



Massachusetts
Institute of
Technology



Under the Radar Screen: How Bugs Trick Our Immune Defenses

Session 4: Ubiquitination and the Proteasome

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In this session

- Paper 1. Identifies a ubiquitin-specific protease (DUB) in Herpes Simplex Virus 1 using activity-based probes, mass spectrometry and proteomic analysis
- Paper 2. Deals with the YopJ virulence factor of *Yersinia*, which is shown to act as DUB, using an NFkB dependent reporter assay and epitope-tagged Ub.

The role of these viral and bacterial gene products in immune evasion through possible rescue from proteasomal degradation and inhibition of NFkB signaling will be discussed

Paper 1

A Deubiquitinating Enzyme Encoded by HSV-1 Belongs to a Family of Cysteine Proteases that Is Conserved across the Family Herpesviridae

Molecular Cell, **19**, pp547-557, (2005)

Lisa M. Kattenhorn, Gregory A. Korbel, Benedikt M. Kessler, Eric Spooner, and
Hidde L. Ploegh

Questions

- A. What does the inclusion of NEM prove in Fig. 1B in the paper?
- B. What mechanisms have been put forward to explain the 47 kD UL37 fragment?
- C. What produces the Gly-GABA modification in Fig. 2B?
- D. Considering Fig. 4 would you say the HSV-1 infected HFFs have now been exhaustively probed for DUBs?
- E. Do the activity assays of Fig. 5 accurately display DUB activity?

Paper 2

Yersinia virulence factor YopJ acts as
a deubiquitinase to inhibit NF- κ B activation

Journal of Experimental Medicine, **202**, pp1327-1332, (2005)

Honglin Zhou, Denise M. Monack, Nobuhiko Kayagaki, Ingrid Wertz,
Jianpin Yin, Beni Wolf, and Vishva M. Dixit

Questions

- A. Can you explain the rationale behind the assay of Fig. 1A and do you think it's properly controlled?
- B. Why do the authors compare siRNA knockdown of Ubc9 with YopJ activity in Fig. 1?
- C. What is the difference between Fig. 2b and Fig. 2c and is it important to show both?
- D. The proteasome inhibitor MG132 shows in Fig. 3c different behavior between YobJ and CYLD. What is the difference between ubiquitin smears with and without MG132?