BIOGRAPHICAL SKETCH

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NAME: Diane L. Barber, PhD

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

POSITION TITLE: Professor and Department 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 California, Davis	B.S.	06/75	Biological Sciences
University of California, Davis	M.S.	06/77	Physiology
University of California, Los Angeles	Ph.D.	03/85	Anatomy

A. Personal Statement

Our research program addresses questions on fundamental biological processes across scales, from molecules to cells to tissues. Our focus is on the regulation of epithelial plasticity, including differentiation and cancer behaviors. Within this context we are determining how protein behaviors and cell functions are regulated by intracellular pH (pHi) and actin filament dynamics. Cancer cells have a higher pHi than normal cells, which as we describe enables metastatic progression (Webb et al., 2011 Nat Rev Cancer 11:671). However, we have limited understanding of how this occurs at the molecular level and can be exploited to limit disease progression. We have unique expertise to resolve regulation by pHi dynamics at molecular, cellular and tissue scales by bridging structural and cell biology. Through our 10-year collaboration with Matthew Jacobson, a computational biologist, we have shown how protonation functions as a post-translational modification to regulate protein structure and function (Schönichen et al., 2013 Ann Rev Biophys 42:289). We showed in molecular detail how increased pHi is necessary for directed cell migration by identifying the design principles and functional significance of selective pH sensors, including guanine nucleotide exchange factors regulating cell polarity (Frantz et al., 2007 J Cell Biol 179:403), cofilin controlling actin assemblies (Frantz et al., 2008 J Cell Biol 183:865, and talin (Srivastava et al., 2008 Proc Natl Acad Sci 105:14436) and the focal adhesion kinase FAK (Choi et al., 2013 J Cell Biol 202:849) controlling cell-substrate adhesion. We are now addressing questions on how increased pHi enables tumorigenic behaviors (Grillo-Hill et al., 2015 eLife 4:e03270), glycolytic enzymes for metabolic programming (Webb et al., 2015 Nature 523:111) and the selection and retention of specific somatic mutations. Contributing to these studies is our distinct and unique expertise in investigating pHi dynamics and pHi-regulated cell processes combined with innovative approaches, including optogenetic tools for modulating pHi, genetically encoded biosensors to rigorously quantify pHi dynamics in single cells and *in vivo*, and computational programs for identifying titrating networks of ionizable residues in proteins as well as amino acid mutation signatures in cancer databases. In addition to cancer, dysregulated pHi is seen in other human diseases and our recent work investigates how lysosomal and cytosolic pH dynamics contributes to Alzheimer's pathologies. For differentiation programs, we found that increased pHi is necessary for epithelial to mesenchymal transition (EMT; manuscript in preparation) and embryonic stem cell differentiation (manuscript in revision). Our work on actin filament remodeling has focused on regulated actin dynamics in cell migration (Denker et al., 2002 J Cell Biol 159:1087; Patel and Barber, 2005 J Cell Biol 169:321; Baumgartner et al., 2006 PNAS 103:13391; Frantz et al., 2008 J Cell Biol 183:865), in EMT (Haynes et al., 2011 Mol Biol Cell 22:4750) and new modes for activation of the Arp2/3 complex (LeClaire et al., 2008 J Cell Biol 182:647; Narayanan et al., PLoS Comput Biol 7:e1002226; LeClaire et al., 2015 J Cell Biol 208:161). In addition to investigative research, I have a strong training and mentoring record, including

receiving the 2012 Outstanding Faculty Mentoring Award from the UCSF Postdoctoral Scholars Association, serving as the primary advisor for 28 former graduate students and postdoctoral scholars, with 13 currently having academic faculty appointments. Additionally, I became an AAAS Fellow in 2012 and in 2016 was selected to serve as Chair of Women in Cell Biology (WICB) for the American Society for Cell Biology (ASCB).

