

Intracellular pH and ribosomal protein S6 phosphorylation: role in translational control in *Xenopus* oocytes

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SUMMARY

The induction of amphibian oocyte maturation with progesterone as well as the activation of sea urchin eggs at the time of fertilization result in increased protein synthesis. The increase in both cases involves the recruitment of maternal mRNA onto polysomes. Further, it has been reported that sea urchin eggs, like full-grown *Xenopus* oocytes, contain no spare translational capacity based on the observation that injected heterologous mRNA is translated only at the expense of endogenous messages. The nature of the limiting component defined by such experiments is not known, but two factors which have been proposed to play a role in regulating protein synthesis are ribosomal protein S6 phosphorylation and intracellular pH. In the current paper, we review the literature and present new evidence on the roles intracellular pH and S6 phosphorylation have in regulating protein synthesis in *Xenopus* oocytes. We report that pH_i does not increase between stage 3 and stage 6, yet the protein synthetic rate increases at least eight fold during the same period. Hence, we conclude that increasing pH_i is not a prerequisite for increasing protein synthesis. Moreover, we present three arguments against increased ribosomal protein S6 phosphorylation being sufficient or necessary for increased protein synthesis in *Xenopus* oocytes. First, the level of S6 phosphorylation does not increase between stages 4 and 6, a period exhibiting a two to three fold increase in protein synthesis. Second, the injection of globin mRNA into stage-4 oocytes increases total protein synthesis two to three fold, but has no effect on S6 phosphorylation. Third, when the injection of globin mRNA into stage-4 oocytes is followed by an injection of MPF, a dramatic increase in S6 phosphorylation is seen, but total protein synthesis is not further stimulated.

INTRODUCTION AND OVERVIEW

Full-grown amphibian oocytes, arrested in meiotic prophase, are induced to complete meiosis as well as undergo a series of morphological and biochemical changes in response to steroid hormones such as progesterone. The events of maturation occur over a period of several hours after progesterone stimulation, but have been conveniently divided into early and late responses (Smith, 1981; Maller, 1983). Many of the events observed during oocyte maturation are the same events which occur after fertilization of sea urchin eggs (Smith, 1981). Unfertilized sea

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urchin eggs also represent an example of a cell type arrested at a particular point in the cell cycle (pre S phase). Moreover, both the stimulus which induces oocyte maturation (steroid hormone) and that which activates eggs at fertilization (sperm) act on a site at or near the surface.

Fertilization of sea urchin eggs results in a dramatic increase in the rate of protein synthesis, an increase resulting in large part from the recruitment and translation of maternal mRNA (Humphreys, 1971; Raff, 1980). However, part of the increase also is the result of a two- to three-fold increase in translational efficiency (Brandis & Raff, 1978; Hille & Albers, 1979). Furthermore, the two processes, recruitment and more efficient translation of maternal mRNA, may be regulated independently of each other.

Numerous examples have appeared in the literature correlating low pH_i with dormancy, while activation of metabolism correlates with increases in pH_i (Nuccitelli & Heiple, 1982). Metabolic activation of the sea urchin egg at the time of fertilization is associated with a large increase in pH_i (Johnson, Epel & Paul, 1976; Shen & Steinhardt, 1978). This correlates with increased protein synthesis which has suggested a causal relationship (Brandis & Raff, 1979; Grainger, Winkler, Shen & Steinhardt, 1979; Winkler, 1982). For example, activation of eggs by ammonia leads to increased protein synthesis without a corresponding increase in translational efficiency (Brandis & Raff, 1979), suggesting that cytoplasmic alkalization affects mRNA recruitment.

Fertilization of sea urchin eggs also results in the phosphorylation of a 40S ribosomal protein, S6, which correlates with the increase in protein synthetic rate (Ballinger & Hunt, 1981). Increases in S6 phosphorylation have been associated with mitogenic stimuli in several cell types (see Ballinger, Bray & Hunt, 1984). However, at least in sea urchin eggs, the role of S6 phosphorylation in regulating increased protein synthetic rates has been questioned in recent experiments (Ballinger *et al.* 1984). The situation in sea urchin eggs has become even more complicated with the observation that globin mRNA injected into eggs and zygotes competes with endogenous mRNAs for translation, suggesting that some component(s) other than mRNA availability also regulates the protein synthetic rate (Hille, Danilchick, Colin & Moon, 1985).

