Endogenous transport systems in the *Xenopus laevis* oocyte plasma membrane

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**Abstract**

Oocytes of the South African clawed frog *Xenopus laevis* are widely used as a heterologous expression system for the characterization of transport systems such as passive and active membrane transporters, receptors and a whole plethora of other membrane proteins originally derived from animal or plant tissues. The large size of the oocytes and the high degree of expression of exogenous mRNA or cDNA makes them an optimal tool, when compared with other expression systems such as yeast, *Escherichia coli* or eukaryotic cell lines, for the expression and functional characterization of membrane proteins. This easy to handle expression system is becoming increasingly attractive for pharmacological research. Commercially available automated systems that microinject mRNA into the oocytes and perform electrophysiological measurements fully automatically allow for a mass screening of new computer designed drugs to target membrane transport proteins. Yet, the oocytes possess a large variety of endogenous membrane transporters and it is absolutely mandatory to distinguish the endogenous transporters from the heterologous, expressed transport systems. Here, we review briefly the endogenous membrane transport systems of the oocytes.

**1. Introduction**

Since the pioneering work of Gurdon et al. in 1971 [1], *Xenopus laevis* oocytes have been extensively used as an heterologous expression system for the plethora of proteins derived from bacteria, plants and virtually all animal tissues [2–4]. The large size of the oocytes and their nearly promiscuous expression of mRNA or cDNA make them an ideal expression system with many advantages when compared with more conventional expression systems such as yeast, cell lines or *Escherichia coli*. Moreover, the oocyte expression system can be used to isolate proteins for which sequence data is not available by a technique termed expression cloning [5]. The oocyte expression system allows functional identification of hitherto unknown proteins, molecular characterization of protein function and determination of functionally relevant residues and domains of membrane proteins including transporters, ion channels and receptors. Many of these proteins have been cloned and are now studied thoroughly as potential targets for drug development. In the recent years several methods of high throughput electrophysiological screening have been developed that are automated and allow simultaneous data acquisition and analysis from multiple oocytes permitting primary screening as well as thorough characterization of new pharmacologically active compounds [6].

Since the oocyte expression system is increasingly used in physiological, pharmacological and neurophysiological research, we describe in this brief review the endogenously expressed membrane transport systems, which should be useful for every research group using oocytes as a heterologous expression system. Because the goal of this review is to give a short, but nevertheless comprehensive overview, the referenced literature is exemplary and we apologize to all colleagues whose work was not cited. We further restricted ourselves to the description of transport systems in the plasma membrane of *X. laevis* defolliculated oocytes.

**1.1. Basic electrical and ionic parameters of the oocyte membrane**

Oocytes of *X. laevis* classified as stage V or VI according the classification of Dumont [7] belong to the largest single cells in the animal kingdom. They have a diameter from 1 to 1.3 mm and a highly folded plasma membrane with an area between 18 and 20 mm² [3,8]. The electrical parameters of the oocytes such as membrane resistance (Rm), potential (Um) and current (Im) are important factors that give information about the “quality” of the oocytes. These parameters differ from oocyte to oocyte within one batch (i.e. derived from the same donor animal) and differences in these parameters can be even larger in the oocytes derived from different donors. Electrical membrane resistance of the oocytes is quite high and ranges usually from some 100 kΩ to 2 MΩ [9]. Oocytes exhibiting significantly lower resistance should not be used for microinjection and subsequent electrophysiological experiments. The search of literature indicates that there are large differences in
the membrane potential of the oocytes ranging from –27 mV to –90 mV, when determined in physiological Ringer solution [10,11]. From our experience \( U_m \) is mostly around –50 mV [12,13]; yet \( U_m \) is strongly dependent of the extracellular K⁺ concentration, diameter and proper handling of the microelectrodes used. Only fine electrode tips and proper impaling prevent unrecoverable damage of the oocyte plasma membrane resulting in low \( R_m \) and \( U_m \) values. Membrane current (\( I_m \)) is also strongly dependent on the ionic environment and is typically zero when determined at \( U_m \). When the plasma membrane is voltage-clamped (to potentials lower or higher than \( U_m \)) the \( I_m \) rises due to activation of voltage-dependent transport systems [9,14]. Intracellular ion concentrations (summarized in Table 1) are very important for the investigation of passive or active transport systems because rate and/or direction of the respective transports are determined by the electrochemical gradients.

