Microtubule Protein Synthesis during Oogenesis and Early Embryogenesis in *Xenopus laevis*

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A method is described which permits the preparation of discrete classes of oocytes of different sizes from all stages of oogenesis in *Xenopus laevis*. This technique is used in the determination of the content of microtubule protein in oocytes during the course of oogenesis. These experiments show that microtubule protein is present in oocytes of all sizes assayed and that the amount is simply related to the volume of the oocyte. In the largest oocytes microtubule protein constitutes 1% of the soluble protein and this amount does not change on maturation and fertilization. These results show that the changes occurring in the oocyte on maturation which allow the cytoplasm to support microtubule polymerization occur as a result of a modification of the pre-existing microtubule protein, not from protein synthesis *de novo*. These experiments also indicate that the synthesis of microtubule protein either from 'masked' mRNA or from newly synthesized mRNA plays an insignificant role in microtubule protein synthesis at maturation, ovulation and immediately post-fertilization.

Gurdon (1968) demonstrated that nuclei injected into oocytes of *Xenopus laevis* will trigger the formation of asters and spindles. This occurs, however, only if the oocytes have been subject to hormone stimulation leading to the maturation of the oocyte. This difference in behaviour between the immature and mature oocyte can be explained in three ways. The non-treated oocyte contains no microtubule protein (hereafter referred to as tubulin) and the hormone causes (1) the synthesis of tubulin message and its subsequent translation or (2) the immature oocyte contains 'masked' message that is translated on stimulus by the hormone. [Although the experiments of Gurdon and his colleagues (Gurdon *et al.*, 1971; Lane *et al.*, 1971; Moar *et al.*, 1971) do not contain all the necessary data, it is possible by using their data to show that the oocyte could probably synthesize the observed quantity of tubulin in the available time between hormone stimulation and ovulation.] In either of these cases tubulin synthesis may give rise to a pool of microtubule subunits, which is drawn on as needed. Alternatively subunit synthesis and polymerization may be closely linked with the result that no pool of subunits arises. (3) The immature oocyte contains tubulin, but this cannot polymerize until the oocyte is mature. This tubulin could be synthesized either during the major part of oogenesis or at some limited period.

Therefore the time and duration of tubulin synthesis is clearly relevant to any consideration of the mechanisms of polymerization of the microtubules.

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As the *Xenopus* oocyte-egg system offers several advantages for the study of the regulation of mitosis, which will be discussed below, experiments were performed to determine the kinetics of tubulin synthesis during oogenesis in this animal. These experiments involved the collection of oocytes of limited and different size ranges and the assay of the supernatant soluble tubulin. Additional experiments were performed to assay tubulin in the early stages of development. For this work the binding of colchicine has been taken as an adequate assay for tubulin. Possible limitations of this method will be discussed below. In an attempt to gain some information about the growth pattern of oocytes the relative distribution into various size classes of growing oocytes in the ovary of a single frog was determined.

Materials and Methods

Biological materials

Female *Xenopus laevis* were kept and induced to lay eggs as described by Gurdon (1967). Eggs were dejellied in a solution of 1% (w/v) papain and 2% (v/v) cysteine hydrochloride in saline (0.182M-NaCl-1.5mM-Tris-HCl, pH8.1). Ovaries were obtained by dissection from decapitated females. Free oocytes were obtained by placing the ovary, with the lobes dissected open, into saline, pH7.6, made 5mM in CaCl₂, containing 1mg of Pronase/ml. While in this solution the ovary was agitated frequently to release the oocytes from the follicular tissue. This process took 15-20min. The released oocytes were still surrounded by a layer of follicle cells. These oocytes

were washed four to five times in saline, pH 7.6, and were then transferred to the column for separation, as described below. Oocytes were prepared from frogs that had been induced to lay 1 or 2 days previously and from animals not treated with hormone. These will be referred to as treated and untreated animals respectively.

