Levels of microtubules during the meiotic maturation of the *Xenopus* oocyte

CATHERINE JESSUS*, CATHERINE THIBIER and RENÉ OZON

Laboratoire de Physiologie de la Reproduction, Groupe Stéroïdes, UA-CNRS 555, Université Pierre et Marie Curie, 4 Place Jussieu, 75252 Paris Cédex 05, France

* Author for correspondence

Summary

The total level of tubulin and the ratio of polymeric tubulin to tubulin dimer were measured by a colchicine filter-binding assay during meiotic maturation of the *Xenopus* oocyte. Although the total level of tubulin remains unchanged (0·12 ± 0·03 μg/oocyte), the level of polymeric tubulin decreases during maturation (25% in prophase oocytes *versus* 20% in metaphase oocytes). The percentage of polymerized tubulin was estimated after drug (nocodazole and taxol) treatments and cold treatment in prophase and progesterone-matured oocytes; in all cases the microtubules present in mature oocyte are less stable than prophase microtubules. The presence of the nucleus modifies neither the level nor the stability of prophase microtubules. Our quantitative results as well as cytological arguments suggest that full-grown *Xenopus* oocytes may contain a cortical microtubular array.

Key words: microtubules, meiotic maturation, oocyte, *Xenopus*.

Introduction

During cell division, drastic changes occur in the cytoskeletal organization, leading to disassembly of interphase microtubules and assembly of spindle microtubules. It has been shown (review by Kirschner & Mitchison, 1986) that the half-life of an interphase microtubule is much longer than the half-life of a spindle microtubule (10 min *versus* 30 s), suggesting that interphase cytoplasm contains microtubule-stabilizing activity when compared with metaphase cytoplasm. The molecular mechanisms involved in this process remain unknown.

The *Xenopus* oocyte is an excellent system for analysing in a whole cell the molecular cascade that governs the prophase/metaphase transition of the cell cycle (Maller, 1983; Ozon *et al.* 1986). It is a particularly suitable system in which to study the regulation of microtubule polymerization/depolymerization during the progression of the cell cycle (Karsenti *et al.* 1984; Jessus *et al.* 1986). In a recent paper Elinson (1985) showed that it was possible to isolate quantitatively, in a stabilizing medium, polymeric tubulin (microtubules) from *Xenopus* eggs. This technique permits the biochemical measurement of the levels of microtubules. It also traps the proteins that may be associated with them and that may regulate their stability. In the work reported here we adapted the stabilizing method described by Elinson (1985), together with a colchicine filter-binding assay, in order to quantify microtubules and to study their properties *in ovo* during the prophase to metaphase transition of the *Xenopus* oocyte.

Materials and methods

Animals

Xenopus laevis adult females (SEREA-CNRS, France) were bred and maintained under laboratory conditions.

Chemicals

[^{35}S]methionine (16 mCi ml^{-1}, 6·8 Ci mg^{-1}) and Ring [C-*methoxyl*-^{3}H]colchicine (5 Ci mmol^{-1}, 1 mCi ml^{-1}) were obtained from Amersham. Nocodazole (methyl-(5-(2-thienyl carbonyl)-1H-benzimidazol-2-yl) carbonate) was obtained from Sigma. Taxol was a gift from Dr M. Suffness of the NIH, Bethesda, MD, USA. Guanosine-5′-triphosphate (GTP) was purchased from Boehringer-Mannheim. Aprotinin and leupeptin were from Sigma.

Oocyte preparation

Animals were anaesthetized with MS222 (Sandoz) at 1 g l^{-1}. Ovaries were removed and transferred to medium A composed of 88 mM-NaCl, 0·33 mM-$Ca(NO_3)_3$, 1 mM-KCl, 0·41 mM-$CaCl_2$, 0·82 mM-$MgSO_4$, 2 mM-Tris, pH 7·4, penicillin (50 000 units l^{-1}) and streptomycin (1 mg l^{-1}).

After dispase (0·4 mg ml^{-1}) digestion for 4 h at laboratory temperature and collagenase (0·8 mg ml^{-1}) digestion for 1 h at 25 °C with continuous stirring, stage VI oocytes, 1·3 mm in diameter (Dumont, 1972), were collected.

Oocytes were induced to mature by addition of 1 μM-progesterone to the external medium. The criterion for maturation was the appearance of a white spot surrounded by pigment on the coloured animal pole of the oocyte. Matured oocytes are referred to as 'metaphase oocytes', and immature oocytes as 'prophase oocytes'.

