Amongst the pro-degenerative factors that have been identified in mammals neurological disease and fetal akinesia deformation syndrome (Ali et al., 2016; as their reduction has been associated with Alzheimer’s disease, paediatric NMNAT2 overexpression is axon-protective so strategies that activate or levels having a regularity role in axon integrity (Mack et al., 2001; Wang et al., 2013). Protection is observed in response to lesion of sciatic nerve and axon-protective effects similar to those observed with loss of SARM1 (Babetto et al., 2013). However, it is now evident that SARM1 mediates its degenerative effect through enzymatic NAD cleavage activity (Essuman et al., 2017). This presents a tangible therapeutic target and inhibitors of SARM1 NAD cleavage activity are in active development (Krauss et al., 2020). PubMed was used to search literature and relevant work in the area of MYCBP2, in consideration of a mini review format, was used as the selection criteria for citations. Dated: October 2021.

**Programmed Axon Degeneration Is Positively Regulated by the Ubiquitin System Component MYCBP2**

An additional pro-degenerative factor is the protein MYCBP2. MYCBP2 is conserved from C. elegans through to mammals and the various orthologues go by a multitude of names (C. elegans Rpm-1, Drosophila Highwire, zebrafish Esrom, mouse Phr1 and human Pam). Collectively they have been coined PHR proteins (Pam/Highwire/Rpm-1) [Grill et al., 2016]. MYCBP2 was first identified as a huge 510 kDa interactor with the proto-oncogene Myc (Guo et al., 1998). Although the physiological significance of this interaction has not been elaborated, genetic screens in Drosophila and C. elegans identified Highwire and Rpm-1 as important regulators of synaptogenesis (Schaefe et al., 2000; Wan et al., 2000; Zhen et al., 2000). A conserved role in synaptic development was also found in vertebrates as nerve terminal morphology is severely disrupted in zebralif and mice constitutively lacking Esrom and MYCBP2, respectively (Burgess et al., 2004; D’Souza et al., 2005; Bloom et al., 2007). In mice the phrenic nerve does not fully innervate the diaphragm resulting in perinatal lethality due to respiratory distress. However, null and hypomorphic mutants of Drosophila Highwire were found to strongly inhibit axonal degeneration after axotomy (Kiong et al., 2012). Furthermore, conditional silencing of MYCBP2 in adult mice is tolerated for at least 6 weeks and confers axon-protective effects similar to those observed with loss of SARM1 (Babetto et al., 2013). Protection is observed in response to lesion of sciotic nerve and containing 1 (SARM1) (Osterloh et al., 2012). Mice constitutively depleted of SARM1 appear healthy and this delays axonal degeneration by several days following axotomy. SARM1 knockout also attenuates Wallerian-like degradation upon exposure to common chemotherapeutic drugs including vincristine and Bortezomb (Gerdt et al., 2013; Geisler et al., 2019; Geisler, 2020). It is now evident that SARM1 mediates its degenerative effect through enzymatic NAD cleavage activity (Essuman et al., 2017). This presents a tangible therapeutic target and inhibitors of SARM1 NAD cleavage activity are in active development (Krauss et al., 2020). PubMed was used to search literature and relevant work in the area of MYCBP2, in consideration of a mini review format, was used as the selection criteria for citations. Dated: October 2021.

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MYCBP2 Is a Novel RING-Cys-Relay Ubiquitin Ligase

In an unexpected discovery we showed that MYCBP2 is the sole member of a highly unusual class of E3 as at its extreme C-terminus resides an unprecedented 30 kDa enzymatic module termed RING-Cys-relay (RCR) (Figure 2A) (Pao et al., 2018). This was surprising because it was generally accepted that the different classes of catalytic E3 module had already been established. This discovery was enabled by chemical biological tools known as activity-based probes (Mulder et al., 2020). The activity-based probes covalently label E3s that demonstrate the transnialtivation activity mediated by E3s that utilize a catalytic cysteine nucleophile (Figure 1) (Pao et al., 2016, 2018). Approximately 45 transnialtivation RCRs were believed to exist and MYCBP2 is a new addition to this subtype.