Separation of oocytes

The oocytes were poured into a column (2 cm × 50 cm) already almost full of saline. The oocytes were allowed to settle, a step that serves to stratify the oocytes coarsely by size. A column of this size was suitable for handling the oocytes from one ovary. Saline, drawn under gravity from a reservoir, was then passed up the column, washing out oocytes of increasing mean diameter as the flow rate was increased. The oocytes were collected from the top of the column by some suitable arrangement. An inverted plastic funnel whose mouth fitted tightly inside the column served very well. The robustness of the oocytes varied from ovary to ovary. The most fragile required very careful handling in the collection and subsequent stages. After separation a given size group was collected and their volume was measured. Samples were removed at this stage, immediately before homogenization, and placed in the fixative, Perenyi's fluid, so that the diameters of the oocytes in each sample could be measured. This was done by using a low-powered microscope with a reticulated eye-piece. The entire procedure from the beginning of dissection of the ovary to the homogenization takes about 90 min. At the end of this period the oocytes had suffered no visible deterioration.

Fertilization in vitro

Embryos were obtained by fertilizing *in vitro* eggs stripped from *Xenopus* primed to lay in the usual way (Gurdon, 1967). The procedure is a modification (J. L. Maller, personal communication) of the method of Wolf & Hedrick (1971). Testes were removed from adult frogs and stored at 4°C in Hanks solution supplemented with 0.1% glucose and 0.035% NaHCO₃, pH 7.8, and 5% (v/v) foetal calf serum, 2 ml of solution being allowed for each testis. For fertilization the testis was minced finely with a pair of forceps in this solution. The eggs, which had been stripped into a tenfold dilution of Hanks solution supplemented with 5% (v/v) foetal calf serum, were drained so that they were just covered by liquid. The sperm suspension was added at a concentration of one-half a testis to 1000 eggs. The dish was swirled and then left for 20–30 min for rotation to occur. The fertilized eggs were then transferred to Barth's solution (Barth & Barth, 1959). The embryos were left to develop at room temperature (22–24°C) until collection for assay. Embryos were dejellied as described and groups (approx. 200) were selected

manually to be of the same stage. The embryos were staged by the criteria of Nieuwkoop & Faber (1956).

Microtubule assay

The collected oocytes or embryos were homogenized in 2–10 times their volume of buffer, 20 mM-potassium phosphate, pH 6.8. At this time a sample was removed for determination of total protein. The homogenates were centrifuged at 27000g (r_{max}) for 50 min. The resulting clear supernatant was carefully removed from below the lipid surface layer and a portion taken for the determination of soluble protein.

It was thought possible that a part of the tubulin content of the homogenized oocytes might have been contributed by the follicle cells that still surround the isolated oocytes. To check this, oocytes were squashed very gently between two glass surfaces and the cytoplasm was collected, leaving behind the largely intact but ruptured follicle layer. This extract was then treated as the others. Although this method does not remove all follicle-cell contamination, it decreases it manyfold.

It is possible that in the homogenization of the embryos some mitotic apparatus might be left intact. These would then be sedimented, leading to an inaccurately low estimate of the soluble tubulin. To guard against this, samples of homogenate were examined microscopically. On no occasion could any intact, or partially intact, mitotic apparatus be seen. This is in accordance with the observation that in the isolation of the mitotic apparatus special steps have to be taken to stabilize them (e.g. Kane, 1965).

