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DNA replication and RNA synthesis in thymocyte nuclei microinjected into the cytoplasm of artificially activated mouse eggs

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Summary

Thymocyte nuclei were microinjected into the cytoplasm of parthenogenetic mouse eggs within 60 min or 3 h after egg activation and DNA replication and RNA synthesis were analysed in remodelled thymocyte nuclei and female pronuclei. We show that thymocyte nuclei which transform into pronucleus-like nuclei (thymocytes injected not later than 60 min after activation) enter S-phase 1 h earlier than the female pronuclei. At the beginning of the first cell cycle they remain transcriptionally silent, but in G2 undertake transcription earlier than the female pronuclei. Partly remodelled thymocyte nuclei (injected 3 h after activation) start to replicate DNA at the same time as the female pronuclei. They reinitiate RNA synthesis within 2 h after transfer and continue to transcribe irrespective of the transcriptional activity of the female pronucleus. We show that the observed transcription is only nuclear, i.e. RNA polymerase II-dependent.

Keywords: DNA replication, Female pronucleus, RNA polymerase II, Thymocyte nucleus, Transcription

Introduction

Formation of cell hybrids has been widely used as a method to study the ability of different types of nuclei (originating from embryonal cells or from fully differentiated somatic cells) to undergo reprogramming under the influence of the cytoplasm of an activated egg. Successful cloning experiments carried out in farm animals such as sheep (Wilmut *et al*., 1997) or cattle (Vignon *et al*., 1998), and in laboratory mammals (mouse: Wakayama *et al*., 1998; Wakayama & Yanagimachi, 1999), were a consequence of those studies. Although they proved that the reprogramming of fully differentiated nuclei is possible, they have not resolved all problems concerning the technique of cloning. The efficiency of normal development of embryos obtained as a result of nuclear transfer is still very low (approximately 1%) and the reasons for such poor development remain unknown. Improvement of the technique and an increase in the efficiency of cloning depend on better understanding of changes that foreign nuclei undergo in the egg cytoplasm.

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It has been shown that the nuclei of different murine somatic cells (thymocytes, erythroleukaemia cells (MEL), embryonic red blood cells) undergo structural remodelling after transfer into the cytoplasm of activated mouse oocytes (Czołowska *et al*., 1984; Szöllösi *et al*., 1986b, 1988, 1998; Borsuk *et al*., 1996). The character of changes in these nuclei depends on the fate of the nuclear envelope after transfer. The nuclei which enter the egg cytoplasm soon after activation (not later than 60 min) undergo nuclear envelope breakdown and subsequently form pronucleus-like nuclei (PLN). The nuclei which are introduced later, after transition of the activated egg to interphase, maintain the nuclear envelope. In this case their remodelling is impaired and the decondensation of chromatin is less pronounced. The structural remodelling of introduced nuclei affects their transcriptional activity. In mouse zygotes and parthenogenetic eggs the activation of RNA synthesis in the pronuclei takes place no earlier than the late 1-cell stage, at the end of S-phase (Bouniol *et al*., 1995; Aoki *et al*., 1997; Bouniol-Baly *et al*., 1997). The transformation of a foreign nucleus into a pronucleus-like nucleus also leads to the termination of RNA synthesis, even if this nucleus was transcriptionally active before transfer. This activity is not restored during 3–6 h of culture of hybrid cells. In contrast, the nuclei which do not transform into pronuclei, continue (MEL) or restart

(thymocytes) RNA synthesis irrespectively of the transcriptional activity of the female pronucleus (Borsuk *et al*., 1996; Szöllösi *et al*., 1998). These studies demonstrated that the ability of foreign nuclei to undergo structural and functional remodelling depends on the activity of cytoplasmic factors operating at the time of cell fusion. However, in the above-mentioned experiments the onset of transcription in the PLN was not determined, nor was it elucidated whether the burst of RNA synthesis observed in foreign nuclei not transforming into pronuclei is only temporary or persists over a long period. It is also not clear whether the observed transcription is both nuclear and nucleolar, i.e. RNA polymerase II- and polymerase I-dependent. In the pronuclei of a mouse zygote, in which nucleoli appear in the form of nucleolus-precursor bodies (NPB; Kopečny *et al*., 1989), only nuclear, i.e. polymerase-II dependent transcription, has been detected (Bouniol *et al*., 1995). In PLN of thymocyte origin nucleoli of similar morphology have been found, which suggests that in this case also only nuclear transcription should be expected. In contrast, in partly remodelled thymocyte nuclei bipartite nucleoli similar to the nucleoli of somatic cells were sometimes observed (Szöllösi *et al*., 1988), but it remains unknown whether rRNA synthesis occurs in these nuclei.

In the present study we injected single thymocyte nuclei into the cytoplasm of activated mouse egg using the method described by Kimura & Yanagimachi (1995) and applied by Wakayama and co-workers (1998) to obtain cloned mice. Injection of DNA and RNA precursors at definite time intervals after egg activation and transfer of the foreign nucleus, permitted us to determine precisely the onset of replication and transcription in the remodelled thymocyte nuclei and in the female pronuclei. Our results suggest that the cytoplasm of the early parthenogenetic 1-cell embryo is transcriptionally permissive and that the ability of the nucleus to start transcription in the first cell cycle depends on its internal readiness to carry on RNA synthesis rather than on the cytoplasmic environment. We show also that transcription observed in the remodelled thymocyte nuclei is exclusively RNA polymerase II-dependent.

