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Identifying the ERAD ubiquitin E3 ligases for viral and cellular targeting of MHC class I

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ABSTRACT

The human cytomegalovirus (HCMV) US2 and US11 gene products hijack mammalian ER-associated degradation (ERAD) to induce rapid degradation of major histocompatibility class I (MHC-I) molecules. The rate-limiting step in this pathway is thought to be the polyubiquitination of MHC-I by distinct host ERAD E3 ubiquitin ligases. TRC8 was identified as the ligase responsible for US2-mediated MHC-I degradation and shown to be required for the cleavage-dependent degradation of some tail-anchored proteins. In addition to MHC-I, plasma membrane profiling identified further immune receptors, which are also substrates for the US2/TRC8 complex. These include at least six α integrins, the coagulation factor thrombomodulin and the NK cell ligand CD112. US2's use of specific HCMV-encoded adaptors makes it an adaptable viral degradation hub. US11-mediated degradation is MHC-I-specific and genetic screens have identified TMEM129, an uncharacterised RING-C2 E3 ligase, as responsible for US11-mediated degradation. In a unique auto-regulatory loop, US11 readily responds to changes in cellular expression of MHC-I. Free US11 either rebinds more MHC-I or is itself degraded by the HRD1/SEL1L E3 ligase complex. While virally encoded US2 and US11 appropriate mammalian ERAD, the MHC-I complex also undergoes stringent cellular quality control and misfolded MHC-I is degraded by the HRD1/SEL1L complex. We discuss the identification and central role of E3 ubiquitin ligases in ER quality control and viral degradation of the MHC-I chain.

1. Introduction

The essential role of the major histocompatibility class I (MHC-I) antigen presentation pathway in immune detection of intracellular pathogens is emphasised by the different strategies used by many viruses to interrupt this pathway (Hansen and Bouvier, 2009; Randow and Lehner, 2009). The ongoing battle between viruses and the immune system has resulted in many viruses co-evolving one or more gene products which inhibit MHC-I presentation, with the presumed aim of preventing viral peptide presentation by MHC-I to cytotoxic T-lymphocytes (CTL). This is exemplified in the herpesvirus family, with human cytomegalovirus (HCMV) providing one of the major paradigms for viral immune evasion. As the largest known human herpesvirus, HCMV encodes ~170 canonical open reading frames of which only 45 are required for viral replication, with many of the remaining genes involved in immune evasion. The downside of this extensive range of viral gene products is that they provide a smorgasbord of potential viral epitopes for MHC-I presentation. In response, HCMV encodes at least four gene products that interfere with classical MHC-I antigen presentation (Hansen and Bouvier, 2009; Loureiro and Ploegh, 2006). HCMV US2 and US11 promote MHC-I reverse translocation from the ER to the cytosol for subsequent proteasomal degradation, US6 blocks TAP-dependent peptide transport, while US3 inhibits tapasin-dependent peptide loading and retains MHC-I in the ER. Here we review our recent findings on the central role of ubiquitination and the identification of independent host ubiquitin E3 ligases used for both viral and cellular regulation of MHC-I assembly within the ER.

2. US2 and US11 hijack mammalian ER-associated degradation (ERAD) to target MHC-I for proteasomal degradation in the cytosol

US2 and US11 are ER-resident type I membrane viral glycoproteins expressed early in HCMV infection. Upon co-translational insertion into the ER, newly synthesised MHC-I is immediately bound by US2 or US11 which induces its rapid degradation with a half-life of 1–10 min. The ER is a protein-folding environment and

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is mainly devoid of proteases or an ubiquitin-proteasome system. In a process now known as retrotranslocation or dislocation, US2 and US11 were shown to induce the reverse translocation of MHC-I from the ER back to the cytosol where the MHC-I is deglycosylated by protein *N*-glycanase (PNGase) and degraded by the proteasome (Wiertz et al., 1996a,b).

Although retrotranslocation was first described in the context of US2/11-mediated MHC-I degradation, it turns out to be part of a common cellular pathway used for the degradation of misfolded secretory proteins from the ER—the so-called 'ER-associated degradation (ERAD)' pathway (Olzmann et al., 2013). The ER provides a folding environment for newly synthesised proteins and only those proteins that acquire their native conformation and pass the ER quality control (ERQC) checkpoint are allowed to exit the ER and traffic through the secretory pathway (Hegde and Ploegh, 2010). ERAD therefore provides a mechanism by which misfolded proteins can be safely removed from the ER by retrotranslocation back to the cytosol and degraded by the proteasome. US2 and US11 hijack this cellular quality control pathway and 'force-feed' it with folding-competent MHC-I to induce its rapid proteasomal degradation. As biological tools, US2 and US11 have been invaluable for the functional characterisation of mammalian ERAD pathways, and allowed constructive comparisons with related degradation pathways in yeast. ERAD is not just responsible for the degradation of misfolded proteins, but also plays a critical role in the regulated turn-over of ER-resident proteins, such as the activated IP_3 receptor and the HMG-CoA reductase, squalene monooxygenase and heme oxygenase-1 (HO-1) enzymes.

