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Review of the PhD Thesis "Structural and Functional Study on Transient Receptor Potential Vanilloid 1 (TRPV1) and Ankyrin Receptor (TRPA1) Channels" by Abdul SAMAD.

In his doctoral thesis, Abdul Samad reports studies on the structure-function relationships of two members of the huge "Transient Receptor Potential" (TRP) family of cation channels, the Vanilloid1 TRP (TRPV1) and the Ankyrin Receptor (TRPA1).
TRP channels are non-selective cation channels that are involved in sensing of a large variety of external stimuli and the subsequent signal transduction. Their activation, gating and desensitization are complex and differ even between members of the same subfamily. Thus it is indeed of great interest to learn about the relationships between structure and (diverse) function(s) of these channels.

The thesis tries to answer three more specific questions:

1. Is the hypothesis that Capsaicin induced TRPV1 desensitization depends on dephosphorylation of Ca-CaM kinase phosphorylated residues correct?

It could be shown using site directed mutagenesis that the proposed desensitization mechanism via dephosphorylation of certain 'normally' phosphorylated residues is probably wrong because at least one of the non phosphorylatable mutant proteins that were generated to answer this question exhibited the same desensitization behavior as the wild type channel.

2. Is the gating mechanism of TRPV1 universal for all TRP channels?

Here Abdul Samad (and his colleagues) demonstrated that the putative S6 inner pore region plays a crucial role in the allyl-isothiocyanate (AITC) induced and voltage dependent gating of TRPA.

3. Are the basic residues in the (putative) intracellular C-terminal tail of TRPA1 important for its function and if yes what is their role?

Also this question could be (at least partially) answered. As shown in the thesis, basic residues within the cytosolic C-terminus of the Ankyrin receptor modulate the gating process in both, response to voltage and chemical stimuli):

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The thesis is divided into two main parts, a kind of review on what is known on the properties of TRP channels and their structure function relationships and a summary of the scientific publications that resulted from the work. Both parts are written in a concise and clear language.

The results obtained during the thesis work were published in four (or five?) peer reviewed scientific articles that all are results of collaborative and interdisciplinary work. In two of these articles Abdul Samad is (equivalent) first author. This clearly demonstrates his scientific capabilities.

Methodologically, the presented work involves many techniques / methods including molecular biology, biochemistry, electrophysiology and also some mathematics (structure modeling). It is clear, that this broad range can only be covered by a team of collaborators. However, from reading the thesis it was not clear for me, which techniques were used and which experiments were carried out by the author personally. I am aware of the fact that the format of the thesis, an introduction plus a summary connecting the publications, does not require a method part. Nonetheless, I personally would have appreciated such a part in which the author could have had explained in some detail the methods and techniques that were used by himself.

Despite the above mentioned very minor restriction, with the present thesis Abdul Samad has clearly proven, that he is able to conduct independent scientific research and that he is also able to communicate his results in a concise and understandable way.

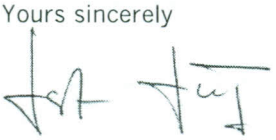
I therefore recommend without reservation the acceptance of his PhD thesis.

For the discussion, I'd suggest to include the following questions/topics

In the thesis Abdul Samad states that the gating of TRPA1 "significantly differs from the mechanisms ***described*** for TRPV1". I'd be interested to learn/discuss, what the gating ***mechanisms*** of TRPV1 are exactly and in how far these mechanisms can be taken for granted.

Homology modeling was carried out using the known structure of the bacterial (inward rectifying) K-channel KcSa as a template to model the TRPV1 region ranging from S5 to S6. Would it make sense to extend and refine this model using the (meanwhile known) structure of a 6-TM-voltage gated K-channel ($K_V1.2$)? Could the results also gain the insight into the structure/function relationships of other TRP channels?

Yours sincerely



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Report on Ph.D. Thesis of Mr. Abdul Samad

This thesis concerns structural-functional studies of two types of transient receptor potential channels. One of the channels is TRPV1, probably the most intensively studied, the other is TRPA1, the most recently discovered member of this family. The thesis is well focused to the pore region and to the adjacent cytoplasmic domain.

Methodically, the thesis covers three areas, homology modeling, site-directed mutagenesis and patch clamp technique. In the absence of crystal structure combination of modeling with mutagenesis followed by phenotype determination can provide invaluable information and this is also the case of this thesis.

The thesis is organized in a manuscript format with introduction followed by five papers. As I am not an expert in the field, I appreciated thorough Introduction into the world of TRP channels. I only missed the list of abbreviations.

