Chapter 5

Functional redundancy in genetic interaction networks

5.1. Introduction

While I have identified functional redundancy between some duplicate gene pairs, the majority of bigenic interactions that were uncovered when systematically mapping synthetic sick and synthetic lethal (SL) interactions in *S. cerevisiae* do not occur between gene duplicates, but rather between genes unrelated at the sequence level.

To date, however, 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 (discussed in Wagner, 2005). Since similar types of non-additive interactions between mutations might underlie multifactorial genetic disease in humans, it is a major open question in genetics whether these individual genetic interactions are conserved between species and thus may be directly predicted in humans using interactions identified in simple model organisms.

I therefore wished to shed light on the evolution of gene networks. To do so, I sought to investigate whether genetic interactions are conserved between the yeast *S. cerevisiae* and the nematode *C. elegans*. Using RNA interference (RNAi) in *C. elegans*, I set out to explore whether individual SL interactions uncovered in yeast are conserved in the worm. Importantly, to date, *C. elegans* is the main animal model in which to carry out systematic functional studies *in vivo* in the context of a developing organism. This study thus allows me to directly compare genetic interaction networks that have been compiled *in vivo* in yeast with *in vivo* genetic interaction networks in the worm.

5.2. Investigating the conservation of synthetic lethal interactions between *S. cerevisiae* and *C. elegans*

I based my study on three *S. cerevisiae* datasets that were compiled using three different technological approaches for the systematic identification of SL interactions: first, a ‘global’ genetic interaction network encompassing ~ 4,000 SL interactions, mapped by using synthetic genetic array (SGA) technology to interrogate synthetic lethality predominantly between deletion alleles of non-essential genes (Tong *et al.*, 2004); second, an essential gene network comprising 567 interactions, including

conditional alleles for almost 300 yeast essential genes, that has been compiled by using SGA analysis (Davierwala *et al.*, 2005); and third, a genome-wide analysis of DNA integrity, providing a network of almost 5,000 SL interactions, which were mapped by employing diploid synthetic lethal analysis by microarray (dSLAM) using the *S. cerevisiae* heterozygous gene deletion collection (Pan *et al.*, 2006). Together, these three screens tested ~850,000 pairwise interactions, covering ~5% of the possible bigenic interaction space, and identified ~9,000 unique interactions including both interactions between null alleles and between hypomorphic mutants. Thus, examining the conservation of these large, diverse and systematically mapped datasets of SL interactions allows me to make firm conclusions about the conservation of genetic interactions between species.

To investigate whether SL interactions are conserved between yeast and worm, I set out to test whether I can detect SL interactions between pairs of *C. elegans* genes that are orthologous to pairs of genes that have been identified as having SL interactions in at least one of these three large-scale screens in *S. cerevisiae*. The INPARANOID algorithm was used to identify *C. elegans* orthologues of *S. cerevisiae* gene pairs (Remm *et al.*, 2001). Considering genetic interactions between yeast gene pairs for which both genes had a single orthologue in *C. elegans* only, 1,148 worm gene pairs were identified.

5.2.1. Using combinatorial RNAi to test whether synthetic lethal interactions are conserved between yeast and worm

Of 1,148 *C. elegans* gene pairs that were orthologous to gene pairs that were reported as having SL interactions in *S. cerevisiae*, 856 gene pairs could be targeted by combinatorial RNAi in the worm using the genome-wide feeding library (Kamath *et al.*, 2003). These gene pairs are listed in Appendix Table 5.1. For each gene pair amenable to analysis by combinatorial RNAi, I compared the phenotype resulting from simultaneously targeting both genes with the phenotypes resulting from targeting each gene individually side by side (as described in Chapter 3 and Materials and Methods; see Figure 3.6). All screens were performed at least twice independently, using the high-

throughput (HTP) RNAi liquid-feeding assay and the RNAi-hypersensitive *rrf-3* background.

First, worms were scored for SL phenotypes in a purely qualitative way. This was done for reasons of throughput. At that stage, thirteen gene pairs had to be excluded from the screen for SL interactions, because RNAi against individual genes resulted in worms that arrested growth at a late larval stage, a phenotype that cannot be enhanced any further. Thus, in total, I was able to screen 843 gene pairs that are orthologous to yeast SL interactions, for synthetic viability defects by using combinatorial RNAi in *C. elegans* (Table 5.1.). These 843 interactions are equivalent to 692 interactions in *S. cerevisiae* between two null alleles, 67 interactions between a hypomorph and a null allele, and 84 interactions between two hypomorphs.

For six out of 843 gene pairs that could be investigated for SL interactions in the worm, phenotypes generated by combinatorial RNAi are qualitatively stronger than the contributions of both individual RNAi phenotypes (Table 5.2.). In four cases, simultaneous targeting of both genes appeared to result either in reduced brood sizes or in reduced embryonic survival rates. RNAi phenotypes for these gene pairs were quantified by manually counting larvae, unhatched eggs, and adults within each experiment and subject to statistical analysis under a multiplicative model, using a Student's t-test (two-tailed distribution, two-sample equal variance; see 'Generating known synthetic lethal phenotypes by combinatorial RNAi' and Materials and Methods for a detailed description) to confirm all four gene pairs as SL interactions (Table 5.3.a and Figure 5.1.a and 5.1.b).

Combinatorial RNAi against two gene pairs (the gene pairs *lis-1* and *cap-1*, and Y6B3A.1 and *tfg-1*) resulted in pronounced synthetic adult lethal phenotypes (Table 5.2., Table 5.3.b and Figure 5.1.c and 5.1.d). Intriguingly, *lis-1* encodes an orthologue of human LIS1, which leads to lissencephaly, a disorder of neural development, when mutated (Online Mendelian Inheritance in Man, OMIM^{TM}; www.ncbi.nlm.nih.gov/omim/). Thus, it would be interesting to investigate whether patients with a severe clinical phenotype also carry a mutant allele of the human orthologue of *C. elegans cap-1*.

