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Review

Ion currents of *Xenopus laevis* oocytes: state of the art

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1. Introduction

Oocytes and eggs of the South African clawed toad *Xenopus laevis* have been extensively used in biological and pharmacological research. These cells are excellently suited for investigations on questions of developmental biology, intracellular signaling cascades, biochemical pathways and transport-related phenomena. Furthermore, several expression systems have been developed, the most favored of which is based on injection of mRNAs or DNAs into *X. laevis* oocytes [1]. When the translated mRNA produces a receptor or a transport protein, it is usually biochemically functional, exhibiting the appropriate pharmacological and electrophysiological properties. However, the oocyte itself is equipped with a whole orchestra of transport systems. This makes the oocyte a suitable model to investigate transport processes on a cellular level. An undisputed advantage of the oocyte is the easiness how the cells can be handled. Oocytes are available at any time within 4–5 h of preparation and do not need elaborate cell culture conditions such as absolutely sterile handling or expansive culture media. The experimenter further profits by the possibility to combine several experimental techniques on a single cell. These advantages of the oocyte led to a tremendous increase in the knowledge about the endogenous transport systems of the oocyte within the last 20 years.

In this review I will focus on the endogenous ion channels of the *X. laevis* oocyte. Since oocytes are increasingly used for the heterologous expression of receptors, transport proteins and ion channels, it is inevitable to know whether the oocytes possess endogenously the protein to be expressed in order to exclude possible distortions. In 1987 Dascal [2] thoroughly reviewed endogenous ion channels of the oocytes. Since then numerous additional ion channels have been discovered and nearly every month new reports on hitherto unknown properties of endogenous ion channels emerge. Interestingly, none of the endogenous ion channels of the *X. laevis* oocyte has been cloned to date. The present overview summarizes the very recent advancements in endogenous ion channels of *X. laevis* that were reported since the comprehensive review by Dascal [2]. Excellent reviews in recent years have discussed related features. The reader is referred to these reviews for areas that are not covered in detail here. Because of space limitations only ion channels from defolliculated oocytes are dealt with and ion channels of follicle-enclosed oocytes are not considered. Furthermore, the given literature is exemplary and I apologize to all colleagues whose work could not be cited.

2. The biology of the oocyte

2.1. Oogenesis and development

Oogenesis in different species is varying, although the fundamental stages are similar. In *X. laevis* oogenesis, namely growth from stage I to stage VI [3], is asynchronous, meaning that all stages of growth are usually found in the ovary at a given time [4]. The most obvious differences between oocytes from stage I and stage VI are their size (100 μM and 1300 μM, respectively) and pigmentation which ranges from colorless to marked polarization into the dark animal hemisphere and the beige vegetative hemisphere. In stage VI oocytes the two hemispheres are divided by an essentially unpigmented equatorial band. The process of oogenesis requires approximately 8 months. Once stage VI is reached, the oocytes do not continue to increase in size but remain at this point for some time before undergoing atresia (death and resorption of the oocytes). During the oogenesis oocytes accumulate ribosomes, yolk, glycogen, lipids and 'maternal' mRNA, the latter becoming responsible for the regulation of protein synthesis and the early embryonic development in later developmental phases [5,6]. Like most vertebrates, *Xenopus* oocytes are physiologically arrested at the G_2/M border in the first meiotic prophase.

A follicular layer consisting of about 5000 cells surrounds each oocyte [7]. Release of gonadotropin stimulates follicle cells to produce progesterone [8]. The prophase block is lifted in response to mitogenic stimulation by insulin, progesterone or IGF_1. Entry into meiosis II is signaled several hours later by germinal vesicle breakdown (GVBD) [9]. Maturation can be achieved in vitro by incubating oocytes with progesterone (10 μM for 30 min) or in vivo by injecting subcutaneous human chorionic gonadotropin [10].

During maturation the bioelectrical properties of

the oocyte membrane change profoundly. The membrane potential (U_m) depolarizes drastically from values around −50 mV (see also Table 1) to approximately −10 mV. This depolarization causes downregulation of ion channels and cotransport systems in the membrane by impairing the driving force for the voltage-dependent carriers [11]. The Na^+/K^+-ATPase is mostly inactivated by internalization thereby contributing further to membrane depolarization [10]. Only some transport systems remain active in maturated oocytes, now called eggs, such as the Ca^{2+}-inactivated Cl^- channel [12]. Eggs pass through the oviduct and are surrounded by a proteinaceous layer, called 'jelly coat', that is essential for fertilization [5,13,14]. The jelly eggs are shed into hypoosmotic pond water (as low as 10 mosm) immediately prior to fertilization. The hypotonic shock initiates partial recovery of the membrane voltage (to about −30 mV), a necessary prerequisite for insemination [15]. Once a sperm has entered the cell, the membrane again depolarizes (to values >+5 mV), triggered by the acrosome reaction [16]. The depolarization occurs within 3 s of insemination and is induced by intracellular Ca^{2+} waves that activate Cl^- channels. This fertilization potential is thought to prevent polyspermy [17]. The further development until the blastula stage is completed under appropriate environmental conditions within 4 h [18].

2.2. Bioelectrical properties of the oocyte

For the most electrophysiological, pharmacological and biochemical purposes oocytes of stages V and IV are used. These cells have a diameter of 1–1.3 mm and can be easily handled with Pasteur pipettes the sharp end of which has been fire polished (for a very informative description of procedures and techniques refer to [19,20]). Usually, the experimental design requires the removal of the follicle cells which can easily be achieved by treatment with collagenase followed by washing for 10 min in Ca^{2+}-free Ringer. However, if only a few oocytes are needed, manual defolliculation with small forceps could be the method of choice. Since follicle cells and the oocyte plasma membrane are connected via gap-junctions, between defolliculation and electrophysiological experiments the oocyte should be given approximately 1 h of recovery. After defolliculation the oocyte plasma membrane is still covered by a proteinaceous vitelline layer that has been secreted by the follicle cells. For patch-clamp experiments the vitelline layer has to be removed by placing the oocytes in hypertonic solution [20,21].

Table 1
Membrane potential of defolliculated oocytes

Membrane potential (mV)	Ref.
−27	[217]
−41	[218]
−44	[54]
−50	[195]
−47	[219]
−59	[220]

The oocyte plasma membrane is highly folded into macro- and microvilli, thereby increasing the apparent surface area by a factor of 4. The actual surface area is reportedly between 18 mm^2 [22] and 20 mm^2 [1] whilst capacitance measurements revealed values of 230 nF [23] and 220 nF [24–26]. The oocyte membrane has a high input resistance that differs with oocyte batches and ranges from several 100 kΩ to 2 MΩ and sometimes even more [27].

Membrane potentials (U_m) of oocytes from a given batch have been shown to be similar, whilst a great variance exists between different batches ranging from −30 to −70 mV, rarely −90 mV can be seen. From our experience, U_m are most of the time around −55 mV. U_m is mainly a K^+ diffusion potential, yet the Na^+/K^+-ATPase contributes significantly to U_m [28]. Table 1 summarizes U_m reported from several groups. However, it has to be noticed that only fine electrode tips and proper electrode impaling guarantee that the plasma membrane recovers completely after puncture and the prick potential (i.e. U_m measured in the moment of inserting the first electrode) is reached again. Reports that the prick potential cannot be reached after electrode insertion (e.g. [2]) seem to come from improper handling. Some groups also reported seasonal variations in U_m [1,21].

Table 2 compiles data on intracellular ionic concentrations. In general, the intracellular ion concentrations tend to be similar in oocytes obtained from the same donor on a given day, but to vary between batches of oocytes from different females. Different equilibrium potentials (E_x) for the three main inor-

Table 2
Intraoocyte concentrations of the most important ions

Ion species	Intraoocyte concentration (mM)	Ref.
Na^+	23	[217]
	6	[221]
	10	[218]
	4	[220]
	9	[101]
K^+	148	[217]
	92	[221]
	110	[218]
	117	[222]
	76	[101]
Cl^-	62	[217]
	33	[221]
	38	[218]
	50	[195]
	54	[222]
	24	[101]
Ca^{2+}	3×10^{-6}	[223]
	30×10^{-6}	[224]
	$50–90\times10^{-6}$	[145]
	400×10^{-6}	[191]
Mg^{2+}	>0.5	[191]

ganic ions (i.e. Na^+, K^+, and Cl^-) have been reported (Table 3). The discrepancies might stem from different estimations of the respective intracellular ion concentration. Therefore, means for the respective intracellular ion concentration were calculated from Table 2 and the expected range for E_x of the three main ions was estimated using the procedure described in detail by Begenisisch [29]. The values are given in the most right column of Table 3.

