Multiple functionalities of molecular chaperones revealed through systematic mapping of their interaction networks

Published, Papers in Press, September 7, 2018, DOI 10.1074/jbc.TM118.002805

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Edited by Norma M. Allewell

Chaperones are a highly interactive group of proteins that function globally in many cellular processes involved in maintaining protein homeostasis. Traditional biochemical assays typically do not provide a complete view of the intricate networks through which chaperones collaborate to promote proteostasis. Recent advances in high-throughput systematic analyses of chaperone interactions have uncovered that chaperones display a remarkable cooperativity in their interactions with numerous client proteins. This cooperativity has been found to be a fundamental aspect of a properly functioning cell. Aberrant formation or improper regulation of these interactions can easily lead to disease states. Herein, we provide an overview of the use of large-scale interaction assays, whether physical (protein–protein) or genetic (epistatic), to study chaperone interaction networks. Importantly, we discuss the ongoing need for such studies to determine the mechanisms by which protein homeostasis is controlled in the cell.

To ensure that proteins reach their native state, cells have evolved a complex machinery of molecular chaperones that assist in the proper folding of newly synthesized polypeptides, as well as in the rescue of existing ones from stress-induced misfolding and aggregation (1). Chaperones are highly conserved across all organisms and are classified into families based on their functional similarities. Some chaperones function with partner proteins, termed cochaperones, that themselves can bind protein clients or can regulate chaperone functions. Here, we term chaperones and cochaperones as CCos.[3]

For CCos to act on a myriad of non-native clients across all cellular compartments in the dense intracellular space, they have adapted to work together in a systematic manner involving a certain functional hierarchy (2–4). This interconnectivity among CCos allows for a rapid and robust response to maintain protein homeostasis in the event of a stress insult (5). High-throughput experiments are needed to elucidate such interconnectivity of CCos. Such experiments determine interactions involving CCos and client proteins or between a CCo and another CCo. In this Review, we summarize efforts to build CCo networks using various model organisms. We also survey the diversity of experimental methods used and what novel aspects of CCo networks they reveal.

Historical perspective of studies on chaperone networks

Earlier work on molecular chaperones mainly involved studying the biochemical and biophysical mechanisms of a single or a small subset of CCo proteins. But CCos rarely function in isolation from each other; instead, they typically function as part of a network. The past 13 years have shown an increased interest in studying CCos at a system-wide level, which particularly concentrated on deciphering client protein repertoires interacting with or coexpressed with a single CCo member or a single CCo family (6–15). Other studies also involved looking at multiple CCo families and various client repertoires in different species (Fig. 1) (16–19). The need to understand the underlying multichaperone functions, such as protein client hand-off between CCos, and the extent of CCo engagement on the proteome and the interplay of CCos acting both in quality control as well as in protein biogenesis has become critical and requires a systems approach.

The emergence of CCo systems studies was driven mainly by the use of the yeast *Saccharomyces cerevisiae* as a model organism because of the ease by which it can be genetically manipulated and the availability of many early high–throughput approaches to study interactions in this organism (20, 21). Yeast cells provided an ideal platform to obtain an overview of how CCos function together in a network. This was demonstrated by our group, which presented the first study of this kind using Hsp90 in 2005 (7) and then using multiple CCos in 2009 (17) (Fig. 1). In the latter work, we uncovered a system of chaperone subnetworks, and by looking at their connectivity, we

This work was supported in part by Canadian Institutes of Health Research Grants MOP-93778 and MOP-81256 (to W. A. H.). This is the seventh article in the JBC Reviews series "Molecular chaperones and protein quality control." The authors declare that they have no conflicts of interest with the contents of this article.

[1] Supported by a Canadian Institutes of Health Research Training Program in Protein Folding and Interaction Dynamics, Principles and Diseases Fellowship, by a University of Toronto Fellowship from the Department of Biochemistry, and by an Ontario Graduate Scholarship.

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[3] The abbreviations used are: CCo, chaperone and cochaperone; PPI, protein–protein interaction; Y2H, yeast two-hybrid; MYTH, membrane yeast two-hybrid; AP-MS, affinity purification coupled with MS; LUMIER, luminescence-based mammalian interactome mapping; GI, genetic interaction; SGA, synthetic genetic array; SILAC, stable isotope labeling with amino acids in cell culture coupled to MS; GCN, gene coexpression network; NAJ, naturally joined; BD, binding domain; AD, activator domain; COX, coexpression; ER, endoplasmic reticulum; APEX, ascorbate peroxidase.

