MODIFICATIONS IN OOCYTE PROTEIN SYNTHESIS INDUCED BY PROGESTERONE IN *XENOPUS LAEVIS*

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1. Introduction

Amphibian oocytes are arrested at the diplotene stage of meiotic prophase. In *Xenopus laevis* the in vitro incubation of isolated oocytes in the presence of progesterone can reinitiate the progression of maturation up to metaphase II after rupture of the germinal vesicle (GVBD) (reviewed in [1–3]). During this process a maturation promoting factor (MPF) appears in the cytoplasm, which is capable of inducing maturation in non-stimulated oocytes [4]; its formation appears to be dependent on prior protein synthesis [5,6].

We have recently found that ornithine decarboxylase activity in progesterone-stimulated oocytes increases within 4 h and requires the synthesis of new enzyme molecules [7]. It is also recognized that during maturation the uptake of amino acids is decreased [8] but overall protein synthesis is definitely increased [2,9]. Electrophoretically detected changes in protein synthesis have been described after progesterone treatment [8,10]. In order to obtain more detailed data on these differences we have used the high-resolution two-dimensional polyacrylamide gel electrophoresis system as in O'Farrell [11]. We have observed early modifications in the rate of synthesis of at least 5 polypeptides out of approx. 600 detectable by this electrophoretic technique under our experimental conditions.

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2. Materials and methods

2.1. *Isolation of oocytes*

Adult *Xenopus laevis* females were decapitated and ovarian tissue subjected to 0.2% collagenase digestion for 2 h at 28°C. After defolliculation oocytes were washed extensively in Barth's medium [12] and stage V–VI oocytes [13] selected. In each series of experiments oocytes were obtained from the same animal.

2.2. *Incubation studies*

Oocytes in groups of 100–150 were incubated in 2 ml Barth's buffer at room temperature. Progesterone was added to the medium to give a final concentration of 10 μM. At the start of each experiment 15 oocytes were withdrawn and incubated for 2 h in 50 μl medium containing [^{35}S]methionine (1 Ci/l, 1095 Ci/mmol, New England Nuclear). Then at 2-h intervals, batches of 15 oocytes were withdrawn and incubated in [^{35}S]methionine medium as before. Pulse-labelling was carried out until GVBD was complete, usually 8–10 h after progesterone treatment.

2.3. *Preparation of samples*

At the end of every 2 h labelling period, the medium was removed and the oocytes homogenized in 10 vols. of lysis buffer A [11]. The homogenates were centrifuged at 2500 rev./min for 15 min at 15°C. Aliquots of the supernatant were taken for trichloroacetic acid precipitation on glass-fibre discs. After washing with 10% trichloroacetic acid and ethanol the discs were counted in 10 ml toluene containing 0.4% (w/v) of 2,5-diphenyloxazole and 0.01% (w/v) of 1,4-bis[2-(5-phenyloxazolyl)]benzene. Aliquots of

the incubation medium were also subjected to trichloroacetic acid precipitation to determine whether ^{35}S-labelled proteins were also secreted.

2.4. *Electrophoresis*

In every experiment equal amounts of trichloroacetic acid-precipitable radioactivity were processed. The two-dimensional electrophoretic system of [11] was used with minor modifications. The first dimension, isoelectric focussing, was carried out in glass tubes 130 mm × 2 mm for a total of 6000–8000 V hours. Usually 5×10^5–1×10^6 cpm representing 1–8 oocytes were applied to the tube gels. After focussing the gels were equilibrated in sample buffer and stored at −70°C.

The second dimension sodium dodecyl sulfate (SDS) electrophoresis was performed on 10–15% linear gradient acrylamide slab gels (acrylamide/bisacrylamide, 200:1). The isoelectric gel was squeezed between the plates 1.5 mm apart onto the stacking gel and this resulted in stretching the tube gel to about 15 cm. Electrophoresis was usually run at 7.5 mA/gel overnight.

2.5. *Fluorography*

The stacking gels were removed after electrophoresis in the second dimension and the running gel impregnated with 2,5-diphenyl oxazole as in [14]. The impregnated gels were dried and exposed to Kodak X-Omat film presensitized with red light. Exposure times varied from 16–48 h.

