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Cullin-RING E3 Ubiquitin Ligase 7 in Growth Control and Cancer

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Abstract

CRL7^{Fbxw8} is an E3 ubiquitin ligase complex, containing cullin7 (CUL7) as a scaffold, the F-box protein Fbxw8 as a substrate receptor, the Skp1 adaptor, and the ROC1/Rbx1 RING finger protein for working with E2 enzyme to facilitate ubiquitin transfer. This chapter provides an update on studies linking CRL7^{Fbxw8} to hereditary human growth retardation disease, as at least 64 *cul7* germ line mutations were found in patients with autosomal recessive 3-M syndrome. CRL7^{Fbxw8} interacts with two additional 3-M associated proteins OBSL1 and CCDC8, leading to subcellular localization of the E3 complex to regions including plasma membrane, centrosome, and Golgi. At least ten mammalian cellular proteins were identified or implicated as CRL7^{Fbxw8} substrates. Discussion focuses on the possible impact of CRL7^{Fbxw8}-mediated proteolytic or non-proteolytic pathways in growth control and cancer.

Keywords

E3 ubiquitin ligase; Cullin 7; 3-M disease; Growth signaling

17.1 The CRL7^{Fbxw8} Complex

The CRL7^{Fbxw8} complex is a member of Cullin-RING E3 ubiquitin ligase (CRL) family (Petroski and Deshaies 2005; Sarikas et al. 2011). CRL7^{Fbxw8} was originally isolated and identified by Dias and colleagues using biochemical affinity purification and mass spectrometry (Dias et al. 2002). It contains four subunits (Fig. 17.1a) including cullin 7 (CUL7, also known as KIAA0076, p185, or p193), the WD40 repeat-containing F-box protein Fbxw8 (also named Fbx29, Fbw6, or Fbw8), the adapter protein Skp1 (S-phase kinase-associated protein 1), and the RING (for Really Interesting New Gene) finger protein ROC1 (also termed Rbx1 or Hrt1). The core CRL7^{Fbxw8} composition (CUL7, Fbxw8, Skp1, and ROC1) was independently identified and reported by Arai et al. (2003).

CRL7^{Fbxw8} has two unique biochemical properties. First, CUL7 is an atypical cullin family protein (Fig. 17.1b). The primary function of CUL7 is to provide a molecular scaffold that organizes an E3 CRL complex. However, human CUL7 contains 1698 amino acids, a size more than double that of a canonical cullin molecule (CUL1–5). As elaborated below, CUL7 appears to comprise multiple protein-protein interaction domains, enabling a range of

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biochemical functions for both proteolytic and non-proteolytic activities. Secondly, while CUL7 assembles an SCF/CRL1-like complex, it exhibits a remarkable selectivity by interacting with Skp1-Fbxw8 predominantly. Since the initial report of the $CRL7^{Fbxw8}$ complex by Dias et al. (2002) and Arai et al. (2003), many independent reports have confirmed the selective CUL7-Fbxw8 association (Bae et al. 2017; Kim et al. 2014; Kong et al. 2012; Li et al. 2017; Litterman et al. 2011; Okabe et al. 2006; Tsunematsu et al. 2006; Wang et al. 2014; Yan et al. 2014). Currently there are no high-resolution structural models of the CUL7-Fbxw8 interactions to help understand the molecular basis for the selectivity.

Despite abundant evidence for $CRL7^{Fbxw8}$ as a dominant E3 form, it may not be the only type of subunit organization. There are independent reports demonstrating interactions between CUL7 and CUL1 (Tsunematsu et al. 2006) or CUL9 (also named PARC; Skaar et al. 2007; Li et al. 2014). Additional CUL7-based E3 complexes have been reported as well (Kong et al. 2019; Luo et al. 2019; Shah and Maddika 2018).

17.2 $CRL7^{Fbxw8}$, 3-M Syndrome, and E3 Subcellular Distribution

3-M is a human autosomal recessive growth retardation syndrome (reviewed by Clayton et al. 2012; Huber et al. 2011). It is characterized by small birth size and postnatal growth restriction associated with a range of minor anomalies (including a triangular-shaped face, flat cheeks, full lips, short chest, and prominent fleshy heels). In 2005 a landmark report from Huber and colleagues (Huber et al. 2005) first linked *cul7* germ line mutations to 3-M syndrome. This connection has since been confirmed and extended by many independent groups concerning patients worldwide (Al-Dosari et al. 2012; Dauber et al. 2013; Hasegawa et al. 2016; Hu et al. 2017; Lugli et al. 2016; Meazza et al. 2013; Simsek-Kiper et al. 2019). To date, 64 3-M-linked *cul7* mutations have been reported and can be readily accessed in the database of HGMD. These mutations span the entire CUL7 coding sequence. Many mutations are expected to disable CUL7 activity by mechanisms of mRNA decay or significant protein truncations. There are, however, substitution mutations that could help CUL7 structural activity relationship analysis. For instance, 3-M-derived missense mutation H1464P resides in the CUL7 cullin domain (Fig. 17.1b) and was shown to cause reduction of the E3 ligase activity (Huber et al. 2005). In addition, CUL7 mutation may be responsible for the 3-M-like, Yakuts short stature syndrome (Maksimova et al. 2007).

