The Role of Neuronal Complexes in Human X-Linked Brain Diseases

Frédéric Laumonnier, Peter C. Cuthbert, and Seth G. N. Grant

Beyond finding individual genes that are involved in medical disorders, an important challenge is the integration of sets of disease genes with the complexities of basic biological processes. We examine this issue by focusing on neuronal multiprotein complexes and their components encoded on the human X chromosome. Multiprotein signaling complexes in the postsynaptic terminal of central nervous system synapses are essential for the induction of neuronal plasticity and cognitive processes in animals. The prototype complex is the N-methyl-D-aspartate receptor complex/membrane-associated guanylate kinase–associated signaling complex (NRC/MASC) comprising 185 proteins and embedded within the postsynaptic density (PSD), which is a set of complexes totaling ~1,100 proteins. It is striking that 86% (6 of 7) of X-linked NRC/MASC genes and 49% (19 of 39) of X-chromosomal PSD genes are already known to be involved in human psychiatric disorders. Moreover, of the 69 known proteins mutated in X-linked mental retardation, 19 (28%) encode postsynaptic proteins. The high incidence of involvement in cognitive disorders is also found in mouse mutants and indicates that the complexes are functioning as integrated entities or molecular machines and that disruption of different components impairs their overall role in cognitive processes. We also noticed that NRC/MASC genes appear to be more strongly associated with mental retardation and autism spectrum disorders. We propose that systematic studies of PSD and NRC/MASC genes in mice and humans will give a high yield of novel genes important for human disease and new mechanistic insights into higher cognitive functions.

The synapse is fundamentally important for neural function because it mediates the interneuron communication that forms the basis of all cognitive activity.[1–4] The majority of synapses in the CNS use glutamate as a neurotransmitter.[5,6] Glutamate is released from presynaptic terminals in response to incoming action potentials, diffuses across the synaptic cleft, and activates receptors embedded in the postsynaptic membrane.[7,8] The main types of glutamate receptors are ion-channel–forming N-methyl-D-aspartic acid (NMDA), α-amino-3-hydroxy-5-methyl-4-isoxazolepropionic acid (AMPA), and G protein–coupled metabotropic (mGluR) receptors (fig. 1*A*). The primary role of AMPA receptors is to mediate the membrane depolarization that is necessary to initiate action potentials in the postsynaptic neuron. By contrast, the NMDA and mGluR receptors do not significantly contribute to the depolarization but do initiate signal transduction–pathway signaling. Moreover, NMDA and mGluR receptors are physically linked by scaffolding proteins and are found within multiprotein complexes, along with signaling enzymes and other proteins.[10–13]

Pharmacological antagonists for the glutamate receptors have been available for >20 years and have been used extensively in animal and human studies, and it is clear that these receptors play a role in a diverse set of behaviors.[14,15] These findings have led to the "glutamate hypothesis" of mental illnesses.[16,17] Although there is no doubt that glutamate receptors are physiologically important, progress in several areas has dramatically expanded our understanding of their role in synapse biology. First, it is known that the receptors physically link to a plethora of proteins and form signaling and trafficking complexes (discussed in detail below); second, synapse proteomics has characterized multiprotein complexes and has discovered hundreds of postsynaptic proteins, many of which are involved with human disease; and, third, genetic manipulation of synapse proteins in mouse has overcome the limited availability of pharmacological antagonists and, hence, has allowed the functional testing of specific genes in behaviors. Given the large amount of available data within these different areas of investigation, it is timely to integrate these data sets and to ask how they might be useful in future human genetic studies of brain diseases.

We will address a number of general issues relevant to any tissue or disease, using the extensive information on synapse proteins and specific multiprotein complexes. Interrogating these lists and models with human genetic data allows several questions to be addressed. First, how many of the genes encoding the components of a complex are involved with human disease? Second, are there similarities in the phenotypes that might indicate that the mutations have interfered with the overall function of the complex? Third, what do the human phenotypes reveal about the physiological or cellular functions of the complex? Fourth, can we confidently use the gene lists to hunt for further disease-causing mutations? Fifth, can understanding the interaction of proteins in the complexes pro-

From the Genes to Cognition Programme, Wellcome Trust Sanger Institute, Hinxton, United Kingdom

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Address for correspondence and reprints: Dr. Seth G. N. Grant, Wellcome Trust Sanger Institute, Genome Campus, Hinxton, Cambridge, CB10 1SA, United Kingdom. E-mail: sg3@sanger.ac.uk

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vide useful models for understanding genetic interactions, such as epistasis, or polygenic disorders? We will address these issues, using data on neurological phenotypes in humans with X-linked disorders and data from studies of proteins found on the postsynaptic side of mammalian brain synapses. This focus provides a more in-depth view from which we can learn lessons used to guide studies on all autosomes as well as larger sets of brain genes.

The Synapse and the Postsynaptic Proteome

By analogy with genome projects that aim to provide comprehensive lists of genes, synapse proteomics aims to produce comprehensive lists of proteins that are found in synapses. The postsynaptic proteome (PSP) is the complement of proteins localized within the postsynaptic terminal, and recent large-scale efforts to characterize the PSP have produced a comprehensive description of its constituents.[18–23] These studies were performed by the biochemical fractionation of the synapse and by subsequent protein identification with the use of mass spectrometry and antibody-based methods. Meta-analysis of these data sets indicates that the PSP contains ~1,180 proteins in a number of distinct structural and functional complexes (fig. 1*B* and table A1 [online only]). The largest of these complexes is the postsynaptic density (PSD), a dense structure directly below the postsynaptic membrane that is visible by electron microscopy[24,25] and that comprises ~1,124 proteins (table A1 [online only] and the Genes to Cognition [G2C] Web site). It is worth noting that these lists are not definitive, since some proteins escape detection and some proteins will be contaminants from the fractionation procedure.

The PSD contains many different classes of proteins representing a broad range of cell biological functions, including membrane-bound receptors (including the glutamate receptors), adhesion proteins and channels, signaling proteins and adaptors, and proteins involved in transport, RNA metabolism, and transcription and translation (table A1 [online only]).[18–23] Compared with the entire mouse proteome, PSD proteins are enriched in protein interaction domains and, in particular, in PDZ (PSD-95, Discs-large, ZO-1) and SH3 (Src homology 3) domains, consistent with the abundance of adaptor and scaffolding proteins. There is also enrichment of kinase, calcium-dependent signaling, and Ras guanosine triphosphatase (GTPase) domains.[20]

The glutamate receptor complexes are subsets of the PSP, and there is considerable overlap between the various complexes (fig. 1*B*). Affinity purification of NMDA receptor complexes (NRC) or affinity isolation of membrane-associated guanylate kinase (MAGUK) proteins, which directly bind NMDA receptors, resulted in 185 proteins.[10,20] These complexes are alternatively known as the "NRC" or the "MASC" (MAGUK-associated signaling complexes), since both isolation procedures result in a similar set of proteins. Within the NRC/MASC can be found the NMDA and mGluR subunits, whereas AMPA-receptor subunits are in separate and smaller complexes (nine proteins).[20] Through affinity isolation, mGluR5 receptor complexes were found to contain 76 proteins.[26] The initial observations that NMDA and mGluR receptors were associated with dozens of proteins were surprising; however, since then, a substantial number of binary protein-interaction studies have mapped the interactions in detail. Moreover, many of the proteins in the NRC/MASC are known to mediate the signaling functions of the receptors.[27,28] As suggested by the Venn diagram in figure 1*B*, the PSP is a

