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Defining the Cellular Interactome of Disease-Linked Proteins in Neurodegeneration

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1. Introduction

Age-related neurodegenerative diseases are recognized as a major health issue worldwide. Due to our aging society, the number of people suffering from dementia is drastically increasing, creating serious challenges for society and the public health system. Our current paucity of effective treatments and total lack of cures, when coupled with this increasing prevalence, makes the exploration of novel strategies for therapeutic interventions of the utmost importance. Although many of the disease-causing proteins have been identified, the molecular mechanisms that underlie disease pathogenesis are still not fully understood. One common feature of neurodegenerative diseases is the accumulation of misfolded proteins into toxic oligomers and aggregates. Gaining extensive knowledge regarding the formation of these cytotoxic species, the cellular machineries that guarantee their persistence or clearance and the basis of their toxicity is essential for the development of both preventive and therapeutic interventions. A powerful approach to increase our knowledge on these disease processes is the use of proteomic tools to define the interaction networks of disease-related proteins. Significant technical progress has been made in the last decade that now allows high-throughput screening for protein-protein interactions on a proteome level. In this book chapter, we review the diverse proteomic approaches that have been used to define the interactomes of disease-linked proteins and the impact of these findings on the understanding of pathogenic processes.

1.1 Cellular mechanisms of neurodegeneration

The intracellular and extracellular aggregation of mutated and/or misfolded proteins into highly ordered β-sheet rich aggregates, termed amyloid, is a common hallmark of many neurodegenerative diseases such as Alzheimer's (AD), Parkinson's (PD) and Huntington's (HD) disease (Fig.1).

AD is the most common progressive neurodegenerative disease, affecting millions of people worldwide. AD patients show neuronal loss, the deposition of extracellular amyloid plaques consisting of amyloid β peptides (Aβ, ($A\beta_{40}$ and $A\beta_{42}$)) and intracellular neurofibrillary

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tangles consisting of hyperphosphorylated and cleaved Tau. Although various models have been proposed to explain the pathogenic processes underlying the disease, the exact mechanisms leading to AD are not fully understood (Mudher & Lovestone, 2002).

The amyloid cascade hypothesis proposes that aberrant cleavage of the amyloid precursor protein (APP) by two different proteases (β/γ-secretases) leads to the accumulation of aggregation-prone Aβ peptides that eventually cause disease through multiple mechanisms, including microglial infiltration, generation of reactive oxygen species and synaptic damage (Hardy & Selkoe, 2002). According to this model, early intracellular Aβ accumulation induces the aggregation of the microtubule associated protein Tau. In familial forms of AD, genetic mutations within the APP or γ-sectretase cause extensive formation of Aβ protofibrils, which leads to neurotoxicity.

The Tau or tangle hypothesis, however, claims that it is the disruption of microtubule binding and aggregation of Tau by phosphorylation or genetic mutations that initiates the disease cascade (Maccioni et al., 2010). The formation of neurofibrillary tangles subsequently leads to disintegration of the neuronal cytoskeleton, which causes the disruption of neuronal transport and cell death.

Although Tau and Aβ became the focus of extensive research, the exact disease mechanisms remain unclear. The complexity of the disease is reflected in the fact that many other susceptibility genes or proteins involved in development and progression of this disease have been identified in the last decade.

After AD, PD represents the second most prevalent neurodegenerative disease. It is characterized by the loss of dopaminergic neurons in the substantia nigra and formation of intracellular inclusions (Lewy bodies) consisting of α-synuclein, a presynaptic protein of unknown function (Martin, I. et al., 2011). Importantly, in idiopathic Parkinson's disease, α-synuclein inclusions first localize to defined brain areas and pathology appears to progress in a topographically predictable manner (Braak et al., 2004). The majority of cases are sporadic and age-related. However the identification of several mutated genes in the small number of early onset familial forms of PD implicate various proteins contributing to disease progression. So far, genetic mutations in α-synuclein (SNCA), parkin (PARK2), ubiquitin carboxyl-terminal esterase L1 (UCHL1), parkinson protein 7 (PARK7, DJ-1), PTEN-induced putative kinase 1 (PINK1), leucine-rich repeat kinase 2 (LRRK2), α-synuclein interacting protein (SNCAIP) and glucosidase, beta, acid (GBA) have been linked to familial forms of PD (Martin, I. et al., 2011).

Polyglutamine expansions within unrelated proteins are the underlying cause of nine different neurodegenerative pathologies, including HD, spinobulbar muscular atrophy and spinocerebellar ataxias (SCAs) (Hands & Wyttenbach, 2010). HD, a dominantly inherited neurodegenerative disease that is caused by an expansion of the polyglutamine tract in the Huntingtin (HTT) protein, is the most widely studied of these diseases. It manifests in movement disorder, psychological disturbances and cognitive dysfunction. Hallmarks of HD are cytoplasmic and nuclear inclusions that consist of N-terminal fragments of expanded HTT. Mutant HTT causes cellular dysfunction and neurodegeneration, probably through a combination of toxic gain-of-function and loss-of-function mechanisms. Many proteins have been found to localize to HTT inclusions and it has been postulated that mutant HTT interferes with the function of a diverse variety of cellular proteins, leading to toxic alterations of many pathways.

Although the disease-related proteins in AD, PD and HD have different identities, common cellular mechanisms underlie the formation of oligomeric complexes and amyloidogenic aggregates. Whether neurotoxicity is caused by a toxic loss-of-function of the disease-associated proteins or a toxic gain-of-function of the built up amyloids is still under debate. Gaining more insights into the cellular and molecular pathways leading to aggregation and neurotoxicity could, thus, help to identify general and specific targets for therapeutic intervention in a variety of neurodegenerative diseases.

1.2 Protein aggregation and neurotoxicity

Maintaining the functionality of the proteome in a highly dynamic cellular environment constantly exposed to physical, metabolic and environmental stresses is essential for cell survival. Protein homeostasis, or "proteostasis", is controlled by a highly interconnected network of different protein quality control pathways that balance protein folding, degradation and aggregation (Kettern et al., 2010). Molecular chaperones are central players in this network. According to the current concept of chaperone function, chaperones act as surveillance factors that scan the cell and recognize non-native proteins. Upon binding to a substrate protein, they prevent its aggregation by facilitating folding or disposal through the proteasomal (CAP – chaperone assisted proteasomal degradation) or autophagic (CMA – chaperone mediated autophagy, CASA – chaperone assisted selective autophagy) machineries. The association of regulatory cochaperones determines the function of the chaperone as a "folding" or "degradation" factor. Inefficient or unsuccessful folding of a substrate protein thereby enhances the probability that degradation-inducing cochaperones will associate and initiate ubiquitination of the client protein. The different protein quality control pathways do not operate independently from each other and their coordinated action allows the cell to adjust to different alterations that endanger the integrity of the proteome.

Aggregation of disease-linked proteins probably represents a second line of defense against cytotoxic effects of misfolded proteins. At this stage, aggregate formation is probably a cytoprotective mechanism by which the cell sequesters misfolded proteins or oligomers that cannot be degraded by the proteasome. It has been shown that aggregate formation is not an uncontrolled process, but can be defined as part of the cellular protein quality control (Tyedmers et al., 2010). Usually, protein aggregates can be cleared by macroautophagy (Martinez-Vicente & Cuervo, 2007). However, this second line of defense is also overtaxed in neurodegenerative diseases, as the enhanced production of misfolded proteins is cumulative. Thus, the continuous presence of large or numerous aggregates at later disease stages probably exerts a negative effect on many cellular functions and enhances toxicity. The inhibition of cell function may be due to either sterical hindrance of cellular processes, such as axonal transport, or to coaggregation of other proteins that are then depleted from the cell (Olzscha et al., 2011). Taken together, understanding the mechanisms that lead to aggregation of disease-linked proteins is crucial for the identification of processes that cause toxicity in neurodegeneration.

Sporadic neurodegenerative diseases are usually age-related and reflect the overtaxing of a large variety of cellular processes that normally control protein homeostasis (Douglas & Dillin, 2011). In contrast hereditary early-onset dementias are caused by a set of genetic mutations that lead to the constant production of misfolded and aggregation-prone proteins. Mutations causing familial neurodegenerative diseases have been shown to increase the aggregation tendency of several disease-associated proteins in different ways.

