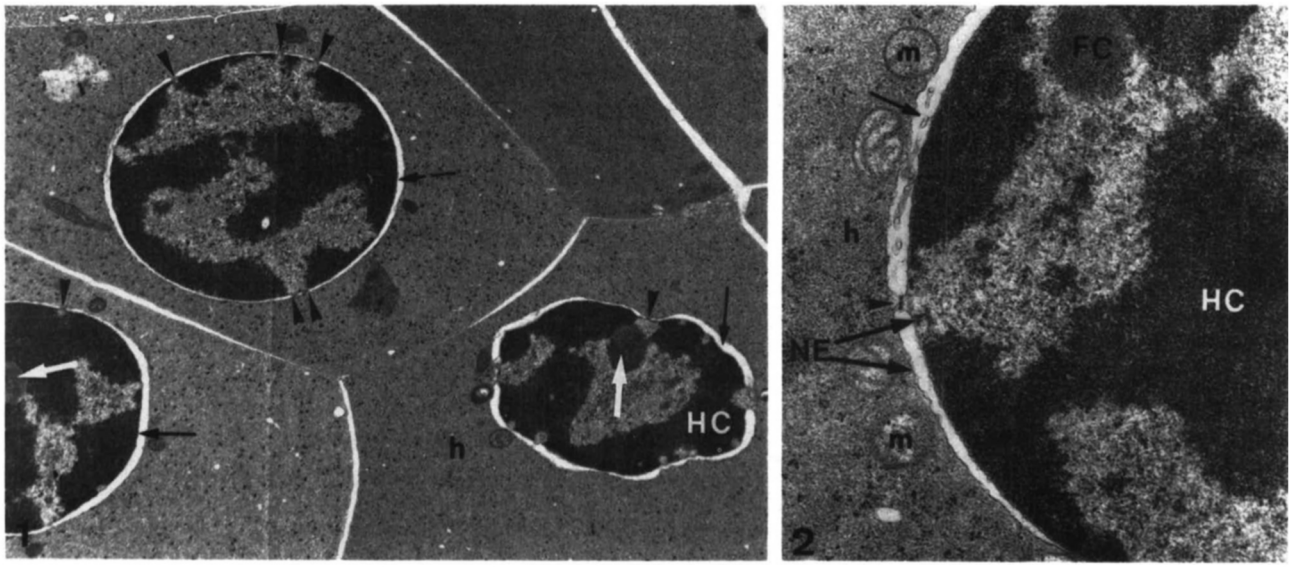

Figure 1 A pellet prepared by centrifugation of freshly collected blood from a 12-day-old mouse fetus. Embryonic red blood cells (e-RBCs) are deformed by the centrifugal force. Electron density of individual cells differs because of different amount of haemoglobin (h) deposited in the cytoplasm and its organelles, as well as in the nucleus. The nucleus is highly heterochromatic (HC) and clearly demarcated by a swollen perinuclear space (arrows) decorated with very few nuclear pores (arrowheads). In two nuclei the fibrillar centre is visible (white arrow). × 7000. **Figure 2** Details of the nuclear structure of an unfused e-RBC. The two membranes that comprise the NE (long arrows), the nuclear pore (arrowhead), the heterochromatin (HC) and the fibrillar centre (FC) are shown. Several mitochondria (m) adhere to the surface of the NE. h, haemoglobin. × 27 600.

and heterokaryons were then cultured for 45 min to 6 h after fusion. Developmental age of heterokaryons at the moment of fixation thus varied: the shortest was 1 h 30 min (fusion 30 min after activation, culture 1 h after fusion) and the longest 8–9 h (fusion 3 h after activation, culture 5–6 h after fusion).

To determine fusion success, some hybrid cells were prepared as whole-mount preparations for light microscopy (Tarkowski & Wróblewska, 1967). For electron microscopy, material was processed as previously described (Szöllösi *et al.*, 1986*a*,*b*).

Assessment of transcriptional activity

Polymerase II (pol II)-dependent transcriptional activity of e-RBC before fusion was assessed according to Wansink *et al.* (1993) by incorporation of BrUTP into permeabilised cells, previously deposited on gelatin-coated slides.

Pol II-dependent transcriptional activity of e-RBC nuclei introduced into activated eggs was examined according to the method of Bouniol *et al.* (1995). MII (series A) and activated oocytes (series B) were microinjected with BrUTP (Sigma; 100 mM solution in 2 mM Pipes with 140 mM KCl, pH 7.4) with the help of an Eppendorf micro-injector. After injection and activation, oocytes were fused with e-RBCs as described above, and cell hybrids were cultured in BAT6 medium (Nasr-Esfahani *et al.*, 1990). Two series of heterokaryons were produced (Table 1). In series A, fusion was performed shortly (25–47 min) after activation, and heterokaryons were cultured for 3–6 h (the protocol which allows for remodelling of donor nuclei into pronuclei). In series B, fusion occurred 5–6 h after activation (i.e. in interphase) and donor nuclei were incubated either 4–5 h (series B1; egg pronucleus transcriptionally inactive) or 6–7 h after fusion (series B2; egg pronucleus initiates transcription) (Bouniol *et al.*, 1995; Aoki *et al.*, 1997).

