Emerging Common Molecular Pathways for Primary Dystonia

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ABSTRACT: The dystonias are a group of hyperkinetic movement disorders whose principal cause is neuron dysfunction at 1 or more interconnected nodes of the motor system. The study of genes and proteins that cause familial dystonia provides critical information about the cellular pathways involved in this dysfunction, which disrupts the motor pathways at the systems level. In recent years study of the increasing number of DYT genes has implicated a number of cell functions that appear to be involved in the pathogenesis of dystonia. A review of the literature published in English-language publications available on PubMed relating to the genetics and cellular pathology of dystonia was performed. Numerous potential pathogenetic mechanisms have been identified. We describe those that fall into 3 emerging thematic groups: cell-cycle and transcriptional regulation in the nucleus, endoplasmic reticulum and nuclear envelope function, and control of synaptic function. © 2013 Movement Disorder Society

Key Words: DYT genes; cell cycle; endoplasmic reticulum; nuclear envelope; synaptic function

The dystonias are a heterogeneous group of hyperkinetic movement disorders characterized by sustained involuntary muscle spasms and postures.1 The most common forms are primary, in which dystonic movements are the only clinical feature and there is no evidence of neurodegeneration. In the majority of these cases the cellular processes that lead to functional abnormalities of neurons, sufficient to disrupt the finely tuned control of movement, are unknown. However, in recent years the identification of genes that cause rare monogenic familial dystonia has given insight into the neurobiology of dystonia and shed light on the molecular mechanisms involved. Further evidence has come from the study of more complex forms of secondary dystonia, in which there is evidence of neurodegeneration or central nervous system (CNS) damage and the dystonic movements are part of a more complex neurological phenotype.

The purpose of this review is to summarize the evidence, predominantly from the study of genetic forms of dystonia, and highlight cellular pathways that are important to the genesis of dystonia. In particular, it focuses on areas where there are common themes to the cellular pathogenesis. Most of this information comes from study of monogenic primary dystonias and the proteins that the various DYT genes encode. The genetic classification of dystonia has subtypes DYT 1–25 and includes pure primary dystonias, dystonia-plus syndromes, in which other features such as myoclonus or parkinsonism are present, and paroxysmal dyskinesias, in which dystonia is often a prominent feature. Table 1 describes the DYT loci, showing key clinical features and information about the protein encoded by the DYT gene. The following sections describe the specific forms of primary and dystonia-plus syndromes for which the gene and protein have been identified and studied and that are discussed in this review.