2. Endogenous transport systems

Transport systems can be roughly grouped into passive and active transport systems according to the energy they use to mediate transport. Passive transport systems depend on the electrochemical driving force of the transported ions or on the chemical gradient alone in case of uncharged molecules [15]. Passive transport systems can only mediate “downhill” transport following the (electro) chemical concentration gradient of the respective substrate. In contrast, the active transport proteins mostly use the energy of ATP to mediate “uphill” transports against the (electro) chemical gradients.

3. Passive transporters

Within this group of transporters in the oocyte plasma membrane we can distinguish two different transport mechanisms, i.e. facilitated diffusion and flow through channel proteins. While oocytes exhibit a large diversity of ion channels, only a few facilitated diffusion and flow through channel proteins. While oocytes can distinguish two different transport mechanisms, i.e.

### Table 1

<table>
<thead>
<tr>
<th>Ion species</th>
<th>Concentration [mM]</th>
<th>References</th>
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<tbody>
<tr>
<td>Na⁺</td>
<td>4</td>
<td>[182]</td>
</tr>
<tr>
<td></td>
<td>6</td>
<td>[183]</td>
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<td>23</td>
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<td>K⁺</td>
<td>76</td>
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<td></td>
<td>92</td>
<td>[183]</td>
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<td>117</td>
<td>[185]</td>
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<tr>
<td></td>
<td>148</td>
<td>[10]</td>
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<tr>
<td>Cl⁻</td>
<td>24</td>
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<td>33</td>
<td>[183]</td>
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<td>54</td>
<td>[185]</td>
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<td></td>
<td>62</td>
<td>[10]</td>
</tr>
<tr>
<td>Ca²⁺</td>
<td>(3 \times 10^{-6})</td>
<td>[186]</td>
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<tr>
<td></td>
<td>(30 \times 10^{-6})</td>
<td>[187]</td>
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<td>(50-90 \times 10^{-6})</td>
<td>[104]</td>
</tr>
<tr>
<td></td>
<td>(400 \times 10^{-6})</td>
<td>[188]</td>
</tr>
<tr>
<td>Mg²⁺</td>
<td>&gt;0.5</td>
<td>[188]</td>
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</table>

In sharp contrast to the poor expression of diffusion carriers, X. laevis oocytes posses a whole plethora of ion channels. In the following we will give a short overview on the endogenous ion channels; for a detailed description of oocyte ion channels please refer to more comprehensive reviews [9,14,28–30].

3.1. Ion channels

Intraoocyte ion concentrations.

### 3.2. Anion channels

Anion channels, namely Cl⁻ channels outnumber by far all other ion conductances of the oocyte plasma membrane. There are at least four different classes of Cl⁻ channels that differ in the mode of their activation. The first class, Ca²⁺-activated Cl⁻ channels (CaCCs) are concentrated at the animal hemisphere of the oocytes creating a functional polarity that is quite important for further developmental changes following fertilization [31–34]. CaCCs play important and diverse roles in almost all cells, ranging from the fast block to polyspermy to the essential functions in transduction of intra- and extracellular signals [35–37]. Direction of Cl⁻ through CaCCs is dependent on the membrane potential [38], the Cl⁻ concentration gradient and the intracellular Ca²⁺ level (for review see [39]). Although it was known that these channels can mediate either excitatory or inhibitory responses, depending on the amplitude of the intracellular Ca²⁺ signal [40] their molecular identity in the oocytes was unclear until the identification of CaCCs as members of the bestrophins, a newly identified family of proteins that can function both as regulators of voltage-dependent Ca²⁺ channels and as Ca²⁺-activated Cl⁻ channels [41]. CaCCs in the oocytes are modulated by phosphorylation and protein kinase C (PKC) [42–44], and can be inhibited by several Cl⁻ channel blockers such as niflumic acid, flufenamic acid, 9-AC (anthracene-9-carboxylic acid) and NPPB [45,46]. Some authors argue for the existence of different populations of CaCCs rather than a single discrete channel population and until now, at least four different classes of CaCCs have been described [36,47–50].

