Modeling the Potential For Complex Genetic Interactions in a Simple Eukaryotic Genome: Actin Displays a Rich Spectrum of Complex Haploinsufficiencies

Brian Haarer, Susan Viggiano, *Olga Troyanskaya, and David C. Amberg
SUNY Upstate medical University, Department of Biochemistry and Molecular Biology, 750 E. Adams St. Syracuse, NY 13210
***Department of Computer Science and Lewis-Sigler Institute for Integrative Genomics, Princeton University, NJ 08544**
corresponding author: ambergd@upstate.edu

Abstract

Multigenic influences are increasingly being appreciated as major contributors to human genetic disorders. Sequences for several human genomes has shown that we are highly polymorphic having different alleles at most loci suggesting that there is a high number of multi-allelic gene pairs in all individuals. We have used a major cellular system (the actin cytoskeleton) of the simplest eukaryotic model (*S. cerevisiae*) to systematically determine the potential for deleterious bigenic interactions in complex heterozygotes. Specifically, we have constructed complex haploinsufficient strains between an actin null allele and null alleles for the non-essential genes and measured their vitality. Remarkably, for ~4800 non-essential genes, we have found that at least 200 have deleterious complex haploinsufficient (CHI) interactions with an *act1*Δ allele. This set is enriched for genes with gene ontology terms that are shared with actin and contains the genes for several known actin binding proteins but also present are many genes not previously connected to actin function and many of unknown function. To determine whether these genes influence actin function we examined the actin cytoskeletons in null haploid strains and found that nearly half have defects in actin organization that in many cases are severe and had not been previously reported. The most common phenotype in these strains was excessive actin assembly suggesting that a depletion of the monomer pool under conditions of limiting actin concentration is the cause of the observed poor growth in the complex heterozygotes, a theory was confirmed using alleles of actin that express F-actin stabilizing forms of the protein. CHI interactions with actin are frequently seen with the genes for multiple components of a complex or genes involved in the same function. For example, many of the genes for the large ribosomal subunit are CHI with *act1*Δ. However, nearly all of the *RPL* genes have paralogs and in almost all cases only one of the pair is CHI with actin. Invariably the null strain for this paralog has severe actin organization defects suggesting that these paralogs are not functionally equivalent. Using the alanine scan alleles of actin, we have asked if CHI interactions can be recapitulated by the reduction in subsets of actin functions. In many cases they can and in fact there is spatial congruence to this data suggesting that the loss of binding of specific actin binding proteins causes subsets of CHI interactions.

Introduction

The genetic influences on human disease have been the subject of many investigations that have naturally focused first on the simple cases of monogenic disorders resulting from either dominant or recessive loss of function alleles. However, dominant alleles are fairly rare and the incidence of homozygosing even common recessive alleles (allele frequencies of ~1;1,000) is fairly rare but clearly observable in large populations. For an increasing number of disorders, researchers have been able to show that there are clear genetic influences that are polygenic in nature. Sequencing of human genomes has shown that we are rich in sequence polymorphisms with estimates that sequence differences occur from every 100-300 base pairs (source-The Human Genome Project website at: http://www.ornl.gov/sci/techresources/Human_Genome/faq/snps.shtml) or up to every 1,200 base pairs (source-The International HapMap project at: http://hapmap.org/whatishapmap.html). Regardless, given the size of human genes, these estimates suggest that any given individual has two different alleles for all genes. With a lower estimate of 30,000 genes in the human genome, this means that in a single person there are: (60,000x60,000)-30,000 or ~$3.6x10^9$ possible binary, complex genetic interactions. Estimates derived from cousin matings indicate that we each have 1-10 recessive loss of function alleles (??REF??) meaning that every person could have as many as 100 complex haploinsufficient (CHI) interactions negatively influencing phenotype. In the work presented here we employed the simplest eukaryotic model, *S. cerevisiae*, to determine the prevalence of deleterious, binary, complex haploinsufficient interactions that can be observed for a major conserved cellular system, the actin cytoskeleton.

The actin cytoskeleton is incredibly functionally diverse participating in many cytoplasmic activities from membrane trafficking, polarized cell growth, cytokinesis, organelle positioning and segregation to name a few. More recently long suspected nuclear functions in chromatin remodeling, nuclear structure and transcription have been confirmed (Bettinger, 2004 #790) (Percipalle, 2006 #944). This functional diversity positions the actin cytoskeleton to be an ideal integrator of sub-cellular systems. In addition, the actin cytoskeleton is superbly sensitive to disturbances in stoichiometry/actin concentration (Wertman, 1992 #27); these two facts, and the extremely high level of conservation of actin in structure and function, make it an ideal model for testing the potential for deleterious complex genetic interactions in the eukaryotic genome.

Results:

The mechanics of a complex haploinsufficient screen in yeast are relatively straightforward. We constructed a haploid strain deleted for the genomic copy of the single conventional actin gene (*ACT1*) in which the actin open reading frame was replaced with the selectable *NAT*^{r} noursethricin resistance gene (the *act1*Δ*::NAT*^{r} allele). Since actin is an essential gene (Shortle, 1982 #4), this allele needed to be covered by a low copy, plasmid borne copy of *ACT1*. In addition, this plasmid carried the *URA3* gene that could be counter-selected against by adding 5-fluoro-orotic acid to the medium. This query strain was mated to the EUROSCARF ordered array of ~4,800 haploid strains, each of which was deleted for a non-essential gene by replacement with the G418 resistance gene, kanamycin (*G418*^{r}*)*. The complex heterozygous knock-out diploids were selected on medium containing both noursethricin and G418, the diploids were then spotted to medium containing 5-FOA to select for cells that had lost the *ACT1*^{wt} bearing plasmid and their relative growth rates at 25° and 35° were scored; 35° is a semi-permissive for the *act1*Δ hemizygote (Wertman, 1992 #27). CHI strains that appeared to have a growth deficit were confirmed by hand mating and streaking for single colonies on 5-FOA medium. Note we confirmed that actin expression in an *act1*Δ hemizygous diploid was lowered to 1/2 of that observed in a wild type diploid strain (data not shown).