**DYT1 Dystonia: TorsinA**

DYT1 dystonia is caused by a heterozygous 3-bp GAG deletion in the TOR1A gene.2 TorsinA is a 332–amino acid protein typical of the AAA+ (ATPases associated with a variety of cellular activities) protein
<table>
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<tr>
<th>Locus</th>
<th>Designation</th>
<th>Clinical features</th>
<th>Gene/inheritance</th>
<th>Protein</th>
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<tr>
<td><strong>Pure dystonia</strong></td>
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<tr>
<td>DYT1 Chr9q34.11</td>
<td>Early-onset primary dystonia</td>
<td>Childhood onset dystonia in limb with generalization</td>
<td>TOR1A Autosomal dominant</td>
<td>TorsinA</td>
<td>AAA⁺ protein, nuclear envelope, ER secretory and stress response, regulation of synaptic function</td>
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<td>DYT2</td>
<td>Early-onset dystonia</td>
<td>Adolescent-onset segmental or generalized dystonia</td>
<td>Autosomal recessive</td>
<td>Unknown</td>
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<tr>
<td>DYT4 Chr19p13.3</td>
<td>Whispering dysphonia</td>
<td>Childhood-onset laryngeal spasm with cervical dystonia</td>
<td>TUBB4a Autosomal dominant</td>
<td>Beta-tubulin 4a</td>
<td>Structural cytoskeleton protein</td>
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<td>DYT6 Chr 8p11.21</td>
<td>AD early-onset focal dystonia</td>
<td>Early-onset dystonia with prominent cervical and laryngeal involvement</td>
<td>THAP1 Autosomal dominant</td>
<td>Thanatos-associated domain-containing apoptosis associated protein 1</td>
<td>Atypical zinc-finger protein; THAP domain is chromatin-binding factor and regulates transcription</td>
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<tr>
<td>DYT7 Chr 8p</td>
<td>Familial focal dystonia</td>
<td>Adult-onset focal dystonia</td>
<td>Unknown Autosomal dominant</td>
<td>Unknown</td>
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<tr>
<td>DYT13 Chr1p36.32-p36.13</td>
<td>Familial cranio cervical dystonia</td>
<td>Focal or segmental dystonia of cranio cervical region and upper limbs</td>
<td>Unknown Autosomal dominant</td>
<td>Unknown</td>
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<tr>
<td>DYT17 Chr 20p11.2-2q13.12</td>
<td>Early-onset AR dystonia</td>
<td>Early-onset focal dystonia progressing to generalized with dysphonia and dysarthria</td>
<td>Unknown Autosomal recessive</td>
<td>Unknown</td>
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</tr>
<tr>
<td>DYT21 Chr 2q14.3-q21.3</td>
<td>Late-onset dystonia</td>
<td>Late-onset multifocal and generalized dystonia</td>
<td>Unknown Autosomal dominant</td>
<td>Unknown</td>
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<tr>
<td>DYT23 Chr9q34.11</td>
<td>Cervical dystonia</td>
<td>Late-onset primary cervical dystonia</td>
<td>CIZ1 Autosomal dominant</td>
<td>Cip1-interacting zinc finger protein 1</td>
<td>Regulation of G1-S cell cycle and DNA replication</td>
</tr>
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<td>DYT24 Chr11p14.2</td>
<td>Late-onset dystonia</td>
<td>Cranial and cervical dystonia</td>
<td>AN03 Autosomal dominant</td>
<td>Anoctamin 3</td>
<td>Calcium-gated chloride channel</td>
</tr>
<tr>
<td>DYT25 Chr18p</td>
<td>Cervical dystonia with local spread</td>
<td>Predominantly late-onset primary cervical dystonia with spread to face</td>
<td>GNAL Autosomal dominant</td>
<td>Alpha subunit of G protein</td>
<td>Probable interaction with D1 and adenosine 2A receptors.</td>
</tr>
<tr>
<td><strong>Dystonia plus syndromes</strong></td>
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<tr>
<td>DYT3 Xq13.1</td>
<td>X-linked dystonia (Lubag)</td>
<td>Segmental or generalized dystonia with parkinsonism</td>
<td>TAF1 X-linked</td>
<td>TATA box-binding protein associated factor 1</td>
<td>Regulation of transcription initiation and cell cycle</td>
</tr>
<tr>
<td>DYT5/14 Chr2q13.2</td>
<td>Dopa-responsive dystonia</td>
<td>Dystonia with parkinsonism, diurnal variation, very good response to L-dopa</td>
<td>GCH1 Autosomal dominant</td>
<td>GTP cyclohydrolase 1</td>
<td>Rate-limiting enzyme in synthesis of tetrahydrobiopterin, key cofactor in monamine synthesis; results in deficient dopamine synthesis</td>
</tr>
<tr>
<td>DYT11 Chr7q21.3</td>
<td>Myoclonic dystonia syndrome</td>
<td>Upper body myoclonic jerks with dystonia; responsive to alcohol</td>
<td>SGC E Autosomal dominant</td>
<td>Epsilon-sarcoglycan</td>
<td>Cell membrane protein that may act as structural platform for other protein interactions</td>
</tr>
<tr>
<td>DYT12 Chr19q13.2</td>
<td>Rapid onset dystonia parkinsonism</td>
<td>Acute-onset generalized dystonia with parkinsonism, rostrocaudal gradient of symptoms</td>
<td>ATP1A3 Autosomal dominant</td>
<td>Alpha 3 subunit of Na/K ATPase</td>
<td>Subunit of Na/K ATPase on neuronal membrane</td>
</tr>
</tbody>
</table>
family; these proteins typically function as oligomers and use the energy of ATP hydrolysis for many functions including protein trafficking, membrane fusion, protein refolding, and degradation.3 The pathogenic codon deletion (ΔE) in torsinA removes a glutamic acid residue from a C-terminal alpha helix believed to be critical for oligomerization and/or tertiary structure.4 TorsinA is ubiquitously expressed in numerous cell types including neurons and glia within the CNS.5–8 TorsinA mRNA is expressed in sensorimotor regions of the brain including the cerebral cortex, striatum, substantia nigra pars compacta, thalamus, hippocampus, midbrain, pons, cerebellum, and spinal cord.5–8 It has been suggested that the neuron-specific effect of mutant torsinA occurs, as there is low expression of other forms of torsin, notably torsinB, and cannot therefore correct for the presence of dysfunctional torsinA.9

### DYT4 Dystonia: Beta-Tubulin 4a

Study of a large family with autosomal dominant whis- pering dysphonia and generalized dystonia revealed a single cosegregating mutation in the beta-tubulin 4a gene.10 The mutation in the beta-tubulin autoregulatory domain was highly expressed in the nervous system and implicates the cytoskeleton in dystonia pathogenesis.

