Integrating Pathogenic Models of Autism: Pathway and Network Analysis

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Introduction

Autism spectrum disorder (ASD) is considered a complex genetic disorder that affects around 1 % of the population. A diagnosis of ASD is dependent on qualitative impairments in communication and social skills and the presence of repetitive behaviors and/or restricted interests. Single-gene mutations and defined chromosomal defects account for over 25 % of ASD cases (Miles 2011), with the remainder often being referred to as idiopathic ASD. Twin and family studies indicate ASD is highly heritable, with heritability estimates of 80–90 %, while spontaneous (de novo) genetic mutations are implicated in ~10 % of cases (Ronald and Hoekstra 2011). At the cellular level, many of the genes implicated in ASD have direct or indirect roles in synapse function, and ASD is sometimes referred to as a "synapsopathy" (Dölen and Bear 2009) or "synaptopathy" (Brose et al. 2010) due to the abnormal synaptic morphology and function detected. However, in addition to the current diagnostic criteria of ASD, a variety of additional neurological phenotypes are frequently detected such as attention-deficit hyperactivity disorder (ADHD), epilepsy, mental retardation (MR), and obsessive–compulsive disorder (see Bishop et al. 2014). Furthermore, biochemical, gastrointestinal, and immune system abnormalities frequently co-occur with ASD (McDougle et al. 2005; Castellani et al. 2009; Buie et al. 2010; Brown and Mehl-Madrona 2011; Suzuki et al. 2011; Benach et al. 2012). As with other common complex genetic disorders, the phenotype of each individual varies and is dependent on which gene or genes are mutated, the individual genetic background, as well the in utero environment before birth (Bishop et al. 2014).

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Autism Genetics

To date, hundreds of genetic mutations have been linked to the development of ASD, and a variety of databases have been established to collate information on emerging and defined "autism genes" (Table 1). This number of genes is typical of complex genetic disorders, including mental retardation (MR), epilepsy, and ADHD. Despite the large number of genes implicated in complex genetic disorders, analysis of key subsets of *ASD* genes (see Table 2) suggests that mutations associated with ASD converge on key shared pathways which, when disrupted, affect synaptic function (Bourgeron 2009; Bill and Geschwind 2009). Interactomic analysis is discussed in more detail elsewhere (Barth and Bishop 2014), and an example incorporating the ASD genes from Table 2 is provided here (Fig. 1), illustrating the complex interactions between ASD genes.

Of note, many of the genes implicated in ASD also increase the risk of a variety of neurological and medical conditions (Bolton 2009; Lichtenstein et al. 2010; Betancur 2011; Talkowski et al. 2012), and it is clear from syndromic ASD cases that a single-gene mutation frequently leads to a phenotype of which ASD is just one part (Benvenuto et al. 2009; Caglayan 2010; Betancur 2011; Barth and Bishop 2014). Evidence from research into a variety of single-gene disorders also indicates different mutations in the same gene can lead to different phenotypes (Antonarakis and Beckmann 2006; Bishop et al. 2014). This phenomenon is due to factors such as the multiple binding partners and pleiotropic roles of many gene products (see Fig. 1). Studying gene product interactions, and the effects of gene products in multiple cellular pathways, is a key tool for increasing understanding of these phenomena.

In the field of ASD research, some genetic mutations are known to directly affect synapse development, morphology, and function (Bourgeron 2009; Bill and Geschwind 2009; Peça and Feng 2012). By contrast, other autism genes appear to indirectly lead to synaptic anomalies and include genes affecting secretory pathway function, calcium signalling, or impacting on poorly understood mitochondrial activities (Krey and Dolmetsch 2007; Bourgeron 2009; Gargus 2009; Palmieri and Persico 2010; Aziz et al. 2011a, 2011b, 2012; Peça and Feng 2012). This chapter discusses how, using specific examples, pathway and network analyses can integrate the functional effects of single-gene mutations with the current pathogenic models of ASD etiology.

Syndromic Autism and the Target of Rapamycin Pathway

The most common single-gene forms of ASD are FXS and tuberous sclerosis (TS), which account for an estimated 7 % and 4 % of ASD cases, respectively. In addition, syndromes associated with PTEN (phosphatase and tensin homolog) gene mutations are found in 1–5 % of ASD patients (Zhou and Parada 2012). Along with neurofibromatosis type 1 (NF1), which accounts for around 0.6 % of

Table 1 Useful databases for autism spectrum disorder genetic research

Database name	Database descriptor	Universal resource locator
1,000Genomes	A deep catalog of human genetic variation	www.1000genomes.org
ACRD	ASD Chromosome Rearrangement Database	http://projects.tcag.ca/autism
AGD	Autism Genetic Database	http://wren.bcf.ku.edu/
AGRE	Autism Genetic Resource Exchange	https://research.agre.org/
ALFRED	ALleleFREquency database	http://alfred.med.yale.edu/alfred/
AutDB	Autism database	www.mindspec.org/autdb.html
AutismKB	Autism Knowledge Base	http://autismkb.cbi.pku.edu.cn
AutWorks	Autism-network analysis tool	http://autworks.hms.harvard.edu
CNVR	Copy Number Variant Resource	http://cnv.chop.edu/
dbSNP	Single Nucleotide Polymorphism database (at NCBI)	www.ncbi.nlm.nih.gov/projects/SNP
DECIPHER	Database of Chromosomal Imbalance and Phenotype in Humans using Ensembl Resources	http://decipher.sanger.ac.uk/
DGV	Database of Genomic Variants	http://projects.tcag.ca/variation/
EVS	Exome Variant Server	http://evs.gs.washington.edu/EVS
FunctionalNet	Probabilistic Functional Gene Networks (from five eukaryotes)	http://www.functionalnet.org/
G2con	Genes to Cognition database	http://www.g2conline.org/
GAD	Genetic Association database	http://geneticassociationdb.nih.gov
GeneAtlas	Gene and Phenotype Database at Paris René Descartes University	http://genatlas.medecine.univ-paris5.fr
GeneCards	Human gene compendium	http://www.genecards.org/
HapMap	International HapMap project	www.hapmap.org
HGMD	Human Gene Mutation Database	www.hgmd.cf.ac.uk
HuGE	Human Genome Epidemiology navigator (includes links to Genopedia and Phenopedia)	http://hugenavigator.net/
HumanNet	Probabilistic Functional Gene Network of Homo sapiens	http://www.functionalnet.org/humannet/about.html
HVP	Human Variome Project	www.humanvariomeproject.org
OMIM	Online Mendelian Inheritance in Man	www.ncbi.nlm.nih.gov/omim
PGC	Psychiatric GWAS Consortium	https://pgc.unc.edu/
SFARI	Simons Foundation Autism Research Initiative gene database	https://sfari.org/resources/sfari-gene
SLEP	Sullivan Lab Evidence Project (psychiatric genetics at CISGen)	http://gbrowse.csbio.unc.edu/cgi-bin/gb2/gbrowse/slep
UCSC	Genome Bioinformatics and Genome Browser	http://genome.ucsc.edu/index.html
UniProt	Universal Protein Resource	www.uniprot.org

Table 2 Key autism spectrum disorder genes

Official gene symbol	Aliases	Chromosomal location	Descriptor(s)	Dataset
AHI1	JBTS3	6q23.3	Abelson helper integration site 1	1
			Jouberin	
AVPR1A	V1aR	12q14-q15	Arginine vasopressin receptor 1A	1
			antidiuretic hormone receptor 1a	
C3orf58	DIA1	3q24	Chromosome 3 open reading frame 58	1
	GoPro49		Deleted in autism-1	
CACNA1C	CAC1C	12p13.3	Calcium channel, voltage-dependent	1
	Cav1.2		L type, alpha 1C subunit	
CADPS2	CAPS2	7q31.32	Ca^{2+}-dependent secretion activator 2	1
CNTN3	PCS	3p12.3	Contactin 3 (plasmacytoma associated)	2
	BIG-1		Plasmacytoma-associated neuronal glycoprotein	
	PANG		Brain-derived immunoglobulin superfamily protein 1	
CNTN4	AXCAM	3p26.3	Contactin 4	2
	BIG-2		Axonal-associated cell adhesion molecule	
			Brain-derived immunoglobulin superfamily protein 2	
CNTNAP2	NRXN4	7q35	Contactin associated protein-like 2	1
	AUTS15			
	CNTP2			
CYFIP1	SRA1	15q11	Cytoplasmic FMR1 interacting protein 1;	1
	SHYC		Specifically Rac1-associated protein 1	
			Selective hybridizing clone	
DHCR7	D7SR;	11q13.4	7-Dehydrocholesterol reductase	1
	SLOS		Smith–Lemli–Opitz syndrome	
	D7SR			
DISC1	SCZD9	1q42.1	Disrupted in schizophrenia 1	1
	C1orf136			
EN2	HME2	7q36.3	Engrailed homeobox 2	1
	AUTS10		Homeobox protein engrailed-2	
FMR1	POF	Xq27.3	Fragile X mental retardation 1	1, 2
	FMRP		Premature ovarian failure 1	
	FRAXA			
GABRB3	GBRB3	15q12	Gamma-aminobutyric acid (GABA) A receptor, beta 3 subunit	1
ITGB3	GP3A	17q21.32	Integrin, beta 3	1
	CD61		Platelet glycoprotein IIIa	
			CD61 antigen	
JAKMIP1	MARLIN1	4p16.1	Janus kinase and microtubule interacting protein 1	1
	GABABRBP		Multiple coiled-coil GABABR1-binding	
			Multiple alpha-helices and RNA -linker	

