Biochemical Journal (2017) **474** 2177–2190
DOI: 10.1042/BCJ20160616

Review Article

Cellular and disease functions of the Prader–Willi Syndrome gene *MAGEL2*

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Melanoma antigen L2 (MAGEL2 or MAGE-L2) is a member of the MAGE family of ubiquitin ligase regulators. It is maternally imprinted and often paternally deleted or mutated in the related neurodevelopmental syndromes, Prader–Willi Syndrome (PWS) and Schaaf–Yang Syndrome (SHFYNG). MAGEL2 is highly expressed in the hypothalamus and plays an important role in a fundamental cellular process that recycles membrane proteins from endosomes through the retromer sorting pathway. MAGEL2 is part of a multi-subunit protein complex consisting of MAGEL2, the TRIM27 E3 ubiquitin ligase, and the USP7 deubiquitinating enzyme. The MAGEL2-USP7-TRIM27 (or MUST) complex facilitates the retromer recycling pathway through ubiquitination and activation of the WASH actin nucleation promoting factor. This review provides an overview of the MAGE protein family of ubiquitin ligases regulators and details the molecular and cellular role of MAGEL2 in ubiquitination, actin regulation and endosomal sorting processes, as well as MAGEL2 implications in PWS and SHFYNG disorders. The physiological functions of MAGEL2, elucidated through the study of *Magel2* knockout mouse models, are also discussed.

The MAGE family

Over 30 years ago, Boon and colleagues cloned the first MAGE gene while studying a melanoma patient who had strong T cell reactivity against autologous tumor cells (reviewed in [1]). Through an elegant unbiased screening approach, they identified the first human tumor antigen, melanoma antigen-1 (MAGE-1, now named MAGE-A1). After their groundbreaking discovery, they and others quickly realized that the gene encoding MAGE-1 belonged to a larger family, now recognized as the melanoma antigen gene family (MAGEs). In humans, the MAGE family consists of approximately 40 unique MAGE genes with additional pseudogenes [2]. Evolutionarily, MAGEs can be traced back to protozoa; however, earlier eukaryotes, including non-placental mammals such as platypus, have only a single MAGE gene [3–5]. The first expansion of the MAGE family occurred in marsupials, but the majority of MAGE genes were added in placental mammals [2,4–6]. MAGE genes continue to evolve rapidly with both expansion and speciation of MAGEs occurring in eutherian mammals.

MAGE genes are broadly divided into two types, type I and II, based on their tissue expression pattern. Two-thirds of MAGEs (*MAGE-A, -B* and *-C* subfamilies) fall into the type I classification and are defined as cancer-testis antigens (CTAs) given their: (1) restricted expression to testis and other reproductive tissues; (2) aberrant re-activation in cancers; and (3) their ability to elicit an immune response. The remaining (*MAGE-D, -E, -F, -G, -H, NECDIN* and *MAGEL2*) are type II MAGEs and have broad expression patterns in many tissues (Figure 1A,B) [2,3,5–7]. MAGE genes are predominantly assembled on the X chromosome, a characteristic of several CTA gene families. However, several type II genes reside in (*MAGEL2* and *NECDIN*) or immediately adjacent to (*MAGE-G1*) the region on chromosome 15 affected in Prader–Willi syndrome patients (Figure 1C) [8]. Type II MAGE genes appeared earlier in evolution and are closer to non-mammalian, ancestral MAGEs [9]. A single copy of a MAGE-like gene is present in zebrafish, fruit fly and chicken, and is expressed in the brain of both the developing embryo and the adult animal and is involved in neurogenesis. Interestingly,