Oocyte treatments

Enucleation procedure. Oocytes were enucleated using a procedure adapted from Ford & Gurdon (1977). Small numbers of oocytes were transferred to medium A diluted 1:10 and containing an additional 10 mM-$MgCl_2$. A small incision was made obliquely at the animal pole, following which the translucent germinal vesicle emerged from the slit. The enucleated oocytes were immediately transferred to the phosphate healing solution (90 mM-potassium phosphate, pH 7·2, 10 mM-NaCl, 1 mM-$MgSO_4$), kept in this solution for 60–90 min and then transferred to medium A. When the enucleation slit was completely eliminated the oocytes were used for experiments.

Taxol injection. A stock solution of 53 μM-taxol was prepared in pure ethanol. Oocytes (total volume, 1 μl; water diffusion volume, about 0·5 μl) were microinjected at the equatorial level, with 75 nl of taxol solution (ethanol, 30°). The final taxol concentration may be estimated to be about 8 μM in the cell water compartment.

Cold treatment. The incubation medium was kept on ice until the temperature had reached 2–3 °C. Oocytes were incubated in this medium for various times (10 s to 90 min), and then removed for quantification of tubulin.

Nocodazole treatment. The oocyte incubation medium was supplemented with nocodazole (10 μg ml^{-1}) for 1 h. To test the reversibility of the drug, oocytes were then washed and incubated for 2 h in the incubation medium prior to tubulin quantification.

^{35}S-labelled protein-labelling procedure. Oocytes were preincubated for 16 h at laboratory temperature (22 °C) with gentle continuous shaking in medium A containing [^{35}S]methionine (166 μCi ml^{-1}, 200 oocytes/ml). Then oocytes were washed eight times in medium A at room temperature.

Gel electrophoresis and autoradiography

SDS-solubilized fractions (20–50 μl) were submitted to electrophoresis, according to Laemmli (1970) adapted for 7 cm × 8·5 cm slab gels (1 mm thick) of 0·1 % SDS–7·5 % polyacrylamide; slab gels were run for 3 h at room temperature at a constant voltage of 160 V and were stained with Coomassie Blue. For the detection of [^{35}S]methionine-labelled proteins, gels were incubated in Amplify (Amersham) at room temperature with agitation for 15–30 min. Gels were dried and exposed in contact with Kodak X-Omat AR film in a Cromex cassette (Du Pont) with intensifying screen, at −70 °C.

Microtubule extraction procedure

Microtubule extraction was done by using a modification of the procedure described by Elinson (1985). In a typical extraction, 60 oocytes were rinsed in 5 ml medium (RM) (100 mM-Pipes, pH 6·9, 1 mM-$MgCl_2$, 10 mM-EGTA, 10 % glycerol). After removing most of the solution, oocytes were mixed with 1·3 ml extraction medium (EM) (100 mM-Pipes, pH 6·9, 10 mM-EGTA, 1 mM-$MgCl_2$, 3 mM-NaN_3, 0·5 % Triton X-100, 30 % glycerol, 1 mM-GTP, 0·5 μM-taxol, 0·5 mM-benzamidine, 2 mM-PMSF, 0·01 mg ml^{-1} leupeptin, 0·02 TIU aprotinin, 100 μg ml^{-1} soybean trypsin inhibitor). The oocytes were homogenized, and the homogenate centrifuged at 1000 ***g*** for 1 min to yield a pellet containing most of the yolk platelet and a post-yolk supernatant. The pellet was washed with 0·5 ml EM and recentrifuged at 1000 ***g*** for 1 min; this 1000 ***g*** supernatant was added to the first post-yolk supernatant. All of these procedures were done at 20 °C. The 1000 ***g*** supernatant was centrifuged at 100 000 ***g*** for 1 h at 20 °C yielding a pellet that was considered to be the polymeric tubulin fraction and a supernatant that was considered to be the subunit fraction. The 100 000 ***g*** pellet was suspended in 400 μl depolymerizing medium (DM) (100 mM-Pipes, pH 6·9, 1 mM-$MgCl_2$, 2 mM-$CaCl_2$, 3 mM-NaN_3, 0·5 mM-benzamidine, 2 mM-PMSF, 0·01 mg ml^{-1} leupeptin, 0·02 TIU aprotinin, 100 μg ml^{-1} soybean trypsin inhibitor). The resuspended pellet and the supernatant were incubated on ice for 30 min before the quantification of tubulin.