Yeast SL dataset	**SL interactions tested in *C. elegans* using combinatorial RNAi**	**SL interactions tested in *C. elegans* using genetic mutants + RNAi**
Tong *et al.*, 2004	**370**[a]	**88**[b]
Null + null	319[a]	71[b]
Hypomorph + null	51	17
Davierwala *et al.*, 2005	**100**	**7**
Null + hypomorph	16	-
Hypomorph + hypomorph	84	7
Pan *et al.*, 2006	**416**[a]	**88**[b]
Null + null	416[a]	88[b]
Total	**843**[a]	**174**[b]

Table 5.1. Overview of synthetic lethal interactions that were tested between *S. cerevisiae* and *C. elegans*
The three yeast data sets my study was based on, the number of interactions between pairs of *C. elegans* genes that are orthologous to pairs of genes identified as having synthetic lethal (SL) interactions in yeast that could be tested by combinatorial RNA interference (RNAi) and by RNAi in a worm strain homozygous for a loss-of-function genetic mutation in a second gene, respectively, and whether these correspond to null or hypomorphic alleles, are shown.
[a] 43 interactions are redundant between the Tong *et al*. and Pan *et al*. datasets.
[b] 9 interactions are redundant between the Tong *et al*. and Pan *et al*. datasets.

Yeast SL dataset	**SL interactions tested in *C. elegans* using combinatorial RNAi**	**Conserved interactions**	**SL phenotype**
Tong *et al.*, 2004	370	*lis-1* + *cap-1* *pfd-6* + C05D11.3	AL BS
Davierwala *et al.*, 2005	100	Y6B3A.1 + *tfg-1*	AL
Pan *et al.*, 2003	416	*rfp-1* + *rack-1* *rfp-1* + *htz1* *rfp-1* + *gfl-1*	BS BS, ES BS, ES
Total	843[a]	6	

Table 5.2. Synthetic lethal interactions are not conserved between *S. cerevisiae* and *C. elegans*

C. elegans gene pairs that were synthetic lethal (SL) in *S. cerevisiae* and that were identified to be SL by combinatorial RNAi are shown ('Conserved interactions'). SL phenotypes are classified as adult lethal ('AL'), reduced brood size ('BS') and reduced embryonic survival ('ES'), respectively.

a)

Interacting gene pairs	Gene1		Gene2		Gene1 & 2		p-value	
	BS	ES	BS	ES	BS	ES	BS	ES
pfd-6 + C05D11.3	49	75	66	72	15	48	6.22E-05	1.04E-01
rfp-1 + *rack-1*	100	90	76	100	28	91	3.81E-19	8.38E-01
rfp-1 + *gfl-1*	96	85	88	98	44	60	1.07E-07	1.51E-05
rfp-1 + *htz-1*	97	94	98	100	60	76	2.30E-10	6.70E-07

b)

Interacting gene pairs	Gene1	Gene2	Gene1 & 2	p-value
	Wt	Wt	Wt	Wt
lis-1 + *cap-1*	100	100	48	6.64E-17
Y6B3A.1 + *tfg-1*	99	95	38	3.93E-18

Table 5.3. Quantitative analysis of synthetic lethal interactions that are conserved between *S. cerevisiae* and *C. elegans*

Synthetic lethal phenotypes in *C. elegans* were verified by quantification and statistical analysis under a multiplicative model (Phillips *et al.*, 2000; Puniyani *et al.*, 2004); percentages of average wild-type brood size ('BS') and embryonic survival ('ES') rate (a), and fractions of animals that appeared wild-type ('Wt'; b) after RNA interference (RNAi) against each gene individually ('Gene1', 'Gene2') and combinatorial RNAi against both genes simultaneously ('Gene1 & 2') are shown. Values presented are the arithmetic mean of two independent experiments performed in the RNAi-hypersensitive *rrf-3* background. A Student's t-test was used to assess the statistical significance of quantitative phenotype data.

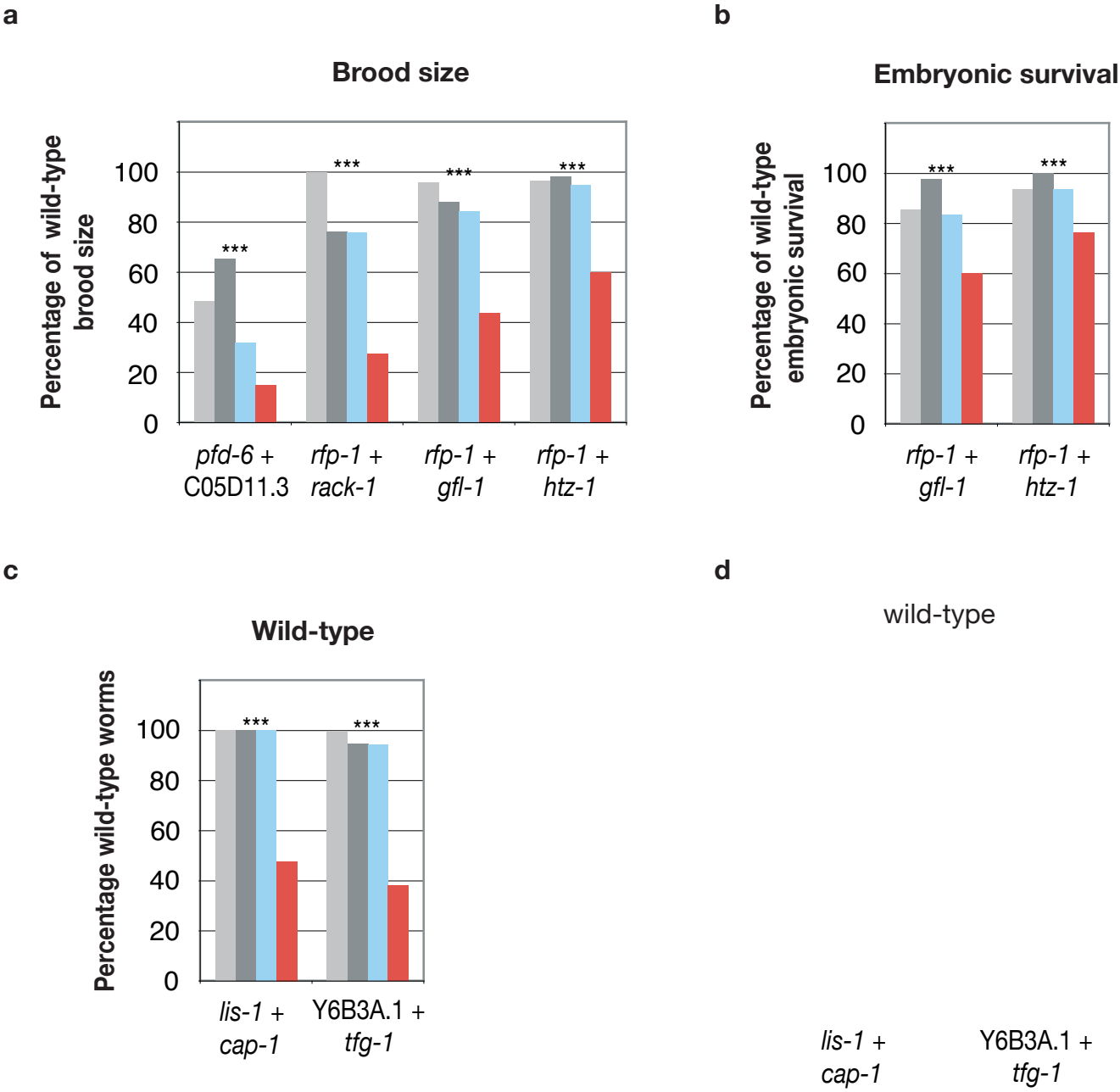

Figure 5.1. Synthetic lethal interactions that are conserved between *S. cerevisiae* and *C. elegans*