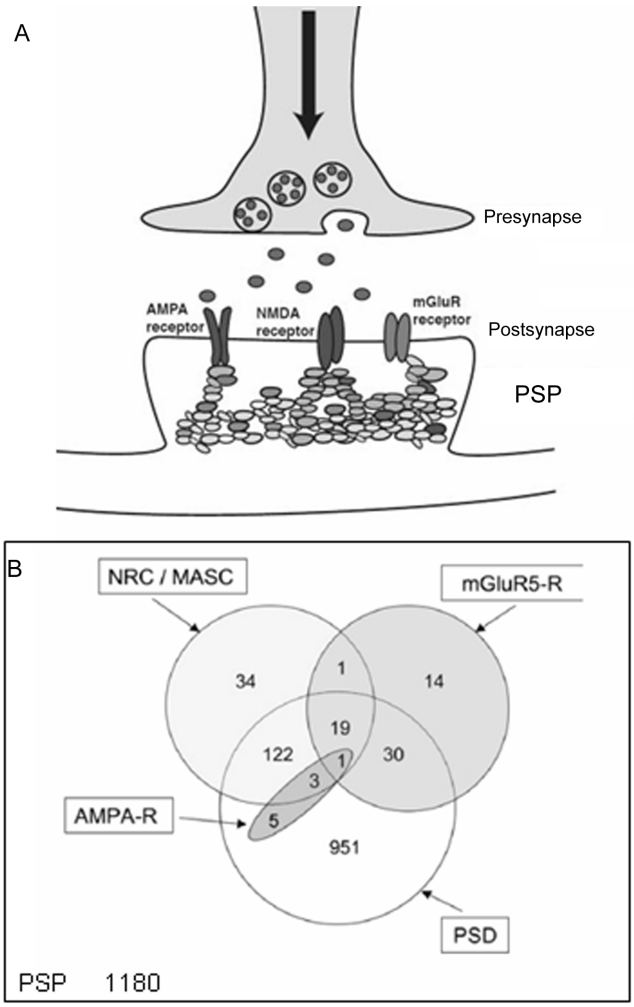

Figure 1. The PSP of a glutamatergic excitatory synapse. *A,* The PSP, the complement of postsynaptic proteins that contains ~1,180 proteins. This set of proteins is organized into complexes of varying sizes (*B*). The function of postsynaptic complexes is to receive and process signals that mediate neuronal communication and synaptic and behavioral plasticity. NMDA, AMPA, and mGLuR subtypes of glutamate receptors are indicated. *B,* Venn diagram of constituent protein complexes of the PSP (adapted from Grant[9]). The total set of PSP (1,180 proteins) is represented as sets of complexes (NRC/MASC, mGLuR5, AMPA, and PSD), and the number of proteins in these sets and overlaps are indicated. Details of the specific proteins are found in table A1 (online only).

set of complexes embedded within the PSD and has often been referred to as a "supramolecular" complex.[29]

The NRC/MASC is the most well studied of these large postsynaptic complexes and can be considered a prototype for the overall PSP. The physiological role of NRC/MASC proteins has been investigated using knockout mice and pharmacological intervention, most typically with the use of brain slices in which synaptic plasticity has been induced. More than 40 NRC proteins are necessary for the process of converting patterns of neuronal activity into long-lasting changes in neuronal function, and a similar number are required for behavioral forms of plasticity in rodents, such as learning or fear conditioning.[27,28,30] These numbers continue to increase as further genes are tested, which reinforces the model that the NRC/MASC is a signaling complex involved with the basic process of neural plasticity.

In addition to the accumulation of mouse genetic and phenotypic data on NRC/MASC proteins, the binary interactions of proteins within the complexes have been mapped and used to generate protein-interaction networks.[27,28] The average number of protein interactions separating any pair of NRC/MASC proteins is very low (average shortest path length 3.3), suggesting that the complex consists of a large network containing multiple clusters of well-connected proteins rather than a system of linear pathways with occasional interconnections. Algorithm-based network cluster analysis indicates that the complex contains 13 clusters, each with distinct functional characteristics and phenotypic associations (fig. 2). The flow of information through the complex is modeled in figure 3. In essence, the glutamate receptors and their proximal associated proteins form "input" modules, which then connect to a large set of general signaling proteins referred to as "processing" modules, which then signal to "output" modules comprising some well-known effector-pathway components, such as the extracellular signal-regulated kinase (ERK)/mitogen-activated protein kinase (MAPK) pathway (see the work of Pocklington et al.[27] and Pocklington et al.[28] for details of the networks). The mouse and human mutations that result in plasticity or behavioral deficits were mapped onto this network, and some interesting distributions of phenotypes were seen in particular modules. Although it is clear that this type of systems-biology approach will benefit from systematic mutational studies like those done in yeast,[31,32] it demonstrates that molecular network maps of synaptic protein complexes can be used to help understand the functional relationships between proteins. At the very least, it provides a logic for assembling a disparate set of genetic studies into a unified model.

These proteomic and mouse genetic studies serve as a driver for human genetic studies, since it would seem likely that many of the proteins would be involved in human disease. Indeed, when the NRC was first characterized, it was recognized that three proteins were well known to be mutated in neurological diseases.[10] A recent data-mining and literature-curation study revealed that 54 NRC/MASC proteins are involved with both psychiatric and neurological conditions.[27,30] A considerable number of these disorders have cognitive components (e.g., autism, schizophrenia, and mental retardation [MR]) consistent with mouse genetic studies showing specific impairments in cognitive function.[27,30]

The X Chromosome as a Model for the Genetics of Cognition

In recent decades, the role of the X chromosome in cognition has been extensively studied. Although it is known that genes influencing cognitive function are distributed throughout the human genome, many more "cognition genes" have been found on the X chromosome than on comparable segments of the autosomes.[33] In parallel with these observations, numerous epidemiological studies performed to evaluate the sex ratio in autism and MR have indicated an excess of males, suggesting a preferential association between genetic defects and cognitive disorders in males.[34–38] Males outnumber females in nearly all surveys of MR, with an excess of ~20%, and numerous families have been reported in which MR segregated in an X-linked inheritance pattern. X-linked MR (XLMR) is a common cause of moderate to severe intellectual disability in males, with a prevalence of 2.6 cases per 1,000 in the general population, accounting for >10% of all cases of MR.[39,40] Although highly heterogeneous, XLMR is usually divided into syndromic forms (MRXS), which have associated musculoskeletal or metabolic symptoms, and nonsyndromic (or nonspecific) forms (MRX) in which MR is the sole feature, although accumulating evidence suggests that this boundary is less evident than was previously expected.[35,41] So far, >140 MRXS conditions have been reported; in almost half of these, causative mutations in genes have been described.[40–42]

