Temperature 1:1, 26–27; April/May/June 2014; © 2014 Landes Bioscience

V_2A_2lidating TRP channel heteromers

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Transient receptor potential (TRP) ion channels play a key role in sensing environmental and endogenous stimuli. Among sensory neurons, different TRP channels are widely expressed, and their expression profiles overlap. Although many TRP channels are homotetramers, we have shown that a functional tetrameric TRP channel can contain two types of monomers.

In our study, we investigated whether human TRPV1 and TRPA1 subunits can associate to form functional heteromeric ion channels.[1] We chose these two TRP channel isoforms because both play a pivotal role in the detection of potentially harmful chemical stimuli, and because there is a substantial overlap in their expression; specifically, TRPA1 is found only in a subset of TRPV1-expressing neurons, and does not occur on its own.

Heteromeric channels with a defined subunit arrangement can be constructed by concatenation of the subunits into a single entity. This approach was first used successfully for a potassium channel.[2]

Comment on: Fischer MJ, Balasuriya D, Jeggle P, Goetze TA, McNaughton PA, Reeh PW, Edwardson JM. Direct evidence for functional TRPV1/TRPA1 heteromers. Pflugers Arch 2014; (Forthcoming); PMID:24643480; http://dx.doi.org/10.1007/s00424-014-1497-z

Keywords: concatemer, dimer, heteromer, TRPV1, TRPA1, pain

Abbreviations: TRP, transient receptor potential

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Submitted: 05/21/2014

Revised: 06/10/2014

Accepted: 06/10/2014

Published Online: 06/24/2014

http://dx.doi.org/10.4161/temp.29548

Before results from these constrained heteromers can be interpreted, it is important to know whether the process of concatenation itself affects channel properties. In the case of TRP channels, concatenation of four TRPM8 subunits was shown previously to generate a channel that behaved identically to a channel formed from four individual subunits.[3] This technique allowed an investigation into the contribution of the monomers to channel gating, as unresponsive mutant subunits could be introduced with a defined stoichiometry and arrangement. In our study, channels formed from concatenated TRPV1::TRPV1 homodimers were compared with channels formed from individual TRPV1 subunits. No differences were observed in responses to chemical and thermal stimuli; further, protein kinase C-based sensitization of the response to TRPV1 activator capsaicin was also similar to that in the wildtype channel. Native TRPV1 was reported to be present in the plasma membrane in the form of dimers, which associate upon activation into tetramers[4]; hence, the TRPV1::TRPV1 homodimer will not cause changes in the physiological behavior of the channel as a concatenated tetramer would do. The occurrence of dimeric TRP channels in the membrane also raises the possibility of an ongoing rearrangement of the channel stoichiometry.

A concatenated heterodimer allows investigation of a tetrameric channel with a 2:2 stoichiometry. A TRPV1::TRPA1 concatemer was responsive to TRPV1 stimuli, including capsaicin, acidic pH or ethanol, but not to TRPA1 stimuli, including electrophilic agonists such as allylisothiocyanate or hydrogen peroxide, and non-electrophilic agonists such as carvacrol. We were not surprised by this lack of responsiveness to TRPA1 stimuli, as all large N-terminal fusions to TRPA1 reported so far have resulted in minimal or no residual channel function.

Heteromeric channel constructs can show different functional characteristics from those of the corresponding homotetramers. This has been shown for the TRPV1::TRPV3 concatamer, in which both subunits are functional.[5] Specifically, channels formed from TRPV1::TRPV3 concatamers have a different single-channel conductance and a different heat threshold from the respective homotetramers; the observed values are between those of the homotetramers but do not match the arithmetic average, supporting the notion that the channel formed from TRPV1::TRPV3 is a new species.

For TRPV1, a Hill slope above one has been reported, indicating some cooperativity in the gating of the subunits by capsaicin.[6] Somewhat surprisingly, the capsaicin concentration-response curves of TRPV1::TRPV1 and TRPV1::TRPA1 were identical to that of TRPV1. In contrast, functional analysis showed a clear difference in the capsaicin-induced shift of the current-voltage relationship. Results for TRPV1::TRPV1 corresponded with those for the native channel. However, a channel formed from TRPV1::TRPA1 affords only two rather than four capsaicin binding sites, and showed a smaller agonist-induced shift, as one might predict. In the concatemer, the presence of non-functional TRPA1 subunits within the channel exerts an inhibitory effect on channel gating through TRPV1 subunits.

Atomic force microscopy imaging of isolated channels assembled from TRPV1::TRPA1 concatemers showed a size similar to that of the wildtype channels, suggesting the formation of channels consisting of four subunits, as expected. The heterodimers assemble primarily in a configuration with identical subunits in diagonally opposite positions (**Fig. 1**). However, less frequently, an adjacent localization of identical subunits was observed. We speculate that the long flexible linker used for concatenation might

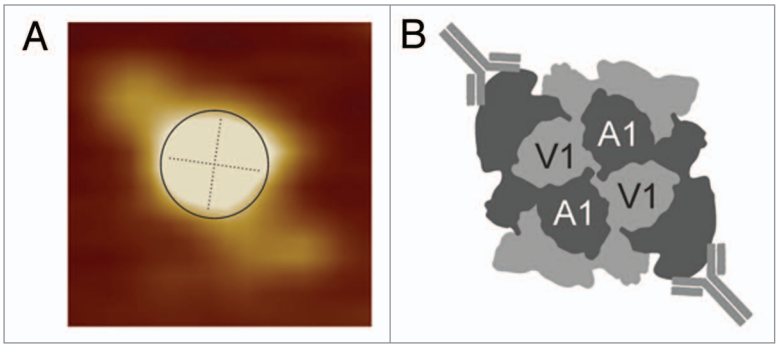

Figure 1. (**A**) Atomic force microscopy image of an isolated channel composed of two TRPV1::TRPA1 concatemers. The channel is decorated by antibodies against epitope tags on TRPA1. (**B**) Corresponding schematic representation. When expressed in cells, this complex responds to the TRPV1 activators capsaicin, heat and acidic pH, but not to activators of TRPA1.

permit this arrangement. A shorter linker or even fusion of terminally truncated proteins might be used to investigate this possibility.

An important issue to be clarified is whether different monomers spontaneously form heteromers. The percentage of heteromers will depend on the relative association constants between identical and different subunits. The sparse information available suggests that the affinities are in fact not vastly different[7]; hence, a considerable proportion of heteromers would be expected. Given the many TRP channel isoforms, functional channels might also be formed from more than two different subunits. In addition, functional channels might even include subunits (e.g., splice variants) that cannot form functional channels alone. Hence, there is a vast array of possibilities to explore. Other combinations of TRP channels investigated so far are TRPP2/TRPC1, TRPP2/TRPV4 and TRPC3/TRPC4.

In our experiments, the TRPV1::TRPA1 concatemers did not respond to TRPA1 agonists, raising the question of whether heteromers formed from monomers would behave in the same way. An answer to this question would have major implications for the existing TRPA1 literature.

Disclosure of Potential Conflicts of Interest

There are no potential conflicts of interest.

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