The colchicine-binding material was assayed by the method of M. Kirschner (personal communication), which is a modification of that of Wilson (1970). A portion (0.2 ml) of the supernatant was taken and 5 μ l of a solution of colchicine added. This solution contains labelled and unlabelled colchicine at concentrations such that the final assay mixture of 0.205 ml contains 30 μ M-colchicine and 0.25 μ Ci of ³H. [*methoxy*-³H]Colchicine (5 Ci/mmol) was from New England Nuclear Corp., Boston, Mass., U.S.A.; colchicine was from Sigma, St. Louis, Mo., U.S.A. The assays were incubated at room temperature (22–24°C) for 1 h. The reaction was terminated by the addition at 0°C of 1 ml of a suspension of TEAE-cellulose (Cellex T; Bio-Rad Laboratories, Richmond, Calif., U.S.A.) in phosphate buffer at the concentration of 2 g/100 ml. In these conditions the tubulin binds to the TEAE-cellulose. The assays were stood on ice for 5 min. They were then filtered through glass-fibre filters (Whatman GF/C; H. Reeve Angel and Co. Ltd., London E.C.4, U.K.). The resulting filters were placed, moist, in 20 ml of scintillant containing NCS [4.0 g of PPO (2,5-diphenyloxazole), 0.3 g of POPOP [1,4-bis-(5-phenyloxazol-2-yl)-benzene], 40 ml of NCS, 960 ml of toluene]. The

vials were heated at 60°C until the cellulose dissolved (usually 2–3h). The cooled vials were counted for radioactivity in a Nuclear–Chicago liquid-scintillation counter with an efficiency of about 25%. The NCS solubilization increases efficiency twofold. All assays were done in triplicate.

Protein determinations

These were performed by the method of Lowry *et al.* (1951). The standards were prepared from bovine serum albumin (Pentex Inc., Kankakee, Ill., U.S.A.).

Measurement of range and frequencies of oocyte diameters

Distribution within an ovary. Isolated oocytes were prepared from a single ovary as described but were not subject to column separation. Four large samples were taken and the frequencies of classes of oocyte diameters were determined for each sample.

Statistical analysis. Methods for statistical analysis are taken from Snedecor & Cochran (1967).

Fig. 2. Frequency of class of oocyte diameter in whole ovary

The histogram is based on results from the measurement of oocyte diameters of four samples, taken from the same ovary preparation, to minimize sampling errors. The samples contained 169, 156, 178 and 186 oocytes. As before, 17 reticule divisions $\equiv$ 1 mm.

Fig. 1. Histogram of class of oocyte diameter against frequency

Free oocytes were prepared as described from the ovary of a hormone-treated frog. Size fractions, (I)–(VI), were collected from the column. Oocyte diameter is measured in reticule divisions, 17 of which are equivalent to 1.0mm. $\bar{x}$, Oocyte mean diam.; S.E., standard error; n, number of oocytes measured in each sample for each size class. At these values of $\bar{x}$ and S.E. the value of n for each size class necessary to be sure that two means $\bar{x}_1$ and $\bar{x}_2$ are significantly different at the 99% confidence level is about 6 (i.e. n need be only 6).

Results

Collection of batches of oocytes of different mean size

Fig. 1 shows a histogram of the frequencies of classes of oocyte diameters for the oocytes from one ovary which were arbitrarily divided into six groups as a result of column fractionation. From this Figure it can be seen that it is possible to obtain groups of oocytes of significantly different means. The smaller spread about the mean in the larger and smaller groups reflects the greater overall numbers of these size classes in the ovary as a whole (see Fig. 2). This experiment enables us to conclude that by using this method it is possible to sample, at a number of particular sizes, the total population of oocytes in an ovary and from this to obtain significant information as to how tubulin content varies with oocyte size.

Size distribution of oocytes in whole ovary

Fig. 2 shows the distribution of oocytes into size classes. The relevance of this result will be discussed below. This result is supported by results (not shown) derived from the size distribution of oocytes obtained for each experiment, an example of which is shown in Fig. 1.

Microscopic examination of whole ovary showed a much larger population of oocytes with a diameter of 0.3mm (5 reticule divisions) or less than is shown in Fig. 2. This indicates that this procedure for isolating oocytes selects against small oocytes. These small oocytes seem to be very tightly associated with the ovarian connective tissue and are probably not released from this tissue by the Pronase digestion. They are thus discarded. Examination of whole ovary

showed that the data in Fig. 2 accurately represent the size distribution of oocytes with a diameter of more than 0.3 mm.

