Topical Review

Endogenous Ion Channels in Oocytes of *Xenopus laevis:* Recent Developments

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Introduction

Xenopus laevis oocytes devoid of their follicular enclosure provide a frequently used expression system for investigating channels, transporters and receptors following injection of the appropriate mRNA or cDNA extracted from a wide variety of tissues including plants (elegantly reviewed by Sigel [118]). However, if one intends to make use of the oocyte expression system, it is absolutely imperative to know whether or not the oocyte endogenously already possesses the transport system under investigation. In 1987 Dascal [27] published an extraordinarily elaborate state of the art review of all ion channels of the oocyte plasma membrane that were known at this time. Since then a whole orchestra of reports dealing with the revealing of former unknown ion channels endogenous to the oocyte plasma membrane has emerged. This review will focus on publications that have appeared since the Dascal review (i.e., after 1987) and is restricted to endogenous ion channels of the defolliculated oocyte of *Xenopus laevis.* Although a large number of excellent reviews covering a broad range of aspects of the amphibian oocyte has been published recently, reflecting the excitement which surrounds the possibilities of the oocyte system, only two comprehensive reviews covered some, but by far not all of the endogenous ion channels of the *Xenopus laevis* oocyte [38, 39]. Because the goal of this review is to be brief, citations to the literature are selective.

Oocytes of *Xenopus laevis* are huge cells with a diameter ranging from 1 to 1.3 mm. Their size allows simple handling with ordinary Pasteur pipettes whose sharp edges have been fire polished. Moreover, the size of the oocytes permits the combination of several techniques on a single cell. So is it rather easy to inject substances into the oocyte while the membrane potential is monitored or while being voltage-clamped [138]. The experimenter could have access to intracellular changes during electrophysiological experiments if it is required by the experimental design. Furthermore, measurements of isotope influxes or effluxes can easily be recorded during voltage-clamp experiments [45]. Binding and flux studies yield the number of copies of the protein under investigation [114]. Even complete control of the ionic composition of the cytoplasmic fluid is possible with a technique that was developed recently and was termed the cut-open technique [91, 125]. Although the previously reported very fast response seen in the early gating current records might have been artifactual [120], the method has the additional advantages of low current noise and allows stable recordings over several hours, as is also the case for voltage- and patch-clamp measurements [51, 122]. A thorough compilation of electrophysiological and biochemical methods for the investigation of ion channels in *Xenopus laevis* oocytes was published not long ago (e.g. [52, 57, 89, 117, 119, 121, 123]).

It should also be mentioned that the oocyte system exhibits some disadvantages that could complicate the experiments and the interpretation of the results [118, 124]. The major drawbacks of the oocytes include seasonal variations reported by some laboratories, biological variance between oocytes of different donors, short life

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Table 1. Endogenous ion channels of *Xenopus laevis* oocytes

Transported substrate	Channel property	References
Na^+	-Activated by longlasting depolarization	[56, 112, 10, 24, 23, 64]
	-NH_4Cl^- inducible	[16]
	-Activated by ATP	[67, 90]
	-Amiloride-sensitive	[137]
K^+	-Blocked by TEA and Ba^{2+}	[54, 129, 18, 20, 80]
	-TEA-sensitive, Ba^{2+}-insensitive	[96, 78]
	-Small K^+ channel (IsK, minK)	[127, 6, 126, 130]
Ca^{2+}	-Voltage-dependent	[30, 88, 73, 28, 69, 30, 77, 101, 13]
	-Store-operated	[149, 76, 43, 109, 61, 93, 95, 94, 107, 133, 31, 106, 92, 50, 81, 41, 5, 14, 21]
Various cations		
-Mechanosensitive	Activated by membrane stretch	[49, 83, 147, 70, 71, 72, 46, 146, 145, 82, 47, 48, 116, 111, 144]
-Nonselective	-Hemi-gap-junctional channels	[35, 153, 3]
	-Palytoxin-induced	[136]
	-NH_4^+-permeable	[17, 19, 25]
	-Maitotoxin-induced	[9, 140]
	-Ca^{2+}-inactivated	[3, 153]
Cl^-	-Ca^{2+}-activated	[32, 85, 98, 58, 11, 65, 97, 8, 143, 129, 7, 84, 100, 42, 104, 47, 11, 50, 79, 44, 12, 22, 99, 34, 33, 113, 60, 37, 108, 92, 68]
	-Ca^{2+}-inactivated	[110, 138, 139]
	-Induced by hyperpolarization	[63, 129, 103, 59, 6]
	-Hypotonicity-activated	[105, 1, 128, 135, 74]
Water		[134, 151, 152, 116]

References are given in the order of their appearance in the text.

time (around 14 days) and strong temperature dependence.

In the following I will give a list of the endogenous ion channels sorted by their favorite substrate. The endogenous ion channels of *Xenopus laevis* oocytes described within the last ten years are summarized in Table 1. However, an unequivocal classification is not possible in each case, as discussed below.

Na^+ Channels

Voltage-dependent Na^+ channels are among the first channels discovered in *Xenopus laevis* oocytes and were described for the first time by Kado et al. in 1981 [56]; they showed that long-lasting depolarization of the oocyte membrane activated a transient slow inward current mediated by Na^+ channels. These channels were blocked by tetrodotoxin at submicromolar concentrations and open time was prolonged by veratrine indicating that these Na^+ channels are similar to those found in nerve, mammalian brain, and muscle cells [102]. The Na^+ currents could amount to amplitudes of 250 nA and more at -10 mV [64]. Patch-clamp studies, using the macro-patch technique revealed that the Na^+ conductances were highly selective for Na^+ and that the intrinsic inactivation could be prevented by MgATP [112]. Recently, it has

been shown that the depolarization-inducible Na^+ channels were sensitive to $[Ca^{2+}]_i$ [10]. Decreasing $[Ca^{2+}]_i$ by injection of Ca^{2+}-chelators significantly reduced the current, while increasing $[Ca^{2+}]_i$ by injecting inositol-1,4,5-trisphosphate ($Ins(1,4,5)P_3$) to release Ca^{2+} from intracellular stores potentiated the Na^+ current. Changes in extracellular Ca^{2+} (in the presence of Mg^{2+}) had no influence on the depolarization-induced Na^+ channel. Phosphorylation of the channel seems to be a fundamental step of Na^+ channel induction since activators of protein kinase C (PKC) resulted in enhanced Na^+ current amplitude, whereas PKC inhibitors decreased the inward current [24]. It seems likely that sustained depolarization of the oocyte plasma membrane leads to mobilization of the intracellular pool of PKC as also described for Ca^{2+} channels in chromaffin cells [4]. In this pathway, leading to activation of PKC, phospholipase C (PLC) seems to be involved [23]. Sustained depolarization might result in the activation of PLC which in turn catalyzes the hydrolysis of inositol-4,5-bisphosphate ($Ins(4,5)P_2$), thereby promoting the generation of diacylglycerol (DAG), an activator of PKC.

The physiological importance of the Na^+ channel could be a contribution to the processes succeeding fertilization which is followed by a massive efflux of Cl^-, causing instant depolarization of the egg membrane and preventing polyspermy [26]. The depolarization acti-

vates the Na^+ currents that help to depolarize the membrane. However, it has to be stressed that more thorough investigations of the depolarization-inducible Na^+ current were complicated by the fact that in the majority of oocytes these channels could not be detected [64].

Recently, another Na^+ conductance in oocytes was reported by Burckhardt et al. [16]. This conductance was induced by extracellular NH_4Cl, could be blocked by flufenamate and conducts Na^+ as well as NH_4. Since this NH_4Cl-inducible Na^+ current was studied in oocytes voltage-clamped to –70 mV, it can be excluded that it is identical with the depolarization-induced Na^+ current. However, its physiological role remains to be elucidated.

