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Extra View

Revealing Hidden Relationships Among Yeast Genes Involved in Chromosome Segregation Using Systematic Synthetic Lethal and Synthetic Dosage Lethal Screens

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synthetic genetic array, synthetic dosage lethality, synthetic lethality, kinetochore, temperature sensitive mutation, *Saccharomyces cerevisiae*

ABBREVIATIONS

SL	synthetic lethal
SDL	synthetic dosage lethal
SGA	synthetic genetic array
CEN	centromere
ts	temperature sensitive
TET	tetracycline

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ABSTRACT

The vast accumulation of knowledge from genome sequencing projects and studies with model organisms has presented a remarkable challenge to biologists: to understand the functions of thousands of highly conserved genes and how they work together to regulate fundamental cellular processes. This challenge is compounded by the inescapable reality that most genes are 'buffered' by other genes that contribute to the same biological processes, limiting the impact of phenotypic studies with single mutants. In budding yeast, functional genomic methods have been developed for the systematic application of established genetic techniques. In particular, the Synthetic Genetic Array (SGA) method allows genome-wide synthetic lethal (SL) and synthetic dosage lethal (SDL) screens thus enabling an unbiased survey of genetic interactions. We have used genes encoding components of the yeast kinetochore as a biological testbed for assaying the utility of SGA-based SL and SDL screens for revealing new pathways and genes involved in chromosome segregation. We identified 211 nonessential deletion mutants that were unable to tolerate either overexpression or loss of function of kinetochore genes. Our study uncovered a wealth of relationships between gene products that functionally interact with the kinetochore, and also highlighted the value of performing genome-wide screens with both hypomorphic and hypermorphic alleles of query genes. Here, we will highlight our recent kinetochore SGA genomic screens, in the broader context of applying complementary genetic screening approaches in the systematic exploration of biological pathways or functional complexes.

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SYNTHETIC GENETIC ARRAY ANALYSIS

For the past decade or so, the budding yeast *Saccharomyces cerevisiae* has been a leading model for the development of genomic methods aimed at systematic discovery of gene function. However, despite the popularity of yeast as a model, approximately one-third of all predicted yeast genes remain classified as coding for proteins of unknown function (http://genome-www.stanford.edu/Saccharomyces), with a proportionate increase in our ignorance as we move into more complex organisms. Systematic gene deletion in budding yeast has revealed a principle reason for the failure of many yeast genes to succumb to standard genetic scrutiny; yeast cells exhibit a striking capacity to tolerate deletion of the majority of individual genes (~5000 of the predicted 6000 genes in budding yeast are dispensable for haploid viability, reviewed in ref. 1).

To surmount this redundancy, a fully automated method, called 'Synthetic Genetic Array' or SGA analysis, has been developed that allows the systematic identification of genetic interactions using the yeast gene deletion collection (reviewed in ref. 2). The first utilization of the SGA method was for the identification of synthetic lethal (SL) genetic interactions.[3] SL screening typically begins with a hypomorphic mutation in a query gene —either a complete loss-of-function (deletion) allele, or a point mutation that compromises the function of an otherwise essential gene - and seeks additional mutations that cause cell death specifically when the function of the query gene is reduced or eliminated (Fig. 1). The SGA method allows a query strain carrying a mutation in a gene of interest to be mated to a nonessential haploid deletion set of the opposite mating type. A series of replica-pinning steps then permits selection of double mutants lacking the query gene of interest and carrying one of the deletion mutants in the haploid deletion array. In brief, genetic markers are used to allow selection and sporulation of diploids, and subsequent recovery of haploid double mutant strains. The double mutant arrays can then be assayed

for a phenotype of interest: for example, the lethality or reduced viability of a double mutant compared to the viability of both single mutants identifies a SL interaction. Directed studies in yeast have previously shown that SL interactions occur between genes involved in the same biological pathway.[4] Proof of principal experiments using SGA analysis confirmed that genes identified in a particular genome-wide SL screen were significantly enriched for genes involved in the same cellular process as the query gene.[3,5] The position and connectivity of genes in the network of genetic interactions that has been created using SL-SGA is also highly predictive of specific molecular function.[5]