Received: 28 November 2016
Revised: 24 March 2017
Accepted: 31 March 2017

Version of Record published:
16 June 2017

Biochemical Journal (2017) **474** 2177–2190
DOI: 10.1042/BCJ20160616

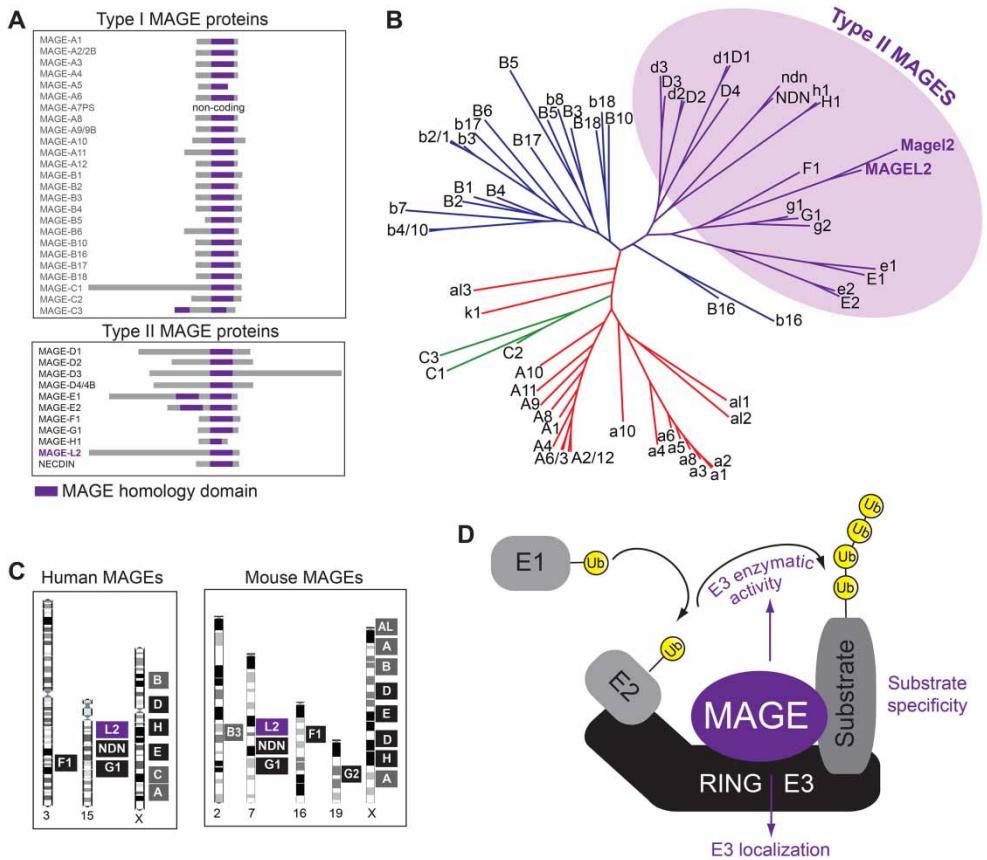

Figure 1. (**A**) Schematic overview of Type I and Type II MAGE protein family members in human, MAGE homology domain (MHD) is depicted in purple. (**B**) Phylogenetic tree of MAGE proteins. Type I MAGE families: MAGE-A (red), -B (blue) and -C (green) and Type II families: MAGE-D, -E, -F, -G, -H, MAGE-L2, and NECDIN. (**C**) Chromosomal location of MAGE genes in human and mouse. (**D**) MAGE proteins regulate RING E3 ubiquitin ligases.

several mammalian type II MAGEs (such as MAGE-D1, Necdin, and MAGEL2) are enriched in the brain and have been found to be involved in neurogenesis and brain function [4,10–12]. By contrast, the yeast and fruit fly MAGE proteins have been implicated in DNA damage response and repair as part of the SMC5/6 complex [13–15]. In mammals, MAGE-G1 (also known as NSMCE3) plays a similar function [16].

MAGE proteins are highly related (approximately 50% sequence identity) and are defined by a conserved 170-amino acid sequence known as the MAGE homology domain (MHD) that is present in both type I and II MAGEs and mediates protein–protein interactions (Figure 1A) [2]. Biophysical and structural studies have shown that the MHD folds into two tandem winged-helix motifs (WH-A and WH-B) that are dynamic and their relative orientation can differ between MAGEs and upon binding to other proteins [17–19]. This flexibility and sequence diversity allows specific MAGEs to interact with distinct partners that confer individualized functions.

MAGE-RING ligases

Based on their specific expression and immunogenic activity, several MAGE proteins have been extensively investigated as targets for cancer immunotherapy [7,20–23]. However, their molecular and physiological functions remained unclear for several years. Recently it was discovered that MAGE proteins serve as regulators of E3 RING ubiquitin ligases (Figure 1D) [17,24,25]. E3 ligases specify substrates for ubiquitination and mediate the transfer of ubiquitin from an E2 ubiquitin-conjugating enzyme to a substrate [26]. The vast majority of E3 ligases belong to the group of RING E3s, encoded by over 600 human genes, which either target substrates for proteasome-dependent proteolysis or modulate their protein function, structure, assembly and/or localization

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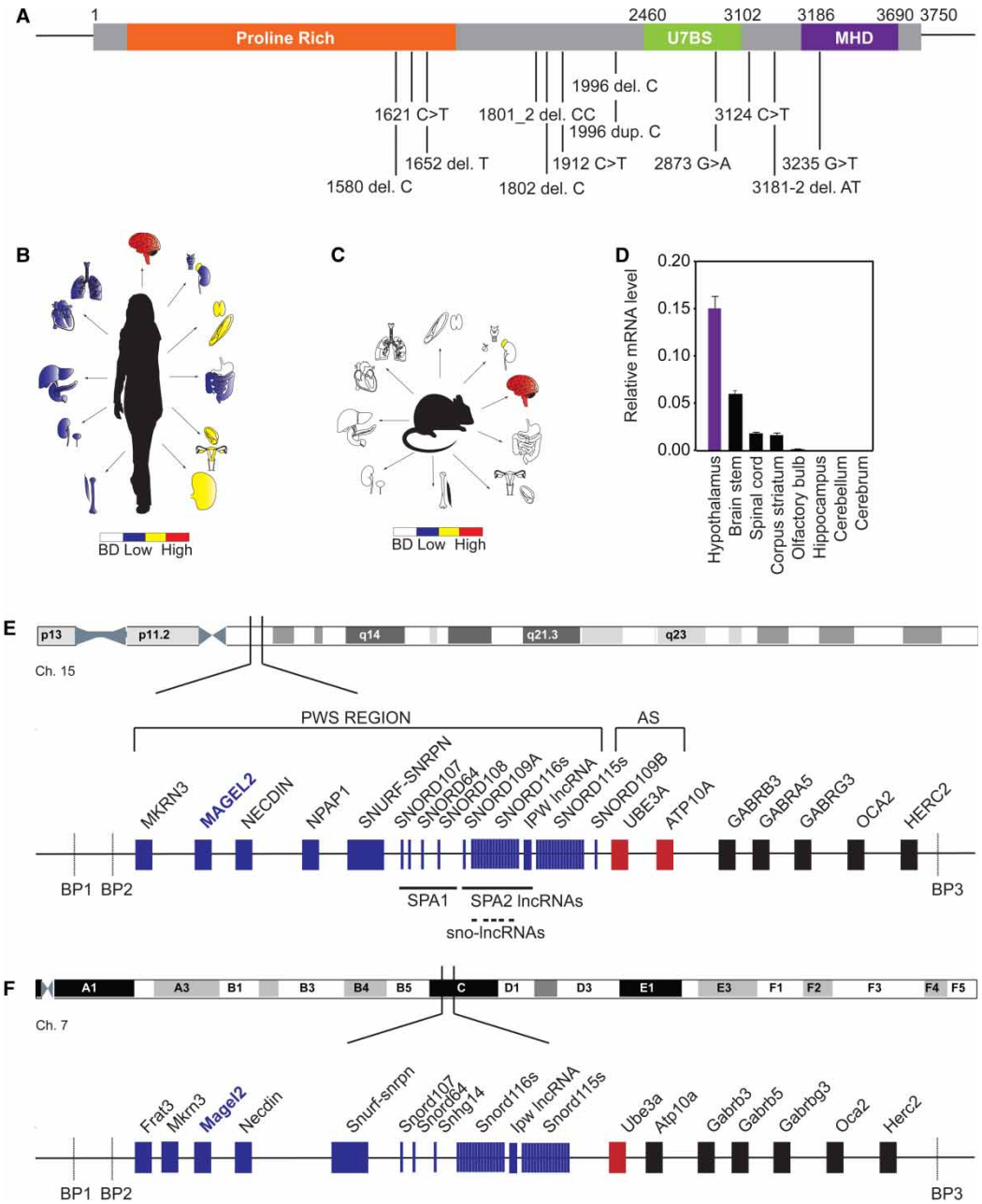

