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Summary. During the first meiotic cell division of the *Xenopus* oocyte, a transient microtubule network appears at the basal part of the disintegrating nucleus. In order to know if this structure plays a role in the formation of the first meiotic spindle, we have studied the actions of Ca^{2+} ions and taxol during the whole maturation process. We now report that when the free cytosolic Ca^{2+} concentration is raised by ionophore A 23187, the perinuclear microtubule array is disrupted and the spindle formation is inhibited.

When taxol is microinjected into maturing oocytes, the perinuclear microtubules become anarchically distributed and the normal organization of the metaphase spindle is blocked. MPF (Maturation promoting factor) activity is maintained in these experimental conditions (Germinal vesicle breakdown is induced). These results indicate that polymerization/depolymerization of perinuclear microtubules are involved in the nuclear envelope breakdown and the subsequent assembly of the first meiotic spindle.

Introduction.

Microtubules are the predominant structural components of the mitotic and meiotic spindles. Nevertheless their precise role in cell division is poorly understood. The amphibian oocyte provides an attractive experimental model for the study of the nuclear envelope breakdown and the assembly of the metaphase spindle. In the ovary, an homogenous population of full grown oocytes is blocked at the diplotene stage of the meiotic prophase. The first meiotic cell division, or meiotic maturation, may be obtained *in vitro* following hormonal treatment. When defolliculated *Xenopus* oocytes are stimulated with progesterone (1 μM) a cytoplasmic factor (MPF) or maturation promoting factor appears (Masui and Clarke, 1979) and the nuclear envelopes break down synchronously (GVBD) ; the formation of the spindles of the first and then the second meiotic metaphases occurs in the absence of centriole in about 6 to 10 hours depending of the tested females (Masui and Clarke, 1979). Thereafter, the matured ovocytes remain blocked at metaphase II until fertilization or activation.

During the late stage of the meiotic prophase (stage VI oocyte, Dumont, 1972), few microtubules have been observed near the germinal vesicle of the amphibian oocyte. Although tubulin is present in rather high concentration [1 % to 2 % of the soluble proteins (Pestell, 1975 ; Heidemann and Kirschner, 1975 ; Jessus C., personal communication)] in these oocytes it is maintained in an unpolymerized form. Furthermore, all treatments known to induce tubulin assembly fail to induce the formation of microtubules in stage VI oocytes. In fact, microinjection of basal bodies, sperm, centrosomes, or taxol (Heidemann and Kirschner, 1975, 1978 ; Elinson, 1977 ; Heidemann and Gallas, 1980 ; Karsenti *et al.*, 1984) does not induce asters in prophase I blocked oocytes. In contrast, although the concentration of the different isoforms of tubulin does not change during maturation, when unfertilized (metaphase II oocyte) eggs are treated with heavy water (van Assel and Brachet, 1966) or microinjected with basal bodies or sperm, or taxol (Heidemann and Kirschner, 1975, 1978 ; Elinson, 1977 ; Heidemann and Gallas, 1980) numerous asters appear in the cytoplasm. Taken together these observations indicate that the critical concentration for tubulin polymerization changes during maturation. A possible explanation for these results is that during its disintegration the nucleus releases regulatory factors necessary for the control of the critical concentration of tubulin for microtubule assembly. Recently we have shown that as soon as the nuclear envelope breaks down numerous microtubules appear basally at the junction between the nucleoplasm and the underlying cytoplasm. These microtubules become organized into a dense microtubular network oriented towards the animal pole. This transient asymmetrical structure which last 30 to 45 min precedes the formation of the first metaphase spindle (Huchon *et al.*, 1981).

In order to know if this transient microtubular structure plays a role in the formation of the first meiotic spindle, we have studied the effect of Ca^{2+} ions and of taxol during the whole maturation process. We now report that when the free cytosolic Ca^{2+} concentration is raised by the ionophore A 23187 the perinuclear microtubule structure is disrupted and spindle formation is inhibited. On the other hand when taxol, which promotes tubulin polymerization and prevents microtubule depolymerization (Schiff *et al.*, 1979) is microinjected into maturing oocytes, the formation of the spindle is blocked depending on the times of drug injection.

Materials and methods.

Animals and chemicals. — *Xenopus laevis* females were purchased from Dr de Rover (The Netherlands). They were bred and maintained under laboratory conditions.

Progesterone, collagenase type 1 were obtained from Sigma, dispase grade II from Boehringer. Ionophore A 23187 was a gift of the Lilly Research Laboratories. Taxol was generously supplied by Dr Pantaloni (CNRS).

Preparation of oocytes. — Ovaries were surgically removed from animals anesthetized with MS 222 (1 g/l ; Sandoz). Stage VI oocytes (Dumont, 1972)

were enzymatically defolliculated by dispase and collagenase digestion (Huchon *et al.,* 1981) and incubated in medium A : 88 mM NaCl ; 0,33 mM Ca $(NO_3)_2$; 1 mM KCl ; 0,41 mM $CaCl_2$; 0,82 mM $MgSo_4$; 2 mM Tris-HCl ; pH 7,4 (Merriam, 1971). Penicillin (50 000 UI/l) and streptomycin (50 mg/l) were added to the medium.

