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A Novel Family of Membrane-Bound E3 Ubiquitin Ligases

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A novel E3 ubiquitin ligase family that consists of viral E3 ubiquitin ligases (E3s) and their mammalian homologues was recently discovered. These novel E3s are membrane-bound molecules that share the secondary structure and catalytic domain for E3 activity. All family members have two transmembrane regions at the center and a RING-CH domain at the amino terminus. Forced expression of these novel E3s has been shown to reduce the surface expression of various membrane proteins through ubiquitination of target molecules. Initial examples of viral E3s were identified in Kaposi's sarcoma associated herpesvirus (KSHV) and murine γ-herpesvirus 68 (MHV-68) and have been designated as modulator of immune recognition (MIR) 1, 2 and mK3, respectively. MIR 1, 2 and mK3 are able to down-regulate MHC class I molecule expression, and mK3 is required to establish an effective latent viral infection *in vivo*. The first characterized mammalian homologue to MIR 1, 2 and mK3 is c-MIR/MARCH VIII. Forced expression of c-MIR/MARCH VIII down-regulates B7-2, a co-stimulatory molecule important for antigen presentation. Subsequently, several mammalian molecules related to c-MIR/MARCH VIII have been characterized and named as membrane associated RING-CH (MARCH) family. However, the precise physiological function of MARCH family members remains as yet unknown.

Key words: antigen presentation, endocytosis, mammalian homologue, ubiquitination, viral immune evasion.

There are several families of known E3s: RING-type E3s, HECT-type E3s, and PHD-type E3s (*1–4*). The RING and PHD E3 families have been shown to function without the need for any other interacting molecules except E2s, ubiquitin conjugating enzymes (*2*, *3*). In contrast, the HECT family functions in the context of an E3 complex, which consists of catalytic domain–containing molecules and adaptor molecules (*2*). E3s are believed to be key molecules in numerous physiological and pathological processes, as E3s determine the substrate specificity for ubiquitination.

Recently, we and other have described a novel family of E3s, first identified as proteins related to immune-evasion strategies in viruses (*5–12*). All members of this new E3 family are membrane-bound and possess a variant type RING domain, named as RING-CH or RING variant (RINGv) domain, because of an interval difference between the third and forth cysteine residues in a putative zinc-binding motif (*13*). This novel family was designated as Modulator of Immune Recognition (MIR) family, based on the name for the first identified family members (*9*). As in the case of RING family, MIR family proteins induce ubiquitination through direct molecular interaction.

MIR family members consist of viral E3s and their mammalian homologues (*14, 15*). Forced expression of MIR family molecules reduces the surface expression of immune regulatory molecules such as MHC class I molecules, B7-2 and ICAM-1 (*5, 6, 8, 14, 15*). In the case of viral E3s, the downregulation of immune regulatory molecules is believed to be an important facet of the viral immune evasion strategy. In contrast, the biological relevance of the phenomenon observed by forced expression of mammalian homologues remains as yet to be identified (*14, 15*).

Initially identified MIR family members: K3, K5 and mK3

In 2000, several groups reported the identification of novel molecules encoded by herpesvirus that down-regulate surface expression of MHC class I. K3 and K5 of KSHV were identified by analysis of all ORFs within the unique region of KSHV genome (*6*). K3 and K5 have 40% amino acid identity and are thought to originate from a gene duplication event. Both K3 and K5 induce unusual down-regulation of MHC class I molecules, but the activity of K3 is stronger than that of K5 (*5, 6*). Moreover, K3 affects the expression of all allotypes of MHC class I molecules: HLA-A, B and C. In contrast, K5 affects only the expression of HLA-A and B (*5*). mK3 of MHV-68 was discovered by searching the MHV-68 genome library (*7*). The expression of MHC class I molecules was initially shown to be down-regulated on MHV-68–infected fibroblast. Also, antigen-specific cytotoxic T lymphocyte (CTL) did not efficiently recognize MHV-68–infected cells. By narrowing down the responsible region in the MHV-68 genome, mK3 was identified as a responsible molecule for MHC I down-regulation in the context of MHV-68 infection. KSHV

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and MHV-68 belong to the same family of herpesvirus: γ-2-herpesvirus, and K3, K5 and mK3 share the same secondary structure and the position of the zinc-finger domain, which was subsequently identified as a catalytic domain in E3 ubiquitin ligases (*13, 16, 17*).