Hybrid cells were fixed in 2% paraformaldehyde in PBS for 20 min, permeabilised in 0.1% Triton X-100 in PBS for 15 min at room temperature, and BrU incorporation was then detected by indirect immunofluorescence using a monoclonal IgG anti-BrdU antibody (Caltag Laboratories) and a fluorescein-conjugated goat anti-mouse IgG (Caltag Laboratories) as described by Bouniol *et al.* (1995). To identify e-RBC nuclei, hybrid cells were labelled with Hoechst 33342 (Sigma, 2 μg/ml PBS) following incubation with the secondary antibody. Heterokaryons were inspected with a microscope equipped for epifluorescence.

Results

Structure of nucleated red blood cells

In order to appreciate the extent of their remodelling after fusion with mouse oocytes, we examined the structure of fetal primitive erythrocytes. e-RBCs are

https://doi.org/10.1017/S0967199400005098 Published online by Cambridge University Press

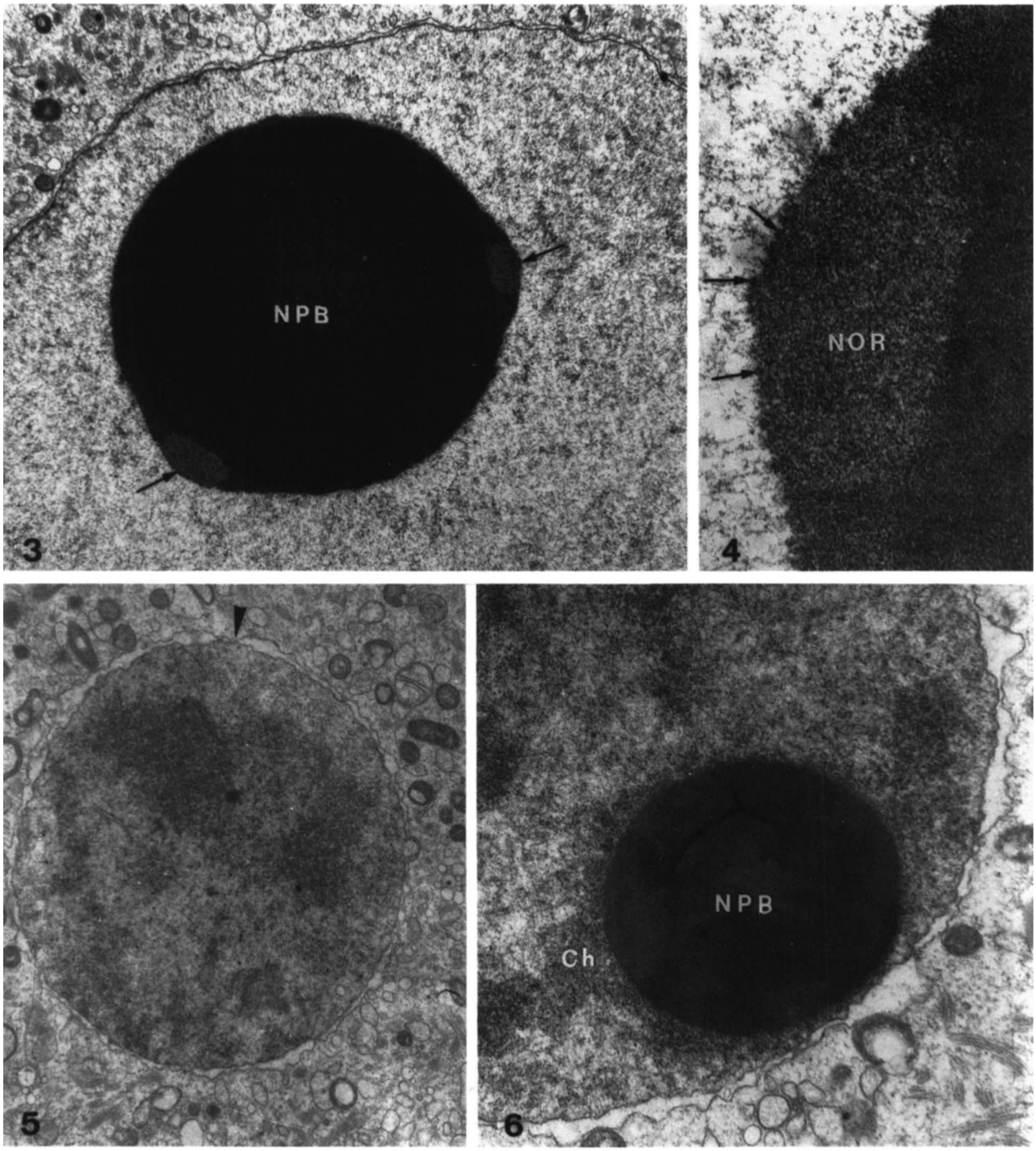

Figure 3 In the e-RBC-derived 'pronucleus', a nucleolus precursor body (NPB) forms. Arrows point to two nucleolar organiser regions (NORs). × 12 200. **Figure 4** A high-magnification electron micrograph showing a part of the NPB with the lenticular body (NOR); both are composed of thin filaments but the electron opacity of the lenticular body is lower than that of the main mass of the NPB. At the surface of the lenticular body there are patches of granules of pre-ribosomal granule dimensions (arrows). × 80 000. **Figure 5** An e-RBC nucleus transferred 2 h after parthenogenetic activation of the oocyte. The NE remains intact and the chromatin decondenses slightly and unevenly. The perinuclear space is irregular, as it was in the control e-RBC, and there are only a few nuclear pores present (arrowhead). × 12 000. **Figure 6** Another e-RBC-oocyte hybrid formed 2 h after activation. The nucleus shows a uniformly electron-dense NPB without any sign of the lenticular body (NOR). The NPB is surrounded by a chromatin halo (Ch). × 23 900.

https://doi.org/10.1017/S0967199400005098 Published online by Cambridge University Press

spherical and measure close to 6 μm in diameter. As they store different amounts of haemoglobin in the nucleus and the cytoplasm (Kovach *et al.*, 1967; Rifkind *et al.*, 1974; Claussen, 1985; Harris, 1986) and also in some cytoplasmic organelles, the individual cells may differ from each other in electron density (Fig. 1).