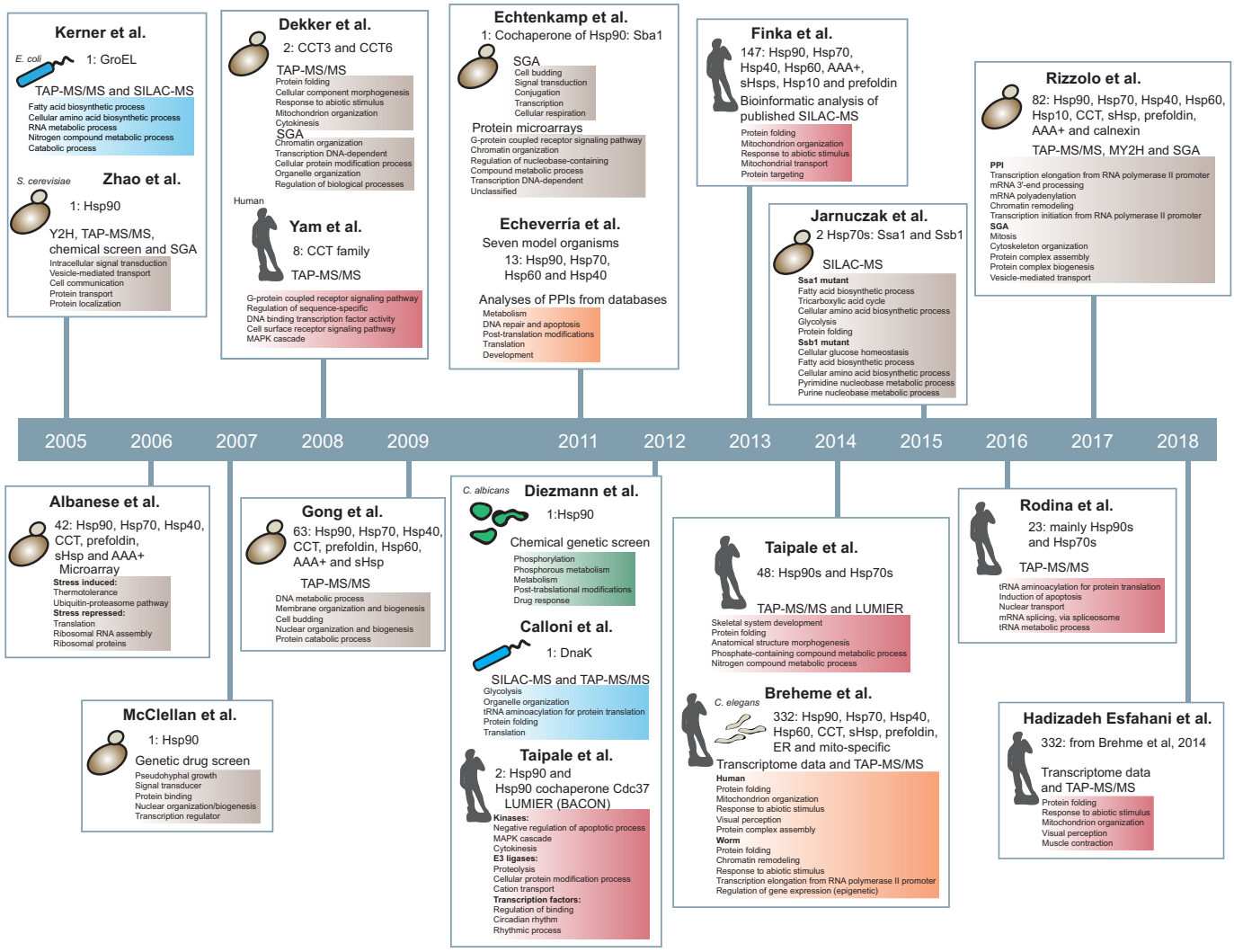

Figure 1. Historical perspective of CCo interaction network studies. Shown is a sample of relevant CCo interaction network studies placed in chronological order. For each study, the number and name of CCo or CCo family, the model organism, the experimental method, and the top five most enriched ($p < 0.05$) interactor Gene Ontology slim bioprocess terms are given. In some cases, the interactor bioprocesses are grouped based on the analysis from the study itself. *Boxes* are colored based on the model organism used. The study by Echeverría *et al.* (18) used the following model organisms: *Schizosaccharomyces pombe*, *Arabidopsis*, yeast, human, *Drosophila*, *Caenorhabditis elegans*, and mouse. For the Taipale *et al.* (24) study, BACON refers to LUMIER with bait control.

were able to group them into functionally specific CCos with less than 200 clients (*e.g.* Hsp60, various Hsp40s, and sHsps) and functionally promiscuous CCos with more than 200 clients (*e.g.* most Hsp70s, Hsp90s, CCTs, and prefoldins).

Within the functionally diverse CCos, some are known to engage with a particular class of protein clients, which is the case of the Hsp90 cochaperone Cdc37 that is capable of mediating folding of almost all of the kinome (22, 23). In 2012, the Lindquist group (24) performed a quantitative survey of clients of the Hsp90 chaperone and its cochaperone Cdc37 using HEK293T cells (Fig. 1). They were able to systematically measure interactions with 60% of the human kinome and found that this was mainly mediated by the Cdc37 cochaperone. Furthermore, these interactions were based mainly on the thermodynamic stability of these client kinases, where Hsp90 engagement depended on the intrinsic stability of the kinase protein. In addition, they found that Hsp90 interacted with 31% of ubiquitin ligases in the cell, most of which contained β-propeller domains such as Kelch and WD40. Surprisingly, only a few interacting transcription factors were found (~7%), which was lower than expected. In 2014, the same group performed a study of the Hsp90 and Hsp70 chaperone networks in the same type of human cells with particular emphasis on their cochaperones (Fig. 1) (25). They found that the cochaperones promote protein complex assembly across a diverse set of processes and hence serve as a recruitment tool for chaperones to act globally. In addition, they found interaction preferences of cochaperones for specific protein client folds such as CDC37 with kinases, SGT1 with leucine-rich repeats, NUDC with β-propeller domains, TRiC/CCT with WD40, among others. Importantly, this work used CCo members from more than a single family as bait to build a more complete CCo network in humans.

In 2016, the Chiosis and Guzman groups (26) (Fig. 1) undertook a large-scale study to understand how CCo–client complexes change in a large set of tumor specimens with the goal of finding new ways for drug-targeting therapies of CCos. They found cancer cells to undergo a considerable biochemical

rewiring of their CCo network upon stress whereby many CCo and client proteins form high-molecular-weight complexes, termed the "epichaperome," to promote cell survival. Specifically, this rewiring was found to be driven by the *MYC* regulator oncogene with the core Hsp90 and Hsp70 systems acting as nucleation sites. Importantly, the epichaperome has implications for diagnostics as well as for drug intervention.

Because of its complexity, so far CCo networks only encompass a few families, and it was not until 2017 when our group built a comprehensive CCo network using all chaperones and cochaperones in yeast (Fig. 1) (27). We used an integrative approach of physical and genetic interaction mapping to uncover CCo–client and CCo–CCo associations across the cell. The comprehensive CCo yeast network uncovered the presence of a large functional multichaperone complex, which we named the naturally joined (NAJ) chaperone complex involving members of the Hsp90, Hsp70, Hsp40, CCT, AAA^+, and the sHsp families clustering together (27). The NAJ chaperone complex provides evidence that CCos function together at a higher hierarchical level in the cell and hence exhibit remarkable connectivity throughout the proteome. To quantify the significance of the NAJ chaperone complex in the whole-yeast proteome, key network topology parameters were evaluated and showed that indeed this complex has significant centrality in the total yeast interaction network (28). Furthermore, this study in yeast suggests that the epichaperome present in cancer cells could be a NAJ chaperone complex in humans to promote tumor proliferation (29).

With the extraordinary efficiency and relatively low cost of applying high-throughput genomic and proteomic techniques, large interaction data are currently available that have not been fully analyzed especially from mammalian cells. Indeed, proteome imbalance in cancer has recently been evaluated by Hadizadeh Esfahani *et al.* (30) using gene expression profiling and protein–protein interaction (PPI) datasets that are publicly available (Fig. 1). The group built an analytical pipeline to profile CCos across many tumors demonstrating the up-regulation of ATP-dependent chaperone families, such as the Hsp90 and Hsp60 in the majority of cancers, and the down-regulation of ATP-independent CCo families, such as the sHsps in kidney chromophobe cell carcinoma, kidney renal papillary cell carcinoma, kidney renal clear cell carcinoma, thyroid carcinoma, and pheochromocytoma and paraganglioma.

Experimental tools to build chaperone networks

Chaperones interact with numerous protein clients and/or other chaperones. For example, it is estimated that CCos mediate the folding of about 62% of the total yeast proteins (31). These client–CCo interactions have a broad range of affinities and typically involve protein complexes of varying protomer number with diverse contact surface sizes (32). Given the varied biochemical nature of chaperone interactions, it is critical to choose the optimal experimental method to decipher these transient spatiotemporal events. In Fig. 2, the most relevant methods employed to date to build chaperone interaction networks are depicted. These involve gene coexpression (Fig. 2*A*) and physical or PPI methods such as yeast two-hybrid (Y2H) (Fig. 2*B*) and membrane yeast two-hybrid (MYTH) (Fig. 2*C*), affinity purification coupled with MS (AP-MS) (Fig. 2*D*), and luminescence-based mammalian interactome mapping (LUMIER) (Fig. 2*E*). Other methods include quantitative proteomic techniques such as stable isotope labeling with amino acids in cell culture coupled to MS (SILAC-MS) (Fig. 2*F*) as well as genetic (epistatic) interactions (GIs) obtained by synthetic genetic array (SGA) assays (Fig. 2*G*).