In three separate screens, 35% overlap was seen per screen. Of those genes identified in at least two screens, ~35% reconfirmed by hand testing and of those hit in a single screen only 20% reconfirmed. Remarkably, 132 genes were identified in these primary and clearly non-saturating screens. These are indicated in Fig. 1 by purple colored lines connecting them to the central actin hub. In this collection are several known actin binding protein genes (labeled ABPs on the left of Fig. 1) including the genes for the Arp2/3 regulator *ABP1,* the the Arp2/3 complex component *ARC18*, the V-ATPase component VMA5, yeast fimbrin (*SAC6)*, yeast cyclase associated protein *SRV2*, the formin *BNR1*, and the only gene previously known to be CHI with actin (Welch, 1993 #187), the transcription factor *ANC1TAF14*. Other genes identified that have been directly implicated in actin function include *SAC1* and *SAC7* (identified as suppressors of actin mutants (Novick, 1989 #13) (Dunn, 1990 #155)), the polarity regulators *BEM1, PEA2,* and *GIC1*, the catalytic subunit for phosphatase 2A *PPH21* and the folding facilitators *GIM3* and *GIM4*. We identified 7 of the 64 gene pairs associated with actin in the *Saccharomyces* Genome Database (SGD).

The fitness of monogenic haploinsufficient strains (from the heterozygous diploid knock-out collection) was recently analyzed in competition growth assays (Deutschbauer, 2005 #897), providing a useful

comparison to the results of our CHI screen. Only 8 genes were identified in both screens (*PAF1, HSL7, RPP1A, RPL6A, RPL13B, RPL14A, RPL35B,* and *RPL43A*). The overlap is clearly biased toward translation as 6 of the 8 encode components of the large ribosomal subunit. The results from the monogenic haploinsuffiency study suggested a bias toward genes required to maintain optimal biosynthetic capacity (e.g. translation and transcription) but the low overlap between these screens (with the exception of 7 of the genes listed above (*PAF1* encodes and RNA polymerase II subunit)) suggests that the CHI screen with actin did not appear to have a similar bias.

The preliminary CHI screens were clearly sub-saturating possibly due to the ability of yeast to generate and tolerate at high frequency, 2N+1 aneuploidies (rate ~10^{-4} (Hartwell, 1985 #945) (Meeks-Wagner, 1986 #946)). Therefore, the primary CHI hits were analyzed, with the help of the SGD, to identify additional genes that could be expected to be CHI with actin. The criteria included shared functions and/or being present in a defined complex with the product of the primary CHI hit. In this way another ?? non-essential genes were targeted for CHI testing against the *act1*Δ allele. ?? of these showed CHI interactions with the *act1*Δ allele; these are indicated in Fig. 1 with an olive green colored line connecting them to the central actin hub. Interconnecting blue lines have been added to this figure to show the physical and functional interactions between gene products so that related clusters can be recognized. In many instances several genes of shared function also show a CHI interaction, some examples include the CCR4-NOT transcription complex (*CCR4, NOT3, NOT5,* and *POP2*; shown on the right of Fig. 1), the tubulin pre-foldin complex (*GIM1, GIM3, GIM5, PAC10, PFD1,* and *YKE2*; shown at the top of Fig. 1), the INO80 complex (*ANC1TAF14, ARP8, IES1,* and *IES3*; shown in the upper left of Fig. 1), and the ESCRT complexes involved in multi-vesicular body protein sorting (*DID4, STP22, VPS20, VPS22, VPS24, VPS25, VPS28, VPS32,* and *VPS36*; shown on the left of Fig. 1). This observation was not universally true even for complexes in which multiple genes are CHI with actin. For example, for the CCR4-NOT complex the *MOT2* gene was not CHI with actin, for the INO80 complex *ARP5* was not CHI with actin and for the ESCRT complexes *SRN2* was not CHI with actin. These results suggest, as one might expect, that not all components of these complexes are sufficiently limiting to see the interaction when gene copy is lowered by 1/2. Note that many essential genes that might be expected to be CHI with actin were not tested.

The identification of multiple large ribosomal subunit genes was curious and therefore we investigated further. Almost all of the large ribosomal

genes have very closely related paralogs and yet in the primary screen we only identified one of each pair. We subsequently tested the non-CHI paralogs, and select additional large ribosomal gene pairs for CHI interactions with actin. Since the ribosomal gene paralogs have generally been considered to be functionally equivalent, we were very surprised to find that in nearly all cases tested only one of the paralogs for each gene pair is CHI with actin. The exceptions were *RPL7* and *RPL35* for which both paralogs gave a strong CHI interaction. To examine possible involvement in actin function, we visualized the actin cytoskeleton by rhodamine-phalloidin staining in haploid knock-out strains for both paralogs of the ribosomal genes. In nearly all cases, the paralog that is CHI with actin had severe actin and cell morphology defects while the paralog that was not CHI with actin had a normal actin cytoskeleton. The exceptions were *RPL43* for which both knock-outs had moderate actin organization defects and *RPL39* (which has no paralog) whose knock-out had an apparently normal actin cytoskeleton. As an example, Fig. 2 shows the CHI testing (panel A) and rhodamine-phalloidin staining (panel B) for *RPL6* and *RPL13* gene paralogs. Note that *rpl6a*Δ and *rpl13b*Δ are CHI with actin and have severe actin organization defects while *rpl6b*Δ and *rpl13a*Δ are normal in both regards and yet the proteins encoded by these loci are predicted to be 88% and 91% identical, respectively. We theorized that perhaps the more highly expressed paralog would be the one that displays the CHI interaction. To address this we examined the calculated protein levels based on GFP fluorescence from the integrated GFP fusions as reported on the O'Shea laboratory web page (http://yeastgfp.ucsf.edu/). Although this is an imperfect way to infer exact protein expression levels, they are expressed under the control of their normal promoters and therefore should be useful for comparisons. However, we found that there was no correlation for a paralog's relative expression level and being CHI with actin. For the case of *RPL13*, it was estimated that there are 133,000 copies of Rpl13Ap per cell and 54,200 copies of Rpl13Bp per cell and so the one that is CHI with actin is in fact less well expressed while for *RPL6* the two paralogs have comparable levels of expression. Although we cannot say at this time why these differences arise, clearly in nearly all cases, the large ribosomal gene paralogs are not functionally equivalent: one set appears to differentially contribute to the function and proper organization of the actin cytoskeleton.