Microinjection procedure. — A stock solution of taxol (2.9 mM) was prepared in pure ethanol. Oocytes (total volume 1 μ l or water diffusion volume 0.5 μl) were microinjected, at the equator level, with 75 nl of diluted taxol solution (1/100) in 30 percent ethanol. The final taxol concentration may be estimated to about 4 μM in the cell water compartment.

Assay for MPF activity by cytoplasmic transfer. — MPF activity was tested by cytoplasmic transfer ; apical cytoplasm (50 nl) from one mature donor oocyte was microinjected into one recipient oocyte (stade VI). Donor oocytes were induced to mature by progesterone 1 μM.

Enucleation procedure. — Enucleation of defolliculated oocytes was performed according to a modification of the method of Ford and Gurdon (1977). A small incision was made obliquely at the animal pole with a tungsten needle. After a few minutes the oocyte was gently squeezed with a needle or forceps until the translucent nucleus popped out of the wound ; if necessary the germinal vesicle was removes from the oocyte. The most successfully enucleated oocytes healed within one to two hours.

Maturation criteria, cytological analysis. — In oocytes induced to mature in the presence of progesterone, the appearance and development of the maturation spot at the animal pole of the oocyte were observed individually every 10 min under a dissection microscope until the time selected for fixation.

In treated oocytes (ionophore incubation or taxol injection) the appearance and the evolution of the normal or abnormal spots were observed individually every 10 min under a dissection microscope. They were fixed when the external morphological appearance (essentially pigment repartition) stabilizes ; generally two hours after the appearance of the maturation spot.

The oocytes were fixed in Smith's solution for 6 to 12 hours, washed, dehydrated, embedded in paraffin and cut into serial 10 μm sections which were stained with the Feulgen reagent and Fast Green.

Results.

I. — *Presence of microtubules during nuclear envelope breakdown.*

During GVBD (germinal vesicle breakdown) a fibrillar network is organized at the basal part of the disintegrating nucleus (Brachet *et al.,* 1970 ; Huchon *et al.,* 1981). This structure appears concomitantly with the basal breakdown of the nucleus and migrates to the oocyte surface as the nuclear envelope disappears (fig. 1, 2). It includes two cytological distinct parts :

— a dense layer tangent to the basal limit of the nucleus, highly stained with fast green ;

— bundles of fibers perpendicular to the dense layer, oriented towards the animal pole ; chromosomes are found in this region.

A granular material accumulates beneath this complex apparatus.

The fibrillar network migrates towards the animal pole, since the distance from the base of the network to the plasma membrane decreases as maturation progresses ; this migration lasts about 30 minutes. At the end of the migration period, condensed chromosomes are observed in an apparently homogeneous area located near the plasma membrane (fig. 3) where metaphase I appears.

We have shown previously by electron microscopy that microtubules are present at the level of the bundles of fibers seen by light microscopy (Huchon *et al.,* 1981). When oocytes were sectioned and examined by immunofluorescence microscopy with antibodies to tubulin, positive staining was seen at this level V (manuscript in preparation). These transitory microtubules were always observed during normal maturation (fig. 4).

II. — *Effect of ionophore A 23187 on formation of the first meiotic spindle.*

A. *Induction of GVBD in the presence of ionophore A 23187.* — As first reported by Wasserman and Masui (1975), this ionophore can induce the formation of MPF and GVBD. The response was dependant upon Ca^{2+} ion concentrations in the medium and varied among the tested females.

1. *Normal Ca^{2+} ion concentration (0.74 mM).* — In the presence of ionophore and a normal Ca^{2+} ion concentration, 73 % maturation was obtained in one female among three tested. In these matured oocytes a typical or almost typical maturation spot was observed. Peripheral metaphase II spindles localized in the cortex were observed in 6 oocytes and only one atypical meiosis figure in deep cytoplasm (table 1).

2. *Increased Ca^{2+} ion concentration (15 mM).* — When the incubation medium was supplemented with an excess of Ca^{2+} ions (15 mM), a low percentage of GVBD was obtained ; in the two females tested (♀ 1, ♀ 2) the percentages were respectively 40 and 23 %. In agreement with the results of Wasserman and Masui (1975), we observed that the animal hemisphere pigment repartition of « maturing » oocytes was abnormal : either marbled appearance of