MHC class I molecule down-regulation by viral MIR family members

MHC class I molecules are a key component of host immune surveillance against viruses. Cells infected by the viruses generate virus-derived short peptides through ubiquitination and degradation of viral proteins through the ubiquitin-proteasome system. Peptides are transported into the ER *via* the transporter associated with antigen processing (TAP), and the assembly of MHC class I–viral peptide complex takes place in the ER. MHC class I–peptide complexes are then transported to the cell surface via endosomal pathways. Finally, CTLs recognize MHC class I–peptide complexes and subsequently eliminate virus-infected cells by cytolysis. In addition, activated CTL releases various cytokines to support the elimination of viruses. Thus, K3 and K5-mediated down-regulation of MHC class I appears to be part of a sophisticated viral strategy for immune evasion. However, normal MHC class I molecule expression is necessary for the protection from natural killer (NK) cells–mediated killing, thus the viral E3s-mediated MHC class I inhibition might render infected cells sensitive to NK cell-mediated killing. We have earlier proposed that KSHV has an additional strategy to evade NK cell–mediated killing through the action of K5 (*18*). K5 also triggers significant down-regulation of ICAM-1 and B7-2, which are NK cell activators, and strongly inhibits killing mediated by NK cells (*18*). Since ICAM-1 and B7-2 are well known as co-stimulatory molecules for antigen presentation, KSHV K5 appears to inhibit both T cell–mediated immune responses and NK cell–mediated killing (*18, 19*). Based on these findings, K3 and K5 were renamed a Modulator of Immune Recognition (MIR) 1 and 2, respectively (*9*).

Pathological relevance of MIR1, MIR2 and mK3 in herpesvirus infection

In the case of KSHV, the findings described above were obtained from experiment in which the examined molecules were expressed without any components required for complete viral propagation. Therefore, the contribution of MIR1 and 2 to KSHV propagation in vivo remains unclear. However, based on the expression kinetics of MIR1 and 2, it is possible to draw a hypothetical picture on the respective roles of MIR1 and 2 in virus propagation in vivo. In primary effusion lymphoma–derived cell lines, MIR1 and 2 were expressed during the immediate-early phase of viral infection (*20*). On the other hand, in primary endothelial cells, MIR2 was expressed at high level at all phases of viral infection, while MIR1 expression was very low (*21*). Thus, the contribution of MIR1 and 2 to KSHV propagation appears to be dependent on the types of infected cells.

Stevenson *et al.* have reported an excellent experiment to examine the above hypothesis using genetically modified MHV-68 lacking the mK3 gene (*22*). The propagation of the mK3-deficient virus was not impaired *in vitro*, and the fibroblasts infected with the mK3-deficient virus were more sensitive to antigen-specific CTL clones than those infected with the wild-type virus. Also, the mK3-deficient virus was not able to down-regulate MHC class I expression efficiently, compared to the wild-type virus. These findings support the hypothesis that viral MIRs function as immune modulators in the context of viral infection. Furthermore, in mice infected with an mK3-deficent virus, the efficiency of the latent infection in the spleen was reduced, while the frequency of virus-specific CTL was increased. Thus, mK3 clearly functions as an immune modulator that helps the virus to evade host immunity *in vivo*. Since MHV-68 does not encode the functional homologue of KSHV K5, the validity of the above hypothesis with respect to K5 has not yet been tested.