In the most general sense, the CHI interactions with actin could be interpreted from two perspectives: 1) The interacting gene could impact on the function and/or organization of the actin cytoskeleton, for example it could encode for an actin binding protein that regulates actin assembly and

dynamics, or 2) The process in which the interacting gene participates could rely upon the actin cytoskeleton for its efficient operation. Experience has told us that most actin regulators are required for proper organization of the actin cytoskeleton as measured by rhodamine-phalloidin staining. To determine this possibility for the CHI gene set, we visualized the actin cytoskeleton in haploid knock-out strains. We reasoned that if a gene is involved in regulating actin, then obvious defects are most likely to be observed in the complete absence of gene activity. Surprisingly, nearly 75% of the CHI gene knock-out strains had discernible defects in actin organization that were as expected, usually accompanied by cell morphology defects. In fact ~40% had severe actin and cell morphology defects and in most cases this aspect of their phenotypes had not previously been reported. Although the nature of the observed defects was diverse, many appeared to share a common feature of excessive actin filament elaboration accompanied by an elongated cell phenotype. Figure 3 shows the rhodamine-phalloidin staining for the knock-outs of several genes of this class: *YDR049w* is a gene of unknown function, *UAF30* is involved in RNA Polymerase I transcription, *SHP1* is involved in ubiquitin mediated protein degradation, *SET5* encodes a SET domain containing protein of unknown function, and *DBF2* encodes a kinase involved in exit from mitosis. Our naïve assumption at the start of this analysis was that the lowered expression in actin hemizygotes would result in a lowered capacity to make the essential filamentous actin structures and that anything that further reduced the capacity to make filaments would create a filament crisis resulting in loss of viability. The observation that many of the null strains for CHI genes had excessive actin assembly seems to argue the converse i.e. that the CHI diploids do not have trouble making sufficient filamentous structures but have a critically limiting pool of monomeric actin and that excessive actin assembly under conditions of limiting actin concentration compromises cell viability. To our knowledge this is a novel hypothesis for explaining the potentially deleterious effects of haploinsufficiency and so we engineered a relatively simple test of this model. The act1-159p mutant (V159N) forms more stable actin filaments by reducing the rate of filament disassembly (Belmont, 1998 #513). A diploid was engineered which contained a null allele of actin over the *act1-159* allele, covered with the *URA3*-marked plasmid bearing the wild type actin allele. This strain was completely inviable on 5-FOA medium that selected against the wild type actin bearing plasmid confirming that under conditions of limiting actin concentration, F-actin stabilization is lethal.

Another example of inappropriate and excessive actin assembly was observed in the strains deleted for components of the ESCRTI, II and III complexes. As shown in Fig. 4, F-actin can be observed to accumulate on an intracellular compartment found in the mother cell body of these *vps36*Δ cells. Strains knocked-out for any of the genes encoding components of the three ESCRT complexes displayed an identical pattern of F-actin accumulation. This localization strongly resembled the class E compartment known to accumulate in these mutants that is an intermediate, pre-vacoular endocytic compartment (Babst, 2005 #947). Although actin's involvement in endocytic trafficking is well established (Ascough, 2004 #948), F-actin accumulation on class E bodies has not been previously reported. Given our results with the *act1-159* allele it is tempting to theorize that excessive F-actin assembly on accumulating class E bodies leads to a consumption of the monomer pool and the observed CHI interactions with ESCRT complex encoding genes. Alternatively, it may indicate a failure to recycle endocytic machinery (including actin-nucleating proteins) to the plasma membrane.

As can bee seen in Fig. 1, the CHI screen with actin identified a large number of yeast genes of unknown or unassigned function (indicated in grey). Certainly one of the goals of genome level screens is to try and assign functions to the still considerable number of yeast genes for which little is known. Statistically, we might expect some of these genes to fall within the well-represented functional categories shown in Fig. 1. For example, we might expect 1-2 new actin binding proteins within this set of genes of unknown function. Since most actin binding proteins display a distinctive localization pattern, we again turned to the GFP localization data for functional clues (http://yeastgfp.ucsf.edu/). Two proteins in particular had very similar localization patterns that looked as if they could overlap with actin cortical patch localization: Lsp1p and Pst2p. Note that actin cortical patches are plasma membrane associated, early endocytic structures. The cortical spots of Lsp1p and Pst2p were different from actin cortical patches in that they were not strictly polarized to the bud or neck cortex. *LSP1* has a paralog (*PIL1*) whose protein shows a similar localization pattern to Lsp1p and *PST2* has two paralogs (*YCP4* and *RFS1*) both of which show similar localization patterns to Pst2p and Lsp1p. All of the paralogs were found to also have CHI interactions with actin and the null strains showed modest defects in actin organization and a low percentage of cells with aberrant cell morphologies. Subsequent to these analyses, the lab of Peter Walter published their identification of pre-endocytic structures they termed eisosomes that contain the Lsp1 and Pil1 proteins (Walther, 2006 #949). The CHI interactions with actin suggest that the cytoskeleton has a role in the

function of these structures. In addition, the localization data further suggests that the Pst2p, Ycp4p, and Rfs1p homologs may also function within the eisosomes.

The primary CHI hits were also analyzed using the Genomica web site to identify possible similarities/correlations. Besides the previously mentioned bias toward cytoskeletal and polarity genes, these programs found a non-random distribution of predicted Sum1p transcription factor binding sites in the promoter regions of several CHI genes (P value=10^{-4}, (Segal, 2005 #950)). This observation predicted that *sum1*Δ should also be CHI with actin and this was in fact confirmed to be the case. Also consistent with a role in the regulation of cytoskeletal genes, are results from the Boone lab reporting that the *sum1*Δ allele is synthetic lethal/sick with several prefoldin complex genes identified in our CHI screen (*GIM3, GIM5, PAC10* and *YKE2*) and several polarity and cytoskeletal genes (*ARP2, ARC40, CDC42, RVS161,* and *RVS167*) (Tong, 2004 #791). Collectively these observations suggest that Sum1p has a particularly important role in the regulation of genes that impact the actin cytoskeleton.