Materials and methods

Recovery and artificial activation of ovulated oocytes

Metaphase II arrested mouse oocytes were recovered from the oviducts of F1(CBA/H × C75BL/10) females induced to ovulate by intraperitoneal injection of 10 IU of PMSG (Folligon, Intervet) followed by 10 IU of hCG (Chorulon, Intervet) 48–56 h later. Females were killed by cervical dislocation 17 h after hCG injection. Ovulated oocytes surrounded by follicular cells were released from the oviducts into a solution of hyaluronidase in Dulbecco A (1 mg/ml; Sigma). After dispersing follicular cells, the oocytes were washed in M2 medium (medium 16 buffered with Hepes; Fulton & Whittingham, 1978). Oocytes were activated 18 h post-hCG injection by 8 min of exposure to 8% ethanol in M2 (Cuthbertson, 1983).

Microinjection of thymocyte nuclei

Thymocytes were obtained from the thymus of 1- to 4-days-old F1(CBA/H × C57B1/10) mice (Czołowska *et al*., 1984). Cells were suspended in 100 µl of M2.

The nuclei of thymocytes were injected individually into the cytoplasm of parthenogenetically activated oocytes according to the method of Kimura & Yanagimachi (1995). A piezo-driven micropipette was used instead of a mechanically driven conventional pipette (MW Piezo Stepper PM 10–1; Leica).

Micromanipulations were performed under an inverted microscope (Diaphot 300; Nikon) equipped with Hoffman modulation contrast optics. The microscope stage was cooled with a temperature-controlling plate (Semic, Kraków, Poland) to about 12–15 °C. Several piezo pulses (speed 100 mm/s, step size 0.5–1.0 µm) were given to allow zona penetration. The oolemma was perforated with one piezo pulse (speed 100 mm/s, step size 0.5–1.0 µm). Thymocyte-injected oocytes were kept for 10 min in the manipulation medium on the cooled stage. They were then transferred to M2 for 10 min at room temperature (21–23 °C) and subsequently incubated in M2 medium at 37.5 °C under 5% CO_2. Thymocytes were injected either 60 min or 3 h after activation of oocytes.

Assessment of replication

To detect replication in the female pronucleus and the introduced thymocyte nucleus, heterokaryons were microinjected with a precursor of DNA replication, digoxigenin-11-dUTP (Dig-11-dUTP, 1 mM solution, undiluted commercial product, Boehringer) 1–6 h after nuclear transfer, using an Eppendorf microinjector. The injected volume was approximately 2 pl. Heterokaryons were fixed and processed for immunofluorescence 1 h after injection of the precursor, according to the procedure described by Bouniol-Baly *et al.* (1997). Dig-11-dUTP incorporation into newly synthesised DNA was detected by indirect immunofluorescence using mouse monoclonal anti-digoxigenin primary antibody (Boehringer) and a fluorescein-conjugated goat anti-mouse IgG (H+L) secondary antibody (Caltag Laboratories). To identify nuclei, heterokaryons were stained with a chromatin-specific dye,

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Hoechst 33342 (2–4 μg/ml PBS), following incubation with the secondary antibody. Immunostaining with the primary antibody omitted was performed to confirm the specificity of the secondary antibody binding. In control experiments the replication in activated eggs and in heterokaryons was blocked by aphidicolin (3 μg/ml of M2, Sigma). Control heterokaryons were injected with Dig-11-dUTP, fixed and processed for immunostaining as the experimental ones.

Assessment of transcription

RNA polymerase II-dependent transcription in the female pronucleus and in the introduced thymocyte nucleus was assessed by immunofluorescent detection of BrU incorporated into nascent transcripts, using BrUTP as a precursor (Wansink *et al*., 1993; Bouniol *et al*., 1995). Heterokaryons were microinjected with 1–2 pl of BrUTP (100 mM solution in injection buffer – 2 mM PIPES, 140 mM KCl, pH 7.4) at different time after injection of the nucleus of a thymocyte (from 1 h to 14 h). They were fixed and processed for immunofluorescence 1 h after injection of the precursor. BrU incorporation was detected by indirect immunofluorescence using mouse monoclonal anti-BrdU antibody (Caltag Laboratories) as a primary antibody and a fluorescein-conjugated goat anti-mouse IgG (H+L) as a secondary antibody (Caltag Laboratories). Following incubation in the solution of the secondary antibody, the nuclei in heterokaryons were stained with Hoechst 33342 dye. The immunostaining without the incubation in primary antibody was performed to confirm the specificity of the secondary antibody binding. In control experiments activated eggs and heterokaryons were cultured in the presence of 5,6-dichlorobenzimidazole riboside (DRB, 200 μM; Sigma) (Zandomeni *et al*., 1986) to block the activity of RNA polymerase II. Control heterokaryons were microinjected with BrUTP, fixed and processed for immunostaining in the same way as the experimental ones.

Observations were performed with the help of a Zeiss inverted microscope (Axiovert 135) using commercial Zeiss filter sets: excitation filter 450–490 mm, with emission filter 520–560 nm for fluorescein. Images were captured with a CCD camera (Variocam, PCO).