The SGA method is not limited to SL screening, but is adaptable to other genetic and functional screens (reviewed in ref. 2). Screens based on increased gene dosage or overexpression of a query gene (typically a hypermorphic state) such as synthetic dosage lethality (SDL) screens have also been successfully utilized to uncover hidden functional relationships. An SDL interaction occurs when overexpression of a query gene has no notable effect on a wild type strain but causes lethality or synthetic sickness when overexpressed in a strain carrying a mutation in a gene in the same biological pathway as the query gene (Fig. 1 and reviewed in ref. 6). We demonstrated that the SGA method can be applied to SDL analysis by performing the first genome-wide SDL screens in yeast.[7] The query genes were introduced into the yeast deletion set on inducible plasmids that carried a regulatable *GAL1* promoter (P_{GAL1}) such that gene expression was repressed on glucose media and induced on galactose media. In brief, we mated query strains carrying the P_{GAL1}-inducible plasmids to the nonessential deletion set and isolated *MAT*a recombinants that contained both a deletion mutant and a P_{GAL1}-inducible plasmid under noninducing conditions (Fig. 2). We then induced expression of each query gene in each deletion mutant and assessed the fitness of the strain compared to a vector control. Similar to SL-SGA analysis, we demonstrated that genome wide SDL screens enrich for genes involved in the same cellular processes as the query gene (see discussion below).

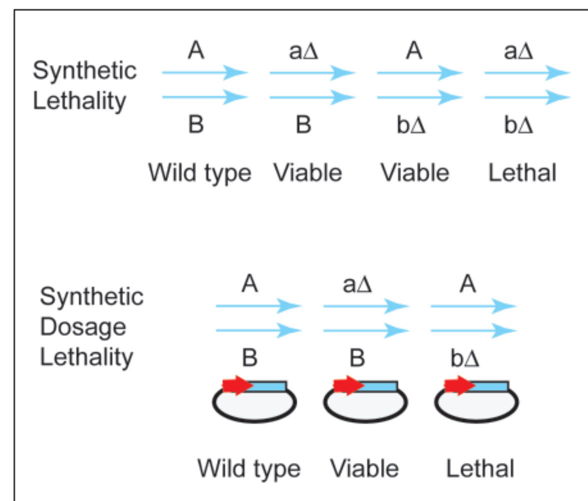

Figure 1. Schematic illustration of synthetic lethality vs synthetic dosage lethality. On the plasmids the red arrow represents the GAL1 promoter, the blue box the query gene.

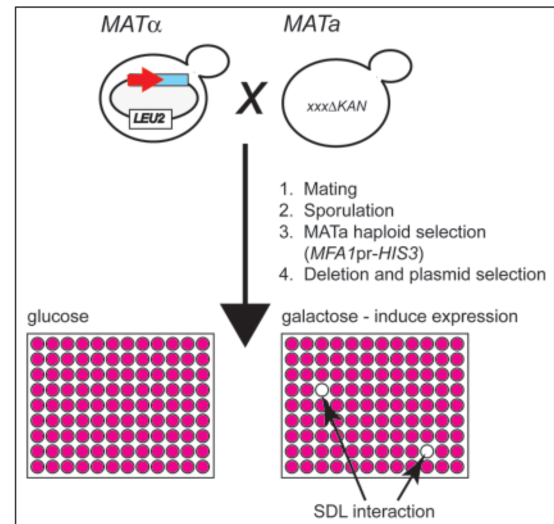

Figure 2. Schematic illustration of systematic synthetic dosage lethality screening. A *MAT*α SGA starting strain carrying a P_{GAL1}-inducible plasmid with the *LEU2* selectable marker and the *MFA1-HIS* and *can1*Δ is crossed to an ordered deletion mutant array. Diploids are selected on media containing G418 (to select for the deletion mutant carrying the *kanMX6* marker) and lacking leucine (to select for the P_{GAL1}-inducible plasmid), sporulated and *MAT*a haploids carrying the plasmid and the deletion mutant are selected on media lacking histidine and leucine and containing G418. Haploids are pinned onto media containing galactose to induce the expression of the *GAL1* promoter regulated gene on the plasmid. Colony growth is compared on galactose and glucose to identify mutants whose growth is compromised (SDL or SDS) upon overexpression of the gene on the plasmid.