In gene coexpression, a correlation score is computed for the expression profile of each pair of genes typically obtained from large-scale transcriptomics to build a gene coexpression network (GCN) (Fig. 2*A*). GCNs are highly dependent on the chosen cell/tissue types where marked differences for CCo gene expression are found. In the Brehme *et al.* (33) study on aging, changes in the CCo GCN were evaluated using superior frontal gyrus brain tissue from diseased and normal individuals between the ages of 22 and 90 years old. This tissue is optimal for CCo GCN analyses because of its highly pronounced aging dynamics. A much larger GCN dataset was used to study CCo changes in cancer by Hadizadeh Esfahani *et al.* (30) using The Cancer Genome Atlas compendium. The authors mapped CCo expression changes across 22 cancers compared with their healthy counterpart from thousands of publicly available patient biopsies. This comprehensive approach to study GCN in cancers revealed a global up-regulation of CCos as a general aspect of proteostasis. By subdividing the changes into up- and down-regulated CCo families, the authors were able to further stratify to specific cancers. It is important to note that GCNs provide correlation information between genes and do not attempt to describe any biochemical association between them; hence, they are typically used in combination with PPIs.

In PPIs, large-scale classical experiments involved the use of Y2H and MYTH methods as well as direct purification of complexes using affinity purification tags followed by MS analyses (AP-MS). Briefly, an interaction in Y2H involves the downstream activation of a reporter gene *lacZ* by the transcription factor *Gal4* whose DNA-binding (BD) and activator (AD) domains are fused separately to the bait (*e.g.* a CCo) and a prey, respectively. When the prey–AD protein binds to the bait–BD, a functional transcription factor protein is formed, and as a result the interaction is detected by monitoring LacZ expression (Fig. 2*B*) (34).

An extension of Y2H is MYTH, which detects interactions between membrane-bound proteins. This technique uses split ubiquitin where the mutant N-terminal "NubG" moiety of ubiquitin is fused to the prey protein, and the C-terminal "Cub" moiety is fused to the bait. NubG has the I13G mutation that prevents the spontaneous association of NubG to Cub (35). When an interaction occurs between bait and prey, cytosolic deubiquitinases cleave off the transcription factor BD from the formed ubiquitin, which consequently leads to the transcription of a reporter gene (Fig. 2*C*) (35). Both Y2H and MYTH pose two important disadvantages for CCo PPI determination. The first is that they are primarily binary interaction mapping techniques and can miss client interactions that require mediating cochaperones or cofactors, except in the case when this bridging component is expressed in yeast. The second is that they will likely miss client proteins that require post-translational modifications for their interaction to occur with a CCo. In addi-

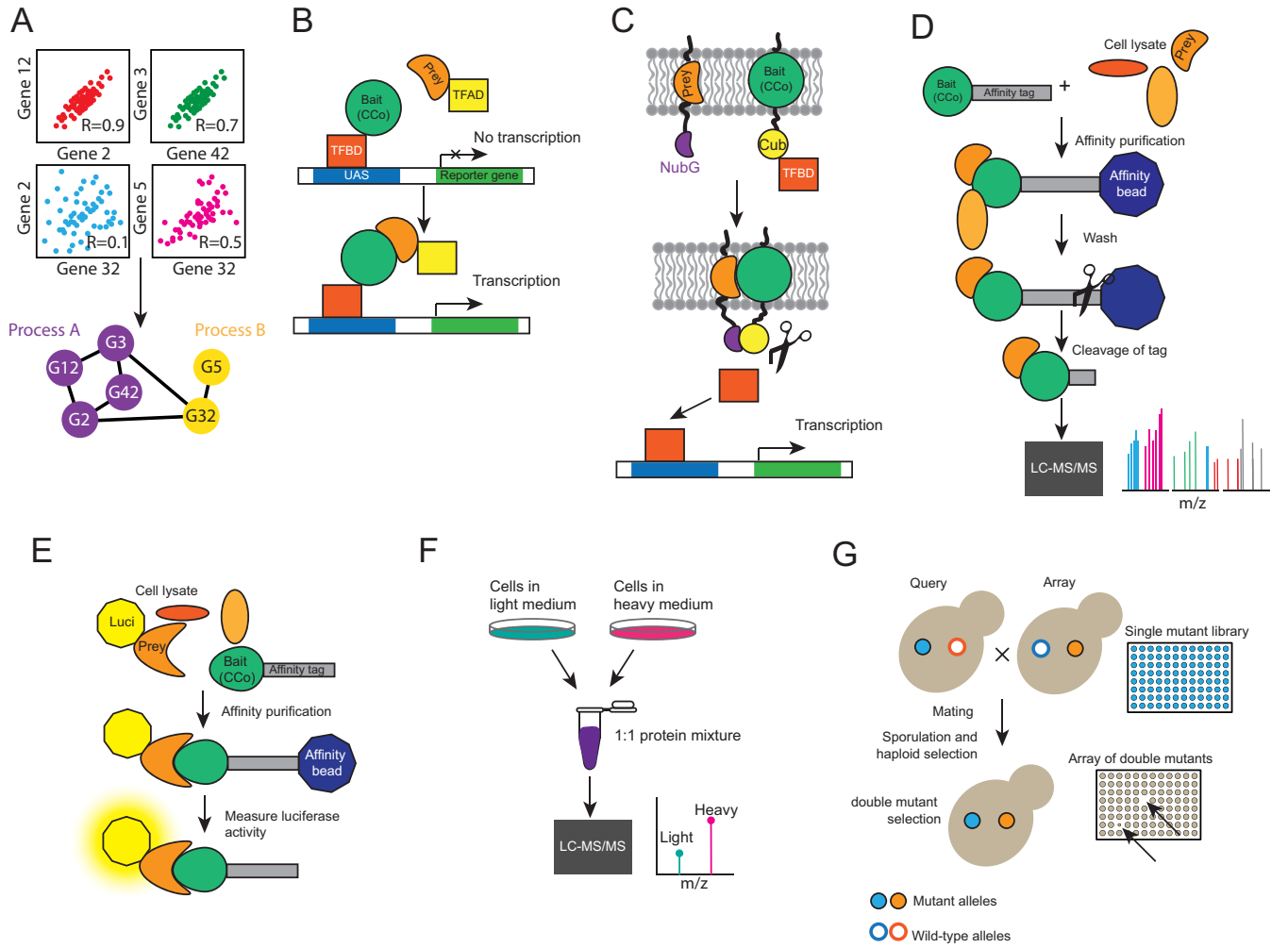

Figure 2. Experimental methods used to build CCo interaction networks. *A*, gene coexpression network analysis. *B*, Y2H (*TFBD,* transcription factor–binding domain; *TFAD,* transcription factor–activating domain; *UAS,* upstream-activating sequence). *C*, MYTH (*NubG,* modified N-terminal fragment of ubiquitin; *Cub-TFBD,* C-terminal fragment of ubiquitin linked to the transcription factor–binding domain). *D*, AP-MS/MS. *E*, LUMIER. *F*, SILAC-MS. Light and heavy media refer to cells grown in specialized media containing light or heavy forms of the essential amino acids lysine and arginine. *G*, SGA.

tion, a more general disadvantage in epitope tag–based techniques known to affect PPIs, in particular with CCos, is the accessibility of the tag for its subsequent targeting or the interference of the tag in the interaction.