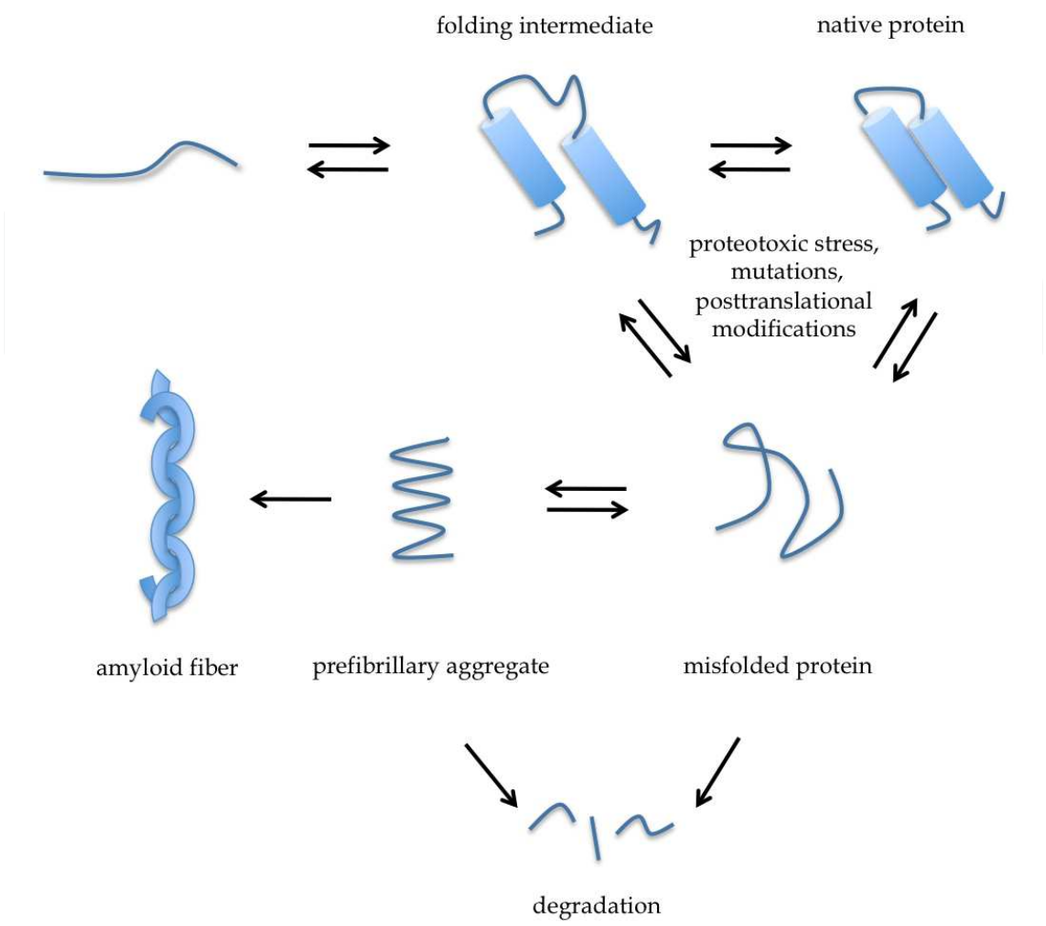

Fig. 1. Protein aggregation in neurodegenerative disease. To be functional, a protein has to fold into an appropriate three-dimensional structure. Aggregation of unfolded cellular proteins is usually prevented by the quality control machinery, which supports the folding process and ensures the removal of unfolded or misfolded proteins. Mutations that enhance the tendency of disease-linked proteins to aggregate, overexpression of aggregation-prone proteins or proteotoxic stress during aging may, however, overwhelm the cellular folding and degradation machineries. Once the quality control machinery is overwhelmed, protein aggregation may be a second line of defense to prevent cytotoxic effects of misfolded proteins or prefibrillary aggregates. Most of the disease-linked proteins in neurodegenerative diseases form highly ordered β-sheet-rich amyloid fibrils. The continued production of aggregation-prone proteins concomitant with decreased protein degradation leads to increased aggregation, likely leading to toxicity at later stages.

Often, mutations in the coding sequence of the disease-causing gene affect the propensity of the protein to misfold and, eventually, aggregate. In HD and several spinocerebellar ataxias (SCAs), a clear correlation between the lengths of the polyglutamine stretch in the disease-causing proteins and the tendency to aggregate has been observed (Martindale et al., 1998). Furthermore, three missense mutations in the α-synuclein gene that are associated with early-onset forms of PD (A30P, E46K and A53T) have been found to enhance the propensity

of α-synuclein to aggregate (Li et al., 2001, Greenbaum et al., 2005). Several mutations in Tau have been linked to various neurodegenerative diseases; while Tau mutations affect different aspects of the protein's function, all result in increased aggregation (Wolfe, 2009).

Importantly, altered gene dosage is sometimes sufficient to cause disease. A genomic duplication or triplication involving the α-synuclein locus has been found to cause some forms of familial early-onset PD (Chartier-Harlin et al., 2004, Singleton et al., 2003). In addition, the higher risk for dementias with neuropathological features of AD in Down's syndrome has been attributed to the triplication of the APP gene in these patients (Rumble et al., 1989).

Mutations can, however, also alter posttranslational modifications of the disease-causing proteins. Proteolytic cleavage of disease-related proteins often precedes amyloid deposition and mutations in the coding region can also affect proteolytic processing of the disease-related protein. Generation of the neurotoxic Aβ peptide by β- and γ-secretase mediated sequential proteolysis of APP plays a central role in AD (O' Brien & Wong, 2011). Pathogenic APP mutations that cause early-onset familial AD are clustered around the α-, β-, γ-secretase cleavage sites and affect the ratio of $A\beta_{40/42}$, the latter having an increased propensity to form amyloid plaques (van Dam & De Deyn, 2006). The role of proteolytic cleavage of Tau in neurodegeneration is less well understood, but proteolysis of Tau can be clearly linked to aggregation and neurotoxicity (Wang et al., 2010). Proteolytic cleavage of several polyglutamine disease-linked proteins liberates toxic protein fragments that can form aggregates (Shao & Diamond, 2007). Finally, hyperphosphorylation of Tau and phosphorylation of α-synuclein and ataxin-1 has been shown to enhance their aggregation. Recently, other posttranslational modifications such as oxidation, sumoylation, ubiquitination or nitration have also been implicated in the aggregation of Tau, α-synuclein and polyglutamine-rich proteins (Beyer, 2006, Martin, L. et al., 2011, Pennuto et al., 2009). In addition, glycosylation affects the processing of APP (Georgopoulou et al., 2001).

2. Interactome mapping in diseases

In the last decades, significant progress has been made uncovering a large number of genetic mutations that are associated with a variety of genetically inherited disorders, summarized in the Online Mendelian Inheritance in Man database (OMIM) (Amberger et al., 2011). Clinical symptomatology, however, is less dependent on single mutations than on how whole organisms and systems are altered.

The pathology of one single gene mutation mapped to a specific disease is rarely caused by the malfunction of just one mutated gene product, but rather reflects perturbations of the whole interaction network in which the altered protein is embedded. This concept is in line with the finding that cellular proteins linked to the same disease exhibit a high tendency to interact with each other (Barabasi et al., 2011). In the interactome, the corresponding disease module, thus, consists of a group of proteins that function together in a cellular pathway or process whose breakdown results in a specific pathophenotype. To understand local network perturbations underlying the diseases phenotype, it is necessary to systematically explore the complex interaction network in which the disease-associated proteins are interconnected. Systems-based approaches to human diseases can, thus, lead to the

identification of new disease genes and pathways. As drug discovery starts to concentrate on network-based targets rather than single gene targets, a deeper understanding of the disease module is crucial for the development of treatments (Morphy & Rankovic, 2007). Moreover the identification of disease modules can help to uncover subnetworks of interacting proteins that are shared between diseases with similar pathologies, such as neurodegeneration. A powerful approach to detect disease modules in neurodegenerative diseases is the interactome mapping of the different disease-linked proteins in combination with the generation of a complete map of the human interactome. The integration of the interactome of a diseases module with gene expression data or structural proteomic data will significantly advance our understanding of the pathophysiology of the disease in search for effective treatments.

