Remodeling of mouse thymocyte nuclei depends on the time of their transfer into activated, homologous oocytes

DANIEL SZÖLLÖSI[1,*], RENATA CZOŁOWSKA[2], MARIA S. SZÖLLÖSI[1] and ANDRZEJ K. TARKOWSKI[2]

[1]*Unité de la Fécondation, INRA, Station Centrale de Physiologie Animale, 78350 Jouy-en-Josas, France*
[2]*Department of Embryology, Institute of Zoology, University of Warsaw, Krakowskie Przedmiescie 26/28, 00921 Warsaw 64, Poland*

* Author for correspondence

Summary

The potential of parthenogenetically activated mouse oocytes to remodel somatic cell nuclei was studied by ultrastructural means using oocyte–thymocyte hybrids. Complete nuclear remodeling, initiated by nuclear envelope breakdown and chromosome condensation (which is followed by formation of pronucleus-like nucleus) is possible only during a short time gap between metaphase II and telophase of meiotic division. Maturation-promoting factor activity is high during this period. The thymocyte nucleus can follow the sequence of morphological changes only in concert with the development of the native nucleus and only after exposure of the chromatin to the ooplasm. If hybridization is effected with pronucleate oocytes, the thymocyte nucleus retains its interphase character but shows particular modifications in nucleolar morphology (identical to changes observed during reactivation of the nucleolus in stimulated lymphocyte) and in the activity of the nuclear envelope (blebbing). Thus the nucleus not exposed to maturation-promoting factor activity may be influenced by a 'programme' specific for oocyte (blebbing) and by a programme inherent in the introduced somatic cell nucleus.

Key words: cell hybrids, mammal, oocyte, thymocyte, nuclear remodeling, nuclear envelope.

Introduction

Fusion of mouse thymocytes with oocytes, close to the time of parthenogenetic activation, leads to extensive remodeling of the thymocyte nuclei. The foreign nuclei follow the course of development that is characteristic of the female nucleus completing meiosis. Nuclear envelope breakdown and chromatin condensation are followed by chromatin decondensation, nuclear envelope reconstitution and nucleologenesis, which take place in parallel with the formation of the pronucleus (Czołowska *et al.* 1984; Szöllösi *et al.* 1986*b*). In contrast, no major remodeling can be induced in thymocyte nuclei that are transferred to pronucleate eggs (unpublished observations cited by Szöllösi *et al.* 1986*b*).

In the present study we show that the oocyte activities responsible for nuclear envelope removal and chromatin condensation gradually disappear from the oocyte cytoplasm after activation and are no longer detectable (at least when thymocyte nuclei are used as probes) in oocytes that are hybridized at about 2 h after activation. Complete remodeling of thymocyte nuclei with reconstitution of pronucleus-like nuclei is thus possible only during a short period between metaphase II and telophase II of meiosis. If thymocytes are fused with pronucleate parthenogenetic eggs, the nuclear envelope of the transferred nuclei remains intact and chromosome condensation is not induced. Such nuclei never develop into typical pronuclei. Rather, they follow an independent course of development involving limited swelling and decondensation as well as conspicuous changes in the nucleolar structure.

Materials and methods

Oocytes were obtained from Swiss albino (random bred) and F1(C57Bl/10 × CBA) mice that were induced to ovulate by intraperitoneal injection of gonadotrophic hormones. Procedures for inducing ovulation, harvesting and culturing oocytes, parthenogenetic activation, isolation of thymocytes, hybridization and fixation of experimental material have been described (Czołowska *et al.* 1984; Szöllösi *et al.* 1986*a*,*b*). In essence, the procedure consisted of ethanol-induced activation of zona-free oocytes (oocytes 15–17½ h after second hormone injection) and culture of oocytes in medium M16 (Whittingham, 1971) or in medium M2 (Fulton & Whittingham, 1978)

for the required time before fusion. Fusion was induced by treating the oocytes, previously agglutinated with thymocytes, with a 45–50% solution of polyethylene glycol (PEG; Fluka, M_r 2000) for 50–60 s. PEG-treated oocyte–thymocyte aggregates were cultured for 1–2½ h thereafter.

Some hybrids were fixed for light microscopy (Tarkowski & Wroblewska, 1967) to determine the hybridization success. For electron microscopy the material was fixed (Szöllösi *et al.* 1986*a*,*b*) and embedded in Epon, sectioned serially at 0·5 μm and stained with Richardson's blue stain (Richardson *et al.* 1960). As soon as nuclear structures could be recognized by light microscopy, sections were prepared for electron microscopy. All hybrids were sectioned in this manner, semi-thin sections alternating with thin sections. The stage of the female chromosome set, and the number and condition of thymocyte nuclei were recorded.

Experimental material was studied in three groups: *group I*, activated oocytes were cultured for 1 h, fused with thymocytes and cultured for another 1 h (5 hybrids studied); *group II*, activated oocytes were cultured 2, 3, 5 and 6 h before hybridization with thymocytes and cultured for a further 1 h (8 hybrids studied); *group III*, activated oocytes were cultured for 5½–6 h before fusion, hybridized with thymocytes and cultured for 2–2½ h (three hybrids studied).

To extend observations made by electron microscopy we examined and recorded by light microscopy the fate of thymocyte nuclei introduced into pronucleate oocytes. For this purpose oocytes were fused with thymocytes: (1) 4–4½ h after activation and cultured 4 h (22 hybrids), and (2) 6–6½ h after activation and cultured for 17 h (17 hybrids).

Results

Group I: thymocytes fused with oocytes between metaphase II and telophase

Two hours after parthenogenetic activation of the oocyte and 1 h after hybrid formation by fusion with PEG, meiosis advances to late anaphase–early telophase. The female chromosomes are either still individual or fused partially into a single telophase chromatin mass. No sign of nuclear envelope (NE) formation is yet seen (Fig. 1). Even though the second polar body (Pb II) is in the process of expulsion, cytokinesis is not yet completed. Under the plasma membrane of forming Pb II, a layer of thin filaments represents actin (Maro *et al.* 1984; Schatten *et al.* 1985). At the transition between oocyte and midbody and the midbody and Pb II, respectively, layers of thin filaments constitute contractile rings (Fig. 2) (Szöllösi, 1970*a*). The spindle microtubules (Mts) are on either side of the midbody and between the separating daughter nuclei. Unfused thymocytes are regularly amassed at the furrow region, some of which appose tightly to the egg plasma membrane as though they would initiate fusion (Fig. 3) (cf. Szöllösi *et al.* 1986*a*). Therefore, cell hybridization procedures and the presence of thymocytes adhering to the oocyte surface apparently do not interfere with normal reorganization of the cytoskeletal system of oocytes during activation, nor with the cortical activities associated with Pb II extrusion.

The thymocyte nuclei, which entered the oocytes by membrane fusion, are found in three different configurations: (1) nuclei that have undergone premature chromosome condensation (PCC) (Fig. 4); (2) partially condensed nuclei (Fig. 5); (3) interphase nuclei with intact but slightly swollen NE (Fig. 6).

The nuclei induced to undergo PCC form either uniformly or irregularly condensed chromatin. No remnants of the NE are present. The group of PCC chromatin is located in the oocyte periphery, within an incorporation cone (Sołtynska *et al.* 1986). The plasma membrane lacks microvilli in the cone and is lined with a layer of filaments similar to actin as is found also in the fertilization cone (Maro *et al.* 1984; Longo & Chen, 1985).

