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Functional and Structural Analyses of an Olfactory Receptor from *Drosophila melanogaster*

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Abstract

In insects, olfaction is mediated by a large family of integral membrane proteins, called olfactory receptors (ORs), that mediate the transduction of odorant binding into a neuronal signal. A functional assay for insect ORs was developed utilising calcium imaging in Sf9 cells. The *Drosophila melanogaster* OR, Or22a, was expressed using transient transfection, and its activity measured by monitoring increased intracellular calcium levels using a calcium–sensitive dye. The interaction of the odorants ethyl butyrate, pentyl acetate and ethyl acetate with Or22a were both dose–dependent and sensitive, with EC_{50} values of 1.53 x 10^{-11} M, 5.61 x 10^{-10} M and 3.72 x 10^{-9} M, respectively. Furthermore, Or22a expressed in Sf9 cells has a similar response profile to a range of odorants previously tested *in vivo*. This assay system will provide a useful tool for the investigation of insect olfactory receptor structure and function.

A consensus of eleven transmembrane (TM) domain prediction algorithms suggested a model for Or22a that contains seven TM domains, reminiscent of GPCRs. To test this model empirically, the membrane topology of Or22a was determined using epitope–tagging of predicted loops followed by immunochemistry. These experiments revealed that Or22a has seven TM domains but that its orientation in the membrane is opposite to that of GPCRs, having a cytoplasmic N–terminus. This orientation was also observed for *Epiphyas postvittana* Or1, which suggests that this inverted topology may be common to all insect ORs.

To test whether Or22a forms higher order structures, fluorescence resonance energy transfer (FRET) between cyan and yellow fluorescent proteins inserted into the intracellular loops of Or22a was employed. The third intracellular loop interacts strongly with itself

in homo–multimers, with interactions between the first and first loops and first and third loops also observed. These experiments show that ligand binding ORs can form multimeric structures in heterologous cells. The co–transfection of Or83b into S2 cells had no impact on these interactions, however Or83b is likely expressed in this cell line. Finally, models of how a ligand binding OR interacts physically with the ion channel Or83b are presented, and approaches that could be used to distinguish between these models are discussed.

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ΔF	Change in fluorescence
°C	Degrees celsius
AC	Adenylyl cyclase
ADP	Adenosine diphosphate
AL	Antennal lobe
ATP	Adenosine triphosphate
BiFC	Bimolecular fluorescence complementation
bp	Base pair(s)
BRET	Bioluminescene resonance energy transfer
cAMP	Cyclic adenosine monophosphate
CCD	Charge–coupled device
cDNA	Complementary deoxyribonucleic acid
CFP	Cyan fluorescent protein
cGMP	Cyclic guanosine monophosphate
CGRP	Calcitonin–gene–related peptide
CRLR	Calcitonin receptor–like receptor
C–terminus	Carboxy–terminus
DAPI	4,6–diamidino–2–phenylindole
DMSO	Dimethyl sulfoxide
DNA	Deoxyribonucleic acid
dNTP	Deoxynucleotide triphosphate
EAG	Electroantennogram
EC_{50}	Half maximal effective concentration
FRET	Fluorescence resonance energy transfer
g	Gravitational constant
GDP	Guanosine diphosphate
GFP	Green fluorescent protein
GPCR	G protein–coupled receptor
G protein	Guanine nucleotide–binding proteins
GRK	G protein–coupled receptor kinase
GTP	Guanosine triphosphate

HEK293	Human embryonic kidney–293
HMM	Hidden Markov model
IP_3	Inositol triphosphate
K_m	Michaelis constant
kDa	kilo Daltons
LDS	Lithium dodecyl sulphate
mRNA	Messenger ribonucleic acid
NMR	Nuclear magnetic resonance
N–termius	Amino–terminus
OBP	Odorant binding protein
ODE	Odorant degrading enzyme
OR	Olfactory receptor
ORN	Olfactory receptor neuron
PAGE	Polyacrylamide electrophoresis
PBP	Pheromone binding protein
PBS	Phosphate–buffered saline
PCR	Polymerase chain reaction
PDB	Protein Data Bank
pH	Potential of hydrogen
PIP_2	Phosphatidylinositol bisphosphate
PIPES	Piperazine-N-N'-bis(2-ethanesulfonic acid)
PLC	Phospholipase C
PVDF	Polyvinylidene fluoride
RAMP	Receptor activity–modifying protein
RANTES	Regulated activation normal T cell expressed secreted
RCSB	Research Collaboratory for Structural Bioinformatics
ROI	Region of interest
RNA	Ribonucleic acid
RTK	Receptor tyrosine kinase
RT–PCR	Reverse transcription polymerase chain reaction
S2	Schneider 2
SDS	Sodium dodecyl sulphate
S.E.M.	Standard error of the mean
Sf9	*Spodoptera frugiperda* 9
SNMP	Sensory neuron membrane protein
TBS	Tris–buffered saline
TM	Transmembrane
Y2H	Yeast two–hybrid
YFP	Yellow fluorescent protein

List of Publications

The research presented in this thesis is my own work, and contributed to the following publications:

- **Aidan Kiely**, Astrid Authier, Andrew V. Kralicek, Coral G. Warr and Richard D. Newcomb. 2007. Functional analysis of a *Drosophila melanogaster* olfactory receptor expressed in Sf9 cells. *Journal of Neuroscience Methods*, **159**(2), 189–194.

- Renee Smart, **Aidan Kiely**, Morgan Beale, Ernesto Vargas, Colm J. Carraher, Andrew V. Kralicek, David L. Christie, Chen Chen, Richard D. Newcomb and Coral G. Warr. 2008. *Drosophila* odorant receptors are novel seven transmembrane domain proteins that can signal independently of heterotrimeric G proteins. *Insect Biochemistry and Molecular Biology*, **38**(8), 770–780.

In addition, the following publications are in review or in preparation:

- Melissa Jordan, Alisha A. Anderson, Doreen Begum, Colm J. Carraher, Astrid Authier, Sean Marshall, **Aidan Kiely**, Laurence Gatehouse, David R. Greenwood, David L. Christie, Andrew V. Kralicek, Stephen Trowell and Richard D. Newcomb. 2008. Odorant receptors from the lightbrown apple moth (*Epiphyas postvittana*) recognize important volatile compounds produced by plants. *Insect Biochemistry and Molecular Biology* (Accepted).

- **Aidan Kiely**, Pablo German, Andrew V. Kralicek, David L. Christie and Richard D. Newcomb. 2008. Analysis of the homodimerisation domains of the insect olfactory receptor Or22a. (In Preparation).