* PLATES, FIGURES AND TABLES *
Table 2.1 Composition of SDS gel solutions.

<table>
<thead>
<tr>
<th>Gel concentration</th>
<th>Separating Gel</th>
<th>Stacking Gel</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>10%</td>
<td>15%</td>
</tr>
<tr>
<td>30% Acrylamide</td>
<td>3.3mls</td>
<td>5.0mls</td>
</tr>
<tr>
<td>0.2% Bisacrylamide</td>
<td></td>
<td></td>
</tr>
<tr>
<td>5x separating gel buffer</td>
<td>2.0mls</td>
<td>2.0mls</td>
</tr>
<tr>
<td>H2O</td>
<td>4.5mls</td>
<td>1.9mls</td>
</tr>
<tr>
<td>Glycerol</td>
<td>0</td>
<td>1.0mls</td>
</tr>
<tr>
<td>10% SDS</td>
<td>0.1mls</td>
<td>0.1mls</td>
</tr>
<tr>
<td>10% Ammonium persulphate</td>
<td>50μl</td>
<td>50μl</td>
</tr>
<tr>
<td>TEMED</td>
<td>10μl</td>
<td>10μl</td>
</tr>
<tr>
<td>Total volume</td>
<td>10mls</td>
<td>10mls</td>
</tr>
</tbody>
</table>
Table 3.1 Total amount of 4S and 5S RNA in different oocyte stages.

<table>
<thead>
<tr>
<th>Oocyte stage</th>
<th>Total RNA μg/oocyte</th>
<th>4S RNA μg/ % of</th>
<th>5S RNA μg/ % of</th>
<th>4S/5S RNA molar ratio</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>oocyte total</td>
<td>oocyte total</td>
<td></td>
</tr>
<tr>
<td>Full grown</td>
<td>4.2</td>
<td>0.052 1.1</td>
<td>0.075 1.8</td>
<td>0.92</td>
</tr>
<tr>
<td>White 1</td>
<td>0.7</td>
<td>0.0375 5.3</td>
<td>0.06 8.5</td>
<td>0.83</td>
</tr>
<tr>
<td>White 2</td>
<td>0.7</td>
<td>0.096 13.7</td>
<td>0.14 20.0</td>
<td>0.9</td>
</tr>
<tr>
<td>Previtellogenic 1</td>
<td>0.035</td>
<td>0.015 42.0</td>
<td>0.013 38.0</td>
<td>1.5</td>
</tr>
<tr>
<td>Ilogenic 2</td>
<td>0.05</td>
<td>0.023 46.0</td>
<td>0.019 38.0</td>
<td>1.6</td>
</tr>
</tbody>
</table>

1. Results of experiment 1 in which 9,000 previtellogenic, 350 white and 500 full grown oocytes were used.

2. Results of experiment 2 in which 5,000 previtellogenic, 500 white and 500 full grown oocytes were used.

Total RNA was extracted from separated oocyte stages, (as described in chapter 2), the 4S and 5S RNA was first separated from 18 and 28S RNA on sucrose gradients. Total full grown oocyte RNA was loaded on 4x14ml 7 to 30% sucrose gradients in NETS buffer total white and total previtellogenic oocyte RNA were each loaded on one 14ml gradient. Gradients were centrifuged at 40K for 4 hours 30 minutes in the MSE.
6x14ml Titanium swing out rotor. Gradients were pumped through the ISCO UV scanner and peaks collected and precipitated with 2 volumes ethanol. 4S RNA was separated from 5S RNA by running on 12% polyacrylamide gels (as described in chapter 2). One gel was run for the total 4S and 5S RNA from each oocyte stage. E.coli 4S RNA and low molecular weight RNA from ribosomes were run as markers to determine the position of 4S and 5S RNA. Actual amounts of RNA were calculated by comparing areas under UV absorbance peaks with the area under the peak of a known amount of E.coli 4S RNA peak. The figures obtained were divided by the actual number of oocytes used in each experiment. Molar ratios were calculated assuming a molecular weight of $3 \times 10^4$ for 4S RNA and $4 \times 10^4$ Daltons for 5S RNA (Ford, 1971).
### Table 3.2 Percentage of RNA in full grown oocytes accumulated by previtellogenic and white stages.

<table>
<thead>
<tr>
<th>Oocyte stage</th>
<th>% of Full Grown oocyte 4S RNA accumulated</th>
<th>% of Full Grown oocyte 5S RNA accumulated</th>
<th>% of Full Grown oocyte total RNA accumulated</th>
</tr>
</thead>
<tbody>
<tr>
<td>Full grown</td>
<td>1 2</td>
<td>1 2</td>
<td>1 2</td>
</tr>
<tr>
<td></td>
<td>100 100</td>
<td>100 100</td>
<td>100 100</td>
</tr>
<tr>
<td>White</td>
<td>72 80</td>
<td>80 87.5</td>
<td>17 18.4</td>
</tr>
<tr>
<td>Previtellogenic</td>
<td>29 19</td>
<td>17.3 11.8</td>
<td>0.8 1.3</td>
</tr>
</tbody>
</table>

1. Calculations from the data of experiment 1 shown in Table 3.1.

2. Calculations from the data of experiment 2 shown in Table 3.1.
Figure 3.1 Separation of isolated oocyte stages into subcellular fractions by sucrose gradient centrifugation.

Oocytes were homogenised and loaded on 17 to 50% sucrose gradients in TKM buffer after a low speed centrifugation. After centrifugation sucrose gradients were pumped through the ISCO 222 UV analyser and fractions collected as indicated. Total RNA was extracted from these fractions. Experimental details are given in chapter 2.

Absorbance traces shown are from;
(a) $10^5$ previtellogenic oocytes loaded on a 14ml gradient,
(b) $10^3$ white oocytes loaded on a 65ml gradient,
(c) 400 full grown oocytes loaded on a 65ml gradient.
FIG. 3.1

SUCROSE GRADIENT CENTRIFUGATION OF ISOLATED OOCYTE STAGES

(a) PREVITELLOGENIC (-15000)

(b) WHITE (1000)

(c) FULL GROWN (400)

1 Soluble fraction
2 42S
3 Mono plus poly-ribosome fraction
Figure 3.2(i) Sucrose gradient centrifugation of total RNA extracted from subcellular fractions of white oocytes.

Total RNA from each of the 3 subcellular fractions collected in figure 3.1(b) was loaded on 14ml 7 to 30% sucrose gradients in NETS buffer. After centrifugation gradients were pumped through the ISCO 222 UV analyser, and the 4 plus 5S RNA peak, 18S and 28S RNA peaks collected and ethanol precipitated.