2.1 The human interactome

To date, most attempts to map protein-protein interaction networks have been made using model organisms such as the yeast *Saccharomyces cerevisiae,* the nematode *Caenorhabditis elegans* and the fruitfly *Drosophila melanogaster*. This model organism usage is due to the better and earlier annotation of their genomes. In the last decade, several attempts to map the human interactome have been made, representing a first crucial step towards the understanding of cellular interconnectivity and the identification of networks that play a key role in human diseases (Vidal et al., 2011). In addition, the combination of high-throughput datasets and literature-based protein-protein interactions into databases helped to extend existing interactome maps and made information obtained from high-throughput screens more accessible (Tab. 1).

Database	**Description**	**Webadress**
BioGRID	*Biological General Repository for Interaction Datasets*	http://thebiogrid.org/
BIND	*Biomolecular Interaction Network Database:* component database of BOND (*Biomolecular Object Network Databank*) integrates a range of component databases including Genbank and BIND, the Biomolecular Interaction Network Database, resource for cross database searches	http://bond.unleashedinformatics.Com/
DIP	*Database of Interacting Proteins*	http://dip.doe-mbi.ucla.edu/dip/Main.cgi
GWIDD	*Genome Wide Docking Database:* integrated resource for structural studies of protein-protein interactions on genome-wide scale	http://gwidd.bioinformatics.ku.edu/

Database	Description	Webadress
Human Protein Inter-action Database	interactions of HIV-1 proteins with host cell proteins, other HIV-1 proteins, or proteins from disease organisms associated with HIV / AIDS	http://www.ncbi.nlm.nih.gov/RefSeq/HIVInteractions/index.html
HPID	*Human Protein Interaction Database*	http://wilab.inha.ac.kr/hpid/
HPRD	*Human Protein Reference Database:* integrates information on domain architecture, post-translational modifications, interaction networks and disease association for each protein in the human proteome	http://www.hprd.org/
HUGE ppi	*Human Unidentified Gene-Encoded large proteins*	http://www.kazusa.or.jp/huge/ppi/
IBIS	*Inferred Biomolecular Interactions Server*	http://www.ncbi.nlm.nih.gov/Structure/ibis/ibis.cgi
IntAct	curated from published protein-protein interaction data	http://www.ebi.ac.uk/intact/
MINT	*Molecular Interaction Database*	http://mint.bio.uniroma2.it/mint/Welcome.do
MIPS	*Mammalian Protein-Protein Interaction Database*	http://mips.helmholtz-muenchen.de/proj/ppi/
NetPro	Comprehensive database of protein-protein and protein-small molecules interaction	http://www.molecularconnections.com/home/en/home/products/NetPro
POINeT	prediction of the human protein-protein interactome based on available orthologous interactome datasets	http://point.bioinformatics.tw/main.do?file=architecture.txt&selectMenu=environment.ini

Table 1. A selection of protein-protein interaction databases.

In the past years, improvement of techniques for high-throughput interaction screening has helped to generate high-quality protein interaction data for many organisms, including humans. In addition, an empirical framework has been proposed to evaluate the quality of data generated by high-throughput mapping approaches and to estimate the size of interactome networks (Venkatesan et al., 2009). Based on empirical sizing, the authors predict the human interactome to consist of ~130,000 interactions. Previous

estimates of human interactome size range from 150,000 – 650,000 interactions (Venkatesan et al., 2009). To date ~23000 human protein-protein interactions have been reported. With the authors' estimation that 42% of these reported interactions represent true positives, only ~8% of the full interactome size has been identified so far (Venkatesan et al., 2009). Comprehensive mapping of the human interactome will require further development of complementary, systematic, unbiased and cost-effective high-throughput mapping approaches.

2.2 Experimental strategies for mapping protein-protein interactions

A large variety of experimental and computational methods can be used to identify protein-protein interactions. Two complementary methods are primarily applied for the large-scale mapping of protein-protein interactions. Mapping of binary interactions was first accomplished by a high-throughput adaption of the yeast two-hybrid (Y2H) system, originally developed by Fields and Song (Fields & Song, 1989). Mapping of indirect protein associations within protein complexes can be carried out by a combination of affinity purification and mass spectrometry (AP/MS) (Rigaut et al., 1999). Unfortunately both techniques have their limitations in terms of quality and coverage. Quality assessment of datasets from different interaction studies has shown that Y2H and AP/MS data provide the same quality but represent different subpopulations of the interactome, resulting in networks with different topologies and biological functions (Seebacher & Gavin, 2011). Binary maps from Y2H screens were enriched for transient signalling interactions and interactions between different protein complexes, whereas data generated from AP/MS experiments preferentially detected interaction within a protein complex. The combinatorial application of both techniques is, therefore, recommended to obtain more complete protein interaction maps. The lack of a complete interactome map can be overcome to a certain extent by using data-mining strategies to identify sub-networks from different incomplete interactome maps.

2.3 The yeast two-hybrid system (Y2H)

Originally developed by Field and Song (Field & Song, 1989) to detect the interactions of two proteins, Y2H has been further adapted to high-throughput screening (Koegl & Uetz, 2007). The Y2H is based on the finding that many transcription factors can be divided into a DNA-binding domain (*BD*) and an activation domain (*AD*) that maintain their functionality when separated and recombined. In the two-hybrid approach the BD (e.g. from the yeast Gal4 or the *E. coli* LexA protein) is fused to a protein of interest to generate the bait (Fig. 2A). The prey is constructed by the fusion of the *AD* (e.g. from the yeast Gal4 *or* the heterologous B42 peptide) with a set of open reading frames (ORFs). Bait and prey fusions are then coexpressed in yeast. When the bait and prey proteins interact, a functional transcription factor is reconstituted. Reconstitution of the transcription factor is detected by measuring the activity of a reporter gene. Several reporter genes have been used so far. In the "classic" two-hybrid, auxotrophic markers such as HIS3 and LEU2 allow selection by growth on selective media lacking histidine or leucine. Another commonly used reporter is the bacterial β-galactosidase. Recently, GFP has been successfully used as a reporter gene and many others are under investigation. Although Y2H has proved valuable in the

confirmation and identification of many protein-protein interactions, there are experimental limitations of this system.