GENETIC STUDY OF THE YEAST KINETOCHORE USING SGA ANALYSIS

The SGA method can be used to study any biological process. We are interested in the mechanisms by which cells are able to stably maintain chromosomes during mitotic cell division and decided to use SGA to develop a genetic interaction map involving yeast kinetochore components.[7] Although chromosome segregation mechanisms have been heavily scrutinized in yeast, we were motivated to undertake unbiased genomic screens due to the general biological importance of proper kinetochore function. For example, mutations in conserved kinetochore proteins have been identified in a variety of tumours hallmarked by chromosome instability, thus highlighting the importance of identifying all proteins involved in kinetochore function (reviewed in ref. 8). *S. cerevisiae* is an excellent model organism for the identification of genes involved in chromosome stability. In recent years great strides have been made in identifying structural components of the multiprotein yeast kinetochore complex that resides on centromere (*CEN*) DNA and links chromosomes to spindle microtubules (reviewed in ref. 9-11). Largely through heroic proteomic approaches, greater than 100 proteins have been localized to *CEN* DNA which has resulted in a substantial increase in the knowledge of proteins implicated in chromosome stability. However, *CEN* localization or physical interaction with the kinetochore is not a requirement of proteins with a role in chromosome stability. In contrast, genetic screening has successfully identified a myriad of proteins that are important for chromosome segregation in yeast, many of which are not structural components of the kinetochore, including DNA replication, cohesion, chromatin-modifying and tubulin-folding proteins.

In an attempt to identify genes implicated in chromosome stability we used SGA technology to perform global SL and SDL screens using the yeast deletion mutant array.[7] We performed 14 genome-wide SL screens using both inner and central kinetochore mutants as our query strains. Our resultant SL data set consisted of 230 genetic interactions among 84 genes of which 33 genes had a previously known role in chromosome segregation. SL interactions were identified with 22 genes and the remaining 62 genes displayed synthetic sickness phenotypes. Remarkably, of the 22 deletion mutants displaying a SL interaction with kinetochore mutants, 13 had been previously implicated in chromosome stability, and six have roles in microtubule dynamics. Therefore, the remaining three deletion mutants that displayed SL interactions, likely have important roles in chromosome transmission fidelity. Global studies support the prediction that at least one of the uncharacterized genes that we identified, *YPR045*c, encodes a protein whose function impinges on chromosome stability. Yeast two-hybrid studies indicate that Ypr045cp interacts with the outer kinetochore protein Dam1p,[12] global localization studies indicate that Ypr045cp localizes to the nucleus[13] and cell cycle microarray studies indicate *YPR045*c transcription peaks in G_2/M.[14]