Absorbance (254nm) traces shown are;
(a) total soluble fraction RNA from white oocytes,
(b) total 42S fraction RNA from white oocytes,
(c) total mono plus polyribosome fraction RNA from white oocytes.
FIG. 3.2 (I)

SUCROSE GRADIENT CENTRIFUGATION OF TOTAL RNA EXTRACTED FROM VARIOUS SUBCELLULAR FRACTIONS OF WHITE OOCYTES
Figure 3.2(ii) Polyacrylamide gel analysis of 4S and 5S RNA from different subcellular fractions of white oocytes.

The 4S plus 5S RNA peak from each subcellular fraction was collected from the sucrose gradients shown in figure 3.2(i) and run on polyacrylamide gels to separate 4S RNA from 5S RNA. A parallel gel was run with a known amount of *E. coli* 4S RNA (sigma) to enable location of the 4S RNA peak and also to allow quantitation of the chart paper (see chapter 2 for full experimental detail).
FIG. 3.2(II)

POLYACRYLAMIDE GEL ELECTROPHORESIS OF 4S PLUS 5S RNA

FROM WHITE OOCYTES:

(a) SOLUBLE FRACTION
(b) 42S
(c) MONO PLUS POLY-RIBOSOME FRACTION
Results shown are the average of three experiments performed using previtellogenic and full grown oocytes. Only one experiment was performed using white oocytes. Homogenates of 5000 to 8000 previtellogenic, 1000 white and 500 to 800 full grown oocytes were prepared and separated into subcellular fractions by sucrose gradient centrifugation as described in chapter 2. Previtellogenic oocytes were loaded on one 14ml gradient, 250 full grown and 1000 white oocytes were loaded per 65ml gradient. Gradients were analysed using the ISCO 222 UV analyser and soluble, 42S and mono plus poly ribosome fractions collected. Total RNA was extracted from each fraction and a maximum of 500ug per gradient loaded on 14ml 7 to 30% sucrose gradients in NETS buffer. The total 4 plus 5S RNA peak from each fraction was collected and separated into component 4S and 5S RNA peaks on 12% polyacrylamide gels (see chapter 2 for details). Gels were scanned at 265nm and areas under peaks calculated. Molar ratios were calculated assuming molecular weights of 4x10^4 for 5S RNA and 3x10^4 Daltons for 4S RNA and utilising the data derived from measuring areas under peaks of 4S and 5S RNA.
Table 3.3 Percentage distribution of 4S and 5S RNA in different subcellular fractions.

<table>
<thead>
<tr>
<th>Subcellular fraction</th>
<th>% of total 4S RNA</th>
<th>% of total 5S RNA</th>
<th>4S/5S RNA molar ratio</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Previtellogenic</strong></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Soluble</td>
<td>5</td>
<td>50</td>
<td>0.22</td>
</tr>
<tr>
<td>42S</td>
<td>85</td>
<td>42</td>
<td>3.70</td>
</tr>
<tr>
<td>mono plus poly ribosomes</td>
<td>10</td>
<td>8</td>
<td>2.20</td>
</tr>
<tr>
<td>total</td>
<td>100</td>
<td>100</td>
<td>1.80</td>
</tr>
<tr>
<td><strong>White</strong></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>soluble</td>
<td>62</td>
<td>71</td>
<td>0.66</td>
</tr>
<tr>
<td>42S</td>
<td>28</td>
<td>10</td>
<td>2.10</td>
</tr>
<tr>
<td>mono plus poly ribosomes</td>
<td>10</td>
<td>19</td>
<td>0.30</td>
</tr>
<tr>
<td>total</td>
<td>100</td>
<td>100</td>
<td>0.76</td>
</tr>
<tr>
<td><strong>Full Grown</strong></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>soluble</td>
<td>96</td>
<td>22</td>
<td>2.70</td>
</tr>
<tr>
<td>42S</td>
<td>-</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>mono plus poly ribosomes</td>
<td>4</td>
<td>78</td>
<td>0.072</td>
</tr>
<tr>
<td>total</td>
<td>100</td>
<td>100</td>
<td>0.65</td>
</tr>
</tbody>
</table>
Using the results from the experiments described in Table 3.3 and total 4S and 5S RNA ng/oocyte calculated from an average of the two experiments shown in Table 3.1, ng of 4S and 5S RNA in different subcellular fractions was calculated. The mean and standard error are shown. Molar ratios were calculated using data derived as described above and assuming a molecular weight of $3 \times 10^4$ Daltons for 4S RNA and $4 \times 10^4$ Daltons for 5S RNA.
Table 3.4 Amount of 4S and 5S RNA per oocyte in different subcellular fractions at various oocyte stages.

<table>
<thead>
<tr>
<th>Subcellular fraction</th>
<th>4S RNA (ng/oocyte)</th>
<th>5S RNA (ng/oocyte)</th>
<th>4S/5S RNA molar ratio</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Previtellogenic</strong></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>soluble</td>
<td>0.93 ±0.07</td>
<td>8.0 ±1.4</td>
<td>0.155</td>
</tr>
<tr>
<td>42S</td>
<td>16.0 ±1.6</td>
<td>6.7 ±1.5</td>
<td>3.2</td>
</tr>
<tr>
<td>mono plus poly ribosomes</td>
<td>1.9 ±0.2</td>
<td>1.2 ±0.1</td>
<td>2.1</td>
</tr>
<tr>
<td>total</td>
<td>18.8</td>
<td>15.9</td>
<td>1.55</td>
</tr>
<tr>
<td><strong>White</strong></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>soluble</td>
<td>42.0</td>
<td>71.0</td>
<td>0.79</td>
</tr>
<tr>
<td>42S</td>
<td>19.0</td>
<td>10.0</td>
<td>2.5</td>
</tr>
<tr>
<td>mono plus poly ribosomes</td>
<td>6.0</td>
<td>19.0</td>
<td>0.42</td>
</tr>
<tr>
<td>total</td>
<td>67.0</td>
<td>100.0</td>
<td>0.88</td>
</tr>
<tr>
<td><strong>Full Grown</strong></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>soluble</td>
<td>84.0 ±0.9</td>
<td>25.0 ±6</td>
<td>4.5</td>
</tr>
<tr>
<td>42S</td>
<td>~</td>
<td>~</td>
<td>~</td>
</tr>
<tr>
<td>mono plus poly ribosomes</td>
<td>3.5 ±0.9</td>
<td>94.0 ±8</td>
<td>0.05</td>
</tr>
<tr>
<td>total</td>
<td>87.5</td>
<td>121.0</td>
<td>0.96</td>
</tr>
</tbody>
</table>
FIG. 3.5
CHANGES IN SUBCELLULAR DISTRIBUTION
OF 4S AND 5S RNA DURING OOGENESIS.

