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INSIGHT

MYRF: A Mysterious Membrane-Bound Transcription Factor Involved in Myelin Development and Human Diseases

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The myelin regulatory factor gene (*MYRF*) encodes a protein evolutionarily conserved from invertebrates to vertebrates, representing a novel type of membrane-bound transcriptional factor (MBTF). In vertebrates, MYRF protein is required for myelin development and maintenance [1, 2]. Unlike other oligodendrocyte-specific transcription factors, it is initially synthesized as a conserved type-II membrane protein, containing a N-terminal proline-rich (Pro) domain, a putative yeast Ndt80-like DNA binding domain (DBD), two nuclear localization signal motifs, an intramolecular chaperone auto-processing (ICA) domain, a transmembrane domain, and a function-unknown C-terminal conserved (C) domain (Fig 1). Among these conserved domains, the DBD directly binds to the promoters of target genes, the transmembrane domain anchors MYRF to the endoplasmic reticulum (ER) membrane, and the ICA domain drives the homo-trimerization of MYRF protein and subsequently induces its self-cleavage *via* its intrinsic peptidase activity [3, 4].

Once cleaved on the ER membrane, the N-terminal trimers of MYRF are released and translocate into the nucleus (Fig 1A, B). ChIP-Seq analyses have identified a consensus DNA binding motif, CTGGYAC [3]. Interestingly, the binding motifs of MYRF N-trimer contain two tandem copies of this consensus motif either in the same or in the opposite orientations, 3- or 7-bp apart [5]. The tandem binding sites theoretically increase the binding specificity of target genes and may explain why MYRF protein functions as a homo-trimer.

MYRF is Required for Oligodendrocyte Differentiation and Myelin Maintenance

MYRF was first discovered as a transcriptional factor essential for the formation of myelin sheaths [1], which are elaborated by oligodendrocytes (OLs) in the vertebrate CNS. During early development, OL differentiation is coordinated by a cohort of factors, including SOX10 and MYRF [5–7]. MYRF is selectively upregulated in OL cells at the onset of differentiation. Loss of *Myrf* in OPCs impairs myelin gene expression and abolishes myelin formation. Conversely, forced expression of MYRF induces the expression of myelin genes such as MBP and PLP [1]. In addition, *Myrf* is also indispensable for myelin maintenance in adult animals [2].

MYRF was recently reported to be a direct target gene of another differentiation factor, SOX10 [8]. During OL development, SOX10 activates the expression of both OPC genes and differentiation-related genes. Once induced by SOX10 at the beginning of OL differentiation, MYRF redirects SOX10 to myelin genes but restrains it from OPC genes simultaneously to ensure its strict target selection [5]. The forward and feedback regulatory loops between MYRF and SOX10 compose an important molecular circuit for myelin development and maintenance. Surprisingly, despite the importance of MYRF in myelin formation and maintenance, so far there are no apparent myelin-related human diseases associated with genetic mutations in this gene.

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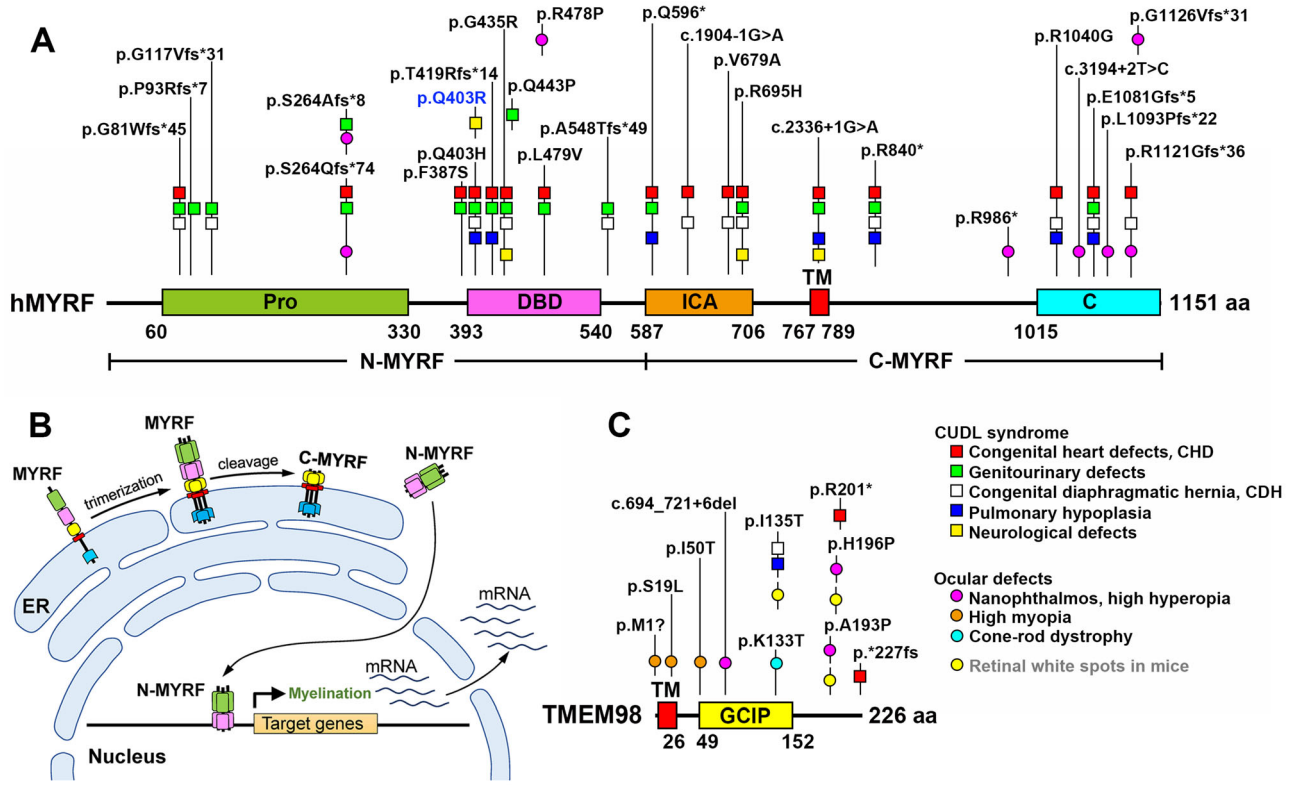

