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in *Xenopus* Oocytes

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Venom glands of young queen bees (*Apis mellifera*) synthesize the toxic peptide melittin as their main product. Melittin is formed by proteolytic cleavage of a precursor, promelittin. Unfractionated RNA prepared from venom glands was injected into *Xenopus* oocytes and was shown to direct the synthesis of a promelittin-like substance. About half of the peptide chain made in oocytes has been sequenced; the 17 amino acid residues identified correspond exactly with sequences found in promelittin from venom gland cells. These results yield final proof that injected messenger RNAs can be read with great fidelity. The translation of a messenger from an insect gland shows that at least some of the translational systems within the oocyte are neither cell-type nor phylum-specific. It seems likely that the oocyte can be used to assay any kind of eukaryotic mRNA.

The conversion of promelittin to melittin could not be detected in oocytes. Moreover, the promelittin synthesized in oocytes differs at the carboxyl end from the product made in gland cells, for the latter terminates with glutamine amide while the oocyte material probably ends with an amino acid with a free α-carboxyl group. Some of the post-translational modifications characteristic of gland cells thus do not seem to take place in oocytes.

1. Introduction

The venom gland of the honey bee, *Apis mellifera*, is a highly specialized organ, and gland cells synthesize the toxic peptide melittin as their main product. The sequence of the 26 amino acids present in melittin has been determined (Habermann & Jentsch, 1967). Melittin is formed by removal of a defined sequence from the N-terminus of a precursor peptide, promelittin (Kreil & Bachmayer, 1971). As isolated from venom glands, promelittin is heterogeneous at the amino end; the main component comprises 34 amino acids (Kreil, 1973). *In vivo* the synthesis of the precursor peptide is inhibited by cycloheximide (Kreil & Bachmayer, 1971), suggesting that promelittin is translated from a messenger RNA *via* the normal ribosomal pathway. The glands of newly emerged queen bees should provide a rich source of promelittin messenger RNA, for at this stage venom production is already proceeding at full capacity (Bachmayer *et al.*, 1972).

The microinjection of mRNA into *Xenopus* oocytes enables one to study the nature and specificity of the translational and post-translational systems of the living cell (Lane *et al.*, 1971,1973; Berns *et al.*, 1972; Marbaix & Lane, 1972; Stevens & Williamson, 1972,1973; Laskey *et al.*, 1972; Lane & Knowland, 1974; Smith *et al.*, 1973). The oocyte system can also be used as a sensitive messenger microassay (Gurdon *et al.*, 1971; Moar *et al.*, 1971).

As is shown in this paper, oocytes injected with unfractionated venom gland RNA synthesize a promelittin-like substance. Experiments with several different radioactive amino acids have permitted the identification of half the amino acid residues, and the partial sequence of the oocyte-derived material can be fitted exactly to the known sequence of venom gland promelittin. In contrast, some of the post-translational modifications which take place in venom gland cells are not detectable in oocytes.

2. Materials and Methods

(a) *Materials*

The purification of melittin and the preparation of defined fragments has been described (Kreil, 1973). Tritiated amino acids of high specific activity were obtained from the Radiochemical Centre (Amersham).

(b) *Isolation of glands*

Queen bees, *Apis mellifera carnica*, were supplied by a breeding station (Singer, Purgstall, Austria) 1 or 2 days before emergence. They were incubated at 34°C in a moist atmosphere. Within 15 h of emergence venom glands were dissected from the abdomen (Snodgrass, 1956) and were stored at −70°C. Only the tubular gland was stored, the venom sac, stinger, etc. being carefully removed.

(c) *Extraction of RNA from whole cells*

Batches of 20 to 30 glands were homogenized at 0°C in 1·5 ml of buffer A (0·1 M-sodium acetate, 0·002 M-EDTA, 2% sodium dodecyl sulphate, pH 5·2) and 1 ml of phenol, which had been treated with 8-hydroxyquinoline. The homogenate was stirred for 10 min and centrifuged for a similar time period at 6000 revs/min. To the aqueous phase, sodium dodecyl sulphate and NaCl were added to yield concentrations of 1% and 4%, respectively. This solution was again extracted with 1 ml of phenol. After the addition of 0·1 vol. of 1 M-NaCl and 2·5 vol. of ethanol, the solution was left to stand overnight at −20°C. The RNA precipitate was collected by centrifugation, redissolved in 0·1 ml of buffer A (without dodecyl sulphate) and the ethanol precipitation step was repeated.