The partially condensed nuclei are also included in an incorporation cone and differ from the nuclei undergoing PCC mainly in the degree of chromatin condensation and the presence of few NE remnants (Fig. 5). The latter consist either of short NE segments with one to two pores or, more frequently, of short smooth membrane vesicles. No nucleolus or nucleolar components (e.g. fibrillar centre) are present. Occasionally Mts penetrate the chromatin and are decorated by ribosome-like particles at the chromatin cytoplasmic border (Fig. 5).

The third type of nuclear structure observed after fusion, the interphase thymocyte nucleus, is spherical. The nuclei of unfused thymocytes, in contrast, are oval, kidney-shaped or bi- or trilobed (Fig. 7). In the hybrid cell the irregularly shaped nuclei round up. The chromatin is uniformly dispersed, although a peripheral heterochromatic rim persists. The nucleolus, represented by a fibrillar centre and dense fibres only (Derenzini *et al.* 1987), is surrounded by heterochromatic aggregates (Fig. 8A). The two leaflets of the NE are separated by an expanded perinuclear space (Fig. 6). Sporadically, pores are present. In contrast to the situation observed in unfused thymocytes sticking to the oocyte (Fig. 8B) no ribosome-like particles remain attached to the cytoplasmic surface of the NE in the hybrid cell. A pair of centrioles, surrounded by pericentriolar material, may be present (not shown). Such interphase-like thymocyte nuclei are not lodged in an incorporation cone.

Group II: thymocytes fused with oocytes in telophase and pronucleate eggs (1 h culture)

After the female pronucleus is formed and becomes spherical it increases in size parallel with the time elapsed between oocyte activation and fusion. The nucleolus(i) is composed exclusively of tightly packed thin filamentous material. Pores are regularly distributed on the NE. Nuclear blebs develop, similar to those reported in the pronuclei of zygotes (Szöllösi & Szöllösi, 1988). The blebs protrude into the perinuclear space. The blebbing process is initiated in the female pronucleus of oocytes cultured 4 h after activation. Pb II is extruded in which a nucleus forms with a continuous NE usually containing a single compact nucleolus, or it may have multiple small nucleoli of the same type as is found in pronuclei. The Pb II nucleus resembles the female pronucleus, but is smaller.

The thymocyte nuclei are similar in morphology irrespective of the time interval between activation and fusion and are never within incorporation cones. They are round to oval, much of the chromatin is in a condensed heterochromatic state either throughout the nucleus

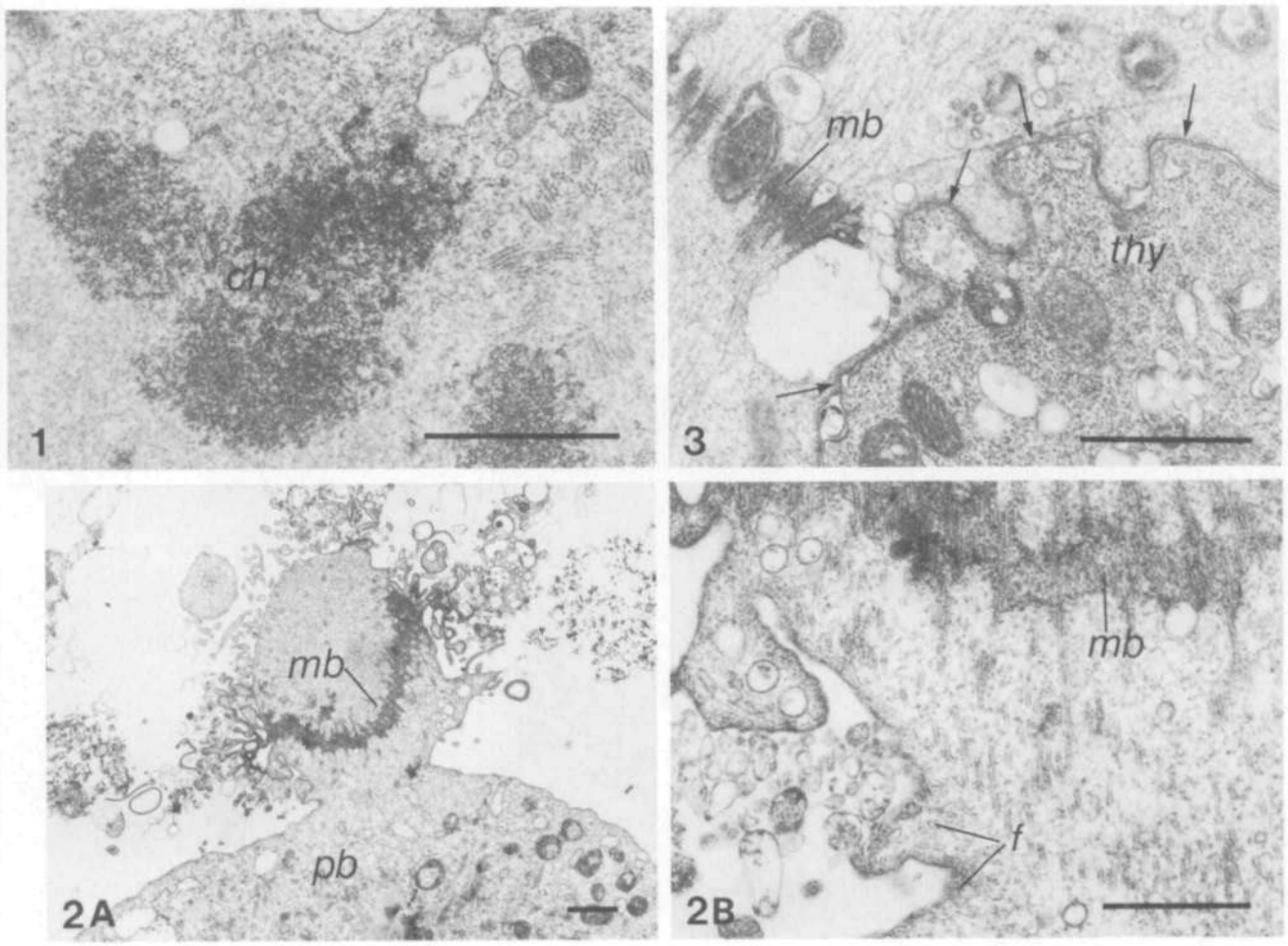

Fig. 1. Group I. Early telophase chromosomes (*ch*) of a parthenogenetically activated oocyte. No sign of NE formation can be detected. ×26000. Bar, 1 μm.

Fig. 2. A. After ethanol-induced activation the midbody (*mb*) maintains a cytoplasmic bridge between oocyte and polar body (*pb*) for several hours. Between both of the respective cells and the midbody, a layer of filaments represents a contractile (actin) ring. The plane of section is slightly oblique to the axis of the midbody and the contractile ring is visible only towards Pb II. ×6300. B. Corresponding to the level of the initiated cytokinesis, thin filaments (*f*) represent the contractile ring towards Pb II. *mb*, midbody. ×40000. Bars: A, 1μm; B, 0·5 μm.