Quantification of tubulin

The colchicine-binding activity has been used to determine the concentration of tubulin, according to the procedure described by Weisenberg *et al.* (1968). Samples of 200 μl of the resuspended pellet (equivalent to 30 oocytes) or of the supernatant (equivalent to 6·66 oocytes) were incubated for 90 min at 37 °C (final volume, 1 ml of the following buffer: 100 mM-Pipes, pH 6·4, 0·5 mM-$MgCl_2$, 1 mM-EGTA, 0·1 mM-EDTA, 0·5 mM-GTP, 1 mM-β-mercaptoethanol, 0·5 mM-benzamidine, 2 mM-PMSF, 0·01 mg ml^{-1} leupeptin, 0·02 TIU aprotinin, 100 μg ml^{-1} soybean trypsin inhibitor) in the presence of 0·6 μCi of [^{3}H]colchicine and variable amounts of unlabelled colchicine (between 10^{-7} and 10^{-4} M). The binding reaction was stopped by adding 10 ml of a phosphate buffer (Na_2HPO_4/NaH_2PO_4 (10 mM), 10 mM-$MgCl_2$, pH 6·4) per assay at 4 °C. Each assay was then slowly filtered at 4 °C under vacuum on DEAE-cellulose discs (Wathman DE81), previously equilibrated in the phosphate buffer. Bound colchicine was retained on the discs. The filters were washed three times with 10 ml of phosphate buffer and radioactivity was measured by liquid scintillation counting. Appropriate control tubes containing comparable amounts of proteins were included in the assay to correct for non-specific binding to filters, but the reaction was immediately stopped by adding phosphate buffer. Specific activity of [^{3}H]colchicine was determined by counting samples of incubation medium. Binding values were plotted using Scatchard's plot. The percentage of polymeric tubulin was estimated by measuring colchicine binding of tubulin in both

the pellet and the supernatant. In routine experiments, total binding was also estimated in the presence of a colchicine concentration of 2×10^{-6} M, and the non-specific binding was determined by measuring the radioactivity bound to the filters at 2×10^{-4} M-colchicine; this value was substracted from the radioactivity bound to the filters at 2×10^{-6} M-colchicine. The percentage of polymeric tubulin was calculated as follows:

Colchicine bound in the pellet (cts min^{-1})/
[Colchicine bound in the pellet (cts min^{-1}) +
colchicine bound in the supernatant (cts min^{-1})]

Results

Validation of the quantitative estimation of polymeric tubulin

As described in Materials and methods, we used an extraction procedure for oocyte tubulin, adapted from Elinson (1985), to quantify the level of tubulin polymerization during *Xenopus* oocyte maturation. A preliminary analysis was, however, necessary to validate the role of taxol (0·5 μM) as a stabilizing agent of oocyte microtubules and also to ascertain that the [^{3}H]colchicine filter-binding assay is a reliable measure of tubulin isolated from the oocyte during maturation.

Taxol (0·5 μM) does not induce tubulin polymerization during extraction. The 100 000 ***g*** supernatant of a post-yolk fraction of prophase oocyte homogenate was prepared in the extraction medium (EM) in the absence of taxol. As shown in Table 1, addition of taxol (0·5 μM) does not induce tubulin polymerization, since no [^{3}H]colchicine binding could be recovered after recentrifugation in the final pellet and since the amount of [^{3}H]colchicine bound remains constant in the supernatants, even after addition of 0·5 μM-taxol. Similar results were obtained with the 100 000 ***g*** supernatant of metaphase oocytes.

Table 1. *Taxol (0·5 μM) does not induce tubulin polymerization during extraction*

	Presence of taxol	Colchicine bound (cts min^{-1} per oocyte)	
		Prophase	Metaphase
Supernatant 1	−	110	82
Pellet 1	−	4	0
Supernatant 2	+	95	80
Pellet 2	+	5	3

A post-yolk fraction (100 000 ***g*** supernatant) was prepared after homogenization in EM without taxol and was divided in two aliquots. The first one was immediately centrifuged at 100 000 ***g*** for 1 h at 22°C, and colchicine binding was measured in the supernatant (supernatant 1) and in the pellet (pellet 1). To the second aliquot taxol was added (0·5 μM final) before centrifugation at 100 000 ***g***, for 1 h at 22°C. Colchicine binding was assayed in the supernatant (supernatant 2) and in the pellet (pellet 2).

