# UBIQUITIN-CONJUGATING ENZYME INVOLVED IN THE IMMUNE RESPONSE CAUSED BY PATHOGENS INVASION

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## ABSTRACT

Ubiquitin-proteasome pathway (UPP) is a significant way of protein degradation and modification in eukaryotic cell and involved in a complex series of intracellular processes. As a key component in UPP, ubiquitin-conjugating enzyme (E2) plays an extremely important role in ubiquitin (Ub) transferring and substrate specific recognition. More and more researches prove that UPP is involved in cells immune reaction caused by pathogens and the attendance of E2 has a significant effect on host cells and pathogen. This review presents an overview of the current research on E2s that involved in immune response caused by viruses and bacteria.

**Keywords:** Ubiquitin-conjugating enzyme, ubiquitin-proteasome pathway, pathogen, immune response

## INTRODUCTION

As a pathway of protein degradation and posttranslational modification (Ciechanover, 1998), ubiquitin-proteasome pathway (UPP) is responsible for the degradation of 80-90% bulk protein in eukaryotic cell (Ying *et al.*, 2009). UPP is involved in a complex series of intracellular processes, such as: gene expression regulation (Yao *et al.*, 2012), cell cycle regulation, DNA damage repair, immune response (Gray and Estelle, 2000), cell apoptosis (Friendman and Xue, 2004) and so on. To prevent cells from undergoing proteotoxic stress, UPP controls the levels of cellular proteome by regulating the complex series of intracellular processes (Lyupina *et al.*, 2012). UPP is a series of complex enzymatic cascade reaction and composed of ubiquitin-activating enzyme (E1), ubiquitin-conjugating enzyme (E2) and ubiquitin-ligating enzyme (E3) (Vierstra, 2003). With the help of these three enzymes and adenosine triphosphate (ATP), UPP finish ubiquitinating the substrate proteins. As the second enzyme of UPP, E2 is highly conserved in different species by the bioinformatics analysis. E2 plays an extremely important role in ubiquitin (Ub) transferring and substrate specific recognition. In recent years, E3 and viral Ub become research focus. There has recently been great progress in the functions of E3s and viral Ub gene. However, up to now, not much research has been performed on the functions of E2s in cells except the transferring of Ub. More and more researchers prove that UPP is involved in cellular immune reactions caused by pathogens. Interestingly, E2s have always been detected in the immune reactions. But the functions of E2s in immune reactions caused by pathogens are still unclear. This review presents an overview of the current researches on different E2s and the immune responses of cells which caused by viruses and bacteria. The purpose of this review is to enhance understanding of the functions of E2s when cells suffer from the invasion of pathogens.

### 1. Ubiquitin-proteasome pathway

UPP is a main way of protein degradation in eukaryotic cells. Ubiquitination of the target protein comprises three steps. First, ATP-dependent E1 activated Ub by C-terminal adenylation, followed by formation of a high-energy thioester bond. Second, activated Ub is then transferred from E1 to the active-site cysteine of E2 preserving the thioester bond. Third, E3 promote transfer of Ub from E2 to the target protein (Ciechanover *et al.*,2000; Zhao *et al.*, 2004; Kim *et al.*, 2007). At last, the targeted proteins are transported to proteasome for degradation (Fig.1). Actually, Ub is a kind of special protein with a very long life span and itself cannot be degraded. Polyubiquitin chain can be dissociated for target proteins with the help of deubiquitinating enzymes (DUBs). The dissociated Ubs can participate in targeting next substrate protein. In previous studies, the connection between the ubiquitination and the activation of the genes, which are involved in the cellular immune responses, has been identified. To control the activity of some key factors regarding immune responses, cells are likely to up-regulate the expression level of genes involved in progress of protein modification, for example the cytokine TNF-α (Li *et al.*, 2003).

