Chapter 6

Discussion

6.1. Introduction

The availability of whole-genome sequences for numerous model organisms and the development of technological tools for generating loss-of-function phenotypes on a genome-wide scale have given us an unprecedented level of insight into eukaryotic gene function. It was found that inactivation of most genes in *S. cerevisiae*, *C. elegans*, and *D. melanogaster* has little discernable effect on viability under laboratory conditions (Bjorklund *et al.*, 2006; Boutros *et al.*, 2004; Giaever *et al.*, 2002; Kamath *et al.*, 2003). Strikingly, however, inactivating specific rare combinations of such non-essential genes under the exact same conditions can have profound effects on an organism's fitness. Such combinatorial effects are termed 'synthetic enhancement interactions'. Synthetic lethality, where mutation of a gene pair leads to non-viability, while inactivation of each gene individually has no discernible effect, represents the most severe form of synthetic enhancement. Synthetic lethal (SL) genetic interactions are classically interpreted as the result of inactivating two functionally redundant pathways in the cell, either of which is individually dispensable. Recently, enormous progress has been made in the yeast *S. cerevisiae*, where functional genomics tools have been established for the systematic mapping of SL interactions on a genome-wide scale (reviewed in Boone *et al.*, 2007). These studies have identified thousands of genetic interactions in yeast and appear to have uncovered an extensive degree of redundancy. However, similar approaches are not currently feasible in any animal, so alternative strategies are needed.

6.2. Combinatorial RNA interference in *C. elegans*

In the nematode *C. elegans*, RNA-mediated interference (RNAi) by bacterial feeding has emerged as a key technique for the genome-scale analysis of individual gene functions *in vivo* (Timmons and Fire, 1998). So far, however, RNAi has only been used extensively to study the loss-of-function phenotypes of single genes. For the systematic identification of genetic interactions by RNAi, I have established and validated robust methods that allow me to target any pairwise combination of *C. elegans* genes in a high-throughput manner ('combinatorial RNAi'). Using this methodology, I was able to generate loss-of-function phenotypes for two genes in the same animal and to identify the

great majority of previously known SL and synthetic post-embryonic genetic interactions. This approach should therefore allow researchers to explore genetic interactions in the worm in a far more systematic way than has been possible in the past.

6.3. Functional redundancy between *C. elegans* gene duplicates can be maintained for extensive evolutionary timescales

I used combinatorial RNAi to begin to investigate functional redundancy in the genome of *C. elegans*. One obvious cause of functional redundancy is gene duplication; duplicated genes that have retained overlapping functions can compensate for inactivation of one another (Force *et al.*, 1999; Lynch and Force, 2000).

Focusing on *C. elegans* genes that correspond to single orthologues in *S. cerevisiae* or *D. melanogaster* genomes, I have provided the first systematic experimental investigation into the redundancy of duplicated genes in any organism. I have identified 16 out of 143 *C. elegans* duplicate gene pairs to be at least partially functionally redundant. Intriguingly, the majority of these redundant gene pairs also do exist as gene duplicates in the related nematode *C. briggsae*, suggesting that these genes have been duplicated in the genome of *C. elegans* before the split from *C. briggsae* more than 80 million years ago. Thus, my findings strongly support models of gene evolution that 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.

6.4. Higher-order redundancy in genetic interaction networks

While I have identified functional redundancy between gene duplicates, most functional redundancy in genetic networks tends to be more complex. The majority of genes that were identified as having SL interactions in genome-scale screens in *S. cerevisiae* do not share sequence similarity, unlike gene duplicates, but rather occur amongst functionally unrelated genes (Tong *et al.*, 2004). This ‘higher-order’ redundancy appears to mechanistically differ from genuine functional redundancy, the redundancy of

gene duplicates. I like to picture this higher-order redundancy as a car. It is possible to tolerate loss of one or other function (i.e. one would be able to prevent an accident, if either the brakes or the steering wheel break), but loss of both functions is catastrophic (i.e. it is probably impossible to direct a car if both brakes and steering wheel are dysfunctional). However, while two functions (i.e. brakes and steering wheel) can somehow compensate for loss of one another, they do not so by simply fulfilling one another's genuine function (i.e. one is not able to steer a car by using the brakes).

6.5. Evolutionary plasticity of genetic interaction networks

In this work, I sought to address a fundamental question in genetics: 'Are SL interactions and thus functional redundant relationships evolutionarily conserved?' I therefore set out to investigate whether SL interactions identified in the yeast *S. cerevisiae* are conserved in the nematode *C. elegans*. I used RNAi to test whether I can detect SL interactions between pairs of *C. elegans* genes that are orthologous to pairs of genes identified as having SL interactions in one of three genome-scale screens in *S. cerevisiae* (Davierwala *et al.*, 2005; Pan *et al.*, 2006; Tong *et al.*, 2004). In total, I screened 843 pairs of *C. elegans* genes for non-additive, synthetic genetic interactions by using combinatorial RNAi. Of these, I also tested 174 pairs by targeting one gene of a pair by RNAi in a worm strain homozygous for a loss-of-function genetic mutation in the second gene; this was the entire set of yeast SL interactions that could be tested by combinatorial RNAi in *C. elegans* and for which a viable mutant strain was available, respectively.

