J. Embryol. exp. Morph. **93**, 213–238 (1986)
Printed in Great Britain © *The Company of Biologists Limited 1986*

Regulation of development in the fully grown mouse oocyte: chromosome-mediated temporal and spatial differentiation of the cytoplasm and plasma membrane

JONATHAN VAN BLERKOM AND HOBART BELL
Department of Molecular, Cellular and Developmental Biology, University of Colorado, Boulder, Colorado, USA

SUMMARY

The relationship between nuclear maturation and the differentiation of the cytoplasm and plasma membrane during resumption of arrested meiosis was investigated by culture of GV- and MII-stage mouse oocytes in the presence and absence of nocodazole. Culture in the presence of nocodazole was associated with dispersal of MI and MII chromosomes throughout the subplasmalemmal cytoplasm. A progression of cortical (thickening of actin filaments) and plasma membrane changes (denudation of microvilli, reduction in cell surface glycoproteins, formation of chromosome-containing evaginations) that normally occurs in proximity to chromosomes associated with intact MI or MII spindles took place only in those regions of the cortical cytoplasm containing the dispersed subplasmalemmal chromosomes. The dispersion and migration of the chromosomes occurred in an apparently random fashion. Fluorescent probe analysis of normal and treated oocytes indicated a stage-specific association between the spatial distribution of chromosomes and mitochondria. Transfer of individual bivalent chromosomes to untreated oocytes at different stages of maturation and to cytoplasts derived from oocytes anucleated prior to GVB demonstrated (1) the necessity of chromosomes for cytoplasmic and plasma membrane differentiation, and (2) that the capacity of the cytoplasm and plasma membrane to differentiate in response to the presence of a chromosome is acquired prior to GVB.

INTRODUCTION

The fully grown mammalian oocyte develops the ability to be fertilized during the period of resumed meiosis that occurs no sooner than several hours prior to ovulation. Fertilizability is the culmination of a rapid progression of intrinsically regulated changes (Van Blerkom, 1985*a*) in nuclear (Zamboni, 1970; Van Blerkom & Runner, 1984), cytoplasmic (Zamboni, 1970; Van Blerkom & Motta, 1979; Van Blerkom & Runner, 1984) and plasma membrane structure and organization (Johnson, Eagery, Muggleton-Harris & Graves, 1975; Nicosia, Wolf & Inoue, 1977; Maro, Johnson, Webb & Flach, 1986). In the laboratory mouse oocyte, cytoplasmic maturation involves changes in the organization and spatial distribution of Golgi complexes (Zamboni, 1970), cortical granules (Nicosia *et al.* 1977) and mitochondria (Van Blerkom & Runner, 1984). At the molecular level, oocyte maturation is characterized both by the cessation of transcription prior to

Key words: mouse oocyte, regulation, nocodazole, nuclear maturation, chromosomes, plasma membrane.

germinal vesicle breakdown (GVB) (Wassarman & Letourneau, 1976) and by the expression of stage-related alterations in quantitative and qualitative patterns of protein synthesis and post-translational modification (Schultz & Wassarman, 1977; Richter & McGaughey, 1983; Van Blerkom, 1985*c*).

Developmental changes in the structure, organization and composition of the plasma membrane and cortical cytoplasm are restricted to the region of the oocyte that contains MI or MII chromosomes that are associated with their respective spindles. The cortical cytoplasm overlying the metaphase spindles is devoid of cortical granules (Nicosia *et al.* 1977), Golgi complexes (Van Blerkom & Motta, 1979) and mitochondria (Zamboni, 1970; Nicosia *et al.* 1977). The plasma membrane in this region is denuded of microvilli (Johnson *et al.* 1975), contains a significantly reduced population of cell surface glycoproteins (Eager, Johnson & Thurley, 1976) and exhibits a marked change in the distribution and organization of diffusible membrane proteins (Wolf & Ziomek, 1983). Differentiation of the subplasmalemmal cytoplasm associated with the presence of the metaphase spindles is indicated by a pronounced, localized thickening of actin filaments (Nicosia *et al.* 1977; Maro, Johnson, Pickering & Flach, 1984). These changes establish an apparent polarity in the preovulatory oocyte that is expressed in the structure of the cytoplasm and plasma membrane that overlies the metaphase spindles (Johnson *et al.* 1975; Longo & Chen, 1985).

Studies of nuclear, cytoplasmic and molecular development during reinitiated meiosis suggest that maturation of the mouse oocyte is regulated by an intrinsic programme directed at the cytoplasmic rather than at the genomic level (Van Blerkom, 1985*a*). The present study examines the relationship between nuclear changes and developmental alterations in the spatial distribution of mitochondria, and the structure and organization of the cortical cytoplasm and plasma membrane during resumed meiosis in the presence of nocodazole, a potent inhibitor of microtubule polymerization (Hoebeke, Van Nigen & De Brabander, 1976). We report that structural and molecular changes in the oocyte cytoplasm, plasma membrane and underlying cortex that normally occur in proximity to MI and MII chromosomes and associated spindles, (1) are mediated or induced by the presence of chromosomes, (2) do not appear to involve direct genomic participation or expression and (3) may require a nuclear-dependent modification of the cytoplasm.

MATERIALS AND METHODS

Oocyte collection and culture

Germinal-vesicle-stage oocytes (GV) were obtained from large ovarian follicles of adult HS mice and freed of surrounding granulosa cells (if present) by repeated passage through a micropipette. Only GV-stage oocytes between 70 and 75 μm in diameter were used. Oocytes were cultured under paraffin oil in groups of 100 in 50–75 μl droplets of Brinster's BMOC-3 supplemented with 4 % BSA and prepared to 280–290 mosmol kg^{-1}. Oocytes matured *in vivo* were harvested from the antral follicles of HS mice previously injected with 5 i.u. of PMSG followed in 48 h by 5 i.u. of hCG. MII-stage oocytes were collected at 10, 12 and 14 h post-hCG. Oocytes were cultured in the presence or absence of nocodazole (methyl 5-[2-thienylcarbonyl-

1H-benzimimidazol-2-yl]carbamate) (Sigma Chemical Co.) at a concentration of 1 or 10 $\mu g\,ml^{-1}$ as follows: GV-stage oocytes were continuously exposed to nocodazole for up to 36 h. MII-stage oocytes, matured *in vivo* or *in vitro*, were exposed to nocodazole for up to 25 h. Oocytes were cultured in humidified chambers maintained at 37 °C in an atmosphere of 90 % N_2, 5 % CO_2, 5 % O_2.

Visualization of mitochondria, chromosomes, actin filaments and surface glycoproteins

Mitochondrial distributions in the cytoplasm of untreated and nocodazole-exposed oocytes were visualized by fluorescence microscopy after staining with 5 $\mu g\,ml^{-1}$ of rhodamine 123 for 3 min at room temperature (Van Blerkom & Runner, 1984). Chromosomal configurations and positions within the cytoplasm were determined by exposure of oocytes to medium containing the fluorescent nuclear DNA probes Hoechst dye 33258 (bisbenzimide, Calbiochem) or DAPI (4′,6-diamino-2-phenyl-indole, Sigma Chemical Co.) for 3 min at room temperature at a concentration of 10 $\mu g\,ml^{-1}$. Either NBD-phallacidin (Barak, Yocum, Nothnagel & Webb, 1980) (Molecular Probes, Inc., Plano, TX) or anti-actin monoclonal antibody was used to visualize cortical actin filaments. All immunostaining procedures utilized PHEM buffer (Schliwa & Van Blerkom, 1981) and were performed at room temperature. For immunostaining, oocytes were washed in PHEM (Schliwa & Van Blerkom, 1981), extracted with 0·15 % Triton X-100 for 4–5 min and rinsed in buffer. Immunofluorescent staining of cells was accomplished using 7 $\mu g\,ml^{-1}$ mouse anti-actin monoclonal antibody followed by fluorescein-labelled anti-rabbit immunoglobin antibodies. Detergent-extracted oocytes were cultured for 10 min in PHEM buffer containing NBD-phallacidin at a concentration of 5 $\mu g\,ml^{-1}$. The distribution of mannose-containing surface glycoproteins was examined by staining unextracted oocytes with FITC-Concanavalin A (700 $\mu g\,ml^{-1}$, Polysciences) for 8 min at room temperature (Johnson *et al.* 1975). Examinations of living oocytes by DIC and fluorescence microscopy involved incremental optical sections (serial) of entire oocytes. Preparation of oocytes and embryos for fluorescent visualization utilized methods previously described (Van Blerkom & Runner, 1984). After fluorescent analysis, oocytes and embryos were examined by differential interference contrast microscopy and at least 20 representative oocytes were prepared for electron microscopy as described by Van Blerkom & Runner (1984).

Chromosomal transfer

GV-stage oocytes cultured continuously in the presence of nocodazole for 24 h frequently contained individual bivalent chromosomes located at the apex of a distinct evagination of the plasma membrane and subjacent cortical cytoplasm. MI chromosomes were transferred to either intact GV-stage oocytes or to cytoplasts derived from oocytes chemically anucleated at 0, 30, 45 and 60 min after reinitiation of meiosis *in vitro*. Chemical enucleation was accomplished by 5 min exposure of GV-stage oocytes to medium containing cytochalasin B (5 $\mu g\,ml^{-1}$) (Schultz, Letourneau & Wasserman, 1978). During this time oocytes underwent pseudocleavage and frequently displayed a nuclear compartment with scant residual cytoplasm and a single, large cytoplasmic fragment – a cytoplast (Van Blerkom, 1985*b*). In the present experiments, chromosome transfers were made only to the large cytoplasts.

Transfer of bivalent chromosomes was accomplished by means of a glass micropipette (Leitz microelectrode glass, coated with Prosil-28, PCR Res. Chem. Inc., Gainesville, FL) prepared on a vertical pipette puller (Koph Instruments, Tujunga, CA). This instrument produced pipettes with a long taper that was sealed at the end. The pipettes were mechanically bevelled to an angle of approximately 45° with an orifice estimated to be approximately 1·5 μm. Holding pipettes were formed by fire polishing one end of a micropipette to reduce the orifice to approximately 20–30 μm.

Transfers were made under phase contrast or differential interference contrast optics, but usually without direct visualization of an individual chromosome. Typically, the micropipette was injected through the zona pellucida and placed in the apical aspect of a cytoplasmic evagination to a depth of approximately 1–2 μm. A Teflon tube attached to the injection pipette was completely filled with heavy paraffin oil (BDH) to within 2 mm of the tip. Once inside the

evagination, cortical contents were withdrawn by means of a microsyringe calibrated in increments of 0·1 μl (Hamilton Co., Reno, NV) until most of the evagination had collapsed around the pipette. The contents of the pipette were transferred to the central portion or to the cortical cytoplasm of a recipient oocyte or cytoplast. The entry made by the micropipette in the zona pellucida was frequently visible after microinjection and served as a positional marker for subsequent analysis. After chromosomal removal, the donor oocytes were fixed in glutaraldehyde (1 % in BMOC-3), stained with DAPI and examined by fluorescence to determine whether chromosomal removal had been accomplished. Recipient oocytes were cultured in the presence or absence of nocodazole and were prepared for examination by light, fluorescence and electron microscopy at 4 h intervals after transfer. Cortical cytoplasm obtained from regions between chromosome-containing evaginations was transferred to the subplasmalemmal cytoplasm of cytoplasts and intact oocytes by means of a micropipette.