2.3. The oocyte at work

The oocyte system allows performing a whole plethora of different techniques for the investigation of ion channels and transporters; even several methods can be applied to a single cell. Electrophysiological techniques comprise two-electrode voltage-clamp [30], cut-open technique [31], patch-clamp in several modes [19–21] including giant patch [32], continuous capacitance measurements to study exo- and endocytotic processes [25,26], intracellular ion measurements with selective electrodes [33], noise analysis [34], pressure clamp [35] and the glass funnel technique [36]. Other techniques that can be applied to the oocyte system include optical [37] and tracer [38] efflux measurements, tracer influx measurements [39], the concentration jump technique [40], confocal microscopy [41], video imaging techniques [42], binding assays [43], gravimetric techniques [44] and volume measurements [45]. If the oocytes are handled properly (i.e. without damaging the membrane) and stored at a constant temperature they can be used for experiments as long as 10–15 days. We found that the best culture temperature is 14°C either for oocytes expressing foreign proteins or for oocytes used for the investigation of endogenous proteins. It is also important to screen the oocytes daily, to remove non-healthy looking or damaged oocytes and to change the culture medium every day.

2.4. Disadvantages of the oocyte system

Although the above-described advantages of the oocyte system predominate over the disadvantages, the major drawbacks of the oocyte should not be concealed. Some laboratories observed seasonal variations of the oocyte quality [28,46], sometimes with periods of fruitless experiments [47]. It also seems that the membrane potential of the oocytes could vary seasonally [1,21]. Results obtained from oocytes of different females might show great variance. Moreover, it is possible that a given endogenous transport system could be measured easily in one batch of oocytes, but might be nearly undetectable in the next. Variations in the expression of endogenous ion currents are observed only sporadically and might be genetically determined; however, plausible explanations for these phenomena are still missing. Fortunately, problems with detection of endogenous

Table 3
Equilibrium potentials for the main inorganic ions

Equilibrium potential	mV	Ref.	Calculated[a] (mV)
E_{Na^+}	46	[217]	47 to 63.5
	61	[218]	
E_{K^+}	−108	[217]	−86.7 to −92.4
	−95	[218]	
E_{Cl^-}	−14	[217]	−16.4 to −23.1
	−28	[218]	

[a]Calculated according Begenisich [29] for a given extracellular concentration (in mM) for $Na^+ = 90$, $K^+ = 3$ and $Cl^- = 95$ based on the intracellular concentrations summarized in Table 2 (means ± S.E.M.; in mM) for $Na^+ = 10.4 \pm 3.3$, $K^+ = 108.6 \pm 12.2$ and $Cl^- = 43.5 \pm 5.8$ ($T = 20$°C).

ion channels arise only for transporters and channels that exhibit rather low activities [11,48,49]. Very rarely it can also be observed that the oocytes of a given batch perish of unknown reasons within 1 or 2 days making measurements impossible. Despite these restrictions, the oocyte system is an extremely suitable model for studying endogenous ion channels and represents an almost unsurpassable expression system.

2.5. The oocyte as an expression system

Although the *X. laevis* oocyte is endogenously equipped with a host of ion channels, transport systems and receptors, it has been proven to be an excellent heterologous expression system for the investigation and characterization of countless transport proteins and receptors (for review see [1]). Fortunately, heterologously expressed ion channels can be clearly distinguished from the endogenous ones. Most of the time, currents produced by the endogenous ion channels are small compared with the currents produced by the expressed channels. In other cases the pharmacological profile of the foreign channel is different from that of the endogenous system. Nevertheless, a comprehensive knowledge of the endogenously occurring ion currents might be helpful to avoid confusion and misinterpretation when expressed channels are characterized.

3. Anion channels

Beside non-selective cation channels (see below), anion channels represent the majority of the ion conductances housed by the oocyte plasma membrane. Although the anion channels possess the ability to conduct organic anions, the lion's share of the anion current is mediated by Cl^- being present in comparatively high concentration within the oocytes (see Table 2). The several classes of Cl^- channels (summarized in Table 4) are introduced and discussed in the following.

3.1. Hyperpolarization-activated Cl^- channels (Cl^-_{hyp})

Oocytes of some but not all donors responded upon hyperpolarization (more negative than −100 mV) with openings of Cl^-_{hyp} [50]. This response was independent of Ca^{2+} and varied greatly in amplitude in oocytes obtained from different animals. The Cl^-_{hyp} currents were around 200 nA in average, activated slowly and did not inactivate in most of the oocytes [51]. Cl^-_{hyp} was not highly selective for Cl^- but accepted several anions in the following selectivity sequence: $I^- > NO_3^- > Br^- > Cl^- >$ propionate > acetate with ratios of 2.5:2:1.4:1:0.35:0.17 [51]. The authors showed further that Cl^-_{hyp} was sensitive to Ba^{2+} and 4-acetamido-4′-isothiocyanatostilbene-2-2′-disulfonic acid (SITS) but insensitive to 4,4′-diisothiocyanatostilbene-2-2′-disulfonic acid (DIDS) and H^+. A member of the vasoactive intestinal peptide (VIP) family, namely the pituitary adenylate cyclase activating polypeptide (PACAP), activated Cl^-_{hyp} [52].

Table 4
Endogenous Cl^- channels

Channel property	Ref.
Hyperpolarization-activated	[50–52]
Volume-sensitive	[53–55,60,62]
Ca^{2+}-activated Cl^- channels	[69]
Asymmetric distribution	[70–73]
Biphasic current	[75,76]
Activation	[77–86]
Activation by foreign proteins	[88–91]
Inhibition	[75,76,92,93]
Different classes of CaCC	[94–98]
Halide channel	[99]

3.2. Volume-sensitive Cl^- channels (Cl^-_{vol})

In most cell types volume regulatory mechanisms have evolved that involve the activation of ionic pathways in order to restore the original volume of the cells after hypotonic challenge. Chen at al. reported that hypotonic solutions caused a twofold increase in the Ca^{2+}-activated Cl^- outwardly rectifying current [53]. However, in a thorough study Ackerman et al. [54] gave conclusive evidence that hypotonic challenge actually induced activation of a Ca^{2+}-independent, volume-sensitive Cl^- channel (Cl^-_{vol}) that could be clearly distinguished from Ca^{2+}-activated Cl^- channels (CaCC; see below). Cl^-_{vol} was only inducible when oocytes were defolliculated manually; however, it was detectable in 99% of the oocytes of different animals contrary to the low

detection rate of only 4% reported earlier [55]. The anion conductivity sequence of Cl^-_{vol} was SCN^- (1.43) > I^- (1.11) ≥ NO_3^- (1.08) = Br^- (1.07) > Cl^- (1) > methylsulfonate (0.67) ≥ HCO_3^- (0.65) ≥ acetate (0.61) > gluconate (0.45) > glutamate (0.38). Cl^-_{vol} could be blocked reversibly by 5-nitro-2-(3-phenylpropylamino)benzoic acid (NPPB), DIDS, SITS and cAMP, but was surprisingly inhibited irreversibly by Gd^{3+} and La^{3+}, while niflumic acid (NFA) had no inhibitory potency. Beside other features, the sensitivity to La^{3+} and the insensitivity to NFA clearly distinguishes Cl^-_{vol} from CaCC. The anion selectivity of Cl^-_{vol} reported by Ackerman et al. [54] coincided with that reported from human airway epithelium [56,57] and differed from that reported for the cystic fibrosis transmembrane conductance regulator (CFTR) [58] and the voltage-activated Cl^- channel [59]. Cl^-_{vol} was also shown to be responsible for the regulatory loss of organic osmolytes upon hypotonic challenge [60].

