$InsP_3$ receptor turnaround

SIR—Two recent papers, one by us[1] and one by Furuichi *et al.*[2], report the similarity in primary structure between the 1,4,5-triphosphate ($InsP_3$) receptor and the ryanodine receptor[3], both gated Ca^{2+} channels. Although the amino-acid sequences reported in the two papers fully agree, there is disagreement about the topology of the $InsP_3$ receptor in the membrane, and the subcellular localization of the protein in cerebellar Purkinje cells.

Although Furuichi *et al.* do not define the number of transmembrane spans of the molecule, they conclude that the C terminus is not located in the cytoplasmic compartment. This contrasts with the cytoplasmic localization of the C terminus of the ryanodine receptor. They also conclude that the $InsP_3$ receptor protein is localized not only in the endoplasmic reticulum (ER), but also on the plasmalemma including postsynaptic densities. Here we point out why the results of our study argue strongly against these conclusions.

The portions of the ryanodine receptor and of the $InsP_3$ receptor close to the C termini show primary structure similarity and are those thought to be involved in Ca^{2+} translocation. Therefore, as pointed out in the News and Views article that discussed both papers[4], an opposite localization with respect to the membrane of the C termini of the two proteins is unexpected. Although not emphasized in our original report, our immunocytochemical experiments[1] unambiguously indicate that the C terminus of the $InsP_3$ receptor, like the C terminus of the ryanodine receptor, is localized in the cytoplasm. Our results show that antibodies raised against a synthetic peptide corresponding to the 19 C-terminus residues of the molecule bind to the cytoplasmic surface of intact (nonpermeabilized) ER tubules and cisternae.

If the $InsP_3$ receptor is concentrated both in the ER and in the plasmalemma, it would be the first example of a transmembrane protein with such a subcellular distribution. The prevailing concept in cell biology is that transmembrane proteins after synthesis are either retained in the ER, or exported to other membranes of the vacuolar apparatus or to the plasmalemma[5]. Our immunocytochemical experiments do not provide any evidence for the presence of the $InsP_3$ receptor (at least the $InsP_3$ receptor in question) at the cell surface including postsynaptic densities. In our immunogold procedure, both the outer and the inner surface of the plasmalemma were accessible to antibody binding, because of mechanical cell dissociation and elution of cytosolic proteins. No immunocytochemical data were shown by Furuichi *et al.* They quote a previous study performed by an immunoperoxidase technique[6]. Because nonspecific labelling of membranes next to immunoreactive sites is a well-known artefact of the immunoperoxidase technique (see ref. 7), the immunoperoxidase results quoted by Furuichi *et al.* are likely to be explained by the presence of $InsP_3$ receptor-containing ER membranes next to the plasmalemma. Incidentally, a previous immunoperoxidase study on the localization of the $InsP_3$ receptor[8] also did not reveal any $InsP_3$ immunoreactivity on the plasmalemma.

To conclude, there is as yet no evidence for assuming that two structurally and functionally similar protein domains have different topologies in the membrane, and rejecting the 'dogma' that a transmembrane protein is destined to be either retained in, or exported from, the ER.

PIETRO DE CAMILLI
KOJI TAKEI
Department of Cell Biology,
and Section of Molecular Neurobiology,
Yale University School of Medicine,
PO Box 3333,
New Haven,
Connecticut 06510, USA

GREGORY A. MIGNERY
THOMAS C. SÜDHOF
Howard Hughes Medical Institute and
Department of Molecular Genetics,
University of Texas Southwestern
Medical Center,
Dallas, Texas 75235, USA

1. Mignery, G.A., Südhof, T.C., Takei, K. & De Camilli, P. *Nature* **342**, 192–195 (1989).
2. Furuichi, T. *et al. Nature* **342**, 32–38 (1989).
3. Takeshima, H. *et al. Nature* **339**, 439–445 (1989).
4. Gill, D.L. *Nature* **342**, 16–18 (1989).
5. Warren, G. *Nature* **327**, 17–18 (1988).
6. Maeda, N. *et al. Devl. Bio.* **133**, 67–76 (1989).
7. De Camilli, P., Harris, S.M., Huttner, W.B. & Greengard, P. *J. Cell Biol.* **96**, 1355–1373 (1983).
8. Ross *et al. Nature* **339**, 468–470 (1989).

Mating calls

SIR—Ryan *et al.*[1] report the basilar papilla tuning of female frogs of the species *Physalaemus coloradorum* to be no different from that of the closely related *P. pustulosus* (Tungara frog), and take that as evidence for a "sensory exploitation" hypothesis for an evolutionary origin of a low frequency male chuck emitted by *P. pustulosus*. The hypothesis is that the tuning of the female ear evolved before the chuck, which has been naturally selected in *P. pustulosus* secondary to the physical characteristics of the female ear. Lack of a chuck in *P. coloradorum* seemed to be evidence against alternative hypotheses that assume the male trait evolved in coordination with the female trait.

The argument depends on the assumption that *P. pustulosus* derived the chucking capacity after species divergence. Alternatively, the female ear tuning and the male chuck could have evolved together in an ancestral species and the chuck would have been lost by differential predation pressure on males of *P. coloradorum*. Ryan demonstrated that there was heavy predation pressure on the Tungara frog from frog-eating bats, which preferentially key on the low-frequency complex chucks rather than on the simpler high-frequency sounds these frogs also make[2]. Selection pressure would be greater on a male vocalizing apparatus than on the silent female ear. Sensitivity to low frequencies is therefore more likely to have become a conserved attribute, with no apparent present use, than emission of the same low frequencies.

This explanation of the results has the advantage of providing a mechanism for the evolution of the female basillar papilla in an ancestral species. Support for the argument comes from the evolutionary history of the Tungara frog, which is hard to study. The divergence apparently began in the late Tertiary, after the formation of the Andes. The two species gained their separate characteristics on either side of the continental divide. No fossil remains of a primordial species are described, much less its vocal apparatus[3].

But the hypothesis that the loss of the trait by predation needs to be clearly disproven before the sensory exploitation hypothesis gains plausibility.

RUSSELL GARDNER
Department of Psychiatry,
University of Texas Medical Branch,
1.200 Graves Building,
Galveston,
Texas 77550,
USA

1. Ryan, M.J. *et al. Nature* **343**, 66–67 (1990).
2. Ryan, M.J. *The Tungara Frog: A Study in Sexual Selection and Communication 83* (Chicago: University Press, 1985).
3. Cannatella, D.C. & Duellman, W.E. *Copeia* 1984: 902–321, 1984.

SIR—In the interesting article by Ryan *et al.*[1], the conclusion that there has been no evolution of female preference follows from the assumption that tuning of the peripheral auditory system accurately predicts female mate preference. A simple way of testing this would be to expose female *Physalaernus coloradorum* to calls of both species in a classic two-way loudspeaker choice experiment. We would expect *P. coloradorum* females to prefer the call of *P. pustulosus* males over conspecifics. Alternatively, both these species may share the same basilar papilla tuning, but differ in female response to auditory stimulation. In that case, female preference would differ in the two species (say, if *P. coloradorum* females ignored chucks), and the relevant selective pres-