Figure 2. (**A**) Schematic structure of the *MAGEL2* gene. Proline-rich region, USP7 binding site (U7BS), MAGE homology domain (MHD) and truncating MAGEL2 mutations reported so far are indicated relative to their positions in the coding sequence of this single-exon gene. (**B** and **C**) The *MAGEL2* gene expression distribution across human and mouse tissues is summarized by the indicated color coding. (**D**) The expression of *Magel2* in the mouse brain as measured by quantitative reverse transcription PCR (RT-qPCR). (**E** and **F**) Schematic representation of the genetic architecture within the PWS cluster in humans (**E**) and the syntenic region in mice (**F**). In the Prader-Willi syndrome (PWS) region, there are several paternal-only (shown in blue) expressed protein coding genes (*MKRN3, MAGEL2, NECDIN, NPAP1 and SNURF-SNRPN*) and paternal-only expressed non-coding RNAs, including snoRNAs and lncRNAs. Additionally, *UBE3A* and *ATP10A* (shown in red) protein coding genes are maternal-only expressed and are related to Angelman syndrome (AS). The cluster of GABA receptor genes (*GABRB3, GABRA5* and *GABRG3*), *OCA* and *HERC2* are not imprinted and have biparental expression (shown in black). The vertical lines denote the common deletion breakpoints (BP1, BP2 and BP3) in PWS and AS.

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[27]. By doing so, E3 RING ubiquitin ligases regulate many important processes in the cell, such as cell division, DNA damage response and immune signaling, and are implicated in the pathogenesis of several diseases, including viral infection, neurodegenerative disorders and cancer.

Diverse binding partners of E3 ligases have emerged as important regulators of the substrate specificity and their enzymatic activity [28]. This includes the MAGE family of proteins that bind to specific E3 RING ubiquitin ligases and form stable complexes referred to as MAGE-RING ligases (MRLs). A number of proteomic studies have identified many MRLs, including those involving both type I and II MAGEs (including MAGE-A1, -A2, -A3, -A6, -B2, -B18, -C2, -D1, -E1, -F1, -G1, -L2 and NECDIN) that bind to specific E3 RING ligases (including, LNX1, NSE1, PRAJA1, RBX1, TRIM27, TRIM28, and TRIM31) [17,25,29–31]. MAGEs bind E3 ligases through their MHD, which is both necessary and sufficient for binding [17]. However, MAGEs do not recognize a common motif on E3 RINGs (such as the RING domain). Instead, MHDs recognize unique regions on E3 RING ligases that allow for specificity in MRLs, such that a single MAGE typically interacts with one or a small number of related E3 ligases [17]. This specificity is probably driven by both sequence diversity of MHDs and structural dynamics in the MHD that determine its 3D shape.

The interaction of MAGEs with E3 ubiquitin ligases is functionally relevant. For example, MAGEs can stimulate TRIM28 auto-ubiquitination and ubiquitination of substrates, such as p53 and ZNF382, leading to their degradation by the proteasome [17,32,33]. In addition, MAGE-A1 and MAGE-G1 can stimulate the ligase activity of their cognate E3 RING ligase, TRIM31 and NSE1, respectively [17,31]. Thus, both type I and II MAGEs can enhance the activity of E3 RING ubiquitin ligases. In addition, MAGEs have been reported to alter substrate specificity of ligases, such that MRL complexes have a different spectrum of targets than the E3 RING alone. Mechanistically, MAGE proteins can directly bind substrates and recruit them to the E3 ligase for ubiquitination, such as MAGE-A3/6 binding AMPK, with ubiquitination mediated by TRIM28 [24]; or change substrate preference, as in the case of MAGE-A11 and Skp2 [34]. In addition, MAGEs can alter the subcellular localization of the ligase, allowing it to find novel substrates, such as MAGEL2 directing TRIM27 to endosomes for ubiquitination WASH [25]. Taken together, these and other studies have concluded that MAGEs function as part of MRL E3 ubiquitin ligase complexes to direct the function of ligases and alter important cellular signaling pathways and processes.

