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Methods

Identification of Potential Interaction Networks Using Sequence-Based Searches for Conserved Protein-Protein Interactions or “Interologs”

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Protein interaction maps have provided insight into the relationships among the predicted proteins of model organisms for which a genome sequence is available. These maps have been useful in generating potential interaction networks, which have confirmed the existence of known complexes and pathways and have suggested the existence of new complexes and or crosstalk between previously unlinked pathways. However, the generation of such maps is costly and labor intensive. Here, we investigate the extent to which a protein interaction map generated in one species can be used to predict interactions in another species.

Protein interaction maps have provided insight into the relationships among the predicted proteins of model organisms for which a genome sequence is available (Froment-Racine et al. 1997, 2000; Flajolet et al. 2000; Ito et al. 2000, 2001; McCraith et al. 2000; Walhout et al. 2000; Rain et al. 2001). Those maps have been useful particularly in generating potential interaction networks. Such networks have confirmed the existence of known complexes and pathways and have suggested the existence of new complexes and or crosstalk between previously unlinked pathways (Froment-Racine et al. 1997, 2000; Uetz et al. 2000). While the knowledge gained from proteome-wide interaction maps is highly valuable, the generation of such maps is costly and labor intensive. Here, we investigate the extent to which a protein interaction map generated in one species can be used to predict interactions in another species. Systematic BLAST searches for pairs of potential orthologs of known interacting protein partners in *Saccharomyces cerevisiae* have been performed to identify potentially conserved interactions, or “interologs”, in *Caenorhabditis elegans*. Starting from a large number of published yeast two-hybrid interactions between yeast proteins, searches for candidate interologs identified networks of potential physical interactions among *C. elegans* proteins. At least 16% of protein interactions in these networks could be detected in a yeast two-hybrid system, suggesting that these interactions are indeed conserved. In addition, many true interologs were amenable to reverse two-hybrid selections (Vidal 1997). Thus, it should be possible to generate reagents such as interaction-defective alleles and/or interaction-dissociating compounds for the further experimental characterization of potential interologs. The above observations suggest that sequence searches for potential interologs and other “comparative proteomics” strategies performed using the protein interaction maps of model organisms will be useful for drug screening programs in parasites, pathogens, and humans.

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The conceptual translation of complete genome sequences into predicted proteomes and the use of this information to provide a first approximation of functional interactions underlying molecular complexes and regulatory pathways are among the most important challenges of the postgenomic era. One general approach is to use in silico methods to compare the proteomes from two or more organisms and predict interactions based upon presumed or demonstrated protein properties. For example, the Rosetta Stone method predicts functional interactions based upon the observation that pairs of functionally related proteins in one organism are sometimes expressed as single-fusion polypeptides in other organisms (Marcotte et al. 1999). Similarly, phylogenetic profiling is based upon the presumption that groups of functionally interacting proteins are selected together during the course of evolution and may have a tendency to co-exist in the same proteomes (Pellegrini et al. 1999).

The approach described here presumes that large numbers of physically interacting proteins in one organism have “coevolved” so that their respective orthologs in other organisms interact as well. This notion of conserved interactions, or “interologs” (Walhout et al. 2000a), is substantiated by the observation that many interactions in signal transduction pathways or molecular machines are conserved between different species. For example, ~7% of interactions currently available in a *C. elegans* protein interaction database have interologs that already have been described in the literature (Walhout et al. 2000a). In addition, searches for potential interologs already have been used to identify the function of several genes. For example, a functional ortholog of the human retinoblastoma protein was found in *Drosophila* using an interolog-based approach (Du et al. 1996). Here, we investigate the extent to which large-scale systematic searches for interologs may be used to identify potential networks of interaction and discuss methods that could be used in studying such potential networks and/or generate therapeutic agents.

RESULTS AND DISCUSSION

To determine the extent to which a protein interaction map generated in *S. cerevisiae* can be used to predict interactions in *C. elegans*, we utilized two large-scale, two-hybrid interaction maps of yeast proteins, which together contain 1195 interac-

tions (Ito et al. 2000; Uetz et al. 2000). We identified potential *C. elegans* interologs by comparing the yeast proteins involved in these interactions to all predicted worm proteins as described in Methods. From 1195 yeast interactions, 257 potential worm interologs involving 282 proteins were identified (see below). Two hundred sixteen worm pairs (corresponding to a total of 276 worm open reading frames [ORFs]) were tested further. A sample of 71 of these potential interologs (corresponding to 72 yeast interactions) was used both to recapitulate the original yeast interactions and to experimentally test the corresponding worm protein interactions using the two-hybrid system (Table 1). The yeast and worm ORFs were cloned into both DNA binding (DB) and activation domain (AD) two-hybrid vectors and scored for interaction in both possible orientations (DB-X/AD-Y and DB-Y/AD-X) (see Methods). Each step of this analysis, beginning with the amplification of ORFs from the original clones, was performed in duplicate on two separate days. Protein pairs conferring at least one of the four yeast two-hybrid phenotypes (Vidal 1997) in both experiments were scored as positive interactions. Of the 72 potential yeast interactions tested, 26% (19) exhibited a detectable interaction in our version of the two-hybrid system (Table 1). It is likely that this number reflects differences in the conditions of yeast two-hybrid systems such as yeast strains, reporter genes, and the procedures for scoring interactions. Of these 19 interactions, six (31%) also scored positive with *C. elegans* proteins (Table 1). In addition, one more worm interaction was detected although the corresponding yeast interaction was undetected. Finally, we tested the remaining 145 worm potential interologs for their ability to mediate a two-hybrid interaction. After combining the two experiments, 35/216 potential interolog pairs (16%) exhibited a detectable interaction (Fig. 1A–D, Fig. 2, and Table 2).