Paulmichl et al. [55] reported expression cloning of a protein that they considered to be the volume-sensitive Cl^- channel (pI_{Cln}). However, in a following study Krapivinski et al. [61] revealed that pI_{Cln} is not an integral membrane protein but an abundant cytoplasmic protein that is a key component of Cl^-_{vol} activation. Since then, a whole plethora of reports emerged demonstrating that pI_{Cln} is not the Cl^-_{vol} but a regulating factor [62–67], while only one group insists on the opposite [68]. Therefore, successful cloning and sequencing of Cl^-_{vol} has to be awaited until comparison with other Cl^- channels will be possible.

3.3. Ca^{2+}-activated Cl^- channels (CaCC)

Originally described by Miledi in 1982 [69], Ca^{2+}-activated Cl^- channels belong to the best investigated ion channels of the oocyte, being present in high numbers and detectable in nearly every oocyte. They are not distributed equally over the oocyte membrane, but are concentrated in the animal (dark) hemisphere [70–72], thereby creating a functional polarization that is important for further developmental events. Clustering of CaCC was further investigated by Parekh [73] who showed that CaCC interacted with store-operated Ca^{2+} channels (SOCC, see below) and that redistribution of the channels induced by uncoupling from the cytoskeleton reduced the CaCC currents.

Activation of CaCC is achieved by elevations of $[Ca^{2+}]_i$ and is independent of external Ca^{2+} [74]. Increase in $[Ca^{2+}]_i$ resulted in a typical biphasic current response with an initial fast peak current followed by rapid decay and subsequent slower decaying current [75,76]. Beside activation through $[Ca^{2+}]_i$, CaCC could be stimulated by several other mechanisms. Rapid cooling [77] and application of rabbit serum to the animal pole [78] evoked CaCC currents. Maitotoxin (MTX), an activator of Ca^{2+} influx and nonselective cation channels, could also stimulate CaCC [79]. Other activators of CaCC act presumably via increase of $[Ca^{2+}]_i$ including chlorpromazine (CPZ) [80], AlF_4^- [81], hyaluronan [82], cytidine-5′-diphosphate-D-glucose (CDPG) [83], trypsin [84], and lysophatidic acid [85]. Activation of CaCC by H_2O_2 was abolished after blocking the endogenous Na^+/Ca^{2+} exchanger by 2′,4′-dichlorobenzamil, $MgCl_2$, $CdCl_2$ or $NiCl_2$ [86].

As also reported for other endogenous ion channels of the oocyte, CaCC could be modulated by expression of foreign proteins. While a heterologously expressed human Cl^- channel, ClC-5 [87], activated CaCC [88,89], expression of annexin [90] or cystic fibrosis transmembrane conductance regulator Cl^- channel (CFTR) [91] suppressed endogenous CaCC. Interestingly, the most common cystic fibrosis causing CFTR mutation ΔF508, characterized by loss of Cl^- channel function, showed also inhibition of CaCC, demonstrating that despite the impaired Cl^- channel function ΔF508-CFTR is capable of regulatory influences on other proteins.

As expected, CaCCs are blocked by several 'typical' Cl^- channel blockers, such as anthracene-9-carboxylic acid (9-AC, $K_{1/2}$ = 110 μM), NFA ($K_{1/2}$ = 17 μM), flufenamic acid (FFA, $K_{1/2}$ = 28 mM) and NPPB ($K_{1/2}$ = 22 μM for the fast component and 68 μM for the slow component) [76,92]. Blocker potency for all these blockers was voltage-dependent. Inactivation of CaCC occurred after Ca^{2+}-triggered activation of protein kinase C (PKC) that could be abolished by the PKC inhibitor H-7 [75]. Interestingly, only Ca^{2+} entering the cell via SOCC (see below) could initiate this inactivating process, whereas Ca^{2+} arriving at the membrane from the inside of the cell could not start this mechanism. CaCC displayed

an average open lifetime of typically around 100 ms [93].

To this point we have assumed that there is one homogenous population of endogenous CaCC. However, controversial reports on the existence of several different CaCC species in the oocyte emerged. Boton et al. proposed the existence of two CaCC with different sensitivities to Ca^{2+} and distinct kinetic and inactivation properties [94]. This was confirmed and extended in a series of papers by the group of Hartzell [95–97]. The authors differentiated a subset of channels that were exclusively activated by store released Ca^{2+}, whereas the second class of CaCC is activated by Ca^{2+} that entered the cell from the outside. Both channels were most abundant on the animal pole of the oocyte. However, in a recent, thorough investigation Kuruma and Hartzell [98] identified a third Ca^{2+}-activated current following injection of inositol trisphosphate ($Ins(1,4,5)P_3$). As discussed in detail by the authors it remains furthermore an open question whether all these observed CaCC are indeed mediated by distinct proteins or whether one class of channels has the ability to operate in several modes.

For completeness, a halide conductance of the oocytes should be mentioned whose nature is obscure [99]. The selectivity sequence of the oocytes' halide uptake (i.e. $I^- > Br^- > Cl^-$) resembled that reported from epithelial cells housing CFTR. Also with regard to the Cl^- channel blocker sensitivity the endogenous halide transporter seems similar to CFTR. However, it was reported that cAMP had no effects on oocyte current demonstrating the absence of any endogenous CFTR-type Cl^- conductance [25,26, 100].

4. Cation channels

4.1. K^+ channels

The oocyte membrane potential (U_m) is mainly determined by the permeability of the plasma membrane to K^+ with minor contributions of Na^+ and Cl^- conductances. It should be mentioned that the Na^+/K^+-ATPase contributes significantly to U_m with an ouabain-sensitive current [28]. Consequently, several endogenous K^+ channels in the oocyte plasma membrane have been described that are summarized in Table 5. A common feature of all endogenous K^+ channels is their sensitivity to quinine, a somewhat unspecific ion channel blocker [101].

One class of K^+ channels in oocytes have been characterized as delayed rectifier K^+ channels that could be distinguished from other K^+ channels by their insensitivity to Ba^{2+}. These channels were activated by depolarization to $U_m > 30$ mV. The peak current produced by these channels proved to be highly variable between oocytes of different animals and ranged from 30 to 400 nA at 30 mV [102]. The channel showed voltage-dependent slow inactivation and was further sensitive to quinine. Beside its Ca^{2+} independence, the K^+ channel was only fairly sensitive to the K^+ channel blockers tetramethylammonium (TEA) and 4-aminopyridine (4-AP) but insensitive to charybdotoxin (CTX) and apamin. Parker and Ivorra [103] examined oocytes obtained from a single donor frog which showed prominent outward currents up to 700 nA at 50 mV. While the current was also independent of Ca^{2+} and insensitive to Ba^{2+} and 4-AP, it was completely inhibited by TEA. However, these somewhat contradictory results do not necessarily prove the existence of two classes of Ba^{2+}-sensitive K^+ channels in the oocyte membrane but could have their origin in the different treatment of the oocytes. Lu et al. [102] kept the oocytes for several hours in Ca^{2+}-free media, thereby seriously influencing the intracellular ion concentrations (see Section 5).

While Ba^{2+}-insensitive K^+ currents could be de-

Table 5
Endogenous K^+ channels

Channel property	Ref.
Ba^{2+}-insensitive	
Insensitive to TEA	[102]
Sensitive to TEA	[103]
Ba^{2+}-sensitive	
H^+-sensitive	[33]
Pump-associated	[104]
ISDN-sensitive	[106]
Partial Ba^{2+} block	[108,110]
Ca^{2+}-activated	[109]
Volume-sensitive	[107]
Stimulation by expression of other proteins	
IsK-stimulated	[111]
CFTR-stimulated	[114,115]

tected only sporadically in the oocytes, far more is known on slightly outwardly rectifying Ba^{2+}-sensitive K^+ channels that are likewise sensitive to TEA. Burckhardt et al. [33] observed an acidification-induced depolarization and a corresponding pH-sensitive outward current that they attributed to a K^+ conductance. Further investigations showed a Ba^{2+}- and H^+-sensitive K^+ current. This Ba^{2+}-sensitive current seems to be closely associated with the Na^+/K^+-ATPase [104]. The activity of the channel 'followed' the activity of the Na^+ pump. Beside a saturable inhibitory component affecting the K^+ outward current, TEA generated a non-saturating inward current, which appeared to be a Ba^{2+}-sensitive electrodiffusion of TEA at positive U_m. However, the effects of TEA on the Na^+/K^+-ATPase which is also directly inhibited by TEA [105] were not tested in this study.

