The Polarized Distribution of poly(A$^+$)-mRNA-induced Functional Ion Channels in the *Xenopus* Oocyte Plasma Membrane Is Prevented by Anticytoskeletal Drugs

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Abstract. Foreign mRNA was expressed in *Xenopus laevis* oocytes. Newly expressed ion currents localized in defined plasma membrane areas were measured using the two-electrode voltage clamp technique in combination with a specially designed chamber, that exposed only part of the surface on the oocytes to channel agonists or inhibitors. Newly expressed currents were found to be unequally distributed in the surface membrane of the oocyte. This asymmetry was most pronounced during the early phase of expression, when channels could almost exclusively be detected in the animal hemisphere of the oocyte. 4 d after injection of the mRNA, or later, channels could be found at a threefold higher density at the animal than at the vegetal pole area. The pattern of distribution was observed to be similar with various ion channels expressed from crude tissue mRNA and from cRNAs coding for rat $GABA_A$ receptor channel subunits. Electron microscopical analysis revealed very similar microvilli patterns at both oocyte pole areas. Thus, the asymmetric current distribution is not due to asymmetric surface structure. Upon incubation during the expression period in either colchicine or cytochalasin D, the current density was found to be equal in both pole areas. The inactive control substance β-lumicolchicine had no effect on the asymmetry of distribution. Colchicine was without effect on the amplitude of the expressed whole cell current. Our measurements reveal a pathway for plasma membrane protein expression endogenous to the *Xenopus* oocyte, that may contribute to the formation and maintenance of polarity of this highly organized cell.

THE *Xenopus* oocyte is not only one of the best characterized preparations for studies of early development (for review see Dawid and Sargent, 1988), but also widely used as a functional expression system for eukaryotic proteins (Gurdon, 1974; Colman, 1984; Sigel 1990). Total tissue mRNA or RNA derived from cDNA may be microinjected into the oocytes. Biosynthesis, co- and posttranslational modification, positioning to the proper cellular compartment, and functional behavior of the coded proteins can easily be monitored (Lane, 1983; Soreq, 1985).

Little is known about the distribution of newly expressed membrane proteins in the surface membrane. Early studies have indicated that the nicotinic acetylcholine receptor channel is predominantly located in the vegetal hemisphere, as shown by localized pressure application of agonist (Barnard et al., 1982; Miledi and Sumikawa, 1982). The influenza virus protein hemagglutinin has been reported to assume random distribution after expression in the *Xenopus* oocyte (Ceriotti and Colman, 1988). Newly expressed receptors, that are known to interact with G-proteins and other signaling components endogenous to the oocytes, have been reported to be predominantly localized in the animal hemisphere (Oron et al., 1988). However, in these experiments, the possibility of a nonrandom distribution of the signaling components distal to the receptor has not been excluded. More recently the same group showed that the distribution of muscarinic acetylcholine binding sites endogenous to the oocyte does indeed agree with the size of current responses measured upon local application of agonist (Matus-Leibovitch et al., 1990). Recent data on the lateral distribution of newly expressed butyrylcholine esterase indicated a patchy distribution with a higher density in the animal hemisphere (Dreyfus et al., 1989). In this case, it is not clear how the protein is associated with the membrane.

We were interested to perform direct measurements of the distribution in the plasma membrane of newly expressed, functional, integral membrane proteins. We found that this distribution is highly polarized. Nonrandom expression of plasma membrane proteins is a feature well known in various other types of polarized cells (for review see Simons and Fuller, 1985; Bartles and Hubbard, 1988; Simons and Wandinger-Ness, 1990). Specific sorting of proteins to the apical membrane in these polarized cells is sensitive to the microtubule disrupting drug colchicine (Achler et al., 1989;

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Durand-Schneider et al., 1987; Rindler et al., 1987). According to a recent study on the intestinal epithelial cell line Caco-2, microtubule perturbation does not affect precision of apical protein sorting, but retards delivery of these proteins to the apical membrane (Matter et al., 1990). In contrast to colchicine, cytochalasin, a drug that prevents actin assembly at the barbed ends of filaments, was without effect on plasma membrane protein sorting (Rindler et al., 1987; Salas et al., 1986). Polarized sorting of membrane protein is not restricted to epithelial cells but has also been described to occur in hippocampal neurones in culture (Dotti and Simons, 1990).

We were interested to see how localized expression of plasma membrane proteins in the *Xenopus* oocyte relates to the one observed in other polarized cells. We additionally report on effects of the anticytoskeletal drugs colchicine and cytochalasin D on the amount of functional plasma protein expression, and on its distribution in the plasma membrane. Preliminary accounts of some of the presented data have previously been published (Peter, A. B., and E. Sigel. 1989. *Experientia [Basel].* 45:A43; Peter, A. B., H. Reuter, and E. Sigel. 1990. *Experientia [Basel]. 46:A17).* The directed expression of foreign plasma membrane proteins may trace a mechanism endogenous to the *Xenopus* oocyte that is involved in the generation and maintenance of asymmetry in this cell.

Materials and Methods

mRNA-induced Channel Expression

Total mRNA was prepared from chick forebrain and leg skeletal muscle of 1-d-old chicks following the procedures of Cathala et al. (1983) with the previously described modifications (Sigel, 1987*a*). *Torpedo* electric organ mRNA was a kind gift from Dr. C. Gundersen (UCLA School of Medicine, Los Angeles). cDNA-derived RNA coding for subunits of the rat brain $GABA_A$ receptor channel were a kind gift from Dr. P. Malherbe (Hoffmann-La Roche, Basel; Malherbe et al., 1990). Injection of the mRNA into the oocytes and the removal of cellular layers surrounding the membrane have been described elsewhere (Sigel, 1987*a*). Unless indicated otherwise, oocytes were injected at the equator, to prevent localized expression due to possible local deposits of mRNA. Measurements were performed on denuded oocytes, i.e., the follicular layers were removed before the current measurement.