- Mono plus poly-ribosome fraction
- 42 S fraction
- Soluble fraction

NG OF RNA PER OOCYTE

4S 5S 4S 5S 4S 5S
PREVITELLOGENIC WHITE FULL GROWN
Table 3.5 Comparison of the recovery of total 4S and 5S RNA from total oocyte RNA extraction (a) and oocyte fractionation experiments (b).

<table>
<thead>
<tr>
<th>Oocyte stage</th>
<th>4S RNA (a)</th>
<th>5S RNA (a)</th>
<th>(b) as a % of (a)</th>
<th>4S RNA</th>
<th>5S RNA</th>
</tr>
</thead>
<tbody>
<tr>
<td>Previte-ilogenic</td>
<td>19</td>
<td>16</td>
<td></td>
<td>12</td>
<td>84</td>
</tr>
<tr>
<td>White</td>
<td>67</td>
<td>56</td>
<td></td>
<td>100</td>
<td>87</td>
</tr>
<tr>
<td>Full Grown</td>
<td>88</td>
<td>26</td>
<td></td>
<td>120</td>
<td>60</td>
</tr>
</tbody>
</table>

(a) Results from experiments in which total RNA was extracted from oocyte homogenates and the amount of total 4S and 5S RNA estimated (see Table 3.1).
(b) Results from experiments in which oocytes were separated into different subcellular fractions and the amount of 4S and 5S RNA in each fraction estimated then summed to obtain a value for the total per oocyte.
Figure 4.1 Optimisation of *Xenopus laevis* liver amino acyl tRNA synthetase preparation.

Reactions were performed using the conditions described in chapter 2. Aminoacylation of *Xenopus laevis* liver tRNA (100μg/ml) was measured by following the appearance of labelled amino acid into CTAB precipitable material (see chapter 2 for experimental details). In (a), (b) and (c) a 3H amino acid mixture containing 11 amino acids of equispecific activity 10 Ci/mmole and 4 of 0.1 Ci/mmole was used and in (d) 3H histidine (58 Ci/mmole) and 3H leucine (51 Ci/mmole) were used. Aminoacylation is plotted as a function of:

(a) enzyme concentration at various time points (using Mg++ 8mM, amino acid mixture 100μCi/ml)

(b) 3H amino acid mixture concentration (enzyme 300μl/ml time 30 minutes, Mg++ 8mM);

(c) Mg++ concentration (3H amino acid mixture 100μCi/ml, enzyme 300μl/ml, time 30 minutes);

(d) Mg++ concentration (3H histidine 58 Ci/mmole 100μl/ml added, 3H leucine 51 Ci/mmole 100μl/ml added, enzyme 300μl/ml, time 30 minutes).

A total reaction volume of 20μl was used.
FIG 4.1 OPTIMISATION OF AMINOACYL tRNA SYNTHETASE

(a) 3H CPM x 10^3 PER 2UL

- 40 mins.
- 30 mins.
- 10 mins.

ENZYME CONC. (µL/mL)

(b) 3H AMINO ACID CONC.

- plus 4S RNA
- minus 4S RNA

(µCi/µL)

(c) 3H CPM x 10^3 PER 6UL

- minus 4S RNA

MG** CONC. (mM)

(d) 3H CPM x 10^3 PER 6UL

- 3H Histidine
- 3H Leucine

- minus 4S RNA

MG** CONC. (mM)
The optimum enzyme conditions determined in figure 4.1 were used (enzyme concentration 300μl/ml, Mg²⁺ 10mM, §H amino acid mixture 100μCi/ml). Reactions were performed as described in chapter 2 and at the various time points indicated 2μl samples were withdrawn, CTAB precipitated, filtered and counted (see chapter 2 for full details).
FIG 4.2  TIME COURSE OF AMINOACYLATION OF VARIOUS 4S RNA CONCENTRATIONS

![Graph showing the time course of aminoacylation of various 4S RNA concentrations.](Image)

- **4S RNA (μg/ml)**
  - (1) 0
  - (2) 200
  - (3) 400
  - (4) 600
Table 4.1 Amino acylation of previtellogenic ovary 4S RNA compared with liver 4S RNA.

<table>
<thead>
<tr>
<th>Source of 4S RNA</th>
<th>p moles $^3$H amino acids incorporated per p mole of 11 amino acid accepting tRNA species</th>
<th>cpm x $10^{-3}$ $^3$H amino acid mixture incorporated into aminoacyl 4S RNA per 2μl sample</th>
</tr>
</thead>
<tbody>
<tr>
<td>X. laevis liver</td>
<td>0.58 ± 0.14</td>
<td>8.75 ± 2.2</td>
</tr>
<tr>
<td>X. laevis PV ovary 42S particle</td>
<td>0.67 ± 0.13</td>
<td>9.30 ± 1.7</td>
</tr>
<tr>
<td>X. laevis PV ovary soluble plus polyosomal</td>
<td>0.51 ± 0.10</td>
<td>7.00 ± 1.0</td>
</tr>
</tbody>
</table>

Aminoacylations were performed in triplicate using the optimum enzyme conditions obtained from previous experiments. In one experiment the effect of addition of 2mM CTP was tested. 2μl samples were withdrawn.
from a reaction volume of 10\(\mu\)l after 30 minutes and cpm \(^{3}\)H into CTAB precipitable material measured (see chapter 2).

If 11 amino acids of equispecific activity 10Ci/mmole (or \(10^{-2}\mu\)Ci/pmole) and 4 of 0.1 Ci/mmole are present in the \(^{3}\)H amino acid mixture, then assuming negligible effect from the 4 amino acids of lower radioactive concentration, 2.2x10\(^{6}\) DPM (disintegrations per minute) per \(\mu\)Ci and a measured counting efficiency of 17% for \(^{3}\)H then one would expect to detect:-

\[
2.2 \times 10^6 \times 10^{-2} \times 0.17 = 3.74 \times 10^3 \text{ cpm/pmole of amino acid}
\]

A 2\(\mu\)l sample from a reaction mixture containing 100\(\mu\)g/ml tRNA will contain:

\[
0.2 \times 10^6 / 3 \times 10^4 = 6.6 \text{ pmoles}
\]

of all 20 amino acid accepting families assuming a molecular weight of 3x10\(^{4}\) for tRNA (Ford, 1971). Assuming equal pool sizes of all 20 families of aminoaccepting tRNA then a 2\(\mu\)l sample will contain 6.6x 11/20 = 3.63pmoles of 11 tRNA families. If these 11 tRNA families were fully aminoacylated using the \(^{3}\)H amino acid mixture one would expect to detect:-

\[
3.63 \times 3.74 \times 10^3 = 13600 \text{ cpm per 2\(\mu\)l sample incorporated into CTAB precipitable material.}
\]

The data in this table is calculated from the observed cpm incorporated into aminoacyl tRNA compared with the expected value if all tRNA molecules were aminoacylated.
Figure 4.3 Optimisation of wheatgerm S-30 cell free system.