Kupitz and Atlas reported that high concentrations (> 0.5 mM) of extracellular ATP activated large Na^+ inward currents in defolliculated oocytes with amplitudes in the range of microamperes. These ATP-induced currents could be blocked completely by the diuretic amiloride [67]. The authors speculated that the putative high amounts of ATP inside the sperm could activate the supposed ATP-sensitive Na^+ channel at the time of fertilization. However, there are major differences between the immature oocytes that the authors used for their investigations and mature eggs. Since the authors failed to show that mature eggs indeed exhibit ATP-sensitive channels and such high ATP concentrations in sperm are unlikely, some doubts on the contribution of ATP-activated ion currents to fertilization processes remain, as also pointed out by Nucitelli and Ferguson [90].

Amiloride, but not its analogues phenamil and benzamil were shown to block another Na^+ conductance in *Xenopus laevis* oocytes [137]. The conductance could be found in about every third oocyte, however, because of its pharmacological profile, the highly selective Na^+ conductance seems to be different from the "classic" amiloride-sensitive epithelial Na^+ channel (ENaC) detectable in most epithelia [40].

K^+ Channels

The membrane potential (V_m) of defolliculated *Xenopus laevis* oocytes is mainly achieved and maintained by the plasma membrane's permeability to K^+ ions. Reducing the electrochemical driving force for K^+ by elevating $[K^+]_o$ induces nearly complete breakdown of V_m and lowers V_m to around 10 percent of its original value at physiological $[K^+]_o$. Oocytes possess several populations of K^+ channels in their plasma membrane, one of which is characterized by its sensitivity to the K^+ channel blockers tetraethylammonium (TEA) and Ba^{2+}. This class of K^+ currents is activated by depolarization. Huang et al. [54] showed that a close link exists between TEA-sensitive K^+ currents and the endogenous Na^+/K^+-ATPase which also has been subject to extensive investigations [131, 132]. However, when using TEA as a K^+

channel blocker one should keep in mind that TEA additionally inhibits Na^+/K^+-ATPase activity in oocytes [36]. TEA itself seems to penetrate the plasma membrane by a Ba^{2+}-sensitive permeability [54]. The Ba^{2+}-sensitive K^+ currents exhibited slow kinetics of activation and deactivation and showed only small current amplitudes of several tens of microamperes despite the powerful depolarizing effect of Ba^{2+}, indicating that the K^+ currents are important contributors to V_m of the oocyte [129]. In some oocytes the Ba^{2+}-sensitive K^+ current appeared to be insensitive to TEA [129]. Extracellular acidification led to inhibition of Ba^{2+}-sensitive K^+ channels causing membrane depolarization. Thus, endogenous K^+ channels are pH-sensitive [18]. Oocyte K^+ channels were sensitive to isosorbiddinitrate (ISDN), a pharmacologically useful organic nitrate, initiating cellular synthesis of the second messenger nitric oxide [20]. cAMP stimulation of human cystic fibrosis transmembrane conductance regulator (CFTR) expressed in oocytes after cRNA-injection activated the Ba^{2+}- and TEA-inhibitable K^+ channel demonstrating regulatory influences of CFTR on endogenous K^+ channels [80].

IsK (or minK) is a 14.5 kDa glycoprotein with one transmembrane domain lacking sequence homology with any other cloned functional channel [127]. Ben-Efraim et al. showed that cytoplasmic or extracellular application of small peptides derived from IsK carboxyl and amino termini activated slow K^+ channels in *Xenopus laevis* oocytes that were sensitive to Ba^{2+} and chlofilium [6]. Their elegant experimental approach gave strong evidence that IsK is a regulatory subunit able to activate pre-existing silent channels rather than to form a functional K^+ channel by itself, as claimed recently by other groups [126, 130]. At this point, it should be mentioned that the oocytes might contain native proteins that could affect heterologously expressed proteins. If a foreign membrane protein is to be expressed, oocytes should be checked carefully for the presence of any endogenous protein, which possibly could influence the newly expressed function (for review *see* [118]).

A further class of endogenous K^+ channels in oocytes seems to be TEA-sensitive while being Ba^{2+}-insensitive. Parker and Ivorra found this K^+ channel species in oocytes obtained from one donor frog [96]. They showed by means of noise analysis that the channels had a unitary conductance of 20 pS and a mean open lifetime of about 300 msec. Although a similar Ba^{2+}-insensitve K^+ current in oocytes from different donors was highly variable in current amplitudes, it could be found routinely [78]. The K^+ currents were insensitive to extracellular Ca^{2+} and showed characteristics similar to those of the delayed rectifier found in some nerve and muscle cells.

For completeness it has to be mentioned that follicle enclosed oocytes additionally possess a K^+ channel

whose activity is lost when the follicle layer is removed [29, 86, 150].

Mechanosensitive Cation Channels (MS Channels)

In addition to a large number of Cl^- channels, oocytes express a particularly high density of MS channels [83] as also described in full detail by a separate review [49]. MS channels, also termed stretch-activated channels (SAC) are distinct from voltage- and ligand-gated channels and are activated mainly by applying positive or negative pressure to patch pipettes in the range of about 10 to 50 $mmHg^{-2}$ [147]. They are predominantly conductive for cations with a selectivity for permeation of $K^+ > NH_4^+ > Cs^+ > Rb^+ Na^+ > Li^+ > Ca^{2+}$ calculated from the slope conductance [147]. Yang and Sachs determined three closed and one open state of the MS channel, the open time being independent of both pressure and voltage [147]. The channel was found to be insensitive to the K^+ channel blocker TEA. The authors further showed that the MS channel possesses two separate binding sites one being an intrachannel site in relation with an additional allosteric site.

Although no high specific blocker of MS channels has been found, amiloride, known to be a blocker of epithelial Na^+ channels, in high concentrations (IC_{50} 500 μM) was shown to evoke voltage-dependent block of inward currents at negative (i.e., physiological) holding potentials mediated by MS channels in *Xenopus laevis* oocytes [70]. However, outward currents of MS channels recorded at positive V_m remained almost unaffected by amiloride. Amiloride block was only voltage-dependent when the inhibitor was applied on the extracellular side; internally applied amiloride exhibited voltage-independent block of MS channels [70]. The amiloride analogues dimethylamiloride (DMA), benzamil, and bromohexamethylamiloride (BrHMA) were shown to block oocytes MS channels in the same highly voltage-dependent manner as amiloride [71]. Yet, all analogues were more potent blockers than amiloride itself. The amiloride analogue fingerprinting, as the authors termed their study, revealed that MS channels of oocytes are distinct form previously described amiloride-sensitive ion transport mechanisms such as ENaC, Na^+/Ca^{2+} exchanger and Na^+/H^+-exchanger [62]. The presence of extracellular Ca^{2+} in physiological concentration (i.e., $\approx$ 1.8 mM) reduced the blocking efficacy of amiloride [72]. Despite the MS channel's preference for K^+ over Na^+, amiloride block was independent of Na^+ or K^+ being the charge carrier [72]. The pharmacology and blocker characteristics of amiloride and its analogues on oocytes MS channels has been thoroughly reviewed elsewhere [46].

Yang and Sachs introduced gadolinium (Gd^{3+}) as another blocker of MS channels in *Xenopus laevis* oocytes [146]. Gd^{3+} decreased channel open time and open channel current and reversibly inhibited channel opening. In the same study the authors showed that Ca^{2+} influenced open time and currents produced by MS channels in the same way than Gd^{3+}. However, Ca^{2+} produced only partial block of MS channels, mainly because the channels accept this ion also as substrate. Mg^{2+} behaves similar as Ca^{2+} by blocking MS channels and by being accepted as a substrate [145].

The introduction of the pressure clamp technique allowed investigations of pressure adaptation kinetics of MS channels and yielded new insights into the relaxation kinetics of MS channels that inactivate with maintained stretch [82]. The technique utilizes step perturbations in pressure and the rate at which the process relaxes to its new equilibrium is measured. Using this technique it could be shown that adaptation is intrinsic to the MS channel and requires intact interactions of the cytoskeleton and the plasma membrane [47]. Sustained stretch led to reduced open probability of the MS channel, while MS channel conductance remained mainly unaffected and repeated stretch pulses induced loss of pressure responsiveness. Another astonishing feature emerging from these studies is the voltage dependence of adaptation: at positive voltages no adaptation could be observed while negative voltages induced rapid adaptation following persistent pressure application [47]. Hamill and McBride compared oocyte MS channels with MS channels found in hair cells of the inner ear [48]. They found some basic common properties such as conductance, selectivity, and blocker profile. However, while in oocytes mechanosensitivity and adaptation are independent of the presence of Ca^{2+}, in hair cells both processes require the attendance of Ca^{2+}.

