

The Role of STIM and Orai Proteins in the Ca²⁺
Release-Activated Ca²⁺ Channel

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ABSTRACT

Store operated Ca^{2+} channels on the plasma membrane are activated by depletion of intracellular Ca^{2+} stores such as the endoplasmic reticulum. The archetypal example of these channels is the Ca^{2+} release-activated Ca^{2+} (CRAC) channel. CRAC channels are expressed in most mammalian cell types, and are required for T cell activation during immune responses, maintenance of muscle tone, and regulation of cell division. The molecular components of the CRAC channel are: STIM1, located on the endoplasmic reticulum membrane, which acts as a luminal Ca^{2+} sensor and store depletion signal; and Orai proteins on the plasma membrane, tetramers of which form the Ca^{2+} permeable pore of the channel.

The first study in this thesis investigated the hypothesis that the relative expression levels of STIM1 and Orai1 determine the biophysical properties of CRAC channels. Changing the transfection ratio of STIM1 to Orai1 containing plasmids resulted in a corresponding change in the relative amount of each protein expressed. Concomitant with this result was a change in a range of channel properties. Most notably, fast Ca^{2+} dependent inactivation (FCDI) of the current was strongest when STIM1 was expressed in relative excess to Orai1. The results of this study demonstrated that STIM1 is a determinant of the CRAC current properties and that it is likely that a variable number of STIM1 peptides can bind to each Orai1 tetramer.

Having defined a new role for STIM1 in the kinetics of CRAC current, mutations of the Orai1 predicted pore reported to affect selectivity and/or gating were investigated to determine whether they may also be affected by the relative expression of STIM1. The results of the second study of this thesis showed that V102I and E190Q Orai1 were both dependent on the relative expression of STIM1, similarly to WT Orai1. A third

mutant, E106D Orai1 produced currents with very strong, accelerated FCDI, independently of the relative expression level of STIM1. In addition, this mutant displayed altered pH dependence. It was concluded that the selectivity centre of Orai1 is also crucial for channel gating and pH dependence.

While the properties and physiological roles of Orai1 have been well described, less is known about its homologues. In order address this, the third study characterised the biophysical properties of channels formed with Orai3. While Orai3 channels were activated by store depletion via STIM1, FCDI was not dependent on the relative expression of STIM1. Surprisingly, an Orai3 current was able to be activated independently of store depletion and STIM1 by external application of the compound 2-APB. Unlike store operated current, the 2-APB activated current was non-selective and displayed no Ca^{2+} dependent kinetics or pH dependence.

The results of this thesis have helped to elucidate the molecular basis underlying many CRAC current properties. Most notably, a now widely accepted understanding was developed that in addition to its role as an intracellular Ca^{2+} sensor, STIM1 is a regulator of CRAC current properties. These findings have the potential to be applied in understanding a broad range of Ca^{2+} signaling dependent cellular processes and disease states.

DECLARATION

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AUTHOR STATEMENTS

Chapter 2:

Properties of Orai1 mediated store-operated current depend on the expression levels of STIM1 and Orai1 proteins

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The authors' responsibilities were as follows:

Nathan Scrimgeour was responsible for the conception and design of the study, collection and assembly of electrophysiological data, data analysis and interpretation, and writing and preparation of the manuscript.

Tom Litjens contributed to the conception and design of the study, and the construction of STIM1 and Orai1 containing plasmids.

Linlin Ma performed the collection and assembly of western blot data, and data analysis and interpretation.

Greg Barritt contributed to the conception and design of the study, data interpretation and preparation of the manuscript.

Grigori Rychkov was responsible for the conception and design of the study, collection of data, data analysis and interpretation, writing and preparation of the manuscript, and acted as the corresponding author.

All authors agreed on the final version of the manuscript. None of the authors had a conflict of interest in relation to this manuscript.

Authors Signatures:

I agree with the author contributions for the manuscript “Properties of Orai1 mediated store-operated current depend on the expression levels of STIM1 and Orai1 proteins”, and give permission for the use of this manuscript in the thesis.

