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Editors

Proteostasis and Disease

From Basic Mechanisms to Clinics

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Preface

Protein homeostasis (proteostasis) is critical for maintaining all cellular functions. Multiple mechanisms are interconnected to preserve the equilibrium of proteins, starting with their synthesis, followed by the performance of their various functions, and finishing with their degradation. When one or more of those mechanisms fail, the functional capacity of the cell is affected, resulting in diverse pathologies depending on the cell type or the affected pathway. Post-translational modifications including phosphorylation, acetylation, or modifications by members of the ubiquitin family play an important role in maintaining protein equilibrium and are indispensable to maintain cellular health and ability to respond to stress. In this book, the focus is on the post-translational modifications by ubiquitin and ubiquitin-like proteins (UbLs). Ubiquitin serves as a signal to control the activation of multiple intracellular signaling pathways, such as cell proliferation by altering the activity of crucial cellular factors (cyclins, JunB, and p53). Changes to UbL-mediated regulation can lead to gain or loss of function of multiple pathways. To address dysregulation of activity or stability mediated by UbLs, most current therapies target the enzymes necessary for adding UbLs to substrates (known as E1 activators, E2 conjugases, or E3 ligases) or the demodifying isopeptidase enzymes that cleave and recycle UbLs.

Chapters in this book are dedicated to the various processes and diseases caused or influenced by proteostasis disequilibrium, such as cancer and neurodegeneration, as well as infectious, developmental, and rare diseases. Finally, the effect of diet on proteostasis modulation is addressed.

Proteostasis disequilibrium in cancer occurs in some of the most aggressive tumors, such as those seen in pancreas, lung, and prostate cancer, but also some of the most common hematologic disorders like acute myeloid leukemia, multiple myeloma, or mantle cell lymphoma. Historically, the first mechanisms studied to understand protein equilibrium were intracellular proteolytic pathways such as the ubiquitin-proteasome systems (UPS) and the autophagy-lysosome (ALS) pathway. For this reason, the first inhibitors developed were directed at the proteasome or autophagy. Bortezomib, ixazomib, and carfilzomib are examples of proteasome inhibitors that have been approved for clinical use, in all cases for multiple myeloma. Whether other cancer types respond to these therapies is under investigation. Furthermore, natural or acquired resistance was observed in some patients. While the UPS is an effective target for drug development, a better understanding of

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TRIM E3 Ubiquitin Ligases in Rare Genetic Disorders

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Germana Meroni

Abstract

The TRIM family comprises proteins characterized by the presence of the tripartite motif composed of a RING domain, one or two B-box domains and a coiled-coil region. The TRIM shared domain structure underscores a common biochemical function as E3 ligase within the ubiquitination cascade. The TRIM proteins represent one of the largest E3 ligase families counting in human more than 70 members. These proteins are implicated in a plethora of cellular processes such as apoptosis, cell cycle regulation, muscular physiology, and innate immune response. Consistently, their alteration results in several pathological conditions emphasizing their medical relevance. Here, the genetic and pathogenetic mechanisms of rare disorders directly caused by mutations in TRIM genes will be reviewed. These diseases fall into different pathological areas, from malformation birth defects due to developmental abnormalities, to neurological disorders and progressive teenage neuromuscular disorders. In many instances, TRIM E3 ligases act on several substrates thus exerting pleiotropic activities: the need of unraveling disease-specific TRIM pathways for a precise targeting therapy avoiding dramatic side effects will be discussed.

Keywords

Tripartite motif, TRIM · RING domain · Ubiquitination · E3 ubiquitin ligases · Rare genetic diseases

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14.1 Introduction

The Tripartite Motif (TRIM) family constitutes the largest subfamily of RING domain-containing proteins [1]. In addition to an N-terminal RING domain, these proteins share the presence of one or two additional Zn-binding domains named B-box (B-box 1 and B-box 2) and a coiled-coil region, hence the term Tripartite Motif (TRIM) or RBCC as acronym to indicate this family (Fig. 14.1a). C-terminal to the Tripartite Motif, the TRIM family members display different domains or domain compositions, which permit an extra subclassification of the family into at least nine groups (Fig. 14.1a) [2].

Possessing a RING domain, the majority of TRIM family members act as E3 ubiquitin ligases within the ubiquitination cascade [3, 4]. Ubiquitination is a posttranslational modification that consists in the covalent bond of Ubiquitin moiety(ies) usually on Lysine residues of the specific targets. This process is regulated through the intervention of a cascade of enzymes for: (1) the activation of the ubiquitin peptide

(E1 ubiquitin activating enzyme); (2) the conjugation of the activated ubiquitin (E2 ubiquitin conjugating enzyme); and (3) the specific transfer of the properly oriented ubiquitin peptide(s) on the substrate (E3 ubiquitin ligase enzyme) [5]. The topologies and distribution of ubiquitin or ubiquitin chains on the targets via the action of this cascade of enzymes determine the fate of the target itself [6] (Fig. 14.1b). Exerting this function, TRIM family members facilitate/catalyze the proper and correct transfer of the ubiquitin moiety to specific targets. In many instances TRIM-mediated ubiquitination of the substrate is meant for proteasome- or lysosome-mediated degradation but signals for the regulation of substrate activity or distribution can also be conveyed (Fig. 14.1c) [7]. Additionally, E3 ligase-independent biological roles of TRIM proteins have been proposed, e.g., RNA-binding [8].