Analysis of chromosomal movements by time-lapse video recording after removal of nocodazole

Chromosomal movements in living oocytes and cytoplasts were continuously monitored by time-lapse video recording utilizing a Panasonic NV-8050 video recorder with an integrated time/event generator. Oocytes were cultured on glass coverslips in a stainless steel chamber containing 2 ml of medium equilibrated with gas and overlaid with light paraffin oil to a thickness of 2 mm. The chamber was sealed at the top with a glass microscope slide but contained two 1 mm ports to allow for exchange of medium or atmosphere. To maintain proper pH and culture conditions, a gentle stream of humidified gas composed of 5 % CO_2, 5 % O_2, and 90 % N_2 was continuously introduced into the chamber. Chromosomal movements were examined by DIC microscopy on a Nikon Diaphot microscope attached to a Dage-MTI video camera. The entire microscope was enclosed by Plexiglas such that the internal temperature could be maintained at 37°C (Nikon NP-2 incubator heater). Recordings were made on high-resolution video tape (HD-PRO, TDK).

RESULTS

Chromosomal movements and mitochondrial distributions in normal and nocodazole-treated oocytes

Oocytes cultured from the GV stage in the presence of 1 or 10 $\mu g\,ml^{-1}$ of nocodazole (Fig. 1A,B) progressed through the normal sequence of nuclear and cytoplasmic changes characteristic of development to the late GVB stage (Van Blerkom & Runner, 1984). For most experiments, nocodazole at a concentration of 1 $\mu g\,ml^{-1}$ was used. Time-lapse video recordings demonstrated identical nuclear and cytoplasmic morphodynamics in normal and treated oocytes. Morphodynamic changes included nucleolar dissolution between 1·0 and 1·5 h, GVB between 1·75 and 2·0 h (Fig. 2B), appearance of cortical Golgi complexes beginning at 2·5 h (Fig. 2D), and aggregation of mitochondria at 3·5 h that resulted in the appearance of small mitochondrial clusters uniformly distributed in the cytoplasm between 4·0 and 4·5 h (Fig. 2A).

The first visual indication of post-GVB nuclear maturation was the presence of bivalent chromosomes (circular bivalent stage, CBV) in untreated oocytes at approximately 3–4 h of culture (Fig. 1C,D). In treated oocytes, bivalent chromosomes did not circularize but rather formed a dense, tightly compacted mass in the central portion of the oocyte (Figs 1E,F, 2D). At 6 h of culture in nocodazole-containing medium, mitochondria were still present in small clusters

in the cytoplasm (Fig. 2C), whereas in untreated oocytes, translocation and perinuclear accumulation of mitochondria occurred (Fig. 1G,H). Untreated oocytes formed the first metaphase (MI) spindle between 6 and 7 h of culture (Fig. 1G,H).

By 11 h of culture, abstriction of the first polar body had taken place in untreated oocytes (Fig. 1I,J), while in treated oocytes two types of chromosomal distribution were observed. Examination of 3400 treated oocytes demonstrated that after 12 h of exposure to nocodazole approximately 2000 (60 %) oocytes had a tightly compacted mass of chromosomes in the cortical region of the cytoplasm

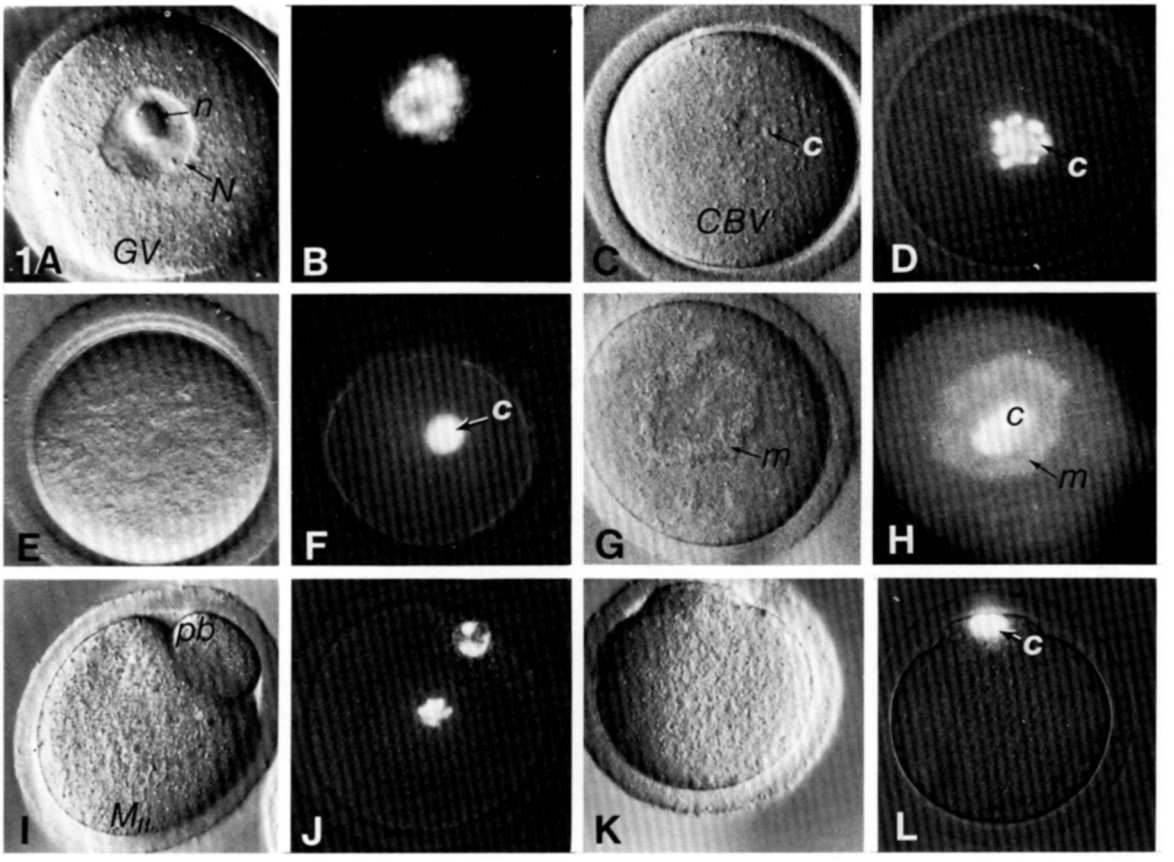


Fig. 1. Differential interference contrast [DIC] (A,C,E,G,I,K) and DAPI-fluorescence images (B,D,F,H,J,L) of mouse oocytes cultured in the presence (E,F,K,L) and absence (A,B,C,D,G,H,I,J) of 10 $\mu g\,ml^{-1}$ of nocodazole. Fig. 1B,D,G demonstrate the appearance of chromatin and chromosomes at the germinal vesicle (*GV*, Fig. 1A), circular bivalent (*CBV*, Fig. 1C) and metaphase II stages (Fig. 1I) of normal oocyte maturation *in vitro*, respectively. Perinuclear aggregation of mitochondria (*m*) is visible both by DIC (Fig. 1G) and fluorescence microscopy (Fig. 1H). Oocytes cultured in the presence of nocodazole (Fig. 1E) displayed a tightly compacted mass of centrally located chromosomes (Fig. 1F). In approximately 80 % of these oocytes, the mass of chromosomes migrated to a cortical location (Fig. 1L) and appeared to initiate polar body formation (Fig. 1K). *N*, nuclear envelope; *n*, nucleolus; *pb*, first polar body; *c*, chromosomes.

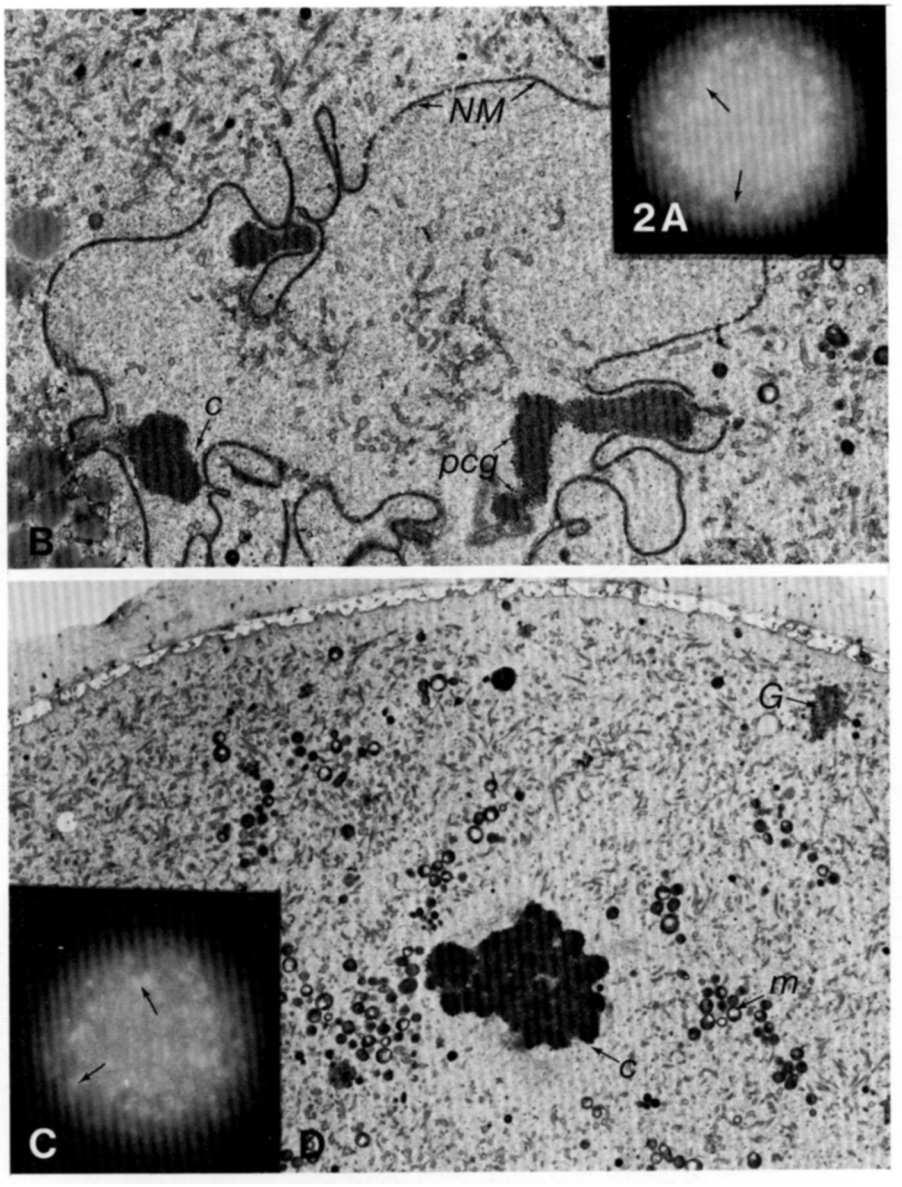


Fig. 2. (A) Rhodamine 123 staining of a living mouse oocyte at 3·0 h after initiation of culture *in vitro*. Fluorescent patterns of rhodamine 123 distribution demonstrate the presence of mitochondrial clusters (arrows) throughout the cytoplasm. (B) Transmission electron micrograph of the oocyte presented in Fig. 2A demonstrates chromosomal condensations (*c*), appearance of perichromatin granules (*pcg*) and discontinuities in the structure of the nuclear membrane (*nm*) in an oocyte undergoing germinal vesicle breakdown. ×6200. (C) Rhodamine 123 staining revealed cytoplasmic clustering of mitochondria (arrows) in this oocyte cultured in the presence of nocodazole for 6 h from the GV stage. Perinuclear aggregation of mitochondria did not occur in the presence of the inhibitor. (D) Transmission electron micrograph of the nocodazole-exposed oocyte shown in Fig. 2C. At 6 h of culture in the presence of nocodazole, a compacted mass of bivalent chromosomes (*c*) occupied the central portion of the oocyte. *m*, mitochondria, *G*, golgi complex. ×4300.