Electrophysiological Experiments

The oocytes were placed into the conically shaped opening (bottom diameter 0.5 mm) of a special bath, which is shown in Fig. 1. We estimate that ~8–12% of the surface, depending on the size of the oocyte, was exposed to the bottom tunnel, which was perfused at a rate of 4 ml/min. For the measurements of the respective ionic currents carried through GABA, kainate, and acetylcholine receptor channels (I_{GABA}, $I_{kainate}$ and I_{nAch})[1], and through sodium channels (I_{Na}), the medium in the upper chamber and the perfusion medium contained 90 mM NaCl, 1 mM KCl, 1 mM $MgCl_2$, 1 mM $CaCl_2$ and 5 mM Hepes-NaOH (pH 7.4). The perfusion with this medium could be switched to an identical medium supplied with GABA (100 μM), kainate (50 μM), acetylcholine (10 μM), or tetrodotoxin (50 nM). For the measurement of barium currents through calcium channels (I_{Ba}), the medium contained 40 mM $Ba(OH)_2$, 50 mM NaOH, 1 mM KOH, 5 mM Hepes, titrated to pH 7.4 using methanesulfonic acid, methanesulfonate being the major anion. $CdCl_2$ (20 μM) was used as an inhibitor. All experiments were carried out at room temperature (20–24°C). Ion currents were measured using a home built high voltage (140 V) two-electrode voltage-clamp amplifier. Oocytes were impaled with two conventional microelectrodes filled with 3 M KCl and displaying an electrical resistance of 2–5 MΩ. The holding potential was set at −100 or −80 mV for voltage- and for ligand-gated channels, respectively. For the measurement of I_{Na} and I_{Ba} the membrane potential was pulsed for 60 ms to −10 mV and for 100 ms to +20 mV, respectively, where the corresponding ion currents were maximal (Sigel, 1987*b*), at a frequency of 0.1 Hz. Ligand-gated ion currents were recorded on a strip chart recorder. Voltage-gated currents were recorded on FM tape and later digitized and analyzed on a standard IBM-compatible PC/AT using Modula-2 (Logitech, Inc., Redwood City, CA). Where applicable, statistical data are given as mean ± SEM. The rate of desensitization of ligand activated currents was independent on the site of measurement and the same as in whole cell current measurements. Therefore, the current amplitudes were read as peak currents, ignoring desensitization. Voltage-dependent current amplitudes were determined assuming a linear relationship between resting current and membrane potential between −100 and −10 mV for measurements of I_{Na} in normal medium, and +20 mV for measurements of I_{Ba} in barium medium.

Determination of Localized Currents

For the measurement of ligand-activated currents, the same oocyte was used for current determinations in the vegetal and animal pole areas. The respective pole areas were exposed to agonist superfusion. Control experiments showed that reimpalement did not alter the size of the measured whole-cell current. To minimize possible errors introduced by agonist diffusion along the oocyte-funnel wall interface, only the asymmetry of expression (see below) was evaluated. In control experiments, it was found that oocytes placed in the special chamber used here (Fig. 1), displayed the identical voltage activated whole cell current amplitudes as the same oocytes placed in a conventional bath. This indicates that the tight contact of the oocyte membrane with the wall of the funnel did not prevent reliable whole cell current measurements. For determination of the fractional, voltage-gated currents localized in the exposed membrane patch, current amplitudes observed before and during superfusion with saturating concentrations of a channel inhibitor were subtracted from each other. The amount of inhibited current was divided by the whole cell current to obtain the fractional inhibition. Fractional inhibition was taken as the fractional current in the drug-exposed patch. In control experiments the time course of I_{Na} inhibition was determined in a conventional bath and compared with the time course observed in the special chamber. While the inhibition in the conventional bath followed a monophasic time course, a biphasic time course was observed in the special chamber. The second phase presumably corresponds to diffusion of the channel inhibitor along the oocyte–funnel wall interface. In order to minimize the effect of drug diffusion, the inhibited current was read 30 s after exposure to the inhibitor. At this time, inhibition in the conventional bath had reached >95%. In some experiments the second phase of inhibition was extrapolated back to the time of medium change. The error introduced by the second phase was <3% of the whole cell current.

Calculation of the Asymmetry of Expression

To minimize the error introduced by agonist and channel inhibitor diffusion along the oocyte funnel wall interface, we calculated the relative currents expressed in the vegetal and the animal pole areas (ratio vegetal/animal). As the relative error caused by diffusion is likely to be the same on either side, this ratio should be independent of diffusion effects. In the case of exclusive localization of functional channels in the animal hemisphere this ratio would be 0.0; in the case of random distribution of channel in the surface membrane the theoretical ratio is 1.0. When measuring ligand-activated currents, individual oocytes were used for the determination of currents at both the vegetal and the animal pole areas. Thus, the ratio (vegetal/animal) could be obtained for individual oocytes. When measuring voltage activated currents the fractional inhibition at the animal and vegetal pole was measured each on different oocytes. The error of the ratio was calculated as the propagated SEM.

Exposure to Anticytoskeletal Drugs

Colchicine and cytochalasin D were obtained from Sigma Chemical Co. (St. Louis, MO). β-lumicolchicine was from Aldrich Chemical Co. (Milwaukee, WI). Colchicine and β-lumicolchicine were dissolved in ethanol at a concentration of 1 and 10 mM, respectively. Cytochalasin D was dissolved in DMSO (10 mM). These stock solutions were kept at −20°C. After

1. *Abbreviations used in this paper*: I_{nAch}, nonspecific cation current activated by acetylcholine; I_{Ba}, voltage-activated barium current through calcium channels; I_{GABA}, chloride current activated by γ-aminobutyrate; $I_{kainate}$, nonspecific cation currents activated by kainate.

injection with mRNA, untreated control oocytes were incubated in modified Barth's solution (Gurdon, 1974) for 50 h, at 18°C. Subsequently, the oocytes were freed from all surrounding cellular layers (Sigel, 1987*a*), and cultured for another 18–24 h. Drug-treated cells were processed identically, except that the culture medium contained the corresponding drug during the first incubation period. Absence of the drugs in the second incubation period did not lead to recovery of the normal morphology, nor did presence of drugs for longer periods of time lead to further changes in channel distribution. The final organic solvent concentration in the incubation was always <0.1%.

Electron Microscopy

After injection with mRNA, oocytes were cultured for 3 d and freed from the surrounding follicular cell layers. Injected and noninjected control oocytes were fixed in 2.5% glutaraldehyde in 20 mM Hepes-NaOH, pH 7.4, containing 90 mM NaCl, 1 mM $CaCl_2$, 1 mM $MgCl_2$, at room temperature, overnight. Subsequently, they were washed in 0.1 M sodium cacodylate-HCl buffer, pH 7.4 at 4°C and equatorially cut in half. Every hemisphere was labeled and processed individually. Oocytes were postfixed in 1% OsO_4 in 0.1 M sodium cacodylate buffer, pH 7.4, washed in 0.05 M maleate-NaOH buffer, pH 7.4 and stained with 0.5% uranyl acetate in the above maleate buffer. After the last wash, the samples were dehydrated in graded ethanol and embedded in Epon, using propylenoxide as intermedium. Thin sections of entire hemispheres were observed with a Phillips 200 electron microscope at 60 kV.