This suggests that, using this approach, the minimal proportion of true interologs that can be detected between two species that are evolutionarily distant by about 900 million years is between 16% and 31%. By comparison, an average of five interactors per bait typically is obtained using a worm AD-cDNA library representing $\sim 19,000$ genes (2.6×10^{-4}) (Walhout et al. 2000a; Davy et al. 2001). Thus, the frequency of detection of interactions through searches for potential interologs is between 600- and 1100-fold higher than that obtained through conventional two-hybrid screens using random libraries. It should be noted that this approach allows direct testing of interactions that might otherwise be difficult to test in a random two-hybrid screen because of the biased representation of cDNA libraries toward highly expressed genes. In addition, the direct testing of an interaction between two proteins can be performed in 96 well setting and therefore is easily amenable to automation.

The E-value reported by BLAST when searching a database is a measure of the likelihood that the observed similarity could have occurred by chance. Thus, biologists can use this value to infer the homology likelihood. The BLAST E-value between the potential orthologous protein pairs tested in this analysis ranged between 10^{-10} and 10^{-151} . One could expect that a potential interolog obtained with two low E-values is more likely to interact. Interestingly, no detectable correlation was found between the likelihood of homology (E-value) and the likelihood of an interaction being conserved between these two organisms (Fig. 1E). However, it is established that the three-dimensional structure of two proteins can be conserved despite considerable primary sequence divergence (Friedberg et al. 2000). In addition, it is possible that two