Taxol stabilizes pre-existing microtubules during extraction procedure. Prophase oocytes were homogenized in EM in the absence or presence of 0·5 μM taxol, or in a depolymerizing medium (DM). Polymeric tubulin was assayed in the 100 000 ***g*** pellet. Table 2 shows first that, in the absence of taxol, about 30 % of microtubules have depolymerized during the whole extraction procedure, and second that little or no tubulin can be recovered in the 100 000 ***g*** pellet after homogenization in DM. Taxol 0·5 μM has the same stabilizing action in metaphase oocytes. An important question is whether polymeric tubulin in the 100 000 ***g*** pellet corresponds to microtubules or to aggregates of tubulin; this last possibility seems unlikely for two reasons: first, because Elinson (1985) using a comparable extraction procedure has already shown by electron microscopy that microtubules are present in the 100 000 ***g*** pellet prepared in EM; and second, because all the *in vitro* and *in vivo* characteristics of oocyte polymeric tubulin correspond to those of microtubules (on the basis of the effects of nocodazole, cold and taxol). Therefore, as already suggested by Elinson (1985), the term 'polymeric tubulin' will refer to microtubules. We further verified by polyacrylamide gel electrophoresis that both α and β tubulins are the major components present in the pellet (Fig. 1). In addition, a protein comigrating with actin and a band of 103 000 M_r were observed.

Quantification of tubulin by [^{3}H]colchicine filter-binding assay. It was also important to confirm the quantitative nature of the colchicine filter-binding assay. As shown in the inset to Fig. 2, the binding of [^{3}H]colchicine increases linearly with the protein concentration in the assay (or oocyte number). The percentage of polymeric tubulin remains constant in the range of concentrations tested (from 10–50 oocytes per assay). Fig. 2 shows a Scatchard plot of the binding data. When the B_{max} value for the 100 000 ***g*** pellet and the B_{max} value for the 100 000 ***g*** supernatant of the same oocyte preparations were added, the total amount of tubulin estimated in these conditions was 0·12–0·03 μg/oocyte. A similar result was also obtained by a Scatchard analysis of the colchicine binding in the

Table 2. *Stabilizing effect of taxol during extraction procedure*

Taxol	Medium	Polymeric tubulin (expressed as % of control)
+	EM	100 (control)
−	EM	70 ± 10 (n = 6)
−	DM	9·4 ± 7 (n = 8)

Oocytes were homogenized in EM (±0·5 μM-taxol) or DM. Polymeric tubulin was assayed in the 100 000 ***g*** pellets. Results are expressed as the mean ± S.D.

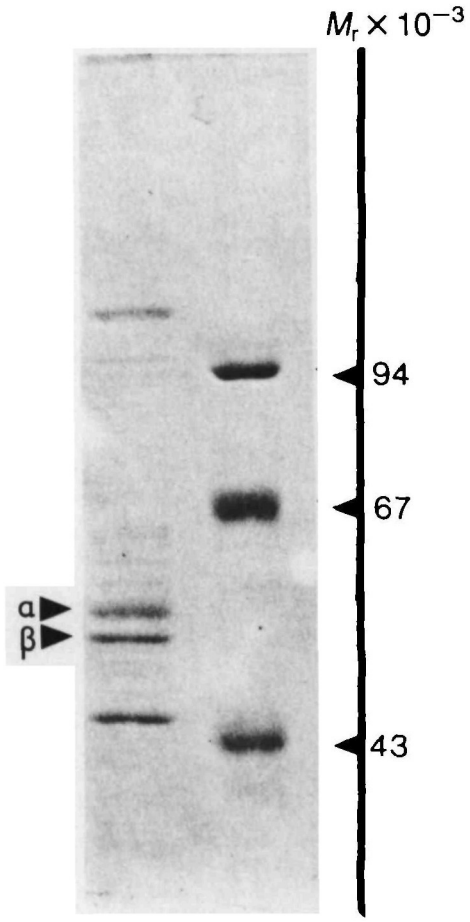

Fig. 1. Polymeric tubulin in the prophase oocyte. Prophase oocytes were homogenized in EM; polymeric tubulin recovered in the 100 000 *g* pellet was resuspended in DM, kept at 2°C for 60 min and again centrifuged at 100 000 *g* at 2°C for 60 min. The resulting supernatant was submitted to polyacrylamide gel electrophoresis as described in Materials and methods. Proteins corresponding to 6·66 oocytes were loaded in the slot. The gel was stained with Coomassie Blue. Left lane, oocyte extract; right lane, M_r markers; α, β, tubulins.