(Fig. 6B) and 1400 (40 %) had a condensed mass of chromosomes occupying the central portion of the cytoplasm (Fig. 6A). In contrast to the findings in untreated oocytes at the CBV stage (Van Blerkom, 1985*a*), fine structural analysis of serial thick (HVTEM) and thin sections (TEM) failed to indicate the presence of cytoplasmic microtubules associated with the mass of condensed, bivalent chromosomes (Fig. 2D). No signs of mitochondrial perinuclear aggregation were observed, but mitochondrial clusters still were distributed throughout the cytoplasm of nocodazole-treated oocytes in an apparently random fashion (similar to the distribution shown in Fig. 2C).

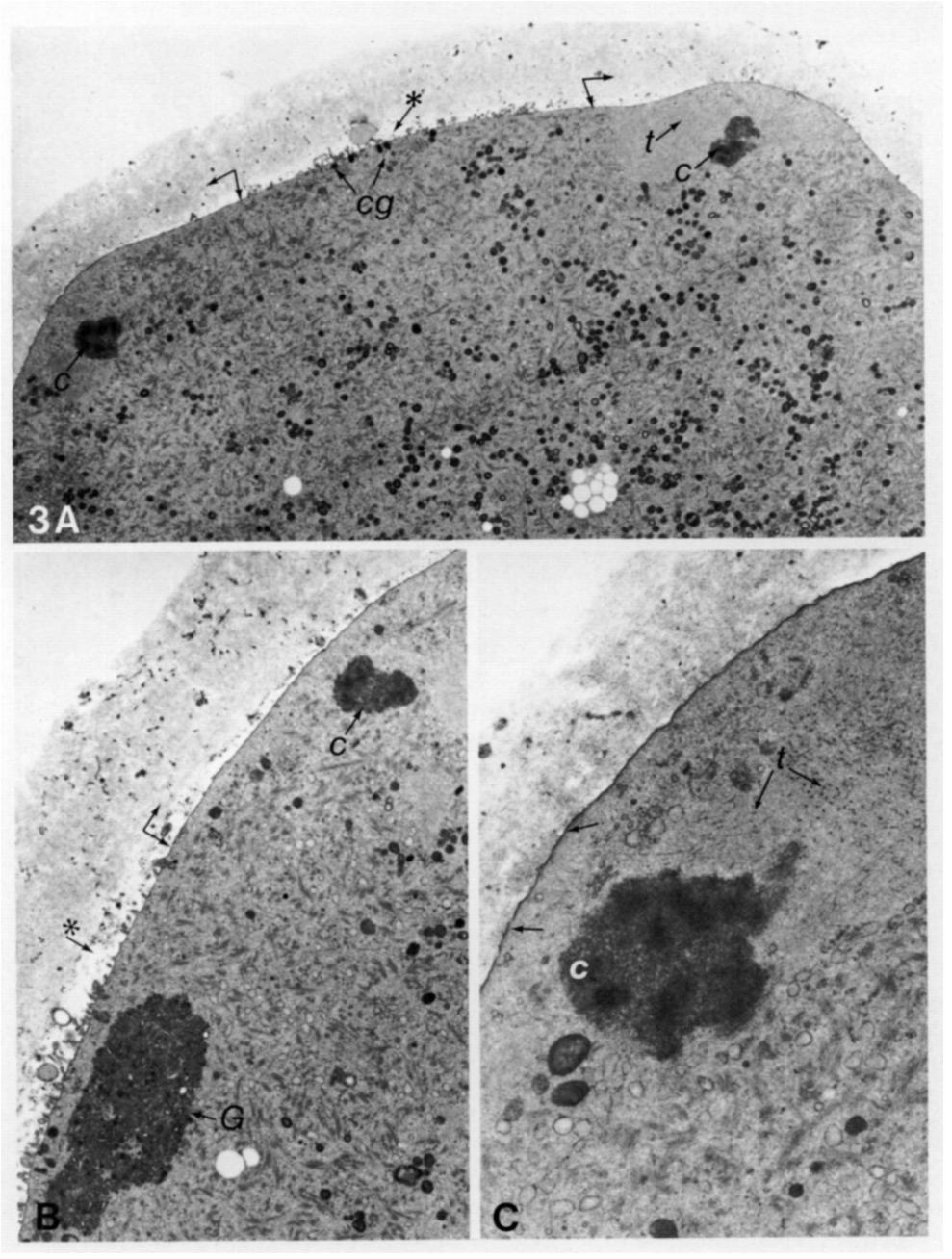
Between 12 and 14 h of culture, movement of the chromosomal mass to the cortical cytoplasm occurred in nearly half of the oocytes that previously showed centrally located chromosomes. At 14–16 h of culture in the presence of nocodazole, an evagination of the plasma membrane similar to the initial stages of polar body formation was observed in 20 % of those oocytes that previously had a single mass of chromosomes located in the cortical cytoplasm (Fig. 1K,L). Abstriction of a polar body, or a polar-body-like structure was not observed in any of the nearly 600 oocytes that presented cortical evaginations similar to that shown in Fig. 1K, even after as many as 36 h of culture.

A striking cytoplasmic distribution of chromosomes occurred between 16 and 18 h of culture in oocytes that had been exposed continuously from the GV stage to nocodazole. At approximately 16 h of culture, the compacted mass of centrally or cortically located chromosomes began to disaggregate. Regardless of the original location of the mass of chromosomes, three patterns of chromosomal disaggregation and dispersion were observed: (1) approximately 30 % (1000) of all oocytes cultured in the presence of nocodazole displayed individual bivalent chromosomes dispersed throughout the cortical cytoplasm (Figs 3A–C, 6D,E,G), (2) 50 % of the oocytes (approximately 1600) exhibited a cortical cytoplasm containing chromosomes present individually and in groups of two to five chromosomes, and (3) approximately 20 % of the 3400 oocytes examined showed no disaggregation or further movement of either a cortically or centrally located mass of chromosomes.

Between 2 and 4 h after bivalent chromosomes first appeared beneath the plasma membrane (20–22 h of culture), the subplasmalemmal cytoplasm and plasma membrane overlying the dispersed chromosomes evaginated (Figs 4A–C, 6K,L). Morphologically, the evaginations resembled the initial stages of polar body formation. However, abstriction of these structures was not observed in any of the oocytes cultured in the presence of nocodazole. For oocytes in which complete disaggregation of the chromosomal mass occurred, fluorescent staining with Hoechst dye 33258 or DAPI demonstrated that the apex of each evagination contained a single bivalent chromosome (Figs 4B, 6L). Although nocodazole perturbs the process of microtubule assembly, reconstructions of serial sections revealed compact bundles of short microtubules attached to dispersed bivalent chromosomes (Figs 3A–C, 4C) in oocytes cultured continuously from the GV stage in the presence of the inhibitor. The pattern of cytoplasmic distribution of

mitochondrial clusters was unaltered during the formation of chromosome-associated evaginations.

Within 1 h of exposure to medium containing nocodazole, approximately 70 % (920) of 1400 metaphase II-stage (MII) oocytes (12 h after initiation of culture)



displayed dissociation of chromosomes from the metaphase spindle. After 2 additional hours of culture, individual or groups of three to eight chromosomes were dispersed within the subplasmalemmal cytoplasm. After 4–6 h of exposure to nocodazole, evaginations of the cortical cytoplasm (Fig. 5A) containing individual MII chromosomes or, more typically, groups of three to eight chromosomes (Fig. 5B,C) were evident. In contrast to MI chromosomes dispersed in the cortical cytoplasm of oocytes exposed to nocodazole from the GV-stage, cortically located MII chromosomes had no associated microtubules (Fig. 5C,D). No specific correlation between the distribution of mitochondria and the location of dispersed MII chromosomes was evident either by fine structural analysis (Fig. 5C) or by fluorescence microscopy (Fig. 5E,F).

Cortical and plasma membrane changes associated with the presence of chromosomes

During the normal maturation of the laboratory mouse oocyte, a depopulation of microvilli and a reduction in the intensity of ConA binding to cell surface glycoproteins occurs in the areas of the plasma membrane overlying chromosomes associated with the first and the second metaphase spindles, the regions of the oocyte from which polar bodies normally emerge. The formation of chromosome-associated surface evaginations in nocodazole-treated oocytes duplicated the initial stages of polar body development in the following manner: (1) complete denudation of microvilli in the region of the cell surface directly overlying dispersed MI and MII chromosomes (Figs 3A–C, 4C, 5C), and (2) a marked reduction in the intensity of ConA binding that was limited to microvilli-free regions of the cell surface overlying dispersed chromosomes (Fig. 6F). The non-uniform staining of ConA is in contrast to the uniform distribution of cell surface ConA fluorescence in treated oocytes containing a compact mass of chromosomes (Fig. 6C). The loss of surface microvilli and the reduction in the intensity of ConA staining occurred rather abruptly between 16–18 h of culture in oocytes that had been exposed to nocodazole continuously from the GV stage and which contained dispersed chromosomes. A very similar sequence of plasma membrane and cell surface differentiation in association with massed or individual chromosomes was

Fig. 3. Transmission electron micrographs of oocytes in which complete dispersal of bivalent chromosomes (*c*) occurred after 19 h of culture from the GV stage in the presence of nocodazole. Compact bundles of microtubules (*t*) originated from bivalent chromosomes and extended into the cytoplasm in a highly directional manner (Fig. 3A,C). The specific orientation of chromosome-associated microtubules was demonstrated by serial section reconstructions. In one plane of section, cortical chromosomes (*c*) appeared to have no attached microtubules (Fig. 3B), whereas in other sections microtubules were clearly present (*t*, Fig. 3C). Fig. 3A–C demonstrate microvilli-free regions of the plasma membrane that were specially associated with the presence of a subplasmalemmal bivalent chromosome. The specificity of this alteration in membrane structure was indicated by adjacent microvillous regions of the plasma membrane (arrows with asterisk, Fig. 3A,B) associated with subplasmalemmal cortical granules (*cg*, Fig. 3A) and Golgi cisternae (*G*, Fig. 3B). An increased density of deposition of lead citrate and uranyl acetate was observed in regions of the plasma membrane that overlaid chromosomes (arrows, Fig. 3C). (A) ×2800; (B) ×4900; (C) ×6800.