Results

We have measured the distribution of poly(A^+)-mRNA induced ion channels in the surface membrane of Xenopus oocytes. The method used here allowed mapping of functional ion channels at low spatial resolution only. We estimate from the geometry of our measurement system (Fig. 1) and of the oocyte that ~10% of the oocyte surface is exposed to drug containing solutions, that either activate ligand-gated channels or inhibit voltage-gated channels. γ-Aminobutyrate was used to activate chloride currents (I_{GABA}) mediated by the $GABA_A$ channel; kainate and acetylcholine to activate nonspecific cation current ($I_{kainate}$ and I_{nAch}) carried through the kainate-sensitive glutamate channel and the nicotinic acetylcholine channel. Voltage-activated sodium current (I_{Na}) and voltage-activated barium current through calcium channels (I_{Ba}) were inhibited by tetrodotoxin and Cd^{2+}, respectively.

Measurement of I_{GABA} and I_{Na} in the Pole Areas

Measurement of the nonrandom distribution of one ligand-activated current and of one voltage-activated current is documented in Figs. 2 and 3. Statistical data are included in additional figures and tables.

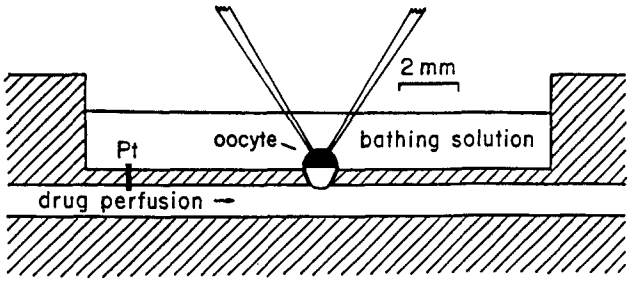

Figure 1. Longitudinal section of the Plexiglas chamber used. The oocyte is placed into a conically shaped opening. Its vegetal pole is exposed to a perfusion channel of a diameter of 1.0 mm. The perfusion medium can be switched to the same medium containing channel agonists or specific inhibitors. *Pt*, platinum filament that forms ground connection between the two compartments of the chamber.

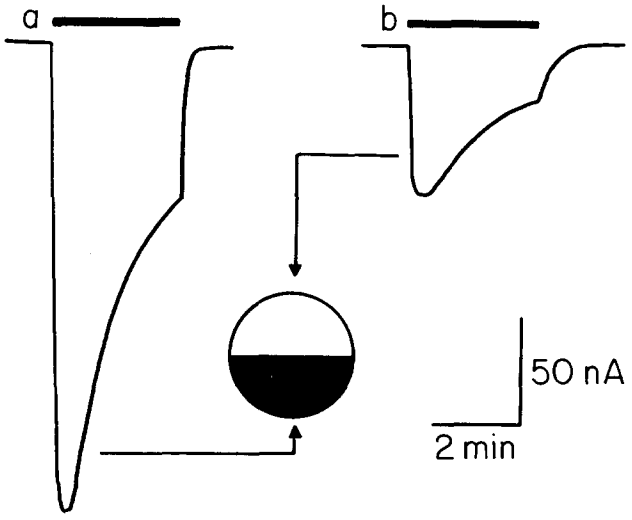

Figure 2. Measurement of local I_{GABA}. Oocytes were injected with chick forebrain mRNA and cultured and prepared for electrophysiological experiments as described in the methods section. 8 d after injection at the vegetal pole, an oocyte was placed with the animal pole pointing downwards into the funnel of the chamber shown in Fig. 1, impaled with two microelectrodes, and the membrane potential was set at −80 mV, using the voltage clamp technique. Subsequently, the continuous perfusion in the lower bore was changed to a medium containing 100 μM GABA (indicated by the bar), and the current resulting from exposure of the animal pole to GABA was recorded (*a*). The same oocyte was rotated by 180° to expose the vegetal pole to the perfusion with GABA (*b*).

Fig. 2 illustrates typical current traces that were obtained by superfusing the animal pole area (Fig. 2 *a*) and the vegetal pole area (Fig. 2 *b*) of an oocyte with 100 μM GABA. In this case the same oocyte was used for the experiments. The measurement was performed 8 d after injection into the vegetal pole of the oocyte with mRNA. The current amplitude recorded from the animal pole area is much bigger than that recorded from the opposite side. The ratio of the currents found at the vegetal and at the animal pole is 0.33.

Fig. 3 illustrates typical sodium current traces obtained in response to depolarization from two different oocytes, 6 d after injection with mRNA at the equator. Traces recorded before and during localized superfusion with the sodium channel inhibitor tetrodotoxin (50 nM) are shown superimposed for each oocyte. The inhibited current fraction at the animal pole area (Fig. 3 *a*) was larger than that at the vegetal pole area (Fig. 3 *b*). This indicates a higher density of active sodium channels at the animal pole than at the vegetal pole area. The membrane resistance decreased upon multiple impalement, making determination at the two poles of the same oocyte difficult. Therefore, different oocytes were used for each local current determination and data were then averaged from a large number of oocytes (see below).

Influence of the Site of mRNA Injection

To prevent any bias of ion channel distribution in the surface membrane caused by possible local mRNA deposits we studied whether the site of injection has an influence on the asym-

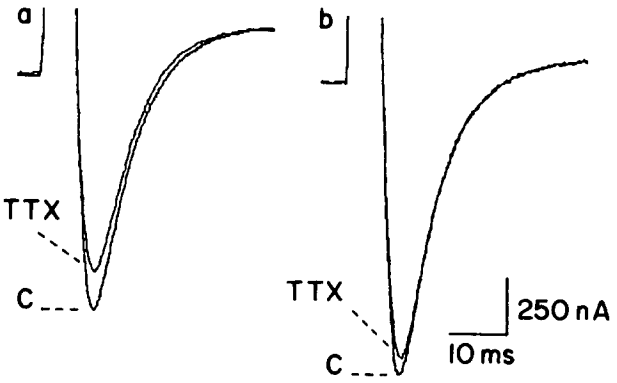

Figure 3. Measurement of local I_{Na}. 6 d after injection at the equator with chick forebrain mRNA, an oocyte was placed with the animal pole pointing downwards into the funnel in the chamber shown in Fig. 1. I_{Na} was measured by short depolarizing pulses to −10 mV from a holding potential of −100 mV. (*a*) Superimposed current traces, recorded before *c* and after perfusion of the animal pole with 50 nM tetrodotoxin (*TTX*). (*b*) The same experiment performed on a different oocyte, except that the vegetal pole was exposed to tetrodotoxin. The fractional current that was inhibited reflects the fraction of active ion channels in the exposed surface area.

metry of newly expressed, functional ion channels. Table I summarizes the data from these experiments. I_{Na} was measured, 2 or 6 d after injection. No significant difference in the localization of the current could be detected, that depended on the site of injection. It is especially interesting to note that, even when the mRNA was injected into the vegetal pole, 2 d after injection there was 12.5 times more current flowing at the animal pole area than at the vegetal pole area (Table I). The difference in the current densities at the two pole areas was much smaller 6 d after injection of mRNA.

