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TRIM-NHL proteins in development and disease

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ABSTRACT

TRIM-NHL proteins are key regulators of developmental transitions, for example promoting differentiation, while inhibiting cell growth and proliferation, in stem and progenitor cells. Abnormalities in these proteins have been also associated with human diseases, particularly affecting muscular and neuronal functions, making them potential targets for therapeutic intervention. The purpose of this review is to provide a systematic and comprehensive summary on the most studied TRIM-NHL proteins, highlighting examples where connections were established between structural features, molecular functions and biological outcomes.

1. The TRIM-NHL proteins: domains and associated molecular functions

The TRIpartite Motif (TRIM) is constituted by three main domains: a Really Interesting New Gene (RING) finger, one or, more commonly, two B-Box-type zinc fingers (BB1 and BB2) and a Coiled-Coil (CC) (Fig. 1A). The TRIM is always located towards the N-terminus of the protein and the order of, as well as the spacing between individual domains, is highly conserved [1]. TRIM-NHL proteins (referred to as "C-VII" subfamily) represent one of the nine subfamilies of TRIM proteins (from "C-I" to "C-IX"), whose classification is based on the presence of an additional domain, NHL, which is positioned C-terminally from the TRIM [2]. The NHL stands for NCL-1/HT2A/LIN-41, the proteins in which the domain was initially described. A filamin (immunoglobulin) domain is often found immediately before the NHL domain [3] (Fig. 1A). Thus, the TRIM-NHL proteins consist of several distinct domains, potentially endowing them with functional flexibility.

The RING domain is defined by a regular series of cysteine (Cys - C) and histidine (His - H) residues, which coordinate two zinc ions in a "cross-brace" fashion, where Cys in positions 1, 2, 5, 6 bind the first zinc ion and Cys and Hys residues in position 3, 4, 7, 8 bind the second one (Fig. 1B; [4–6]). Conserved Cys and His residues are located in the core of the domain and their binding to the zinc ions is essential for maintaining the domain structure [4,5]. The RING domain can act as an E3 ubiquitin ligase (Fig. 1C). On one hand, it directly interacts with an E2 conjugating enzyme, which receives the ubiquitin peptide from an E1 ubiquitin-activating enzyme. On the other hand, it associates with a protein substrate, bringing it to the proximity of E2. Notably, not all RING domains can act as E3 ubiquitin ligases. To possess E3 activity, a RING domain must include a proline immediately after the Cys residue in position 7 [7] and this residue is missing in nematode LIN-41 proteins [8]. Furthermore, untypical TRIM proteins – Brat and Wech – lack the RING domain, suggesting that the other domains can function independently of the RING domain.

The B-Boxes (BBs) are also zinc-binding motives that come in two flavors (type I and type II), presenting similar, although distinct, consensus sequences (Fig. 1B; [1,5,9]). The BBs resemble the RING, ZZ and U-box domains of E3 and E4 ubiquitin ligases, suggesting that the BBs may, in principle, either act as E3s *per se* or enhance the E3 RING domain activity [10]. Similarly to the RING domain, BBs also coordinate their two zinc ions in a "cross-brace" fashion [9–11]. Although the precise function of BBs remains to be demonstrated, they have been proposed, together with the CC domain, to provide the binding site for a substrate ubiquitinated *via* the RING domain [10].

The coiled-coil domain consists of roughly hundred amino acids, whose amino acid sequence is not conserved. Despite that, the secondary structure is usually partitioned into two or three coiled-coil motives, mainly constituted by α-helices that form a "rope-like" structure, stabilized by hydrophobic interactions, often mediated

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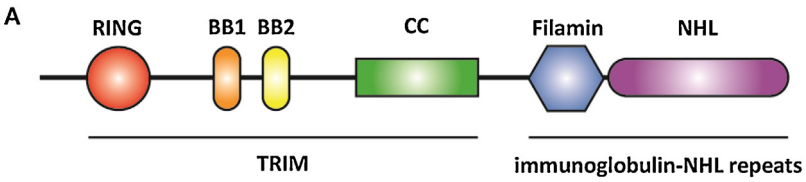

