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Research Article

Isoforms of soluble α-tubulin in oocytes and brain of the frog (genus *Rana*): changes during oocyte maturation

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Abstract. *Rana* oocytes have previously been shown to contain much more soluble tubulin than does the brain, suggesting different assembly and disassembly dynamics of frog oocyte tubulin compared to that in brain. By using centrifugation, SDS-PAGE, two-dimensional gel electrophoresis and Western blots, probed with anti-α-tubulin monoclonal antibodies, polymorphic α-tubulins (isoforms) were compared in brains and follicle-enclosed oocytes of northern (*Rana pipiens*) and southern (*R. berlandieri*) frogs. Oocyte tubulin in both species had isoforms with greater ranges of isoelectric point (pI) than those of brain tubulins; in particular, the oocyte tubulin pIs ranged further into the acidic region of the isoelectric-focusing gels than corresponding brain tubulin. This difference may, in part, be responsible for the previously reported assembly differences between oocyte tubulin (undetectable assembly) and brain tubulin (high assembly). Isoforms of α-tubulin with relatively acidic pI were more abundant in northern frog brain and oocyte soluble extracts than in analogous extracts from southern frogs. Furthermore, additional acidic α-tubulin isoforms were found in progesterone-treated oocytes (i.e., eggs), indicating increased heterogeneity of acidic α-tubulin isoforms during oocyte meiotic maturation. Among northern frog oocyte soluble components fractionated on Superose-6b columns, tubulin complexes with apparent molecular mass of about 1800 kDa were found to contain acidic α-tubulin isoforms while the putative oligomeric tubulins with an apparent molecular mass of about 250 kDa contained an additional relatively basic α-tubulin isoform. The acidic α-tubulin isoforms, therefore, are proposed to be associated with cold-adaptable cells of brain and oocytes, and may also be involved in stabilization of large soluble tubulin complexes in oocytes of the northern frog.

Key words. Microtubule; SDS-PAGE; two-dimensional electrophoresis; isoelectric focusing; DM1A; species specificity.

Microtubules, composed of α- and β-tubulin subunits, are subcellular structures found in all eukaryotic cells. They participate in a wide variety of fundamental cellular functions involving transportation processes, cell motility, chromosome segregation, and maintenance of cell shape [1]. In vivo and in vitro, microtubules can continually polymerize and depolymerize, a property termed dynamic instability which involves exchange between soluble and polymeric pools of tubulin [2, 3]. Many of the basic functions of different cells depend upon specialized structures containing microtubules, e.g., mitotic spindles, cilia, and neuronal axons. The diversity of microtubule-based structures has led to the hypothesis that functionally distinct tubulin isoforms are used in their assembly. These distinct isoforms in cells may result from the expression of multiple tubulin genes and from several

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posttranslational modifications [4, 5]. For the purpose of this paper, an isoform is defined as tubulin which shares common sequence homology in certain consensus regions, but differs in other regions; these differences include posttranslational changes such as acetylation, glutamylation or deglutamylation, phosphorylation, and detyrosination or tyrosination. Thus, a working definition for α-tubulin isoforms is described here as tubulin which reacts with a specific monoclonal antibody, i.e., DM1A, in one of its consensus regions, but upon isoelectric focusing shows heterogeneity in pI. Despite evidence that the distribution of some tubulin isoforms is often tissue specific to a some extent, paradoxically, in other cell types various isoforms may be found in the same structures, especially when assembled in vitro [5–7]. However, mounting evidence indicates that isoform differences, such as α- and β-tubulin multigene expression products and posttranslational modification and acidification of α-tubulins, do play an in vivo role in determining microtubule functions in a wide variety of cell types [8–12].

The regulation of microtubule dynamics has not been fully elucidated in cells. In vitro biochemical studies have suggested at least three possible mechanisms that may control microtubule polymerization dynamics: (i) regulation of gain or loss of the stabilizing cap during microtubule assembly/disassembly [2, 13]; (ii) interaction of microtubule-associated proteins (MAPs) with microtubule surfaces and ends [14, 15]; (iii) tubulin isotype or isoform itself may contribute to the intrinsic properties of microtubule stability [16].