The nucleus is constituted of densely packed thin filaments of heterochromatin not resolvable in usual electron microscopic preparations (Fig. 2). The nuclear pores are widely scattered and the perinuclear space is wide (Figs. 1, 2). These features, already described by other authors (Kovach *et al.*, 1967; Rifkind *et al.*, 1974; Harris, 1986), distinguish the nuclei of e-RBC from those of any other somatic and embryonic cells. A solitary, highly compacted fibrillar centre is the only recognisable nucleolar component (Figs. 1, 2); it structurally resembles the fibrillar centre of small, transcriptionally inactive lymphocytes (Derenzini *et al.*, 1987; Hozâk *et al.*, 1989; Ochs & Smetana, 1989).

Red blood cell nuclei in activated oocytes

Nuclei transferred 25–45 min after activation (group 1)

e-RBC nuclei are readily remodelled when transfered into oocytes shortly after their activation. The nuclear envelope breaks down and the chromatin condenses as an integral mass displaying areas of higher and lower density that are probably the remnants of the original chromatin organisation of a primitive erythrocyte nucleus. A dense circular fibrillar structure can be seen associated with e-RBC chromatin and corresponding to the rudimentary fibrillar centre in the control e-RBC nucleus (not shown). At this stage e-RBC chromatin becomes synchronised with the oocyte chromatin, which reaches telophase II about 90 min after activation (in the PEG-treated heterokaryons telophase II is attained later than in non-treated activated oocytes). Between 4 and 5 h later the female pronucleus is formed, which contains one to several nucleolus precursor bodies (Kopečny *et al.*, 1989) constituted of thin fibrils. e-RBC chromatin transforms into a similarly organised 'pronucleus' also containing one to three nucleolus precursor bodies composed of thin fibrils (Fig. 3). Lenticular bodies corresponding to peripheralised nucleolar organiser regions (NORs) (Takeuchi & Takeuchi, 1986; Szöllösi *et al.*, 1986*b*) develop at the surface of nucleoli of both the egg pronucleus and e-RBC 'pronucleus' (Fig. 3). A few granules associated with some portions of the nucleolus precursor bodies and lenticular bodies (Fig. 4) have an appearance and dimensions comparable with those of pre-ribosomal granules, which may suggest minimal pre-rRNA transcription. Blebbing of the reconstituted nuclear envelope of the e-RBC-derived 'pronucleus' follows the timing and extent of the blebbing activity of the female pronucleus (Szöllösi & Szöllösi, 1988).

Table 1 Transcriptional activity of pronuclei and e-RBC nuclei in activated mouse oocytes (number of heterokaryons)

Experiment:	Series A	Series B1	Series B2
Fusion p.a.:	<47 min	5–6 h	5–6 h
Culture p.f.:	3–6 h	4–5 h	6–7 h
Total	22	52	35
With transcribing pronuclei	0	0	6 (17%)
With transcribing e-RBC nuclei	0	22 (42%)	17 (49%)
Total		39 (45%)	

p.a., post-activation; p.f., post-fusion.
In series A oocytes were injected with BrUTP at metaphase II stage.
In series B oocytes were injected after activation.