The publication of the genomic sequence of the human X chromosome provided a comprehensive data set of the genes and their organization.[43,44] Approximately 1,100 genes have been annotated on the X chromosome, of which at least 800 are protein coding.[43,45,46] The features of the gene organization on the X chromosome are low gene number and density and enrichment for genes expressed in testis, brain, skeletal muscle, ovary, and placenta.[43,47] Although it accounts for only 4% of all identified human genes, this chromosome includes genes responsible for almost 10% of diseases with known Mendelian inheritance.[43] The mammalian X chromosome is very different from the autosomes because of its unequal representation in males (1 copy) and females (2 copies). To compensate for this unequal dose, females inactivate one copy of the X chromosome in every cell. Thus, this chromosome is of particular interest for medical genetics, since numerous disease conditions have been associated with the X chromosome because the phenotypic conse-

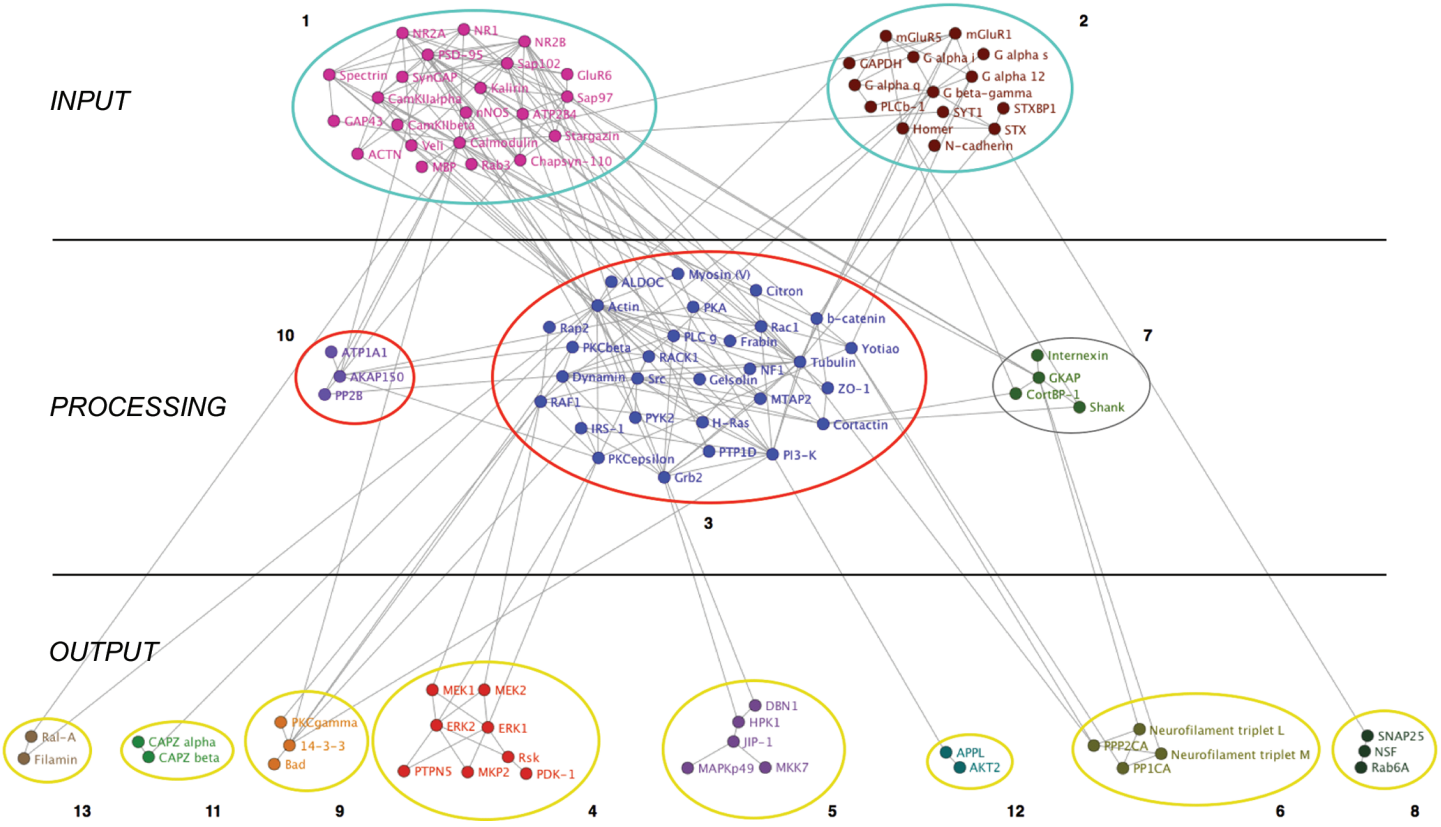

Figure 2. Protein-interaction network of the NRC/MASC complex. A network of binary interactions between NRC/MASC proteins was clustered into "modules" with the use of algorithms.[28] These 13 numbered clusters are grouped into three layers: "input," representing the neurotransmitter receptors and proximal interacting proteins; "processing," general signaling proteins; and "output," downstream sets of signaling proteins such as ERK/MAPK pathways. Reprinted with permission from Molecular Systems Biology.

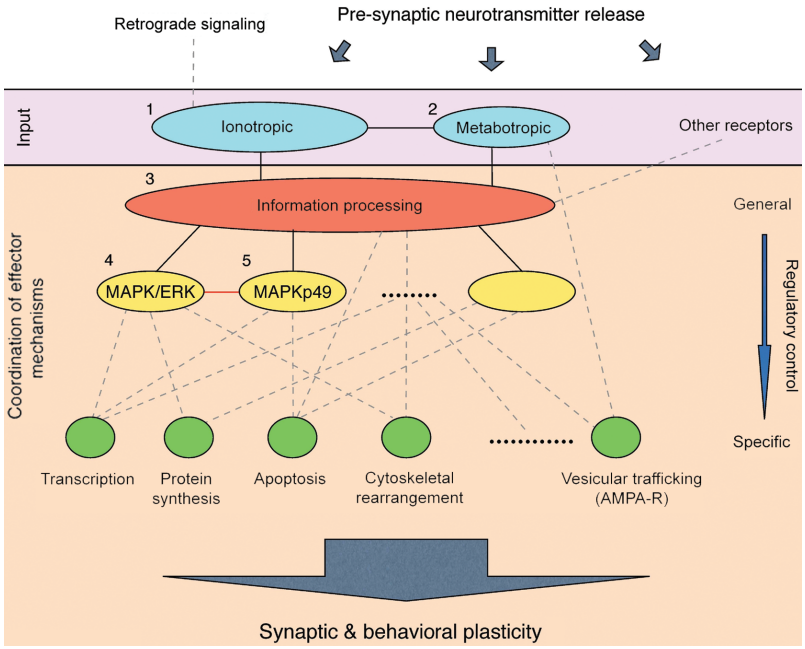

Figure 3. Modular signaling mechanisms of postsynaptic complexes. The modules of clustered proteins are organized into layers of signaling, with synaptic cleft at the top. Presynaptic information, in the form of a neurotransmitter, enters the postsynaptic signaling machinery via activation of ionotropic and metabotropic transmembrane receptors that are in modules of proximal signaling proteins (*blue*). From there, signals are passed to a large information-processing module (*red*) and then are distributed to effector mechanism networks (*green*), which mediate a functional outcome (*dark blue arrow*).[28] This signaling machinery provides a high degree of signal integration by protein interaction and orchestration of output responses.

quences of a recessive mutation are revealed directly in males.[41,44,48]

In the last Ensembl data release (National Center for Biotechnology Information database release 39) and with the use of the large-scale data-mining tool BioMart (MartView software), we found that >500 genes located on the X chromosome are expressed in the human brain, thus representing numerous potential candidates for X-linked brain diseases. Many classes of proteins are represented, ranging from transcription factors, channels and receptors, and DNA/RNA–binding proteins to scaffolders, enzymes, and signal-transduction proteins. These large data sets, rich in human genetic data, as well as representing many brain genes of different functions, provide an ideal tool for systematically analyzing the postsynaptic proteins and their signaling complexes.

