Genome-scale identification of cellular pathways required for cell surface recognition

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This dissertation is submitted for the degree of

Doctor of Philosophy

Trinity College

December 2017

DECLARATION

I hereby declare that the contents of this thesis are original and have not been submitted in whole or in part for consideration for any other degree or qualification in this, or any other, University. This thesis is the result of my own work and includes nothing which is the outcome of work done in collaboration, except where specifically indicated in the text. This thesis does not exceed the word limit set by the Faculty of Biology.

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December 2017

ACKNOWLEDGEMENTS

First, I would like to express my sincere gratitude to my advisor Dr. Gavin Wright for the guidance, motivation, patience and the encouraging words all through my PhD. Thank you mostly for encouraging me to appreciate technology development related research! Thank you also to the members of my thesis committee, Dr. Julian Rayner and Dr. Kosuke Yusa for their insightful comments and encouragement, and also for providing a different perspective to my research.

Deepest gratitude to the members of the Wright Lab who have been a cherished combination of colleagues and friends. Special thank you to Josefin, who has provided instrumental support to my research throughout the PhD; Nicole, Shan, Laura and Kirsty for readily sharing their reagents and technical abilities; and Cecile and Enrica whose openness and scientific insight I always admire. I would also like to thank the members of the flow cytometry core-facility (Bee Ling Ng, Jennifer Grahm, Chistopher Hall and Sam Thompson) for their flow cytometry training and advice; Mandy Sanders for help with sequencing sample submission; and Sanger Institute Core Sequencing facility for sequencing.

Most of all, I would like to thank my parents, whose encouragement and love has made possible my journey from the hills of Rohote to Cambridge! Also, thanks to my beloved siblings, Rashmi and Prashant, for their guidance and unwavering support. Thank you to James- you have been amazingly patient and always been there to support me. Thanks to my friends, John, Katie and Tom who have made my time during my PhD a very enjoyable one.

ABSTRACT

A range of biochemically diverse molecules located in the plasma membrane—such as proteins, glycans, and lipids—mediate cellular recognition events, initiation of signalling pathways, and the regulation of processes important for the normal development and function of multicellular organisms. Interactions mediated by cell surface receptors can be challenging to detect in biochemical assays, because they are often highly transient, and membrane-embedded receptors are difficult to solubilise in their native conformation. The biochemical features of low-affinity extracellular protein interactions have therefore necessitated the development of bespoke methods to detect them.

Here, I develop a genome-scale cell-based genetic screening approach using CRISPR-Cas9 knockout technology that reveals cellular pathways required for specific cell surface recognition events. Using a panel of high-affinity monoclonal antibodies, I first establish a method from which I identify not only the direct receptor but also other required gene products, such as co-receptors, post-translational modifications, and transcription factors contributing to antigen expression and subsequent antibody-antigen recognition on the surface of cells. I next adapt this method to identify cellular factors required for receptor interactions for a panel of recombinant proteins corresponding to the ectodomains of cell surface proteins to the endogenous surface receptors present on a range of cell lines. In addition to finding general cellular features recognised by many ectodomains, I also identify direct interaction partners of recombinant protein probes on cell surfaces together with intracellular genes required for such associations.

Using this method, I identify IGF2R as a binding partner for the R2 subunit of GABAB receptors, providing a mechanism for the internalisation and regulation of GABAB receptor signalling. The results here demonstrate that this single approach can identify the molecular nature and cell biology of surface receptors without the need to make any prior assumptions regarding their biochemical properties.

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