# Generally Physiological

## Of Juno, neuropeptide release, and light-activated chloride channels

This month's installment of *Generally Physiological* explores the identification of an oocyte cell surface receptor required for fertilization, the role of calcium stores in large dense core vesicles in neuropeptide secretion, and the conversion of a channelrhodopsin to a light-activated chloride channel.

### Making a molecular match

The ability of a sperm and an egg to fuse to form the diploid zygote depends on their recognizing each other, which in turn depends on interactions between cell surface receptors. The sperm protein Izumo1, which translocates to the plasma membrane during the process whereby sperm become competent to fertilize eggs, is required for sperm–egg fusion; its binding partner on the egg surface, however, has remained unclear (see Wassarman, 2014). Using an iterative expression cloning approach, Bianchi et al. (2014) probed HEK293 cells transfected with mouse oocyte cDNA with an oligomerized mouse Izumo1 ectodomain and identified a folate receptor paralogue, folate receptor 4 (Folr4), as an Izumo1-binding partner. After determining that Folr4, unlike Folr1 and Folr2, failed to bind folate, the authors renamed it Juno. Noting that an egg receptor essential for fertilization is anchored by glycophosphatidylinositol (GPI), the authors determined that cell surface Juno immunoreactivity was lost after treatment with phosphoinositide phospholipase C, indicative of anchoring by GPI. Oocyte pretreatment with a monoclonal antibody directed against Juno blocked Izumo1 binding in an in vitro

fertilization; moreover, female mice lacking Juno were infertile, whereas $Juno^{-/-}$ males were not, and $Juno^{-/-}$

> An oocyte cell surface receptor required for fertilization, the role of calcium stores in LDCVs in neuropeptide secretion, and the conversion of a ChR to a light-activated chloride channel
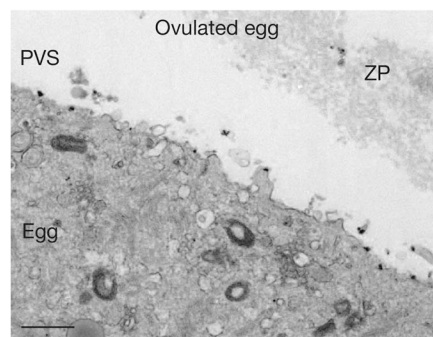
eggs could not be fertilized in vitro. Cell surface Juno was lost after fertilization, undergoing redistribution to extracellular vesicles, suggesting a possible mechanism for the membrane block to polyspermy that takes place subsequent to fertilization. Izumo1 and Juno orthologues exist in all sequenced mammalian genomes,



Immunogold labeling shows that, in fertilized eggs, Juno redistributes from the cell surface to extracellular vesicles. (Reprinted by permission from Macmillan Publishers, Ltd. E. Bianchi, B. Doe, D. Goulding, G.J. Wright. 2014. *Nature.* http://dx.doi.org/10.1038/nature13203, copyright 2014.)

and binding between the ectodomains of Izumo1 and Juno orthologues was conserved in humans,

pigs, and opossums. The authors thus conclude that Juno is the Izumo1 receptor and that, like Izumo1, it is required for fertilization of mammalian eggs.

### Defining a local calcium source?

Although calcium influx through voltage-gated channels provides the primary trigger for neuropeptide secretion, calcium released from internal stores may play a modulatory role. Noting that the large dense core vesicles (LDCVs) in which neuropeptides are stored contain substantial calcium, in this issue, McNally et al. explored the possibility that calcium released from LDCVs gives rise to the ryanodine-sensitive calcium transients that occur in neurohypophysial terminals, providing a local source of calcium to modulate neuropeptide release. Immunofluorescence analysis of neurohypophysial terminals revealed substantial colocalization of ryanodine receptors (RyRs) with LDCV markers, and immunogold labeling in conjunction with electron microscopy showed
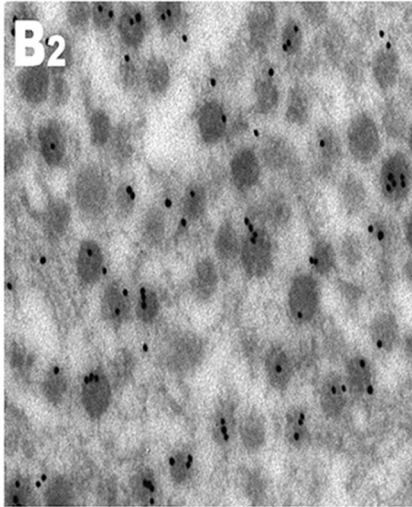
**Immunogold labeling indicates that RyR2 localizes to neurosecretory granules in neurohypophysial slices. (From McNally et al., 2014.)**