Isosorbide dinitrate (ISDN) is an organic nitrate like nitroglycerine that is needed for cellular synthesis of nitric oxide (NO). Busch et al. [106] studied the effects of ISDN on the endogenous K^+ channels. They reported inhibition of the endogenous K^+ channels by ISDN and showed that the effect was not additive to Ba^{2+} inhibition. However, since other NO donors such as isosorbide mononitrate (ISMN) and *S*-nitrocysteine (SNOC) had no effect on the K^+ current, the observed inhibition was independent of the intracellular second messenger NO. Very recently it was shown that the ISDN-sensitive K^+ current could also be activated by hypoosmolality [107].

Depolarization-induced K^+ channels exhibited a time-dependent and a time-independent component and were active in the presence of La^{3+} [108]. In this study, K^+ channels showed only partial inhibition by Ba^{2+}. K^+ channels were activated by increasing $[Ca^{2+}]_i$ after pool depletion and were possibly permeable for Ca^{2+} [109]. It is only recently that endogenous K^+ channels have been shown to possess two different binding sites for Ba^{2+}, one being in the external vestibule of the channel and the second one lying within the channel [110]. The authors came to this conclusion from their findings that the block of the K^+ peak current was voltage-independent while the late current after 80 ms was both time- and voltage-dependent.

Endogenous K^+ channels seem to be responsive to the expression of foreign proteins. Ben-Efraim et al. [111] used small peptides derived from the carboxyl or amino termini of the so-called small K^+ channel (I_sK). Injection into the oocytes resulted in an activation of Ba^{2+}-sensitive endogenous K^+ channels. From these findings the authors concluded that I_sK is a member of a family of short bitopic membrane proteins that are capable of regulating endogenous K^+ channels. However, as also discussed below, it could be shown that a wide variety of (non-channel) proteins are able to activate currents in the oocyte and that endogenous K^+ channels are not exclusively activated by other K^+ channel fragments [112]. Activation seems to be only dependent on the amount of expression rather than the nature of the expressed proteins. The CFTR is basically a Cl^- channel, yet it becomes more and more clear that its regulatory functions on other transport systems might excel the Cl^- transport function [113]. Injection of CFTR cRNA and subsequent stimulation with cAMP activated not only Cl^- currents but also a Ba^{2+}-sensitive K^+ channel [114]. The authors concluded that CFTR may have the capability to endow cAMP sensitivity to an endogenous K^+ channel. This would imply a regulatory function of CFTR as a transducer of the signal from cAMP-dependent protein kinase to alter ion channel activity. Mall et al. [115] observed activation of an I_sK-type K^+ current in oocytes after expression of CFTR that was sensitive to Ba^{2+}, TEA and chromanol 293B and could be activated by cAMP. A similar cAMP- and chromanol 293B-sensitive K^+ conductance has also been described in rat colonic mucosa [116].

4.2. Na^+ channels

Oocytes are endowed with several classes of Na^+ conductances (compiled in Table 6) that are similar to those found in nerve and muscle cells. While one class of Na^+ channels was insensitive to the Na^+ channel blocker tetrodotoxin (TTX) [23,117], another Na^+ current, induced by long lasting depolarization to U_m beyond -40 mV, was characterized by its sensitivity to TTX ($K_{1/2} < 10$ nM). Veratrine, known to increase Na^+ permeability of excitable membranes prolonged the TTX-sensitive Na^+ current in oocytes [118]. The Na^+ current showed broad variations between oocytes from different animals and it could be possible that genes encoding different Na^+ channels

Table 6
Endogenous Na^+ channels

Channel property	Ref.
Depolarization-induced	
Insensitive to TTX	[122,123]
Sensitive to TTX	[118,119]
$[Ca^{2+}]_i$-sensitive	[125,127]
MgATP-prevented inactivation	[120,121]
NH_4^+-induced	[101,128]
Amiloride-sensitive	[49]
ATP-activated	[131]

may be expressed in oocytes from different donors. While all oocytes of a given Na^+ current-positive donor frog exhibited the Na^+ current, only in oocytes of 5% of all donors investigated for this study the Na^+ current could be found [119]. The Na^+ channel reached a maximal current amplitude of 280 nA at $U_m = -10$ mV and a reversing potential between 40 and 50 mV. Channel inactivation could be described by two exponentials with τ values of 2 and 15 ms [119]. While the Na^+ conductance could not be detected using conventional patch-clamp methods, the giant patch technique proved to be successful [120,121]. Channel inactivation in excised giant patches could be prevented by cytosolic MgATP but not by Mg^{2+} or ATP alone. It could be shown that the Na^+ conductance had a single-channel conductance below 1 pS awaking doubts whether the conductance is a 'real channel' or an unspecific Na^+ permeability of the membrane [121].

Treatment with the PKC activators 12-myristate 13-acetate (PMA), oleyl-acetyl-glycerol (OAG) or a synthetic structural diacylglycerol (DAG) analogue resulted in enhanced depolarization-induced, TTX-insensitive Na^+ currents [122,123]. The antibiotic neomycin, known to prevent the phospholipase C (PLC) mediated production of DAG, reduced the Na^+ current significantly. From these data the authors proposed a possible model of Na^+ channel activation in the oocytes: sustained depolarization of the oocyte plasma membrane could trigger the activation of PLC that catalyzes the hydrolysis of $Ins(4,5)P_2$ and promotes the generation of DAG. Subsequent activation of PKC by DAG leads to phosphorylation of silent Na^+ channels or closely related regulatory proteins thereby activating the Na^+ current. These considerations were further supported by the observation that direct inhibition of PKC by staurosporine and tamoxifen caused essential reduction of the Na^+ current. In light of this elegant model former speculations on the direct effect of U_m on channel structure as the main mechanism for the induction of the current [124] seem unattractive.

Bossi et al. [125] investigated the role of Ca^{2+} on the depolarization-induced Na^+ channel in the oocytes. They found that the Na^+ channels were independent on $[Ca^{2+}]_o$ but were regulated by $[Ca^{2+}]_i$. Increasing $[Ca^{2+}]_i$ by injection of $Ins(1,4,5)P_3$ to release Ca^{2+} from intracellular stores led to potentiated induction of Na^+ channels while chelating intracellular Ca^{2+} with EGTA decreased the Na^+ current activity. Activation of Ca^{2+}-activated Cl^- channels by injection of the poorly hydrolyzed D-3-deoxy-3-fluoro-*myo*-inositol 1,4,5-trisphosphate (3-F-$InsP_3$) also activated a Na^+ current with an apparently slowly inactivating behavior [126]. These findings were confirmed very recently by Charpentier and Kado [127]. In their paper the authors showed that Ca^{2+} release from $Ins(1,4,5)P_3$-sensitive stores was needed to produce the depolarization-induced activation of the Na^+ channel.

Addition of NH_4Cl to the oocyte bathing solution resulted in decreased membrane resistance and increased conductance [101,128]. Burckhardt et al. attributed these changes to a NH_4^+-induced Na^+ inward current that was sensitive to the non-steroidal anti-inflammatory drugs FFA and NFA, but insensitive to the K^+-sparing diuretic amiloride, indicating that an epithelial-type Na^+ channel was not involved. The authors proposed that NH_4^+ caused depolarization of the membrane thereby activating Na^+ channels that were also permeable for NH_4^+. Similar effects were evoked by the local anesthetic procaine [129]. However, it could not be excluded that the positively charged protonated form of procaine entered the cell and triggered the observed effects.

Recently, Weber et al. [49] reported the existence of another class of Na^+ channels that were active at resting U_m and needed no depolarization to be activated. In a combination of voltage-clamp and $^{22}Na^+$-influx experiments the authors showed that the Na^+ conductance being present in oocytes of every third animal was amiloride- and phenamil-sensitive. Surprisingly, the amiloride analogue benzamil failed to

inhibit the perfectly selective Na^+ channel. This unique pharmacological profile demonstrated that the amiloride-sensitive Na^+ conductance in the oocytes is different from the epithelial Na^+ channel (ENaC) that is inhibited by benzamil with even higher affinity [130].