Fig. 3. Tubulin content of oocytes plotted against oocyte radius on a double logarithmic plot

The tubulin content is expressed as the c.p.m. of ^3H -labelled colchicine bound in the standard assay per oocyte. For the sake of clarity not all the experimental data are plotted. However, all data are included in the statistical calculations summarized in Table 1. The number of observations for each type of experiment is given by the number n in column 6 of that Table. The curves in this Figure and those of Figs. 4 and 5 were calculated by the method of least squares. Curve A: $\circ$, data for oocytes from frogs that had not received prior treatment with hormone; $\odot$, data from experiments designed to minimize follicle-cell contamination. Curve B ($\bullet$): data from oocytes from frogs that had been treated with hormone.

Tubulin content of oocytes

Fig. 3 shows the tubulin content of oocytes, expressed as c.p.m. of colchicine bound per oocyte, plotted against oocyte radius as a double logarithmic plot. Data for hormone-treated and untreated oocytes are shown. Fig. 3 includes data obtained from oocytes prepared in the manner to minimize follicle cell contamination (see under 'Microtubule assay'). The distribution of these data is identical with that of the data for oocytes prepared without any attempt to minimize follicle-cell contamination. From this it can be concluded that the follicle cells do not contribute any detectable quantity of tubulin in the experiments described in this paper. From the analysis of the data in Table 1, three conclusions can be deduced. (1) By inspection it is clear that $\log$ (c.p.m.) is dependent on $\log$ (radius) and from t_{exp} , (Table 1, column 3) it is shown that this correlation is very tight ($P < 0.001$). Thus we can conclude that the diameter of an oocyte is very significantly related to its tubulin content. This is true in both treated and untreated oocytes. (2) It is clear from Table 1 (column 5) that the slopes of the two lines are significantly different from each other ($P \leq 0.001$). Thus we can conclude that the hormone treatment leads to a change in the relationship between size and tubulin content. (3) if the tubulin content of an oocyte was simply a function of the volume of the oocyte, the slope of curve A in Fig. 3 should be 0.333. From Table 1 (column 5) it can be seen that this value falls within the determined range for the untreated oocytes. At the 90% confidence level the value of 0.333 still falls within the range of $b \pm t \cdot Sb$, which is 0.293–0.363. Thus it can be concluded that this simple relationship does exist between volume and tubulin content in this type of oocyte. In conjunction with the second consideration it can be said that in the

Table 1. Statistical data for the various graphs discussed in the text

t_{exp} is the value of t calculated from the experimental data. The value of t quoted for the 0.001 probability level is for the appropriate number of degrees of freedom. It can be seen that the regression coefficient is significant in excess of the 99.9% confidence level in all cases. b is the true population regression coefficient and $b \pm t \cdot Sb$ is an estimate of the range, at a particular confidence level, in which the true population regression coefficient will be found. The value of t in this expression is chosen for a particular confidence level at the appropriate number of degrees of freedom. Thus for the data of Fig. 3, curve A, t is for a probability of 0.001 for 35 degrees of freedom. n is the number of experimental observations in each category.

Figure	Column Curve	1 Regression coefficient (b)	2 Sample s.d. of the regression coefficient (Sb)	3 t_{exp}	4 t for P of 0.001	5 $b \pm t \cdot Sb$	6 n
3	A	0.328	0.021	15.57	3.59	0.271–0.385	37
	B	0.228	0.014	16.29	3.97	0.187–0.269	19
4	A	0.360	0.013	27.69	3.57	0.334–0.386	45
	B	0.278	0.030	9.27	3.97	0.215–0.341	19
	C	0.251	0.011	22.82	3.75	0.228–0.274	26
5	A	0.838	0.149	5.61	3.67	0.585–1.091	30
	B	0.590	0.133	4.45	4.02	0.329–0.851	18

oocytes from the hormone-treated animals, this relationship no longer holds.

