BIOGRAPHICAL SKETCH

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NAME: Robert A. Nicholas

eRA COMMONS USER NAME (credential, e.g., agency login): ROBERT_NICHOLAS

POSITION TITLE: Professor and Vice Chair

EDUCATION/TRAINING *(Begin with baccalaureate or other initial professional education, such as nursing, include postdoctoral training and residency training if applicable. Add/delete rows as necessary.)*

INSTITUTION AND LOCATION	DEGREE *(if applicable)*	Completion Date MM/YYYY	FIELD OF STUDY
University of Illinois, Urbana-Champaign	B.S. w/ high distinction	1977	Biochemistry
University of California, San Diego	Ph.D.	1984	Chemistry/Biochemistry
Harvard University, Cambridge, MA (Postdoctoral Training)	- - -	1984-1988	Biochemistry

A. Personal Statement

At UNC, I have built a research group that has defined at the molecular level the mechanisms of chromosomally mediated resistance to β-lactam antibiotics and tetracycline in *N. gonorrhoeae*. We have established experimental systems to define unequivocally the roles of individual resistance determinants, as well as their synergism, in the acquisition of chromosomally mediated antibiotic resistance. My laboratory has expertise in a wide range of approaches, including genetics, bacteriology, and biochemistry, and also in the biochemical and physiological characterization of altered mosaic alleles of *penA* encoding Penicillin-Binding Protein 2 (PBP2). I also have a research project to develop new antibiotics for *N. gonorrhoeae* against a novel target, LpxC, an essential enzyme in the lipid A biosynthesis pathway.

I also have an additional research focus on the molecular pharmacology of P2Y nucleotide receptors. These receptors respond to extracellular nucleotides and are important signaling proteins in most major organ systems. In particular, we are investigating the molecular mechanisms of ADP-promoted platelet aggregation. The $P2Y_1$ receptor is coupled to Gq and Ca^{2+} mobilization, and it undergoes rapid desensitization in the presence of agonists. Our current approach is to examine the mechanism of this desensitization in a mouse model, and to determine if this process is critical for proper clot size and hemostasis.

a. Fedarovich A, Cook E, Tomberg J, Nicholas RA, Davies C. (2014) Structural effect of the Asp345a insertion in penicillin-binding protein 2 from penicillin-resistant strains of *Neisseria gonorrhoeae*. Biochemistry 53(48):7596-603. **PMC4263433**
b. Tomberg J, Unemo M, Ohnishi M, Davies C, Nicholas RA (2013) Identification of the amino acids conferring high-level resistance to expanded-spectrum cephalosporins in the *penA* gene from the *Neisseria gonorrhoeae* strain H041. Antimicrob Agents Chemother 57(7):3029-36. **PMC3697319**
c. Tomberg J, Temple B, Fedarovich A, Davies C, Nicholas RA (2012) A highly conserved interaction involving the middle residue of the SXN active-site motif is crucial for function of class B penicillin-binding proteins: mutational and computational analysis of PBP 2 from *Neisseria gonorrhoeae.* Biochemistry 51(13):2775-84. **PMC3338128**
d. Tomberg J, Unemo M, Davies C, Nicholas RA (2010) Molecular and structural analysis of mosaic variants of penicillin-binding protein 2 conferring decreased susceptibility to expanded-spectrum cephalosporins in *Neisseria gonorrhoeae*: role of epistatic mutations. Biochemistry 49(37):8062-70. **PMC2939205**
e. Powell AJ, Tomberg J, Deacon AM, Nicholas RA, Davies C. (2009) Crystal structures of penicillin-binding protein 2 from penicillin-susceptible and penicillin–resistant strains of *Neisseria gonorrhoeae* reveal an unexpectedly subtle mechanism for antibiotic resistance. J Biol Chem 284(2):1202-12. **PMC2613624**

B. Positions and Honors

Positions and Employment

1988-1994	Assistant Professor, Department of Pharmacology, University of North Carolina at Chapel Hill
1994-2002	Associate Professor, Department of Pharmacology, University of North Carolina at Chapel Hill
2002-present	Professor, Department of Pharmacology, University of North Carolina at Chapel Hill
2006-present	Vice-Chair, Department of Pharmacology, University of North Carolina at Chapel Hill
2008-present	Professor, Department of Microbiology and Immunology, University of North Carolina at Chapel Hill
2013-present	Associate Director of Graduate Education, University of North Carolina at Chapel Hill
	Faculty Director, Biological and Biomedical Sciences Program, University of North Carolina at Chapel Hill

Other Experience and Professional Memberships

1992-present	Member, American Society for Microbiology
1993-1996	American Heart Association Research Review Committee, North Carolina Affiliate
1996-1998	American Heart Association Research Committee, North Carolina Affiliate
1996-present	Member, American Society for Pharmacology and Experimental Therapeutics (ASPET)
1998-2000	Member, ASPET Program Committee
1999-present	Editorial Board, Molecular Pharmacology
1999-2004	Director of Graduate Studies, Dept. of Pharmacology
2001-2003	Secretary-Treasurer of ASPET Division for Molecular Pharmacology (elect, current, and past)
2005-2007	President of ASPET Division for Molecular Pharmacology (elect, current, and past)
2007-present	Executive Committee, ASPET Division for Molecular Pharmacology
2005-present	Editorial Board, American Journal of Physiology