Fertilization or parthenogenetic activation of frog eggs increases protein synthesis minimally (Shih, O'Conner, Keem & Smith, 1978), although fertilization does cause a pH_i increase of as much as 0.3 units (Webb & Nuccitelli, 1981). On the other hand, the induction of oocyte maturation results in at least a two-fold increase in protein synthetic rate (Shih *et al.* 1978; Wasserman, Richter & Smith, 1982), and, in this case, an increase in pH_i does correlate with the change in protein synthetic rate (Lee & Steinhardt, 1981; Houle & Wasserman, 1983; Cicirelli, Robinson & Smith, 1983). There has been considerable discussion in the literature as to whether or not this increase in pH_i plays a role in regulating protein synthesis, with both protagonists (Houle & Wasserman, 1983; Wasserman & Houle, 1984) and antagonists (Lee & Steinhardt, 1981; Cicirelli *et al.* 1983; Stith & Maller, 1985).

It has been well documented that a burst in cAMP-independent phosphorylation occurs as a late event during oocyte maturation, and this phosphorylation event correlates well with increased protein synthesis (Maller, Wu & Gerhart, 1977). Furthermore, when maturation is induced by injection of maturation promoting factor (MPF), the rate of protein synthesis increases almost immediately (Wasserman *et al.* 1982) as does the burst in phosphorylation (Maller *et al.* 1977). One protein which is phosphorylated in both circumstances is ribosomal protein S6 (Hanocq-Quertier & Baltus, 1981; Neilsen, Thomas & Maller, 1982; Kalthoff *et al.* 1982; Kruppa, Darmer, Kalthoff & Richter, 1983). These kinds of studies have led to the suggestion that S6 phosphorylation may be necessary, if not sufficient, for increased protein synthesis during oocyte maturation.

Attempts to elucidate the role of various events such as S6 phosphorylation in regulating protein synthesis are complicated when studies are performed on full-grown oocytes since, in stage-6 oocytes, several factors may be involved. Unlike the situation with sea urchin eggs, there is no significant change in ribosome packing density nor in rates of amino acid polymerization when oocytes are induced to mature; translational efficiency is constant. Thus, the increase in protein synthesis which occurs in mature oocytes must result from an increase in the number of messages being translated (Richter, Wasserman & Smith, 1982; Taylor & Smith, 1985). However, other experiments with injected mRNAs have shown that mRNA availability is not the only limiting component for protein synthesis in stage-6 oocytes (Laskey, Mills, Gurdon & Partington, 1977; Richter & Smith, 1981). Since injected messages compete with endogenous mRNAs for translation, some component(s) of the protein synthetic machinery, as well as mRNA, must become available in order for protein synthesis to increase during maturation. The situation in stage-6 oocytes is complicated even further since the level of protein synthesis in control oocytes can vary substantially, dependent on the physiological status of individual females (Wasserman *et al.* 1982; Stith & Maller, 1985). Thus, the magnitude of the increase in protein synthesis during maturation can be relatively small.

Stage-4 oocytes do not respond to progesterone but such oocytes do exhibit germinal vesicle breakdown, as well as increased protein synthetic rate, after injection with MPF (Hanocq-Quertier *et al.* 1976; Wasserman, Houle & Samuel, 1984). Injection of MPF into stage-4 oocytes (8 μ m diameter) also is reported to elevate pH_i as well as stimulate phosphorylation of ribosomal protein S6 (Wasserman *et al.* 1984). Recently, we (Taylor, Johnson & Smith, 1985) observed further that growing stage-4 *Xenopus* oocytes translate injected mRNA. However, unlike the situation in stage-6 oocytes, the injected mRNA did not compete with endogenous messages in stage-4 oocytes. Rather, total protein synthesis increased to a level usually seen in stage-6 oocytes. Thus, stage-4 oocytes apparently contain spare translational capacity. This result suggests that the role of various factors in regulating translation, independent of mRNA recruitment, might be studied best in growing stage-4 oocytes. In what follows, we discuss the results of recent experiments

concerned with the role that changes in pH, and S6 phosphorylation have in regulating protein synthesis in stage-4 oocytes injected with globin mRNA.

MATERIALS AND METHODS

Animals and collection of oocytes

Large adult *X. laevis* females were purchased from South Africa (Snake Farm, Fish Hoek, Cape Province), Michigan (Kelly Evans, Ann Arbor, 716 Northside), or Nasco (Fort Atkinson, Wisc.). These animals were maintained as described by Webb, La Marca & Smith (1975). Ovaries were surgically removed from hypothermically anaesthetized females. Dumont (1972) stage-3 to -6 oocytes were manually defolliculated with watchmaker's forceps and cultured in OR-2 medium (Wallace, Jared, Dumont & Toga, 1973). Maturation was induced in stage-6 oocytes with progesterone ($10 \mu\text{g ml}^{-1}$) purchased from Sigma and scored by the appearance of a white spot at animal pole. GVBD was confirmed in stage-4 oocytes injected with MPF by manual dissection after fixation in 0.5 N-perchloric acid.