Subsequent studies have discovered that mutations in obscurin-like 1 (OBSL1) (Hanson et al. 2009; Huber et al. 2010) and coiled-coil domain containing 8 (CCDC8) (Hanson et al. 2011) contribute to 3-M syndrome as well. To date, 22 and 3 3-M-linked *obsl1* and *ccdc8* mutations, respectively, have been reported (HGMD). CUL7 appears to be the major gene responsible for 3-M syndrome. The prevalence of 3-M mutations was around 70% in CUL7, 20% in OBSL1, and below 10% in CCDC8 (Huber et al. 2011; Hanson et al. 2012).

CUL7, OBSL1, and CCDC8 appear to be associated physically as well. Early co-immunoprecipitation studies revealed interaction between CCDC8 and OBSL1 (Hanson et al. 2011). It was subsequently found that OBSL1 binds to CUL7 (Litterman et al. 2011). Yan et al. (2014) then provided evidence that CUL7, OBSL1, and CCDC8 are in a complex designated as 3-M complex that also include Fbxw8. OBSL1 is a cytoskeletal adaptor

protein linking the internal cytoskeleton of cells to the cell membrane. CCDC8 contains multiple protein-protein interaction domains capable of interacting with OBSL1, then $CRL7^{Fbxw8}$, as well as additional proteins through its C-terminally located PxLPxL motif (Nie et al. 2015).

In a more recent study (Wang et al. 2019), Xiong and colleagues have shown that CCDC8 was localized on the plasma membrane exclusively. Phosphorylation of CCDC8 by CK2 and GSK3 enabled binding to OBSL1 and then CUL7, resulting in assembly of the membrane-associated 3-M complex. These authors further identified the plasma membrane protein LL5β as a substrate of 3-M complex. Inhibition of the CCDC8 phosphorylation by Wnt signaling caused disruption of membrane localization of the 3-M complex and accumulation of LL5β. Such defects were also observed in cells expressing CUL7 or OBSL1 carrying 3-M-derived mutations. Deletion of *Ccdc8* in mice caused defects in trophoblast migration and placental development and exhibited intrauterine growth restriction and perinatal lethality.

17.3 Role of $CRL7^{Fbxw8}$ in Growth Control

The link of *cul7* mutations to human hereditary syndromes 3-M and Yakuts (Huber et al. 2005; Maksimova et al. 2007) strongly suggests a role for $CRL7^{Fbxw8}$ in growth control. Consistent with human genetics evidence, targeted disruption of the *cul7* gene in mice resulted in severe intrauterine growth retardation (IUGR) with significantly smaller fetuses at later gestational stages and placenta anomalies (Arai et al. 2003). Interestingly, the CUL7 gene is upregulated up to 10 times in IUGR and 15 times in preeclampsia associated with IUGR (Gascoin-Lachambre et al. 2010). Dysregulation of the growth hormone signaling appears to be a feature of 3-M syndrome (Hanson et al. 2012). For example, fibroblast cells from 3-M patients carrying *cul7* or *ccdc8* mutations showed impairment in IGF1 or growth hormone signaling, respectively. 3-M fibroblasts containing *obsl1* mutations exhibited impairment in both pathways (Hanson et al. 2012).

Disruption of the *fbxw8* gene resulted in a less severe phenotype with abnormalities mainly restricted to the placenta and growth (Tsunematsu et al. 2006; Tsutsumi et al. 2008). Approximately 30% of the homozygous *fbxw8*−/− offspring reached adulthood, even though their body sizes were smaller than wild-type littermates throughout postnatal development. Thus, CUL7 and Fbxw8 have overlapping function in growth control, consistent with a hypothesis that CUL7 employs Fbxw8 to mediate proliferative activity. On the other hand, the more severe phenotype of the *cul7*−/− mice implicates Fbxw8-independent functions.

A few proteolytic mechanisms have been proposed to explain the role for $CRL7^{Fbxw8}$ in growth control as summarized below.