Results

Thymocyte nuclei were injected into 479 artificially activated eggs; 465 survived the operation and were injected with Dig-11-dUTP or BrUTP. Thymocyte nuclei were found in 419 heterokaryons (94.1%). In the cytoplasm of activated eggs the thymocyte nuclei underwent transformation into nuclei of two types: (i) fully transformed pronucleus-like nuclei (PLN) and (ii) partly remodelled nuclei. Since these two types of remodelled thymocyte nuclei have already been described (light microscopy: Czołowska *et al*., 1984; ultrastructure: Szöllösi *et al*., 1986, 1988), here we only briefly discuss the structure of their chromatin after staining with Hoechst dye.

Thymocyte nuclei which were injected within 60 min after egg activation underwent nuclear envelope breakdown and subsequently transformed into PLN. Their chromatin was generally uniformly decondensed and resembled the chromatin structure in the pronuclei of mouse zygotes. However, in contrast to true pronuclei, in the proximity of the nucleolar precursor bodies (NPB) of PLN, thicker patches of highly condensed chromatin were frequently visible (Fig. 1*A*). In heterokaryons fixed in the second half of the first cell cycle the PLN had overgrown the female pronuclei and usually had more NPB (Fig. 2*A*).

Thymocyte nuclei microinjected 3 h after activation (at this time the activated eggs are already in interphase) rounded up, but swelling was less pronounced than in the nuclei injected within 60 min after activation. These partly remodelled nuclei never reached the size of female pronuclei. Chromatin was only partly decondensed, with many patches of more condensed chromatin scattered in the karyoplasm. The size of nuclei, and some details of their morphology, changed during the *in vitro* culture of heterokaryons. In small nuclei (2–3 h after thymocyte injection) the nucleoli were poorly visible (Fig. 1*C*). In bigger nuclei, present in heterokaryons cultured for at least 4 h, the nucleoli were well visible, although they were never surrounded by condensed chromatin, which is characteristic for female pronuclei and PLN (Fig. 3*A*, arrow).

Onset of DNA replication in heterokaryons

Detection of Dig-dU in heterokaryons injected with Dig-11-dUTP revealed intensive, punctuate fluorescence over both thymocyte nuclei and female pronuclei. Sites of incorporation of DNA precursor are localised outside NBP or nucleoli (Fig. 1*B*, *D*). In each experimental variant (thymocytes injected 60 min or 3 h after activation) six heterokaryons were treated with aphidicolin (inhibitor of DNA polymerase α) and fixed 8 h after activation. In all 12 heterokaryons both types of nuclei, i.e. female pronuclei and thymocyte-derived nuclei, were unlabelled while in untreated heterokaryons at least one nucleus was positively labelled. This confirms that the observed signal represented incorporation of Dig-dU into newly synthesised DNA. Lack of labelling was also recorded when heterokaryons were injected with Dig-11-dUTP and cultured under standard conditions but were stained without incubation in the primary antibody.

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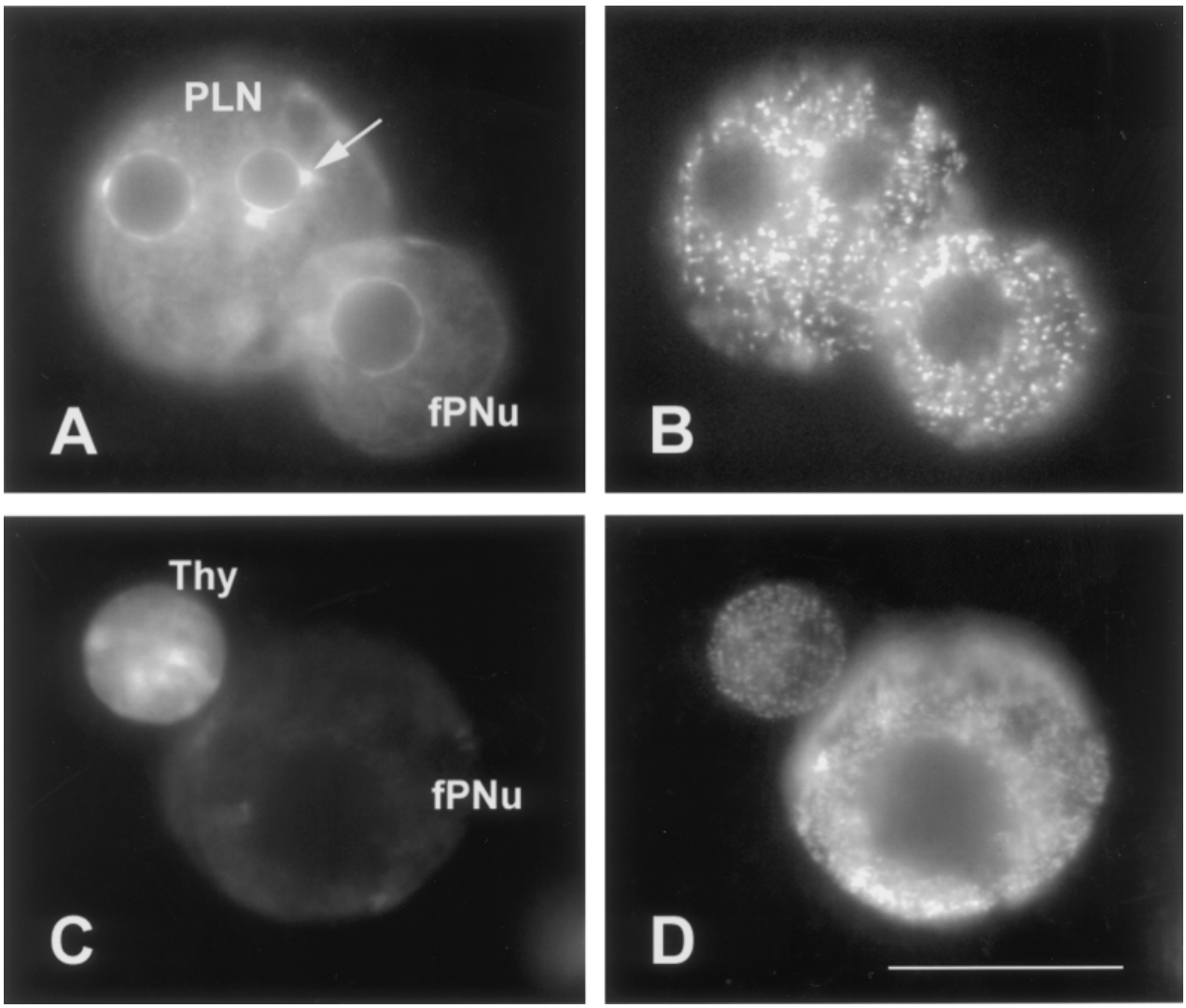