As ubiquitination regulates the stability and activity of many, if not all, proteins, it is not surprising that TRIM family members are implicated in a variety of cellular processes, from transcription to apoptosis, from cell cycle regulation to signal transduction [4, 9]. This involvement, often associated with spatial and temporal specific expression, implicates TRIM proteins in many physiological processes and, following their alteration, in several pathological conditions. The pathologies in which TRIM proteins are implicated are manifold and frequently each TRIM family member can have pleiotropic functions. By regulating the stability of oncogenes and tumor suppressors, several TRIM proteins participate in neoplastic processes [10, 11]. Further, the majority of TRIM proteins are implicated in innate immunity pathways either as positive or negative regulators of the cellular response to invading pathogens, whether viruses or bacteria [12–14] and in autoimmune disorders [15]. In addition, Genome-Wide Association Studies (GWAS) highlighted the contribution of polymorphic variants of TRIM genes to the genetic susceptibility to multifactorial disorders.

The involvement in the pathologies above is addressed elsewhere; this chapter will focus on the direct implication of *TRIM* genes in Mendelian rare genetic diseases. Indeed, the possibility to exploit genomic massive sequencing of affected and non-affected individuals in families with inherited diseases boosted the identification of mutations in *TRIM* genes in several forms of genetic disorders. Here below, the state-of-the-art in this field will be summarized.

14.2 Genetic Diseases Caused by Mutations in Class I *TRIM* Genes

Subclass I TRIM family members are characterized by the presence of both B-box 1 and 2 within the tripartite motif and by a complex C-terminal portion that includes: a COS domain, employed by these proteins to associate with the microtubules [2], followed by a fibronectin type III repeat (FN3) and a PRY-SPRY domain (Fig. 14.1a). The members of this subgroup are mainly expressed during embryonic development and are involved in the definition of the midline in several developing structures. Accordingly, mutations in some of these genes are associated with pediatric pathologies caused by developmental malformations.

14.2.1 *TRIM18/MID1* and Opitz G/BBB Syndrome

The first *TRIM* gene to be associated with a genetic disease was *TRIM18*, most commonly dubbed *MID1* (from here onward, *MID1*), which is the gene responsible for the X-linked form of Opitz G/BBB Syndrome (XLOS; OMIM 300000). XLOS is a disorder characterized by defects in the development of embryonic midline structures and caused by loss-of-function (LOF) mutations in the *MID1* gene [16–18]. The most characteristic clinical features of XLOS are facial anomalies, which include ocular hypertelorism, broad nasal bridge, frontal bossing and cleft lip and/or palate, as well as laryngo-tracheo-esophageal abnormalities and hypospadias. Imperforate anus and congenital heart defects are also present [17]. Neurologically, XLOS patients may also present with developmental delay and intellectual