X-Linked Diseases and Postsynaptic Complexes

To address the role of the NRC/MASC and the PSD in X-linked brain diseases, we annotated the X-chromosome genes encoding components of these complexes. Table 1 summarizes the total numbers and functional classification of PSD and NRC/MASC genes, those on the X chromosome, and those that are mutated in XLMR. The X chromosome was not enriched for NRC/MASC or PSD genes, since 3.7% (39 of 1,124) of NRC/MASC and 3.2% (6 of 186) of PSD genes were encoded on X, which is similar to the 4% of all coding genes found on this chromosome. A high proportion of the X-linked genes coding PSD and NRC/MASC proteins were found to be mutated in psychiatric disorders: 19 (49%) of 39 PSD genes and 6 (85%) of 7 NRC/MASC genes. These proportions are likely to increase, since some of these genes that have no associated human disorder are known to result in abnormal phenotypes in knockout mice (table 2). A specific list of these genes is provided in table 3, since they are suitable targets for future resequencing efforts.

Multiple functional groups of proteins were represented, with the most abundant being signaling molecules and enzymes representing 10 proteins, which were >25% of the total X-linked PSD proteins. Of the 10 specific functional categories of PSD proteins, 8 were encoded on the X chromosome, and there are human mutations in all 8 categories (table 1). We noted that all (6 of 6) cytoskeletal and adhesion proteins (PLP1 [MIM 300401], DMD [MIM 300377], FLNA [MIM 300017], L1CAM [MIM 308840], NLGN3 [MIM 300336], and NLGN4 [MIM 300427]) and all (2 of 2) serine/threonine kinases (CDKL5 [MIM 300203] and RPS6KA3 [MIM 300075]) were mutated in brain diseases (tables 1 and 2).

The multiple classes of proteins involved in XLMR and the heterogeneity of proteins in the PSP allow a unification of the two data sets. In other words, if one were to consider

Table 1. Functional Classification of Synapse Proteome Proteins and Their Representation on the X Chromosome

	No. of Proteins in[b]					
	PSD			NRC/MASC		
Functional Classification[a]	Total	X-Linked	Mutated	Total	X-Linked	Mutated
Channels and receptors	80	4	2	12	0	0
MAGUKs/adaptors/scaffolders	54	4	1	20	1	1
Ser/thr kinases	46	2	2	21	1	1
Tyr kinases	3	0	0	2	0	0
Protein phosphatases	18	0	0	7	0	0
G proteins and modulators	77	3	2	19	0	0
Signaling molecules and enzymes	278	10	3	40	0	0
Transcription and translation	119	5	2	5	0	0
Cytoskeletal and cell-adhesion molecules	153	6	6	35	4	4
Synaptic vesicles and protein transport	159	3	1	22	1	0
Novel	107	2	0	3	0	0
Other	30	0	0	0	0	0
Summary	1,124	39	19	186	7	6

[a] Proteins found in PSD and NRC/MASC profiling experiments were classified into functional groups (see the work of Collins et al.[11]).

[b] The total number of proteins in the complex (Total), the number on the X chromosome (X-linked), and the number known to be mutated in XLMR (Mutated) are indicated for each protein class. Note that almost 50% (19 of 39) of the X-linked genes coding for PSD proteins and 85% (6 of 7) of MASC genes are mutated in nervous-system diseases.

the genetic data alone, then the heterogeneity would be accounted for in terms of a diverse range of mechanisms contributing to XLMR. However, the fact that many components from this heterogeneous list are found within the NRC/MASC or the PSD indicates that the mutations may have a single general mechanism at the level of their function in the multiprotein complex. In other words, the overall function of the complex can be impaired by mutation in different proteins in the complex. This does not mean that the mutations should have identical functions, since the specific role of the proteins in the complexes will differ, although they have a common overall function. This model is strongly supported by mouse genetic data and synaptic physiology for core components of NRC/MASC, where mutations in different classes of proteins result in changes in synaptic plasticity.[49–51]

Functions of Specific X-Linked NRC/MASC and PSD Genes

We will now address the details of specific genes and their phenotypes in humans and mice. Table 2 provides further details of the 39 PSD X-linked genes, including their functions, human disorders, and mouse models. With a focus on the seven X-linked NRC/MASC genes (*DLG3, RPS6KA3, PLP1* [MIM 300401], *L1CAM, SLC25A5* [MIM 300150], *NLGN3,* and *NLGN4*), six are associated with brain diseases. Only the *SLC25A5* gene, which encodes a mitochondrial adenosine diphosphate or triphosphate (ADP/ATP) translocase, has not been involved in XLMR, and it is possible that this translocase is a contaminant of the proteomic experiment. These proteins and other NRC/MASC proteins are schematically represented in figure 4, which also illustrates the genes implicated in human and mouse diseases.

The *DLG3* (discs large, *Drosophila,* homolog of, 3) gene encodes the synapse-associated protein 102 (SAP102), a member of the MAGUK protein family.[53,54] SAP102 and other MAGUK proteins (e.g., PSD-95 and PSD-93/chapsyn-110) are multidomain scaffold proteins that bind the NMDA receptor and other signaling and cytoskeletal proteins.[1,7] Human mutations in *DLG3* are associated with MR,[55] and mouse knockouts result in learning deficits and alterations in the MAPK signaling pathway.[56]

Mutations in *NLGN3* and *NLGN4* (neuroligin 3 and 4) were found in XLMR and/or autism.[57,58] A wide spectrum of phenotypes, ranging from mild MR without communication deficits to Asperger syndrome with normal or supranormal intelligence, were reported.[58] Functional analyses performed in hippocampal neuronal cultures where *Nlgn 1, 2,* and *3* were knocked down showed altered dendritic spines and a reduction in dendritic branching and arborization.[59] Others studies suggest that neuroligins affect, in combination with PSD-95, the direction of development into an inhibitory or excitatory synapse in vitro.[60–62] Interestingly, the recent publication[63] of the triple-knockout mouse for *Nlgn 1–3* showed that the animals die shortly after birth because of respiratory failure. However, Varoqueaux et al. noticed that the density of synaptic contacts is not altered in neuroligin-deficient brains, indicating that neuroligins are required for proper synapse maturation and brain function but not for the initial formation of synaptic contacts.[63]