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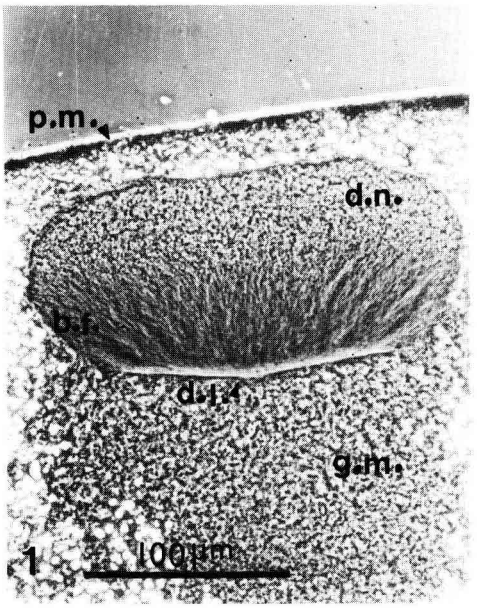
FIG. 1-2. — *Meiotic maturation of* Xenopus *oocyte : Nuclear envelope breakdown, organization of the fibrillar network :* (1) *well developed fibrillar network ;* (2) *peripheral localization of the network.* Phase contrast image. ▶

FIG. 3. — *Gathering of chromosomes in an homogeneous area, where metaphase I appears.* b.f. = bundle of filaments ; ch. = chromosome ; d.l. = dense layer ; d.n. = disintegrating nucleus ; g.m. = granular material ; h.a. = homogeneous area ; p.m. = plasma membrane.

FIG. 4. — *Ultrathin section of microtubules* (arrows, mt.) *at the level of the fibrillar network.* G = 28 000.
(Unpublished observation by G. Steinert, Brussel).


p.m.
d.n.
b.f.
d.l.
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1
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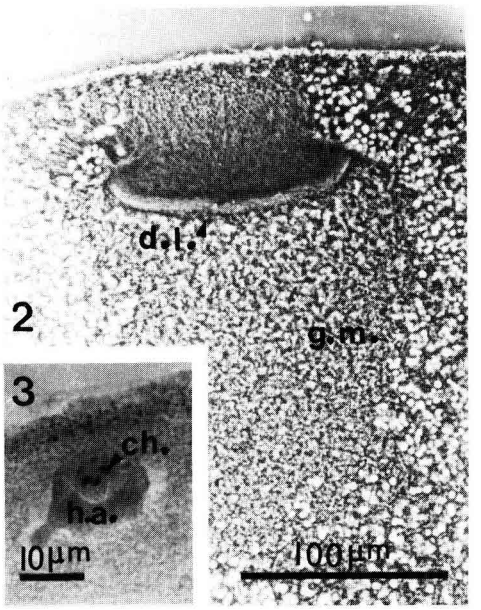
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g.m.
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3
ch.
h.a.
10µm

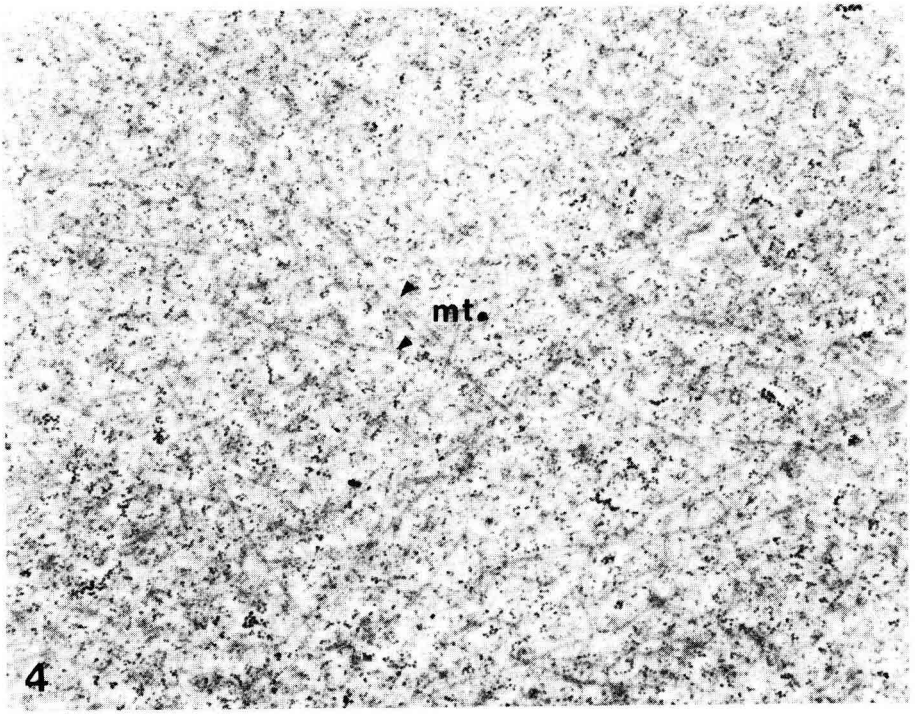
mt.
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TABLE 1

Ca^{2+} ionophore A 23187 induced maturation : cytological analysis.