Asymmetry of Current Densities at Different Times after Injection with mRNA

Fig. 4 summarizes data on the localization of I_{GABA} obtained from oocytes at different days after injection with mRNA at the vegetal pole. During the early phase of expression, ion currents at the animal pole were >10 times bigger than the ones recorded from the vegetal pole area, indicating a pronounced asymmetry of functional $GABA_A$ channel expression. At later stages of expression the measured asymmetry was less pronounced, but even 14 d after injection with

Table I. Lack of Influence of the Site of mRNA Injection on the Asymmetry of I_{Na}

Site of injection	Ratio (mean ± SEM) 2 d after injection	6 d after injection
	n	
Animal pole	–	0.42 ± 0.11 (6)
Equator	0.05 ± 0.01 (13)	0.32 ± 0.04 (18)
Vegetal pole	0.08 ± 0.04 (4)	0.36 ± 0.05 (14)

The ratio indicates the fractional current found at the vegetal divided by that at the animal pole area. The ratios were determined 2 or 6 d after injection with mRNA. *n* indicates the number of oocytes evaluated at each pole area. Each ratio was obtained from measurements using oocytes from at least two different donor animals.

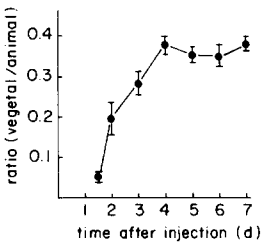

Figure 4. Time dependence of the asymmetry of I_{GABA} expression. Experiments as shown in Fig. 2 were performed on a large number of oocytes from two different donor animals, at different times after injection with mRNA, at the vegetal pole. I_{GABA} found at the vegetal pole was divided by that measured at the animal pole of the same oocyte to obtain the ratio (vegetal/animal). Each time point represents the mean ± SEM of the ratio in at least nine oocytes.

mRNA the relative current density at the animal pole was still ~2.2-fold higher at the animal than at the vegetal pole.

Fig. 5 summarizes data on I_{Na} measured at different days after injection of the oocytes with mRNA. The data were pooled from different batches of oocytes that were injected either at the equator or at the vegetal pole. No significant difference was observed for the two injection sites (Table I). Fig. 5 *a* shows the time course of the whole-cell current expression. In Fig. 5 *b* the fractional current inhibition observed at the animal and at the vegetal pole area is shown. Whereas on the second day after injection, current inhibition at the vegetal pole could hardly be detected, ~30% of the total current could be inhibited at the animal pole area. Measurements at earlier times could not be carried out, because the current amplitudes were too small for precise determinations. At all stages of expression the observed current inhibition at the animal pole area remained above and at the vegetal pole area below the expected value for random distribution. In Fig. 5 *c* the same data are plotted as the ratio of current inhibition in the vegetal pole area divided by the one in the animal pole area. The asymmetry of sodium current distribution was time-dependent and followed a similar time course as that for I_{GABA}.

Distribution of I_{Na} in Other Parts of the Surface

It was of interest to see whether ion channels were evenly distributed within the respective hemispheres with a sharp boundary at the equator, or whether there was gradual change in channel density between the two pole areas. Fig. 6 illustrates the results of experiments carried out on oocytes 3 d after injection with mRNA. Local currents found in the indicated areas are expressed as percentage of the respective whole cell currents. The result clearly shows that there are gradual changes in current densities between animal and vegetal poles, and no sharp density changes were observed at the equator.

Channel Specificity of Asymmetric Current Expression

We wanted to know whether the asymmetric ion channel distribution described above was specific for I_{GABA} and I_{Na}, or for brain tissue. Therefore, we performed similar experiments with the $I_{kainate}$ and I_{Ba}, both expressed from chick brain mRNA, and with I_{Na} expressed from chick skeletal muscle mRNA. The results are summarized in Table II. Pooled data collected between days 4 and 7 after injection are shown for total mRNA injected oocytes, with the exception of I_{nAch}. In the latter case, currents were mapped 3 d after injection. All ion channels tested showed a similar asym-

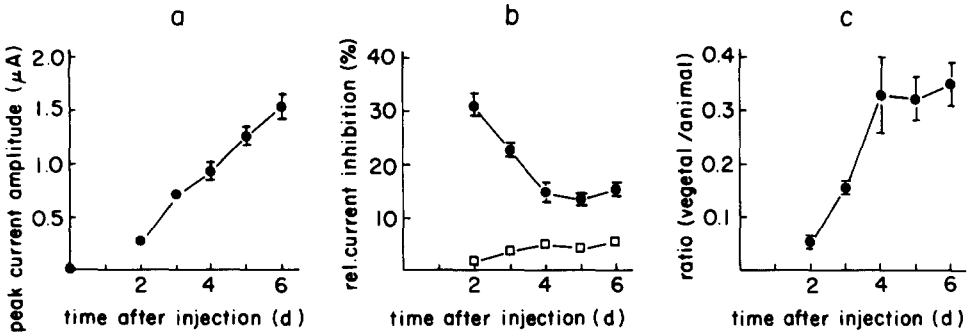

Figure 5. Time dependence of the asymmetry of neuronal I_{Na} expression. (*a*) Time dependence of the whole cell current. Each point indicates the mean of measurements carried out with 19 to 93 oocytes from at least two batches of oocytes. (*b*) Fractional current inhibited by TTX in the animal (●) and in the vegetal (○) pole area, respectively. Each point is derived from measurements with 9 to 47 oocytes, originating from at least two different batches of cells. Data from oocytes injected at the vegetal pole and at the equator are pooled. (*c*) From the data shown under *b*, the fractional current found at the vegetal pole was divided by the one found at the animal pole. All data are given as mean ± SEM.

metry of expression, irrespective of channel type and tissue of origin. The low ratio of I_{nAch} is presumably due to the short expression period and the ratio is in good agreement with I_{GABA} and I_{Na} at the same time after injection of mRNA (Figs. 4, 5).

To further eliminate the possibility that the crude mRNA could contribute information to the positioning of ion channels in the membrane, we also studied the distribution of cloned $GABA_A$ receptor channels. Measurements were performed at days 2 and 3 after injection of cDNA drived RNAs. The results show that these cloned ion channels are distributed in a similar way as ion channels expressed from total mRNA during the late phase of expression. cDNA derived RNA coding for this ion channel displays a time course of expression faster than for total tissue derived mRNA (data not shown).