The second class of anion channels found in the oocyte plasma membrane is represented by volume sensitive Cl⁻ channels (Cl⁻vol) that are activated by hypotonic solutions [51–53]. Cl⁻vol can be clearly distinguished from CaCCs, yet the channel density per oocyte is somewhat controversial [51,54]. Cl⁻vol in X. laevis oocytes is probably identical with the human CIC-3 [55], an intracellular Cl⁻ channel found in endosomes and synaptic vesicles [56]. The sensitivity of Cl⁻vol to La³⁺ and the insensitivity to niflumic acid makes it easy to distinguish between Cl⁻vol and CaCC [57,58]. The ion selectivity of Cl⁻vol is SCN⁻ > I⁻ > NO₃⁻ > Cl⁻ = HCO₃⁻ > acetate [59] differs from the selectivity of hyperpolarization-activated Cl⁻ channels [60], the third class of anion channels found in X. laevis oocytes. These channels are activated by hyperpolarization to more than – 100 mV and exhibit greatly variable current amplitudes [61,62]. Recently, a novel outwardly rectifying anion current has been described [63] that showed some
similarities with the human CIC-5 Cl channel, an endosomal channel involved in renal endocytosis [56].

The fourth class of Cl− channels is represented by the Ca2+-inactivated Cl− channels termed CaCl [12,64–66] reviewed thoroughly in [67]. Although it has been claimed earlier that divalent cation-free solutions cause irreversible damage to the oocytes [68], it has been clearly shown that the removal of Ca2+ and other divalent cations from the extracellular solution reversibly activates large CaCl currents that can be blocked by various Cl− channel blockers such as nitric acid, flufenamic acid and 9-AC [66]. In contrast, an addition of divalent cations leads to immediate inactivation without any damage to the oocyte. The molecular identity of this type of channel is not known, yet it has been demonstrated that CaCl mediate the efflux from intracellular Cl− to the external medium [66]. CaCl is not downregulated during maturation of the oocyte. In contrast, the majority of the other transport systems in the oocyte plasma membrane is downregulated during oocyte maturation (as demonstrated by whole-cell and single-channel data), which indicates that they play an important role in the further development of the oocyte, possibly in the generation of the fertilization potential and subsequent depolarization of the egg membrane to prevent polyspermy [65].

3.3. Cation channels

3.3.1. K+ channels

Several types of endogenous K+ channels in the oocyte plasma membrane have been described. These channels in combination with the Na+/K−/ATPase work to establish and maintain the oocyte membrane potential. All classes of K+ channels are sensitive to the natural alkaloid quinine [69]. Some of the K+ channels are sensitive to TEA (tetraethylammonium) [70] while other classes are TEA-insensitive [71]. Most of the endogenous K+ channels are sensitive to Ba2+ while Ba2+-insensitive K+ channels can be found only sporadically [70,71]. Some of the endogenous K+ channels are activated by the expression of foreign peptides [72] and proteins [73]. For instance, expression of CFTR, a human Cl− channel of secretory epithelia induced activation of K+ channels when activated by cAMP [74–76]. Two classes of K+ channels can be classified by their electrophysiological properties, namely delayed rectifier K+ channels and outwardly rectifying K+ channels. Delayed rectifier channels are insensitive to Ba2+ and are activated by depolarization to U > 30 mV yielding highly variable currents between 30 and 400 nA [70,71]. These channels activate Ca2+-independently and can be inhibited by TEA. Outwardly rectifying K+ channels can be activated by acidification-induced depolarization [24] and seem to be closely associated with the Na+/K−/ATPase [77]. This type of K+ channels can only be partially inhibited by Ba2+ [57,78] and are permeable for Ca2+ [78]. It has been shown that these channels exhibit two different binding sites for Ba2+ [79].

3.3.2. Na+ channels

During sustained strong depolarization of the oocyte plasma membrane an inward Na+ current is slowly elicited. The induction of Na+ current involves phospholipase C and mobilization of intracellular Ca ions [80–82]. The Na+ current is sensitive to the Na+ channel blocker TTX (tetrodotoxin) [83,84] although a TTX-insensitivity was reported by one group [85,86]. Na+ currents evoked by the prolonged strong depolarization are carried by a highly selective, low conductance Na+ channel that have been observed in giant patches [87,88] and cut-open oocyte clamp experiments [89]. Once activated, the channels behave like voltage-dependent Na+ channels from excitable tissue. The local anesthetic lidocaine reversibly blocks the channels when they are in their open configuration but not in the closed state [90].