Females		♀1	♀2	
Ca^{2+} ion concentrations (mM)		15	0,74	15
Total number of oocytes		N = 18	N = 7	N = 19
Cytological stages	basal GVBD	N = 5	N = 0	N = 3
	GVBD nuclear envelope remnants ; condensed chromosomes associated	N = 1	N = 0	N = 3
	GVBD nuclear envelope remnants ; some chromosomes ; reduced fibrillar network	N = 0	N = 0	N = 2
	GVBD reduced fibrillar network	N = 0	N = 0	N = 1
	GVBD anormal meiotic spindle in deep cytoplasm	N = 5	N = 1	N = 9
	GVBD chromosomes not found	N = 2	N = 0	N = 1
	GVBD normal or subnormal peripheral metaphase	N = 5	N = 6	N = 0

70 oocytes from ♀ 1 and 130 oocytes from ♀ 2 were incubated with the ionophore (24 μM) and Ca^{2+} (0.74 or 15 mM). They were divised in two batches : one for cytological analysis and one for estimation of GVBD.

The percents of maturation (absence of germinal vesicle), as judged by dissection of water-boiled fixed oocytes, were : ♀ 1 (Ca^{2+} 15 mM) : 40 % ; ♀ 2 (Ca^{2+} 0.74 mM) : 73 % ; (Ca^{2+} 15 mM) : 23 %.

The cytological analysis was performed only in oocytes which showed a maturation spot or pigment modification at the animal pole. It was verified that in the absence of pigment modification no GVBD had occurred.

Control oocytes were incubated in the presence of progesterone (1 μM) ; 100 % of maturation were induced in both females.

N = number of analyzed oocytes. GVBD : germinal vesicle breakdown.

the reduced pigmented animal hemisphere or dark island of pigment at the apex of the oocyte.

In the 37 oocytes, from both females (♀ 1 and ♀ 2), subjected to cytological analysis, the nuclear envelope had broken down after treatment with ionophore and Ca^{2+} ions (15 mM). As shown in table I, different stages of germinal vesicle breakdown were observed. Remnants of the nuclear envelope (fig. 5) were only found in 14 oocytes only among 37 oocytes which were sectioned. The normal fibrillar network was not generally observed. In a few cases (3 oocytes) a vestigial fibrillar structure was found with or without (fig. 6) fragments of the nuclear envelope. Abnormal meiotic spindles were present in the deep cytoplasm in

14 oocytes (fig. 7, 8). Peripheral spindle was observed in only 5 oocytes from ♀ 1.

B. *Induction of maturation in the presence of progesterone.* — In another series of experiments, oocytes were induced to mature by progesterone 1 μM. They were treated with the ionophore and Ca^{2+} ions (15 mM) at the onset of the visible expression of maturation, *i.e.* when irregular dark pigment deposits appeared at the animal pole (stage a from Huchon *et al.*, 1981). Two hours later the oocytes were fixed. The results obtained (table 2) were comparable to those described when oocytes were induced to mature by ionophore and Ca^{2+} ions alone.

Taken together these cytological observations show that when the free intracellular Ca^{2+} concentration is increased, rupture of the nuclear envelope occurs. Although this treatment inhibits, in most cases, the normal formation of the spindle I, it permits blockage at different stages of the transition prophase → metaphase I. These stages were atypical and may represent abortive steps of the first meiotic cell division. The results presented in table II show that late elevation of the free Ca^{2+} concentration at the onset of the rupture of the nuclear envelope is sufficient to perturb the whole process.