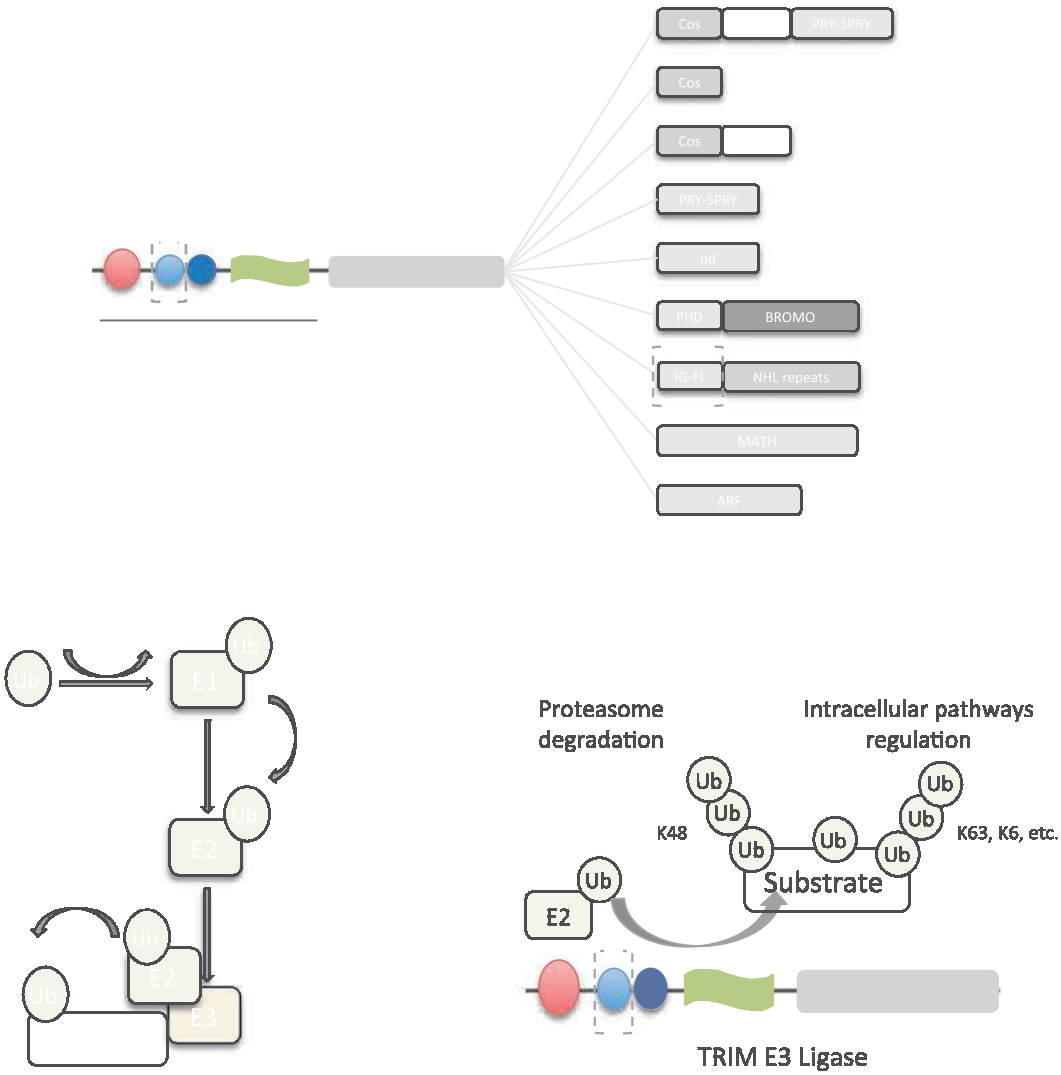

Fig. 14.1 TRIM E3 ubiquitin ligases. (**a**) Domain composition of the tripartite motif proteins and subclassification (class I to IX) of TRIM proteins based on C-terminal domains (right-hand side). Dashed brackets indicate that some of the TRIM members might not display the included domain. *R* RING domain, *B1* B-box 1 domain, *B2* B-box 2 domain, *CC* Coiled-coil region. (**b**) Ubiquitination cascade. *Ub* ubiquitin. (**c**) Scheme of TRIM E3 ligase activity

disabilities and brain abnormalities, such as cerebellar hypoplasia and agenesis of the corpus callosum. Cerebellar development defects are observed also in the murine *Mid1* knockout mouse model [19]. XLOS is a disorder presenting with highly variable expressivity.

More than 80 different mutations have been detected in *MID1* to date; they are represented by either complete/partial deletions of the gene or point mutations (missense, nonsense, splice site mutations), the latter especially within the C-terminal region [18]. In cells, MID1 is associated with the microtubular apparatus and missense and truncating mutations, at least those tested experimentally, result in decreased affinity of the protein for the microtubules suggesting that the LOF mechanism apply to the activity exerted on this cytoskeletal structure (Fig. 14.2a)

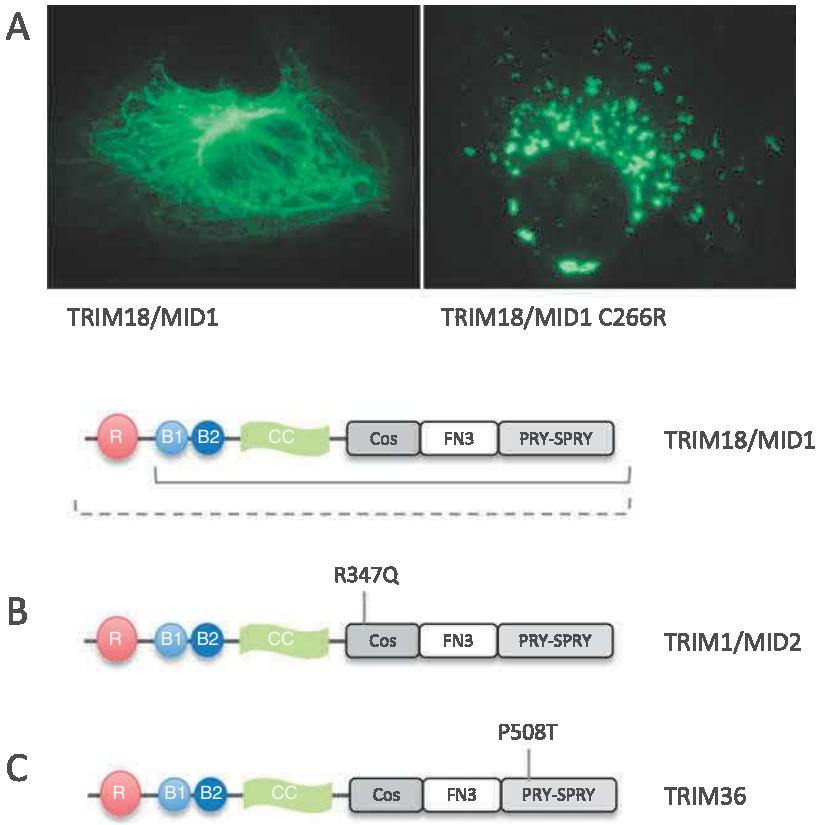

Fig. 14.2 Class I TRIM proteins in genetic diseases. (**a**) Microtubular localization of GFP-TRIM18/MID1 in Cos-7 cells (left-hand panel); decreased affinity for the microtubule of GFP-TRIM18/MID1 carrying a Cys266Arg XLOS mutation (right-hand panel). Bottom, schematic representation of TRIM18/MID1 domain composition; dashed line indicates the presence of XLOS deletions encompassing the entire gene while full line indicates the region where point mutations have been detected. (**b**) Schematic representation of TRIM1/MID2 domain composition and the nature and position of the COS domain missense mutation found in MRX101 patients. (**c**) Schematic representation of TRIM36 domain composition and the nature and position of the PRY-SPRY missense mutation found in anencephaly patients

[20, 21]. Interestingly, point mutations are distributed along the entire length of the protein with the exception of the RING domain, which is involved only when complete or large deletions/duplications are concerned. This may suggest that RING mutations, if occurring, can exert a different role with possible implication in different pathological conditions (Fig. 14.2a).

Since the identification of *MID1* as the gene implicated in XLOS, several biochemical and biological findings have been reported. Despite this, the pathogenesis of the disease is still enigmatic. Biochemically, in vitro ubiquitin E3 ligase activity of MID1 in cooperation with several E2 conjugating enzymes has been described [22, 23]. In biological context, the first reported target of MID1 E3 activity was the catalytic subunit of serine/threonine protein phosphatase 2A (PPP2CA) [24]. MID1 directly interacts, through the B-box 1 domain, with Alpha4 (α4) that is one of the atypical regulatory subunits of PPP2CA driving the latter to ubiquitin-mediated proteasomal degradation [24–27]. Later on, α4 was also reported to be a MID1 substrate [28, 29] and the mechanism of self-regulation of the MID1/α4/PP2Ac complex involves a series of ubiquitination and dephosphorylation events that have been long studied but still remain to be

completely unraveled. Whatever the mechanism, the MID1/α4/PP2Ac complex influences PP2Ac stability [30]. Altered PP2Ac activity affects mTORC1 complex formation and signaling [31] and, further, MID1 assembles with factors involved in mRNA transport and protein translation [30, 32]. This pathway can play a significant role in the pathogenesis of XLOS although the underlying mechanisms are still unraveled.