In our network study of Hsp90 (7), we tried to circumvent tag-related issues by expressing yeast strains with not only the full-length Hsp90 as bait, but also the N-terminal, middle-, C-terminal, and middle+C-terminal tagged domains of Hsp90 were mated with an array library of 6,084 tagged ORFs. We identified 90 ORFs that were specifically and reproducibly interacting with Hsp90, and the strongest interactions were found against the middle and C-terminal domains with most of these interactors being Hsp90 cochaperones such as Cns1, Cpr6, Cpr7, and Ppt1. These results were used to complement the Hsp90 interactions obtained by other techniques.

In a subsequent study, we used MY2H to corroborate interactions of the ER membrane–bound Hsp40, Sec63 (27). Sec63, a J-domain protein that is a core member of the translocon, was endogenously tagged with the ubiquitin fragment Cub at its C terminus and screened against yeast prey libraries expressing proteins with the N-terminal fusion of ubiquitin fragment NubG, and positive preys were subsequently verified (Fig. 2*C*). This MYTH experiment validated different Sec63 interactions.

The most popular technique used to obtain CCo PPIs is AP-MS, which also relies on the expression of the bait protein (CCo) coupled to an epitope tag or on the use of antibodies that target the endogenous bait protein allowing its purification along with any associated proteins present (preys) (Fig. 2*D*) (36). AP-MS can be utilized in an overexpression strategy followed by a single-affinity purification step or tandem-affinity purification. The advantage of an overexpression system is that it may facilitate detection of weak CCo associations, although it can be less optimal for accurate definition of stoichiometry in such interactions. In contrast, the use of bait CCo proteins that are expressed endogenously in the host genome provide better coverage and accuracy in identifying the protein complexes involved (37, 38). However, as mentioned previously, one major challenge in epitope tag–based techniques is the possibility of the tag interfering with protein stability, assembly, or interaction. A clear example of this is the CCT complex in yeast (a double-ring consisting of subunits CCT1–8) where both the N and C termini of the different subunits are buried in the com-

plex. In our 2009 study (17), we found that pulling down the C-terminally tagged CCT4 subunit only recovered CCT2 and CCT6 but not the other subunits. Moreover, if the tag is strategically placed in a loop in the apical domain of the CCT3 subunit, which is located on the outside of the double-ring assembly, the complete complex was pulled down (9, 39). In this AP-MS study, CCT complex interactors were identified as part of the nuclear pore complex, chromatin remodeling, protein degradation, and the septin complex.

Some of the other major challenges for AP-MS is that it is biased toward capturing only stable complexes. However, many CCo–client interactions are known to be transient. In addition, chaperone clients are typically expressed at very low levels in the cell, which can make the detection of a *bona fide* client difficult. To try and get around these problems, the LUMIER method (40) was used to quantify interactors of Hsp90 and Hsp70 (24, 25). In brief, a bait (CCo) protein is tagged with an epitope, and the other prey (client) protein is tagged with *Renilla* luciferase. These proteins are then transiently coexpressed in cells, and the cell lysates are added to a 96-well plate that contains anti-FLAG beads for immunoprecipitation. Subsequently, the interactions are quantified by luminescence (Fig. 2*E*). In the Hsp90 and Hsp70 LUMIER studies, this technique provided a quantitative window into the interaction network of these versatile chaperones with one study reporting 400 client protein interactions of human Hsp90 and Cdc37 (24) and the other study reporting 800 interactions of human Hsp90 and Hsp70 (25). This method allows for quantitative profiling of interactions providing correlations between CCos.

Another quantitative method used for CCo proteomics is based on metabolically incorporating stable isotope-labeled amino acids into the entire proteome under different conditions and identifying changes by MS in the technique called SILAC-MS (Fig. 2*F*). In the study by Finka and Goloubinoff (19), SILAC-MS data were obtained for 11 different immortalized human cancer cell lines. The authors found that, on average, CCos were eight times more abundant than non-CCo polypeptides. The main abundant CCos were found to be Hsp110, Hsp90, Hsp70, and Hsp60. SILAC-MS has also been used to quantify changes in the proteome upon chaperone perturbation. For example, pharmacological inhibition of Hsp90 was found to result in the induction of the heat-shock response (41).

Mapping GIs is an alternative method used to build a CCo network. In GI experiments, it is examined how mutations introduced into two genes interact to modulate a phenotype. SGA analysis is a systematic method developed in yeast for GI analysis (42). SGA involves a series of replica-pinning steps in which mating and meiotic recombination are used to convert a single mutant input library into an output array of double mutants (Fig. 2*G*). Double-mutant cells that have a more severe fitness defect than the expected multiplicative effect of combining the individual mutants are defined to demonstrate a negative GI, whereas double mutants with a less severe defect in fitness than expected demonstrate a positive GI. In addition, a GI profile can be built for a given gene. Such profiles are composed of a set of positive and negative GIs with other genes in the genome. Two genes with similar GI profiles are likely to be part of the same complexes or function in similar pathways (43, 44). In yeast, the number of GIs obtained is more than double that of PPIs making GIs an excellent tool for building the CCo network (27). It is worth noting that CCo gene alterations tend to produce more than one phenotype (pleiotropy) and hence they tend to have many GIs with different genes from multiple pathways. Therefore, even though pleiotropic genes often are hubs in the GI network, they seldom show functional enrichment with specific pathways/processes, and this makes the assignment of a CCo's function in the cell GI interaction network difficult (45).

Integrating multiple interaction datasets to build the CCo network

Given the highly interactive nature of CCos, the determination of interactions using a single experimental approach yields incomplete results. Thus, the use of a combination of multiple experimental techniques is needed to aid in characterizing CCo interactions and to rule out false positives (25, 27, 30, 33). To facilitate the access to the various CCo datasets from large-scale proteomic and genomic studies, various groups and consortia have built public databases that make the data accessible. Some notable examples are the Biological General Repository for Interaction Datasets (BioGRID), the Universal Protein Resource (UniProt), Search Tool for the Retrieval of Interacting Genes/Proteins (STRING), GeneMANIA, Database of Interacting Proteins (DIP), and the Molecular Interactions Database (MINT), as well as databases specific to model organisms such as the *Saccharomyces* Genome Database (SGD), Human Protein Reference Database (HPRD), The Cancer Genome Atlas, and WormBase among others.

As an example of the use of these databases, Brehme *et al.* (33) combined gene coexpression (COX) data of aging human cells with human PPIs to build a PPI–COX network with 7% of the PPI pairs containing a significant coexpression profile (Fig. 3*A*). The authors describe communities of proteins clustered into induced and repressed gene COX categories, which confirmed previously reported changes in the proteostasis network based on disease states.