IRS1 and mTORC1 Negative Feedback Loop

Insulin, or insulin-like growth factor (IGF), stimulates growth by initiating binding to their receptors. The ligand-bound receptor tyrosine kinases then phosphorylate the insulin receptor substrate (IRS) such as IRS1 at multiple tyrosine residues. The resulting phospho-tyrosines provide docking sites capable of recruiting SH2 (Src homology 2)-containing signaling proteins that include PI3-K (phosphoinositide 3-kinase) and Grb2 (growth factor

receptor-bound protein 2), thus activating the downstream Akt (protein kinase B; via *PI3-K*) and RAS (through Grb2) pathways, respectively. Activated Akt inhibits the TSC1/2 (tuberous sclerosis 1/2) complex, thereby liberating the small G-protein Rheb (Ras homologue enriched in brain). This leads to activation of mTORC1 (protein kinase mechanistic target of rapamycin complex 1; Laplante and Sabatini 2012; Zoncu et al. 2011) and its downstream effector kinase S6K1 (s6 kinase 1), resulting in elevated ribosome biogenesis and cell growth (Copps and White 2012; Harrington et al. 2005; Shah and Hunter 2005). Hyper-activated mTORC1/S6K1 catalyze multisite IRS1 seryl-phosphorylation, which suppresses IRS1's ability to interact with the insulin/IGF-1 receptors and promotes proteasomal degradation (Zhande et al. 2002). This mTORC1/IRS1 negative feedback attenuates the strength or duration of PI3-K activity to ensure optimal mTORC1 signaling (Harrington et al. 2005; Shah and Hunter 2005).

Several lines of evidence suggest a role for $CRL7^{Fbxw8}$ in the mTORC1/IRS1 negative feedback control by targeting IRS1 for ubiquitin-dependent degradation. Xu et al. (2008) have shown that Fbxw8 binds to IRS1 and promotes its ubiquitination and proteasomal degradation; inactivation/deletion of Fbxw8 and CUL7, respectively, accumulates IRS1. Importantly, Fbxw8-induced degradation of IRS1 depends on mTORC1 activity. In a support of these observations, embryonic fibroblasts of *cul7*−/− mice were found to accumulate IRS1 and exhibit increased activation of IRS1 downstream pathways Akt and MEK/ERK. It was proposed that hyper-activated mTORC1/S6K1 spark multisite seryl-phosphorylation of IRS1, triggering the binding of IRS1 to $CRL7^{Fbxw8}$, resulting in IRS1 ubiquitination and degradation, and in turn causing attenuation of the PI3-K/Akt activities.

Additional biochemical (Xu et al. 2012) and physiological (Scheufele et al. 2014) evidences were provided in follow-up studies. IRS1 degradation signal sequence was mapped to its N-terminal 574 amino acid residues. Within this segment, Ser-307/Ser-312 and Ser-527 constitute S6K1 phosphorylation consensus sites, which were found indispensable for supporting $CRL7^{Fbxw8}$-mediated degradation (Xu et al. 2012). Using in vitro reconstitution system, the ubiquitination of bacterially expressed IRS1 N-terminal fragment by $CRL7^{Fbxw8}$ was stimulated by S6K1 albeit at low levels. In contrast, $CRL7^{Fbxw8}$ supported efficient ubiquitination of IRS1 N-terminal fragment in hyper-phosphorylated form, which was isolated from infected insect cells. These data suggest requirement of additional phosphorylation by kinases yet to be identified. It was proposed that the requirement of multisite phosphorylation in the N terminus of IRS1 for its turnover might ensure that complete IRS1 degradation occurs only when mTORC1 and S6K1 reach exceedingly high levels. In addition, enhanced AKT and MAP kinase phosphorylation were observed in *cul7*−/− mouse embryonic fibroblasts upon insulin stimulation (Scheufele et al. 2014). Consistent with this, CUL7 knockdown by RNA interference in C2C12 myotubes led to elevated levels of insulin signaling pathways and cellular glucose uptake. The CUL7 depletion decreased the capacity of these cells to mediate insulin-induced degradation of IRS1. In mouse models, heterozygosity of either *cul7* or *fbxw8* elevated PI3-K/AKT activation in skeletal muscle tissue upon insulin stimulation when compared to the wild-type controls. Finally, enhanced insulin sensitivity and plasma glucose clearance were observed in *cul7*+/− or *fbxw8*+/− mice.

An independent investigation has revealed an mTORC2-dependent feedback inhibition of IRS1 by directly phosphorylating Fbxw8, resulting in enhanced stability of this F-box protein that promotes IRS1 degradation (Kim et al. 2012). Collectively, these studies have implicated roles for $CRL7^{Fbxw8}$ in impacting both mTORC1 and mTORC2 signaling.