Other members of the viral MIR family

Recently, several other viral molecules related to MIR1, MIR2, and mK3 have been discovered and characterized in detail (Table 1). MV-LAP/M153R encoded by rabbit myxoma virus which belongs to the poxvirus family down-regulates MHC class I molecules, Fas and CD4 (*10, 23*). In the case of orf12 encoded by herpesvirus saimiri, there is no evidence of down-regulation of MHC class I molecules so far. The contribution of MV-LAP to viral immune evasion has been suggested, based upon results obtained in experiments using a MV-LAP–deficient virus (*23*). While wild-type virus reduces the surface expression of MHC class I molecules and inhibits cytolysis by antigen-specific CTLs, a MV-LAP–deficient virus did not reduce MHC class I molecule expression and was unable to inhibit cytolysis by CTLs. Furthermore, European rabbits infected with MV-LAP–deficient virus did not show severe symptoms, as compared to those infected with wild-type virus. Both

Table 1. **Viral MIR family members.**

Genes	Virus	Target molecules	Refs.
K3/MIR1	KSHV	MHC I, CD 1d	(*5*) (*6*) (*46*)
K5/MIR2		MHC I, ICAM-1, B7-2, CD 1d	(*18*) (*19*) (*46*)
MV-LAP/M153R	Myxoma virus	MHC I, Fas-CD95, CD4	(*23*) (*10*)
mK3	Murine gammaherpesvirus 68	MHC I	(*7*) (*30*)
ORF 12	Herpesvirus saimiri	N/D	(*47*)
IE-1A, IE-1B	Bovine herpesvirus 4	N/D	(*48*)
S153R	Shope fibroma virus	N/D	(*49*)
C7L	Swinepox virus	N/D	(*50*)
5L	Yaba-like disease virus	N/D	(*51*)
010	Lumpy skin disease virus	N/D	(*52*)

wild-type virus and MV-LAP–deficient virus induced primary myxomas at the inoculated site and secondary myxomas at the face and ears in an equal fashion. However, secondary myxomas did not severely distribute in the case of MV-LAP deficient, compared with wild-type virus. Also the mortality rates of the rabbits infected with MV-LAP–deficient virus were less than those of animals infected with wild-type virus. Although MV-LAP was shown to reduce Fas expression, no differences in the incidence of apotosis in vivo were observed. Unfortunately, an analysis of the frequency of viral CTLs in the infected animals was not undertaken. Thus, although available data clearly demonstrate that MV-LAP is a virulence molecule, the pathological connection between MV-LAP function and CTL escape remains yet to be elucidated.

Mammalian homologues of viral MIR family members

Large DNA virus, especially herpesviruses, have been shown to encode viral homologues of mammalian proteins. For instance, the KSHV genome encodes homologues of many human proteins [*e.g.* viral interleukin-6 (v-IL-6), viral macrophage inflammatory protein (v-MIP)] (*24*). Through a database search, several candidates for functional mammalian homologues to MIR family members were extracted (*25, 26*). The first characterized mammalian MIR family member was cellular-MIR (c-MIR) (*14*). c-MIR shares a secondary structure and the catalytic domain for E3 with viral E3s. Overall c-MIR has 12% and 18% amino acid identity to MIR1 and MIR2, respectively. Based on their structural characters MIR homologues, including c-MIR, have been designated as membrane-associated RING-CH (MARCH) family members (*15*). Thus, c-MIR is now also known as MARCH VIII (*15*).

In the MARCH family, there are three pairs that consist of closely related molecules (*15*) (Table 2). These pairs are MARCH-I and c-MIR/MARCH VIII, MARCH-II and -III, and MARCH-IV and -IX. Both MARCH I and c-MIR/MARCH VIII down-regulate the expression of B7-2, Fas and transferrin receptor, while forced expression of MARCH IV and MARCH IX down-regulates the expression of MHC class I molecules and of CD4. In contrast, forced expression of MARCH II, but not of MARCH III, down-regulates B7-2 expression. At present, the physiological relevance of these findings remains unclear, although some MARCH members have interesting expression profiles *in vivo*. MARCH I and MARCH IV seem to be expressed abundantly in secondary lymphoid tissues, and in the placenta, respectively. Currently, genetically modified mice models to study the physiological roles of MARCH family members are under development.

