Xenopus Oocytes to Study Fully-Processed Membrane Proteins

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The use of *Xenopus* oocytes in electrophysiological and biophysical research constitutes a long and successful story, providing major advances to the knowledge of the function and modulation of membrane proteins, mostly receptors, ion channels, and transporters. These cells are capable of correctly expressing heterologous proteins after injecting the corresponding mRNA or cDNA. The *Xenopus* oocyte has become an outstanding host–cell model to carry out detailed studies on the function of fully-processed foreign membrane proteins after their microtransplantation to the oocyte.

Keywords: Xenopus oocytes ; membrane protein biophysics ; neuroreceptor microtransplantation ; proteoliposomes ; membrane protein-lipid interactions

1. Background

Xenopus laevis, the South African clawed frog, is a laboratory animal that is especially suitable for diverse biological studies since, unlike other anurans, their ovaries always contain oocytes in all stages of development (**Figure 1**) and new cycles of oogenesis can be initiated during any season by hormonal treatment. In the last five decades, the use of *Xenopus* oocytes has grown exponentially and has become a chief tool in many laboratories around the world in studies dealing with molecular, cellular, and developmental biology [1][2][3][4][5]. In fact, since the last decade of the 20th century, the *Xenopus* oocyte constitutes one of the most widely used cell model in molecular biology [3][4]. Furthermore, *Xenopus* oocytes are particularly useful cells to carry out electrophysiological recordings to study both native ion channels and receptors [6][7] (reviewed in [2][8][9][10]) and foreign proteins transplanted to this convenient host cell after the injection of either the corresponding mRNA or lipid vesicles containing the purified protein (reviewed in [2][9][11][12][13]). *Xenopus* oocytes have also become the reference model for biophysical studies dealing with structure to function relationships, addressed to unravel the functional consequences of specific mutations of membrane receptors, channels, and transporters (reviewed by [13][14]). Moreover, transplanting foreign, fully-processed membrane proteins to *Xenopus* oocytes is a methodology more recently used to look deeper into the role played by ion channels and neuroreceptors in the pathogenesis of different diseases as well as for drug screening using electrophysiological recordings and/or fluorescence assays [13][15][16]. Altogether, this has led to the *Xenopus* oocyte being regarded as a “living test tube” [17].

Figure 1. Micrographs of *Xenopus* oocytes. (**A**) Image of an opened and extended fragment of the *Xenopus* ovary lobule, showing oocytes at different stages of development. (**B**) Full-grown, immature oocyte, and the plasma membrane (together with its vitelline envelope) from another oocyte, manually isolated and stained with ink for better observation.

2. Advantages and Drawbacks of Using *Xenopus* Oocytes to the Study of Membrane Proteins

As stated by August Krogh [18], an outstanding physiologist awarded the Nobel Prize, for every biological problem there is an organism (model) on which it can be most conveniently studied. In this sense, the *Xenopus* oocyte model, introduced by Ricardo Miledi, constitutes an excellent paradigm of the Krogh principle to carry out detailed studies on the function of foreign membrane proteins, as was the squid giant axon to understand the ionic basis of the action potential, the frog end-plate junction and the squid stellate ganglion to unveil the basis of synaptic transmission or the *Aplysia* ganglia, which allowed for the molecular mechanisms of learning and memory to be dealt with. Some of the advantages that the oocyte fulfills to study foreign proteins are:

(a)*Xenopus* oocytes have a continuous and asynchronous development, allowing a year-round use of these cells [1].

(b)Large size, up to 1.3 mm, and roughly spherical shape [1], which facilitates electrophysiological recordings, even allowing the introduction of additional micropipettes for microinjecting compounds [2]. Furthermore, its size enables simultaneous biochemical and optical techniques with quite high spatio-temporal discrimination [19].

(c)Oocytes constitute an excellent factory for the adequate synthesis and processing of most heterologous proteins [20][21][22][23] (reviewed in [2][13][14][24][25][26][27]).

(d)These cells are easy to manage and cheap to maintain. Moreover, these cells can be kept alive in an inorganic buffer for long periods (up to several weeks) after their isolation from the ovary, without the need of a specific sterile serum or medium [2][14][16]. Nevertheless, oocytes progressively uncouple from the surrounding follicular cells after their separation from the ovary, losing responsiveness to certain hormones and neurotransmitters.

(e)The osmotic water permeability of oocytes is quite low (circa 4×10^{-4} cm/s), which allowed for the first functional characterization of exogenous aquaporins [28][29].

(f) The oocyte plasma membrane can be quite easily isolated either manually ([30][31]; see **Figure 1**) or by simple biochemical procedures [32]. The isolation of plasma membranes enables one to determine the presence of the microtransplanted protein at the oocyte plasma membrane [31][33] and allows studies concerning both the targeting of membrane proteins and the quantitation of the ratios between functional and the total number of ion channels/receptors incorporated into the cell membrane [12][33][34].