### DYT6 Dystonia: THAP1

Dystonia-causing mutations in THAP1 (Thanatos-associated protein domain containing apoptosis associated protein 1) have been widely reported.11,12 THAP1 is a transcription factor and a member of the THAP protein family that contains an evolutionarily conserved zinc-dependent DNA-binding domain. Mutations in THAP1 include several reported deletions and nonsense mutations, including one that removes the start codon. Many of the mutations occur in the DNA-binding domain and several appear to disrupt the nuclear localization signal.13–15 In addition, at least 1 mutation appears to cause disease through recessive inheritance.16 Considered together, these observations suggest that THAP1 may cause dystonia through a loss-of-function mechanism.

Before its identification as a dystonia-causing gene, THAP1 was reported to regulate cell proliferation via regulation of pRB/E2F cell-cycle target genes, and another report pointed to a potential proapoptotic role related to localization in promyelocytic leukemia nuclear bodies.17,18

### DYT11 Myoclonus Dystonia: Epsilon-Sarcoglycan

Epsilon-sarcoglycan (SGCE) is a member of the sarcoglycan protein family that comprises plasma transmembrane proteins. Most SGCE mutations are deletions or nonsense mutations that eliminate gene function.19 The SGCE gene is maternally imprinted, meaning that individuals are dependent on expression from the paternal allele. Nearly all myoclonus-
Dystonia patients inherit a loss-of-function allele from their father and therefore lack functional SGCE protein.

Sarcoglycans are components of the dystrophin-glycoprotein complex (DGC), a membrane-spanning complex that makes connections with the extracellular matrix and the intracellular actin cytoskeleton. Although DGC function is typically described in striated muscle, where its dysfunction is linked to several muscular dystrophies, DGC proteins are also expressed in the CNS. SGCE is found in many brain regions and is associated with dopaminergic neurons in the substantia nigra and ventral segmental areas. In neurons, DGC proteins (including SGCE) concentrate at postsynaptic sites and have been localized to GABAergic inhibitory synapses. This association with GABAergic synapses may contribute to the deficient inhibition that is observed in many forms of primary dystonia.

**DYT16 Dystonia-Parkinsonism: PRKRA**

DYT16 is less prevalent than DYT1 and DYT6, having been described in only a small number of families. The gene encodes the protein PRKRA (protein kinase, interferon-inducible double-stranded RNA-dependent activator), also known as PACT, which regulates activity of protein kinase R.

**DYT23 Dystonia: Cip1-Interacting Zinc Finger Protein 1**


CIZ1 encodes Cip1-interacting zinc finger protein 1, a DNA replication factor. CIZ1 was first identified through its interaction with p21Cip1/Waf1 (CDKN1A), a cyclin-dependent kinase inhibitor involved in G1-S cell-cycle regulation and cellular differentiation. CIZ1 is expressed in the cerebellum, cerebral cortex, substantia nigra, and putamen of the adult human brain.

**DYT24 Dystonia: Anoctamin 3**

Exome sequencing in an autosomal dominant family with craniocervical dystonia identified a putative mutation in the anoctamin 3 (ANO3) gene, and additional mutations were found in other familial and sporadic cases of cervical dystonia. ANO3 is expressed highly in the striatum, but also the neocortex, hippocampus, and amygdala. Anoctamin 3 is believed to act as a calcium-gated chloride channel, and functional studies using Ca$^{2+}$ imaging in case and control fibroblasts demonstrated abnormalities in endoplasmic reticulum–dependent Ca$^{2+}$ signaling.

**DYT25 Dystonia: GNAL**

Recently, mutations in GNAL have been identified in 11 multiplex families (autosomal dominant) predominantly with cervical and segmental dystonia. GNAL encodes the stimulatory alpha subunit Gs, first identified as a G-protein that mediates odorant signaling in the olfactory epithelium, coupling D1 and A2a receptors to adenylyl cyclase and histone H3 phosphorylation.

The following sections focus on mechanisms that have been derived from the study of a number of the studied genes described above but will also consider pathogenesis of selected forms of secondary dystonia. Three key areas can be identified in which there appear to be shared molecular pathways: (1) cell cycle and transcription, (2) nuclear envelope/endoplasmic reticulum (ER) interface and ER secretory pathways, and (3) synaptic function.

**Defects at the G1-S Cell-Cycle Checkpoint**

The eukaryotic cell cycle consists of 4 distinct phases: gap 1 (G1), synthesis (S), gap 2 (G2), and mitosis (M). In addition, terminally differentiated cells like neurons enter a quiescent state called gap 0, or G0. The G1 phase is commonly known as the growth phase. During G1, cells prepare for the DNA synthesis that will occur in the S phase. DNA is synthesized and chromosomes are replicated during the S phase. The G1-S cell-cycle checkpoint pathway controls the transition from the end of G1 to S. The G1-S cell-cycle checkpoint ensures that the cell is fully prepared for DNA synthesis. Numerous factors such as DNA damage from irradiation, contact inhibition, TGFβ, and oxidative stress can inhibit transition from G1 to S, and the G1-S checkpoint has also been implicated in the molecular pathology of dystonia.