These examples (the ribosomal genes, the ESCRT genes, the eisosome genes, and *SUM1*) have been highlighted to point out the some of the novel biological information that can begin to be derived from our dataset. We certainly expect that more intensive bioinformatics analysis will suggest additional, biologically relevant hypotheses.

We have at our disposal an extremely useful set of reagents for studying structure/function relationships within actin: a set of actin mutants specifically designed by alanine scanning to neutralize clusters of charged residues on the surface of the actin protein (Wertman, 1992 #27). We theorize that many of the CHI interactions with the actin null allele may not merely result from a 50% reduction in total actin activity but in fact the loss of subsets of specific actin functions, for example a reduction in the capacity to bind specific actin binding proteins. The alanine scan alleles are perfectly suited to address this hypothesis. As a preliminary test, we constructed query haploid strains carrying six different alanine scan alleles at the normal actin locus covered with the wild type actin gene on the counter-selectable plasmid. These strains were hand tested for a deleterious complex heterozygous (unlinked non-complementation) interactions with null alleles for the strains known to be CHI with the *act1*Δ allele from our primary screens. As can be seen in Fig. 5 B, some of the CHI interactions can be recapitulated as complex heterozygous interactions with these alleles. As one might expect the severity of the alleles (as indicated by the growth deficits of strains in which the mutant is the only source of actin) correlates

with the number of interactions. For example strains bearing both the *act1-105* and *act1-111* alleles are barely viable while strains expressing *act1-120, 124,* and *129* are temperature sensitive for growth and strains expressing *act1-102* are wild type for growth (Wertman, 1992 #27). Our first indication that CHI interactions may reflect the loss of localized activities on the surface of actin came from an apparent spatial congruity in the data. Alleles that alter residues near each other on the surface of actin (such as *act1-105* and *act1-111*; see Figure 5A) share a large number of interactions while alleles that alter distant residues do not (such as *act1-111/105* versus *act1-124*). These observations suggest that CHI interactions with actin might be attributable to reductions in the binding of specific actin interacting proteins and would therefore be able to be phenocopied by null alleles of these actin interacting proteins. For example, 29 genes show CHI interactions with only the *act1-105* and *act1-111* alleles predicting that there are one or more actin binding proteins that are unable to bind to either the act1-112 or the act1-105 proteins and that it is the failure of these interactions that could be the cause of the CHI interactions with these genes. However, *VPS75* shows CHI interactions with *act1-105, 111,* and *129* suggesting the existence of another actin binding protein that cannot interact with not only act1-105p and act1-111p but act1-129p as well. These possibilities can be tested by analyzing null alleles for all known actin binding protein genes for their ability to phenocopy the complex heterozygous interactions of the actin alleles and analyzing this data in light of the actin mutants that are unable to interact with that actin binding protein. In essence we expect to be able to ultimately create a genetically based structure/function map of the actin surface that correlates genetic interactions to actin binding protein footprints on the surface of actin. Theoretically we should be able to map the actin binding sites for known actin binding proteins and in fact infer possible actin binding sites for as yet discovered actin-binding proteins.

Discussion

The yeast model has proven to be extremely useful for determining the prevalence of multigenic influences on phenotype in a eukaryotic genome. Systematic screening for synthetic lethality has shown that most genes display multiple deleterious interactions with genes that are functionally related. However, synthetic lethality in the human genome would require homozygosing loss of function alleles for two genes, clearly a more rare event than inheriting heterozygous gene pairs.

In this study we modeled the extreme case of inheriting two null alleles and found that quite frequently this can have an adverse effect on cell function. Actin is arguable one of the most centrally important proteins in eukaryotic cells and it does display haploinsufficiency on its own, so we cannot say that all genes have the same potential for complex haploinsufficient interactions. However, these results argue that complex heterozygous gene interactions may have the greatest affect on genetically based phenotypic diversity. In fact, haploinsufficiency has been gaining in appreciation as an important influence in human disease. For example, simple haploinsufficiency in a large number of transcription factors has been found to contribute to severe and diverse genetic disorders such as glaucoma, diabetes, and muscular dystrophy to name a few (for a review see (Seidman and Seidman, 2002)) and haploinsufficiency has also been found to contribute to cancer development in mouse models (Spring *et al*., 2002) (Goss *et al*., 2002), defects in liver regeneration in mice (Kalinichenko *et al*., 2003), disease related defects in platelet production in humans (Deutsch *et al*., 2003), cardiomyopathy in mice and humans (Carrier *et al*., 2004) (Yu *et al*., 1998) (Andersen *et al*., 2004), defects in neuronal migration (Kholmanskikh *et al*., 2003) and synaptogenesis (Boeckers *et al*., 2002). Furthermore, complex haploinsufficiency is also gaining recognition as an important contributor to oncogenesis (Ma *et al*., 2005) (Santarosa and Ashworth, 2004) and a very recent paper reports that mice that are hemizygous for the Bub3 mitotic checkpoint gene in conjunction with being hemizygous for the Rae1 nucleoporin-encoding gene display an early aging phenotype (Baker *et al*., 2006). Given the high levels of functional conservation between yeast and human genes, we might expect that complex haploinsufficient networks will be conserved as well. If this is the case, then modeling these networks in yeast could be highly informative for guiding human geneticists working on multigenic disorders for which the contributing genes have eluded identification.

