is adenylydroxymethyldiethylene glycol. The yield of tritiated fragments obtained in this way is sufficient to account for all the 3'-hydroxyl termini of the mRNA.

Pancreatic ribonuclease and combined ribonuclease T₁-pancreatic ribonuclease digests of haemoglobin mRNA show a characteristic pattern of oligo(A) fragments ranging from A₅ to A₁₀. This is not found in ribonuclease T₁ digests and they result from partial degradation of longer poly(A) sequences by pancreatic ribonuclease. It is also not obtained from rRNA, nor from the 9S contaminants found in some preparations of haemoglobin mRNA obtained from sodium dodecyl sulphate-treated polyribosomes (Lanyon et al., 1972). This result further confirms that these contaminants are not mRNA. The quantity of oligo(A) fragments obtained from haemoglobin mRNA is enough to provide an average of 60 residues/mRNA molecule. Since haemoglobin mRNA contains a total of about 140 adenylate residues (Williamson et al., 1971) it may be expected that the coding region of the molecule will be deficient in these residues. This prediction is borne out by examination of the ‘fingerprints’ of ribonuclease T₁ and pancreatic ribonuclease digests of the molecule.

It is concluded that mouse haemoglobin mRNA molecules have at their 3'-hydroxyl ends a sequence consisting exclusively of adenylate residues. The length of this region falls into two size classes of about 75 and 50 residues.


Changes in the Protein-Synthetic Activity of Male *Xenopus laevis* Liver after Hormonal Induction of the Formation of Egg-Yolk Protein

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After a single injection of oestradiol-17β into adult male or female *Xenopus laevis* the egg-yolk-protein precursor vitellogenin is synthesized and secreted by the liver in large amounts (Wallace & Dumont, 1968; Follett et al., 1968; Dolphin et al., 1971). This has been demonstrated *in vivo* and in organ cultures of liver explants from hormone-treated animals (Wallace & Jared, 1969; Clemens et al., 1972). By using the latter system we have been able to show that secretory-protein synthesis accounts for an increasing proportion of the total amino acid incorporation, reaching a maximum at 12 days after a 1 mg dose of oestradiol given to male frogs (Table 1).

In parallel with the observed changes in the protein-synthetic pattern of the cultured liver from hormone-treated *Xenopus* the activity of isolated polyribosomes in cell-free amino acid-incorporation systems also rises when endogenous protein synthesis is assayed under standard conditions (Table 2). The amino acid composition of the radioactive product synthesized *in vitro* indicates that this material is particularly rich in serine relative to phenylalanine. Authentic vitellogenin has a serine/phenylalanine molar ratio of approx. 3.3:1 (Redshaw & Follett, 1971).

The increased protein-synthetic capacity of *Xenopus* ribosomes translating endogenous mRNA *in vitro* is observed even in the presence of inhibitors of peptide-chain initiation, suggesting that more ribosomes are already attached to translatable mRNA *in vivo* (and hence in isolated polyribosomes).

A number of reports indicate that the process of initiation is rate-limiting for protein synthesis (Fan & Penman, 1970; Vaughan et al., 1971; Singer & Penman, 1972), and if this is the case in *Xenopus* liver it is likely that the increased requirement for protein synthesis after hormonal induction of the formation of egg-yolk proteins is met by an increased rate or extent of initiation. This prediction is borne out when the ability
Table 1. Labelling of secreted and non-secreted protein by Xenopus liver in culture
Explants were cultured as described previously (Clemens et al., 1972). [14C]Serine was added to the culture medium at 0.5 μCi/ml and time-courses of its incorporation into secretory and non-secretory proteins were determined over a 24h labelling period. Incorporation is expressed as d.p.m./h per mg of tissue for the period between 6 and 24 h after addition of the labelled amino acid. The percentage of the total incorporation that is in secreted protein has been corrected for the high serine content of vitellogenin relative to that of tissue protein.

<table>
<thead>
<tr>
<th>Time after oestradiol (days)</th>
<th>Rate of protein labelling (d.p.m./h per mg of tissue)</th>
<th>Secreted protein (% of total)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Tissue protein</td>
<td>Secreted protein</td>
</tr>
<tr>
<td>0</td>
<td>27</td>
<td>25</td>
</tr>
<tr>
<td>3</td>
<td>28</td>
<td>286</td>
</tr>
<tr>
<td>6</td>
<td>39</td>
<td>430</td>
</tr>
<tr>
<td>12</td>
<td>20</td>
<td>788</td>
</tr>
<tr>
<td>18</td>
<td>61</td>
<td>238</td>
</tr>
</tbody>
</table>

Table 2. Endogenous protein synthesis by Xenopus liver ribosomes in vitro
Ribosomes were isolated as described previously (Clemens & Tata, 1972) and incubated in a cell-free system under conditions optimal for amino acid incorporation into protein. Rat liver cell sap was used as the source of soluble factors and either [14C]phenylalanine or [14C]serine was the radioactive precursor. Incorporation is expressed as the final number of pmol of labelled precursor in hot-acid-insoluble material at the end of a 40 min incubation period.