For some preparations, glands were homogenized in a buffer of higher pH (buffer B: same as buffer A plus 0·01 M-Tris·HCl, pH 7·4) and extracted twice with phenol/chloroform (1:1, v/v) (modified from Perry *et al.*, 1972). The RNA was precipitated as described above. Both RNA preparations were about equally effective in the oocyte system. Using these procedures up to 10 µg of total RNA can be obtained per gland.

RNA prepared by either of these methods still contains traces of dodecyl sulphate. Microinjection of detergent-like substances may cause inhibition of protein synthesis. To remove residual dodecyl sulphate the RNA was suspended in 0·5 ml of buffer B (containing no dodecyl sulphate) and was extracted twice with 2 vol. of n-butanol. The dodecyl sulphate goes into the organic layer leaving the RNA in the aqueous phase. After extracting the residual butanol with ether, the RNA was again precipitated with ethanol.

(d) *Oocyte microinjection*

Clumps of oocytes were injected by a modified nuclear transplantation procedure (Elsdale *et al.*, 1960; Lane *et al.*, 1971; Gurdon *et al.*, 1971). The micropipettes and all other glassware used were heated overnight at 150°C. The saline solution (88 mM-NaCl,

Fig. 1. Amino acid sequence of promelittin from venom gland cells (Kreil, 1973). The fragments obtained after digestion with pepsin are indicated by arrows marked (P) drawn above the sequence. Additional cleavages with trypsin (T), chymotrypsin (C), thermolysin (Th) and dilute HCl (HCl) are marked by arrows drawn below the structure. Melittin starts at the glycine residue written entirely in capitals. The code used for the peptic fragments of melittin is written above the sequence.

1 mM-KCl, 2·4 mM-NaHCO$_3$, 0·3 mM-Ca(NO$_3$)$_2$, 0·41 mM-CaCl$_2$, 0·82 mM-MgSO$_4$, 15 mM-Tris·HCl, pH 7·6, containing 0·01 g each of sodium benzylpenicillin and streptomycin sulphate per litre) had also been prepared with ribonuclease-free reagents and glassware. Individual oocytes each received 30 to 70 nl of a solution of venom gland RNA dissolved in injection medium (88 mM-NaCl, 15 mM-Tris, 1 mM-KCl, pH 7·6). Batches of approximately 20 oocytes were then incubated at 22°C, for either 8 or 22 h, in about 500 μl of saline solution containing 0·5 to 1·5 mCi of the appropriate radioactive amino acid.

(e) Characterization of the products of translation

After incubation, oocytes were stored on solid CO$_2$. The fractionation procedures used for oocytes were essentially the same as those employed for the isolation of promelittin from venom glands (Kreil & Bachmayer, 1971). Promelittin was eluted from the chromatogram using the following solvent: 40 ml n-propanol, 60 ml water, 2 μl acetic acid, adjusted to pH 6·7 by the addition of pyridine. After proteolytic digestion, peptides were analysed using the techniques described previously (Kreil & Bachmayer, 1971; Kreil, 1973). Additional details are given in the legends to the Figures.

3. Results

Batches of oocytes were injected with total RNA from venom glands and were then cultured in a saline solution containing a single species of radioactive amino acid. Control oocytes were treated similarly, except that the venom gland RNA was omitted from the injection mixture. For most experiments an incubation time of 20 to 22 hours was chosen, but shorter periods (about 8 h) also gave positive results. Experiments with three different labelled amino acids have shown that sequences characteristic of promelittin are detectable in oocytes injected with venom gland RNA but not in control oocytes. The complete amino acid sequence of honey bee promelittin is shown in Figure 1.

(a) Oocytes injected with gland RNA and labelled with [^{3}H]leucine

RNA-injected oocytes were homogenized in 0·1 N-ammonia and were then extracted with n-butanol. The aqueous phase was fractionated by descending paper chromatography on Whatman 3MM paper (Kreil & Bachmayer, 1971). Control oocytes were injected with saline solution containing no gland RNA. Figure 2 shows that the presence of gland RNA in the injection medium leads to the formation of a radioactive species that migrates between the origin and free lysine. The R_F-value of this compound was found to vary slightly; nonetheless, this radioactive species was observed as a separate component in all of the 14 experiments analysed in this way.