Table 2. **MARCH family members.**

Genes	Target molecules	Refs.
MARCH I	Fas-CD95, TfR	(*15*)
MARCH II	TfR, B7-2	(*15*)
MARCH III	N/D	
MARCH IV	MHC class I, CD4	(*15*)
MARCH V	N/D	
MARCH VI	N/D	
MARCH VII	N/D	
c-MIR/MARCH VIII	B7-2, Fas-CD95, TfR	(*14*) (*15*)
MARCH IX	MHC class I, CD4	(*15*)

Altered trafficking of target molecules by MIR family members

To date, a number of important phenomena induced by viral MIR family members have been well characterized. Through the expression of KSHV MIR1 and 2, MHC class I molecules are rapidly endocytosed from the cell surface and transported to the lysosome for degradation (*5, 6*). Experiments with a CD8-MHC class I chimera model showed that the transmembrane and cytoplasmic tail of MHC class I proteins are sufficient for down-regulation by MIR1 and 2 (*5*). Further, the transmembrane domain of MIR2 was shown to determine target specificity; MIR1 molecules with a MIR2 transmembrane domain are capable to down-regulate specific targets of MIR2, such as B7-2 or ICAM-1, but not of MIR1 (*27*). These results suggest that the molecular interaction between the transmembrane domains of target molecules and MIRs is necessary for downregulation, but this hypothesis has not been experimentally verified. In the case of MV-LAP/M153R, the mechanism of CD4 down-regulation was examined in detail (*10*). Like KSHV MIR1 and 2, expression of MV-LAP/M153R induces rapid endocytosis and lysosomal degradation of CD4.

What about MARCH family members? Among MARCH family members, c-MIR/MARCH VIII, MARCH-IV and IX were intensively analyzed (*14, 15*). Forced expression of c-MIR/MARCH VIII induces rapid endocytosis of B7-2, but not of MHC class I molecules (*14*). Pulse-chase analysis revealed that c-MIR/MARCH VIII rapidly degrades B7-2, a process that can be blocked by an inhibitor of vacuolar ATPase, bafilomycin A1. Using CD8 chimeras, the transmemebrane and cytoplasmic tail of B7-2 was shown to be sufficient for down-regulation and to determine substrate specificity by c-MIR/MARCH VIII. While a CD8 chimera with the transmembrane domain and cytoplasmic tail of B7-2 is efficiently down-regulated, this is not the case for CD8 chimeras with transmembrane domains and cytoplasmic tails from MHC class I molecules. Thus, c-MIR/MARCH VIII seems to utilize the strategies similar to those of viral MIR family members (*c.f.* KSHV MIR1 and 2). Similar results have been obtained in case of MARCH IV and IX. Forced expression of MARCH-IV or IX induces rapid endocytosis of MHC class I molecules, while an inhibitor of vacuolar ATPase, concanamycin A, suppresses the function of MARCH-IV or IX (*15*).

In contrast, mK3 utilizes a different way to inhibit the expression of MHC class I molecules. Expression of mK3 induces the retrograde transport of newly synthesized MHC class I molecules from the ER, and MHC class I molecules transported to the cytosol are degraded in the proteasome (*7*). mK3 is able to down-regulate the peptide-free form of murine MHC class I molecules, but not their peptide-bound form (*28*). In consistence with this finding, mK3 is not able to efficiently inhibit the expression of the K^k allele of MHC class I molecules, which are rapidly associated with newly entering peptides into the ER (*28*). Intensive experiments revealed that mK3 associates with the peptide-loading complex, which includes TAP and tapasin