(g)Although the membrane of *Xenopus* oocytes might present certain ion channels and receptors, the expression of endogenous proteins from their own mRNA is low (about 5% of their stored mRNA, [13]). Thus the oocyte plasma membrane usually lacks significant levels of neuronal voltage-dependent Na^+ and K^+ channels and many neuroreceptors and transporters [10].

(h)The oocyte has powerful intracellular signaling cascades, mostly involving $InsP_3$ synthesis and Ca^{2+} release from intracellular stores after phospholipase-C activation. Thus, heterologous expression of certain metabotropic receptors can be simply monitored by recording Ca^{2+}-dependent Cl^- currents, since the Ca^{2+}-dependent Cl^- channel, anoctamin 1 (TMEM16A), is highly expressed in the oocyte membrane [2][27][35][36].

(i) Oocytes are convenient cells to screen potential drugs and to determine their relative efficacies against specific targets. These studies have been boosted by the introduction of devices for the automated voltage-clamping of these cells [13][16].

Nevertheless, *Xenopus* oocytes also present some disadvantages when studying membrane proteins including:

(a)In the ovary, oocytes are found as follicles, constituted by several cellular and acellular layers surrounding the oocyte, from inner to outer: (i) the oocyte; (ii) a fibrous vitelline membrane; (iii) a monolayer of follicular cells, which is coupled to microvilli of the oocyte membrane by gap junctions [37]; (iv) a theca, containing mainly collagen, fibrocytes and small blood vessels; and (v) a layer of epithelial cells. The layers surrounding the oocyte might constitute a handicap to study certain transplanted proteins since follicular cells express ion channels and receptors of their own and are electrically coupled to the oocyte. However, the layers surrounding the oocyte can be removed either manually or by enzymatic means.

(b)There is a certain variability in the expression efficiency of oocytes, which has been related to seasonal differences [27]. Actually, some laboratories stop working with oocytes during summer because of their low quality. Furthermore, noise

and other vibrations should be restricted around aquarium facilities since *Xenopus* are quite sensitive to them.

(c)As previously mentioned, oocytes randomly express certain ion channels and receptors, which might be confused with those heterologously expressed [13]. Thus, it becomes necessary to keep in mind the channels and receptors that can be endogenously expressed by oocytes (excellent reviews regarding this issue are provided in [2][8][9][10][24][25]), though some of them can be pharmacologically blocked. Nevertheless, other cell models commonly used for the heterologous expression of ion channels and transporters such as human embryonic kidney cells (HEK) also have endogenous ion channels [38].

(d)The large size of the oocyte together with the presence of several surrounding layers (cellular and acellular) constitute a limitation to obtaining fast ligand-applications, particularly when using large molecules [39], and consequently, to record fast-kinetics currents (it hinders the resolution of ligand-elicited currents at resolutions below hundred milliseconds; [40]). Moreover, when follicular cells remain attached to the oocyte, certain measured pharmacological values (i.e., half maximal effective concentration, EC_{50}, or half maximal inhibitory concentration, IC_{50}) might be inaccurate [39]. In addition, several factors such as the oocyte large size, the presence of numerous microvilli at its plasma membrane, and its electrical coupling to follicular cells contribute to eliciting extremely large capacitive artifacts, which prevent resolving fast voltage-dependent currents below a few milliseconds [14].

3. Transplant of Fully Processed Membrane Proteins to the *Xenopus* Oocyte

As previously indicated, *Xenopus* oocytes have allowed for the biophysical characterization of many ion channels, neurotransmitter receptors, and transporters thanks to their ease of use, amenability for electrophysiological recordings and their capability to efficiently and faithfully translate most heterologous mRNAs (reviewed in [2][24][25]). It is known that *Xenopus* oocytes are able to make many post-translational modifications of the proteins coded by the exogenous mRNA (as glycosylation, phosphorylation, acetylation, or folding), and to correctly assemble oligomeric receptor/channel complexes. However, occasionally, they do not mimic the post-translational modifications carried out by the cells that used to express them natively. Most likely, these differences account for the failed or altered function of some foreign proteins expressed in oocytes and, perhaps, also for the variability observed between oocytes [27]. For instance, *Torpedo* nicotinic acetylcholine (ACh) receptors (nAChRs) expressed in oocytes after injecting the corresponding mRNAs have an altered pattern of glycosylation [41], and neuronal sympathetic nAChRs (α3β4 and α4β4 subunits) do not exhibit certain properties of the native receptors, putatively because oocytes fail to correctly assemble their different subunits or because of the post-translational channel modifications [42]. Similarly, native brain and heterologously expressed rat α4β2 nAChRs showed significant pharmacological differences [43]. In this regard, it should be considered that certain membrane proteins including ion channels are modulated by their interaction with accessory subunits, which might be lacking in the host cell membrane and are not incorporated when mRNA is used to express specific foreign proteins in the oocyte [13][27][43][44]. Additionally, specific lipid requirements of certain membrane proteins might constitute a severe handicap to the faithful functional expression of some heterologous proteins [45]. Moreover, it has been shown that different mutations in the M4 (the outermost lipid-facing α-helix of the transmembrane domain) of α4β2 nAChRs reduced or abolished the function of these receptors when expressed in the HEK cells, but not when expressed in *Xenopus* oocytes [46]. The differences in the functional activity of these mutant nAChRs between both cell types seem to be due to the different lipid composition of their respective plasma membranes [46].