In 1992 Kupitz and Atlas [131] published a paper that became highly controversial in the following. They reported that oocytes responded with large Na^+ inward currents to extracellular application of ATP in high concentration (i.e. 3 mM). The putative Na^+ current was inhibited by amiloride, GTP and non-hydrolyzable analogues of GTP. From the observation that GTP and amiloride inhibited fertilization they constructed the following model: ATP is released in high amounts from the sperm and binds to a receptor in the egg membrane, thereby promoting the activation of Na^+ channels. However, as also pointed out by Nuccitelli and Ferguson in a convincing comment to the paper [132], Kupitz and Atlas [131] failed to demonstrate that mature eggs in fact exhibit ATP-sensitive Na^+ channels. Moreover, Kupitz and Atlas [131] implied that an increase in Na^+ permeability occurs at fertilization. However, this does not occur in frog eggs where the sperm activates Ca^{2+}-gated Cl^- efflux that provides a block to polyspermy [15]. Furthermore, it seems implausible that a sperm secretes micromolar amounts of ATP during a period in which it is in urgent need of this energy source to find its way through the 0.5 mm thick jelly coat surrounding the egg. Therefore, the conclusions of Kupitz and Atlas [131] have to be considered with appropriate caution.

4.3. Ca^{2+} channels

To study Ca^{2+} channels in oocytes, Ca^{2+} is usually replaced by Ba^{2+} since there is no inactivation of Ca^{2+} channels by Ba^{2+}. On the other hand, Ba^{2+} prevents activation of Ca^{2+}-dependent Cl^- channels and eliminates largely K^+ currents [133]. Possible activation of Na^+ currents (see above) can be excluded by addition of TTX (100 nM). Table 7 summarizes the literature on endogenous Ca^{2+} channels in the oocytes.

The first reports on endogenous *voltage-dependent Ca^{2+} channels (VDCC)* were more or less by-products of studies that used the oocytes mainly as an expression system for foreign Ca^{2+} channels rather than for the investigation of endogenous ion channels. VDCCs belong to the smallest conductances of the oocytes with a peak current usually less than 10 nA [134], yet in some oocytes peak amplitudes of up to 50 nA [135] and sometimes even up to 100 nA [136] were measured. Fewer than 30% of the oocytes exhibited the channels that inactivated within 0.5 s and were 50% inactivated at U_m of −55 to −60 mV [137]. The Ca^{2+} channel opened at U_m more positive than −30 mV and had its peak amplitude at about 10 mV. Cd^{2+} (1 mM) or Co^{2+} (2 mM) completely suppressed the tiny inward current. Injection of $Ins(1,4,5)P_3$ into the oocytes obviously modulated another class of Ca^{2+} channels in a way that they opened and mediated Ca^{2+} influx following membrane hyperpolarization [138]. Dascal et al. [136] showed that the endogenous Ca^{2+} channel is sensitive to Ni^{2+} (40–100 μM) and insensitive to dihydropyridines and ω-conotoxin. This pharmacological profile of the oocyte VDCCs would distinguish these channels clearly from the previously described P-, T- and N-type Ca^{2+} channels, while they would share some similarities with L-type Ca^{2+} channels. In the same study, it was demonstrated that expression of proteins from rat skeletal muscle enhanced the activity of the endogenous Ca^{2+} channels. VDCC were further reversibly inhibited by Mn^{2+} [139].

The usual small currents carried by endogenous VDCC could be elevated by injection of cAMP or incubation with a phorbol ester (PMA), another hint

Table 7
Endogenous Ca^{2+} channels

Channel property	Ref.
Voltage-dependent Ca^{2+} channels	[133–135,137,138]
L-type	[136,139,140]
T-type	[141]
N-type	[141,142]
Store-operated Ca^{2+} channels	[143,144]
Ca^{2+} waves	[145–147]
Regulation	[150–154]
Stimulation	[155–160]
Modulation	[144,146,161,162]
Selectivity	[163]
Pharmacological profile	[166,225]
Pro Ca^{2+} influx factor	[109,168,169,171]
Contra Ca^{2+} influx factor	[170]

for a possible relation to L-type Ca^{2+} channels [140]. However, Lacerda et al. [141] published single-channel recordings that revealed both T- and N-type Ca^{2+} channels with conductances of 9 and 18 pS, respectively. In this study, the authors also reported amplification of the endogenous VDCC by expression of foreign Ca^{2+} channel subunits. Further evidence for the existence of endogenous N-type Ca^{2+} channels came from studies by Allen et al. [142].

SOCC represent the second major class of endogenous Ca^{2+} channels in *Xenopus* oocytes. There is far more literature on SOCCs than on the above described VDCC. Basic findings on this important feature of the oocyte were contributed by Berridge [143] who showed that Cl^- channel mediated oscillations in membrane current reflected oscillatory release of intracellularly sequestered Ca^{2+}. Ins(1,4,5)P_3 was identified as one of the key molecules triggering the release of Ca^{2+} from intracellular stores. (In most of the studies cited in the following a poorly metabolized analogue of Ins(1,4,5)P_3, namely 3-F-InsP_3, was used (e.g. [144]).) Two years later it was demonstrated that the Ins(1,4,5)P_3-mediated oscillatory Ca^{2+} release propagated the forming of so-called Ca^{2+} waves spreading with a complex spatio-temporal pattern across most of the cell and annihilating upon collision [145–147]. *Xenopus* oocytes are in particular well suited for Ins(1,4,5)P_3-related investigations since they express only one subtype of the three known Ins(1,4,5)P_3 receptors [148,149].

Beside Cl^- channels, elevation of $[Ca^{2+}]_i$ by store depletion activates Ca^{2+} channels, namely store-operated Ca^{2+} channels allowing Ca^{2+} influx from the outside. An increase in $[Ca^{2+}]_i$ in turn modulates Ins(1,4,5)P_3-mediated Ca^{2+} release [150] thereby contributing to the oscillatory nature of the Ca^{2+} release. In a following study it could be shown that under certain conditions Ca^{2+} potentiated the Ca^{2+} release process [151,152]. Petersen and Berridge [153] confirmed that Ca^{2+} both potentiated and inhibited its own entry. They further showed that SOCC was modulated by PKC-mediated phosphorylation as also reported by Parekh and Terlau [154]. The potentiation of Ins(1,4,5)P_3-evoked Ca^{2+} release by Ca^{2+} influx could be mimicked by Ba^{2+} and Sr^{2+} [151].

While injection of Ins(1,3,4,5)P_4 was not sufficient to stimulate Ca^{2+} influx [155], Ins(1,3,4,6)P_4 stimulated Ca^{2+} influx with high potency and facilitated the responses to Ins(1,4,5)P_3 in a caffeine-sensitive way [156–159]. It was suggested that Ins(1,4,5)P_3 alone plays the crucial role in the activation of capacitative Ca^{2+} entry as also confirmed by Verjans et al. [160]. Modulation of SOCC was shown to be responsible for the promotion of synchronous intracellular Ca^{2+} liberation across wide areas of the oocyte cytoplasm. Moreover, the steady state Ca^{2+} flux through SOCCs modulated spike frequency and Ca^{2+} wave velocity [144]. Only when Ins(1,4,5)P_3 concentration exceeded a certain threshold which was estimated to be approx. 60 nM, Ca^{2+} store depletion with subsequent activation of SOCC appeared [161,162]. The oscillatory system evoked by Ins(1,4,5)P_3 comprises rhythmic activation and inactivation of the SOCC in a concerted teamwork with CaCCs [146].

In an exceptionally extensive study Yao and Tsien [163] characterized the Ca^{2+} channels that were activated by store depletion. The authors used Ca^{2+} chelators such as EGTA and BAPTA to block Ca^{2+}-dependent Cl^- channels since inhibitors of the anion currents (i.e. NFA) also influenced SOCC. The SOCC exhibited high selectivity for Ca^{2+} over Na^+ and K^+ and produced currents around -90 to -160 nA at $U_m = -60$ mV. Sr^{2+} and Ba^{2+} permeated SOCC in the way Ca^{2+} did, but tended to inactivate the current faster. The SOCC current reversed at 40 mV, displayed inward rectification and could be inhibited by metal ions with a potency sequence of $Mg^{2+} < Ni^{2+} \approx Co^{2+} \approx Mn^{2+} < Cd^{2+} < Zn^{2+} < La^{3+}$. SOCC current appeared to be sensitive to PKC regulation, demonstrated by the ability of PMA to inhibit the current. From these findings it can be concluded that SOCC is similar but not identical with the Ca^{2+} release activated Ca^{2+} current originally described by Hoth and Penner [164] and termed I_{Crac}.