For each gene pair that yielded reproducible synthetic effects by combinatorial RNA interference (RNAi), phenotypes were quantified: brood size (a), embryonic survival rates (b), and percentage of wild-type worms (c), resulting from targeting each gene individually (light- and dark-grey bars) were compared with that generated by targeting both genes of a pair simultaneously (red bars) and with the calculated product of the single gene measurements (blue bars). Values plotted in (a) and (b) represent percentages of typical wild-type brood sizes, and embryonic survival rates, respectively. Data shown are the arithmetic mean of two independent experiments. Synthetic lethality was assessed under a multiplicative model (Phillips *et al*., 2000; Puniyani *et al.,* 2004). ***, P < 1.0E-03; Student's t-test. Representative images of synthetic adult lethal phenotypes resulting from combinatorial RNAi against *lis-1* and *cap-1*, and Y6B3A.1 and *tfg-1*, respectively (d). Scale bars: 0.1 mm. All experiments were performed in the RNAi-hypersensitive strain *rrf-3*.

5.2.2. Using RNAi in genetic mutants to test whether synthetic lethal interactions are conserved between yeast and worm

In addition to screening 843 pairwise orthologues of *S. cerevisiae* SL interactions by combinatorial RNAi for synthetic lethality in the worm, I sought to further test all possible gene pairs for synthetic lethality 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. We have previously used this approach in our laboratory for systematically investigating ~65,000 gene pairs with functions in signal transduction and transcriptional regulation for their ability to genetically interact (Lehner *et al.*, 2006). I was able to analyse 174 gene pairs for synthetic viability defects by using RNAi in 35 *C. elegans* strains carrying defined homozygous genetic mutations; this was the entire set for which a viable mutant strain was publicly available (see Appendix Table 5.2.).

I therefore compared RNAi phenotypes observed in the genetic mutants with the RNAi phenotypes of wild-type worms and with the phenotypes of the genetic mutants fed on bacteria expressing a dsRNA that does not target an expressed portion of the *C. elegans* genome (Ahringer library clone Y95B8A_84.g; see Figure 5.2.) side by side. Worms were screened for SL phenotypes in duplicates in at least two independent experimental setups, using the HTP RNAi liquid-feeding assay.

In few cases, brood size and embryonic survival rates, respectively, after RNAi in a genetic mutant appeared reduced as compared to RNAi against these genes in wild-type worms (Table 5.4.). These homozygous mutant strains, however, had reduced brood sizes and embryonic survival rates, respectively, on their own. I therefore sought to investigate whether the enhanced phenotypes that I observed when targeting a second gene by RNAi in these genetic mutants were the results of true synthetic lethality or rather caused by purely non-specific additive effects of the phenotypes of mutant strains and RNAi phenotypes. To examine this, I fed genetic mutants on additional RNAi clones that produced RNAi phenotypes similar to the phenotype of the putatively interacting gene in wild-type worms. I observed severely enhanced phenotypes after RNAi against all control genes in these four mutant strains carrying defined lesions. I thus considered these putative genetic interactions to be the results of non-specific additive effects between the

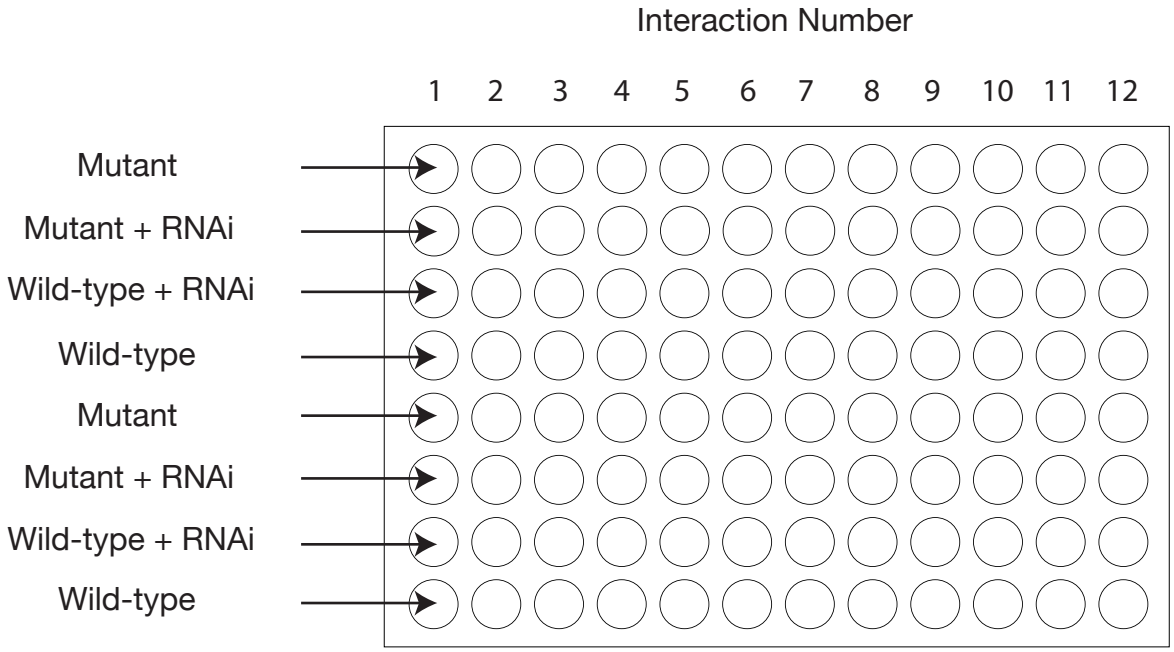

Figure 5.2. Overview of the setup for genetic interaction screens using RNA interference in a genetic mutant
When screening for genetic interactions by targeting one gene by RNA interference (RNAi) in a *C. elegans* strain carrying a homozygous loss-of-function allele of a second gene, RNAi phenotypes of the genetic mutants were compared to the RNAi phenotypes of wild-type worms and to the phenotypes of genetic mutants and wild-type worms, respectively, fed on non-targeting double-stranded RNA-expressing bacteria side by side. By using this setup, genetic interaction screens were performed in duplicates within independent experiments.

