Minireview

Protein-protein interaction networks in the spinocerebellar ataxias

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Published: 10 August 2006

Genome ***Biology*** 2006, **7:**229 (doi:10.1186/gb-2006-7-8-229)

The electronic version of this article is the complete one and can be found online at http://genomebiology.com/2006/7/8/229

Abstract

A large yeast two-hybrid study investigating whether the proteins mutated in different forms of spinocerebellar ataxia have interacting protein partners in common suggests that some forms do share common pathways, and will provide a valuable resource for future work on these diseases.

The spinocerebellar ataxias are a group of heritable human neurodegenerative disorders that result in the loss of cerebellar Purkinje cells; patients have difficulties with balance and coordination. There are a number of different forms, in both humans and mouse models, with similar phenotypes but with different genes mutated. Given the similar phenotypes of these disorders, it would be of interest to know whether the proteins known to be mutated in the different forms interact with any of the same protein partners. In work published recently, Zoghbi and colleagues [1] have now addressed this question using the yeast two-hybrid protein-protein interaction system.

Building resources

The yeast two-hybrid system allows the identification of potential binary protein-protein interactions by exploiting the characteristics of transcription factors that are composed of separable DNA-binding domains and transcriptional transactivation domains. Typically, in one vector - the 'bait' - the potential protein target is fused to the DNA-binding domain from a transcriptional activator such as yeast Gal4 or bacterial LexA. In a second vector, the transcriptional activation domain of Gal4 or LexA is fused in-frame to a library of complete or partial open reading frames or cDNAs, called the 'preys'. The preys represent the potential interaction partners for the bait. When the bait interacts with a prey in the yeast nucleus, the transactivation and DNA-binding domains are brought together, reconstituting a functional transcriptional activator. This event is assayed using appropriate (and in some cases, multiple) reporter genes. Automation, together with refinements in the yeast two-hybrid methodology that have reduced the previously high false-positive hit rates, make it possible to perform such studies on a large scale, using libraries of thousands of baits and preys [2]. This has led to detailed genome-wide studies of potentially interacting proteins in model organisms - delineating the protein 'interactome' - and the first studies of the interactome in humans [3,4]. Along with such genome-wide work, there have also been influential studies based on a single target. For instance, Wanker and colleagues [5] have focused on the interactors of huntingtin, the protein mutated in Huntington's disease.

The new work from Lim *et al*. [1] on the spinocerebellar ataxias is an interesting variation on the theme of the targeted interactome strategy. The authors took 23 proteins that are mutated in dominant or recessive forms of spinocerebellar ataxias in humans or mice, along with 31 other proteins known to interact with some of these primary disease proteins, and used yeast two-hybrid technology to place them into a protein-protein interaction network. They identified 770 protein-protein interactions, many of these involving more than one protein (Figure 1). This network was further expanded using additional data found in the literature.

This spinocerebellar ataxia interactome study [1] and related projects provide data resources of great value to biological