3. A central role for E3 ubiquitin ligases in the ERAD pathway

Ubiquitination is central to ERAD and membrane-bound ERAD E3 ubiquitin ligases form the core of the retrotranslocation machinery. These ligases are surrounded by numerous ERAD factors required for substrate recognition, dislocation and membrane extraction. Misfolded protein substrates are recognized in the ER by a series of chaperones including OS-9, XTP-3B, EDEM1-3 and BiP and are recruited to the ligase via membrane components such as SEL1L and HERP. The substrate is then ubiquitinated by the ERAD E3 ligase and dislocated into the cytosol with the cytosolic AAA ATPase p97/VCP supplying the energy required for dislocation and membrane extraction. p97 is recruited to the ERAD machinery by membrane components including Derlin-1 and UBXD8. Although the exact nature and identity of the retrotranslocation channel remains unclear, the membrane-bound ERAD E3 ligase and components like Derlin-1 might form part of the retrotranslocon. The dislocated cytosolic substrate is finally deglycosylated by p97-associated PNGase and routed to the proteasome for degradation.

Despite the absolute requirement for ubiquitination in the proteasome-mediated degradation of ERAD substrates, its exact role in dislocation remains unclear. Depletion of the cognate E3 ligase with the resulting loss of ubiquitination inhibits dislocation of many ERAD substrates. However, as ubiquitination only occurs on the cytosolic side of the ER, partial retrotranslocation must necessarily precede ubiquitination for luminal ERAD substrates. Membrane extraction might again be ubiquitin-dependent as the AAA ATPase p97 and its co-factors Ufd1/Npl4 are thought to recognize substrates by their ubiquitin moiety. Yeast contain a minimal ERAD machinery with only three E3 ligases: Hrd1p which is thought to target proteins with a luminal and membrane folding defect (ERAD-L/-M); Doa10p which targets proteins with a cytosolic defect (ERAD-C); and the Asi complex which targets proteins of the nuclear envelope. The evolutionary diversification from yeast to higher eukaryotes greatly expanded the repertoire of ERAD E3 ligases which now include the Hrd1p homologues HRD1 and Gp78, the Doa10p homologue MARCH6/TEB4, RNF5/RMA1, RNF170, as well as TRC8 and TMEM129 (discussed below) and the cytosolic E3 ligase CHIP. The driving force for this diversity and the requirement for an increased number of ligases is not well understood, but increased complexity of the secretory system and cell–cell communication might underlie this phenomenon. US2/11 illustrate the increased complexity of the mammalian ERAD system. They both hijack the mammalian ERAD machinery to induce MHC-I degradation, yet these viral gene products function independently and co-opt distinct ERAD pathways. Despite their early role in defining mammalian ERAD, the host E3 ligases hijacked by HCMV US2 and US11 have only recently been identified.

4. The TRC8 E3 ligase ubiquitinates MHC-I and is rate-limiting in US2-mediated degradation

To identify the E3 ligase required for US2-mediated degradation, we used a flow cytometry based siRNA screen in US2-expressing HeLa cells using GFP-tagged MHC-I as the optical read out. Of the 373 predicted E3 ubiquitin ligases tested, depletion of only the TRC8 (RNF139) E3 ligase rescued MHC-I rescue from US2-mediated degradation (Stagg et al., 2009). TRC8 encodes an ER resident polytopic RING E3 ligase with a C-terminal RING-H2 and a unique N-terminal sterol sensing domain (SDD) and was previously identified as a result of a 3;8 translocation associated with familial renal clear cell carcinoma. TRC8 binds the cytoplasmic tail of US2 resulting in rapid MHC-I polyubiquitination, which triggers full retrotranslocation into the cytosol and subsequent proteasomal degradation of the MHC-I (Hsu et al., 2015; Stagg et al., 2009) (Fig 1A). Although US2-induced degradation is lysine dependent, lysines in the cytoplasmic tail of MHC-I are dispensable, suggesting TRC8 ubiquitinates the luminal domain of the MHC-I heavy chain (Furman et al., 2003). Partial dislocation would therefore precede ubiquitination, as seen in the dislocation of misassembled MHC-I in the absence of US2/11 (Burr et al., 2013). Interestingly, following TRC8 depletion, the presence of US2 is insufficient to retain MHC-I in the ER which escapes to the cell surface, suggesting the US2-MHC interaction is transient and unable to retain MHC-I in the ER in the absence of degradation (Stagg et al., 2009).

A key TRC8 interaction partner is the intramembrane cleaving aspartyl protease, signal peptide peptidase (SPP). Although SPP was suggested to be essential for US2-mediated degradation (Loureiro et al., 2006), neither a traditional homologous recombination-mediated somatic cell knock-out of SPP nor CRISPR-mediated SPP deletions in different cell lines affected US2-mediated MHC-I degradation (Boname et al., 2014). Furthermore, the absence of SPP did not affect the US2-TRC8 interaction. SPP does not therefore appear to be required for the US2-mediated dislocation of MHC-I. However, the finding of an intramembrane cleaving protease associated with an ERAD E3 ligase does provide a potentially attractive mechanism for ER protein turnover. In a subsequent proteomics study we found the tail anchored (TA) protein HO-1 accumulates in the absence of SPP (Boname et al., 2014). Tail anchored proteins are inserted into the ER membrane via a C-terminal transmembrane domain and adopt a type II membrane orientation, making them ideal substrates for SPP which only cleaves membrane proteins with a type II orientation, a conserved feature of all known SPP substrates. The HO-1 transmembrane domain is indeed cleaved by SPP thereby releasing it for TRC8 dependent proteasomal degradation (Fig 1B) (Boname et al., 2014). This mechanism of degradation is not unique to HO-1 but shared by a selection of other TA proteins. In the absence of an extended luminal domain, the release and degradation of these proteins might proceed with-