However, conflicting reports have appeared. Ponyeam and Hagen (2012) failed to observe accumulation of IRS1 in cells depleted of CUL7 although the phosphorylation status of IRS1 was not examined. More recently, Yoneyama et al. (2018) have identified human IRS1 S422 as a residue critical for phosphorylation by mTORC1 that appears to trigger interactions with $SCF/CRL1^{\beta TrCP}$ for degradation. Future work is needed, however, to provide evidence for direct binding of ßTrCP to the IRS1 S422 degron peptide, which differs significantly from the well-defined ßTrCP substrate-binding consensus motif.

TBC1D3 and Growth Factor Signaling

Hominoid-specific TBC1D3 oncoprotein enhances growth factor receptor signaling and subsequently promotes cellular proliferation and survival. TBC1D3 is degraded in response to growth factor signaling, thereby constituting a growth factor-driven negative feedback loop (Kong et al. 2012). Multiple lines of evidence suggest that $CRL7^{Fbxw8}$ targets TBC1D3 for ubiquitination and degradation in response to serum and growth factor stimulation.

Hippo Signaling and Cardiomyocyte Proliferation

Using the cardiomyocyte model, it was revealed that inhibition of cardiomyocyte proliferation may be related to the accumulation of the Hippo kinases Mst1 and Lats1/2, suggesting a role for Hippo-YAP signaling in cardiac development. CUL7 was shown to be involved in controlling the abundance of Mst1 and therefore participates in Hippo-Yap signaling and cardiomyocyte proliferation (Zou et al. 2018).

17.4 Role of $CRL7^{Fbxw8}$ in Cancer

p53

Kasper et al. (2006) and Andrews et al. (2006) reported the CUL7-p53 interactions that were mapped to the CUL7 CPH domain (Fig. 17.1b) and p53's tetramerization domain. A follow-up NMR study by Kaustov et al. (2007) provided high-resolution structural model for the interactions between CUL7's CPH and p53's tetramerization domains. Based on available evidence, the consequence of the CUL7-p53 interactions appears to antagonize p53's tumor suppressor activity. Cell culture studies have shown that CUL7 expression resulted in decrease of p53 transcription activity (Andrews et al. 2006), increase of the rate of cell proliferation in a manner that requires intact p53 (Andrews et al. 2006), and inhibition of p53 activation in response to DNA damage (Jung et al. 2007). Additional evidence includes the effects of CUL7 in suppressing Myc-induced apoptosis, although whether such an effect depends on the CUL7-p53 interactions has not been addressed (Kim et al. 2007). Thus far there is no evidence that $CRL7^{Fbxw8}$ plays a role in modifying p53 by ubiquitin that leads to changes in p53 stability (Andrews et al. 2006; Jung et al. 2007).

SV40 T Antigen and Transformation

CUL7 was originally identified by immunoprecipitation studies as a host cell protein p185 (Kohrman and Imperiale 1992) or p193 (Daud et al. 1993) that was associated with simian virus 40 large T antigen in early 1990s, long before its eventual recognition as a component of an E3 ubiquitin ligase complex (Arai et al. 2003; Dias et al. 2002). Studies by Decaprio and colleagues have mapped the CUL7 binding site to T antigen amino acids 69–83 (Kasper et al. 2005). Intriguingly, T antigen mutant defective in binding to CUL7, while still capable of interacting with p53 and pRb, was unable to induce proliferation in mouse embryo fibroblasts. These data suggest that the ability of T antigen to transform requires not only p53 and pRB but also inactivation of CUL7 activity. These results imply a role for CUL7 as a tumor suppressor, at least in the presence of the potent oncoprotein T antigen.

In an effort to substantiate these studies, Hartmann et al. (2014) have shown that wild-type T antigen, but not the mutant (Δ69–83) deficient in binding to CUL7, inhibited the degradation of the $CRL7^{Fbxw8}$ substrate IRS1 by the 26S proteasome. Accumulation and prolonged half-life of IRS1 were observed in cells expressing T antigen. Consistent with this, $CRL7^{Fbxw8}$-dependent IRS1 ubiquitination in vitro was inhibited by purified T antigen. Moreover, cells expressing T antigen, or depleted of CUL7 by RNA interference, showed enhanced activation of IRS1 downstream signaling pathways PI3-K/AKT and Erk mitogen-activated pathway kinase, as well as upregulation of the downstream target gene c-fos. Finally, elevated IRS1 protein levels and activation of downstream signaling were detected in T antigen-positive carcinoma of carcinoembryonic antigen 424/SV40 LT transgenic mice. Altogether, these results suggest a role for T antigen in protecting IRS1 from degradation by $CRL7^{Fbxw8}$. Such viral activity may play a role in sustaining high levels of pro-mitogenic IRS1 downstream signaling pathways.