MS channels are sensitive to volume changes as described by Schütt and Sackin [116]. This volume sensitivity involved cytoskeleton elements as the authors could show using vesicles derived from excised inside-out patches of oocyte membranes. The results confirmed and extended the observations of Hamill and McBride that functional cytoskeleton elements adhering to the plasma membrane rather than the presence of freely diffusible components of the oocyte cytoplasm are a prerequisite for proper MS channel function [47].

Recently, data from our laboratory demonstrated that MS channels could exhibit spontaneous open activities without the need of externally applied pressure [111]. These spontaneous openings were found in about half of all patches and were strongly dependent on temperature and voltage. In the same study the authors further showed that MS channels needed far higher Gd^{3+} concentrations (i.e., 100 μM Gd^{3+} blocked 90% of MS current) for complete block than reported earlier [146].

In a very extensive study Wilkinson et al. [144] investigated the role of MS channel activity on oocyte development. In a clever series of experiments Hamill's

group showed that inhibiting MS channels with Gd^{3+}, amiloride or gentamicin had no serious influence on oocyte growth, maturation, fertilization or embryogenesis. From these data they concluded that other mechanisms could compensate MS channel activity or that the channels somehow managed to become insensitive to the blockers.

Nonselective Cation Channels (NSCC)

Depolarization of the oocyte plasma membrane activated nonselective endogenous hemi-gap-junctional cation channels [35]. These channels were sensitive to external divalent cations such as Ca^{2+} and Mg^{2+}; increasing $[Ca^{2+}]_o$ or $[Mg^{2+}]_o$ evoked marked reduction of channel current amplitudes. Furthermore, increasing $[Ca^{2+}]_o$ accelerated the time course of channel deactivation and reduced clamp current at negative V_m. In the same paper, it was also demonstrated that these hemi-gap-junctional channels in the nonjunctional oocyte membrane were strongly temperature-dependent. Raising temperature by only some degrees reversibly elevated current amplitudes produced by the channels. Injection of antisense oligonucleotides complementary to the sequence of a *Xenopus* connexin reduced the activity of the endogenous hemi-gap-junctional cation channel [35]. Connexin seems to be an endogenous protein that has strong regulatory influences on ion channels as also reported by Zhang et al. [153].

These hemi-gap-junctional channels share some similarities with divalent cation sensitive NSCC described by two other groups including slow activation kinetics, block by external divalent cations, and linear *IV* relationship [3, 153]. Arrellano et al. showed that external Ca^{2+} inhibited a poorly selective cation channel which accepted K^+ and Na^+ as a charge carrier [3]. Ca^{2+} block was voltage-dependent and had an IC_{50} of 61 μM at -60 mV and 212 μM at 0 mV. Oocyte maturation substantially reduced the current. More recently, another group showed that the channel inactivated by extracellular Ca^{2+} is also conductive for large organic monovalent cations and glucose with permeability ratios of 1 : 0.45 : 0.35 : 0.2 : 0.2 : 0.2 for K^+ : $NMDG^+$: TEA^+ : TPA^+ : TBA^+ : glucose [153]. Cl^- was permeant with nearly the same rate as glucose. The channels could be blocked by amiloride, gentamicin, Gd^{3+} and by two Cl^- channel blockers such as flufenamic acid and niflumic acid. The latter blockers has also been shown to block Ca^{2+} inactivated Cl^- channels in *Xenopus laevis* oocytes [138, 139]. Zhang et al. [153] further reported downregulation of the Ca^{2+}-inactivated poorly selective ion conductance following progesterone-induced maturation of the oocytes. However, as discussed in the Cl^- channel section, removal of external divalent cations seems to release block from different channel populations including the above mentioned nonselective cation channels and Cl^- channels.

Several other compounds were reported to induce activation of nonselective cation channels in oocytes. Palytoxin (PTX), the most potent nonpeptidic toxin known to date is able to stimulate a 7–8 pS poorly selective cation conductance by binding to a specific state of the *Xenopus* Na^+/K^+-ATPase [136]. NH_4^+ enters the oocyte via a pathway that could be blocked by typical inhibitors of nonselective cation channels such as La^{3+}, diphenylamine-2-carboxylate (DPC), and p-chloromercuribenzoate (pCMB) [17]. The NH_4^+ uptake by NSCC was activated by trimethylamine [19]. These results were confirmed and extended by Cougnon et al. [25] who also showed that NH_4^+ permeation occurred through multiple pathways including DPC-sensitive nonselective cationic conductance(s). The authors further argued for some ways of NH_4^+ uptake different from NSCC such as the Na^+/K^+-ATPase or a K^+/H^+-antiporter.

Heterologous expression of cloned proteins is able to cause activation of endogenous silent ion conductances as described for a CFTR-induced K^+ channel (please *see* K^+ channel section). Expression of high levels of human or *Drosophila* K^+ channels in oocytes stimulated endogenous silent nonselective cation channels [130]. The NSCC could be activated by hyperpolarization, was blocked by 4,4′diisothiocyanostilbene-2-2′-disulphonic acid (DIDS) and TEA, was further activated by chlofilium and sensitive to changes in external pH.

More recently, Bieldfeld-Ackermann et al. [9] described activation of NSCC in oocytes by maitotoxin (MTX, 50 pM to 1 nM), one of the most potent marine toxin derived from dinoflagellate *Gambierdiscus toxicus*. The current was sensitive to amiloride, benzamil and Gd^{3+}, while other NSCC blockers, such as flufenamic acid, niflumic acid or 3′,5′-dichlorodiphenylamine-2-carboxylic acid (DCDPC) had no significant effects on the conductance. Possible mechanisms of NSCC activation by MTX involve phosphoinositide signaling pathways, Ca^{2+} influx or release from intracellular stores and exocytotic delivery and functional insertion of silent NSCC from intracellular membranes into the plasma membrane. Recently, MTX-induced functional insertion of NSCC into the oocyte plasma membrane has been demonstrated in an abstract [140].

However, comparing all recent reports on NSCC and MS channels while working on this review, it became increasingly obvious that it might not always be possible to undoubtedly distinguish between MS channels and NSCC. Things have been further complicated by a recent report demonstrating that MS channels exhibit spontaneous opening without externally applied pressure working as NSCC [111]. The authors con-

cluded that one ion channel protein could operate in two modes and switch, depending on the environmental conditions, from MS to NSCC mode of operation. On the other hand, one can speculate that activating effects could target several ion transport pathways including MS channels and NSCC at one time.

Ca^{2+} Channels

VOLTAGE-DEPENDENT Ca^{2+} CHANNELS (VDCC)

Xenopus laevis oocytes are endowed with voltage-dependent Ca^{2+} channels firstly described by Dascal et al. [30]. Transient Ca^{2+} entry through VDCCs could be found in fewer than 30% of oocytes, was evoked by depolarization and produced small currents ranging from 20 nA [88] to 50 nA [73] although oocytes of a few toads exhibited large currents up to 100 nA [28]. However, under physiological conditions Ca^{2+} conductance of oocytes is rather low. Almost all groups who studied Ca^{2+} channels in oocytes replaced Ca^{2+} by Ba^{2+} to prevent activation of Ca^{2+}-dependent Cl^- channels and to block K^+ channels which could contaminate the results; some groups additionally added TTX (100 nM) to exclude influence of endogenous Na^+ channels. Patch clamp analysis of single channel events revealed the existence of two classes of Ca^{2+} channels namely a 18 pS N-type and a 9 pS T-type Ca^{2+} channel [69]. Ca^{2+} channels were totally blocked by Cd^{2+} in a reversible way [30] and were sensitive to some organic Ca^{2+} channel blockers such as ω-conotoxin [69]. A detailed pharmacological profile [77] revealed that Cd^{2+} block (0.1 mM) was more effective than Ni^{2+} (1 mM), Mn^{2+} and Co^{2+} (10 mM each). 2,3-butanedione monoxime (BDM) exhibits phosphatase action and was shown to inhibit N-type Ca^{2+} channels in oocytes after upregulation of VDCC by exogenous L-type Ca^{2+} channel subunits [2].