C. elegans strain	RNAi clone
TJ1049	C43E11.9
DS77	C39E9.13
CX51	H20J04.d R151.9 T06G6.9 F21C3.5
RB1457	R05D3.4 B0205.3

Table 5.4. *C. elegans* strains with non-specifically enhanced RNA interference phenotypes

C. elegans strains carrying a defined genetic lesion that showed non-specifically enhanced phenotypes when targeted by RNAi clones (represented by Ahringer library RNA interference (RNAi) clone gene pairs names) are shown.

phenotypes of the genetic mutant and the RNAi phenotypes and do not represent informative SL interactions. The logic behind excluding these interactions is analogous to excluding physical interactions between 'sticky' proteins.

5.3. Synthetic lethal interactions are not conserved between *S. cerevisiae* and *C. elegans*

Taken together, I have investigated 843 pairwise orthologues of genes that were identified as having SL interactions in *S. cerevisiae* for synthetic viability defects in *C. elegans* by combinatorial RNAi and a further 174 pairs by single-gene RNAi in worm strains carrying defined homozygous genetic mutations. Strikingly, I only identified 6 gene pairs (0.7%) to show synthetic lethal phenotypes when targeted by combinatorial RNAi in the worm (Table 5.2. and Figure 5.1.). This observed degree of conservation between SL interactions in *S. cerevisiae* and *C. elegans* is not significantly different to the frequency of SL interactions that we have detected in a systematic large-scale study in *C. elegans*: screening for synthetic synthetic lethality between genes with roles in signaling and transcriptional regulation, we found on average 0.6% of tested gene pairs to genetically interact in the worm (Lehner *et al.*, 2006; $\chi^2 = 0.201$, $P = 0.6538$, 1 degree of freedom). These data thus imply that individual SL interactions are not conserved between *S. cerevisiae* and *C. elegans* more than is expected by chance.

I do not see any functional similarities in the small set of genes that interact that distinguish them from the non-conserved interactions (Table 5.5.), nor is there any correlation between which yeast study the SL interaction derived from and whether it is also found in *C. elegans* (Table 5.2.). Therefore, neither interactions between null alleles nor interactions between hypomorphs appear conserved between these two species.

Moreover, the frequency of SL interactions that can be detected in yeast and worm is very similar (Lehner *et al.*, 2006), hence the non-conservation cannot simply be explained by a reduction in the number of SL interactions. Neither can it be explained by increased functional redundancy as a result of gene duplication in the worm, because I only tested for genetic interactions between gene pairs for which both genes had a single orthologue in *C. elegans*.

S. cerevisiae	***C. elegans***	**Gene1**	**Gene2**
PAC1 + CAP1	*lis-1* + *cap-1*	Orthologue of human lissencephaly gene[a] with functions in spindle organization and biogenesis[b]	F-actin capping protein, alpha subunit[c]
SEC7 + LAS17	Y6B3A.1 + *tfg-1*	ADP ribosylation factor (ARF) guanine nucleotide exchange factor[c]	Human TFG related[d], putative apoptotic suppressor in *C. elegans*[e]
YKE2 + PLP1	*pfd-6* + C05D11.3	Prefoldin subunit 6, KE2 family[c]	Essential for proper microtubule organization and function[f]
BRE1 + ASC1	*rfp-1* + *rack-1*	E3 ubiquitin ligase required for H2B ubiquitination[c]	Homolog of mammalian RACK1 (Receptor of Activated C Kinase)[b]
BRE1 + HTZ1	*rfp-1* + *htz-1*	E3 ubiquitin ligase required for H2B ubiquitination[c]	Histone variant H2AZ homolog[d]
BRE1 + YAF9	*rfp-1* + *gfl-1*	E3 ubiquitin ligase required for H2B ubiquitination[c]	Transcription initiation factor IIF, auxiliary subunit[c]

Table 5.5. Molecular functions of *C. elegans* gene pairs with synthetic lethal RNA interference phenotypes

Molecular roles of synthetic lethal gene pairs that are conserved between *S. cerevisiae* and *C. elegans* are shown. *C. elegans* genes ('Gene1', 'Gene2') are represented by their [a] WormBase descriptions (www.wormbase.org); [b] Gene Ontology descriptions (Ashburner *et al.*, 2000); [c] NCBI eukaryotic orthologous groups (Koonin *et al.*, , 2004); [d] WormBase ID (www.wormbase.org); [e] functions as described in Chen *et al.*, 2004; [f] functions as described in Ogawa *et al.*, 2004.

Strikingly, the lack of conservation of non-additive, synthetic genetic interactions between yeast and worms is in stark contrast to the conservation of single gene functions. Using an identical assay, I found 61% of *C. elegans* genes that are orthologous to an essential gene in *S. cerevisiae* to have a non-viable RNAi phenotype (see 'Transferring gene functions between *S. cerevisiae* and *C. elegans*'). Furthermore, 31% of tested protein interactions were found to be conserved between *S. cerevisiae* and *C. elegans* (Matthews *et al.*, 2001). Thus, although the functions of individual genes and the physical interactions between gene products are well conserved between yeast and worms, non-additive, synthetic genetic interactions are not. Consequently, SL interactions identified in *S. cerevisiae* cannot be used to directly predict genetic interactions in the nematode *C. elegans* and thus are very unlikely to be predictive of genetic interactions in humans.

5.4. 'Induced essentiality' as alternative model for the interpretation of synthetic lethal interactions

However, beyond the direct practical implications for using *S. cerevisiae* data to predict SL interactions in humans, I believe these results may also have important implications for the mechanistic interpretation of SL interactions. The classic interpretation of a SL interaction between two genes (gene A and B) is that the genes (or the pathways in which they act) are at least partially functionally redundant (Kelley and Ideker, 2005; reviewed in Hartman *et al.*, 2001). In this model, loss of one gene has little effect since the alternative, redundant pathway can compensate for this loss (see Figure 1.5.). This situation is most apparent for recently duplicated genes that, by their nature, are highly redundant: in some cases the loss of one duplicate has little effect on fitness, but loss of both duplicates together is catastrophic (Ihmels *et al.*, 2007; Tischler *et al.*, 2006). Most SL interactions do not take place between duplicated genes, however, but between genes that do not share sequence similarity (Tong *et al.*, 2004).