Isolation of globin mRNA

Globin mRNA was prepared as described by Taylor *et al.* (1985). The purity of these RNAs was verified by *in vitro* translation and SDS gel electrophoresis. All RNAs were suspended in sterile water at a concentration of $1\text{--}2 \text{ mg ml}^{-1}$.

Injection of oocytes

Stage-4 and -6 oocytes were injected with 10–20 nl of either the RNA suspension, MPF (cytoplasm from a mature oocyte), or sterile water and were incubated for a period of 2–10 h in OR-2 followed by an injection of 15 nl of [^{32}P]orthophosphate ($10 \text{ mCi } 40 \mu\text{l}^{-1}$, ICN). In some cases an additional injection involving either MPF or sterile water was performed prior to ^{32}P -treatment. Oocytes were then either exposed to progesterone or cultured in OR-2 for 4–8 h. Oocytes in groups of 10 were analysed for ribosomal protein S6 phosphorylation; oocytes run in parallel were used to measure rates of protein synthesis (see below). Stage-6 oocytes injected with MPF did not activate when subsequently microinjected with radioisotope using micropipets with tip diameters $\leq 5 \mu\text{m}$.

Preparation of ^{32}P -labelled ribosomes for one-dimensional SDS-PAGE.

^{32}P -labelled ribosomes were isolated according to Wasserman & Houle (1984). Ribosomal pellets were dissolved in sample buffer and one-dimensional SDS-polyacrylamide gel electrophoresis was carried out as described by Laemmli (1970). Recovery of ribosomes was estimated by measuring the amount of RNA contained in the ribosomal pellets and comparing that to total RNA expected from previous measurements of RNA content in stage-4 and stage-6 oocytes (Taylor & Smith, 1985); rRNA is about 90 % of total RNA (Scheer, 1973). Recovery of ribosomes

from oocytes at both stages was about 72 %. RNA was extracted according to Anderson & Smith (1977) and the quantity estimated by U.V. absorption at 260 nm. RNA extraction efficiency was monitored by adding ^{14}C -labelled RNA at the time of pellet homogenation. Recovery of RNA was 80 % for both stages. Gels were dried down and exposed to preflashed X-ray film (XR-5, Kodak). Densitometric scans were performed on autoradiographs with a Quick Scan integrating densitometer (Helena Laboratories).

Measurement of protein synthesis

Rates of protein synthesis were measured according to Taylor & Smith (1985).

ATP pool specific activity

The ^{32}P ATP precursor pool specific activity was determined using the luciferase assay described previously (Dolecki, Anderson & Smith, 1976). Oocytes were injected with 2.5×10^6 c.p.m. of ^{32}P orthophosphate and processed 8 h later. Recovery of ATP was monitored by addition of a 50-fold excess of ATP to oocyte homogenates not used for specific activity determinations. Recovery of ATP was found to be 14.6 % and 13.5 % for stage-4 and stage-6 oocytes, respectively.

Electrophysiological methods

The pH microelectrodes were constructed using the neutral ion carrier of Ammann *et al.* (1981). Electrodes with tip diameters of $0.5\text{--}2.0\ \mu\text{m}$ were pulled on a vertical pipet puller (Kopf Instruments, Tujunga, CA) from $1.5\ \text{mm}$ O.D. thin-walled glass tubing (WPI, New Haven, Conn.) baked at 200°C overnight, then silanized with dichlorodimethylsilane vapour, and baked for 1 h at 200°C . A 2 mm column of ion exchange resin (WPI) was injected into the shank of the microelectrode and pressure applied to fill the tip of the microelectrode. The remainder of the microelectrode was backfilled with a solution of 0.015M-NaCl , 0.023M-NaOH , $0.04\text{M-KH}_2\text{PO}_4$, $\text{pH } 7.0$. Reference microelectrodes were pulled from $1.5\ \text{mm}$ O.D. "Kwik-Fil" glass capillaries (WPI) and filled with 3M-KCl . The resistance of these electrodes was $10\text{--}30\ \text{M}\Omega$.

An AM-4 differential amplifier (Biodyne Co., Santa Monica, Calif.) with a high impedance probe was used. The outputs of the reference electrode amplifier and of the differential amplifier were displayed on a storage oscilloscope (Tektronix 5111) and on a two-channel recorder (Brush, Model 220, Gould Inc., Cleveland, Ohio) giving a record of the intracellular pH and the membrane potential.