Another important point that emerged from the study by Yao and Tsien [163] is the unreliability to use CaCCs of the oocyte as monitor for the intracellular Ca^{2+} concentration. As the authors clearly pointed out, $[Ca^{2+}]_i$ and the activity of CaCC show no linear relationship for several reasons. Parker and Yao [165] showed that CaCC currents corresponded better to the rate of rise of intracellular free Ca^{2+} than to its steady state level. The unsuitability of CaCC as a Ca^{2+} indicator was further demonstrated

by Centiano et al. [126]. If investigating SOCC the technique of chelating intracellular Ca^{2+} to avoid contaminating CaCC currents should be the method of choice.

Activation of SOCC is independent of vesicle-mediated insertion of new membrane material into the plasma membrane as shown by the insensitivity of Ins(1,4,5)P_3 stimulation of SOCC to primaquine, an inhibitor of vesicle trafficking [166]. Surprisingly, disruption of microtubules and microfilaments did not alter the stimulation, indicating that an intact cytoskeleton is not a necessary prerequisite for channel activation. SOCCs show in their pharmacological profile some similarities with neuronal L-type VDCCs. The VDCC antagonists ω-conotoxins and flunarizine inhibited SOCC [167]. However, as long as the molecular basis for store-operated Ca^{2+} entry remains unclear, it is pure speculation that VDCC and SOCC share structural homologies.

In 1993, two groups independently reported about a diffusible factor that could activate SOCC [109,168]. The putative soluble mediator of a human tumor lymphocyte cell line was termed Ca^{2+} influx factor (CIF) and was shown to have hydroxyls on adjacent carbons, a phosphate and a mass below 500 [168]. Parekh et al. [109] showed that CIF in *Xenopus* oocytes was phosphatase-sensitive and might be phosphorylated itself, but was not an inositol phosphate [169]. They speculated that the molecule might be a novel kinase that gated Ca^{2+} influx through a phosphorylation/dephosphorylation cycle. The existence of CIF in oocytes is not generally accepted since Petersen and Berridge [170] showed that potentiation of SOCC current by okadaic acid was mediated by PKC and thus was not necessarily related to the activity of a CIF. However, the results obtained by the sophisticated patch-cramming experiments of Parekh et al. [109] seem convincing: after excision of the patch they observed rapid SOCC rundown that could be reversed by cramming the patch back into the oocyte.

While Randriamampita and Tsien [168] ruled out Ins(1,4,5)P_3 as a possible CIF candidate, Lupu-Meiri et al. [171] postulated an inositol phosphate receptor in the oocyte plasma membrane that mediated the regulation of the voltage-independent Ca^{2+} entry. Yet, the molecular proof for this possibility is still missing.

4.4. Non-selective cation channels (NSCC)

NSCC (compiled in Table 8) represent a largely heterogeneous group of ion channels and sometimes it is hard to distinguish between the different members of this family and other classes of ion channels. The heterogeneity of NSCCs is also reflected in the oocytes. In principal, NSCC are characterized by their low preference for the transported ion species and their more or less developed impermeability to divalent cations.

Burckhardt and Frömter [172] observed NSCCs in the oocyte plasma membrane that were permeable for NH_4^+, recorded as a depolarization of U_m and a decrease in membrane resistance. They showed that the NH_4^+-permeant NSCC could be inhibited by La^{3+}, DPC and *p*-chloromercuribenzoate (pCMB). These findings were further extended by a report of the same group showing that primary, secondary and tertiary amines could also enter the oocyte through this NSCC [173]. In a very recent study they confirmed and broadened their earlier observations by showing that NH_4^+ permeates through NSCCs that could be partly inhibited by ISDN, glibenclamide, Ba^{2+} and TEA [107]. Similar results were published by Cougnon et al. [101] who further suggested that NH_4^+ is additionally transported by the Na^+/K^+-ATPase and/or a K^+/H^+ antiporter. They also demonstrated NSCC inhibition by 3′,5′-dichlorodiphenylamine-2-carboxylic acid (DCDPC).

Palytoxin (PTX), the most potent non-peptidic toxin of the blue humphead parrotfish, is proposed to convert Na^+/K^+-ATPase molecules into cation-selective ion channels. PTX induced depolarization of U_m by increasing the oocyte membrane's permeability to monovalent cations [174]. The authors showed that PTX converted the Na^+/K^+-ATPase into a NSCC by binding to a specific state of the pump, probably the E2 conformation or an E2 sub-

Table 8
Non-selective cation channels

Channel property	Ref.
NH_4^+ permeable	[101,107,172,173]
Induced by marine toxins	
PTX	[174]
MTX	[24,79,175]
Induced by expression of foreign proteins	[112]

strate. The formation of the channel could be prevented by ouabain or by high external K^+, thereby driving the pump away from the E2 conformation. Another potent marine toxin, namely MTX, activated NSCC leading to strong membrane depolarization [175]. Although a very recent report favored the view that MTX opened Ca^{2+}-activated Cl^- channels of the oocyte by increasing $[Ca^{2+}]_i$ [79], Bielfeld-Ackermann et al. [175] clearly demonstrated that the pharmacological profile of the MTX-activated conductance argues for a NSCC that was sensitive to amiloride, benzamil and Gd^{3+}, while FFA, NFA and DCDPC did not influence the current. These findings were supported by Weber et al. who used continuous capacitance and conductance measurements to demonstrate that MTX induced NSCC and probably mechanosensitive cation channels (MSCC) via exocytotic delivery of new channel proteins and functional insertion into the plasma membrane, thereby increasing the oocyte surface area [24].

As already shown for K^+ channels, NSCC in oocytes can be activated by heterologous expression of foreign proteins [112]. High level expression of six different, functionally distinct proteins (i.e. three different K^+ channels, an amino acid transporter, the dopamine D2 G-protein-coupled receptor and the cytoplasmic protein β-galactosidase) led to induction of NSCC. This NSCC induction was usually not detected until high levels of heterologous expression of the respective protein was achieved. NSCCs could be activated by depolarization and were specifically blocked by DIDS and TEA. The antiarrhythmic drug clofilium increased the NSCC-mediated current, while increasing pH_o decreased the current. The current was not detectable in some oocytes when the proteins were expressed in moderate levels, yet high level expression consistently induced the NSCC current that occasionally also occurred in non-injected control oocytes [112]. From these data it could be speculated that mRNAs encoding for different proteins contain one or more sequences that induce the formation of a regulatory factor which induces NSCC at hyperpolarization.

4.5. Mechanosensitive cation channels (MSCC)

Stretch-activated or MSCC respond to membrane stress by changes in open probability (P_o). The application of suction via a patch pipette increases channel activity without significant effects on current amplitude or channel conductance [176]. Therefore, the mechanical force affects channel gating and not the channel conductance. The plasma membrane of *X. laevis* oocytes is endowed in high density with MSCCs [177] which belong to the most extensively described endogenous ion channels (for separate reviews see [176,178]). The channel is normally quiescent, yet application of suction increases P_o and decreases the duration of the longer closed state.

Originally described in 1985 by Sakmann et al. [179], the first detailed study on an oocyte MSCC has been published in 1989 by Yang and Sachs [180]. The authors described block of MSCCs by Gd^{3+} (10 μM) and Ca^{2+} (>10 μM). While Gd^{3+} blocked the MSCC current completely and was impermeable, Ca^{2+} caused only a partial block because Ca^{2+} proved to be highly permeable. Furthermore, it was shown that Gd^{3+} blocked MSCCs in three distinct, concentration-dependent ways: channel opening was reversibly prevented, open channel currents were reduced and channel open time was decreased. In a subsequent study Yang and Sachs [181] introduced TEA as a tool to distinguish between currents produced by K^+ channels and MSCCs since MSCCs are not sensitive to the K^+ channel blocker. The authors showed further that MSCCs were cation-selective inward rectifiers with an ion selectivity for permeation of $K^+ > NH_4^+ > Cs^+ > Rb^+ > Na^+ > Li^+ > Ca^{2+}$. Analyzing single-channel data the authors found three closed states and one open state of the MSCC that was equally sensitive to negative or positive pressure. In the same study, two ion binding sites were identified, one being an intra-channel site and the other being an allosteric site.

