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Resource

A Systematic Approach for the Genetic Dissection of Protein Complexes in Living Cells

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SUMMARY

Cells contain many important protein complexes involved in performing and regulating structural, metabolic, and signaling functions. One major challenge in cell biology is to elucidate the organization and mechanisms of robustness of these complexes in vivo. We developed a systematic approach to study structural dependencies within complexes in living cells by deleting subunits and measuring pairwise interactions among other components. We used our methodology to perturb two conserved eukaryotic complexes: the retromer and the nuclear pore complex. Our results identify subunits that are critical for the assembly of these complexes, reveal their structural architecture, and uncover mechanisms by which protein interactions are modulated. Our results also show that paralogous proteins play a key role in the robustness of protein complexes and shape their assembly landscape. Our approach paves the way for studying the response of protein interactomes to mutations and enhances our understanding of genotype-phenotype maps.

INTRODUCTION

Mapping the relationships between genes (the genotype) and cellular processes (the phenotype) requires a determination of how proteins interact with one another (Diss et al., 2013; Vidal et al., 2011). Central to interactomes are protein complexes that perform a large number of regulatory, metabolic, structural, and signaling functions (Alberts, 1998). One of the most pressing challenges in cell and systems biology is to understand how these complexes are structurally organized in vivo, how they are regulated, and how they are affected by genetic perturbations (Ideker and Krogan, 2012). This would provide insights into genotype-phenotype maps by showing how mutations affect molecular pathways and complexes, and how these in turn affect cellular functions.

One powerful way to assess the architecture and robustness of interactomes is to measure their response to genetic perturbations (Ideker and Krogan, 2012). For instance, one can ask how gene A affects the protein-protein interaction (PPI) between proteins B and C by deleting A and measuring the B-C interaction (Figure 1A). One could then use these perturbations to study the architecture of protein complexes, most of which have no known structure (Benschop et al., 2010), by identifying dependencies among subunits (Lee et al., 2011). These perturbations would also reveal the molecular mechanisms underlying genotype-phenotype maps at intermediate levels. In previous studies, investigators mostly studied the robustness of protein complexes indirectly by measuring the fitness effects of single and double gene deletion of subunits (Bandyopadhyay et al., 2008; Baryshnikova et al., 2010a). Accordingly, robustness has not been assessed at the molecular level, i.e., these studies did not reveal how the complexes themselves respond to perturbations in terms of their structural organization.

The yeast *Saccharomyces cerevisiae* is the best-suited model for addressing these questions. In principle, one can apply any of the methods designed for PPI detection by deleting genes (80% of genes are not essential; Giaever et al., 2002) and comparing PPIs in wild-type and deletion strains. However, this is a challenging task because the potential number of gene-by-PPI interactions is immensely large, even when only known PPIs are considered (Diss et al., 2013). Furthermore, it would be more informative to study PPIs among endogenously expressed proteins and in living cells in order to preserve the stoichiometry of interactomes. Indeed, the cellular context may be key to understanding the regulatory and structural roles of proteins in PPIs and the corresponding responses to perturbations (Figure 1A).

Here, we adapted the yeast DHFR protein-fragment complementation assay (DHFR-PCA) (Tarassov et al., 2008) to meet these criteria. We examined the effects of perturbations on two contrasting protein complexes: one small, nonessential complex, the retromer, and one large, essential complex, the nuclear pore complex (NPC). The retromer is a pentameric complex that recycles endosomal receptors back to the *trans*-Golgi network (Seaman et al., 1998; Figure 1B) and plays key roles in neurodegenerative diseases in mammals (Wen et al., 2011). Although its architecture has been investigated using a variety of methods (reviewed in McGough and Cullen (2011)), the mode of assembly of the retromer in living cells and the role of each subunit remain to be completely described (Collins, 2008; Norwood et al., 2011). The yeast NPC (Figure 1C) is a large protein complex that comprises 27 core proteins (Alber et al., 2007) and 25 associated proteins (Cherry et al., 2012). The NPC selectively transports cargos across the nuclear envelope, and its architecture, mode of assembly, and evolutionary history are particularly challenging to assess, given its large size (Fernandez-Martinez and Rout, 2012).

Using our systematic approach, we showed how the genetic perturbation of these protein complexes may serve to