<table>
<thead>
<tr>
<th>Time after oestradiol (days)</th>
<th>Amino acid incorporation (pmol/μg of rRNA)</th>
<th>Serine/phenylalanine molar ratio</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Serine</td>
<td>Phenylalanine</td>
</tr>
<tr>
<td>0</td>
<td>0.06</td>
<td>0.06</td>
</tr>
<tr>
<td>1</td>
<td>0.20</td>
<td>0.15</td>
</tr>
<tr>
<td>3</td>
<td>0.29</td>
<td>0.18</td>
</tr>
<tr>
<td>7</td>
<td>0.23</td>
<td>0.15</td>
</tr>
</tbody>
</table>

of preincubated Xenopus liver ribosomes to translate poly(U) at low Mg2+ concentration (5 mM) is assayed. It has been shown that under these conditions polyphenylalanine synthesis is dependent on two initiation factors (Shafritz et al., 1970; Grummt & Bielka, 1971). Ribosomes isolated from male Xenopus 5–6 days after oestrogen treatment and then preincubated to run them off endogenous mRNA are approximately twice as active as controls in the translation of poly(U) (Clemens & Tata, 1973). Furthermore this apparently intrinsic increase in ribosome activity is completely stable to a 0.5 M-KCl washing procedure after preincubation and is not influenced by exposure of the ribosomes to puromycin during run-off followed by salt-washing. Two explanations are possible to account for these results. First, there may be tight binding of initiation factors to the ribosomes during the period of hormone-induced protein synthesis. If this is the case the interaction between these factors and the ribosomes must be stronger than in many eukaryotic cells. Alternatively the ribosomes may be altered structurally in such a way that they are able to utilize initiation factors from the cell sap (in these experiments rat liver supernatant was employed) more rapidly or more efficiently. Mechanisms of this kind may operate in a number of developmental situations, where a pool of inactive ribosomes is mobilized to cope with a large increase in the demand for
new protein synthesis. Obvious examples of such cases are fertilization of sea-urchin or \textit{Xenopus} eggs (Brown & Littna, 1964) and effects of anabolic hormones such as growth hormone (Korner, 1965) or insulin (Wool, 1972) on their target tissues.

In addition to the increased activity of the ribosomes, which we have tentatively attributed to an enhanced ability to initiate, we also find a greater capacity of \textit{Xenopus} liver cell sap to support protein synthesis \textit{in vitro} after oestrogen treatment. We have evidence suggesting that this effect results from increased activity or concentrations of one or both elongation factors. This may be a cellular adaptation mechanism to ensure that the rate of peptide-chain extension on polyribosomes does not become the overall rate-limiting step for protein synthesis (in view of the increased rate of initiation referred to above). Additional findings (M. J. Clemens, unpublished work) show that aminoacyl-tRNA synthetase activities also rise after hormone treatment, and a similar argument can be applied in this case.

Finally it should be mentioned that \textit{Xenopus} liver adapts to the need for greatly increased secretory-protein production by synthesizing new ribosomes, most of which are membrane-bound. Greater ribosome activity together with a greater ribosome content per cell thus constitute two major ways in which the oestrogen-induced synthesis of the egg-yolk proteins is fully expressed.

M. J. C. is a Beit Memorial Research Fellow.


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**Corticosteroid Receptors and the Control of Enzyme Synthesis in Rat Liver**

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The major corticosteroid-receptor protein present in rat liver has been shown (Snart \textit{et al.}, 1970, 1972) to be a basic protein (mol.wt. 100000); it has no subunit characteristics and a $K_{\text{ass}}$, value for corticosterone binding of $5 \times 10^{8} \text{M}^{-1}$ (at 4°C). This receptor protein may readily be distinguished from transcortin by the use of DEAE-cellulose chromatography and in terms of its specificity for corticosterone binding. Saturation of these receptor sites has been associated (Dalton & Snart, 1970) with stimulated tyrosine aminotransferase activity in liver. The second set of receptor sites ($K_{\text{ass}}$, $10^{10} \text{M}^{-1}$) has not been identified in chromatographic separations of liver cytosol. However, an acidic protein fraction may be obtained from chromatin preparations that specifically binds corticosterone (Dastugue \textit{et al.}, 1971) and could correspond to our more limited high-affinity binding sites. Saturation of these receptors has been associated with an increased...