In another example of using multiple interaction datasets, we constructed an integrated yeast CCo network by combining PPIs from four large-scale AP-MS studies (27). We designed a custom integration method to consolidate PPI confidence scores from each dataset to avoid any bias in PPIs involving CCos. The final PPI network was picked by selecting the threshold score that yielded the highest number of CCo interactions while maintaining high precision. Subsequently, GIs involving CCos obtained from large-scale SGA assays in yeast were added to the network. To do this, the first step consisted of predicting protein complexes from the PPI network, which is based on the premise that densely connected regions suggest that the associated proteins have a similar function or are part of the same complex (Fig. 3*B*). The second step involved the overlay of interactions based on CCo GI profile correlation similarities onto the predicted PPI protein complexes. The complexes were connected based on the average inter-complex GI profile correlation similarities score between the genes in each complex (Fig. 3*B*). This integrated network revealed that smaller, more specialized CCo-containing protein complexes are more con-

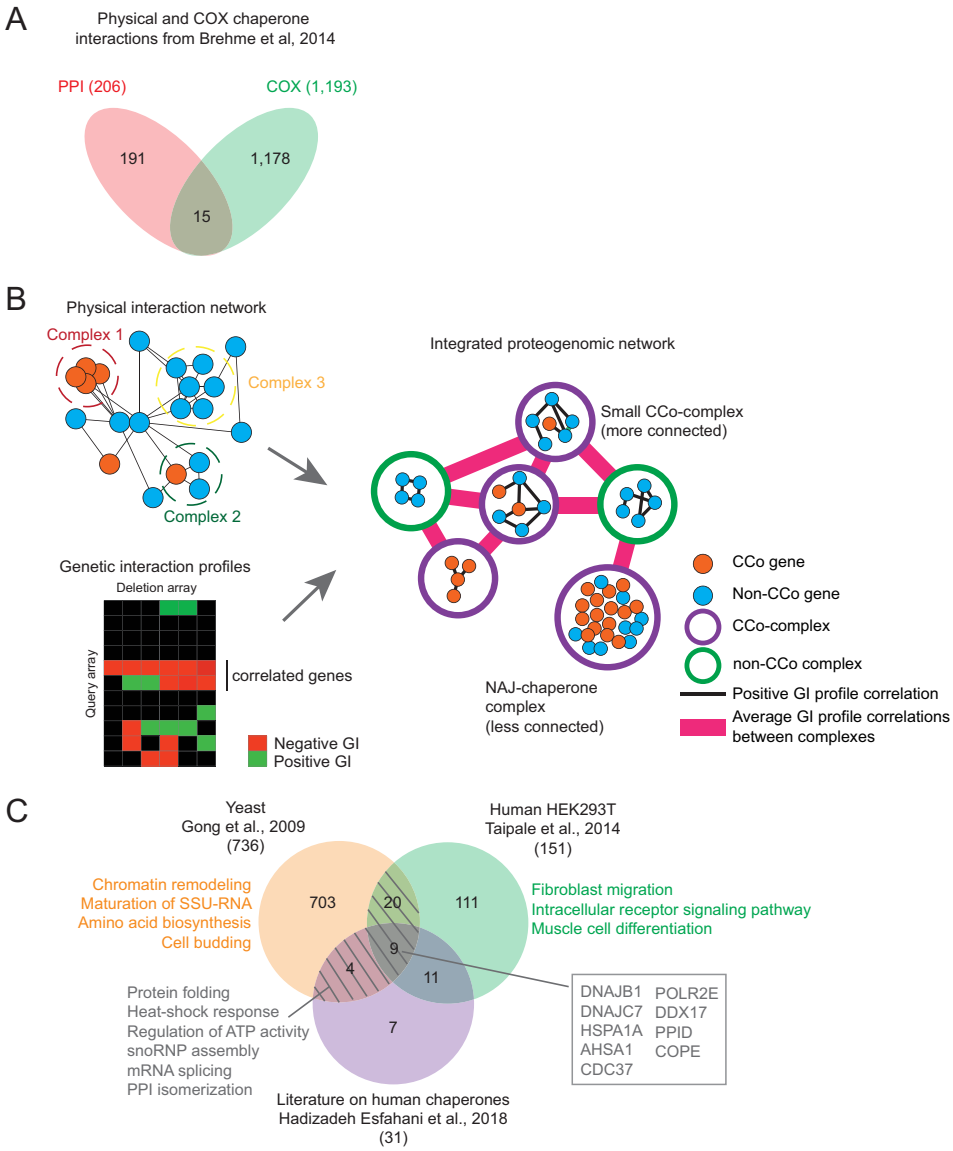

Figure 3. Integration of multiple techniques to build the CCo interaction network. *A,* overlap of the physical (*PPI*) and coexpression (*COX*) datasets from Brehme *et al.* (33). *B,* shown is a schematic describing the integration of the PPI network with the genetic interaction profile correlations network from Rizzolo *et al.* (27). Protein clusters of the PPI network represent proteins that are functionally related. Small CCo complexes with a higher number of inter-complex connections and the large NAJ– chaperone complex with a lower number of inter-complex connections are highlighted. *C,* overlaps of interactors of the Hsp90 chaperone and cochaperones from yeast (17) and human (25, 30) are shown. The most significantly enriched Gene Ontology bioprocesses ($p < 0.05$) in the overlapping and nonoverlapping regions are listed as indicated.

nected. However, most chaperones clustered in one large complex that we named the NAJ complex. This complex had very low connectivity with other complexes, which is reflective of its generalized functionality (Fig. 3*B*) (27). Hence, this analysis clearly shows the unique functionality of CCos as a "jack of all trades, master of none" in the cell.

A glimpse into the evolution of chaperone interaction networks across species

The Hsp90 chaperone family is highly conserved across species and has been extensively studied. To provide an indication of how the Hsp90 chaperone network evolved in different species, here a comparison is provided of the yeast and human Hsp90 interactomes using data from three studies that identified interactors of yeast Hsp90 (17), human Hsp90 in human embryonic kidney 293T cells (25), and human Hsp90 from various databases (30). For the yeast Hsp90 interaction set, the respective human orthologs were identified obtaining a total of 736 interactors. For the human studies, a total of 151 and 31 interactors were obtained by Taipale *et al.* (25) and Hadizadeh Esfahani *et al.* (30), respectively (Fig. 3*C*). Of the 865 interactors compiled from the three studies, we find that surprisingly only 33 are common to both species. These 33 proteins are mainly chaperones of the Hsp40 and Hsp70 families as well as cochaperones of Hsp90, proteins involved in RNA-related processes like RNA helicase complexes and RNA polymerase, peptidyl-prolyl isomerases, and proteins involved in the transport from the ER to Golgi (Fig. 3*C*). Hence, the Hsp90 interaction network seems to vary among species. Nevertheless, one of the conserved functions of Hsp90 between the two species seems to be