Hyperpolarization following injection of Ins-(1,4,5)P_3 into the oocytes also induced Ca^{2+} influx through Cd^{2+}-sensitive VDCC [101]. Furthermore, VDCC are regulated by PKA and PKC as shown by marked increase in Ca^{2+} current amplitude and a slowing of the inactivation time course after injection of cAMP or bath application of phorbol ester [13].

STORE-OPERATED Ca^{2+} CHANNELS (SOC)

Depletion of intracellular Ca^{2+} stores by injection of Ca^{2+} chelators, Ins(1,4,5)P_3, ionomycin or incubation with thapsigargin induced Ca^{2+} influx through Ca^{2+}-selective channels termed store-operated Ca^{2+} channels (SOC) [149]. These currents were similar in most but not all aspects to Ca^{2+} release-activated Ca^{2+} channels (I_{CRAC}) originally described in mast cells [53]. SOCs in

oocytes could be blocked by di- and trivalent cations with the following potency sequence: $Mg^{2+} << Ni^{2+} \approx Co^{2+} \approx Mn^{2+} > Cd^{2+} >> Zn^{2+} >> La^{3+}$ and by the Cl^- channel blocker niflumic acid [149]. Other blockers of SOCs were ω-conotoxins [76] and primaquine [43].

The mechanism by which SOCs sense and respond to Ca^{2+}-store depletion remains controversial: one model postulates the involvement of a messenger molecule that activates Ca^{2+} entry in response to store depletion; in an alternative model, Ins(1,4,5)P_3 receptors in the stores are linked with SOC. While in human tumor lymphocytes a low-molecular-weight Ca^{2+}-influx factor (CIF) was identified [109], a recent paper provided evidence for the coupling hypothesis in HEK293 cells stably expressing SOC [61]. However, so far there is no evidence suggesting the existence of a low-molecular-weight CIF in oocytes [93]. The most convincing data on a possible signal transduction mechanism came from a study designed by Parekh et al. [95]. The authors demonstrated, that okadaic acid stimulated store-operated Ca^{2+} influx after receptor stimulation and explained their observations postulating a diffusible messenger which might be a novel kinase, gating Ca^{2+} influx through a phosphorylation/dephosphorylation cycle [95]. Further evidence for a diffusible messenger was introduced also by Parekh et al. [94]. In a very detailed experimental work they showed that cramming a patch back into the oocyte returned the SOC current that was lost on excision of the patch.

On the other hand, capacitative Ca^{2+} entry was shown to be predominantly localized to the region of stimulation arguing against a highly diffusible Ca^{2+} influx factor [107]. In this study, injection of cytoplasm from oocytes with depleted calcium stores into untreated oocytes failed to activate Ca^{2+} influx suggesting that no diffusible messenger was involved. Overexpression of Ins(1,4,5)P_3-kinase inhibited SOC, leading to the suggestion that Ins(1,4,5)P_3 alone plays the crucial role in the activation of capacitative Ca^{2+} entry by emptying intracellular stores [133]. An argument against this view came from the observation that oocytes do not express the type 3 Ins(1,4,5)P_3 receptor when examined using Western blots [31]. Yet, in the light of these controversial data the crucial experiments identifying the molecular nature of the postulated diffusible activator are to be awaited.

Low level activation of PKC resulted in potentiation of Ca^{2+} entry through SOCs while higher levels of PKC activity reduced capacitative Ca^{2+} entry [106]. Parekh [92] showed that SOC interacted with Ca^{2+}-dependent Cl^- channels by clustering Cl^- channels in the plasma membrane. Further analysis revealed that oocytes possess two distinct Ca^{2+}-dependent Cl^- channels: one was activated by both Ca^{2+} influx and by Ca^{2+} release while the other was activated preferentially by capacitative

Ca^{2+} influx through SOCs [50]. Ca^{2+} was shown to control capacitative Ca^{2+} influx itself by a negative feedback mechanism involving the Ca^{2+}/calmodulin-dependent protein kinase II pathway [81].

SOCs are involved in formation of so-called Ca^{2+} waves in *Xenopus laevis* oocytes [41]. Complex intracellular Ca^{2+} signals control various cellular processes. $Ins(1,4,5)P_3$ opens its receptor channel on internal stores and controls Ca^{2+} influx via SOCs thereby modulating frequency and velocity of Ca^{2+} waves through the oocytes [5, 14, 21].

Water Channels

While using oocytes as an expression system two groups independently found endogenous water channels which shall be mentioned in order to be complete. Zhang and Verkman discovered low water permeability by exposing oocytes to osmotic gradients and subsequent measurements of oocyte volume [134, 151, 152]. Expression of human CFTR in oocytes activated an endogenous water channel as measured by volume increase [115]. Water conductance was sensitive to pCMBS and phloretin, commonly known as a blocker of glucose-facilitated diffusion carrier. However, since it has been reported that glucose transporters can be water permeable [75] and oocytes reportedly own at least two glucose transport systems [141, 142], it cannot be ruled out that water uses these carriers to enter the cell.

Cl$^-$ Channels

The predominant class of ion channels in *Xenopus laevis* oocytes includes several types of Cl$^-$ channels which are by far the most abundant channels of the oocytes. Most of the recent papers about endogenous ion channels of the oocyte are dealing with Cl$^-$ channels all of which cannot be addressed here because of space limitations.

Ca^{2+}-ACTIVATED Cl$^-$ CHANNELS (CaCC)

Initially, it was found that activation of muscarinic receptors or receptors for serotonin and glutamate induced a transient Cl$^-$ current followed by sustaining oscillatory Cl$^-$ currents evoked by Ca^{2+} release from intracellular stores via an $Ins(1,4,5)P_3$- and $Ins(1,3,4,5)P_4$-sensitive pathway [32, 85, 98]. CaCC currents were drastically reduced when oocytes were stored at 4 °C for 3 days and had a selectivity sequence of $I^- > Br^- \geq Cl^-$ [58]. The channels were sensitive to DPC, 9-AC and intracellular injection of Ca^{2+} chelators [11, 58]. Caffeine was shown to inhibit the $Ins(1,4,5)P_3$-mediated Ca^{2+} release, thereby abolishing CaCC currents [65, 97]. However, while inhibiting CaCC, caffeine did not affect the early

$Ins(1,4,5)P_3$ response [8]. Niflumic acid and flufenamic acid exhibited direct interaction with the CaCC protein and were described as reversible high affinity CaCC blockers [143]. La^{3+} and Gd^{3+} produced almost irreversible block of CaCC while Cd^{2+}, Co^{2+}, Ni^{2+}, and Mn^{2+} allowed partially channel recovery [129].

CaCC were asymmetrically distributed in the oocyte plasma membrane having the highest density in the animal pole [7, 65, 84, 100]. Using patch-clamp analysis of inside-out macropatches, Gomes-Hernandez et al. could show that the current density on the animal pole was ten times higher leading to functional polarity of the oocyte [42]. Therefore, caution in data interpretation is necessary when CaCCs are utilized as the only monitor of $[Ca^{2+}]_i$.

Parker and Yao first argued for a single CaCC population with changing kinetic behavior [104, 148]. However, Boton et al. already in 1987 speculated on the existence of more than one Ca^{2+}-activated Cl$^-$ channel with different Ca^{2+} dependence of activation and inactivation and different time course of inactivation [11]. Several papers brought more evidence for the 2-channel model: Hartzell demonstrated that one current was activated preferentially by Ca^{2+} influx through SOCs and that the other one could be activated by store released Ca^{2+} as well as by Ca^{2+} influx [50]. The different activation pattern of these two CaCCs could be mediated by asymmetrical distribution of SOC and CaCC as thoroughly investigated recently by the same group [79]. Yet, it should be noted that results can also differ dependent of the recording methods. Grygorczyk et al. reported that bioluminescence data show other results than electrophysiologically obtained data because of the different sensitivity of the techniques [44].