The response of the pH microelectrode used was determined by putting it in a $\text{pH } 6.8$ and then a $\text{pH } 7.8$ OR-2 solution. A $58\ \text{mV}$ change was measured both before and after the measurements on the oocytes. Full response of the electrode upon addition of the 1M-HCl to change the pH of the bathing medium by $0.5\ \text{pH}$ units occurred in about 20 sec.

The electrodes were placed in holders which were in turn mounted in micropositioners arranged so that the electrodes could be inserted in the oocytes at

a small angle to the vertical. This was done with the aid of a dissecting microscope. The entire apparatus was enclosed in a Faraday cage to minimize pickup by the high-impedance pH electrode.



RESULTS

Protein synthesis in growing oocytes



Previous studies have shown that the rate of protein synthesis in growing oocytes increases approximately as a function of the volume as calculated from oocyte diameter (Taylor & Smith, 1985). Thus, the rate of protein synthesis in stage-6 oocytes averages two- to three-fold greater than that in stage-4 oocytes, depending on whether or not early *versus* late stage-4 oocytes are compared with stage 6. This is documented more fully by the data in Table 1, which also shows that injection of stage-4 oocytes (800 μm diameter) with either globin mRNA or with MPF raises total protein synthesis  approximately the same level as seen in stage-6 control oocytes. Perhaps more significantly, in stage-4 oocytes injected with globin mRNA and then with MPF, there is no additional increase in total protein synthesis. This observation supports the suggestion (Wasserman *et al.* 1982) that MPF is involved at the level of mRNA recruitment and not in modifications of the protein synthetic machinery.

Table 1. *Protein synthetic rates in stage-4 and -6 oocytes injected with mRNA or MPF*

Oocyte stage and treatment [†]	Protein synthesis rate* (ng h ⁻¹ oocyte ⁻¹)
St.4 + distilled water	9.8 8.7 + .9
St.4 + 20 ng globin mRNA	18.6 18.4 + 1.8
St.4 + 20 nl of MPF	19.7
St.4 + 20 ng globin mRNA + 20 nl of MPF	20.1
St.6 + distilled water	19.9 20.2 + 2.0
St.6 + 20 ng globin mRNA	20.9
St.6 + 20 nl of MPF	43.7
St.6 + progesterone 	37.9 35.3 + 3.3

*Determined according to Taylor & Smith (1985).

†The experiments were conducted on oocytes from three different females. The single values listed under protein synthesis rate were all on oocytes from one female, and correspond to data in Fig. 2. The values listed as mean + standard deviation were conducted on oocytes from the other two females and one group corresponds to data in Fig. 1.

*Table 2. ATP pool size and specific activity in stage-4 and -6 oocytes**

Oocyte stage	Diameter [†] (μm)	ATP pool [‡] size (n moles oocyte ⁻¹)	Ratio of ATP pool size to st.4	ATP pool [‡] specific activity (c.p.m. p mole ⁻¹)	Ratio of Spec. Activ. to St.6
4	800 ± 15	.53 ± .20(2) ^δ	1.00	1486.5 ± 579(2)	2.57
6	1250 ± 20	1.55 ± .11(2)	2.92	579.5 ± 40(2)	1.00

*Stage-4 and stage-6 oocytes were injected with 2.5×10^6 c.p.m. of [³²P] orthophosphate, incubated for 8 h in OR-2, and processed as described in Materials and Methods.

[†]Mean of a group of 10 oocytes.

[‡]Values represent the mean ± standard deviation.

^δNumbers in parentheses represent number of females examined.

Phosphorylation of ribosomal protein S6 during oogenesis

Since the data described above show that spare translational capacity exists in stage-4 oocytes, we conducted experiments to estimate the level of S6 phosphorylation prior to and after stimulation of protein synthesis. Initial experiments were conducted to determine the specific activity of the ATP in both stage-4 and stage-6 oocytes injected with [³²P]orthophosphate and incubated for 8 h. As shown in Table 2, the size of the ATP pool increased almost three fold in comparing stage-4 with stage-6 oocytes. This agrees with the increase in GTP pool size known to occur between stage-4 and stage-6 oocytes (LaMarca, Smith & Strobel, 1973). Moreover, our value for the total quantity of ATP present in stage-6 oocytes agrees well with that reported by Maller *et al.* (1977). Since both stage-4 and stage-6 oocytes were injected with equal amounts of radioactivity, one would predict that the pool specific activity of ATP in stage-4 oocytes would be about three fold greater than in stage 6. The actual measured values are not greatly different from this (Table 2).

