Supplemental Table 1. Specific activities of marker enzymes measured in *Xenopus laevis* oocytes whole homogenate and in two membrane fractions isolated by using standard and silica beads methods.

All values are means ± SE of three different oocytes preparations.

Enzymes assayed	Whole homogenate[a]	Standard isolated membranes[b]	Silica beads isolated membranes[c]
	Enzyme activity[d] (μmol/mg protein) (% respect to homogenate)		
Alkaline phosphodiesterase (plasma membrane)	16.05 ± 0.7046 100 %	126.33 ± 2.44 787%	63.48 ± 2.66 395.5%
Cytochrome c oxidase (mitochondria)	0.838 ± 0.0143 100 %	0.00782 ± 0.0022 0.93%	0.0198 ± 0.0078 2.36%
NADH cyt c reductase (endoplasmic reticulum)	0.2003 ± 0.0327 100%	0.0103 ± 0.0270 5.14%	0.0091 ± 0.075 4.54%

[a] Oocytes whole homogenate was extracted as described by Vera-Estrella *et al*. (1). Thirty oocytes were homogenized in 500 ml of protein extraction buffer (250 mM mannitol, 10% (w/v) glycerol, 10 mM Tris/MES (pH 8), 1 mM EDTA, 5 mM DTT, 1 mM benzamidine, 1 mM PMSF, and 5% (w/v) insoluble PVP) and vortexed for 1 min. The homogenate was centrifuged at 10,000 × *g* for 15 min using a table top Beckman GS15R centrifuge (Beckman, Mexico) to remove cellular debris. Samples were frozen in liquid N_2 for further use.

[b] Standard isolation of oocyte membranes was done as described in Experimental Procedures.

[c] For the isolation of oocyte plasma membrane with silica beads we followed a procedure described by Kamsteeg and Deen (2). Fifteen oocytes were rotated in 1% colloidal silica, ludox C1 (Sigma, Mexico) in MES buffered saline (MBS, 20 mM MES, 80 mM NaCl, pH 6.0) for 20 min at room temperature (RT), washed three times with MBS, rotated in 0.1% polyacrylic acid (Sigma, Mexico) in MBS for 20 min at RT and washed three times in MBS. Subsequently, plasma membranes were isolated at 4°C using the standard procedure described by Wall and Patel (3). The oocytes were homogenized in 1.2 ml of HbA (20 mM Tris pH 7.4, 5 mM $MgCl_2$, 5 mM NaH_2PO_4, 1 mM EDTA, 80 mM sucrose, 1 mM PMSF, 5 μg/ml leupeptin and pepstatin) and centrifuged for 20 s at 13.5 × *g*, then, 1 ml of the supernatant was removed and 1 ml of HbA was added. This centrifugation and exchange was repeated two times at 13.5 × *g*, once at 24 × *g* and once at 38 × *g*. After the last centrifugation, HbA was removed and plasma membranes were spun down for 20 min at 14 000 × *g* and resuspended in HbA.

[d] All the enzyme assays were measured spectrophotometrically. Alkaline phosphodiesterase (PDE) activity (plasma membrane marker) was measured as described by Warren *et al*. (4). Thirty μg of protein were added to 200 μl reaction buffer (40 mM carbonate buffer pH 10.5, 0.1% (vol/vol) Triton X-100, and 2 mM thymidine-5'-monophosphate-p-nitrophenol ester). Samples were incubated at 37°C for 1 h and the reaction was stopped by the addition of 200 μl of 10% trichloroacetic acid (TCA). After adding 1.5 ml of 2 M NaOH the absorbance was read at 400 nm and the values were calculated by using standards from 0.25 mM p-nitrophenol in 5% TCA. Cytochrome c oxidase (mitochondria marker) and NADH cytochrome c reductase (endoplasmic reticulum marker) activities were measured by reduction or oxidation of cytochrome c, respectively, as described by Askerlund *et al*. (5). The values were calculated by using the cytochrome c extinction coefficient of 18.5 mM/cm read at 550 nm.

Alkaline PDE assays showed an activity of 126.33 ± 2.44 μmol p-nitrophenol/mg protein in membranes isolated with the standard method, revealing a 7.87-fold enrichment of plasma membrane

compared to the whole homogenate (16.05 ± 0.7 μmol p-nitrophenol/mg protein), and a 1.99-fold enrichment compared to membranes isolated with the silica beads method (63.48 ± 2.66 μmol p-nitrophenol/mg protein). The quantities of mitochondria and endoplasmic reticulum in the membrane fractions isolated with the standard and the silica beads methods are negligible respect to the whole homogenate and values reported in the literature (6,7). Based on these results and the greater enrichment of plasma membrane we used the standard method for subsequent experiments.

REFERENCES

1. Vera-Estrella, R., Barkla, B.J., Bonhert, H.J., and Pantoja, O. (1999) *Planta* 207, 426-435
2. Kamsteeg, E-J., and Deen, P.M.T. (2001) *Biochem Biophys Res Comm* 282, 683-690
3. Wall, D.A., and Patel, S. (1989) *J Membr Biol* 107, 189-201
4. Hill, W.G., Southern, N.M., MacIver, B., Poter, E., Apodaca, G., Smith, C.P., and Zeidel, M.L. (2005) *Am J Phyiol Renal Physiol* 289, F217-F224
5. Askerlund, P., Larsson, C., Widell, S., and Moller, I.M. (1987) *Physiol Plantarum* 71, 9-19
6. Chatterjee, S.K., Kim, U., and Bielat, K. (1976) *Br J Cancer* 33, 15-26
7. Mishra, S.K., Gard, N.K., and Kidwai, A.M. (1985) *J Biosci* 7, 365-373