Oocytes seem to have the ability to sense where Ca^{2+} is coming from leading to fine tuning of the response. Ca^{2+} entering the oocyte from the external space activated a Ca^{2+}-dependent putative PKC causing subsequent inactivation of CaCCs while Ca^{2+} released from intracellular stores did not initiate an inactivating process [12]. The slow inducible Na^+ current seemed to be involved in the inactivation mechanism [22].

Experiments utilizing photorelease of $Ins(1,4,5)P_3$ from a caged precursor revealed that a threshold concentration of $Ins(1,4,5)P_3$ (60 nM) is required for Ca^{2+} release and CaCC activation [99]. External lysophosphatidic acid (LPA) induced activation of CaCC in a TTX-sensitive manner suggesting the participation of a LPA-specific Ca^{2+}-mobilizing membrane receptor linked to a TTX-sensitive G protein [34]. CaCC activation was also achieved with the protease trypsin and the authors explained this effect by the existence of a trypsin-specific receptor in the oocyte plasma membrane [33]. Following removal of external Na^+, endogenous Na^+/Ca^{2+}-exchangers reversed their mode of operation by extrud-

ing Na^+ from the cytoplasm and importing Ca^{2+} into the cell, leading to CaCC activation [113]. Since then, several other CaCC activation pathways were reported, including injection of pyrimidine nucleotide-glucose conjugates [60], external application of hyaluronan [37], $AlF4^-$ [87], or the antipsychotic drugs chlorpromazine and trifluoroperazine [108]. 5-hydroxytryptamine induced a fast transient inward current probably carried by SOCs followed by a slowly activating current attributed to CaCCs [92].

Expression and cAMP activation of human CFTR caused inhibition of CaCC in oocytes demonstrating a further regulatory function of CFTR [66]. Annexins, a family of Ca^{2+} binding proteins derived from a mouse tumor cell line also caused block of CaCC [55].

It remains an open question how many different types of CaCCs are expressed in oocytes. Kuruma and Hartzell [68] observed three currents after injection of $Ins(1,4,5)P_3$. They discuss whether these different currents were mediated by different channels or one channel operating in different modes. Although they favor the one channel theory, further work has to be done to identify the molecular identity of the channel(s).

Ca^{2+}-INACTIVATED Cl^- CHANNELS (CaIC)

As already mentioned in the cation section removal of external Ca^{2+} unblocks several ion conductances [3, 153]. While these authors identified the unblocked conductance as a nonselective cation channel, data from our laboratory identified a Cl^- channel that was activated by removal of external Ca^{2+} and consequently was termed CaIC [110, 138, 139]. The Ca^{2+}-inactivated ion conductances described by the three groups mentioned above share some features, however CaIC differs in some crucial properties: (i) CaIC was still fully functional after maturation, shown by whole cell measurements [138] and by single channel events [110] obtained from maturated oocytes (eggs). While the vast majority of ion transporters in oocytes are inactivated during maturation, the active CaIC in eggs seems to play a crucial physiological role in fertilization events. (ii) Single channel analysis of CaIC undoubtedly revealed a 90 pS Cl^- channel that could be inhibited via pipette perfusion of Ca^{2+} or Cl^-channel blockers such as flufenamic acid, niflumic acid, DPC and 9-AC. Unfortunately, single channel data for the Ca^{2+}-inactivated NSCC are not available. (iii) CaIC was active even in the absence of any external permeable cation. (iv) Removal of external Ca^{2+} led to considerable efflux of Cl^- through CaICs which could be measured as quenching of fluorescent Cl^--sensitive dye 6-methoxy-N-(sulfopropyl)quinolinium (SPQ). It can be further speculated that the different Ca^{2+}-inactivated channels could use a common extracellular Ca^{2+} sensing receptor whose existence was reported from bovine para-

thyroid [15]. Table 2 summarizes the most important features of the Ca^{2+}-inactivated ion conductances described up to now. However, from Table 2 it also becomes clear that there is a lack of some important data on the Ca^{2+}-inactivated ion channels reported by the three groups.

At the moment there is controversy about the ionic nature of the Ca^{2+}-inactivated currents. The observed reversal potentials (V_{rev}) around -10 mV do not allow an accurate classification of the currents since they differ from the calculated V_{rev} for K^+ (-100 mV), Na^+ (60 mV) and Cl^- (-30 mV). One explanation for the obvious discrepancies could be that removal of Ca^{2+} activates different ion channels that might change their selectivity depending on the ionic environment. It could be also that several endogenous channels need Ca^{2+} as a "cofactor" in their selectivity pore and removal of the cofactor changes the conductive behavior of the channels normally kept closed by Ca^{2+}. Therefore, the apparent contradictory results of the three groups may indicate that the macroscopic Ca^{2+}-sensitive current is mediated by a family of more or less specific cation- and anionselective conductances whose interactive regulation is unclear. Depending on the environmental conditions one channel type might be masked so that the other channel type dominates. The gap-junctional hemichannel described by Ebihara could also be involved [35]. The channel is blocked by external divalent cations and shares some properties with Ca^{2+}-inactivated cation channels (please *see also* the section on NSCC). However, the hemichannel can be clearly distinguished from CaIC by its low current amplitude (i.e., 100 nA compared to > 1 μA for CaIC) and its low Ca^{2+} sensitivity: while 90% of CaIC inactivate at $[Ca^{2+}]_o$ of 200 μM, hemichannel currents are maximal at this $[Ca^{2+}]_o$ [35].

Another explanation for the discrepancies observed by the different groups could be the existence of a single channel population of poorly selective channels that hardly discriminate between anions and cations. Depending on the environmental conditions the channel is permeable to cations or anions. The fact that the channel is sensitive to almost every blocker makes this hypothesis a viable alternative.

The physiological role of the Ca^{2+}-inactivated ion channels remains also as mysterious as the fact that Arellano et al. [3] and Zhang et al. [153] saw downregulation of the channels following maturation while we always had active channels in eggs. However, it remains to be elucidated, how these Ca^{2+}-inactivated ion conductances are relating on each other and how they might be interacting with each other.

HYPERPOLARIZATION-INDUCED Cl^- CHANNELS (Cl^-_{hyp})

Hyperpolarizing pulses slowly activated persistent Cl^- currents that were sensitive to SITS, Ba^{2+} and La^{3+}, and

Table 2. Properties of the Ca^{2+}-inactivated ion channels

Channel property	Arellano et al. [3]	Weber et al. [139] [110, 138]	Zhang et al. [153]
Ca^{2+}-dependence at V_m = −60 mV, $K_{1/2}$, μM	~60	20–30	n.a.
Selectivity	Na^+, $K^+ \gg Cl^-$	$I^- > Br^- > Cl^- \gg Na^+$, K^+	$Na^+ = K^+ \gg Cl^-$
Permeability to large organic cations and anions	n.a.	n.a.	+
Transport direction at physiological V_m	Cation absorption	Cl^- secretion	Cation absorption
Sensitivity to Cl^- channel blockers	n.a.	+ (FFA, NFA, DPC, NPPB, AZT 9-AC, SITS)	+ (FFA, NFA) − (9-AC, SITS)
Sensitivity to cation channel blockers	+	+ (Unpublished data)	+
Activity in absence of extracellular permeant cations	n.a.	+	n.a.
Sensitivity to Gd^{3+}	+	+ (Unpublished data)	+
Reversal potential, mV	−8.2 ± 3.8	−12.2 ± 0.8	−9.5 ± 3
Conductance, pS	n.a.	90	n.a.
V_m-dependence of $K_{1/2}$ for Ca^{2+}	+	−	n.a.
Activity in eggs	−	+	−
Activation by cyclic nucleotides	n.a.	+ (cAMP, cGMP)	n.a.
Regulation by connexin	n.a.	n.a.	+
Methods applied	Voltage-clamp	Voltage-clamp patch-clamp optical Cl^-efflux measurements	Voltage-clamp

Abbreviations: +, observed; −, not observed; 9-AC, anthracene-9-carboxylic acid; AZT, 3′azido-3′deoxythymidine; DPC, diphenylamine-2-carboxylate; FFA, flufenamic acid; n.a., data not available; NFA, niflumic acid; NPPB, 5-nitrophenyl-aminopropyl-benzoic acid; SITS, 4-4′-acetoamido-4′isothiocyanatostilbene-2,2′-disulfonicacid

insensitive to external or cytoplasmic Ca^{2+}, external Na^+ or pH [63, 129]. Strong hyperpolarization (> −100 mV) gave rise to well maintained Cl^- currents in oocytes from some donors [103]. Current amplitude was highly variable between oocytes of different donors and showed a selectivity sequence of $I^- > NO_3^- > Br^- > Cl^- >$ propionate > acetate [63]. Cl^-_{hyp} channels exhibited inward rectification and could be stimulated by external pituitary adenylate cyclase activating polypeptide (PACAP) [59]. Peptides derived from the amino-terminal domain of putative K^+ channel forming protein IsK (or minK, *see also* K^+ channel section) were claimed to induce Cl^-_{hyp} in oocytes [6]. However, it became not quite clear whether IsK itself formed a channel or endogenous Cl^-_{hyp} were activated.

