Article Addendum

Exploring the conservation of synthetic lethal genetic interaction networks

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High-throughput studies have enabled the large-scale mapping of synthetic lethal genetic interaction networks in the budding yeast *Saccharomyces cerevisiae* (*S. cerevisiae*). Recently, complementary high-throughput methods have been developed to map genetic interactions in the fission yeast *Schizosaccharomyces pombe* (*S. pombe*), enabling comparative analyses of genetic interaction networks between *S. pombe* and *S. cerevisiae*, two species separated by hundreds of millions of years of evolution. The resultant data has providing our first view of a possible core genetic interaction network shared between two distantly related eukaryotes, and identified numerous species-specific interactions that may contribute to the unique biology of these two different organisms. These and other results suggest that comparative interactomic studies will provide novel insights into the structure of genetic interaction networks.

High-throughput genetic interaction mapping projects seek to identify all the genetic interactions between a given query gene and a set (or 'array') of hundreds or thousands of target genes. Various combinations of query and array gene function have been examined, including null (both homozygous and heterozygous) and hypomorphic gene mutations, as well as overexpression of wild-type genes.[1-6] These data can be used to identify genes that function in common, opposing or compensatory pathways, predict the function of uncharacterized gene products on the basis of their spectrum of interactions and infer the composition of multi-protein complexes.[3,7-9] There is substantial interest in improving the performance of existing systems, developing new methods and systems to examine genetic interactions

in different organisms and combining the resultant knowledge to expand our understanding of eukaryotic cell biology.

Yeast Leads the Way

High-throughput mapping of genetic interactions was pioneered in the budding yeast *S. cerevisiae*, where the Synthetic Genetic Array (*Sc*SGA) analysis technique was developed to investigate genetic interactions between a given query gene mutation and each of the ~4,700 nonessential genes.[3,10] In this technique, two parental strains harboring single gene deletions are manipulated to create recombinant double mutant progeny. The growth of the double mutant colony is compared to the growth of the individual single mutant colonies to identify those combinations of mutations that display a greater than expected (synthetic) fitness defect manifested by completely defective (lethal) or slow growth (sick) phenotypes. This method is readily automated and the requisite colony manipulations can be performed on a solid agar surface at high densities (1,536 individual colonies per plate is standard). *Sc*SGA synthetic lethal analysis has been used to study the pathways regulating secretion, sister chromatid cohesion, DNA synthesis and repair and tRNA export, as well as to elucidate the global architecture of genetic interaction networks.[7,10-14] In addition to *Sc*SGA, several related methods have been developed that also enable pairwise genetic interactions to be mapped systematically in *S. cerevisiae*.[15-17] Moreover, conceptually similar large-scale methods have been used to examine genetic interaction networks in a multi-cellular organism, the nematode worm *C. elegans*.[4,5] In these approaches, RNA interference (RNAi) is used to inactivate the function of one or both genes under study. The future application of RNAi-based methodologies to study genetic interactions in mammalian cell culture models is eagerly anticipated.

Despite advances in the mapping of genetic interactions in metazoan organisms, single-celled yeasts remain the premier model system for high-throughput genetic network mapping for three reasons. First, the existence of genome-wide deletion libraries that specifically eliminate the function of a target gene ensures that all observed phenotypes in the corresponding mutant are 'on target' and thus highly reproducible. Second, simple, automated methods to manipulate yeast strains enable massive parallelization and truly genome-wide datasets to be collected efficiently. Third, the emergence of quantitative methods to evaluate mutant phenotypes permit

the detection and classification of double mutant phenotypes as additive, synthetic or epistatic.[18-21] Together, these advantages enable the high confidence and high throughput detection of subtle genetic interactions that would be misclassified or elude detection altogether 'by eye' alone.

Building upon the conceptual and technical advances made in *S. cerevisiae*, new SGA-like techniques have emerged that enable high-throughput analysis of genetic interactions in other single-celled species including the bacterium *E. coli* (eSGA and GIANT-Coli)[22,23] and the fission yeast *Schizosaccharomyces pombe* (PEM and *Sp*SGA).[18,24,25] These methods open the way to large-scale 'comparative interactomic'[26,27] studies of genetic networks between different species.