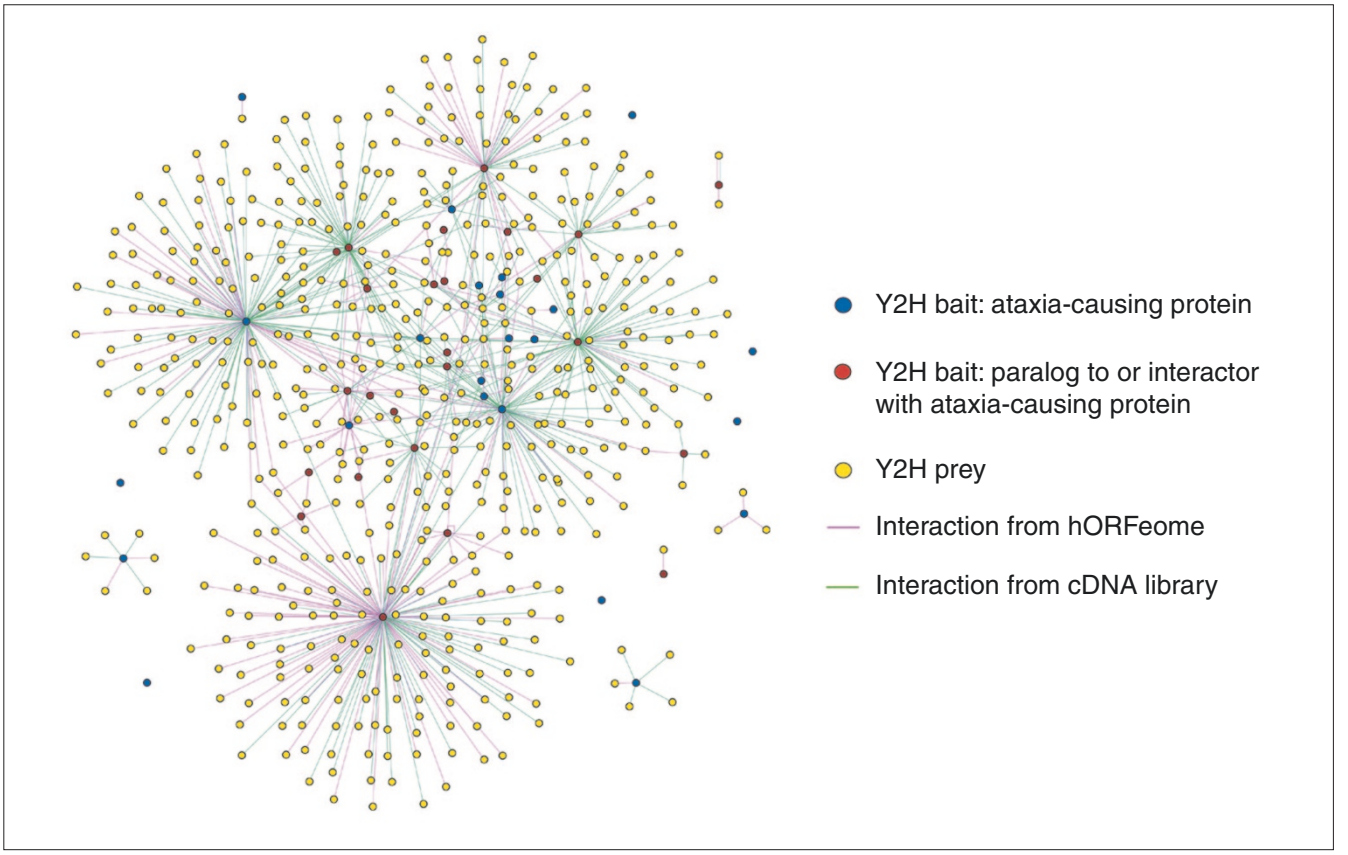

Figure 1
An interaction network of proteins involved in spinocerebellar ataxias. The yeast two-hybrid interaction data of Lim *et al.* [1] reveal one large interconnected network consisting of 752 protein-protein interactions between 36 ataxia-associated proteins and 541 prey proteins. Circles (nodes) represent proteins, and any two proteins connected by a line have been shown to interact in the yeast two-hybrid (Y2H) screen. Blue circles depict protein baits corresponding to the proteins known to be mutated in ataxias; red circles depict protein baits that are paralogs of ataxia-causing proteins or known interactors with them. The yellow circles depict prey proteins tested in the yeast two-hybrid screen and come from two sources. Those connected by a purple line to a node come from the human open reading frame library (the hORFeome), while those connected by a green line come from a human brain cDNA library. All lines represent either first- or second-order interactions to ataxia-causing proteins. First-order interactions are direct interactions, while second-order interactions occur via an intermediary protein. Reproduced with permission from Elsevier [1].

scientists. A large number of likely binary protein-protein interactions are revealed, along with information on interactors of interactors. In a general sense, this provides a powerful set of starting points for further studies leading to the understanding of the biological functions of the various proteins. The availability of large resources such as this study gives us a powerful tool that I suspect will increasingly change the way we approach problems in cell biology.