The promelittin-like substance derived from RNA-injected oocytes was eluted from the paper chromatogram, as was the equivalent region from the control (fractions 3 to 5 of Fig. 2). Each of the two samples was then mixed with unlabelled melittin and digested with pepsin. The resulting hydrolysate was subjected to paper electrophoresis at pH 4·7 (see Fig. 3).

Both melittin and promelittin contain four leucine residues, all of which are internal (see Fig. 1). After peptic hydrolysis and fractionation by paper electrophoresis, two leucine residues are recovered in a slightly basic heptapeptide (Mel$_{7-13}$), whilst the other two residues are present in the neutral tripeptides Ala-Val-Leu and Pro-Ala-Leu.
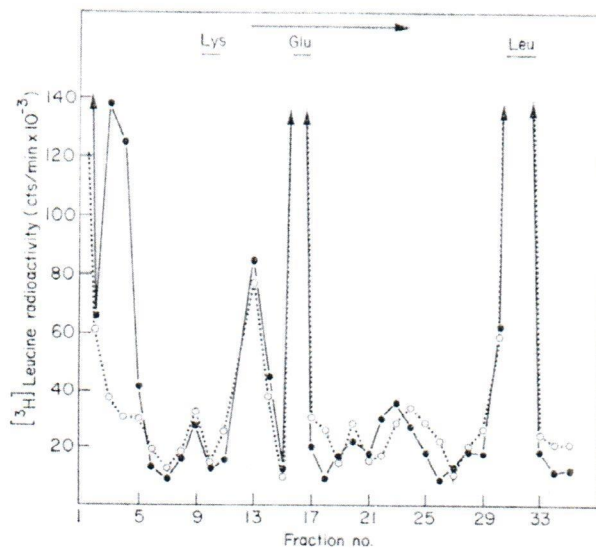
FIG. 2. Paper chromatography of the extract of oocytes injected with venom gland RNA and labelled with [³H]leucine. Batches of about 20 oocytes were suspended in 1 ml of 0·1 N-ammonia and extracted 3 times with an equal volume of n-butanol. The aqueous layer was fractionated by paper chromatography (Whatman 3MM paper, n-butanol/acetic acid/water, 4:1:2, by vol.). The chromatogram was then cut into strips and the distribution of radioactive material was determined by liquid scintillation counting. The profiles obtained are shown in this Figure. The closed circles (—●—●—) denote material from oocytes injected with venom gland RNA whilst material from control cells is represented by open circles (. . .○. . .○. . .). The bars represent the position of amino acids used as references.
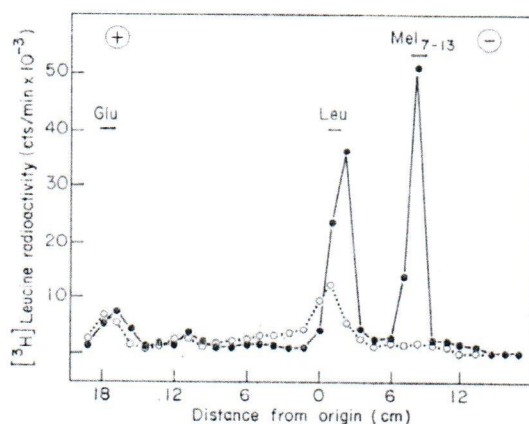

FIG. 3. Paper electrophoresis of the digestion products obtained by treating promelittin from leucine-labelled oocytes with pepsin. Fractions 3 to 5 of Fig. 1 were eluted and digested at pH 1·8 for 2 h with 0·1 mg pepsin in the presence of 1 mg of unlabelled melittin. The resulting fragments were separated by high voltage paper electrophoresis at pH 4·7 (1% pyridine/acetic acid buffer). The closed circles (—●—●—) represent material from oocytes injected with gland RNA, whilst the open circles (. . .○. . .○. . .) correspond to material from control oocytes. The bars (—) represent the electrophoretic mobility of various markers, namely glutamic acid, leucine and a basic heptapeptide (Mel₇₋₁₃) obtained by peptic digestion of insect melittin.

Figure 3 shows that digestion of the promelittin-like material from oocytes yields the expected pattern. About half of the total radioactivity was found to co-migrate with the reference heptapeptide Lys-Val-Leu-Thr-Thr-Gly-Leu. The rest of the radioactive material was found in the neutral region. The latter was subjected to paper electrophoresis at pH 1·7 and paper chromatography. About equal amounts of radioactive material were found co-migrating with the tripeptides Ala-Val-Leu and Pro-Ala-Leu. These radioactive peptides yielded the expected dipeptides after the first, and free leucine after the second Edman cycle.