Fig. 1 Structure and function of MYRF. **A** Structural motifs conserved in human and mouse MYRF. The black vertical lines indicate variants in MYRF associated with human CUDL syndrome and nanophthalmos. Note that these mutations occur across the entire molecule. The p.Q403R mutation has been identified in patients with reversible myelin vacuolization, which is considered to be a neurological disorder here. Pro, proline-rich domain; DBD, Ndt80-like DNA binding domain; ICA, intramolecular chaperone auto-processing domain; TM, transmembrane domain; C, conserved C-terminal domain. **B** Diagram of MYRF cleavage, nuclear translocation, and transcriptional activation. ER, endoplasmic reticulum. **C** Variants in the C-terminal of TMEM98 often cause nanophthalmos in humans. while mutations in the N-terminal usually lead to myopia.

Variants in MYRF are Associated with a Wide Spectrum of Human Disorders

Besides oligodendrocytes, MYRF is also expressed in the retinal pigment epithelium (RPE), a single layer of pigmented epithelial cells between the choroid and retina. The RPE functions as blood-retinal barrier and is required for normal vision. Recently, a series of heterozygous variants in MYRF have been linked to inheritable human ocular defects (Fig 1A), such as nanophthalmos [9–12]. For instance, nanophthalmos has been found to be caused by a mutation in the splice site of *MYRF (MYRF c.3376-1G>A)*, leading to the production of a truncated protein lacking the C-terminal [9]. Unexpectedly, nanophthalmos patients do not develop obvious myelin phenotypes, possibly because myelin development is less sensitive to gene dosage changes caused by heterozygous mutations.

Increasing evidence demonstrates that variants in MYRF are also associated with several human autosomal dominant inheritable disorders, including congenital heart defects, urogenital anomalies, congenital diaphragmatic hernia, and pulmonary hypoplasia (cardiac-urogenital-diaphragm-lung syndrome; CUDL syndrome, or MYRF-related congenital anomalies syndrome) [13–15]. Like the situation in nanophthalmos, MYRF mutations in these diseases have been found in both the N- and C-fragment of MYRF, including the Pro domain, DBD domain, ICA domain, and the ER luminal region (Fig 1A). Notably, all the CUDL syndrome-related MYRF variants are heterozygous and usually lead to premature death in humans, suggesting an essential role for MYRF in visceral development. To our surprise, individuals with CUDL syndrome do not display myelin defects, and adult nanophthalmos patients usually do not develop a systemic phenotype of CUDL syndrome. In view of these phenotypic variances in different tissues, it is plausible that the myelin and ocular disorders may be missed in those individuals with systemic disorders due to premature death.