Fig. 3. A thymocyte (*thy*) adheres very closely and uniformly to the plasma membrane at the midbody (*mb*). A layer of membrane-associated thin filaments on the side of the Pb is indicated by arrows. ×22500. Bar, 1 μm.

(Fig. 9) or along the NE and around the fibrillar centre. The nucleolus may be present in two different configurations, represented by fibrillar centres and dense fibres (Fig. 10), as in the thymocytes prior to hybridization, or in various degrees of development of a bipartite nucleolus. The latter is made up of the dense, compact thin filament-containing part and the granular part. There are euchromatic nuclear areas between the peripheral and perinucleolar heterochromatin (Figs 11, 12). The NE has the usual structure, but the perinuclear space is sometimes slightly swollen. As in the hybrids from group I containing interphase nuclei, polysomal clusters are not attached to the NE of any of the thymocyte nuclei present in the ooplasm. No region was found in the hybrid cytoplasm that was rich in ribosome-like particles. The small volume of the thymocyte cytoplasm evidently dispersed rapidly leaving only a pair of centrioles and the morphologically characteristic mitochondria as a recognizable thymocytic contribution to the hybrid cytoplasm (not shown). Occasionally a small Mt bundle can be seen in the proximity of the thymocyte NE (Fig. 12).

Two hybrids represent rare cases in which a thymocyte fused with Pb II. These thymocyte nuclei behave similarly to those present in the oocyte. Their volume is smaller, however, the chromatin is denser and it has a more homogeneous appearance.

Group III: thymocytes hybridized with pronucleate oocytes (2–2½h culture)

In comparison with group II, hybrid formation in this group was initiated only 5–6 h after activation, but they were cultured for a longer time after initiation of cell fusion (2–2½ h instead of 1 h). No structural changes are observed in the female pronucleus. Nor are significant modifications observed in the chromatin structure of the

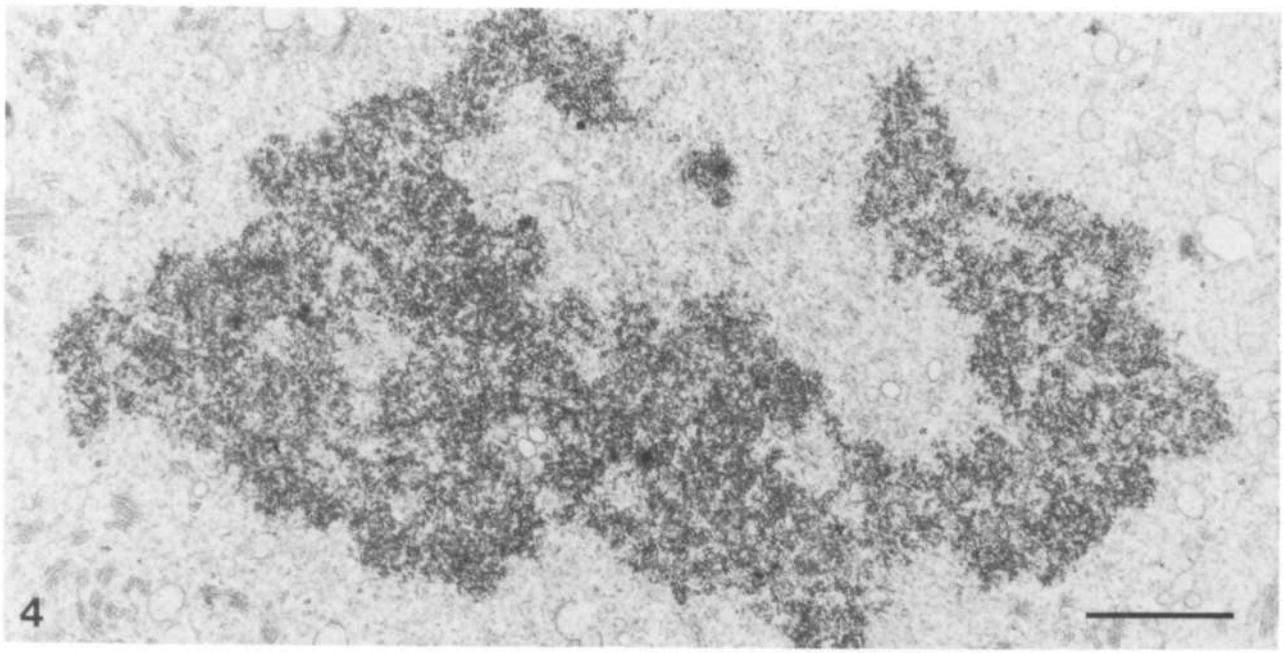

Fig. 4. Group I hybrid: thymocyte chromatin denuded of its NE during PCC. ×19 000. Bar, 1 μm.

