Published in final edited form as:

Biochem J. ; 474(13): 2177–2190. doi:10.1042/BCJ20160616.

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

Klementina Fon Tacer and Patrick Ryan Potts*

Department of Cell and Molecular Biology, St. Jude Children's Research Hospital, Memphis, TN 38105-3678

Abstract

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 and its physiological functions elucidated through the study of *MageI2* knockout mouse models.

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* belongs 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* in eutherian mammals.

*Correspondence: Patrick Ryan Potts, 262 Danny Thomas Pl, Memphis, TN 38105-3678 ryan.potts@stjude.org (901) 595-8791.

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

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 pattern 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 (*MAGEG1*) 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 MAGE-like gene in zebrafish, fruitfly and chicken are present in a single copy in those organisms and are expressed in the brain of the developing embryo and adult animal and involved in neurogenesis (10-13). Interestingly, 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 (10-13). In contrast, the yeast and fruit fly MAGE proteins have been implicated in DNA damage response and repair as part of the SMC5/6 complex (14-16). In mammals *MAGE-G1* (also known as *NSMCE3*) plays a similar function (17).

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 (18-20). This flexibility and sequence diversity allow specific MAGEs to interact with distinct partners that confer individualized functions.

MAGE-RING Ligases (MRLs)

Based on their specific expression and immunogenic activity, several MAGE proteins have been extensively investigated as targets for cancer immunotherapy (7, 21-24). However, their molecular and physiological functions remained unclear for several years. Recently it was uncovered that MAGE proteins serve as regulators of E3 RING ubiquitin ligases (Figure 1D) (18, 25, 26). E3 ligases specify substrates for ubiquitination and mediate the transfer of ubiquitin from an E2 ubiquitin-conjugating enzyme to a substrate (27). The vast majority of E3 ligases belong to the group of RING E3s, encoded by over 600 human genes, that either target substrates for proteasome-dependent proteolysis or modulate their protein function, structure, assembly, and/or localization (28). 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 (29). 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) (18, 26, 30-32). MAGEs bind E3 ligases through their MHD that is both necessary and sufficient for binding (18). 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 (18). This specificity is likely driven by both sequence diversity of MHDs and structural dynamics in the MHD that determines its three-dimensional 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 (18, 33, 34). In addition, MAGE-A1 and MAGE-G1 can stimulate the ligases activity of their cognate E3 RING ligase, TRIM31 and NSE1, respectively (18, 32). 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 and mediating its ubiquitination by TRIM28 (25) or change substrate preference in the case of MAGE-A11 and Skp2 (35). Also, 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 (26). 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 enriched in the mouse brain and specifically within the hypothalamus (Figure 2C-D) (36). 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 (36, 37). Around mid-gestation, *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 central nervous system in the adult animal, specifically to the hypothalamus (36, 37). 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, 18, 25, 26, 38), MAGEL2 binds to the E3 RING ubiquitin ligase TRIM27 (18, 26). Further *in vitro* studies revealed that like the architecture of several other MRL complexes (18), the MHD of MAGEL2 directly interacts with the coiled-coil domain of TRIM27 (18, 26). 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-protein interaction motif. TRIM27 has been implicated in a number of cellular and disease processes, including PTEN, AKT, and NF- κ B signaling, transcription, T-cell activation, apoptosis, innate immunity, spermatogenesis, muscle atrophy, cancer, and autism (14, 39-46). 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 (26).

Intriguingly, a fraction of MAGEL2 and TRIM27 are co-localized in discrete cytoplasmic puncta (26, 47). 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 (26). 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) (48, 49). In the absence of retromer, its cargo proteins are sorted through the default multi-vesicular body trafficking route leading to degradation in the lysosome (48, 49). Retromer is recruited to endosomes in a Rab7-dependent manner where it or its associated SNX proteins bind to specific membrane proteins in endosomes and recycles these to the TGN or plasma membrane sparing them from degradation (50-55). 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) (52, 56-59). 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 that facilitates formation of F-actin on endosomes (49, 60, 61). The precise function of F-actin on endosomes is still debated, but 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 (49, 60, 61). 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 (62). Importantly, cells depleted of MAGEL2 or TRIM27 have diminished endosomal F-actin and Arp2/3 due to compromised WASH activity (26, 36). WASH exists in a macromolecular complex termed the WASH regulatory complex (SHRC) (60, 61). 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) (63, 64). The C-terminal FAM21 tail mediates the recruitment of WASH to endosomes through binding the VPS35 subunit of retromer (60, 61, 65, 66). 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 that has been proposed to work as a timing mechanism in which sufficient retromer binding to endosomes is necessary before WASH can be recruited to endosomes (49, 61, 65, 66).

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 (63, 64, 67-69). 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 (70), including small GTPases CDC42 and RAC1, PIP2 and PIP3 phospholipids, and phosphorylation by the SRC, ABL, and CDK family of kinases (67-72). 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 (73). For example, K63-linked polyubiquitin chains are critical for EGFR lysosomal sorting and are involved in protein sorting in yeast (74, 75). 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 to activate it. 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 to ubiquitous WASH, suggesting that MAGEL2 serves as a tissue specific regulator for WASH activation and additional forms of

regulation to activate WASH likely exist, such as regulation by small GTPases, inositol 4,5-bisphosphate, phosphorylation, and interaction with other binding partners (76).

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 (77, 78). Interestingly, MAGE-L2 and TRIM27 form a stable, stoichiometric complex with the ubiquitin-specific protease 7 (USP7; also known as herpesvirus-associated ubiquitin-specific protease, HAUSP) deubiquitinating enzyme through multiple intricate interactions (36). We term this the MUST complex for MAGE-L2-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 (79-81). USP7 regulates the ubiquitination of many proteins, for example the MDM2-p53 pathway, and impacts multiple cellular and disease processes, such as DNA repair, transcription, immune responses, viral replication, and cancer (79, 80). 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* (82). In addition to regulating the MAGE-L2-TRIM27 MRL, USP7 has also been shown to be involved in MAGE-L2-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 (83).