High-Throughput Comparative Analysis of Genetic Interactions

Some genes are common to most if not all eukaryotes,[28] while others are specific to different branches of the evolutionary tree. Similarly, one would predict that certain genetic interactions *between* genes will be broadly conserved between species while others will be specific to, and help define, a given evolutionary sub-group. The combinatorial possibilities inherent in genetic (and protein-protein) interaction networks suggests that the way genes and gene products are wired together is likely as important in defining cell function as the function of individual genes themselves. Thus, an important goal is to define conserved and species-specific (as well as family, genus, phylum-specific, etc.,) genetic interaction networks.

The first experimental comparisons of genetic interaction networks were made between *S. cerevisiae* and *C. elegans*. Intersection of the high-throughput genetic interaction networks for these two organisms found less than 5% conservation.[4,5,29] On the other hand, a recent smaller-scale study determined that a significant fraction (9/21 or 43%) of tested genetic interactions between genes involved in mitotic spindle assembly are conserved between *S. cerevisiae* and *C. elegans*.[30] One possibility is that the conservation of genetic interactions between species is limited to certain processes, such as spindle formation, even when the genes for other processes are conserved. It is also possible that previous comparisons may have failed to turn up more significant degree of conservations between species for technical reasons (discussed in ref. 18).

We were recently able to begin addressing these questions through a comparative study of genetic interactions in two different yeast species, *S. cerevisiae* and *S. pombe*. These two species are separated by ~1,000 million years of evolution[31] and both offer all the technical advantages of single-celled organisms with respect to the quantitative mapping of genetic interaction networks (see above). First, we performed a large-scale curation of the *S. pombe* literature to catalogue all known genetic interactions for this organism. By integrating this information with an existing, independent literature curation effort[32] we were able to determine that 23% of literature-curated synthetic sick/synthetic lethal (SS/SL) genetic interactions were conserved between *S. pombe* and *S. cerevisiae*.[18] We then developed a new method, which we call *S. pombe* Synthetic Genetic Analysis (*Sp*SGA), that enables mutants harboring null alleles of two non-essential genes to be isolated rapidly and scored quantitatively in this organism.[18] We applied both *Sc*SGA and *Sp*SGA methods to examine genetic interactions between a matrix of approximately
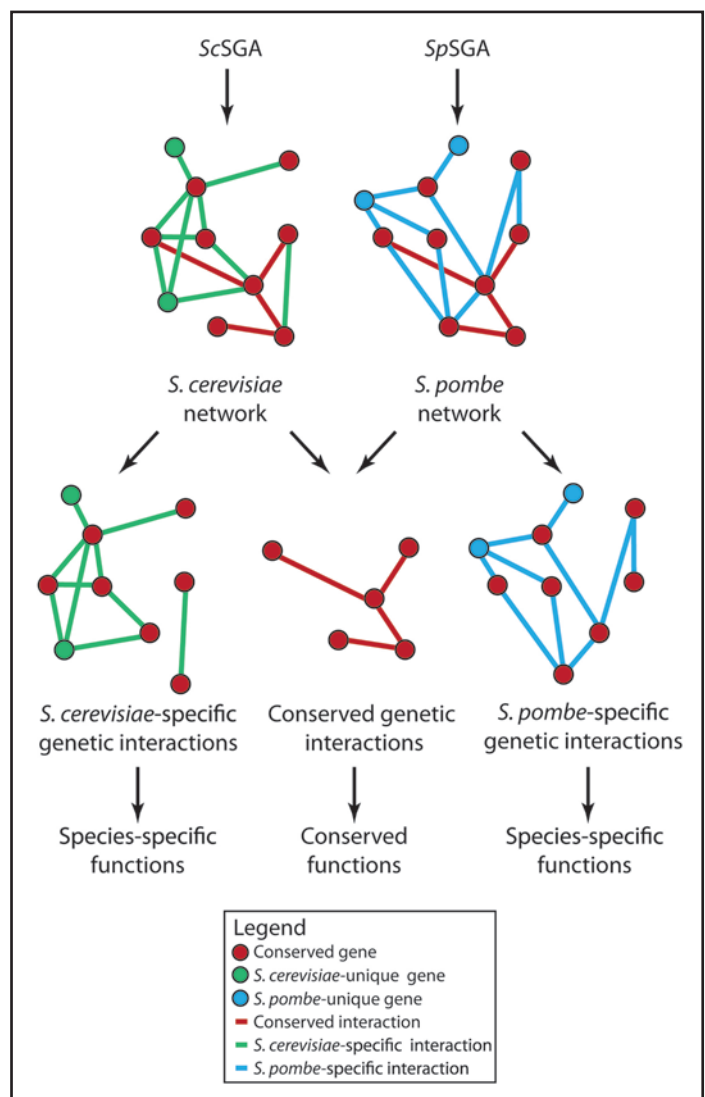