(*continued*)

Table 2 (continued)

Official gene symbol	Aliases	Chromosomal location	Descriptor(s)	Dataset
KCNJ6	GIRK2	21q22.1	Potassium inwardly rectifying channel, subfamily J, member 6	1
	BIR1			
	KATP2			
	IRK6			
MECP2	RTT	Xq28	Methyl CpG binding protein 2	1, 2
	AUTSX3			
MET	HGFR	7q31	*Met* proto-oncogene	1
	c-Met		Hepatocyte growth factor receptor	
	RCCP2			
	AUTS9			
NF1	NF-1	17q11.2	Neurofibromin 1	2
NLGN3	AUTSX1	Xq13.1	Neuroligin 3	1, 2
NLGN4X	HNLX	Xp22.31	Neuroligin 4, X-linked	1, 2
	NLGN4		Neuroligin X	
	AUTSX2			
NRXN1	NRX1B	2p16.3	Neurexin 1	1, 2
			Neurexin I-beta	
OXTR	OXYR	3p25.3	Oxytocin receptor	1
	OT-R			
PCDH9	PCD9	13q21.32	Protocadherin 9	2
PCDH10	PCD10	4q28.3	Protocadherin 10	2
PLAUR	UPAR	19q13.31	Plasminogen activator, urokinase receptor	1
	CD87		CD87 antigen	
			Urokinase plasminogen activator surface receptor	
PRKCB	KPCB	16p12.2	Protein kinase C, beta	1
PTEN	MMAC1	10q23.31	Phosphatase and tensin homolog	1, 2
RBFOX1	A2BP1	16p13.3	RNA binding protein, fox-1 homolog	1
RELN	RL	7q22	Reelin	1
	LIS2			
SERPINE1	PAI1	7q22.1	Serpin peptidase inhibitor, clade E, member 1	1
	PLANH1			
SHANK1	SSTRIP	19q13.3	SH3 and multiple ankyrin repeat domains 1	2
			Synamon	
SHANK3	PSAP2	22q13.3	SH3 and multiple ankyrin repeat domains 3	1, 2
	SHAN3		Proline-rich synapse-associated protein 2	
	ProSAP2			
SLC25A12	ARALAR1	2q31.1	Solute carrier family 25, member 12	1
			Calcium-binding mitochondrial carrier protein Aralar 1	

(*continued*)

Table 2 (continued)

Official gene symbol	Aliases	Chromosomal location	Descriptor(s)	Dataset
SLC6A4	5HTT	17q11.2	Solute carrier family 6, member 4	1
	SERT		Serotonin transporter	
			5-Hydroxytryptamine transporter	
TSC1	LAM	9q34	Tuberous sclerosis 1	1, 2
			Hamartin	
TSC2	TSC4	16p13.3	Tuberous sclerosis 2	1, 2
			Tuberin	
UBE3A	E6AP	15q11.2	Ubiquitin protein ligase E3A	1
	ANCR		Human papilloma virus E6-associated	
	HPVE6A			

Dataset 1 represents 33 genes discussed by Bill and Geschwind (2009)
Dataset 2 represents 16 genes discussed by Bourgeron (2009)

ASDs, these three disorders represent around 15 % of ASD cases. These key syndromic forms of ASD are caused by mutations in the *FMR1* gene (FXS), *TSC1* or *TSC2* genes (TS), *NF1* (NF1), and the *PTEN*-hamartoma tumor syndromes (PHTS) and autism with macrocephaly (AM) by mutations in the *PTEN* gene (see Table 2). The genes each localize to different human chromosomes, as indicated in Fig. 2. The *TSC1* gene encodes a protein known as TSC1 or hamartin, while TSC2 encodes TSC2, also known as tuberin (Fig. 3). The *FMR1* gene encodes the fragile X mental retardation protein (FMRP) and *PTEN* encodes the phosphatase, PTEN (phosphatase and tensin homolog) (see Fig. 4). The NF1 gene encodes a large protein, known as neurofibromin-1 (NF1) (Fig. 5), and each protein has multiple known motifs and domains that contribute to their function. TS, PHTS, and NF1 are autosomal-dominant neurocutaneous disorders, presenting with neurologic and dermatologic abnormalities, and the protein products are known to act as tumor suppressors (Lodish and Stratakis 2010). By contrast FXS is an X-linked disorder (see Fig. 2) and the gene is not known to have a role in tumorigenesis. Readers are referred to the Online Mendelian Inheritance in Man (OMIM) database, GeneCards, and/or UniProt (see Table 1) for additional genetic and phenotypic information.

Strikingly, most of the genes causative of the syndromic forms of ASD discussed above (*PTEN*, *NF1*, *TSC1*, or *TSC2*) encode products functioning as upstream regulators of a cellular signalling pathway, known as the TOR (target of rapamycin) pathway (see Fig. 6). By contrast, the *FMR1* gene product, FMRP, functions downstream in the TOR signalling pathway (Fig. 6). TS, or tuberous sclerosis complex disorder (TSCD), is of particular interest, as it is caused by the mutation of one of two genes. Mutations in either the *TSC1* or *TSC2* gene clearly give rise to a sufficiently similar phenotype that patients are diagnosed with the same disorder. This similarity in phenotype is due to the two gene products interacting with each other within cells to form a functional protein complex (Fig. 6). The resulting protein complex, known as the tuberous sclerosis complex (TSC), negatively regulates the kinase activity of the TOR complex, TORC1. The TSC regulates