A highly unusual characteristic of MYCBP2 is that unlike other classes of transnialtivation E3 it contains a second, downstream catalytic cysteine residue (Figure 2B). Once the initial catalytic ligase-ubiquitin intermediate has been formed, the Ub molecule is intramolecularly “relaxed” over a distance of ~24 Å to a downstream site. This is the downstream site that is tasked with substrate modification (Pao et al., 2018). Both of the catalytic cysteines reside within an experimentally confirmed on-binding protein domain termed the tandem cysteine domain (Figure 2C). Further irregularities pertain to MYCBP2 because E3s studied previously invariably couple Ub to lysine amino groups via an isopeptide bond. In a striking digression from this dogma, MYCBP2 couples Ub to hydrolytic groups via an ester bond, where a strong preference is observed for threonine over serine residues in model substrates. Not only does this relaxation of the ubiquitin conjugation ordering of the upstream site. This might serve as an “entropic spring” allowing the ubiquitin molecule to be relayed to a downstream catalytic cysteine. Although not experimentally confirmed, the working model is that Fbox45 and Skp1 bond to the distal N-terminal FBD1 region. They are responsible for binding substrates, including NMNAT2, and position them proximal to the downstream cysteine within the RCR module. The downstream site efficiently ubiquitinates hydroxy amino acids rather than conventional lysine residues. This unconventional mechanism of ubiquitination involving the two catalytic cysteines, termed RING-Cys-Relay (RCR), is central to neuronal development and its loss confers neurite protection in primary superior cervical ganglion cultures.

RING-Cys-Relay Ubiquitin Ligase Activity Promotes Wallerian Degeneration

Notably, in addition to the neurodevelopmental phenotypes being recapitulated in the homoygous knock-in mouse model, so were the axon-protection effects. Despite the reduction in neurite outgrowth, loss of RCR ubiquitin ligase activity attenuated the degeneration of superior cervical ganglia explants following axotomy (Mabbitt et al., 2020). In addition, exogenous NMNAT2 was stabilized in embryonic fibroblasts from homoygous and homozygous knock-in model, reminiscent of the phenotypes observed with MYCBP2 silencing in mammals (Burgess et al., 2004; D’Souza et al., 2005; Bloom et al., 2007). Indeed, loss of RCR ubiquitin ligase activity in late-stage embryos also resulted in a marked reduction in diaphragm innervation by the phrenic nerve (Mabbitt et al., 2020). These observations indicated that the newly discovered RCR ubiquitin ligase mechanism is associated with the previously reported neurodevelopmental phenotypes.
should allow these aspects to be investigated further. Taken together, these observations suggest that the neurodevelopmental and pro-degenerative effects of MYCBP2 are largely dependent on its unusual RCR E3 activity.

Structural and Biochemical Insights into RING-Cys-Relay Ubiquitin Ligase Activity

Enabling the discovery of small molecule inhibitors of the RCR ubiquitin ligase machinery in MYCBP2 would be its structural and biochemical characterizations, by determining the binding of an ubiquitin-loaded enzyme, impairing the ubiquitin relay process, or blocking the downstream site would be expected to abolish MYCBP2 ubiquitin ligase activity. Structural studies initially revealed the general architecture of the RCR module and its domain organization (Pao et al., 2018). Consistent with the role of F-box domains being direct substrate receptors, the FBD1 region in MYCBP2 is the only receptor module assigned to MYCBP2. The fact that MYCBP2, a non-Cullin family member, also utilizes a dedicated substrate receptor is a dynamically exchange substrate receptor modules (Wang et al., 2020), Fbxo45 interacts with NMNAT2 (Babetto et al., 2013; Desbois et al., 2018). Consistent with the role of F-box domains being direct substrate receptors, the FBD1 region in MYCBP2 is the only receptor module assigned to MYCBP2. The fact that MYCBP2, a non-Cullin family member, also utilizes a dedicated substrate receptor is a dynamically exchange substrate receptor modules (Wang et al., 2020), Fbxo45 interacts with NMNAT2 (Babetto et al., 2013; Desbois et al., 2018). Consistent with the role of F-box domains being direct substrate receptors, the FBD1 region in MYCBP2 is the only receptor module assigned to MYCBP2. The fact that MYCBP2, a non-Cullin family member, also utilizes a dedicated substrate receptor is a dynamically exchange substrate receptor modules (Wang et al., 2020), Fbxo45 interacts with NMNAT2 (Babetto et al., 2013; Desbois et al., 2018).