The MAGEL2 protein

Type I MAGEs have recently been reviewed [7], and thus here we will focus on the type II MAGEL2 that has important cell biological functions and implications in physiology and disease. The *MAGEL2* gene encodes one of the largest MAGE proteins (1249 amino acids in human and 1284 in mouse). The MHD is located at the C terminus, from amino acid 1027–1195 in human (Figure 2A), whereas the N-terminal half of MAGEL2 is highly proline rich (>30%). *MAGEL2* is a maternally imprinted, GC-rich, single-exon gene that is exclusively expressed from the unmethylated paternal allele. Examination of >20 human tissues revealed that *MAGEL2* expression is highly enriched in the brain (Figure 2B). Furthermore, from more than 50 mouse tissues analyzed, *Magel2* mRNA is also shown to be enriched in the mouse brain and, specifically, within the hypothalamus (Figure 2C,D) [35]. This is consistent with preceding analyses of embryonic and adult expression of *Magel2* by knock-in of the *LacZ* gene into the *Magel2* chromosomal locus [35,36]. Around mid-gestation, the *Magel2* transcript is restricted to the central nervous system (CNS), in particular the neural tube, forebrain, midbrain and embryonic hypothalamus. In non-neuronal tissues, *Magel2* is expressed in the genital tubercle, midgut region and placenta, but becomes more restricted to CNS in the adult animal, specifically to the hypothalamus [35,36]. In the adult brain, the majority of *Magel2*-positive neurons reside in the hypothalamic region with the most prominent *Magel2* expression in the suprachiasmatic (SCN), the paraventricular (PVN) and supraoptic (SON) nuclei, in particular in vasopressin-positive neurons, where it shows a circadian expression pattern.

MAGEL2-TRIM27 and endosomal protein recycling

Consistent with MAGEs regulating E3 RING ubiquitin ligases [7,17,24,25,37], MAGEL2 binds to the E3 RING ubiquitin ligase TRIM27 [17,25]. Further *in vitro* studies revealed that, like the architecture of several other MRL complexes [17], the MHD of MAGEL2 directly interacts with the coiled-coil domain of TRIM27 [17,25]. TRIM27 (also known as Ret finger protein; RFP) is a member of the tripartite motif (TRIM) containing E3 RING ubiquitin ligase family that have a conserved architecture at their N-terminus and a variable C-terminal region. TRIM27 contains an N-terminal RING motif that binds to E2 ubiquitin-conjugating enzymes followed by a zinc-containing B-box motif, coiled-coil multimerization motif, and a C-terminal PRY-SPRY protein–

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DOI: 10.1042/BCJ20160616

protein interaction motif. TRIM27 has been implicated in a number of cellular and disease processes, including PTEN, AKT, and NF-kB signaling, transcription, T-cell activation, apoptosis, innate immunity, spermatogenesis, muscle atrophy, cancer, and autism [13,38–45]. MAGEL2 binds only a fraction of the total cellular pool of TRIM27 and it remains to be determined which of the known TRIM27 activities are regulated by MAGEL2 [25].

Intriguingly, a fraction of MAGEL2 and TRIM27 are co-localized in discrete cytoplasmic puncta [25,46]. These subcellular structures were determined to be specialized endosomes containing the cargo sorting retromer complex that recruits the MAGEL2-TRIM27 MRL to endosomes through direct interaction between MAGEL2 and the VPS35 retromer subunit [25]. The retromer is an endosomal coat-like complex that regulates the recycling of specific membrane cargo proteins from endosomes back to the trans-Golgi network (TGN) or plasma membrane (Figure 3A) [47,48]. In the absence of retromer, its cargo proteins are sorted through the default multi-vesicular body trafficking route leading to degradation in the lysosome [47,48]. Retromer is recruited to endosomes in a Rab7-dependent manner where it or its associated SNX proteins bind to specific