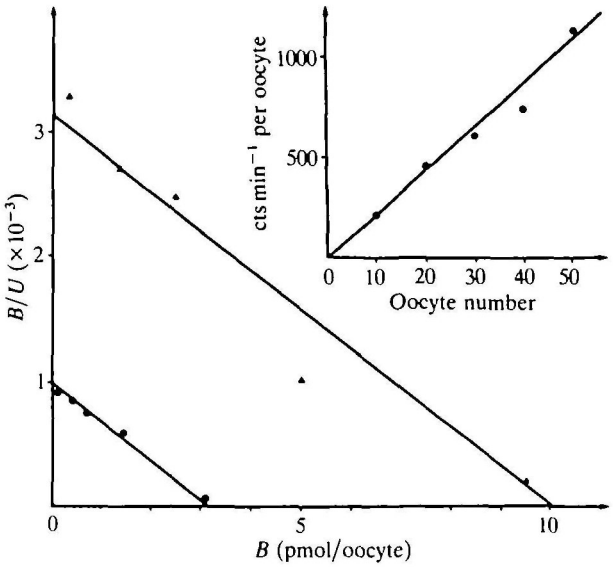

Fig. 2. Scatchard plot of colchicine binding to tubulin. Prophase oocytes were homogenized in EM and colchicine binding was tested in both 100 000 *g* pellet and 100 000 *g* supernatant at different concentrations of colchicine in the assay. B, colchicine bound per 10 oocytes; U, unbound colchicine; (●——●) pellet; (▲——▲) supernatant. Inset: colchicine binding at different protein concentrations. Prophase oocytes were homogenized in EM (60 oocytes per 1·8 ml) and colchicine binding was assayed in the 100 000 *g* pellet.

100 000 *g* supernatant prepared in the depolymerization medium. These values appear low when compared with those estimated after immunoblotting of tubulin by Elinson (1985), but compare well with values found with the *Xenopus* egg by Heidemann & Kirschner (1975). We cannot exclude the possibility that the colchicine filter-binding assay might underestimate the total amount of tubulin; in any case our results show that it permits a reliable measurement of the ratio polymeric/subunit tubulin during the maturation process (see below).

Polymeric tubulin during the course of maturation

The total amount of tubulin estimated by the colchicine filter-binding assay remains constant during the whole maturation process (0·12 ± 0·03 μg/oocyte). Table 3 indicates that the percentage of polymeric tubulin is significantly higher in prophase oocytes (25 ± 5·8 %) than in metaphase oocytes (20·2 ± 6 %). When oocytes are at the stage of GVBD (germinal vesicle breakdown) according to the morphological and cytological criteria defined by Huchon *et al.* (1981), the percentage of polymeric tubulin was found to be variable (data not shown), suggesting that the number of microtubules in the oocyte may increase or decrease during this short period. This interpretation is consistent with cytological observations (Huchon *et al.* 1981; Jessus *et al.* 1986).

Table 3. *Percentage of polymeric tubulin during meiotic maturation in control and taxol microinjected oocytes*

Prophase		Metaphase	
Control	Taxol microinjected	Control	Taxol microinjected
25 ± 5·8 % ($n = 28$)*	30 ± 9·8 % ($n = 4$)	20·2 ± 6 % ($n = 28$)*	41·3 ± 14·1 % ($n = 4$)

Control oocytes (prophase and metaphase) and taxol microinjected prophase and metaphase oocytes (taxol: 53 μM, 75 nl/oocyte) were homogenized in EM 1 h after the microinjection. The percentage of polymeric tubulin was estimated by colchicine filter binding assay. Results are expressed as the mean ± S.D. * $P < 0·05$.

Effects of drug and cold on the in ovo *level of polymeric tubulin*

Since the main objective of this research was to use the level of polymeric tubulin as a tool to estimate microtubule properties during the transition from prophase to metaphase, we have analysed the effects in cultured oocytes of different agents known to induce tubulin polymerization/depolymerization in whole cells.

Taxol. In the first series of experiments, the effect of taxol *in ovo* was studied by microinjection. Prophase and metaphase oocytes were microinjected with taxol (53 μM; 75 nl per oocyte). One hour later oocytes were homogenized in EM, and polymeric tubulin was estimated. In these conditions, there is an increase in the level of polymeric tubulin (Table 3) at both stages of oocyte development.