Table 1. Summary of Yeast Two-Hybrid Results

| yeast pairs | positive | <i>C. elegans</i> | interolog | positive |
|---------------|----------|-------------------|-------------|----------|
| SMT3 UBC9 | x | K12C11.2 | F29B9.6 | x |
| LSM4 LSM1 | | F32A5.7 | F40F8.9 | |
| LSM4 CKB2 | | F32A5.7 | T01G9.6A | |
| LSM2 LSM1 | | T10G3.6 | F40F8.9 | |
| LSM2 DHH1 | | T10G3.6 | C07H6.5 | |
| TEM1 SMX3 | x | C39F7.4 | ZK652.1 | |
| YPT1 PEP12 | | C39F7.4 | F56A8.7 | |
| TEM1 HMO1 | | C39F7.4 | T20B12.8 | |
| YPT31 PEP12 | | F53G12.1 | F56A8.7 | |
| GCN3 GCN3 | x | ZK1098.4 | ZK1098.4 | x |
| CKA2 CKB2 | x | B0205.7 | T01G9.6A | x |
| CDC7 DHH1 | | C34G6.5 | C07H6.5 | |
| RPT3 RPT4 | | F23F12.6 | F23F1.8 | |
| RPT3 RPT3 | | F23F12.6 | F23F12.6 | |
| RPT3 RPT5 | x | F23F12.6 | F56H1.4 | |
| VTI1 PEP12 | | Y57G11C.4 | F56A8.7 | |
| YLR432W GCN3 | | T22D1.3 | ZK1098.4 | |
| YRB1 NAP1 | | F59A2.1 | D2096.8 | |
| CPA1 CKB2 | | D2085.1 | T01G9.6A | |
| LSM4 LSM7 | | F32A5.7 | ZK593.7 | x |
| SKP1 MET30 | x | F46A9.5 | F55B12.3 | x |
| SKP1 RUB1 | | F46A9.5 | F45H11.2 | |
| SKP1 BDF1 | | F46A9.5 | F13C5.2 | |
| SKP1 SGT1 | | F46A9.5 | D1054.3 | |
| LSM2 LSM5 | | T10G3.6 | F28F8.3 | |
| LSM2 LSM7 | | T10G3.6 | ZK593.7 | |
| LSM2 SMD2 | | T10G3.6 | C52E4.3 | |
| TEM1 ECI1 | x | C39F7.4 | R06F6.9 | |
| TEM1 DMC1 | | C39F7.4 | Y43C5A.6 | |
| TEM1 LAC1 | | C39F7.4 | C09G4.1 | |
| TEM1 MNS1 | | C39F7.4 | ZC410.3 | |
| TEM1 SOR1 | x | C39F7.4 | R04B5.5 | |
| TEM1 KIN3 | | C39F7.4 | F19H6.1 | |
| TIF35 GNA1 | | F22B5.2 | B0024.12 | |
| SNF4 GAL83 | x | F55F3.1 | Y111B2C.H | |
| CDC7 YOR006C | | C34G6.5 | F52C12.2 | |
| RPT3 YGR232W | x | F23F12.6 | F40G9.1 | |
| LSM5 LSM7 | x | F28F8.3 | ZK593.7 | x |
| GDH1 LSM1 | x | ZK829.4 | F40F8.9 | |
| RAD51 UBC9 | | Y43C5A.6 | F29B9.6 | |
| RAD51 YMR233W | | Y43C5A.6 | T24G10.2 | |
| CLB2 CKS1 | | T06E6.2 | C09G4.3 | |
| CLB3 CKS1 | x | T06E6.2 | C09G4.3 | |
| CLB2 MUS81 | | T06E6.2 | C43E11.2 | |
| CLB2 FPR1 | | T06E6.2 | Y18D10A.19C | |
| CLB2 YHR035W | | T06E6.2 | Y113G7A.3 | |
| SRP1 CAR1 | | F32E10.4 | T21F4.1 | |
| SRP1 ICL1 | | F32E10.4 | C05E4.9 | |
| SRP1 PHO13 | | F32E10.4 | F44E7.2 | |
| SRP1 SOR1 | x | F32E10.4 | R04B5.5 | |
| SRP1 YMR226C | | F32E10.4 | F28H7.2 | |
| SRP1 MET17 | | F32E10.4 | ZK1127.10 | |
| SRP1 TSA1 | | F32E10.4 | F09E5.2 | |
| FZF1 CKB2 | | F53F8.1 | T01G9.6A | |
| CLB1 CKS1 | | H31G24.4 | C09G4.3 | |
| YMR269W GCN3 | | F49H12.5 | ZK1098.4 | |
| YDL246C SOR1 | x | R04B5.5 | R04B5.5 | x |
| HTA1 NAP1 | x | C50F4.13 | D2096.8 | |
| PPA2 GCN3 | | C47E12.4 | ZK1098.4 | |
| GPD2 GNA1 | | K11H3.1 | B0024.12 | |
| PRP46 PEP12 | x | D1054.15 | F56A8.7 | |
| PRE10 GNA1 | | ZK945.2 | B0024.12 | |
| SNF4 COQ5 | | Y111B2C.H | ZK652.9 | |
| SNF4 YDL214C | | Y111B2C.H | R02C2.1 | |
| SNF4 YMR291W | x | Y111B2C.H | K12C11.4 | |
| RPS8B CKS1 | | F42C5.8 | C09G4.3 | |
| RPS8B GNA1 | | F42C5.8 | B0024.12 | |
| PPG1 HSC82 | | Y75B8A.30 | C47E8.5 | |
| PPG1 PPT1 | | Y75B8A.30 | Y39B6B.FF | |
| PPG1 CPR6 | | Y75B8A.30 | Y75B12B.5 | |
| NAB2 GCN3 | | T21B6.3 | ZK1098.4 | |
| UBC9 UBC9 | x | Y59A8C.A | F29B9.6 | |

Summary of yeast two-hybrid results for a random set of 72 yeast interactions from two large-scale two-hybrid interaction maps of yeast proteins (Ito et al. 2000; Uetz et al. 2000) and of their corresponding *C. elegans* potential interologs. Interactions that were detected in both organisms are highlighted blue. Interactions detected only in yeast are highlighted green. One interaction was detected in *C. elegans* although undetected in yeast, and is highlighted orange.

