Curriculum Vitae

Stephen J. Juris

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Harvard Medical School
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Education

Postdoctoral Research — June 2002 - Present
Harvard Medical School, Boston, MA
Advisor: R. John Collier, Ph.D.
Title: *Identification of novel inhibitors of anthrax toxin action*

Ph.D., Biological Chemistry — June 2002
The University of Michigan, Ann Arbor, MI
Advisor: Jack E. Dixon, Ph.D.
Thesis Title: *Analysis of the function of the Yersinia proteins kinase YpkA and its role in bacterial pathogenesis*

B.S., Biochemistry — May 1996
Boston College, Chestnut Hill, MA

Research Experience

Harvard Medical School, Boston, MA — June 2002 - Present
R. John Collier, Ph.D.: Identification of inhibitors of anthrax toxin action.

The University of Michigan, Ann Arbor, MI — September 1996 – June 2002
Jack E. Dixon, Ph.D.: The role of the *Yersinia* protein kinase YpkA in pathogenesis.

Yale University, New Haven, CT — Summer 1995
Gerald I. Shulman, MD, Ph.D.: The role of free fatty acids on GLUT4 translocation to the plasma membrane in type II diabetes.

Bayer Pharmaceuticals, West Haven, CT — Summer 1994
Charles Maniglia, Ph.D.: The role of kinases in the onset of osteoarthritis.

Bayer Pharmaceuticals, West Haven, CT — Summer 1993
Charles Maniglia, Ph.D.: Analysis of levels of TGF-β in osteoarthritic cartilage.

Teaching Experience

The University of Michigan, Department of Biological Chemistry 2000
Tutor for individual: Dental Biochemistry

The University of Michigan, Department of Biological Chemistry 1997
Tutor for undergraduates: Descriptive Biochemistry

The University of Michigan, Department of Biological Chemistry 1997
Teaching Assistant: Introductory Biochemistry Laboratory

The University of Michigan, Department of Biological Chemistry 1996
Teaching Assistant: Introductory Biochemistry

Honors and Awards

Boston College Merit Scholarship (1992-1996)
Molecular Mechanisms of Microbial Pathogenesis Training Fellowship (1999-2001)

Publications

1. **Stephen J. Juris**, Amy E. Rudolph, Don Huddler, Kim Orth, and Jack E. Dixon. 2000. A distinctive role for the *Yersinia* protein kinase: actin binding, kinase activation, and cytoskeleton disruption. *Proc. Natl. Acad. Sci. U.S.A.* 97: 9431-9436.

2. **Stephen J. Juris***, Feng Shao*, and Jack E. Dixon. 2002. *Yersinia* effectors target mammalian signaling pathways. *Cell. Microbiol.* 4: 201-211.

3. Darran J. Wigelsworth, Bryan A. Krantz, Kenneth A. Christensen, D. Borden Lacy, **Stephen J. Juris**, and R. John Collier. 2004. Binding stoichiometry and kinetics of the interaction of a human anthrax toxin receptor, CMG2, with protective antigen. *J. Biol. Chem.* 279: 23349-23356.

4. Bryan A. Krantz*, Roman A. Melnyk*, Sen Zhang, **Stephen J. Juris**, D. Borden Lacy, Zhengyan Wu, Alana Finkelstein, and R. John Collier. 2005. A phenylalanine clamp catalyzes protein translocation through the anthrax toxin pore. *Science.* 309: 777-781.

5. **Stephen J. Juris**, Kavita Shah, Kevan Shokat, Jack E. Dixon, and Panayiotis O. Vacratsis. 2005. Identification of otubain 1 as a novel substrate for the *Yersinia* protein kinase using chemical genetics and mass spectrometry. *Chem. Biol.* (submitted).

6. **Stephen J. Juris**, Bryan A. Krantz, Darran J. Wigelsworth, and R. John Collier. 2005. Modification of the N-terminal domain of lethal factor alters translocation via protective antigen. (in preparation).

7. **Stephen J. Juris**, Su Chiang, Brian C. Kraybill, and R. John Collier. 2005. Identification of small molecule inhibitors of anthrax toxin translocation. (in preparation).

8. **Stephen J. Juris**, Roman A. Melnyk, and R. John Collier. 2005. Unraveling the mechanism of anthrax toxin translocation: traversing the membrane one by one. (in preparation).

* These authors contributed equally to the work.

Presentations

1. **Stephen J. Juris**, Amy Rudolph, Kim Orth, and Jack E. Dixon. Identification of a cytosolic activator of the *Yersinia* outer protein (Yop) effector YpkA. Cold Spring Harbor Meeting in Microbial Pathogenesis and Hosts Response, Cold Spring Harbor, New York, September 1999.

2. **Stephen J. Juris** (Invited Speaker), Amy E. Rudolph, Don Huddler, Kim Orth, and Jack E. Dixon. A distinctive role for the *Yersinia* protein kinase: actin binding, kinase activation, and cytoskeleton disruption. Protein Phosphorylation and Molecular Recognition, Experimental Biology 2001 (ASBMB), Orlando, Florida, March 2001.

3. **Stephen J. Juris** (Invited Speaker), Su Chiang, Ruth-Anne Pimental, and R. John Collier. Identification of small molecule inhibitors of anthrax toxin translocation. Regional Centers for Defense and Emerging Infectious Disease Research, Second Annual Meeting, Galveston, Texas, March 2005.

4. **Stephen J. Juris**, Roman A. Melnyk, and R. John Collier. Unraveling the mechanism of anthrax toxin translocation: traversing the membrane one by one. Cold Spring Harbor Meeting in Microbial Pathogenesis and Host Response, Cold Spring Harbor, New York, September 2005.

Other Skills

Detailed understanding of Macintosh operating systems and standard desktop publishing tools (Word, Excel, Powerpoint, Photoshop, Illustrator).