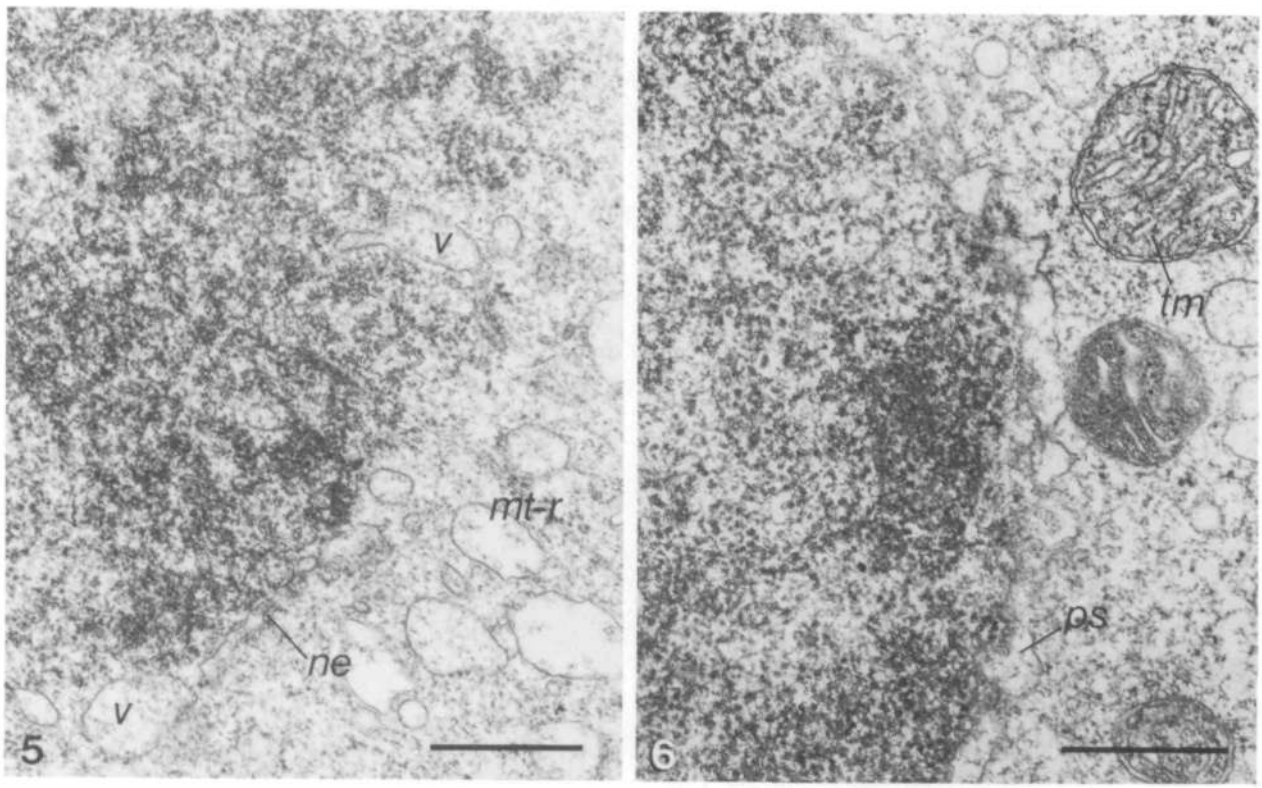

Fig. 5. Group I hybrid: cytoplasmic smooth-membraned vesicles (*v*) surround a partially condensed, denuded thymocyte chromatin. Note the presence of some NE remnants (*ne*); close to the chromatin peripheral microtubules are decorated by ribosome-like particles (*mt-r*) for a short distance. ×40 000. Bar, 0·5 μm.

Fig. 6. Group I hybrid: thymocyte nucleus is slightly swollen. The chromatin is fairly compact and the perinuclear space (*ps*) is irregular. Nuclear pores are rarely identifiable. The arrow indicates a thymocyte mitochondrion (*tm*). ×50 000. Bar, 0·5 μm.

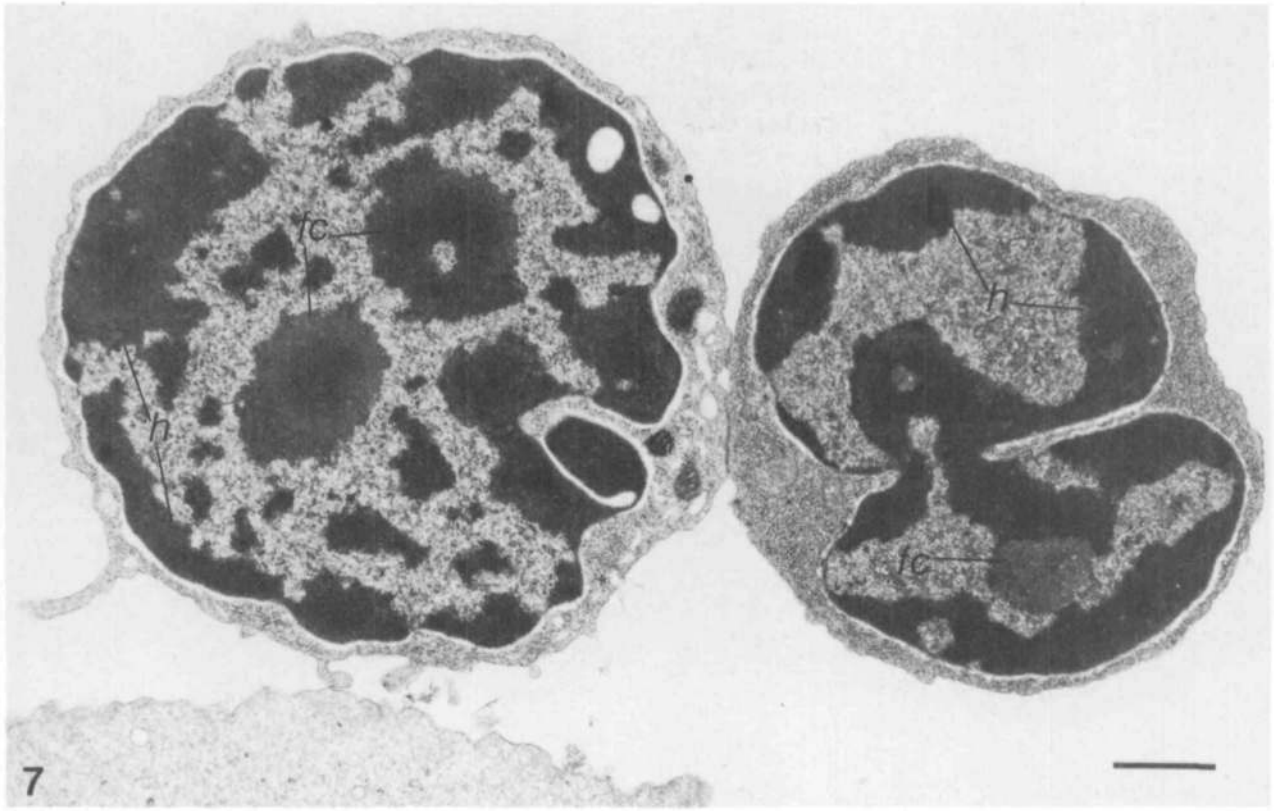

Fig. 7. Unfused thymocyte nuclei are rich in heterochromatin (*h*) (often peripheral); one of two fibrillar centres (*fc*) are the only recognizable nucleolar element. The nuclei are either kidney-shaped or are bi- or trilobed. ×14 000. Bar, 1 μm.

thymocyte nuclei: most of the chromatin remains heterochromatic along the NE and around the nucleolus. Surprising modifications observed in the thymocyte nucleoli in hybrids from group II are accentuated by further development of nucleoli, the expansion of a fibrillogranular outer portion around a compact fibrillar centre and dense fibres (Figs 13, 14). This may occur also in the rare cases in which a thymocyte successfully formed a hybrid with Pb II (Fig. 15). The Pb II nucleus resembles the female pronucleus in every respect, but it is slightly smaller. Four small nucleoli are present, composed exclusively of thin filaments. Two small nucleoli have a small, peripheral, filamentous centre. Regions of the perinuclear space enlarge and blebs and empty membrane vesicles occasionally form in them. The Thy nucleus is electron-opaque and forms a fibrillogranular, complex nucleolus as described above. Centrioles were never observed in this group of hybrids.

Further fate of thymocyte nuclei in pronucleate oocytes (light-microscopic observations)

Thymocyte nuclei introduced into pronucleate oocytes (4 h and 6 h after activation) and incubated in the oocytes for a longer time (4 h and 17 h, respectively) are in two different interphase conditions. In more than 90% of hybrids nuclei swell and decondense but are completely distinguishable from the female pronucleus by their darkly stained karyoplasm. These thymocyte nuclei never increase in diameter to the same extent as the female pronucleus. They are characterized by a varying number of nucleoli (the female pronucleus always has one compact nucleolus at this time) with a central core surrounded by a darker staining rim. In hybrids approaching the first cleavage division some thymocyte nuclei initiate mitotic condensation concomitantly with the female pronucleus.

Decondensed nuclei are often accompanied by small, round heterochromatic nuclei, which may correspond to round condensed nuclei described in hybrids from group II.