B. Positions and Honors

Academic Positions

1977-1980	Lecturer, Department of Biology, University of California, Los Angeles
1980-1985	Predoctoral Fellow, Department of Anatomy, University of California, Los Angeles
1985-1987	NIH Postdoctoral Fellow (NRSA), Department of Physiology, University of Massachusetts Medical Center, Worcester, MA
1987-1991	Assistant Professor, Department of Surgery/Section of Anatomy, Yale University
1991-1995	Assistant Professor, Departments of Stomatology and Surgery, UCSF
1995-2001	Associate Professor, Departments of Stomatology and Surgery, UCSF
2001-2004	Professor, Department of Stomatology, UCSF
2005-2010	Professor and Vice-Chair, Department of Cell and Tissue Biology, UCSF
2010-2013	Professor and Interim Chair, Department of Cell and Tissue Biology, UCSF
2013-present	Professor and Chair, Department of Cell and Tissue Biology, UCSF

Awards and Recognitions

1985-1987	NIH Individual NRSA Postdoctoral Fellowship
1986	Joseph P. Healy Research Award, University of Massachusetts Medical Center
1995-2000	Established Investigator, American Heart Association
1996-2013	NIH Study Sections (1996-1998 GMA2; 2001-2004 CDF3; 2005 2 sessions CSF; 2008 MIST, 2009 NCDS, 2009-2011 EUREKA, 2012-2014 ZRG1, 2015 ZGM1)
1997-present	Editorial Board service American Journal of Physiology: Cell Physiology, American Society of Biochemistry and Molecular Biology, Journal of Molecular Signaling
1998	Innovation in Basic Sciences Award, UCSF
2001-2003	Vice-chair/Chair, Gordon Research Conference on Molecular Pharmacology
2008	Annual Student Invited Speaker, Molecular and Cellular Biochemistry, University of Kentucky
2010-present	Leland A. and Gladys K. Barber Endowed Chair in Dentistry
2012	Elected AAAS Fellow
2012	Outstanding Faculty Mentor Award, UCSF Postdoctoral Scholars Association
2013-present	UCSF Discovery Fellows Advisory Council
2013	Faculty Research Lecturer Award, UCSF School of Dentistry
2014	Keynote Speaker, Bay Area Postdoctoral Symposium
2015	Invited participant (of 25) for Telluride Workshop on Protein Electrostatics
2016-2019	Chair, Women in Cell Biology (WICB) for American Society of Cell Biology (ASCB)
2016	Invited participant (of 20) for Company of Biologists International Workshop on Metabolism in Development and Disease
2016	NIH Workshop on Advancing Women in Independent Careers, Speaker

C. Contributions to Science

1. Functional significance of intracellular pH (pHi) dynamics and design principles of pH sensors

We have pioneered elucidating the role of intracellular pH (pHi) dynamics in regulating diverse cell processes. We have also generated new insights on a molecular understanding of how pHi dynamics regulates protein structure and function. In collaboration with Matthew Jacobson at UCSF, more than 10 years ago we began using the term “pH sensor” for proteins with activities and ligand binding affinities that are regulated within the narrow pHi range (1a) and this term has been widely adopted in the field. We also had a major impact on the view of post-translational modification by protons as a regulatory mechanism for protein structure and function, analogous to post-translational modification by phosphorylation and acetylation, as highlighted in our invited publication in Annual Reviews of Biophysics (1b). Determining protein regulation by pHi dynamics has a number of challenges because protonation/deprotonation cannot be detected by mass spectrometry or antibodies and is not catalyzed by enzymes. However, our work has established that protein regulation by physiological pH changes includes classically defined modes such as specificity, allostery, coincidence detection and cooperativity. Moreover, because pHi dynamics can regulate multiple proteins in unison it can

coordinate complex cell behaviors, as described below for directed cell migration (Contribution 2), and as we highlighted for the effects of dyregulated pHi dynamics in cancer (1c,d). Through our work on cell behaviors regulated by pHi dynamics we developed innovative approaches for modulating pHi, including optogenetic tools, and for rigorously quantifying pHi dynamics, including biosensors for *in vivo* analysis (1d).

1.1. Srivastava, J, Barber, DL, and Jacobson, MP. 2007 Intracellular pH sensors: design principles and functional significance. *Physiology* 22:30-39.