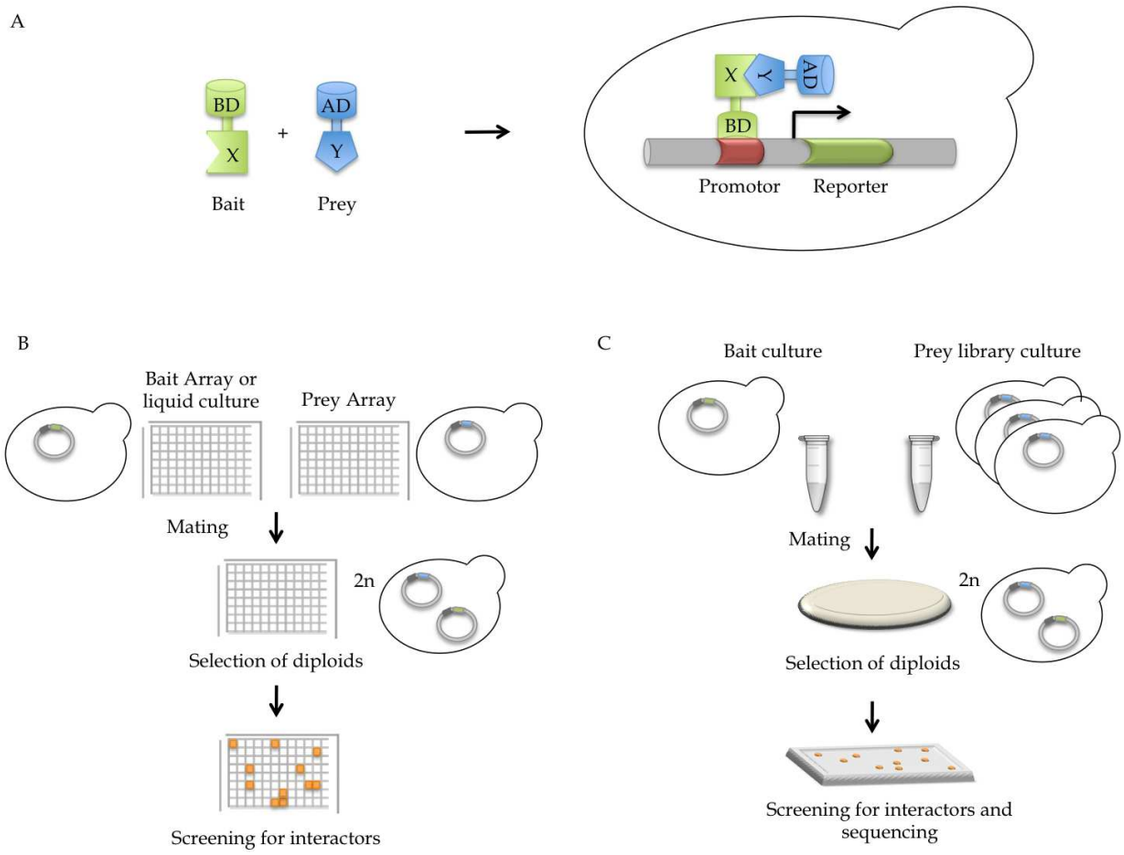

Fig. 2. The yeast two-hybrid system and high-throughput adaptions.
A Principle of the yeast two-hybrid system. To test for a direct interaction, proteins X and Y are coexpressed as fusions with the binding domain (*BD*), or bait, and the activation domain (*AD*), or prey, of a transcription factor (e.g. Gal4) in yeast. If protein X and Y interact directly a functional transcription factor is reconstituted which induces transcription of a reporter gene that allows detection of X-Y interaction (e.g. GFP) or selection for X-Y interaction (e.g. HIS3).
B Workflow of the matrix or array approach for high-throughput interaction screening. A prey array is generated by dispensing yeast clones that each express a different *AD* fusion in a multiwell plate. In an automated step, the prey array is then pinned on a multiwell plate containing yeast clones that express the bait (*BD* fusion). Prey and bait clones are allowed to mate and diploids are selected based on the expression of selection genes. If the bait and prey proteins interact directly, then the expression of a reporter gene that allows screening for interactors of the bait protein is induced.
C Principle of the exhaustive or random library screen for high-throughput screening. In this screen, a bait fusion is screened against a pooled prey library consisting of ORFs or ORF fragments. Diploids and positive interactors are selected based on growth of selection plates. In contrast to the array approach each positive clone has to be picked and sequenced after selection to identify the prey protein.

To obtain worthwhile Y2H results, the bait and prey fusion proteins must properly fold and not be hindered from proper interaction. Their interaction must be stable and not require posttranslational modification. Furthermore, the interaction in the Y2H takes place in the nucleus, though this restriction can be circumvented by the use of protein fragments that fold more efficiently and are able to translocate to the nucleus. *AD* and *BD* fusions alone can also sometimes auto-activate reporter gene expression. This is excluded by performing a self-activation assay of the bait and prey constructs. In this test, a control plasmid coding for an unrelated *BD* or *AD* fusion-protein is added along with the construct of interest. The auto-activation background of a HIS3 reporter can be reduced by the addition of the HIS3 inhibitor, 3-AT. The usage of the opposite fusion construct or a different protein fragment is also often effective in suppressing auto-activation. In addition, several alternative genetic screening techniques have been developed to allow the detection of interactions for transcription activators and membrane proteins (Auerbach et al., 2002).

2.3.1 Large-scale yeast two-hybrid screens

The most powerful application of the Y2H system, one that can generate comprehensive protein-protein interaction maps, is the unbiased screening of whole libraries. Two adaptions of the "classical" Y2H for high-throughput screening are the "matrix approach" or "array approach" and the "exhaustive library screening" or "random library screening" approach (Fig. 2B and 2C) (Koegl & Uetz, 2007).

In the array approach, a set of defined prey proteins is tested for interaction with a bait protein (Fig. 2B). Bait and prey constructs are individually transformed into isogenic reporter strains of opposite mating types. Yeast clones expressing a single *AD* fusion are dispensed into single wells of a multi-well plate, generating a matrix of *AD* fusions. The array of *AD* fusions is then spotted onto another multi-well plate containing one yeast clone expressing a single *BD* fusion for mating. In this way, all *BD* fusions are mated to *AD* arrays. A positive interaction is then detected by the ability of a diploid cell to activate the reporter gene (e.g. growth on selective media). As multiple assays can be performed with the same system under identical conditions, they can later be compared. To exclude false positives, experiments are usually performed in duplicates and only interactions found in both experiments are considered to be true. As whole genomes are available as ordered clone sets, each component of the array has a known identity and no sequencing is required after identifying a positive interaction. To accelerate the screening procedure, a pooling strategy can be applied. In the pooled array screen, *AD* fusions of known identity are tested as pools of *AD* fusions against the *BD* strains. This means that the identification of interaction partners from a positive pool requires retesting of all members. It is also possible to use pools of baits against an *AD* array.

A second approach to analyse the interactome of whole proteomes is the "exhaustive library screening" or "random library screening" approach (Fig. 2C). In this assay, a *BD* fusion is screened against a library of *AD* fusions of full Length ORFs or ORF fragments. For this approach, it is not necessary to know the sequence of the whole genome, as random prey libraries can be generated from randomly cut genomic DNA. In addition a large number of cDNA libraries is commercially available. Like the array screen, the bait and prey constructs are individually transferred into isogenic yeast strains of opposing mating types. However, in contrast to the matrix approach, the different *AD* fusions are not separated on an array.

Instead each *BD* fusion strain is mated with pooled *AD* fusion strains. After mating of the two strains, the diploid yeast cells are plated on selective media to screen for interactions. The identity of the *AD* fusion has to be determined by yeast colony PCR of positive colonies followed by sequencing.

In terms of quality assessment, rigorous evaluation and filtering of the raw data will enhance the quality and reliability of results. Routine testing of positive interactions is performed in duplicate and protein interactions that are not reproducible are discarded. From interactome studies, it is estimated that the coverage of array-based two-hybrid screens is only ~20% (Rajagopala et al., 2011). This high false negative rate is probably caused by the technical limitations of the system. Furthermore, different attempts to map the yeast interactome show very little overlap with each other and with annotated protein-protein interactions, probably due to the usage of different Y2H systems (Uetz et al., 2000, Ito et al., 2001). Recent attempts to generate a high quality dataset of the yeast interactome have revealed over 1000 new interactions (Yu et al., 2008). Further technical development and improvement is needed to increase the coverage of interactome screens. However, as the Y2H only covers a subset of interactions, a complementary AP/MS approach is necessary to generate a comprehensive map of the interactome (Yu et al., 2008).

2.4 Affinity purification coupled to mass spectrometry

Most proteins function in cellular processes as multi-subunit protein complexes. A well-established method to identify protein co-complexes is based on affinity purification followed by mass spectrometry (Fig.3) (Bauer & Kuster, 2003). The classical coimmunoprecipitation protocol is commonly used to detect whether two proteins interact in cellular systems, but can be also used to identify new interaction partners. In this approach, one protein is affinity captured along with its associated proteins by a specific antibody immobilized on Protein A or G sepharose. Experimental details like the affinity tag, lysis conditions, incubation time and washing conditions have a significant impact on the output and need to be optimized depending on the protein complex stability and localization. To preserve protein-protein interactions and native conformations, relatively mild conditions should be used during lysis. TritonX-100 or NP-40 are widely used in cell lysis buffers as non-ionic detergents and efficiently lyse membranes but are mild enough to preserve protein-protein interactions. Other variables that can influence the outcome of the affinity purification are salt concentrations, divalent cation concentrations and pH. The purified complex is rinsed several times to remove unspecifically bound proteins and complexes are eluted from the resin by low or high pH, high salt concentrations, by competition with a counter ligand or by adding Laemmli buffer to the beads. After purification, the isolated protein complex is usually separated on 1D or 2 D gels and protein lanes or spots are digested by trypsin and subsequently analyzed by mass spectrometry. It is also possible to precipitate eluted protein complexes and trypsinize the pellet. Comparison of the proteins detected in the sample with a negative control leads to the identification of specific interaction partners. Although low background binding is favoured, the stringency of washing steps needs to be adjusted to preserve more dynamic or transient interactions. Nonspecific binding can be reduced by adding low levels of detergent or by adjusting the salt concentration.