Protein content of oocytes

Fig. 4 shows the protein content of oocytes, both soluble and total, plotted against oocyte size, again as a double logarithmic plot. Hormone treatment affects the relationship between size and soluble protein content, but has no effect on the total protein content. From Table 1, by the same type of reasoning, one can reach the following conclusions. (1) The diameter of the oocyte is significantly related to the protein content, both total and supernatant. (2) With reference to Fig. 4, curve A is significantly different from curves B and C (a little less than at the 95% level for curve B, considerably in excess of 99% for curve C); curves C and B are not significantly different in slope, but obviously are in relative position. (3) Although it is possible that the true population regression coefficient might be 0.333 for curve A it is not significantly probable ($P < 5\%$). Therefore the soluble protein content of non-hormone-treated oocytes is not simply related to volume.

In the smallest oocytes assayed (diam. approx. 0.3mm) the soluble protein constitutes about 50% of the total protein. In the largest oocytes the proportion has fallen to about 10%. Between these limits the proportion of the total protein constituted by the soluble protein is linearly related to the diameter of the oocyte.

Fig. 4. Total and soluble protein content of oocytes plotted against radius on a double logarithmic plot

See the legend to Fig. 3 for details. Curve A (○), soluble protein for non-hormone-treated oocytes. Curve C (△), insoluble protein for non-hormone-treated oocytes. Curve B (●), soluble protein for hormone-treated oocytes. Curve D (▲) insoluble protein for hormone-treated oocytes.

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Fig. 5. Diameter of oocytes plotted against specific activity (soluble protein) for treated and untreated oocytes

See the legend to Fig. 3 for details. Curve A (○), untreated oocytes; curve B (●), treated oocytes.

Specific activity of oocytes

Fig. 5 shows the relation between specific activity, calculated for soluble protein, as a function of oocyte size. Again from Table 1 (column 5) it can be seen that there is a significant relationship between those parameters. Comparing relative rates of change of tubulin and soluble protein with oocyte diameter for non-treated and treated oocytes (Fig. 3, curve A, and Fig. 4, curves A and B, and Fig. 5, curve B) it can be expected that the specific activity of the tubulin of the hormone-treated oocytes should increase less rapidly as a function of size than that of the untreated oocytes. Fig. 5 shows that this expectation is fulfilled. It can be seen from Table 1 (column 3) that diameter is definitely correlated with specific activity ($P > 0.001$). However, column 5 (Table 1) shows that the regression coefficients of the two lines are not significantly different even at the 90% confidence limit. That is to say that the rate of change of specific activity with diameter is not significantly different for treated and untreated oocytes.

Tubulin content of early embryos

Fig. 6 shows the tubulin content of embryos at various stages up to about 10h after fertilization. Also included on this Figure are values for full-sized non-hormone-treated oocytes and unfertilized eggs. From this experiment one can conclude that there is no significant difference in the tubulin content of mature oocytes, unfertilized eggs and developing embryos. Not only are the average values comparable within the range of variation expected from different animals and from sampling error, but samples from the same population show fluctuations in tubulin content of comparable magnitudes during development. The tubulin content of mature oocytes and

Fig. 6. Tubulin content of early-stage embryos

The preparation of the embryos has been described in the Materials and Methods section. The value for the tubulin content of the mature oocytes (untreated) is an average ($n = 6$, s.e. = 9.1, range 29.5–53.4), as is that for the unfertilized eggs ($n = 5$, s.e. = 11.0, range 35.4–64.7). The Figure also includes data for the specific activity of tubulin at the different developmental stages. ○, Specific activity; ●, colchicine bound.

unfertilized eggs, expressed here in terms of radioactivity bound, is equal to about 1% of the soluble protein of the cell, assuming that one molecule of colchicine is bound per 120000-molecular-weight tubulin molecule.