Electron Microscopic Analysis of the Surface Membrane

The asymmetric distribution of ion channels described above was measured at a macroscopic level as ionic currents. Therefore, the macroscopically asymmetric distribution could, in principle, reflect a nonrandom increase in surface area due to microvilli together with a microscopically random distribution of channels. Therefore, we analyzed the ultrastructure of the oocyte plasma membrane for surface enlargement at the two pole areas. Fig. 7 shows typical examples of electron micrographs of the animal (Fig. 7 *a*) and the vegetal pole area (Fig. 7 *c*) of the same oocyte. Previous injection with mRNA did not affect the microvilli pattern at either pole of the oocyte (Fig. 7, *b* and *d*). Preliminary morphometric analysis indicates that the surface enlargement caused by the microvilli is 9- to 11-fold in both pole areas of oocytes, irrespective of whether the oocytes had previously been injected with chick brain mRNA or not (Schittny, J. C., and E. Sigel, unpublished observations).

Thus, the asymmetric distribution of plasma membrane proteins is not just a consequence of an asymmetric surface structure caused by a different microvilli pattern. In other polarized cells asymmetric expression of plasma membrane proteins is dependent on an intact microtubule network. Therefore, we were interested to investigate whether the polarized distribution in the oocyte was sensitive to anticytoskeletal drugs.

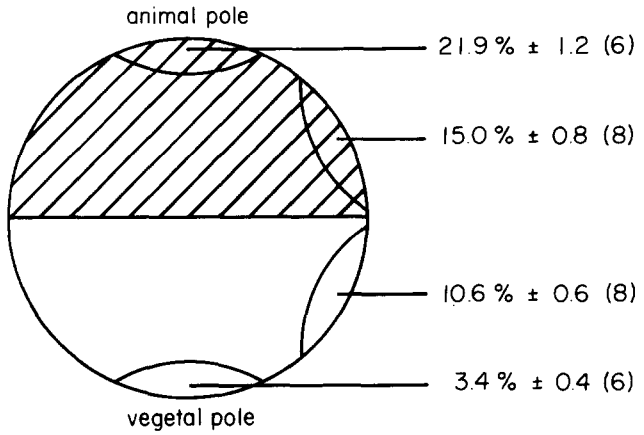

Figure 6. Longitudinal mapping of neuronal I_{Na}. Currents were mapped at the two pole areas and at sites adjacent to the equator. The numbers in the figure indicate the percentage of the whole-cell current found in the respective area (mean ± SEM). The fractional currents found adjacent to the equator were in each case statistically different from the fractional current found at the pole in the respective pole area ($P < 0.001$).

Table II. Asymmetry of Current Expression Induced by mRNA Originating from Different Sources

Current	Source of mRNA	Time	Ratio
		d	
$I_{kainate}$	Chick brain	4–7	0.36 ± 0.03 (7/2)
I_{GABA}	Chick brain	4–7	0.37 ± 0.01 (36/4)
I_{GABA}	cDNA coding for rat subunit isoforms (mixtures)	2–3	
	$\alpha 1\beta 1$		0.39 ± 0.08 (7/1)
	$\alpha 3\beta 1$		0.46 ± 0.09 (6/1)
	$\alpha 1\beta 1\gamma 2$		0.35 ± 0.08 (4/1)
I_{Ba}	Chick brain	7	0.36 ± 0.08 (6/2)
I_{Na}	Chick brain	4–7	0.34 ± 0.03 (59/8)
I_{Na}	Chick skeletal muscle	5–7	0.35 ± 0.06 (10/1)
I_{nAch}	*Torpedo* electric organ	3	0.13 ± 0.02 (4/1)

The ratio is a measure of the asymmetry of functional channel expression and indicates the fractional current found at the vegetal divided by the one found at the animal pole area. Data were pooled from different experiments carried out at different days after injection of the oocytes with mRNA (time). For the determination of the ratio for cDNA derived RNA, measurements of the fractional currents at the vegetal and animal pole were carried out using different oocytes. Ratios are given as mean ± SEM (number of oocytes/number of different batches of oocytes).