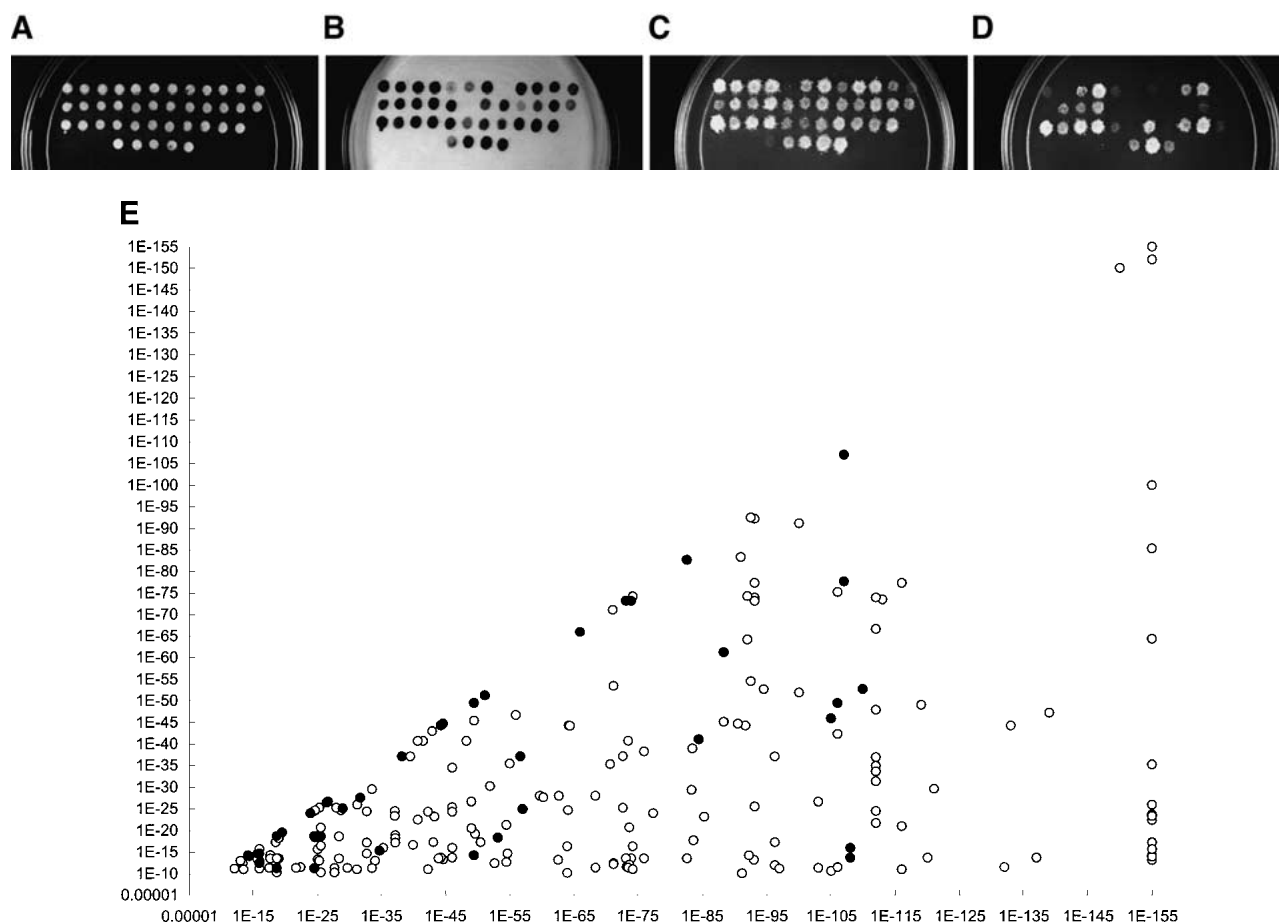


Figure 1 Experimentally verified interactions between *Saccharomyces cerevisiae* and *Caenorhabditis elegans*. (A–D) Yeast diploid cells expressing each of 35 *C. elegans* potential interologs. Pairs are arranged in the order described in Table 2. The five patches at the bottom are controls (negative control on the left side and controls of increasing interaction strength towards the right side). See Vidal (1997) for a detailed description of these controls. (B) β -Galactosidase assay to detect the expression of *GAL1::lacZ*. (C) Growth assay on SC-Leu-Trp-His, +20 mM 3AT plates to detect the expression of *GAL1::HIS3*. (D) Growth assay on SC-Leu-Trp-Ura plates to detect the expression of *SPAL10::URA3*. (E) Conservation of interactions. Each *C. elegans* protein pair tested was plotted according to two *E*-values. The first *E*-value corresponds to the conservation between the X (from yeast) and X' (from *C. elegans*) proteins while the second *E*-value corresponds to the conservation between the Y (from yeast) and Y' (from *C. elegans*) proteins. The smaller of the two *E*-values was plotted on the X-axis and the greater on the Y-axis. The *C. elegans* protein pairs that tested positive in the two-hybrid system are labeled in black.

interacting proteins may have coevolved such that only discrete interacting domains were conserved.

The data described above suggest that the approach of sequence-based searches for candidate interologs can be used globally to identify potential networks of interactions. However, such networks only can be considered as biological hypotheses. Hence, we investigated methods to generate reagents that can be used to study potential interaction networks identified by interolog searches. The reverse two-hybrid system provides a genetic selection that allows the rapid identification of *cis*-acting mutations or *trans*-acting molecules that dissociate potential interactions (Vidal 1997). The two-hybrid *SPAL10::URA3* inducible reporter gene (Vidal et al. 1996) confers sensitivity to 5-Fluoroorotic acid (5-FOA). The dissociation of the yeast two-hybrid interaction confers a selective advantage allowing screens for dissociating compounds or for mutations that prevent the normal association of a protein pair using positive selection. Such reagents can be used back in vivo to characterize the role of the corresponding protein-protein interactions (Endoh et al. 2001).

To test the degree to which the reverse two-hybrid system can be applied to our network of identified interologs, we determined the percentage of the interactions described above that could be counter-selected on media containing 5-FOA (Vidal 1997). Starting from the 35 true worm interologs described above, 77% (27/35) of *C. elegans* interactions were detected as 5-FOA sensitive (Fig. 3). Because the reverse two-hybrid system can be automated (Endoh et al. 2001), it is possible that relatively large numbers of yeast two-hybrid interactions that emerge from interolog searches could indeed be tested back in the relevant biological settings.