In the common leopard frog, *Rana pipiens,* differences between brain and oocyte tubulin and microtubules have been reported; tubulin from oocytes could not be purified by warm-cold cycle microtubule assembly-disassembly, while it could be easily purified from brain [17]. In addition, soluble tubulin was found to predominate over polymeric tubulin in oocytes of *R. pipiens* and *R. berlandieri* [17, 18]. Microtubules were characterized by cold stability in brain and oocytes of the former species [17]; normal renal cells of *R. pipiens* have also been reported to have cold-stable microtubules [19]. In addition, preliminary data suggested that these cold-stable microtubules were developmentally regulated during oogenesis [20]. Furthermore, the majority of the soluble tubulins from oocytes eluted from Superose-6b gel filtration columns, indicating that soluble oocyte tubulin exists primarily as an array of large complexes [18]. Subsequently, the large complexes were found to be associated with γ-tubulin and at least five other proteins thought to be organized into γ-tubulin ring complexes (γ-TuRCs) [21]. Soluble brain tubulin, by contrast, was found to exist primarily as dimers [18].

Oocytes arise by the process of oogenesis, and in lower vertebrates such as frogs, this process includes incorporation of nutritional, informational, and cell machinery components [22]. Thus, microtubules and their constituent tubulin may be considered components needed for some oocyte machinery, including that required by the future embryonic cells. Any diversity of tubulin isoforms in oocytes may play a role in microtubule assembly and disassembly, including the dynamics of putative oligomers or soluble complexes of tubulin. Oocyte maturation involves major microtubule changes as the cell progresses from prophase I to metaphase II of meiosis. Therefore, the identification of tubulin isoforms and their relative abundance in soluble pools, including the large, soluble tubulin complexes in oocytes [18, 21] and eggs [23] becomes important in understanding oocyte maturation, and cell division in general.

The present study focused on soluble α-tubulin isoforms in the brain and oocytes from southern frogs, *R. berlandieri*, and northern frogs, *R. pipiens*. By comparing the oocyte tubulins to those of the more widely studied brain tubulins, better understanding of the basis for tubulin dynamics in the female gamete may be reached. The heterogeneities of α-tubulins from oocytes and brain of the two species were compared by SDS-PAGE, two-dimensional (2-D) gel electrophoresis, and Western blots probed with DM1A. This antibody was chosen because it is specific for a consensus sequence ($_{426}$AALEKDY-EEVGVDSV-E-GEGEEEGEE$_{450}$) in the C-terminal region of α-tubulin [24, 25], and routinely reacts only with *Rana* proteins that comigrate with authentic tubulin standards [17, 26]. Soluble α-tubulin isoforms in oocytes at the prophase I and metaphase II stage were also compared. Tubulin isoforms present in brain and oocytes of these two frog species, which differ greatly in habitat and climatic temperatures exposure, were examined with the hope that the tissue- and species-specific context could provide insights into the functions of tubulin isoforms contributing to microtubule dynamics in different tissues and cell types.

Materials and methods

Animals

Gravid female adult northern leopard frogs (*R. pipiens*), obtained in fall and winter months from Hazen Inc. (Alberg, Vt.), were kept in trays of tap water, held at 4 °C with water changes every 2–3 days. Gravid female adult southern frogs (*R. berlandieri),* obtained in summer months from Carolina Biological Inc. (Burlington, N. C.) were kept in aquarium tanks with tap water circulating at room temperature (25 °C). Frogs were identified to species according to Conant [27]. The two species have non-overlapping distributions: *R. pipiens* is found in the northern US and Canada, while *R. berlandieri* is localized to southern Texas and Mexico [27]. The frogs were

decapitated and the spinal cord was pithed for tissue sampling.

Oocyte and brain preparations

Ovaries were removed immediately after sacrifice and placed in amphibian Ringer's solution (pH 7.4) containing 114 mM NaCl, 1.36 mM $CaCl_2$, 2.02 mM KCl, 2.38 mM $NaHCO_3$, 30 mg/l penicillin and 50 mg/l streptomycin. Type I fully grown oocytes [28] were immediately isolated using watchmaker's forceps No. 4 in amphibian Ringer's solution under a dissecting microscope. Fully grown oocytes of both species have diameters of approximately 1.7 mm.