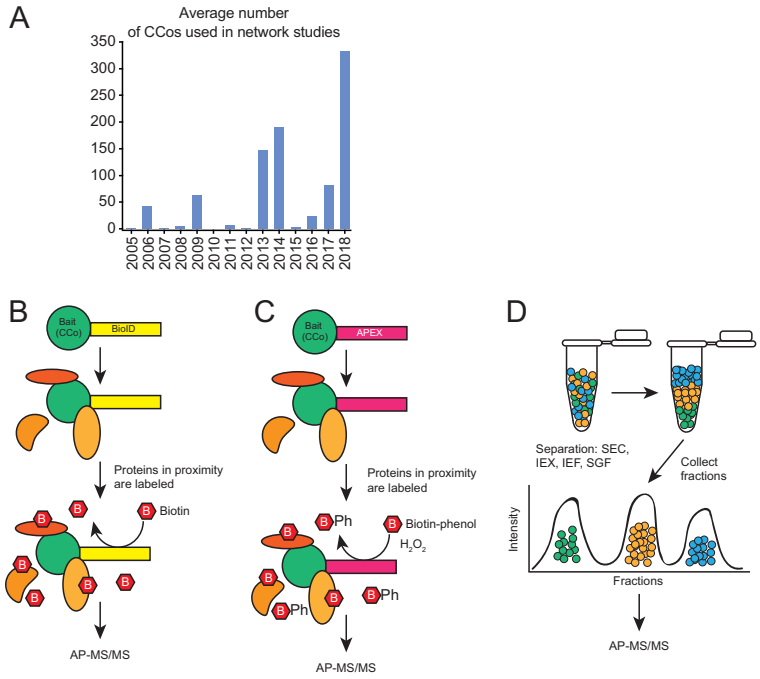

Figure 4. Novel experimental methods to map CCo networks. *A,* average number of CCos used in network studies by year from Fig. 1 (total CCos divided by number of papers per year). *B,* proximity-dependent biotin labeling using biotin ligase-based biotin identification (*BioID*). The ligase is fused to a protein of interest and is expressed in cells, where it biotinylates proximal endogenous proteins. The biotinylated proteins are then pulled down using streptavidin beads and identified by MS. *C,* APEX-mediated biotin labeling. This is a method similar to BioID. In this approach, APEX is either fused to a protein of interest or targeted to a specific cellular compartment or location. To initiate labeling, cells are then incubated with hydrogen peroxide and a biotinylated tyramide derivative (*B-Ph,* biotin–phenol), both of which are membrane-permeant. Biotin–phenol is converted to a phenoxyl radical by APEX upon H_2O_2 treatment. The hyper-reactivity of the biotin–phenoxyl radical results in a short half-life to favor labeling of nearby proteins that are then subsequently identified by MS. *D,* cofractionation coupled to MS. Cell extracts are extensively fractionated using various biochemical techniques: *SEC,* size-exclusion chromatography; *IEX,* ion-exchange chromatography; *IEF,* isoelectric focusing; *SGF,* sucrose density gradient purification. Coeluting proteins are then identified by MS.

to promote assembly of multiprotein complexes in many different cellular compartments (Fig. 3*C*).

Future directions

The past decade has seen a dramatic increase in studies on protein homeostasis using high-throughput approaches involving an increasing number of CCos (Fig. 4*A*). However, there is currently an important need to study the evolution of such CCo networks across species (46), to understand how these highly-connected networks operate within the complex proteome, and to determine how these networks are modified in response to stress, disease state, or in aging.

In aging cells, a well-characterized consequence is the loss of protein homeostasis capacity, but the reasons as to why this happens remains largely unknown. Recent work on metazoans has suggested that aging is a regulated process whereby the decline in protein homeostasis occurs in early adulthood, and it is the activation of stress-response factors that leads to life-span extension, albeit at the cost of reducing fecundity (47, 48). Indeed, as mentioned above, the CCo expression network has been shown to change in aged human brains where ATP-dependent CCos are up-regulated and ATP-independent are down-regulated (33). In the case of yeast, CCos have been shown to be central to the formation and sorting of the various misfolded proteins into compartments. The decline in these storage systems during aging might contribute to pathogenesis (49). Together, these studies highlight the need to fully characterize CCo network rearrangements in response to aging.

To further study CCo network fitness, future networks need to be built from cells that have been systematically treated by some proteostasis disruptor such as a drug or an abiotic stress to screen for response signatures that may elucidate novel condition-dependent members of the CCo network. The results need to be complemented by studies on different human disease cell types ultimately yielding a wealth of information regarding chaperone network robustness. The mapping of CCo networks in whole animals would be a great advance for this field.

Novel high-throughput approaches can be used in the near future to identify and enhance the CCo network resolution. These improvements will not only come from uncovering weak transitory interactions, but also by using techniques that are capable of capturing interactions that are specific to CCos working in organelles such as the ER or mitochondria. For example, many ER chaperone clients remain unknown due to limitations in experimental techniques capable of capturing them. It should be noted that the concept of chaperone networks was first discussed in terms of the chaperones in the ER, where it was proposed that a "matrix" of chaperones is present in the ER that can bind to unfolded proteins (50–52).

Newer high-throughput approaches include proximity-dependent biotin labeling using mutant biotin ligase (Fig. 4*B*, *BioID*) or ascorbate peroxidase (Fig. 4*C*, *APEX*) to screen for immediate neighbors. Another approach is the global proteomic profiling of soluble protein complexes obtained by chro-

matographic separation of cell extracts that are subsequently analyzed by MS (Fig. 4*D*).

In conclusion, obtaining high-resolution CCo networks will serve as a tool that will help both in the development of novel diagnostics for patient care, as well as in the identification of new therapeutics targets for different diseases.