The basic heptapeptide, obtained from the initial peptic digestion of the promelittin-like substance, was further cleaved with chymotrypsin and the resulting fragments as well as the intact peptide were subjected to three cycles of Edman degradation. After each step the resulting fragments were separated by paper electrophoresis at pH 1·7 and the corresponding fragments isolated from insect melittin were run alongside as markers.

In a further experiment with labelled leucine, the promelittin-like material from oocytes was first digested with trypsin and subsequently with pepsin. The resulting digest was separated by paper electrophoresis at pH 4·7; of the total radioactivity present, 19·6% was found to migrate with the marker fragment Ala-Val-Leu-Lys which had been isolated from melittin by the same procedure. After further hydrolysis with thermolysin a radioactive peptide indistinguishable from Leu-Lys was found to be present.

These experiments with labelled leucine show that, in response to injected venom gland RNA, the oocyte synthesizes a promelittin-like substance containing the following amino acid sequences (N stands for a neutral amino acid, B for lysine or arginine): N-Val-Leu, N-N-Leu-B, B-Val-Leu-N-N-Gly-Leu, and N-Ala-Leu. All these fragments correspond to parts of the sequence

<div align="center">Ala-Val-Leu-Lys-Val-Leu-Thr-Thr-Gly-Leu-Pro-Ala-Leu</div>

which is found in both melittin and its precursor. Thus, one can conclude that the promelittin-like material made in oocytes contains four leucine residues in the exact positions expected of the four leucine residues found in promelittin or melittin from bee glands. Moreover, the residues adjacent to the labelled leucines could be identified by comparing the mobilities of dipeptides with marker dipeptides of known composition; and the results obtained showed that the neighbouring residues were the same as those in the material from venom glands.

(b) *Oocytes injected with gland RNA and labelled with [³H]isoleucine*

The substance made in oocytes under the direction of gland RNA resembles promelittin as opposed to melittin since it cannot be extracted into *n*-butanol at alkaline pH. Labelling with [³H]isoleucine and [³H]proline yields information about the N-terminal region of the promelittin molecule and thus confirms that the oocyte synthesizes the precursor rather than the toxin itself.

The promelittin-like component was isolated from oocytes labelled with [³H]-isoleucine. Paper electrophoresis of the peptic digest yields three major radioactive peaks (see Fig. 4). Small amounts of other fragments were also detected, but these have not been analysed further.

Tentative conclusions about the termini of the product made in oocytes can be drawn from the results shown in Figure 4. On the anodic side, several negatively
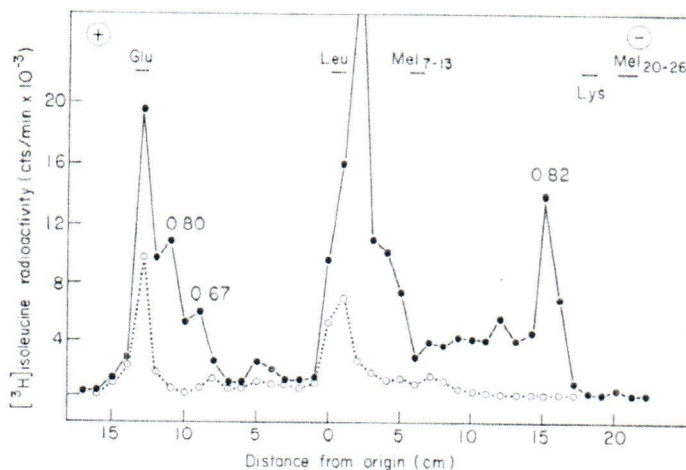
FIG. 4. Paper electrophoresis of a peptic digest of oocyte-derived promelittin labelled with [³H]isoleucine. Promelittin was prepared from injected oocytes as described in Fig. 1. Again, the closed circles (—●—●—) represent material from oocytes treated with gland RNA, and the open circles (...○...○...) show the profile of labelled material from controls. Amino acids and fragments of melittin were used as reference substances (bar markers). Digestion and electrophoretic separations were performed as in the experiment shown in Fig. 2. Numbers refer to mobilities relative to glutamic acid and lysine for acidic and basic peptides, respectively.

charged peptides are present with mobilities, relative to glutamic acid, ranging from 0·67 to about 1·0. However, as shown below, mild acid hydrolysis yields only one major fragment containing labelled isoleucine in the penultimate position. We interpret this as evidence that these acidic peptides differ only at their amino ends and hence that the product made in oocytes, like that from venom glands, exhibits heterogeneity at its N-terminus.