(*29*). mK3 is not able to inhibit the expression of MHC class I molecules in fibroblast cells derived from either TAP or tapasin-deficient mice. The association of mK3 to the peptide-loading complex contributes to its stability and enhances its function. Thus, the peptide-loading complex seems to be the key structure for mK3 to induce an effective inhibition of MHC class I molecule expression (*30*). Furthermore, mK3 was shown to degrade the peptide-loading complex itself (*31*). The expression of mK3 degrades tapasin and TAP, and mK3 is able to interact with TAP1 in the absence of TAP2 and tapasin. mK3-mediated degradation of TAP provides resistance to the upregulation of MHC class I molecules induced by IFN-γ. Therefore, it is proposed that mK3 degrades the peptide-loading complex through binding to TAP1, and this effect might be important for viral immune evasion in the lymphoid tissue (*12, 31*).

MIR family members act as E3 ubiquitin ligase

As described above, all MIR family members have been shown to alter the trafficking of membrane proteins. Considerable progress has been made in recent years on the elucidation of the molecular basis for MIR family members action. MIR family members possess a putative zinc-finger motif whose structure resembles the plant homeodomain (PHD) domain. Initially, based on this observation, the zinc-finger motif of MIR family was named the bovine herpesvirus 4, Kaposi's sarcoma–associated herpesvirus, and Swinepox virus (BKS)-PHD domain (*32*). However, the analysis of the crystal structure of this domain revealed a similarity with the RING domain that is well known as the catalytic domain of E3 ubiquitin ligases. On this basis, this domain has been renamed as RING-CH domain (*13*). In several MIR family members, their RING-CH domains were demonstrated to have E3 activity *in vitro* (*10, 14–17*). Mutant forms of MIR1 and 2 whose putative zinc binding sites within the RING-CH domain are disrupted, are unable to down-regulate target expression (*16*). Also, the lysine residues presented in the cytoplasmic tail of B7-2 were identified as targets for MIR2 mediated down-regulation (*16*). These finding strongly suggested that MIR2 functions as an E3 for B7-2 and MHC class I molecules, since lysine residues are well characterized as responsible residues for ubiquitination. As expected, the expression of MIR1 or 2 strongly induced ubiquitination of MHC class I molecules, while MIR2 also induced ubiquitination of B7-2 (*16*). An *in vitro* ubiquitination assay, using GST-fused RING-CH domain of MIR2, UbcH5a, one of E2s, was shown to support ubiquitination (*16*). Importantly, MIR2 also down-regulated the expression of a modified B7-1 molecule with lysine residues at its cytoplasmic tail and, further, induced ubiquitination of this modified B7-1 molecule (*16*). Since B7-1 is not degraded by MIR2, these results demonstrate that MIR1 and 2 function as E3 ubiquitin ligase, and that ubiquitination is necessary for MIR family–mediated down-regulation of target molecules.

Similar results were reported in the case of CD4 and MHC class I molecule down-regulation by MV-LAP/M153R, B7-2 down-regulation by c-MIR/MARCH VIII, and MHC class I molecule down-regulation by MARCH IV and IX (*10, 14, 15*). MV-LAP/M153R induces ubiquitination of CD4 and, further, down-regulates CD4 surface expression; effects that are dependent upon the presence of lysine residues at the cytoplasmic tail of CD4 (*10*). c-MIR/MARCH-VIII also induces ubiquitination of B7-2, and degradation of B7-2 was shown to depend upon binding of c-MIR/MARCH-VIII to B7-2 (*14*). Findings in an *in vitro* ubiquitination assay suggested that each MARCH family member utilizes a different E2: UbcH2 for MARCH-II, IV and VIII; UbcH3 for MARCH-I and II; UbcH5a for MARCH-I, II, IV and VIII (*15*). In this ubiquitination assay, a specific E2 for MARCH IX was not identified (*15*). The status of ubiquitination induced by MARCH-IV or IX seems to be different from other cases. MARCH-IV and IX induce monoubiquitination of MHC class I molecules, but MIR1 and 2 of KSHV, MV-LAP/M153R and c-MIR/MARCH VIII were reported to induce polyubiquitination of target molecules. However, it has to be noted that the possibility of monoubiquitination at multiple sites on target molecules was not excluded in the above experiments.