Considering the classic model for the interpretation of SL interactions, in which synthetic lethality is considered a consequence of inactivating two functionally redundant genes or pathways, one might expect SL interactions to be conserved if individual gene functions are conserved. My findings, however, do not support this theory, but rather led

me to suggest an alternative model to explain SL interactions. I consider that genetic networks that underlie viability are not constant but flexible to change under different environmental conditions. This flexibility allows biological systems to adopt a range of alternative viable states, each with their set of essential genes. Thus, a gene that is non-essential under normal laboratory growth conditions may be absolutely critical for survival in a different environmental condition. Increasing experimental evidence supports this notion (Chang *et al.*, 2002; Davis-Kaplan *et al.*, 2004; Dudley *et al.*, 2005; Enyenihi and Saunders, 2003; Kuepfer *et al.*, 2005; Martinez *et al.*, 2004; Smith *et al.*, 2006). I propose that loss of gene A results in a rearrangement of the genetic network into an alternative viable state, where gene B is now an essential gene (Figure 5.3.). In this view, gene B is required under a condition caused by the loss of gene A. The functions of gene A and gene B, or the pathways in which they act, are not redundant or related — gene B is simply required under a condition caused by loss of gene A. In this model — which I have termed 'induced essentiality' — I consider SL interactions to represent a special form of conditional lethality, where loss of gene A partly mimics the response of the cell to an environmental condition. In light of this hypothesis, SL interactions are highly unlikely to be conserved — the range of environments that yeast cells need to respond to are very different to those that affect cells in an intact animal. Furthermore, regulatory networks governing cellular responses evolve very rapidly (Maslov *et al.*, 2004; Odom *et al.*, 2007). Thus, while in the classical model, SL interactions are interpreted as the consequence of inactivating functionally redundant genes or pathways, I propose SL interactions to be the consequence of the ability of genetic networks to rearrange into alternative viable states driven by the evolution of adaptive responses to environmental changes.

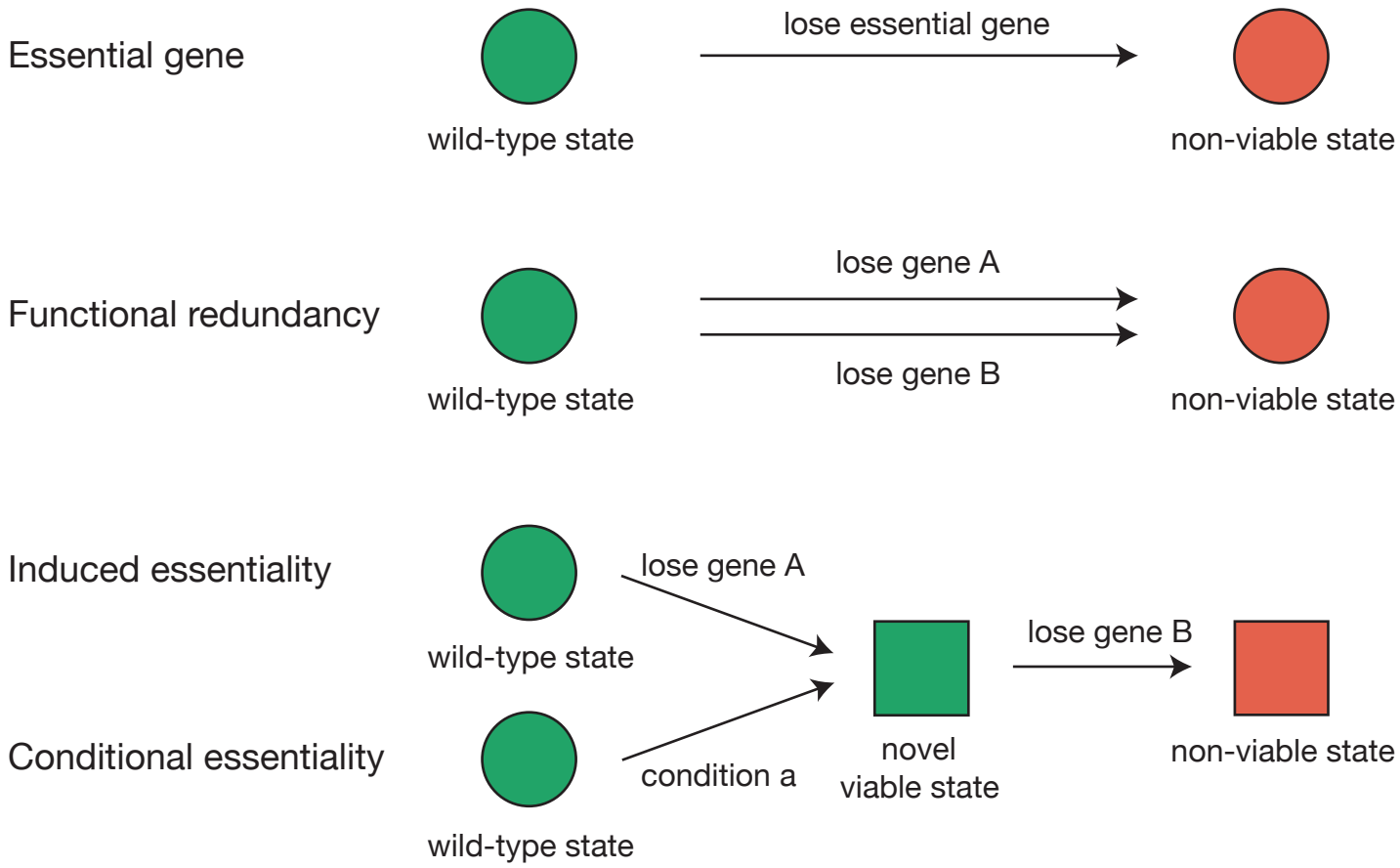

Figure 5.3. Two models for the interpretation of synthetic lethal interactions
In the classical model, SL interactions occur between two redundant genes or pathways ('Functional redundancy'; here gene A and gene B) that can compensate for the loss of one another. In the alternative model ('Induced essentiality'), loss of one gene (gene A) results in a rearrangement of the genetic network into a novel network; this rearrangement may mimic the response to an environmental condition. In this new network, the other gene (gene B) is now an essential gene. The rapid evolution of synthetic lethal interactions compared with individual gene functions favours this second model.