Discussion

Mouse oocyte cytoplasmic factor(s) inducing remodeling of thymocyte nuclei persists during the second meiotic division

The use of thymocyte nuclei appears to be a very sensitive tool in studying the presence of active factors in the cytoplasm of mammalian oocytes. Following fusion with ovulated mouse oocytes, the thymocyte nucleus is exposed to the influence of ooplasmic factors (Czołowska *et al.* 1984; Szöllösi *et al.* 1986*a*) as is the case for the sperm nucleus during sperm penetration (Szöllösi & Ris, 1961). The thymocyte nucleus is accompanied by very few cytoplasmic organelles in comparison with the oocyte and they disperse rapidly in the cytoplasm of the hybrid. Using thymocytes as donor test nuclei, we were able to demonstrate the presence of oocyte activities ('factors')

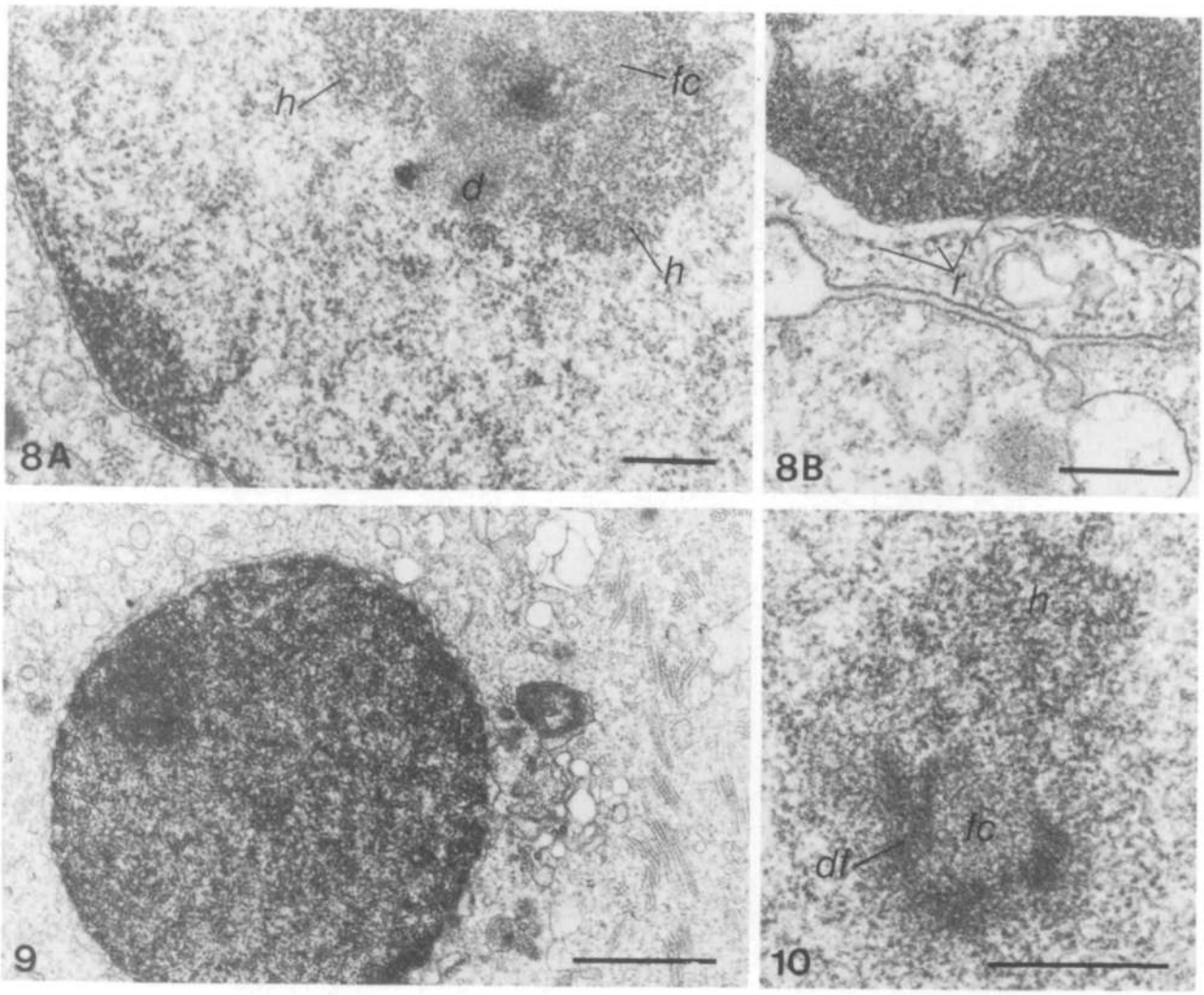

Fig. 8. A. Group I hybrid: within the rounded, slightly swollen thymocyte nucleus, the fibrillar centre (*fc*) and a small dense (*d*) area are surrounded by perinucleolar heterochromatin (*h*). ×36 000. B. The nuclear envelope of a thymocyte adhering to, but not fused with, an oocyte. Ribosome-like particles (*r*) are attached in large numbers to the nuclear envelope. ×64 000. Bars: A, 0·5 μm; B, 0·25 μm.

Fig. 9. Group II hybrids: thymocyte nucleus became spherical but its chromatin retains its dense packing. The NE changes little and has many pores. ×20 000. Bar, 1 μm.

Fig. 10. Only the fibrillar centre (*fc*), dense fibres (*df*) and some 'perinucleolar' heterochromatin (*h*) represent the nucleolus in thymocyte nuclei in group II hybrids. ×50 000. Bar, 0·5 μm.

responsible for chromatin condensation and decondensation, for NE removal and reconstitution, as well as a factor promoting formation of compact nucleoli usually found in pronuclei (Szöllösi, 1970*b*). The coming-into-play of an ordered series of factors results in remodeling of transferred nuclei according to the pronuclear pattern (Czołowska *et al.* 1984; Szöllösi *et al.* 1986*b*). All factors operate in ovulated mouse oocytes close to the time of activation (20–50 min), that is, at the onset of the first cell cycle (Szöllösi *et al.* 1986*b*). In the present series of experiments, we show that the factors required for the complete morphological remodeling of thymocyte nuclei do not persist beyond the period delimited by the completion of the second meiotic division. The factors disappear gradually from the cytoplasm after oocyte activation.

The most distinct expression of nuclear changes involved in remodeling comprises breakdown of the nuclear envelope and condensation of the chromatin. These nuclear changes must be attributed to MPF (maturation-promoting factor, M-phase promoting factor), a 'factor' probably composed of the sum of activities of a number of different factors, which still operate in metaphase II oocytes (Bałakier, 1979; Tarkowski & Bałakier, 1980; Sörensen *et al.* 1985). While the PCC reaction is a measure of high MPF activity, the enlargement of the perinuclear space can be considered as the weakest response (group I). The enlargement of the perinuclear space may have been caused by the removal of the pores from a part of, or from the totality of, the NE. In fact the disappearance of nuclear pores during germinal vesicle breakdown (GVBD; Calarco *et al.* 1971; Szöllösi *et al.*