Figure 1 DNA replication in pronucleus-like nuclei (PLN), partly remodelled thymocyte nuclei (Thy) and female pronuclei (fPNu) of heterokaryons. (*A*), (*C*) DNA stained with Hoechst 33342. (*B*), (*D*) Immunofluorescent detection of Dig-11-dUTP incorporated into newly synthesised DNA. (*A*), (*B*) Thymocyte nucleus injected 60 min after activation, hetarokaryon cultured for 7 h; arrow indicates patch of condensed chromatin in the proximity of the nucleolus precursor body of PLN. (*C*), (*D*) Thymocyte nucleus injected 3 h after activation, heterokaryon cultured for 7 h. Scale bar represents 20 μm.

We observed that DNA replication in female pronuclei of heterokaryons begins approximately 6 h after egg activation. Depending on the experimental variant (thymocyte nuclei injected 60 min or 3 h after activation; respectively Table 1 and Table 2), in 33.3% or 19.2% of heterokaryons the female pronculei were replicating. Numerous bright spots of Dig-dU incorporation were always observed scattered in the karyoplasm, which suggests that replication starts simultaneously in many sites. The number of pronuclei in S-phase was increasing with time. Eight hours after activation the female pronuclei were replicating in the majority of heterokaryons (Tables 1, 2).

The PLN of thymocyte origin started DNA replication 1 h earlier than the female pronuclei. Five hours

Table 1 Initiation of replication in female pronuclei and pronucleus-like nuclei (PLN) of thymocyte origin (thymocytes injected 60 min after egg activation)

Time after activation/time after thymocyte injection (h)	% of replicating nuclei		Total no. of heterokaryons
	Female pronuclei	PLN of thymocyte origin	
5/4	0	29.4	17
6/5	33.3	66.7	18
7/6	75	83.3	24
8/7	100	100	28

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after activation in 29.4% of heterokaryons the replicating PLN were accompanied by non-replicating female pronuclei (Table 1). The proportion of replicating PLN increased with time and 8 h after activation both female pronucleus and thymocyte nucleus synthesised DNA in all heterokaryons. The pattern of fluorescence in PLN was identical to that observed in female pronuclei, i.e. numerous bright spots were scattered in the nucleoplasm outside the NPB (Fig. 1*B*).

In contrast, the beginning of DNA replication in partly remodelled thymocyte nuclei took place 1 h later than in female pronuclei, i.e. 7 h after activation. Six hours after activation, when 19.2% of female pronuclei were already replicating, there was only one heterokaryon (3.8%, Table 2) in which the thymocyte nucleus was also replicating. Seven hours after activation (4 h after injection of the thymocyte) in 55.5% of heterokaryons both nuclei, i.e. the female pronucleus and the partly remodelled thymocyte nucleus, were in the S-phase. At this time in most thymocyte nuclei chromatin decondensation was well advanced, and the nucleoli were clearly visible. However, in two cases also smaller nuclei with more condensed chromatin synthesised DNA, and the pattern of fluorescence was identical to that observed in more decondensed thymocyte nuclei, as well as in the female pronuclei. Interestingly, 8 h after activation the partly remodelled nuclei were replicating in all heterokaryons, even when the female pronucleus was not in S-phase (4 cases; Table 2).

Transcriptional activity of female pronuclei and thymocyte-derived nuclei in heterokaryons

In this part of the study we wanted to determine the onset of transcription in PLNs and female pronuclei in heterokaryons obtained after injection of thymocyte nuclei into newly activated eggs (60 min after activation). For this purpose BrUTP was injected every hour between the fifth and eighth hour after activation and heterokaryons were fixed 1 h later. Immunofluorescent analysis of BrU incorporation revealed that the female pronuclei start transcription no earlier than 8 h after activation (2 cases, 12.5%; Table 3). In heterokaryons fixed 16 h after activation all female pronuclei were transcriptionally active.