Optimisations were carried out using the reaction conditions described in chapter 2 with a previtellogenic ovary poly A⁺ RNA (50µg/ml) template. 2µl samples were withdrawn from a reaction volume of 20µl and protein synthesis assayed by measuring incorporation of ³⁵S methionine into TCA precipitable material. A control without added RNA was included in all experiments. Protein synthesis is plotted as a function of:

(a) Wheatgerm extract concentration (Mg⁺⁺ 2.25mM, K⁺ 80mM, spermidine 0.25mM, ³⁵S methionine 100µCi/ml, time 30 minutes).

(b) Magnesium concentration (K⁺ 80mM, wheatgerm extract 200µl/ml, time 30 minutes, no spermidine).

(c) Spermidine concentration (K⁺ 80mM, Mg⁺⁺ 2.8mM minus an equal concentration to the added spermidine concentration, wheatgerm extract 200µl/ml, time 30 minutes, ³⁵S methionine 200µl/ml).

(d) Magnesium concentration (K⁺ 80mM, spermidine 0.35mM, wheatgerm 200µl/ml, time 30 minutes, ³⁵S methionine 200µCi/ml).

(e) Potassium concentration (Mg⁺⁺ 2.75mM, spermidine 0.35mM, wheatgerm 200µl/ml, time 30 minutes, ³⁵S methionine 200µCi/ml).
FIG. 4.3 OPTIMISATION OF WHEATGERM S-30 EXTRACT

(a) 35S C.P.M. x 10^3 PER 2 μL

- poly A+ RNA (50 μg/ml)
- no added RNA

(b) 35S C.P.M. x 10^4 PER 2 μL

(c) 35S C.P.M. x 10^6 PER 2 μL

(d) 35S C.P.M. x 10^7 PER 2 μL

- poly A+ RNA (50 μg/ml)
- no added RNA

WHEATGERM CONC. (μL/ml)

SPERMIDINE CONC. (mM)

Mg++ CONC. (mM)
FIG 4.3

35 S CPM $\times 10^{-4}$ PER 2$\mu$L

K$^+$ CONC. (mM)
Figure 4.4 Translation in the wheatgerm system of poly A⁺ RNA from previtellogenic (PV) ovary and full grown (FG) oocytes plus and minus previtellogenic ovary 4S RNA.

Cell free translations were performed using the optimum wheatgerm conditions determined in previous experiments (K⁺ 80mM, wheatgerm 200µl/ml, Mg⁺⁺ 2.75mM, spermidine 0.35mM). 2µl samples were withdrawn from a total reaction volume of 25µl at the time points indicated and incorporation into ³⁵S methionine TCA precipitable cpm measured. Time courses of protein synthesis are shown with added:

(a) PV ovary poly A⁺ RNA (50µg/ml) plus and minus PV ovary soluble plus polysomal 4S RNA (50µg/ml).

(b) PV ovary poly A⁺ RNA (50µg/ml) plus and minus PV ovary 42S particle 4S RNA (50µg/ml).

(c) FG oocyte poly A⁺ RNA (100µg/ml) plus and minus PV ovary soluble plus polysomal 4S RNA (50µg/ml).

(d) FG oocyte poly A⁺ RNA (100µg/ml) plus and minus PV ovary 42S particle 4S RNA (50µg/ml).

In all experiments a control with no added RNA was also included and ³⁵S methionine was added at a concentration of 200µg/ml.
FIG 4.4 TRANSLATION OF POLY A⁺ RNA PLUS AND MINUS 4S RNA

(a) PREVITELLOGENIC (P.V.) OVARY POLY A⁺ RNA (50 µg/ml) ± SOLUBLE PLUS 4S RNA POLYSOMAL

(b) ± 42S 4S RNA

(c) FULL GROWN OOCYTE POLY A⁺ RNA (100 µg/ml) ± SOLUBLE PLUS 4S RNA POLYSOMAL

(d) ± 42S 4S RNA

polyA⁺RNA  4S RNA (50 µg/ml)
Plate 4.1 Comparison of wheatgerm cell free translation products directed by previtellogenic ovary and full grown oocyte poly A⁺ RNA template, plus and minus previtellogenic ovary 4S RNA.

Cell free translations were performed as described in chapter 2. Time courses of protein synthesis are plotted in Figure 4.4. Samples containing 5x10⁵ TCA precipitable ³⁵S methionine cpm were prepared for electrophoresis and run on one dimensional polyacrylamide SDS gradient gels as described in chapter 2. Molecular weight markers were run in parallel tracks. Gels were stained, dried down and autoradiographed for 5 days as described in chapter 2. Tracks contain products directed by:

(a) Previtellogenic (PV) ovary poly A⁺ RNA.
(b) PV ovary poly A⁺ RNA with added soluble plus polysomal 4S RNA from PV ovary.
(c) PV ovary poly A⁺ RNA with added 42S particle RNA from PV ovary.
(d) Full grown (FG) oocyte poly A⁺ RNA.
(e) FG oocyte poly A⁺ RNA with added soluble plus polysomal 4S RNA from PV ovary.
(f) FG oocyte poly A⁺ RNA with added 42S particle 4S RNA from PV ovary.
Plate 4.2 Comparison of products from wheatgerm cell free translations without added RNA and with added previtellogenic ovary poly A⁺ RNA.

Cell free translations were carried out as described in chapter 2. 7.5x10⁵ and 2.5x10⁵ ³⁵S methionine TCA precipitable cpm respectively of, (a) PV ovary poly A⁺ RNA products and (b) wheatgerm endogenous products were run on one dimensional SDS gradient gels. Gels were stained, dried down and exposed to X-ray film for (a) 4 days and (b) 10 days. Full experimental procedures are described in chapter 2. Autoradiographs shown are from the same gel.
PLATE 4.2

(a) PV ovary poly A⁺ RNA (50 μg/ml)

(b) No added RNA
Figure 4.5 Transfer of $^3$H amino acids from preaminoacylated 4S RNA into protein during cell free translation in the wheatgerm system.