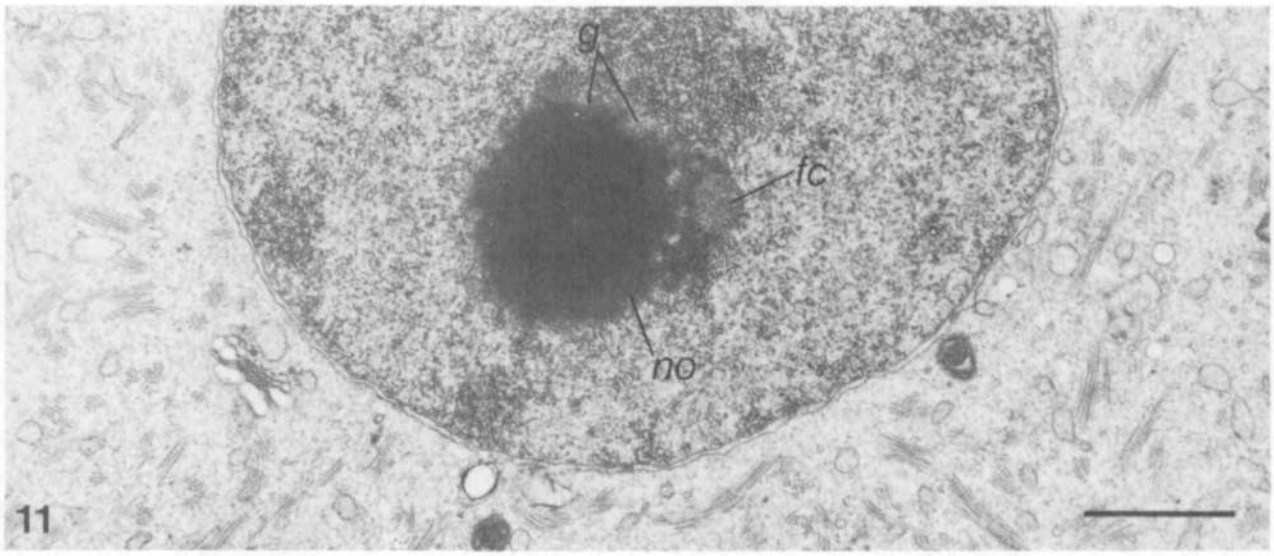

Fig. 11. Structurally very complex nucleoli (*no*), typical of nuclei actively engaged in production of pre-ribosomal granules (*g*) and the usual fibrillar centres (*fc*) are often formed in group II hybrids. ×20 000. Bar, 1 μm.

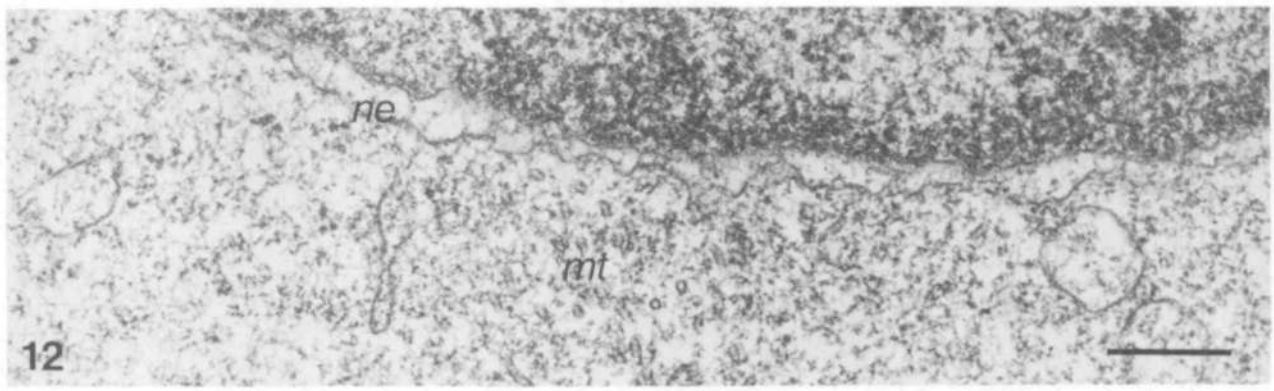

Fig. 12. Ribosome-like particles are not adhering to the NE (*ne*) in group II hybrids. A microtuble bundle (*mt*) is seen near the NE. ×63 000. Bar, 0·25 μm.

1988) and mitotic somatic and primordial germ cells (Szöllösi *et al.* 1972) has been reported just prior to NE breakdown and promises to be a general phenomenon in the cell cycle. It is evident that if an activated oocyte is cultured longer than the time during which MPF is effective, the original NE of the thymocyte will be conserved. Differential behaviour of nuclei in oocytes from group I may reflect individual developmental progression of activation, or may be an effect of asynchronous fusions and/or dilution of 'factors' by many competing nuclei (cf. Szöllösi *et al.* 1986*b*; Clarke & Masui, 1986, 1987).

Thymocyte nuclei that are first induced to PCC develop into typical 'pronuclei' and synthesize DNA (unpublished experiments). In zygotes transcription is in general blocked (Howlett & Bolton, 1986; Clegg & Piko, 1983). Mammalian somatic cell nuclei (hepatocytes) transplanted to *Xenopus* (Brun, 1973) or to hamster oocytes (Naish *et al.* 1987) at the time of oocyte activation enter into S-phase concomitantly with the female pronucleus. Frog erythrocyte nuclei also synthesize DNA after 'conditioning' and transfer into *Rana* oocytes (Leonard *et al.* 1982). Morphological and functional remodeling seems to be possible only after exposure of denuded chromatin to the ooplasm. The significance and necessity of exposure of the transplanted somatic nuclei to MPF (ooplasmic factors) containing cytoplasm for modification of the developmental potential of the nucleus has been emphasized by DiBerardino and her associates (reviewed by DiBerardino *et al.* 1984; DiBerardino, 1986). The serial nuclear transfer (the passaging of donor nuclei through the cytoplasm of Meta I oocytes) developed by them in fact increases the time of access of the cytoplasmic proteins of the host cytoplasm to the donor chromatin. These proteins, which may be trapped with the chromatin when the NE is reconstituted, may represent the key factors responsible for the genetic reprogramming of nuclei when introduced into oocytes. The participation of cytoplasmic regulatory elements on expression of 'muscle genes' in a human amniotic cell fused with a multinucleated, mouse myotube has been clearly demonstrated (Blau *et al.* 1983).

