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Note

Transcriptional Compensation for Gene Loss Plays a Minor Role in Maintaining Genetic Robustness in *Saccharomyces cerevisiae*

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ABSTRACT

If a gene is mutated and its function lost, are compensatory genes upregulated? We investigated whether genes are transcriptionally upregulated when their synthetic sick or lethal (SSL) partners are lost. We identified several new examples; however, remarkably few SSL pairs exhibited this phenomenon, suggesting that transcriptional compensation by SSL partners is a rare mechanism for maintaining genetic robustness.

THE ability to tolerate random mutation is critical to an organism's fitness (Kitano 2004). This tolerance often relies on genes or pathways that can compensate for the loss of one another (Tong *et al.* 2001, 2004). A key indicator of a compensatory relationship is a synthetic sick or lethal (SSL) interaction, in which mutation of two genes in combination yields a more deleterious phenotype than either single mutation alone.

The existence of genetic compensation is well accepted (Nowak *et al.* 1997; Winzeler *et al.* 1999; Wagner 2000; Tong *et al.* 2001, 2004; Gu *et al.* 2003), but the mechanism by which this compensation is achieved remains unclear. Are compensatory genes, expressed at wild-type levels, sufficient to buffer gene loss? Or does the cell detect loss of a gene and respond by upregulating compensatory genes/pathways (*i.e.*, the SSL interaction partners of the deleted gene)? Previously, Lesage *et al.* (2004) suggested that transcriptional compensation among SSL partners is rare. Now large mRNA expression and genetic interaction data sets offer an opportunity to address this question on a genome-wide level.

Investigating transcriptional compensation among SSL gene pairs: To investigate whether compensatory genes are transcriptionally upregulated in response to gene loss, we employed a large data set of *Saccharomyces cerevisiae* mRNA expression profiles of single-gene mutants (Winzeler *et al.* 1999; Hughes *et al.* 2000a) and a systematically generated data set of gene pairs assessed for SSL interaction (Tong *et al.* 2001, 2004). For each transcriptionally profiled mutant, we considered expression levels of genes previously assessed for SSL interaction with the mutated gene. For example, if the mutant of gene *M* was transcriptionally profiled and gene *M* was also assessed for SSL interaction with a gene *G*, we considered the expression level of *G* in the mutant of *M*. More specifically, for each *M:G* pair, we compared expression of *G* in mutant and wild-type cells using log ratios [log (expression in mutant/expression in wild type)]. We excluded profiles of known aneuploid strains (Hughes *et al.* 2000b) and took averages of log ratios in the cases of duplicate measurements (<10%). We then separately considered *M:G* pairs with (872) and without (112,686) SSL interaction. The distributions of log ratios for SSL and non-SSL pairs were similar (Figure 1). Normalizing the log ratios using a gene-specific error model (Hughes *et al.* 2000a) produced similar distributions (data not shown). These data suggest that transcriptional regulation of compensatory genes does not play a global role in maintaining robustness.

We next investigated whether transcriptional compensation played a greater role among SSL *M:G* pairs with homology in comparison to nonhomologous SSL pairs. Because homologous genes were more likely than nonhomologous genes to overlap in function, we hypothesized that they may exhibit more pronounced compensatory transcriptional effects. Our data did not support this hypothesis (see supplementary material at http://www.genetics.org/supplemental/).

Transcriptional compensation among a minority of gene pairs: Although transcriptional compensation for gene loss did not appear to be a global phenomenon,

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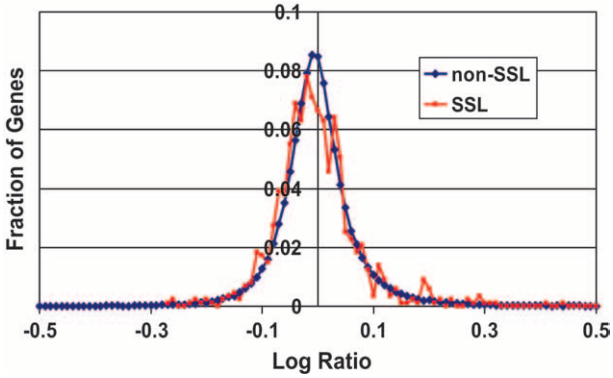

FIGURE 1.—Log ratios of expression for each gene, *G*, in a gene *M* mutant strain relative to wild type. *M:G* gene pairs with an SSL interaction are plotted separately from non-SSL pairs.

it has been previously observed for several SSL gene pairs (TERASHIMA *et al.* 2000; LESAGE *et al.* 2004; KAFRI *et al.* 2005). To systematically identify additional cases, we again considered *M:G* pairs previously assessed for SSL interaction. Among genes significantly changed in an *M* mutant (according to significance threshold $P < 0.001$), SSL partners of *M* were more likely than non-SSL partners to be transcriptionally upregulated ($P = 0.02$) (see supplementary material at http://www.genetics.org/supplemental/). Therefore, although transcriptional compensation is rare, our results confirm that it does occur for a minority of SSL pairs.