In the thymocyte-derived PLN the onset of RNA synthesis occurred earlier than in the female pronuclei. The first transcriptionally active PLN was noticed 7 h after activation (6.3%; Table 3), and 1 h later transcription was observed in as many as 68.8% of heterokaryons. Nine hours after activation RNA synthesis in PLN was detected in all heterokaryons (Table 3). PLN and female pronuclei revealed a similar pattern of fluorescence, with sites of BrU incorporation scattered in the karyoplasm. In the PLN those sites seemed to be more numerous, and often stronger signal was observed in the proxmity of some of the NPB (Fig. 2*B*, arrows). Such areas of intense BrU incorporation were never noticed in the female pronuclei.

We have shown earlier (Borsuk *et al.*, 1996) that partly remodelled thymocyte nuclei derived from thymocytes injected 3 h after egg activation were able to start transcription independently of the transcriptional status of the female pronuclei. In the present study BrUTP was injected every hour, between the fourth and eighth hour after activation, in order to find out how soon RNA synthesis starts after transfer of the thymocyte nucleus, and how long it lasts. The analysis of BrU incorporation revealed that 5 h after activation (2 h after thymocyte injection) all nuclei of thymocyte origin were transcribing (Table 4). These nuclei were rounded up but still very small, and the chromatin was only slightly decondensed. The entire area of the nucleus outside the nucleoli was filled with sites of BrU incorporation. One hour later the percentage of heterokaryons in which transcribing thymocyte nuclei were recorded decreased to 45.5%. In the remaining heterokaryons (55.5%) the karyoplasm of thymocyte nuclei was unlabelled, except for two or three discrete spots localised near the patches of more condensed chromatin surrounding the nucleoli (Fig. 4*B*). We have not observed any differences in the structure of

Table 2 Initiation of replication in female pronuclei and thymocyte nuclei undergoing partial remodelling (thymocytes injected 3 h after egg activation)

Time after activation/time after injection of the thymocyte (h)	% of replicating nuclei		Total no. of heterokaryons
	Female pronuclei	Thymocyte nuclei	
5/2	0	0	18
6/3	19.2	3.8	26
7/4	55.5	55.5	18
8/5	75	100	16

Table 3 Initiation of transcription in female pronuclei and pronucleus-like nuclei (PLN) of thymocyte origin (thymocytes injected 60 min after activation)

Time after activation/time after thymocyte injection (h)	% of replicating nuclei		Total no. of heterokaryons
	Female pronuclei	PLN of thymocyte origin	
6/5	0	0	31
7/6	0	6.3	16
8/7	12.5	68.8	16
9/8	17.6	100	17
16/15	100	100	16

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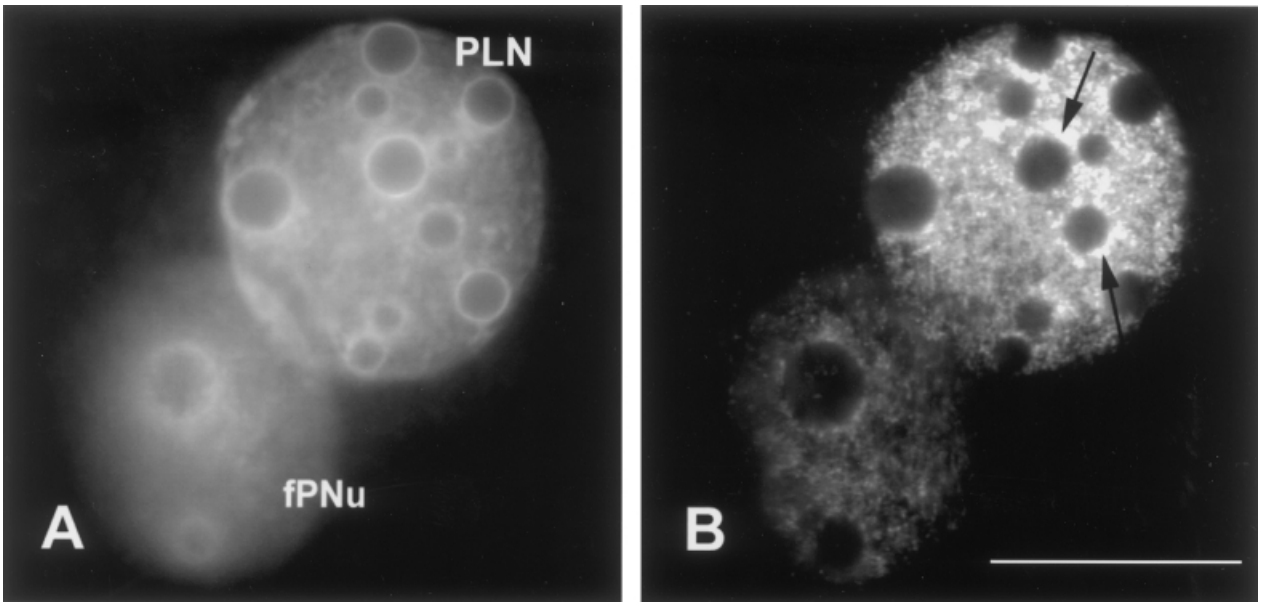

Figure 2 Transcriptional activity of pronucleus-like nuclei (PLN) of thymocyte origin and female pronucleus (fPNu). Thymocyte nucleus injected 60 min after activation, heterokaryon cultured for 14 h. (*A*) DNA stained with Hoechst 33342. (*B*) Immunofluorescent detection of BrU incorporation into nascent RNA; arrows indicate the sites of intense fluorescence in the proximity of nucleolus precursor bodies of PLN. Scale bar represents 20 μm.