The *L1CAM* gene encodes the L1 protein, which is a highly conserved member of the immunoglobulin-like family of cell-adhesion glycoprotein molecules. During

development, L1 is expressed in neurons throughout the brain and is involved in neurite outgrowth, axonal guidance, synaptogenesis, myelination, and fasciculation.[64–66] Mutations in L1 affect development of the nervous system in human and mouse: they are responsible for a form of MRXS described as "X-linked hydrocephalus," "MASA syndrome" (MR, aphasia, shuffling gait, and adductus thumbs), or "spastic paraplegia type I" (SPG1).[64] L1 disruption in mice also produces a cognitive defect, and abnormalities of the hippocampus and the morphology of individual neurons were reported.[67] A recent study showed that the MAPK pathway (involving mitogen-activated protein kinase kinase [MEK] and ERK) regulates L1CAM-mediated nerve growth by phosphorylating L1CAM and then modulating its interaction with ankyrin B (see fig. 4).[68]

The *RPS6KA3* gene encoding the 90-kDa ribosomal S6 serine-threonine kinase-2 (RSK2) is mutated in both MRXS (e.g., Coffin-Lowry syndrome)[69] and MRX.[70] In the CNS, the ERK signaling pathway activates RSK2 and leads to protein kinase A (PKA)–dependent activation of cyclic adenosine monophosphate response element–binding (CREB) protein in the hippocampus and to the regulation of neuronal synaptic plasticity (fig. 4).[71] Interestingly, a study performed by Naisbitt et al. suggested that RSK2 directly binds by a C-terminal motif and/or phosphorylates Shank1 (SH3 and multiple ankyrin-repeat domains 1 [MIM 604999]), Shank3 (MIM 606230), Magi-1 (MAGUK, WW and PDZ domain–containing 1 [MIM 602625]) and Grip1 (glutamate receptor–interacting protein 1 [MIM 604597]), which are then tethered to the NRC/MASC by Shank interactors, such as guanylate kinase–associated proteins (GKAP) or Homer, which belong to the PSP.[72] They also demonstrated that signaling via RSK2 seems to regulate AMPA-receptor transmission (such as the X-linked GRIA3 protein).[73]

The proteolipid protein 1 (PLP1) is the major integral membrane protein of adult CNS myelin.[74] PLP1 is synthesized at the endoplasmic reticulum (ER) and then is transported to the cell surface, where it is incorporated into the myelin membrane. The primary role of PLP1 in myelin formation is currently thought to be the adhesion and stabilization of the extracellular surfaces of the myelin membrane, although some evidence suggests that PLP1 may function as a channel-forming protein.[75] Mutations in the *PLP1* gene are associated with forms of MRXS, including Pelizaeus-Merzbacher disease and X-linked SPG1. Mutant forms of PLP1 are retained in the ER, and the resulting accumulation of mutant protein is thought to be a direct cause of oligodendrocyte cell death.[76] The PLP1 protein has been found in different PSD purification studies, thus suggesting a postsynaptic subcellular localization in the neuronal cell.[19–21,23] Recently, Gudz et al. found that, after agonist activation of the AMPA receptor, the PLP1, $\alpha_v\beta_3$ integrin, and the AMPA receptor proteins form a complex.[77] These data are particularly interesting, since this protein is not typically considered to be postsynaptic. Another link between PLP1 and NRC/MASC may be provided by the recent observation that oligodendrocytes express NMDA receptors, which are important for aspects of ischemia.[78,79]

Turning our attention from the NRC/MASC, which can be considered a signaling complex embedded within a much larger set of proteins forming the PSD, we address the functions of specific PSD genes involved in human X-linked disorders. The channel and receptors group includes the *GRIA3* (glutamate receptor, ionotropic, AMPA 3 [MIM 305915]) and *IL1RAPL1* (interleukin 1 receptor accessory protein-like 1 [MIM 300206]) genes. The *GRIA3* gene encodes the AMPA receptor GLUR3, which mediates fast synaptic transmission in the CNS.[80] This gene has been previously described in a female with MR and bipolar affective disorder and a balanced X-autosome translocation truncating the *GRIA3* gene.[81] Very recently, three missense mutations at evolutionarily conserved positions of the *GRIA3* gene have been characterized in three unrelated males with MR.[82] Analyses of knockout mice for *Gria3* revealed an enhanced long-term potentiation of synaptic transmission, which indicates a role of GRIA3 in the regulation of synaptic plasticity.[80]

The *IL1RAPL1* gene, mutated in several families with MRX,[83] codes for a receptor protein that interacts with the neuronal calcium sensor-1 protein (a member of a large Ca^{2+}-binding protein family) through its cytoplasmic C-terminal domain.[84] IL1RAPL may be involved in the regulation of calcium-dependent exocytosis and, therefore, in synaptic activity.[84]

The *HADH2* (hydroxyacyl-CoA dehydrogenase, type II [MIM 300256]), MAOA (monoamine oxidase A [MIM 309850]), and *PRPS1* (phosphoribosylpyrophosphate synthetase I [MIM 311850]) genes encode signaling enzymes with a role in the degradation of branched-chain fatty acids and isoleucine, serotonin metabolism, and purine synthesis, respectively. They are involved in variable forms of MRXS.

Two G protein–modulator genes, *GDI1* (guanosine diphosphate dissociation inhibitor 1 [MIM 300104]) and *ARHGEF9* (Rho guanine nucleotide exchange factor 9 [MIM 300429]), appear to be mutated in MRX.

Very recently, Klauck et al. identified mutations in the *RPL10* gene (ribosomal protein L10 [MIM 312173]) in individuals with autism and MR.[85] RPL10 belongs to the L10e family of ribosomal proteins and is a component of the 60S large ribosomal subunit, which links the 40S and 80S subunits. Human mutant RPL10 proteins may exhibit altered translation of synaptic proteins, which may be important for synthesis of many NRC/MASC and PSD proteins.

Although the cellular function of many of these X-linked PSD proteins has been extensively studied, it is noteworthy that several are not considered to be postsynaptic, either because of the lack of specific functional studies in neurons or because of the expected noncytoplasmic subcellular localization (transcription or translation factors). For instance, several proteins involved in the

Table 2. Proteins of the PSP Encoded by X-Chromosomal Genes

Protein Class[a] and Gene	Function	Psychiatric Disorder(s)	MIM Number	Knockout Model[b]
Channels and receptors:				
GRIA3	Glutamate receptor	MRXS, autism, bipolar disorder	305915	Yes
PGRMC1	Progesterone receptor	…	300435	…
ATP2B3	Ca^{2+} ATPase	…	300014	…
IL1RAPL1	IL1 receptor accessory proteinlike	MRX	300206	…
MAGUKs/adaptors/scaffolders:				
CASK	Calcium/calmodulin–dependent serine protein kinase	…	300172	Lethal
CNKSR2	Connector enhancer of kinase suppressor of Ras 2	…	…	…
DLG3	Synapse-associated protein 102	MRX	300189	Yes
SH3KBP1	SH3-domain kinase-binding protein (regulator of receptor endocytosis and lysosomal degradation)	…	300374	…
Ser/thr kinases:				
CDKL5	Ser/thr–protein kinase	MRXS, autism, infantile spasms	300203	…
RPS6KA3	Ribosomal protein S6 kinase	MRXS, MRX	300075	Yes
G proteins:				
SEPT6	GTP-binding protein, cytokinesis	…	…	Yes
G modulators:				
ARHGEF9	Cdc42 guanine nucleotide exchange factor	MRX, epilepsy	300429	…
GDI1	Rab GDP dissociation inhibitor	MRX	300104	Yes
Signaling molecules and enzymes:				
Protease/protease inhibitor:				
PSMD10	Regulatory subunit of the 26S proteasome	…	603480	…
Other enzymes:				
DDX3X	RNA helicase (transcription, splicing, translation)	…	…	…
HADH2	Hydroxyacyl-CoA dehydrogenase	MRXS, choreoathetosis	300256	…
MAOA	MAOA	MRXS, aggressive behavior	309850	Yes
OGT	O-linked N-acetylglucosamine transferase	…	300255	Yes
PRPS1	Phosphoribosyl pyrophosphate synthetase	MRXS, gout	311850	Yes
RP2	Involved in beta-tubulin folding	…	312600	…
Mitochondrial enzymes:				
IDH3G	Isocitrate dehydrogenase 3 (NAD^+) gamma	…	300089	…
PDCD8	Oxidoreductase, apoptosis-inducing factor	…	300169	Yes
PDHA1	Pyruvate dehydrogenase (lipoamide) alpha 1	…	300502	Yes