Groups of 20 oocytes from northern frogs were induced to mature by addition of 10 µg/ml progesterone (Sigma, St. Louis, Mo.) in Ringer's solution at 20 °C and incubated for 24–26 h until the white spot on the surface of the oocyte animal pole could be seen under a dissecting microscope. Meanwhile, the same volume (10 µl) of steroid vehicle (propylene glycol : EtOH, 1 : 1) was added to other groups of oocytes as a control. After incubation, some replicate groups of oocytes were heat-fixed in a microwave oven (800 W) for 15–20 s. After hemisection, the fixed oocytes were examined to determine if germinal vesicle dissolution (GVD) had occurred by scoring for the disappearance of the conspicuous oocyte nucleus (i. e., germinal vesicle or GV). Other replicate groups of living, unfixed, oocytes were used to prepare extracts for tubulin determinations as described below. Oocytes of southern frogs could not be induced to mature in vitro by progesterone and only fully grown prophase I stage oocytes were sampled for tubulin determination in this species.

Frog brains were removed immediately after sacrifice and weighed to the nearest 0.01 g. Whole brain tissue, immediately prior to homogenization, was washed three times with PBS buffer (140 mM NaCl, 2.7 mM KCl, 9.66 mM Na_2HPO_4, 1.47 mM KH_2PO_4 and 0.1 mM NaN_3).

Extraction of soluble tubulins

After being washed with PBS three times, oocytes, either progesterone-matured oocytes (i. e., eggs) or non-treated oocytes (vehicle control), were homogenized with 1 oocyte/10 µl PIPES buffer (0.1 M PIPES, 5 mM EGTA, 1% Triton X-100, 1 mM $MgCl_2$, 0.9 M glycerol, 1 mM dithiothreitol, 2 mM PMSF, 2 mg/l leupeptin, 1 mM TAME, pH 6.6) in microhomogenizers (KONTES, Vineland, N. J). The brain tissue was also homogenized with 1 µl PIPES buffer per milligram brain wet weight. The crude homogenate was then centrifuged at 16,000 g for 3 min in a Beckman E centrifuge. After centrifugation, aliquots of crude homogenate, supernatant and pellet, resuspended in PIPES with the same volume as sampled supernatant, were added to solubilization buffer (10 M urea, 5% mercaptoethanol, 4 mM EDTA, and 5% sodium dodecyl sulfate) with a 1 : 8 ratio in volume and stored at –20 °C for further SDS-PAGE and Western blot analysis.

SDS-PAGE and isoelectric focusing

Isoelectric focusing electrophoresis (IEF) was carried out as described by Sinclair and Rickwood [29]. Aliquots (15 µl) of the prepared extracts or column fractions were loaded on Wiretrol 100-µl capillary tubes (Drummond Scientific, Broomall, Pa.) filled to the calibrated 100-µl mark with 4% polyacrylamide gel containing 9.5 M urea, 5% 2-mercaptoethanol, 2% Nonidet p-40, 1.6% pH 5–7 Ampholines (Sigma), 0.4% pH 3.5–10 Ampholines (Sigma). In addition, some IEF runs (narrow range) used 2% pH 4–6 Ampholines (Sigma). Prior to loading, the gels were pre-run overnight at 300 V to establish a gradient. Electrophoresis of the proteins was performed for 18–20 h at 300 V in a capillary tube electrophoresis apparatus (Idea Scientific, Minneapolis, Minn.) containing 10 mM H_3PO_4 in the lower reservoir which was connected to the positive terminal and 20 mM NaOH in the upper reservoir which was connected to the negative terminal. The pH gradient in IEF gels was assessed with mini-pH electrodes applied to the gel surface.

SDS-PAGE was performed according to the method of Laemmli [30] with 10% polyacrylamide mini-separating gel and 3.5% stacking gel in a mini-gel apparatus (Idea Scientific). For 2-D electrophoresis, the IEF rod gel was transferred to the top of the mini-slab gel and electrophoresis was run in the second dimension at 50 V for about 3 h. For 1-D SDS-PAGE, the proteins were run at 100 V for about 1.5 h. Two sets of gels were run with identical samples: one gel was silver stained [31] and the other was used for immunoblot analysis. Bovine brain tubulin (Molecular Probes) was used as a tubulin standard. Pre-stained protein standard or Kaleidoscope protein standards (BioRad) were used as molecular-weight markers in gels.