Table 4 Transcription in female pronuclei and thymocyte nuclei undergoing partial remodelling (thymocytes injected 3 h after activation)

Time after activation/time after thymocyte injection	% of replicating nuclei		Total no. of heterokaryons
	Female pronuclei	Thymocyte nuclei	
5/2	0	100	14
6/3	0	45.5	55
7/4	0	62.5	16
8/5	2	60.7	54
9/6	5.3	94.7	19

transcribing and non-transcribing nuclei stained with Hoechst dye. The proportion of transcribing nuclei decreased only temporarily, and from 7 h after activation started to increase (62.5%), to reach 94.7% in heterokaryons fixed 9 h after activation.

In order to check out whether the transcriptional activity of thymocyte-drived nuclei is only nuclear, i.e. RNA polymerase II-dependent, or also nucleolar, i.e. RNA polymerase I-dependent, 1 h after thymocyte injection the heterokaryons were transferred into M2 medium containing DRB, a specific inhibitor of RNA polymerase II activity. Heterokaryons were injected with BrUTP 8 h after activation and fixed 1 h later. This protocol was applied to 30 heterokaryons obtained after injection of thymocyte nuclei 60 min after activation and to 20 heterokaryons obtained after injection of thymocyte nuclei 3 h after activation. None of 50 heterokaryons treated with DRB exhibited any signs of BrU incorporation. This result shows that independently of the type of remodelling the thymocyte nuclei exhibit only RNA pol II-dependent transcription.

Discussion

In the present study we have shown that the regulation of transcription of a somatic (thymocyte) cell nucleus injected into the cytoplasm of an activated egg is dependent on the type of its remodelling. The nuclei which undergo nuclear envelope breakdown and subsequently form PLN follow the pattern of transcriptionally silent and active periods, similarly to the female pronuclei. In contrast, the thymocyte nuclei which retain their original nuclear envelope transcribe independently of the transcriptional activity of the female pronucleus.

It was shown earlier that PLN originating from murine somatic cells other than thymocytes (erythroleukaemia (MEL), embryonal red blood cells (eRBC) or fibroblasts (3T3 cells)), when transferred into activated eggs, remain transcriptionally inactive at the beginning of the first cell cycle of heterokaryons (Borsuk *et al*., 1996; Szöllösi *et al*., 1998; Waksmundzka & Debey, 2001). Here we extend these observations by showing that the thymocyte-derived PLN start transcription in the first cell cycle of heterokaryons 1–2 h sooner than the female pronuclei. Some asynchrony in

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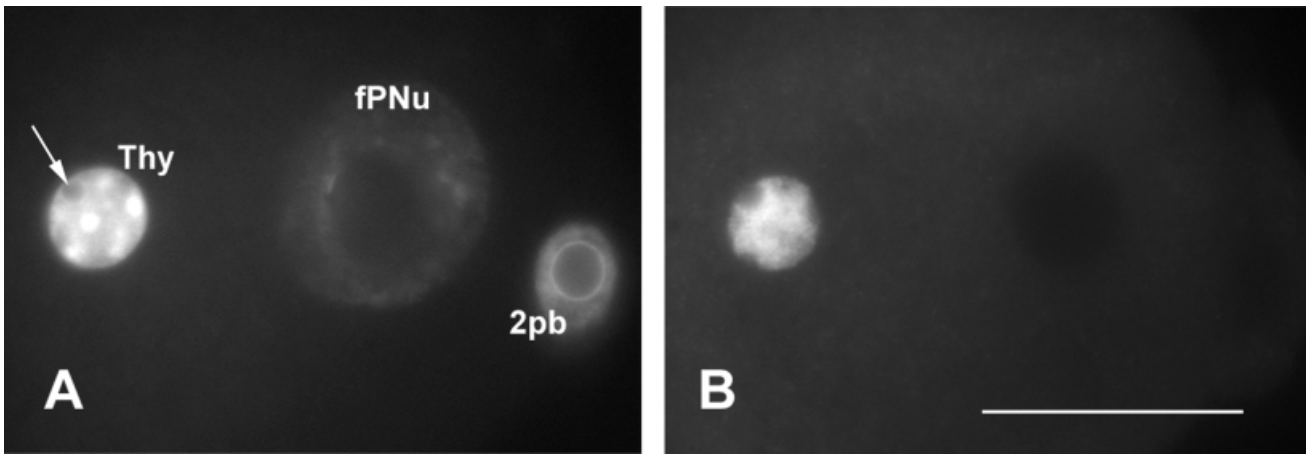

Figure 3 Transcriptional activity of partly remodelled thymocyte nucleus (Thy) and female pronucleus (fPNu). Thymocyte nucleus injected 3 h after activation, heterokaryon fixed 7 h after activation. (*A*) DNA stained with Hoechst 33342; 2pb, second polar body; arrow indicates nucleolus in the partly remodelled nucleus. (*B*) Immunofluorescent detection of BrU incorporation; female pronucleus does not show incorporation of BrU, i.e. is not transcribing. Scale bar represents 20 μm.