In order to identify ribosomal protein S6, proteins isolated from ³²P-labelled ribosomes were electrophoresed on one-dimensional SDS polyacrylamide gels. Relative mobility and the pattern of phosphorylation for stage-6 oocytes were compared with gel patterns previously published by several investigators (Nielsen *et al.* 1982; Wasserman & Houle, 1984; Wasserman *et al.* 1984). Figure 1 shows an autoradiograph of ³²P-labelled ribosomal proteins isolated from stage-4 and stage-6 oocytes. Densitometric scans from two such experiments indicate that the intensity of the band at approximately 32×10^3 relative molecular mass, corresponding to ribosomal protein S6, is 2.6 times greater in stage-4 oocytes than in stage-6 oocytes. However, after correction for differences in ATP pool specific activity, the level of S6 phosphorylation remains approximately constant between stages 4 and 6. Additional support for this view is presented in Fig. 2 which also shows an autoradiograph of ³²P-labelled ribosomal proteins from stages-4 and -6 oocytes. However, in this case the ATP pool size in the stage-4 oocytes was one-fourth that in stage-6 and the stage-4 oocytes were injected with one-fourth as much label;



Fig. 1. Autoradiograph of ^{32}P -labelled ribosomal proteins isolated from control stage-4 oocytes (lane 1), stage-4 oocytes injected with 20 ng of globin mRNA (lane 2), control stage-6 oocytes (lane 3), and stage-6 oocytes induced to mature in progesterone (lane 4). All oocytes were injected with 2.5×10^6 c.p.m. of ^{32}P orthophosphate.

pool specific activities were equal. Densitometric scans revealed that S6 phosphorylation in the stage-4 oocytes \triangle 95 % that observed in stage-6 oocytes. Since the protein synthesis measurements run in parallel with these studies (Table 1) show an increase of at least two fold between stages 4 and 6, it seems clear that an increase in the level of S6 phosphorylation during oogenesis is not necessarily linked to increased synthetic activity.

Phosphorylation of S6 in oocytes injected with mRNA or MPF

Nielsen *et al.* (1977) initially reported that ribosomal protein S6 is preferentially phosphorylated during maturation and that this phosphorylation coincides with the increase in protein synthesis observed in maturing oocytes (Wasserman *et al.* 1982). We also find that the level of S6 phosphorylation is increased \triangle in stage-6 oocytes induced to mature with progesterone (Figs 1 and 2). Densitometric scans revealed

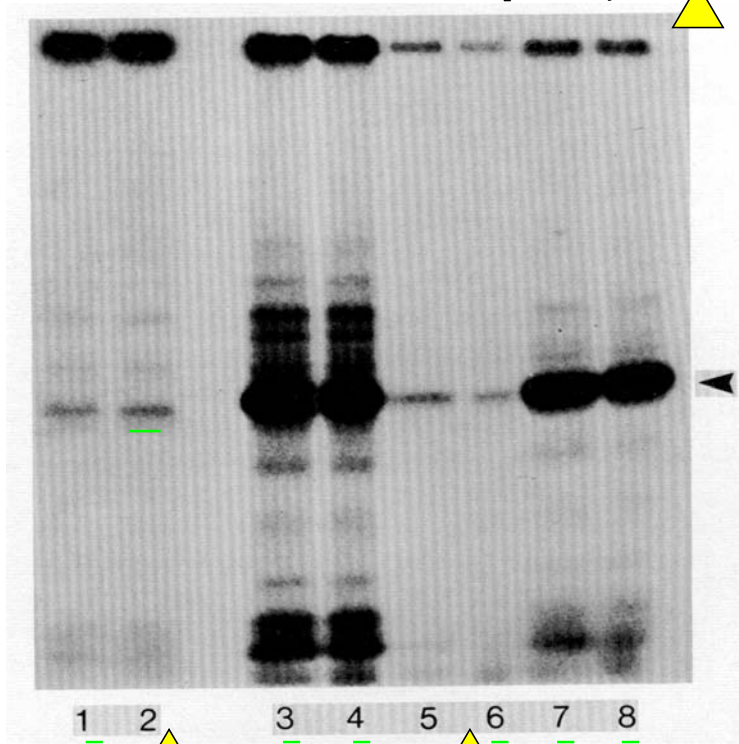


Fig. 2. Autoradiograph of ^{32}P -labelled ribosomal proteins isolated from control stage-4 oocytes (lane 1), stage-4 oocytes injected with 20 ng of globin mRNA (lane 2), stage-4 oocytes injected with 20 nl of MPF (lane 3), stage-4 oocytes injected with 20 ng of globin mRNA followed by 20 nl of MPF (lane 4), control stage-6 oocytes (lane 5), stage-6 oocytes injected with 20 ng globin mRNA (lane 6), stage-6 oocytes injected with 20 nl of MPF (lane 7), and stage-6 oocytes induced to mature in progesterone (lane 8). Stage-4 oocytes were injected with 6.25×10^5 c.p.m. of ^{32}P orthophosphate, while stage-6 oocytes were injected with 2.5×10^5 c.p.m. of ^{32}P orthophosphate.