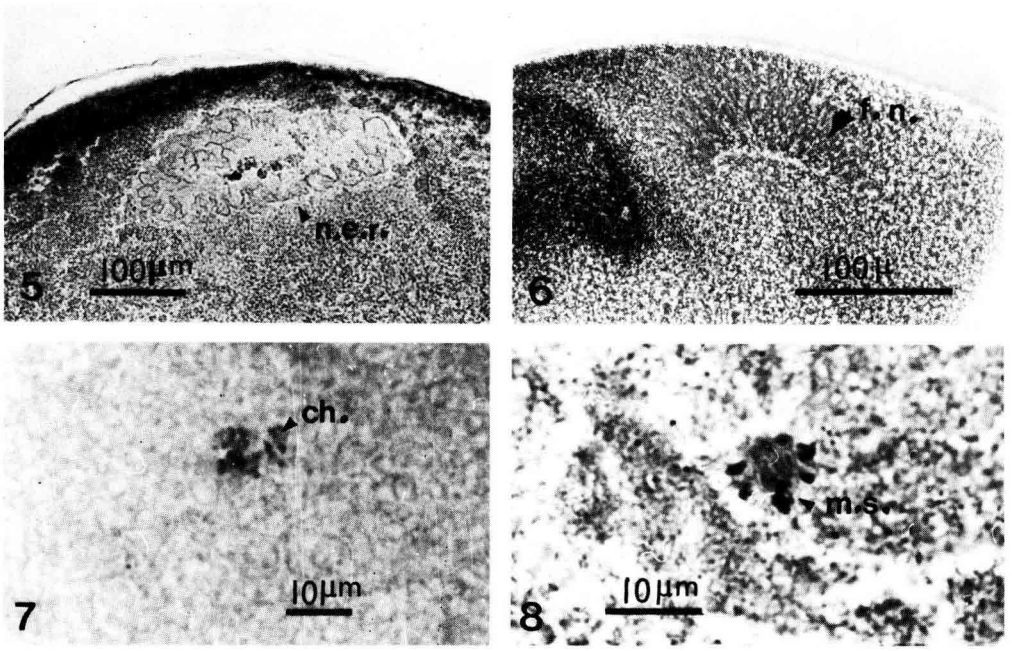


FIG. 5-6-7-8. — *Induction of GVBD by ionophore A 23187 and Ca^{2+} ions 15 mM : cytological analysis of different observed stages* (table 1) : (5) *remnants of nuclear envelope* (n.e.r.) ; (6) *reduced fibrillar network* (f.n.) ; (7) *condensed chromosomes* (ch) *in deep cytoplasm* ; (8) *abnormal meiotic spindle* (m.s.) *in deep cytoplasm.*

TABLE 2

Effect of Ca^{2+} ionophore A 23187 on progesterone induced maturation : cytological analysis.

Females		♀1	♀2
Total number of oocytes		N = 9	N = 16
Cytological stages	GVBD nuclear envelope remnants, some condensed chromosomes associated	N = 3	N = 8
	GVBD anormal meiotic spindle in deep cytoplasm	N = 3	N = 0
	GVBD some chromosomes with few fibrils in deep cytoplasm	N = 0	N = 1
	GVBD chromosomes not found	N = 1	N = 7
	GVBD normal or subnormal meiosis figures	N = 2	N = 0

Oocytes were induced to mature in the presence of progesterone (1 μM). The incubation medium was supplemented with the ionophore (24 μM) and Ca^{2+} (15 mM) at stage a (*i.e.* when irregular dark pigment deposits appeared at the animal pole).

The tested females (♀ 1, ♀ 2) were the same as in former experiments.

N = number of analyzed oocytes. GVBD : germinal vesicle breakdown.

The Ca^{2+} ionophore experiments suggest that the polymerization/depolymerization of the microtubules observed at the lower part of the nuclear envelope may be involved in the gathering of the chromosomes and the assembly of a normal metaphase I. To test further this possibility we microinjected taxol, which is known to promote tubulin polymerization and/or to inhibit microtubule depolymerization, into oocytes at different periods of the maturation process.

III. — *Effect of taxol on formation of the first meiotic spindle.*

A. *Progesterone induced maturation.* — Oocytes were microinjected with taxol (75 nl, 2 ng) and then continuously treated with progesterone 1 μM. Control oocytes were induced to mature with progesterone alone. When a typical maturation spot appeared in control oocytes, no pigment changes or atypical pigment repartition was observed in progesterone and taxol treated oocytes. However taxol treated oocytes contained transferable MPF activity (table 3).

The main morphological modifications induced by taxol were at the level of the fibrillar network. As reported in table IV, oocytes were blocked at 4 different steps. The cytological aspects of these arrested stages were abnormal as compared to control maturing oocytes.

1) The nucleus was disrupted only at its basal pole. Bundles of fibers, perpendicular to the basal part of the disintegrating germinal vesicle, were

oriented both towards the nucleus and the cytoplasm (fig. 9, 10). The dense layer that typically stained with fast green was not observed. Apical and lateral segments of the nuclear envelope and the basal bundles of fibers still enclosed the nucleoplasm. No cytaster was present in the cytoplasm (fig. 9).

TABLE 3

Effect of taxol treatment on MPF activity.

	Donor oocytes taxol + progesterone	Recipient oocytes	Oocyte maturation
♀1	N = 10	N = 10	90 %
♀2	N = 11	N = 11	100 %

MPF activity was tested by cytoplasmic transfer ; cytoplasm (50 nl) from each mature donor oocyte, was microinjected into a recipient oocyte (Stage VI). Donor oocytes were induced to mature, after taxol treatment, by progesterone. Ten mature donor oocytes (with atypical maturation spot) were individually tested.

Controls for these two females : — progesterone induced maturation, percent of maturation : ♀ 1 = 100 % ; ♀ 2 = 100 % ; — MPF induced maturation, percent of maturation : ♀ 1 = 100 % ; ♀ 2 = 100 %.

N = Number of tested oocytes.

2) The nucleus has totally disappeared. Brush-like cytasters were found in the area of the disintegrated nucleus. Numerous, well-developped asters were seen in the whole animal hemisphere whereas fibrous areas were present near the equator in the deep-ooplasm (fig. 11). No chromosomes were observed.

3) The nucleus has totally disappeared. A microtubular apparatus near the oocyte surface showed a crescent-like configuration. Numerous well-formed asters were seen in the animal hemisphere and fibrous areas were observed in the deep ooplasm (fig. 12). A few condensed chromosomes were associated with the fibrillar structure.