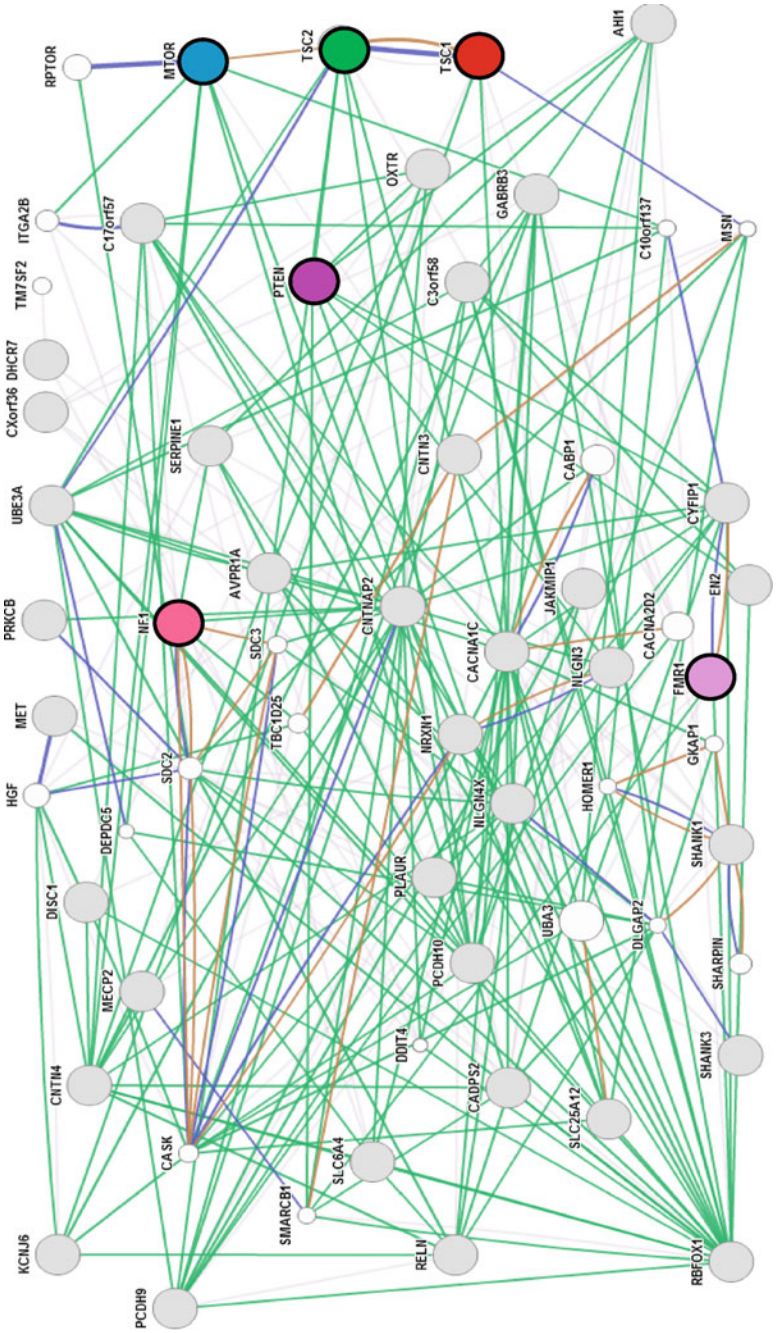

Fig. 1 **Emerging functional links between autism gene products**. Physical (*blue*), predicted (*brown*), co-expression (*grey*), and genetic (*dark green*) interaction network show complex interactions between the genes from Table 2. *Red circle* = TSC1; *green circle* = TSC2; *blue circle* = mammalian TOR. Syndromic ASD gene products, NF1, PTEN, and FMRP/FMR1 are indicated in *pink*

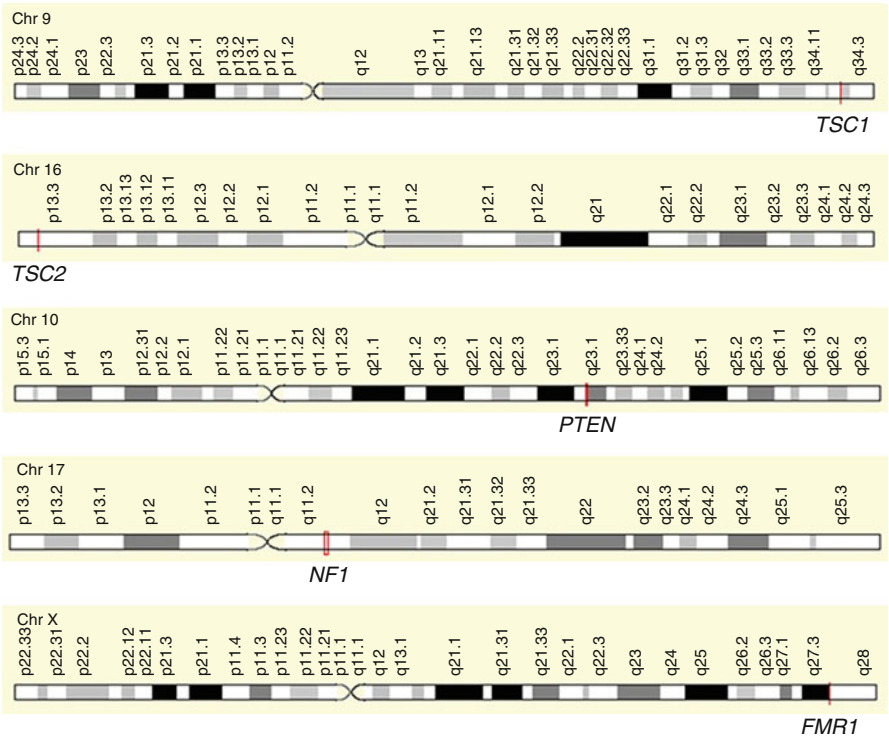

Fig. 2 Chromosome localization of key syndromic autism genes. Human *TSC1* and *TSC2* localize to chromosome 9, at position 9q34.13, and chromosome 16, position 16p13.3 (*red lines*), respectively. Human *PTEN* is found on chromosome 10, position 10q23.3, NF1 on chromosome 17, position 17q11.2, while *FMR1* is an X-linked gene at position Xq27.3 (Data derived from GeneCards, www.genecards.org)

TORC1 activity by the activity of a mutual binding partner, Rheb (Ras homologue enriched in brain). Rheb can activate TORC1 when in the GTP-bound form (see Fig. 6), and this in turn is modulated by the GTPase-activating protein (GAP) domain of TSC2 (see Fig. 3), which is dependent on binding to TSC1.

As is well known for ASD, the neurodevelopmental phenotype of patients with TS, NF1, PTHS, and fragile X syndrome (FXS) is heterogeneous, and reasons underlying this heterogeneity are discussed elsewhere in this book (Bishop et al. 2014), and the mechanism underlying the association of ASD and these syndromes is also unclear. Current data on the etiology of TS, however, indicate that mutation of *TSC1* or *TSC2* directly influences the development of ASD, rather than it being a secondary effect of MR, epilepsy, or tumors in these patients (Smalley 1998; Bishop et al. 2014; Jülich and Sahin 2014). Therefore, ASD and TS are currently thought to share a pathobiological factor(s). Understanding the multiple downstream effects of the TOR pathway is essential, to explain how a single-gene mutation such as that of *TSC2* or *PTEN* can lead to a complex phenotype of

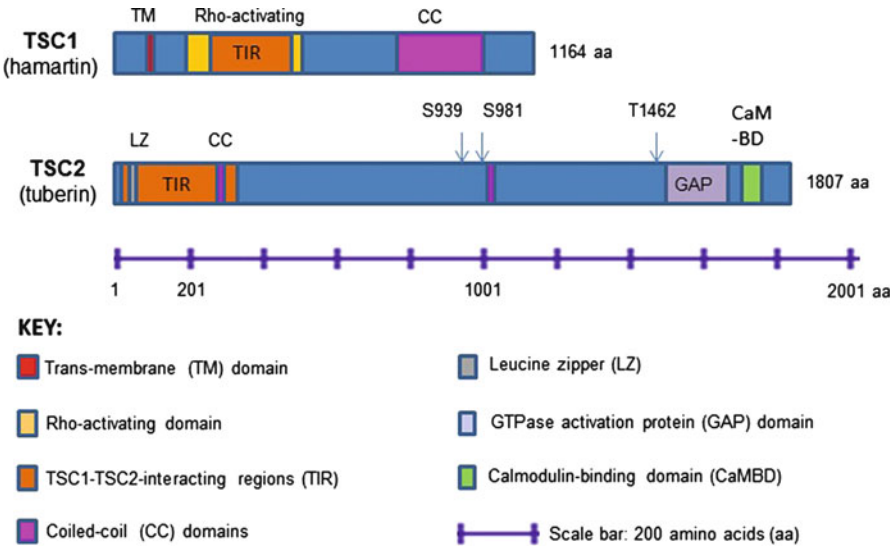

Fig. 3 Schematic representation of the *TSC1* and *TSC2* gene products. *TSC1* and *TSC2* encode the 1,164 amino acid (aa) gene product TSC1 (hamartin), and the 1,807 aa gene product, TSC2 (tuberin), respectively. Key domains and motifs are indicated, with inhibitory serine (S) or threonine (T) kinase sites on TSC2 for PKB (alias AKT) indicated (*arrows*). Further details are available from the GeneCards database (www.genecards.org)

hamartomas, MR, epilepsy, and ASD. Based on current models of the pathobiology of ASD, it is expected that the downstream effects of *TSC1*, *TSC2*, *NF1*, *PTEN*, or *FMR1* mutation must alter the function of the cell secretory pathway (including the ER and/or Golgi apparatus), calcium signalling, and/or mitochondrial function, ultimately leading to alterations in synapse function (Krey and Dolmetsch 2007; Bourgeron 2009; Gargus 2009; Palmieri and Persico 2010; Aziz et al. 2011a, b, 2014; Peça and Feng 2012). Evidence supporting these models of ASD pathogenesis is discussed next, with reference to TS and other syndromic forms of ASD.

