



**Karolinska  
Institutet**

**Department of Cell and Molecular Biology**

# **Regulation of internalization and replication of intracellular bacterial pathogens**

**AKADEMISK AVHANDLING**

som för avläggande av medicine doktorsexamen vid Karolinska  
Institutet offentligen försvaras i hörsal Petrén, Nobels väg 12B

**Fredagen den 10 june, 2011, kl 09.30**

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**Stockholm 2011**

# ABSTRACT

The capacity of intracellular bacteria to cause disease depends on their ability to invade and replicate within eukaryotic host cells. These characteristics also allow preferential invasion and replication by facultative anaerobic bacteria in solid tumours, which can be exploited to design delivery vectors for cancer therapy.

The aim of this thesis is to study the molecular mechanisms that regulate two parameters of bacterial invasion: internalization and escape from the host innate immune response.

We show that the deubiquitinating enzyme UCH-L1 promotes internalization of *Listeria monocytogenes* and *Salmonella enterica* in epithelial cells. Knockdown of UCH-L1 reduces the uptake of both bacteria in UCH-L1-positive epithelial cells, while expression of the catalytically active enzyme promotes internalization in the UCH-L1-negative HeLa cell line. This effect is dependent on modulation of the actin cytoskeleton dynamics, alteration of clustering and activation of the *L. monocytogenes* receptor Met, a receptor tyrosine kinase (RTK). Actin cytoskeleton re-arrangement and RTK signalling share a common effector protein: the focal adhesion kinase (FAK), a key regulator of focal adhesion complexes. We found that UCH-L1 interacts with components of the focal adhesion and cadherin complexes: FAK, paxillin, vinculin,  $\beta$ -catenin and p120, and further regulates the activation of FAK and the formation of focal adhesion complexes, leading to an increase of adhesive capacity and motility of the cells.

These findings highlight an unrecognized involvement of the ubiquitin cycle in bacterial entry. Considering that UCH-L1 is highly expressed in malignant cells, this may represent one of the mechanisms by which intracellular facultative anaerobic bacteria preferentially localize within solid tumours.

Intracellular bacteria replication is controlled by the activation of a broad array of defensive mechanisms, but mainly relies on compartmentalization followed by lysosomal destruction of the invading microorganisms in professional phagocytic cells, macrophages and neutrophils. Several pro-inflammatory cytokines enhance the bactericidal capacity of the host cells. We demonstrated that the *bona fide* cytokine Thioredoxin (Trx) 80, a truncated form of Thioredoxin 1, induces monocytes activation and inhibits replication of intracellular pathogens by trapping the bacteria into the lysosomal compartment, thus promoting their destruction. Our results show that Trx80 potentiates the bactericidal activities of professional phagocytes, and contributes to the first line of defense against intracellular pathogens.