Lane et al. introduced amiloride as a blocker of MSCCs [182]. Amiloride ($K_{1/2}$ = 500 μM) produced a flickery block of MSCCs from both sides of the membrane in a voltage-dependent way when applied from the external side and in a voltage-independent mode when added internally. Amiloride analogues such as dimethylamiloride (DMA, $K_{1/2}$ = 370 μM), benzamil ($K_{1/2}$ = 95 μM) and bromohexamethylamiloride (BrHMA, $K_{1/2}$ = 34 μM) blocked MSCCs with even higher affinity in a voltage-dependent way [183,184]. Amiloride block was shown to be reduced

in presence of external Ca^{2+} (1.8 mM) while it was independent on the charge carrier (Na^+ or K^+, respectively) [185].

It is an intrinsic property of several MSCCs to exhibit a time-dependent rundown of P_o when exposed to a constant or repeated pipette suction. This adaptation to the pipette pressure can be distinguished from channel inactivation since the MSCC remains sensitive to mechanical stimuli, but with a reduced stretch sensitivity. MSCCs in oocytes were shown to adapt rapidly to repeated stimulation by suction steps [186]. The adaptation was voltage-dependent and most effective at resting or hyperpolarized U_m, while absent at depolarized U_m. Reactivation of MSCCs appeared upon stronger stimulation. The ability of Ca^{2+}-independent adaptation and MSCC activity is lost following over-stimulation probably due to uncoupling the plasma membrane from the cytoskeletal elements [186]. The mechanism underlying adaptation was further investigated using the pressure-clamp technique in conjunction with the patch-clamp technique [187]. The pressure-clamp technique utilizes steps of positive or negative pressure to study the relaxation kinetics of the oocyte MSCCs. Using this technique, McBride et al. could show that specific properties of oocyte MSCCs, e.g. high sensitivity and rapid adaptation are dependent on intact plasma membrane-cytoskeleton association(s). The same group could further demonstrate that membrane tension rather than the applied pressure per se gated the MSCCs [188].

Volume sensitivity of the MSCCs was evaluated using an elegant technique of patch-clamping small vesicles derived from the oocyte plasma membrane [189]. It could be clearly shown that MSCCs were volume- as well as stretch-sensitive. Moreover, it was demonstrated convincingly that the volume sensitivity was dependent on cytoskeletal elements adhering to the plasma membrane and independent of freely diffusible components in the oocyte cytoplasm.

MSCCs displayed inward rectification in Ca^{2+}-free solutions [181,182]. It was proposed that this rectification might be caused by intrinsic properties of the *Xenopus* oocyte MSCC involving asymmetries in its energy barrier profile [181] as also reported from *Rana* oocytes [190]. Further evidence for this model came from a previous study by Wu et al. [191] studying the relations of Mg^{2+} block and rectification.

The potential physiological role of MSCCs was thoroughly investigated in a study involving effects of MSCCs on oocyte growth, maturation, fertilization and embryogenesis [192]. The MSCC blockers Gd^{3+}, gentamicin and amiloride were used to knock out MSCCs and development was studied. Surprisingly, the blockers had little if any effect on the developmental events. It was speculated that other systems of the oocyte can take over when MSCC functions were blocked or that the oocytes in long-term presence of the blockers developed mechanisms to circumvent possibly negative influences of the blockers. No matter how, the putative physiological role of MSCCs in oocyte development remains unclear as is the case for most of the oocyte ion channels.

A possible link between NSCC and MSCC of the oocytes came very recently from Reifarth et al. [193]. They gave evidence for a stretch-independent mode of operation of the MSCC thereby behaving like NSCC. The authors proposed a model involving one protein that is responsible for NSCC and MSCC currents with the ability to operate either in a stretch-dependent or a stretch-independent mode. In the same study it was shown that only far higher concentrations of Gd^{3+} (i.e. 500 μM) than previously reported could produce complete block of MS cation channels (see above).

5. Ca^{2+}-inactivated ion channels

Since the ionic nature of Ca^{2+}-inactivated ion channels is still controversial and it seems possible that an anion channel as well as cation channel(s) contribute to the currents observed after removal of external divalent cations, these channels, summarized in Table 9, will be discussed in their own section.

Until recently it was generally believed that solutions free of divalent cations cause irreversible damage to the oocyte membrane by inducing leakages [194]. However, early in 1995 the first report on a Ca^{2+}-inactivated ion channel, namely the Ca^{2+}-inactivated Cl^- channel (CaIC), was published [195]. The authors demonstrated that removal of all external divalent cations activated a Cl^- channel showing whole-cell and single-channel data. The CaIC was sensitive to common Cl^- channel blockers such as

Table 9
Ca^{2+}-inactivated ion channels

Channel property	Ref.
Ca^{2+}-inactivated Cl^- channel	[12,37,195,200]
Monovalent cation channel	[201]
Non-selective cation channel	[202]
Hemi-gap-junctional channel	[204]

NFA and FFA, had a reversal potential near the Cl^- equilibrium potential (−12 mV to −15 mV, see also Table 3) and had a single-channel slope conductance of 90 pS. 20 μM external Ca^{2+} were sufficient to block half of the CaIC currents that also could be observed in the absence of any external permeable cations. While most of the membrane transport systems of the oocyte are downregulated during maturation, CaIC currents were active in mature eggs [195].

In the following, Weber et al. described the newly discovered Ca^{2+}-inactivated Cl^- channel in more detail [12]. Using single-channel analysis in combination with pipette perfusion they showed that the $[Ca^{2+}]_i$-independent CaIC current, detectable in every oocyte of all donors, was sensitive to almost all known Cl^- channel blockers (i.e. DPC, NPPB, AZT, 9-AC), yet surprisingly was further activated by SITS and DIDS. CaIC was stimulated by cAMP but not by cGMP, regulated by PKC and dependent on intact cytoskeleton. To prove that Ca^{2+} removal actually stimulates an efflux of Cl^- the Cl^--sensitive dye 6-methoxy-*N*-(sulfopropyl)quinolinium (SPQ) was used. The fluorescence of SPQ was quenched significantly after CaIC activation by the efflux of Cl^- in the millimolar range, yet in the presence of external Ca^{2+} no change in emission intensity occurred [37]. In this study the authors also showed that the CaIC was poorly selective for anions as it is typical for maxi Cl^- channels [196,197]. Furthermore, inside-out patches revealed that the CaIC was also able to accept cations as substrate (transport ratio Cl^-:$NMDG^+$ (*N*-methyl-D-glucamine) = 1.0:0.31). This selectivity pattern allowed the classification of the CaIC as a rather unselective maxi Cl^- channel that had also been described from rat skeletal muscle [198] and rat hippocampal neurons [199]. More recently the same group demonstrated that Gd^{3+} and metal ions such as Cu^{2+} and Zn^{2+} blocked CaIC currents [200]. Surprisingly, CaIC was also blocked by amiloride, but not by its analogue benzamil [200]. Dynamic capacitance measurements to monitor the oocyte surface revealed that CaIC protein is abundant in large numbers in the plasma membrane and that no new channel proteins are inserted into the membrane by exocytosis upon CaIC stimulation as previously reported for another Cl^- channel [25]. All the data presented so far doubtlessly point out that removal of external divalent cations triggers reversible activation of ion channels, one of which is the Ca^{2+}-inactivated Cl^- channel.

To date, Ca^{2+}-inactivated channels form a fascinating and far from complete saga. However, things have been complicated since late in 1995 Arellano et al. [201] published a report on a monovalent cation conductance that is inactivated by external Ca^{2+}. Deducted from whole-cell data, the authors reported that removal of external Ca^{2+} activated a current that was carried by Na^+ and K^+. The currents could also be unblocked by depolarization of the membrane even in the presence of millimolar Ca^{2+} or Mg^{2+}. In vitro maturation abolished the current. Unfortunately, the authors missed the crucial experiment to remove Ca^{2+} in the total absence of any permeable external cations and to discuss their data in context with the data published on the Ca^{2+}-inactivated Cl^- channel.