Dendritic Spine Morphology and the TSC–TOR Pathway

Neurons are highly polarized cells, with a long axon for action potential propagation and release of neurotransmitters and several dendrites for responding to the signals from an adjoining axon. Dendrites and axons of mammalian neurons typically have a branching morphology of varying complexity, referred to as axonal or dendritic branching, or arborization, dependent on location. The exact morphology of neurons is highly regulated, and their structure, once thought static at maturation, is now known to be highly dynamic throughout life. Excitatory synapses in the brain are characterized by presynaptic axonal boutons (protrusions) apposing "spines" protruding from dendritic arbors. In particular, dendritic spines and excitatory synapses (such as

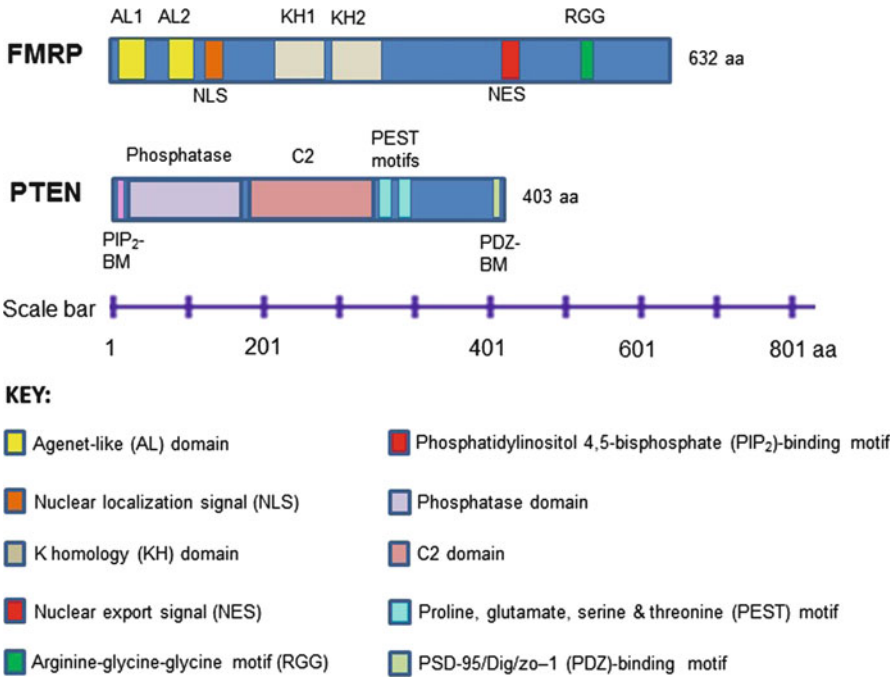

Fig. 4 Schematic representation of the FMR1 and PTEN gene products. *FMR1* and *PTEN* encode the 632 amino acid (aa) gene product *FMRP* (fragile X mental retardation protein), and the 403 aa gene product, PTEN (phosphatase and tensin-homolog). Key domains and motifs are indicated. Further details are available from the GeneCards database (www.genecards.org)

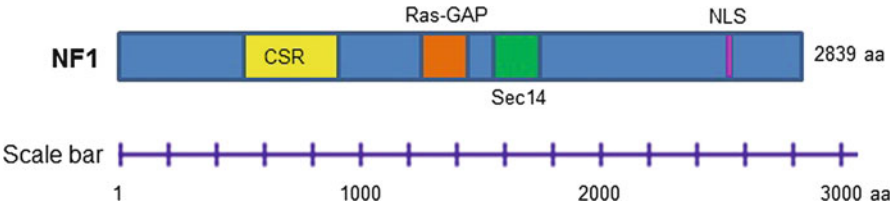

Fig. 5 Schematic representation of the NF1 gene product. *NF1* encodes a 2,839 amino acid (aa) gene product NF1 (*neurofibromin* 1), which acts as a *negative regulator* of Ras, due to its GTPase-activating protein *(GAP)* activity. The Sec14 domain is also known as the CRAL-TRIO domain, and the *functional role* of nuclear-targeted NF1 is unknown. Further details are available from the GeneCards database (www.genecards.org)

glutamatergic brain synapses) are highly dynamic, and the term "synaptic plasticity" is used to describe the ability of synaptic connections between two neurons to change in "strength" in response to neuronal activity (Alvarez and Sabatini 2007). Dendritic spines change in number, size, and shape and therefore correlate with modifications in synaptic strength (Jan and Jan 2010).

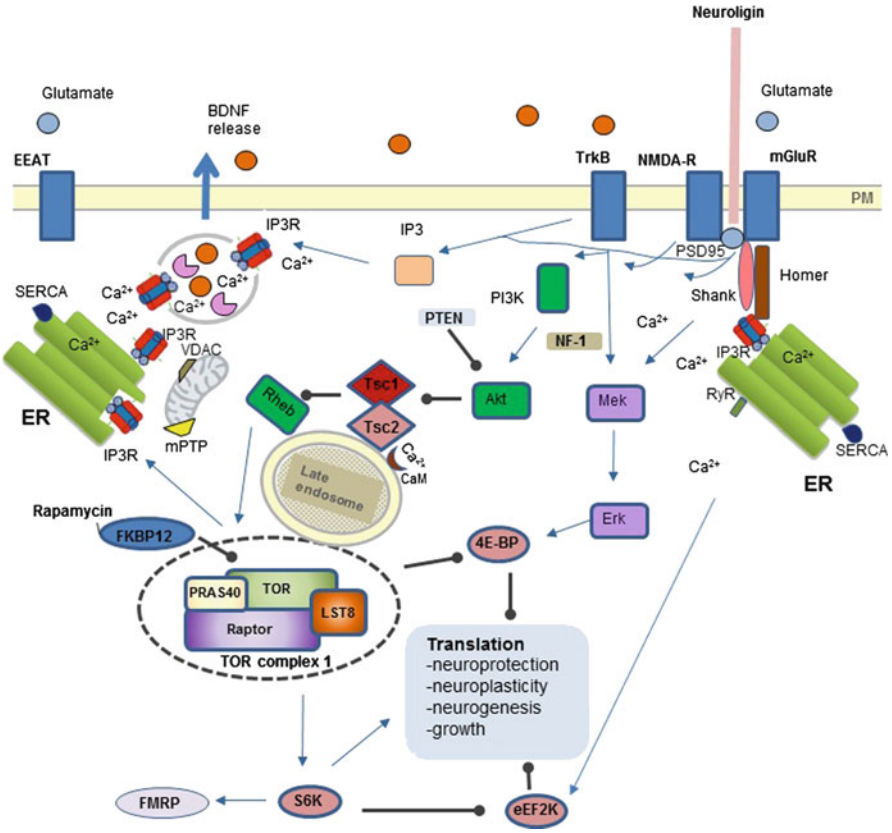

Fig. 6 Schematic of key components of the TSC–TOR pathway. Neuronal receptors and channels activate downstream signalling pathways leading to mammalian *TOR* complex 1 activation. TORC1 activation regulates several downstream effectors of translation: S6K, 4E-BP, and eEF2K. Presynaptic stimulation also leads to increased cytosolic levels of Ca^{2+} (from intracellular stores) and release of BDNF (brain-derived neurotrophic factor). *PM* plasma membrane; *CaM* Calmodulin (see Fig. 2)

Dendrite morphogenesis is dependent on a wide variety of cellular processes, including specific transcriptional regulators; factors affecting local translation in dendrites; cytoskeletal motors and regulators; secreted proteins, cell-surface receptors, and adhesins; regulators of the secretory and endocytic pathways; and signalling pathways that regulate these processes, including the TOR pathway (Kennedy and Ehlers 2006; Jan and Jan 2010; Troca-Marín et al. 2012). Dendritic spines are particularly dynamic structures. Although most dendritic spines are transient, long-term adjustments, such as the addition/loss of stable spines and synapses, are required for learning and memory. These long-term changes are referred to as long-term potentiation (LTP) and long-term depression (LTD) (Alvarez and Sabatini 2007). Of relevance to autism, abnormalities in dendritic spine size, shape, or number are found in many neurodevelopmental and neuropsychiatric

disorders and have been documented in both idiopathic and syndromic cases of ASD (reviewed by Penzes et al. 2011).

While the TSC–TOR signalling pathway is well known as a regulator of cell growth and the formation of tumors, it has only recently been identified as an important pathway in nervous system development, by controlling factors such as cell migration, axon guidance, synaptic expansion, and dendritic arborization (Swiech et al. 2008). Nonetheless, differences in dendritic morphology and spine number have been reported in several syndromic forms of ASD, including those involved in TOR pathway processes. For example, in FXS an increase in dendritic spine density is detected, although these spines are long and immature. Likewise, in PHTS, increased dendritic spine density and hypertrophic dendritic arbors are detected, while in TS, aberrant dendrite and spine morphology is detected, specifically an increase in spine size, but a decrease in spine density (Peça and Feng 2012; Troca-Marín et al. 2012). The abnormal appearance of dendrites in patients is likewise found in animal models of syndromic forms of autism, and these morphological anomalies are associated with deficits in synaptic plasticity, including LTD and LTP, underpinning learning and memory (Penzes et al. 2011; Ehninger 2013; Gipson and Johnston 2012).