SDL screens in which proteins of the inner kinetochore complex have been over expressed in a subset of deletion mutants have been particularly successful in identifying mutants defective in chromosome segregation.[15] Therefore, in a parallel effort to our SL-SGA screens, we developed a genome-wide SDL screen to identify deletion mutants that could not tolerate overexpression of inner kinetochore genes (*CTF13*, *NDC10* and *SKP1*) at three different temperatures (16°C, 25°C, 37°C). One-hundred and forty-one deletion mutants displayed one or more SDL or synthetic dosage sickness (SDS) genetic interactions upon increased dosage of *CTF13*, *NDC10*, and/or *SKP1*, resulting in a total of 382 genetic interactions. The majority of these interactions were SDS (358) while the remaining 24 SDL interactions occurred in 13 deletion mutants. Of the 141 genes identified in our SDL-SGA screen, only 30 genes had previously identified roles in genome stability, six had roles in microtubule dynamics, and four had roles in cell cycle progression. Of these 30 genes, seven displayed SDL interactions. The enrichment of genes displaying SDL interactions that are involved in chromosome segregation suggests that strong SDL interactions may be of particular interest. However, 33 genes with known roles in chromosome segregation displayed SDS phenotypes upon overexpression of the inner kinetochore genes. Additional SDL-SGA screens using query genes of diverse biological processes will be required in order to determine whether the strength of the interaction (SDL vs SDS) is indicative of the connectivity of the genetic interactions and a predictor of biological function.

In total, our SL and SDL screens identified 211 nonessential gene deletion strains that could not tolerate either loss-of-function or overexpression of kinetochore components. Remarkably, only 16 known kinetochore or *CEN* localizing proteins were identified. Therefore our study dramatically increased our knowledge of genes and pathways that have functional links to chromosome stability. We were also surprised to find that when we compared our SDL and SL datasets, only 14 genes were identified in both screens. Below, we discuss the use of multiple alleles for SL screening and the implications of our nonoverlapping datasets from SDL and SL screens in light of our kinetochore genomic study and other recent SGA studies.

SL-SGA SCREENING WITH FUNCTIONALLY DISTINCT ALLELES

SL-SGA screening using multiple point mutations of a gene may provide information about the functional domains of the query protein. For example, in our screens, two temperature sensitive (ts) alleles of the essential inner kinetochore gene *CEP3*, *cep3-1* and *cep3-2*, were used as query strains[16] resulting in the identification of 14 and 22 genetic interactions respectively with an overlap of 7 genes. Though the exact nature of the two *cep3* mutations used in our study remains unknown (Strunnikov A, personal communication), the limited overlap in genetic interaction may indicate that different Cep3p functions are compromised in the two alleles. Alternatively, the profile differences may reflect variation in allele function at the screening temperature. Analogous genetic studies with different alleles of *SPC24*, which encodes a component of the Ndc80 central kinetochore complex, bolster the view that screens with different alleles can be biologically informative, and may reflect the nature of the mutation.[17] An SGA screen using *spc24*-9, which has a high rate of chromosome loss, as a query mutation uncovered a large number of genetic interactions with kinetochore mutants at 25°C. *spc24-9* carries a single ts mutation in the C terminus of Spc24p and recent structural work suggests that the C-terminal domain is likely required for Spc24p interaction with the kinetochore.[18] In contrast, *spc24-8* and *spc24-10*, which carry ts mutations in the N-terminal coiled-coiled domain and have low rates of chromosome loss, do not display genetic interactions with kinetochore mutants at 25°C.

Davierwala et al have also explored the use of essential ts alleles in systematic SL screens. In this study, the genetic interactions seen with a ts allele were compared to those seen when the essential query gene was conditionally expressed using the tetracycline (TET) promoter.[19] In TET-promoter strains, the expression of the gene is controlled by an inserted, heterologous promoter and is shut off or lowered by the addition of doxycycline. The TET-alleles utilized in SGA screens are likely mimicking lowered gene dosage or a hypomorphic state. The genetic interactions of five different query genes with an array of strains carrying either TET-promoter or ts-alleles of 42 essential genes were explored. More than half of the genetic interactions detected occurred with both the conditional promoter and the ts-allele. These results suggest that, in the cases tested, the ts mutation was producing a loss-of-function phenotype at the restrictive temperature, analogous to the repressed TET-allele. Genetic interactions that are specific to a particular allele, however, may provide insight into functional attributes of the query gene. These results suggest that SL-SGA screens with well-characterized alleles of essential genes could provide a uniquely integrated view of cellular networks —those that interweave genetic and physical interaction networks with structural information. For example, yeast actin and tubulin are excellent models, given the availability of considerable physical interaction network and structural information, and mutated versions of actin and tubulin that are structurally designed.[20,21]