References

1. Saibil, H. (2013) Chaperone machines for protein folding, unfolding and disaggregation. *Nat. Rev. Mol. Cell Biol.* **14,** 630–642 CrossRef Medline
2. Maslov, S., and Sneppen, K. (2002) Specificity and stability in topology of protein networks. *Science* **296,** 910–913 CrossRef Medline
3. Barabási, A. L., and Oltvai, Z. N. (2004) Network biology: understanding the cell's functional organization. *Nat. Rev. Genet.* **5,** 101–113 CrossRef Medline
4. Young, J. C., Agashe, V. R., Siegers, K., and Hartl, F. U. (2004) Pathways of chaperone-mediated protein folding in the cytosol. *Nat. Rev. Mol. Cell Biol.* **5,** 781–791 CrossRef Medline
5. Balch, W. E., Morimoto, R. I., Dillin, A., and Kelly, J. W. (2008) Adapting proteostasis for disease intervention. *Science* **319,** 916–919 CrossRef Medline
6. Kerner, M. J., Naylor, D. J., Ishihama, Y., Maier, T., Chang, H. C., Stines, A. P., Georgopoulos, C., Frishman, D., Hayer-Hartl, M., Mann, M., and Hartl, F. U. (2005) Proteome-wide analysis of chaperonin-dependent protein folding in *Escherichia coli*. *Cell* **122,** 209–220 CrossRef Medline
7. Zhao, R., Davey, M., Hsu, Y. C., Kaplanek, P., Tong, A., Parsons, A. B., Krogan, N., Cagney, G., Mai, D., Greenblatt, J., Boone, C., Emili, A., and Houry, W. A. (2005) Navigating the chaperone network: an integrative map of physical and genetic interactions mediated by the hsp90 chaperone. *Cell* **120,** 715–727 CrossRef Medline
8. McClellan, A. J., Xia, Y., Deutschbauer, A. M., Davis, R. W., Gerstein, M., and Frydman, J. (2007) Diverse cellular functions of the Hsp90 molecular chaperone uncovered using systems approaches. *Cell* **131,** 121–135 CrossRef Medline
9. Dekker, C., Stirling, P. C., McCormack, E. A., Filmore, H., Paul, A., Brost, R. L., Costanzo, M., Boone, C., Leroux, M. R., and Willison, K. R. (2008) The interaction network of the chaperonin CCT. *EMBO J.* **27,** 1827–1839 CrossRef Medline
10. Yam, A. Y., Xia, Y., Lin, H. T., Burlingame, A., Gerstein, M., and Frydman, J. (2008) Defining the TRiC/CCT interactome links chaperonin function to stabilization of newly made proteins with complex topologies. *Nat. Struct. Mol. Biol.* **15,** 1255–1262 CrossRef Medline
11. Echtenkamp, F. J., Zelin, E., Oxelmark, E., Woo, J. I., Andrews, B. J., Garabedian, M., and Freeman, B. C. (2011) Global functional map of the p23 molecular chaperone reveals an extensive cellular network. *Mol. Cell* **43,** 229–241 CrossRef Medline
12. Calloni, G., Chen, T., Schermann, S. M., Chang, H. C., Genevaux, P., Agostini, F., Tartaglia, G. G., Hayer-Hartl, M., and Hartl, F. U. (2012) DnaK functions as a central hub in the *E. coli* chaperone network. *Cell Rep.* **1,** 251–264 CrossRef Medline
13. Diezmann, S., Michaut, M., Shapiro, R. S., Bader, G. D., and Cowen, L. E. (2012) Mapping the Hsp90 genetic interaction network in *Candida albicans* reveals environmental contingency and rewired circuitry. *PLoS Genet.* **8,** e1002562 CrossRef Medline
14. Jarnuczak, A. F., Eyers, C. E., Schwartz, J. M., Grant, C. M., and Hubbard, S. J. (2015) Quantitative proteomics and network analysis of SSA1 and SSB1 deletion mutants reveals robustness of chaperone HSP70 network in *Saccharomyces cerevisiae*. *Proteomics* **15,** 3126–3139 CrossRef Medline
15. Jarnuczak, A. F., Albornoz, M. G., Eyers, C. E., Grant, C. M., and Hubbard, S. J. (2018) A quantitative and temporal map of proteostasis during heat shock in *Saccharomyces cerevisiae*. *Mol. Omics* **14,** 37–52 CrossRef Medline
16. Albanèse, V., Yam, A. Y., Baughman, J., Parnot, C., and Frydman, J. (2006) Systems analyses reveal two chaperone networks with distinct functions in eukaryotic cells. *Cell* **124,** 75–88 CrossRef Medline
17. Gong, Y., Kakihara, Y., Krogan, N., Greenblatt, J., Emili, A., Zhang, Z., and Houry, W. A. (2009) An atlas of chaperone–protein interactions in *Saccharomyces cerevisiae*: implications to protein folding pathways in the cell. *Mol. Syst. Biol.* **5,** 275 Medline
18. Echeverría, P. C., Bernthaler, A., Dupuis, P., Mayer, B., and Picard, D. (2011) An interaction network predicted from public data as a discovery tool: application to the Hsp90 molecular chaperone machine. *PLoS ONE* **6,** e26044 CrossRef Medline
19. Finka, A., and Goloubinoff, P. (2013) Proteomic data from human cell cultures refine mechanisms of chaperone-mediated protein homeostasis. *Cell Stress Chaperones* **18,** 591–605 CrossRef Medline
20. Zhu, H., Bilgin, M., and Snyder, M. (2003) Proteomics. *Annu. Rev. Biochem.* **72,** 783–812 CrossRef Medline
21. Verghese, J., Abrams, J., Wang, Y., and Morano, K. A. (2012) Biology of the heat-shock response and protein chaperones: budding yeast (*Saccharomyces cerevisiae*) as a model system. *Microbiol. Mol. Biol. Rev.* **76,** 115–158 CrossRef Medline
22. Mandal, A. K., Lee, P., Chen, J. A., Nillegoda, N., Heller, A., DiStasio, S., Oen, H., Victor, J., Nair, D. M., Brodsky, J. L., and Caplan, A. J. (2007) Cdc37 has distinct roles in protein kinase quality control that protect nascent chains from degradation and promote posttranslational maturation. *J. Cell Biol.* **176,** 319–328 CrossRef Medline
23. Caplan, A. J., Mandal, A. K., and Theodoraki, M. A. (2007) Molecular chaperones and protein kinase quality control. *Trends Cell Biol.* **17,** 87–92 CrossRef Medline
24. Taipale, M., Krykbaeva, I., Koeva, M., Kayatekin, C., Westover, K. D., Karras, G. I., and Lindquist, S. (2012) Quantitative analysis of HSP90–client interactions reveals principles of substrate recognition. *Cell* **150,** 987–1001 CrossRef Medline
25. Taipale, M., Tucker, G., Peng, J., Krykbaeva, I., Lin, Z. Y., Larsen, B., Choi, H., Berger, B., Gingras, A. C., and Lindquist, S. (2014) A quantitative chaperone interaction network reveals the architecture of cellular protein homeostasis pathways. *Cell* **158,** 434–448 CrossRef Medline
26. Rodina, A., Wang, T., Yan, P., Gomes, E. D., Dunphy, M. P., Pillarsetty, N., Koren, J., Gerecitano, J. F., Taldone, T., Zong, H., Caldas-Lopes, E., Alpaugh, M., Corben, A., Riolo, M., Beattie, B., *et al.* (2016) The epichaperome is an integrated chaperome network that facilitates tumour survival. *Nature* **538,** 397–401 CrossRef Medline
27. Rizzolo, K., Huen, J., Kumar, A., Phanse, S., Vlasblom, J., Kakihara, Y., Zeineddine, H. A., Minic, Z., Snider, J., Wang, W., Pons, C., Seraphim, T. V., Boczek, E. E., Alberti, S., Costanzo, M., *et al.* (2017) Features of the chaperone cellular network revealed through systematic interaction mapping. *Cell Rep.* **20,** 2735–2748 CrossRef Medline
28. Rizzolo, K., Kumar, A., Kakihara, Y., Phanse, S., Minic, Z., Snider, J., Stagljar, I., Zilles, S., Babu, M., and Houry, W. A. (2018) Systems analysis of the genetic interaction network of yeast molecular chaperones. *Mol. Omics* **14,** 82–94 CrossRef Medline
29. Joshi, S., Wang, T., Araujo, T. L. S., Sharma, S., Brodsky, J. L., and Chiosis, G. (2018) Adapting to stress–chaperome networks in cancer. *Nat. Rev. Cancer* **18,** 562–575 CrossRef Medline
30. Hadizadeh Esfahani, A., Sverchkova, A., Saez-Rodriguez, J., Schuppert, A. A., and Brehme, M. (2018) A systematic atlas of chaperome deregulation topologies across the human cancer landscape. *PLoS Comput. Biol.* **14,** e1005890 CrossRef Medline
31. Brownridge, P., Lawless, C., Payapilly, A. B., Lanthaler, K., Holman, S. W., Harman, V. M., Grant, C. M., Beynon, R. J., and Hubbard, S. J. (2013) Quantitative analysis of chaperone network throughput in budding yeast. *Proteomics* **13,** 1276–1291 CrossRef Medline
32. Freilich, R., Arhar, T., Abrams, J. L., and Gestwicki, J. E. (2018) Protein–protein interactions in the molecular chaperone network. *Acc. Chem. Res.* **51,** 940–949 CrossRef Medline
33. Brehme, M., Voisine, C., Rolland, T., Wachi, S., Soper, J. H., Zhu, Y., Orton, K., Villella, A., Garza, D., Vidal, M., Ge, H., and Morimoto, R. I. (2014) A chaperome subnetwork safeguards proteostasis in aging and neurodegenerative disease. *Cell Rep.* **9,** 1135–1150 CrossRef Medline
34. Suter, B., Kittanakom, S., and Stagljar, I. (2008) Two-hybrid technologies in proteomics research. *Curr. Opin. Biotechnol.* **19,** 316–323 CrossRef Medline
35. Snider, J., Kittanakom, S., Damjanovic, D., Curak, J., Wong, V., and Stagljar, I. (2010) Detecting interactions with membrane proteins using a mem-