Glutamate Receptors and the TOR Pathway

As regulation of the number and density of neurotransmitter receptors is a key mechanism underlying various forms of synaptic plasticity, including LTP and LTD, a change in number of even a few receptors in dendritic spines can affect neurotransmission (Kennedy and Ehlers 2006). Efforts have been made to measure effects of mutations on neurotransmission and to quantify receptors within key dendritic regions, known as postsynaptic densities (due to their distinctive electron-dense appearance by microscopy). In FXS model mice, enhanced metabotropic glutamate receptor-dependent long-term depression (mGluR-LTD) has been detected, which has been attributed to effects of the *Fmr1* mutation on cellular translation events essential for LTD, and the mutant phenotype in fragile X model organisms can be corrected by genetic or pharmacological inhibition of mGluR5 (Auerbach and Bear 2010). Surprisingly, the opposite has been detected in models of TS, as mGluR-LTD is impaired, not enhanced, indicating that TS and FXS have divergent synaptic plasticity phenotypes (Auerbach et al. 2011; Chévere-Torres et al. 2012).

Neuronal mGluR receptors are G-protein-coupled receptors (GPCRs) that are predominantly postsynaptic and, along with the ionotropic glutamate receptors, are activated by glutamate, the major excitatory amino acid neurotransmitter in the brain. The mGluRs, particularly mGluR5, have received considerable attention as key players in neuronal development, synaptic plasticity, LTP/LTD, and seizure activity. Stimulating cell-surface mGluRs leads to activation of phospholipase C (PLC) and the formation of the second messengers, InsP3 (inositol 1,4,5-trisphosphate) and DAG (diacyl glycerol). InsP3 production triggers Ca^{2+} release

from the ER via InsP3 receptors (IP3Rs), while DAG activates protein kinase C (PKC), known to reduce oxidative stress and cell damage (Byrnes et al. 2009) (Fig. 6). Calcium released from the ER triggers further amplification of Ca^{2+} release, by activating ryanodine receptors (RyRs) on the ER, major cellular mediators of intracellular calcium-induced calcium release (CICR) (Mattson et al. 2008).

Interactomic and pathway analyses indicate that IP3R1 (*ITPR1* gene product) interacts with Homer proteins and both are part of a multivalent complex which also includes mGluRs, Shank, and PSD-95 (postsynaptic density protein-95) (Tu et al. 1999; Xiao et al. 2000). These binding partners are key components of the postsynaptic density (see Fig. 6), the electron-dense protein network in dendritic spines. The interactions between mGluRs, ER, and PSD molecules are essential for the correct organization of Ca^{2+} sensors and effectors in the postsynapse and suggest that Ca^{2+} signalling in dendritic spines has a high degree of spatiotemporal organization (Tu et al. 1999). Shank and PSD-95 also provide links between mGluR and ionotropic glutamate receptor signalling.

Although coupled to the same InsP3 second-messenger pathway, mGluR1 and mGluR5 serve different physiological functions, which remain poorly understood. What is known, however, is that mGluR1 and mGluR5 traffic differently on interaction with Homer (Kuwajima et al. 2007) and a larger proportion of mGluR5 is found on intracellular membranes than at the cell surface (Kumar et al. 2012). Intracellular mGluR5 receptors remain targets for activation by glutamate, due to the presence of cell-surface glutamate transporters, known as EEATs (excitatory amino acid transporters, also known as glutamate transporters), part of the solute carrier family 1A (*SLC1A*) gene family, that facilitate glutamate uptake into the cytoplasm. Indeed, TORC1 signalling induces the upregulation of EAAT2, also known as glutamate transporter 1 (GLT1), while the TOR inhibition (by rapamycin) decreases EEAT2 protein and mRNA levels (Wu et al. 2010). Intracellular glutamate can then activate intracellular mGluR5, which induces a sustained calcium-response. By contrast, stimulation of cell-surface mGluR5 triggers a rapid, transient cytoplasmic calcium rise. These data indicate intracellular mGluR5 is critical for LTP and synaptic plasticity (Xiao et al. 2000; Kumar et al. 2012).

Therefore, in tuberous sclerosis, mGluR5 signalling via Ca^{2+} release from intracellular stores is impaired, leading to abnormal dendrite morphology and impairments in LTD. Mechanisms by which mGluR5 signalling may become impaired in patients with *TSC1* or *TSC2* mutations, which over-activates TORC1 signalling, will be discussed further below.

TSC Mutations: Effects on Calcium Homeostasis and Signalling

Intracellular Ca^{2+} storage and release has multiple cellular effects and affects several functions of the secretory pathway. It is important to note that IP3Rs are not only localized to the ER but are also found on the Golgi apparatus, and stimulation of cells with agonists, or IP3, results in significant Ca^{2+} release from Golgi stores (Lin et al. 1999). More recently, IP3R has also been localized to

secretory vesicles, and IP3-mediated Ca^{2+} release from secretory granules has now been demonstrated in many cell types (Yoo 2010, 2011). The amount of IP3R localizing to each cellular compartment varies depending on the cell type, and in cells with a high secretory load, secretory granules contain the majority of intracellular calcium (Yoo 2010, 2011). For example, in the neuronal-like PC12 cells, secretory granules are responsible for the majority of IP3-mediated cellular Ca^{2+} release into the cytoplasm (Yoo 2010, 2011).

The unusually high calcium-storing capability of secretory granules (dense-core vesicles) is due to the presence of high concentrations of the Ca^{2+} storage proteins chromogranin A, chromogranin B, and chromogranin C. These proteins, abbreviated as CgA, CgB, and CgC, are also known as parathyroid secretory protein, secretogranin-I, and secretogranin-II and are encoded by the *CHGA*, *CHGB*, *SCG2* genes, respectively. They are found in many secretory cell types, and it is well documented that neurons and astrocytes both have secretory granules containing chromogranins and IP3Rs (Yoo and Hur 2012). Most of the intravesicular calcium is bound to these proteins, which participate directly in secretory granule biogenesis, at least in part by facilitating condensation and packaging of proteins into secretory granules (Yoo 2010). Elevation of intracellular Ca^{2+} is known to stimulate the phosphorylation of CgA, which regulates protein packaging into secretory granules and subsequent secretion (Yanagihara et al. 1996). Of note, phosphorylation of CgA, CgB, and CgC is carried out by the Golgi casein kinase, an activity defective in a majority of ASD patients (Castagnola et al. 2008). Processing of proproteins, such as prohormones, in the secretory pathway is also regulated by luminal Ca^{2+} levels, as calcium is necessary for prohormone-processing enzyme activity (Austin and Shields 1996).

In addition to IP3R and SERCA, Calnuc, is another protein involved in regulating the secretory-pathway Ca^{2+} stores (Lin et al. 1999). Calnuc, also known as nucleobindin-1 and encoded by the *NUCB1* gene, is a calcium-binding protein localizing to the lumen of the Golgi apparatus and secretory vesicles. Calnuc plays a key role in the Golgi lumen, controlling many aspects of the unfolded protein (UPR) response, as it regulates the activation of ATF6 (activating transcription factor) via proteolytic processing in the Golgi apparatus (Tsukumo et al. 2007). ATF6 is an ER membrane-anchored transcription factor, which is transported to the Golgi apparatus and cleaved, as part of the UPR. Calnuc also regulates proteolytic processing of other proproteins, occurring prior to secretion, such as that of APP (amyloid precursor protein) (Lin et al. 2007). Overexpression of Calnuc enhances regulated, but not constitutive or basal, secretion, e.g., secretion of ACTH (Lin et al. 2009). The related calcium-binding gene product, NUCB2, is similarly implicated in regulation of secretion by controlling the releasable Ca^{2+} store in the ER and Golgi apparatus, regulating signalling of GPCRs and mediating the exocytosis of secretory granules (Kalnina et al. 2009). The IP3-induced release of Ca^{2+} from secretory granules is sufficient to initiate exocytosis of secretory granules (Yoo 2010).