Although estimates indicate that human individuals may have as many as 100 complex haploinsufficient gene pairs, clearly partial loss of function

alleles are much more common. Therefore, we examined whether our CHI interactions with actin could be recapitulated as complex heterozygous interactions with viable point mutations in actin. Although the number of interactions correlated with the severity of the actin allele, many of the CHI interactions could be observed using actin mutants that only partially compromise actin function and even a few interactions could be observed with alleles that have slight effects on actin function (based on the growth rates of mutant haploids). In this regard the mutants discriminated between the CHI genes, although if two alleles alter residues near each other on the actin surface, there was extensive overlap for their CHI interactions. This spatial congruity suggests to us that CHI interactions may result from the loss of interactions between actin and actin binding proteins. If this is the case then null alleles of actin binding protein genes should phenocopy subsets of CHI interactions with the actin alleles. Conversely, the genetic interaction overlap between the actin alleles and actin binding protein genes might be used to map the binding sites for these actin binding proteins or to infer the binding sites for as yet identified actin binding proteins. By using the large set of actin mutants at our disposal we expect to be able to derive a CHI-based structure-function map of the actin surface; a long term goal of this project.

Genetic interaction analysis in yeast has been very fruitful for discovering functional relationships between genes, initially suppression analysis but more recently synthetic lethality has been extremely useful. Our results suggest that CHI screens will become an important addition to genomic scale genetic interaction analysis. The screen was clearly biased toward genes already known to be involved in actin related functions and identified several well known actin binding protein genes. The set of CHI genes displayed in Fig. 1 seems extremely functionally diverse, perhaps leading one to conclude that the screen is not very specific. However, actin is clearly the most functionally diverse eukaryotic protein and many functional categories are over-represented in this list. Furthermore, 50% of the genes identified are required for normal actin organization so there is an apparent over-representation of genes in our list that impact actin function. In addition, if the screen lacked specificity we would expect that genes with shared functions would not all be CHI with actin, but in fact the converse was observed for several multi-protein complexes. Clearly the most interesting examples of this were the genes for the large ribosomal subunit. Our finding that the paralogs for these genes are not equivalent (for a CHI interaction with actin or their effect on the organization of the actin cytoskeleton) suggests functional specialization that was not previously

appreciated. Since we failed to identify any small ribosomal subunit genes, this functional specialization appears to be focused purely in the large subunit. We do not currently understand the biological basis for this functional specialization but two possibilities come to mind: 1) The cell constructs two (or more) flavors of ribosomes with different paralog compositions and some of these specialized ribosomes have a greater role in translating cytoskeletal proteins, or 2) *RPL* paralogs are under different regulatory constraints. For example, some *RPL* genes may be able to auto-regulate in response to reductions in expression of their paralog and that limiting production of functionally equivalent ribosomes selectively affects the actin cytoskeleton.

We hypothesize that CHI analysis will not only be useful for uncovering disease-relevant networks of genetic interactions but as with other methods of genetic interaction analysis, it will uncover novel aspects of cell biology. Two aspects that distinguish CHI analysis are 1) The relative ease with which these screens can be performed in contrast to the very labor intensive procedures that synthetic lethality or suppression analysis entail, and 2) A greater relevance to the polygenic influences that shape human phenotype.

Figure Legends

Fig. 1 Actin shows complex haploinsufficiency (CHI) with a large number of non-essential yeast genes. Hemizygosity of the indicated genes causes a growth deficit if the strain is also hemizygous for the actin gene *ACT1* (example genotype *yfg1*Δ/*YFG1*^{wt} *ACT1*^{wt}/*act1*Δ). Some of these genes were identified by screening of the non-essential gene knock-out collection (indicated by purple connecting lines to the central hub) while others were tested because they were implicated by the results of the primary screens (indicated by olive green connecting lines to the central hub). Physical interactions between CHI gene products are indicated by connecting blue lines. Genes that encode known actin binding proteins are labeled on the left. The genes are color coded based on their primary Gene Ontology assignment (key on the bottom right). Genes in brackets are listed by virtue of CHI hits of an overlapping deletion of a dubious orf in the EUROSCARF deletion collection.

Fig. 2 Large ribosomal subunit gene paralogs make differential contributions to actin function. (A) Strains of the genotype *ACT1*^{wt}/*act1*Δ *rpl13a*Δ/*RPL13A*^{wt} , *ACT1*^{wt}/*act1*Δ *rpl13b*Δ/*RPL13B*^{wt}, *ACT1*^{wt}/*act1*Δ *rpl6a*Δ/*RPL6A*^{wt} and, *ACT1*^{wt}/*act1*Δ *rpl6b*Δ/*RPL6B*^{wt} were constructed by mating *act1*Δ strains carrying a low copy plasmid expressing wild type actin and the *URA3* gene product to strains deleted for the *RPL6* and *RPL13* paralogs. These complex heterozygotes were streaked for single colonies and replicated to medium containing 5-FOA to select for cells that had lost the Ura3p expressing plasmid. *rpl13a*Δ and *rpl6b*Δ are not CHI with actin while *rpl6a*Δ and *rpl13b*Δ are strongly CHI with *act1*Δ. (B) Haploid strains deleted for the *RPL6* and *RPL13* paralogs were stained with rhodamine-phalloidin and their actin cytoskeletons were visualized by fluorescence microscopy.

Fig. 3 Many of the genes that are CHI with actin are required for proper organization of the actin cytoskeleton. Haploid strains deleted for the indicated genes were strained with rhodamine-phalloidin and their actin cytoskeletons were visualized by fluorescence microscopy.

Fig. 4 Deletion of genes encoding ESCRT complex components causes F-actin to accumulate on the Class E endocytic compartment. A *vps32*Δ strain was stained with rhodamine-phalloidin and its actin cytoskeleton was visualized by fluorescence microscopy.

Fig. 5 CHI interactions can be attributed to the loss of subsets of actin functions. (A) The front surface of actin is shown with the locations of actin alanine scan alleles color coded by growth phenotype (green=wild type, yellow=recessive conditional, red=recessive lethal, blue=presumed dominant lethal). The locations of the residues changed by the six alleles tested for unlinked non-complementation with the CHI genes are indicated with arrows. Note the residues changed by the *act1-129* allele reside on the back surface, directly behind the residues changed by the *act1-105* allele. (B) The hubs show actin alleles tested against the CHI gene set for complex heterozygous interactions and the nodes are those genes that were found to interact with these alleles.