This work suggests that interaction maps from one species may be useful in predicting interactions in another species and may provide insight into the function of otherwise uncharacterized proteins. In addition, the identification of an interolog provides additional support for the validity of the initial interaction found in the “reference” species. This may be most meaningful if the only evidence for the original interaction comes, itself, from a high-throughput experiment. When the function of one of the proteins in the starting spe-

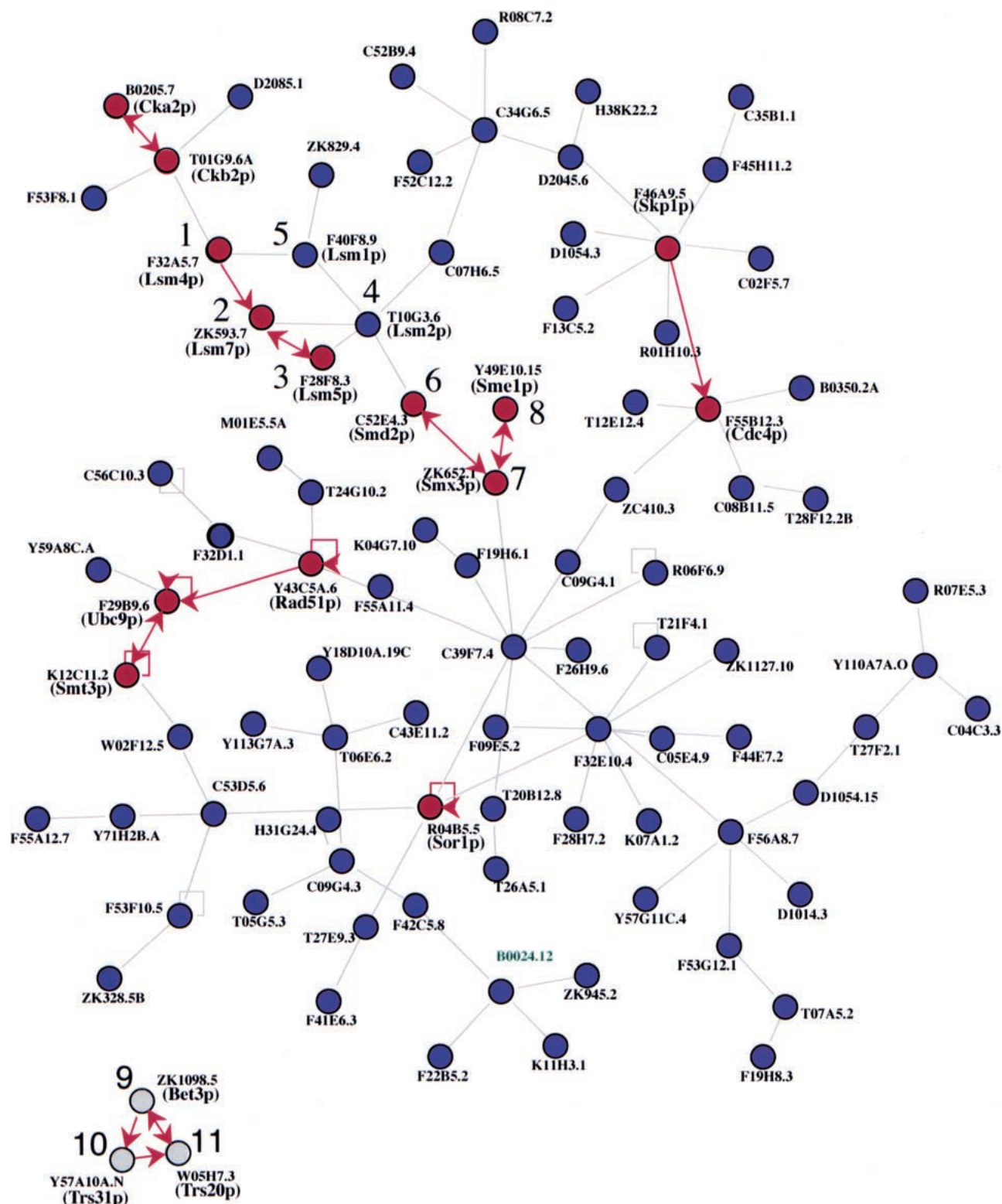


Figure 2 A predicted network of *Caenorhabditis elegans* protein interactions involving a subset of the 216 pairs of protein interactions tested in our analysis. Corresponding yeast proteins names are shown in parentheses. Nodes represent proteins and edges represent an interaction between two yeast proteins. Interactions also detected in *C. elegans* are represented by red arrows. Interactions detected in both orientations are represented by double-headed red arrows. Interactions involving proteins 1–8 have been implicated in RNA processing in yeast and the interaction cluster (Nos. 9–11) involves three proteins whose potential yeast orthologs have been found in TRAPP complexes.