brane two-hybrid assay in yeast. *Nat. Protoc.* **5,** 1281–1293 CrossRef Medline
36. Morris, J. H., Knudsen, G. M., Verschueren, E., Johnson, J. R., Cimermancic, P., Greninger, A. L., and Pico, A. R. (2014) Affinity purification-mass spectrometry and network analysis to understand protein–protein interactions. *Nat. Protoc.* **9,** 2539–2554 CrossRef Medline
37. von Mering, C., Krause, R., Snel, B., Cornell, M., Oliver, S. G., Fields, S., and Bork, P. (2002) Comparative assessment of large-scale data sets of protein–protein interactions. *Nature* **417,** 399–403 CrossRef Medline
38. Collins, S. R., Kemmeren, P., Zhao, X. C., Greenblatt, J. F., Spencer, F., Holstege, F. C., Weissman, J. S., and Krogan, N. J. (2007) Toward a comprehensive atlas of the physical interactome of *Saccharomyces cerevisiae*. *Mol. Cell. Proteomics* **6,** 439–450 CrossRef Medline
39. Pappenberger, G., McCormack, E. A., and Willison, K. R. (2006) Quantitative actin folding reactions using yeast CCT purified via an internal tag in the CCT3/γ subunit. *J. Mol. Biol.* **360,** 484–496 CrossRef Medline
40. Barrios-Rodiles, M., Brown, K. R., Ozdamar, B., Bose, R., Liu, Z., Donovan, R. S., Shinjo, F., Liu, Y., Dembowy, J., Taylor, I. W., Luga, V., Przulj, N., Robinson, M., Suzuki, H., Hayashizaki, Y., *et al.* (2005) High-throughput mapping of a dynamic signaling network in mammalian cells. *Science* **307,** 1621–1625 CrossRef Medline
41. Sharma, K., Vabulas, R. M., Macek, B., Pinkert, S., Cox, J., Mann, M., and Hartl, F. U. (2012) Quantitative proteomics reveals that Hsp90 inhibition preferentially targets kinases and the DNA damage response. *Mol. Cell. Proteomics* **11,** M111.014654 CrossRef Medline
42. Boone, C., Bussey, H., and Andrews, B. J. (2007) Exploring genetic interactions and networks with yeast. *Nat. Rev. Genet.* **8,** 437–449 CrossRef Medline
43. Tong, A. H., Lesage, G., Bader, G. D., Ding, H., Xu, H., Xin, X., Young, J., Berriz, G. F., Brost, R. L., Chang, M., Chen, Y., Cheng, X., Chua, G., Friesen, H., Goldberg, D. S., *et al.* (2004) Global mapping of the yeast genetic interaction network. *Science* **303,** 808–813 CrossRef Medline
44. Costanzo, M., VanderSluis, B., Koch, E. N., Baryshnikova, A., Pons, C., Tan, G., Wang, W., Usaj, M., Hanchard, J., Lee, S. D., Pelechano, V., Styles, E. B., Billmann, M., van Leeuwen, J., van Dyk, N., *et al.* (2016) A global genetic interaction network maps a wiring diagram of cellular function. *Science* **353,** aaf1420 CrossRef Medline
45. Kumar, A., Rizzolo, K., Zilles, S., Babu, M., and Houry, W. A. (2018) Computational analysis of the chaperone interaction networks. *Methods Mol. Biol.* **1709,** 275–291 CrossRef Medline
46. Lindquist, S. (2009) Protein folding sculpting evolutionary change. *Cold Spring Harb. Symp. Quant. Biol.* **74,** 103–108 CrossRef Medline
47. Labbadia, J., and Morimoto, R. I. (2015) The biology of proteostasis in aging and disease. *Annu. Rev. Biochem.* **84,** 435–464 CrossRef Medline
48. Ben-Zvi, A., Miller, E. A., and Morimoto, R. I. (2009) Collapse of proteostasis represents an early molecular event in *Caenorhabditis elegans* aging. *Proc. Natl. Acad. Sci. U.S.A.* **106,** 14914–14919 CrossRef Medline
49. Sontag, E. M., Samant, R. S., and Frydman, J. (2017) Mechanisms and functions of spatial protein quality control. *Annu. Rev. Biochem.* **86,** 97–122 CrossRef Medline
50. Tatu, U., and Helenius, A. (1997) Interactions between newly synthesized glycoproteins, calnexin and a network of resident chaperones in the endoplasmic reticulum. *J. Cell Biol.* **136,** 555–565 CrossRef Medline
51. Meunier, L., Usherwood, Y. K., Chung, K. T., and Hendershot, L. M. (2002) A subset of chaperones and folding enzymes form multiprotein complexes in endoplasmic reticulum to bind nascent proteins. *Mol. Biol. Cell* **13,** 4456–4469 CrossRef Medline
52. Jonikas, M. C., Collins, S. R., Denic, V., Oh, E., Quan, E. M., Schmid, V., Weibezahn, J., Schwappach, B., Walter, P., Weissman, J. S., and Schuldiner, M. (2009) Comprehensive characterization of genes required for protein folding in the endoplasmic reticulum. *Science* **323,** 1693–1697 CrossRef Medline

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J. Biol. Chem. 2019, 294:2142-2150.
doi: 10.1074/jbc.TM118.002805 originally published online September 7, 2018