It has previously been demonstrated that the acidic fragments derived from the amino end of promelittin all contain an Asp-Pro bond which is susceptible to treatment with dilute HCl. Consequently, the acidic peptides from oocyte-derived promelittin were partially hydrolysed in 0·01 N-HCl at 100°C. Electrophoresis now shows that the bulk of the radioactivity is recovered in a less acidic fragment (R_{Glu} = 0·39). Using stepwise Edman degradation of this latter fragment, we could assign the following sequence to this peptide: N-Glu-N-N-Ile-Gly. The corresponding fragment isolated from insect promelittin has been shown to have the sequence: Pro-Glu-Ala-Gly-Ile-Gly (Kreil, 1973).

The neutral fraction of Figure 4 was further resolved by paper electrophoresis at pH 1·7. A radioactive fragment was detected co-migrating with Ile-Ser-Trp, a peptic fragment of melittin. The radioactive Ile was N-terminal.

The basic fragment that can be isolated from a peptic digest of the promelittin-like material synthesized in oocytes has an R_{Lys} value of 0·82 (see Fig. 4). It is thus distinctly less basic than the C-terminal heptapeptide (Mel$_{20-26}$) of melittin and promelittin, for this has an R_{Lys} of 1·05 and a net charge of +5. The difference probably arises because the oocyte lacks the enzyme(s) required to amidate the C-terminal glutamine residue: this inability would result in a heptapeptide of net charge +4. However, other explanations, for example the presence of extra residues at the

30

carboxyl end, cannot be ruled out. Using tryptic digestion and Edman degradation, it could be shown that the oocyte-derived fragment starts with the sequence Ile-B-B (where B stands for Lys or Arg) as does the C-terminal heptapeptide of venom gland promelittin.

(c) Oocytes injected with gland RNA and labelled with [³H]proline

In a further experiment, the promelittin-like substance was isolated from oocytes incubated in the presence of radioactive proline. Figure 5 shows paper electrophoresis of a peptic digest of this material. The bulk of the radioactivity present in the neutral region electrophoreses at pH 1·7 with the marker tripeptide Pro-Ala-Leu. This assignment was substantiated by paper chromatography and by end-group analysis.

The acidic peptides shown in Figure 5 were cleaved with dilute HCl, yielding the same fragment as was observed in the experiment with [³H]Ile. Edman degradation revealed the expected N-terminal proline. Moreover, dipeptidyl aminopeptidase I liberated a labelled fragment identified as Pro-Glu.
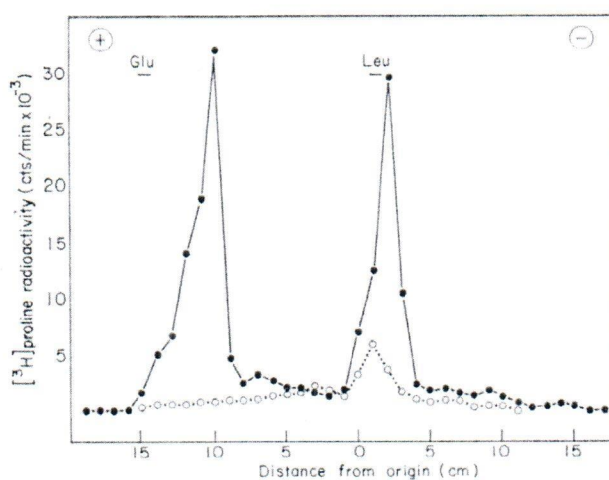

Fig. 5. Paper electrophoresis of a peptic digest of proline-labelled promelittin isolated from oocytes. The material was obtained from a chromatogram similar to the one shown in Fig. 2. The profile with open circles represents the control.

Hydrolysis with dilute HCl produced additional, negatively charged proline-containing fragments, but these were not analysed further.