42S particle 4S RNA (500µg/ml) from previtellogenic ovary was aminoacylated, with $^3$H amino acids and cold amino acids to make up the full complement required for protein synthesis, using the X. laevis liver aminoacyl tRNA synthetase preparation. After 30 minutes the aminoacyl tRNA was extracted and precipitated with ethanol. The redissolved aminoacyl tRNA was added to wheatgerm cell free incubations at a concentration of 50µg/ml as the only source of added amino acid. Protein synthesis with added previtellogenic ovary poly A+ RNA (50µg/ml) template and without added poly A+ RNA was followed by measuring incorporation of $^3$H amino acid into TCA precipitable material at the time points indicated. $^3$H amino acyl tRNA at each time point was also measured by performing CTAB precipitations on aliquots removed from the reaction mixture. A plot of cpm lost from the initial level of $^3$H amino acyl tRNA is also included in the figure (see chapter 2 for full experimental details).
FIG. 4.5  TRANSFER OF LABEL FROM AMINOACYL tRNA INTO PROTEIN

![Graph showing the transfer of label from aminoacyl tRNA into protein over time.](image)

- **poly A⁺ RNA**  
- **aminoacyl 4S RNA**  
- cpm. protein  
- $t_n - t_0$ mins. (cpm$_0$ = 650)

-  
-  
-  
-  
-  
-  
-  
- cpm. aminoacyl tRNA  
- $t_0 - t_n$ mins. (cpm$_0$ = 11500)
Figure 4.6 Translation in the wheatgerm system of previtellogenic (PV) ovary and full grown (FG) oocyte poly A⁺ RNA plus and minus preaminoacylated PV ovary 4S RNA.

4S RNA (500µg/ml) was aminoacylated, with ³⁵S methionine and unlabelled amino acids to make up the full complement required for protein synthesis, using the X. laevis liver aminoacyl tRNA synthetase preparation (reaction conditions were the optimum conditions determined for the ³H amino acid mixture). After 30 minutes the amino acyl tRNA was extracted and ethanol precipitated. The redissolved amino acyl tRNA was added to wheatgerm cell free translations at a range of concentrations along with additional ³⁵S methionine and unlabelled amino acids. Time courses are shown of protein synthesis stimulated by:

(a) PV ovary poly A⁺ RNA (50µg/ml) plus and minus aminoacyl PV ovary soluble plus polysomal 4S RNA (50µg/ml).

(b) PV ovary poly A⁺ RNA (50µg/ml) plus and minus aminoacyl PV ovary 42S particle 4S RNA (50µg/ml).

(c) FG oocyte poly A⁺ RNA (100µg/ml) plus and minus aminoacyl PV ovary soluble plus polysomal 4S RNA (50µg/ml).

(d) FG oocyte poly A⁺ RNA (100µg/ml) plus and minus aminoacyl PV ovary 42S particle 4S RNA (50µg/ml).
FIG. 4.6 TRANSLATION OF POLYA\(^+\) RNA PLUS AND MINUS AMINOACYL 4S RNA

PREVITELLOGENIC OVARY POLYA\(^+\) RNA (50 \(\mu\)g/ml)

FULL GROWN OOCYTE POLYA\(^+\) RNA (100 \(\mu\)g/ml)

polyA\(^+\)RNA 4S RNA (50 \(\mu\)g/ml)
Plate 4.3 Comparison of wheatgerm cell free translation products directed by previtellogenic (PV) ovary and full grown (FG) oocyte poly A⁺ RNA template plus and minus preaminoacylated PV ovary 4S RNA.

Cell free translations were performed and SDS gradient gels run as described in chapter 2. Time courses of protein synthesis are shown in Figure 4.6. The Plate shows tracks, where the following RNAs were added to cell free translation incubations:

I (a) PV ovary poly A⁺ RNA 7.5x10⁵ cpm ³⁵S methionine TCA precipitable cpm were loaded.
(b) PV ovary poly A⁺ RNA plus preaminoacylated 42S particle 4S RNA from PV ovary 10⁶ cpm.

II (a) PV ovary poly A⁺ RNA 7.5x10⁵.
(b) PV ovary poly A⁺ RNA plus preaminoacylated PV ovary soluble plus polysomal 4S RNA 10⁶ cpm.

III (a) FG oocyte poly A⁺ RNA.
(b) FG oocyte poly A⁺ RNA plus preaminoacylated 42S particle 4S RNA from PV ovary.

IV (a) FG oocyte poly A⁺ RNA 3.75x10⁵ cpm.
(b) FG oocyte poly A⁺ RNA plus preaminoacylated soluble plus polysomal 4S RNA from PV ovary 5x10⁵ cpm.

Gels shown on Plate 4.3(I) and (II) were exposed for three days and those on Plate 4.3(III) and (IV) for five days. Autoradiographs shown in I, II, III, IV are from different gels.
Two immature ovaries (average oocyte diameter 96μm) and 15 full grown oocytes were incubated at 21°C in 100μl and 30μl respectively of modified Barth X medium containing 600μCi/ml $^{35}$S methionine for 40 hours. Follicle cells were removed and protein samples prepared for IEF gel electrophoresis as described in chapter 2 section 2. $5 \times 10^5$ $^{35}$S methionine TCA precipitable cpm were loaded per gel from:

(a) previtellogenic oocytes (12.5% of total),
(b) full grown oocytes (20% of total).

Electrophoresis and fluorography were performed as described in chapter 2. Dried down gels were exposed to X-ray film for 3 days.

Proteins detected only, or synthesised relatively more intensely in this stage.

Proteins or the position of proteins which are relatively more intensely synthesised in, or which are detectable only in the other oocyte stage.
Figure 5.1 Diagram showing changes in the pattern of acidic and neutral protein synthesis between previtellogenic and full grown oocyte stages.

(a) A tracing is shown from the fluorograph in Plate 5.1(a).
- Proteins detectable in previtellogenic but not full grown oocytes.
- Proteins which are relatively more intensely synthesised in previtellogenic compared with full grown oocytes.

(b) A tracing from the fluorograph in Plate 5.1(b) is shown.
- Proteins detectable in full grown but not previtellogenic oocytes.
- Proteins which are relatively more intensely synthesised in full grown compared with previtellogenic oocytes.

(c) A tracing from the fluorograph in Plate 5.1(b) showing proteins whose synthesis is relatively constant in previtellogenic and full grown oocytes.
PLATE 5.1(a) PREVITELLOGENIC

(b) FULL GROWN
FIG 5.1(c)
Plate 5.2 Two dimensional gel analysis of acidic and neutral protein synthesis in white oocytes compared with a mixture of previtellogenic and full grown oocyte proteins.

500 white oocytes (isolated from mature ovary by collagenase treatment) were incubated at 21°C in 50μl of modified Barth X medium containing 600μCi/ml 35S methionine for 20 hours. Protein samples were prepared for IEF gel electrophoresis, gels were run and fluorography carried out as described in chapter 2. Gels were exposed to X-ray film for 3 days. 5x 10^5 35S methionine TCA precipitable cpm were loaded per gel from:

(a) White oocytes (20% of total)

- Proteins which show major changes of synthesis between previtellogenic and white stages.