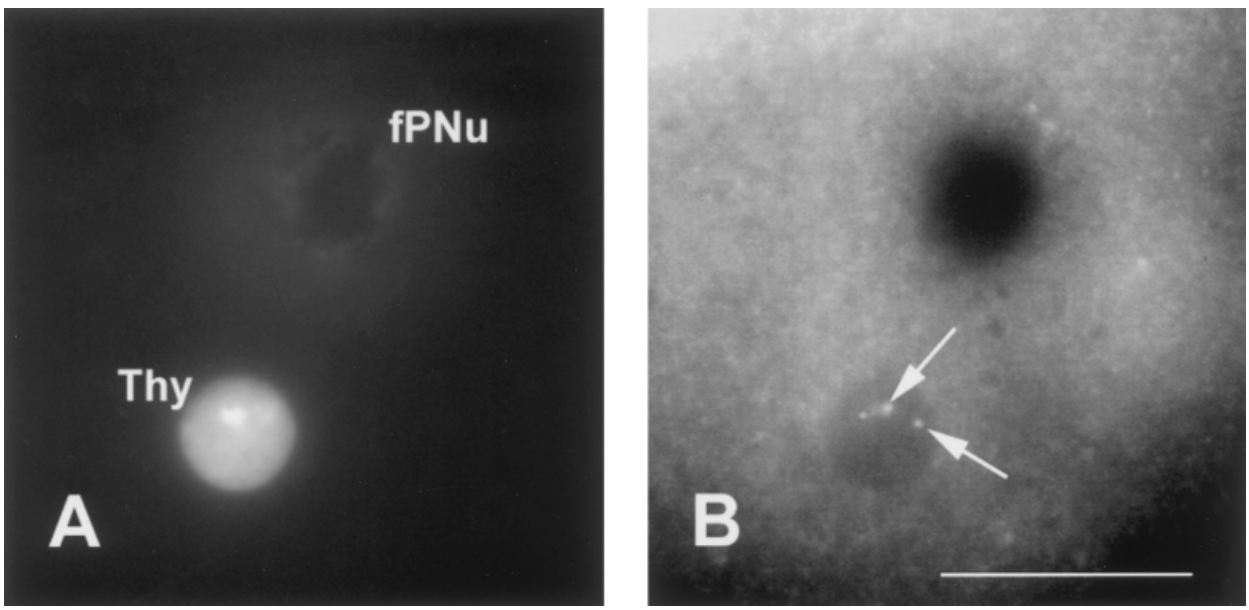

Figure 4 Heterokaryon obtained after injection of a thymocyte nucleus into an activated egg 3 h after activation and fixed 6 h after activation. (*A*) DNA stained with Hoechest 33342; Thy, partly remodelled thymocyte nucleus; fPNu, female pronucleus (*B*) Immunofluorescent detection of BrU incorporation. Neither nuclei are labelled except for three spots (arrows) in the karyoplasm of Thy localised near the patches of more condensed chromatin. Scale bar represents 20 μm.

the initiation of transcription was reported for the pronuclei in the zygote of the mouse. It was shown that the male pronucleus starts transcription sooner (Bouniol *et al*., 1995; Bouniol-Baly *et al*., 1997), and is more active in RNA synthesis, than the female pronucleus (Aoki *et al*., 1997). Different levels of hyperacetylated histones in male and female pronuclei has been proposed to be the possible explanation for the observed differences (Adenot *et al*., 1997). It is suggested that the replacement of protamines by histones, which takes place during decondensation of the sperm nucleus, permits the introduction of cytoplasmic factors that promote faster development of the male pronucleus. This explanation, although probable in the case of the male pronucleus, fails to explain the faster development and early initiation of transcription in the PLN of thymocyte origin. It was observed that thymocyte nuclei undergoing premature chromatin condensation after transfer into newly activated eggs, transit to interphase earlier and grow faster than female pronuclei (Czołowska *et al*., 1984; Borsuk *et al*., 1996). Timing of the transition to interphase can determine the timing of initiation of transcription, as was observed in parthenogenetic eggs, in which formation

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of pronuclei was accelerated by 6-DMAP treatment and in which the onset of transcription occurred sooner than in non-treated eggs (Borsuk & Maluchnik, unpublished data). Apparently, a nucleus which reforms earlier after egg activation, more quickly achieves the stage which allows initiation of transcription. One of the events which play an important role in the onset of transcription is DNA replication. Although the inhibition of the first round of replication does not prevent the initiation of transcription in mouse zygotes and parthenogenetic 1-cell embryos, it reduces the total amount of transcription (Bouniol-Baly *et al*., 1997; Aoki *et al*., 1997). It has also been shown that the expression of some polypeptides during major genome activation at the 2-cell stage depends on the DNA replication which takes place at the 1-cell stage (Davis *et al*., 1996; Davis & Schultz, 1997). On the basis of these results it has been proposed that the first round of replication provides the opportunity for the maternally derived transcription factors to gain access to DNA and, in consequence, to reprogramme the expression of genes. Most probably, the timing of the onset of replication also affects the timing of the beginning of transcription in the first cell cycle. In mouse zygotes transcription in pronuclei starts at the end of the S-phase (Bouniol-Baly *et al*., 1997). The male pronucleus initiates DNA replication sooner than the female pronucleus and, consequently, it starts to transcribe earlier (Bouniol *et al*., 1997). A similar relationship was observed in heterokaryons described in the present paper, in which the thymocyte-derived PLN were able to initiate replication and transcription sooner than the female pronuclei.