The results obtained with three different labelled amino acids show that the injection of venom gland RNA into oocytes causes the synthesis of a peptide that is probably identical in amino acid sequence to the promelittin of venom glands. The sequence homology was shown directly in the case of four leucine, three isoleucine, two proline and, from an unpublished experiment with [³H]lysine, two lysine residues. Moreover, additional residues could be assigned with great certainty from the mobility of dipeptides containing one known labelled amino acid. Thus, of the 34 residues present in the main component of promelittin, a total of 17 have been

Sequence of promelittin (A)   Glu-Pro-Glu-Asp-Pro-Glu-Ala-Gly-Ile-Gly-

Oocyte product, deduced sequence (B)   . . . . . Pro-Glu- N - N -Ile-Gly/

Fragments analysed from oocyte
product (C)

Pro-Glu- N . . . . . .
N -Glu- N - N -Ile-Gly

(A) -Ala-Val-Leu-Lys-Val-Leu-Thr-Thr-Gly-Leu-Pro-Ala-Leu-Ile-Ser-Trp-Ile-Lys-Arg-Lys-Arg-Gln—Gln—NH$_2$

(B) / N -Val-Leu-Lys-Val-Leu- N - N -Gly-Leu-Pro-Ala-Leu/Ile- N - N /Ile-Lys- B . . . . .

(C) N -Val-Leu   Pro- N - N   Ile- B - B . . . . .

N - N -Leu- B   N -Ala-Leu   N -Lys- B . . . . .

B -Val-Leu - N - N - Gly-Leu   Ile- N - N

Lys- N - N . . . . .

Fig. 6. Comparison of the amino acid sequence of promelittin from honey bees with the partial structure deduced for the compound made in oocytes. Partial sequences of promelittin fragments from oocytes labelled with [³H]leucine, [³H]isoleucine and [³H]proline, as described in the present paper, and 2 fragments derived from [³H]lysine-labelled oocytes (unpublished experiment) have been ordered along the known sequence of insect promelittin. Melittin starts at the first glycine residue (no. 9). In the fragments the residue containing the label is underlined, N standing for a neutral amino acid and B representing either lysine or arginine. A bar (/) indicates the lack of an overlapping sequence. Except for the C-terminus, which is glutamine amide in promelittin but probably just glutamine in the product made in oocytes, no sequence differences could be detected.

found to be correctly inserted in the compound made in oocytes. Figure 6 shows a comparison between the structure of promelittin from venom glands and the partial sequence of the product made in oocytes.

(d) Promelittin synthesis in oocytes: efficiency of translation

Most of the labelled proteins from oocytes are not soluble in the mixture of n-butanol and aqueous ammonia used for the extraction of promelittin. Of the soluble part, only a portion is promelittin (e.g. fractions 3 to 5 of Fig. 2). In the experiments with different labelled amino acids, as described in the previous sections, 0·6 to 1·6% of the total label recovered in the oocytes was present in the promelittin-like material. Two types of RNA preparations have been used in this study; both were found to be about equally efficient in directing promelittin synthesis in oocytes.

(e) The search for melittin in oocytes injected with gland RNA

Oocytes labelled with [³H]isoleucine or [³H]leucine were tested for the presence of melittin. With the extraction conditions used for oocytes, melittin would be recovered in the upper layer consisting of n-butanol saturated with 0·1 M-ammonia (Kreil & Bachmayer, 1971). The organic layer was fractionated by paper chromatography in parallel with the aqueous phase. Except for free amino acids, few other radioactive compounds were present in the butanol extract. Consequently, traces of labelled melittin, amounting to only 1 or 2% of the promelittin synthesized, would be revealed; but extracts of injected oocytes contained no detectable amounts of melittin.

4. Discussion

The injection of messenger RNA into a living cell can yield information of two kinds: first, certain properties of the cell are revealed, such as the specificity of the translational machinery and the presence of post-translational modification systems; second, the nature and amount of the injected mRNA can be tested. Moreover, if the RNA is injected with another molecular species their interaction can be studied (see review by Lane & Knowland, 1974). In the present paper we demonstrate the translation of promelittin mRNA from insect venom glands in oocytes of the frog *Xenopus laevis*. This suggests that the oocyte system is quite general and can be used to study any kind of eukaryotic mRNA. However, the oocyte does not seem to be capable of carrying out certain post-translational modifications that occur in venom gland cells.

The venom glands of young queen bees synthesize promelittin, a peptide of 34 amino acids, as their main product. Consequently, the messenger for promelittin is likely to be one of the most abundant species of gland cell mRNA. Oocytes injected with unfractionated venom gland RNA synthesize a product that can be purified using the procedures worked out for promelittin from venom gland cells.