Another Na+ selective conductance is activated by short (<1 s) strong depolarization greater than +40 mV that is different from the Na+ channel conductance activated by prolonged depolarization and is somewhat more unspecific allowing permeation of monovalent cations such as K+, Rb+, Cs+, TMA (tetramethylammonium) and choline [89].

The third Na+ conductance in the oocyte plasma membrane is clearly different from the depolarization activated Na+ conductances and is characterized by its sensitivity to amiloride, a potent blocker of the epithelial Na+ channel ENaC [91]. This conductance is present in every third oocyte, produces whole-cell currents in the range of 10–30 nA and is insensitive to the high affinity amiloride analogue benzamil. This indicates that this Na+ conductance in the oocyte plasma membrane is different from ENaC.

3.3.3. Ca2+ channels

Different Ca2+ channels were described for the first time by Dascal et al. [92] when the authors used oocytes for the heterologous expression of Ca2+ channels. They realized that oocytes are endowed with voltage-dependent Ca2+ channels (VDCC). The experimental setup for studying these channels makes it necessary to substitute Ca2+ for Ba2+ thereby preventing activation of CaCCs and largely eliminating K+ currents; Na+ conductances can be inhibited with low amounts of TTX (usually 100 nM) [93–95]. VDCC currents are usually rather small ranging from 10 to 100 nA [92,94,96], are stimulated by Ins(1,4,5)P3 [97], cAMP [98] and are sensitive to Mn2+ [99]. It has been reported that there are various endogenous VDCCs belonging to the L-type Ca2+ channels [96,98,99], T-type [100], and N-type [100,101].

The second major class of Ca2+ channels in the oocytes is represented by store-operated Ca2+ channels (SOCCs). These channels are activated by elevation of the intracellular Ca2+ concentration evoked by Ins(1,4,5)P3 mediated store depletion [102–108]. Ca2+ release from intracellular stores and subsequent increase in the intracellular Ca2+ concentration in turn modulates Ins(1,4,5)P3 release, thereby contributing to the oscillatory nature of the Ca2+ signaling [109–114]. This Ca2+, released from intracellular stores permits Ca2+ to enter the cell through Ca2+-selective SOCCs in a caffeine sensitive way [115–119]. The Ins(1,4,5)P3 evoked Ca2+ oscillatory system compromises rhythmic activation and inactivation of membrane SOCCs in a concerted cooperation with CaCCs [105,120,121]. Yao and Tsien [122] showed that capacitative Ca2+ entry via SOCCs in the X. laevis plasma membrane behave like Ca2+ release activated Ca2+ channels (CRACs) in mast cells [123]. Activation of SOCC does not involve membrane insertion of additional SOCCs stored in vesicles [124] as reported for other channels such as CFTR Cl− channels [125,126]. Up to now, the molecular identity of the SOCCs is still a mystery.

3.4. Nonselective cation channels (NSCCs)

These channels form a quite heterologous family of channels in the oocyte plasma membrane and it is really difficult, if not impossible to define how they differ from the other ion channels. For proper investigation of NSCCs the Ca2+-activated Cl− channels should be blocked to prevent contamination of the observed currents mediated by the influx of Ca2+ and Mg2+ through NSCCs under physiological conditions [127]. NSCCs can be stimulated by hyperpolarization to membrane potentials lower than −150 mV, intracellular acidification or heterologous expression of foreign proteins and can be clearly distinguished from SOCCs [62,73,127,128]. Furthermore, it was found that NSCC can be activated by different substances including bisphosphonates [129], palytoxin (PTX), the non-peptidic toxin of the blue humphead parrotfish [130] and the marine toxin maitotoxin [131,132]. By measuring membrane capacitance changes that reflect changes in
membrane surface area, we showed that maitotoxin induced the exocytotic insertion of NSCC from the intracellular pools into the plasma membrane [131]. NSCC can be blocked by DIDS and TEA [73], yet as it is also the case for most of the endogenous ion channels, the molecular identity of NSCCs remains a mystery.