When fusion occurs 45–60 min after activation, about half the donor nuclei are remodelled as described above while the other half do not show these changes. Between 45 and 60 min after activation (which is the time when the female chromatin approaches telophase II) thus represents the critical time after which e-RBC nuclei can not be remodelled into pronuclei.

Nuclei transferred 1–7 h after activation (group 2)

In these cell hybrids, the nuclear envelope of e-RBC nuclei remains intact (Fig. 5) and the number of nuclear pores does not change. The donor nucleus initiates a different and much simpler route of morphological changes. The chromatin decondenses, but the nucleus never expands as much as it does in the case of the pronucleus-like remodelling. The compact fibrous nucleolus precursor body develops (Fig. 6) but nucleolar organiser regions are never seen at its surface.

Pol-II-dependent transcriptional activity of e-RBC transferred to activated eggs

Before fusion, e-RBC are slightly active in transcription (not shown). The signal intensity obtained after immunodetection of incorporated BrU was similarly low in all nuclei, but consistently above the signal in controls that had incorporated non-modified uridine or not been treated with the first antibody.

Table 1 shows the results of three series of experiments in which activated eggs fused with e-RBCs

https://doi.org/10.1017/S0967199400005098 Published online by Cambridge University Press

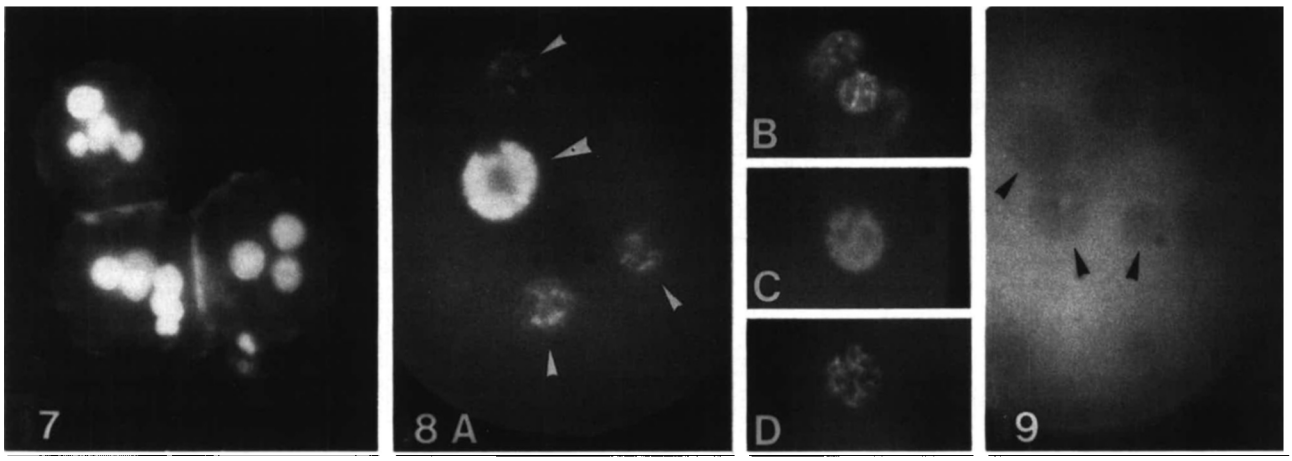

Figure 7 A group of three eggs fused with e-RBCs 3 h after activation and cultured until the end of the cell cycle. Multiple e-RBC nuclei stained with Hoechst 33342. ×310. **Figure 8** Fluorescent staining of BrU-labelled RNA in e-RBC nuclei incubated in parthenogenetic mouse eggs. Activated eggs fused with e-RBCs a few hours after activation and cultured few hours post PEG treatment (series B). (*A*) Strongly stained female pronucleus (large arrowhead) and three e-RBCs showing punctate staining (small arrowheads). (*B–D*) Pattern of fluorescent staining of e-RBC nuclei in three different oocytes; (*B*) shows a group of nuclei, whereas (*C*) and (*D*) show single nuclei. ×496. **Figure 9** Activated oocyte fused with e-RBCs according to the same protocol as in Fig. 8. There is no staining reaction. e-RBC nuclei are visualised as black holes (arrowheads). ×496.

were assayed for the occurrence of nuclear transcription using the immunostaining method of Bouniol *et al.* (1995).

Staining with Hoechst 33342 revealed that 1 to 19 e-RBC nuclei could reside in a given egg besides the female pronucleus (Fig. 7). In cell hybrids in which e-RBC have undergone complete remodelling into 'pronuclei' (series A), all nuclei, whether of maternal or somatic origin, were negative in transcription.