4) The nucleus has disappeared. Chromosomes were found near the plasma membrane in a large spherical structure whose fibers were anarchically oriented (fig. 13). No typical spindles were formed. Numerous cytasters and fibrous areas were observed in the animal hemisphere.

In another series of experiments, oocytes were induced to mature by progesterone (1 μM), then taxol (75 nl, 2 ng) was injected into oocytes at stage a of external expression of maturation. All oocytes were arrested at the intermediary stage 2 (table 4).

B. *MPF induced maturation.* — Taxol (75 nl, 2 ng) was microinjected into oocytes before transfer of cytoplasm from mature oocytes. Arrested oocytes were fixed two hours after MPF transfer. Oocytes from ♀ 1 were blocked at the intermediary stage 4 ; in the two other females (♀ 4, ♀ 5) oocytes were arrested at stage 3 (table 4).

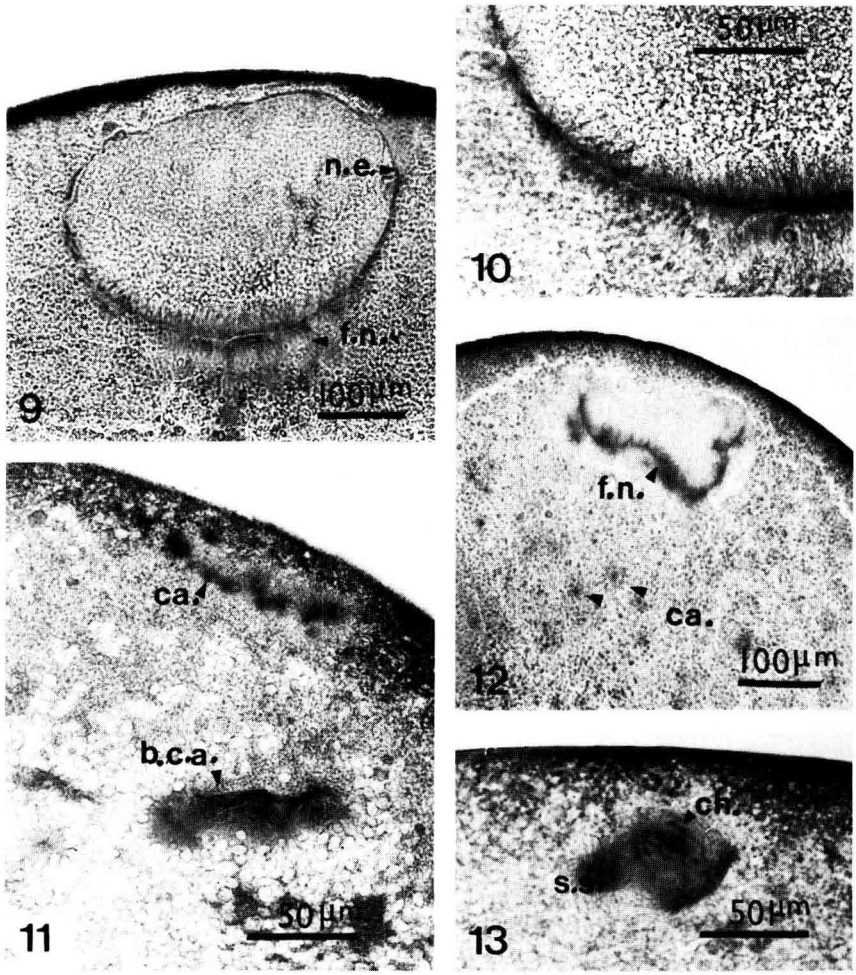
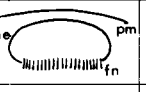
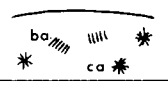
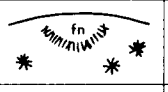
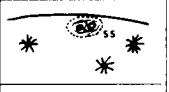


FIG. 9-10-11-12-13. — *Effect of taxol microinjection on GVBD and the formation of the first meiotic spindle :* 4 stages were observed (table 4). (9) *general view of a disintegrating nucleus blocked at stage I ;* (10) *detail of the basal part of the nucleus at stage I ;* (11) stage 2 ; (12) *stage 3 ;* (13) *stage 4.*
Oocytes were microinjected with taxol (2 ng) then continuously treated with progesterone 1 µM. b.ca. = brush-like-cytaster ; ca. = cytaster ; ch. = chromosome ; f.n. = fibrillar network ; s.s. = spherical structure.

In control experiments (♀ 3, ♀ 4) taxol was also microinjected into prophase I blocked oocytes. No asters were observed. In contrast, as already reported by Heidemann and Gallas (1980), microinjection of taxol induced numerous asters in metaphase II oocytes (MPF or progesterone induced maturations — not shown).

TABLE 4

Effect of taxol on maturation : cytological analysis.