1.2 Schönichen, A, Webb, BE, Jacobson, MP, and Barber, DL. 2013 Considering protonation as a posttranslational modification regulating protein structure and function. *Ann Rev Biophys.* 42:289-314. PMID:23451893

1.3 Webb, BE, Chimenti, M, Jacobson, MP and Barber, DL. 2011 Dysregulated pH: a perfect storm for cancer progression. *Nat Rev Cancer*. 11:671-677. PMID: 21833026

1.4 Grillo-Hill, BK, Choi, CC, Jimenez-Vidal, M and Barber, DL. 2015 Increased H+ efflux is sufficient to induce dysplasia and necessary for viability with oncogene expression. eLife 4:e03270. PMID:25793441

2. Molecular understanding of pHi-dependent directed cell migration

Prior to our work, a number of publications established that increased pHi enables cell migration for a broad range of functions, including development, immune cell responses and cancer metastasis. However, a molecular understanding of how this occurs remained unknown. Our 2002 publication in J Cell Biology (2a; > 7,000 downloads and cited in 320 articles) shows that increased pHi is necessary for efficient directional migration of mammalian fibroblasts by promoting cell polarity, actin filament assembly and focal adhesion remodeling. In 3 original publications we also show evolutionary conservation of increased pHi being necessary for *Dictyostelium* chemotaxis. And in 4 original publications we identified pH sensors regulating cell migration, including guanine nucleotide exchange factors (GEFs) activating Cdc42 at the leading edge for polarity (Frantz, J Cell Biol 2007, 179:403), cofilin for actin filament assembly at the leading edge (2b), and the focal adhesion proteins talin (2c) and focal adhesion kinase (FAK) (2d) for cell-substrate adhesion remodeling. Our publications on these pH sensors includes detailed cell and biochemical analyses as well as pH-dependent molecular dynamics simulations (in collaboration with Matthew Jacobson) and NMR (in collaboration with Mark Kelly). Collectively, this work also reveals distinct signaling modes whereby pH regulates these proteins, including specificity (GEFs and FAK), coincidence detection (cofilin) and allostery and cooperativity (talin).

2.1 Denker, SP and Barber, DL. 2002 Cell migration requires both ion translocation and cytoskeletal anchoring by the Na-H exchanger NHE1. *J Cell Biol.* 159:1087-1096. (Highlighted in Journal [Using acid to find direction. J Cell Biol 2002 159:911] and cited in Faculty of 1000; Factor 13)

2.2 Frantz, C, Barreiro, G, Dominguez, L, Chen, X, Eddy, R, Condeelis, J, Kelly, M, Jacobson, MP and Barber, DL. 2008 Cofilin is a pH sensor for actin free barbed end formation. *J. Cell Biol.* 183:865-879 (Highlighted in Journal; Cited in Faculty of 1000, Factor 8.0) PMID:19029335

2.3 Srivastava, J, Barreiro, G, Groscurth, S, Gringas, AR, Goult, BT Critchley, DR, Kelly, MJS, Jacobson. MP and Barber, DL. 2008 Structural model and functional significance of pH-dependent talin-actin binding for focal adhesion remodeling. *Proc Natl Acad Sci.* 105:14436-14441. PMID:18780792

2.4 Choi, CC, Webb, BA, Chimenti, MS, Jacobson, MP and Barber, DL. 2013 pH sensing by FAK-His58 regulates focal adhesion remodeling. *J Cell Biol*. 202:849-59. [Commentary: C. Lawson and D. D. Schlaepfer, "pHocal adhesion kinase regulation is on a FERM foundation", *J. Cell. Biol*., 202:833-836.] [Commentary: K. Legg, "Factoring pH into FAK phosphorylation", Cell Migration Gateway.]; cited in Faculty of 1000 Prime) PMID:24043700