The above-mentioned handicaps were overcome when Marsal et al. [47] found that foreign membranes carrying neurotransmitter receptors and ion channels could be incorporated into the *Xenopus* oocyte membrane, which allowed for the microtransplantation of fully processed proteins retaining their natural environment. Since then, several groups have followed this approach of the intracellular injection of plasma membranes to study foreign membrane proteins in *Xenopus* oocytes (reviewed in [12]). Thus, many neuroreceptors, transporters, and ion channels have been transplanted from their original cells to the oocyte membrane including: (i) nAChRs from *Torpedo* electroplaques [33][47], muscle fibers [48][49], brain neurons [50], and from cell lines overexpressing heterologous neuronal nAChRs [26]; (ii) gamma-aminobutyric acid receptor type A ($GABA_AR$) from the brain synaptosomal membranes [15][26][51][52][53][54][55][56][57][58][59][60][61]; (iii) glutamate (AMPA, α-amino-3-hydroxy-5-methyl-4-isoxazolepropionic acid/Kainate/NMDA, N-methyl-D-aspartate) receptors [26][52][62][63][64]; (iv) voltage-activated Na^+ and Ca^{2+} channels from the human brain [34][65]; (v) Cl^- channels from *Torpedo* electroplax [33][47] and from human syncytiotrophoblast microvillous membranes [66]; and (vi) membrane transporters such as P-glycoprotein [31] and the Cl^- transporters, KCC1 (K^+–Cl^- cotransporter type 1) and NKCC2 (Na^+–K^+–Cl^- cotransporter type 2; [55]).

The intracellular injection of plasma membranes to insert foreign proteins into the oocyte membrane was later extended by microtransplanting functional proteins after their purification and reconstitution in lipid vesicles of defined composition

[33][67][68][69]. The main steps followed to microtransplant purified and reconstituted nAChRs from *Torpedo* electroplaques to the *Xenopus* oocyte membrane are shown in **Figure 2**.

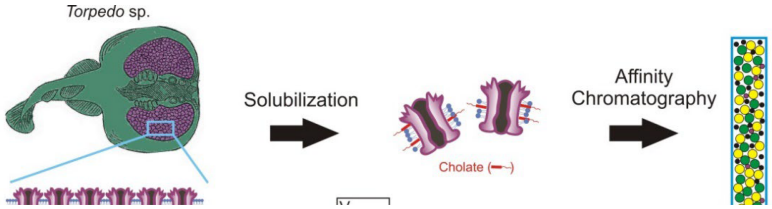

Figure 2. Methodology followed to microtransplant foreign membrane proteins to oocytes. Scheme of the steps to microtransplant the purified and reconstituted nicotinic acetylcholine (ACh) receptors (nAChRs) to the *Xenopus* oocyte membrane to carry out detailed functional studies.

It should be noted that the goal of this experimental approach is not to use oocytes as a factory to generate proteins, but as a convenient cellular system to carry out detailed functional studies of the transplanted membrane proteins. Nevertheless, the use of purified and reconstituted proteins, instead of fragments of cellular membranes, has several advantages including: (i) it allows for the study of single molecular entities; (ii) it does not require the transplanted protein to be highly expressed in the plasma membrane, although the presence of a large amount of protein simplifies its purification; and (iii) it makes it possible to study the influence that the lipid composition of the reconstitution matrix has on both the function of the transplanted protein and the process of fusion between the vesicular and cellular membranes. This later point is of special relevance since many proteins need to interact with specific lipids for developing their full functional activity [70][71]. Consequently, the microtransplantation of purified and reconstituted proteins into the *Xenopus* oocyte membrane arises as an excellent way to unravel the lipid–protein interactions, since it allows researchers to both insert proteins bound to specific lipids, which can even be labelled, and to selectively modify the lipid content of the cell membrane. Using this approach, it is possible to change not only the ratio of different phospholipids surrounding the protein to determine their functional relevance, but also the charge or the length of the acyl chains to induce local changes in the bilayer thickness and elasticity, which might also be important for the protein activity [72][73]. Thus, this approach constitutes a very useful extension to the classical use of cDNA or mRNA for the functional study of ion channels and neurotransmitter receptors.

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