Collectively, these studies may reconcile the CUL7 oncogene/tumor suppressor paradox (Sarikas et al. 2008). In normal cells, the mTORC1/IRS1 feedback functions to ensure proper mTOR signaling (Harrington et al. 2005; Shah and Hunter 2005). Loss of CUL7 results in sustained mTOR signaling, leading to senescence (Xu et al. 2008). This is in keeping with a role for $CRL7^{Fbxw8}$ in growth control. However, the potent oncoprotein T antigen commands high levels of cell proliferation. Breaking the mTORC1/IRS1 negative feedback loop by T antigen-mediated inhibition of IRS1 degradation may be necessary to sustain pro-mitogenic signaling, thereby meeting proliferative demands.

Cyclin D1 and Cell Cycle

Cell cycle progression into S phase requires removing cyclin D1 through re-localization and degradation. Okabe et al. (2006) have provided evidence suggesting that sustained MAPK signaling, a feature unique to cancer cells, resulted in cyclin D1 phosphorylation at T286, which triggered interactions with $CRL7^{Fbxw8}$ leading to ubiquitin-dependent proteasomal degradation. Fbxw8 knockdown caused a significant accumulation of cyclin D1, as well as cytoplasmic sequestration of CDK1, leading to a severe reduction of cell proliferation. Constitutive nuclear expression of cyclin D1-T286A reversed these effects. These findings support a role for $CRL7^{Fbxw8}$ in cancer cell proliferation through proteolysis of cyclin D1. However, mouse embryonic fibroblasts (MEFs) from *fbxw8*−/− mice or the wild type

showed similar rate of cyclin D1 degradation. These genetic analyses raised questions on a significant role for Fbxw8 in cyclin D1 degradation during normal cell cycle progression (Kanie et al. 2012).

HPK1, MAPK, and Pancreatic Cancer

Hematopoietic progenitor kinase 1 (HPK1) inhibits MEK1/2-mediated ERK activation and is lost in >95% pancreatic cancer through proteasome-mediated degradation. HPK1 may function as a novel tumor suppressor, and loss of HPK1 plays a critical role in the development of pancreatic cancer. $CRL7^{Fbxw8}$ targets HPK1 for degradation in a manner that requires HPK1 autophosphorylation (Wang et al. 2014). Knockdown of Fbxw8 restores endogenous HPK1 protein expression and inhibits cell proliferation of pancreatic cancer cells. These findings suggest a role for $CRL7^{Fbxw8}$ in constituting a negative feedback loop to restrain the growth-inhibitory activity of HPK1 and that $CRL7^{Fbxw8}$ promotes pancreatic cancer cell proliferation.

CUL7, CUL9, and Microtubule Dynamics

Yan et al. (2014) have linked CUL7, OBSL1, and CCDC8 to the control of microtubule dynamics. It was observed that CUL7 depletion results in altered microtubule dynamics, prometaphase arrest, tetraploidy, and mitotic cell death. Importantly, these defects were observed in CUL7 mutated 3-M cells as well and were rescued by expression of the wild-type CUL7, but not by 3-M-derived mutants. Similar defects were observed in cells depleted of OBSL1 or CCDC8. It was proposed that CUL7, OBSL1, and CCDC8 proteins form a 3-M complex that functions in maintaining microtubule, genome integrity, and normal development.

The CUL7/microtubule dynamics appears to be connected with cullin 9 (CUL9) (Li et al. 2014). *Cul9* null mice develop spontaneous tumors in multiple organs. It was observed that the microtubule and mitosis defects caused by knockdown of CUL7 or OBSL1 were rescued by depletion of CUL9. It was shown that CUL7 inhibits the CUL9-mediated ubiquitination and degradation of survivin. It was proposed that a 3M-CUL9-survivin pathway is critical for maintaining microtubule and genome integrity, normal development, and tumor suppression.

17.5 Role of $CRL7^{Fbxw8}$ in Stem Cell Self Renewal

Nanog

Nanog regulates human and mouse embryonic stem (ES) cell self-renewal activity. Activation of ERK signaling inhibits ES cell self-renewal and induces differentiation. It was shown that this inhibition is mediated by the ability of ERK1 to phosphorylate Nanog, which leads to binding to Fbxw8 and ubiquitination-mediated degradation (Kim et al. 2014).

OCT4

The POU transcription factor OCT4 is critical for maintaining the undifferentiated state of embryonic stem cells (ESCs) and generating induced pluripotent stem cells (iPSCs). It was observed that c-Jun N-terminal kinases (JNKs) directly phosphorylated OCT4 at serine 347,

which triggered the binding of Fbxw8, leading to increased OCT4 proteasomal degradation (Bae et al. 2017).