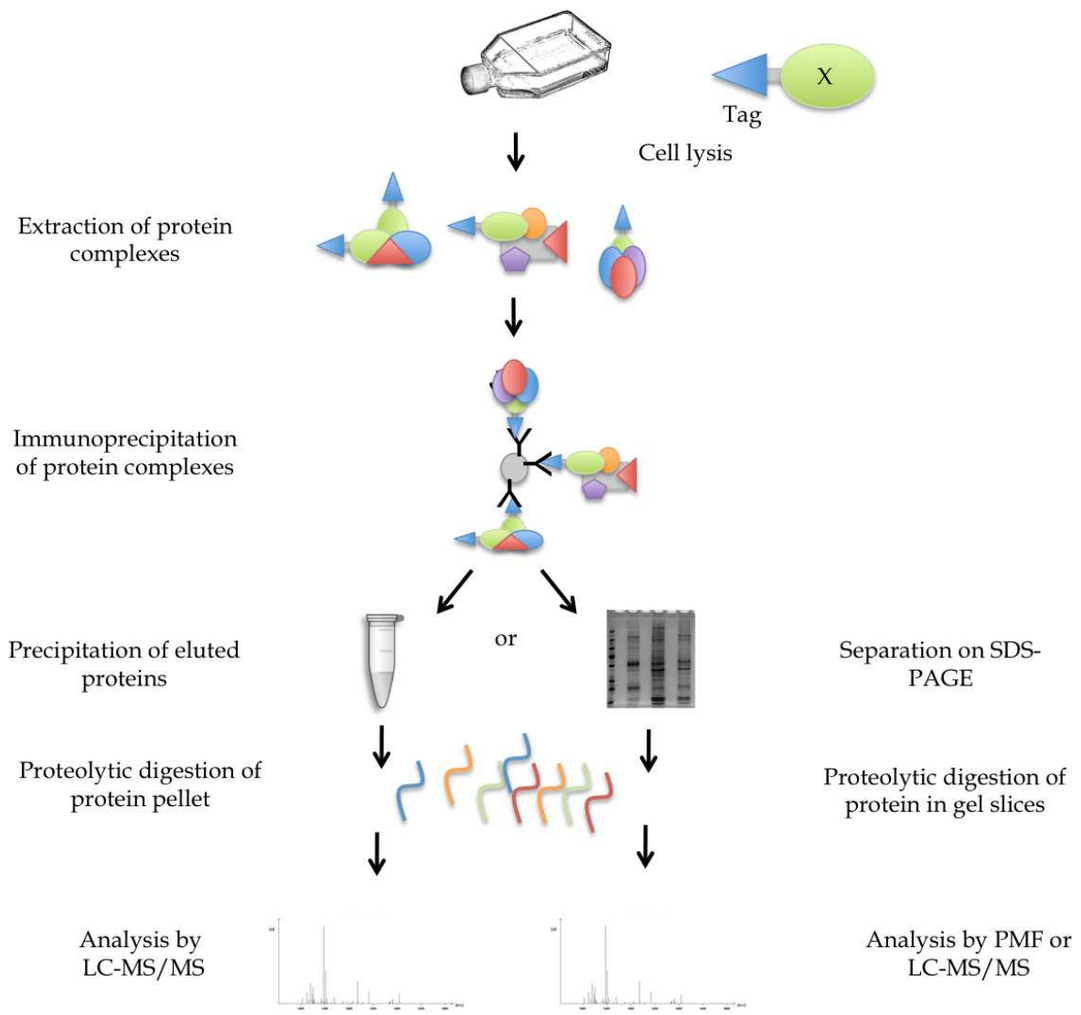

Fig. 3. Workflow of AP/MS for high throughput screening. Cells that express an epitope-tagged form of the protein of interest are lysed to extract protein complexes. The protein complexes are then isolated using a tag- specific antibody. The antibody-bound protein complex is immobilized on Protein A or Protein G sepharose and non-specific interactors are eliminated by several wash steps. As a control, the immobilized antibody is incubated with an untagged cell extract or a cell extract containing a tagged control protein (e.g. GFP). After purification, the protein complexes can be either separated on a gel or eluted and precipitated. The gel slices or the protein pellet are then digested to generate peptide fragments that can be analyzed by mass spectrometry to identify co-complexed proteins.

2.4.1 Large scale AP/MS screens

Proteome wide interaction studies need to meet certain criteria, such as reproducibility and low background binding. To circumvent the need of a specific antibody, the bait protein can be fused to an epitope tag that allows the pull down of different protein complexes using the same antibody under standardized conditions. A large variety of different epitopes has been used in the past and are commercially available (e.g. HA, FLAG, MYC). One disadvantage

of this technique, however, is that the epitope-tagged proteins are overexpressed in the cell. Cytotoxicity induced by overexpression can be prevented by the usage of inducible expression systems. The tag might also interfere with protein folding and localization. Despite these limitations, coimmunoprecipitation via an epitope tag has been successfully used in proteome wide screens (Gavin et al., 2002, Ho et al., 2002).

Another coimmunoprecipitation protocol that is frequently used for high-throughput experiments is the tandem affinity purification (TAP) developed by Rigaut (Rigaut et al., 1999). The basic concept is similar to the coimmunoprecipitation of epitope tagged proteins. The main difference, however, is the use of two tags. One commonly used TAP tag consists of an IgG binding protein (Protein A) linked to a calmodulin-binding domain (CBD) via a TEV protease recognition site. Protein complexes are purified by the incubation of lysates with IgG sepharose beads that capture the Protein A tag. After washing the immobilized complex is eluted by TEV cleavage. Eluted protein can then be bound to a calmodulin sepharose column via their CBD domain. As the binding to calmodulin is calcium dependent, immobilized protein complexes can be eluted by addition of EDTA. The major advantage of this technique over one-step purification is increased specificity, reducing background binding to levels suitable for large-scale analysis (Gavin et al., 2002, Krogan et al., 2006). However, the long purification protocol preserves only stable interactions. For this reason, one-step purification with an epitope-tagged protein of interest is preferable if a broader range of interactions should be captured. Although the stringency of the purification protocol needs to be adjusted, the high sensitivity of mass spectrometry requires a minimum of non-specific binding to avoid laborious post-experimental filtering, especially in the case of large-scale experiments. An alternative approach to eliminate non-specific interactors without loosing low abundant or transiently interacting proteins is the use of quantitative mass spectrometry (Kaake et al., 2010).

The analysis of the isolated protein complex typically starts from separation of the complex components on 1D SDS-PAGE gels based on their molecular weight or 2D gels based on their charge and molecular weight followed by protease digestion. Usually, trypsin or Lys-C are employed to cleave the protein in peptide fragments because they generate peptides that have basic amino acids at their C-termini, which is favourable for detection and sequencing by mass spectrometry. In large-scale experiments, however, all purified proteins are precipitated and digested together to avoid time-consuming separation steps by gel electrophoresis and instead protein samples are fractionated by liquid chromatography (LC) after tryptic digest. Depending on the complexity of the sample, different approaches for protein identification by mass spectrometry can be used (Bauer and Kuster 2003). Complex peptide mixtures are analyzed by liquid chromatography in combination with tandem mass spectrometry (LC-MS/MS), whereas protein samples that have been separated on 1D SDS-PAGE or 2D gels and have therefore a lower complexity are analyzed by peptide mass fingerprinting (PMF).