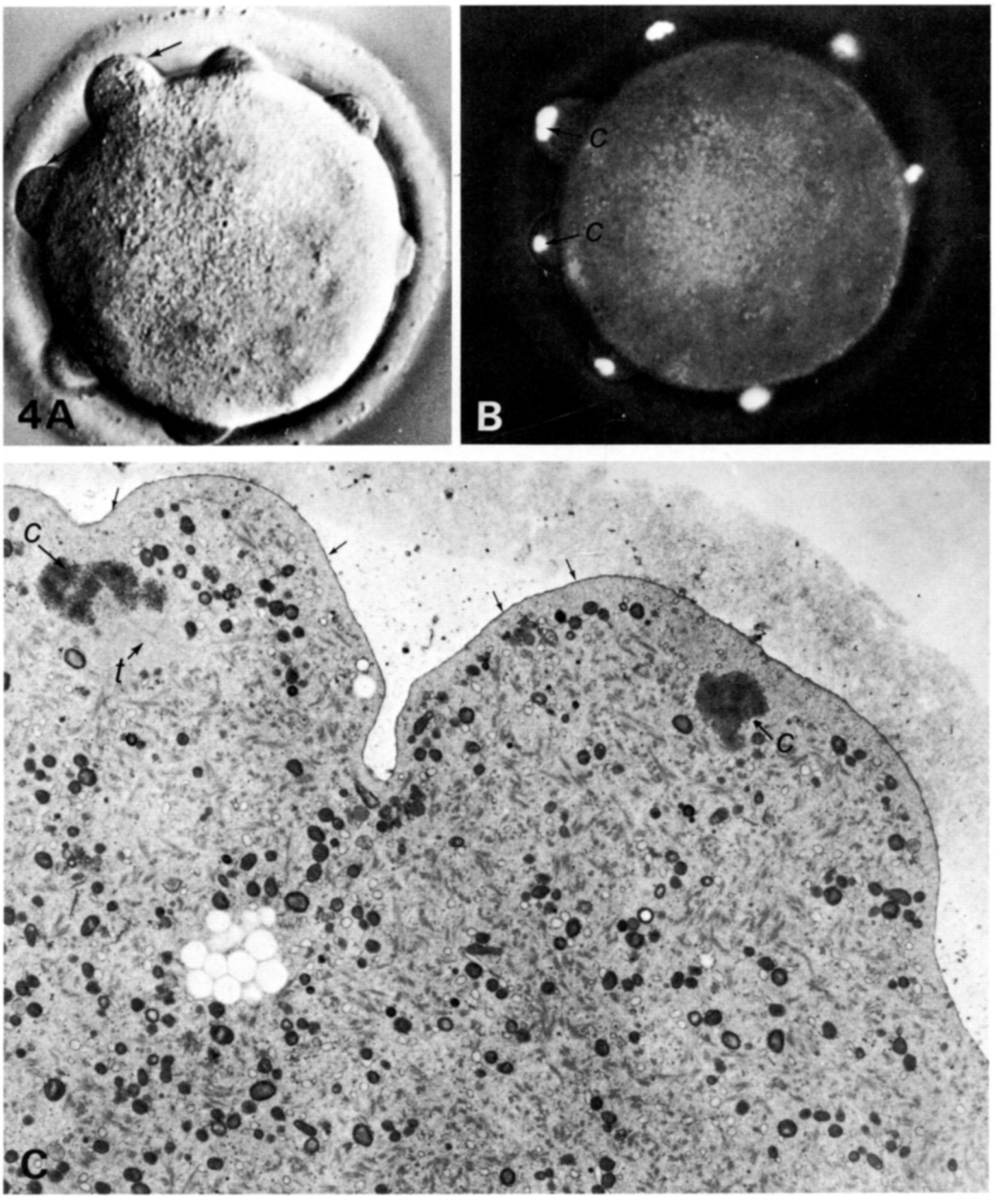


Fig. 4. DIC (Fig. 4A), DAPI-fluorescence (Fig. 4B) and transmission electron microscopic images (Fig. 4C) of an oocyte that developed bivalent chromosome-containing evaginations of the plasma membrane and cortical cytoplasm after 22 h of culture from the GV-stage in nocodazole-containing medium. Optical sections of entire DAPI-stained oocytes demonstrated that each bivalent chromosome was contained in a separate evagination (Fig. 3B). Electron microscopy confirmed the absence of microvilli (arrows, Fig. 4C) in the regions of the plasma membrane associated with subjacent chromosomes (*c*, Fig. 4C). *t*, microtubules. (C) ×5400.

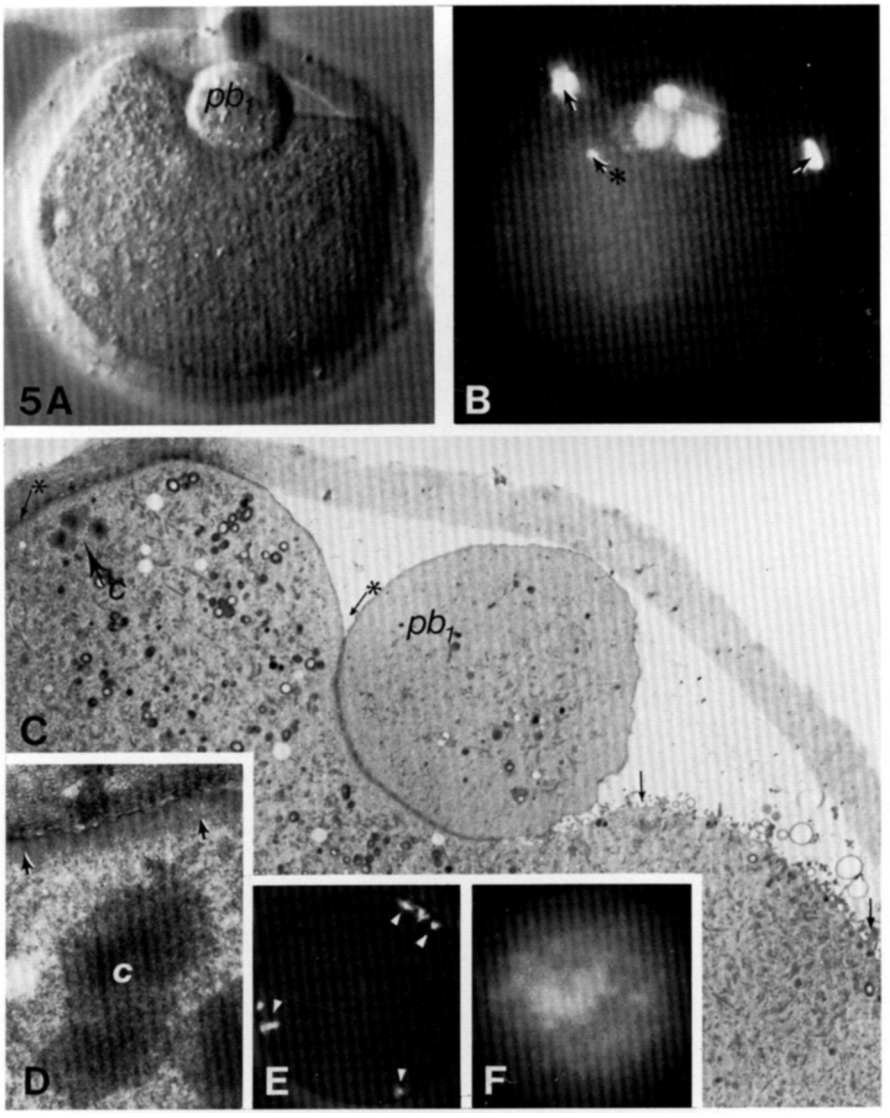


Fig. 5. DIC (Fig. 5A), fluorescence (Fig. 5B,E,F) and transmission electron microscopic (Fig. 5C,D) images of an oocyte that had matured to MII and was subsequently cultured for 2·5 h in medium containing nocodazole. Owing to the fact that chromosomes were not in the same optical plane, the identification of all individual chromosomes in a single fluorescent image was difficult. However, movement of the optical plane through the entire oocyte permitted all the fluorescent chromosomes to be accounted for. For example, in Fig. 5B four chromosomes were present in the two membranous evaginations adjacent to the first polar body [pb_1], and a single chromosome was visible in the subjacent cytoplasm (arrow with asterisk). Fluorescent analysis of a different plane of this oocyte revealed the presence of seven other chromosomes (arrowheads, Fig. 5E). Transmission electron microscopy demonstrated that the plasma membrane overlying cortical chromosomes (*c*) was devoid of microvilli (arrows with asterisks, Fig. 5C). The highly localized depopulation of microvilli is demonstrated in Fig. 5C where the plane of the thin section revealed a microvillous portion of the plasma membrane (arrows, Fig. 5C) that was lateral to the mass of cortical chromosomes present in the oocyte (see the area on the right in Fig. 5B). The subplasmalemmal actin filament network was thicker in those regions overlying chromosomes (*c*, Fig. 5C; arrows, Fig. 5D) than in chromosome-free regions of the cortical cytoplasm. The relatively uniform cytoplasmic distribution of mitochondria was indicated by staining with rhodamine 123 (Fig. 5F). (C) ×2500; (D) ×15 000.

observed in (1) treated oocytes in which the compact mass of bivalent chromosomes was cortically positioned prior to chromosomal dissociation and dispersal (Fig. 1K,L), and (2) MII oocytes approximately 2–4 h after exposure to nocodazole and cortical dispersal of chromosomes. The specificity of chromosome-mediated differentiation was demonstrated by the presence of microvilli and comparatively intense ConA fluorescence in areas of the plasma membrane associated with subjacent cortical granules (Fig. 3A) and Golgi complexes (Fig. 3B) and that were also adjacent to microvilli-free regions.

A pronounced thickening of cortical actin filaments was both temporally and spatially correlated with a depopulation of microvilli and a reduction in the intensity of ConA fluorescence in the regions of the plasma membrane associated with a single subplasmalemmal chromosome or groups of chromosomes (Fig. 5C,D). The relationship between the presence of a chromosome and a focal thickening of cortical actin filaments was evident by fluorescence microscopy after exposure of oocytes to anti-actin antibody (Fig. 6G,H) or NBD-phallacidin (Fig. 6I,J). Chromosome-associated thickening of cortical actin was observed in oocytes exposed to nocodazole continuously from the GV-stage and in oocytes treated with nocodazole at MII. Alterations in the structure and organization of the cell surface, plasma membrane and cortical cytoplasm were initiated when a chromosome(s) was approximately 5–8 μm from the plasma membrane.

Fine structural analysis of selected oocytes that had been continuously monitored by time-lapse video recording indicated that the formation of plasma membrane evaginations occurred when a chromosome was within 1 to 2 μm of the plasma membrane. For oocytes exposed to nocodazole from the GV stage, evaginations formed rather abruptly at 20 h of culture and emerged randomly over the surface of the oocyte in a virtually simultaneous fashion over a 10 min period (Figs 4A,B, 6K,L). *In vivo* and *in vitro* matured oocytes exposed to nocodazole at MII (12 h post-hCG or after initiation of culture) developed chromosome(s)-containing evaginations approximately 4–6 h later (Fig. 5A,B).

