Minireview

Connecting the dots in Huntington's disease with protein interaction networks

Flaviano Giorgini* and Paul J Muchowski*†

Addresses: *Department of Pharmacology and †The Center for Neurogenetics and Neurotherapeutics, University of Washington, Seattle, WA 98195, USA.

Correspondence: Paul J Muchowski. E-mail: mucho@u.washington.edu

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Abstract

Analysis of protein-protein interaction networks is becoming important for inferring the function of uncharacterized proteins. A recent study using this approach has identified new proteins and interactions that might be involved in the pathogenesis of the neurodegenerative disorder Huntington's disease, including a GTPase-activating protein that co-localizes with protein aggregates in Huntington's disease patients.

Huntington's disease (HD) is an autosomal dominant neurodegenerative disorder characterized by motor dysfunction, cognitive impairment, and psychiatric abnormalities. It is the most prevalent among at least nine related inherited neurodegenerative diseases that involve expansion of CAG repeats that encode polyglutamine (polyQ) tracts. In the case of HD, an expanded CAG repeat in the gene *IT-15* leads to an expansion of the polyQ region in the protein huntingtin (Htt) [1]. Beyond a critical threshold of about 37 glutamines this leads to the hallmarks of HD: aggregation of mutant Htt in insoluble neuronal 'inclusion bodies' and specific degeneration of neurons in the cerebral cortex and striatum. Although HD has been investigated intensively by many researchers since *IT-15* was cloned, no pharmacological treatment is yet available that effectively prevents progression of disease in patients, in large part because of a lack of understanding of the pathological mechanisms of the disease.

Most evidence indicates that mutant Htt exerts its pathological effect in a true dominant manner and that Htt with an expanded polyQ tract is cytotoxic. As the vast majority of HD patients have one normal copy and one mutant copy of *IT-15,* it is thought that the dominant effect of mutant Htt is due to novel abnormal protein interactions that cause toxicity and ultimately lead to the neurodegeneration seen in HD. Recent observations suggest, however, that depletion of wild-type Htt protein and loss of normal protein interactions involving Htt may also contribute to the pathology of HD [2,3]. In order to understand better the pathological mechanism of HD and the normal function of Htt, it is critical to elucidate the interaction partners of both wild-type and mutant Htt. Towards that goal, Goehler *et al.* [4] report in a recent paper in *Molecular Cell* the generation of a protein-protein interaction network for HD that has revealed many new interactions and identified several uncharacterized proteins, all of which may help in devising novel hypotheses about disease mechanisms and potential strategies for therapeutic intervention.

Known interaction partners of Htt

Htt is a large protein of about 3,144 amino acids with a polyQ region of variable length located at the amino terminus. Immediately carboxy-terminal to the polyQ repeat are two proline-rich regions, which are required for many protein-protein interactions [5,6], for sequestration of vesicle-associated proteins in Htt inclusion bodies [7], and for modulating the toxic conformations of a mutant Htt fragment when transfected into yeast (M. Duennwald, S. Jagadish, F.G., S. Willingham, S.L. Lindquist and P.J.M., unpublished observations). Htt also contains ten highly conserved HEAT repeats, which are found in many proteins

involved in intracellular transport and chromosomal segregation [8,9]. Many interaction partners for both wild-type and mutant Htt have been isolated in the past decade by several methods, including the yeast two-hybrid system, affinity chromatography, and immunoprecipitation [5,6]. These protein partners have shed light on both the pathological mechanism of mutant Htt and the roles that wild-type Htt may play in many cellular processes, including gene transcription, vesicle trafficking, endocytosis, and intracellular signaling [5]. The large size of Htt and its apparent role in several cellular processes has raised the possibility that Htt serves as a scaffold that arranges protein complexes by modulating the binding of accessory factors [6]. The apparent complexity of the pathological mechanisms that underlie HD may be attributed in part to the loss (and gain) of many of these diverse protein-protein interactions. From the perspective of developing drug therapies for HD, this complexity is particularly daunting, as researchers will have to validate individually the importance of many of these protein-protein interactions by genetic or pharmacological approaches.