HYPOTONICITY-ACTIVATED Cl^- CHANNELS (Cl^-_{vol})

Paulmichl et al. reported that only 4% of collagenase-treated oocytes exhibited endogenous volume-sensitive Cl^- channels [105]. However, when oocytes were defolliculated manually, hypotonicity-activated, voltage-independent but volume-sensitive Cl^- channels could be detected in nearly every oocyte [1]. In contrast to the Ca^{2+}-dependent Cl^- channels, these channels were insensitive to Ca^{2+} and niflumic acid but sensitive to La^{3+} [1]. Activation of the Cl^-_{vol} channel was dependent on an abundant cytoplasmic regulatory protein termed pI_{Cln} [128]. The authors showed that a monoclonal antibody recognizing pI_{Cln} blocked volume-sensitive Cl^- channels and suggested that pI_{Cln} might be involved in linking the channels to actin-bound cytoskeletal elements. However, as shown more recently, the pI_{Cln} induced Cl^- current is different from the endogenous volume-sensitive Cl^- channel [135]. The extensive patch-clamp study of Voets et al. [135] revealed differences in rectification, selectivity, inactivation kinetics, and extracellular pH. Moreover, pI_{Cln}-induced Cl^- currents could only be found in follicle-enclosed or manually defolliculated oocytes. These findings clearly show that the pI_{Cln}-induced

current and the volume-sensitive Cl⁻ current are different conductances.

Further evidence against the hypothesis that pI_{Cln} is the Cl^-_{vol} channel came from work done by Li et al. [74]. The authors reconstituted recombinant pI_{Cln} into artificial and biological membranes and showed that the channel was at least seven times more selective for cations than for anions. Therefore, the physiological role of pI_{Cln} remains unknown and controversial.

Concluding Remarks

The advantages and possibilities of *Xenopus laevis* oocytes attract more and more scientists to use the oocytes for the expression and characterization of foreign proteins. The result has been an explosion of knowledge regarding the physiology of cellular transport systems. Although not applicable for the expression of all transport systems (e.g., electroneutral transporters), the use of expression cloning in oocytes proved to be enormously helpful when transport systems are present in too small a number to be successfully investigated in native tissues [118]. Especially, the rapid development of powerful tools using molecular biology and the combination with electrophysiological methods on *Xenopus laevis* oocytes brought fascinating insights into ion channel function. However, the native oocyte itself is a fascinating object for the study of transport systems. It has provided and will provide new insights on ion transporter function, regulation and channel-channel interactions. And despite the large amount of work that has been done on endogenous transport proteins of the oocyte, which I tried to compile at least partly with the present review, much additional work will be required. As repeatedly mentioned above, some basic ionic transport phenomena remain unexplained and the solution of those problems will not only help to understand the physiology of oocytes but of mammalian cells on the whole.

References

1. Ackerman, M.J., Wickman, K.D., Clapham, D.E. 1994. *J. Gen. Physiol.* **103:**153–179
2. Allen, T.J.A., Mikala, G., Wu, X.-P., Dolphin, A.C. 1998. *J. Physiol.* **508:**1–14
3. Arellano, R.O., Woodward, R.M., Miledi, R. 1995. *J. Physiol.* **484:**593–604
4. Artalejo, C.R., Hoffmann, P.C., Rossie, S., Perlman, R.L., Fox, A.P. 1992. *Neurosci. Abstr.* **18:**1271 (*Abstr.*)
5. Atri, A., Amundson, J., Clapham, D.E., Sneyd, J. 1993. *Biophys. J.* **65:**1727–1739
6. Ben-Efraim, I., Shai, Y., Attali, B. 1996. *J. Biol. Chem.* **271:**8768–8771
7. Berridge, M.J. 1988. *J. Physiol.* **403:**589–599
8. Berridge, M.J. 1991. *Proc. R. Soc. Lond. B* **244:**57–62
9. Bielfeld-Ackermann, A., Range, C., Korbmacher, C. 1998. *Pfluegers Arch.* **436:**329–337
10. Bossi, E., Centinaio, E., Moriondo, A., Peres, A. 1998. *J. Cell. Physiol.* **174:**154–159
11. Boton, R., Dascal, N., Gillo, B., Lass, Y. 1989. *J. Physiol.* **408:**511–534
12. Boton, R., Singer, D., Dascal, N. 1990. *Pfluegers Arch.* **416:**1–6
13. Bourinet, E., Fournier, F., Nargeot, J., Charnet, P. 1992. *FEBS Lett.* **299:**5–9
14. Brooker, G., Seki, T., Croll, D., Wahlestedt, C. 1990. *Proc. Natl. Acad. Sci. USA* **87:**2813–2817
15. Brown, E.M., Gamba, G., Riccardi, D., Lombardi, M., Butter, R., Kifor, O., Sun, A., Hediger, M.A., Lytton, J., Hebert, S.C. 1993. *Nature* **366:**575–580
16. Burckhardt, B.C., Burckhardt, G. 1997. *Pfluegers Arch.* **434:**306–312
17. Burckhardt, B.C., Frömter, E. 1992. *Pfluegers Arch.* **420:**83–86
18. Burckhardt, B.C., Kroll, B., Frömter, E. 1992. *Pfluegers Arch.* **420:**78–82
19. Burckhardt, B.C., Thelen, P. 1995. *Pfluegers Arch.* **429:**306–312
20. Busch, A.E., Kopp, H.G., Waldegger, S., Samarzija, I., Süßbrich, H., Raber, G., Kunzelmann, K., Ruppersberg, J.P., Lang, F. 1996. *J. Physiol.* **491:**735–741
21. Camacho, P., Lechleiter, J.D. 1993. *Science* **260:**226–229
22. Centinaio, E., Bossi, E., Peres, A. 1997. *Cell. Mol. Life Sci.* **53:**604–610
23. Charpentier, G., Behue, N., Fournier, F. 1995. *Pfluegers Arch.* **429:**825–831
24. Charpentier, G., Fournier, F., Behue, N., Marlot, D., Brule, G. 1993. *Proc. R. Soc.* **254937:**15–20
25. Cougnon, M., Bouyer, P., Hulin, P., Anagnostopoulos, T., Planelles, G. 1996. *Pfluegers Arch.* **431:**658–667
26. Cross, N.L., Elinson, R.P. 1980. *Develop. Biol.* **75:**187–198
27. Dascal, N. 1987. *Crit. Rev. Biochem.* **22:**317–387
28. Dascal, N., Lotan, I., Karni, E., Gigi, A. 1992. *J. Physiol.* **450:**469–490
29. Dascal, N., Lotan, I., Lass, Y. 1987. *Pfluegers Arch.* **409:**521–527
30. Dascal, N., Snutch, T.P., Lübbert, H., Davidson, N., Lester, H.A. 1986. *Science* **231:**1147–1150
31. DeLisle, S., Blondel, O., Longo, F.J., Schnabel, W.E., Bell, G.L., Welsh, M.J. 1996. *Am. J. Physiol.* **270:**C1255–C1261
32. DeLisle, S., Krause, K.-H., Denning, G.M., Potter, B.V.L., Welsh, M.J. 1990. *J. Biol. Chem.* **265:**11727–11730
33. Durieux, M.E., Salafranca, M.N., Lynch, K.R. 1994. *FEBS Lett.* **337:**235–238
34. Durieux, M.E., Salafranca, M.N., Lynch, K.R., Moorman, J.R. 1992. *Am. J. Physiol.* **263:**C896–C900
35. Ebihara, L. 1996. *Biophys. J.* **71:**742–748
36. Eckstein-Ludwig, U., Rettinger, J., Vasilets, L.A., Schwarz, W. 1998. *Biochim. Biophys. Acta* **1372:**289–300
37. Fraser, S.P. 1997. *FEBS Lett.* **404:**56–60
38. Fraser, S.P., Djamgoz, M.B.A. 1992. *In: Xenopus* Oocytes: Endogenous Electrophysiological Characteristics. Current Aspects of the Neurosciences. N.N. Osborne, editor. pp. 267–315. Macmillan Press
39. Fraser, S.P., Moon, C., Djamgoz, M.B.A. 1997. *In:* Electrophysiology of *Xenopus* Oocytes: An Expression System in Molecular