**CIZ1**

The cellular role and neural localization of CIZ1 are compatible with current themes in dystonia research. CIZ1, TORIA, THAP1, and genes associated with neurodegenerative dystonia seen in DYT3 dystonia (TAFl) and ataxia telangiectasia (ATM) are involved in G1–S cell-cycle regulation (Fig. 1).

In cell-free systems, CIZ1 is able to promote DNA replication after replication complex formation. The C-terminal domain anchors CIZ1 to the nonchromatin nuclear matrix, whereas DNA replication activity resides in the N-terminal half of the protein. The C-terminal domain of CIZ1 also recognizes the consensus DNA sequence ARYSR(0–2)YYAC. Studies of GFP-tagged CIZ1 have shown that formation of
FIG. 1. G1/S cell-cycle checkpoint and dystonia. Dystonia-associated proteins are shaded red. Indirect, multistep, and putative pathways are denoted with hashed lines. In general, arrows indicate excitatory interactions, and stops mark inhibitory interactions. However, some relationships are nonlinear and the result of combinatorial actions of heteromeric complexes and posttranslational modifications. \( +p \), Phosphorylation; ATM, ataxia telangiectasia mutated; ATR, ataxia telangiectasia and Rad3-related protein; CDC25A, cell division cycle 25 homolog A; CDK2, cyclin-dependent kinase 2; CDK4, cyclin-dependent kinase 4; CDK6, cyclin-dependent kinase 6; CDKN1A, p21/WAF1 or cyclin-dependent kinase inhibitor 1; CHK1, checkpoint kinase 1; CIZ1, Cip1-interacting zinc finger protein; E2F, transcription factor E2F; E2F/TFDP1 Target Genes; DNA Damage; Histone H3; G1 Phase; G2/M Checkpoint; G0; R, restriction point; Rb, retinoblastoma protein; SMAD2, mothers against decapentaplegic homolog 2; SMAD3, mothers against decapentaplegic homolog 3; S phase, synthesis phase; TAF1, transcription initiation factor TFIID subunit 1; TFDP1, transcription factor Dp1; TGFβ, transforming growth factor β.
subnuclear particles or foci requires both the N- and C-terminal domains.34

The N-terminal region of CIZ1, including the first zinc finger motif, binds to the N-terminal CDK2-interacting domain of CDKN1A, and this interaction is disrupted by overexpression of CDK2.35 When CIZ1 and CDKN1A are individually overexpressed, they localize primarily to the nucleus. However, overexpression of CIZ1 can induce cytoplasmic distribution of CDKN1A, suggesting that CIZ1 regulates the cellular localization of CDKN1A.

In cell-free experiments, CIZ1 increases the number of nuclei that initiate DNA replication, and in intact wild-type and CDKN1A-null cells, CIZ1 stimulates DNA synthesis. Consistent with a role in DNA replication, endogenous CIZ1 was found to colocalize with proliferating cell nuclear antigen (PCNA) during S phase, and targeted depletion of CIZ1 represses cell proliferation by inhibiting entry into S phase.31 CIZ1-depleted cells accumulate chromatin-bound minichromosome maintenance complex component 3 MCM3 and PCNA but fail to synthesize DNA efficiently.31

CIZ1 is an estrogen-responsive gene.36 CIZ1 coregulates estrogen receptor alpha (ERα) by enhancing ERα transactivation activity and promoting the recruitment of the ERα complex to target genes. CIZ1 overexpression confers estrogen hypersensitivity and promotes growth rate, anchorage independence, and tumorigenic properties in breast cancer cells. It is tempting to speculate that aberrant interactions between ERα and CIZ1 or other, as yet unidentified dystonia-related proteins contribute to the relative increased prevalence of cervical dystonia in females.

**THAP1**

THAP1 plays an important role in transcriptional regulation in the context of cell proliferation and pRB/E2F cell-cycle pathways.18,37,38 Both RNAi silencing and overexpression of THAP1 inhibit G1-S progression. Overexpression of THAP1 in primary human endothelial cells inhibited proliferation, and gene expression profiling showed that this effect was a result of repression of pRB/E2F cell-cycle target genes. The antiproliferative effects of THAP1 on endothelial cells were not dependent on apoptosis. THAP1 appears to inhibit cell-cycle progression at the G1-S transition, localizes to promyelocytic leukemia nuclear bodies with the proapoptotic leucine-zipper protein Par-4, and potentiates TNFα-induced apoptosis.17