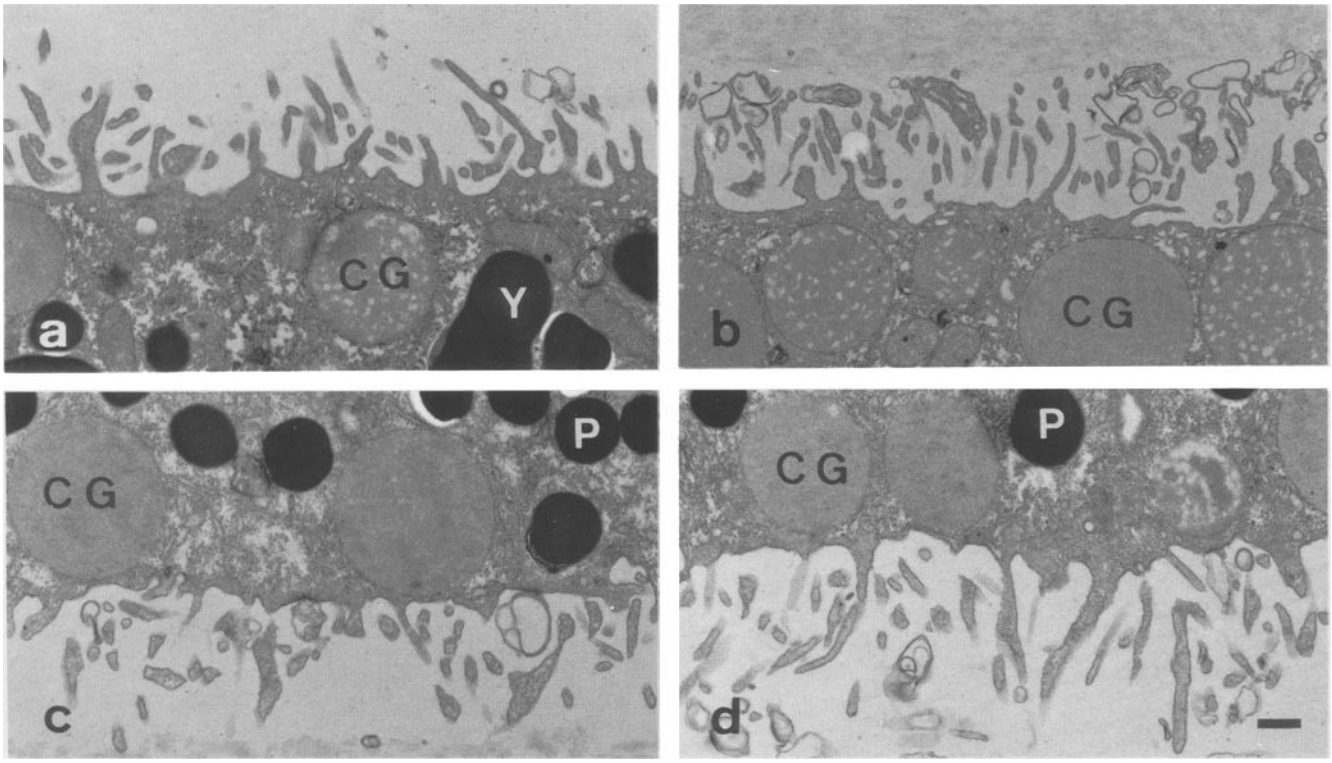

Figure 7. Electron micrograph of the oocyte plasma membrane. After 3 d in culture, oocytes were freed from their surrounding cellular layers, and equatorially cut after fixation. The figure documents the high degree of similarity in microvilli number and structure at either pole of the same oocyte, irrespective of whether or not the oocyte was injected with mRNA. (*a*) Vegetal and (*c*) animal pole area of the same noninjected control oocyte. (*b*) Vegetal and (*d*) animal pole area of the same mRNA injected oocyte. *CG*, cortical granule; *P*, pigment granule; *Y*, yolk granule. Bar, 1 μm.

Effect of Colchicine and Cytochalasin D on Oocyte Morphology

Colchicine and cytochalasin D each led to distinct changes in the distribution of the pigment granules, that give the animal hemisphere of the oocyte its distinct black appearance (not shown). Exposure to 10 μM colchicine released the nucleus from its microtubule mediated anchoring, and the floating nucleus displaces pigment granules as previously observed at much higher concentrations of colchicine (Colman et al., 1981). At 1 and 10 μM colchicine, ~26 and 68% of the cells showed a characteristic whitish spot. The control substance β-lumicolchicine, which does not depolymerize microtubules, did not affect the pigmentation pattern. Cytochalasin D (2 μM) caused redistribution of the granules, partly to the vegetal half, similar as previously reported for 50 μM cytochalasin B (Colman et al., 1981).

Effect of Colchicine and Cytochalasin D on Functional Expression of I_{Na}

For the experiments with anticytoskeletal agents we chose neuronal sodium channels expressed from crude chick brain mRNA. Colchicine (10 μM) and cytochalasin D (2 μM) were tested individually, for immediate effects on I_{Na}. None of the drugs affected voltage dependence or amplitude of the current within 15 min of application (not shown).

For all further studies, oocytes were exposed for 50 h to anticytoskeletal drugs. At the concentrations used in this study, the drugs did not affect membrane potential or membrane resistance (not shown). Exposure of the oocytes to the microtubule dissociating drug colchicine, even at the highest concentration used (10 μM), did not affect the total amount of I_{Na} expressed. The current expressed in treated oocytes was 103 ± 7% (mean ± SEM; n = 15 oocytes from three different batches) of untreated control oocytes (n = 15).

The actin network disrupting drug cytochalasin D was used at a concentration of 2 μM. At this concentration cytochalasin D is known to have pronounced effects on actin organization in cell culture systems (Cooper, 1987). Long-term exposure of the oocytes to cytochalasin D (2 μM) reduced the total amount of current expressed to 65 ± 9% (mean ± SEM; n = 15 oocytes from three different batches) of the control.

Colchicine and Cytochalasin D Affect the Distribution of I_{Na}

In preliminary experiments we observed that I_{Na} in oocytes exposed to long-term treatment (50 h) with 10 μM colchicine did not show the typical asymmetrical current distribution. In contrast exposure of oocytes to the control substance β-lumicolchicine, that does not affect microtubule integrity, had no effect as compared with experiments carried out in the absence of drug.

Fig. 8 summarizes data obtained with oocytes from at least three different batches. Measurements were carried out three days after injection of the oocytes with mRNA. In the absence of anticytoskeletal drugs, or in the presence of β-lumicolchicine, ~3% of the whole cell I_{Na} could be inhibited by exposure of the vegetal pole area to tetrodotoxin, and ~22%

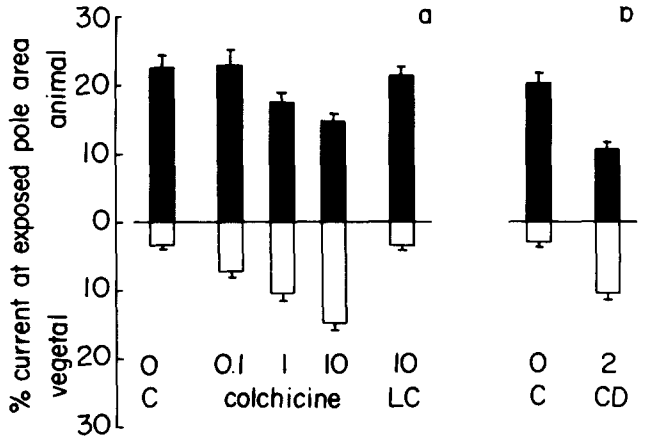

Figure 8. Effect of anticytoskeletal drugs on the fractional current detected in the oocyte pole areas. From each of three batches of oocytes, a minimum of three oocytes was used, exposing the respective pole areas to 50 nM tetrodotoxin. Fractional inhibition of I_{Na} by tetrodotoxin at the animal pole area is indicated with black columns pointing upwards, inhibition at the vegetal pole area with open columns pointing downwards. Mean ± SEM of the respective fractional current inhibitions are given. The numbers below the columns indicate the drug concentrations in micromolar. (*a*) Oocytes were incubated for 50 h, in control medium (*C*), in different concentrations of colchicine, or in β-lumicolchicine (*LC*). (*b*) The same experiment with cytochalasin D (*CD*).

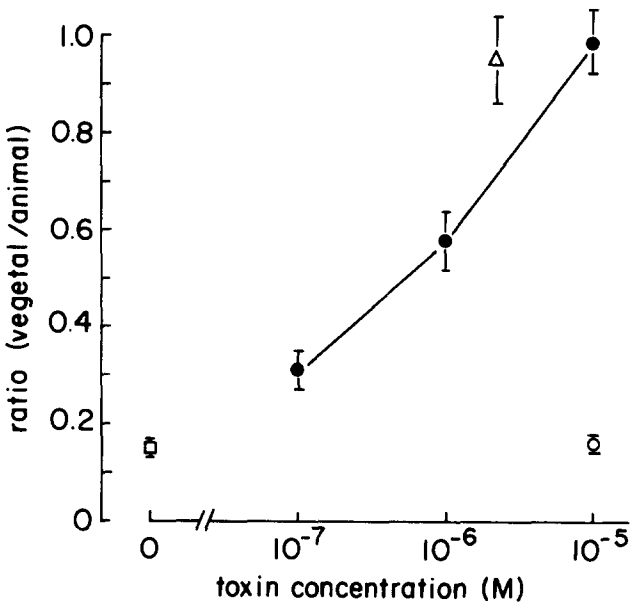

Figure 9. Ratio of the respective fractional I_{Na} at the vegetal and animal pole areas. For random expression, the expected ratio is 1.0. □, control; ●, colchicine; ○, β-lumicolchicine; △, cytochalasin D. Ratios are derived from data shown in Fig. 8, and are given as mean ± propagated SEM.

could be inhibited at the animal pole area (Fig. 8 *a*), indicating a relative current density at the vegetal to animal pole area of 0.14 (Fig. 9). Incubation of the oocytes during the whole expression period with 10 μM colchicine led to randomization of functional channel expression (Figs. 8 *a* and *9*). This effect was dose dependent (EC_{50} ~1 μM). The specificity of the drug action is shown by the absence of an effect of β-lumicolchicine (10 μM) (Figs. 8 *a* and *9*).