In the case of MHV-68 mK3, it is not clear which residues are responsible for mK3-mediated ubiquitination of MHC class I molecules. Like other MIR family members, mK3 ubiquitinates MHC class I molecules through its RING-CH domain, and the cytoplasmic tail of MHC class I is necessary for degraded by mK3 (*17*). Moreover, using proteasome inhibitors, ubiquitination has been shown to be crucial for down-regulation of MHC class I molecule expression (*17*). Interestingly, Wang *et al*. report that lysine-less MHC class I molecules are still ubiquitinated and efficiently degraded (*33*). The ubiquitination observed in this report might reflect a partial dislocation of MHC class I molecules to the cytosol (*33, 34*). Alternatively, unknown residues within the cytoplasmic tail of MHC class I molecules might be target sites for ubiquitination and thus responsible sites for downregulation. Recently, it has been reported that KSHV MIR1 ubiquitinates HLA-B7 molecules that lack a lysine residues on their cytoplasmic tail. In this experiment, a cysteine residue on the cytoplasmic tail was shown to be the responsible residue for ubiquitination (*35*). Thiol-ester bonds were suggested as basis for this interaction (*35*).

Other functional domains of MIR family members

RING-CH domain is a catalytic domain for E3 activity, but the existence of other functional domains was suggested by results obtained from a mutagenesis analysis of MIR1 (*36*). A conserved region in MIR1 and 2 in the C-terminal portion has been identified (*27, 36*). This conserved region includes an YXXV/N (a putative tyrosine-based) motif, a NTRV motif, a proline rich region, and an acidic cluster region (Fig. 1). Among these, all putative domains, except the proline rich region, seem to be involved in the down-regulation of MHC class I molecules. The YXXV/N and NTRV motifs are necessary for the induction of rapid endocytosis of MHC class I molecules, and the acidic cluster region is necessary for the transport of MHC class I molecules to the lysosome. At present, the involvement of these conserved regions of MIR1 and 2 in the ubiquitination of target molecules remains yet to be shown. Interestingly, c-MIR/MARCH IX and MARCH I also have two YXXΦ motifs, which are important for B7-2

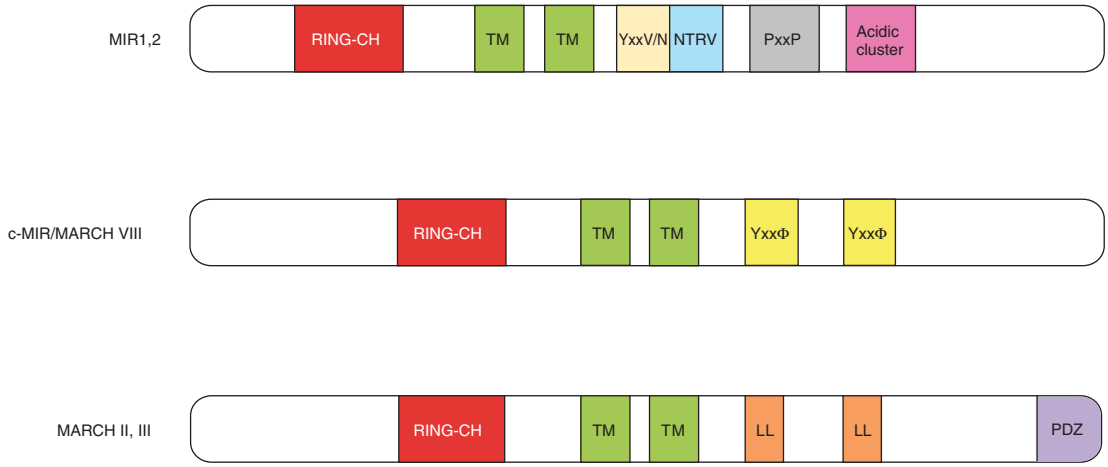

Fig. 1. **Structure of MIR family members.** The domain organization of MIR family members is shown. *TM*, transmembrane region; *PXXP*, proline rich region; *YXX*Φ, tyrosine-based motif; *LL*, dileucine motif; *PDZ*, PDZ binding domain.