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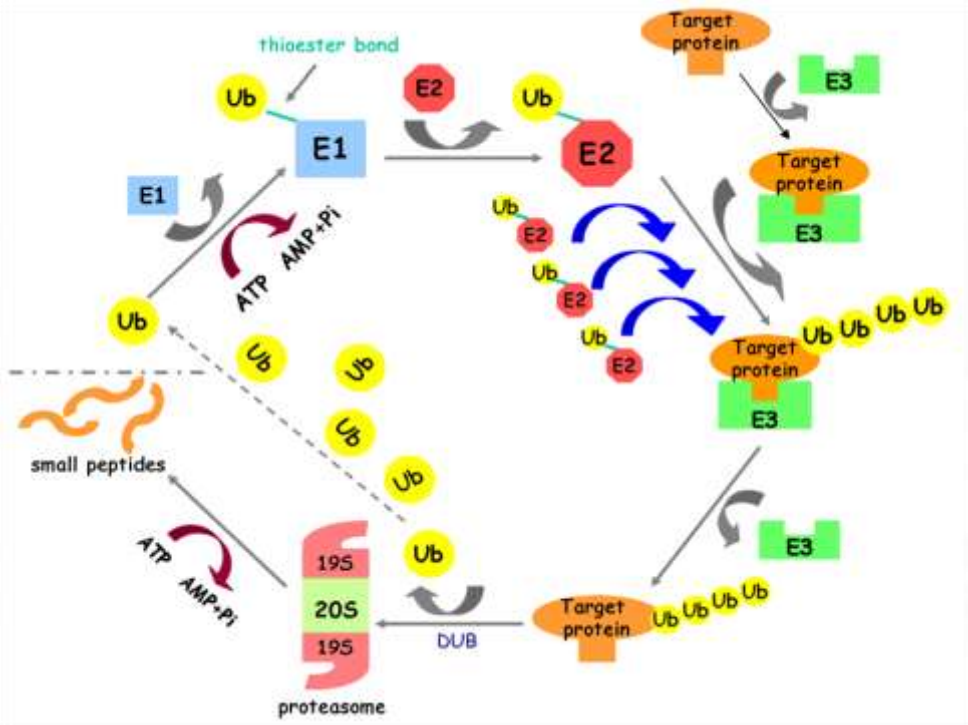


Fig.1. Ubiquitin-proteasome pathway (UPP). ATP-dependent E1 activated Ub by C-terminal adenylation, followed by formation of a high-energy thioester bond; activated Ub is then transferred from E1 to the active-site cysteine of E2 preserving the thioester bond; E3 promote transfer of Ub from E2 to the target protein. Polyubiquitin chain can be dissociated for target proteins with the help of deubiquitinating enzymes (DUBs). The dissociated Ubs can participate in targeting next substrate protein.

**2. The characteristics and classes of E2**

Eukaryotic genomes encode large families of E2s and eukaryotic E2 originate from the *Guillardia theta* nucleomorph about 2500 million years ago (Ying *et al.*, 2009). E2 is mainly vertically inherited over eukaryotic evolution (Lespinet *et al.*, 2002). In *Guillardia theta* nucleomorph, a lot of genes may have been lost due to its condition, and the genes only necessary for survival are kept. However, three E2 genes have been identified among 632 reference sequences (Douglas *et al.*, 2001). Up to now, 17 kinds of E2s have been found in yeast and 13 in *Arabidopsis thaliana* (Hegde and Upadhya, 2011). Human genome encodes 60-70 E2s. The diversification of E2s is quite essential to the specificity of substrates. E2s all have a conservative UBC domain composed of 150 amino acids and within the UBC domain E2s possess a specific active site cysteine residue. The structure of E2 owns an N-termianal α-helix, three C-termianal α-helix and a four-stranded antiparallel β-sheet (Houben *et al.*, 2004). Most of the E2s are small molecular weight proteins (van Wijk and Timmers, 2010). Based on structural analysis, E2s can be divided into four groups: Class I only consists of the conserved UBC domain (Zhang *et al.*, 2003); Class II has additional C-terminal extensions and Class III has additional N-terminal extensions (Binggang *et al.*, 1997; Muller *et al.*, 1998); Class IV possesses both N and C- terminal extensions (Miura *et al.*, 1999). Different classes of E2s are very meaningful to different target proteins of UPP.

**3. E2s and cellular immune responses**

Whether bacterium or virus, the invasion of pathogens can give rise to a series of cellular immune responses. UPP is chief pathway of cellular protein hydrolysis. Nowadays, in order to enhance understanding of the activities of pathogens in cells, many researches are focused on the changes of enzymes involved in UPP when the pathogens invade host cells. E2 is a key enzyme of UPP and it can directly or indirectly conjugate Ub to bulk protein. Recently, that E2 can interact with the proteins of pathogens have been identified in some studies. Not only do the research results provide evidences for the interaction between the host proteins and pathogens, but the results lay the foundation for the further functions of E2s. At present, the researches of E2s are largely focused on several model organisms, such as yeast, Arabidopsis thaliana and mouse. Furthermore, some E2s researchers lay a lot of emphasis on the aspect of tumor. Using DNA microarrays, an ubiquitin-conjugating enzyme E2C gene (UBE2C) was found to be the most highly overexpressed gene in both the primary tumors and the liver metastases (Takahashi *et al.*, 2006). Current studies have shown the increase of E2 expression levels challenged to some pathogens, such as bacteria and

viruses. In the review, we sum the research results about interaction between E2s and pathogens recently and discuss the functions of E2s in cellular immune responses caused by viruses and bacteria.

**3.1 Immune responses caused by viruses**

The virus is a kind of highly parasitic microorganism. Besides, virus itself cannot live alone and it must live on some organisms. By inhibiting the protein synthesis and metabolism or degradation of host proteins, virus plunders and exploits substrate for the proliferation of progeny virus. However, the invasion of virus can lead to a series of immune responses of cell. As a significant pathway of protein degradation, UPP is always used by some viruses. Some key enzymes of UPP are usually targeted by virus, for example, E2s. By interacting with the E2s, virus can modify some cellular protein allowing them to escape antiviral defense systems.