Clouaire and colleagues (2005) identified a consen-
sus DNA-binding sequence (AGTACGGGCCA) recog-
nized by the THAP domain of THAP1.39 Nucleotide positions upstream of the core motif appear to modu-
late the strength and affinity of the GGCA/THAP interaction. Other THAP zinc fingers, including human THAP2 and THAP3, share structural homology but do not recognize the same DNA target sequence. Protein–protein interactions, including multimerization, mediated by the coiled-coil domain of THAP1 may increase binding of the THAP zinc finger. Human THAP1, human THAP9, and Drosophila THAP bind DNA through a bipartite interaction using both the major and minor grooves.40

Overexpression of THAP1 in endothelial cells has been used as an indirect means of identifying THAP1 targets.18 A total of 16 genes were upregulated >1.5-fold, and 80 downregulated. Of the latter group, the genes were concentrated in classes related to cell-cycle/ cell proliferation, and the majority were also regulated by the pRB/E2F pathway. The cellular effects of THAP1 knock-down with RNAi were similar to the effects of overexpression. In addition, THAP1 knock-down was associated with decreased expression of 8 pRB/E2F cell-cycle target genes: RRM1, Mad2, survivin, HMMR, RRM2, CDC2, cyclin B1, and DLG7. RRM1 was shown to be a direct transcriptional target of THAP1. Potential THAP1 binding sites were also identified in the promoters of other genes: RRM2, BIRC2, survivin, and cyclin B1.

Studies have also focused on a potential functional interaction with torsinA. THAP1 was reported to bind to and activate the promoter of the Tor1a gene that encodes torsinA, although these studies did not demonstrate changes in torsinA mRNA following manipulation of THAP1.41,42 Moreover, there do not appear to be sequence alterations in the genes encoding THAP1 or torsinA that explain disease penetrance in DYT1 dystonia.43,44

Two hybrid studies have identified several protein interactions for THAP1.17,45 Analysis of the set of interacting proteins suggests that THAP1 may contribute to transcription, splicing, and, possibly, RNA transport. Interestingly, several interacting proteins indirectly implicate THAP1 in cerebellar development. For example, NKAP is a transcriptional repressor acting on Notch target genes and is required for T-cell development. Notch 1 is required for neuronal and glial differentiation in the cerebellum.46 FXR2, a homologue of the fragile X mental retardation protein (FMRP), is involved in mRNA transport. Deletion of FMRP in mice is associated with cerebellar ultrastructural abnormalities and motor learning deficits.47 Finally, DVL2 appears to be involved in the Wnt signaling pathway, which is critical for cerebellar morphogenesis.48 There is an increasing body of evidence that implicates the cerebellum in the genesis of dystonic movements,49 and these data suggest that THAP1 could lead to subtle functional abnormalities of cerebellar functions.

Although often considered a neurodegenerative disorder, some patients with Lubag (DYT3) manifest isolated focal (including cervical) or segmental dystonia years before the development of parkinsonism.50
DYT3 is associated with deficiency of a neuronal isoform of TAF1 (N-TAF1) that is expressed preferentially in medium spiny neurons of the striosomes compartment. TAF1 (TATA box-binding protein [TBP] associated factor 1) forms part of the TFIID transcriptional complex that is composed of TBP and up to 13 additional TAFs. TFIID binds to TATA boxes and participates in transcriptional initiation. Deficiency of defective TAF1 could exert deleterious effects on cell-cycle control via multiple mechanisms. For instance, TAF1 induces G1-S progression by phosphorylating p53 at threonine-55. In TAF1-defective cell lines, ATR localizes to subnuclear foci and contributes to phosphorylation of downstream targets such as p53 and CHK1, which induces cell-cycle arrest. Finally, TAF1 has been shown to undergo alternative splicing in response to developmental or DNA damage signals.

**TorsinA**

TorsinA binds the KASH domain of nesprin-3, which spans the nuclear envelope (NE) outer membrane, and TA is concentrated at the nuclear envelope in its mutant form. TorsinA appears to play a role in interactions between the nucleus and cytoskeleton. These interactions may be important for cell polarity and/or transcription. Alternatively, torsinA may participate in transcriptional regulation and/or G1-S cell-cycle regulation via the TGFβ pathway. Dro sophila larvae that overexpress mutant ΔE torsinA exhibit overt ultrastructural defects at the neuromuscular junction similar to defects reported for mutants with defective TGFβ signaling. Overexpression of SMAD2, a downstream effector of the TGFβ pathway, corrects morphological and behavioral defects associated with expression of mutant torsinA.