5.5. Investigating higher-order similarities in synthetic lethal interactions between *S. cerevisiae* and *C. elegans*

Having found that SL interactions are not conserved between *S. cerevisiae* and *C. elegans* more than expected by chance despite the very high degree of conservation of individual gene functions, I considered the possibility that there might be higher-order similarities in SL interactions between yeast and animals. I hypothesized that although gene networks might not have been conserved between species at the level of individual SL interactions, they might have been conserved at a higher level, such as at the level of pathways or molecular machines. For example, while in yeast, gene A (e.g. a specific component of the DNA repair pathway) is SL with gene B (e.g. a gene with a role in DNA replication) in worm, another gene (other than the orthologue of yeast gene A) that functions in DNA repair might be SL with another component (other than the orthologue of yeast gene B) that is involved in the process of DNA repair.

In order to test this hypothesis, I set out to screen for genetic interactions in *C. elegans* within the same set of genes that have been screened for SL interactions in *S. cerevisiae* and *C. elegans*. To do so, I selected two query genes and screened for novel genetic interactions between these and all 1,046 single orthologues between *S. cerevisiae* and *C. elegans* that could be targeted by an RNAi clone in the Ahringer library (as discussed in Chapter 4). This approach allowed me to systematically investigate genetic interactions in the worm within the same set of genes that have been screened for SL interactions in yeast. I chose *lis-1*, the *C. elegans* orthologue of *S. cerevisiae PAC1*, which is encoding for a component of the dynein/dynactin pathway and *mdf-2*, orthologous to yeast *MAD2*, a gene encoding for a component of the spindle-assembly checkpoint complex, respectively, as query genes (Table 5.6.). Using our HTP liquid-feeding assay, I compared the phenotypes resulting from simultaneously targeting two genes by combinatorial RNAi with the RNAi phenotypes of both genes individually in the RNAi-hypersensitive strain *rrf-3* in duplicates within two independent experimental setups.

When screening for genetic interactions with *mdf-2* as query, I found combinatorial RNAi against *mdf-2* and *tbg-1*, a gene encoding for gamma-tubulin, to

S. cerevisiae	***C. elegans***	**Gene 1**	**Gene 2**
PAC1 + CAP2	*lis-1* + *cap-2*	Orthologue of human lissencephaly gene[a] with functions in spindle organization and biogenesis[b]	F-actin capping protein, beta subunit[c]
MAD2 + TUB4	*mdf-2* + *tbg-1*	Spindle assembly checkpoint protein[c]	Gamma tubulin[c]

Table 5.6. Molecular functions of novel synthetic lethal interactions between *C. elegans* orthologues of *S. cerevisiae* genes

Molecular roles of novel synthetic lethal gene pairs that were identified in *C. elegans* are shown. *C. elegans* genes ('Gene1', 'Gene2') are represented by their [a] WormBase descriptions (www.wormbase.org); [b] Gene Ontology descriptions (Ashburner *et al*., 2000); [c] NCBI eukaryotic orthologous groups (Koonin *et al*., 2004).

result in reduced brood and embryonic survival rates (Table 5.6., Table 5.7., and Figure 5.4.a and 5.4.b). Using *lis-1* as query gene, I identified both *cap-1* and *cap-2*, encoding for alpha- and beta-subunits, respectively, of the F-actin capping protein hetero-dimer, to result in synthetic adult lethal phenotypes with *lis-1* (Table 5.6., Table 5.7., and Figure 5.4.c and 5.4.d). While both *cap-1* and *cap-2* showed the same combinatorial RNAi phenotype with *lis-1*, combinatorial RNAi against *cap-1* and *cap-2* did not result in an enhanced phenotype as compared to each single-gene RNAi phenotype alone (Figure 5.4.e). This finding supports the notion that SL interactions are mostly uncovered between components of different molecular pathways, rather than between genes functioning within the same pathways (Bader *et al.*, 2003; Kelley and Ideker, 2005; Ye *et al.*, 2005). While I had already verified the SL interaction between *C. elegans lis-1* and *cap-1*, I further confirmed the two novel SL interactions between worm genes *lis-1* and *cap-2*, and *mdf-2* and *tbg-1*, respectively, by quantification and statistical analysis under a multiplicative model (Table 5.7. and Figure 5.4.; as discussed in Chapter 3).

Thus, by systematically screening for SL interactions in *C. elegans* within the same set of genes that have been screened for SL interactions in *S. cerevisiae*, I uncovered two novel genetic interactions and confirmed one previously identified SL interaction in *C. elegans* by using combinatorial RNAi. While the yeast genes *PAC1* and *CAP2*, which are orthologous to the worm genes *lis-1* and *cap-2*, might not have been assayed for synthetic lethality in the large-scale screen by Tong *et al.* (2004), in which the yeast genes *PAC1* and *CAP1* were identified as an SL pair, this novel interaction in *C. elegans* might be explained functionally, considering that *cap-1* and *cap-2* encode for alpha- and beta-subunits, respectively, of a capping protein heterodimer (Table 5.6.).

Intriguingly, when searching BIOGRID, a database for all compiled interaction data for model organisms (Stark *et al.*, 2006), I found *DYN2*, a gene encoding for a microtubule motor protein, to be amongst the reported SL interaction partners of the *S. cerevisiae* gene *CAP2*. Both *PAC1* — the yeast orthologue of *C. elegans lis-1*, which I identified as being SL with both *cap-1* and *cap-2* — and *DYN2* are encoding for components of the dynein pathway. These data support the hypothesis that even though individual SL interactions are not conserved between *S. cerevisiae* and *C. elegans* more

a)

Interacting gene pair	Gene1		Gene2		Gene1 & 2		p-value	
	BS	ES	BS	ES	BS	ES	BS	ES
mdf-2 + tbg-1	77	95	30	38	12	11	2.23E-03	1.82E-03

b)

Interacting gene pair	Gene1	Gene2	Gene1 & 2	p-value
	Wt	Wt	Wt	Wt
lis-1 + cap-2	100	100	25	4.42E-20

Table 5.7. Quantitative analysis of novel synthetic lethal interactions between *C. elegans* orthologues of *S. cerevisiae* genes

Synthetic lethal phenotypes that were identified between *C. elegans* orthologues of *S. cerevisiae* genes were quantified and subject to statistical analysis under a multiplicative model (Phillips *et al.*, 2000; Puniyani *et al.*, 2004). Percentages of average wild-type brood size ('BS'), and embryonic survival ('ES') rates, respectively (a) and fractions of worms with a wild-type ('Wt') phenotype (b) after simultaneously targeting both genes of a pair by combinatorial RNA interference (RNAi) ('Gene1 & 2') and after RNAi against each gene alone ('Gene1', 'Gene2') are shown. Numbers listed are the arithmetic mean of two independent biological repeats performed in the RNAi-hypersensitive strain *rrf-3*. p-values were assigned by comparing measurements obtained after combinatorial RNAi with the calculated product of measurements for both individual genes using a Student's t-test.