3. Results

The patterns of protein synthesis as seen in the two-dimensional gels were very reproducible in many aspects. Although there were a number of modifications only those which were consitently seen were recorded. A total of 7 experiments with progesterone and the appropriate controls were performed.

3.1. *Protein increase/induction*

The synthesis of polypeptides in the absence of progesterone is shown in fig.1. This protein map is almost identical to that observed in [15]. Their 'house-keeping' proteins include actin and tubulin and are indicated in fig.1.

Fig.2 shows the patterns of protein synthesis during a 4–6 h treatment with progesterone. There was 32% GVBD at 6 h. Polypeptide labelled 3 has

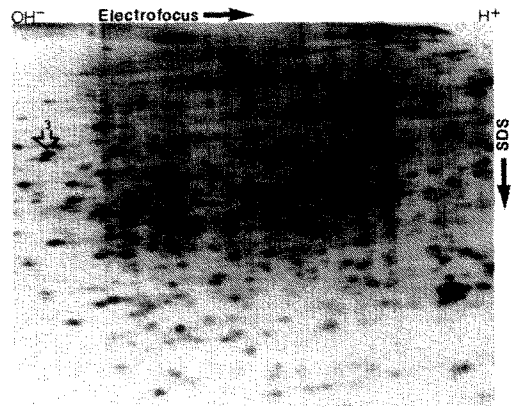

Fig.1. Patterns of polypeptide synthesis in *Xenopus laevis* oocytes not subjected to any treatment. 15 oocytes were incubated for 2 h at 22°C in 50 μl Barth's medium containing 1 Ci/l [^{35}S]methionine. 'House-keeping' proteins are encircled, marker proteins are labelled 1a, 2a, A-actin, T-tubulin. Arrows indicate where the major consistent modifications occur.

increased in intensity. Polypeptides 1, 2 and 5 were not present in controls. Polypeptides 1 and 2 became most evident between 2–4 h of progesterone treatment and remained until after GVBD was complete. Polypeptide 1 which could be single or grouped, had the same M_r-value as the triplet with a slightly higher p*I* (1a; fig.1). Polypeptide 2 had a higher M_r-value than the marker 2a and is not completely separated

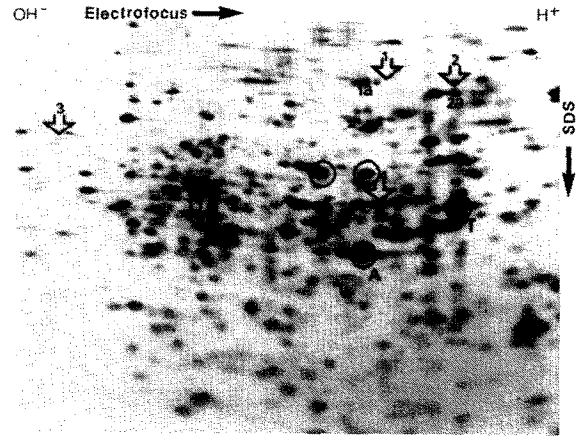

Fig.2. Patterns of polypeptide synthesis in *Xenopus laevis* oocytes after 4 h treatment with progesterone followed by 2 h labelling in [^{35}S]methionine-containing medium. GVBD was 32%. Polypeptides 1–3 and 5 represent increased/induction and polypeptide 4, a repression. Note that polypeptide 2 has a slightly higher M_r-value than the marker 2a.

Table 1
A typical experiment showing the amount of trichloroacetic acid-precipitable proteins in the incubation medium compared to that of oocyte homogenate

Time (h)	%GVBD	^{35}S-Labelled proteins in medium (cpm/μl)	^{35}S-Labelled proteins oocyte homogenate (cpm/μl)
0–2	0	180	41 700
2–4	0	250	33 800
4–6	32	310	49 800
6–8	64	220	30 300
Control	0	250	37 900

At the end of each 2 h incubation (15 oocytes in 50 μl Barth's buffer containing 1 Ci/l [^{35}S]methionine) the medium was removed and the oocytes homogenized in 150 μl O'Farrell's [11] lysis buffer A. A total of 5 μl of incubation medium and 2 μl of oocyte homogenate were subjected to TCA precipitation

from it. Polypeptides 3 and 5 appeared within the first 2 h of incubation with progesterone and remained evident until GVBD. Polypeptide 5 appeared in 6 experiments. In 1 experiments where the oocytes matured very slowly the inductions of polypeptides 1–3 were not manifest until 4–6 h post-progesterone treatment (GVBD was 50% at 10 h).