As stated above, one of the many proposed 'normal' functions of Htt as determined by analysis of protein interactions is a role in transcriptional regulation. Indeed, a large body of work indicates that transcriptional dysregulation may be important for the pathogenesis of HD [10,11]. Htt binds several nuclear transcription factors, including the cAMP response-element binding protein (CREB)-binding protein (CBP), specificity protein 1 (SP1), and p53 [5,6]. CBP is critical for expression of neural genes and neuronal function [5]; it acts as a histone acetyltransferase as well as a transcription factor. Interactions of mutant Htt with CBP abrogates the acetyltransferase activity of this protein *in vitro*, reducing the level of acetylated histones [12] and probably thereby decreasing the transcription of target genes *in vivo*. In addition, pharmacological inhibition of histone deacetylases reverses neurodegeneration in fly models of polyQ disease [12] and improves motor deficits in a mouse model of HD [13,14]. It is interesting to note that a double-knockout mouse lacking CREB and the related transcription factor CREM develops a HD-like phenotype of neurodegeneration in striatal cells [15]. Given that Htt interacts with many other transcription factors, the role of transcriptional dysfunction in HD is most likely to be much more complex than a simple interaction between CBP and Htt, but the characterization of this interaction has provided some tantalizing clues to the role of mutant Htt in HD pathogenesis, showing the importance of identifying and characterizing Htt interaction partners.

Generating a protein interaction network for HD

Functional genomic strategies have gained in importance in recent years with the flood of information provided by the genome sequences available for many organisms. One of these approaches involves the analysis of interaction networks to infer the function of each uncharacterized protein from the functions of known proteins that are in the same local interaction cluster within the network (Figure 1) [16-18]. In an excellent example of this approach, Schwikowski *et al*. [16] generated a genome-wide protein-protein interaction network for *Saccharomyces cerevisiae* by synthesizing information from two high-throughput genomic yeast two-hybrid studies [19,20] and many smaller interaction studies. In total, this group analyzed 2,709 interactions among 2,309 yeast proteins. The authors [16] found that when these interactions were mapped, only one large interaction network was obtained, containing 2,358 interactions among 1,548 proteins. The majority of proteins with known functions or subcellular localization clustered together in smaller local networks within the interaction network, and the functions of 72% of the characterized proteins with at least one known interaction partner could be correctly predicted on the basis of this network [16]. This shows that protein-protein interaction networks can be used to predict, at a high level of accuracy, the function of uncharacterized proteins within clusters and the functional relationship between these clusters [17].

Goehler *et al*. [4] used a similar approach on a smaller scale to generate an interaction network of human proteins for HD, in order to elucidate better the role of Htt in the cell and

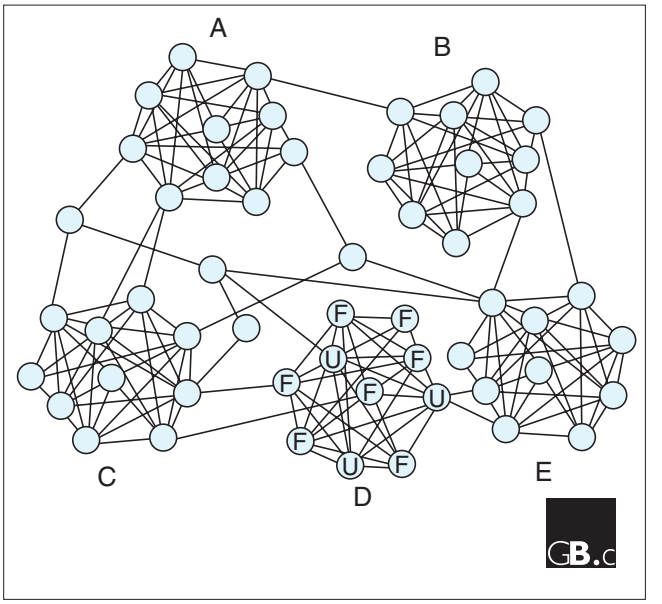

Figure 1
A schematic representation of a hypothetical protein-protein interaction network. Each sphere represents a protein and the connecting lines represent protein-protein interactions. Within an interaction network, smaller local interaction networks or 'clusters' may form (A-E). Proteins in clusters generally have similar functions, allowing prediction of the cellular function of uncharacterized proteins (U in cluster D) from the function of characterized proteins within the cluster (F).