3.5. Mechanosensitive cation channels (MSCC)

MSCC, also termed stretch activated cation channels (SAC) activate following mild stretching of the oocyte membrane by gentle suction via a patch pipette [133]. Activation of MSCC represents an increase in channel open probability ($P_o$) without significant changes in channel current or conductance. Originally described by Sakmann et al. [134], MSCC reside in high density in the oocyte membrane with up to $3 \times 10^6$ channels per oocyte [135–137]. Under physiological conditions, i.e. in the absence of membrane stretch and the presence of Ca$^{2+}$, the channels are quiescent [138]. The channels are inhibited by Gd$^{3+}$ (10 μM), high concentrations of amiloride [139–141] and an extracellular Ca$^{2+}$ concentration higher than 10 μM [142]. MSCCs are permeable for a wide range of cations with a selectivity of K$^+$ > NH$_4^+$ > Cs$^+$ > Rb$^+$ > Na$^+$ > Li$^+$ > Ca$^{2+}$ [142]. Extensive studies using patch-clamp recordings revealed three closed states and one open state [138] and demonstrated that MSCCs are present in plasma membrane vesicles and membrane plebs devoid of cytoskeleton structures [143].

MSCC of the oocyte membrane have the ability to adapt to sustained membrane stretch and respond with a time-dependent rundown of the open probability. The channels are not inactivated by this rapid adaptation, rather their sensitivity to mechanical stimuli is reversibly decreased [144–147]. Although, the use of simultaneous recordings of membrane currents and fluorescence-labeled ATP showed that the activation of MSCC triggers the release of ATP from the oocyte [148,149], the physiological role of the ATP release remains to be elucidated. The MSCC mediated influx of Ca$^{2+}$ can subsequently activate the CaCC [150]. MSCCs exhibit volume-sensitivity that is dependent of cytoskeletal structures connected to the plasma membrane and independent of the existence of freely diffusible cytosolic components [151]. The fact that oocyte growth, maturation, fertilization and embryogenesis proceed in the presence of MSCC inhibitors indicates that further developmental processes of the oocytes do not depend on the activity of MSCC [152].

3.6. Water channels

Water transport across membranes is a vital function of many cells. Although water can pass many membranes by diffusion, some membranes are virtually impermeable for water and specific proteins termed ‘water channels’ or ‘aquaporins’ mediate water permeability ($P_l$) of the plasma membrane [153]. For a long time the existence of aquaporins in X. laevis oocytes was a matter of controversial discussions [14,29]. Yet, in 2002 Virkki et al. [154] reported cloning and functional characterization of an aquaporin that belongs to the subfamily of aquaglyceroporins Some other reports describe the existence of aquaporins in oocytes with different functional properties, yet the overall abundance of $P_l$ of the oocyte membrane is rather low [155–157].

4. Primary and secondary active transporters

This family of transport proteins can be further divided into primary active and secondary active systems. Primary active systems are directly driven by the energy stored in the high-energy phosphate bond of ATP and are used to push the ions against their concentration gradients. Secondary active transport systems use ion gradients that are generated by primary active transporters. Most secondary active transporters are driven by Na$^+$ or H$^+$ gradients, yet only Na$^+$-dependent transport systems have been described in the oocytes.

4.1. Primary active (direct) transporters

The most prominent primary active transport system in X. laevis oocytes is the Na$^+$/K$^+$-ATPase, also nicknamed the Na$^+$ pump. The P-type Na$^+$/K$^+$-ATPase ‘pumps’ 3 Na$^+$ out of the cell and 2 K$^+$ into the cell against their electrochemical concentration gradients consuming one molecule ATP per pump cycle. Since the Na$^+$/K$^+$-ATPase is responsible for creating and maintaining the gradients of K$^+$ and Na$^+$ across the plasma membranes of animal cells, it is not surprising that it could be found in all animal cells (excellently reviewed in: [158]). In view of the fact that one net charge (i.e. 1 Na$^+$) is transported per pump cycle, the Na$^+$/K$^+$-ATPase is electrogenic (or rhogenic) and contributes significantly to the membrane potential. Its contribution to the membrane potential in oocytes is about 30%, the rest being mainly a K$^+$-diffusion potential. Conversely, the Na$^+$/K$^+$-ATPase is voltage dependent being more active at more negative membrane potentials [159,160]. The minimal functional unit of the pump is a heterodimer membrane molecule consisting of α- and β-subunits [161]. The α-subunits contain binding sites for ATP and Na$^+$ on the cytosolic side while the binding sites for K$^+$ are located at the external side of the molecule. The glycosylated β-subunits are located outside the α-subunits, yet the exact functions of the β-subunits are still matter of discussions.