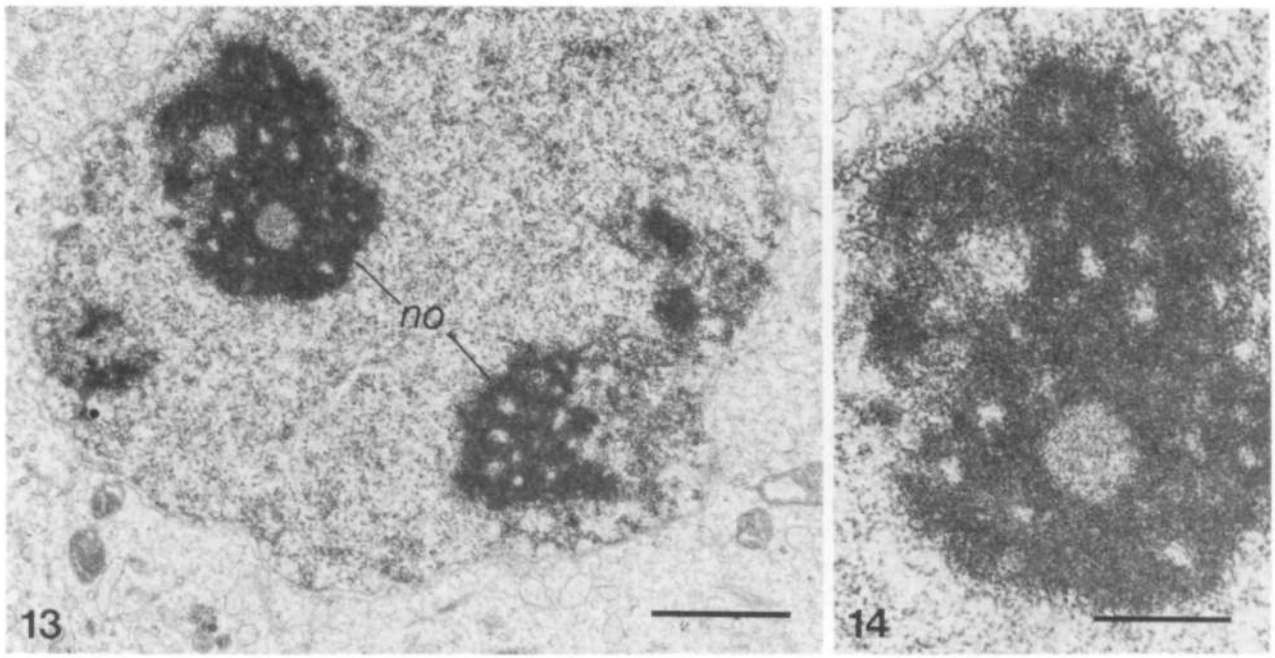

Fig. 13. In the hybridized thymocyte nucleus complex, large, fibrillogranular nucleoli (*no*) develop in group III hybrids. ×18000. Bar, 1 μm.
Fig. 14. Higher magnification view of one of the nucleoli in Fig. 13. ×37000. Bar, 0·5 μm.

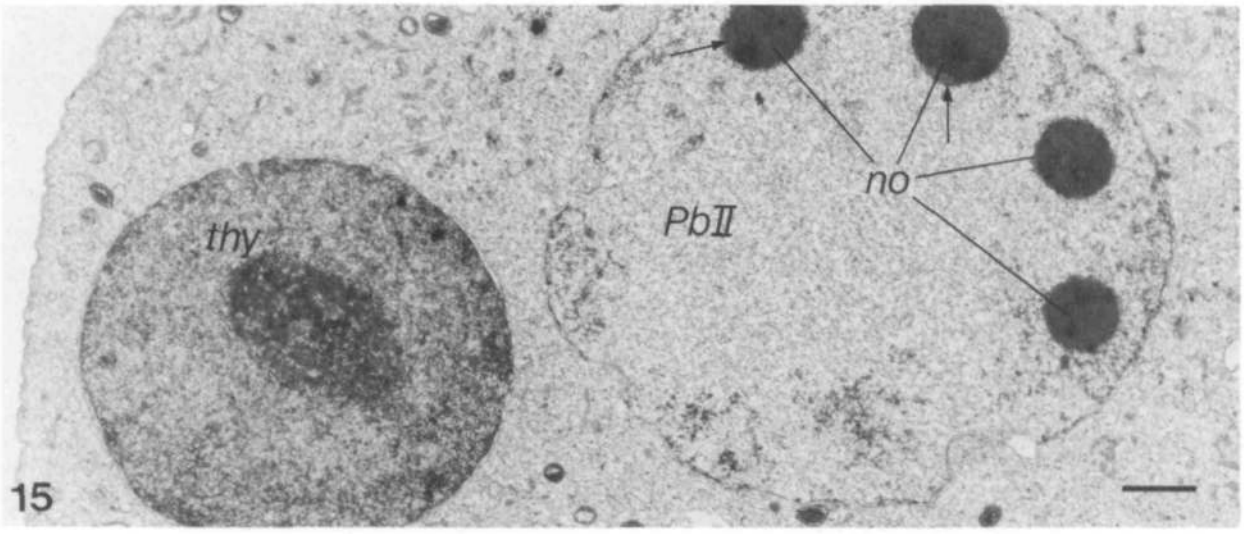

Fig. 15. In rare cases a Pb II and thymocyte hybrid is formed. The Pb II nuclear chromatin is decondensed (*Pb II*) and has four dense, fibrillar, pronucleus-type nucleoli (*no*). The arrows indicate a peripheralized fibrillar centre (*fc*) along two pronucleus-type fibrillar nucleoli. The thymocyte nucleus (*thy*) is electron-opaque and has a large, reticulated nucleolus. ×9500. Bar, 1 μm.

During the formation of the new nuclei, as is also the case in late telophase, some of the cytoplasmic proteins reprogramme nuclei during mitosis (Swanson & McNeil, 1987).

The timing of developmental events triggered by artificial (ethanol) activation is not significantly modified by the hybridization procedures applied here. Pb II abstriction is sometimes delayed if hybridization follows activation within a short interval (Szöllösi *et al.* 1986*b*). Disappearance of remodeling factors (MPF), timed in respect of the oocyte's developmental age, occurs at the final stage of the second meiotic division, i.e. early telophase, and may depend on the increasing activities of inhibitors of the mitotic factors (M) (Adlakha & Rao, 1986). The critical time period within which thymocyte nuclei may remodel (metaphase II – telophase) is in fact the period 'reserved' for remodeling of the sperm nucleus to form the male pronucleus. Remodeling factor activity, therefore, should be understood as the oocyte's contribution to the facilitation of fertilization. What is unexpec-

ted, however, is the fact that the oocyte cytoskeletal elements react to a foreign, denuded somatic cell chromatin by forming the incorporation cone (see also Sołtynska *et al.* 1986) and that this chromatin is treated (remodeled) in a similar way to a sperm nucleus (except for the removal of the sperm nuclear protamines).

In oocytes that were hybridized when the female pronucleus was completely formed after activation, the thymocyte nuclei retain their interphase character. The nuclear envelope is intact and nuclear pores are present. The NE breakdown activity of the ooplasm either disappears in pronucleate eggs or is no longer demonstrable in the system using interphase nuclei as a probe.

Interphase organization of thymocyte nuclei can be modified also in the absence of MPF

The MPF is either drastically modified or disappears entirely after the oocyte has completed the second meiotic division (MPF may be destroyed by the same mechanism as in somatic cells; see review by Adlakha & Rao, 1986) that permits the donor nucleus to retain interphase. The overall structure of thymocyte nuclei transferred to pronucleate eggs was only slightly modified by the transformation of the originally lobed or kidney-shaped nuclei into a smooth sphere. Transformation into a sphere may be the result of a limited uptake of substances, primarily those with small water-soluble molecules, i.e. salts, amino acids, nucleosides and small peptides. In somatic cell hybrids a slight nuclear swelling also takes place at the time of hybridization (Ringertz & Savage, 1976). The thymocyte nuclei retaining their nuclear envelope swell to a limited extent after longer culture (see Materials and methods) but do not decondense to the same degree as those thymocyte nuclei from which the envelope has been removed and a new one reconstituted. In the latter case, thymocyte nuclei are approximately the same size as fully grown pronuclei (Szöllösi *et al.* 1986*b*). On the other hand, the swelling, sometimes extensive, that nuclei of embryonic cells undergo after hybridization with activated oocytes does not depend on NE breakdown (unpublished experiments). It seems, therefore, that the intact thymocyte nuclear envelope, or any NE for that matter, constitutes a barrier to massive protein migration and exchanges and, in turn, to chromatin reorganization. Only after exposure of denuded thymocyte chromatin to the ooplasmic environment does the major shift in nuclear behaviour appear possible (Szöllösi *et al.* 1986*b*). Similar conclusions have been reached by embryologists using an amphibian oocyte system for reprogramming transplanted erythrocyte nuclei (reviewed by DiBerardino *et al.* 1984).