3. New regulatory mechanisms for actin filament dynamics

We identified a previously unrecognized and critical mechanism for activation of the Arp2/3 complex, the molecular machine that generates branched actin filaments to drive membrane protrusion for cell migration, move intracellular vesicles and propel pathogen transmission in host cells. Prior to our findings the binding of nucleation promoting factors (NPFs) of the WASP and WAVE families was thought to be sufficient for stimulating Arp2/3 nucleating activity. However, we showed that phosphorylation of the Arp2 subunit is necessary for activation and that NPF binding is not sufficient for Arp2/3 complex activity in the absence of pArp2 (3a). We showed an evolutionarily critical role for pArp2 in mammalian (3a,d), *Drosophila* (3a) and *Dictyostelium* (3c) cells and identified the Ste20 kinase NIK (MAP4K4) as the first kinase shown to phosphorylate Arp2 and increase Arp2/3 complex nucleating activity. Moreover, in collaboration with Matthew Jacobson we used molecular dynamics simulations and biochemical analyses to reveal a mechanism for how pArp2 primes the Arp2/3 complex for activation by NPFs (3b). Our resolved mechanism showed that

phosphorylation of Arp2 destabilizes a network of salt-bridge interactions at the interface of the Arp2, Arp3, and ARPC4 subunits to allow a 20 Å reorientation of Arp2 relative to the Arp3 subunits. The impact of this newly identified mechanism also included identifying for the first time that electrostatic interactions between subunits retain an autoinhibited inactive Arp2/3 complex. Collectively, our work on phosphorylation as a previously unrecognized regulatory mechanism for Arp2/3 complex activity opened entirely new directions for understanding the control of actin filament dynamics and the many cell processes and behaviors regulated by actin polymerization. Moreover, our work on NIK phosphorylation of Arp2 together with our previous publication showing that NIK phosphorylates the ERM proteins ezrin, radixin and moesin to form stable plasma membrane protrusions (*Proc Natl Acad Sci.* 2006 103:13391-13396) contributes mechanistic insights for recent findings from other groups that NIK is upregulated in cancers and promotes metastasis.

3.1 LeClaire, LL III, Baumgartner, M, Iwasa, JH, Mullins, RD and Barber, DL. 2008 Phosphorylation of the Arp2/3 complex is necessary to nucleate actin filaments. *J Cell Biol.* 182:647-654. (Highlighted in Journal and cited in Faculty of 1000; Factor 8.0) PMID:22125478

3.2 Narayanan, A, LeClaire, LL, Barber, DL, and Jacobson, MP. 2011 Phosphorylation of the Arp2 subunit relieves auto-inhibitory interactions for Arp2/3 complex activation. PLoS Comput Biol. 7:e1002226. PMID:22125478

3.3 Choi, CC,* Thomason, PA,* Zaki, M, Insall, RH, and Barber, DL. 2013 Phosphorylation of actin-related protein 2 (Arp2) is required for normal development and cAMP chemotaxis in *Dictyostelium. J Biol Chem*. 288(4):2464-74. (*authors contributed equally) PMID:23223240

3.4 LeClaire, LL, Rana, MK, Baumgartner, M and Barber, DL. 2015 Arp2 phosphorylation: Functional significance and regulation by the Nck-interacting kinase NIK. *J. Cell. Biol*. 208:161-170. PMID:25601402 (Highlighted commentary in Journal 208:138)

4. Direct regulation of ion transport proteins

We made major contributions to understanding how signaling mechanisms directly regulate the activity of plasma membrane ion transporters. We first showed that the ubiquitously expressed Na-H exchanger NHE1 anchors actin filaments by directly binding the ERM proteins ezrin, radixin and moesin (4a; 300+ citations) and subsequent work from other groups confirmed a similar anchoring mechanism for other NHE isoforms as well as other plasma membrane ion transport proteins. This landmark study also showed that ERM binding/actin anchoring is necessary to localize NHE1 to the distal margin of membrane protrusions, which in turn is necessary for pHi dynamics to enable membrane protrusions and cell migration. We also first identified that NHE1 is a substrate for the Rho kinase ROCK (4b) and the kinases NIK/MAP4K4 (*J. Biol. Chem.* 2001 276:31349-31356.) and Akt (4d), including identifying distinct phosphorylated serine residues in NHE1 for each of these kinases as well as functional significance for growth factor and GPCR signaling and actin filament dynamics. We also first identified and cloned cDNA encoding a previously unknown calcium-binding protein we named CHP (calcineurin B homologous protein) through a genetic screen for NHE1-interacting proteins (4d). This finding generated new directions that resulted in recognition of a CHP family of three distinct members belonging to a superfamily of calcium-binding proteins that includes calmodulin, calcineurin B, revoerin, Kv channel interacting proteins, frequinins, neruocalcins and integrin binding proteins. Moreover, work by our group and others has shown diverse regulatory functions for CHP family members in the activity of several ion transport proteins, intracellular vesicle trafficking and gene transcription.