In contrast, 39 of 87 eggs (*c.* 45%) fused with e-RBCs 5–6 h after activation and submitted to 4–7 h of culture (series B) had 1 to 6 positively stained e-RBC nuclei (Fig. 8). In most of these heterokaryons, 30–85% of e-RBC nuclei out of the total number of nuclei present in a heterokaryon were positive, which indicates that transcribing (Fig. 8*A*) and non-transcribing nuclei (Fig. 9) can coexist within the same cytoplasm. Among 39 heterokaryons carying transcriptionally positive e-RBC nuclei (series B1 and B2), only 6 (15%) also had the female pronucleus positive. They belonged to subgroup B2, cultured for a longer time and fixed about 12 h 30 min after activation.

Discussion

Remodelling of e-RBC nuclei into pronuclei

Our present work demonstrates that if activation precedes fusion by no more than 45 min, e-RBC nuclei remodel readily into pronuclei. The remodelling of the e-RBC nuclei followed the pathway described for thymocyte and blastomere nuclei transferred to recently activated mouse oocytes (Szöllösi *et al.*, 1988; Czołowska *et al.*, 1992; Borsuk *et al.*, 1996). Nuclear envelope breakdown was followed by chromatin condensation, and then, after slight chromatin decondensation, the nuclear envelope was formed anew. In an activated oocyte, maturation promoting factor (MPF) activity decreases and disappears almost entirely at telophase II (Weber *et al.*, 1991; Szöllösi *et al.*, 1993) This is the stage (reached in our heterokaryons about 90 min after activation) at which the two chromatins (recipient and donor) became synchronised. In the newly formed nuclear envelope segments, the nuclear pores appeared and pronuclear growth was initiated. In the expanding pronucleus, nucleolus precursor bodies formed with associated lenticular bodies (nucleolar organiser regions). It should be noted that the lenticular bodies, which exist only in the germinal vesicle of oocytes and in zygotes (they are no longer observed after the 2-cell stage), were also found in 'pronuclei' obtained from all cell types that were remodelled after fusion with oocytes within 45 min of activation (Table 2), with the exception of MELs (Borsuk *et al.*, 1996). The reconstituted diploid e-RBC 'pronuclei' were identical in many respects (morphology, rate of formation and growth, appearance of the nucleolus precursor bodies) to the female pronucleus, including the lack of pol-II-dependent transcription at the beginning of the first cell cycle. All e-RBC nuclei that remodelled into pronuclei remained transcriptionally inactive during 3–6 h after fusion. This is consistent with the results of

https://doi.org/10.1017/S0967199400005098 Published online by Cambridge University Press

Table 2 Remodelling of embryonic and somatic nuclei in activated mouse oocytes

Origin (type) of nucleus Phase of cell cycle	Fusion < 60 min post-activation (remodelling into pronucleus-like nucleus)				Fusion > 60 min post-activation (interphase nucleus maintained)		
	Nucleolar precursor bodies			pol II-dependent transcription	Nucleolus		pol II-dependent transcription
	NEBD	LB	GC		NEBD		
Blastomere (embryonic) S/G_2 (Czołowska *et al.*, 1992; Borsuk, unpublished)	+	+	—	—	—	Bipartite[b]	+
MEL (somatic) $G_1/S/G_2$ (Borsuk *et al.*, 1996)	+	—	+	—	—	Bipartite[b]	+
Thymocyte (somatic) G_0 (Szöllösi *et al.*, 1986*b*, 1988; Borsuk *et al.*, 1996)	+	+	—	—[a]	—	Bipartite[b] or nucleolar precursor bodies	+
e-RBC (somatic) G_0 (this study)	+	+	—	—	—	Nucleolar precursor bodies	+

NEBD, nuclear envelope breakdown; LB, lenticular body (NOR); GC, granular component.
[a] Transcription starts together with the female pronucleus at G_2 (Borsuk *et al.*, unpublished).
[b] Bipartite nucleolus contains a fibrillar centre, dense fibrillar component and granular component.

experiments by Borsuk *et al.* (1996), who found that neither thymocyte nor MEL nuclei display any transcriptional activity after remodelling into pronuclei. This is also consistent with the data of Bouniol *et al.* (1995) on the time of appearance of endogenous pol-II-dependent transcription in the fertilised egg, approximately 14 h after fertilisation. As e-RBC nuclei sustained a low transcriptional activity before fusion we must conclude that it is the cytoplasmic environment provided by an oocyte just after activation that makes the transferred nuclei, as well as endogenous nuclei (pronuclei), transcriptionally silent.