Table 2. Summary of Yeast Two-Hybrid Results

| Protein 1 | | Protein 2 | | | | | | | | |
|---|-------------------|---|---|------|-------|---------|-----------|-----------|------|-------|
| <i>S. cerevisiae</i> | <i>C. elegans</i> | <i>S. cerevisiae</i> | <i>C. elegans</i> | | | | | | | |
| % identity | E value | Extent of alignment in shortest protein | E value | | | | | | | |
| Extent of alignment in shortest protein | % identity | <i>S. cerevisiae</i> | Extent of alignment in shortest protein | | | | | | | |
| % identity | E value | <i>C. elegans</i> | % identity | | | | | | | |
| SNRNP complex | SMX3 | ZK652.1 | 2.00E-19 | 87% | 48.9% | SMD2 | C52E4.3 | 8.00E-27 | 99% | 49.2% |
| SNRNP complex | SME1 | Y49E10.15 | 2.00E-19 | 94% | 48.5% | SXM3 | ZK652.1 | 2.00E-19 | 85% | 48.2% |
| SNRNP complex | LSM4 | F32A5.7 | 4.00E-13 | 84% | 21.6% | LSM7 | ZK593.7 | 1.00E-16 | 85% | 36.0% |
| SNRNP complex | LSM5 | F28F8.3 | 2.00E-15 | 96% | 37.4% | LSM7 | ZK593.7 | 1.00E-16 | 87% | 36.0% |
| Polyadenylation factor I complex | YTH1 | F11A10.3 | 2.00E-36 | 96% | 7.2% | FIP1 | F32D1.9 | 5.00E-16 | 93% | 13.8% |
| TRAPP complex | TRS20 | W05H7.3 | 2.00E-20 | 57% | 32.0% | BET3 | ZK1098.5 | 3.00E-26 | 96% | 32.8% |
| TRAPP complex | TRS31 | Y57A10A.n | 5.00E-12 | 79% | 16.5% | TRS20 | W05H7.3 | 2.00E-20 | 93% | 32.0% |
| TRAPP complex | TRS31 | Y57A10A.n | 5.00E-12 | 79% | 16.5% | BET3 | ZK1098.5 | 3.00E-26 | 94% | 32.8% |
| vesicular transport | VPS27 | C07G1.5 | 1.00E-30 | 96% | 19.3% | YHL002W | C34G6.7 | 8.00E-27 | 96% | 24.1% |
| Recombinosome | RAD51 | Y43C5A.6 | 2.00E-84 | 95% | 46.0% | RAD51 | Y43C5A.6 | 2.00E-84 | 96% | 46.0% |
| Recombinosome/protein degradation | RAD51 | Y43C5A.6 | 1.00E-106 | 96% | 46.0% | UBC9 | F29B9.6 | 3.00E-51 | 96% | 50.0% |
| protein degradation | UBC9 | F29B9.6 | 3.00E-51 | 85% | 50.0% | UBC9 | F29B9.6 | 3.00E-51 | 96% | 50.0% |
| protein degradation | SMT3 | K12C11.2 | 6.00E-15 | 85% | 36.3% | UBC9 | F29B9.6 | 3.00E-51 | 85% | 50.0% |
| protein degradation | SMT3 | K12C11.2 | 6.00E-15 | 74% | 36.3% | SMT3 | K12C11.2 | 6.00E-15 | 95% | 36.3% |
| protein degradation | LAP4 | F01F1.9 | 2.00E-46 | 96% | 23.6% | LAP4 | F01F1.9 | 2.00E-46 | 96% | 23.6% |
| protein degradation | NPL4 | F59E12.5 | 3.00E-86 | 87% | 28.0% | UFD1 | F19B6.2 | 9.00E-43 | 95% | 33.6% |
| Proteasome 19s and 26s proteins | RPN11 | K07D4.3 | 1.00E-107 | 100% | 61.9% | RPN8 | R12E2.3 | 2.00E-79 | 96% | 38.7% |
| Protein modification/conjugation | ULA1 | C26E6.8 | 5.00E-20 | 99% | 25.3% | UBA3 | F11H8.1 | 6.00E-55 | 97% | 27.2% |
| Farnesyltransferase complex | RAM1 | F23B12.6 | 9.00E-59 | 95% | 33.5% | RAM2 | R02D3.5 | 1.00E-26 | 82% | 26.2% |
| GIM complex (protein folding) | GIM5 | R151.9 | 9.00E-15 | 83% | 28.0% | YKE2 | F21C3.5 | 4.00E-15 | 94% | 30.2% |
| eIF2B complex | GCN3 | ZK1098.4 | 5.00E-46 | 63% | 35.4% | GCN3 | ZK1098.4 | 5.00E-46 | 77% | 35.4% |
| SCF-Cdc4p-Sic1p complex | SKP1 | F46A9.5 | 7.00E-39 | 93% | 40.1% | CDC4 | F55B12.3 | 2.00E-58 | 98% | 21.1% |
| Protein kinase CKII complex | CKA2 | B0205.7 | 1.00E-110 | 95% | 51.3% | CKB2 | T01G9.6a | 2.00E-54 | 88% | 39.0% |
| SNF1 protein kinase complex | SNF1 | T01G8.1 | 1.00E-108 | 78% | 35.3% | GAL83 | F55F3.1 | 2.00E-14 | 45% | 20.7% |
| Transcription factor complex | HAP3 | W10D9.4 | 3.00E-27 | 99% | 14.6% | HAP5 | F23F1.1 | 2.00E-20 | 99% | 8.9% |
| carbohydrate metabolism | SOR1 | R04B5.5 | 7.00E-75 | 83% | 41.2% | SOR1 | R04B5.5 | 1.00E-75 | 83% | 41.2% |
| carbohydrate metabolism | YJR024C | ZC373.5 | 3.00E-28 | 88% | 29.2% | YJR024C | ZC373.5 | 3.00E-28 | 88% | 29.2% |
| nucleotide metabolism | CDD1 | C47D2.2 | 3.00E-21 | 84% | 36.0% | CDD1 | C47D2.2 | 3.00E-21 | 99% | 36.0% |
| nucleotide metabolism | PRS5 | R151.2 | 1.00E-47 | 97% | 32.1% | PRS2 | R151.2 | 1.00E-105 | 97% | 50.1% |
| nucleotide metabolism | YLR328W | F26H9.4 | 1.00E-25 | 99% | 17.9% | YLR328W | F26H9.4 | 1.00E-25 | 99% | 17.9% |
| nucleotide metabolism | YNK1 | F25H2.5 | 6.00E-53 | 93% | 61.7% | YNK1 | F25H2.5 | 6.00E-53 | 92% | 61.7% |
| amino acid metabolism | PRO3 | M153.1 | 2.00E-28 | 100% | 27.4% | PRO3 | M153.1 | 1.00E-28 | 100% | 27.4% |
| metabolism (?) | FOL2 | F32G8.6 | 1.00E-67 | 95% | 50.8% | FOL2 | F32G8.6 | 1.00E-67 | 95% | 50.8% |
| unknown function | YJR072C | C34E10.2 | 5.00E-90 | 91% | 40.0% | YLR243W | Y75B8A.14 | 6.00E-63 | 97% | 41.7% |
| unknown function | YDR267C | Y18D10A.9 | 2.00E-33 | 94% | 29.3% | YHR122W | F45G2.10 | 3.00E-29 | 84% | 29.3% |