that the increase ranges between 3.6- and 5.0-fold. Moreover, the increase in S6 phosphorylation during maturation is accompanied by approximately a twofold increase in protein synthesis. Injection of MPF into stage-4 oocytes also resulted in an increase (five to six fold) in the level of S6 phosphorylation. Again, this correlates with an increase in the rate of protein synthesis of about two fold induced by the MPF injection. However, the data in Table 1 show that it is possible to increase the protein synthetic rate in stage-4 oocytes without a concomitant change in the level of S6 phosphorylation. This is clearly the case when stage-4 oocytes are injected with globin mRNA (Table 1, Figs 1, 2). Furthermore, the data in Fig. 2 and Table 1 show that when stage-4 oocytes are injected first with an amount of globin mRNA sufficient to saturate the oocyte translational machinery (Taylor *et al.* 1985), and then with MPF, the level of S6 phosphorylation increases dramatically; the rate of protein synthesis is unchanged (Table 1). We conclude from these kinds of experiments that increases in the level of S6 phosphorylation are not sufficient, and probably not necessary, for increased protein synthetic activity.

Table 3. *Intracellular pH during oogenesis**

Frog	Oocyte stage			
	St.3	St.4	St.5	St.6
1	—	—	7.39 ± .01(3)	7.39 ± .03(3)
2	7.33 ± .09(4)	7.37 ± .02(3)	7.36 ± .05(4)	7.35 ± .03(3)
3	7.30 ± .01(3)	7.28 ± 0.02(6)	7.26 ± .04(6)	7.24 ± .04(6)
4	7.47 ± .10(5)	7.47 ± .10(10)	7.43 ± .04(7)	7.45 ± .01(2)
5	7.35 ± .06(3)	7.37 ± .05(6)	7.22 ± .04(3)	7.35 ± .03(6)
6	7.47 ± .05(4)	7.41 ± .06(3)	7.42 ± .04(3)	7.43 ± .04(5)
Average	7.39 ± .07(19)	7.39 ± .06(28)	7.35 ± .04(25)	7.35 ± .03(25)

*The pH values represent the mean ± standard deviation. Numbers in parentheses are oocytes sampled.

Intracellular pH during oogenesis

The intracellular pH (pH_i) of the oocyte is operationally defined as the value that we obtained 0.5 to 2 h after dissection from the follicle, and corresponds to the time period after electrode potentials have stabilized. The data from six experiments are summarized in Table 3. The average intracellular pH measurements of stage-3 to -6 oocytes were found to be 7.39 ± 0.07 (Stage 3), 7.39 ± 0.06 (Stage 4), 7.35 ± 0.04 (Stage 5), and 7.35 ± 0.03 (Stage 6). Clearly, these minor differences are not statistically significant (Student's test).

The average pH_i value for stage-5 oocytes compares favourably with the values previously reported for full-grown oocytes by Lee & Steinhardt (1981) and Cicirelli *et al.* (1983). Further, the absence of any significant pH_i difference between stage-4 and stage-6 oocytes confirms the observation made by Stith & Maller (1984) using the DMO method, and does not support the view that progressive alkalinization of the oocyte cytoplasm during oogenesis plays any role in regulating protein synthetic rates (Houle & Wasserman, 1983; Wasserman & Houle, 1984).

DISCUSSION

While some component of the protein synthetic apparatus in full-grown oocytes is clearly limiting, the nature of this component is not known. Full-grown oocytes are known to contain ribosomes far in excess of the number needed. As Woodland (1974) and Taylor & Smith (1985) reported, only about 2% of the oocyte's ribosomes are actually found in polysomes. This number has increased about 15% by the blastula stage. Since RNA synthesis does not occur during the time between GVBD and the mid-blastula transition (Gelfand & Smith, 1983; Newport & Kirschner, 1982a, b), this result by itself implies that the quantities of all species of RNA involved in translation are not limited in the oocyte. Furthermore, since *Xenopus* oocytes are capable of aminoacylating exogenous tRNAs injected into

them at concentrations greatly exceeding the endogenous tRNA content (Gatica, Tarrago, Allende & Allende, 1975; Bienz, Kubli, deHenan & Grosjean, 1980), it would appear the availability of aminoacyl-tRNA synthetases is not limiting in the oocyte. These kinds of data support the view that, quantitatively, the various components of the translational machinery are present in excess amounts relative to the needs of control oocytes. This is not to say, however, that all of the components are fully competent to participate in protein synthesis.