The specificity of chromosome-associated cortical and plasma membrane differentiation is demonstrated by the transfer of cytoplasm and chromosomes

The specific involvement of chromosomes in the differentiation of the cortical cytoplasm and plasma membrane was demonstrated by the physical transfer of individual bivalent chromosomes to (1) intact, untreated GV-stage oocytes, (2) untreated oocytes at the CBV stage, and (3) cytoplasts obtained from pseudocleaved GV-stage oocytes exposed to cytochalasin B at 15 min intervals after collection from the ovary. 1178 transfers were attempted with individual bivalent chromosomes derived from 391 donor oocytes that had been cultured from the GV stage for 22 h in the presence of nocodazole. These studies included oocytes in which the number of evaginations visible by light microscopy indicated that complete dispersion of individual bivalent chromosomes had occurred (e.g. Fig. 4A). Subsequent light and fluorescence microscopic analysis of the recipient oocytes demonstrated successful chromosomal transfer on 130 occasions (62

transfers to cytoplasts and 68 to GV- and CBV-stage oocytes). Cortical cytoplasm obtained from regions between chromosome-containing evaginations of nocodazole-treated oocytes was transferred to the cortical region of 46 cytoplasts (45 and 60 min after initiation of culture), 28 untreated GV- and 37 CBV-stage oocytes. Cytoplasmic transfers were accomplished under DIC optics. The success of transfer was indicated both by the depletion of the contents of the micropipette and by local expansion of the cortical cytoplasm in the recipient oocyte or cytoplast. The design of the transfer experiments is presented in diagrammatic form in Fig. 7.

The fluorescent image obtained from an oocyte (cultured in the presence of nocodazole for 22 h) immediately after removal of a chromosome and staining with DAPI is shown in Fig. 8B. The chromosome was transferred to the centre of a cytoplast derived from a GV-stage oocyte that had been exposed to cytochalasin B 45 min after harvesting from the ovary. The electron microscopic appearance of the bivalent chromosome, which at 5 h after transfer was in proximity to the cortical cytoplasm, is shown in Fig. 8A. Six bivalent chromosomes (present as a cluster in the donor oocyte) were transferred to the centre of the cytoplast shown in Fig. 8C,D. This cytoplast was obtained from an oocyte exposed to cytochalasin B 60 min after initiation of culture. At approximately 18 h of culture in the presence of nocodazole, five surface evaginations were evident (Fig. 8D). Fluorescence microscopy confirmed that each evagination contained a single bivalent chromosome (Fig. 8C). One chromosome apparently failed to migrate significantly and remained in the pericortical region of the cytoplast (white arrow, Fig. 8C).

The temporal dependency of plasma membrane and cortical differentiation was most clearly demonstrated by chromosomal transfers to cytoplasts derived from pseudocleaved GV-stage oocytes exposed to cytochalasin B at 0 min (immediately after harvesting from the ovary), and at 15 min intervals up to 60 min. No indication of cortical, cell surface and plasma membrane differentiation or evagination was observed after as many as 36 h of culture in any of the 28 cytoplasts derived from oocytes exposed to cytochalasin B at 0 and 30 min after harvesting that also received a bivalent chromosome(s) at these times. Fluorescence microscopy demonstrated no apparent migration of the transferred chromosome from the original site of deposition in the central portion of the cytoplast. By contrast, 31 of 34 recipient cytoplasts obtained from oocytes exposed to cytochalasin B at 45 and 60 min after harvesting showed cortical migration of the transferred chromosome, and progressive differentiation of the cortical cytoplasm and plasma membrane, i.e. focal thickening of cortical actin filaments, depletion of microvilli, reduction in intensity of ConA fluorescence and development of a plasma membrane evagination after 18 h of culture.

Reversibility of nocodazole-associated inhibition of chromosomal maturation

After replacement of nocodazole-containing medium with normal medium, some of the GV-stage oocytes cultured in the presence of the inhibitor for as many

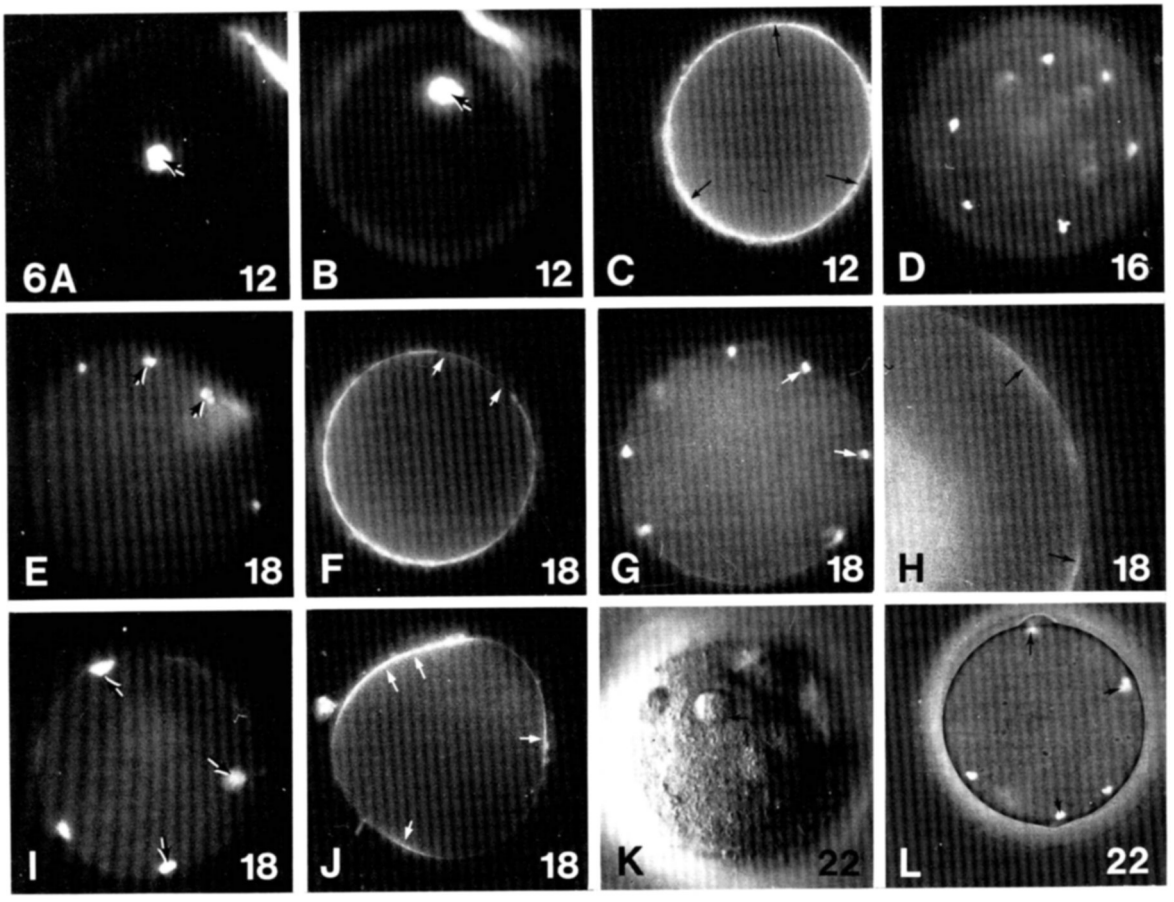


Fig. 6. Relationship between the spatial distribution of chromosomes and (1) the intensity of binding of concanavalin A to cell surface glycoproteins and (2) differential thickness of cortical actin filaments. The relationship was demonstrated by fluorescent probe analysis of oocytes cultured from the GV stage in the presence of nocodazole for up to 22 h (h of culture are presented in the lower right-hand portion of the panels). At 12 h of culture, compacted masses of bivalent chromosomes stained with Hoechst dye 33258 were either centrally (Fig. 6A) or cortically located (Fig. 6B). FITC–concanavalin A fluorescence was uniformly distributed over the oocyte surface at 12 h of culture (Fig. 6C). At 16 h, oocytes were frequently encountered in which individual bivalent chromosomes were dispersed throughout the cortical cytoplasm (Fig. 6D). FITC–concanavalin A staining of these oocytes revealed a significant reduction in fluorescence in those regions of the plasma membrane (arrows, Fig. 6F) associated with subplasmalemmal chromosomes (Fig. 6E). Anti-actin monoclonal antibody (Fig. 6H) or NBD-phallacidin (Fig. 6J) staining of oocytes containing dispersed bivalent chromosomes (Fig. 6G,I) demonstrated an increased intensity of subplasmalemmal actin filament fluorescence (arrows, Fig. 6H,J) that was specifically localized to regions of the cortical cytoplasm that contained individual chromosomes (arrows, Fig. 6G,I). Fig. 6E and F, G and H, and I and J represent three different oocytes stained with two fluorescent probes and photographed at approximately the same optical plane. The emergence of evaginations of the cortical cytoplasm and plasma membrane (Fig. 6K) that contained dispersed bivalent chromosomes (Fig. 6L) occurred rather abruptly between 20 and 22 h of culture in the presence of nocodazole.

as 36 h were able to resume meiotic maturation, form an MI spindle and subsequently develop and abstrict a polar body. Time-lapse video recordings of oocytes that had developed chromosome-containing surface evaginations at approximately 18–20 h of culture (Fig. 9A) demonstrated that approximately 8 min after the removal of nocodazole, cortically located bivalent chromosomes displayed saltatory motion. Chromosomal motion of this nature was not observed during the period of time that chromosomes were contained in evaginations of the cortical cytoplasm and plasma membrane.

The extent to which nuclear (meiotic) maturation resumed and progressed was closely related to the degree of chromosomal dispersion that had occurred during

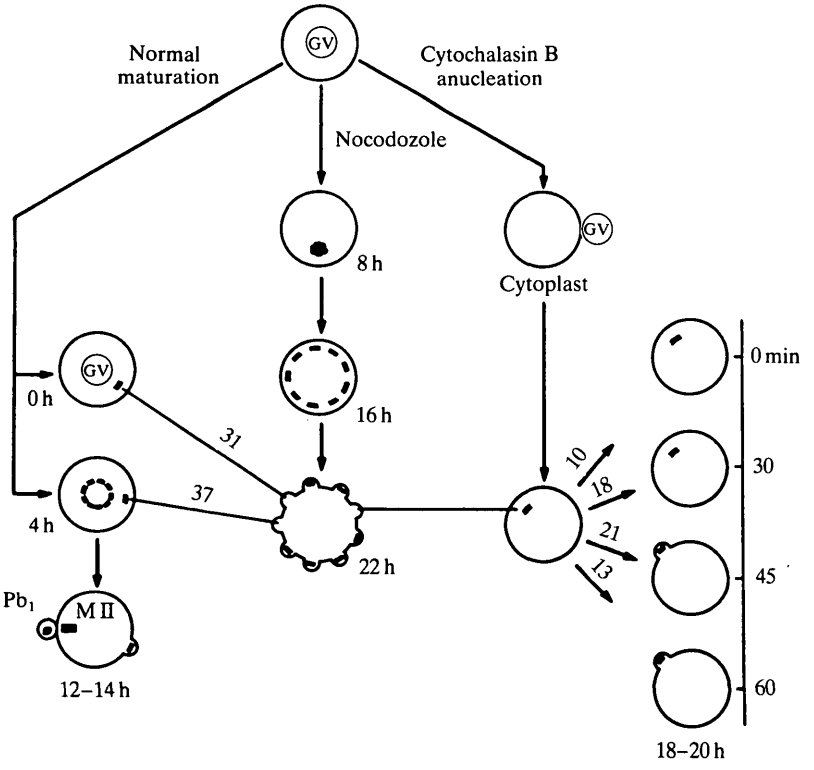


Fig. 7. Design and results of the chromosome transfer experiments. The numbers of successful chromosomal transfers are indicated on the lines radiating from the nocodazole-treated oocyte (22 h) and from the cytoplast. Bivalent chromosomes were derived from oocytes that exhibited chromosome-containing evaginations after 22 h of culture in the presence of nocodazole, and were transferred to either (1) intact GV- (0 h) or CBV- (4 h) stage oocytes, or (2) cytoplasts obtained from oocytes anucleated by exposure to cytochalasin B at 15 min intervals after harvesting from the ovary. Approximately 10 h after placement in GV- or CBV-stage oocytes, the transferred bivalent chromosomes were contained within individual evaginations of the plasma membrane. The host oocyte also frequently developed and abstricted a normal first polar body. Chromosome-containing evaginations formed in cytoplasts approximately 20 h after transfer, but only in cytoplasts derived from oocytes that had been anucleated between 45 and 60 min after collection from the ovary.