Neurobiology. Electrophysiology, A Practical Approach. D.I. Wallis, editor. pp. 65–86. Oxford University Press

40. Garty, H., Palmer, L.G. 1997. *Phys. Rev.* **77:**359–396
41. Girard, S., Clapham, D.E. 1993. *Science* **260:**229–232
42. Gomez-Hernandez, J.-M., Stühmer, W., Parekh, A.B. 1997. *J. Physiol.* **502:**569–574
43. Gregory, R.B., Barritt, G.J. 1996. *Biochem. J.* **319:**755–760
44. Grygorczyk, R., Feighner, S.D., Adam, M., Liu, K.K., LeCouter, J.E., Dashkevicz, M.P., Hreniuk, D.L., Rydberg, E.H., Arena, J.P. 1996. *J. Neuroscience Meth.* **67:**19–25
45. Grygorczyk, R., Schwarz, W., Passow, H. 1987. *J. Membrane Biol.* **99:**127–136
46. Hamill, O.P., Lane, J.W., McBride, D.W. 1992. *Trends Pharmacol. Sci.* **13:**373–376
47. Hamill, O.P., McBride, D.W. 1992. *Proc. Natl. Acad. Sci. USA* **89:**7462–7466
48. Hamill, O.P., McBride, D.W. 1994. *News Physiol. Sci.* **9:**53–59
49. Hamill, O.P., McBride, D.W. 1996. *Pharmacol. Rev.* **48:**231–252
50. Hartzell, H.C. 1996. *J. Gen. Physiol.* **108:**157–175
51. Heinemann, S.H., Conti, F., Stühmer, W. 1992. *Meth. Enzymol.* **207:**353–368
52. Hilgemann, D.W., Lu, C.-C. 1998. *Meth. Enzymol.* **293:**267–280
53. Hoth, M., Penner, R. 1992. *Nature* **355:**353–356
54. Huang, H., St.-Jean, H., Coady, M.J., Lapointe, J.-Y. 1995. *J. Membrane Biol.* **143:**29–35
55. Jorgensen, A.J., Bennekou, P., Eskesen, K., Kristensen, B.I. 1997. *Pfluegers Arch.* **434:**261–266
56. Kado, R.T., Marcher, K., Ozon, R. 1981. *Develop. Biol.* **84:**471–476
57. Kaneko, S., Akaike, A., Satoh, M. 1998. *Meth. Enzymol.* **293:**319–331
58. Katayama, Y., Widdicombe, J.H. 1991. *J. Physiol.* **443:**587–599
59. Kato, M., Hanaoka, Y., Tatemoto, K., Kimura, C. 1997. *Reg. Peptides* **70:**167–172
60. Kim, H.Y., Thomas, D., Hanley, M.R. 1996. *Mol. Pharmacol.* **49:**360–364
61. Kiselyov, K., Xu, X., Mozhayeva, G., Kuo, T., Pessah, I., Mignery, G., Zhu, X., Birnbaumer, L., Muallem, S. 1998. *Nature* **396:**478–482
62. Kleyman, T.R., Cragoe, E.J. 1988. *J. Membrane Biol.* **105:**1–21
63. Kowdley, G.C., Ackerman, S.J., John, J.E., Jones, L.R., Moorman, J.R. 1994. *J. Gen. Physiol.* **103:**217–230
64. Krafte, D.S., Volberg, W.A. 1992. *J. Neuroscience Meth.* **43:**189–193
65. Kristian, T., Kolaj, M., Poledna, J. 1991. *Gen. Physiol. Biophys.* **10:**265–280
66. Kunzelmann, K., Mall, M., Briel, M., Hipper, A., Nitschke, R., Ricken, S., Greger, R. 1997. *Pfluegers Arch.-Europ. J. Physiol.* **435:**178–181
67. Kupitz, Y., Atlas, D. 1993. *Science* **261:**484–486
68. Kuruma, A., Hartzell, H.C. 1999. *Am. J. Physiol.* **273:**C161–C175
69. Lacerda, A.E., Perez-Reyes, E., Wei, X., Castellano, A., Brown, A.M. 1994. *Biophys. J.* **66:**1833–1843
70. Lane, J.W., McBride, D.W., Hamill, O.P. 1991. *J. Physiol.* **441:**347–366
71. Lane, J.W., McBride, D.W., Hamill, O.P. 1992. *Br. J. Pharmacol.* **106:**283–286
72. Lane, J.W., McBride, D.W., Hamill, O.P. 1993. *Br. J. Pharmacol.* **108:**116–119
73. Leonard, J.P., Nargeot, J., Snutch, T.P., Davidson, N., Lester, H.A. 1998. *J. Neurosci.* **7:**875–881
74. Li, C., Breton, S., Morrison, R., Cannon, C.L., Emma, F., Sanchez-Olea, R., Bear, C., Strange, K. 1998. *J. Gen. Physiol.* **112:**727–736
75. Loike, J.D., Hickman, S., Kuang, K., Xu, M., Cao, L., Vera, J.C., Silverstein, S.C., Fischbarg, J. 1996. *Am. J. Physiol.* **271:**C1474–C1479
76. Lomax, R.B., Herrero, C.J., García-Palermo, E., García, A.G., Montiel, C. 1998. *Cell Calcium* **23:**229–239
77. Lory, P., Rassendren, F.A., Richard, S., Tiaho, F., Nargeot, J. 1990. *J. Physiol.* **429:**95–112
78. Lu, L., Montrose-Rafizadeh, C., Hwang, T.-C., Guggino, W.B. 1990. *Biophys. J.* **57:**1117–1123
79. Machaca, K., Hartzell, H.C. 1998. *Biophys. J.* **74:**1286–1295
80. Mall, M., Kunzelmann, K., Hipper, A., Busch, A.E., Greger, R. 1996. *Pfluegers Arch.* **432:**516–522
81. Matifat, F., Fournier, F., Lorca, T., Capony, J.P., Brûlé, G., Collin, T. 1997. *Biochem. J.* **322:**267–272
82. McBride, D.W., Hamill, O.P. 1993. *Trends Neurosci.* **16:**341–345
83. Methfessel, C., Witzemann, V., Takahashi, T., Mishina, M., Numa, S., Sakmann, B. 1986. *Pfluegers Arch.* **407:**577–588
84. Miledi, R., Parker, I. 1989. *J. Physiol.* **415:**189–210
85. Miledi, R., Parker, I., Sumikawa, K. 1987. *J. Physiol.* **383:**213–229
86. Miledi, R., Woodward, R.M. 1989. *J. Physiol.* **416:**601–621
87. Moon, C., Fraser, S.P., Djamgoz, M.B.A. 1997. *Cell. Signal.* **9:**497–504
88. Moorman, J.R., Zhou, Z., Kirsch, G.E., Lacerda, A.E., Caffrey, J.M., Lam, D.M.K., Joho, R.H., Brown, A.M. 1998. *Am. J. Physiol.* **253:**H985–H991
89. Nowak, M.W., Gallivan, J.P., Silverman, S.K., Labarca, C.G., Dougherty, D.A., Lester, H.A. 1998. *Meth. Enzymol.* **293:**504–529
90. Nuccitelli, R., Ferguson, J.E. 1994. *Science* **263:**988 (*Abstr.*)
91. Olcese, R., Neely, A., Qin, N., Wei, X., Birnbaumer, L., Stefani, E. 1996. *J. Physiol.* **497:**675–686
92. Parekh, A.B. 1995. *Pfluegers Arch.* **430:**954–963
93. Parekh, A.B., Penner, R. 1997. *Phys. Rev.* **77:**901–930
94. Parekh, A.B., Terlau, H. 1996. Pfluegers Arch. **432:**14–25
95. Parekh, A.B., Terlau, H., Stühmer, W. 1993. *Nature* **364:**814–818
96. Parker, I., Ivorra, I. 1990. *Proc. R. Soc. Lond. B* **238:**369–381
97. Parker, I., Ivorra, I. 1991. *J. Physiol.* **433:**229–240
98. Parker, I., Ivorra, I. 1991. *J. Physiol.* **43:**207–227
99. Parker, I., Ivorra, I. 1992. *Am. J. Physiol.* **262:**C154–C165
100. Parker, I., Miledi, R. 1987. *Proc. R. Soc. Lond. B* **232:**59–70
101. Parker, I., Miledi, R. 1987. *Proc. R. Soc. Lond. B* **231:**27–36
102. Parker, I., Miledi, R. 1987. *Proc. R. Soc. Lond. B* **232:**289–296
103. Parker, I., Miledi, R. 1988. *Proc. R. Soc. Lond. B* **233:**191–199
104. Parker, I., Yao, Y. 1994. *Cell Calcium* **15:**276–288
105. Paulmichl, M., Li, Y., Wickman, K.D., Ackerman, M.J., Peralta, E., Clapham, D.E. 1992. *Nature* **356:**238–241
106. Petersen, C.C.H., Berridge, M.J. 1994. *J. Biol. Chem.* **269:**32246–32253
107. Petersen, C.C.H., Berridge, M.J. 1996. *Pfluegers Arch.* **432:**286–292
108. Quamme, G.A. 1997. *Biochim. Biophys. Acta* **1324:**18–26
109. Randriamampita, C., Tsien, R.Y. 1993. *Nature* **364:**809–814
110. Reifarth, F.W., Amasheh, S., Clauss, W., Weber, W.-M. 1997. *J. Membrane Biol.* **155:**95–104
111. Reifarth, F.W., Clauss, W., Weber, W.-M. 1999. *Biochim. Biophys. Acta* **1417:**63–76
112. Rettinger, J. 1995. *Pfluegers Arch.* **429 (Suppl. 6):**R65
113. Schlief, T., Heinemann, S.H. 1995. *J. Physiol.* **486:**124–130
114. Schmalzing, G., Eckard, P., Kröner, S., Passow, H. 1990. *Am. J. Physiol.* **258:**C179–C184

