JGP Journal of General Physiology

RESEARCH NEWS

A new model struts its stuff

Caitlin Sedwick

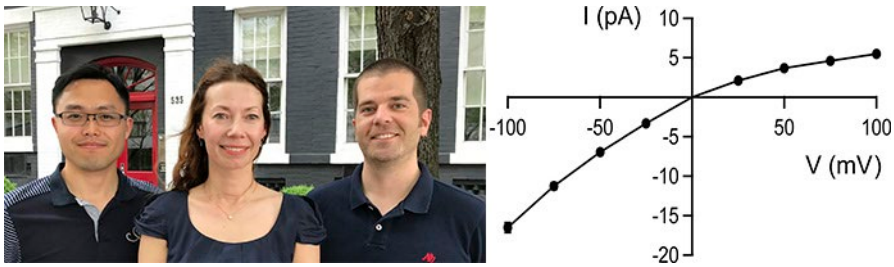
JGP paper presents a model for studying cyclic nucleotide–modulated channels.

Cyclic nucleotide–modulated ion channels are involved in diverse cellular processes. Eukaryotes have two structurally related but functionally distinct subtypes of these channels: cyclic nucleotide–gated (CNG) channels and hyperpolarization-activated and cyclic nucleotide–modulated (HCN) channels (1). Some bacteria also possess channels resembling CNG and HCN. Channel activity is affected by binding of cyclic nucleotides (cAMP and/or cGMP), but little is known about how binding causes changes in channel activity. In this issue of the *Journal of General Physiology*, Schmidpeter et al. approach this problem through studies of the bacterial cyclic nucleotide–modulated channel SthK (2).

"There is no good model for cyclic nucleotide–modulated channels at this time," says Dr. Crina Nimigean, an Associate Professor at Cornell University. "Eukaryotic channels are quite difficult to express and purify in eukaryotic systems. They're also very expensive to produce in the amounts that would be necessary to perform structural and functional assays. On the other hand, we don't really know much about the prokaryotic channels."

Studies with bacterial channels have yielded structural information, but corresponding functional data are lacking because no ionic current could be detected passing through these channels (3, 4). In search of a better model protein, Nimigean's group began working with the bacterial channel SthK, whose predicted structure suggested it might behave similarly to a eukaryotic CNG channel. When expressed in frog oocytes, SthK is selective for potassium and activated by cAMP but inhibited by cGMP (5). Nimigean's laboratory worked to study the channel in more detail.

"We found that we can produce a lot of SthK protein, that we can purify it homogeneously, and that we can use it for many functional and biophysical assays," explains postdoc Philipp Schmidpeter, who, together with fellow postdoc Xiaolong Gao, spearheaded the group's work with SthK.



Co–first authors Philip Schmidpeter (right) and Xiaolong Gao (left), senior author Crina Nimigean (middle), and colleagues characterize the bacterial cyclic nucleotide–modulated channel SthK, whose current-voltage relationship is shown in the graph at right. Photo courtesy of the authors.

Schmidpeter et al. placed purified SthK protein in synthetic liposomes and used a radioactivity-based assay to screen for channel activity. In the presence of cAMP and select lipids, the authors easily observed SthK channel activity. As with eukaryotic CNG channels, cyclic nucleotides were absolutely required for SthK channels to open.

"We expected cAMP would be a full agonist in line with eukaryotic CNG channels, where cyclic nucleotide binding leads to full activation of the channel," says Nimigean. Surprisingly, however, cAMP turned out to be only a partial agonist for SthK. In addition, measurement of the kinetics of channel opening using fluorescence-based stopped-flow assays showed that channel activation by cAMP was biphasic, with a rapid but partial initial activation that gradually increased with time.

For more information on SthK's behavior, the researchers recorded single-channel potassium currents and obtained the channel's current-voltage relationship. These data showed that unlike eukaryotic CNGs, SthK was voltage dependent, opening for much longer at positive voltages than at negative ones.

In a further surprise, data from single-channel recordings showed cGMP was not an antagonist of SthK; instead, it was a very weak partial agonist. Earlier studies were performed in a different system and had not used single-channel studies (5), which might explain the different conclusions reached in this work.

Why do cAMP and cGMP evoke different responses from SthK? One possibility is that cGMP binds SthK less well than cAMP, but ligand binding assays demonstrated that cGMP and cAMP bound with similar affinities. Structural information (6) indicates that interactions between cAMP and the channel's cytoplasmic nucleotide-binding domain may drive structural changes that open the channel's pore.

The next step for Nimigean's group will be to seek mechanistic explanations for this and other questions that the SthK functional studies have raised. "SthK is also a very promising target for structural analyses such as cryo-EM," notes Schmidpeter.

1. James, Z.M., and W.N. Zagotta. 2018. *J. Gen. Physiol.* 150:225–244.
2. Schmidpeter, P.A.M., et al. 2018. *J. Gen. Physiol.* https://doi.org/10.1085/jgp.201812023
3. Kowal, J., et al. 2018. *Structure.* 26:20–27.e3.
4. James, Z.M., et al. 2017. *Proc. Natl. Acad. Sci. USA.* 114:4430–4435.
5. Brams, M., et al. 2014. *Proc. Natl. Acad. Sci. USA.* 111:7855–7860.
6. Kesters, D., et al. 2015. *PLoS One.* 10:e0116369.

csedwick@gmail.com.

https://doi.org/10.1085/jgp.201812116