The Na$^+$/K$^+$-ATPase is an allosteric protein with two alternative conformations, namely E1 and E2 [162] with the E1 conformation being open to the inside of the cell and high affinity for Na$^+$, whereas E2 opens to the extracellular space with high affinity for K$^+$. Na$^+$-triggered phosphorylation of the pump by MgATP stabilizes the protein in the E2 conformation, while K$^+$-triggered dephosphorylation stabilizes the pump in the E1 form. A transport cycle involves initial binding of intracellular Na$^+$ to the E1 form triggering phosphorylation of the α-subunit by MgATP, resulting in a conformational transformation from E1 to E2. This conformational change moves Na$^+$ through the membrane to the extracellular space, where they are released into the extracellular space. K$^+$ binding sites are now exposed to the outside of the cell and the α-subunits are then occupied by extracellular K$^+$. Binding of K$^+$ triggers dephosphorylation of the α-subunits and drives another conformational change that moves K$^+$ through the membrane to the inner surface, where the K$^+$ are released into the cytosol leaving the pump ready for the next cycle. Cardiac glycosides, such as ouabain, digoxine and digitoxine are potent inhibitors of the Na$^+$/K$^+$-ATPase binding to special sites on the α-subunits [162]. Another inhibitor of the Na$^+$/K$^+$-ATPase is tetraethylammonium that blocks the pump in a voltage-dependent manner [163]. The serum and glucocorticoid-dependent kinase SGK1 was reported to initiate translocation of preformed pump molecules from intracellular pools into the plasma membrane upon physiological needs [164]. When the oocyte undergoes progesterone induced maturation major parts of the pump molecules are removed from the plasma membrane and internalized in the intracellular stores [165], a process that can also be induced by activation of protein kinase C [8].

The Na$^+$/K$^+$-ATPase seems to be the only prominent primary active transport system in the oocyte plasma membrane although one report suggested the existence of a Ca$^{2+}$-ATPase in the oocyte membrane [166]. Yet, a functional proof for the existence of these Ca$^{2+}$-ATPases in the oocyte plasma membrane is missing.

4.2. Secondary active (indirect) transporters

The Na$^+$/K$^+$-ATPase of the oocytes creates and maintains a Na$^+$ gradient that energizes a subpopulation of secondary active trans-
port systems in the oocyte plasma membrane. Na\(^{+}\)-dependent cotransport systems for glucose and amino acids are the best studied secondary active transport systems in the oocytes. The Na\(^{+}/\)glucose cotransporter in *X. laevis* oocytes has a stoichiometry of 2 Na\(^{+}\) and 1 glucose molecule [20] and can be clearly distinguished from Na\(^{+}/\)glucose cotransporters from other species, e.g. from rat small intestine [167]. The Na\(^{+}/\)glucose cotransporter in the oocyte membrane is highly specific for glucose (the structure isomer mannose is not accepted as a substrate) and is specifically blocked by phloretin with a \(K_{i}/2\) of 1.2 \(\mu\)M [20]. The Na\(^{+}/\)glucose cotransporter is strongly potential-dependent and is downregulated by the matura-
tion-induced depolarization. Yet, up to now it is not known whether the Na\(^{+}/\)glucose cotransporter in the oocytes mediates simultaneous uptake of water molecules (>250) per molecule glu-
cose as described for the human Na\(^{+}/\)glucose cotransporter SGLT1 [168].

There are several Na\(^{+}\)-dependent cotransport systems for the uptake of amino acids extensively reviewed by Van Winkle [169]. The uptake of alanine is mediated by an electrogenic and volt-
age-dependent Na\(^{+}/\)alanine cotransporter that is downregulated 
simultaneous uptake of water molecules (>250) per molecule glu-
cose as described for the human Na\(^{+}/\)glucose cotransporter SGLT1 
[168].

5. Concluding remarks

The use of *X. laevis* oocytes as an expression system for the char-
acterization of pharmacologically interesting proteins including ion 
channels and receptors has been increased dramatically in the last 10 years. And especially since the automated injection 
and measure systems such as OpusXpress™ or Robocyte™ became 
available, high throughput screening of computer 
designed compound libraries has become possible [6,180,181]. These new technologies and interests make the knowledge of the 
endogenous transport systems absolutely mandatory. These new technologies and interests make the knowledge of the 
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