115. Schreiber, R., Greger, R., Nitschke, R., Kunzelmann, K. 1997. Pfluegers Arch. **434:**841–847

116. Schütt, W., Sackin, H. 1997. Pfluegers Arch. **433:**368–375

117. Shih, T.M., Smith, R.D., Toro, L., Goldin, A.L. 1998. *Meth. Enzymol.* **293:**529–556

118. Sigel, E. 1990. *J. Membr. Biol.* **117:**201–221

119. Stampe, P., Begenisich, T. 1998. *Meth. Enzymol.* **293:**556–564

120. Stefani, E., Bezanilla, F. 1998. *Meth. Enzymol.* **293:**300–318

121. Steffan, R., Hennesthal, C., Heinemann, S.H. 1998. *Meth. Enzymol.* **293:**391–419

122. Stühmer, W. 1992. *Meth. Enzymol.* **207:**319–339

123. Stühmer, W. 1998. *Meth. Enzymol.* **293:**280–300

124. Stühmer, W., Parekh, A.B. 1995. *In:* Electrophysiological recordings from *Xenopus* oocytes. Single-Channel Recording. B. Sakmann and E. Neher, editors. pp. 341–356. Plenum Press, New York

125. Taglialatela, M., Toro, L., Stefani, E. 1992. *Biophys. J.* **61:**78–82

126. Takumi, T., Moriyoshi, K., Aramori, I., Ishii, T., Oiki, S., Okada, Y., Ohkubo, H., Nakanishi, S. 1991. *J. Biol. Chem.* **266:**22192–22198

127. Takumi, T., Ohkubo, H., Nakanishi, S. 1988. *Science* **242:**1042–1045

128. Tauc, M., Bidet, M., Puojeol, P. 1996. *J. Membrane Biol.* **150:**255–273

129. Tokimasa, T., North, R.A. 1996. *J. Physiol.* **496:**677–686

130. Tzounopoulos, T., Maylie, J., Adelman, J.P. 1995. *Biophys. J.* **69:**904–908

131. Vasilets, L.A., Schwarz, W. 1993. *Biochim. Biophys. Acta* **1154:**201–222

132. Vasilets, L.A., Schwarz, W. 1994. *Cell. Physiol. Biochem.* **4:**81–95

133. Verjans, B., Petersen, C.C.H., Berridge, M.J. 1994. *Biochem. J.* **304:**679–682

134. Verkman, A.S., Van Hoek, A.N., Ma, T., Frigeri, A., Skach, W.R., Mitra, A., Tamarappoo, Bi.K., Farinas, J. 1996. *Am. J. Physiol.* **270:**C12–C30

135. Voets, T., Buyse, G., Tytgat, J., Droogmans, G., Eggermont, J., Nilius, B. 1996. *J. Physiol.* **495:**441–447

136. Wang, X., Horisberger, J.-D. 1997. *FEBS Lett.* **409:**391–395

137. Weber, W.-M., Liebold, K.M., Clauss, W. 1995. *Biochim. Biophys. Acta* **1239:**201–206

138. Weber, W.-M., Liebold, K.M., Reifarth, F.W., Clauss, W. 1995. *J. Membrane Biol.* **148:**263–275

139. Weber, W.-M., Liebold, K.M., Reifarth, F.W., Uhr, U., Clauss, W. 1995. *Pfluegers Arch.* **429:**820–824

140. Weber, W.-M., Popp, C., Clauss, W., Van Driessche, W. 1999. *Pfluegers Arch.* **437:**R89

141. Weber, W.-M., Püschel, B., Steffgen, J., Koepsell, H., Schwarz, W. 1991. *Biochim. Biophys. Acta* **1063:**73–80

142. Weber, W.-M., Schwarz, W., Passow, H. 1990. *J. Membrane Biol.* **111:**93–102

143. White, M.M., Aylwin, M. 1990. *Mol. Pharmacol.* **37:**720–724

144. Wilkinson, N.C., Gao, F., Hamill, O.P. 1998. *J. Membrane Biol.* **165:**161–174

145. Wu, G., McBride, D.W., Hamill, O.P. 1998. *Pfluegers Arch.* **435:**572–574

146. Yang, X.-C., Sachs, F. 1989. *Science* **243:**1068–1071

147. Yang, X.-C., Sachs, F. 1990. *J. Physiol.* (*Lond.*) **431:**103–122

148. Yao, Y., Parker, I. 1993. *J. Physiol.* (*Lond.*) **468:**275–296

149. Yao, Y., Tsien, R.Y. 1997. *J. Gen. Physiol.* **109:**703–715

150. Yoshida, S., Plant, S. 1991. *J. Physiol.* (*Lond.*) **443:**651–667

151. Zhang, R., Logee, K.A., Verkman, A.S. 1997. *J. Biol. Chem.* **265:**15375–15378

152. Zhang, R., Verkman, A.S. 1991. *Am. J. Physiol.* **260:**C26–C34

153. Zhang, Y., McBride, D.W., Hamill, O.P. 1998. *J. Physiol.* **508:**763–776