Western blot analysis

Proteins were electrophoretically transferred to PVDF membranes (Millipore) using a 'Genie' electroblotter (Idea Scientific) at 12 V for 1.5 h. Before Western blotting, PVDF membranes were washed first in 100% methanol, then with distilled water and finally equilibrated in blotting buffer (Tris-glycine pH 8.3 in 20% methanol) for 10–15 min. The blots were blocked with 5% milk protein (Carnation) in 'Zip-lock' bags for 30 min at room temperature while the blotted gels were stained with Coomassie Brilliant Blue R-250 to determine the transfer efficiency. Bovine brain tubulin standard was undetectable in gels after electroblotting, indicating high transfer efficiency. After washing twice with PBS, the blocked membranes were incubated with a 1 : 200 dilution of anti-α-tubulin monoclonal antibody (DM1A; Accurate Chemical Company, Westbury, N. Y.)

including 1% goat serum for 2 h at room temperature. After rinsing three times with PBS, the blots were incubated with a 1:100 dilution of colloidal gold-conjugated goat anti-mouse immunoglobin G (GAM-BL; Amersham) overnight at 4 °C. The membrane was then washed three times with PBS and the gold probe was silver-enhanced with IntenSE II reagents (Amersham).

Gel filtration on a Superose-6b column

The 16,000 g supernatants from 100 oocytes/batch (about 1 ml) were fractionated on Superose-6b columns (2 × 100 cm) (Pharmacia) at room temperature. Proteins were eluted in CSF buffer (0.25 M sucrose, 0.2 M NaCl, 2.5 mM EGTA, 10 mM sodium phosphate, pH 6.5 with 0.02% sodium azide) [10]. Proteins in each 2-ml fraction were then precipitated with 2 ml 100% acetone and centrifuged at 3500 rpm in a desktop centrifuge (Beckman GTE) at 25 °C for 15 min. After the supernatant in each fraction had been carefully removed, the resulting protein pellet was resuspended in 100 µl solubilization buffer, heated in boiling water for 3 min and stored at –20 °C for later use. Dextran blue (2000 kDa), thyroglobulin (669 kDa), apoferritin (433 kDa), alchohol dehydrogenase (150 kDa), and carbonic anhydrase (29 kDa) (all from Sigma) were used to calibrate the gel column for determining the molecular mass of fractions.

Results

Tubulin isoforms in prophase I oocytes: species comparison

To compare the tubulin isoforms in oocytes from both species, the soluble components were analyzed with 2-D electrophoresis and Western blotting with the anti-α-tubulin antibody DM1A. The soluble tubulins were analyzed exclusively because they represent the majority of the tubulin present in the oocyte [18] and running the IEF portion of 2-D gels on the yolky pellet material is technically difficult. In oocytes, three major tubulin isoform groups were found in both species (fig. 1) using wide-range IEF gels. A pool of basic α-tubulin isoforms was dominant in southern frog oocytes, while in northern frogs, two loci of more acidic α-tubulin isoforms were dominant, as shown in Western blots probed with DM1A (fig. 1). Silver-stained 2-D gels routinely showed a number of candidate spots in the general area of tubulin migration (fig. 1C).

Isoforms of α-tubulin in prophase I oocytes and eggs

Type I fully grown oocytes, in prophase I as shown by the presence of a GV (oocytes), from northern frogs were induced to mature with 10 µg/ml progesterone in vitro. The latter are defined here as 'eggs' and had progressed to metaphase II of meiosis as indicated by the appearance of a 'white spot' and GVD as described above. The 16,000 g supernatants from oocytes and eggs were both analyzed by 2-D electrophoresis and Western blot. IEF gels which were immunoblotted showed reduced acidic α-tubulin isoforms in the egg supernatant compared to that of oocytes (fig. 2). Overloaded gels (upper panel fig. 2) showed a major acidic isoform, a basic isoform, and at least one isoform with an intermediate pI. Egg supernatant, in contrast, (lower panel fig. 2) lacked the most acidic isoforms seen in the oocyte samples. Similar IEF gels were run on SDS-PAGE in the second dimension to improve resolution of the isoforms. In the egg soluble supernatant, a broad band containing three or more α-tubulin isoforms appeared in relatively acidic pI positions suggesting high heterogeneity (fig. 3). In the oocyte soluble fraction, however, the α-tubulin isoform loci on the blot with relatively acidic pI values appeared as a narrow band indicating more homogeneity (fig. 3). The isoforms with intermediate pI values seemed to vary in heterogeneity from one female to another as seen when comparing the northern frog oocyte depicted in fig. 1 (middle panel) with that of another female, shown in fig. 3 (upper