Another signaling pathway in which MID1 was reported to be involved is the Sonic Hedgehog (Shh) pathway. MID1 leads, in a direct or indirect way, to the proteolytic cleavage of the kinase Fu [33] and controls the nucleus–cytoplasmic shuttling of the Shh target GLI3 [34]. Although the mechanism of action of MID1 in this pathway is still poorly investigated, the involvement of midline structure definition in embryonic development can be more easily interpreted through alteration of this pathway. Indeed, as demonstrated during very early stage chicken development, Mid1 and Shh have cross-repressive relationship [35] and the two genes in human, *MID1* and *SHH*, when mutated result in opposite phenotypes: an enlargement (XLOS) versus a narrowing (Holoprocencephaly, OMIM 142945) of the midline, respectively.

Another mechanism in which MID1 is likely involved is the control of cytokinesis. One of the assessed substrates, BRAF35, has a role during neuronal differentiation [36] and cytokinesis [37, 38] and MID1 is associated with the midbody at the conclusion of the mitosis [20, 39]. MID1-dependent BRAF35 ubiquitination might regulate its subcellular localization [40]. MID1 and BRAF35 are both expressed in proliferative compartments during embryonic development and their interaction, either implicated in the repression of neuronal genes or in the regulation of cell division/cell cycle, or both, can be relevant for the pathogenesis of the Opitz syndrome. Recently, MID1 has been found in association with the microtubule-organizing and midbody protein Astrin, consistently MID1 silencing leads to cytokinetic defects [39, 41].

It is possible that MID1 implication in mTORC1 and Shh pathways and in the process of cytokinesis might be different sides of the same coin, which warrants further investigation. Interestingly, the implication of MID1 in cytokinesis through the Astrin protein involves principally the closest TRIM18/MID1 paralogue, TRIM1/MID2, addressed in the following section.

14.2.2 *TRIM1/MID2* and X-Linked Intellectual Disability

TRIM1, also known as *MID2* (from here onward, *MID2*), is the closest *TRIM18/MID1* paralogue and is located as *MID1* on the X chromosome in human and mouse [42, 43]. The two gene products interact forming a microtubular-associated complex but the precise stoichiometry and dynamics of the heteromeric complex is presently unknown [27]. Despite being in complex with MID1, MID2 has never been found mutated in XLOS patients. However, it is implicated in a form of X-linked recessive intellectual disability (Mental Retardation, X-Linked 101—MRX101, OMIM 300928). Out of 11 affected males of a large Indian family, 6 were genetically evaluated and a missense mutation in *MID2* was found to segregate with the pathological condition. The mutation was found in the heterozygous state in carrier females who are unaffected [44]. The onset of the signs occurs at birth and this intellectual pathology is characterized by global developmental delay, impaired cognition, poor or lack of speech, and in some patients the occurrence of seizures. Some affected individuals present with long face and large ears, squint and strabismus, underscoring a broader developmental involvement [44].

The missense mutation segregating in this family, Arg347Gln, involves a very conserved amino acid in the COS domain of MID2 (Fig. 14.2b). This domain is implicated in the association of the protein with the microtubules and, consistently, a mimic of the mutated protein does not present the classical filamentous distribution but is found in cytoplasmic bodies and aggregates [44]. MID2 controls the abovementioned Astrin through direct E3 ligase activity regulating its protein levels on exit from the cell cycle [39]. The Arg347Gln MID2 is still

able to localize at the midbody, bind and ubiquitinate Astrin, thus depriving of pathogenetic hints in MRX101 [39].

In the pathological conditions described so far, the same cellular consequence of the mutant protein is thus observed for both *MID2* and *MID1*. Opitz syndrome is also characterized by the presence of intellectual disability but whether the onset of this sign in the two conditions is linked to the formation of MID1-MID2 complex function is still to be determined.

14.2.3 *TRIM36* and Anencephaly

Another member of the TRIM subgroup I implicated in a birth defect is *TRIM36*, which maps on chromosome 5q22.3 and is responsible for anencephaly, an extreme form of neural tube defect (OMIM 206500). Anencephaly is characterized by the absence of cranial vault and brain tissues in the fetus and is incompatible with life. Alteration of *TRIM36* was detected in a 20-week-old fetus, born to consanguineous parents: a homozygous Pro508Thr missense mutation identified in the PRY-SPRY domain (Fig. 14.2c). The mutation, detected by whole-exome sequencing, segregated with the disorder in the family [45].

Exogenous expression of the mutated form of TRIM36 leads to disrupted microtubules, disorganized spindles, loosely arranged chromosomes, abnormal cytokinesis, decreased cell proliferation, and increased apoptosis compared to the wild-type protein in different cell lines [45]. Similar results were obtained by cellular knockdown of TRIM36 using siRNA suggesting a complex mechanism, involving precise dosage of the wild-type product instead of a classic loss-of-function usually presented by recessive disorders, inducing proliferation defects during neurulation underlying this condition [45]. The function of TRIM36 during development was studied in Xenopus, where the frog orthologue is implicated in cortical rotation and consequently in dorsal axis formation [46]. How this function is related to neural tube closure is it not known; however, the microtubular apparatus is crucial for the morphogenetic movements necessary for neural tube closure and the xMid1/xMid2 complex was also found implicated in neural tube closure in the same species [47, 48]. So far, no data on TRIM36 E3 ubiquitin ligase activity and possible substrates are known.