Honors

1989-1991	Faculty Development Award in Basic Pharmacology from the Pharmaceutical Manufacturers Association Foundation
1992	Jr. Faculty Development Award from the University of North Carolina at Chapel Hill
1994-1999	Established Investigator of the American Heart Association
2003-2004	Recipient, Teaching Excellence Award in Pharmacology
2004-2005	Recipient, Teaching Excellence Award in Pharmacology

C. Contribution to Science

1. My early work in chromosomally mediated antibiotic resistance in the pathogenic organism *Neisseria gonorrhoeae* focused on identifying the molecular basis of resistance. My lab was the first to identify the mutations responsible for resistance to β-lactam antibiotics and tetracyclines conferred by *penB*, *ponA*, and *rpsJ*. Screening for these mutations in clinical isolates is now commonplace in clinical microbiology labs throughout the world. These studies also revealed the complex nature of high-level antibiotic resistance and the synergistic interactions of different resistance determinants. For example, we showed that resistance due to mutations in the outer membrane porin PIB encoded by *penB* require the presence of the *mtr* determinant that increases expression of the MtrC-MtrD-MtrE efflux pump, that a mutation in PBP1 it depends on other factors to increase resistance, and that *rpsJ* requires the *mtr* and *penB* determinants to achieve donor levels of resistance to tetracycline.
    a. Ropp PA, Hu M, Olesky M, Nicholas RA. (2002) Mutations in *ponA*, the gene encoding penicillin-binding protein 1, and a novel locus, *penC*, are required for high-level chromosomally mediated penicillin resistance in *Neisseria gonorrhoeae*. Antimicrob Agents Chemother, 46(3):769-77. **PMC127492**.
    b. Olesky M, Hobbs M, Nicholas RA. Identification and analysis of amino acid mutations in porin IB that mediate intermediate-level resistance to penicillin and tetracycline in *Neisseria gonorrhoeae*. (2002) Antimicrob Agents Chemother, 46(9):2811-20. **PMC127413**.
    c. Olesky M, Zhao S, Rosenberg RL, Nicholas RA. (2006) Porin-mediated antibiotic resistance in

Neisseria gonorrhoeae: ion, solute, and antibiotic permeation through PIB proteins with *penB* mutations. J Bacteriol 188(7):2300-8. **PMC1428387**.

d. Hu M, Nandi S, Davies C, Nicholas RA. (2005) High-level chromosomally mediated tetracycline resistance in *Neisseria gonorrhoeae* results from a point mutation in the *rpsJ* gene encoding ribosomal protein S10 in combination with the *mtrR* and *penB* resistance determinants. Antimicrob Agents Chemother, 49(10):4327-34. **PMC1251527**.

2. Our more recent work with chromosomally mediated antibiotic resistance in *N. gonorrhoeae* has focused on understanding the mechanisms of this resistance. These papers are widely cited, and are the definitive studies in the field for understanding chromosomally mediated resistance to the expanded-spectrum cephalosporins, ceftriaxone and cefixime. The studies also include our discovery of the role of the secretin PilQ in mediating influx of antibiotics into the cell.
   a. Zhao S, Duncan M, Tomberg J, Davies C, Unemo M, Nicholas RA. (2009) Genetics of chromosomally mediated intermediate resistance to ceftriaxone and cefixime in *Neisseria gonorrhoeae*. Antimicrob Agents Chemother 53(9):3744-51. **PMC2737842**
   b. Zhao S, Tobiason D, Seifert HS, and Nicholas RA (2005) The *penC* mutation conferring antibiotic resistance in *Neisseria gonorrhoeae* arises from a mutation in the PilQ secretin that interferes with multimer assembly and prevents antibiotic influx. Mol Microbiol, 57:1238-51. **PMC2673695**
   c. Nandi S, Swanson S, Tomberg J, Nicholas RA. Diffusion of antibiotics through the PilQ secretin in *Neisseria gonorrhoeae* occurs through the immature, SDS-labile form (2015) J Bacteriol, Jan 20;PubMed PMID: 25605303. **PMC4372736**
3. We have also investigated the regulation of the nucleotide-activated P2Y receptors in signaling, trafficking and internalization. These studies established the distribution of receptors in polarized epithelial cells and were the first to systematically investigate the mechanisms of P2Y receptor distribution at the molecular level. We defined a novel targeting sequences in $P2Y_1$, $P2Y_2$, and $P2Y_4$ receptors, and our studies on targeting of $P2Y_2$ receptors were the first to show the existence of an extracellular apical targeting signal. We also defined the residues involved in receptor internalization of the $P2Y_1$ receptor, which has paved the way for our more recent studies investigating the desensitization of these receptors in platelets.
   a. Wolff SC, Qi, AD, Harden TK, Nicholas RA (2010) Charged residues in the C-terminus of the $P2Y_1$ receptor constitute a basolateral-sorting signal. J Cell Sci 123(14):2512-20. **PMC2894661**
   b. Qi, AD, Wolff, SC, and Nicholas, RA (2005) The apical targeting signal of the $P2Y_2$ receptor is located in its first extracellular loop. J Biol Chem, 80:29169-75.
   c. DuBose DR, Wolff SC, Qi AD, Naruszewicz I, Nicholas RA (2013) Apical targeting of the $P2Y_4$ receptor is directed by hydrophobic and basic residues in the cytoplasmic tail. Am J Physiol Cell Physiol 304(3):C228-39. **PMC3566436**
   d. Wolff, SC, Qi, AD, Harden, TK, Nicholas, RA. (2005) Polarized expression of human P2Y receptors in epithelial cells from kidney, lung, and colon. Amer J Physiol (Cell Physiol), 288(3):C624-32.
   e. Qi AD, Houston-Cohen D, Naruszewicz I, Harden TK, Nicholas RA (2011) Ser352 and Ser354 in the carboxyl terminus of the human $P2Y_1$ receptor are required for agonist-promoted phosphorylation and internalization in MDCK cells. Br J Pharmacol 162: 1304-13. **PMC3058163**