17.6 Role of CRL7^{Fbxw8} in Neurons

Golgi

Litterman et al. (2011) reported that CRL7^{Fbxw8} is Golgi associated as a result of CUL7-OBSL1 interactions. Inactivation of CRL7^{Fbxw8} through depletion of Fbxw8 impairs Golgi structure and function and dramatically inhibits the elaboration and growth of dendrites in primary neurons and in the developing rat cerebellum in vivo. CRL7^{Fbxw8} targets the Golgi protein Grasp65 for ubiquitination and degradation, thereby critically regulating the structural integrity and function of the Golgi apparatus and dendrite development in neurons.

Eag1 Potassium Channels

Hsu et al. (2017) have observed interactions between CUL7 and rat Eag1, both of which appear to co-localize at synaptic regions in neurons. CUL7 appears to target endoplasmic reticulum- and plasma membrane-localized rat Eag1 to the proteasome and the lysosome, respectively, for protein degradation. These findings suggest a role for CUL7 in quality control of Eag1 channels.

17.7 Concluding Remarks

Since its discovery in 2002, CRL7^{Fbxw8} has been shown or implicated in control of the stability of more than ten protein substrates. Table 17.1 provides a summary of these substrates with key biological role(s) and identified/implicated kinase (s) involved in the proteolytic signaling. Note that six CRL7^{Fbxw8} substrates have played roles in growth control (Fig. 17.2). The discovery of two additional 3-M-linked proteins OBSL1 and CCDC8 and their physical association with CRL7^{Fbxw8} underscore the significance of distinct subcellular locations of the E3 complex (Fig. 17.3) in its biological function and role in growth retardation disease.

It remains unclear whether the biological defects observed in mouse *cul7* knockout (Arai et al. 2003) and/or human 3-M syndrome bearing *cul7* mutations (Huber et al. 2005) can be attributed to aberrant accumulation of any of the CRL7^{Fbxw8} substrates discovered to date (Table 17.1). It is possible that we have not yet identified the CRL7^{Fbxw8} substrate that plays a predominant growth regulatory role and that, when dysregulated, leads to 3-M growth retardation. Alternatively, 3-M may be a disease caused by dysregulation of multiple proteolytic pathways affected by CRL7^{Fbxw8}. It should also be mindful that CUL7 has non-proteolytic functions (such as binding to p53, Fig. 17.1b) that may play significant role in cell proliferation and 3-M syndrome.

It is hoped that continuing efforts using genetic and biochemical approaches will lead to better understanding of the role of CRL7^{Fbxw8} in growth control and cancer. For example, advanced mouse models may be created to resemble the human 3-M syndrome and to more precisely define the role of CRL7^{Fbxw8} in cell proliferation signaling pathways. In-depth characterization of the proteolytic and non-proteolytic functions of CRL7^{Fbxw8} may yield

new mechanistic insights. It is hopeful that such discoveries would enable the birth of innovative therapeutic approaches for the treatment of 3-M and related growth retardation syndromes.

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Abbreviation

3-M
An autosomal recessive disorder characterized by pre- and postnatal growth retardation and named after the initials of three researchers (Miller, McKusick, and Malvaux) who first identified the disease

Akt
Protein kinase B

CCDC8
Coiled-coil domain containing 8

CDK
Cyclin-dependent kinase

CRL
Cullin-RING E3 ubiquitin ligase

$CRL7^{Fbxw8}$
A member of CRL family that contains four proteins known as cullin7, Fbxw8, Skp1, and ROC1/Rbx1