Remodelling of interphase e-RBC nuclei in interphase eggs

Our present experiments demonstrate that remodelling of e-RBC nuclei is less extensive when nuclear transfer follows oocyte activation by one to several hours. There was no evidence of nuclear envelope breakdown and chromatin condensation – thus the chromatin of e-RBC nuclei was not exposed to the ooplasm. The nucleolus appeared in the form of the nucleolus precursor bodies composed of thin fibrils only. Such morphology that indicates the absence of transcription of ribosomal genes in the transferred e-RBC nucleus can be expected since activated mouse eggs do not transcribe ribosomal RNA until the 2-cell stage (Telford *et al.*, 1990). In chick erythrocyte nuclei, nucleologenesis and rRNA synthesis can be reactivated only by fusion with cells **active** in rRNA transcription – the process lasting a few days and requiring migration of host proteins (such as polymerase I) into erythrocyte nuclei (somatic cell hybrids; Hernandez-Verdun & Bouteille, 1979; Scheer *et al.*, 1983). Surprisingly, when a thymocyte nucleus was incubated in the interphase ooplasm of an activated egg, the nucleolus was associated with a cluster of pre-ribosomal granules after 1 h of culture, which suggests rapid activation of ribosomal genes (Szöllösi *et al.*, 1988). In contrast to erythrocytes, thymocytes are arrested in G_0 only temporarily, and reactivate readily in response to antigens. Reactivation of nucleolus and nucleolar rRNA synthesis following mitogenic stimulation of a lymphocyte *in vitro* takes about 2 days, however, and depends on the translocation of polymerase I from the cytoplasmic to the nuclear compartment (Setterfield *et al.*, 1983; Halleck *et al.*, 1989). Since the nucleolar changes noted in oocyte–thymocyte hybrids occurred very fast one it can be assumed that remnants of the transcriptional machinery for ribosomal synthesis are still present in the resting thymocyte nucleus, and can be activated in conditions permitting rapid chromatin expansion.

Our previous experiments have also shown that the nuclei active in rRNA synthesis (from 8-cell stage blastomeres and MEL cells) preserved the organisation typical of a nucleus transcribing ribosomal genes (bipartite nucleolus, i.e. with both granular and fibrillar components) during several hours after

https://doi.org/10.1017/S0967199400005098 Published online by Cambridge University Press

transfer to interphase mouse eggs (Czołowska *et al.*, 1992; Borsuk *et al.*, 1996). Similar temporary 'freezing' of nucleolar structure and nucleolar rRNA synthesis occurs in nuclear transfer embryos produced by fusion of a 16- to 32-cell stage blastomere with enucleated activated bovine oocytes (King *et al.*, 1996).

In the present work we demonstrate that e-RBC nuclei incubated in interphase egg cytoplasm reinforce pol-II-dependent transcription. Transcriptionally positive e-RBC nuclei were found in 45% of hybrid cells examined and coexisted with transcriptionally silent nuclei within the same heterokaryon. In negative nuclei, transcriptional activity could have been lost either due to to a detrimental effect of experimental manipulations on particular eRBCs, or a dilution of transcription factors in the egg cytoplasm, and/or competition for these factors between endogenous (pronucleus) and exogenous (e-RBC) nuclei. Activation of mRNA synthesis in e-RBC nuclei may involve globin genes since these are among first genes transcribed by avian erythrocyte nuclei after fusion with transcriptionally active somatic cells (Linder *et al.*, 1981; Bruno *et al.* 1981).

Poly-II-dependent transcription has been detected in nuclei of different types incubated in cytoplasm of interphase mouse eggs, regardless of whether donors were initially active (blastomeres and MEL cells) or inactive (thymocytes) in this transcription (Borsuk *et al.*, 1996; and unpublished observations). It is particularly intriguing that these nuclei resumed or continued transcription at the period when the native egg pronucleus was transcriptionally silent (transcription starts only at the end of the first cell cycle; Bouniol *et al.*, 1995; Aoki *et al.*, 1997). This might suggest that in normal development transcription is regulated at the level of pronucleus, not cytoplasm.

Results of experiments described in the present work and in our previous publications are summarised in Table 2. It is evident that removal of the nuclear envelope and exposure of the chromatin to the ooplasm leads consistently to the formation of a 'pronucleus' irrespective of the type and activity of the nucleus introduced into activated mouse oocytes. The situation is more complicated when donor nuclei are transferred to interphase eggs and the nuclear envelope is preserved. The nuclear envelope barrier differentiates forthcoming nuclear changes. If the nuclei originate from actively transcribing cells, they become structurally and functionally 'frozen' for at least several hours. If the nuclei originate from differentiated cells arrested in G_0, they undergo limited structural modification that coincides with renewal or reinforcement of transcriptional activity. Whether this burst of transcription is only temporary or denotes true nuclear reactivation remains to be examined. Comparing the behaviour of nuclei of various differentiation and proliferation states (G_0 thymocytes, G_0 embryonic red blood cells, cycling murine erythroleukaemia cells and embryonic 8-cell blastomeres) may furnish an interesting basis for understanding the respective roles of nuclear and ooplasmic factors in nuclear reprogramming and genome activity in early mammalian development.