Fig. 1

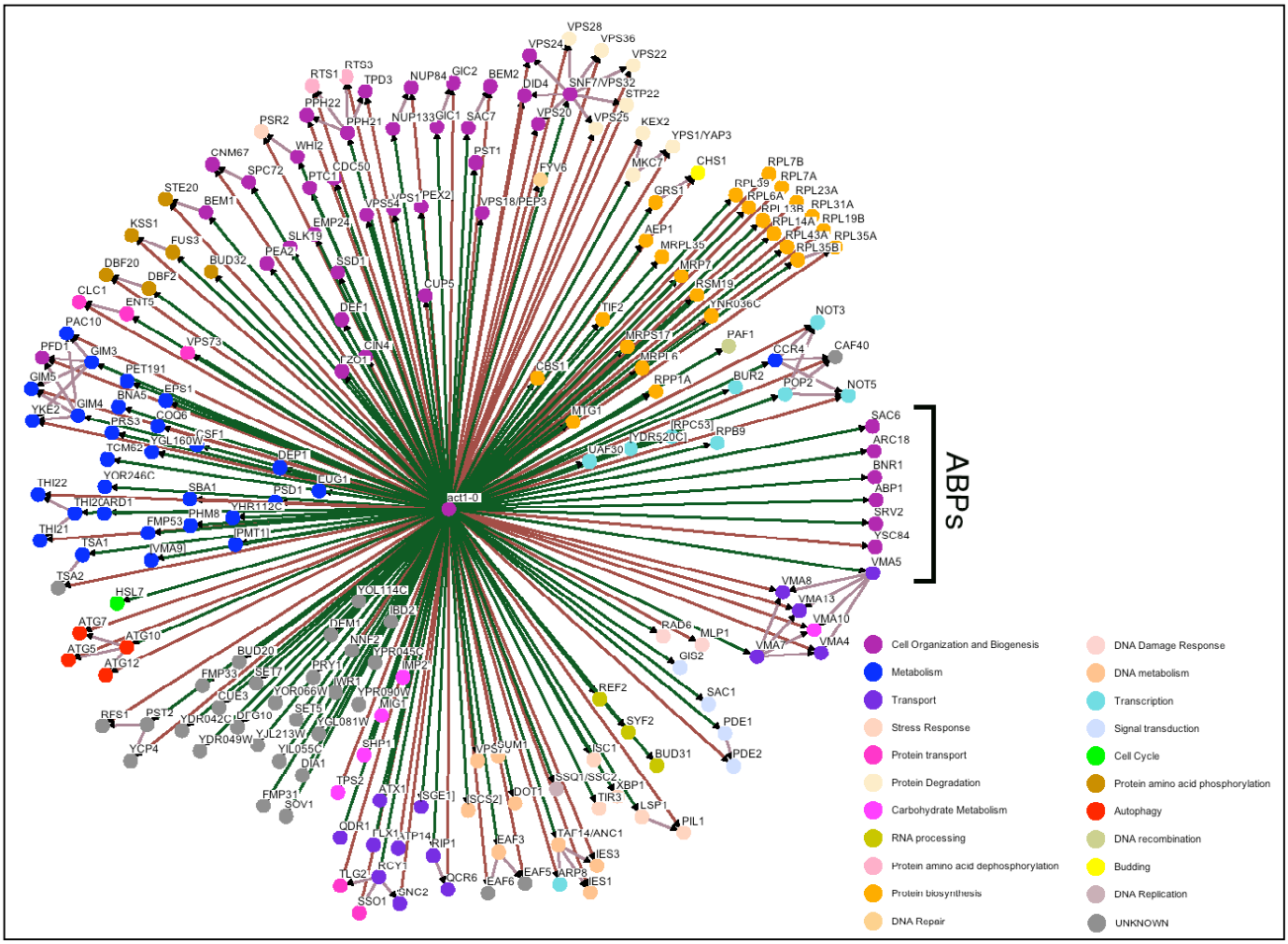
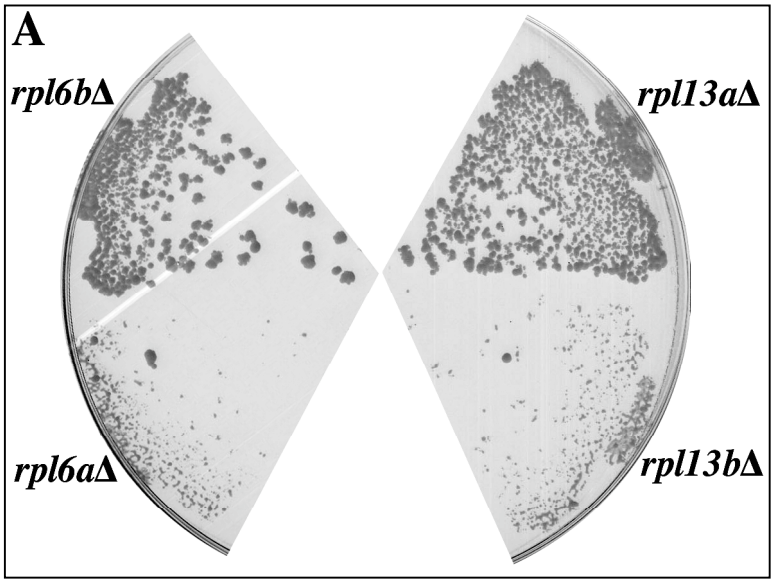

ABPS
act1-0
Cell Organization and Biogenesis
Metabolism
Transport
Stress Response
Protein transport
Protein Degradation
Carbohydrate Metabolism
RNA processing
Protein amino acid dephosphorylation
Protein biosynthesis
DNA Repair
DNA Damage Response
DNA metabolism
Transcription
Signal transduction
Cell Cycle
Protein amino acid phosphorylation
Autophagy
DNA recombination
Budding
DNA Replication
UNKNOWN