Recently, it has been reported that sea urchin eggs, like full-grown *Xenopus* oocytes, contain no spare translational capacity. This is based on the observation that sea urchin eggs or zygotes injected with globin mRNA translated the heterologous mRNA only at the expense of endogenous messages (Hille *et al.* 1985). In approaching the question of what component(s) might be limiting for protein synthesis, they presented evidence suggesting as one possibility that egg ribosomes are repressed. The nature of the repression is unclear but could involve modification in the phosphorylation state of ribosomal proteins. Alternatively, Hille *et al.* (1985) suggest that conversion of ribosomes from an inactive state to a functional one is dependent on alkalization of the cytoplasm.

In sea urchin eggs, protein synthesis can be partially stimulated by weak bases such as ammonia, but Ballinger *et al.* (1984) have shown that ammonia neither stimulates ribosomal protein S6 phosphorylation nor does it inactivate a specific S6 phosphatase. In amphibian oocytes, Stith & Maller (1985) point out circumstances in which an increase in protein synthesis does not correlate with increased S6 phosphorylation. This includes the observation that oocytes obtained from PMSG-primed females exhibit increased rates of protein synthesis, compared with stage-6 oocytes from unprimed females, but show no change in the basal level of S6 phosphorylation. In the current study, we present additional examples in which protein synthetic rates can be elevated with no coincident increase in S6 phosphorylation. Further, we have presented evidence that increased S6 phosphorylation can be brought about with no effect on protein synthesis. Taken together, the evidence from both sea urchin and amphibian studies suggests that S6 phosphorylation is neither sufficient nor necessary for increased protein synthesis.

Phosphorylation of S6 protein accompanies the action of several kinds of growth stimuli, including serum, epidermal growth factor (EGF), viral infection, insulin-like growth factor (IGF) and fertilization (Ballinger & Hunt, 1981; Leader, 1980; Traugott, 1981). If the role of ribosomal protein S6 phosphorylation is not to regulate the quantitative aspects of protein synthesis, i.e. the overall cellular rate of translation; perhaps it plays a role in regulation of the qualitative aspects, i.e. the selection of specific mRNA. Support for this view comes from studies in which it has been shown that heat shock of *Drosophila* cells results in rapid S6 dephosphorylation and ribosomes that are unable to translate specific mRNAs *in vitro* (Glover, 1982; Scott & Pardue, 1981). Lastly, the isolation of a S6 protein kinase activity from *Xenopus* eggs (Maller *et al.* 1985) should facilitate understanding of the roles S6 phosphorylation plays in cell growth and proliferation.

Several studies have correlated low intracellular pH with dormancy and activation of metabolism with increases in pH_i (see Nuccitelli & Heiple, 1982). One of the most thoroughly studied examples is the sea urchin egg in which an increase in pH_i at the time of fertilization is thought to play a major role in the activation of protein synthesis (Johnson *et al.* 1976; Shen & Steinhardt, 1978; Grainger *et al.* 1979; Winkler, Steinhardt, Grainger & Minning, 1980). The mechanism by which cytoplasmic alkalinization might function in regulating protein synthesis remains unknown. Hille *et al.* (1985) have suggested that the conversion of ribosomes from an 'inactive state' to a functional one is dependent on the pH change in the cytoplasm. Winkler, Nelson, Lashbrook & Hershey (1985) have reported recently that the formation of preinitiation complexes containing a 40S ribosomal subunit and methionyl-tRNA was increased at $\text{pH} 7.4$ compared to $\text{pH} 6.9$ using a sea urchin egg cell-free translation system. Whatever the case, echinoid eggs appear to differ substantially from amphibian oocytes in the role of pH_i in stimulating protein synthesis.

Two kinds of evidence have been used to support the view that cytoplasmic alkalinization during oocyte maturation is involved in the regulation of protein synthesis. First, several laboratories have reported that pH_i increases by between 0.2 and 0.5 units at approximately the same time prior to GVBD that protein synthesis increases (Lee & Steinhardt, 1981; Houle & Wasserman, 1983; Cicirelli *et al.* 1983). Related to this, the pH_i of growing oocytes is reported to increase during a period of oogenesis (stage 4–stage 6) when the total protein synthetic rate is increasing (Wasserman *et al.* 1984). Second, treatment of oocytes with agents which cause alkalinization (weak bases) or acidification (sodium acetate) of oocyte cytoplasm results in a stimulation or repression, respectively, of the protein synthetic rate (Houle & Wasserman, 1983; Wasserman *et al.* 1984).