Although the mechanisms underpinning the pathogenesis of the three disorders described above are not defined yet, it is fascinating that members of subclass I are all implicated in developmental disorders, often related to central nervous system and midline structures. The remaining 3 members (TRIM9, 46, and 67) have not been so far associated to any genetic disorders; however, they are all microtubular proteins some of which have roles in midline and CNS development in mouse models and have been associated with cerebellar disorders [49–52]. It may be interesting to evaluate their possible cooperation, likely through direct interaction, in some of these processes.

14.3 Genetic Diseases Caused by Mutations in Class VII *TRIM* Genes

Class VII TRIM family members are defined by the presence of a C-terminal 6-bladed β-propeller-like structure composed of 6 NHL (NCL-1, HT2A, LIN41) repeats, in some members preceded by a Filamin-type immunoglobulin domain (IGFLMN) (Fig. 14.1a) [1, 43]. Within their tripartite motif, some members present both B-box 1 and B-box 2 whereas some others have retained only B-box 2. Members of this group have been implicated in genetic diseases that are illustrated here below.

14.3.1 *TRIM2* and Charcot–Marie–Tooth

Charcot–Marie–Tooth (CMT) disease represents a subgroup of inherited peripheral neuropathies characterized by symptoms typically starting from the feet and progressing in a distal to proximal pattern. CMT patients present with muscle weakness and atrophy in the lower limbs resulting in gait difficulties and can develop skeletal abnormalities (e.g., pes cavus, scoliosis). The

onset of the clinical signs occurs in the first 2 decades of life with the tendency to start early in childhood and to show a more severe course and complex phenotype in the autosomal recessive forms. Type 2 forms, also known as axonal CMT, contrary to type 1, are characterized by normal or moderately slow nerve conduction velocity [53]. To date, more than 50 genes are implicated in CMT forms.

Mutations in *TRIM2* on chromosome 4q31.3 have been found in the axonal type CMT type 2R (OMIM 615490), a recessive condition characterized by onset in early childhood. One of the patients, born to consanguineous parents, carries a homozygous Asp667Ala missense variant localized just before the fifth NHL repeat and leading to a change of a highly conserved residue in evolution [54] (Fig. 14.3a). In the other reported patient, a compound heterozygous mutation was identified: Glu227Val, within the tripartite coiled-coil domain, and a 1 bp deletion leading to a frameshift and a premature truncation of the TRIM2 protein, Lys567Argfs7* at the NHL domain level [55] (Fig. 14.3a). In the case of the variants detected in the compound heterozygote, the patient's fibroblasts showed that TRIM2 is highly unstable indicating loss-of-function as pathogenetic mechanisms. The patients with *TRIM2* mutations present peripheral axonal neuropathy, muscle weakness and atrophy, slow motor nerve conduction velocities, loss of myelinated fibers, accumulation of neurofilaments within axons, and swollen myelinated fibers. In the more severely affected patient, deceased before 3 years of age, respiratory insufficiency, tracheomalacia, and vocal cord paralysis were also present [54].

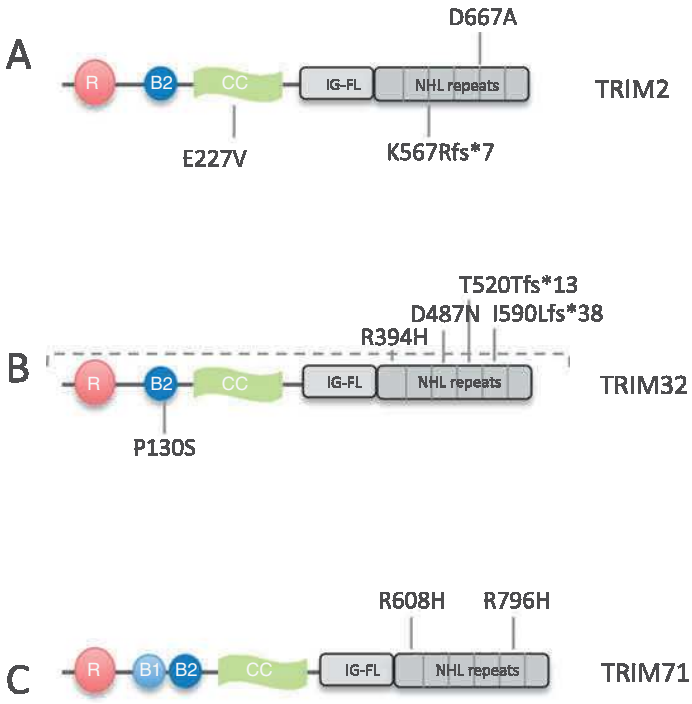

Fig. 14.3 Class VII TRIM proteins in genetic diseases. (**a**) Schematic representation of TRIM2 domain composition with the homozygous mutation found in one CMT2R patient (top) and the compound heterozygous mutations found in another patient (bottom). (**b**) TRIM32 domain structure and position of some of the point mutations found in LGMD8R patients (top); dashed line indicates the presence of LGMD8R-associated deletions encompassing the entire gene. The single mutation found in BBS11 patients is shown (bottom). (**c**) Schematic representation of TRIM71 domain structure and the nature and position of the NHL missense mutation found in Congenital Hydrocephalus patients

The *Trim2* gene trapped (null) knockout mouse line shows gait ataxia and neurodegeneration accompanied by axonal swelling and development of neurofilament aggregates, a phenotype that recapitulates CMT although, differently from human, implicates not only the peripheral but also the central nervous system [56]. This axonopathy is characterized by disorganized intermediate neurofilaments and accumulation of NF-L in axons followed by progressive neurodegeneration [56]. Indeed, NF-L was found to be a substrate of TRIM2 ubiquitin ligase activity explaining its accumulation in *Trim2* knockout mouse axons and possibly elucidating the axonal defects in *TRIM2*-mutated CMT patients [56].

