Systematic analysis of the evolution and conservation of genetic interactions using *C. elegans* as a model system

This dissertation is submitted in accordance with the requirements of the University of Cambridge for the degree of Doctor of Philosophy

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To my parents, with love

Preface

This thesis describes my work undertaken in the laboratory of Andrew G. Fraser at the Wellcome Trust Sanger Institute while member of Clare Hall, University of Cambridge. It is submitted in fulfilment of the requirements for the degree of Doctor of Philosophy. This dissertation 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. The work described here has not been submitted for any degree, diploma, or other qualification. This thesis does not exceed 300, single-sided pages of double spaced text, not including the bibliography and appendices.

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Cambridge, September 2007

Abstract

Systematic analyses of loss-of-function phenotypes have been carried out for the majority of genes in *S. cerevisiae, C. elegans,* and *D. melanogaster*. While these studies greatly expand our knowledge of individual gene functions, they do not address redundancy in genetic networks nor do they attempt to identify genetic interactions. Developing tools for the systematic mapping of genetic interactions is thus a crucial step for exploring gene networks.

I established protocols for simultaneously targeting multiple genes by RNA interference (RNAi) in *C. elegans* using bacterial feeding (‘combinatorial RNAi'). This approach allows me to examine interactions between any pair of genes and to detect the great majority of previously known synthetic lethal (SL) and post-embryonic synthetic genetic interactions. I used this technique to provide the first large-scale analysis in any organism of the redundant functions of gene duplicates. Focusing on genes that have been duplicated in the genome of *C. elegans* since divergence from either *S. cerevisiae* or *D. melanogaster,* I identified 16 out of 143 of duplicated gene pairs amenable to analysis by combinatorial RNAi to be at least partially functionally redundant. Intriguingly, the majority of these redundant gene pairs were duplicated before the split of *C. elegans* and *C. briggsae* 80-110 million years ago. My findings support population genetics models, which suggest that redundancy is not just a transient side effect of recent gene duplication but is instead a phenomenon that can be maintained over substantial periods of evolutionary time.

While I have identified functional redundancy between gene duplicates, most redundancy in genetic networks tends to be more complex. The majority of synthetic lethal interactions that were uncovered in *S. cerevisiae* occur between genes unrelated at the sequence level. To date, there is still much debate about how such ‘higher-order’ functional redundancy might arise, whether it is a selectable trait, and whether such redundancy can be conserved throughout evolution. Thus, to shed light on the evolution of genetic interactions, I investigated the conservation of gene networks between *S. cerevisiae* and *C. elegans*. Using an RNAi-based approach, I set out to explore whether

individual synthetic lethal interactions uncovered in *S. cerevisiae* are retained in *C. elegans*. I found synthetic lethal interactions to be poorly conserved between yeast and worm — despite the very high degree of conservation of individual gene functions — demonstrating a substantial evolutionary plasticity of complex gene networks. My results suggest that SL interactions are unlikely to be explained by simple models of genetic redundancy and led me to propose a novel model for the interpretation of SL interactions. In this view ('induced essentiality'), SL interactions represent a special form of conditional essentiality.

Publications

Publications arising from the work described in this thesis at the time of submission:

Tischler, J, Lehner, B., Fraser, A. G. (2007). Evolutionary plasticity of genetic interaction networks. *Submitted.*

Lehner, B., **Tischler, J.**, Fraser, A. G. (2006). RNAi screens in *C. elegans* in a 96-well liquid format and their application to the systematic identification of genetic interactions. *Nat Protoc.* **1**, 1617-1620.

Tischler, J,, Lehner, B., Chen, N., Fraser, A. G. (2006). Combinatorial RNA interference in *C. elegans* reveals that redundancy between gene duplicates can be maintained for more than 80 million years of evolution. *Genome Biol,* **7**, R69.

Lehner, B., Crombie, C., **Tischler, J**., Fortunato, A., Fraser, A. G. (2006). Systematic mapping of genetic interactions in *Caenorhabditis elegans* identifies common modifiers of diverse signaling pathways. *Nat Genet*, **38**, 896-903.

Lehner, B., Calixto, A., Crombie, C., **Tischler, J.**, Fortunato, A., Chalfie, M., Fraser, A. G. (2006). Loss of LIN-35, the *Caenorhabditis elegans* ortholog of the tumor suppressor p105Rb, results in enhanced RNA interference. *Genome Biol,* **7**, R4.

Lehner, B., **Tischler, J.**, Fraser, A. G. (2005). Systems biology: where it's at in 2005. *Genome Biol*,. 6, 338.

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