PMF is an analytical method in which the absolute masses of peptides from a tryptic digest can be measured by matrix-assisted laser desorption/ionization time-of-flight mass spectrometry (MALDI-TOF). The measured masses of the sample are then looked up in a database that contains predicted peptide masses that have been generated by *in silico* digests for every protein. The results are statistically analyzed to find a significant overlap between the experimentally generated and predicted peptide masses. The identification method is

based on the assumption that it is very unlikely that the exact same combination of peptide masses is found in more than one protein. Although this method can be applied to protein mixtures, the presence of different proteins complicates the analysis. Therefore, PMF is usually applied to identify proteins or protein mixtures isolated from 1 or 2D gels. Mixtures of more than five proteins require the additional use of tandem mass spectrometry for reliable protein identification.

Tandem mass spectrometry, uses a combination of sequence and mass information for the identification of proteins (MS/MS). In a first step, the masses of peptides from the tryptic digest are measured. Single peptides are then isolated from the mixture in the mass spectrometer and collided with inert gas molecules, which leads to fragmentation of the peptide backbone. Peptide fragments generated by this method differ in length by one amino acid. Measurement in the mass spectrometer allows determination of mass differences between two adjacent fragments that indicate a particular amino acid residue. The peptide mass, fragment mass and sequence is then compared against one or more databases to identify the proteins in the sample. Each peptide is individually identified and attributed to a protein in the mixture. For the analysis of complex samples, tandem mass spectrometry is generally combined with liquid chromatography to fractionate the peptide mixture before analysis by tandem mass spectrometry (LC-MS/MS).

Due to the sensitivity of the method and depending on the stringency of the purification protocol, post-experimental filtering is necessary to eliminate non-specific interactors. The ultimate goal of post-experimental analysis is the maximum reduction of false positives while maintaining the maximum coverage. Usually proteins that coimmunoprecipitate with a protein of interest are compared to proteins that are detected in a control sample to define unspecific interactions. Each of the experiments is commonly done at least in duplicate to identify high confidence interacting proteins. Whereas reproducibility seems to be a good indicator for true positive interactions, the quality assessment by comparisons with existing datasets depends on the selected gold standard. An ideal gold standard reference data set would be confirmed by other sources and should be generated by a comparable method to that being applied. Yu et al. (Yu et al., 2008) used an approach in which they tested several reference databases using various techniques to select a good gold standard.

2.5 Quality assessment of interactome mapping datasets

The identification of disease-modules and the interactome mapping of disease-linked proteins relies on the quality of the available reference datasets and the filtering of the experimentally generated interaction data. In early studies the precision of interactome maps was estimated by the integration of other biological attributes, such as gene ontology, or comparison with literature curated datasets. However, these methods suffer limitations in the estimation of data quality, as they need to be complete and unbiased. Recent efforts to establish an empirical framework for protein interaction maps will improve the estimation of accuracy and sensitivity for interaction maps generated by high-throughput interaction screens (Venkatesan et al., 2009). This empirical framework evaluates four different quality parameters of the currently used methods to estimate quality:

1. *Screening completeness*: the fraction of overall possible ORF pairs that are tested to generate the interaction map.
2. *Assay sensitivity*: the fraction of all interactions that could be identified by the applied experimental method.
3. *Sampling sensitivity*: the fraction of all detectable interactions that are identified in one trial of an assay under specific experimental conditions.
4. *Precision:* the fraction of known true positive pairs in the dataset.

Estimation of these parameters offers a quantitative idea of the coverage and accuracy of an interaction map and, when used in a standardized way, enables the comparison of different datasets.

3. Interactome mapping of disease-linked proteins in neurodegenerative diseases

Although great efforts have been made to uncover specific and shared pathways underlying neurodegeneration, many cellular mechanisms involved remain to be uncovered. As many disease-linked proteins function in multisubunit complexes and are functionally embedded in large cellular networks, gaining extensive knowledge of the architecture and composition of these complexes and networks is crucial to identify disease-linked pathways and to establish new disease markers. Based on this knowledge, diagnosis of early-onset dementias could be significantly improved and new therapeutic strategies could be developed. For age-related dementias, the discovery and characterization of shared pathways could pave the way towards common therapeutic interventions. To uncover the networks and complexes in which disease-linked proteins are embedded, interaction studies of many disease linked proteins, such as AD–associated (Chen et al., 2006, Krauthammer et al., 2004, Liu et al., 2006, Norstrom et al., 2010, Perreau et al., 2010, Soler-Lopez et a., 2010, Tamayev et al., 2009), PD-associated (Engelender et al., 1999, Meixner et al., 2011, Schnack et al., 2008, Suzuki, 2006, Woods et al., 2007, Zheng et al., 2008), HD-associated (Goehler et al., 2004, Kaltenbach et al., 2007) and ataxia-associated proteins (Kahle et al., 2011, Lim et al., 2006) as well as of prion protein (PrP) (Nieznanski, 2010) have been conducted. In addition, an interactome study that utilizes artificially designed amyloid-like fibrils has been performed and focused more on the general mechanisms underlying the toxic gain-of-function of β-sheet rich proteins (Olzscha et al., 2011).

3.1 Interactome mapping of disease- linked genes by large-scale Y2H

Several Y2H studies have been conducted to identify interaction partners of disease-related proteins in neurodegenerative diseases. In the following section, we highlight several studies that have contributed significant insight into disease networks by using new approaches or developing new ideas.

3.1.1 Generating the AD interaction network

An interesting approach to generate a complete interaction map for AD has been employed by Soler-Lopez et al. (Soler-Lopez et al., 2010). The authors elegantly combined different strategies to establish the most complete AD interaction network to date by exploring the interactomes of all known AD-linked proteins. The integration of their data

with literature-based information provides new insights into the molecular interplay of different functional modules within the AD network and has helped to identify new candidate genes. Whereas most of the other interactome mapping approaches only focused on one prominent disease-linked protein, this study underlines the need to study the complete set of disease-associated genes in order to get the whole picture of a complex disorder like AD.

Although extensively studied, the cellular mechanisms that underlie the neurophathological changes associated with AD remain elusive. Despite the central role of APP and Tau, AD is a genetically complex disease and several genetic risk factors have been identified in the last decades. As it has been shown that the susceptibility/causative genes for many diseases are often interconnected within the same biological module, Soler-Lopez et al. applied different network biology strategies in their recently published study to build the most complete AD related interactome (Soler-Lopez et al., 2010).

The authors used available information from the Online Mendelian Inheritance in Man database to assemble a set of 12 well established AD related genes that they termed "seed". To be classified as a seed gene at least one mutation of the gene must be associated with AD in the OMIM Morbid Map. Interestingly, quantification of the degree of connectivity by computing the minimal path length between seed genes shows that the seed genes are connected by three links on average, whereas control sets (randomly picked genes and randomly picked disease causing genes from different disorders) were connected by more then four links on average. Starting from their defined AD seed genes, the authors developed a strategy to discover new disease genes. For this purpose they aimed to identify proteins that match at least one of the following criteria: 1.) the encoded proteins must directly interact with the seed genes, 2.) the gene must locate to a known genetic susceptibility locus and 3.) the gene must have altered expression in AD.

To define the interaction network of their selected seed proteins, they performed Y2H interaction screens. Baits were constructed for nine genes from the seed (three ORFs were not available) and yeast clones for the individual baits were transformed with an adult brain cDNA prey library. Each experiment was done in five replicates. 72 positive interactions resulted from this screen and were retested by pairwise cotransformant Y2H arrays. 32 high confidence interactors for the seed proteins could be validated.

In a second approach, the authors performed a pairwise candidate screen based on published genome wide association studies that identified four chromosomal regions (7q36, 10q24, 19q13.2, 20p) containing unknown AD susceptibility genes. They focused on 185 genes that are located in chromosomal regions linked to AD. Of those, 44 candidate genes known to be coexpressed with AD genes and suitable for Y2H screening were tested for their interaction with the 9 seed proteins. A systematic matrix-based Y2H was performed to identify interactors of the AD seed among the candidates extracted from the AD linked chromosomal regions. Two different technical approaches, a mating and a cotransformation screen, were used to perform the Y2H. With the identified interactions from the two Y2H, the authors generated a high-confidence interaction core (HC). The interactors identified in the library screen had to be validated to be included in the HC and the interactors from the pairwise candidate screen had to activate at least two reporter genes to be defined as high-confidence interaction. The final HC contained 8 seed genes (no HC interactors were

identified for one seed gene) and 66 interactors, 27 from the library screen and a further 39 from the pairwise candidate screen. Interestingly, the different interactor set showed no overlap and only a low overlap with other studies, highlighting the importance of utilizing multiple approaches to increase coverage.