In somatic cells replication and transcription can occur simultaneously in different nuclear domains (Wansink *et al*., 1994). In contrast, in the pronuclei of mouse zygotes transcription is initiated when DNA replication is almost terminated (Bouniol-Baly *et al*., 1997). Comparison of data presented in Tables 1 and 3 suggests that in many PLN of thymocyte origin transcription can occur simultaneously with replication, as in the nuclei of somatic cells. Eight hours after activation DNA synthesis was detected in 100% of PLN, and they exhibited the pattern of labelling characteristic for the early or middle stages of the process (evaluated on the basis of patterns of S-phase progression in zygotes as presented by Bouniol-Baly *et al*., 1997). At the same time transcription was visualised in 68.8% of thymocyte-derived nuclei. These results suggest that, even when the formation of a remodelled nucleus is preceded by the breakdown of the nuclear envelope and premature chromosome condensation (Czołowska *et al*., 1984; Szöllösi *et al*., 1986, 1988), its chromatin maintains some abilities characteristic of somatic nuclei rather than pronuclei.

Thymocyte nuclei which undergo only partial remodelling and remain enclosed in intact nuclear envelopes (Szöllösi *et al*., 1986, 1988) are able to reinitiate RNA synthesis independently of the activity of the female pronucleus (Borsuk *et al*., 1996). Here we show that the activation of transcription occurs as soon as 2 h after the transfer of the foreign nucleus. It has been shown previously that the nuclei of thymocytes, freshly liberated from the thymus of 1- to 4-day-old mice, are rich in heterochromatin (Szöllösi *et al*., 1988) and do not exhibit detectable polymerase II-dependent transcriptional activity (Borsuk *et al*., 1996). Under the influence of the cytoplasm of an activated egg these nuclei undergo chromatin decondensation and reinitiate RNA synthesis. We suppose that the transcriptional machinery is already present in the thymocyte nucleus and that the process can start as soon as the chromatin becomes decondensed. Most probably the acquisition of chromatin structure which is permissive for transcription takes place even sooner than 2 h after transfer of the nucleus. Reactivation of transcription occurs in all thymocyte nuclei irrespective of the size and the degree of decondensation of chromatin they have achieved. Surprisingly, 3 h after thymocyte injection RNA synthesis is stopped again in about half the heterokaryons. The reasons for such behaviour of transferred nuclei remain unknown. Such a phenomenon has not been observed previously (Borsuk *et al*., 1996; Szöllösi *et al*., 1998) because BrUTP was not introduced as a pulse, and the overall incorporation of the precursor was detected at the end of the culture period. Moreover, when somatic nuclei were transferred by PEG-mediated cell fusion, usually many cells fused with a single activated egg, which made the interpretation of results difficult. It was observed that in e-RBC the reactivation of transcription did not occur in all heterokaryons and often in the same heterokaryon the transcribing nuclei coexisted with the non-transcribing nuclei (Szöllösi *et al*., 1998). This was explained by accidental damage to the nuclei of some of the PEG-treated cells or by competition for transcription factors between nuclei. None of these hypotheses can explain the temporary lack of RNA synthesis observed in some of the partly remodelled thymocyte nuclei as described in the present paper. We have not found any distinct differences in the morphology of transcribing and non-transcribing nuclei after staining chromatin with Hoechst dye. Probably the population of thymocytes obtained from the thymus glands of newborn mice is not fully uniform. In favourable cytoplasmic conditions they are all able rapidly to initiate RNA synthesis using factors and enzymes stored in the nucleus. Later on, when their remodelling progresses and the uptake of some proteins is necessary to sustain transcriptional activity, in some nuclei silencing of transcription takes place.

It has been shown in previous studies that remodel-

ling of thymocyte nuclei includes changes in the morphology of their nucleoli. The analysis of their ultrastructure has revealed that PLN possess filamentous and compact nucleoli similar to the NPB of the pronuclei of the mouse zygote (Szöllösi *et al*., 1988; Borsuk *et al*., 1996). In partly remodelled thymocyte nuclei, in some cases bipartite nucleoli were observed which contained a fibrillar centre, a dense fibrillar component and a granular component (Szöllösi *et al*., 1998). In other cases only nucleoli of the NPB type were found (Borsuk *et al*., 1996). These results suggest that at least in the case of bipartite nucleoli the reinitiation of polymerase I-dependent rRNA synthesis is possible. In the present study we show that both types of remodelled thymocyte nuclei, i.e. PLN and partly remodelled nuclei, exhibit only polymerase II-dependent transcription. It is possible that, under the conditions of the present experiment, nucleoli of the NPB type, in which rRNA synthesis does not take place, were formed in all thymocyte nuclei. Another explanation of the observed phenomenon is that the initiation of rRNA synthesis cannot take place in 1-cell embryo, even when the nucleoli are built of all the components typical for nucleoli of somatic cells. Transcription of ribosomal genes in mouse embryos occurs no earlier than the 2-cell stage (Telford *et al*., 1990). It has been shown also that RNA polymerase I and nucleolar protein fibrillarin, engaged in rRNA synthesis and processing of pre-rRNA, are not present in the pronuclei of 1-cell mouse embryos or the nuclei of early 2-cell mouse embryos (Cuadros-Fernandez & Esponda, 1996). The lack or inaccessibility of such important factors in artificially activated mouse eggs may explain the inability of remodelled thymocyte nuclei to reinitiated rRNA synthesis.

In general, the results presented in this study indicate that the cytoplasm of parthenogenetic early 1-cell embryos is transcriptionally permissive and that the initiation of polymerase II-dependent RNA synthesis is regulated at the level of the nucleus (pronucleus) rather than controlled by cytoplasmic factors.

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