The yeast protein pairs involved in the 35 conserved interactions could be divided into several general functional groups of core biological processes as described in the Yeast Protein Database YPD (<http://www.proteome.com>): (1) RNA metabolism, (2) vesicular transport, (3) protein metabolism (synthesis, modification, degradation), and (4) general metabolism. The *C. elegans* and *S. cerevisiae* protein names, their corresponding BLAST E-values, and the extent of the alignment in the shortest protein for each pair is shown.

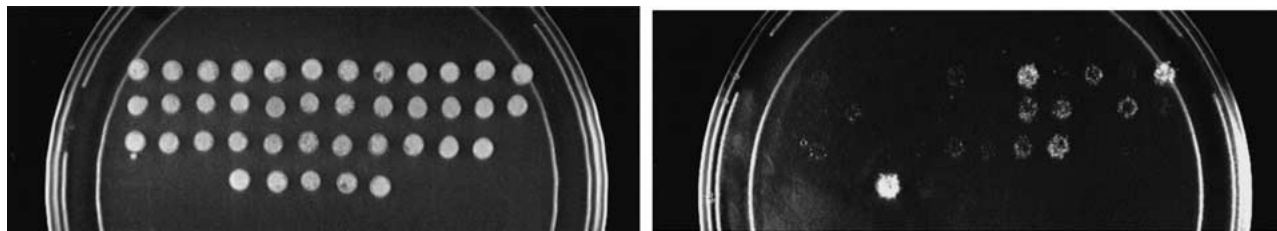


Figure 3 Assessment of the reverse two-hybrid system as a method to further study *Caenorhabditis elegans* protein interactions. Reverse two-hybrid system. Each yeast diploid expressing one of the 35 conserved interacting protein pairs from *C. elegans* was tested to identify those that are sensitive to 5-Fluoroorotic acid (5-FOA). Pairs are arranged in the order described in Table 2. Diploids were replica plated onto both SC-Leu-Trp (A) and SC-Leu, -Trp + 0.2% 5-FOA (B). Twenty-seven of thirty-five pairs (77%) were 5-FOA sensitive.

cies has not been characterized, a “guilt by association” annotation also can be applied with more confidence. One potential therapeutic application of this approach would be to identify interactions conserved between a well-characterized model organism and a related pathogen. The majority of the interactions conserved between the distantly related species *S. cerevisiae* and *C. elegans* seem to be involved primarily in core metabolic processes. However, it is reasonable to predict that certain interactions conserved between more closely related organisms such as *Drosophila* and other insects such as mosquitoes, or *C. elegans* and other nematodes such as *Ascaris lumbricoides*, would be specific to the very closely related species. These interactions could be subjected to the reverse two-hybrid analysis (Vidal 1997) to identify reagents that are capable of dissociating them (Young et al. 1998). Such reagents might be valuable candidates for therapeutic agents.