14.3.2 *TRIM32* and Limb Girdle Muscular Dystrophy

TRIM32 is an NHL-containing family member mutated in patients with an autosomal recessive form of Limb Girdle Muscular Dystrophy (LGMD Type 8R, formerly known as 2H, OMIM 254110) [57]. The clinical manifestations in LGMD8R range from mild muscular impairment to severe muscle weakness in wheelchair-bound patients. This disorder is heterogeneous; indeed some of the same TRIM32 LGMD8R mutations can be associated with a more severe form of muscular disease, Sarcotubular Myopathy (STM) [57]. Histological analyses of LGMD8R patients' muscles revealed the presence of rounded fibers with internal nuclei, presence of atrophic and hypertrophic fibers, degenerated myofibrils and Z-lines, presence of enlarged vacuoles [58].

All mutations associated with LGMD8R and STM are either point mutations within the NHL region, leading to missense or premature truncation of the protein, or deletions of large portions of the gene [59] (Fig. 14.3b). Reproduction of either complete LOF (null *Trim32*) or of an NHL-contained missense mutation (Asp489Asn, corresponding to the Asp487Asn human mutation) in murine models recapitulate the progression of the human disease as described above [60, 61]. Further, these models revealed the presence of disorganized sarcomeres and autophagic double-membrane vacuoles. In addition, a neurological component with altered neurofilaments was identified. The murine models, assessing the instability of the missense mutation, indicate LOF as a mechanism underlying the LGMD8R. While this is intuitive with large gene deletions that completely abolish TRIM32 activity, it is likely that the NHL-contained point mutations will alter the binding to the target substrates. As for the latter, several TRIM32 E3 ligase activity substrates have been reported to date. Some of them are clearly related to the muscular fiber structure and physiology (actin, desmin, dysbindin) whereas others are important cell cycle regulators (c-Myc, p53) [11, 59]. The two different groups of targets indicate that TRIM32 might be implicated in both muscle atrophy and regeneration, with implication in the biology of satellite cells [62, 63].

Interestingly, a missense mutation in TRIM32 B-box 2 domain (Pro130Ser) has been associated with a form of Bardet–Biedl Syndrome (BBS11; OMIM 209900), a ciliopathy characterized by multi-organ abnormalities clinically diverse from muscular dystrophies [64] (Fig. 14.3b). The role of TRIM32 at the primary cilium is currently unclear but this finding further highlights the pleiotropic role of TRIM32 and of its specific mutations.

14.3.3 *TRIM71* and Congenital Hydrocephalus

Congenital Hydrocephalus (CH) is a condition characterized by enlarged brain ventricles, progressive distension of the cerebral ventricular system arising from failed cerebrospinal fluid passage and homeostasis [65]. The genetics underlying this condition is difficult and still unclear. Recently, a large cohort of CH probands was screened for de novo mutations and among the genes mutated in these patients, *TRIM71* was found [66]. The patients with *TRIM71* mutations exhibited neurodevelopmental delay and epilepsy, and in one case open schizencephalic clefts, in addition to CH.

Two heterozygous missense mutations were found in three CH patients: p.Arg608His and p. Arg796His [66]. Interestingly, these variants are

at homologous positions in different NHL repeats where arginine residues are evolutionary conserved and histidine substitution are predicted to alter folding of the blade of the propeller domain (Fig. 14.3c). The recurrent mutation in two patients and the analogy between the two missense variants detected in heterozygous state suggest that a gain-of-function mechanism underlies the congenital defect in these patients. Indeed, *Trim71* null mouse line exhibits a different and worse phenotype, exencephaly and embryonic lethality [67]. Besides the E3 ligase activity [68], TRIM71 also mediates posttranscriptional silencing of mRNAs through direct interactions with UTRs of target genes via the NHL domain and the latter might be hampered in these patients [69]. Interestingly, *Trim71* is expressed in the developing neuroepithelium and ventricular zone and in particular in the ciliated neuroepithelium as are the other genes found mutated in CH. This suggests a common physiological/embryological function, the alteration of which leads to Congenital Hydorcephalus.

The descriptions above highlighted a common theme among class VII TRIM members: the disease-causing mutations affect mainly the NHL domain. In several instances, this domain is necessary to bind the target substrates of TRIM-NHL E3 ligase activity. However, the NHL domain mediates both protein–protein and protein–RNA interactions. The *Drosophila* TRIM-NHL member BRAT binds single-stranded RNA and the mammalian members of this group are involved in miRNA binding through the top surface of the NHL β-propeller structure [8]. It will be interesting to further investigate these properties and the relationship between TRIM-NHL-mediated RNA regulation and ubiquitin E3 ligase activity.

14.4 Genetic Diseases Caused by Mutations in Other Classes of *TRIM* Genes

Class I and VII list several genes associated with genetic disorders, mainly with pediatric and teenage onset, as these subclasses includes genes with strong and often specific temporo-spatial expression during development [1]. Additional rare genetic diseases are caused by mutations in *TRIM* genes from other subgroups and they are briefly described here below.