Transcription and translation:				
Transcription elements:				
ATRX	Transcriptional regulator	MRXS, NS-MR, microcephaly, hypotonic facies	300032	Yes
Other nuclear/DNA binding:				
HNRPH2	Component of the heterogenous nuclear ribonucleoprotein (hnRNP) complexes	…	601036	…
SMARCA1	Transcriptional activator	…	300012	…
Ribosomal:				
RPL10	60S ribosomal protein L10	MR, autism	312173	…
RPS4X	40S ribosomal protein S4	…	312760	…
Cytoskeletal and cell adhesion:				
Myelin				
PLP1	Component of myelin	MRXS, spastic paraplegia, Pelizaeus-Merzbacher disease	300401	Yes
Other cytoskeletal:				
DMD	Dystrophin; bridges the inner cytoskeleton (F-actin) and the extracellular matrix	MRXS, muscular dystrophy	300377	Yes
FLNA	Actin-binding protein	MRXS, epilepsy, brain anomalies	300017	…
Other cell-adhesion molecules:				
L1CAM	L1 cell–adhesion molecule, axonal glycoprotein	MRXS, MASA syndrome, hydrocephalus	308840	Yes
NLGN3	Neuronal cell–surface protein	Autism	300336	…
NLGN4	Neuronal cell–surface protein	MRX, autism	300427	…
Synaptic vesicles/protein transport:				
Synaptic vesicle:				
SYN1	Synaptic vesicle–associated protein	MRXS, epilepsy, macrocephaly, aggressive behavior	313440	Yes
SYP	Integral protein of small presynaptic vesicles	…	313475	Yes
Transporters:				
SLC25A5	Mitochondrial carrier; adenine nucleotide translocator	…	300150	…
Uncharacterized/novel:				
MGC4825	Unknown	…	…	…
IQSEC2	Unknown	…	300522	…

[a] Proteins have been classified according to their function and involvement in X-linked psychiatric disorders.

[b] Knockout mouse models are indicated, and further details are obtainable from the Mouse Genome Informatics database version 3.5.

Table 3. X-Linked Genes of the PSP That Are Not Currently Known to Have Mutations in Human Cognitive Disorders

Gene	Locus	KIAA or MGC Full-Length Clone	Cellular Function
PGRMC1	Xq24	MGC8891	Transmembrane receptor
ATP2B3	Xq28	NA	Transmembrane receptor
CASK	Xp11.4	MGC150920	Scaffolder
CNKSR2	Xp22.12	KIAA0902	Scaffolder
SH3KBP1	Xp22.12	MGC9446	Scaffolder
SEPT6	Xq24	KIAA0128	GTP-binding protein potentially involved in cytokinesis
PSMD10	Xq22.3	MGC9114	Involved in the ATP-dependent degradation of ubiquitinated proteins
DDX3X	Xp11.4	MGC20129	Regulation of transcription, splicing and translation
OGT	Xq13.1	MGC22921, MGC39117	Protein amino acid O-linked glycosylation
RP2	Xp11.3	KIAA0215	Involved in beta-tubulin folding
IDH3G	Xq28	MGC5393, MGC2102	Involved in citric acid cycle in mitochondrion
PDCD8	Xq25	MGC111425	Mitochondrial apoptosis–inducing factor
PDHA1	Xp22.12	MGC8609	Glycolysis, gluconeogenesis, acetyCoA metabolism
HNRPH2	Xq22.1	...	Heterogenous nuclear ribonucleoprotein complex (pre-mRNA maturation)
SMARCA1	Xq25	MGC151056	Chromatin remodeling, regulation of transcription
RPS4X	Xq13.1	MGC8636, MGC87857	Translation
SYP	Xp11.23	MGC70359	Presynaptic vesicle
SLC25A5	Xq24	MGC65136	Ion transporter
MGC4825	Xp22.11	MGC4825	Unknown
IQSEC2	Xp11.22	KIAA0522	Unknown

NOTE.—These genes represent priority targets for future testing and resequencing in X-linked cognitive disorders. Note that *CASK* and *CNKSR2* also belong to the MASC complex. This list provides the reference of cloned full-length coding cDNA sequences of most of the genes. NA = not available.

regulation of transcription or translation, such as CDKL5 and RPL10, have been identified in PSD complexes. Interestingly, the subcellular localization of the CDKL5 protein is primarily nuclear, as shown in several reports,[86,87] but a weak cytoplasmic signal suggests that this protein can also exhibit some protein-protein interactions in this compartment. Indeed, the presence of four SH3-binding sites at its C-terminal part could lead to the formation of specific interactions with numerous PSD proteins that possess such SH3 domains. Dystrophin is not generally considered to be present at the PSD (e.g., in Duchenne muscular dystrophy), but several studies showed that this protein is localized subcellularly to the PSD.[88–90] Furthermore, Kim et al. demonstrated that dystrophin was absent from the PSD proteins in the brain of a patient with Duchenne muscular dystrophy but was present in the brain of an age-matched control.[91] It is also important to note that the PSD and NRC/MASC isolation and mass spectrometry methods are not perfect and that lists of proteins will contain a low level of contaminants, as well as exclude some proteins that failed to be detected. Thus, refinement in methods and sample preparation will lead to updated lists of PSP proteins.

XLMR Genes outside the PSP

Clearly, not all XLMR genes encode for proteins in postsynaptic complexes; of the 70 XLMR genes identified to date, 19 (27%) fall into the PSP categories. It is interesting to consider how, if at all, the non-PSP XLMR genes are functionally connected to the PSP XLMR genes. Clearly, NRC/MASC proteins could be regulated by enzymes and pathways outside the synapse (e.g., trafficking and post-translational modifications), and we can consider that the effects of mutation in non-PSP XLMR mediate their effects by alteration of NRC/MASC and PSD. Toward this, it is known that a significant proportion of non-PSP XLMR genes code for proteins involved in signal transduction and regulation of transcription and those that impact synaptic function and dendrite development.