Strikingly, only 6/843 (0.7%) of the tested gene pairs that were SL in *S. cerevisiae* also resulted in a synthetic viability defect in *C. elegans*. This is not significantly different to the frequency of SL interactions that we have detected by systematically investigating ~65,000 *C. elegans* gene pairs with roles in signal transduction and transcription for their ability to genetically interact. Thus, these findings demonstrate that individual SL interactions are not conserved between *S. cerevisiae* and *C. elegans* more than expected by chance.

Moreover, this observed interaction frequency does also not differ from the average interaction density in yeast gene networks. Hence, this non-conservation of genetic interactions between *S. cerevisiae* and *C. elegans* cannot simply be explained by a reduction in the number of SL interactions.

The observed non- conservation of SL interactions between yeast and worms is in marked contrast to the conservation of single gene functions. Using the same experimental platform, I identified 61% of *C. elegans* genes corresponding to an essential gene in yeast to show a non-viable RNAi phenotype, suggesting that these genes also play an essential role in the worm. Moreover, I found 28% of *C. elegans* gene duplicates related to an essential gene in yeast to have a SL RNAi phenotype. Furthermore, 31% of a test set of protein interactions were shown to be conserved between yeast and worm (Matthews *et al.*, 2001). I thus conclude that while the knowledge of an essential gene function in yeast can strongly predict the essential function of an orthologous gene in the worm, and also — albeit to a lesser extent — the essential function covered by a pair of duplicated genes in the genome of *C. elegans*, SL interactions identified in yeast cannot be used to directly predict candidate genetic interactions in the worm.

6.6. Non-conservation of synthetic lethal interactions and its implications for multigenic human disease

Most obviously, if SL interactions are not conserved between yeast and worm, it is highly unlikely that they will be conserved between yeast and human. Thus, while identifying the function of a single gene in yeast is likely to be predictive of the function of its orthologue in humans, one cannot transfer genetic interactions between species so directly. For example, it is highly unlikely that yeast SL data can be used to directly identify genes that when inhibited will selectively kill cancer cells carrying a mutation in a tumour suppressor gene (Kaelin, 2005). Considering that increasing numbers of human diseases are identified as resulting from combinations of mutations in multiple genes that alone have little effect (reviewed in Badano and Katsanis, 2002), alternative integrated approaches will be required to predict modifier genes in complex genetic diseases in humans.

6.7. Synthetic lethal interactions and predictions of gene functions

Finally, I want to emphasize that although I have shown here that SL interactions are not conserved between yeast and a multicellular organism more than expected by chance, genetic interaction screens in *S. cerevisiae* are nonetheless informative for understanding multicellular biology. Clustering yeast genes according to their profiles of genetic interactions is a very powerful method for defining their precise molecular functions (Wong *et al.*, 2004). Thus, despite the lack of direct conservation of SL interactions between yeast and animals, SL screens in yeast are still highly informative for understanding general principles of how genes combine in non-additive modes. (Collins *et al.*, 2007; Pan *et al.*, 2006; Schuldiner *et al.*, 2005; Tong *et al.*, 2004; Wong *et al.*, 2004).

6.8. 'Induced essentiality' model for the interpretation of synthetic lethal interactions

Beyond the direct practical implications for the use of SL interaction data, my findings led me to suggest a novel model for the interpretation of SL interactions. In the classic model, SL interactions between two genes (gene A and B) are considered to be the result of inactivating two functionally redundant genes or pathways in which the genes act (reviewed in Guarente, 1993; Hartman *et al.*, 2001). However, I suggest that there is an alternative explanation for SL interactions, which I have termed 'induced essentiality'. In this view, the phenomenon of synthetic lethality is considered a side-effect of the evolution of adaptive responses to different environmental conditions. In my proposed model, loss of gene A induces the genetic network to rearrange so the organism's viability is maintained. In this novel network, gene B becomes indispensable. Thus, inactivating both gene A and gene B results in synthetic lethality, without gene A and gene B being functionally redundant. I believe that my finding that SL interactions are not conserved favours the 'induced essentiality' model.

6.9. Conclusion

In summary, during my PhD training, I have established robust methods (‘combinatorial RNAi’) to study genetic interactions between any pair of genes in *C. elegans*. I used this technique to provide the first extensive systematic analysis in any organism of the potentially redundant functions of duplicated genes and found that redundancy between some *C. elegans* gene duplicates has been maintained for long evolutionary timescales. When investigating whether SL interactions are conserved between yeast and worms, I found that genetic interaction networks evolve much faster than both the functions of individual genes and protein-protein interaction networks.

I thus consider the current hypothesis that SL interactions represent functional redundant relationships to be very unlikely. Instead, I propose a novel model for the interpretation of SL interactions. In this view, SL interactions are suggested to represent a special form of conditional essentiality (‘induced essentiality’).