Cold effect. The cold stability of microtubules present in the whole oocyte was then analysed. After exposure to cold (4°C) for short times, the kinetics of tubulin depolymerization were always faster in metaphase oocytes than in prophase oocytes. Interestingly, an exposure to cold as short as 10 s induces the depolymerization of 70% of polymeric tubulin in metaphase oocyte. At 60 min, more than 80% of polymeric tubulin has disappeared in both types of oocytes (Fig. 3). Altogether, these results show: first, that prophase and metaphase oocytes contain microtubules whose cold stability differs; and second, that few microtubules (no more than 20%) remain stable after cold treatment (4°C). Microtubules that have depolymerized after exposure to cold for 1 h can repolymerize to nearly the same levels found in untreated oocytes (Fig. 3, inset). Again a striking difference was observed in the kinetics of repolymerization: prophase microtubules are re-formed earlier than metaphase microtubules.

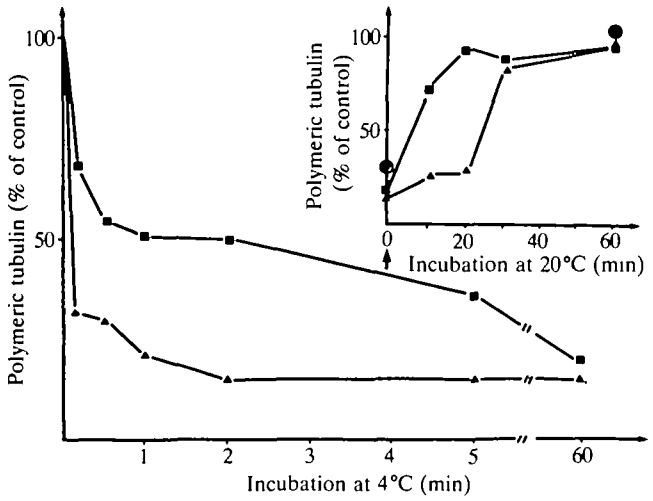

Fig. 3. Effect of cold on the level of polymeric tubulin in prophase, enucleated and metaphase oocytes. Oocytes were exposed to cold (4°C) for various times, then polymeric tubulin was estimated by the colchicine binding assay, as described in Materials and methods. Control oocytes (prophase, enucleated and metaphase oocytes at 22°C) are referred to as 100%. Inset: oocytes were exposed for 60 min to cold (4°C) and then transferred at room temperature (20°C) for various times (↑). Polymeric tubulin was estimated by the colchicine binding assay. (■——■) Prophase oocytes; (▲——▲) metaphase oocytes; (◆——◆) enucleated oocytes.

Nocodazole. Nocodazole is a drug known to induce depolymerization of microtubules in whole cells (Hoebeke *et al.* 1976). We therefore incubated oocytes in the presence of nocodazole (10 μg ml^{-1}) 60 min before homogenization. This treatment strongly decreases the level of polymeric tubulin (Fig. 4); again it must be noticed that it is more efficient in metaphase oocytes than in prophase oocytes. After removal of the drug, and incubation for a further 120 min, we did not observe repolymerization of microtubules, suggesting that the drug concentration remains high in the oocyte even after extensive rinsing (Fig. 4).

Effect of the nucleus on the level of polymeric tubulin during maturation

Does the nucleus affect the level of polymeric tubulin in prophase oocytes? To answer this question, the total amount of tubulin and the percentage of polymeric tubulin were estimated in nucleated or enucleated oocytes. They were found to be similar. Incubation of nucleated or enucleated oocytes at low temperature (4°C) induces tubulin depolymerization in a similar manner (Fig. 3). After removal from the cold and incubation for 120 min at room temperature tubulin repolymerizes again to control levels, indicating that the nucleus does not have a major effect either on the

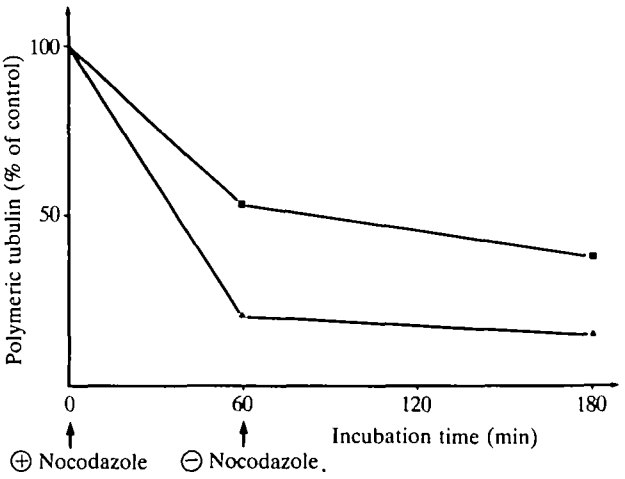

Fig. 4. Effect of nocodazole on the level of polymeric tubulin in prophase and metaphase oocytes. Nocodazole was added to the incubation medium (10 μg ml^{-1}) for 60 min (⊕) and extensively washed out (⊖). Polymeric tubulin was measured at 60 min and 180 min. Results are expressed as in Fig. 3. (■——■) Prophase oocytes; (▲——▲) metaphase oocytes.