USP7 has a complex role in regulation of the MAGE-L2-TRIM27 MRL and retromer-mediated endosomal recycling (Figure 3B) (36). First, USP7 deubiquitinates TRIM27 to prevent its own auto-degradation and thus stabilizes the complex (36). Second, USP7 controls WASH ubiquitination to prevent over-activation of WASH and ensure proper levels of endosomal actin are generated (36). This function is critical given that over-production of endosomal actin has been shown to be detrimental (36, 61). Thus, USP7 functions as a rheostat for MAGE-L2-TRIM27 function that enable fine-tuning of actin nucleation. USP7 is not the only DUB implicated in protein trafficking through the endocytic pathway as Cezanne, 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 case of USP8 work in the retromer pathway (84, 85). Further studies on the enzymatic activity of this intricate E3-DUB complex are warranted and in particular what regulatory mechanisms and signaling pathways feed into controlling its activity.

MAGE-L2 in Prader-Willi and Schaaf-Yang Syndromes

MAGE-L2 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) (37, 86, 87). 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 first years of life, followed by a global developmental delay, breathing defects, specific behavior and multiple endocrine dysfunctions (Table 1)

(88). 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 its consequences (89, 90). In addition, patients exhibit several other behavioral problems, and a subset of them meet full diagnostic criteria for autism spectrum disorder (91-93).

Interestingly, close to 50 children with a nonsense mutations in the paternal copy of *MAGEL2* have been identified with a PWS-like syndrome known as Schaaf-Yang syndrome (SHFYNG, OMIM #615547) (Figure 2A) (94-97). 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 (94-97), which are considered the hallmark features of PWS (89). In addition, joint contractures are almost uniformly present among SHFYNG infants whereas they are less frequently seen in PWS (95, 97-99). Furthermore, patients with truncating mutations in *MAGEL2* have a higher prevalence of autism spectrum disorder (ASD) compared to classical PWS (94-96, 100). 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 (101).

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* (102, 103). 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 (102, 103). 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 (104, 105). Alternatively, *MAGEL2* is coded by a single exon and therefore mutations in *MAGEL2* likely 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 syndrome. (36). 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 syndrome (Table 1). However, children with *USP7* mutation often present with seizures which is less common in PWS and SHFYNG syndrome (89, 94), suggesting that MAGEL2-independent cellular functions of USP7 and possibly TRIM27 contribute to the phenotypes seen in patients with USP7 haploinsufficiency (14, 39-46, 79, 80, 82). A number of additional cases have been identified that await further characterization and will help better understand 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 (106, 107). 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 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 (108). 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 (109). Additionally, retromer has been implicated in several neurodegenerative diseases, including Alzheimer's and Parkinson's disease (110, 111). 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 that is also maternally imprinted (Figure 2F) (37, 86). *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 (112, 113). Neonatal *Magel2* null mice fail to thrive, have 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) (112-115). These mice have blunted circadian rhythm leading to general hypo-activity associated with abnormal feeding behavior, resembling symptoms seen in PWS patients (112-114, 116). 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 (117). *Magel2* deficient mice present with several endocrine dysfunctions resembling ones seen in patients, including hypothalamic dysfunction which is consistent with the expression pattern of

Magel2 (Figure 2) (112). In line with disrupted sleep-wake cycles in mice and patients, *Magel2* knockout mice have decreased orexin level, accompanied by increase in prohormone prepro-orexin, suggesting a defect in the maturation process of the hormone (112). *Magel2* knockout mice also have decreased levels of diverse neurotransmitters, including serotonin, dopamine and catecholaminergic biogenic amines in various regions of the brain (114, 118) and manifest several abnormal behaviors, such as an increase in anxiety in novel environment, altered social phenotypes, and a deficit in preference for social novelty (114, 118, 119). Notably, low central nervous system (CNS) serotonin levels have been associated with impulsive, aggressive and self-injurious behavior that is exemplified in PWS patients (120).

Consistent with the hypothalamic defects and phenotypes seen in patients, *Magel2* knockout mice have also compromised fertility (112-114), impaired ghrelin and growth hormone axis (115, 121), insulin and leptin insensitivity, increased corticosterone levels and deregulated glucose and cholesterol homeostasis (113, 115, 121). 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 and increased adiposity distribution of muscle fiber types and bone mineral content (114, 122-125). This suggests that MAGEL2-dependent pathways may contribute to hypotonia, altered body composition and a high incidence of musculoskeletal abnormalities in PWS and SHFYNG (97, 126-129).

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 (130). These *Magel2* deficient mice showed higher neonatal mortality due to suckling defects and impaired feeding. In line with the previous model, the surviving male mice manifest deficits in appreciation of social novelty, spatial learning and memory. One of the most prominent neuroendocrine dysfunction in these *Magel2*-deficient mice was impaired production of oxytocin and the neonatal lethality and the social deficits could be rescued by oxytocin injections (130, 131). 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 (112, 113). 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 manifested phenotypes in PWS and SHFYNG syndrome 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 (37, 86, 87, 94-97). Additionally, its biochemical and cellular functions have begun to be illuminated that implicate it in the regulation of actin assembly on endosomes and retromer-dependent endosomal protein recycling (26, 36). Going forward, we need to continue to uncover the cellular and molecular pathways that are regulated by MAGEL2 to fully understand the

phenotypes seen in patients and mice. In do so, we will hopefully enable better targeted therapies to improve quality of life of people affected with MAGEL2 and USP7 disorders and discover fundamental aspects of basic cell biology.

Acknowledgments

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 apologies for our inability to discuss and reference all work in the field due to space limitations.