Treatment	females	CYTOLOGICAL STAGES			
		STAGE 1	STAGE 2	STAGE 3	STAGE 4
					
Progesterone and taxol at the same time	♀1	N = 1	N = 0	N = 0	N = 11
	♀3	N = 3	N = 7	N = 0	N = 0
	♀4	N = 1	N = 2	N = 6	N = 0
Taxol at stage a of progesterone induced maturation	♀3	N = 1	N = 10	N = 0	N = 0
Taxol and MPF at the same time	♀1	N = 0	N = 0	N = 1	N = 11
	♀4	N = 0	N = 2	N = 11	N = 0
	♀5	N = 0	N = 0	N = 13	N = 0

For progesterone induced maturation, oocytes were incubated continuously in the presence of hormone (1 μM) ; taxol (50 nl) was microinjected either before progesterone addition either at stage a (onset of the maturation spot).

For MPF induced maturation, oocytes were injected with taxol (75 nl), and then with cytoplasm (50 nl) taken from mature donor oocytes.

Controls : For each female mature control oocytes (ten for progesterone, ten for MPF) were analyzed ; metaphase II spindle were always observed.

N = Number of analyzed oocytes. b.a. = brush-aster ; c.a. = cytaster ; f.n. = fibrillar net work ; n.e. = nuclear envelope ; p.m. = plasma membrane ; s.s. = spherical structure.

IV. — *Effect of taxol in enucleated oocytes.*

Well healed enucleated oocytes from two females were induced to mature by transfer of MPF. Taxol was microinjected into enucleated oocytes at a time when 100 % of the nucleated MPF-stimulated oocytes from the same female had matured. One hour after taxol injection, only well healed oocytes were fixed (30 oocytes). Depending upon the presence and the number of cytasters in the cytoplasm, three cases were observed : no aster ; one to three asters ; several cytasters (table 5). A correlation could be established between the size of the wound at the time of enucleation and the presence of cytasters. Enucleated oocytes with a large wound did not produce asters or very few when injected with taxol. In contrast, enucleated oocytes with a small wound produced numerous asters when injected with taxol. These results suggest that nucleoplasm may have leaked during enucleation in small wound oocytes. In ten prophase I blocked oocytes, from one female, the nucleus was opened with a tungsten needle and then microinjected with taxol. No cytaster was observed.

TABLE 5

Effect of taxol treatment on MPF induced maturation in enucleated oocytes : cytological analysis.

Wound size at the enucleation time		large		small	
Cytasters or fibrillar area :		no	1 to 3	1 to 3	more than 50
females	$♀_4$	N = 5	N = 3	N = 1	N = 4
	$♀_6$	N = 12	N = 4	N = 1	N = 0

Enucleated oocytes (large and small wound size) were microinjected with MPF. Taxol (75 nl) was then injected at the time when MPF microinjected control nucleated oocytes has matured.

In control matured oocytes taxol did produce at least 50 cytasters of various size. This estimation was calculated from 5 oocytes per female (not shown).

N = number of cytological analyzed oocytes.

Discussion.

During the first meiotic cell division of the *Xenopus* oocyte an asymmetrical microtubular array appears at the basal part of the disintegrating nucleus. In order to know whether these transient microtubules play a role in the maturation process we have investigated the effects of two agents which are known to perturb *in vivo* microtubular structures : the Ca^{2+} ionophore A-23187 and taxol. In no instance did these *in ovo* treatments inhibit MPF appearance. Our results show that :

1) the Ca^{2+} ionophore inhibits the assembly of the juxtanuclear microtubules when the extracellular Ca^{2+} concentration is maintained at high level (15 mM) and no metaphase spindles were found in the oocyte cortex.

2) In the presence of taxol, an atypical microtubular network appears at the basal part of the disintegrating nucleus. The change in this network varies among oocytes (fig. 9, 11, 12). In no instance did a normal metaphase occurs ; in about 25 % of treated oocytes, condensed chromosomes were observed, near the plasma membrane, associated with bundles of microtubules anarchically oriented (fig. 13 and table 4).

These results indicate that microtubule assembly/dissassembly play a critical role during a short period (30 min) which takes place between the beginning of the nuclear membrane breakdown and the organization of the first metaphase spindle.

In progesterone stimulated oocytes microtubules are seen at the onset of germinal vesicle breakdown, in the area where nucleoplasm and cytoplasm are mixed at the basal pole of the disrupting nucleus. At this period of the maturation process MPF can be transfered by injection into recipient oocyte. This cytological observation raises a fundamental question : why are microtubules formed in this region and what are the mechanisms controlling their assembly ? As reported in the Introduction very few microtubules are present in the vicinity of the

nuclear region in full-grown prophase I blocked oocytes. Furthermore these oocytes are unable to support tubulin polymerization in all tested conditions : either after basal bodies microinjection, or centrosomes microinjection and or taxol treatments (Heidemann and Kirschner, 1975, 1978 ; Karsenti *et al.,* 1984 ; Heidemann and Gallas, 1980). We assume that the critical concentration for microtubule assembly at this stage is high enough to prevent polymerization of tubulin.