Discussion

Separation of oocytes

The method described here for selecting different-sized oocytes (the method was first suggested by Dr. Max Birnstiel) offers several advantages over others recently proposed (e.g. Mairy & Denis, 1971). It allows large quantities of material to be handled in a gentle manner. Also, although no attempt was made in these experiments to regulate the flow in the column, if this were done it would be possible to select a given size of oocyte with great reproducibility. The method, however, does not give a size distribution which reflects the natural size distribution of oocytes in the ovary over the entire range, as most of the very small oocytes are lost. However, as the

primary object of this paper was to determine the tubulin and protein content of individual size classes of oocytes, this method of obtaining oocytes did not distort the conclusions. A different method, or a modification of the one described here, will have to be used to extend observations to oocytes of diameters less than 0.3 mm. Nevertheless, the method would seem to have wide application in the field of the biochemistry of oogenesis.

Colchicine-binding assay

The ability to bind colchicine is a well-established property of tubulin and is therefore a useful basis for an assay procedure (Borissy & Taylor, 1967a,b; Shelanski & Taylor, 1967; Wilson & Friedkin, 1967). However, it is possible that colchicine can bind to other than tubulin. Work has shown that colchicine-binding material in liver can be precipitated by centrifugation (Stadler & Franke, 1972). However, those experiments were interpreted as showing that microtubule material was attached to membranes and other precipitable material. So far no evidence

has been presented to show that colchicine definitely binds to other than tubulin. Therefore it has been accepted as the basis for assay in these experiments. However, because of the lack of knowledge about its specificity of binding the results have been presented in the form of the amount of radioactivity bound. In this way the data relate directly to the quantity measured. Thus, in this paper, the colchicine-binding material has been referred to as tubulin for convenience.

A number of attempts were made to label microtubule protein directly. These experiments involved incubating *in vitro* pieces of ovary with labelled amino acids and also the injection of labelled amino acids into a living frog at intervals for several weeks. Subsequent isolation of tubulin by column chromatography failed to demonstrate any radioactivity associated with the tubulin peak, as identified by its ability to bind colchicine. For this reason the colchicine-binding assay was used as a measure of the tubulin present in the oocytes.

Tubulin content of oocytes

The experiments reported here show that tubulin is present at all stages of oogenesis for which material was obtained. These experiments do not exclude the hormone-stimulated synthesis of tubulin *de novo* or the translation of pre-existing mRNA (hypotheses 1 and 2 of the introduction section) as contributing to the tubulin present in the oocyte at ovulation. However, they indicate that the hypothesis that immature oocytes contain tubulin that is converted into a polymerizable form at maturation is a sufficient explanation for the relevant observations in *Xenopus*. Moreover, for the following reasons, it seems unlikely that (1) or (2) play more than a minor role. The tubulin found in a full-sized oocyte (untreated) seems to have accumulated as a result of a single process operating throughout oogenesis. As the full-sized oocyte and the unfertilized egg contain similar quantities of tubulin, there does not seem to be room for a new synthetic process between these two stages, without postulating the breakdown of as yet unused tubulin. Similarly, if tubulin can participate in a number of rounds of polymerization, there is no requirement for synthesis up to stage 9.

Recent work on sea urchins (Raff *et al.*, 1971, 1972) has shown that in these organisms the oocytes and unfertilized eggs also contain a pool of tubulin. However, in addition, there is a pool of 'masked' message which is translated after fertilization. The reasons for this difference are not known. One can speculate that it may be related to the fact that *Xenopus* has only one functional requirement for tubulin, that is to form the mitotic apparatus, whereas sea urchins in addition require tubulin for cilia. This may require tubulin of two different synthetic histories.

As has already been pointed out, the tubulin content of oocytes increases simply with the volume. Depending on how the oocyte grows this has interesting implications on the control of tubulin synthesis. Two extreme models can be proposed. Either (1) the volume increases linearly with time, when the rate of tubulin synthesis would remain constant, or (2) the radius increases linearly with the time, and thus the rate of tubulin synthesis would increase exponentially. These two models make distinct predictions about the rate of growth of the oocytes. In the first the rate of increase in radius would fall off exponentially, in the second the rate of increase is constant. As the population of oocytes in an ovary have all grown to their present radius through the smaller intermediate stages, the two alternatives make distinguishable predictions about how long an oocyte will spend in a given size class. In case (1), there should be larger numbers of oocytes in the larger size classes falling off exponentially as size decreases. In the second case oocytes should be distributed evenly in the different size classes. To test this the distribution of oocytes into various size classes in an ovary was investigated (Fig. 2). Although it was expected that the clear predictions of the two hypotheses would be less clear in practice, the resulting distribution cannot be fitted to either scheme.