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

References

1. Jager E, Knuth A. The discovery of cancer/testis antigens by autologous typing with T cell clones and the evolution of cancer vaccines. *Cancer Immun.* 2012; 12:6. [PubMed: 22896751]
2. Chomez P, De Backer O, Bertrand M, De Plaen E, Boon T, Lucas S. An Overview of the MAGE Gene Family with the Identification of All Human Members of the Family. *Cancer Research.* 2001; 61(14):5544–51. [PubMed: 11454705]
3. Barker PA, Salehi A. The MAGE proteins: Emerging roles in cell cycle progression, apoptosis, and neurogenetic disease. *Journal of Neuroscience Research.* 2002; 67(6):705–12. [PubMed: 11891783]
4. Lopez-Sanchez N, Gonzalez-Fernandez Z, Niinobe M, Yoshikawa K, Frade JM. Single mage gene in the chicken genome encodes CMage, a protein with functional similarities to mammalian type II Mage proteins. *Physiol Genomics.* 2007; 30(2):156–71. [PubMed: 17374844]
5. Zhao Q, Caballero OL, Simpson AJ, Strausberg RL. Differential evolution of MAGE genes based on expression pattern and selection pressure. *PLoS One.* 2012; 7(10):e48240. [PubMed: 23133577]
6. Katsura Y, Satta Y. Evolutionary history of the cancer immunity antigen MAGE gene family. *PLoS One.* 2011; 6(6):e20365. [PubMed: 21695252]
7. Weon JL, Potts PR. The MAGE protein family and cancer. *Current Opinion in Cell Biology.* 2015; 37:1–8. [PubMed: 26342994]
8. Chibuk TK, Bischof JM, Wevrick R. A necdin/MAGE-like gene in the chromosome 15 autism susceptibility region: expression, imprinting, and mapping of the human and mouse orthologues. *BMC Genetics.* 2001; 2:22-. [PubMed: 11782285]
9. Watrin F, Le Meur E, Roeckel N, Ripoche MA, Dandolo L, Muscatelli F. The Prader-Willi syndrome murine imprinting center is not involved in the spatio-temporal transcriptional regulation of the Necdin gene. *BMC Genetics.* 2005; 6(1):1. [PubMed: 15634360]
10. Bischof JM, Ekker M, Wevrick R. A MAGE/NDN-like gene in zebrafish. *Developmental Dynamics.* 2003; 228(3):475–9. [PubMed: 14579385]
11. Nishimura I, Sakoda JY, Yoshikawa K. Drosophila MAGE controls neural precursor proliferation in postembryonic neurogenesis. *Neuroscience.* 2008; 154(2):572–81. [PubMed: 18479827]
12. Nishimura I, Shimizu S, Sakoda Jy, Yoshikawa K. Expression of Drosophila MAGE gene encoding a necdin homologous protein in postembryonic neurogenesis. *Gene Expression Patterns.* 2007; 7(3):244–51. [PubMed: 17084677]
13. López-Sánchez N, González-Fernández Z, Niinobe M, Yoshikawa K, Frade JM. Single mage gene in the chicken genome encodes CMage, a protein with functional similarities to mammalian type II Mage proteins. *Physiological Genomics.* 2007; 30(2):156–71. [PubMed: 17374844]
14. Lee JT, Shan J, Zhong J, Li M, Zhou B, Zhou A, et al. RFP-mediated ubiquitination of PTEN modulates its effect on AKT activation. *Cell Res.* 2013; 23(4):552–64. [PubMed: 23419514]
15. Pebernard S, McDonald WH, Pavlova Y, Yates JR 3rd, Boddy MN. Nse1, Nse2, and a novel subunit of the Smc5-Smc6 complex, Nse3, play a crucial role in meiosis. *Mol Biol Cell.* 2004; 15(11):4866–76. [PubMed: 15331764]
16. Potts PR. The Yin and Yang of the MMS21-SMC5/6 SUMO ligase complex in homologous recombination. *DNA Repair (Amst).* 2009; 8(4):499–506. [PubMed: 19217832]