to help inform strategies for combating HD pathogenesis. The authors used a combination of library and matrix yeast two-hybrid screens to place Htt within the context of an interaction network. The yeast two-hybrid system takes advantage of the modular nature of transcription factors by separating the DNA-binding domains and transcriptional-activation domains of transcription factors and independently fusing these domains with candidate interacting proteins [21,22]. Constructs encoding these fusion proteins are transformed into yeast cells; if the two candidate proteins interact *in vivo*, a functional transcription factor is reconstituted and expression of a reporter gene is activated (Figure 2a). In library yeast two-hybrid screening, one candidate fusion protein is designated the 'bait' and is used to screen a collection (or library) of 'prey' fusions proteins for interactions (Figure 2b). Matrix yeast two-hybrid screening is a modification of the standard screening method whereby many strains containing distinct bait and prey proteins are arrayed and brought together by mating, such that all pairwise interactions in a group of proteins can be tested (Figure 2c).

Goehler *et al.* [4] began by screening a fetal brain library using the yeast two-hybrid method and identified new interacting proteins using a total of 52 baits. These baits included proteins involved in cellular processes associated with Htt, proteins known to interact with Htt, and five different amino-terminal fragments of Htt itself. Using this approach, 55 interactions were identified among 23 bait and 51 prey proteins. An additional 23 baits were generated from some of the prey cDNAs that encoded proteins with verified interactions. This tool-chest of 51 prey proteins and 46 bait proteins allowed the authors to perform the central experiment in this body of work, the pairwise testing of baits and preys using the matrix two-hybrid system (a remarkable total of 2,360 combinations) [4]. The bait and prey proteins were individually expressed in strains of opposite mating type, which were mated to test for potential interactions. All 55 two-hybrid interactions from the library screens were reproduced, and 131 new protein-protein interactions were found, generating a total of 186 interactions among 35 bait and 51 prey proteins, including 165 novel potential interactions. Co-immunoprecipitation experiments were used to test 54 of these interactions, of which around 65% were validated.

Among the plethora of proteins in the resulting network of interactions, 19 proteins were identified that interact directly with Htt, of which only four had been previously identified as Htt interactors - huntingtin-interacting protein 1 (HIP1), the transcription-elongation factor CA150, the SH3-domain-containing Grb2-like protein SH3GL3, and the spliceosome protein HYPA [6]. Of the 19 Htt partners identified, six are involved in transcription, four in transport, and three in cell signaling, lending more support to a role for Htt in these processes. In addition, six novel Htt-interacting proteins of unknown function were isolated (designated HIP5, HIP11, HIP13, HIP15, HIP16, and CGI-125).