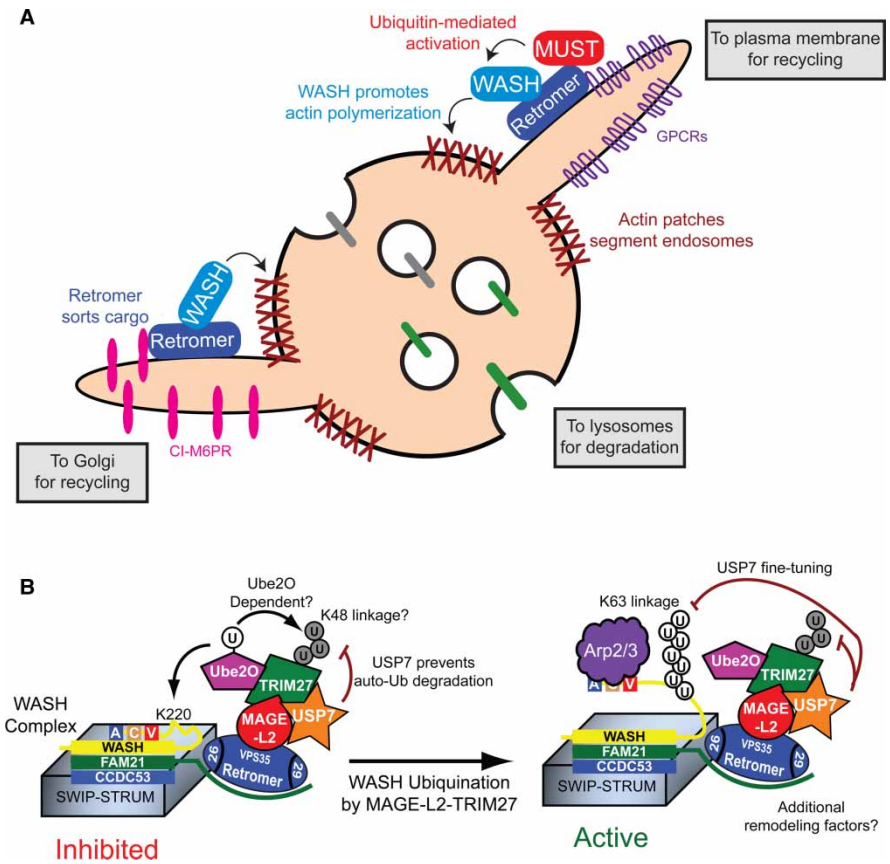

Figure 3. (**A**) The MUST complex (MAGEL2-USP7-TRIM27) is important for the retromer-mediated endosomal protein recycling pathway. The retromer coat-like complex recognizes membrane protein cargo on endosomes and facilitates their trafficking back to the plasma membrane or trans-Golgi network, sparing them from degradation in lysosomes. Sorting of retromer cargo into tubules emanating from the endosome is facilitated by deposition of actin by the WASH and Arp2/3 complexes to demarcate distinct endosomal domains. Non-degradative ubiquitination of WASH by the MUST complex facilitates WASH activity and endosomal actin accumulation. (**B**) Molecular mechanism of how the MUST complex regulates WASH activity. Retromer recruits the the WASH regulatory complex (SHRC) complex (WASH, FAM21, CCDC53, SWIP, and Strumpellin) through binding FAM21 and recruits the MUST complex by binding MAGEL2. The MUST complex promotes the activation of SHRC by K63-linked polyubiquitination of WASH leading to VCA (Verprolin homologous, Central hydrophobic, Acidics) exposure and Arp2/3 and actin binding to generate endosomal F-actin. USP7 deubiquitinating activity functions to prevent TRIM27 auto-ubiquitination and degradation, as well as fine-tuning WASH ubiquitination levels. Too little or too much WASH ubiquitination and thus endosomal F-actin levels are both detrimental to recycling. GPCRs, G-protein-coupled receptors.

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membrane proteins in endosomes and recycle these to the TGN or plasma membrane, sparing them from degradation [49–54]. A number of membrane proteins have been shown to flow through this recycling pathway, including housekeeping proteins (such as cation-independent mannose-6-phosphate receptor; CI-M6PR), signaling proteins (such as Wntless and G-protein coupled receptors), and bacterial toxins (such as cholera toxin) [51,55–58]. Importantly, disruption of the MAGEL2-TRIM27 MRL impairs recycling of proteins (including CI-M6PR and CTxB) through this pathway, leading to their degradation.

MAGEL2-TRIM27 ubiquitination of WASH

Build-up of retromer on endosomes results in the recruitment of the WASH actin nucleation promoting factor, which facilitates formation of F-actin on endosomes [48,59,60]. The precise function of F-actin on endosomes is still debated, but it has been hypothesized to stabilize the cargo-filled retromer-coated tubules, segregate endosomes to allow cargo enrichment in emanating tubules, and pinch off tubules by pushing against the endosome at the point of tubule attachment [48,59,60]. In addition, endosome-associated actin was proposed to contribute to the sequence-dependent selectivity of protein recycling by stabilizing the specialized endosomal tubules and providing the machinery for active concentration of specific cargo proteins [61]. Importantly, cells depleted of MAGEL2 or TRIM27 have diminished endosomal F-actin and Arp2/3 due to compromised WASH activity [25,35]. WASH exists in a macromolecular complex termed the WASH regulatory complex (SHRC) [59,60]. It consists of five subunits, WASH, FAM21, CCDC53, SWIP and Strumpellin. Based on the known structure of the related WASP family member, WAVE, it is thought that WASH, CCDC53 and the N-terminus of FAM21 form an alpha helical bundle that sits on a base of SWIP and Strumpellin (Figure 3B) [62,63]. The C-terminal FAM21 tail mediates the recruitment of WASH to endosomes through binding the VPS35 subunit of retromer [59,60,64,65]. This recruitment is accomplished through a series of low-affinity interactions between multiple L-F-[D/E](3-10)-L-F repeat elements in the FAM21 C-terminal tail and VPS35, which have been proposed to work as a timing mechanism in which sufficient retromer binding to endosomes is necessary before WASH can be recruited to endosomes [48,60,64,65].

Like other WASP family members, including WASP/N-WASP, WAVE/SCAR, WHAMM/JMY, WASH contains a C-terminal VCA (verprolin homologous or WH2, central hydrophobic, and acidic) motif that binds to actin and the Arp2/3 complex to stimulate actin filament nucleation. It has been shown that the VCA motifs of WASP/N-WASP, WAVE and WASH are auto-inhibited through both intra-molecular and inter-molecular interactions [62,63,66–68]. A number of signals feed into activation/exposure of the VCA motifs of WASP family members to regulate nucleation of F-actin in a temporal and spatial manner [69], including small GTPases CDC42 and RAC1, PIP2 and PIP3 phospholipids, and phosphorylation by the SRC, ABL and CDK family of kinases [66–71]. We have shown that, like other WASP family members, WASH gets activated by post-translational modification whereby the MAGEL2-TRIM27 MRL ubiquitinates WASH on K220 in the WASH regulatory loop (Figure 3B). Importantly, MAGEL2-TRIM27 promotes K63-linked polyubiquitination of WASH. Unlike many of the other forms of ubiquitin linkages, K63-linked ubiquitination does not generally target proteins for degradation by the proteasome, but instead acts as a signaling event similar to phosphorylation [72]. For example, K63-linked polyubiquitin chains are critical for EGFR lysosomal sorting and are involved in protein sorting in yeast [73,74]. K63-linked polyubiquitination of WASH by the MAGEL2-TRIM27 MRL acts as a signal to activate the auto-inhibited SHRC (Figure 3B). However, it still remains to be determined how this ubiquitination event leads to activation. Two simple hypotheses include: (1) that the ubiquitin chain physically disrupts the auto-inhibitory mechanisms of SHRC allowing VCA exposure for Arp2/3 and actin binding; or (2) the K63-linked polyubiquitin chain acts as a recruitment platform for additional factors that facilitate remodeling of the SHRC in order to achieve activation. It should also be noted that MAGEL2 is only conserved in mammals, whereas WASH is found in all eukaryotes. In addition, MAGEL2 is expressed in a more restricted manner compared with ubiquitous WASH, suggesting that MAGEL2 serves as a tissue-specific regulator for WASH activation and that additional forms of regulation to activate WASH probably exist, such as regulation by small GTPases, inositol 4,5-bisphosphate, phosphorylation and interaction with other binding partners [75].