17. Taylor EM, Copsey AC, Hudson JJ, Vidot S, Lehmann AR. Identification of the proteins, including MAGEG1, that make up the human SMC5-6 protein complex. *Mol Cell Biol.* 2008; 28(4):1197–206. [PubMed: 18086888]
18. Doyle JM, Gao J, Wang J, Yang M, Potts PR. MAGE-RING protein complexes comprise a family of E3 ubiquitin ligases. *Mol Cell.* 2010; 39(6):963–74. [PubMed: 20864041]
19. Newman JA, Cooper CD, Roos AK, Aitkenhead H, Oppermann UC, Cho HJ, et al. Structures of Two Melanoma-Associated Antigens Suggest Allosteric Regulation of Effector Binding. *PLoS One.* 2016; 11(2):e0148762. [PubMed: 26910052]
20. Hagiwara Y, Sieverling L, Hanif F, Anton J, Dickinson ER, Bui TT, et al. Consequences of point mutations in melanoma-associated antigen 4 (MAGE-A4) protein: Insights from structural and biophysical studies. *Sci Rep.* 2016; 6:25182. [PubMed: 27121989]
21. Goldman B, DeFrancesco L. The cancer vaccine roller coaster. *Nat Biotech.* 2009; 27(2):129–39.
22. Brichard VG, Lejeune D. GSK's antigen-specific cancer immunotherapy programme: Pilot results leading to Phase III clinical development. *Vaccine.* 2007; 25(20):B61–B71. [PubMed: 17916463]
23. Scanlan MJ, Gure AO, Jungbluth AA, Old LJ, Chen YT. Cancer/testis antigens: an expanding family of targets for cancer immunotherapy. *Immunological Reviews.* 2002; 188(1):22–32. [PubMed: 12445278]
24. van der Bruggen P, Traversari C, Chomez P, Lurquin C, De Plaen E, Van den Eynde B, et al. A gene encoding an antigen recognized by cytolytic T lymphocytes on a human melanoma. *Science.* 1991; 254(5038):1643–7. [PubMed: 1840703]
25. Pineda Carlos T, Ramanathan S, Fon Tacer K, Weon Jenny L, Potts Malia B, Ou YH, et al. Degradation of AMPK by a Cancer-Specific Ubiquitin Ligase. *Cell.* 2015; 160(4):715–28. [PubMed: 25679763]
26. Hao YH, Doyle Jennifer M, Ramanathan S, Gomez Timothy S, Jia D, Xu M, et al. Regulation of WASH-Dependent Actin Polymerization and Protein Trafficking by Ubiquitination. *Cell.* 2013; 152(5):1051–64. [PubMed: 23452853]
27. Metzger MB, Pruneda JN, Klevit RE, Weissman AM. RING-type E3 ligases: Master manipulators of E2 ubiquitin-conjugating enzymes and ubiquitination. *Biochimica et Biophysica Acta (BBA) - Molecular Cell Research.* 2014; 1843(1):47–60. [PubMed: 23747565]
28. Deshaies RJ, Joazeiro CA. RING domain E3 ubiquitin ligases. *Annu Rev Biochem.* 2009; 78:399–434. [PubMed: 19489725]
29. Vittal V, Stewart MD, Brzovic PS, Klevit RE. Regulating the Regulators: Recent Revelations in the Control of E3 Ubiquitin Ligases. *J Biol Chem.* 2015; 290(35):21244–51. [PubMed: 26187467]
30. Reddy EM, Chettiar ST, Kaur N, Ganeshkumar R, Shepal V, Shanbhag NC, et al. Dlxin-1, a member of MAGE family, inhibits cell proliferation, invasion and tumorigenicity of glioma stem cells. *Cancer gene therapy.* 2011; 18(3):206–18. [PubMed: 21109781]
31. Hao J, Song X, Wang J, Guo C, Li Y, Li B, et al. Cancer-testis antigen MAGE-C2 binds Rbx1 and inhibits ubiquitin ligase-mediated turnover of cyclin E. *Oncotarget.* 2015; 6(39)
32. Kozakova L, Vondrova L, Stejskal K, Charalabous P, Kolesar P, Lehmann AR, et al. The melanoma-associated antigen 1 (MAGEA1) protein stimulates the E3 ubiquitin-ligase activity of TRIM31 within a TRIM31-MAGEA1-NSE4 complex. *Cell Cycle.* 2015; 14(6):920–30. [PubMed: 25590999]
33. Xiao TZ, Suh Y, Longley BJ. MAGE proteins regulate KRAB zinc finger transcription factors and KAP1 E3 ligase activity. *Arch Biochem Biophys.* 2014; 563:136–44. [PubMed: 25107531]
34. Xiao TZ, Bhatia N, Urrutia R, Lomberg GA, Simpson A, Longley BJ. MAGE I transcription factors regulate KAP1 and KRAB domain zinc finger transcription factor mediated gene repression. *PLoS One.* 2011; 6(8):e23747. [PubMed: 21876767]
35. Su S, Chen X, Geng J, Mingos JT, Grossman G, Wilson EM. Melanoma antigen-A11 regulates substrate-specificity of Skp2-mediated protein degradation. *Mol Cell Endocrinol.* 2017; 439:1–9. [PubMed: 27720894]
36. Hao YH, Fountain Michael D Jr, Fon Tacer K, Xia F, Bi W, Kang S-Hae L, et al. USP7 Acts as a Molecular Rheostat to Promote WASH-Dependent Endosomal Protein Recycling and Is Mutated in a Human Neurodevelopmental Disorder. *Molecular Cell.* 2015; 59(6):956–69. [PubMed: 26365382]

37. Lee S, Kozlov S, Hernandez L, Chamberlain SJ, Brannan CI, Stewart CL, et al. Expression and imprinting of MAGEL2 suggest a role in Prader-will syndrome and the homologous murine imprinting phenotype. *Hum Mol Genet.* 2000; 9(12):1813–9. [PubMed: 10915770]
38. Mouri A, Noda Y, Watanabe K, Nabeshima T. The roles of MAGE-D1 in the neuronal functions and pathology of the central nervous system. *Rev Neurosci.* 2013; 24(1):61–70. [PubMed: 23314527]
39. St Pourcain B, Whitehouse AJ, Ang WQ, Warrington NM, Glessner JT, Wang K, et al. Common variation contributes to the genetic architecture of social communication traits. *Mol Autism.* 2013; 4(1):34. [PubMed: 24047820]
40. Zaman MM, Nomura T, Takagi T, Okamura T, Jin W, Shinagawa T, et al. Ubiquitination-deubiquitination by the TRIM27-USP7 complex regulates tumor necrosis factor alpha-induced apoptosis. *Mol Cell Biol.* 2013; 33(24):4971–84. [PubMed: 24144979]
41. Zheng Q, Hou J, Zhou Y, Yang Y, Xie B, Cao X. Siglec1 suppresses antiviral innate immune response by inducing TBK1 degradation via the ubiquitin ligase TRIM27. *Cell Res.* 2015; 25(10):1121–36. [PubMed: 26358190]
42. Cao T, Shannon M, Handel MA, Etkin LD. Mouse ret finger protein (rfp) proto-oncogene is expressed at specific stages of mouse spermatogenesis. *Dev Genet.* 1996; 19(4):309–20. [PubMed: 9023983]
43. Dho SH, Kwon KS. The Ret finger protein induces apoptosis via its RING finger-B box-coiled-coil motif. *J Biol Chem.* 2003; 278(34):31902–8. [PubMed: 12807881]
44. Joung H, Eom GH, Choe N, Lee HM, Ko JH, Kwon DH, et al. Ret finger protein mediates Pax7-induced ubiquitination of MyoD in skeletal muscle atrophy. *Cell Signal.* 2014; 26(10):2240–8. [PubMed: 25025573]
45. Shimono Y, Murakami H, Hasegawa Y, Takahashi M. RET finger protein is a transcriptional repressor and interacts with enhancer of polycomb that has dual transcriptional functions. *J Biol Chem.* 2000; 275(50):39411–9. [PubMed: 10976108]
46. Zha J, Han KJ, Xu LG, He W, Zhou Q, Chen D, et al. The Ret finger protein inhibits signaling mediated by the noncanonical and canonical IkappaB kinase family members. *J Immunol.* 2006; 176(2):1072–80. [PubMed: 16393995]
47. Harbers M, Nomura T, Ohno S, Ishii S. Intracellular localization of the Ret finger protein depends on a functional nuclear export signal and protein kinase C activation. *J Biol Chem.* 2001; 276(51):48596–607. [PubMed: 11591718]
48. Burd C, Cullen PJ. Retromer: a master conductor of endosome sorting. *Cold Spring Harb Perspect Biol.* 2014; 6(2)
49. Seaman MN, Gautreau A, Billadeau DD. Retromer-mediated endosomal protein sorting: all WASHed up! *Trends Cell Biol.* 2013; 23(11):522–8. [PubMed: 23721880]
50. Fjorback AW, Seaman M, Gustafsen C, Mehmedbasic A, Gokool S, Wu C, et al. Retromer binds the FANSHY sorting motif in SorLA to regulate amyloid precursor protein sorting and processing. *J Neurosci.* 2012; 32(4):1467–80. [PubMed: 22279231]
51. Rojas R, van Vlijmen T, Mardones GA, Prabhu Y, Rojas AL, Mohammed S, et al. Regulation of retromer recruitment to endosomes by sequential action of Rab5 and Rab7. *J Cell Biol.* 2008; 183(3):513–26. [PubMed: 18981234]
52. Steinberg F, Gallon M, Winfield M, Thomas EC, Bell AJ, Heesom KJ, et al. A global analysis of SNX27-retromer assembly and cargo specificity reveals a function in glucose and metal ion transport. *Nat Cell Biol.* 2013; 15(5):461–71. [PubMed: 23563491]
53. Strohlic TI, Setty TG, Sitaram A, Burd CG. Grd19/Snx3p functions as a cargo-specific adapter for retromer-dependent endocytic recycling. *J Cell Biol.* 2007; 177(1):115–25. [PubMed: 17420293]
54. Temkin P, Lauffer B, Jager S, Cimermancic P, Krogan NJ, von Zastrow M. SNX27 mediates retromer tubule entry and endosome-to-plasma membrane trafficking of signalling receptors. *Nat Cell Biol.* 2011; 13(6):715–21. [PubMed: 21602791]
55. Hierro A, Rojas AL, Rojas R, Murthy N, Effantin G, Kajava AV, et al. Functional architecture of the retromer cargo-recognition complex. *Nature.* 2007; 449(7165):1063–7. [PubMed: 17891154]

