Translation of exogenous messenger RNA coding for nicotinic acetylcholine receptors produces functional receptors in Xenopus oocytes

BY E. A. BARNARD, F.R.S.,† R. MILEDI, F.R.S.,‡ AND K. SUMIKAWA§

† Department of Biochemistry, Imperial College, London SW7 2AZ, U.K.
‡ Department of Biophysics, University College London, Gower Street, London WC1E 6BT, U.K.
§ Molecular Genetics Department, Searle Research and Development, Lane End Road, High Wycombe, Buckinghamshire, HP12 4HL, U.K.

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Messenger RNA extracted from the electric organ of Torpedo was injected into Xenopus oocytes. This led to the synthesis and incorporation of functional acetylcholine receptors into the membrane of the oocyte. When activated by acetylcholine these Torpedo acetylcholine receptors in the oocyte membrane opened channels whose ionic permeability resembled that of nicotinic receptors in other cells.

INTRODUCTION

The methods of molecular biology offer a new and powerful approach to the study of membrane-bound proteins. For instance, injection of heterologous messenger RNA (mRNA; isolated from the electric organ of Torpedo) into Xenopus oocytes led to the synthesis and assembly of the multi-subunit acetylcholine (ACh) receptor molecule (Sumikawa et al. 1981). These ACh receptors were capable of binding α-bungarotoxin (α-BuTX), but we did not know if the receptors coded by Torpedo mRNA were incorporated into the membrane of the oocyte, or if they were capable of being activated by ACh. The experiments briefly reported here were designed to answer these questions.

METHODS

mRNA was extracted from fresh electric organ of Torpedo marmorata, or from human fibroblasts to use as control. In each case the poly(A) mRNA fraction was isolated (Houghton et al. 1980) and injected into Xenopus oocytes (ca. 20 ng per cell), which were then cultured (cf. Gurdon 1974). Other details were as given previously (Sumikawa et al. 1981).

At various times after incubation injected or non-injected oocytes were placed in normal frog Ringer solution (NaCl 115 mM, KCl 1 mM, CaCl₂ 1.8 mM and Heps or Tris buffer 5 mM, pH 7.2) and examined with electrophysiological techniques. For voltage-clamping the membrane, two microelectrodes were inserted into the oocyte and ACh was applied by iontophoresis from a micropipette.
RESULTS

As found previously (Sumikawa et al. 1981) after injection of Torpedo mRNA into oocytes, ACh receptors, as defined by the specific binding of α-BuTX, accumulated in the cells. Table 1 shows the production of $[^{125}I]α$-BuTX-binding component in some of the oocytes used for the present experiments. Table 1 shows further that some of the translation product is probably inserted into the oocyte membrane, because it can be labelled by incubating intact oocytes with α-BuTX which presumably cannot enter the cell. To examine if ACh receptors are indeed inserted into the surface membrane, the oocytes were studied electrophysiologically.

TABLE 1. α-BuTX-binding in injected oocytes

(After injection of oocytes with mRNA and incubation, either (i) the translation products were totally extracted from the cells into the medium containing 1% Triton X-100 (Sumikawa et al. 1981), or (ii) the culture medium alone was taken. Alternatively, (iii) intact oocytes were incubated with $[^{125}I]α$-BuTX to label surface ACh receptors (as a control, intact oocytes injected with water were also incubated with $[^{125}I]α$-BuTX). In each case the specific α-BuTX-binding was measured. The control mRNA was extracted from human fibroblasts (Houghton et al. 1980).)

<table>
<thead>
<tr>
<th>injection</th>
<th>fraction</th>
<th>$[^{125}I]α$-BuTX specific binding per oocyte/fmol</th>
</tr>
</thead>
<tbody>
<tr>
<td>20 ng Torpedo mRNA</td>
<td>(i) total Triton extract</td>
<td>13.7–20</td>
</tr>
<tr>
<td></td>
<td>(ii) aqueous extracellular medium</td>
<td>not detectable</td>
</tr>
<tr>
<td></td>
<td>(iii) surface membrane</td>
<td>4.8–6.3</td>
</tr>
<tr>
<td>20 ng control mRNA</td>
<td>total Triton extract</td>
<td>not detectable</td>
</tr>
</tbody>
</table>

After injection, the resting potential, current–voltage relation and other electrical characteristics of the oocyte membrane were normal (cf. Kusano et al. 1977, 1982) in many oocytes. In some oocytes the resting potential and membrane resistance were low, but even these yielded useful information.

When ACh was applied to the surface of oocytes injected with Torpedo mRNA a response was elicited in practically all the oocytes examined more than 2 days after injection. It has been shown previously (Kusano et al. 1977, 1982) that the membrane of some Xenopus oocytes already contains ACh receptors; but their response to ACh differs in several respects from that observed in the injected oocytes. The response in normal oocytes occurs after a long delay (figure 1 a) and is ‘oscillatory’ in nature (cf. Kusano et al. 1977, 1982). In contrast, the membrane potential (or current) change induced by ACh applied iontophoretically to oocytes injected with Torpedo mRNA starts practically without delay and has the smooth shape (figure 1 b) usually seen when ACh is applied in this way to the muscle membrane.