- Proteins which show major changes of synthesis between white and full grown stages.

(b) An equal mixture of previtellogenic and full grown oocyte labelled proteins prepared as described in the legend to Plate 5.1.

- Proteins which increase in relative synthesis gradually over the course of oogenesis.

- Proteins which decrease in relative synthesis gradually over the course of oogenesis.
Figure 5.2 Diagram comparing acidic and neutral protein synthesis in white oocytes with that in previtellogenic and full grown oocytes.

(a) A tracing from the fluorograph shown in Plate 5.2(a) comparing protein synthesis in white oocytes with that in previtellogenic oocytes.
- Proteins whose synthesis increases between previtellogenic and white oocyte stages.
< Proteins whose synthesis decreases between previtellogenic and white oocyte stages.
- Proteins whose synthesis is detected in white but not previtellogenic oocytes.
- The position of proteins whose synthesis is detectable in previtellogenic but not white oocytes.

(b) A tracing from the fluorograph shown in Plate 5.2(a) comparing protein synthesis in white oocytes with that in full grown oocytes.
- Proteins whose synthesis increases between white and full grown oocyte stages.
< Proteins whose synthesis decreases between white and full grown oocyte stages.
- Proteins whose synthesis is detected in white but not full grown oocytes.
- The position of proteins synthesised in full grown but not white oocytes.
FIG 5.2(a) WHITE compared with PREVITELLOGENIC

FIG 5.2(b) WHITE compared with FULL GROWN
Isolated white and full grown oocytes and previtellogenic ovaries were incubated at 21°C in modified Barth X medium containing $^{35}$S methionine 400μCi/ml for 20 hours. After defolliculation oocytes (15,000 previtellogenic (average diameter 96 to 144μm), 500 white and 30 full grown) were homogenised and separated into subcellular fractions by sucrose gradient centrifugation on 14ml gradients as described in chapter 2. After centrifugation gradients were pumped through the ISCO 22 UV analyser and 0.5ml fractions collected. A 10μl sample from each fraction was TCA precipitated and counted the remainder of the fractions were pooled as indicated and protein extracted as described in chapter 2.
SUCROSE GRADIENT CENTRIFUGATION
OF ISOLATED OOCYTE STAGES

(a) PREVITELLOGENIC (15000)
(b) WHITE (500)
(c) FULL GROWN (30)

1 Soluble fraction
2 42 S
3 Mono plus poly-ribosome fraction
Plate 5.4 Protein synthesis in previtellogenic oocytes analysed by acid urea triton/SDS polyacrylamide gel electrophoresis.

(a) Total oocyte homogenate.

Two previtellogenic ovaries (average oocyte diameter 96μm) were incubated for 20 hours at 21°C in modified Barth X medium containing 600μCi/ml $^{35}$S methionine. After defolliculaton protein samples were prepared for electrophoresis on the acid urea triton gel system as described in chapter 2. 2x$10^5$ $^{35}$S methionine TCA precipitable cpm was loaded.

(b)(c)(d) Separated subcellular fractions.

$^{35}$S methionine labelled proteins were recovered from subcellular fractions (prepared as described in Figure 5.3 legend) and prepared for gel electrophoresis as described in chapter 2. Equal proportions of the TCA precipitable cpm recovered from each fraction were loaded on gels;

(b) soluble fraction ($10^5$ cpm),
(c) 42S fraction ($2x10^4$ cpm),
(d) mono plus polyribosome fraction ($4x10^4$ cpm).

Gels were run, stained, photographed and fluorographed (exposure to X-ray film was 3 days for (a) and 6 days for (b) (c) (d)) as described in chapter 2.

Figure 5.4 Shows tracings of the fluorographs illustrated in Plate 5.4. Proteins have been classified so that S=soluble, R=ribosomal, 42S=42S particle and mRNP=mRNP particle. (a)-(d) are as labelled in Plate 5.4.
FIG 5.4 (C) PV42S (label)

(d) P.V. ribosome fraction (label)
PLATE 5.4(c) P.V. 42 S (label)

(d) P.V. ribosome fraction (label)
Plate 5.5 Pattern of stained protein in previtellogenic oocytes analysed by acid urea triton/SDS gel electrophoresis.

Photographs of the staining pattern of protein for the gel fluorographs illustrated in Plate 5.4 are shown. Gels were run with protein from:
(a) total oocyte homogenate,
(b) soluble fraction,
(c) 42S fraction,
(d) mono plus polyribosome fraction.

Figure 5.5 Diagram of stained protein in previtellogenic oocytes analysed by acid urea triton/SDS gel electrophoresis.

Tracings of the gels illustrated in Plate 5.5 are shown. Proteins have been classified according to the following scheme; S=soluble, R=ribosomal, 42S=42S RNP particle, mRNP= mRNP particle. Gels were loaded with protein isolated from:
(a) total oocyte homogenate,
(b) soluble fraction,
(c) 42S fraction,
(d) mono plus polyribosome fraction.
PLATE 5.5(a) PV total (stain)

PLATE 5.5(b) PV soluble (stain)
FIG 5.5(c) PV 42S (stain)
PLATE 5.5(c) PV. 42S (stain)

(d) PV. ribosome fraction (stain)
Plate 5.6 Protein synthesis in white oocytes analysed by acid urea triton/SDS gel electrophoresis.

1000 white oocytes were incubated for 20 hours in 100μl of modified Barth X medium containing 600μCi/ml $^{35}$S methionine. 500 were separated into subcellular fractions before protein extraction (see figure 5.3) and protein was extracted from the total oocyte homogenate of the other 500. Sample preparation, gel electrophoresis and fluorography were performed as described in chapter 2. About 20% of the total recovered TCA precipitable cpm were loaded on the following gels:

(a) total oocyte homogenate (2x10^5 cpm),
(b) soluble fraction (4x10^4 cpm),
(c) 42S fraction (3.5x10^4 cpm),
(d) mono plus polyribosome fraction (12x10^4 cpm).

Gels were stained and photographed before fluorography (exposure to X-ray film for 3 days).

Figure 5.6 Diagram of protein synthesis in white oocytes analysed by acid urea triton/SDS gel electrophoresis.

Tracings of the fluorographs illustrated in Plate 5.6 are shown. Proteins have been classified according to the following scheme; S=soluble, R=ribosomal, 42S=42S particle, mRNP=mRNP particle. Gels were loaded with protein isolated from;

(a) total oocyte homogenate,
(b) soluble fraction,
(c) 42S fraction,
(d) mono plus polyribosome fraction.
FIG 5.6(a) white total (label)

FIG 5.6(b) white soluble (label)
PLATE 5.6

(a) white total (label)

(b) white soluble (label)
FIG. 56 (c) white 42S (label)

(d) white ribosome fraction (label)
PLATE 5.6(c) white 42 S (label)

(d) white ribosome fraction (label)
Plate 5.7 Pattern of stained protein in white oocytes analysed by acid urea triton/SDS gel electrophoresis.