**3.1.1 Plant viruses**

On the aspect of ubiquitination, the best researched of plant is Arabidopsis thaliana. In plant, there are approximately 1400 genes, coded about 5% total protein, involved in UPP. What's more, 37 of those genes can code E2s and 8 code E2-like proteins (Smalle and Vierstra, 2004). Now the research slows that Cotton leaf curl Multan virus (CLCuMV), one of Geminiviruses, can encodes a single gene βC1.βC1 is a kind of pathogenicity protein and important for symptom expression (Cui *et al.,* 2004; Qian and Zhou, 2005). It has been reported that the DNA-β-specific symptoms of the host plans was induced by the interaction between βC1 and the tomato E2 (S1UBC3) (Eini *et al.,* 2009). In order to modify the host UPP, βC1 bind to S1UBC3 on the mytistoylation-like motif. The result shows that the E2 is a target of virus. With the help of the target, virus can succeed in escaping host defenses or exploiting host biological pathway.

**3.1.2 Animal viruses**

The studies on the interaction between animal viruses and the E2s are mainly focused on mammal in recent years. Researchers try to clarify the way in which the viruses plunder and exploit substrates for the proliferation in host cells by investigating the interaction between host UPP and viruses. As the etiological agent of adult T-cell leukemia, Human T-cell leukemia virus type 1 (HTLV-1) encodes Tax, which is a kind of regulatory protein (Yao and Wigdahl, 2000). It has been proved that E2 Ubc13 is important to the ubiquitination Tax. The ubiquitination of Tax oncoprotein can regulate the mechanism that promotes the activation of NF-κB (Shembade and Harhaj, 2007). What's more vital is that the activation of NF-κB is able to facilitate T cells transformation (Harhaj and Harhaj, 2005). T cell transformation is beneficial to the virus itself. But the transformation is disadvantageous to the host cells. This situation has also been reported in the process of HIV infected cells. Tsg101, a kind of E2 variant protein, has been shown to interact specifically with the p6 region of HIV-1 Gag protein in mammalian cells. The interaction is relevant to the life cycle of virus (Ve-Plank *et al.,* 2001). These research results are similar to the results in the researches of plants. Based on the current study results, some E2s are beneficial to viruses survival and proliferation during viruses invasion. In other words viruses are good at making use of host enzymes. But not all the E2s can be exploited by the virus. A kind of *Bombyx mori* E2, involved in antivirus against nucleopolyhedrovirus, has been found by fluorescent differential display in our laboratory (data not published). The expression level of the E2 in the resistance strain was higher than the susceptible strain (data not published). These results imply that various E2s possess different functions. It may be one of the reasons of biodiversity.

**3.2 immune responses caused by bacteria**

The bacterium is a class of etiological agent and the bacterial invasion can result in a series of cellular immune reactions. That E2s can interact with bacteria and help bacteria to invade host cells have been also reported in current studies. The overexpression of UBE2 gene challenged to *Vibrio anguillarum* in *Concholepas concholepas* (Nunez-Acuna *et al.,* 2012). Besides, *Shigella flexneri* is responsible for shigellosis of humans. By targeting E2 of the host cell, *Shigella flexneri* effector OspG interferes with immune responses (Kim *et al.,* 2005). Interestingly, ectopic expression of the E2 from wild rice OgUBC1 in *Arabidopsis thaliana* confers resistance against Botrytis cinerea on the OgUBC1-expressing plants (Jeon *et al.,* 2012). Apparently, OgUBC1 is involved in the ubiquitination process of cellular immune response against Botrytis cinerea in plants. Taken together, the functions of E2s in cellular immune responses caused by bacteria are dissimilar. The diversification of the functions of E2s may be decided by the various structures of E2s.

**Conclusions**

In this review, we focused on showing the different functions of E2s in cellular immune responses caused by viruses and bacteria. As a key component of UPP, E2 is crucial for the protein degradation and modification.

Previous studies have reported that pathogens interacted with cellular immune responses by targeting host E2s. Successful viruses or bacteria have evolved superior strategies to escape host defenses or exploit host biological pathways. Some kinds of E2s, to a certain extent, assist viruses or bacteria to survive and proliferate in host cells. However, the E2s perform distinct functions in cellular responses. Some E2s are likely to be concerned with resistance against pathogens. By enhancing some key genes related to immune responses activity, some E2s can interfere with the survival and proliferation of pathogens. Therefore, the functions of E2s family in cell are not uniform. The difference of E2s makes the research of E2 family become difficult. But, with the tireless efforts, we have obtained great achievements on E2s researches. The researches on E2s not only supplement the functions of E2s family, but also help us to clarify the association between pathogens and host UPP. What's more, the association will assist us to understand the exploitation process of metabolic substrates actualized by pathogens and help us find some potential targets to cure some diseases.

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