Six of the 13 SSL gene pairs that we found to exhibit transcriptional upregulation were previously noted (Table 1) (ZHAO *et al.* 1998; LESAGE *et al.* 2004), confirming our method of analysis. Five of these 6 were noted by LESAGE *et al.* (2004), who reported transcriptional upregulation for 10 of the SSL pairs that we investigated. That we observed compensation for only 5 of these 10 pairs may reflect experimental error or differences in mutant strains or growth conditions of the mRNA expression data sets.

For seven pairs, transcriptional upregulation in the context of SSL interaction had not been previously noted (Table 1). However, for the pair *CDC42:GIC2*, compensation at the protein expression level was previously observed; the level of *GIC2* protein increased in the *CDC42*-null mutant (JAQUENOUD *et al.* 1998). For the remaining six pairs, our observations may help define functional relationships between genetic interactors that exhibit compensatory transcriptional upregulation.

The SSL pair *FKS1:RIM21* exhibited transcriptional compensation. Little information on the relationship between the 1,3-β-D-glucan synthase *FKS1* and *RIM21*, a protein of unknown molecular function and cellular component, is available. Our observation of transcriptional compensation by *RIM21* in the *FKS1*-null mutant, in the context of genetic interaction, may help determine the function of *RIM21*.

Another example, *SHE4*, is the SSL partner of *CHS7*. According to the Saccharomyces Genome Database, *CHS7* is a protein of unknown function involved in chitin biosynthesis (DOLINSKI *et al.* 2004) and was transcriptionally upregulated in the *SHE4*-null mutant.

Surprisingly, one gene, *FKS2*, was downregulated in the deletion mutant of its SSL partner, *FKS1* (Table 1). This contrasts with the compensatory transcriptional upregulation seen previously (TERASHIMA *et al.* 2000; LESAGE *et al.* 2004) and may represent an experimental error (see discussion of cross-hybridization below) or differences in the *FKS1* mutants or conditions profiled (HUGHES *et al.* 2000a; LAGORCE *et al.* 2003).

In summary, we explored transcriptional upregulation as a mechanism of compensation for gene loss. While we confirmed a handful of previously observed cases and identified several new ones, our data suggest that transcriptional compensation among SSL partners is rare.

Our conclusion agrees with a qualitative observation from a smaller study, which considered SSL partners of three genes involved in β-1,3-glucan assembly and used a different expression data set (LAGORCE *et al.* 2003; LESAGE *et al.* 2004). By contrast, we addressed this question quantitatively and examined the SSL interaction partners of 18 query genes. Therefore, our conclusion has broader scope, encompassing additional biological processes (see supplementary Table S1 at http://www.genetics.org/supplemental/ for query genes examined).

We have likely missed some instances of upregulation of compensatory genes. For example, microarrays are not sensitive enough to detect all transcriptional changes. Furthermore, expression experiments were conducted in rich medium, while SSL interaction was assessed in near minimal medium, so pairs exhibiting compensatory upregulation in minimal but not rich media were missed. In addition, cross-hybridization between paralogous genes may mask cases of compensatory upregulation or may cause the appearance of compensatory downregulation (HUGHES *et al.* 2001). However, even using a permissive definition of homology (BLAST *E*-value $<10^{-3}$), only 2% of SSL gene pairs are paralogous (TONG *et al.* 2004), so this source of decreased sensitivity to compensatory upregulation is unlikely to have affected our overall conclusion. Finally, the SSL interaction data set has a false-negative rate of 17–41% (TONG *et al.* 2001, 2004), causing us to incorrectly consider some pairs as non-SSL. However, given the remarkably low frequency of compensatory upregulation observed here, increased sensitivity to SSL interaction is unlikely to change our overall conclusion.

Compensation by upregulation may be even rarer than we report here. In some of the 13 cases that we report, the observed upregulation may not be *required* to achieve compensation. Thus, we may have conservatively

TABLE 1

SSL *M:G* pairs in which expression of gene *G* is significantly changed in the null mutant of gene *M* ($P < 0.001$)