It should be pointed out that the pH_i of *Xenopus* stage-6 oocytes varies considerably in oocytes from different females, and not all oocytes elevate basal pH_i in response to progesterone (Cicirelli *et al.* 1983). Protein synthesis varies much less and always increases during maturation. Further, the elevated pH_i observed prior to GVBD decreases to control levels after GVBD, but protein synthesis remains elevated. Finally, alterations in pH_i by simple changes in pH of the medium in which oocytes are incubated has virtually no effect on protein synthetic rates (Cicirelli *et al.* 1983). Finally, the current data show clearly that pH_i does not increase between stage 3 and stage 6, in contrast to the results of Wasserman *et al.* (1984), yet the protein synthetic rate increases at least eight fold during the same period (Taylor & Smith, 1985). We conclude from such data that increasing pH_i is not a prerequisite for increasing protein synthesis (also Stith & Maller, 1985), although the possibility exists, first suggested by Lee & Steinhardt (1981), that a certain threshold value must be obtained in order for increased protein synthesis to occur.

In the current paper, we have discussed and presented evidence suggesting that a number of components associated with translational regulation in many systems

do not play a role in regulating translation in *Xenopus oocytes*. Thus, the nature of the component that limits translation in oocytes ranging from stage 4 through stage 6 remains unknown. However, Lingrel & Woodland (1974) first suggested that oocytes contain a 'recruitment factor' that is in limited supply but which acts as a preinitiation level ender mRNA translatable. Richter & Smith (1981) supported this view in a series of experiments involving the injection of heterologous mRNAs into oocytes. Related to this, a series of papers, Thach and colleagues (see Godefroy-Colburn & Thach, 1981) in studying competition between viral and host cell mRNAs suggested that messages compete for a 'discriminatory factor' which in limiting amount determines which mRNA is translated.

While the current experiments have not resolved possibilities of the type raised above, they do suggest experimental approaches with which to address questions concerned with mRNA recruitment. For example, Richter *et al.* (1983) have demonstrated that injection of adenovirus 5 mRNA into stage-6 oocytes results in a stimulation of endogenous protein synthesis, presumably via a protein(s) translated from one or more of the adenovirus messages. Similarly, we (Wasserman *et al.* 1982) have suggested that proteinaceous MPF increases protein synthesis by operating at the level of mRNA recruitment. This suggestion is confirmed by the data (Table 1) showing that MPF does not further stimulate protein synthesis in stage-4 oocytes previously injected with saturating amounts of mRNA. Thus, we suggest that in stage-4 oocytes, MPF acts only to make mRNA available for translation, while in stage-6 oocytes it also results in an increase in the putative limiting component(s). Perhaps, studies concerned with effects of MPF on protein synthesis at the two stages will allow discrimination between effects on recruitment *versus* the translational machinery.

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DISCUSSION

Speaker: D. Smith (Purdue)

Question from A. Durston (Utrecht):

Can you say something about the evidence for the recruitment factor hypothesis?

Answer:

There are two kinds of evidence. One is that if one injects a variety of different kinds of messenger RNA into stage-6 oocytes they are not competitive with each other in terms of class. In other words, if you inject messages which are normally translated on rough endoplasmic reticula, those don't compete with messages which are normally translated on free polysomes. This suggested to us at that time that there must be proteins which discriminate between those two classes of message. The other evidence for recruitment proteins is almost by default since neither initiation nor any other aspect of the association of message with polysomes, nor subsequent elongation, appear to be limited.

In terms of the repressor proteins, what we were able to identify were a class of proteins which are able to bind message with about a twenty-fold greater affinity than any other nucleic acid. Those proteins are very concentrated in stage 1 and 2 oocytes and progressively disappear through oogenesis. When messenger RNA is recombined with those protein *in vitro* to form a particle, and then injected back into the oocyte, the proteins prevent translation. You can re-extract the message from the particles and show that it is perfectly happy.

Question H. Woodland (Warwick):

All this work you talk about is on *Xenopus*. Do you know what happens in a more reasonable frog when you can understand what is happening with the oocytes?

Answer:

We know what happens with respect to rates of protein synthesis. We have not looked at pH changes. But, even in the case of *Rana pipiens* which was the species we originally worked with, an increase in the rate of synthesis in response to steroid is only about two-fold.

Question (H. Woodland):

What about the message injection experiments?

Answer:

No one has really done these. There is a simple explanation: we can't get good *Rana* any more so we switched to *Xenopus*. It is still hard to get the animals, but it is an interesting question and it could be done.