Variant ataxia-telangiectasia from recessive mutations in ATM may present with dystonia. This is similar to THAP1 dystonia, with frequent craniocervical and upper limb involvement, and can occur in the absence of cerebellar atrophy on MRI or ataxia on clinical examination. ATM (ataxia telangiectasia, a process whereby misfolded proteins are removed from the ER lumen and degraded by the proteasome. Interestingly, the DYT16 protein PRKRA (PACT) may also play a role in regulating ER stress via induction of protein kinase R.
TorsinA also functions at the nuclear envelope. A “substrate trap” version of torsinA that prevents it from uncoupling from protein partners causes it to accumulate abnormally in the NE. Similarly, disease mutant ΔE-torsinA concentrates abnormally at the nuclear membrane, indicating that the DYT1 mutation may lead to an abnormal interaction with an NE partner, possibly lamina-associated polypeptide 1 (LAP1). The neuronal nuclear membranes from torsinA null of ΔE-homozygous knock-in mice exhibit abnormal nuclear envelope membranes, providing in vivo evidence for an NE role for torsinA. Considered together, these observations suggest that the DYT1 mutation impairs normal torsinA function.

The role of torsinA within the NE is not well understood but may involve regulating connections between proteins that tether the nucleus to the cytoskeletal networker transcriptional regulation at the inner NE membrane. These functions may intersect with that of epsilon-sarcoglycan, a protein that participates in nucleo-cytoskeletal connections, and the transcription factor THAP1 (Fig. 2).

**Epsilon-Sarcoglycan**

The connection of epsilon-sarcoglycan (SCGE) function to the actin cytoskeleton, which itself has links to the nuclear membrane, points to a possible connection between torsinA and SCGE. TorsinA has been implicated in regulating connections between the nucleus and the cytoskeleton, in particular through its reported interactions with the nesprins, a family of outer NE proteins that themselves can interact with the actin cytoskeleton. TorsinA has been reported to interact with nesprin-3, which mislocalizes in cells lacking torsinA or that harbor DYT1 mutant torsinA. Several reports have demonstrated that nesprin-3 can connect to the actin cytoskeleton, consistent with a potential functional connection between torsinA and SCGE. This connection between torsinA and SCGE may also be in part direct, as torsinA has been reported to interact with and promote the degradation of SCGE mutants in the ER. Abnormalities of the actin cytoskeleton could account for observations of neural process abnormalities in cell lines overexpressing torsinA.

**THAP1**

Although THAP1 does not appear to have a direct effect on torsinA expression, there are other ways that nuclear-localized THAP1 could potentially interact with torsinA-related pathways. The nuclear membrane is increasingly recognized as a site of transcriptional regulation through several mechanisms. It is possible that THAP1 targets are dysregulated in DYT1 dystonia, in which mutant torsinA mislocalizes to the nuclear membrane. It is also possible that THAP1 and torsinA pathways connect via pRB, as this is a THAP1-binding partner that also indirectly connects to torsinA via laminA and LAP1. Direct testing of these possibilities awaits the identification of THAP1 target genes in neurons, which can then be assessed in DYT1 and forms of primary dystonia.

**Synaptic Function**

Primary dystonia and the dystonia-plus syndromes are characterized by the absence of neurodegeneration, implying a functional neuronal defect that leads to the
abnormal movement command. This may be a neurochemical defect, possibly for multiple neurotransmitter pathways. The hypothesis that primary dystonia is a result of disturbance of neurotransmitters or synaptic transmission is an attractive one, and there is evidence to support this, particularly involving the dopaminergic pathway (Fig. 3). The clearest example is from a study of dopa-responsive dystonia in which mutations encoding proteins critical for dopamine (DA) biosynthesis, including GTP-cyclohydrolase 1 and tyrosine hydroxylase, cause dystonia.92,93 Most cases of DRD are due to GTPCH1 mutations; GTPCH1 is the rate-limiting step in production of tetrahydrobiopterin, which is a key cofactor in the synthesis of monoamines, particularly dopamine. Reduced levels of functional GTPCH1 lead to reduced DA levels and dystonia. DRD, therefore, responds very well to l-dopa therapy, which corrects the presynaptic deficit of DA.94

D2 receptor antagonists can cause tardive dystonia,95 and dystonia is also seen in Parkinson’s disease, particularly young onset,96 and Lesch–Nyhan syndrome, in which there is preferential loss of nigral dopaminergic neurons.97 Furthermore, other DA-related abnormalities have been found in various forms of focal primary dystonia, including reduced basal ganglia D2 receptor binding in imaging studies and possible association of dystonia with polymorphisms in the D5 receptor gene.98,99