USP7 is an essential component of the MAGE-L2-TRIM27 MRL

Like other post-translational modifications, ubiquitination is reversible and can be removed by the action of deubiquitinating enzymes [76,77]. Interestingly, MAGEL2 and TRIM27 form a stable, stoichiometric complex

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with the deubiquitinating enzyme (DUB), ubiquitin-specific protease 7 (USP7; also known as herpesvirus-associated ubiquitin-specific protease, HAUSP), through multiple intricate interactions [35]. We term this the 'MUST' complex for MAGEL2-USP7-TRIM27. USP7 is a deubiquitinating enzyme in the ubiquitin-specific protease family that can cleave multiple chain linkages, including K48- and K63-linked ubiquitin chains [78–80]. USP7 regulates the ubiquitination of many proteins (e.g., the MDM2-p53 pathway) and impacts multiple cellular and disease processes, such as DNA repair, transcription, immune responses, viral replication and cancer [78,79]. It is worth noting that mice in which *Usp7* has been knocked out are early embryonic lethal and cannot be rescued by p53 knockout, suggesting additional important developmental roles of Usp7 [81]. In addition to regulating the MAGEL2-TRIM27 MRL, USP7 has also been shown to be involved in MAGEL2-independent functions of TRIM27, such as TNF-α-induced apoptosis where TRIM27 ubiquitination of USP7 regulates degradation of receptor-interacting protein 1 (RIP1), resulting in the positive regulation of TNF-α-induced apoptosis [39].

USP7 has a complex role in regulation of the MAGEL2-TRIM27 MRL and retromer-mediated endosomal recycling (Figure 3B) [35]. First, USP7 deubiquitinates TRIM27 to prevent its own auto-degradation and thus stabilizes the complex [35]. Second, USP7 controls WASH ubiquitination to prevent over-activation of WASH and to ensure proper levels of endosomal actin are generated [35]. This function is critical given that over-

Table 1: Overlapping and differential symptoms seen in patients with Prader-Willi Syndrome (PWS), Schaaf-Yang Syndrome (SHFYNG), and USP7 haploinsufficiency are compared to the relevant phenotypes seen in the two *Magel2* knockout mouse models.
For details and references see the main text.

	Patients				Magel2 KO mouse models		
	Symptoms	**PWS**	**SHFYNG**	**USP7**	**Phenotypes**	**Wevrick**	**Muscatelli**
PWS major criteria	Neonatal hypotonia	+	+		Neonatal mortality	10%	50%
	Poor suck	+	+		Suckling defects		+
	Feeding problems in infancy, failure to thrive	+			Neonatal failure to thrive and pre-wean growth retardation	+	+
	Hyperphagia, lack of satiety	+			Increased leptin plasma levels, reduced leptin sensitivity	+	
	Obesity	+			Weight gain, decreased lean mass with increased adiposity after weaning	+	
	Developmental delay and intellectual disability	+	+	+	Abnormal behavior in novel environments	+	+
	Hypogonadism	+	+	+	Progressive infertility and decreased olfactory discrimination	+	
	Hypotonia	+	+	+	Decreased locomotor activity, muscle dysfunction and decreased bone mineral content	+	
	Characteristic facial features	+					
PWS minor criteria	Infantile lethargy and decreased fetal movement	+	+				
	Short stature	+	+			+	
	Small hands	+			Growth hormone axis impairment		
	Narrow hands	+					
	Eye abnormalities	+	+				
	Hypopigmentation	+					
	Thick saliva	+					
	Characteristic behavior	+	+				
	Speech articulation defects	+	+	+	Decreased oxytocin	+	+
	Skin picking	+	+		Decreased serotonin levels	+	
	Sleep apnea	+	+		Blunted circadian rhythm, decreased orexin levels	+	
Other	ASD		+	+	Abnormal in novel environments	+	+
	Contractures		+				
	Seizures			+			

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production of endosomal actin has been shown to be detrimental [35,60]. Thus, USP7 functions as a rheostat for MAGEL2-TRIM27 function that enables fine-tuning of actin nucleation. USP7 is not the only deubiquitinating enzyme implicated in protein trafficking through the endocytic pathway, as Cezzane, USP2, USP6, USP8 and USP9X have all been reported to regulate the sorting of a diverse set of cargo (such as EGFR, WNT receptor Frizzled, and LDLR) and, in the a case of USP8, work in the retromer pathway [82,83]. Further studies on the enzymatic activity of this intricate E3-deubiquitinating enzymes complex are warranted and, in particular, the regulatory mechanisms and signaling pathways that feed into controlling its activity require elucidation.