This indicates that the material from the two sources is basically similar. By using standard methods for sequencing peptides, we have demonstrated that there is more than a general resemblance between the two molecular species: the position of at least 17 residues has been determined in the oocyte-derived promelittin and each assignment has proved correct in terms of the known amino acid sequence of

insect promelittin. This shows that the informational content of the codons for a variety of amino acids is the same in cells from different phyla. Our results also yield final proof that at least part of an injected messenger RNA can be translated faithfully and there is no evidence for the occurrence of translational errors.

The translation of an insect gland cell messenger in *Xenopus* oocytes demonstrates that some at least of the translational systems of these frog cells are neither phylum nor cell-type specific. The possibility that tissue-specific restrictions on translation are in themselves species specific is ruled out by the translation of *Xenopus* globin mRNA in *Xenopus* oocytes (H. R. Woodland, unpublished observations). The possibility that other messengers present in the injected RNA might give rise to gland-specific factors is rendered unlikely by the observation that partially purified promelittin mRNA, isolated from the 4 to 9 S region of a sucrose gradient, is translated in oocytes (unpublished experiment). Since promelittin is probably not synthesized in vertebrate cells, it is unlikely that in oocytes specific factors are required to translate the promelittin messenger.

Post-translational systems that are present in specialized vertebrate cells but are absent in oocytes have yet to be discovered (Lane & Knowland, 1974). In promelittin isolated from venom glands, both ends of the molecule are changed by secondary modifications. Our results show that two reactions that occur in the venom gland cells of an invertebrate are not detectable in the oocyte, whilst a third modification reaction may occur in the oocyte as well as in the gland cell.

Promelittin and melittin, like many other peptides synthesized in animal cells, have at the C-terminal end an amide rather than a free α-carboxyl group. Peptic digests of melittin or its precursor contain a highly basic heptapeptide which is derived from the C-terminus. This fragment is not detectable in the corresponding hydrolysate of the compound made in oocytes (see Fig. 3); instead, a less basic peptide is present which probably contains a free α-carboxyl group and possibly extra residues at the C-terminus as well. Even though the C-terminal sequence of oocyte-derived promelittin is not known, our results suggest that the oocyte lacks the enzyme(s) required to make the C-terminal structure found in gland cell material.

The oocyte also appears unable to effect the conversion of promelittin to melittin. Under our extraction conditions, melittin is recovered in the butanol layer. Nonetheless, in a number of experiments paper chromatography failed to reveal the presence of melittin in the organic phase. Thus, whilst enzymes within the oocyte appear able to cleave the immunoglobulin light chain precursor (Mach *et al.*, 1973) and will also cleave the encephalomyocarditis virus precursor protein correctly in five places (Laskey *et al.*, 1972), there appear to be no enzymes that will catalyse the conversion of promelittin to melittin.

At the amino end, promelittin isolated from venom gland cells is heterogeneous. The experiments with injected oocytes labelled with isoleucine and proline both demonstrated the presence of several peptic fragments with a net negative charge, a pattern also encountered in the analysis of insect promelittin (Kreil, 1973). Thus the oocyte-derived promelittin, like the material from venom glands, appears to have a frayed amino end. If one assumes that there is only one species of promelittin mRNA, this heterogeneity could arise for two reasons: first the oocyte may contain proteases of the correct specificity which can catalyse what may be the first steps in the activation of the precursor; or second, the RNA preparation may also have contained the messengers coding for the requisite enzymes.

With the present approach it may be possible to determine terminal sequences and finally the entire structure of the promelittin-like compound made in oocytes. Since the assay for promelittin is both simple and sensitive, the translation of RNA from venom glands could also be studied in cell-free systems. Preliminary evidence suggests that promelittin mRNA can also be translated in the system described by Schreier & Staehelin (1973). Furthermore, the mRNA for promelittin merits attention since it codes for a peptide of rather low molecular weight.

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Note added in proof: Since the completion of this manuscript additional results have been obtained from experiments with [³H]serine, [³H]threonine, [³H]alanine, and [³H]tryptophan. Several residues could be identified and the structure of the oocyte product shown in Fig. 6 can now be partially completed to yield the following sequence:
. . . Pro-Glu-Ala-N-Ile-Gly/Ala-Val-Leu-Lys-Val-Leu-Thr-Thr-Gly-Leu/Pro-Ala-Leu/Ile-Ser-Trp/Ile-Lys-B . . .