As has been pointed out in the Results section, the very low numbers of small oocytes represented in Fig. 2 is probably an artifact. However, the two peaks in Fig. 2 seem to reflect the true distribution of oocyte size classes. This, which is contrary to both hypotheses, prevents us from drawing any simple conclusions about the pattern of growth of the oocytes.

There are two processes that could lead to an increase in the specific activity of the tubulin (relative to the soluble protein), as a function of oocyte size. The rate of synthesis of tubulin could increase relative to the rates of synthesis of other soluble proteins or non-tubulin soluble proteins could be lost, possibly by incorporation into insoluble protein, as the oocytes grow. The first of these alternatives has not been investigated. The second leads to the expectation that the concentration of soluble protein per unit volume will decrease as oocyte size increases. Observations made during the course of this work (not reported) suggest that this may be true, but not unequivocally.

Finally, it remains to discuss the difference between the data for treated and untreated oocytes in Figs. 3, 4 and 5. Both types of oocyte were studied in view of the effect that hormone is known to have on large oocytes (see above).

It has been shown (Wallace & Jared, 1968, 1969) that hormone treatment of female *Xenopus* causes the synthesis of yolk-protein precursors by the liver. These precursors are actively incorporated by the

growing oocytes. The results already discussed in the present paper indicate that there is a very close correlation between the protein and, more specifically, tubulin content of oocytes and their size. This correlation, together with the fact that no information has been obtained that relates directly to the temporal development of the oocytes, makes it impossible to follow directly the incorporation of yolk precursors into the hormone-treated oocytes. There are two ways in which the data for the treated oocytes could be displaced in the way observed. The hormone treatment could cause an increase in volume of the oocytes without a concomitant increase in soluble protein. Alternatively, there may be a decrease in soluble protein with no change in volume. The first hypothesis seems to be excluded because there is no observable difference in the data for the total protein between treated and untreated oocytes, where a similar effect would be observed if there was a change in volume. Therefore it seems probable that there is a loss of soluble protein and tubulin in particular. The mechanism for this loss is not known. It could result from degradation or by leakage from the oocytes. Such a leakage should be reflected also in the total protein content of the oocytes. However, over the larger part of the experimental range of oocyte size, the total protein is present in considerably greater quantity than the soluble protein (see above). Thus a proportionally large loss of soluble protein would only represent a small fraction of the total protein, which might not be detectable under the experimental conditions used here. Thus a leakage of a small quantity of soluble protein would be compatible with the fact that there is no observable difference in position of the graph for total protein for treated and untreated oocytes in Fig. 4.

It is not known to what extent the situation described in this paper for tubulin is representative of less-specialized cells. It is known that the *Xenopus* oocyte does accumulate pools of materials for use at later stages of development. The accumulation of yolk is an obvious example of this. Others are the accumulation of ribosomes (Brown & Gurdon, 1964) and deoxynucleotides (Woodland & Pestell, 1972). This type of accumulation does not seem to be typical of somatic cells.

However, having established that maturation leads to a change in the microtubule protein that, given the correct stimulus, enables it to polymerize, it will be possible to investigate this phenomenon further. This can be done by utilizing the ability of *Xenopus* oocytes to remain viable after microinjection. In this way the internal environment of the cell can be modified and its effect on the tubulin can be determined. In this way it should be possible to determine which events in maturation are involved in this change in properties of the tubulin. Such observations should be directly relevant to the understanding of the changes occurring

in the microtubule subunits in the mitoses occurring in less-specialized cells.

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