CUL
Cullin

Grb2
Growth factor receptor-bound protein 2

HPK1
Hematopoietic progenitor kinase 1

IGF
Insulin-like growth factor

IRS
Insulin receptor substrate

IUGR
Intrauterine growth retardation

MAPK/ERK
Mitogen-activated protein kinase

MEK
Mitogen-activated protein kinase

mTOR
Mechanistic target of rapamycin

OBSL1
Obscurin-like 1

PI3-K
Phosphoinositide 3-kinase

Rheb
Ras homologue enriched in brain

S/Ser
Serine

S6K1
s6 kinase 1

SH2
Src homology 2

TSC1/2
Tuberous sclerosis 1/2 complex

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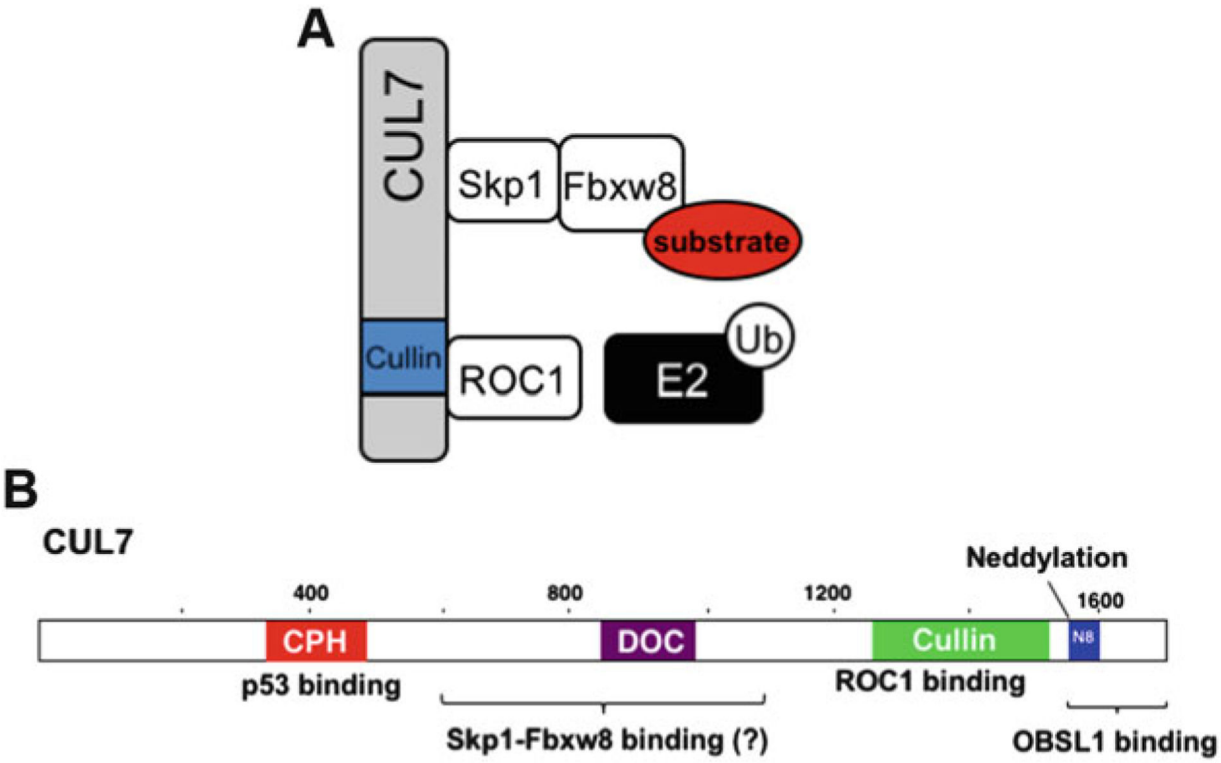

Fig. 17.1.

(**a**) Organization of $CRL7^{Fbxw8}$. While Fbxw8 is a substrate receptor, ROC1 works with an E2 ubiquitin-conjugating enzyme for transferring ubiquitin to the bound substrate. (**b**) Domain organization of CUL7. The Fbxw8 binding site is derived from an early study (Huber et al. 2005). Future work is needed to precisely locate the binding interface. The requirement for the CUL7 C-terminus for binding to OBSL1 was shown by Litterman et al. (2011)

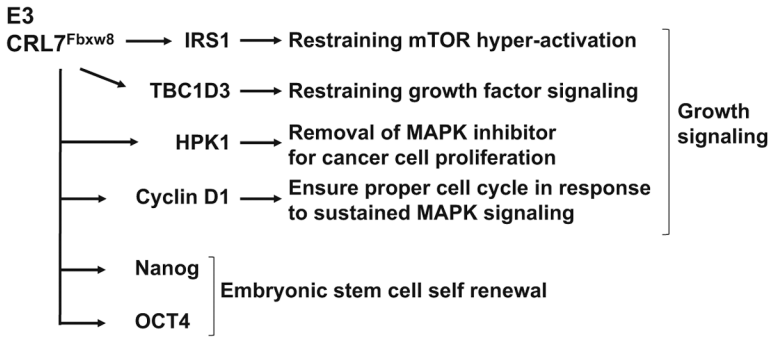

Fig. 17.2.

Proteolytic pathways of $CRL7^{Fbxw8}$ in growth signaling. Shown are six $CRL7^{Fbxw8}$ substrates in cell proliferation and stem cell self-renewal