In the first study, the hypothesis that capsaicin induced TRPV1 desensitization is solely dependent on phosphorylation state of two amino acid residues was challenged and an important functional role of two amino acid residues close to the TRP box in regulating the activity of the channel was shown.

In the second study, focused on the pore-forming region of TRPA1, several amino acid residues were found by alanine mutagenesis to influence channel gating. Convincing was the demonstration of the importance of proline P949 in the middle of the pore. This residue could be functionally replaced by "flexible" glycine found at the same position in some other channels.

In the third study, 27 basic residues of the C-terminus of TRPA were subjected again to alanine mutagenesis followed by analysis of the changes in agonist gating. One third of the residues was shown to have a role in the recognition of chemical and voltage stimuli.

In conclusion, this is a high quality thesis that clearly meets the requirements of the Ph.D. degree.

Questions for the author:

In Annotation, the author is claiming that key amino acid residues in the TRP box interacting with the phospholipid were found. I have not found this information in Paper I.

It is probably my ignorance, but I do not understand the sentence in Introduction (p. 22) stating that SCAM would be a good method to determine pore diameter of TRPA1.

Can TRPs be expressed heterologously in some other organisms. Would this expression provide some advantages to expression in mammalian cells?

Alanine scanning mutagenesis is a routine approach using alanine as structurally "neutral" amino acid. Does the author think that using some less "boring" amino acid like glutamic acid could provide significantly different results in scanning of the C-terminus of TRPA1?

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Opponent's review of the PhD Thesis

of Abdul Samad entitled „Structural and Functional Study on Transient Receptor Potential Vanilloid 1 (TRPV1) and Ankyrin Receptor (TRPA1) Channels"

The PhD thesis presented by Abdul Samad deals with the study of structural and functional relationships of rat transient receptor potential cation channel, subfamily V, member 1 (TRPV1, also known as the capsaicin receptor), and human transient receptor potential cation channel, subfamily A, member 1 (TRPA1). The TRPV1 channel is a nonselective cation channel that is activated by vanilloid compounds like capsaicin, low pH, noxious heat, and depolarizing voltages. The TRPA1 channel is a sensory neuron-specific channel that is gated by various proalgesic agents, low temperature or highly depolarizing voltages. Both these cation channels are an important component of the transduction machinery with still unclear mechanism of activation and desensitization.

In the presented thesis the capsaicin induced Ca^{2+}-dependent desensitization of TRPV1 was studied and key amino acid residues in the C-terminal domain important for the decelerated gating kinetics of the desensitized channel were identified. Another topic studied in this thesis is the role of putative inner-pore S6 region of TRPA1 in channel gating and the identification of amino acid residues important for channel opening and closing. In addition, several regions within the C-terminus of TRPA1 important for both the chemical and voltage sensitivity were identified.

The formal structure of presented thesis follows the division into two main parts. The first one describes the biological background of TRP proteins, the structural aspects of TRPV1 and TRPA1 channels, and general structural and functional relationships of TRP channels. The second one consists of a summary of attached papers accompanied by five publications where Mr. Samad is the first author or co-author. Four publications were published in international journals with impact factor. Mr. Samad is the first author on two of them. The formal and graphical quality of presented thesis is good. The occurrence of typing

errors is minimal. Concerning the formal structure of the thesis I missed at least a brief overview of techniques used by the author himself in the presented work. Since all included papers are based on collaboration of several groups, the author's contribution to each paper should be clarified during the thesis defense (e.g. which experiments were performed by him etc.).

For discussion sake, I have following questions related to the presented thesis:

1. TRP channels are known to be phosphorylated on multiple Ser/Thr sites by several kinases (e.g. PKA, PKC, CaMKII). Such phosphorylations could induce the binding of various adaptor and regulatory proteins. Could that also be the case of TRP channels?

2. The author mentioned the hypothesis that calmodulin (CaM) can "crosslink" both termini of TRPV1 channel (page 29). CaM is a small monomeric protein than binds only one target protein. Could you explain this hypothesis in more details, for example, how CaM could crosslink two polypeptide chains?

In conclusion, the PhD thesis presented by Abdul Samad represents significant contribution to the study of structural and functional relationships of TRPV1 and TRPA1 cation channels. The thesis is written carefully in intelligible English, and all results were published in international journals. Mr. Samad clearly demonstrated that he is able of independent scientific work.

Since the thesis presented by Abdul Samad satisfies all requirements for PhD thesis I fully recommend its acceptance.

Prague, November 17th 2010

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