cold stability of prophase microtubules or on repolymerization of tubulin after cold treatment (Fig. 3). In order to determine if the nucleus and its breakdown were necessary for the appearance of microtubules in matured oocytes, oocytes were enucleated; after progesterone treatment (8 h) of nucleated or enucleated oocytes, the levels of polymeric tubulin were found to be comparable in both types of oocytes. This surprising result indicates that the nucleus has a negligible effect on the appearance of microtubules in matured oocytes, and that the spindle microtubules represent only a minor fraction of the total amount of microtubules in these oocytes.

Electrophoretic and autoradiographic analysis of oocyte microtubules

Since we have shown that the stability of polymeric tubulin varies between prophase and metaphase oocytes, a major question was whether the association of specific proteins with microtubules could be responsible for this differential stability. We therefore analysed the electrophoretic patterns of the polymeric tubulin isolated after [^{35}S]methionine-labelling of prophase and metaphase oocytes. Fig. 5 shows the autoradiography of radioactive proteins present in the 100 000 ***g*** pellets. Various proteins were found associated with the tubulin doublet, among them actin. The major reproducible difference was observed at the level of two bands in the high M_r region, present in higher concentration in prophase microtubules. Further studies are necessary to determine whether these proteins correspond to microtubule-associated proteins (MAPs). In preliminary experiments, we have shown that two proteins present in the high M_r region from the 100 000 ***g*** pellets can be immunoprecipitated by a polyclonal antibody against rat brain MAP2, and a monoclonal antibody against rat brain MAP1.

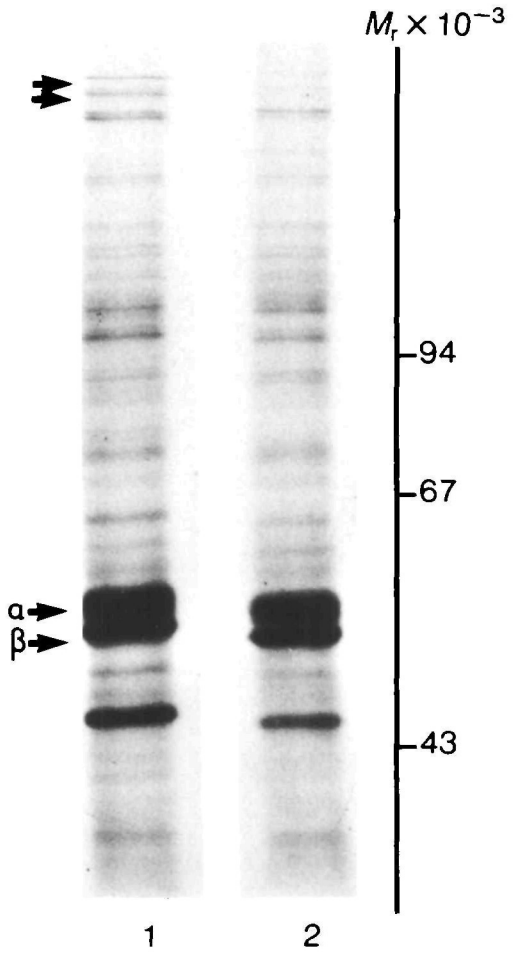

Fig. 5. [^{35}S]methionine-labelled proteins recovered in polymeric tubulin pellets of prophase and metaphase oocytes. Oocytes were labelled with [^{35}S]methionine as described in Materials and methods. After extensive washing, oocytes were homogenized in EM and the 100 000 ***g*** pellet, which contains polymeric tubulin, was resuspended in DM, kept at 2°C for 60 min, and again centrifuged at 100 000 ***g***, for 60 min at 2°C. The resulting supernatant was submitted to electrophoresis and then to autoradiography. Proteins corresponding to the same amount of oocytes (6·66 oocytes) and the same amount of radioactivity (21 000 cts min^{-1}) were loaded in each slot. Lane 1, prophase oocytes; lane 2, metaphase oocytes.