A complete bibliography can be found through my NCBI at the following URL: http://www.ncbi.nlm.nih.gov/sites/myncbi/10ap9uOKouf/bibliography/40331898/public/?sort=date&direction=descending

D. Research Support

Ongoing Research Support

5 R01 AI094475 (PI: Zhou, P) NIH/NIAID 04/01/11 – 03/31/16

"LpxC inhibitors as a novel class of antibiotics against *N. gonorrhoeae*"

The long-term goal of this proposal is to develop LpxC inhibitors with good pharmacokinetic and pharmacodynamic properties that are potent and efficacious against *N. gonorrhoeae* both *in vitro* and *in vivo*.

Role: Co-PI

2 R01 GM066861 (PI: Davies, C) NIH/NIGMS 01/01/12 – 12/31/16

"Molecular Targets in Peptidoglycan Synthesis"

The goals of this project are i) to determine the role of protein dynamics in penicillin resistance mediated by mutations in PBP 2, ii) to elucidate the mechanism of cephalosporin resistance conferred by mosaic variants of PBP 2, and iii) to determine the substrate specificities of AmiC and the biological function of its N-terminal domain.

Role: Co-Investigator

1U19AI113170-01 (CRC PI: Jerse, A) NIH/NIAID 08/01/2014 – 07/31/2019

The Atlantic Coast Sexually Transmitted Infection Cooperative Research Center (AC STI CRC)

Project 5: "Impact of Gonococcal Antibiotic Resistance Mechanisms on In-Host Fitness"

The goals of this project are: i) to identify the mutation(s) in compensatory mutants of FA19 *penA41* and elucidate the mechanisms by which these mutations increase fitness, ii) to define the role of *mtr* mutations in fitness of gonococci with a mosaic *penA* allele and to identify the changes in transcription following acquisition of a mosaic *penA* allele, and iii) to examine if genes known to be important in gonococcal resistance to cationic antimicrobial peptides (CAMPs) or other genes can contribute to fitness advantages to wild-type and or Cro^R *penA* mutant strains.

Role: PI, Project 5

1U19AI113170-01 (CRC PI: Jerse, A) NIH/NIAID 08/01/2014 – 07/31/2019

The Atlantic Coast Sexually Transmitted Infection Cooperative Research Center (AC STI CRC)

Project 1: "Gonococcal Modulation of Host Immune Responses during Single and Dual Infections"

The goals of this project are: i) to identify mechanisms by which *N. gonorrhoeae* PorB and OMV inhibit DC-induced T cell proliferation, ii) to determine if gonococcal LtgA and LtgD enzymes interfere with host detection, and iii) to determine which aspects of *N. gonorrhoeae* host immune manipulation enhance chlamydial infection.

Role: Co-investigator, Project 1 (PI: Duncan, J)

AHA 14GRNT20480121 Grant-in-Aid American Heart Association 07/01/14 – 06/31/16

"Regulation of Platelet Aggregation through Rapid Desensitization of the $P2Y_1$ Receptor"

The aims of this proposal are to understand how desensitization of the $P2Y_1$ receptor limits platelet aggregation and thrombus formation.

Role: Principal Investigator

5-T32-GM007040 (PI: Nicholas, RA) NIH/NIGMS 07/01/1975 – 06/30/2015

"Predoctoral Training in Pharmacological Sciences"

Role: PI/Program Director

Pending Research Support

2 R01 GM066861 (PI: Davies, C) NIH/NIGMS 01/01/16 – 12/31/21

"Molecular Targets in Peptidoglycan Synthesis"

The goals of this project are i) to determine the mechanisms by which specific mutations in PBP2 contribute to cephalosporin resistance, ii) to determine how PBP2 discriminates against cephalosporins and not PG substrate, and iii) to determine whether molecular dynamics underpins cephalosporin resistance.

Role: Co-Investigator

Impact Score: 30 Percentile: 15%