When low doses of taxol (final concentration 4 μM, *i.e.* a dose three times lower than that used by Heidemann and Gallas, 1980) are microinjected either at the time of progesterone treatment or in maturing oocytes just before the beginning of the nuclear envelope breakdown, the formation of the microtubular array present at the basal pole of the germinal vesicle is modified. In some oocytes the lateral and apical disruption of the nuclear envelope is arrested and the nucleoplasm does not leak out of the nucleus and interestingly no cytaster was induced (fig. 9, 10). In all other cases the nuclear envelope disappears and the mixing of the nucleoplasm and the cytoplasm occurs. Well developed brushes of microtubules are seen together with numerous asters (fig. 11, 12). Therefore two conditions seem to be required for tubulin assembly during GVBD. First, the presence of the MPF activity in the cytoplasm ; second, the release of regulatory factors from the disintegrating nucleus. Apparently none of these conditions is sufficient in itself. No juxtanuclear microtubules appear after the mechanical disruption of the nuclear envelope in stage VI oocyte and no cytasters are formed in the presence of taxol. Also, enucleation prevents cytaster formation in MPF and taxol treated oocytes. These results are in good agreement with the observations of Heidemann and Kirschner (1978) that showed that oocytes acquired the ability to form asters upon basal bodies injection only at the time of germinal vesicle breakdown. They also confirm experiments by Hanocq-Quertier *et al.* (1978) indicating that D_2O induced cytasters in the basal part of the nucleus where it starts to breakdown.

In a recent investigation Karsenti *et al.* (1984) microinjected karyoplasts, nuclei devoid of centrosomes, into the cytoplasm of inactivated *Xenopus* eggs. They showed that these nuclei assembled a microtubule array around them after the breakdown of their nuclear envelope. These observations, in agreement with our results, again suggest that the nucleus locally reduces the critical concentration for tubulin polymerization when MPF activity is present in the cytoplasm.

The ionophore experiments strengthened the role of microtubules during maturation. In a very few oocytes from rare females the ionophore induces normal maturation by itself (incubation medium containing normal Ca^{2+} 0,74 mM). The whole maturation process appears cytologically normal, indicating that intracellular Ca^{2+} levels do not reach sufficient concentrations to perturb microtubule assembly/dissassembly. In contrast, when extracellular Ca^{2+} is increased (15 mM) the ionophore totally inhibits the formation of the microtubular array and the subsequent metaphase assembly without preventing the breakdown of the germinal vesicle. This result confirms the observation of Baltus *et al.* (1977). Interestingly, very short microtubular structures were observed around the

condensed chromosomes, indicating that some microtubules near or associated with the chromosomes may remain stable even in the presence of high Ca^{2+} level.

Taken together our results indicate that the « basal nuclear array » is the first manifestation of normal microtubule assembly during maturation ; this array may control the assembly and the migration of the chromosomes towards the animal pole.

An essential unanswered question remains : why does this transient microtubule structure appears in this precise region ? At least two hypotheses, which are not at all exclusive, may be proposed. It exists in this area a local accumulation of MAPs (microtubule associated proteins) that, in the presence of nucleoplasm and under the control of MPF, induces the tubulin assembly. In preliminary experiments we have microinjected fluorescent MAPs into prophase blocked oocyte ; two hours after microinjection the fluorescent MAPs are localized exclusively at the basal part of the nucleus (Jessus *et al.,* 1984). It is also possible that the presence of numerous reticulum vesicles beneath the basal part of nuclear envelope (Huchon *et al.,* 1981) which actively sequester Ca^{2+} (Cartaud *et al.,* 1984), may locally regulate the Ca^{2+} concentration necessary for microtubule polymerization.

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Résumé. *Les microtubules pendant la rupture de la vésicule germinative (GVBD) de l'ovocyte de Xénope : effets de l'ionophore Ca^{2+} A. 23187 et du taxol.*

Au cours de la 1re division méïotique de l'ovocyte de *Xenopus,* un appareil microtubulaire transitoire apparaît à la base du noyau en voie de désintégration. Pour savoir si cette structure joue un rôle dans la formation du 1er fuseau méïotique, nous avons étudié l'action des ions Ca^{2+} et du taxol au cours de la maturation. Quand la concentration cytosolique en Ca^{2+} libre est augmentée par l'ionophore A. 23187, l'appareil microtubulaire n'apparaît pas et la formation du fuseau est inhibée. Quand du taxol est injecté dans des ovocytes en cours de maturation, les microtubules périnucléaires sont distribués d'une façon anarchique et l'organisation du fuseau de métaphase I est bloquée. L'activité MPF (Maturation Promoting Factor) est maintenue dans nos conditions expérimentales (rupture de l'enveloppe nucléaire induite). Ces résultats indiquent que la polymérisation/dépolymérisation des microtubules périnucléaires est impliquée pendant la rupture du noyau et la formation du 1er fuseau de division méïotique.

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