56. Arighi CN, Hartnell LM, Aguilar RC, Haft CR, Bonifacino JS. Role of the mammalian retromer in sorting of the cation-independent mannose 6-phosphate receptor. *J Cell Biol.* 2004; 165(1):123–33. [PubMed: 15078903]
57. Eaton S. Retromer retrieves wntless. *Dev Cell.* 2008; 14(1):4–6. [PubMed: 18194646]
58. Seaman MN. Cargo-selective endosomal sorting for retrieval to the Golgi requires retromer. *J Cell Biol.* 2004; 165(1):111–22. [PubMed: 15078902]
59. Duleh SN, Welch MD. Regulation of integrin trafficking, cell adhesion, and cell migration by WASH and the Arp2/3 complex. *Cytoskeleton.* 2012; 69(12):1047–58. [PubMed: 23012235]
60. Derivery E, Sousa C, Gautier JJ, Lombard B, Loew D, Gautreau A. The Arp2/3 activator WASH controls the fission of endosomes through a large multiprotein complex. *Dev Cell.* 2009; 17(5):712–23. [PubMed: 19922875]
61. Gomez TS, Billadeau DD. A FAM21-containing WASH complex regulates retromer-dependent sorting. *Dev Cell.* 2009; 17(5):699–711. [PubMed: 19922874]
62. Puthenveedu MA, Lauffer B, Temkin P, Vistein R, Carlton P, Thorn K, et al. Sequence-Dependent Sorting of Recycling Proteins by Actin-Stabilized Endosomal Microdomains. *Cell.* 2010; 143(5):761–73. [PubMed: 21111236]
63. Chen Z, Borek D, Padrick SB, Gomez TS, Metlagel Z, Ismail AM, et al. Structure and control of the actin regulatory WAVE complex. *Nature.* 2010; 468(7323):533–8. [PubMed: 21107423]
64. Jia D, Gomez TS, Metlagel Z, Umetani J, Otwinowski Z, Rosen MK, et al. WASH and WAVE actin regulators of the Wiskott-Aldrich syndrome protein (WASP) family are controlled by analogous structurally related complexes. *Proc Natl Acad Sci U S A.* 2010; 107(23):10442–7. [PubMed: 20498093]
65. Jia D, Gomez TS, Billadeau DD, Rosen MK. Multiple repeat elements within the FAM21 tail link the WASH actin regulatory complex to the retromer. *Mol Biol Cell.* 2012; 23(12):2352–61. [PubMed: 22513087]
66. Harbour ME, Breusegem SY, Seaman MN. Recruitment of the endosomal WASH complex is mediated by the extended ‘tail’ of Fam21 binding to the retromer protein Vps35. *Biochem J.* 2012; 442(1):209–20. [PubMed: 22070227]
67. Ismail AM, Padrick SB, Chen B, Umetani J, Rosen MK. The WAVE regulatory complex is inhibited. *Nat Struct Mol Biol.* 2009; 16(5):561–3. [PubMed: 19363480]
68. Kim AS, Kakalis LT, Abdul-Manan N, Liu GA, Rosen MK. Autoinhibition and activation mechanisms of the Wiskott-Aldrich syndrome protein. *Nature.* 2000; 404(6774):151–8. [PubMed: 10724160]
69. Miki H, Sasaki T, Takai Y, Takenawa T. Induction of filopodium formation by a WASP-related actin-depolymerizing protein N-WASP. *Nature.* 1998; 391(6662):93–6. [PubMed: 9422512]
70. Padrick SB, Rosen MK. Physical mechanisms of signal integration by WASP family proteins. *Annu Rev Biochem.* 2010; 79:707–35. [PubMed: 20533885]
71. Eden S, Rohatgi R, Podtelejnikov AV, Mann M, Kirschner MW. Mechanism of regulation of WAVE1-induced actin nucleation by Rac1 and Nck. *Nature.* 2002; 418(6899):790–3. [PubMed: 12181570]
72. Lebensohn AM, Kirschner MW. Activation of the WAVE complex by coincident signals controls actin assembly. *Mol Cell.* 2009; 36(3):512–24. [PubMed: 19917258]
73. Komander D, Rape M. The ubiquitin code. *Annu Rev Biochem.* 2012; 81:203–29. [PubMed: 22524316]
74. Huang F, Zeng X, Kim W, Balasubramani M, Fortian A, Gygi SP, et al. Lysine 63-linked polyubiquitination is required for EGF receptor degradation. *Proceedings of the National Academy of Sciences.* 2013; 110(39):15722–7.
75. Lauwers E, Jacob C, André B. K63-linked ubiquitin chains as a specific signal for protein sorting into the multivesicular body pathway. *The Journal of Cell Biology.* 2009; 185(3):493–502. [PubMed: 19398763]
76. Bompard G, Caron E. Regulation of WASP/WAVE proteins: making a long story short. *The Journal of Cell Biology.* 2004; 166(7):957–62. [PubMed: 15452139]
77. Komander D, Clague MJ, Urbe S. Breaking the chains: structure and function of the deubiquitinases. *Nat Rev Mol Cell Biol.* 2009; 10(8):550–63. [PubMed: 19626045]