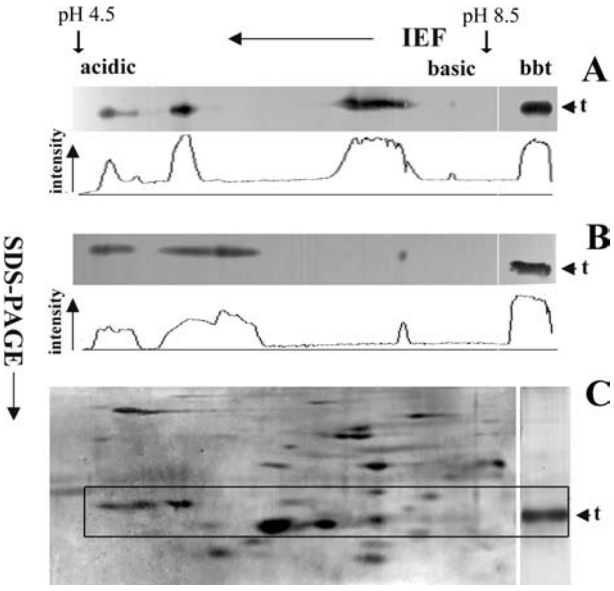

Figure 1. Two-dimensional gel and immunoblot of α-tubulin from soluble extracts from oocytes of southern and northern frogs (*R. berlandieri* and *R. pipiens*, respectively) probed with DM1A. Northern frog oocytes (*B*) contained more acidic α-tubulin isoform heterogeneity than did southern frog oocytes (*A*). Instead, southern frog oocytes had a dominant pool of basic α-tubulin isoforms. There were three major tubulin isoform groups in both species. Tubulin isoforms were separated by 2-D electrophoresis and probed with DM1A. The lower panel (*C*) is a silver-stained gel of northern frog oocyte soluble proteins, similar to that used to prepare the blot above (*B*). The gel area enclosed in the box is the corresponding area shown in the blots above and in blots presented in other figures. t, tubulin; arrowhead indicates position both of bovine brain tubulin standard and the densitometry tracing shown below each blot; 1-D-only lane bbt, bovine brain tubulin (1 µg).

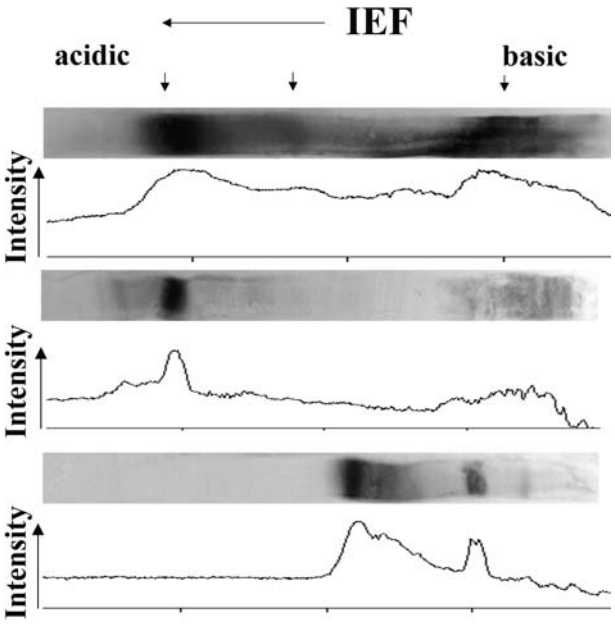

Figure 2. IEF gels were Western blotted and probed with DM1A; the upper two panels are 16,000 g supernatant of northern frog (*R. pipiens*) oocytes, 2 and 0.5 oocyte equivalents, respectively. A densitometry tracing is shown below each blot. The bottom panel represents a similar sample but of egg supernatant (0.5 oocyte equivalents). An overloaded gel (top panel) shows two major peaks and one or more minor intermediate-pI peaks.