MAGEL2 in Prader–Willi and Schaaf–Yang syndromes

MAGEL2 is one of the protein-coding genes in the maternally imprinted region on chromosome 15 (15q11–13) whose paternal copy is lost in children with Prader–Willi syndrome (Figure 2E) [36,84,85]. Prader–Willi syndrome (PWS, OMIM#176270) is a complex neurodevelopmental disorder with a worldwide prevalence of 1 in 15 000–30 000 births that is characterized by neonatal hypogonadism and severe hypotonia with feeding problems and failure to thrive during the first years of life, followed by a global developmental delay, breathing defects, specific behavior and multiple endocrine dysfunctions (Table 1) [86]. PWS children develop mild to moderate intellectual disability, hypothyroidism, short stature with small hands due to growth hormone and adrenal insufficiency, pain insensitivity and a characteristic behavioral profile. Most notably, they drastically change their feeding behavior towards hyperphagia, leading to severe obesity and the associated consequences [87,88]. In addition, patients exhibit several other behavioral problems, and a subset of these meet full diagnostic criteria for autism spectrum disorder [89–91].

Interestingly, close to 50 children with a nonsense mutation in the paternal copy of *MAGEL2* have been identified with a PWS-like syndrome known as Schaaf-Yang syndrome (SHFYNG, OMIM #615547) (Figure 2A) [92–95]. This includes both *de novo* mutations and familial cases, with a mutational hotspot at nucleotides c.1990–1996. SHFYNG children present with several symptoms typically seen in PWS, such as developmental delay and intellectual disability, neonatal hypotonia, feeding difficulties and failure to thrive in early childhood as well as a characteristic behavior profile with violent outbursts, suggesting an important contribution of MAGEL2 in the pathogenesis of PWS (Table 1). However, SHFYNG patients experience some symptoms that are not typically seen in classic PWS. In particular, most of them do not develop severe hyperphagia and morbid obesity [92–95], which are considered the hallmark features of PWS [87]. In addition, joint contractures are almost uniformly present among SHFYNG infants, whereas they are less frequently seen in PWS [93,95–97]. Furthermore, patients with truncating mutations in *MAGEL2* have a higher prevalence of autism spectrum disorder (ASD) compared with those with classical PWS [92–94,98]. Recently, a new case of *de novo* nonsense mutation in MAGEL2 was reported in an infant diagnosed with Opitz trigonocephaly C syndrome characterized by craniofacial anomalies, intellectual and psychomotor disability, and cardiac defects with a high mortality rate [99].

In contrast to the *MAGEL2* point mutation cases, individuals with atypical PWS deletions that include *MAGEL2* but not *SNORD116* cluster were reported to have milder phenotypes than seen in PWS or SHFYNG with point mutations in *MAGEL2* [100,101]. Although these patients displayed some symptoms of PWS and SHFYNG, such as motor and developmental delays, mild feeding difficulties, no joint contractures, signs of autism, or hyperphagia were reported in cases of *MAGEL2* whole gene deletion [100,101]. It is intriguing that a deletion of the entire gene leads to a milder phenotype than a truncating mutation. It is possible that the deletion of the complete paternal copy of the gene and its promoter, as seen in the latter cases, could lead to the leaky expression of the maternal copy of the *MAGEL2* gene, as shown in the recent finding of stochastic loss of silencing of the imprinted *Ndn* allele in mice [102,103]. Alternatively, MAGEL2 is coded by a single exon and therefore mutations in *MAGEL2* probably do not cause nonsense-mediated decay of the mRNA and may result in aberrant truncated protein products with potential dominant negative or other neomorphic effects.

In addition to MAGEL2 mutations, USP7 is also mutated in a subset of children with similar phenotypes to those seen in PWS and SHFYNG, providing further evidence that MAGEL2 with its binding partners regulate biological pathways that are affected in patients with PWS and SHFYNG [35]. The original seven infants identified with *USP7* haploinsufficiency manifested several symptoms of PWS and SHFYNG and a high prevalence of ASD, similar to that seen in SHFYNG (Table 1). However, children with *USP7* mutation often present with seizures, which is less common in those with PWS and SHFYNG [87,92], suggesting that MAGEL2-independent cellular functions of USP7 and possibly TRIM27 contribute to the phenotypes seen in patients with USP7 haploinsufficiency [13,38–45,78,79,81]. A number of additional cases have been identified

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DOI: 10.1042/BCJ20160616

that await further characterization and will help to improve understanding of the molecular mechanisms underlying the observed phenotypes. Furthermore, for a number of years, USP7 has been seen as a high-value drug target, given its important regulation of a number of cellular pathways relevant to cancer, including p53. Biotech and pharmaceutical companies have developed highly potent and specific USP7 inhibitors for the treatment of a variety of cancers [104,105]. Identification of a neurodevelopmental disorder caused by mutation/deletion of one copy of USP7 raises concerns over the potential use of USP7 inhibitors, especially in pregnant women and children.

Given the implications of MAGEL2 and USP7 in neurodevelopmental disorders, knowing the cargo proteins that depend on this pathway will be essential in order to fully understand the physiological function and pathological implications of the MAGEL2-TRIM27 MRL and potentially treat this devastating disease. Substantial evidence now points to the nervous system as being highly sensitive to retromer dysfunction, and retromer-endosomal trafficking is critical for neuronal plasticity [106]. It has been shown that retromer trafficking contributes to both sides of synaptic functions, including being important for the presynaptic release of neurotransmitters and receptor density in the postsynaptic membrane [107]. Additionally, retromer has been implicated in several neurodegenerative diseases, including Alzheimer's and Parkinson's disease [108,109]. Given the CNS-enriched expression of MAGEL2, it will be of interest in the future to explore whether the MAGEL2-TRIM27 MRL functions in the pathogenesis of neurodegenerative diseases through regulation of the retromer recycling pathway.