Comprehensive understanding of Lasergene DNA sequence management and sequence analysis software.

Experience using sequence databases, including BLAST and PSI-BLAST alignments.

DISSERTATION RESEARCH SUMMARY

Pathogenic species of *Yersinia* utilize a type III secretion apparatus to translocate six effector proteins into the host cell. These proteins downregulate the immune response of the host, thereby insuring bacterial survival. One effector protein, YpkA, contains a eukaryotic serine/threonine protein kinase domain. The experiments described here analyze the role of YpkA in *Yersinia* pathogenesis and identify its target within the host cell.

Transient overexpression of YpkA in mammalian cells and *in vitro* kinase assays revealed that YpkA is expressed as an active kinase in mammalian cells. However, recombinant YpkA was completely inactive *in vitro*, suggesting that mammalian cells contain an activator of YpkA. Indeed, addition of eukaryotic cell extracts to recombinant YpkA resulted in the stimulation of YpkA kinase activity. Biochemical purification identified actin as an activator of YpkA. Addition of purified actin to YpkA resulted in a dose-dependent activation of YpkA, with maximal activity measured at stoichiometric ratios of YpkA and actin. Deletion of the C-terminal amphipathic helix in YpkA disrupted both YpkA kinase activity and the interaction between YpkA and actin. Transient overexpression of YpkA in HeLa cells resulted in a cytotoxic phenotype with a complete disruption of actin stress fibers. *In vitro* kinase assays revealed that both G- and F-actin are substrates for YpkA. Although, phosphorylation of actin does not inhibit *in vitro* polymerization, it may inhibit actin dynamics *in vivo*. Biochemical characterization of actin phosphorylation revealed that YpkA phosphorylated actin on serine 33, which is located in subdomain 2 of actin. This domain is important for ATPase activity and monomer interaction in growing actin filaments, suggesting that phosphorylation of this serine may alter these functions.

To identify novel YpkA substrates, a technique termed chemical genetics was employed. This technique identified a novel 36-kDa substrate for YpkA within mammalian extracts. This 36-kDa protein was identified as human protein FLJ20113 using matrix-assisted laser desorption ionization-time of flight (MALDI-TOF) mass spectrometry. FLJ20113 is not only an *in vitro* substrate for YpkA, but it also co-immunoprecipitates with YpkA and the actin cytoskeleton. FLJ20113 has been identified as otubain 1, a member of a family of deubiquitinating enzymes, suggesting a role for a novel protease and ubiquitination in the regulation of actin dynamics. Collectively, these studies reveal a mechanism of YpkA regulation and clarify its role in *Yersinia* pathogenesis.

POSTDOCTORAL RESEARCH SUMMARY

The causative agent of anthrax is the Gram-positive rod-shaped bacterium *Bacillus anthracis*. There are two main virulence factors of *B. anthracis*: a capsule, which inhibits phagocytosis, and a toxin. The toxin belongs to the AB family of toxins, each member of which has a moiety that acts within the target cell (the A moiety) and a moiety that binds to the target cell and translocates the A moiety into the cytosol (the B moiety). Anthrax toxin consists of a single B moiety called protective antigen (PA) and two A moieties: edema factor (EF) and lethal factor (LF). Translocation of EF and LF into the host cell by PA requires the binding of PA to the anthrax toxin receptor, a ubiquitously expressed transmembrane protein, and cleavage of PA by furin or a furin-like protease. After cleavage, the C-terminal 63 kDa fragment of PA heptamerizes and binds EF and LF. These assembled complexes are endocytosed, and at low pH within the endosome, a conformational change is induced in the heptamer that involves formation of a 14-stranded transmembrane beta-barrel. Translocation of EF and LF into the target cell cytosol is dependent on channel formation, but the exact nature of the dependence is unknown. Indeed, the process of translocation is poorly understood for any AB toxin.

To elucidate the structural constraints on the enzymatic moieties during translocation, I have engineered multimeric forms of the N-terminal domain of LF (LFn) and tested their ability to translocate across planar lipid bilayers. Crosslinked LFn is incapable of translocating in a PA-dependent manner, demonstrating that the PA channel cannot accommodate two enzymatic moieties at the same time during translocation. As crosslinked LFn cannot translocate, I tested its potential as an inhibitor of toxin translocation into cells by testing its ability to inhibit the translocation of the fusion protein LFn-DTA (a fusion between LFn and the catalytic domain of diphtheria toxin) into CHO cells. Whereas LFn-DTA translocation into cells was inhibited by LFn with an IC_{50} value of 4 nM, crosslinked LFn was capable of inhibiting LFn-DTA translocation 80-200 fold better (IC_{50}s between 20 and 50 pM). These data suggest that multimeric LFn competitively binds to PA prepores and acts to block the normal translocation function of PA. These studies reveal a novel approach to generating anthrax therapeutics. I have also designed an assay to identify small molecule inhibitors that will inhibit translocation of anthrax toxin in order to identify potential therapeutics for anthrax. I have screened over 50,000 small molecules for inhibition of LF_N-DTA cytotoxicity and have identified small molecule inhibitors of this pathway (1,084 hits). A number of these molecules (17) inhibit both LF_N-DTA and LF cytotoxicity, suggesting that these molecules inhibit toxin translocation at steps earlier than enzymatic action within the intoxication pathway. Currently, the strongest inhibitors of this screen, as well as the compounds that inhibit both LF_N-DTA and LF cytotoxicity, are being verified and tested in biochemical *in vitro* assays to determine their point of action within the anthrax intoxication pathway. Inhibitors identified can provide insight into anthrax toxin translocation and a starting point for application of combinatorial chemistry to modify inhibitors to maximize potency and bioavailability.

References

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