Fig. 2


A
rpl6bΔ
rpl13aΔ
rpl6aΔ
rpl13bΔ

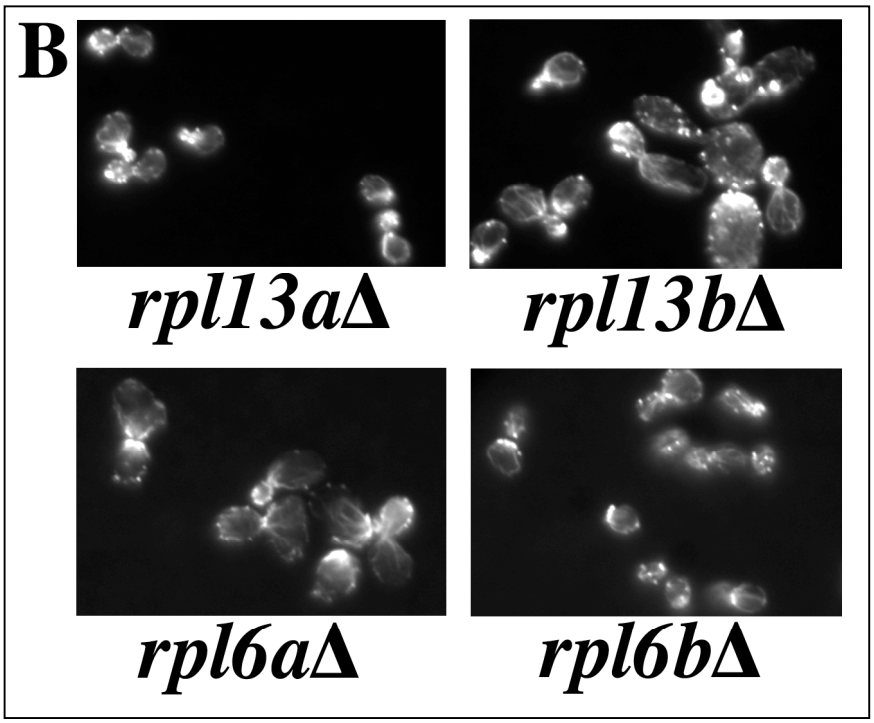

B
rpl13aΔ
rpl13bΔ
rpl6aΔ
rpl6bΔ

Fig. 3

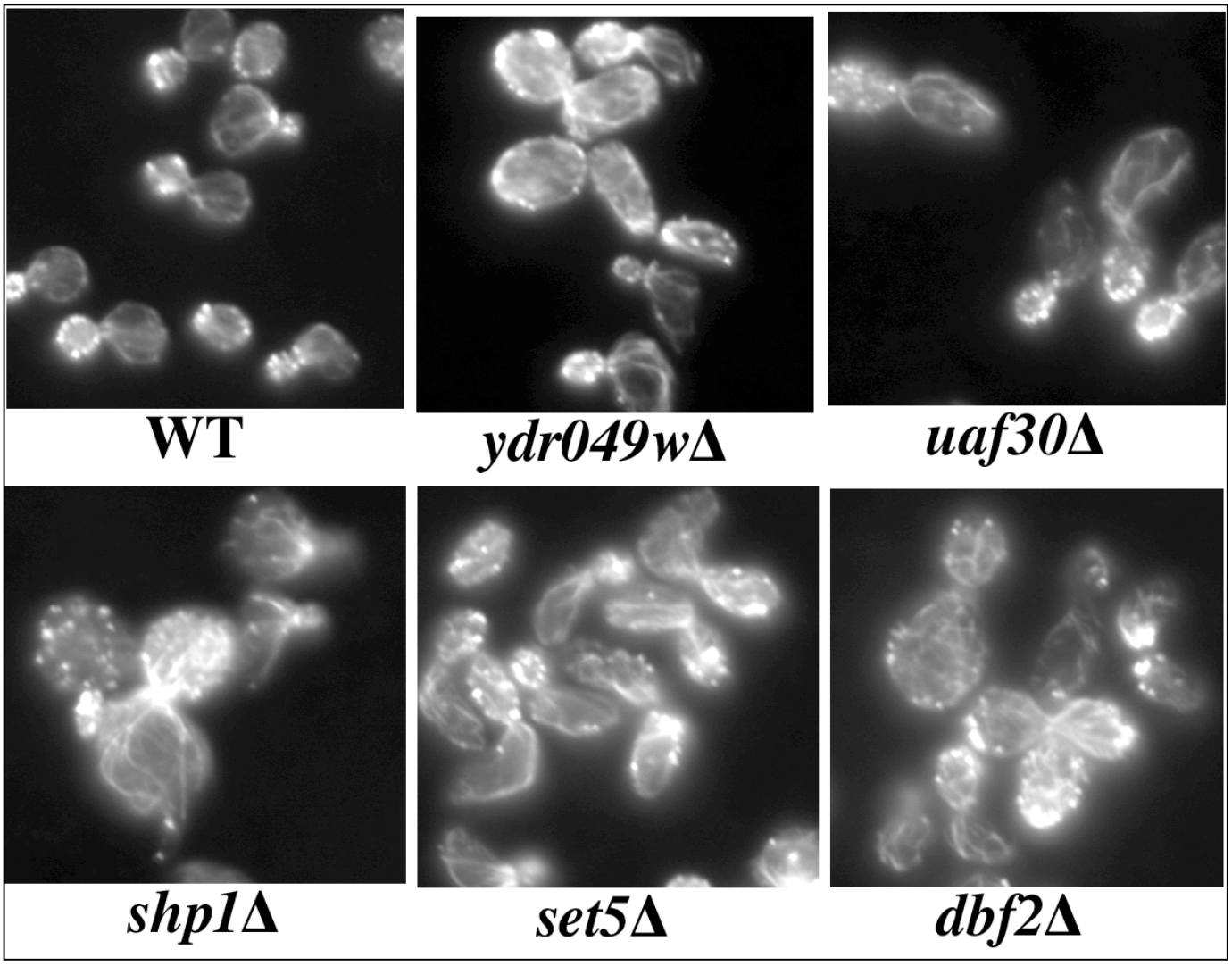


Fig 4.