Gene *M* (the mutated gene)			Gene *G* (for which mRNA expression was measured)			Homology	Expression change	*P*-value(s)	Transcriptional compensation previously noted
Gene IDs	Protein	Description	Gene IDs	Protein	Description				
BNI1 YNL271C S000005215	Formin, involved in spindle orientation	Formin, nucleates the formation of linear actin filaments, involved in cell processes such as budding and mitotic spindle orientation, which require the formation of polarized actin cables, functionally redundant with BNR1.	SLT2 YHR030C S000001072		Suppressor of lyt2; serine/threonine MAP kinase.		Up	4.2E-4	
CDC42 YLR229C S000004219	Rho subfamily of Ras-like proteins	Small rho-like GTPase essential for establishment and maintenance of cell polarity; mutants have defects in the organization of actin and septins.	GIC2 YDR309C S000002717		Protein of unknown function involved in initiation of budding and cellular polarization; interacts with Cdc42p via the Cdc42/Rac-interactive binding domain.		Up	6.9E-5	
FKS1 YLR342W S000004334	1,3-β-D-glucan synthase	Catalytic subunit of 1,3-β-D-glucan synthase, functionally redundant with alternate catalytic subunit Gsc2p; binds to regulatory subunit Rho1p; involved in cell wall synthesis and maintenance; localizes to sites of cell wall remodeling.	FKS2 GSC2 YGR032W S000003264	1,3-β-D-glucan synthase catalytic component	Catalytic subunit of 1,3-β-D-glucan synthase with similarity to an alternate catalytic subunit, Fks1p (Gsc1p); Rho1p encodes the regulatory subunit; involved in cell wall synthesis and maintenance.	Yes	Down	2.1E-8	Tereshima *et al.* (2000) and Lesage *et al.* (2004) observed compensatory upregulation.
FKS1 YLR342W S000004334			KAI1 MID2 YLR332W S000004324		Protein required for mating		Up	3.2E-4	
FKS1 YLR342W S000004334			PAL2 RIM21 YNL294C S000005238	Unknown function	Regulator of IME2		Up	8.8E-4	
FKS1 YLR342W S000004334			RLM1 YPL089C S000006010		Serum response factor-like protein that may function downstream of MPK1 (SLT2) MAP-kinase pathway; serum response factor-like protein.		Up	1.2E-5 2.7E-4	Zhao *et al.* (1998)
FKS1 YLR342W S000004334			SLT2 YHR030C S000001072		Suppressor of lyt2; serine/threonine MAP kinase.		Up	1.5E-5 9.9E-6	Lesage *et al.* (2004)
GAS1 YMR307W S000004924	Cell surface glycoprotein (115–120 kDa)	β-1,3-glucanosyltransferase required for cell wall assembly; localizes to the cell surface via a glycosylphosphatidylinositol anchor.	CHS3 YBR023C S000000227	Chitin synthase 3	Chitin synthase III, catalyzes the transfer of *N*-acetyl-glucosamine to chitin; required for synthesis of the majority of cell wall chitin, the chitin ring during bud emergence, and spore wall chitosan.		Up	7.4E-4	Lesage *et al.* (2004)

(*continued*)

TABLE 1

(Continued)

Gene *M* (the mutated gene)			Gene *G* (for which mRNA expression was measured)			Homology	Expression change	*P*-value(s)	Transcriptional compensation previously noted
Gene IDs	Protein	Description	Gene IDs	Protein	Description				
GAS1 YMR307W S000004924			KRE11 YGR166W S000003398		Protein involved in biosynthesis of cell wall β-glucans; subunit of the TRAPP (transport protein particle) complex, involved in the late steps of endoplasmic-reticulum-to-Golgi transport.		Up	1.2E-4	Lesage *et al.* (2004)
GAS1 YMR307W S000004924			SLT2 YHR030C S000001072		Suppressor of lyt2; serine/threonine MAP kinase.		Up	6.0E-7	Lesage *et al.* (2004)
GAS1 YMR307W S000004924			YMR316C-A S000004933				Up	5.0E-6	
GAS1 YMR307W S000004924			YAL053W S000000049				Up	1.5E-5	Lesage *et al.* (2004)
SHE4 YOR035C S000005561		Protein containing a UCS (UNC-45/CRO1/SHE4) domain; binds to myosin motor domains to regulate myosin function; involved in endocytosis, polarization of the actin cytoskeleton, and asymmetric mRNA localization.	ARC40 YBR234C S000000438		Arp2/3 complex subunit, 40 kDa; component of Arp2/Arp3 protein complex.		Up	3.0E-4	
SHE4 YOR035C S000005561			CHS7 YHR142W S000001184		Protein of unknown function, involved in chitin biosynthesis by regulating Chs3p export from the ER.		Up	8.2E-6	

Presented are the gene identifiers, the protein, and a description of gene *M*, followed by the same information for gene *G*; whether *M* and *G* proteins are homologous (BLAST $E < e\text{-}3$); direction of expression change of gene *G* in M null mutant compared to wild type; *P*-value for significance of change in expression taken from Hughes *et al.* (2000a); and whether transcriptional compensation for *M:G* was previously noted in the context of genetic interaction.

overcounted cases of compensation by transcriptional upregulation.

Our study has an interesting parallel to a previous study. Giaever *et al.* (2002) identified genes required to survive a change *in environmental condition*, while we examined genes required to survive a change *in genotype* (*i.e.*, the synthetic lethal partners of deleted genes). In both cases, genes required for surviving the change are often not transcriptionally upregulated.

Collectively, our results and previous ones suggest that transcriptional "retuning" in response to change, environmental or genotypic, is rare. Given the rarity of gene loss, a regulatory network that detects and responds to gene loss may be a large target for mutation with only a weak selection for its maintenance. Furthermore, the potential consequences of a misregulated network may be too costly to justify its benefit. Regardless of the explanation, our study suggests that robustness is generally achieved without a change in mRNA expression of compensatory genes.

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