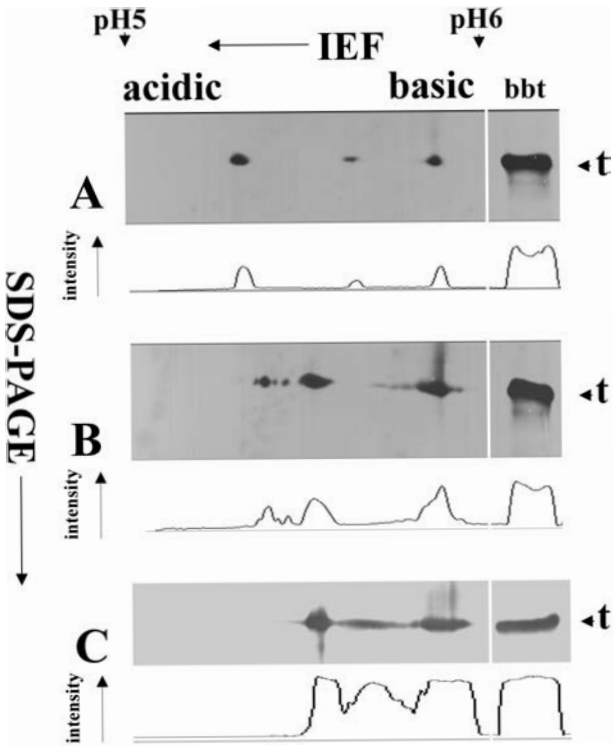

Figure 3. Heterogeneity of α-tubulin isoforms with an acidic pI value was enhanced in northern frog (*R. pipiens*) eggs (*B*) compared with oocytes (*A*). Tubulin isoforms were determined by 2-D electrophoresis and Western blots probed with DM1A. For comparison, purified bovine brain tubulin was subjected to 2-D electrophoresis, blotted and probed with DM1A (*C*). t, tubulin; arrowhead indicates position both of bovine brain tubulin standard and the densitometry tracing shown below each blot; 1-D-only lane bbt, bovine brain tubulin (1 µg).

panel). Nevertheless, acidic properties of tubulin isoforms and isoform heterogeneity were enhanced in eggs compared to sibling oocytes from the same female. As a general comparison, pure bovine brain tubulin standard was similarly subjected to isoform analysis (fig. 3, bottom panel); both eggs and oocytes contained some isoforms more acidic than those found in the standard preparation.

Isoforms in oligomeric tubulins with different molecular mass

The soluble tubulin fraction from northern frog oocytes contained a large proportion of tubulins which eluted from gel filtration columns as very large complexes [18]. To further characterize these putative oligomeric tubulins, the soluble fraction from *R. pipiens* oocytes was separated on a Superose-6b column. Fractions eluting at volumes corresponding to either 1800-kDa molecular mass or 250-kDa molecular mass fractions were analyzed by 2-D electrophoresis and Western blotting. In 1800-kDa fractions, only one α-tubulin isoform group was found when probed by DM1A (fig. 4). However, in the 250-kDa fraction, an additional α-tubulin isoform with a more basic relative pI value was present and, in fact, seemed, quantitatively, to be the dominant α-isoform found in the lower molecular-weight fraction (fig. 4). The acidic isoforms in both fractions showed similar relative pI values.

Isoforms of α-tubulin in *Rana* brain: species and tissue specificity

A high degree of heterogeneity of tubulin isoforms was found in brains from both species. Three major α-tubulin isoform groups were present in the brains of both frog species (fig. 5). The most acidic α-tubulin isoform from the brain was found predominately in the northern compared to the southern frog (fig. 5).

Discussion

Tissue specificity of α-tubulin: oocyte versus brain

Tissue differences between brain and oocytes are obvious at the gross morphological level and these differences underlie the very diverse functions executed by these two tissues. These differences also extend to the molecular level as might be expected. For example, in vitro, purification of tubulins from oocytes using a warm-cold cycle was not achieved in *Rana* [17] or in *Xenopus* [32], while northern frog brain tubulins are easily assembled and thus