Common pathways

From a disease perspective, the study by Lim *et al.* [1] suggests that there may be common pathways shared by different disease proteins. For instance, their screen revealed a possible link between Purkinje cell atrophy associated protein-1 (Puratrophin-1) and the protein (ataxin-1) mutated in spinocerebellar ataxia type 1 through interactions with Coilin-interacting protein. Recently, Puratrophin-1 was implicated in a form of autosomal dominant spinocerebellar ataxia linked to 16q22.1 [6]. In addition, some of the newly identified partner proteins interact with more than one ataxia protein. Indeed, the interaction network created using the spinocerebellar ataxia proteins shows greater connectivity, shorter interaction path lengths linking different proteins, and more proteins showing multiple interactions compared with control networks created from a list of proteins associated with a phenotypically diverse group of disorders [1]. This reinforces the likelihood that similar biological pathways are perturbed in certain spinocerebellar ataxias caused by different mutated genes. If such pathways turn out to be critical to neurodegeneration, this may point to tractable therapeutic targets that are shared among a range of diseases - an enticing prospect. A corollary to this is that certain proteins in this network may be excellent functional candidates for as-yet unidentified ataxia loci, if they map to the appropriate genetic intervals.

The current study [1], when viewed in the context of previous genetic modifier screens in *Drosophila* models of spinocerebellar ataxia types 1 and 3, suggests that enhanced or decreased function of some of the interacting proteins can modulate the severity of spinocerebellar ataxia type 1 [7,8]. For instance, wild-type ataxin-2 and the *Drosophila* Couch Potato protein have previously been shown to be modifiers of mutant ataxin-1 toxicity in flies. Lim *et al*. [1] have now confirmed the human orthologs of these modifiers as ataxin-1 interactors. As the previous genetic modifier screens were not saturating, other interactors in the network may also be considered as potential modifiers.

Limitations

Such large datasets are not without caveats. About 80% of a sample of the yeast two-hybrid hits in the spinocerebellar ataxia study were confirmed using coaffinity purification, a high success rate for this type of study [1]. Nevertheless, this suggests that about 20% of untested yeast two-hybrid interactions may be false positives. In addition to technical false positives, one can also see biological false positives: for instance, when two proteins genuinely interact *in vitro* or in the yeast nucleus but are never found in the same cell compartment or the same cell type, and thus cannot interact *in vivo*. The proportion of biological false positives may be low, but it needs to be borne in mind.

The large-scale mammalian protein-protein interaction networks reported to date are only partially complete [3,4]. The prey libraries only partially cover the genome and some of the baits may not have been efficient, either because they were not functional or properly folded in yeast, or because they could not interact with partners in the yeast nucleus, a prerequisite for yeast two-hybrid screens. Thus, the currently available mammalian studies will probably serve as starting frameworks for future, more comprehensive screens using both yeast two-hybrid and complementary approaches for identifying protein-protein interactions.

What are the challenges for the future? In general, there will be major benefits if one can move towards datasets with even fewer false-positive interactions and more real interactions, some of which may need to be captured with alternative technologies such as affinity purification followed by mass spectrometry. Studies based on the concept pioneered by Lim *et al*. [1] are likely to investigate other diseases with similar phenotypes but different gene mutations, and may reveal novel shared pathways. For instance, Zoghbi and colleagues [1] suggest that such studies may be useful in diabetes, Parkinson's disease and hypertension.

One of the key issues is distilling functional sense out of these large datasets. In the context of disease studies like that on spinocerebellar ataxia [1] or the huntingtin interactome [5], specific hypotheses can often be readily tested by confirming interactions and then assessing whether they modulate the functions of the wild-type or disease proteins. Indeed, this has been demonstrated for one of the huntingtin interactors, GIT1, a G-protein-coupled receptor kinase-interacting protein, which enhances huntingtin aggregation by recruiting it into membrane vesicles [5]. In this context, the existing studies represent real gifts to researchers working on these diseases.

Ideally, we would like to be able to move from papers reporting large lists of interacting proteins of uncertain functional significance to a situation where the interaction networks form part of a representation of functional networks in cells. I suspect that such data may evolve from the integration of interactome data with gene-expression profiles and studies of single and double knockouts in model organisms or mammalian cells. Along with such 'wet-lab' experiments comes the need for user-friendly databases that allow efficient and reliable interpretation of protein-protein interactors and integrated datasets. In the meantime, the wealth of data in the public domain resulting from these large scale studies is a resource that is likely to fuel many exciting new studies on the biological significance of specific binary interactions.

Acknowledgements

I thank the Wellcome Trust for funding.

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