78. Reyes-Turcu FE, Ventii KH, Wilkinson KD. Regulation and cellular roles of ubiquitin-specific deubiquitinating enzymes. *Annu Rev Biochem.* 2009; 78:363–97. [PubMed: 19489724]
79. Li M, Chen D, Shiloh A, Luo J, Nikolaev AY, Qin J, et al. Deubiquitination of p53 by HAUSP is an important pathway for p53 stabilization. *Nature.* 2002; 416(6881):648–53. [PubMed: 11923872]
80. Nicholson B, Suresh Kumar KG. The multifaceted roles of USP7: new therapeutic opportunities. *Cell Biochem Biophys.* 2011; 60(1-2):61–8. [PubMed: 21468693]
81. Schaefer JB, Morgan DO. Protein-linked ubiquitin chain structure restricts activity of deubiquitinating enzymes. *J Biol Chem.* 2011; 286(52):45186–96. [PubMed: 22072716]
82. Kon N, Kobayashi Y, Li M, Brooks CL, Ludwig T, Gu W. Inactivation of HAUSP in vivo modulates p53 function. *Oncogene.* 2010; 29(9):1270–9. [PubMed: 19946331]
83. Zaman MMU, Nomura T, Takagi T, Okamura T, Jin W, Shinagawa T, et al. Ubiquitination-Deubiquitination by the TRIM27-USP7 Complex Regulates Tumor Necrosis Factor Alpha-Induced Apoptosis. *Molecular and Cellular Biology.* 2013; 33(24):4971–84. [PubMed: 24144979]
84. Clague MJ, Urbé S. Integration of cellular ubiquitin and membrane traffic systems: focus on deubiquitylases. *The FEBS Journal.* 2017:n/a–n/a.
85. MacDonald E, Urbé S, Clague MJ. USP8 Controls the Trafficking and Sorting of Lysosomal Enzymes. *Traffic.* 2014; 15(8):879–88. [PubMed: 24894536]
86. Boccaccio I, Glatt-Deeley H, Watrin F, Roeckel N, Lalande M, Muscatelli F. The human MAGEL2 gene and its mouse homologue are paternally expressed and mapped to the Prader-Willi region. *Hum Mol Genet.* 1999; 8(13):2497–505. [PubMed: 10556298]
87. Holm VA, Cassidy SB, Butler MG, Hanchett JM, Greenswag LR, Whitman BY, et al. Prader-Willi Syndrome: Consensus Diagnostic Criteria. *Pediatrics.* 1993; 91(2):398–402. [PubMed: 8424017]
88. Cassidy SB, Driscoll DJ. Prader-Willi syndrome. *Eur J Hum Genet.* 2008; 17(1):3–13. [PubMed: 18781185]
89. Cassidy SB, Schwartz S, Miller JL, Driscoll DJ. Prader-Willi syndrome. *Genetics in Medicine.* 2012; 14(1):10–26. [PubMed: 22237428]
90. Butler MG. Prader-Willi Syndrome: Obesity due to Genomic Imprinting. *Current Genomics.* 2011; 12(3):204–15. [PubMed: 22043168]
91. Greaves N, Prince E, Evans DW, Charman T. Repetitive and ritualistic behaviour in children with Prader-Willi syndrome and children with autism. *J Intellect Disabil Res.* 2006; 50(Pt 2):92–100. [PubMed: 16403198]
92. Koenig K, Klin A, Schultz R. Deficits in social attribution ability in Prader-Willi syndrome. *J Autism Dev Disord.* 2004; 34(5):573–82. [PubMed: 15628610]
93. Dykens EM, Lee E, Roof E. Prader-Willi syndrome and autism spectrum disorders: An evolving story. *Journal of Neurodevelopmental Disorders.* 2011; 3(3):225–37. [PubMed: 21858456]
94. Schaaf CP, Gonzalez-Garay ML, Xia F, Potocki L, Gripp KW, Zhang B, et al. Truncating mutations of MAGEL2 cause Prader-Willi phenotypes and autism. *Nat Genet.* 2013; 45(11):1405–8. [PubMed: 24076603]
95. Soden SE, Saunders CJ, Willig LK, Farrow EG, Smith LD, Petrikin JE, et al. Effectiveness of exome and genome sequencing guided by acuity of illness for diagnosis of neurodevelopmental disorders. *Science Translational Medicine.* 2014; 6(265):265ra168–265ra168.
96. Fountain MD, Aten E, Cho MT, Juusola J, Walkiewicz MA, Ray JW, et al. The phenotypic spectrum of Schaaf-Yang syndrome: 18 new affected individuals from 14 families. *Genet Med.* 2016
97. Mejlachowicz D, Nolent F, Maluenda J, Ranjatoelina-Randrianaivo H, Giuliano F, Gut I, et al. Truncating Mutations of MAGEL2, a Gene within the Prader-Willi Locus, Are Responsible for Severe Arthrogryposis. *The American Journal of Human Genetics.* 2015; 97(4):616–20. [PubMed: 26365340]
98. Denizot S, Boscher C, Le Vaillant C, Roze JC, Gras Le Guen C. Distal Arthrogryposis and Neonatal Hypotonia: an Unusual Presentation of Prader-Willi Syndrome (PWS). *J Perinatol.* 24(11):733–4. 0000.
99. Bigi N, Faure JM, Coubes C, Puechberty J, Lefort G, Sarda P, et al. Prader-Willi syndrome: is there a recognizable fetal phenotype? *Prenatal Diagnosis.* 2008; 28(9):796–9. [PubMed: 18661490]