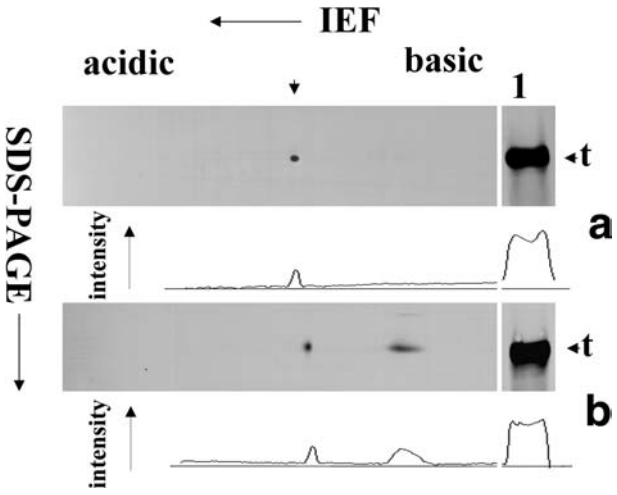

Figure 4. Tubulin isoform profiles in *R. pipiens* oocyte oligomeric tubulin pools were detected using 2-D electrophoresis and Western blot probed with the α-tubulin antibody DM1A. The oligomeric tubulins were extracted by 16,000 g centrifugation and fractionated by Superose-6b column. Oligomeric tubulins with an apparent molecular mass of 1800 kDa contained only an isoform with an acidic pI value (*a*); oligomeric tubulin with an apparent molecular mass of 250 kDa had one additional isoform with a basic pI value which was more prevalent than the acidic isoform (*b*). t, tubulin; arrowhead indicates position both of bovine brain tubulin standard and the densitometry tracing shown below each blot; 1-D only lane 1, 1 µg bovine brain tubulin.

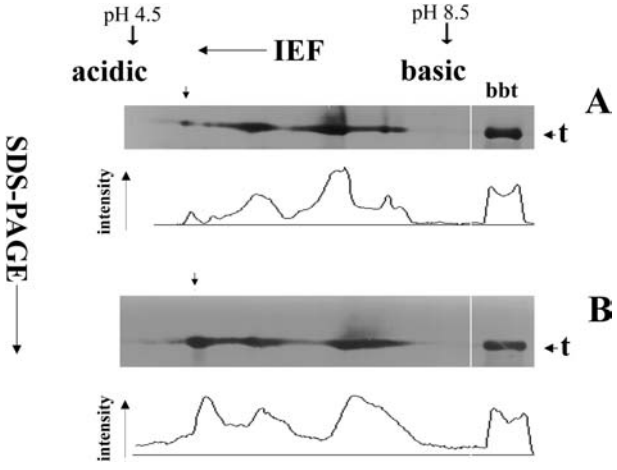

Figure 5. Two-dimensional immunoblot of α-tubulin from soluble brain extracts from southern and northern frogs (*R. berlandieri* and *R. pipiens*, respectively) probed with DM1A. An acidic α-tubulin isoform from brain (downward arrow point) was found predominately in northern (*B*) compared to the southern (*A*) frog. The α-tubulin isoforms were detected by 2-D electrophoresis and Western blot probed with DM1A. There were three major isoform groups with similar migration patterns and high heterogeneity in both species. t, tubulin; left-pointing arrowhead indicates position both of bovine brain tubulin standard and the densitometry tracing shown below each blot; 1-D-only lane bbt, 1 µg bovine brain tubulin.

purified [17]. The oocyte-brain difference in tubulin dynamics suggests that assembly and disassembly of microtubules is controlled in a tissue-specific fashion. Results presented here indicate differences between isoforms found in oocytes and brain: in both species, oocytes contained acidic isoforms which ranged further toward the acidic extreme than did those in corresponding brain samples (fig. 1 vs 5). These results support the hypothesis that extremely acidic isoforms may assemble less readily and thus contribute to the differences already noted between oocyte and brain tubulin assembly. In *Rana* oocytes, qualitatively more acidic and intermediate-pI α-tubulin isoforms were observed in northern frog compared to southern frog oocytes, whereas basic isoforms predominated in the latter (fig. 1). In brain tissues, quantitatively more acidic α-tubulin isoform was found in northern frogs compared to southern frogs, although both species had similar qualitative isoform loci in 2-D electrophoresis (fig. 5). Acidic isoforms may be an intrinsic mechanism favoring the cold stability of microtubules in frog brain and oocytes. In Antarctic fish, however, basic α-tubulins predominate compared with both mammalian and Atlantic cod α-tubulin; thus basic α-tubulins have been suggested to be significant for cold adaptation [33, 34]. In contrast, in another cold-adapted animal, the Atlantic cod, the brain contained large amounts of several acidic α-tubulins [34]. Thus, whether the basic or acidic nature of tubulin contributes to the stability of microtubules in a temperature-dependent manner remains to be elucidated.