down-regulation (unpublished data) (Fig. 1). MARCH II and III contain a dileucine motif, which is known as sorting motif, and a PDZ-binding motif (Fig. 1). Involvement of the PDZ-binding motif in MARCH-II, III-mediated inhibition of transferrin uptake was demonstrated (*37, 38*). In summary, several domains are likely to support the down-regulation of target molecule expression by MIR family members, but further analysis is necessary to demonstrate how these domains contribute to the mechanisms of action of MIR family members.

How does ubiquitination contribute to alteration of trafficking by MIR family members?

Ubiquitination was demonstrated to be required at several steps during the process of endocytosis. In the case of yeast, it was clearly shown that endocytosis of plasma membrane proteins requires ubiquitination of their cytoplasmic tails by the E3 ubiquitin ligase, Rsp5p, and that ubiquitinated membrane molecules are targeted for lysosomal degradation (*39*). In the latter step, ubiquitinated membrane molecules are directed to vesicles inside multivesicular bodies (MVBs) through interaction with class E vacuolar protein-sorting (vps) proteins, and the vesicles containing ubiquitinated targets are then transported into the lysosome. So far, 17 class E vps genes in yeast have been characterized. Among these, 12 genes form four endosomal sorting complexes required for transport, termed ESCRT complexes 0, I, II and III (*40–44*). In contrast, in mammalian cells, several cytokine receptors are ubiquitinated and endocytosed upon binding of their ligands, but it remains unclear whether ubiquitination triggers the endocytosis of these receptors (*39*).

Most MIR family members induce rapid endocytosis and lysosomal degradation of target molecules. Further, most known MIR family members function as E3 ubiquitin ligases. These characteristics strongly suggest that MIR family members utilize a molecular machinery similar to that found in yeast. Several experiments to test this hypothesis have been undertaken. Knock-down experiments of TSG 101, a component of ESCRT I, highlighted TSG 101 as a mediator for MHC class I down-regulation by MIR1 (*45*). In the case of MV-LAP/M153R, the lysosomal degradation of CD4 was significantly blocked by inhibition of Hrs, a component of ESCRT 0 (*10*). Also, inhibition of Vps4, which is required for the dissociation of the ESCRT complex from MVBs, prevented the down-regulation of MHC class I by MARCH-IV or IX (*15*). Thus, MVBs appears to be involved in the down-regulation of target molecules by MIR family members.

Furthermore, the contribution of ubiquitination to endocytosis was suggested by results of mutagenesis analysis in the cytoplasmic tail of target molecules. A mutant HLA-A2 molecule whose cytoplasmic lysine residue is mutated to an arginine residue was shown to be resistant to MIR1-induced endocytosis (*45*). Unfortunately, the detailed mechanisms of MIR family–mediated endocytosis remain elusive. A hypothetical model for the mechanisms of action of MIR family members is given in Fig. 2.

Perspectives

Over the past few years, many fascinating phenomena induced by MIR family members, a novel E3 ubiquitin ligase family, have been identified. Some of these phenomena explain how viruses escape host immunity, but in many cases, especially, the biological relevance of the phenomenon observed by forced expression of MARCH family remains yet to be elucidated. Also, the molecular mechanisms underlying these new immune suppression phenomena are not fully understood and there remain as yet many open questions. For instance, how are ubiquitinated membrane proteins endocytosed? Where are target molecules ubiquitinated? Further efforts are necessary to answer these questions. In conclusion, our understanding of the biology of MIR family members is still at an early stage. Future research into this intriguing class of proteins will provide new insight into the molecular mechanisms of immune regulation.