Magel2 mutant mouse models reveal insights into its physiological function

Mice with a targeted deletion of *Magel2* recapitulate fundamental aspects of PWS and SHFYNG, additionally implying an important role of MAGEL2 in the pathogenesis of these disorders. The mouse *Magel2* gene is localized in a syntenic region of the human PWS cluster on mouse chromosome 7, which is also maternally imprinted (Figure 2F) [36,84]. *Magel2* loss of function has been studied in two animal models. The first mouse model was created by replacing the *Magel2* coding sequence with a *LacZ* reporter leaving the original promoter intact [110,111]. Neonatal *Magel2* null mice fail to thrive, have a slight increase in embryonic mortality and exhibit growth retardation in early life that is followed by weight gain after weaning and increased adiposity with disrupted metabolism and endocrine homeostasis (Table 1) [110–113]. These mice have a blunted circadian rhythm leading to general hypoactivity associated with abnormal feeding behavior, resembling symptoms seen in PWS patients [110–112,114]. At the cellular level, the MAGEL2 protein was shown to interact with and modulate the activity of the core components of the circadian clock, further suggesting an important role for MAGEL2 in regulating circadian rhythm [115]. *Magel2*-deficient mice present with several endocrine dysfunctions that resemble those seen in PWS and SHFYNG patients, including hypothalamic dysfunction, which is consistent with the expression pattern of *Magel2* (Figure 2) [110]. In line with disrupted sleep–wake cycles in mice and PWS and SHFYNG patients, *Magel2* knockout mice have decreased orexin level, accompanied by an increase in prohormone prepro-orexin, suggesting a defect in the maturation process of the hormone [110]. *Magel2* knockout mice also have decreased levels of diverse neurotransmitters, including serotonin, dopamine and catecholaminergic biogenic amines in various regions of the brain [112,116] and manifest several abnormal behaviors, such as an increase in anxiety in novel environments, altered social phenotypes, and a deficit in preference for social novelty [112,116,117]. Notably, low CNS serotonin levels have been associated with impulsive, aggressive and self-injurious behavior that is exemplified in PWS patients [118].

Consistent with the hypothalamic defects and phenotypes seen in PWS patients, *Magel2* knockout mice also have compromised fertility [110–112], impaired ghrelin and growth hormone axis [113,119], insulin and leptin insensitivity, increased corticosterone levels and deregulated glucose and cholesterol homeostasis [111,113,119]. However, despite compromised hypothalamic circuits, mice do not develop hyperphagia or severe obesity, but they do display changes in body composition, such as decreased lean mass, increased adiposity, changes in the distribution of muscle fiber types, and decreased bone mineral content [112,120–123]. This suggests that MAGEL2-dependent pathways may contribute to hypotonia, altered body composition and a high incidence of musculoskeletal abnormalities in PWS and SHFYNG [95,124–127].

Muscatelli et al. developed a second mouse model in which the *Magel2* promoter and most of the gene is deleted, leaving the last 1165 bp of the 4226 bp coding sequence [128]. These *Magel2*-deficient mice showed higher neonatal mortality due to suckling defects and impaired feeding. In line with the previous model, the

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surviving male mice manifest deficits in appreciation of social novelty, spatial learning and memory. One of the most prominent neuroendocrine dysfunctions in these *Magel2*-deficient mice was impaired production of oxytocin; neonatal lethality and the social deficits could be rescued by oxytocin injections [128,129]. Detailed analysis of the Magel2 knockout hypothalamus revealed that oxytocin maturation from a pro-hormone is compromised and is consistent with the orexin maturation defects in the mouse model developed by Wevrick and collaborators [110,111]. These studies suggest that proteolytic processing in the secretory pathway may require MAGEL2. Despite the phenotypic differences, the two described mouse models recapitulate several of the manifested phenotypes in PWS and SHFYNG and will provide an important opportunity to examine the physiological basis for symptoms that affect children with deletion or mutation of the *MAGEL2* gene.

Conclusions and future perspectives

MAGEs are an interesting family of ubiquitin ligase regulators that have been implicated in a number of cellular, physiological and pathological processes. The type II MAGE gene, MAGEL2, has been linked to PWS and SHFYNG genetic diseases [36,84,85,92–95]. Additionally, its biochemical and cellular functions have begun to be illuminated, which implicate it in the regulation of actin assembly on endosomes and retromer-dependent endosomal protein recycling [25,35]. Going forward, we need to continue to uncover the cellular and molecular pathways that are regulated by MAGEL2 in order to fully understand the phenotypes seen in patients and mice. In doing so, we will hopefully enable improved targeted therapies to improve the quality of life of people affected with MAGEL2 and USP7 disorders as well as discovering fundamental aspects of basic cell biology.

Funding

This work was supported by NIGMS grant R01GM111332 and Foundation for Prader-Willi Syndrome Research grant 342604 to P.R.P.

Acknowledgements

We would like to thank members of the Potts lab for advice and critical discussions. Additionally, we would like to thank Françoise Muscatelli, Christian Schaaf, and Rachel Wevrick for the helpful discussions and critical feedback. We apologise for our inability to discuss and reference all work in the field due to space limitations.

Competing Interests

The Authors declare that there are no competing interests associated with the manuscript.

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