METHODS

Potential Ortholog Identification

To identify potential orthologs of yeast proteins in *C. elegans*, we used BLASTP to search a *C. elegans* database generated from the ORF predictions in version WS7 of ACeDB. Only matches with *E*-values lower than 10^{-10} were considered. Although other studies (Snel et al. 1999) have considered as potential orthologs two proteins that are each other's best match in their respective genomes, we decided to systematically select as potential orthologs the best *C. elegans* matches for the yeast proteins. Indeed, orthology can be a one-to-many or many-to-many relationship (Tatusov et al. 1997). Thus, in the few cases where the same *C. elegans* protein is the best match for two distinct yeast proteins, we consider this *C. elegans* protein to be the potential orthologs for both yeast proteins. We recognize that this procedure allowed us to evaluate only one potential *C. elegans* ortholog per yeast protein.

PCR and Cloning

Complete *C. elegans* ORFs that correspond to 282 potential *C. elegans* orthologs were PCR amplified from a mixed-stage cDNA library (Walhout et al. 2000b). The control *S. cerevisiae* ORFs were amplified from S288C genomic DNA. PCR products were generated using ORF-specific primers designed by the program OSP (Hillier and Green 1991) and tailed with the AttB1 (5') and AttB2 (3') Gateway recombinational cloning sequences (Walhout et al. 2000a,b; Reboul et al. 2001). *C. elegans* and yeast ORFs were cloned into the Gateway Entry vector (INVITROGEN) as described with several modifications (Reboul et al. 2001).

Each ORF was PCR amplified from its corresponding Entry clone for subsequent cloning into both Gal4p DNA binding domain-fusion (DB) and Gal4p activation domain-fusion

(AD) yeast two-hybrid vectors pGBT9 (Clontech) and pACT2 (Endoh 2000), respectively. ORFs to be cloned into the DB-fusion vector were amplified from Entry clones using the primers DB-B1: 5'TAGTAACAAAGGTCAAAGACAGTTGACTGTATCGTTCGAGGTTGTACAAAAAAGCAGGCT-3' and PGBT9.B2-TERM: 5'-AAATCAT AAATCATAAGAAATTCGC-CCGGAATTAGCTTGGTTGTACAAGAAAGCTGGGT-3'. ORFs to be cloned into the AD vector were amplified from Entry clones using the primers AD-B1: 5'-CTATTCGATGATGAAGATACCCACCAAACCCAAAAAAGAGTTGTACAAAAAGCAGGCT-3' and pACT2.B2-Term: 5'-TGAAGTGAACCTGCGGGGTTTTTTCAGTATCTACGATTCATTTGTACAAGAAAGCTGGGT-3'.

Yeast Transformation/Gap-Repair and Mating

Yeast transformation/gap-repair reactions were performed in 96-well plates as described elsewhere (Walhout and Vidal 2001) with several modifications. MaV103 (*MATa*) cells were transformed with the 2 μ m AD-fusion vector pACT2-GFP and the corresponding AD-PCR fragments. MaV203 (*MAT α*) cells were transformed with the 2 μ m DB-fusion vector pGBT9 and the corresponding DB-PCR fragments. Five microliters of each transformation mix were spotted in 96-spot format onto synthetic complete (SC) plates lacking leucine or tryptophane (SC-Leu or SC-Trp). Yeast carrying the AD-fusion constructs were mated to yeast cells carrying the DB-fusion constructs in “96-spot” format by replica plating both onto YEPD plates. To identify self-activator DB-fusion proteins, each transformant bearing a DB-fusion protein construct was mated to a transformant carrying an empty AD vector. Following 1 d of growth at 30°C, mating plates were replica plated to SC-Leu-Trp plates to select for diploids.

Yeast Two-Hybrid Analysis

Diploids were transferred to a 15-cm nylon filter on a YEPD plate for subsequent β -galactosidase assays and to an SC-Leu-Trp-Ura plate and an SC-Leu-Trp-His plate supplemented with 20 mM 3-aminotriazole (3AT) to assay for the *SPAL10::URA3* and *GAL1::HIS3* reporter gene activity, respectively. The identity of both hybrid proteins from diploids exhibiting a positive yeast two-hybrid phenotype was determined by sequencing the corresponding ORFs. The identity of all ORFs tested was verified by sequencing.

5-FOA Sensitivity

The two-hybrid inducible *SPAL10::URA3* reporter gene drives expression of the *URA3* gene, which is involved in uracil biosynthesis, and which also is able to catalyze the conversion of 5-FOA into a toxic compound. Thus, yeast diploids expressing interacting partners fused to AD and DB are able to grow on medium lacking uracil, but do not grow on medium containing 5-FOA. This allows for the screening of either dissociating compounds or interaction-defective alleles that will confer a

positive growth selection. The 5-FOA negative selection is, however, dependent on the strength of the two-hybrid interaction. We identified interactions that are 5-FOA sensitive and thus suitable for reverse two-hybrid screening, by replica plating diploids onto both SC-Leu-Trp and SC-Leu-Trp + 0.2% 5-FOA.

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