Figure 1. A ball and stick representation of the interactions (edges) between different genes (nodes) in two model genetic interaction networks: one for *S. cerevisiae* generated using the *Sc*SGA method and one for *S. pombe* generated using *Sp*SGA. Conserved interactions are presumed to contribute to processes shared between both species, while species-specific interactions contribute to species-specific functions; however, this remains to be demonstrated.

225 x 225 orthologous genes involved in a wide variety of biological processes in these two divergent yeasts (Fig. 1). Similar methods were also applied to a distinct set of *S. pombe* and *S. cerevisiae* genes by another group.[24] Importantly, both studies report similar results with respect to the overall number of conserved synthetic sick/synthetic lethal (SS/SL) genetic interactions, on the order of ~30%. Together, these results support the hypothesis that a significant number of genetic interactions are conserved between these distantly related species, consistent with previous observations that 65% of *S. cerevisiae* essential genes retain their essential function in *S. pombe*.[33] Nevertheless, it is clear from these results that the majority of SS/SL genetic interactions and a significant number of essential genes are species-specific, implying substantial rewiring of the genetic interaction network of these two species. Approximately 75% of *S. pombe*

genes have a recognizable ortholog in *S. cerevisiae* (*S. pombe* GeneDB, www.genedb.org/genedb/pombe/). Thus, to a first approximation, these data suggest that significant diversity between single-celled organisms may be generated throughout evolution by rearranging the 'wiring' between genes.

The field of comparative genetic interaction analysis is in its infancy and the results obtained to date raise many questions for which there are currently no good answers. First, how and why do species-specific genetic interactions arise between genes that are broadly conserved (Fig. 1 and reviewed in ref. 18)? Are these interactions truly species-specific, or simply context-dependent? For example, it is known that certain genetic interactions may only become apparent under particular nutrient conditions,[34] and therefore it is possible that assaying genetic interactions under different conditions (e.g., in more naturalistic environments) will reveal differences in the rate of conservation. Crucially, how do species-specific genetic interactions relate to species-specific biology? Second, how are species-specific genes integrated into conserved genetic interaction networks formed between highly conserved genes (Fig. 1 and reviewed in ref. 18)? Third, do certain classes of genes, whether defined by functional category, sequence conservation, or some other measure, retain their interactions to a greater degree than other classes? Fourth, how does the degree of network overlap correlate with evolutionary distance? Finally, can we use our knowledge of conserved and species-specific genetic interactions either alone, or combined with other types of comparative data (co-expression, protein-protein interactions, etc.,),[35,36] to predict whether or not two genes are likely to interact with one another in any given species? To answer these questions and others we will require more comprehensive coverage of genetic interaction networks in *S. cerevisiae* and *S. pombe*, the examination of genetic interactions in other yeast species, and the further development of techniques to map genetic interactions rapidly in key metazoan model organisms such as *C. elegans*, Drosophila, *M. musculus* and human tissue culture models.

Conclusions

In the near term, genetic interactions documented in simple, experimentally tractable model organisms such as yeast will improve our understanding of basic eukaryotic cell function, reveal how conserved gene products are functionally interconnected, show how this wiring is rearranged during evolution to generate different cell types and organisms and help predict genetic interactions in more complex species. In the longer term, a better understanding of conserved and species-specific genetic interaction networks could allow us to infer genetic networks for ancestral or extinct species, improve our ability to rationally modify existing organisms for biotechnology purposes and, potentially, contribute to the design of synthetic organisms containing the appropriate genetic 'wiring' necessary to sustain life or execute a given task.

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