Recent nuclear transfer experiments of Campbell *et al.* (1996) and Wilmut *et al.* (1997) have proved that live mammalian offspring can be produced from established cell lines of embryonic and adult origin that were induced to quiescence. Embryonic red blood cell nuclei may be considered as potential donors for nuclear transfer. They constitute a population of synchronised dormant (G_0) cells having a compacted nucleus that lacks the majority of nuclear proteins. Such a nucleus can easily interact with the ooplasm of an activated egg and remodel into a true pronucleus. It should be recalled that in amphibians, erythrocyte nuclei are able to promote development of enucleated oocytes up to advanced larval stages after serial transplantations (DiBerardino & Hoffner Orr, 1992). Developmental potency of mammalian embryonic red blood cell nuclei awaits experimental confirmation.

Acknowledgements

This work was carried out within the framework of the INSERM East/West Cooperation program No. 5 REW 01, and was partly financed through a French–Polish scientific exchange program (ATP 12) and by grant No. 6PO4C 029 09 from the Polish State Committee of Scientific Research. WHO small Institutional Grant to the Department of Embryology, University of Warsaw, is kindly acknowledged. Mrs Denise Huneau has greatly contributed by preparation of thin sections. Mr F. Fort and Mr R. Scandolo deserve our thanks for preparing the photographs.

References

Aoki, F., Worrad, D.M. & Schultz, R.M. (1997). Regulation of transcriptional activity during the first and second cell cycles in the preimplantation mouse embryo. *Dev. Biol.* **181**, 296–307.

Borsuk, E., Szöllösi, M.S., Besombes, D. & Debey, P. (1996). Fusion with activated mouse oocytes modulates the transcriptional activity of introduced somatic cell nuclei. *Exp. Cell Res.* **225**, 93–101.

Bouniol, C., Nguyen, E. & Debey, P. (1995). Endogenous transcription occurs at the 1-cell stage in the mouse embryo. *Exp. Cell Res.* **218**, 57–62.

Bruno, J., Reich, N. & Lucas, J.J. (1981). Globin synthesis in hybrid cells constructed by transplantation of dormant avian erythrocyte nuclei into enucleated fibroblasts. *Mol. Cell Biol.* **1**, 1163–76.

Campbell, K.H.S., McWhir, J., Ritchie, W.A. & Wilmut, I. (1996). Sheep cloned by nuclear transfer from a cultured cell line. *Nature* **380**, 64–6.

Claussen, C.P. (1985). Cell organelles involved in hemoglobin biosynthesis in vertebrates. *Fortsch. Zool.* **30**, 397–400.

Cuthbertson, K.S.R. (1983). Parthenogenetic activation of mouse oocytes *in vitro* with ethanol and benzyl alcohol. *J. Exp. Zool.* **226**, 311–14.

Czołowska, R., Szöllösi, D. & Szöllösi, M.S. (1992). Changes in embryonic 8-cell nuclei transferred by means of cell fusion to mouse eggs. *Int. J. Dev. Biol.* **36**, 543–53.

Derenzini, M., Farabegoli, F., Pession, A. & Novello, F. (1987). Spatial redistribution of ribosomal chromatin in the fibrillar centres of human circulating lymphocytes after stimulation of transcription. *Exp. Cell Res.* **170**, 31–41.

DiBerardino, M.A. & Hoffner-Orr, N. (1992). Genomic potential of erythroid and leucocytic cells of *Rana pipiens* analyzed by nuclear transfer into diplotene and maturing oocytes. *Differentiation* **50**, 1–13.

Fulton, B.P. & Whittingham, D.G. (1978). Activation of mammalian oocytes by intracellular injection of calcium. *Nature* **273**, 149–51.

Halleck, M.S., Schlegel, R.A. & Rose, K.M. (1989). Cytoplasmic to nuclear translocation of RNA polymerase I is required for lipopolisaccharide-induced nucleolar RNA synthesis and subsequent DNA synthesis in murine B lymphocytes. *J. Cell Sci.* **92**, 101–9.

Harris, J.R. (1986). Blood cell nuclei: the structure and function of lymphoid and erythroid nuclei. *Int. Rev. Cytol.* **102**, 53–168.

Hernandez-Verdun, D. & Bouteille, M. (1979). Nucleologenesis in chick erythrocyte nuclei reactivated by cell fusion. *J. Ultrastruct. Res.* **69**, 164–79.

Hozák, P., Novák, J.T. & Smetana, K. (1989). Three-dimensional reconstructions of nucleolus-organizing regions in PHA-stimulated human lymphocytes. *Biol. Cell* **66**, 225–33.

King, W.A., Shepherd, D.L., Plante, L., Lavoir, M.-C., Looney C.R. & Barnes, F.L. (1996). Nucleolar and mitochondrial morphology in bovine embryos reconstructed by nuclear transfer. *Mol. Reprod. Dev.* **44**, 499–506.