Similar experiments as with colchicine were performed with cytochalasin D (2 μM). At the concentration used, cytochalasin D completely randomized functional current expression (Figs. 8 *b*, 9).

The fractional current expected at both pole areas with complete randomization is ~8–12%. This is proportional to the fractional surface area exposed to the tetrodotoxin containing solution. Although the data obtained with cytochalasin D are in line with the expectation, fractional inhibition at both poles obtained with colchicine (10 μM) is slightly larger (Fig. 8 *a*). This is probably due to the exposure of a larger fractional area of the oocytes to tetrodotoxin, since the stiffness of the oocytes may have changed in the presence of colchicine.

Discussion

Polarized Distribution of Newly Expressed Ion Channels

We have described a nonrandom distribution of foreign, poly(A^+)-mRNA induced ion currents in the *Xenopus* oocyte. The methods used allow mapping at low spatial resolution only. They do not give any information about possible clustering of channels in hot spots. Furthermore, only active ion channels are detected. Therefore, our study reflects the true distribution of ion channels only, if their availability to open is the same, throughout the membrane. For example differential activity of protein kinase C in different areas of the oocytes could inactivate I_{Na} and I_{GABA} (Sigel and Baur, 1988) locally. However, different types of exogenous ion channels expressed either from poly(A^+)-mRNA extracted from various tissues, or from cDNA, all follow a common pattern of expression in the surface membrane of the oocyte (Table II). It is very unlikely that all the proteins expressed underly regulation by the same hypothetical, inhomogenously distributed mechanism of modulation.

If we assume that the percentage of functional ion channels is the same in different regions of the oocyte, our results indicate that the newly expressed ion channels are non-randomly distributed in the surface membrane, because the macroscopic currents are very different in the two pole areas of the cell. It may be extrapolated from our data that, during the initial phase of expression, channels are exclusively expressed around the animal pole. All ion channels investigated here followed the same distribution pattern, independently of the type of channel or tissue of origin (Table II). Even ion channels expressed from pure cDNA-derived mRNAs distributed in the same fashion. This raises the interesting possibility that by studying the localized expression of foreign ion channels in the *Xenopus* oocyte, we may be tracing a pathway for membrane protein expression, endogenous to this complex cell.

Earlier reports on expression of the nicotinic acetylcholine receptor channel in the *Xenopus* oocyte after injection with mRNA from *Torpedo electroplax* (Barnard et al., 1982) and from denervated cat muscle (Miledi and Sumikawa, 1982) have mentioned that pressure application of the agonist elicited larger currents at the vegetal pole than at the animal pole. The reason for this discrepancy with our results is not clear.

Recent observations concerning the muscarinic acetylcholine receptor endogenous to oocytes indicate that a subpopulation of frogs produces oocytes with a strongly asymmetric distribution of these receptors, while oocytes from other donors do not display hemispheric asymmetry (Matus-Leibovitch et al., 1990).

Relationship to Endogenous Asymmetry

In the oocyte there is an unequal distribution of the sites of protein synthesis. About 60% of the ribosomal RNA is located in the animal hemisphere, a fact that can be attributed to the high density of yolk granules in the vegetal half (Nieuwkoop and Faber, 1967). The value of 60% agrees with the cytoplasmic space available for large molecules that do not enter the nucleus (Drummond et al., 1985). In stages V and VI oocytes used here (Nieuwkoop and Faber, 1967), the Golgi apparatus exists as a widely dispersed organelle in the accessible cytoplasm (Colman et al., 1985). With random insertion after delocalized synthesis throughout the accessible cytoplasm, this spatial arrangement would result in an asymmetric plasma membrane protein distribution with a ratio (vegetal/animal) = 0.67. In our study, we have observed a more pronounced asymmetry, that can not be accounted for by this asymmetry in the distribution of components endogenous to the oocyte.

Preliminary morphometric data of the surface membrane indicate a 9–11-fold enlargement of the area caused by microvilli (Schittny, J. C., and E. Sigel, unpublished observations). Using electrophysiological techniques, this increase has earlier been estimated to be four to fivefold (Methfessel et al., 1986). According to our measurements, the surface increase is very similar at the vegetal and at the animal pole area. Thus, the possibility can be excluded that the observed asymmetry of current expression at the macroscopic level is a consequence of an asymmetric surface enlargement in combination with a microscopically random distribution of ion channels.

Earlier studies have addressed the question of the mobility and speed of distribution of microinjected mRNA and newly synthesized foreign protein. It has been demonstrated that poly(A^+)-mRNA coding for plasma membrane proteins shows little movement in the cytoplasm within 48 h after injection at the animal pole (Drummond et al., 1985). In contrast, when injected at the vegetal pole, RNA was found in similar amounts in the two hemispheres, after this period of time (Drummond et al., 1985). This suggests preferential movement of microinjected mRNA towards the animal pole. Within the first few hours after microinjection of mRNA, local synthesis at the site of injection of exogenous proteins has been observed after injection of poly(A^+)-mRNA at the vegetal pole and at the animal pole (Ceriotti and Colman, 1988). The plasma membrane protein influenza virus hemagglutinin synthesized within the first 3 h after injection of mRNA into the vegetal pole remains for at least another 20 h in this half of the oocyte (Ceriotti and Colman, 1988). This indicates that transport of newly formed plasma membrane protein to the opposite hemisphere is slow. It is interesting to note that in our experiments, little functional appearance of ion channel was detected within the first 24 h, and that the rate of functional appearance in the plasma membrane increased only at a later time (Fig. 5 *a*). In our study, asymmetry of current expression was observed for all the channel types studied, and at any time after injection of mRNA. However, in the early phase of expression asymmetry was most pronounced. Whether this is the consequence of a migration of mRNA or of newly synthesized protein is not clear from our experiments.