The HC interactions were validated by complementary strategies, such as GST pull down assays, coimmunoprecipitations and colocalization studies. Due to the high stringency applied for the identification of HC interactors, 87% of the interactors could be validated. The analysis of the HC identified four novel direct interactions between well-established AD related proteins (APP, A2M, APOE, PSEN1, PSEN2), implicating a possible link between plaque formation and inflammatory processes and providing insights into the regulation of APP cleavage. The assembled network showed an enrichment of 3 biological processes (oxidation reduction, regulation of apoptosis and negative regulation of cell motion), 5 molecular functions (protein binding, mono-oxygenase activity, oxygen binding, actin binding and integrin binding) and 6 cellular compartment terms (cytoplasm, pseudopodium, platelet alpha granule lumen, cytosol, cytoskeleton and internal side of plasma membrane) as well as altered expression of 17 out of 66 interactors, based on microarray data (Blalock et al., 2004). Furthermore, 6 out of 58 direct interactors were listed in the AlzGene database, whereas none of the non-interacting proteins were found in the database. Network analysis suggests a role for the programmed cell death 4 protein (PDCD4) in regulating neuronal death. PCDC4 was found to bind to the seed proteins PSEN2 and APOE and is located in a functionally homogenous network module enriched for translational elongation. As PCDC4 is upregulated in AD brain tissue, the authors suggest that the protein plays a role in Aβ toxicity. Another interesting member in the AD interactome network (AD-PIN) is ESCIT (evolutionary conserved signalling intermediate in Toll pathway), which links the redox signalling and immune response modules. Based on published data, the authors hypothesize that ESCIT may represent a molecular link between mitochondrial processes and AD lesions. Taken together, newly identified genes in the HC are likely related to AD onset or progression.

To generate a complete AD-PIN, the interaction partners of the HC were retrieved from literature-curated databases. The AD-PIN was then used to identify functional modules within the network that help to link processes potentially involved in AD and to identify the relationship between new candidate genes.

Taken together, this integrative approach combined stringent interaction screening and extensive validation by complementary strategies to build a comprehensive disease interaction map and highlight the importance of a network view of disease and the necessity of data integration from different sources when exploring disease interactomes.

3.1.2 The ataxia-ome

Disorders having a common clinical presentation likely also have common altered pathways. Examples of such diseases are familial spinocerebellar ataxias. A multitude of disease-associated mutations have been discovered, each leading to gain or loss of normal protein function. These mutations all inevitably lead to loss of Purkinje cells through an as yet unknown common pathway. An interesting study by Lim et al. used an Y2H approach to explore the interaction network of 54 proteins involved in 23 ataxias (Lim et al., 2006).

This study nicely showed that interactome mapping of different disease related genes can help to identify common pathways shared by diseases with similar presentation, such as neurodegenerative disorders.

Similar to the AD interactome study, the authors first defined a set of 23 ataxia-causing genes (11 recessive and 12 dominant, including the polyglutamine-mediated SCAs) whose mutations are linked to ataxias in humans or mice. Paralogs and an additional 31 directly interacting proteins were grouped together as ataxia-associated proteins. Bait and prey constructs for each of the genes were constructed to perform matrix-based mating type reciprocal screens against the human ORFeome. To minimize false positives, they used a stringent version of the Y2H system that expresses fusion proteins at low levels. Yeast clones containing single ataxia baits were mated with yeast clones of the opposite mating type containing 188 different ORF minilibrary pools (Rual et al., 2005). In a second screen, reciprocal interactions were tested between human ORFeome baits and ataxia preys to exclude effects caused by misfolding of the fusion protein and to include autoactivation baits. The overlap of the two screens comprises only 5.2% of the observed interactions, which is typical for reciprocal studies (Rual et al., 2005). To include a tissue specific library, the authors screened a human brain cDNA library using the same experimental setup. 29 interactions were overlapping in both screens, indicating that screening different types of libraries can enhance the coverage of the interactome map, as splice forms of a single gene are often spread across cDNA libraries. 83% of the interactions were validated experimentally by GST pull down assays from HEK293 cells with randomly picked interaction pairs. Analysis of the interaction sets found that 72% of those with compartment annotations colocalize together and 98% of the interactors share a GO branch, demonstrating the high quality of the Y2H generated interaction network.

A large interconnected network between the 36 ataxia genes and 541 preys was revealed for ataxia processes. In addition, 13 ataxia causing baits were linked directly or through common interacting proteins, indicating that the proteins in this interconnected network are functionally linked.

To build the ataxia network, Y2H data were integrated with interaction data from available databases. The network was then divided into a direct ataxia network, which contains first-order protein interactions, and the expanded ataxia network, which contains additional second- or third-order interactions. Further comparison of this ataxia network with networks generated from unrelated disease proteins showed that ataxia network nodes have a shorter path length, with a higher number of single hub interconnections. Surprisingly, 18 out of 23 ataxia causing proteins interacted directly or indirectly.

The ataxia interactome assembled by Lim et al. shows that for different ataxias, the similar pathophenotype is indeed caused by the alteration of shared pathways and processes. Main hubs in this ataxia network are involved in DNA repair and maintenance, transcription, RNA processing and protein quality control. Taken together, this kind of approach shows that genes and proteins that are involved in the same or related disorders are highly interconnected, often operating in the same pathways. In this way, interactome studies can prove valuable in identifying the shared pathways underlying related phenotypes.

3.2 Interactome mapping of disease-linked genes by AP/MS

Several AP/MS approaches have been successfully applied to identify interaction partners of disease-linked proteins in different neurodegenerative diseases. The following section summarizes two quantitative approaches that aim to identify distinct disease-relevant interaction networks.

3.2.1 QUICK screen to identify a PD-associated interaction network

Although PD represents a complex disorder for which several genetic risk factors have been identified, no complementary PD network has yet been generated, unlike for AD (Soler-Lopez et al., 2010). To explore the physiological function of the disease associated leucine-rich repeat kinase 2 (LRRK2), Meixner et al. used a new AP/MS protocol to identify the interaction network under stoichiometric constraints, called QUICK (quantitative immunoprecipitation combined with knockdown) (Meixner et al., 2011, Selbach & Mann, 2006). The QUICK screen aims to detect protein-protein interactions at endogenous levels and in their normal cellular environment by using a combination of SILAC, RNA interference, coimmunoprecititation and quantitative MS. For the QUICK approach, NIH3T3 cells were transduced with a lentiviral shRNA construct to knock down endogenous LRRK2. WT and LRRK2 knock down cells were then grown in SILAC medium containing either normal heavy isotope labeled lysine or arginine. For coimmunoprecipitation, an LRRK2 specific antibody was crosslinked to Protein G sepharose. Cells were lysed in buffer containing 1% NP-40 and phosphatase inhibitors. Equal numbers of heavy and light SILAC labeled cells were incubated with LRRK2 sepharose and then pooled. Purified LRRK2 complexes were eluted after several washing steps in Laemmli buffer. Separation of the proteins was achieved by SDS-PAGE gel electrophoresis, followed by tryptic digestion of gel slices. Interaction partners were identified by subsequent LC-MS/MS. Identified interaction partners were verified using the same approach, without prior SILAC labeling. Using this protocol, 36 proteins were identified as high confidence LRRK2 interactors. Bioinformatic analysis and integration of interaction data from different databases showed that these proteins are mainly members of the actin family and actin-associated proteins, pointing to an important role for LRRK2 in actin cytoskeleton based processes. Additional experiments demonstrated that LRRK2 binds to F-Actin *in vitro* and regulates its assembly. Knockdown of LRRK2 leads to morphological alterations and shortened neurite processes in primary neurons, further indicating a physiological role for LRRK2 in cytoskeletal organization. As the experimental strategy of the QUICK approach aims to identify interactors at physiological conditions (endogenous expression of the target protein) with specific antibodies, this method is well suited to explore the physiological function of other disease-linked proteins. Moreover, using knock-down or knock-out cells for control immunoprecipitations with a specific antibody proved as a suitable control. So far, this approach is not suited for large-scale applications involving several candidate proteins, but is of interest for generating interactome maps for one protein.