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Chapter 3:

Glutamate 106 in the Orai1 pore contributes to fast Ca²⁺-dependent inactivation and pH dependence of Ca²⁺ release-activated Ca²⁺ (CRAC) current

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David Wilson contributed to the conception and design of the study, data interpretation and preparation of the manuscript.

Grigori Rychkov was responsible for the conception and design of the study, data analysis and writing and preparation of the manuscript.

All authors agreed on the final version of the manuscript. None of the authors had a conflict of interest in relation to this manuscript.

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ABBREVIATION LIST

| <i>Abbreviation</i> | <i>Full term</i> |
|------------------------------------|--|
| 2-APB | 2-aminoethoxydiphenylborate |
| ARC | arachadonate-regulated Ca ²⁺ |
| BAPTA | 1,2-bis(o-aminophenoxy)ethane-N,N,N',N'-tetraacetic acid |
| [Ca ²⁺] _c – | cytoplasmic Ca ²⁺ concentration |
| CaM | calmodulin |
| CBD _{Orai1} | calmodulin binding domain of Orai1 |
| CC | coiled-coil |
| CAD | CRAC activation domain |
| CIF | Calcium Influx Factor |
| CMD | CRAC modulatory domain |
| CRAC | Ca ²⁺ -release activated Ca ²⁺ |
| CRACR2A | CRAC Regulator 2A |
| DMEM | Dulbecco's modified Eagle's Medium |
| DVF | divalent free |
| EAE | experimental autoimmune encephalomyelitis |
| ECCE | excitation-coupled Ca ²⁺ entry |
| eGFP | enhanced green fluorescent protein |
| EGR1 | Early Growth Response 1 |
| EGTA | ethylene glycol tetraacetic acid |
| ER | endoplasmic reticulum |
| FBS | fetal bovine serum |
| FCDI | fast Ca ²⁺ dependent inactivation |
| FLC | fetal liver chimera |
| GFP | green fluorescent protein |
| HEK293 | human embryonic kidney 293 cell line |
| I _{CRAC} | Ca ²⁺ -release activated Ca ²⁺ current |
| I _{SOC} | store operated channel current |
| IP ₃ | inositol 1,4,5-trisphosphate |
| I-V | current-voltage |
| MS | Multiple Sclerosis |
| NFAT | Nuclear Factor of Activated T cells |
| NMDG ⁺ | N-methyl-D-glucamine |
| PLC | phospholipase C |
| PMCA | Plasma Membrane Ca ²⁺ ATPase |

| | |
|----------------|--|
| P _o | apparent open probability |
| RBL | rat basophilic leukaemia |
| SAM | sterile α -motif |
| SCID | Severe Combined Immunodeficiency |
| SERCA | Sarcoplasmic/Endoplasmic Reticulum Ca ²⁺ ATPase |
| siRNA | short interfering RNA |
| SOC | store operated channel |
| SOCE | store operated Ca ²⁺ entry |
| SR | sarcoplasmic reticulum |
| STIM | Stromal Interacting Molecule |
| TRP | Transient Receptor Potential |
| TRPC | Canonical Transient Receptor Potential |
| VSMC | vascular smooth muscle cell |
| WT1 | Wilms Tumour Suppressor |

CONFERENCE PRESENTATIONS DURING CANDIDATURE

International

- 2011** 55th Annual Biophysical Society Meeting
Baltimore, MD, USA
- Poster Presentation:** The glutamate 106 of Orai1 regulates fast Ca^{2+} dependent inactivation and pH dependence of CRAC current

National

- 2010** Australian Physiological Society/Australian Biophysics Society Joint Meeting Adelaide, SA
- Oral Presentation:** pH dependence of the CRAC channel
- 2010** Australian Neuroscience Society/Australian Physiological Society Joint Meeting
Sydney, NSW
- Oral presentation:** Altered CRAC channel gating in the Orai1 E106D mutant
- 2009** Curtin Conference on Ion Channels and Transporters in honour of Peter Gage, Canberra, ACT
- Poster presentation:** The effect of point mutations in the Orai1 pore on CRAC channel gating
- 2008** Australian Physiological Society Meeting 2008, Melbourne, VIC
- Student award:** Best poster presentation
- Poster presentation:** Store independent activation and properties of Orai3/STIM1 mediated current.