The Role of STIM and Orai Proteins in the Ca^{2+} Release-Activated Ca^{2+} 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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Scrimgeour NR, Wilson DP & Rychkov GY (2012). Glutamate 106 in the Orai1 pore contributes to fast Ca^{2+}-dependent inactivation and pH dependence of Ca^{2+} release-activated Ca^{2+} (CRAC) current. *Biochem J* **441,** 743-753.

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.

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Chapter 3:

Glutamate 106 in the Orai1 pore contributes to fast Ca^{2+}-dependent inactivation and pH dependence of Ca^{2+} release-activated Ca^{2+} (CRAC) current

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Nathan Scrimgeour was responsible for the conception and design of the study, generation of Orai1 mutants, collection and assembly of electrophysiological data, data analysis and interpretation, and writing and preparation of the manuscript.

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

Abbreviation	*Full term*
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.