Regulation of MYRF Self-Cleavage and Function by TMEM98

Membrane-bound transcription factors (MBTFs), such as Notch, SREBPs, and ATF6 often undergo ligand- or stress-regulated intramembrane proteolysis to release their nuclear-targeted products. However, some previous studies suggested that MYRF proteolysis is constitutive, as prokaryotically-expressed MYRF protein with the ICA domain undergoes efficient self-cleavage in *E. coli* cells [3]. However, this does not exclude the possibility of negative regulation of MYRF self-proteolysis in cells that normally express it. Following this idea, we recently identified TMEM98, another ER membrane-bound transmembrane protein, as a MYRF binding protein through a biochemical approach [16]. Subsequent experiments revealed that TMEM98 acts as a negative regulator of MYRF intramolecular self-cleavage. Overexpression of TMEM98 blocks the self-cleavage of MYRF and inhibits its induction of myelin gene expression both *in vitro* and *in vivo*.

In support of their strong regulatory and functional relationship, TMEM98 and MYRF are strictly co-expressed in multiple tissues, including oligodendrocytes, RPE cells, and coelomic epithelium-derived cells [9, 14, 16]. Like MYRF, TMEM98 has been implicated in several ocular diseases in humans (e.g. nanophthalmos and high myopia) and CUDL syndrome (Fig 1C) [15, 17–21]. In addition, ablation of TMEM98 in mouse RPE cells also leads to retinal and RPE abnormalities and eye enlargement, with ectopic localization and activation of MYRF [22]. Unexpectedly, phenotypes caused by mutations in MYRF and TMEM98 are not identical. While individuals with MYRF mutations usually develop nanophthalmos, different TMEM98 mutations cause opposite refractive error phenotypes in humans. For instance, p.A193P and p.H196P mutations in TMEM98 cause nanophthalmos with hyperopia (small eyes, farsightedness) [22], whereas mutations in the N-terminal region of TMEM98 lead to high myopia (nearsightedness) (Fig 1C) [21]. In addition, our previous studies showed that the C-terminal of MYRF is not required for its binding to TMEM98 [16], and yet heterozygous mutations in this region are still linked to nanophthalmos and CUDL syndrome (Fig 1A), suggesting a TMEM98-independent mechanism for MYRF in regulating human organ development. Therefore, the functional relationship between MYRF and TMEM98 in organ development and human diseases is somewhat complicated, and the exact regulatory mechanisms remain to be defined in future investigations.

Conclusions and Remaining Questions

To date, all studies support the notion that MYRF functions as an MBTF on the ER membrane. Following its intramolecular self-proteolysis mediated by the ICA domain, the N-terminal fragment is translocated to the nucleus, and acts as a transcriptional activator both *in vitro* and *in vivo* [3, 4]. At present, it remains unknown why MYRF is synthesized as an ER-bound membrane protein before its N-terminal fragment is cleaved and translocated into the nucleus to activate gene expression. Is ER localization necessary for its post-transcriptional modification? Also, what is the function of the C-terminal region? Emerging evidence suggests that the C-fragment also plays a critical role in cell and tissue development, as many point mutations in the C-terminal of MYRF lead to various types of human disorder, similar to the variants in the N-terminal region (Fig 1). Interestingly, a recent study elegantly demonstrated that the ER-resident MYRF fragment contributes to the maintenance of ER homeostasis in highly secretory pancreatic ductal adenocarcinoma cells, implying that the C- MYRF fragment may help to reduce ER stress during the rapid production of myelin proteins [23].

Another intriguing puzzle is why different mutations in the same gene cause distinct types of human disorders. While some variants in MYRF cause CUDL syndrome to different degrees, others only result in non-fatal nanophthalmos phenotypes (Fig 1A). Surprisingly, despite its importance in myelin development, there is only one report that MYRF p.Q403R is linked to reversible myelin vacuolization, an innocuous myelin defect associated with mildly impaired consciousness (Fig 1A) [24]. In this regard, RPE cells or visceral cells appear to be more sensitive to MYRF mutations, possibly because these cells are more sensitive to the dosage effect of MYRF protein. Hence, it will be interesting and important to elucidate how different variants affect the normal function of MYRF in different cell types during development and in human disorders.

Finally, what is the biological significance of the physical interaction between MYRF and TMEM98? Although we demonstrated that TMEM98 strongly inhibits the self-cleavage of MYRF *in vitro* [16], we know almost nothing about its *in vivo* role in regulating MYRF cleavage and function, given that the expression level of endogenous TMEM98 in tissue is fairly low compared to that in overexpression studies. In TMEM98-expressing OLs and RPE cells, immunostaining of N-MYRF exhibited a predominant nuclear localization [3, 22], repudiating an complete repression effect of TMEM98 on MYRF self-cleavage *in vivo*. Based on these findings, it is tempting to hypothesize that TMEM98 functions as a brake on MYRF

self-cleavage, allowing MYRF a longer time window for undergoing proper post-translational processing and/or protein folding in the ER membrane before being released into the nucleus.

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Conflict of interest The authors declare no competing financial interests.

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