100. Bennett JA, Germani T, Haqq AM, Zwaigenbaum L. Autism spectrum disorder in Prader-Willi syndrome: A systematic review. *American Journal of Medical Genetics Part A*. 2015; 167(12): 2936–44.
101. Urreiziti R, Cueto-Gonzalez AM, Franco-Valls H, Mort-Farre S, Roca-Ayats N, Ponomarenko J, et al. A De Novo Nonsense Mutation in MAGEL2 in a Patient Initially Diagnosed as Opitz-C: Similarities Between Schaaf-Yang and Opitz-C Syndromes. *Sci Rep*. 2017; 7:44138. [PubMed: 28281571]
102. Kanber D, Giltay J, Wiczorek D, Zogel C, Hochstenbach R, Caliebe A, et al. A paternal deletion of MKRN3, MAGEL2 and NDN does not result in Prader-Willi syndrome. *Eur J Hum Genet*. 2008; 17(5):582–90. [PubMed: 19066619]
103. Buiting K, Di Donato N, Beygo J, Bens S, von der Hagen M, Hackmann K, et al. Clinical phenotypes of MAGEL2 mutations and deletions. *Orphanet Journal of Rare Diseases*. 2014; 9(1): 40. [PubMed: 24661356]
104. Matarazzo V, Muscatelli F. Natural breaking of the maternal silence at the mouse and human imprinted Prader-Willi locus. *Rare Diseases*. 2013; 1(1):e27228. [PubMed: 25003016]
105. Rieusset A, Schaller F, Unmehopa U, Matarazzo V, Watrin F, Linke M, et al. Stochastic Loss of Silencing of the Imprinted Ndn/NDN Allele, in a Mouse Model and Humans with Prader-Willi Syndrome, Has Functional Consequences. *PLoS Genet*. 2013; 9(9):e1003752. [PubMed: 24039599]
106. Kessler BM. Selective and reversible inhibitors of ubiquitin-specific protease 7: a patent evaluation (WO2013030218). *Expert Opinion on Therapeutic Patents*. 2014; 24(5):597–602. [PubMed: 24456106]
107. McClurg UL, Robson CN. Deubiquitinating enzymes as oncotargets. *Oncotarget*. 2015; 6(12)
108. Zhang D, Isack NR, Glodowski DR, Liu J, Chen CC, Xu XZ, et al. RAB-6.2 and the retromer regulate glutamate receptor recycling through a retrograde pathway. *J Cell Biol*. 2012; 196(1):85–101. [PubMed: 22213799]
109. Small SA, Petsko GA. Retromer in Alzheimer disease, Parkinson disease and other neurological disorders. *Nat Rev Neurosci*. 2015; 16(3):126–32. [PubMed: 25669742]
110. Bonifacino JS, Rojas R. Retrograde transport from endosomes to the trans-Golgi network. *Nat Rev Mol Cell Biol*. 2006; 7(8):568–79. [PubMed: 16936697]
111. Small SA. Retromer sorting: A pathogenic pathway in late-onset alzheimer disease. *Archives of Neurology*. 2008; 65(3):323–8. [PubMed: 18332244]
112. Kozlov SV, Bogenpohl JW, Howell MP, Wevrick R, Panda S, Hogenesch JB, et al. The imprinted gene Magel2 regulates normal circadian output. *Nat Genet*. 2007; 39(10):1266–72. [PubMed: 17893678]
113. Bischof JM, Stewart CL, Wevrick R. Inactivation of the mouse Magel2 gene results in growth abnormalities similar to Prader-Willi syndrome. *Hum Mol Genet*. 2007; 16(22):2713–9. [PubMed: 17728320]
114. Mercer RE, Wevrick R. Loss of magel2, a candidate gene for features of Prader-Willi syndrome, impairs reproductive function in mice. *PLoS One*. 2009; 4(1):e4291. [PubMed: 19172181]
115. Tennese AA, Wevrick R. Impaired hypothalamic regulation of endocrine function and delayed counterregulatory response to hypoglycemia in Magel2-null mice. *Endocrinology*. 2011; 152(3): 967–78. [PubMed: 21248145]
116. Camfferman D, Doug McEvoy R, O'Donoghue F, Lushington K. Prader Willi Syndrome and excessive daytime sleepiness. *Sleep Medicine Reviews*. 2008; 12(1):65–75. [PubMed: 18201664]
117. Devos J, Weselake SV, Wevrick R. Magel2, a Prader-Willi syndrome candidate gene, modulates the activities of circadian rhythm proteins in cultured cells. *J Circadian Rhythms*. 2011; 9(1):12. [PubMed: 22208286]
118. Luck C, Vitaterna MH, Wevrick R. Dopamine pathway imbalance in mice lacking Magel2, a Prader-Willi syndrome candidate gene. *Behav Neurosci*. 2016; 130(4):448–59. [PubMed: 27254754]
119. Fountain MD, Tao H, Chen CA, Yin J, Schaaf CP. Magel2 knockout mice manifest altered social phenotypes and a deficit in preference for social novelty. *Genes Brain Behav*. 2017