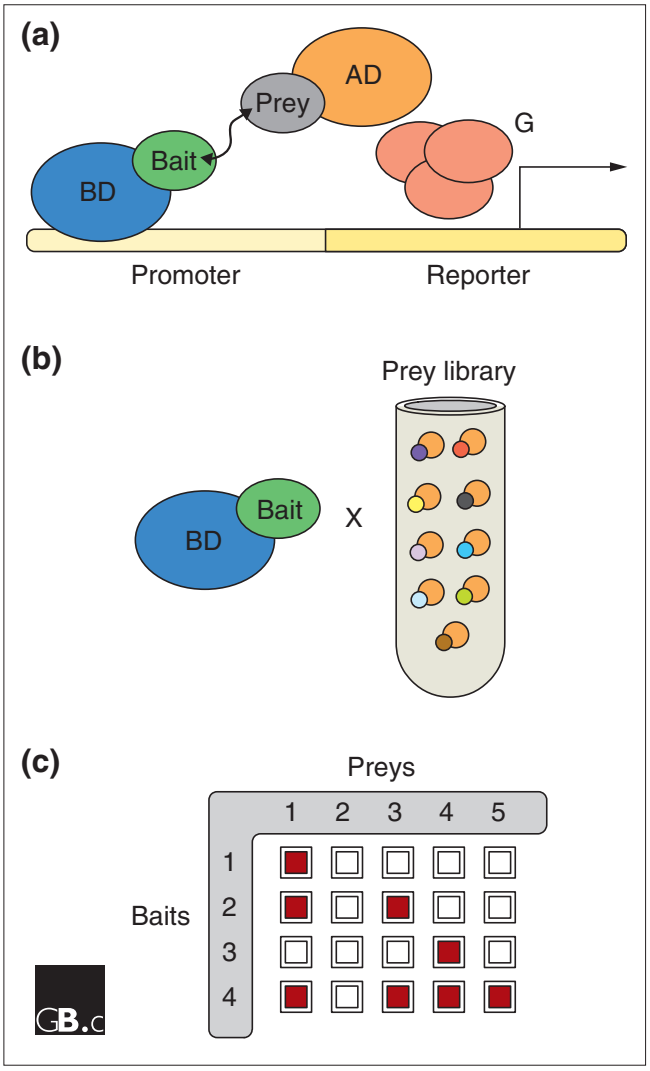

Figure 2
Schematic representations of library and matrix yeast two-hybrid screens. **(a)** A model of the yeast two-hybrid system. The DNA-binding domain (BD) and transcriptional activation domain (AD) from a transcription factor are independently fused with candidate interacting proteins (the bait and prey, respectively). If the bait and prey proteins interact (curved line) within a cell expressing both fusions, the resulting functional transcription factor can bind the promoter of a reporter gene and activate its transcription by interacting with the general transcription machinery (G). **(b)** A library yeast two-hybrid screen. A collection of preys are screened with a bait of interest by transforming yeast cells with plasmids encoding the constructs in order to isolate its interaction partners. **(c)** A matrix yeast two-hybrid screen used to generate a protein-protein interaction network. Several baits and preys are arrayed in 96-well microtiter plates and the fusion proteins are brought together by mating. Diploids containing both bait and prey are isolated on selective plates and protein-protein interactions are ascertained by expression of the reporter gene. The dark squares indicate an interaction between the bait given at the end of the row and the prey indicated at the top of the column.

The power of protein-protein interaction networks is highlighted by the discovery of G-protein-coupled receptor kinase interactor 1 (GIT1) as an interaction partner of Htt

[4]. GIT1 is a GTPase-activating protein that modulates actin polymerization, synapse formation, spine morphology, and plasticity in neurons [23,24]. The authors found that GIT1 not only promotes Htt aggregation but is required for this aggregation [4]. In the brains of HD patients, GIT1 co-localized to Htt aggregates and was amino-terminally truncated, ostensibly by a disease-specific process [4]. In addition to Htt, GIT1 was observed to interact with BARD1, a RING-domain protein associated with the breast-cancer protein BRCA1, and HIP5, a previously uncharacterized protein. In combination, BARD1 and HIP5 have 27 interactions within the network in addition to their interactions with GIT1; these will provide many avenues of inquiry into the role of GIT1 in Htt aggregation and the abnormal accumulation of amino-terminally truncated GIT1 in the brains of HD patients. It is worth noting that if a role for GIT1 in HD pathogenesis can be validated by genetic methods, inhibition of its proteolysis may be an excellent approach to therapy of this disorder.

As is often asked with such 'fishing expedition' approaches, how does one deal with this deluge of information? And how will the identification of these new protein-protein interactions lead to a better understanding of HD? Although this work [4] is an important first step, the challenge ahead is in determining which of the novel proteins and interactions merits additional functional analysis, such as molecular genetic dissection in mouse models of HD. One method would be to validate the candidates using models of polyQ disease in organisms such as fruit flies, yeast, and the nematode *Caenorhabditis elegans,* which have already yielded many genetic modifiers of polyQ toxicity [25-29]. Analysis in these simpler model organisms may also discern the role of the novel proteins and interactions in cellular processes and thus help validate the functional predictions from the interaction clusters described by Goehler *et al.* [4]. In addition, as the normal function of the novel proteins and the roles they may play in HD can now be inferred from clustering within the HD protein-protein interaction network, a more directed research strategy can be used when investigating these proteins.

The recent study by Goehler *et al.* [4] showcases the potential of the interaction network approach to provide candidate targets for research into human disease. Although more than 1,000 human disease genes have been documented [30], most of them remain functionally uncharacterized. Application of this approach - as well as other genomic and proteomic strategies such as gene-expression and protein profiling and genetic screens in model systems - to other human diseases will provide a wealth of new candidate targets for drug intervention and will give further insights into the pathogenic mechanisms of these disorders.

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