
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 P2Y₁ receptor is coupled to Gq and Ca²⁺ 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, Tobaison 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 P2Y₁, P2Y₂, and P2Y₄ receptors, and our studies on targeting of P2Y₂ 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 P2Y₁ 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 P2Y₁ 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 P2Y₂ 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 P2Y₄ 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 P2Y₁ receptor are required for agonist-promoted phosphorylation and internalization in MDCK cells. Br J Pharmacol 162: 1304-13. **PMC3058163**

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