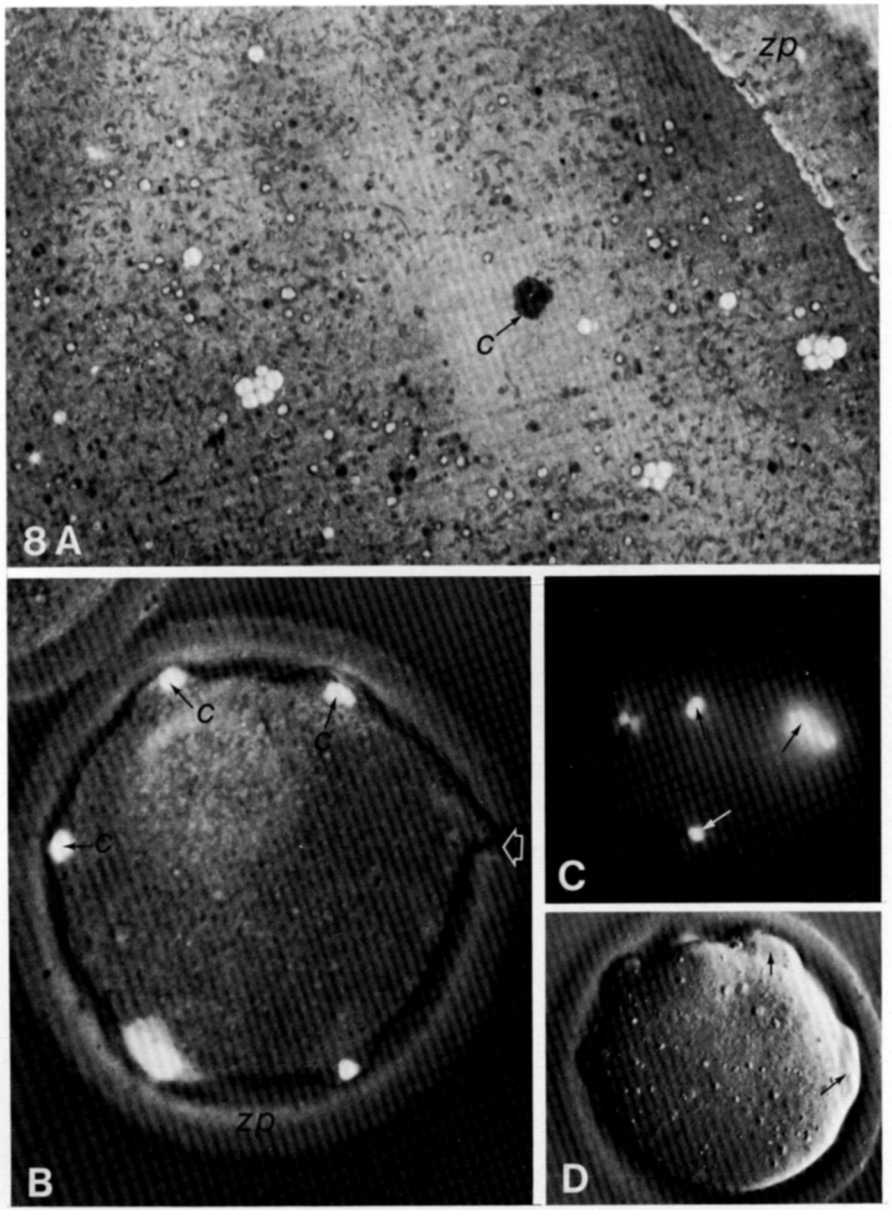


Fig. 8. The distribution of bivalent chromosomes in a donor oocyte (Fig. 8B) and host cytoplast(s) (Fig. 8A,C,D) used in chromosomal transfer experiments. The absence of DAPI fluorescence in an evagination after withdrawal of the micropipette indicated successful removal of a bivalent chromosome (white arrow, Fig. 8B). Occasionally, a small tear in the zona pellucida (*zp*) remained for several minutes after removal of the transfer pipette. Fig. 8A shows the appearance of the cytoplasm and the pericortical location of a bivalent chromosome (*c*) that had been deposited in the centre of this cytoplast 5 h earlier. DAPI fluorescence (Fig. 8C) and DIC microscopic images (Fig. 8D) of a cytoplast approximately 18 h after receiving a mass of six bivalent chromosomes. The black arrows indicate surface evaginations containing subplasmalemmal chromosomes. A single transferred chromosome (white arrow, Fig. 8C) remained in the interior of the cytoplast after deposition. (A) ×4600.

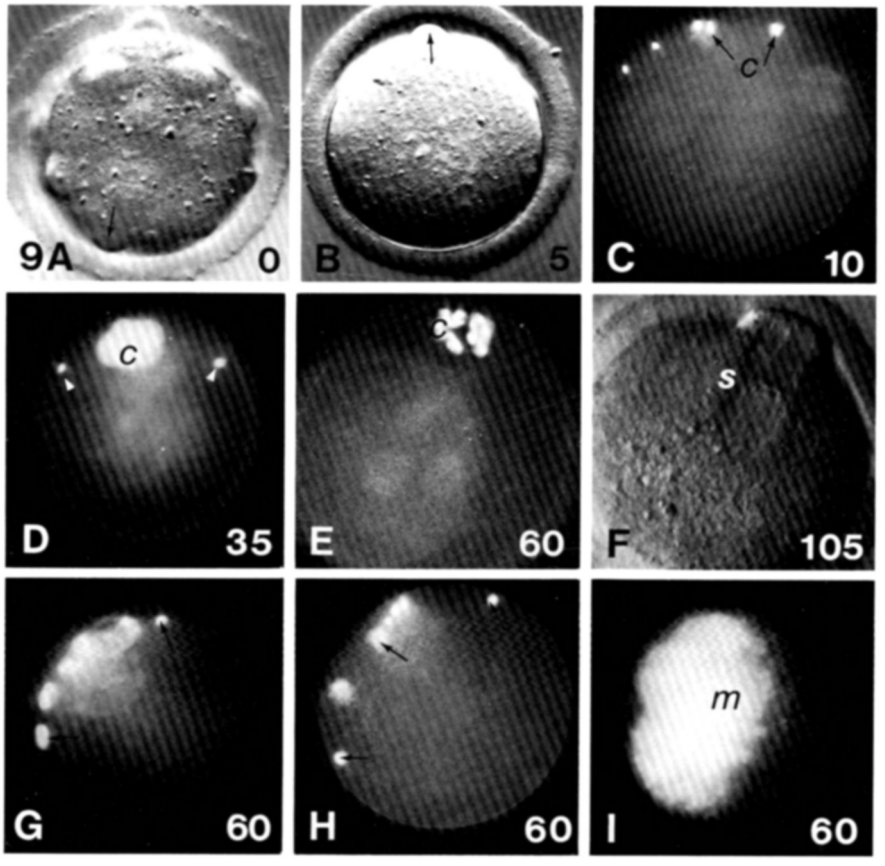


Fig. 9. The temporal sequence of cellular reorganizations resumes after replacement with normal medium in oocytes cultured for 22 h in the presence of nocodazole and which displayed incomplete chromosomal dispersion. Fig. 9A–C, D–F and G–I are derived from three separate oocytes. The chromosome-containing evaginations (arrows, Fig. 9A,B) present at 22 h of culture (indicated as 0 min in Fig. 9A) were nearly all resorbed within 5 min of replacement of medium (Fig. 9B). At approximately 10 min, DAPI fluorescence demonstrated the distribution of subplasmalemmal chromosomes (*c*, Fig. 9C). By 35 min of culture in normal medium, a large mass of cortical chromosomes had formed (*c*, Fig. 9D). The presence of individual chromosomes that had failed to migrate to the larger chromosomal aggregate was evident after DAPI staining (arrows, Fig. 9D). The development of a first metaphase spindle was evident by 60 min of culture (Fig. 9E) and by 105 min, the spindle (*s*) had rotated and the process of polar body formation had begun (Fig. 9F). Fig. 9G,H shows the spatial association that developed between cortical chromosomes and cytoplasmic mitochondria 60 min after removal of nocodazole from oocytes previously exposed to the inhibitor for 22 h (from the GV-stage). At least 13 bivalent chromosomes that previously had been contained within evaginations were localized in one region of the oocyte (arrows, Fig. 9G,H). Mitochondrial clusters (*m*) that were uniformly distributed throughout the cytoplasm during culture in the presence of nocodazole (see Fig. 5F, for example) aggregated in the region of the cytoplasm that contained the majority of the dispersed chromosomes (Fig. 9I).

culture in the presence of nocodazole. For oocytes in which chromosomal dispersion was complete, i.e. each bivalent was contained in an individual evagination, removal of nocodazole was followed by resorption of the evaginations and initiation of chromosomal motion. However, movements of individual chromosomes were confined to the region of the cortical cytoplasm that formed after resorption of the evaginations. By contrast, for oocytes in which chromosomal dispersion was incomplete (i.e. bivalent chromosomes, both individually and in clusters, were contained in single evaginations), a first metaphase spindle formed only when a mass of at least six to eight bivalents was present in the cortical cytoplasm, or could develop as a result of the cortical migration and aggregation of chromosomes. Time-lapse video recordings demonstrated that individual bivalents typically migrated to a mass of four or more chromosomes. However, even when such a cluster of bivalent chromosomes was present, the lateral distance from a chromosomal cluster over which a single bivalent chromosome was observed to migrate and join to a larger aggregate was restricted to approximately 10 μm.

The temporal sequence of cellular reorganizations that occurred in oocytes with incomplete chromosomal dispersion after removal of nocodazole is shown in Fig. 9. Within 5 min of introduction of normal culture medium (Fig. 9A), surface evaginations had resorbed (Fig. 9B) and chromosomes, either individually or in groups were located in the subplasmalemmal cytoplasm (Fig. 9C). Movement of individual bivalents to larger, proximal masses of chromosomes took place within 45 min (Fig. 9D). Between 1 and 1·5 h after the resorption of the evaginations, a first metaphase spindle formed (Fig. 9E,F). Approximately 1–2 h after spindle formation, single or multiple polar bodies were abstricted. Polar body abstriction was complete and occurred in an apparently normal fashion over a period of 5–10 min. The ability to resume chromosomal maturation after removal of nocodazole and to develop and abstrict a polar body was directly related to the number of bivalent chromosomes that could aggregate and form a metaphase spindle. Fluorescence microscopic examination of oocytes with incomplete chromosomal dispersion demonstrated the presence of individual, cortically located, bivalent chromosomes that had not participated in the formation of a functional metaphase spindle (Fig. 9D). None of the evaginations containing a single chromosome that had been transferred to a cytoplast or to an intact GV or CBV-stage oocyte showed any signs of abstriction. Multiple polar bodies were observed to develop only when six or more bivalents were present.