The described RCR ubiquitin ligase module resides at the extreme C-terminus of the giant MYCBP2 protein but a region in the middle of the protein, known as the FBD1 region, has a conserved function in synapse formation reveals Phr1 as a candidate gene for Caenorhabditis elegans PHR protein RPA-1 to perisynaptic regions. Dev Dyn 237:630-639.

Substrate Recognition Is Achieved through a Multi-Subunit MYCBP2 Complex

The described RCR ubiquitin ligase module resides at the extreme C-terminus of the giant MYCBP2 protein but a region in the middle of the protein, known as the FBD1 region, has a conserved function in synapse formation reveals Phr1 as a candidate gene for Caenorhabditis elegans PHR protein RPA-1 to perisynaptic regions. Dev Dyn 237:630-639.

The FBD1 region in MYCBP2 is ~2000 residues N-terminal of the RCR ubiquitin ligase module (Figure 2A) (Saiga et al., 2009; Desbois et al., 2018). Knock-down of Skp1 or Fbxo45 phenocopies MYCBP2 silencing as it confers axon-protction in response to both physical and chemical injury (Yamagishi and Tessier-Lavigne, 2016). Furthermore, this has been directly linked to stabilization of NMNAT2 (Yamagishi and Tessier-Lavigne, 2016). Consistent with the role of F-box domains being direct substrate receptors, Fbxo45 interacts with NMNAT2 (Babetto et al., 2013; Desbois et al., 2018). Interestingly, disruption of the interaction between Rpn-1 and Fsn-1 in C. elegans with a transgenically expressed peptide inhibitor recapitulates the synaptic defects observed with null worms (Sharma et al., 2014). This suggests that the substrate receptor sub-complex is a potential therapeutic target. Taken together, these findings point toward a huge multi-subunit ligase machine, where the C-terminal RCR module is the catalytic engine, being central to normal neurodevelopment and post-developmental programmed axon degeneration (Figure 2C). Hence, inhibitors that disrupt not only the RCR module, but also the formation of the multi-subunit complex, should stabilize NMNAT2 and confer axon protective effects in response to injury. Further structural characterization should enable the visualization of this complex and determine how the C-terminal RCR module catalysis the downstream target. This might then act like an “entropic spring” enabling the ubiquitin molecule to be catapulted to the downstream site (Figure 2C) (Mabbitt et al., 2020).

By further leveraging the activity-based probe technology a stabilized form of the otherwise labile primary E2-E3 ubiquitin transfer intermediate was prepared and isolated (Mabbitt et al., 2020). This permitted high-resolution structure determination by X-ray crystallography. The structure revealed the molecular contacts required for E2-E3 ubiquitin transfer. In the earlier isolated structures, containing the upstream cysteine terminus, the modiglycin loop, was too flexible to be observed (Pao et al., 2018). The new structure of the stabilized transfer complex revealed that the region containing the upstream cysteine forms a transiently ordered helical configuration during E2-E3 ubiquitin transfer (Figure 2C). The study also provides insights into how the intramolecular ubiquitin relay step works. Proline scanning experiments supported a model where the energy required to facilitate the striking ubiquitin relay process is generated by the transient ordering of the upstream site. This might then act like an “entropic spring” enabling the ubiquitin molecule to be catapulted to the downstream site (Figure 2C) (Mabbitt et al., 2020).

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