Kopečny, V., Fléchon J-E., Camous S. & Fulka J. Jr (1989). Nucleologenesis and the onset of transcription in the eight-cell bovine embryo: fine-structural autoradiographic study. *Mol. Reprod. Dev.* **1**, 79–90.

Kovach, J.S., Marks, P.A., Russell, E.S. & Epler, H. (1967). Erythroid cell development in fetal mice: ultrastructural characteristics and hemoglobin synthesis. *J. Mol. Biol.* **25**, 131–42.

Linder, S., Zuckerman, S.H. & Ringertz, N.R. (1981). Reactivation of chicken erythrocyte nuclei in heterokaryons results in expression of adult chicken globin genes. *Proc. Natl. Acad. Sci. USA* **78**, 6286–9.

Nasr-Esfahani, M., Johnson, M.H. & Aitken, R.J. (1990). The effect of iron and iron chelators on the *in vitro* block to development of the mouse preimplantation embryo: BAT6 a new medium for improved culture of the mouse embryos *in vitro*. *Hum. Reprod.* **5**, 997–1003.

Ochs, R.L. & Smetana, K. (1989). Fibrillar center distribution in nucleoli of PHA-stimulated human lymphocytes. *Exp. Cell Res.* **184**, 552–7.

Rifkind, R.A., Bank, A. & Marks, P.A. (1974). Erythropoiesis. In *The Red Blood Cell*, ed. D. Mac N. Surgenor, vol. 1, pp. 51–89. New York: Academic Press.

Scheer, U., Lanfranchi, G., Rose, K.M., Franke, W.W. & Ringertz, N.R. (1983). Migration of rat RNA polymerase into chick erythrocyte nuclei undergoing reactivation in chick-rat heterokaryons. *J. Cell Biol.* **97**, 1641–3.

Setterfield, G., Hall, R., Bladon, T., Little, J. & Kaplan, J.G. (1983). Changes in structure and composition of lymphocyte nuclei during mitogenic stimulation. *J. Ultrastruct. Res.* **82**, 264–82.

Szöllösi, M.S. & Szöllösi, D. (1988). 'Blebbing' of the nuclear envelope of mouse zygotes, early embryos and hybrid cells. *J. Cell Sci.* **91**, 257–67.

Szöllösi, D., Czołowska, R., Sołtyńska, M.S. & Tarkowski, A.K. (1986*a*). Ultrastructure of cell fusion and premature chromosome condensation (PCC) of thymocyte nuclei in metaphase II mouse oocytes. *Biol. Cell* **56**, 239–50.

Szöllösi, D., Czołowska, R., Sołtyńska, M.S. & Tarkowski, A.K. (1986*b*). Remodelling of thymocyte nuclei in activated mouse oocytes: an ultrastructural study. *Eur. J. Cell Biol.* **42**, 140–51.

Szöllösi, D., Czołowska, R., Szöllösi, M.S. & Tarkowski, A.K. (1988). Remodelling of mouse thymocyte nuclei depends on the time of their transfer into activated, homologous oocytes. *J. Cell Sci.* **91**, 603–13.

Szöllösi, M.S., Kubiak, J.Z., Debey, P., de Pennard, H., Szöllösi, D. & Maro, B. (1993). Inhibition of protein kinases by 6-dimethylaminopurine accelerates the transition to interphase in activated mouse oocytes. *J. Cell Sci.* **104**, 861–72.

Takeuchi, I.K. & Takeuchi, Y.K. (1986). Ultrastructural localization of Ag-NOR proteins in full- grown oocytes and preimplantation embryos of mice. *J. Electron Microsc.* **35**, 280–7.

Tarkowski, A.K. & Wróblewska, J. (1967). Development of blastomeres of mouse eggs isolated at the 4- and 8-cell stage. *J. Embryol. Exp. Morphol.* **18**, 155–80.

Telford, N.A., Watson, A.J. & Schultz, G.A. (1990). Transition from a maternal to embryonic control in early mammalian development: a comparison of several species. *Mol. Reprod. Dev.* **26**, 90–100.

Wansink, D.G., Schul, W., van der Kraan, I., van Steensel, B., van Driel, R. & de Jong, L. (1993). Fluorescent labeling of nascent RNA reveals transcription by RNA polymerase II in domains scattered throughout the nucleus. *J. Cell Biol.* **122**, 283–93.

Weber, M., Kubiak, J.Z., Arlinghaus, R.B., Pines, J. & Maro, B. (1991). c-*mos* proto-oncogene product is partly degraded after release from meiotic arrest and persists during interphase in mouse zygotes. *Dev. Biol.* **148**, 393-7.

Wilmut, I., Schnieke, A.E., McWhir, J., Kind, A.J. & Campbell, K.H.S. (1997). Viable offspring derived from fetal and adult mammalian cells. *Nature* **385**, 810–3.