TorsinA

The finding of torsinA mRNA in neurons of the substantia nigra led to neurochemical analysis of dopamine and its metabolites in postmortem brain tissue.5,100 However, these studies were inconclusive.101,102 Similarly, studies in various transgenic mouse models provided inconsistent results regarding metabolite levels.103–106 However, more recent work in a model using the TH promoter to drive expression of wild-type or mutant torsinA has suggested a defect in DA reuptake implicating the DA transporter (DAT).107,108 This has been supported by findings of reduced DA reuptake in hMT-CMV mice109 and a recent study in DYT knock-in mice showing that TH-positive substantia nigra neurons were slightly reduced in number and increased in size,110 and evidence of a direct interaction between torsinA and DAT71 as well as vesicle monoamine transporter 2 (VMAT2).111 Recent work using more sensitive voltammetry to measure extracellular DA, however, found evidence to suggest that it was release not reuptake that was impaired, arguing against DAT dysfunction.107,112

Further evidence for involvement of presynaptic vesicles comes from cellular studies. It has been detected that torsinA is associated with vesicles in axons and presynaptic terminals, and biochemical fractionation analysis showed enrichment of torsinA in the fraction containing synaptosomal membranes.113 TorsinA has also been found to colocalize with snapin (SNARE-associated protein) on dense-core granules at the tips of differentiated PC12 cells.114 Functional analysis of SH-SY5Y cells expressing wild-type or mutant torsinA has shown that it regulates the degradation of snapin and stonin-2 (synaptotagmin specific endocytic adapter) by the proteosome, with mutant torsinA leading to reduced levels and compromised synaptic vesicle recycling.115

Abnormalities in synaptic vesicle recycling has been supported by recent work in cultured hippocampal neurons from a knock-in mouse model of DYT1 dystonia that suggested that torsinA regulates recycling at a level at or upstream of the rise in calcium concentration in nerve terminals and that the regulation is influenced by neuronal activity.116 Furthermore, using patch-clamp electrophysiology, it was found that neurons with mutant torsinA had more frequent miniature glutamate release, which may underlie the excitability of the CNS in DYT1 dystonia.117 The authors have not looked at other neurons, potentially more relevant to basal ganglia dysfunction.

Postsynaptic defects in hMT-CMV-derived striatal slices showed that activation of D2 receptors (D2Rs) led to abnormal activation and inappropriate firing of cholinergic interneurons118 and GABAergic medium spiny neurons.119 In addition, medium spiny neurons from hMT-CMV mice had decreased expression of surface D2Rs with impaired G-protein coupling, despite normal levels of D2R mRNA.120 It was suggested that there was a posttranslational defect in receptor processing. This may occur in the ER for TA, where it acts as a molecular chaperone, leading to abnormal folding or oligomerization of the D2R, and a direct interaction between TA and the D2R has previously been demonstrated.71 In support of this hypothesis are data from [11C]-raclopride PET studies that showed reduced levels of D2R binding in patients with DYT1 and DYT6 dystonia, suggesting a pathogenetic link between these 2 forms and a D2R defect.121,122 For DYT1, reductions in radioligand binding were found in the caudate, putamen, and ventrolateral thalamus.123 Further work needs to be performed to clarify this area, not least is whether the potential D2R defect in DYT6 dystonia is also at a posttranslational level or relates to disrupted transcription.

The abnormal synaptic plasticity in transgenic models of primary dystonia has been reviewed recently and highlighted disruption of synaptic scaling, with facilitation of synaptic potentiation, together with loss of synaptic inhibitory processes.124 In the hMT-CMV model described above, impaired D2R postsynaptic function was suggested by the inability of the D2R agonist to reestablish normal corticostriatal synaptic plasticity. Interestingly, blockade of A2A receptors
fully restored the impairment of synaptic plasticity. A2A receptors and D2 receptors oppose each other in the induction of bidirectional synaptic plasticity, with D2Rs promoting long-term depression and A2A receptors favoring induction of long-term potentiation. The effect of the A2AR antagonist suggests the deficit in D2R function in the model can be reversed by eliminating the negative tone exerted by A2ARs on this. The possible role of A2ARs in genetic dystonia is also implicated from preliminary work on GNAL.

**GNAL**

G-proteins link seven-transmembrane-domain receptors to downstream effectors and function as heterotrimers composed of α, β and γ subunits. Evidence suggests that Gα(olf) acts in medium spiny neurons to couple dopamine type 1 receptors (D1Rs) of the direct pathway and adenosine A2A receptors (A2ARs) of the indirect pathway to the activation of adenylate cyclase type 5. A2ARs and Gα(olf) are also expressed in striatal cholinergic interneurons. The mutations identified in GNAL appear to lead to loss of function and implicate abnormalities in D1R and/or A2AR transmission in the pathogenesis of dystonia.