Given that Ca^{2+} and TOR both control many aspects of cell homeostasis, it not surprising that further links between the cellular processes regulated have recently been found. For example, TOR has been found not only to interact with, and

phosphorylate, the IP3R but also to facilitate IP3R-mediated Ca^{2+} release (Frégeau et al. 2011). Indeed, cell treatment with rapamycin, or nutrient deprivation, which acts to inhibit TOR activity, leads to an increase in IP3-induced release of intracellular Ca^{2+} (Frégeau et al. 2011). These results clearly identify TOR as a modulator of intracellular Ca^{2+} signalling (see Fig. 6).

A central role for Ca^{2+} is also emerging at the earliest stages of the secretory pathway, where Ca^{2+} is emerging as a key regulator of ER function. It is well known that a Ca^{2+} concentration increase in the cytosol affects levels in the ER which, in turn, affects the function of a number chaperones within the ER lumen. ER chaperones, such as calreticulin, calnexin, BiP (alias Grp78), and endoplasmin (Grp94), are Ca^{2+}-binding proteins, and changes in free Ca^{2+} concentration in the lumen of the ER profoundly affect their functional activity (Verkhratsky 2005). A decrease in luminal Ca^{2+} renders ER chaperones inactive and prevents correct protein folding and secretion. Under conditions of ER stress, chaperone expression is upregulated, while global translation is inhibited. Of these chaperones, calreticulin is the most abundant in neurons and contributes greatly to ER Ca^{2+}-buffering capacity, as well as regulating SERCA pumps, in addition to its chaperone function. An important property of the SERCA2b calcium transporter (encoded by isoform 1 of the *SERCA2* gene, which is expressed in brain), relevant for ER Ca^{2+} homeostasis, is that its activity is regulated by the free Ca^{2+} concentration in the ER lumen, which is largely mediated via calreticulin and ERp57 (the *PDIA3* gene product, alias Grp58). Calreticulin "senses" luminal Ca^{2+} levels and binds SERCA2b, to activate Ca^{2+} uptake whenever luminal Ca^{2+} concentrations decrease. In turn, ERp57 regulates Ca^{2+} uptake by modulating the redox state of SERCA2b in a Ca^{2+}-dependent manner and inhibits SERCA2b pump activity when luminal Ca^{2+} levels are high (Verkhratsky 2005). Of interest, both BiP and ERp57 are substrates of the Golgi casein kinase, implicated as defective in as many as 70 % of ASD patients (Castagnola et al. 2008), and phosphorylation of BiP is known to prevent substrate binding (Gaut 1997). Therefore, regulation of intracellular Ca^{2+} stores has multiple effects on cells, is modulated by TOR signalling, and also plays several roles in the regulation of exocytosis and protein secretion.

The discussions above indicate that Ca^{2+}-regulated processes are expected to be disrupted in TS patients, similar to those reported in idiopathic ASD individuals (Krey and Dolmetsch 2007). While this is not a well-studied aspect in TS, very recently, *CADPS2*, an autism gene required for Ca^{2+}-dependent secretion of neuropeptides, was found to be downregulated in cells from TS patients (Tyburczy and Kaminska 2012). These data support a role for dysregulated calcium signalling in TS.

TSC Mutations and Mitochondria

Neural stimulation induces the recruitment of organelles with important roles in secretion/exocytosis and/or calcium homeostasis: the ER, Golgi outposts, and mitochondria, to the relevant regions. These organelles help neurons respond and change in response to neural activity (Valenzuela et al. 2011). Mitochondrial fission

and mitochondrial movement into dendritic spines are part of the intracellular phenomena required to regulate spine morphogenesis and regulate neuronal plasticity (Mattson 2007). Of note, these processes are also regulated by the TOR and AMPK signalling pathways, and inhibition of AMPK (AMP-activated protein kinase) leads to a TOR-dependent increase in mitochondrial biogenesis (D'Souza et al. 2007). AMPK is involved in signalling events that bridge calcium signalling pathways to the nutrient- and growth factor-sensing mTOR pathway (Shaw et al. 2004; Tamás et al. 2006). Recent research has identified novel TOR signalling mechanisms that modulate mitochondrial biogenesis, and TOR over-activation (which occurs in TS) leads to increased mitochondrial biogenesis, as well as cell growth (Dunlop and Tee 2009). Therefore, as detected in patients with idiopathic ASD, mitochondrial function may be indirectly affected in TS, and it is possible this may contribute to the neurological symptoms associated with tuberous sclerosis.

Mitochondrial biogenesis is controlled by the intimate relationship of mitochondria with the ER, which is mediated by regions known as MAM (mitochondria-associated membranes), and the interaction of the ER with mitochondria regulates mitochondrial metabolism and energy production. The GTPase, mitofusin-2 (MFN2), is important for the close apposition of mitochondria and the ER, and is enriched in the interacting membranous regions (de Brito and Scorrano 2009). Mfn2 ablation in mouse models causes alterations in mitochondrial morphology, mitochondrial dysfunction, as well as defective calcium homeostasis, leading to ER stress (Sebastián et al. 2012). The interactions between mitochondria and the ER are reversible and are regulated by physiological cytosolic Ca^{2+} levels (de Brito and Scorrano 2010). Indeed, the interaction between the ER and mitochondria is essential for both Ca^{2+} homeostasis and lipid biosynthesis. These findings have led to a growing understanding that a significant proportion of Ca^{2+} released from the ER is taken up by mitochondria via a "quasi-synaptic" mechanism between the two organelles (de Brito and Scorrano 2010). As mentioned above, when TSC is mutated, mGluR-LTP is impaired, less of the second-messenger IP3 is produced, and therefore less Ca^{2+} will be released from intracellular stores. Conversely, inhibition of TOR (by starvation) also causes morphological changes in mitochondria, specifically mitochondrial elongation (Gomes et al. 2011). Therefore the ER and mitochondria and Ca^{2+} signalling are intimately entwined and their relationship is essential for the correct cellular responses to neural activity. Therefore, secondary mitochondrial dysfunction would not be unexpected in TS patients.

Further confirming a role of *TSC1* and *TSC2* mutations in mitochondrial dysfunction, tissue from TS tumors contains cells with swollen mitochondria (Yamamoto et al. 2002), and increased numbers of mitochondria are found in postmortem brains of TS patients (Sarnat et al. 2011). Giant cells, the hallmark cells in the brain of tuberous sclerosis complex patients, have numerous lamellar mitochondria (Jozwiak et al. 2005). By contrast, *TSC1* deficiency in T cells leads to decreased mitochondrial content and function, and this leads to a reduced numbers of both conventional and invariant natural killer T cells (O'Brien et al. 2011). These, albeit sparse, morphological data also indicate that disruption of the TSC–TOR pathway affects not only calcium signalling and ER function, but also mitochondrial function.

Cellular Exocytotic and Secretion Defects in Tuberous Sclerosis

The secretion of a number of peptide hormones is modulated by TSC–TOR signalling, and secretory defects are emerging as a key contributor to the multiple phenotypes characteristic of TS and in ASD pathogenesis. Effects on secretion/exocytosis are to be expected, in part due to effects of the TOR pathway on calcium homeostasis at multiple points, and also due to regulation of expression of key genes regulating exocytosis/secretion itself (Johnson et al. 2002). As discussed above, luminal ER, Golgi, and secretory vesicle Ca^{2+} levels regulate secretory pathway function and play an important role in the posttranslational processing, sorting, and packaging of secreted proteins. A rise in cytosolic Ca^{2+} concentration is also necessary to induce regulated secretion in most cell types, although in neurons, dense granule exocytosis requires a lower increase in cytosolic Ca^{2+} concentration for fusion than do synaptic vesicles (Scheenen et al. 1998). Hallmark giant TS cells are also characterized by a prominent Golgi apparatus, displaced rough ER, and large numbers of dense-core granules, suggestive of global changes in secretory traffic (Jozwiak et al. 2005). However, the effects of the TSC–TOR pathway on exocytosis/secretion remain the most poorly understood aspect of TSC–TOR signalling. Despite this scarcity of information, effects of the TSC–TOR pathway on five different secretory cargoes are known, as discussed next.

The first cargo for discussion is ghrelin, best known for effects on appetite and metabolism, but which also affects pituitary hormone secretion and sleep regulation (Portelli et al. 2012). Ghrelin is a peptide hormone, and emerging evidence suggests that decreased levels of ghrelin are associated with epilepsy and that ghrelin has anticonvulsant action (Portelli et al. 2012). It has also recently been suggested that ghrelin could be a therapeutic target for patients for both epilepsy and ASD, and ghrelin has been shown to promote LTP and memory formation (Portelli et al. 2012). Of relevance to TS, a reciprocal relationship has been found between TOR signalling and ghrelin. For example, in a mouse model, inhibition of TOR signalling by fasting leads to increased levels of ghrelin expression, increased gastric preproghrelin synthesis, and increased levels of secreted ghrelin. By contrast, activation of TOR signalling (which is the phenomenon occurring in TSC patients) decreases the expression of preproghrelin and the levels of secreted plasma ghrelin (Xu et al. 2009, 2010).