The problem of Ca^{2+}-inactivated ion channels in *X. laevis* oocytes is particularly vexing since a previous report attributed the effects of Ca^{2+} removal to a non-selective cation channel [202]. Zhang et al. [202] reported permeability ratios of 1:0.99:0.45:0.35:0.24:0.2:0.2:0.2 for K^+:Na^+:$NMDG^+$:TEA^+:Cl^-:TPA^+:TBA^+:gluconate$^-$ (TPA = tetrapropylammonium; TBA = tetrabutylammonium). The currents inactivated by external Ca^{2+} were sensitive to FFA, NFA, Gd^{3+} and amiloride and disappeared after maturation. 80% of the current was abolished after preinjection of oocytes with antisense oligonucleotide against connexin 38, the major hemi-gap-junctional protein in the *Xenopus* oocyte [203]. Zhang et al. compared and discussed their findings with data published by the two other groups (see above). They ruled out that the Ca^{2+}-inactivated currents could be mediated by subsets of cation- and anion-selective channels and attributed the current blocked by external Ca^{2+} to the hemi-gap-junctional channel.

Ebihara came to the same conclusion and suggested that the Ca^{2+}-inactivated current was due to the opening of endogenous hemi-gap-junctional channels [204]. While Ebihara discussed the data of Arellano et al. [201] the findings on the Ca^{2+}-inactivated Cl^- channel were ignored. However, CaIC can be clearly distinguished from the hemi-gap-junctional channel that needed depolarization to be activated and produced only small currents in the range of 100 nA, while the CaIC currents are in the magnitude of several microamperes. Moreover, the single channel conductance of the hemi-gap-junctional channel is an order of magnitude lower than that reported by Weber et al. [12] for the CaIC (i.e. 1.5 pS and 90 pS, respectively). Furthermore, the fact that the hemi-gap-junctional channel is active in the presence of Mg^{2+} and even 200 μM Ca^{2+} clearly shows that there exist at least two channel populations that can be easily distinguished.

Comparison of the data reported by Arellano et al. [201], Weber et al. [12,37,195,200], and Zhang et al. [202] revealed some differences in the experimental conditions. The most obvious difference in the above mentioned investigations is the composition of the Ca^{2+}-free solutions that were used. While Weber et al. always chelated Ca^{2+} with EDTA, EGTA or BAPTA (each 5 mM), both of the other groups used only virtually Ca^{2+}-free solutions, i.e. without Ca^{2+} chelator. Another difference in the experimental design could be seen in the membrane potential the oocyte membrane was clamped to. Arellano et al. [201] reported prevailing activation with depolarized membranes (i.e. membrane potential ≤ -10 mV). Under these conditions the driving force for anions is reduced. Around the physiological membrane potential (i.e. between -50 and -70 mV) the CaIC is independent of the membrane voltage. The fact that CaIC is active even in the absence of any external cations, the observation that the cation channel is activated by depolarization even in the presence of Ca^{2+}, the lack of inward rectification by the CaIC, the single-channel data on oocytes and eggs and the optical Cl^- efflux measurements led us to propose the following explanations.

Two possible models for the phenomena caused by removal of extracellular Ca^{2+} seem plausible. At first, one population of ion channels could exist in oocytes that are able to operate in different modes according to the ionic environment. Depending on the ionic composition of the surrounding medium the channel responds to Ca^{2+} removal with cation influx or anion efflux. This capability could make sense since the oocyte is seriously challenged during development by changing ionic compositions of the environment after being spawned into hypoosmotic pond water. However, the broad spectrum of ion channel inhibitors that are effective in blocking the Ca^{2+}-inactivated ion channels and the data by Zhang et al. [202] argue against the one-channel different-modes option.

A more attractive explanation for all the discrepancies described by the several groups is the existence of at least two different channel populations that are inactivated by extracellular Ca^{2+}. In this model more or less specific ion channels could change their selectivity according to the environmental demands. The relatively low ion specificity reported from single-channel analysis by Weber et al. [12] and from whole-cell studies by Arellano et al. [201] and Zhang et al. [202] argue for this kind of explanation. It could be further possible that these channels use a putative common extracellular Ca^{2+}-sensing receptor as described from other cells [205]. Moreover, it also seems plausible that the Ca^{2+}-inactivated ion channels use extracellular Ca^{2+} and/or Cl^- as regulative cofactors and that the respective channel population becomes active upon the availability of these ions. As described above, Ca^{2+}-inactivated ion channels are sensitive to a wide variety of ion channel blocking mechanisms. This is another potential argument in favor of the existence of at least two different ion channel populations.

From the current state of the art, it is impossible to dispel all discrepancies that have come up in the last years on Ca^{2+}-inactivated ion channels of *X. laevis* oocytes. However, since at least the CaIC is not downregulated during maturation and can still be found active in eggs as demonstrated by whole-cell and single-channel data, it should be obvious that there is a physiological role for the channel in the developmental fate of the oocyte. Since the jelly coat, that is surrounding the eggs keeps the Ca^{2+} concentration in the range where CaIC activates [14], potential roles in the generation of the fertilization potential and subsequent depolarization of the egg membrane to avoid polyspermy seem plausible.

6. Water channels

Water transport is an essential property of living cells. In some cell types water uptake is mediated by specific water transporting proteins also referred to as 'water channels' or 'aquaporins' [45]. Water permeability (P_f) of *X. laevis* oocytes is quite low and was found to be insensitive to water channel blocking agents such as $HgCl_2$, phloretin and cAMP [206]. The absolute values reported for P_f vacillate between 1 μm/s [207], 4 μm/s [208], 12 μm/s [209] and 14 μm/s [210]. This low water permeability is typical of P_f in lipid bilayers not containing water channels, indicating that water channels are absent in the oocyte plasma membrane [206]. The absence of endogenous water channels makes the oocyte an almost ideal expression system for this channels. Schreiber et al. [44] used oocytes to express CFTR and found that CFTR activated endogenous water uptake that could be inhibited by phloretin and *p*-chloromercuribenzene sulfonate (pCMBS). However, the authors came to the conclusion that the observed water influx was mediated by CFTR itself rather than by endogenous water channels. Indeed, the intrinsic water permeability of CFTR was also reported by Hasegawa et al. [211]. Therefore, a putative possibility that CFTR acted as a regulator of water channels in *X. laevis* oocytes can be ruled out because of the absence of endogenous water channels.

7. Conclusion and future perspectives

In the recent years a permanently growing number of reports has emerged that attributed severe human diseases to intrinsic defects of ion channels including e.g. cystic fibrosis [212], kidney stones [213], epilepsy [214,215], Liddle's disease [216] and Dent's disease [213]. Therefore, it is imperative to understand the elemental properties of ion channels.

During the last decade the *X. laevis* oocyte expression system has been used increasingly in efforts to obtain new insights in structure and behavior of some of the major ion channels. Since then, numerous ion channels, transporters and receptors have been cloned and sequenced employing the *X. laevis* oocyte expression system. In many instances, fundamental knowledge of the mechanisms and functions of ion channels in health and disease was gained with help of the oocytes. However, as compiled in this review, *X. laevis* oocytes are endowed with a whole plethora of endogenous ion channels. Furthermore, in addition to these ion channels, oocytes possess a large variety of active and passive transport systems that were not subject of this paper. Therefore, whenever the *X. laevis* oocyte is chosen as an heterologous expression system, it is absolutely imperative to check the oocytes for the endogenous existence of the ion channel or transporter that is to be expressed. The knowledge of endogenous ion channels in *X. laevis* oocytes may help to avoid false interpretations when expressed ion channels and transporters are characterized. It is the author's hope that the present review could be of needful assistance in this respect.

Beside its usefulness as an expression system the oocyte itself has proved to be an interesting model for the study of ion channels. The progress in the identification of formerly unknown ion channels in *X. laevis* oocytes and in the characterization of these channels has been quite remarkable in the last few years. Research on endogenous ion channels in the oocytes yielded fascinating findings in the molecular mechanisms of ion transport that can be transferred to other biological systems. However, despite this progress, many unresolved questions on endogenous ion channels of the *X. laevis* oocyte remain to be elucidated.

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