**DYT12: Alpha-3 Subunit of Na+/K+ ATPase**

Neuronal dysfunction leading to abnormal neurophysiology has been implicated for the DYT12 protein, alpha-3 subunit of the Na+/K+ ATPase, which belongs to the group of P-type ATPases, which utilize energy liberated during the hydrolysis of ATP for active transport of cations across cell membranes. Biochemical enzyme assays have revealed that mutations in α3 cause a reduction in both Na+ affinity and extrusion of intracellular Na+, leading to disrupted electrochemical ionic gradients across the neuronal
cell membrane.\textsuperscript{126} It is possible that this may lead to downstream abnormalities of synaptic function. A phenotypic model of RDP was recently generated by chemically inhibiting the α3 isoform of the ATPase function in selected brain regions using the targeted infusion of ouabain, which selectively reduces α3-ATPase function in a dose-dependent manner in genetically wild-type mice.\textsuperscript{127} Ouabain infusions in the basal ganglia and cerebellum induced a parkinsonism-like or dystonic-like phenotype, respectively, but only concomitant infusions in both structures yielded a stress-inducible phenotype resembling features of RDP. In the mouse model, dystonic postures were reduced following transient inhibition of cerebellar input by GABA injection, again underlining the emerging importance of cerebellar dysfunction in dystonia and RDP.

Further evidence to support a dopaminergic etiology for dystonia comes from the study of dopamine transporter deficiency syndrome.

**Dopamine Transporter Deficiency Syndrome**

Dopamine transporter deficiency syndrome is an autosomal recessive condition caused by loss-of-function mutations in the \textit{DAT} gene.\textsuperscript{128,129} DAT is a transmembrane protein exclusively expressed in dopaminergic neurons, in which it mediates the reuptake of dopamine into presynaptic terminals after synaptic transmission. This rapid recycling of neurotransmitter is crucial to synaptic function, as it replenishes dopamine stores in the presynaptic terminal and prevents desensitization of the postsynaptic terminal. Children presented in infancy with either hyperkinesia, parkinsonism, or a mixed hyperkinetic and hypokinetic movement disorder. Some individuals had previously been misdiagnosed with cerebral palsy. During childhood they developed severe dystonia-parkinsonism associated with an eye movement disorder and pyramidal tract features. Investigations revealed raised ratios of homovanillic acid to 5-hydroxyindoleacetic acid in cerebrospinal fluid. DAT SCAN imaging in 1 patient showed complete loss of dopamine transporter activity in the basal nuclei, although a trial of l-dopa had no effect on either the patient’s symptoms or CSF parameters.

**Common Themes in Dystonia Molecular Pathways**

The increasing knowledge of proteins whose mutant forms cause dystonia has implicated a large number of neurobiological pathways that lead to dystonic movements. A number of themes have emerged that have been identified in this review—from abnormal transcription and cell cycle because of the nuclear effects of dystonia genes to ER dysfunction and synaptic abnormalities. It is right to seek common pathways that may represent targets for therapeutic strategies for this group of incurable movement disorders. However, it may also lead to oversimplification in the search for unifying mechanisms. Most cases of dystonia are primary and not associated with neuronal death. Thus, the pathogenic mechanisms may be subtle and only cause relatively mild defects in the relevant pathways, leading to abnormal processing of the motor command within the CNS.\textsuperscript{70}

There is increasing awareness of the role of abnormal inhibition and plasticity affecting sensorimotor pathways in dystonia.\textsuperscript{1} This may be a template laid down in early life, supporting the view that primary dystonia is a neurodevelopmental circuit disorder. In support of this are the developmental patterns of expression of the best-studied primary dystonia gene products: torsinA and THAP1. TorsinA expression in the mouse was highest during prenatal and early postnatal development, particularly in the cortex, striatum, thalamus, and cerebellum.\textsuperscript{130} In the human brain, torsinA protein is detectable at 4–8 weeks postnatally in the cerebellum (Purkinje cells), substantia nigra hippocampus, and basal ganglia.\textsuperscript{131} Similarly, THAP1 is expressed in the rat in early development, particularly in the cerebellum (Purkinje cells), cortical pyramidal neurons, relay neurons in the thalamus, medium and cholinergic striatal neurons, dopaminergic substantia nigra neurons, and hippocampal neurons.\textsuperscript{132} This developmentally regulated expression of 2 dystonia-associated proteins suggests a role in terminal regulation and establishment of key circuits involved in motor control. The themes identified in this review would have a significant effect on neurodevelopment in terms of altered transcription of key genes at the nuclear level, protein processing and trafficking through the ER, or altered ER stress response in important periods of neural cell differentiation, through to abnormal synaptic function affecting neurotransmission or synaptic plasticity. Any or all of the above mechanisms could lead to abnormal patterns or responsiveness of sensorimotor circuits, leading to a susceptibility to developing an abnormal “dystonic state.”

It may be that with the increasing number of DYT loci and better understanding of recently identified genes encoding CIZ1, GNAL, and ANO3, the key cellular pathogenetic mechanisms involved in the genetics of dystonic movement will become clearer.

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