In the second example, the TSC–TOR pathway has been found to regulate secretion of neurotensin, another peptide hormone, which has been mostly intensively studied using cultured enteroendocrine cells, using nutrients to activate TOR. In this system, neurotensin secretion is negatively regulated in nutrient-rich medium in a TOR signalling-dependent manner (Li et al. 2011). By contrast, inhibition of TOR signalling, for example, using rapamycin, enhances both neurotensin expression and neurotensin secretion (Li et al. 2011). Of relevance, serum neurotensin levels in children with idiopathic ASD have recently been found to be higher than those of control children (Angelidou et al. 2010), and neurotensin has also been proposed as a potential target for novel ASD therapeutics (Ghanizadeh 2010).

Thirdly, in *TSC2*-deficient cells, intracellular trafficking of polycystin-1 (PC1), the product of the *PKD1* gene, is disrupted, and PC1 is found sequestered within the Golgi apparatus, rather than being delivered to the cell surface. Re-expression of *TSC2* restores the correct membrane localization of PC1 (Boletta 2009). Deletion of the *PKD1* gene encoding PC1 frequently occurs in TS patients, due to its chromosomal location adjacent to *TSC2*. Deletion of PKD1 leads to a kidney disorder known as ADPCK (autosomal-dominant polycystic kidney disease) and 85 % of ADPCK patients also have tuberous sclerosis (Boletta 2009). However, ADPCK patients with deleted *TSC2* have a more severe form of kidney disease, due to the additional TSC2 mutation modifying the ADPCK phenotype. This is because *TSC2* loss affects the intracellular trafficking of PC1, leading to the retention of PC1 in the Golgi apparatus, rather than being delivered to the cell surface. Studies in animal models reveal that if *Tsc2* function is restored, PC1 delivery to the cell surface is likewise restored (Boletta 2009). These data identify TSC2 as a determinant of PC1 function and, potentially, ADPKD severity.

Fourthly, caveolin-1, a membrane-associated scaffolding protein with multiple roles in signalling and traffic from the PM, is also mislocalized in cells lacking *Tsc2* (Jones et al. 2004). In cells lacking *TSC2*, most caveolin-1 is displaced from the PM and is found on a Brefeldin-A-sensitive, post-Golgi compartment, distinct from the endosomes and lysosomes. Reintroduction of TSC2, but not a disease-causing mutant, reverses the caveolin-1 localization to the PM. Therefore, similar to the defect in PC1 delivery to the cell surface described above, when *Tsc2* is deleted, caveolin-1 is retained in post-Golgi secretory vesicles and not delivered to the PM.

Finally, the vesicular stomatitis virus G protein (VSV-G), a viral glycoprotein usually targeted to the PM, is also retained in distinct post-Golgi vesicles and not transported to the PM in *Tsc2*-deficient cells (Jones et al. 2004). Together, these data suggest a role of TSC2 in regulating post-Golgi transport without affecting protein sorting, and the presence of mislocalized cell-surface proteins and secreted factors in *TSC2*-mutated cells is expected to contribute to the overall phenotype of TS. This has also been suggested as an etiological mechanism for idiopathic ASD (Aziz et al. 2014).

These findings are not unexpected, as a large body of evidence indicates that long-term synaptic plasticity, particularly of glutamatergic synapses, is critically dependent on cellular trafficking. For example, synaptic plasticity is crucially dependent on trafficking of metabotropic and ionotropic glutamate receptors, secretion of neuropeptides and hormones, while delivery of the postsynaptic density protein, PSD-95 (encoded by the *DLG4* gene), to the synapse also requires vesicular transport (Yoshii et al. 2011). In addition, the well-characterized ASD genes encoding neurexins and neuroligins also highlight an expected role of secretory pathway deficits in other individuals with ASD. Originally, neurexins and neuroligins were considered to simply facilitate adhesion between the pre- and postsynapse; however, emerging evidence supports an essential role for neurexins in regulated secretion from both neurons and endocrine cells. Deletion of other "adhesion" genes, such as neural cell adhesion molecules (NCAMs) and cadherins (CDHs), also causes secretory deficits (Dudanova et al. 2006). These observations

provide further support for the idea that cellular trafficking, including secretion and exocytosis, is of vital importance for long-term synaptic plasticity.

A comprehensive picture of how TSC1/TSC2 regulates the secretory pathway is still emerging, although regulation of calcium homeostasis via TOR signalling is likely to be a major contributor. In addition, some data indicates disruption of TSC1/TSC2 also affects the cytoskeletal network, which may inhibit secretory vesicle movement (Jones et al. 2004). More indirect effects of the TSC–mTOR pathway on the secretory pathway are also feasible. For example, TCS2 and Rheb (see Fig. 6) can be detected on ER and Golgi membranes (Wienecke et al.1996; Jones et al. 2004; Buerger et al. 2006), as can a large proportion of TOR (Drenan et al. 2004; Liu and Zheng 2007). Rheb also directly interacts with TOR (Fig. 6), and the Golgi localization of Rheb is essential for stimulation of TOR activity (Buerger et al. 2006). It appears that the ER and Golgi are crucial for signalling by TOR and central for TOR function and regulation of calcium homeostasis and cellular secretion. Therefore, TSC–TOR-dependent signalling pathways regulate protein synthesis, modulate metabolism, as well as control post-Golgi traffic of secretory cargo. This latter step will affect cellular secretion of neuropeptides, peptide hormones, and may affect delivery of transporters and receptors, to the cell surface.

Immune Deficits in TS and ASD

Recent evidence suggests the inflammatory immune response is significantly altered in TS patients, compared to controls (Haidinger et al. 2010). Ghrelin also plays an important role in cytokine secretion and immune function (Portelli et al. 2012). Cytokines, such as IL-1beta and/or IFN-gamma, in turn deplete the ER of Ca^{2+} and further activate the ER stress pathway (Cardozo et al. 2005; Matsuda et al. 2006) and can affect dendritic outgrowth and synapse formation (Kim et al. 2002; Ben Achour and Pascual 2010). Inhibition of TOR by rapamycin promotes production of proinflammatory cytokines, while deletion of *TSC2* reverses this effect. In vivo, inhibition of TOR also regulates the inflammatory response and protects genetically susceptible mice against lethal infection (Weichhart et al. 2008). Therefore, the TSC–TOR pathway is a key regulator of innate immune homeostasis, and regulation of this pathway has clinical implications for infectious autoimmune diseases and cancer (Weichhart et al. 2008; Weichhart and Säemann 2008). Modification of cellular signalling via this pathway has broad implications for ASD, cancer etiology, infectious disease, and autoimmune disorders (Weichhart et al. 2008; Weichhart and Säemann 2008).

Of importance, these findings have parallels with patients with idiopathic ASD, where immune alterations are frequently detected, with multiple studies detecting increased levels of cytokines in serum and brains of individuals with ASD (Pardo et al. 2005; Vargas et al. 2005; Ashwood et al. 2011; Suzuki et al. 2011). These similarities further support the validity of studying syndromic forms of ASD to increase our understanding of idiopathic ASD. The abnormal levels of cytokine

secretion detected in ASD patients may not only contribute to comorbid conditions in these individuals but may also contribute to the severity of ASD phenotype.

Gastrointestinal Dysfunction in TS and ASD

Gastrointestinal involvement has been reported in tuberous sclerosis (Moulis et al. 1992; Hizawa et al. 1994; Leung and Robson 2007) and in idiopathic ASD. While gastrointestinal dysfunction in TS may be due to cellular overgrowth, the dysregulated TOR signalling pathway also affects the secretory pathway (see above), nutrients are well known as activators of TORC1 activity and, overall, TORC1 signalling is known to have impact on digestion, gut motility, inflammation, appetite, and satiety signalling.