Discussion

We have developed a reliable and simple method for measuring the ratio of polymeric tubulin/free tubulin subunits in the whole *Xenopus* oocyte by the extraction procedure originally described by Elinson (1985). We added a rapid quantification of tubulin by [^{3}H]colchicine binding, in order to run numerous parallel assays. The results obtained by this method have yielded valuable information regarding the characteristics of microtubules during the prophase/metaphase transition of the *Xenopus* oocyte. (1) Microtubules are present in prophase oocytes. (2) Taxol induces tubulin polymerization in prophase oocytes as well as in metaphase oocytes. (3) The stability of prophase microtubules to cold or drug treatments is higher in prophase oocytes than in metaphase oocytes. (4) Two proteins of high molecular weight are associated with microtubules, mainly in prophase oocytes.

The observation that nearly 25% of tubulin is present in a polymerized state in prophase oocytes was rather unexpected; in fact, the apparent complete inability of immature *Xenopus* oocytes to support microtubule polymerization (Heidemann & Kirschner, 1975, 1978; Heidemann & Gallas, 1980; Huchon & Ozon, 1985) and the apparent absence of microtubules as observed by electron microscopy (Brachet *et al.* 1970) had led to the conclusion that microtubules were absent from this oocyte. Two recent studies, however, indicate that this conclusion is incorrect: Heidemann *et*

al. (1985) showed that microtubules can be observed at the ultrastructural level in the ooplasm of stratified immature oocytes; Jessus *et al.* (1986) demonstrated both by electron microscopy and immunocytology that microtubules are present around the nuclear envelope of the prophase oocyte. Our results directly confirm these cytological findings and, furthermore, show that the amount of polymeric tubulin (25% of the total tubulin pool) is astonishingly high in these oocytes. A major question therefore is where is polymeric tubulin localized in this giant cell? It is improbable that the scattered microtubules observed near the nuclear envelope can account for the total polymeric tubulin measured by our quantitative assay. Preliminary immunocytological analysis indicates that microtubules may be also present in the oocyte cortex; it may therefore be possible that the *Xenopus* oocyte cortex, like the cortex of the immature echinoderm oocyte (Otto & Schroeder, 1984), contains a microtubular network. Altogether, these results confirm and extend the recent conclusion of Heidemann *et al.* (1985) that microtubules are present in immature oocytes and that taxol stimulates microtubule assembly in this cell. Interestingly, when prophase oocytes are cold-treated, a small proportion (20%) of the microtubules remains stable; after immunofluorescence staining with anti-tubulin antibody, fluorescence remains unchanged around the nuclear envelope (Jessus *et al.* 1986); this may suggest that perinuclear microtubules are cold-resistant, whereas other cytoplasmic microtubules can depolymerize after prolonged exposure to cold.

The effect of taxol, as well as the reversibility of cold treatment, indicates that prophase cytoplasm does support microtubule polymerization. However, unlike metaphase cytoplasm, the prophase cytoplasm is unable to organize cytasters. These observations unequivocally show that the spatial distribution of nucleation centres for tubulin polymerization changes during meiotic maturation, but not the ability of tubulin to polymerize. At the same time as new microtubule organizing centres appear where the nuclear envelope breaks down, the stability of microtubules in the cytoplasm changes; the higher stability of prophase microtubules is particularly well illustrated after cold treatment (Fig. 3).

Enucleation experiments indicate that the nucleus does not appear to be involved in the maintenance of prophase microtubules or in their repolymerization after cold treatment. It has been shown that enucleation prior to the induction of maturation suppresses the ability of the mature cytoplasm to initiate aster formation (Heidemann & Kirschner, 1978; Huchon & Ozon, 1985), suggesting the absence of sites for tubulin polymerization in enucleated matured oocytes. Surprisingly, however, our results show that microtubules are present in these oocytes. This unexpected observation indicates that besides asters others sites for tubulin polymerization may exist in matured oocytes.

When *in vivo* stabilized microtubules from [^{35}S]methionine-labelled prophase and metaphase oocytes were subjected to gel electrophoresis and autoradiography, many proteins were found associated with the tubulin dimer. Interestingly, two proteins of high molecular weight were present mainly in prophase-stabilized microtubules. These preliminary observations raise the possibility that different proteins are associated *in vivo* with the different types of microtubules.

In conclusion, the quantitative estimation of microtubules isolated after stabilization from the whole oocyte has brought further understanding of the mechanisms that control the prophase/metaphase transition; it also raises new questions, among which are the following: do new MAPs appear at the time of germinal vesicle breakdown? are post-transcriptional modifications of tubulin involved during this period? what is the role of the nucleoplasm in the formation of aster microtubules?

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