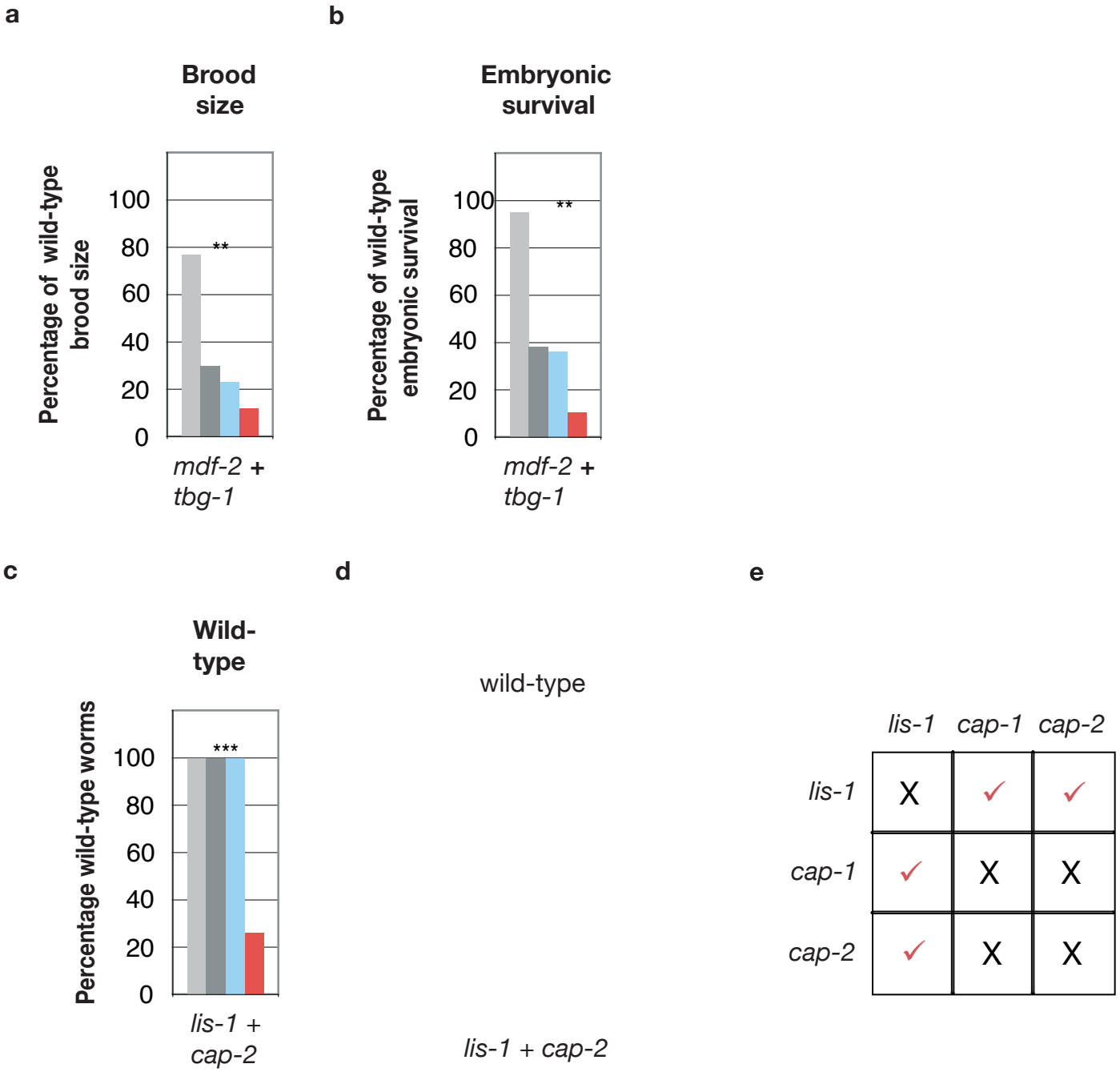

Figure 5.4. Identification of novel synthetic lethal interactions between *C. elegans* orthologues of *S. cerevisiae* genes

See next page for Figure legend.

Figure 5.4. Identification of novel synthetic lethal interactions between *C. elegans* orthologues of *S. cerevisiae* genes

Brood size (a) and embryonic survival rates (b) after combinatorial RNA interference (RNAi) against *mdf-2* and *tbg-1* are represented as percentages of typical wild-type measurements. Fraction of wild-type worms after combinatorial RNAi against *lis-1* and *cap-2* are shown (c). Brood size, embryonic survival, and percentages of wild-type worms, respectively, were compared after targeting both genes individually (light- and dark- grey bars), with the values after targeting both genes simultaneously (red bars) and the calculated product of both single-gene measurements (blue bars). Values plotted are the arithmetic mean of two independent experiments. Statistical significance of quantitative phenotype data was assessed under a multiplicative model (Phillips *et al.*, 2000; Puniyani *et al.*, 2004), using the Student's t-test. ***, $P < 1.0E-03$; **, $P < 1.0E-02$. Representation of synthetic adult lethal phenotype generated by the simultaneous targeting of *C. elegans lis-1* and *cap-2* by combinatorial RNAi (d). Scale bars: 0.1 mm. Schematic showing the results of targeting all pairwise combinations of *C. elegans* genes *lis-1*, *cap-1*, and *cap-2* by RNAi (e). ✓ represents a synthetic lethal phenotype. X denotes that combinatorial RNAi phenotypes did not deviate from single-gene RNAi phenotypes. All screens for genetic interactions were performed in the RNAi-hypersensitive *rrf-3* background.

than expected by chance, there might be higher-order similarities in SL interactions between yeast and worm.