The ACh receptors present in normal oocytes are muscarinic in nature and, when activated by ACh, cause a movement of Cl ions across membrane channels (Kusano et al. 1977, 1982). In contrast the experiments done so far indicate that Na and K ions are mainly responsible for the currents induced by activation of the ACh
receptors that appear after injection of *Torpedo* mRNA. Because of this, the equilibrium potential for ACh acting on the two types of ACh receptors is different. This is illustrated in figure 2, which shows the responses to ACh applied when the membrane potential was set at different levels, in an oocyte that had been injected with *Torpedo* mRNA. It can be seen that at $-31$ mV (figure 2a) both the early and the late responses to ACh depolarized the membrane. At $-28$ mV (figure 2b) the late response disappeared, and at $-24$ mV it was inverted (figure 2c). In contrast, the early response to ACh was still a depolarizing one even at that level, and it did not reverse direction until the membrane potential reached about $-10$ mV. This is illustrated in figure 3 obtained, under voltage clamp, from another oocyte also injected with *Torpedo* mRNA.

A further difference between the ACh receptors present normally in some oocytes and those induced by injection of *Torpedo* mRNA lies in their reaction to antagonists: atropine blocks the former but not the latter, while the opposite occurs when (+)-tubocurarine or α-BuTX is used. Thus the newly induced ACh receptors belong to the so-called nicotinic class. It is clear then that we are dealing with two
different types of receptors, which, when activated by ACh, open membrane channels with different ionic permeabilities. The ‘Torpedo ACh receptors’ incorporated into the oocyte membrane are a direct consequence of the injection of Torpedo mRNA, because control oocytes injected with water or, better still, oocytes injected with fibroblast mRNA did not produce these ACh receptors and the nicotinic response to ACh could not be elicited in them.

These results and others will be presented in more detail at a later date, but a few more need to be mentioned here. First, the receptors coded by Torpedo mRNA are in the oocyte membrane and not in the enveloping follicular cells, because the nicotinic responses could be obtained after the follicular cells were removed by collagenase treatment. Secondly, many Torpedo ACh receptors are inserted in the
plasma membrane with the ACh-recognition site in the external medium and few, if any, can be activated from the cell’s interior, because intracellular application of ACh did not evoke a response. Thirdly, the nicotinic response of injected oocytes persists for a long time: it was previously known that normal oocytes can be maintained for over 1 month (R. Miledi, unpublished) and in the present experiments nicotinic responses could still be obtained 4 weeks after injection of Torpedo mRNA. Lastly, preliminary experiments with α-BuTX and protein synthesis inhibitors suggest that synthesis and incorporation of ACh receptors may still occur more than 3 weeks after injection of mRNA.

Figure 3. Voltage dependence of ACh current in an oocyte 25 days after injection of Torpedo mRNA. From top of figure, ACh-induced currents obtained with the membrane potential clamped at −60, −40, −20 and 0 mV. The equilibrium potential for ACh acting on the ‘Torpedo ACh receptors’ was −8 mV after correcting for the electrode tip potential.

**Discussion**

These results show that Xenopus oocytes injected with mRNA, extracted from a cell normally carrying ACh receptors on its surface membrane, develop ACh receptors and associated ionic channels that resemble those of the original cell. Altogether the evidence leads us to conclude that foreign mRNA coding for the ACh receptor proteins is translated in the oocyte; the products are then subjected to appropriate post-translational processing and result in the translocation to, and correct insertion into, the oocyte membrane, where they form a functional ACh–receptor–channel complex. Since the oocyte already has the systems necessary for the incorporation of various neurotransmitter receptors into the membrane (Kusano et al. 1977, 1982), it may be that the only exogenous mRNA required to induce the appearance of nicotinic ACh receptors in the oocyte membrane is that specifically coding for the receptor proteins. However, the possibility still remains that some of the mRNA injected might be involved in the assembling and addressing of receptors to the surface membrane.

The present results are interesting, because they provide the necessary proof that the mRNA under study codes for a complete receptor. Previous evidence was insufficient: the receptor subunits synthesized in an *in vitro* system did not bind
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α-BuTX (Mendez et al. 1980; Anderson & Blobel 1981); and toxin-binding seen after translation in the oocyte (Sumikawa et al. 1981) does not necessarily mean that receptors are functional.

The well established versatility of the amphibian oocyte in the translation of exogenous mRNA, and in the post-translational processing and secretion of products (Gurdon 1974; Lane et al. 1979), is now extended to the capacity of directing to the membrane, and inserting therein, a foreign trans-membrane protein. This protein is not appreciably secreted from the cell (cf. Table 1); on the contrary, it comes oriented within the membrane so that a functional receptor unit is formed. Further work may shed some light on the molecular processes involved in the insertion of ACh receptors into the membrane. It already appears that this is not a random process, for, although receptors can occur all over the oocyte's surface, the 'Torpedo ACh receptors' are preferentially inserted in the vegetal hemisphere, whereas the oocyte's own muscarinic receptors were found more frequently in the opposite (animal) side (Kusano et al. 1982).

The oocyte system provides a new method that will help to identify and characterize, both chemically and functionally, receptors for neurotransmitters and other substances. An mRNA coding for any cell membrane receptor may lead to the production of receptors and their appropriate drug responses, simply by injection into the oocyte. We have already done this with fish ACh receptors and with cat muscle ACh receptors (unpublished) and there is no reason to suspect that attempts with other receptors will not be equally successful.

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References