At least five proteins are directly involved in synaptic function and activity: FMRP, which binds to and is involved in the metabolism of neuronal mRNAs, including PSD-95, whose localization and regulated translation play central roles in neurite outgrowth and synaptic plasticity (S. G. N. Grant, unpublished data)[92]; OPHN1 (oligophrenin 1 [MIM 300127]); PAK3 (p21-activated kinase 3 [MIM 300142]); FGD1 (FYVE, RhoGEF, and PH domain–containing protein 1 [MIM 300546]); and ARHGEF6 (Rho guanine nucleotide exchange factor 6 [MIM 300267]), which are required for the regulation of the RhoGTPase signaling pathway. These proteins integrate extracellular and intracellular signals to orchestrate coordinated changes in the actin cytoskeleton, which is essential for directed neurite outgrowth and the regulation of synaptic connectivity.[93]

Very recently, Tarpey et al. identified mutations in the gene encoding the sigma 2 subunit of the adaptor protein 1 complex (*AP1S2* [MIM 603532]) causing XLMR.[94] *AP1S2* encodes an adaptin protein that constitutes part of the adaptor protein complex found at the cytoplasmic face of coated vesicles located at the Golgi complex. The complex mediates the recruitment of clathrin to the vesicle membrane. Tarpey et al.[94] propose that aberrant endocytic

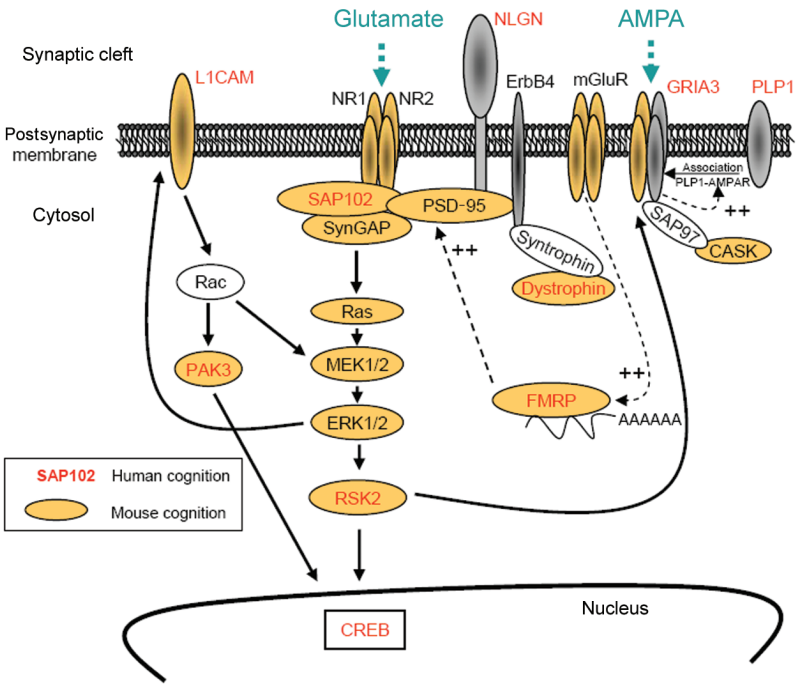

Figure 4. Human and mouse mutations and the cognitive disorders affecting specific NRC/MASC signaling pathways and other postsynaptic proteins. The NMDA receptor subunits (NR1 and NR2) are linked to MAGUK proteins (SAP102 and PSD-95) that bind SynGAP, which regulates the Ras-ERK-RSK pathway. This pathway regulates transcription (e.g., CREB), cell adhesion (via L1CAM), and AMPA receptors. MAGUKs, including DLG3/SAP102, coordinate the postsynaptic signaling response to NMDA receptor (NR1 and NR2) activation. The MAP kinase pathway is an important limb of this response, leading to changes in transcription factors such as RSK2, which, in turn, send feedback to modify AMPA receptor function and thus produce synaptic plasticity. Note that FMRP, encoded by the *FMR1* gene, does not belong to this complex but is involved in the regulation of PSD-95 translation via mGluR activation.[52] Note that the NRC/MASC–associated signaling pathway is involved in MRX as well as MRXS. The molecules are shaded in yellow if there is a known mouse mutation that results in cognitive dysfunction, and red letters indicate if mutation is in a human gene.

processing through disruption of adaptor protein complexes is likely to result from the *AP1S2* mutations identified in the families with XLMR and that such defects may cause abnormal synaptic development and function. Interestingly, we previously identified[11] several PSD proteins that participate in the formation of the clathrin vesicle (see table A1 [online only]).

Many of the non-PSP genes code for proteins involved in chromatin remodeling and regulation of transcription, which would mean that controlled activation/repression of their targeted genes may be crucial for cognitive function. A good example is the *MECP2* gene (methyl-CpG–binding protein 2 [MIM 300005]), which causes Rett syndrome and XLMR and acts as a transcriptional silencer of neuronal genes. Particularly, *MECP2* regulates the expression of the gene encoding brain-derived neurotrophic factor (*BDNF* [MIM 113505]), a secreted protein that has crucial roles in survival, development, and synaptic plasticity in the nervous system.[95] Thus, it is not surprising that defects affecting these signaling cascades going from the postsynaptic membrane to the nucleus, which are pivotal for the formation of learning and the memory, lead to cognitive impairments.

Autosomal PSP Genes and MR-Associated Diseases

The high percentage of X-linked genes in NRC/MASC (85%) and PSD (49%) is indicative of similar roles of autosomal genes in MR. Of the 1,180 genes coding PSP proteins, >1,000 are located on the autosomes and can be considered potential candidates in psychiatric disorders; thus, the expectation is that the majority of MR genes will be found on autosomes and will include many NRC/MASC genes. Indeed, we curated literature on autosomal NRC/MASC genes in disease, and almost 50 genes were involved in various brain disorders.[30]

Several autosomal genes have already been implicated in autosomal MRX and MRXS.[40,47] Interestingly, recent studies have pointed out the involvement of some PSD ion channels in MR associated with autistic disorder. Splawski et al. found gain-of-function mutations in the *CACNA1C* (calcium channel, voltage-dependent, L type, alpha-1C subunit [MIM 114205]) gene in individuals affected by multiorgan dysfunction, cognitive abnormalities, and autism.[96] The encoded protein is involved in calcium-induced calcium release and the calcium intracellular signaling pathway. Recently, the haploinsuffi-

ciency of the *KCNMA1* (potassium channel, calcium-activated, large conductance, subfamily M, alpha member 1 [MIM 600150]) gene has been involved in autism and MR.[97] The knockout mouse model for this gene showed some behavioral alterations and cerebellar Purkinje neurons dysfunction.[98]

Thanks to the development of genomewide screening technologies such as BAC–comparative genomic hybridization arrays, the association of de novo chromosomal disorders associated with autosomal MR (microdeletions, duplications, or translocations) has led to the definition of genomic territories encompassing genes of PSD proteins. For instance, Willatt et al. described a form of MRXS associated with a 1.5-Mb 3q29 microdeletion.[99] The deletion encompasses 22 genes, including *PAK2* (p21-activated kinase 2 [MIM 605022]) and *DLG1* (discs large, *Drosophila,* homolog of, 1 [MIM 601014]), which are autosomal homologues of two known XLMR genes, *PAK3* and *DLG3*. The encoded DLG1 protein (also named "SAP97") belongs to the NRC/MASC complex and is also linked to AMPA receptors (table A1 [online only] and fig. 4). Very recently, Shaw-Smith et al. reported that a recurrent 500- to 650-kb microdeletion located at 17q21.3 is associated with developmental delay and learning disability.[100] Interestingly, this deletion encompasses the *MAPT* (microtubule-associated protein tau [MIM 157140]) gene, which encodes a protein present in the PSD complex.