Possible Mechanisms Leading to Asymmetric Channel Distribution

(a) Localized Insertion at the Animal Pole Followed by Lateral Diffusion. Early expression is almost exclusively found in the animal pole area. Therefore, it may be hypothesized that the observed lateral pattern of distribution of ion channels, and the changes in the extent of asymmetry during the expression period, are due to an initial insertion of ion channels at the animal pole only. This would either require a transport of the injected mRNA into the animal hemisphere, localized protein synthesis and membrane insertion, or alternatively, protein synthesis and subsequent transport of the newly synthesized protein to the animal hemisphere. After insertion into the surface membrane at the animal pole, the ion channels could then diffuse laterally in the plasma membrane and reach other localizations. The rate of lateral diffusion in a planar lipid bilayer may be estimated by using the equation $r^2 = 4Dt$, where r is the mean migration distance, t is the time elapsed, and D is the diffusion constant of the integral protein in the lipid bilayer. For the nicotinic acetylcholine receptor, D has been found $<1\text{–}3\cdot10^{-9}$ cm^2/s (Poo, 1982). For our calculation we assume a higher mobility $D = 5\cdot10^{-9}$ cm^2/s. It has to be taken into account that the surface membrane of the oocyte is folded and, according to our preliminary morphometric analysis, ~10 times larger than expected from the spherical appearance of the cell. Therefore, the nominal diameter of the oocyte must be assumed to be ~3.5 mm for our calculation. From the above equation and the binomial distribution, it may be estimated, that within 3 d <6% of the membrane proteins starting at time zero at the animal pole diffuse half way to the equator. The time course of functional channel expression in the surface membrane (Fig. 5 *a*) indicates that a large percentage of channels functionally detectable at the third day after injection of mRNA, is synthesized at that day. Therefore, average diffusion time of the channels must be considerably <3 d. We conclude that the model assuming membrane insertion at the animal pole followed by lateral diffusion can not explain our experimental data. Based on similar calculations, we can also exclude insertion in the entire animal hemisphere, followed by diffusion into the vegetal hemisphere.

(b) Electrophoresis. Currents recorded from *Xenopus* oocytes using a noninvasive technique have indicated that electrical current enters the animal hemisphere and leaves the vegetal hemisphere (Robinson, 1979). This current is expected to create an electric field in the oocyte, along the animal/vegetal axis, which is positive at the animal pole. Such electric fields have been hypothesized to have an important organizing role in developing systems (Jaffe, 1985). The electric field would provide a force driving for any negatively charged entity directed towards the animal pole. This would include poly(A^+)-mRNA, membrane vesicles budding from the Golgi compartment and glycoproteins in the plasma membrane. In the absence of any estimate of the size of the electric field in the oocyte, it is difficult to prove or disprove

this model. It may be noted that not all glycoproteins show an uneven distribution in the surface membrane (Ceriotti and Colman, 1988). However, it could always be argued that such components are immobilized by some trapping mechanism.

(c) Directed Transport to the Surface. We could postulate a transport process in the oocyte for newly synthesized proteins directed from the post-Golgi compartment to the animal hemisphere of the oocyte. The experiments using cDNA-derived mRNAs indicate that this putative transport process is endogenous to the oocyte. Such a transport appears to depend both on microtubules and actin filaments. Interestingly, secretory proteins newly synthesized in the *Xenopus* oocyte, shortly after injection with mRNA into the animal and vegetal poles, have a larger tendency to move from the vegetal to the animal hemisphere than vice versa (Drummond et al., 1985). A directed transport of vesicles emerging from the post-Golgi compartment would explain this phenomenon.

Effect of Anticytoskeletal Drugs

We report here that colchicine (10 μM) and cytochalasin D (2 μM) lead to randomized distribution of newly expressed sodium channels in *Xenopus* oocytes, without greatly altering the extent of functional expression in the surface membrane. This distribution is highly asymmetric in the absence of drugs. Functional microtubules and actin networks thus appear to be involved in localized expression of foreign ion channels in *Xenopus* oocytes. It is not clear from our experiments whether the intact cytoskeleton is needed for intracellular transport of mRNA or of newly synthesized protein.

The fate of actin filaments and microtubuli upon exposure to disrupting drugs has, to our knowledge, not been characterized in the *Xenopus* oocytes. However, it has been shown that colchicine, even at a concentration of 2 mM, does not result in a change of the microvillus surface (Colman et al., 1981).

Colchicine strongly affected the positioning of neuronal sodium channels to specific membrane domains in the *Xenopus* oocyte without interfering with the amount of channels expressed functionally in the surface membrane. This sensitivity to colchicine is reminiscent of the apical pathway in epithelial cells (Achler et al., 1989; Rindler et al., 1987) and in liver cells (Durand-Schneider et al., 1987). In these cells, colchicine has been shown to interfere with the localized expression of plasma membrane proteins destined to the apical surface membrane domain, but not with expression in surface membranes per se. In the human epithelial cell line Caco-2, colchicine has been reported to leave sorting unaffected, but to delay delivery of proteins destined to the apical membrane (Matter et al., 1990). On the other hand, the pathway of membrane protein expression in the *Xenopus* oocyte must differ from the apical pathway in epithelial cells. In contrast to *Xenopus* oocytes cytochalasin fails to affect the apical pathway of membrane protein expression in epithelial cells (Rindler et al., 1987; Salas et al., 1986). In liver cells, the actin filament-stabilizing drug phalloidin has been reported to interfere with the domain specific distribution of plasma membrane proteins (Durand-Schneider et al., 1987).

It has previously been shown that secretion of foreign, mRNA induced proteins by the oocyte is not affected by 10 μM colchicine, or by 250 μM cytochalasin B (Colman et al., 1981). In hepatoma cells plasma membrane insertion of integral membrane proteins has been proposed to follow the same pathway as protein secretion (Strous et al., 1983). Whether or not there is polarized secretion of mRNA induced proteins in the *Xenopus* oocyte is not known.

Conclusions

In conclusion, we have found a pathway for polarized delivery of membrane proteins in the *Xenopus* oocyte, which depends on functional integrity of microtubules and actin filaments. This pathway of protein expression may be endogenous to the *Xenopus* oocyte and may be involved in the generation and/or maintenance of polarity of the oocyte. Thus, it could represent a key factor in the polarized organization of this cell, which is vital during early development. Due to their size and easy accessibility, *Xenopus* oocytes may offer advantages for certain types of studies over in vivo systems used for the investigation of plasma membrane targeting to plasma membrane domains, such as liver and intestinal epithelia, and also over epithelial cell culture systems. For those studying plasma membrane proteins expressed in *Xenopus* oocytes, it may be useful to know that newly expressed membrane proteins can be expected to be nonrandomly distributed in the plasma membrane.

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