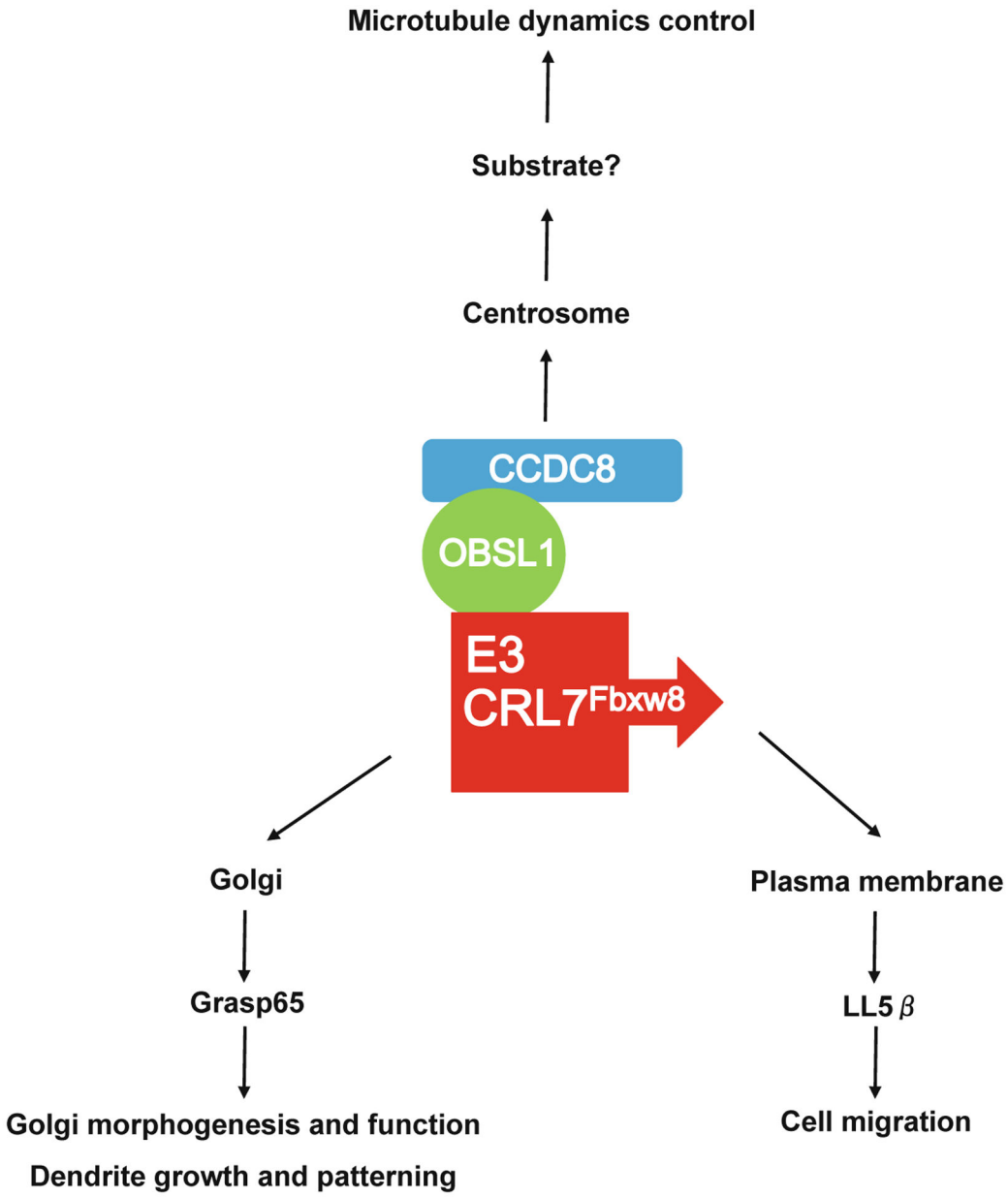

Fig. 17.3.

Subcellular localization of $CRL7^{Fbxw8}$ as a result of interactions with OBSL1 and CCDC8. Shown are a few distinct subcellular locations of $CRL7^{Fbxw8}$ due to recruitment through interactions with OBSL1 and CCDC8

Table 17.1

$CRL7^{Fbxw8}$ substrates

Substrates	Biological role	Kinase(s)	Reference
• IRS1	Insulin/IGF-1 signaling	S6K, mTORC1	Xu et al. (2008, 2012)
• Cyclin D1	Cell cycle progression	MAPK	Okabe et al. (2006)
• HPK1	Inhibition of MEK1/2-mediated activation of ERK	HPK1, autophosphorylation	Wang et al. (2014)
• TBC1D3	Growth factor receptor signaling, proliferation, and survival	Unknown	Kong et al. (2012)
• Mst1[a]	Hippo signaling		Zou et al. (2018)
• Nanog	Embryonic stem cell self-renewal	ERK1	Kim et al. (2014)
• OCT4	Embryonic stem cell self-renewal, cell differentiation	JNK	Bae et al. (2017)
• Grasp65	Neuronal morphogenesis, dendrite growth and patterning, Golgi function	Unknown	Litterman et al. (2011)
• Eag1[a]	Neuron, potassium (K+) channels		Hsu et al. (2017)
• HIV-1 Gag	HIV-1 assembly		Wei et al. (2015)
• MRFAP1	Anaphase-telophase transition, genomic stability	Unknown	Li et al. (2017)

[a]The abundance of these proteins was regulated by CUL7 although the role of Fbxw8 was not reported