In the pancreas, TORC1 signalling regulates intestinal hormone secretion and the secretion of digestive enzymes from pancreatic acinar cells (Williams 2010; Xu et al. 2010; Li et al. 2011). For example, inhibition of TORC1 activity stimulates the expression of gastric ghrelin mRNA and protein and leads to an increase in concentration of plasma ghrelin, while gastrin synthesis and secretion are inhibited (Xu et al. 2010). In addition, hormones secreted from enteroendocrine cells, including ghrelin, leptin, and melatonin, also stimulate secretion of pancreatic digestive enzymes (Jaworek et al. 2010). The brain reciprocally regulates pancreatic exocrine function, and systemically circulating hormones have a complex interaction affecting the pancreas, gut, and the brain, with the latter often referred to as the "gut-brain axis" (Jaworek et al. 2010).

Another example of an enteroendocrine cell hormone whose secretion is regulated by TORC1 signalling is neurotensin (Li et al. 2011). Neurotensin regulates a number of digestive processes including gastrointestinal motility and pancreatic and biliary secretion. It exerts growth-promoting effects on normal gastrointestinal tissues and cancer cells and affects inflammatory mechanisms (Kalafatakis and Triantafyllou 2011). Neurotensin levels are elevated in patients with idiopathic ASD, and this has been proposed to stimulate immune cells, especially mast cells, and/or have direct effects on brain inflammation in ASD (Angelidou et al. 2010). Ghrelin likewise participates in crosstalk between the immune and neuroendocrine systems (Baatar et al. 2011). Therefore, the TOR pathway has multiple effects on gastrointestinal function.

From Disease Models to Therapeutic Agents: Implications for ASD

The new millennium has led to major breakthroughs in our understanding of the biology of ASDs and in progress towards effective therapeutics. To date, the most exciting findings have been those in animal models of ASD indicating that, even in adulthood, the phenotype is fully reversible (Ehninger et al. 2008; Silva and Ehninger 2009). Effective treatment of ASD in animal models has been achieved

both with therapeutic drugs (see Barth and Bishop 2014) and by using "gene therapy" to change the expression of "modifier genes" that ameliorate the phenotype induced by the ASD mutation (see Bishop et al. 2014). Understanding the synaptic anomalies in TS, NF1, *PTEN*-associated ASD, FXS, and other syndromic forms of ASD has led to the development of animal models of disease, the testing of therapeutic drugs in these organisms, and has now proceeded to clinical trials. Many excellent review articles discuss the rationales behind these therapeutic approaches further (see Gipson and Johnston 2012; Hampson et al. 2012; Sahin 2012).

In the case of TS, the abnormal neuronal plasticity and memory defects in *TSC2*-deficient adult mice can be rescued by treating mice with rapamycin (known clinically as sirolimus) (Silva and Ehninger 2009). In humans, rapamycin was first used as an antifungal therapeutic but is now licenced for use as an immunosuppressant and antitumor agent (Ehninger and Silva 2011; Gipson and Johnston 2012). Rapamycin or analogs ("rapalogs"), such as everolimus, act on TOR signalling and partially compensate for the effect of *TSC1/TSC2* mutation, which inappropriately activates TORC1 signalling (Fig. 6). Exciting recent research indicates the therapeutic benefits of rapamycin/rapalogs extend well beyond their roles as tumor suppressors, due to the pleiotropic effects induced by TORC1 hyperactivation. For example, in human cell-culture and mouse models, rapamycin therapy normalizes the innate immune-response deficits of TS (Weichhart et al. 2008), while in a mouse model of TS, a 3-day treatment of adult mice rescued synaptic plasticity and behavioral deficits (Ehninger et al. 2008). Several TS patients have been reported to show improvement in behavioral phenotypes on treatment with rapalogs (Chung et al. 2011), and a placebo-controlled, double-blind clinical trial is underway to assess the effects of everolimus on neurocognition, autistic features, epilepsy, and sleep habits (clinicaltrials.gov: NCT01289912). Therefore, therapeutics for TS and some other syndromic forms of ASD are looking promising. Of greater relevance, however, is the finding that many other ASD genes functionally interact with the TSC-TORC1 pathway (see Fig. 1), and indicates that therapies developed for syndromic ASD may be applicable to a subset(s) of other patients with ASD (discussed further in Barth and Bishop 2014).

Conclusion

In the past 20 years, impressive insights have been made into the genetic and cellular abnormalities of syndromic and nonsyndromic ASD. Understanding syndromic forms of ASD, such as TS, and the underlying dysregulation of the TOR pathway, dovetails with the many current models of ASD pathobiology, including dysregulated cellular calcium homeostasis, mitochondrial dysfunction, secretory pathway abnormalities, abnormal immunological findings, and gastrointestinal anomalies. Increased understanding of the TOR pathway and the contribution of the secretory pathway to neuronal function may lead to an improved understanding of the pathogenesis of ASD and illuminate novel targets for

therapeutics. Furthermore, pathway analysis suggests a single therapeutic may be able to address both the core and noncore symptoms present in individuals with ASD. It is clear that significant progress has been made toward understanding the molecular pathways underlying TS and other syndromic forms of ASD; however many questions still need addressing if the links between genes, synapse function, and the ASD phenotype are to be fully understood.

Key Terms

Axons. Long protrusions from the main body of neuronal cells, which transmit signals to other cells.

Dendrites. Branched, treelike protrusions emanating from the main body of neuronal cells, which receive signals emitted from the axons of adjoining neurons.

Long-term potentiation. A long-lasting increase in synaptic signalling, thought to underpin learning and memory.

Long-term depression. A long-lasting decrease in synaptic signalling.

Synaptic plasticity. Changes in synaptic transmission leading to an increase or decrease in the efficacy of the synapse.

Key Facts of the TSC–TOR Pathway

- The *TSC1* and *TSC2* gene products can interact to form the tuberous sclerosis complex (TSC).
- Mutation in either the *TSC1* or *TSC2* gene causes the genetic disorder, tuberous sclerosis (TS), and is characterized by benign tissue growths, epilepsy, and autism.
- The TSC regulates a lipid-anchored, membrane-associated GTPase, Rheb (Ras homolog enriched in brain).
- TSC inactivation of Rheb leads to inactivation a serine/threonine protein kinase, referred to as TOR (target of rapamycin) or mTOR (mammalian TOR).
- TOR is a key cellular nutrient, energy, and stress sensor and is part of two protein complexes: TORC1 or TORC2.
- TORC1 is the only known target of activated Rheb.
- TSC2 stimulates the conversion of Rheb–GTP (active) to Rheb–GDP (inactive), thereby inhibiting TORC1.
- The best-characterized effect of TORC1 activation is an increase in protein translation due to phosphorylation of eIF4EBP1 (eukaryotic initiation factor 4E-binding protein 1) and p70S6K (p70 ribosomal S6 kinase).
- TSC negatively regulates TORC1, and in TS patients TOR signalling via TORC1 is hyperactivated.
- Of relevance to autism, TOR signalling pathways are not only linked to tumorigenesis but also affect synaptic plasticity and neuroendocrine function.

- Mutations in genes affecting upstream and downstream TORC1 signalling molecules are also implicated in autism.
- Rapamycin (sirolimus) inhibits TORC1 signalling and can reverse the effects of *TSC1* or *TSC2* mutations in model systems.
- Rapamycin, widely used as an immunosuppressant, is now licenced for use as a cancer therapeutic and is in clinical trials to assess therapeutic benefits on epilepsy and autism.

Summary Points

- Mutation of single genes causing syndromic ASD, such as those causing tuberous sclerosis (TS), affects synapse morphology and plasticity, a phenomenon common to other forms of ASD.
- Understanding the cellular pathways and networks of interacting gene products can explain noncore phenotypes detected in individuals with syndromic forms of ASD.
- The TSC-TORC1 pathway is dysregulated in TS and regulates many downstream cellular processes.
- The TSC-TORC1 pathway regulates intracellular calcium signalling, and calcium-regulated processes are also reported to be affected in other types of ASD.
- Mitochondrial morphology and function may also be aberrant in TS and may contribute to synaptic dysfunction in TS, as suggested for idiopathic ASD.
- Secretion of several peptide hormones and targeting of cell-surface proteins is dysregulated in TS, and secretory pathway anomalies are also documented in idiopathic ASD.
- The TSC-TORC1 pathway also regulates the innate immune response, another dysfunction reported in idiopathic ASD.
- Gastrointestinal involvement has been reported in TS and idiopathic ASD and the TSC-TORC1 pathway is known to impact on digestion, gut motility, appetite, and satiety.
- Therefore both ASD and a range of additional signs and symptoms can be caused by mutation in a single gene, because many genes act in complex cellular pathways.
- Understanding the cellular pathways affected by ASD genes will help understand the noncore symptoms of individuals with ASD, the mechanisms causative of the core ASD phenotype, and in the design of effective therapeutics.

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