Conclusions

Network Organization and Integration of Postsynaptic Signaling

The function of NRC/MASC in synaptic physiology and behavior has been studied using both reductionist single-gene strategies and, more recently, large-scale systems-biology approaches.[27,28] As discussed above, the phenotypes of single gene mutations (in humans and mice) have supported the biochemical model that the NRC/MASC complex is important as an overall structure in cognition. At the cell biological level, where it has been extensively studied using mutations and drugs in brain slices, the NRC/MASC complex is necessary for the process of induction of synaptic plasticity. This induction involves the detection of patterns of synaptic activity and the initiation of biochemical pathways that lead to changes in the property of the synapse and other parts of the neuron. The fact that NRC/MASC proteins are involved with activation of local synaptic events, such as changes in AMPA receptors and dendritic spine structure, as well as distant events at the nucleus, indicates that the complex must coregulate these processes.

The coregulation of multiple cell biological processes (e.g., receptor trafficking and protein translation and transcription) by NRC/MASC can be accounted for by the network properties of the protein interactions within the complexes (figs. 2 and 3).[28] In this model, activation of kinases and phosphatases and other enzymes is followed by a high degree of cross talk and interaction between proteins and pathways. In this way, signals are integrated and many proteins play a role in the final outcome, which includes the driving and orchestration of the downstream biological processes. This model has been supported by statistical and experimental data. Another important feature of the networks is that they can account for the property of robustness to perturbation; that is, the loss of any single gene only partially interferes with the overall process that the complex is involved with, such as induction of synaptic plasticity. The severity of the phenotype of the single-gene mutation is a reflection of the degree of connectivity of the protein with other parts of the complex. More details of the robustness are described in the work by Pocklington et al., in which the connectivity of proteins was plotted as a function of the mutational phenotype.[27,28]

These network models provide a logical process to extend the human genetics of cognition beyond finding individual genes and toward understanding gene interactions. When the view that more than one gene is involved in function of the complex (and cognition) is considered, then the relative position (connectivity) of those two genes will influence the phenotype. This principle is valid for NRC/MASC genes, since epistasis was observed among three genes when tested using double knockouts.[101] One future approach is to examine the frequency of alleles of NRC/MASC genes and their combinations in groups of individuals with cognitive deficits and disorders. An additional exciting dimension to these network models of genetic disorders is the prospect that therapeutic interventions can be predicted for molecular targets that could rescue the mutation. For example, if a mutation affects a component of a signaling pathway within the complex, then a drug may be able to activate some compensatory pathway that is connected via a set of local protein interactions. These approaches may open new therapeutic opportunities for some of these currently untreated conditions of XLMR.

The model of a postsynaptic signaling network is simplified here, and it should be noted that the effects of mutations on this network will result not only in altered signaling at diseased synapses but also in changes in neuronal structure. The NMDA receptor is a well-known regulator of synaptic morphology, as are many postsynaptic proteins.[1,5,6] Furthermore, dendritic spines that contain the NRC/MASC are irregularly shaped and have abnormal densities in a number of cognitive disorders characterized by MR, such as MRX, Down syndrome, Angelman syndrome, and autism.[102–105] It is also important to consider that some PSD gene deficits lead to MR with or without brain abnormalities. This would mean that not only can the PSD complex be important for the establishment and maintenance of synaptic connection and activity but that it can also be crucial for the structure of some brain regions; for instance, *L1CAM* mutations are associated

with agenesis of the corpus callosum and hydrocephalus (table 2), and *ATRX* and *SYN1* mutations cause microcephaly. The relative contribution to morphological changes and their differences in brain regions may also reflect the developmental and regional expression profiles of these genes.

The molecular networks described for the NRC/MASC can now be extended to the other proteins in the PSD and to non-PSP genes, to provide more-comprehensive molecular neuronal networks. The landscape of these networks and the locations of disease genes may ultimately provide logic to the behavioral phenotypes of patients.

Cognition and the X Chromosome

Beyond disease genetics, the molecular understanding of synaptic processes and the X chromosome may shed light on wider issues in behavioral science. Genes on the X chromosome not only influence general intelligence but also have relatively specific effects on social cognition and emotional regulation. Zechner et al. suggest that the X chromosome has been engaged in the development of sexually selected characteristics for at least 300 million years and that natural selection has favored the development of X-linked genes that are associated with higher cognitive abilities.[106] Moreover, Skuse proposes that male and female brains may differ not only because of their contrasting genetic constitutions but also because of their sex-steroid environments and that differences in cognitive and social abilities between the sexes could be directly linked to the influence of X-chromosome genes.[33] Another study pointed out that the contribution of X-linked genes to cognition is significantly higher than would be expected.[47] Interestingly, a recent study reported that X-linked genes are highly expressed in brain tissues of several mammalian species, and it showed a greater proportion of highly expressed X-linked genes in human versus mouse brain.[107] These data suggest that, through evolution, the X chromosome has become a repository for genes highly expressed in brain and that such genes may have a role in enhancing cognitive functions.

Summary

Here, we have integrated studies of human X-linked diseases with mouse genetic and proteomic studies of the PSP. It is clear that proteomic profiling of NRC/MASC and PSD proteins provides a rich source of disease-relevant genes. The data from human and mouse genetic studies also support the model that the NRC/MASC is necessary for cognitive function. In the near term, we suggest two important future directions: (i) comprehensive and systematic studies of NRC/MASC and PSP genes in human brain disorders with the use of genetic methods and (ii) systems biology–based approaches to the synapse with the use of large-scale data sets integrated with bioinformatics approaches. These approaches, which are being pursued in the G2C project, should provide a novel foundation for future therapeutic developments aimed at treating common cognitive disorders. Detailed lists of the proteins described here are available in table A1 (online only), together with curated data on human genetic disorders and mouse mutations in the G2C database. This neurobiological approach of integration of proteomics, mouse and human genetics, and network biology is generally applicable to any biological or pathological condition.

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Web Resources

The URLs for data presented herein are as follows:

G2C, http://www.genes2cognition.org/db.html
MartView software, http://www.ensembl.org/Multi/martview/
Mouse Genome Informatics, http://www.informatics.jax.org/
Online Mendelian Inheritance in Man (OMIM), http://www.ncbi.nlm.nih.gov/Omim/ (for PLP1, FLNA, L1CAM, NLGN3, NLGN4, CDKL5, RPS6KA3, *DLG3, RPS6KA3, PLP1, L1CAM, SLC25A5,* Shank1, Shank3, Magi-1, Grip1, *GRIA3, IL1RAPL1, HADH2,* MAOA, *PRPS1, GDI1, ARHGEF9, RPL10,* OPHN1, PAK3, FGD1, ARHGEF6, AP1S2, MECP2, *BDNF, CACNA1C, KCNMA1, PAK2, DLG1, MAPT,* genes in table 1, and proteins in table A1 [online only])
PPID (Protein-Protein Interactions Database), http://defiant.inf.ed.ac.uk:8000/
Swiss Prot database, http://www.ebi.ac.uk/swissprot/access.html#srs
UniGene, http://www.ncbi.nlm.nih.gov/entrez/query.fcgi?db=unigene

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