The rapid development of a specific association between mitochondrial clusters and cortical chromosomes after removal of nocodazole was revealed by staining with rhodamine 123. Within 45 min after the introduction of normal medium, migration of proximal mitochondrial clusters to aggregated masses of chromosomes was evident by fluorescence microscopy (Fig. 9G,H,I). This association was more apparent with clusters of three or more chromosomes than with individual bivalents. Coincident with the accumulation of perichromosomal mitochondria, fine structural analysis demonstrated the presence of microtubules that radiated

from the kinetochore region of the bivalent chromosomes. Although microtubules did polymerize in association with chromosomes after the removal of nocodazole, a similar relationship between the spatial distribution of mitochondria and dispersed MII chromosomes was not observed.

DISCUSSION

Changes in mitochondrial and chromosomal organization

The cellular development of the maturing oocyte, which in the mouse involves mitochondrial redistributions and modifications in the structure and composition of the cortical cytoplasm and plasma membrane, are temporally correlated with progressive changes in nuclear (chromosomal) organization (Van Blerkom, 1985*a*,*b*). A sequence of mitochondrial redistributions during resumed meiosis in mouse oocytes has been reported by Van Blerkom & Runner (1984). Mitochondria that are uniformly distributed throughout the cytoplasm in the GV-stage oocyte form small clusters during GVB. These clusters migrated to the perinuclear region during the CBV-stage and formed a dense, ring-like aggregate of mitochondria that envelop the developing MI spindle. After abstriction of the first polar body, the perinuclear mass of mitochondria disaggregate and return to a relatively uniform distribution throughout the cytoplasm.

Van Blerkom (1985*a*) proposed that the translocation of mitochondrial clusters to the perinuclear region of the mouse oocyte may be mediated by microtubules that radiate into the cytoplasm from perinuclear microtubule-organizing centres (MTOCs) (Wassarman & Fujiwara, 1978) that develop in proximity to the germinal vesicle envelope (Calarco, Donahue & Szollosi, 1972). In the presence of nocodazole, a rapidly reversible inhibitor of microtubule polymerization, oocytes cultured from the GV stage underwent cytoplasmic clustering of mitochondria, GVB and chromosomal condensation according to the same temporal schedule as that observed in untreated oocytes (Van Blerkom & Runner, 1984). However, the CBV stage did not develop and chromosomes remained for several hours after condensation as a dense mass in the central portion of the oocyte. In approximately 30 % of the treated oocytes examined, the mass of centrally located chromosomes migrated to a peripheral location and subsequently disaggregated in such a manner that bivalent chromosomes, both individually and in small groups, were dispersed in the cortical cytoplasm. For oocytes in which the mass of chromosomes remained centrally located, continued culture in the presence of nocodazole was accompanied by disaggregation of the mass and migration of individual chromosomes, or groups of chromosomes, away from the centre of the oocyte to the cortical cytoplasm.

The present findings support the suggestion of microtubule participation in mitochondrial translocation during resumed meiosis. In oocytes cultured continuously in the presence of nocodazole from the GV stage, no specific association could be established between the spatial distribution of mitochondrial clusters and (1) compacted masses of centrally or peripherally positioned chromosomes,

(2) small groups or individual chromosomes during their migration to the cortical cytoplasm, and (3) bivalent chromosomes either resident in the cortical cytoplasm or contained in evaginations of the plasma membrane. A similar result occurred in oocytes exposed to nocodazole after abstriction of the first polar body. In these MII-stage oocytes, disaggregation of the meiotic spindle was followed by cortical migration and random distribution of chromosomes. During culture of MII oocytes in the presence of nocodazole, no specific association between mitochondria and subplasmalemmal chromosomes was observed.

Electron microscopic analysis of nocodazole-treated oocytes at the late GV and GVB stages failed to demonstrate the presence of cytoplasmic microtubules of perinuclear origin. No cytoplasmic microtubules were observed to originate from the compacted mass of bivalent chromosomes that formed after GVB in treated oocytes. However, small, compact bundles of relatively short microtubules were associated with MI chromosomes that had disaggregated from the original condensed mass and were either in the process of migration to the cortex, or had become resident in the cortical cytoplasm.

Electron microscopic examination of oocytes exposed to nocodazole after abstriction of the first polar body revealed an absence of microtubules and mitochondria associated with MII chromosomes that had dissociated from the metaphase spindle and dispersed within the cortical cytoplasm. Similar findings derived from observations of chromosomes in mouse oocytes exposed to nocodazole after ovulation have been reported by Maro *et al.* (1986). The sensitivity to nocodazole of microtubules associated with MII chromosomes is very similar to that observed in mitotic cells (Zieve, Turnbull, Mullins & McIntosh, 1980). The association of microtubules with MI but not MII chromosomes in treated oocytes may indicate developmental variation in stability and/or sensitivity of meiotic microtubules to nocodazole. Alternatively, oocytes cultured from the GV stage had been exposed to nocodazole for a longer period of time than oocytes obtained at MII. Rather than a manifestation of a developmental difference, the association of microtubules with MI but not MII chromosomes could be a consequence of a reduction in the influence of nocodazole during extended periods of culture.

The relationship between the spatial distribution of mitochondrial clusters and chromosomes was most clearly demonstrated after the removal of nocodazole and replacement with normal culture medium. The removal of nocodazole presumably restores intracellular conditions that are permissive for the normal process of microtubule polymerization. As a consequence of exposure of mature oocytes to nocodazole (Maro *et al.* 1986), chromosomes dissociate from the MII spindle, disperse into the cortical cytoplasm and have no attached microtubules. However, approximately 30 min after the removal of the inhibitor, microtubules arising from the kinetochore region were observed. Although microtubules did polymerize in association with dispersed MII chromosomes, mitochondria did not migrate to or aggregate in the vicinity of the chromosomes. This finding is in contrast to the situation that existed in treated oocytes containing dispersed MI chromosomes

where, after removal of nocodazole, mitochondrial clusters became associated with bivalent chromosomes. However, these results are similar to observations on the behaviour of mitochondria during the GVB, CBV, MI and MII stages of oocyte maturation in the mouse (Van Blerkom & Runner, 1984): (1) the perinuclear concentration of mitochondria develops during MI and disperses after abstriction of the first polar body and formation of the MII spindle, and (2) the mitochondria associate neither specifically nor extensively with the MII spindle.

Changes in the cytoplasmic distribution of mitochondria may be determined by the spatial orientation and the developmental state of meiotic chromosomes, as well as by the nature of the association between chromosomes and microtubules. In this respect, it may also be reasonable to speculate that chemical changes in the outer mitochondrial membrane may occur during oocyte maturation (Kruip, Cran, Van Beneden & Dieleman, 1983), and these changes may impart to mitochondria a stage-specific capacity to interact with chromosome-associated microtubules.

Chromosomal mediation of cortical and plasma membrane differentiation

Cortical and plasma membrane differentiation during resumption of arrested meiosis in the mouse oocyte occurs in association with the metaphase spindles. In the present study, the migration of individual bivalent chromosomes to the cortical cytoplasm was accompanied by the same process of cortical and plasma membrane differentiation normally associated with the presence of the spindle-associated meiotic chromosomes. The area of cortical and plasma membrane differentiation associated with the presence of an underlying chromosome has been clearly delineated by fluorescence, transmission and scanning electron microscopy (Longo & Chen, 1985; Maro *et al.* 1986). In the present study, the subplasmalemmal cytoplasm, under the apparent influence of a chromosome, also was characterized by the absence of mitochondria, cortical granules and Golgi cisternae. The specificity of chromosome-associated differentiation was shown by the absence of altered cortical, plasma membrane and cell surface characteristics in regions of the oocyte surface adjacent to Golgi complexes, cortical granules and mitochondria.

Individual bivalent (MI) chromosomes dispersed throughout the cortical cytoplasm as a consequence of exposure of GV-stage oocytes to nocodazole were associated with small, compact bundles of relatively short microtubules. This observation raises the possibility that cortical and plasma membrane differentiation may be influenced by microtubules rather than by chromosomes. However, the same clearly delineated progression of cortical, plasma membrane and cell surface differentiation observed in the presence of MI chromosomes occurred in those regions of oocyte subplasmalemmal cytoplasm that contained dispersed MII chromosomes devoid of associated microtubules. These results and interpretations also are consistent with the findings of similar light, fluorescent and electron microscopic studies involving GV- and MII-stage (ovulated) mouse

oocytes cultured in the presence of colchicine (Longo & Chen, 1985), and MII-stage mouse oocytes cultured in the presence of nocodazole (Maro *et al.* 1986).

The temporal and spatial relationship between the presence of a chromosome and the occurrence of cortical and plasma membrane differentiation was clearly demonstrated in chromosome transfer experiments. Transfer of a single bivalent chromosome to a GV- or CBV-stage oocyte resulted in the same sequence of cortical actin thickening, microvilli depopulation and reduction in cell surface ConA binding observed in association with the endogenous MI spindle. However, this sequence of changes frequently occurred in a region of the oocyte surface distant from the normal MI spindle. In spite of the fact that a normal first polar body was formed in the same oocyte, evaginations that contained individual, transferred chromosomes did not abstrict. This finding suggests that the presence of a chromosome is sufficient to establish the localized intracellular changes requisite to polar body formation at any region of the oocyte surface, but may require a critical mass of chromosomes or a functional spindle, or both, before abstriction of the polar body can occur. The findings of Maro *et al.* (1986) support this interpretation by demonstrating that relatively large clusters of MII chromosomes dispersed in the cortical cytoplasm in treated oocytes are capable, after removal of nocodazole, of inducing the formation of multiple spindles that rotate and yield multiple abstricted polar bodies containing varying numbers of chromosomes.

The same spatial relationship that existed between the presence of a chromosome and the occurrence of a sequence of differentiative events in the cortical cytoplasm and overlying plasma membrane was also observed in cytoplasts that received one or more bivalent chromosomes. The specificity of chromosomal mediation of this sequence was further indicated by transfer of cytoplasm from nocodazole-treated oocytes to cytoplasts and to intact, untreated GV- and CBV-stage oocytes. No evidence of cortical or plasma membrane differentiation was obtained in any of the studies involving cytoplasmic transfers. Taken together, the above findings demonstrate that the cortical and plasma membrane polarity established in the MI- and MII-stage mouse oocyte is induced rather than pre-existing. The results also indicate that the entire surface of the mouse oocyte is equally able to differentiate in response to the presence of a chromosome(s). The polarity and compartmentalization of the cortical cytoplasm occur during normal oocyte maturation by virtue of the fact that the chromosomes arrive at the plasma membrane as a package rather than, as shown in the present study, as single chromosomes in disaggregation.

Chromosomal but not genomic participation in the autonomous expression of an intrinsic developmental programme in the mouse oocyte

The expression of a developmental sequence of molecular and cellular changes (cytoplasmic maturation) in the mouse oocyte appears to be initiated just prior to or at the time of GVB. GV-stage oocytes cultured in the presence of dibutryl cyclic AMP (dbCAMP) for up to 16 h do not undergo GVB or cytoplasmic clustering of

mitochondria (Van Blerkom & Runner, 1984). The temporal sequence of dynamic change at the nuclear and cytoplasmic levels occurs in an apparently normal fashion after the removal of dbCAMP (Van Blerkom & Runner, 1984). The reversible inhibition of oocyte maturation suggests that cytoplasmic changes may be initiated or regulated by factors present in the germinal vesicle and exported to the cytoplasm prior to or at the time of GVB. Wassarman, Schultz & Letourneau (1979) reported that specific proteins, some of which were phosphorylated, accumulated in high concentrations in the nucleoplasm and were released into the cytoplasm around the time of GVB. A more recent study by Schultz, Montgomery & Belanoff (1983) described changes in patterns of protein phosphorylation and dephosphorylation that were initiated approximately 30 min after placement of GV-stage mouse oocytes (1–1·5 h prior to GVB) in conditions that permitted the spontaneous resumption of arrested meiosis. These results suggest that translocation of preformed nuclear proteins to the cytoplasm, or changes in protein synthesis and modification, or both, may begin well in advance of GVB and chromosomal condensation (Schultz *et al.* 1983). The above observations suggest the possibility that molecular changes at the nuclear level may initiate a cascade of events leading to GVB and to a developmental alteration of the cytoplasm in preparation for the presence of chromosomes (Van Blerkom & Runner, 1984; Van Blerkom, 1985*a*).