Relationship to oocyte maturation

Progesterone triggers the resumption of meiotic maturation during which GVD occurs, in association with changes in microtubule dynamics. In *Xenopus*, a predominant basic α-tubulin isoform present in prophase oocytes decreased during meiotic maturation, and maturation-promoting factor (MPF) was hypothesized to induce the acidification of this isoform [35]. The data presented here agree that acidic isoforms of α-tubulin increase during amphibian oocyte maturation, but this may not be at the expense of basic isoforms. During GVD, reorganization of microtubules may involve disassembly and assembly of microtubules via the centrosome-nucleated mechanism including α-tubulins [36]. Tubulin isoforms may contribute to microtubule dynamics during the maturation process, such as reduction of acetylated α-tubulin and basic α-tubulin in *Xenopus* [12, 35]. Thus, increased acidic α-tubulin isoform heterogeneity during maturation may play a role to stabilize putative oocyte oligomeric tubulins. The oocytes may have stable oligomeric tubulins, associated with γ-tubulins, acting as microtubule-nucleation centers in microtubule dynamics [23, 37, 38]. Whether these acidic isoforms are derived from new gene expression or from the acidification of

basic isoforms by progesterone treatment is worthy of future study. Signaling molecules such as $p34^{cdc2}$, cyclin, and c-mos may be directly involved in tubulin dynamics [10].

α-tubulin isoforms in oocyte oligomeric tubulins

Putative oligomeric tubulins with a molecular mass ranging from 200 kDa (tubulin tetramer) to more than 2000 kDa (>20 tubulin dimers) were found in soluble cellular components such as *Rana* oocytes [18] and cultured cells [10]. In oocytes but not in brain tissue, putative oligomeric tubulins constitute the major proportion of soluble tubulins. The oligomeric tubulins were proposed to play a role in regulation of microtubule stability [18]. Further characterization of the large oocyte tubulin complexes is thus necessary to understand microtubule behavior in these cells. As detected by 2-D electrophoresis and Western blots probed with DM1A, high-molecular-mass fractions contained only an acidic isoform, while an additional major basic isoform occurred in low-molecular-mass fractions (fig. 4). Therefore, in northern frog oocyte 16,000 g soluble fractions, two major α-tubulin isoforms could be accounted for by the acidic isoform found in the high-molecular-weight putative oligomeric tubulins, plus the acidic and basic isoforms found to be components in the low-molecular-weight soluble tubulins. Presumably, the other isoforms demonstrated in oocytes (fig. 3) would be accounted for by the intermediate-sized putative oligomers and perhaps the small dimer pool. But the possibility that other minor α-tubulin isoforms exist, with different pI values, which could not be detected by this method, should not be excluded. These results suggested that frog oocyte microtubules that contain both basic and acidic α-tubulin isoforms tend to disassemble readily, while homogeneous acidic α-tubulin isoforms could contribute to a stable oligomeric tubulin pool.

In progesterone-treated mature oocytes from northern frog, three more acidic isoforms than found in immature controls were demonstrated in 2-D electrophoresis and Western-blots (fig. 5). The heterogeneity of acidic α-tubulin isoforms was thus enhanced in oocyte putative oligomeric tubulins during maturation. Our earlier research showed that a size shift of putative oligomeric tubulins to higher-molecular-weight fractions occurred after progesterone treatment [18]. Thus the acidification of α-tubulin may occur to increase the stability of oligomeric tubulins in mature oocytes. Progesterone might switch basic tubulin pools to acidic tubulins since high-molecular-weight putative oligomeric tubulin complexes have predominately acidic tubulins. In *Xenopus*, a predominant basic α-tubulin isoform present in prophase oocytes decreased during meiotic maturation, and MPF was hypothesized to induce the acidification of this isoform [35].

Potential temperature effects on tubulin dynamics: species habitat differences

The process of elongation and shrinkage of microtubules can be enhanced in some species by warm and cold temperature, respectively. In nature, northern leopard frogs can survive well for months at 4–6 °C, temperatures which southern frogs cannot tolerate. Thus, northern frogs must have microtubules that can fulfill their basic cellular functions at low temperatures [19]. The acidic isoforms of α-tubulins detected in northern frog extracts from brain and oocytes could account, in part, for the cold-adapted characteristics of microtubules in temperate-climate animals [39]. Cold-adapted microtubules were also found in Atlantic and Antarctic fish that live in the cold. In these species, microtubules can even assemble at 8 °C [34]. Nevertheless, the exact role of tubulin isoforms in differential assembly within various tissues, and the relationship to cold adaptation remain to be elucidated.

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