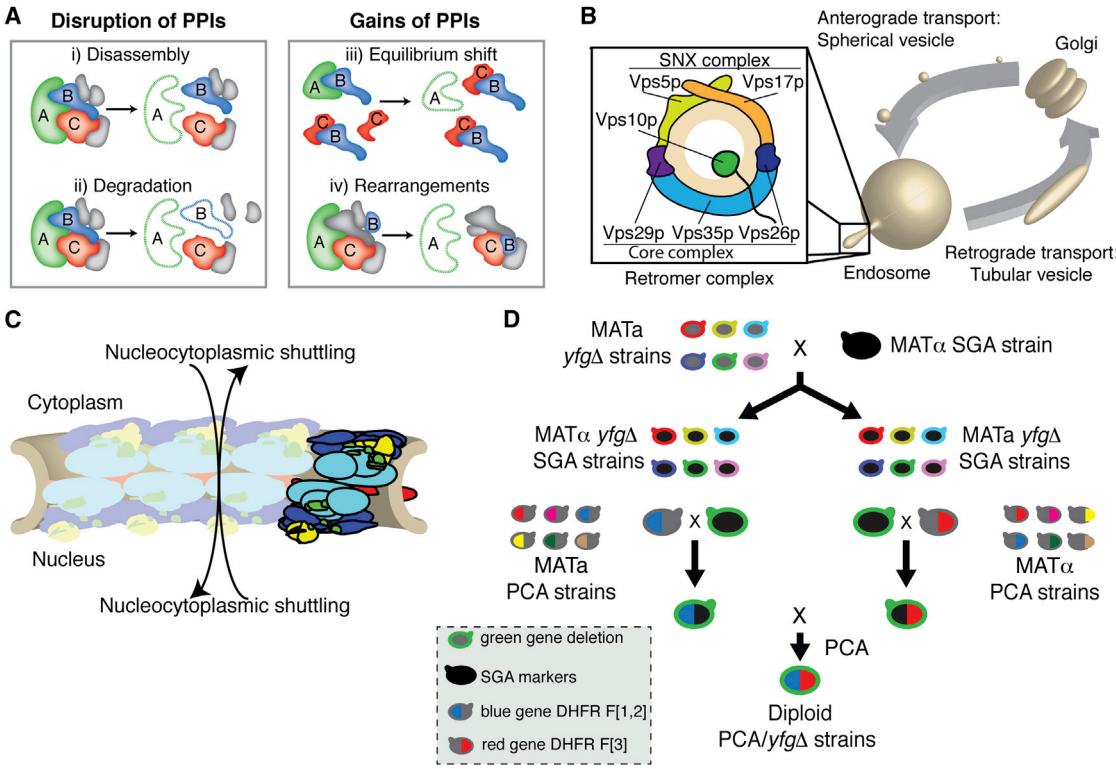

Figure 1. Genetic Dissection of Protein Interactomes

(A) Changes in PPIs could arise through different, nonexclusive mechanisms. The deletion of gene A (dashed line) could lead to the disruption of the B-C interaction if (i) protein A is an adaptor protein for protein B and C or (ii) protein A stabilizes protein B and/or C (left). The deletion of A could lead to new B-C interactions if (iii) proteins C and A normally compete for B or (iv) a complex can adopt a new configuration upon the deletion of A (right). Empty shapes are absent proteins.

(B) Current model of the architecture of the retromer and the associated transmembrane sorting receptor Vps10p (Seaman et al., 1998).

(C) Current model of the architecture of the NPC. Subcomplexes are organized into five different rings: membrane (red), inner (cyan), linker (yellow), outer (dark blue), and FG-nucleoporins (green) (Alber et al., 2007).

(D) Systematic strain construction. SGA markers are introduced in the yeast deletion collection (4,293 strains). SGA deletion strains are crossed with PCA strains to introduce gene deletions and the DHFR-fused genes into the same haploid background. The resulting haploid strains are crossed to measure PPIs in a deletion background. PPIs can also be measured in strains that are heterozygous for one or two deletions (not shown). Control strains (wild-type, *HO* deletion) follow the same procedure.

See also Figure S1 and Tables S1, S5, S6, and S7.

reconstruct their architecture. In addition, we showed that paralogous genes contribute to the structural robustness of the NPC by limiting the impact of perturbations on PPIs and thus influencing the genotype-phenotype map at the molecular level.

RESULTS

Systematic Perturbation of Protein Interactomes

The DHFR-PCA (henceforth called PCA) is an in vivo survival assay that allows one to measure direct and near-direct (close-proximity) PPIs on a large scale (Gagnon-Arsenault et al., 2013; Tarassov et al., 2008; Figure S1A) and in a quantitative manner (Freschi et al., 2013). We combined PCA with synthetic genetic array (SGA) tools (Costanzo et al., 2010) to introduce gene deletions (Giaever et al., 2002) into the PCA strains (Figures 1D and S1B). The yeast deletion collection (4,293 strains) was first mated with a strain harboring the SGA markers that allow high-throughput ploidy and mating type selection (Costanzo et al., 2010; Figures 1D and S1B) and then with PCA strains. In principle, this approach allows one to measure any PPI in both homozygous and heterozygous deletion strains (at one or two loci), and consequently to detect potential dosage effects on PPIs.

Systematic Perturbation of the Retromer

We first used our approach to perform a systematic screen involving the five subunits and the associated receptor of the retromer as PCA baits (Figure 1B). As preys, we used all of these proteins plus 19 others that are putatively associated with the complex (Stark et al., 2011; Table S1). We measured 147 PPIs in 28 genetic backgrounds, corresponding to the wild-type (*ho*Δ, the mock deletion) and the six retromer subunit deletion backgrounds in all possible homozygous, heterozygous, and double-heterozygous genotypes, for a final, high-quality set of 3,831 unique deletion-bait-prey combinations (Table S2).

We measured colony size on high-density arrays to estimate PCA signal. We computed an interaction score (I score, average