type 2 RyRs localized to LDCVs in neurohypophysial slices. LDCVs bear a nonspecific cation channel with biophysical properties resembling those of RyRs, and pharmacological analysis revealed that these channels respond appropriately to an RyR agonist (ryanodine) and antagonist (ruthenium red). Intriguingly, calcium-evoked release of vasopressin from permeabilized neurohypophysial terminals was potentiated by ryanodine at agonist concentrations and inhibited by ruthenium red or higher (antagonist) concentrations of ryanodine. The authors thus propose that calcium released from ryanodine-sensitive stores in LDCVs may act to modulate neuropeptide secretion from neurohypophysial terminals.

### Creating a chloride channel

Heterologous expression of the channelrhodopsins (ChRs), a group of light-gated cation channels derived from algae, enables the precise activation of specific populations of neurons by light. Although light-driven chloride and proton pumps have also been identified, and used to hyperpolarize cells, these microbial opsins, which pump only a single ion per photon and do not decrease the input resistance, are relatively inefficient for inhibiting neuronal activity (see Hayashi, 2014). Now, two groups have exploited insights obtained from the recent determination of the high-resolution crystal structure of a ChR1/ChR2 hybrid to engineer ChRs that act as chloride channels. Wietek et al. (2014) replaced a single glutamate located in the ChR central gate with arginine; hypothesizing that ChR cation selectivity depends on a negative electrostatic potential surrounding the pore and vestibule, Berndt et al. (2014) mutated a series of residues so as to reverse this polarity. Both groups expressed their chloride-conducting ChRs in neurons and showed that they could block action potential generation. The use of microbial opsins to activate excitable cells has provided remarkable insights into neurophysiology; the introduction of inhibitory ChRs should provide an exciting addition to the optogenetic toolkit.
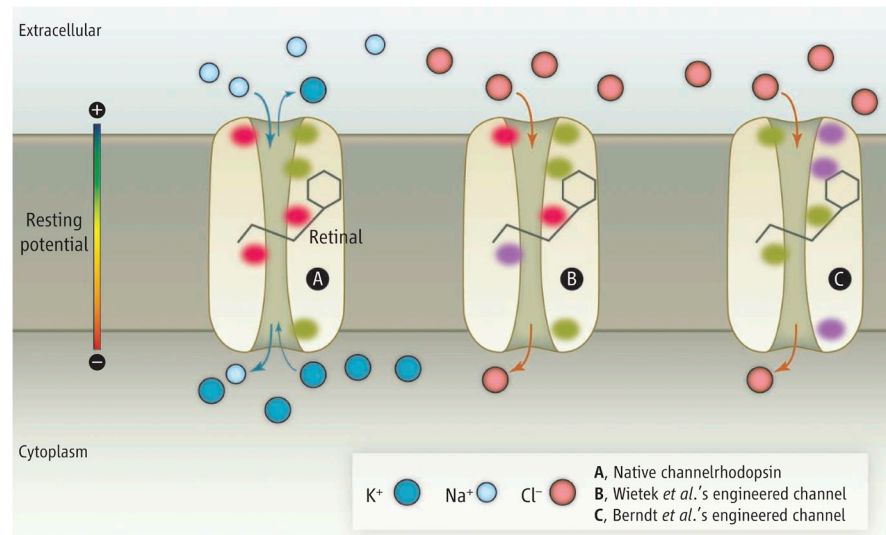
Elizabeth M. Adler
Executive Editor, *JGP*
eadler@rockefeller.edu

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**ChR residues substituted by Wietek et al. (2014) (B) and Berndt et al. (2014) (C) to create chloride-conducting ChRs. (A) Native ChRs, with mutated residues indicated in red (acidic), purple (basic), and green (neutral). Mutated residues in which polarity is unchanged are not shown. (From Hayashi. 2014. *Science.* http://dx.doi.org/10.1126/science.1253616. Reprinted with permission from AAAS.)**