COMPARISON OF SL AND SDL SCREENING

When we embarked on our project, we expected the overlap between our kinetochore SDL and SL screens might be substantial and enriched for biologically relevant interactions. We were surprised to find that the overlap between the kinetochore SL and SDL data sets was comprised of only 14 genes out of 211 total genes identified between the two data sets. Though the overlapping data set was significantly enriched for genes encoding kinetochore proteins, the

lack of overlap indicates that SDL- and SL-SGA screens mechanistically probe different genetic interactions. Papp et al. proposed the "balance" hypothesis in which an imbalance of the normal stochiometry of a protein-protein complex is deleterious to the cell.[22] The balance hypothesis predicts that overexpression phenotypes mimic haploinsufficient phenotypes in which one copy of a gene is deleted in a diploid cell. Therefore, according to the balance theory, hypermorphic (SDL) and hypomorphic (SL) genomic screens should identify similar deletion mutants. However, since the majority of the genes in our kinetochore SL and SDL data sets do not overlap, these screens must assess different genetic relationships.

A recent systematic exploration of the mechanism by which gene overexpression induces toxicity supports this view.[23] Sopko et al. assembled an ordered array comprised of 5280 yeast strains, each containing an inducible copy of a different yeast gene, covering >80% of the genome. Remarkably overexpression of 769 genes in wild type cells resulted in a slow growth phenotype. This collection of strains expressing 'toxic' genes was phenotypically assessed, using a variety of morphological descriptors. Of those genes that caused an obvious cell biological phenotype when overexpressed, only a small subset produced a phenotype that mimicked that produced by deletion of the same gene. This subset, however, was enriched for protein complexes, consistent with the balance hypothesis. The majority of the tested phenotypes that occurred upon overexpression did not resemble those of the null mutant (for nonessential genes) or the TET-repressible promoter strains (for essential genes). Sopko et al. conclude that the majority of overexpressed genes exhibit either a hyperactive gain-of function phenotype or a novel unregulated effect. Thus, while some of the SDL interactions identified in our kinetochore screen are due to "balancer" effects, (kinetochore mutants that are sensitive to overexpression of the kinetochore proteins), it is likely that the majority of deletion mutants identified do not tolerate a kinetochore gain-of-function phenotype.

CONCLUSION

Is systematic exploration of particular biological pathways using parallel SL and SDL screens a generally valuable approach? Though presently the number of genome-wide SDL screens are few, the limited overlap in the SDL and SL datasets, coupled with the identification of unique regulators of chromosome stability in both types of screens, suggests that the results of SDL and SL screens will generally be complementary in nature. Despite this complementary nature, studies of genes present in the overlapping dataset between SDL and SL screens may prove particularly useful. For example, our kinetochore SL screens identified the iron responsive transcription factor *RCS1/AFT1*. Rcs1p is a well characterized protein that regulates expression of genes involved in iron uptake and studies suggest that Rcs1p only has a role under iron-depleted conditions (reviewed in ref. 24). Due to the well-established role of Rsc1p in a process apparently unrelated to kinetochore function, *RCS1* would not have been our first choice among the 84 genes identified in the kinetochore SL-SGA screen for further characterization of its role in chromosome stability. However *RCS1* was also isolated in our SDL screens—we knew the limited list of genes in the overlap between the SL and SDL screens was significantly enriched for genes involved in chromosome stability, predicting that Rsc1p had a previously unappreciated role in chromosome stability. Indeed, we found that Rcs1p is required for high fidelity chromosome transmission under iron-replete conditions, localizes to *CEN* regions and has both genetic and physical interactions with the inner kinetochore protein Cbf1p. This case illustrates the potential power of combining both SL-SGA and SDL-SGA screens to uncover hidden relationships and predict function of genes, a pattern likely to be replicated in future studies.

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