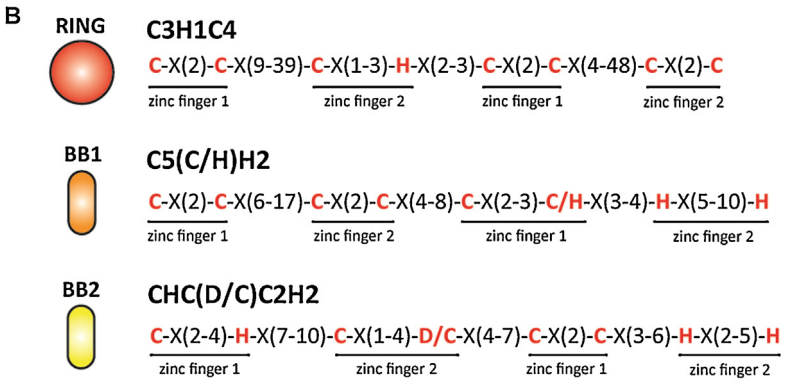

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Domain (protein)	Function	Substrate/interactor	Reference
RING (TRIM2)	Ubiquitination	NF-L	*Balastik et al., 2008*
RING (TRIM3)	Ubiquitination	p21	*Raheja et al., 2014*
RING (LIN-41)	Ubiquitination	Ago2	*Rybak et al., 2009*
BBs (Brat)	Protein-protein interaction	β-catenin/Armadillo	*Komori et al., 2014*
BBs (LIN-41)	Subcellular localization		*Chang et al., 2012*
BBs+CC [+NHL] (Wech)	Protein-protein interaction	Talin and ILK	*Löer et al., 2008*
CC (LIN-41)	Protein-protein interaction	Ago2	*Rybak et al., 2009*
CC (LIN-41)	Post-transcriptional regulation		*Chang et al., 2012*
CC+Filamin (LIN-41)	Post-transcriptional regulation	No mRNA binders	*Loedige et al., 2013*
NHL (Brat)	Post-transcriptional regulation	mRNAs	*Sonoda and Wharton, 2001*
NHL (Brat)	Post-transcriptional regulation	mRNAs	*Loedige et al., 2014*
NHL (TRIM2)	Protein-protein interaction	myosin V	*Ohkawa et al., 2001*
NHL (LIN-41)	Subcellular localization		*Chang et al., 2012*
NHL (LIN-41)	miRNA binding	miR302, miR290	*Chang et al., 2012*
NHL (LIN-41)	Post-transcriptional regulation	mRNAs	*Loedige et al., 2013*

Fig. 1. Functional domains of TRIM-NHL proteins. (A) Architecture of a typical TRIM-NHL protein. Composition of the TRIM (RING, BB1/2 and CC) and the immunoglobulin-NHL (Filamin and NHL) portions of the protein. The domains are aligned from the N- (left) to the C-terminus (right) as they normally occur in TRIM-NHL proteins. (B) Consensus zinc finger motives present in the TRIM domain. The types of motives are in bold, key residues of the sequences are in red: RING, BB1 and BB2. "C" stands for cysteine, "H" for histidine, "D" for aspartic acid and "X" for any amino acid. Numbers in brackets represent the range of a certain residue. (C) List of described molecular functions for specific domains of TRIM-NHL proteins. Specific substrates and/or associated proteins and RNAs are indicated. (For interpretation of the color information in this figure legend, the reader is referred to the web version of the article.)

by leucines [10,12]. The CC domain allows the formation of homo- or heterodimers, promotes the formation of protein complexes (*e.g.*, recruiting the substrate for ubiquitination) and can help to define certain subcellular compartments [1]. The precise function of this domain in TRIM-NHL proteins awaits clarification, but the domain has been implicated in mRNA regulation (Fig. 1C; [13]).

The filamin domain is often associated with *the NHL repeats* at the C-termini of TRIM-NHL proteins [3], potentially indicating a shared molecular function. The filamin domain exhibits a classic immunoglobulin-like structure, constituted by seven β-strands arranged in two antiparallel β-sheets [8,14], whose function, in the context of TRIM-NHL proteins, has been recently linked to mRNA regulation. Specifically, the filamin domain, together with the CC, was proposed to recruit proteins regulating mRNA translation [13].

The NHL domain consists of five or six repeats, of roughly forty residues each, and folds into a barrel-like β-propeller structure. The NHL repeats have been generally regarded as structural units involved in protein binding [15,16]. However, in TRIM-NHL proteins, one of the two propeller surfaces is highly positively charged and has been proposed to directly associate with the negatively charged RNA phosphate backbone (Fig. 1C; [17–19]). How the specificity of RNA binding is achieved remains an important problem for the future research.

Below, we discuss biological functions of most-studied TRIM-NHL family members, highlighting examples where their mutations have been linked to human diseases (Table 1).

2. Most studied family members: biological roles and associated human diseases

2.1. Brat, Mei-P26, NCL-1 and NHL-2

Several TRIM-NHL family members have been examined in model organisms (Fig. 2). The *Drosophila melanogaster* Brat is perhaps the most studied family member. The name *brat*, BRAin Tumor, comes from the larval *brat* phenotype [20]. In normal development, Brat controls the decision between differentiation and self-renewal in larval neuroblast lineages, which give rise to adult neurons. During asymmetric division of a neuroblast Brat, together with the transcription factor (TF) Prospero, segregates into one