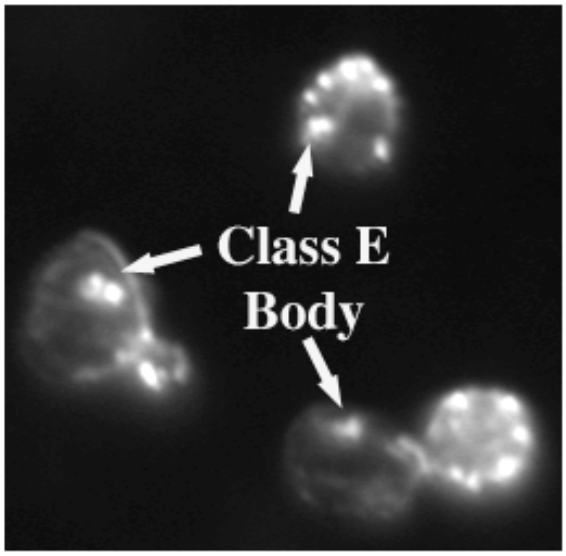
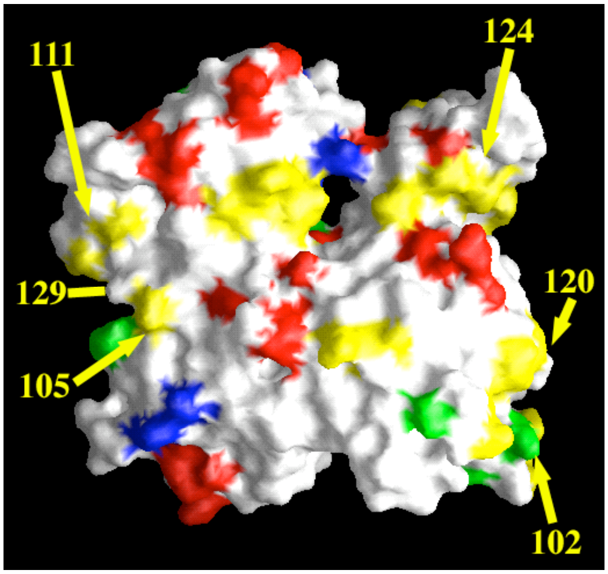
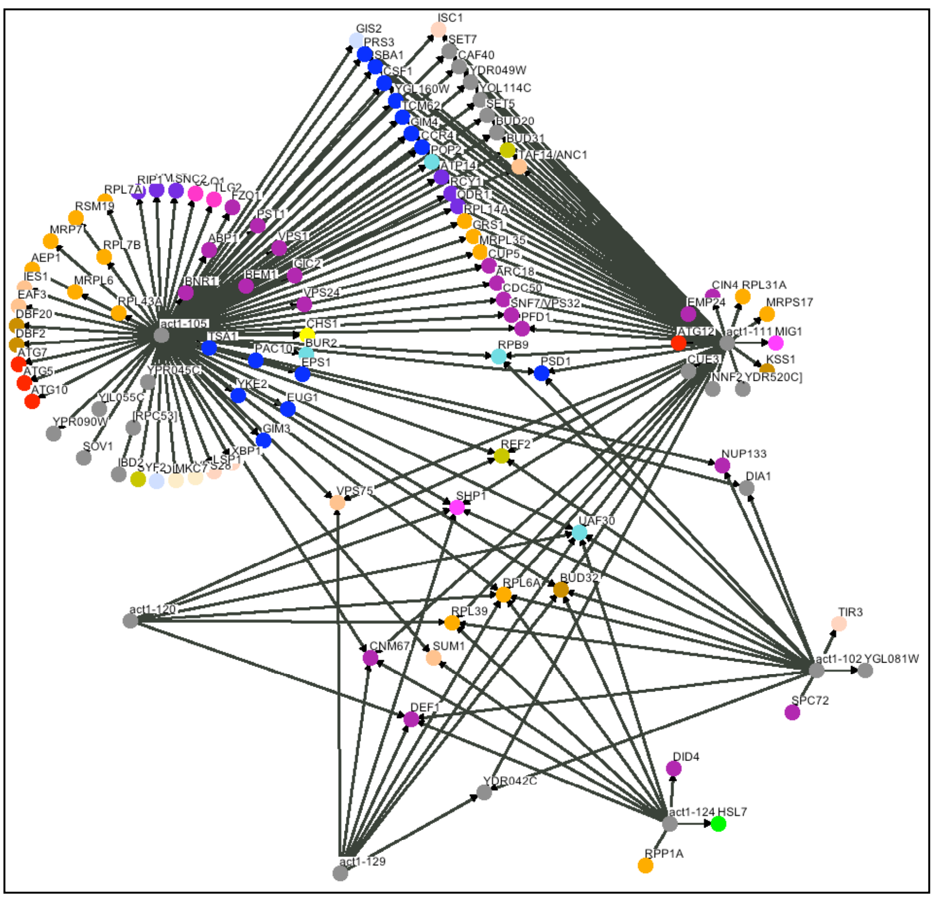


Fig. 5

A


111
124
129
120
105
102

B


act1-105
act1-111
act1-120
act1-102
act1-124
act1-129
ISC1
SET7
GIS2
PRS3
CAF40
TAF14/ANC1
CIN4
RPL31A
MRPS17
MIG1
KSS1
NUP133
DIA1
REF2
VPS75
SHP1
UAF30
RPL6A
BUD32
RPL39
CNM67
SUM1
DEF1
TIR3
YGL081W
SPC72
DID4
YDR042C
HSL7
RPP1A
ATG7
ATG5
ATG10
YPR090W
SOV1
RPL7B
RSM19
MRP7
AEP1
MRPL6
RPL43A
VPS24
CHS1
BUR2
EPS1
YKE2
EUG1
GIM3
RPB9
PSD1
PFD1
SNF7/VPS32
CDC50
ARC18
CUP5
MRPL35
GRS1
RPL14A