3.2.2 Interactome mapping of amyloid-like aggregates

Ordered proteinacious aggregates with high ß-sheet content, termed amyloid, are a characteristic of many neurodegenerative diseases. Whether this aggregation is cytoprotective or cytotoxic is still under debate. One hypothesis for aggregate toxicity is that these aggregates sequester cellular proteins, thereby leading to functional impairment (Chiti & Dobson, 2006).

To uncover the gain-of-function toxicity of amyloid-like fibrils in general, Olzscha et al. defined the interactome of artificial β-sheet proteins designed to form amyloids (West et al., 1999). The advantage of this method is that none of the artificial proteins have endogenous interaction partners, which allows for the identification of common amyloid interacting proteins independent of the identity of the aggregating protein. β4, 17 and 23, which differ in their β-sheet propensity, with β23 having the highest tendency to form β-sheets, were selected for interactome mapping. As a control, the authors used an α-helical protein with a similar amino acid composition (α-S284). Interaction analysis of the different β-sheet proteins was performed using SILAC followed by LC-MS/MS. Constructs coding for MYC-labelled proteins were transfected into HEK293 cells and proteins were labelled with light, medium or heavy arginine and lysine isotopes. Different experimental setups were chosen: (1) empty MYC tag vector, MYC α-S284, MYC β23; (2) MYC α-S284, MYC β4, MYC β17; (3) MYC β4, MYC β17, MYC β23 for quantitative MS. Lysates from heavy, medium and light labelled cells were mixed in a 1:1:1 ratio and amyloidogenic aggregates were isolated using anti-MYC coupled magnetic beads to avoid loss of the protein aggregates due to centrifugation. After purification the bound proteins were eluted and processed for LC-MS/MS analysis. Experiments were performed in triplicate. Proteins were defined as interactors if they were enriched relative to the α-S284 control or one of the β proteins with >95% confidence in two of the three repeats. Interactors were validated by coimmunoprecipitation and western blotting or immunofluorescence analysis. Bioinformatic analysis of the amyloid interactomes revealed that aggregates sequester a large amount of large, unstructured proteins that occupy essential hub position in housekeeping functions such as transcription and translation, chromatin regulation, vesicular transport, cell motility and morphology, as well as protein quality control. The authors hypothesize that amyloidogenic aggregation coaggregates a metastable subproteome, thereby causing perturbations in essential cellular networks leading to toxicity.

3.3 Interactome mapping by combinatorial approaches: The HD interactome

As Y2H and AP/MS approaches identify different groups of interactors, the combination of the two experimental strategies may prove valuable for the generation of comprehensive interaction maps of disease-linked proteins. In a study on HD, Kaltenbach et al. used these two approaches to generate a comprehensive HD interactome, which was then tested for modulators of polyglutamine toxicity. Importantly, the two approaches revealed distinct but biologically relevant interactions (Kaltenbach et al., 2007).

In this study the authors performed Y2H and AP/MS approaches in parallel to generate a comprehensive HTT interactome. Assuming that HTT and its interactors are functionally linked and regulate each other, the HTT interactome should be enriched for genetic modifiers of neurotoxicity. To test this hypothesis, the authors generated a *Drosophila* model of polyglutamine toxicity. For the AP/MS, different recombinant TAP-tagged HTT fragment baits were constructed (HTT 1-90: Q23, Q48, Q75; HTT 443-1100 and HTT-2758-3114) and purified from bacteria for pull down assays with mouse or human brain tissue and mouse muscle tissue. Purified complexes were then analyzed by MS. HTT fragments 1-90 were also used to probe HeLa, HEK293 and M17 neuroblastoma cell lysates. The interaction lists from the different pull downs were filtered by excluding proteins observed in a control pull down with the TAP tag alone and subjected to statistical analysis. The interaction lists were compared to a database of high-scoring peptides from 88 other unrelated pull downs. By

using this approach, the authors generated a list of 145 mouse and human specific interactors with WT and expanded HTT fragments. In a complementary assay, a set of additional HTT interactors was identified by Y2H with HTT fragment baits (WT: 23Q and mutant: >45Q) against prey libraries from 17 different human tissue cDNA sources. Again, only baits generated from N-terminal fragments gave reproducible results. Filtering of the data was performed to generate a high confidence interaction dataset. Only interactions that were observed at least three times in the Y2H were integrated into the dataset; interactors were further compared to a database of Y2H interaction screens such that promiscuous interactors could be excluded. Finally, all positive prey constructs were retested by cotransformation into yeast strains expressing bait constructs. Previously published HTT interactors were included regardless of whether they matched the quality assessment criteria. A total of 104 interactors were identified by this Y2H approach.

The interactome was tested for possible genetic interaction in a fly model of polyglutamine toxicity. In this model, the N-terminal fragment of human HTT cDNA, including a 128Q expansion in exon 1, is expressed in the eye. The resulting neurodegenerative phenotype is visible by examining the retinal histology. Interactors that have orthologues in the fly and for which suitable fly stocks for overexpression or partial loss-of-function were available were selected from both high-confidence datasets. 60 interactors divided equally between interactors from the Y2H and AP/MS approach were tested as modifiers of polyglutamine mediated toxicity. 80% of the tested interactors altered the readout of more than one allele in a single background or a single allele in multiple backgrounds. The high confidence interaction dataset was further validated by coimmunoprecipitation studies using lysates from WT and HD mice. Data from this study strongly suggest that Y2H and AP/MS are of comparable quality in identifying biologically relevant interactions. Gene ontology analysis demonstrated that modifiers cluster into different groups, such as cytoskeletal organization, signal transduction, synaptic transmission, proteolysis and regulation of transcription and translation. Based on their experimentally generated interaction datasets, the authors built an interaction map of HD by integrating interaction data from different databases.

Interacting proteins of the HD network that were confirmed as modifiers in the fly model were shown to have diverse biological functions, such as synaptic transmission, cytoskeletal organization, signal transduction and transcription. In addition the authors revealed an unknown association between the HTT fragment and components of the vesicle secretion apparatus, suggesting that modulation of SNARE-mediated neurotransmitter secretion may be a physiological function of HTT. The involvement of HTT in these processes suggests a model in which mutant HTT interferes with different cellular pathways, leading to pathology.

4. Summary

Proteins do not operate alone, but instead function as a large interconnected network. Disease-related mutations, consequently, disrupt not only the function of individual proteins, but also the larger network in which these proteins function, only thereby leading to clinically relevant pathology. While many complex diseases are caused by an array of mutations in any of a multitude of genes, these all lead to a similar pathology. Focusing not just on individual proteins but, rather, on the network of proteins altered in

diseases will prove essential to both identifying new candidate genes for this and related disease, as well as to provide new targets for the development of therapeutic interventions. A combination of different experimental and bioinformatic approaches has proved valuable in the network analysis of neurodegenerative diseases. Further studies are necessary to generate high quality, comprehensive datasets, which can be used to identify shared disease pathways.

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6. References

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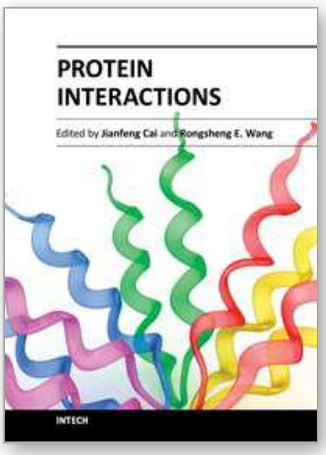

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Protein interactions, which include interactions between proteins and other biomolecules, are essential to all aspects of biological processes, such as cell growth, differentiation, and apoptosis. Therefore, investigation and modulation of protein interactions are of significance as it not only reveals the mechanism governing cellular activity, but also leads to potential agents for the treatment of various diseases. The objective of this book is to highlight some of the latest approaches in the study of protein interactions, including modulation of protein interactions, development of analytical techniques, etc. Collectively they demonstrate the importance and the possibility for the further investigation and modulation of protein interactions as technology is evolving.

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