14.4.1 *TRIM8* and Epileptic Encephalopathy

Early-Onset Epileptic Encephalopathy (EOEE) is a heterogeneous group of neurodevelopmental disorders characterized by intractable pharmacoresistant seizures and unfavorable developmental outcome. Many forms are described and variants in several genes have been reported [70]. Up to now, chromosome 10q24.32 located *TRIM8* was reported mutated in 6 EOEE diagnosed patients. In addition to epileptic episodes, the patients present with growth delay, dysmorphic facial appearance, and skeletal malformations. They often present with difficulties or complete lack of meaningful words and social smiling.

All patients carry de novo truncating mutation within exon 6 of TRIM8. The variants are represented either by insertion or deletion of 1 bp causing frameshifts or by nonsense substitution (Fig. 14.4a) [70–72]. All the variants leave an intact tripartite motif, the stability of which is not investigated, and lack the C-terminal portion that in TRIM8, belonging to class V, does not present any homology with other domains and whose function is not known (Fig. 14.1a) [2, 43]. If stable the truncated TRIM8 product might function in a dominant negative manner thus impacting on the function of wild-type TRIM8.

TRIM8 plays divergent roles in many biological processes and signaling pathways. TRIM8 is a nuclear protein that exerts either a tumor suppressor action, playing a prominent role in regulating p53 tumor suppressor activity, or an oncogene function, through the positive regulation of the NF-κB pathway. Moreover, TRIM8 is aberrantly expressed in glioblastoma and its expression suppresses cell growth and induces a significant reduction of clonogenic potential in glioblastoma cell lines [73, 74]. How these

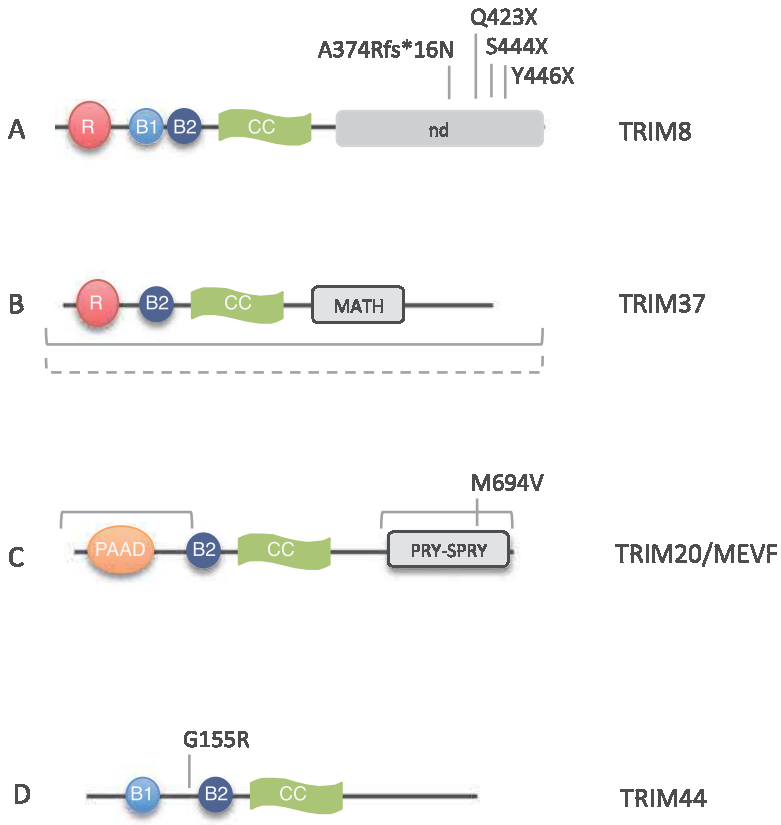

Fig. 14.4 Other classes of TRIM proteins in genetic diseases. (**a**) Schematic representation of TRIM8 domain composition and position of some of the mutations found in EOEE patients. (**b**) TRIM37 domain structure; dashed line indicates the presence of MULIBREY-associated deletions encompassing the entire gene while full line indicates the presence of point mutations along the entire length of the product. (**c**) Schematic representation of TRIM20 domain structure and the positions of the missense mutations found in FMF patients (continuous lines). The most common mutation is also indicated. (**d**) TRIM44 domain structure and position and nature of the missense mutation detected in aniridia patients

findings can help in understanding the pathogenesis of EOEE is still unknown but, in this respect, involvement of TRIM8 in the regulation of stemness in neural tissues is intriguing.

14.4.2 *TRIM37* and MULIBREY Nanism

MULIBREY (MUscle, LIver, BRain, and EYes) nanism (OMIM 253250) is caused by homozygous or compound heterozygous mutation in the *TRIM37* gene, which encodes a peroxisomal and nuclear protein [75, 76]. Consistently with a ubiquitous expression of the *TRIM37* gene, this disorder affects several tissues in particular of mesodermal origin. MULIBREY nanism patients present with severe pre- and postnatal growth impairment including occasional progressive cardiomyopathy, characteristic facial features, failure of sexual maturation, insulin resistance with type 2 diabetes, and an increased risk for tumors. Patients often display short stature and muscular hypotonia, and dysmorphic facial features. The neurological involvement concerns large cerebral ventricles and cisternae but normal intelligence is observed. Hepatomegaly and metabolic alterations are also associated with the disease as well as both male and female infertility [77].

To date, more than 20 *TRIM37* mutations have been found associated with this disorder. These mutations are mainly represented by frameshift, splice site alteration and stop codon, all leading to premature truncation of the protein product along its entire length, even proximal to the N-terminus. Intragenic rearrangements and complete gene deletion are also reported, likely promoted by repetitive Alu elements numerous in this genomic region [77]. Most patients are from Finland where a major mutation due to founder effect is the most represented [75]. A *Trim37* knockout mouse line

recapitulates several features of the human disease suggesting a loss-of-function as pathogenetic mechanism [78].