Photographs of the staining pattern of proteins for the gel fluorographs illustrated in Plate 5.6 are shown. Gels were loaded with protein isolated from;
(a) total oocyte homogenate,
(b) soluble fraction,
(c) 42S fraction,
(d) mono plus polyribosome fraction.

Figure 5.7 Diagram of stained protein in white oocytes analysed by acid urea triton/SDS gel electrophoresis.

Tracings of the stained gels shown in Plate 5.7 are shown. Proteins have been classified according to the following scheme; S=soluble, R=ribosomal, 42S=42S RNP particle, mRNP=mRNP particle. Gels were loaded with protein isolated from;
(a) total oocyte homogenate,
(c) 42S fraction,
(d) mono plus polyribosome fraction.
FIG. 5.7(c) White 42S stain

(d) White ribosome fraction (stain)
PLATE 5.7(c) white 42S (stain)

(d) white ribosome fraction (stain)
Plate 5.8 Protein synthesis in full grown oocytes analysed by acid urea triton/SDS gel electrophoresis.

Full grown oocytes were incubated at 21°C for 20 hours in modified Barth X medium containing $^{35}$S methionine 400μCi/ml. Protein was extracted from the total homogenate of 15 oocytes and separated subcellular fractions of 50 oocytes prepared by sucrose gradient centrifugation. Sample preparation and gel electrophoresis were carried out as described in chapter 2. Equal proportions of the TCA precipitable cpm recovered from various subcellular fractions were loaded on gels which were stained and photographed before fluorography was performed. Gels were exposed to X-ray film for 3 days. Protein loaded was isolated from:

(a) total oocyte homogenate (2x10⁵ cpm loaded),
(b) soluble fraction (10⁵ cpm loaded),
(c) 42S fraction (5x10⁴ cpm loaded),
(d) mono plus polyribosome fraction (1.4x10⁵ cpm loaded).

Figure 5.8 Diagram of protein synthesis in full grown oocytes analysed by acid urea triton/SDS gel electrophoresis.

Tracings of the fluorographs illustrated in Plate 5.8 are shown. Proteins have been classified according to the following scheme; S=soluble, R=ribosomal, mRNP=mRNP particle. (a)-(d) are as labelled in Plate 5.8.
PLATE 5.8(a) FG total (label)

(b) FG soluble (label)
FIG. 5.8 (c) FG 42S (label)

(d) FG ribosome fraction (label)
PLATE 5.8(c) FG 42 S (label)

(d) FG ribosome fraction (label)
Plate 5.9 Pattern of stained protein in full grown oocytes analysed by acid urea triton/SDS gel electrophoresis.

Photographs of the staining pattern of proteins for the following fractions are shown, (no proteins could be detected by stain in the 42S fraction);
(a) total oocyte homogenate,
(b) soluble fraction,
(d) mono plus polyribosome fraction.

Figure 5.9 Diagram of stained protein in full grown oocytes analysed by acid urea triton/SDS gel electrophoresis.

Tracings of the gels illustrated in Plate 5.9 are shown. Proteins have been classified according to the following scheme; S=soluble, R=ribosomal, mRNP=mRNP particle. No stained protein was detected in the 42S fraction. Tracings are of;
(a) total oocyte homogenate,
(b) soluble fraction,
(d) mono plus polyribosomal.
PLATE 5.9(a) FG total (stain)

(b) FG soluble (stain)
PLATE 5.9(d) FG ribosome fraction (stain)

FIG. 5.9(d) FG ribosome fraction (stain)
Plate 5.10 Acid urea triton/SDS gel analysis of $^{35}$S methionine labelled protein from:
(a) full grown oocyte total homogenate,
(b) full grown oocyte total homogenate plus mono plus polyribosome fraction of previtellogenic oocytes,
(c) previtellogenic oocyte mono plus polyribosome fraction.
Sample preparation and gel electrophoresis were performed as described in chapter 2. Gels were stained and photographed before fluorography was carried out. (Gels were exposed to X-ray film for 3 days). An enlargement of the area of the gel on which the 42S and mRNP proteins run is shown.

Figure 5.10

A tracing of Plate 5.10 is shown from,
- full grown oocyte proteins,
- a mixture of full grown and previtellogenic oocyte proteins,
- previtellogenic oocytes mono plus poly ribosome fraction.
PLATE 5.10 (a) FG (label)

(b) FG + PV 42 S (label)
PLATE 510(c) PV 42S (label)

FIG. 5.10
Plate 5.11 Acid urea triton/SDS gel electrophoresis of $^{35}$S methionine labelled protein from full grown and previtellogenic oocyte total cell homogenate mixed at the initial homogenisation step.

Full grown oocytes were incubated for 20 hours in modified Barth X medium containing $^{35}$S methionine 600µCi/ml. 15 oocytes were homogenised then previtellogenic oocyte total homogenate containing $1.5 \times 10^5$ TCA precipitable cpm added (equivalent to about 100 oocytes and comprising about 7.5% of the total full grown oocyte cpm) and the extraction continued as usual. $2 \times 10^5$ cpm was loaded on the gel. Electrophoresis and fluorography were performed as described in chapter 2. (The gel was exposed to X-ray film for 3 days).
Plate 6.1 Comparison of acidic and neutral protein synthesis in previtellogenic oocytes of *Xenopus laevis* and *Xenopus borealis*.

Previtellogenic ovaries (average oocyte diameter 96um) were incubated at 21°C for 20 hours in modified Barth X medium containing $^{35}$S methionine 600μCi/ml. Oocytes were isolated and freed from follicle cells then protein samples prepared for IEF gel electrophoresis (see chapter 2). $5 \times 10^5$ TCA precipitable cpm were loaded per gel of:

(a) *X. borealis* previtellogenic oocyte proteins,
   ⇆ some *X. borealis* specific proteins.

(b) *X. laevis* previtellogenic oocyte proteins,
   ⇆ some *X. laevis* specific proteins.

(c) a mixture of *X. laevis* and *X. borealis* previtellogenic oocyte proteins,
   ⇆ *X. laevis* specific proteins marked in (a)
   ⇆ *X. borealis* specific proteins marked in (b).

Gel electrophoresis, staining and fluorography (gels were exposed to X-ray film for 3 days) were performed as described in chapter 2.
PLATE 6.1(b) X. laevis

(c) X. borealis + X. laevis
Figure 6.1 Diagram showing conserved and species specific acidic and neutral proteins synthesised by *X.laevis* and *X.borealis*.