4.1 Denker, SP. Huang, DC, Orlowski, J, Furthmayr, H and Barber, DL. 2000 Direct binding the Na-H exchanger NHE1 to ERM proteins regulates the cortical cytoskeleton and cell shape independently of H^+ translocation. *Mol Cell.* 6:1425-1436.

4.2 Tominaga, T, Ishizaki, T, Narumiya, D, and Barber, DL. 1998 p160ROCK mediates RhoA activation of Na-H exchange. *EMBO J.* 17:4712-4722.

4.3 Meima, ME, Webb, BE, Witkowska, HE, and Barber, DL. 2009 The Na-H exchanger NHE1 is an Akt substrate necessary for actin filament reorganization by growth factors. *J Biol Chem.* 284(39):26666-75. PMID:19622752

4.4 Lin, X and Barber, DL. 1996 A calcineruin homologous protein inhibits GTPase activation of Na-H exchange. *Proc. Natl. Acad. Sci.* 93:12631-12636.

5. Molecular analysis of the glycolytic enzyme phosphofructokinse-1: Crystal structure and cancer-associated mutations

We resolved two critical limitations for investigating mammalian phosphofructokinase -1 (PFK1), the first rate-limiting enzyme and “gatekeeper” of glycolysis. First is recombinant protein that has properties, including allosteric regulation, like those of native enzyme to permit site-directed mutagenesis. We used recombinant

protein to generate and test functionally for the first time mutations identified in human cancers. Second is the first structure of the mammalian tetrameric enzyme, which we showed in complex with ATP as well as ADP. This recently published work will substantially benefit studies to understand the structure and function of this key, highly allosteric regulated enzyme as well as the role of glycolysis in metabolic diseases, including diabetes and cancer.

5.1 Webb BA*, Forouhar F*, Szu FE, Seetharaman J, Tong L**, Barber DL**. 2015 Structures of human phosphofructokinase-1 and atomic basis of cancer-associated mutations. *Nature* 523:111-114. (*co-first authors, **co-corresponding authors)

Full publication list in NCBI My Bibliography
http://www.ncbi.nlm.nih.gov/sites/myncbi/1zyn8uk9wTQQB/bibliography/40099561/public/?sort=date&direction=ascending

D. Additional Information: Research Support

Ongoing Support
1R01 GM116384 (D. Barber and T. Nystul) 07/01/2015 – 06/30/2019
NIH / NIGMS
Regulation of Epithelial Plasticity
Role on Project: Co PI with Todd Nystul

Paul Allen Family Foundation 10/01/2015 – 09/30/2018
(A. Kao, D. Barber, M. Jacobson, T. Wittmann)
Dysregulation of pH dynamics in Alzheimer's Disease Pathogenesis

1R01 CA197855-01 (Barber) 06/01/2016-05/31/2021
06/01/2016 - 05/31/2021
Roles for intracellular pH dynamics in cancer.

Pending Support
None

Completed Support (last 5 years)
5R21CA178706 (Barber) 07/01/2013 – 06/30/2015
NIH / NCI
Retention of somatic mutations in cancers by changes in pH sensing
Role on Project: Principal Investigator (PI)

5R01GM047413 (Barber) 01/01/1993 – 05/31/2015
NIH / NIGMS
Mechanisms of Receptor Regulated Na+-H+ Exchange
Role on Project: Principal Investigator (PI)

5R01GM058642 (Barber) 08/01/1999 – 11/30/2013
NIH / NIGMS
Actions of the Sodium-Hydrogen Exchanger Subtype, NHE1
Role on Project: Principal Investigator (PI)

5R01GM071526 (Barber) 02/01/2007 – 09/30/2011
NIH / NIGMS
Mechanotransduction in Fibroblast
Role on Project: Principal Investigator (PI)