Incubation in activated ooplasm has a clear effect on the association of the ribosome-like particle with thymocyte NE. Ribosome-like particles decorating the NE of free thymocytes are no longer observed in oocyte–thymocyte hybrids containing interphase nuclei. The particle-removing capacity of ooplasm must appear as an effect of oocyte activation, because in non-activated, ageing oocytes, which are characterized by a weakened MPF activity and gradual shifting to interphase (Czołowska *et al.* 1986; Eichenlaub-Ritter *et al.* 1986; Kubiak, personal communication), ribosome-like particles remain attached to the outer leaflet of NE fragments retained by thymocyte nuclei (unpublished results).

Thymocyte nuclei introduced into pronucleate eggs respond to a longer incubation in ooplasm (2–2½ h; group III) by particular modifications of the interphase appearance. The first modification involves development of a bipartite nucleolus with a fibrillogranular peripheral portion. This occurs in a nucleus that, at the time of fusion, consisted only of the fibrillar centre and dense fibres, lacking all other nucleolar components. Moreover, it occurs in a cell, the activated oocyte, that has no RNA-synthetic activity. Mouse embryonic nuclei become active in ribosomal RNA (rRNA) transcription not earlier than the third cell cycle (Hughes *et al.* 1979). The fibrillogranular nucleolar component formed then for the first time (Hillman & Tasca, 1969; Szöllösi, 1970*b*). It is a matter of speculation whether our observation may be taken as favouring the presence of 'inducers' of RNA synthesis in the one-celled mouse embryo, which may be unable to operate under the usual conditions when the compact filamentous nucleolus, typical of pronucleate eggs, is present. This particular nucleolar organization is accomplished by association of the nucleolus with basic proteins at the end of oogenesis (Sörenson & Wassarman, 1976). A further hypothesis, which we consider to be more likely, is that the thymocyte nucleus entering the oocyte cytoplasm is endowed at the moment of its entry with all the necessary enzyme systems for the synthesis of pre-rRNA, the role it was programmed to perform as its original function after recognition of a new antigen in the circulation (Harris, 1986). Small, water-soluble molecules, the essential substrates for the synthetic activities, which enter freely into the thymocyte nucleus, are provided by the oocyte. In contrast large, diffusible molecules, e.g. multienzyme complexes and cytoplasmic factors, do not enter freely into the nucleus. The permeability rate is unrelated to molecular size or charge (Brachet, 1987; DeRobertis *et al.* 1977). The karyophilic molecules must bear a 'recognition' marker for entry (Dingwall *et al.* 1986; Newmeyer & Forbes, 1988; Richardson *et al.* 1988). Most of the bidirectional molecular traffic takes place *via* the pores (Milligan, 1986). By the use of fluorescein-labelled dextrin the size limit for free molecular diffusion between the cytoplasmic and nuclear compartments was set to be about $10^3 M_r$. Larger molecules ($>40\times10^3 M_r$) were found to have access to the nucleus only when the nuclear envelope was not intact (Swanson & McNeil, 1987). DNA re-replication of an entire genome in a *Xenopus* oocyte cytosol system is also only possible after its passage through mitosis and a new S-phase or permeabilization of the NE (Blow & Laskey, 1988).

The modification of nuclear structure described for groups II and III, primarily concerning the development of the bipartite nucleoli in thymocyte nuclei, which are structurally comparable to nucleoli of any somatic cells actively involved in transcription of the ribosomal genome (Derenzini *et al.* 1987; Fakan, 1986), is independent of, or even in spite of, the host oocyte cytoplasm.

Rapid morphological remodeling of thymocyte nucleoli after their fusion with the oocyte compares favourably with changes described in circulating lymphocytes in which rRNA synthesis was stimulated by phytohaemagglutinin (PHA) (Derenzini *et al.* 1987). The time scale with which these changes transpire is, however, quite different. Large increase in rRNA synthesis concomitant with nucleolar development is observed 20 h after PHA stimulation in lymphocytes, while in our hybrids nucleolar remodeling is induced after 1–2 h (groups II and III, respectively) following cell hybridization (RNA-synthetic activities are currently under investigation).

A total of 1–2 h is also required for expression of 'muscle genes' in human amniocyte–mouse myotube heterokaryon formation (Blau *et al.* 1983), a delay of very similar duration to the appearance of the complex nucleolus in the thymocyte nucleus. Since rRNA synthesis in mouse development is observed first at the four-cell stage, and in significant quantities only in the subsequent cell cycle, the nucleolar transformations, the fragmentation of the fibrillar centres and the development of the extensive granular component must be due only to the presence in the thymocyte of all the enzyme systems necessary for the activation and transcription of the rRNA genome.

It should be recalled that thymocyte nuclei that have been induced to PCC and remodeled in mouse oocytes reconstitute interphase nuclei with compact, pronucleus-like nucleoli (Szöllösi *et al.* 1986*b*). During induction of PCC the thymocyte nucleolus (only fibrillar centre and dense fibres were recognized in small thymocytes) disperses in the same manner as it does in mitotic or meiotic prophase. Formation of the pronucleus-type of compact nucleolus depends therefore on the exposure of denuded chromatin to the ooplasm, becoming 'conditioned' by its active factors to develop according to the cytoplasm-specific nucleolus. On the other hand, development of the bipartite nucleolus is comparable only with nucleolar activation in somatic cell hybrids, formed between interphase cells (Hernandez-Verden & Bouteille, 1979; Derenzini *et al.* 1987; Setterfield *et al.* 1983). The speed with which the bipartite nucleolus develops in oocyte–thymocyte hybrids (2 h) is impressive in comparison with nucleolar reactivation in nuclei of chicken erythrocytes hybridized with rat epithelial cells (minimum 24 h; Dupuy-Coin *et al.* 1976).

Recapitulating, in our experimental system only those thymocyte nuclei that were 'injected' into it by PEG hybridization close to the time of its parthenogenetic activation conform to the host egg's cytoplasmic activities. Thymocyte nuclei introduced after this time no longer conform to the activities of the host oocyte as is the case with small lymphocytes injected into *Xenopus* oocytes (Gurdon & Brown, 1965; Gurdon, 1968, 1986). The cytoplasmic factors are thus either less active or totally destroyed.

The second modification of the interphase thymocyte nucleus submitted to a longer incubation in the ooplasm (group III) is blebbing of the nuclear envelope, a stage-specific phenomenon of pronuclear activity of the mouse zygote, has been published (Szöllösi & Szöllösi, 1988). Blebbing occurs in the pronucleus of parthenogenetically activated oocyte as soon as the pronucleus forms and the chromatin decondenses ($3\frac{1}{2}$–4 h in mouse). In thymocyte nuclei, in contrast, blebbing is induced after 2–$2\frac{1}{2}$ h exposure to the cytoplasm of the haploid, gynogenetic parthenote. Blebbing represents an excellent marker for initiation of new functions by the transferred nucleus in concert with the activated oocyte programme.

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