Tracings from Plate 6.1(c) are shown indicating;

(a) proteins which are conserved in electrophoretic mobility and which have similar relative rates of synthesis in the two species,

(b) proteins which are species specific for,

- *X.laevis*
- *X.borealis*

or which are synthesised at a relatively greater rate in one species compared with the other,

- proteins synthesised at a relatively greater rate in *X.laevis*
- proteins synthesised at a relatively greater rate in *X.borealis*.
Figure 6.2 Subcellular fractionation of previtellogenic oocytes from *X. borealis* by sucrose gradient centrifugation.

Previtellogenic ovaries were incubated at 21°C for 20 hours in modified Barth X medium containing 400μCi/ml $^{35}$S methionine. Oocytes freed from follicle cells were homogenised and loaded (10000 size 72 - 168μm diameter per gradient) on 14ml 17 to 50% sucrose gradients in TKM buffer after a low speed centrifugation as described in chapter 2. Gradients were pumped through the ISCO 222 UV analyser, 0.5ml fractions were collected, a 10μl aliquot from each fraction was TCA precipitated and counted, the remainder of the fractions were pooled as indicated and protein was extracted from the pooled fractions. Experimental procedures are described in detail in chapter 2.
FIG. 6.2  SUCROSE GRADIENT CENTRIFUGATION OF *X. BOREALIS*

PREVITELLOGENIC OOCYTES

1 SOLUBLE FRACTION
2 42S
3 MONO PLUS POLY-RIBOSOME FRACTION
Plate 6.3 Protein synthesis in *X. borealis* previtellogenic oocytes analysed by acid urea triton/SDS gel electrophoresis.

(a) Total oocyte homogenate. Previtellogenic ovaries were incubated at 21°C for 20 hours in modified Barth X medium containing 600μCi/ml $^{35}$S methionine. Protein was extracted from the total homogenate and samples prepared for electrophoresis as described in chapter 2. $2 \times 10^5$ $^{35}$S methionine TCA precipitable cpm were loaded.

(b), (c), (d) Subcellular fractions. An equal proportion of the $^{35}$S methionine TCA precipitable cpm recovered from subcellular fractions as indicated in Figure 6.2 were loaded on gels from;

(b) soluble fraction (78,000 cpm),
(c) 42S fraction (46,000 cpm),
(d) mono plus polyribosome fraction (71,000 cpm).

Gel electrophoresis, staining and fluorography were carried out as described in chapter 2. Gels were exposed to X-ray film for 3 days.

Figure 6.3 Diagram showing protein synthesis in previtellogenic oocytes of *X. borealis* analysed by acid urea triton/SDS gel electrophoresis

Tracings of the fluorographs illustrated in Plate 6.3 are shown on which proteins have been classified according to the following scheme; S= soluble, R= ribosomal, 42S= 42S RNP particle, mRNP= mRNP particle. (a)-(d) are as labelled in Plate 6.3.
(a) X.Borealis total (label)

(b) X.Borealis soluble (label)
PLATE 6.3(a) *X. Borealis* total (label)

PLATE 6.3(b) *X. Borealis* soluble (label)
FIG. 6.3 (c) X.Borealis 42S (label)

(d) X.Borealis ribosome fraction (label)
PLATE 6.3(c) X. Borealis 42S (label)

(d) X Borealis ribosome fraction (label)
Plate 6.4 Pattern of stained protein in previtellogenic oocytes of X.borealis.

A photograph of the stained pattern of proteins from Plate 6.3(a) is shown.

Figure 6.4 Diagram showing the pattern of stained protein in previtellogenic oocytes of X.borealis.

A tracing of Plate 6.4 is shown on which proteins have been classified according to the following scheme; S=soluble, R=ribosomal, 42S=42S RNP particle, mRNP=mRNP particle.
PLATE 6.4 X. Borealis total (stain)

FIG 6.4 X. Borealis total (stain)
Plate 6.5 Comparison of protein synthesis, analysed by acid urea triton/SDS gel electrophoresis in previtellogenic oocytes of *X. laevis* and *X. borealis*.

$^{35}$S methionine labelled protein from total homogenate of isolated defollicled oocytes was prepared as described in the legends to Plates 6.3(a) and 5.4. $2 \times 10^5$ $^{35}$S methionine TCA precipitable cpm were loaded per gel of proteins from:

- (a) *X. borealis* total oocyte homogenate,
- (b) *X. laevis* total oocyte homogenate,
- (c) an equal mixture of *X. laevis* and *X. borealis* total homogenate.

Gel electrophoresis, staining and fluorography (gels were exposed to X-ray film for 3 days) were performed as described in chapter 2. An enlargement of the area of gel on which the 42S and mRNP proteins run is shown.

Figure 6.5 Diagram comparing protein synthesis in previtellogenic oocytes of *X. laevis* and *X. borealis* analysed by acid urea triton/SDS gel electrophoresis.

Tracings of the gel fluorographs illustrated in Plate 6.5 are shown on which proteins have been classified according to the following scheme; 42S=42S RNP particle, mRNP=mRNP particle. Gels were loaded with;

- (a) *X. borealis* total proteins,
- (b) *X. laevis* total proteins,
- (c) a mixture of *X. laevis* and *X. borealis* total proteins.
PLATE 6.5(a) x Borealis

(b) x Laevis
PLATE 6.5(c) X. Borealis + X. Laevis

FIG 6.5(c) X. Borealis + X. Laevis
Plate 6.6 Comparison of the pattern of stained protein analysed by acid urea triton/SDS gel electrophoresis in previtellogenic oocytes of X.laevis and X.borealis.

Photographs of the pattern of stained protein in the gel fluorographs illustrated in Plate 6.5 are shown. Proteins loaded were from:
(a) X.laevis total oocyte homogenate,
(b) an equal mixture of X.laevis and X.borealis total oocyte homogenate.

Figure 6.6 Diagram comparing the pattern of stained protein analysed by acid urea triton/SDS gel electrophoresis in previtellogenic oocytes of X.laevis and X.borealis.

Tracings of the gels shown in Plate 6.6 are shown. Proteins have been classified according to the following scheme; S=soluble, R=ribosomal, 42S=42S RNP particle, mRNP=mRNP particle. Samples were prepared from;
(a) X.laevis total oocyte homogenate,
(b) a mixture of X.laevis and X.borealis proteins.
FIG. 6.6a X. Laevis (stain)

(b) X. Laevis + X. Borealis (stain)
PLATE 6.6 (a) *X. Laevis* total (stain)

*X. Laevis* + *X. Borealis* total (stain)