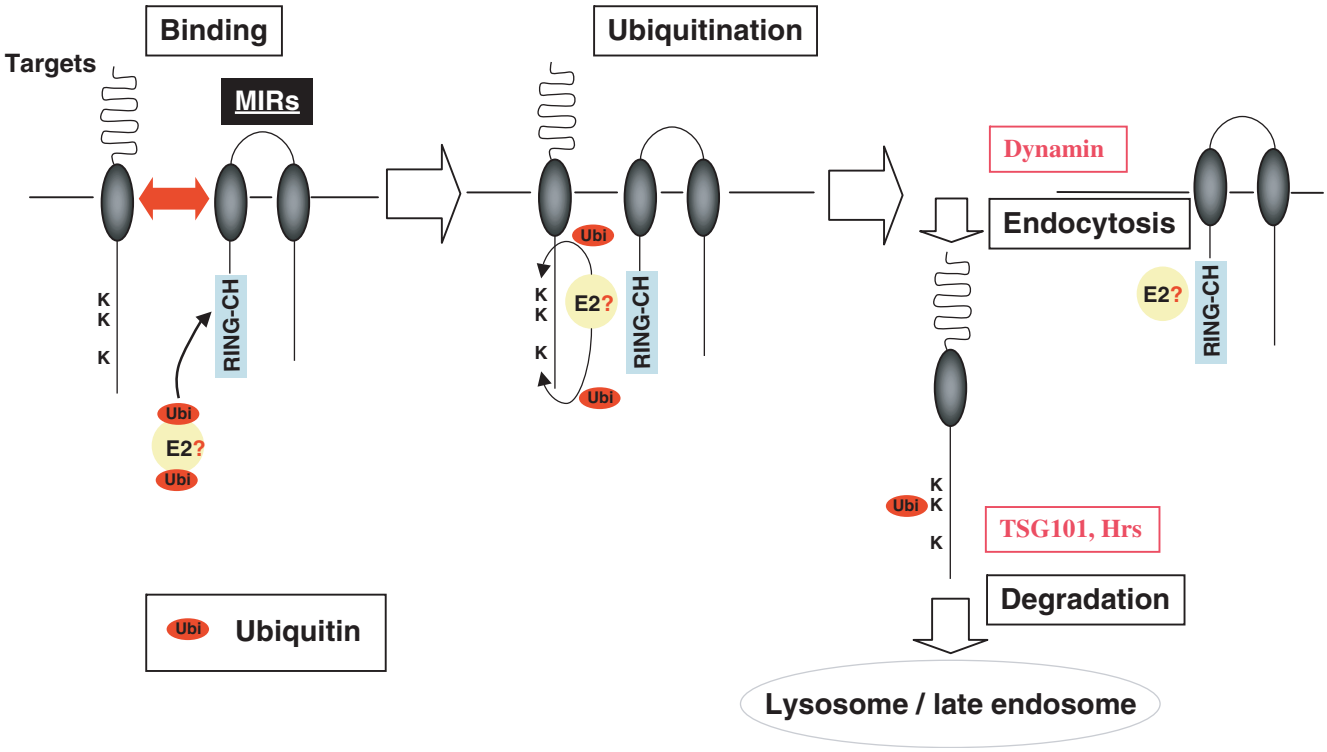

Fig. 2. **Hypothetical model for down-regulaiton of targets by MIR family members.** MIR family members (MIRs) bind to target molecules via their transmembrane regions. E2, ubiquitin conjugation enzyme, is recruited to the RING-CH domains of MIRs. Cytoplasmic tails of targets are ubiquitinated and targets are endocytosed from plasma membrane through the dynamin dependent pathway. Ubiquitinated targets are transported into the lysosome through binding to class E vps proteins and are subsequently degraded.

Addendum in Proof: L.M. Duncan *et al*. (*EMBO J*. 2006 Apr 19; 25(8): 1635–45) recently reported that UbcH56/c and Ubc13 are E2 ubiquitin-conjugating enzymes for MIRI.

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