120. Hellings JA, Warnock JK. Self-injurious behavior and serotonin in Prader-Willi syndrome. *Psychopharmacology bulletin*. 1994; 30(2):245–50. [PubMed: 7831463]
121. Mercer RE, Michaelson SD, Chee MJS, Atallah TA, Wevrick R, Colmers WF. Magel2 Is Required for Leptin-Mediated Depolarization of POMC Neurons in the Hypothalamic Arcuate Nucleus in Mice. *PLoS Genetics*. 2013; 9(1):e1003207. [PubMed: 23341784]
122. Goldstone AP, Brynes AE, Thomas EL, Bell JD, Frost G, Holland A, et al. Resting metabolic rate, plasma leptin concentrations, leptin receptor expression, and adipose tissue measured by whole-body magnetic resonance imaging in women with Prader-Willi syndrome. *The American Journal of Clinical Nutrition*. 2002; 75(3):468–75. [PubMed: 11864851]
123. l'Allemand D, Eiholzer U, Schlumpf M, Torresani T, Girard J. Carbohydrate Metabolism Is Not Impaired after 3 Years of Growth Hormone Therapy in Children with Prader-Willi Syndrome. *Hormone Research in Paediatrics*. 2003; 59(5):239–48.
124. Maillard J, Park S, Croizier S, Vanacker C, Cook JH, Prevot V, et al. Loss of Magel2 impairs the development of hypothalamic Anorexigenic circuits. *Human Molecular Genetics*. 2016
125. Kamaludin AA, Smolarchuk C, Bischof JM, Eggert R, Greer JJ, Ren J, et al. Muscle dysfunction caused by loss of Magel2 in a mouse model of Prader-Willi and Schaaf-Yang syndromes. *Human Molecular Genetics*. 2016
126. Reus L, van Vlimmeren LA, Staal JB, Janssen AJWM, Otten BJ, Pelzer BJ, et al. Objective evaluation of muscle strength in infants with hypotonia and muscle weakness. *Research in Developmental Disabilities*. 2013; 34(4):1160–9. [PubMed: 23380578]
127. Edouard T, Deal C, Van Vliet G, Gaulin N, Moreau A, Rauch F, et al. Muscle-Bone Characteristics in Children with Prader-Willi Syndrome. *The Journal of Clinical Endocrinology & Metabolism*. 2011; 97(2):E275–E81. [PubMed: 22162467]
128. Capodaglio P, Menegoni F, Vismara L, Cimolin V, Grugni G, Galli M. Characterisation of balance capacity in Prader-Willi patients. *Research in Developmental Disabilities*. 2011; 32(1):81–6. [PubMed: 20884170]
129. Nakamura Y, Murakami N, Iida T, Ozeki S, Asano S, Nohara Y, et al. The characteristics of scoliosis in Prader-Willi syndrome (PWS): analysis of 58 scoliosis patients with PWS. *Journal of Orthopaedic Science*. 2015; 20(1):17–22. [PubMed: 25253244]
130. Schaller F, Watrin F, Sturny R, Massacrier A, Szeppetowski P, Muscatelli F. A single postnatal injection of oxytocin rescues the lethal feeding behaviour in mouse newborns deficient for the imprinted Magel2 gene. *Hum Mol Genet*. 2010; 19(24):4895–905. [PubMed: 20876615]
131. Meziane H, Schaller F, Bauer S, Villard C, Matarazzo V, Riet F, et al. An Early Postnatal Oxytocin Treatment Prevents Social and Learning Deficits in Adult Mice Deficient for Magel2, a Gene Involved in Prader-Willi Syndrome and Autism. *Biological Psychiatry*. 2015; 78(2):85–94. [PubMed: 25599930]

Author Manuscript

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-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 RT-QPCR. (E-F) Schematic representation of the genetic architecture within the PWS cluster in humans (E) and the syntenic region in mice (F). In the 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*), *OCA2* 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.

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 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 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.

Overlapping and differential symptoms seen in patients with PWS, SHFYNG, and USP7 haploinsufficiency are compared to the relevant phenotypes seen in the two *Mage12* knockout mouse models. For details and references see the main text.

Table 1

	Patients			MAGE-L2 mouse KO models	
	PWS	SHFYNG	USP7	Wevrick et al.	Muscattelli et al.
PWS major criteria	Neonatal hypotonia, poor suck			10% prenatal mortality	50% postnatal mortality and suckling defects
	Feeding problems in infancy, failure to thrive			Neonatal failure to thrive and pre-wean growth retardation.	Impaired feeding and failure thrive
	Hyperphagia, lack of satiety			Increased leptin plasma levels and reduced leptin sensitivity	
	Obesity			Weight gain, decreased lean mass with increased adiposity after weaning	
	Developmental delay and intellectual disability			Abnormal behavior in novel environments	Deficits in novel object exploration, spatial learning and memory
	Hypogonadism			Progressive infertility and decreased olfactorydiscrimination	
	Hypotonia			Decreased locomotor activity, muscle dysfunction and decreased bone mineral content	
	Characteristicfacial features				
	Infantile lethargy and decreased fetal movement				
	Short stature			Growth hormone axis impairment	
PWS minor criteria	Small hands				
	Narrow hands				
	Eye abnormalities				
	Hypopigmentation				
	Thick saliva				
	Characteristic behavior			Abnormal in novel environments	Decreased oxytocin levels
	Speech articulation defects				
	Skin picking			Decreased serotonin levels.	
	Sleep apnea			Blunted circadian rhythm, decreased orexin levels	
		ASD		Abnormal in novel environments	Decreased oxytocin levels
Other					

	Patients			MAGE-L2 mouse KO models	
PWS		SHEYNG	USP7	Wevrick et al.	Muscattelli et al.
		Contractures			
			Seizures		