3.2. *Protein regression*

Polypeptide 4 was repressed under the influence of progesterone but the repression was evident only after 2 h of treatment. Although other polypeptides seem to be repressed, this one was most consistent.

3.3. *Protein secretion*

The secretion of ^{35}S-labelled proteins into the medium was relatively low compared to incorporation into oocyte proteins. Since there were variations in the uptake of [^{35}S]methionine and speed of maturation the data from 1 typical experiment is shown in table 1.

4. Discussion

The technique of two-dimensional (2D) electrophoresis had been used for the analysis of specific protein synthetic changes during maturation of mammalian oocytes [16–18]. As far as we are aware this is the first report of high-resolution electrophoretic analysis of progesterone-induced changes in oocyte polypeptide synthesis in an amphibian species. Using a similar technique it was reported that follicles from human chorionic gonadotropin-treated *Xenopus laevis* synthesized 4 new [^{35}S]methionine-labelled proteins [19] but it was not determined whether these were follicular cell or oocyte proteins. On the other hand, no new proteins were detected in stimulated *Xenopus laevis* oocytes before maturation although many of the more than 700 proteins detected by 2D electrophoresis became greatly reduced or undetectable [20]. Novel protein synthesis was first observed during gastrulation [20]. The differences in treatment and duration of labelling preclude proper comparisons.

In mammals there appears to be a net decrease in protein synthesis during spontaneous maturation tested in vitro [21] and inhibitors of protein synthesis do not inhibit the progression to germinal vesicle breakdown in isolated oocytes [22]. The progesterone-reinitiated maturation of *Xenopus laevis* oocytes on the other hand, is dependent on protein synthesis since cycloheximide would inhibit maturation [5,6]. In spite of decreased uptake of amino acids [8] and the relative impermeability of the oocyte membrane to amino acids [22] the evidence to data suggest that there is a net increase in protein synthesis during oocyte maturation [3,9]. Here, using a highly sensitive technique, we were able to demonstrate 5 modifications in polypeptide synthesis during progesterone-induced maturation, 4 represent inductions and 1 repression. The data also indicate that methionine can be very effectively taken up from the medium and be incorporated into protein.

The question arises as to whether the increase of proteins indicate peptide synthesis or post-transla-

tional modifications. From the data of the 7 experiments it would appear that protein 3 is synthesized at a faster rate whereas proteins 1, 2 and 5 were newly synthesized or transformed polypeptides. Polypeptide 1 could be a phosphorylated protein since it appears to belong to a group of proteins with the same M_r-value. Polypeptide 2 may be formed from 2a (fig.1) which has a lower M_r-value. Polypeptide 4 seems to be a form of a glycoprotein since there are several spots of decreasing p*I* and identical M_r-values. These modifications are different from those seen in mouse [16] except for polypeptide 3.

Post-translational modification concomitant with a change in rate of protein synthesis could account for some of the modifications observed. Protein phosphorylation is a potentially important event [24,25] and does occur during maturation of *Xenopus laevis* oocytes [26]. Polypeptide 1 could be a likely candidate as a phosphorylated protein, even though the labelling period was 2 h.

Since the total low-speed supernatant of oocyte homogenate was analyzed, it was not possible to determine which cellular compartment was responsible for the modified proteins. It is known that the protein composition of the nucleus is different from that of the cytoplasm [27]. Moreover, it has been shown that the synthesis and phosphorylation of a protein found in the germinal vesicle occurs during spontaneous maturation of mouse oocyte in vitro [28].

In conclusion, although it is premature to decide whether the modifications induced by progesterone are crucial for the completion of GVBD, they are nevertheless very early changes which occur with great consistency.

Acknowledgements

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