Interestingly, a recent study by Cuschieri *et al.* (2006) had uncovered the yeast gene *MAD2* to be synthetic lethal with *TUB4*. These genes are orthologous to *C. elegans mdf-2* and *tbg-1*, respectively, which I had identified as SL gene pair in the worm. Thus, even though on a global scale, SL interactions are not conserved between *S. cerevisiae* and *C. elegans*, isolated individual functional interactions may be conserved throughout evolution.

In summary, while I had found SL interactions to be poorly conserved between *S. cerevisiae* and *C. elegans*, I identified two novel genetic interactions in the worm when screening for SL interactions within *C. elegans* orthologues of *S. cerevisiae* using two genes with roles in mitosis as query genes. One of these, the interaction between *lis-1* and *cap-2* might be similar in function to the genetic interaction between *lis-1* and *cap-1*, which I found to be conserved between yeast and worm. The other one, an interaction between orthologues of the worm genes *mdf-2* and *tbg-1* has lately also been revealed in a small-scale study in the yeast *S. cerevisiae*.

Therefore, extending the search for SL interactions in *C. elegans* between the same set of genes that have been investigated for SL interactions in *S. cerevisiae* to a larger scale might provide further insights into the complexity and global organization of SL interactions in different species.

5.6. Comparison of literature-curated genetic interaction data

Having found that SL interactions between *S. cerevisiae* and *C. elegans* are not conserved more than expected by chance by using a directed systematic approach, I wished to supplement my experimental data with data compiled from literature. I therefore set out to compare all previously known SL data between *S. cerevisiae* and *C. elegans*. To do so, I extracted all 9,175 unique yeast SL interactions from BIOGRID, a database storing genetic interaction data for model organisms (Stark *et al.*, 2006), and all 1,006 known genetic interactions in *C. elegans* from WormBase (www.wormbase.org).

Using the INPARANOID algorithm, I identified single *C. elegans* orthologues for *S. cerevisiae* genes. I found 1,293 pairwise worm orthologues to correlate with yeast SL interactions. None of these gene pairs, however, had been previously shown to genetically interact in *C. elegans*, supporting my experimental data (Table 5.8.).

I further extended this analysis to also include literature data available on genetic interactions in *D. melanogaster* (Crosby *et al.*, 2007). Of 1,575 fly gene pairs that I identified as being orthologous to yeast SL interactions, 3 gene pairs had previously been found to also genetically interact in *D. melanogaster* (see Appendix Table 5.3.).

While I found no overlap between previously known SL interactions in yeast and worm, I next investigated whether I can find an overlap of known genetic interactions between worm and fly. I identified 212 pairwise *D. melanogaster* orthologues corresponding to previously known genetic interactions in *C. elegans*; of these, 23 were reported to also genetically interact in *D. melanogaster* (see Appendix Table 5.3.). However, it has to be noted that the nature of genetic interactions is not specified in the compiled datasets of known genetic interactions both for *C. elegans* and *D. melanogaster*. Hence, these datasets might contain both additive and non-additive genetic interactions. Therefore, the observed higher degree of overlap of genetic interactions between *C. elegans* and *D. melanogaster* than between *S. cerevisiae* and *C. elegans* might — at least in part — be explained by additive genetic interactions.

Moreover, I have to consider likely ascertainment biases in the genes that have been investigated for genetic interactions in either species when comparing literature-curated data that have not been compiled in a comprehensive way. While enormous efforts have been made to map SL interactions on a genome-wide scale in *S. cerevisiae*, SL screens have not yet been extended to genome-scale studies in other model systems. In our laboratory, we have provided the first systematic large-scale analysis of genetic interactions in *C. elegans*. We investigated ~65,000 gene pairs with functions in the signaling and transcriptional networks that regulate development for their ability to genetically interact. Focused small-scale genetic interaction screens have also been performed to gain further insights into DNA repair and posterior patterning in *C. elegans* (Baugh *et al.*, 2005; van Haaften *et al.*, 2004).

S. cerevisiae SL	*C. elegans* orthologous gene pairs	*C. elegans* genetic interactions
9,175	1,293	0

S. cerevisiae SL	*D. melanogaster* orthologous gene pairs	*D. melanogaster* genetic interactions
9,175	1,575	3

C. elegans genetic interactions	*D. melanogaster* orthologous gene pairs	*D. melanogaster* genetic interactions
1,006	212	23

Table 5.8. Comparison of genetic interaction data compiled from literature
Literature-curated synthetic lethal interactions ('SL') from *S. cerevisiae* and previously known genetic interactions between orthologous gene pairs in *C. elegans* and *D. melanogaster* were compared. Numbers for genetic interactions, their respective orthologous gene pairs in another species, and the overlap of genetic interactions are shown.

Thus, while genetic interactions have been mapped on a global scale in yeast, genetic interactions in more complex organisms have — with few exceptions — mostly been compiled on a case-by-case basis. Consequently, even though this comparative study supports my finding that SL interactions between *S. cerevisiae* and *C. elegans* are not conserved more than expected by chance, I cannot make firm conclusions about the considerable degree of conservation of genetic interactions between *C. elegans* and *D. melanogaster*.

5.7. Conclusion

In summary, in this chapter, I have addressed a major open question in genetics: 'Are synthetic lethal interactions evolutionarily conserved?' I set out to investigate whether SL interactions that have recently been mapped on a genome-wide scale in the yeast *S. cerevisiae* are conserved in the nematode *C. elegans*. To do so, I examined 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 at least one of three large-scale screens in *S. cerevisiae*. In total, I investigated 843 pairs of *C. elegans* genes for 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, I found that SL interactions are not conserved despite a high degree of conservation of individual gene functions and protein interactions. These results, however, do not rule out the possibility that there might be higher-order similarities in SL interactions between *S. cerevisiae* and *C. elegans*. To test this hypothesis, I extended my search for genetic interactions to all *C. elegans* orthologues of *S. cerevisiae* genes and identified two novel interactions in the worm. I believe that a systematic large-scale analysis of the same set of genes that had been tested for SL interactions in yeast for their ability to genetically interact in the worm will provide deeper insights into the structure and general properties of complex genetic interaction networks.

Together, my findings imply that SL interactions are unlikely to be explained by simple models of redundancy and led me to propose a novel model to explain SL interactions. In this view, synthetic lethality represents a special form of conditional essentiality (‘induced essentiality’). In conclusion, I suggest a substantial evolutionary plasticity in genetic interaction networks.