The hypothesis that GVB and the completion of chromosomal condensation are preceded by activation of cytoplasmic maturation and do not require concurrent genomic input has been suggested by previous molecular and cellular studies of anucleate fragments (cytoplasts) derived from GV-stage mouse oocytes at 0, 15, 20, 45 and 60 min after the resumption of meiosis *in vitro*. Qualitative analysis of protein expression in cytoplasts cultured for as many as 36 h after enucleation demonstrates the occurrence of changes in protein synthesis (Schultz *et al.* 1978; Van Blerkom, 1985*c*) and modification (Van Blerkom, 1985*a*) that is associated with maturation of the normal mouse oocyte. However, these changes take place only in cytoplasts derived from oocytes that have been enucleated between 30 and 60 min after the initiation of culture.

In the present study, chromosomes were transferred to cytoplasts produced at various times after the initiation of oocyte culture. The results indicate that the cytoplasm of cytoplasts derived at 0 and 30 min does not have the ability to promote chromosomal migration, and that the cortical cytoplasm and plasma membrane may not have the capacity to differentiate in response to the presence of a chromosome, even after 36 h of culture. By contrast, chromosomes placed in the central portion of cytoplasts enucleated after approximately 45 and 60 min of maturation *in vitro* migrated to the cortex and initiated the same sequence of cortical, plasma membrane and cell surface differentiation that normally occurs in proximity to an intact metaphase spindle.

An alternative interpretation of the findings, based on the observation that cytochalasin B-induced pseudocleavage is accompanied by a transient and total loss of cortical microfilaments and microvilli (Wassarman *et al.* 1977), is that the

differential response of cytoplasts to the presence of a transferred chromosome could be the result of (1) the inability of a chromosome to migrate to the cortex and, or, (2) the absence of cortical microfilaments that can respond to the presence of a chromosome. The migration of chromosomes in oocytes appears to be a microfilament-mediated process because drugs that interfere with microfilament organization, such as cytochalasin D, inhibit the migration of even nocodazole-disaggregated meiotic chromosomes (Van Blerkom & Bell, unpublished data; Maro *et al.* 1986). The presence of a chromosome in the cortical cytoplasm was insured in some of the transfer experiments by the cortical placement of a chromosome in cytoplasts obtained 30 min after the initiation of culture. However, no evidence of cortical or cell surface alteration was observed in these cells after as many as 36 h of culture. This observation is in contrast to the situation that prevailed in cytoplasts obtained at 45 and 60 min after the initiation of culture (see Fig. 7 for summary). Microvilli and cortical microfilaments reappear uniformly after the completion of pseudocleavage, but only in the anucleate compartment (Wassarman *et al.* 1977). This result strongly suggests that microvilli and cortical microfilaments had reformed and were uniformly distributed so as to be able to respond to the presence of a chromosome. Collectively, these observations suggest that a change in the responsiveness of the cytoplasm to the presence of a chromosome occurs approximately 45 min after the resumption of meiosis.

The precise nature of the signal(s) and the actual mechanism that may be involved in the activation of the oocyte cytoplasm during the resumption of meiosis remain to be determined. Van Blerkom (1985*a*) suggested that proteins exported from the GV may activate cytoskeletal-associated enzymes that function in the stage-related post-translational modification of oocyte proteins. The activation of enzymes, such as kinases and phosphatases (Schultz *et al.* 1983), could rapidly alter the cytoarchitectural state of the cytoplasm to allow the movement of chromosomes and the redistribution of organelles. Although the process by which a chromosome can mediate or induce local changes in cytoplasmic and plasma membrane structure and organization is unknown, it is conceivable that chromosome-associated proteins or ions may be involved. The ability to transfer chromosomes between oocytes, and the occurrence of well-defined and precisely timed developmental events during the maturation of the laboratory mouse oocyte offers an extremely useful approach to understanding both the nature of interaction between nucleus and cytoplasm, and the mechanism of autonomous expression of an intrinsic developmental programme.

We thank Dr Michael Klymkowsky for his helpful comments on the manuscript and for providing anti-actin monoclonal antibody. This work was supported by grants from the National Institutes of Health (HD-13500; RR 07013-19, BRSG Program, Division of Research Resources).

REFERENCES

BARAK, L. S., YOCUM, R. R., NOTHNAGEL, E. A. & WEBB, W. W. (1980). Fluorescence staining of the actin cytoskeleton in living cells with 7-nitobenz-2-oxa-1,3,-diazole phallacidin. *Proc. natn. Acad. Sci., U.S.A.* **77**, 980–984.

CALARCO, P. G., DONAHUE, R. P. & SZOLLOSI, D. (1972). Germinal vesicle breakdown in the mouse oocyte. *J. Cell Sci.* **10**, 369–385.

EAGER, D., JOHNSON, M. H. & THURLEY, K. W. (1975). Ultrastructural studies on the surface membrane of the mouse egg. *J. Cell Sci.* **22**, 345–353.

HOEBEKE, J., VAN NIGEN, G. & DE BRABANDER, M. (1976). Interaction of Oncodazole (R17934), a new anti-tumor drug, with rat brain tubulin. *Biochem. Biophys. Res. Commun.* **69**, 319–342.

JOHNSON, M. H., EAGER, D., MUGGLETON-HARRIS, A. L. & GRAVES, H. M. (1975). Mosaicism in the organization of concanavalin A receptors on surface membrane of mouse eggs. *Nature, Lond.* **257**, 321–322.

KRUIP, T. A. M., CRAN, D. G., VAN BENEDEN, T. H. & DIELEMAN, S. J. (1983). Structural changes in bovine oocytes during final maturation *in vivo*. *Gamete Res.* **8**, 29–48.

LONGO, F. J. & CHEN, D. Y. (1985). Development of cortical polarity in mouse eggs: Involvement of the meiotic apparatus. *Devl Biol.* **107**, 382–394.

MARO, B., JOHNSON, M. H., PICKERING, S. J. & FLACH, G. (1984). Changes in actin distribution during fertilization of the mouse egg. *J. Embryol. exp. Morph.* **81**, 211–237.

MARO, B., JOHNSON, M. H., WEBB, M. & FLACH, G. (1986). Mechanism of polar body formation in the mouse oocyte: an interaction between the chromosomes, the cytoskeleton and the plasma membrane. *J. Embryol. exp. Morph.* **92**, 11–32.

NICOSIA, S. V., WOLF, D. P. & INOUE, M. (1977). Cortical granule distribution and cell surface characteristics in mouse eggs. *Devl Biol.* **57**, 56–74.

RICHTER, J. D. & MCGAUGHEY, R. W. (1983). Patterns of polypeptide synthesis in mouse oocytes during germinal vesicle breakdown and during maintenance of the germinal vesicle stage by dibutryl cAMP. *Devl Biol.* **83**, 188–192.

SCHLIWA, M. & VAN BLERKOM, J. (1981). Structural interaction of cytoskeletal components. *J. Cell Biol.* **90**, 222–235.

SCHULTZ, R. M. & WASSARMAN, P. M. (1977). Specific changes in the pattern of protein synthesis during meiotic maturation of mammalian oocytes *in vitro*. *Proc. natn. Acad. Sci., U.S.A.* **74**, 538–541.

SCHULTZ, R. M., LETOURNEAU, G. E. & WASSARMAN, P. M. (1978). Meiotic maturation of mouse oocytes *in vitro*: Protein synthesis in nucleate and anucleate oocyte fragments. *J. Cell Sci.* **30**, 251–264.

SCHULTZ, R. M., MONTGOMERY, R. & BELANOFF, J. R. (1983). Regulation of mouse oocyte meiotic maturation: Implication of a decrease in oocyte cAMP and protein dephosphorylation in commitment to resume meiosis. *Devl Biol.* **97**, 264–273.

VAN BLERKOM, J. (1985*a*). Extragenomic regulation and autonomous expression of a developmental program in the early mouse embryo. *Ann. N.Y. Acad. Sci.* **442**, 58–72.

VAN BLERKOM, J. (1985*b*). Protein synthesis during oogenesis and early embryogenesis in the mammal. In *Biology of Fertilization*, vol. 3 (ed. C. B. Metz & A. Monroy), pp. 379–399. New York: Academic Press.

VAN BLERKOM, J. (1985*c*). Posttranslational modification of proteins during resumption of arrested meiosis and early embryogenesis in the mammal. In *Control of Cell Growth and Proliferation* (ed. C. M. Veneziale), pp. 67–86. Princeton, New Jersey: Van Nostrand, Reinhold, Co.

VAN BLERKOM, J. & MOTTA, P. M. (1979). *The Cellular Basis of Mammalian Reproduction.* Baltimore: Urban and Schwarzenberg.

VAN BLERKOM, J. & RUNNER, M. N. (1984). Mitochondrial reorganization during resumption of arrested meiosis in the mouse oocyte. *Amer. J. Anat.* **171**, 335–355.

WASSARMAN, P. M. & LETOURNEAU, G. E. (1976). RNA synthesis in fully grown mouse oocytes. *Nature, Lond.* **261**, 73–74.

WASSARMAN, P. M., UKENA, T. E., JOSEFOWICZ, W. J., LETOURNEAU, G. E. & KARNOVSKY, M. J. (1977). Cytochalasin B-induced pseudocleavage of mouse oocytes *in vitro*. II. Studies of the mechanism and morphological consequences of pseudocleavage. *J. Cell Sci.* **26**, 323–337.

WASSARMAN, P. M. & FUJIWARA, K. (1978). Immunofluorescent anti-tubulin staining of spindles during meiotic maturation of mouse oocytes *in vitro*. *J. Cell Sci.* **29**, 171–188.

WASSARMAN, P. M., SCHULTZ, R. M. & LETOURNEAU, G. E. (1979). Protein synthesis during meiotic maturation of mouse oocytes *in vitro*: Synthesis and phosphorylation of a protein localized in the germinal vesicle. *Devl Biol.* **69**, 94–107.

WOLF, D. E. & ZIOMEK, C. A. (1983). Regionalization and lateral diffusion of membrane proteins in unfertilized and fertilized mouse eggs. *J. Cell Biol.* **96**, 1786–1790.

ZAMBONI, L. (1970). Ultrastructure of mammalian oocytes and ova. *Biol. Reprod. (Suppl.)* **2**, 44–63.

ZIEVE, G. W., TURNBULL, D., MULLINS, M. & MCINTOSH, J. R. (1980). Production of large numbers of mitotic mammalian cells by use of the reversible microtubule inhibitor nocodazole. *Expl Cell Res.* **126**, 397–405.

(*Accepted 18 November 1985*)