The product of the *TRIM37* gene is unique within the TRIM family as it presents the MATH domain C-terminal to the tripartite motif (Fig. 14.4b). This 133-residue-domain is involved in the formation of oligomeric structures and is shared with the TRAF E3 ubiquitin ligases [79]. Interestingly, like TRAF proteins also TRIM37 is involved in innate immunity and antiviral defense. TRIM37 ubiquitin ligase activity was demonstrated against PEX5 and K646 for the regulation of peroxisomal import suggesting MULIBREY as a peroxisomal biogenesis disorder [80]. Recently, TRIM37 has been found implicated in centriole formation through its E3 ligase activity and this can also open novel avenues to understand MULIBREY nanism pathogenesis [81].

14.4.3 RING-less TRIM Family Members and Genetic Diseases

Two rare genetic disorders are caused by mutations in non-orthodox members of the TRIM family in that they do not possess a RING domain, although evolutionarily they are closely related to the classical TRIM proteins [43]

14.4.3.1 *TRIM20* and Familial Mediterranean Fever

In the case of TRIM20, better known as MEVF or Pyrin, the position of the RING domain is taken by the PAAD or PYRIN domain (Fig. 14.4c). Proteins containing a Pyrin domain are frequently involved in inflammation and innate immunity processes and include intracellular pathogen receptors. Therefore, it is not surprising that mutations in this gene, mainly expressed in blood cells, cause Familial Mediterranean Fever (OMIM 249100, AR; 134,610, AD) [82, 83]. Familial Mediterranean fever (FMF) is an autosomal disorder with onset in childhood or adolescence and characterized by recurrent attacks of fever lasting 24–48 h, which may occur several times per week to once per year, and inflammation in the peritoneum, synovium, or pleura. This disorder is prevalent in Arabic, Turkish, Armenian, and Sephardic Jewish populations, hence the name, and the ancestral M694 V mutation account for the majority of the mutated alleles. The pathogenetic variants fall into two clusters, one at the N-terminus and one within the PRY-SPRY domain and depending on whether monoallelic or biallelic mutations are detected they are involved in the dominant or recessive form, respectively [84]. They are mainly missense mutations suggesting that the periodic nature of inflammatory attacks in FMF is consistent with a protein that functions adequately at steady state but decompensates under stress. *TRIM20* product, like several innate immunity TRIM family members, senses perturbation in intracellular homeostasis leading to the activation of the inflammasome complex and downstream activation of pro-inflammatory pathways [85, 86].

14.4.3.2 *TRIM44* and Aniridia

TRIM44 does not present a domain N-terminal to the B-box 1 and its incomplete tripartite motif is composed of B-box 1 and B-box 2, unusually separated by a long stretch of acidic residues, and coiled-coil region (Fig. 14.4d). Heterozygous mutations in the *TRIM44* gene have been detected in the autosomal dominant form of Aniridia, AN3 (OMIM 617142). In a 4-generation-family with affected individuals showing decreased progressive visual acuity, bilateral defects of the iris, cataract, and, in some patients, glaucoma, a missense mutation in the *TRIM44* gene leading to Gly155Arg substitution segregated with the disease [87]. This mutation has been shown to affect PAX6 expression, a master gene for eye field development and whose mutations are considered the major cause of aniridia [87]. TRIM44 has been shown involved in tumorigenesis through the activation of the AKT/mTOR pathway [88] and molecularly it has been shown to stabilize another TRIM family member, TRIM17, possibly through a USP-like function [89]. How these processes underlie the control of PAX6 in aniridia is at present not known.

14.5 Conclusions and Future Perspectives

The number of TRIM family members implicated in rare genetic diseases is growing but unfortunately our knowledge of the pathogenesis is not increasing at the same pace. Even when findings are reported, the mechanisms are not dissected at a level needed to clearly understand the molecular pathogenetic processes involved and to propose a therapeutic approach. Gene therapy, sometimes offered for LOF genetic diseases would not be advisable in many of the cases addressed above due to the lack of the necessary therapeutic window in disorders very often arising during embryonic development. The design of targeting approaches requires more information on the pathogenetic role of genetic disease-TRIM protein and many limitations should be overcome.

First, the E3 ligase activity should be correlated more strictly to the pathogenetic process. In many instances, we lack information on the natural substrates and even when targets are known, the topology of the ubiquitin modification and hence the fate of the target and the ubiquitination machinery involved are not assessed. Further complicating matters, for some TRIM proteins, several substrates are reported and in addition to their role in the genetic disorder, they are shown to be involved in other physiological (immunity, miRNA processing) and pathological (cancer, HIV infection) processes. These pleiotropic roles are currently representing an obstacle to the design of specific therapeutic approaches targeting these molecules but avoiding dramatic undesirable effects. In addition, other biochemical functions, associated or not with the E3 ligase activity, might play a role in the disease pathogenesis.

In the particular case of TRIM proteins, an issue still to be addressed properly is their homo- and hetero-interaction dynamics [90]. Through the formation of different homo- and hetero-complexes, the details of which are completely lacking, these proteins can diversify their cellular and physiological roles. Besides, also the pathogenetic variants can play a role in this dynamics thus impacting differently on the diseases.

In addition, lack of solved TRIM protein structures is a hurdle toward therapy. Despite the importance of TRIM E3 proteins in many diseases, the published structures include a handful of single or tandem domains of TRIM proteins. Structural biology studies, tightly coupled with biochemical analyses, will provide additional information to further dissect TRIM complex stoichiometry, the E3–E2 interactions, and the structural basis for substrate selection in physiological and pathological conditions.

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