BIOGRAPHICAL SKETCH

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NAME: Kenneth D. Irvine

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

POSITION TITLE: Distinguished Professor

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
Williams College, Williamstown MA	B.A.	06/1985	Chemistry
Stanford University, Stanford CA	PhD	06/1991	Biochemistry
Princeton University, Princeton NJ		07/1995	Developmental Biology

A. Personal Statement

I have over 27 years of experience as a PI, and throughout this time have established a strong record of productive leadership and mentorship of a research team. I am dedicated to mentoring the students and post-docs in my lab, and have successfully mentored 15 students through completion of their PhD plus 2 currently in the lab, and 16 post-docs who have continued on in their research careers, including many that obtained faculty positions both in the US and abroad. I have also mentored 47 undergraduate students, and many of these have gone on to careers in research or medicine. I have taken on academic leadership positions at Rutgers, including Director of the Cell & Developmental Biology Graduate Program and Interim Director of the Waksman Institute, an interdisciplinary research institute at Rutgers. I have been active in the *Drosophila* community, including a 5-year term on the National *Drosophila* Board as President-Elect, President, and Past-President, during which I led revisions of the *Drosophila* board charter and White paper, and co-organized a meeting on *Drosophila* resources and community. Although not currently an NIH study section member, I have served on 6 different NIH review panels over the past 4 years. My labs' research has focused on analysis of signaling pathways and their roles in development, with a particular emphasis on molecular mechanisms that control growth and morphogenesis to form organs of correct size and shape. Much of our research has focused on the Notch, Dachsous-Fat, and Hippo pathways. We are responsible for many discoveries in the field, including the regulation of Notch signaling by glycosylation and its contribution to boundary formation, discovery and placement of key genes within the Fat-Hippo pathway (eg *dachs*, *discs overgrown*, *lowfat*, *zyxin, vamana, early girl*), the discovery of key cellular processes in Fat-Hippo signaling (eg the polarized localization of Dachs, tension-dependent regulation of Jub and Warts), and key biochemical processes (eg the kinase activity of Four-jointed, the phosphorylation of Fat by Dco, the phosphorylation of Ajuba proteins by JNK and ERK), and discovery of the first molecular mechanism by which cytoskeletal tension can regulate Hippo signaling (via Jub). We have also made important contributions to the understanding of transcriptional regulation by Hippo signaling through analysis of Yorkie regulation and activity, and to the regulation and roles of Dachsous-Fat planar cell polarity. Although much of our research employs *Drosophila*, we also have experience with studies that employ mice or cultured mammalian cells as experimental models. For example, we created a gene-targeted mutation in murine *Dchs1* and published several studies on murine *Dchs1* and *Fat4* mutant phenotypes. Our studies employ a wide range of approaches and models, and a distinctive feature of our research is our demonstrated ability to employ and integrate multiple levels of analysis, including imaging, genetic, cellular, and biochemical approaches.

<u>Current Research Support</u>

1. NIH R35 GM131748-01 to -05 Irvine (PI) 5/1/19 – 4/30/24

Regulation of Growth and Morphogenesis
This grant investigates focusses on 1) Mechanisms of Hippo signaling, 2) Biomechanical Hippo signaling, 3) Modulation of cytoskeletal tension and morphogenesis through Jub and Step, 4) Control of *Drosophila* wing size and shape by Ds-Fat signaling and cytoskeletal tension.

4 Selected Recent and Relevant publications:
a) Ibar, C., Chinthalapudi, K., Heissler, S.M., and Irvine, K.D. 2023. Competition between myosin II and β_H-Spectrin regulates cytoskeletal tension. Biorxiv 10.1101/2022.12.01.518662. (provisionally accepted at **eLIfe**)
b) Venkatramanan, S., Ibar, C. and Irvine, K.D. 2021. TRIP6 is required for tension at adherens junctions. **J Cell Science,** *134*, jcs.247866
c) Zhou, Z. Alégot, H., and Irvine, K.D. 2019. Oriented cell divisions are not required for Drosophila wing shape. **Current Biology**, *29*, 856-864.
d) Rauskolb, C., Sun, S., Sun, G., Pan, Y. and Irvine, K.D. 2014. Cytoskeletal Tension inhibits Hippo signaling through an Ajuba-Warts complex. **Cell**, *158*, 143-156. PMCID: PMC4082802

B. Positions, Scientific Appointments, and Honors

Positions:

1995 - 2001 Assistant Professor,
2001 - 2006 Associate Professor,
2006 - 2012 Professor,
2012 - Distinguished Professor, Waksman Institute and Department of Molecular Biology and Biochemistry, and Member, Cancer Institute of New Jersey, Rutgers University, NJ.

2000 - 2004 Assistant Investigator,
2005 - 2016 Investigator, Howard Hughes Medical Institute

2018 - 2020 Director, Cell & Developmental Biology Graduate Program, Rutgers

2019 - Interim Director, Waksman Institute, Rutgers

Scientific Appointments (within past 15 years):

Grant Review

2003-2009 NIH Study Section Member, DEV-1
2008-2009 Chair, NIH DEV-1 Study Section
2011 Odysseus Research Foundation
2011 NIH Study Section ad hoc, DEV-2
2011, 2015, 2017, 2018 Reviewer for Welcome Trust
2011, 2012 Human Frontiers Science Program
2013 NIH, NCI Special Emphasis Panel
2013,2016, 2019 Ad hoc reviewer for grants submitted to NSF
2014 Simons Foundation
2014,2021 Israel Science Foundation
2014 NIH CB-C Special Emphasis Panel
2014 NIH, NICHD UCC Site Visit Review Panel
2015-2018 Rutgers Aresty Program for Undergraduate Research
2016 Reviewer for MRC Research Councils UK
2016,2017,2020 NIH, NICHD Developmental Mechanisms of Human Structural Birth Defects P01
2017 NIH, NHGRI U41 Genomic Resources Panel
2017 NIH, NIGMS special emphasis panel
2019 NIH special emphasis panel
2020 NIH special emphasis panel
2020 BBSRC
2021 NICHD special emphasis panel, chair
2022 NIH F05 Fellowship panel for Cell Biology, Developmental Biology and Bioengineering
2023 NIH CSRS study section, ad hoc

Editorial

Ongoing	Review 15 - 20 manuscripts per year for various journals
2002-2018	Member, Faculty of 1000
2009	co-Editor, Current Opinion in Genetics and Development August issue
2011	co-Editor, Developmental Dynamics Special Issue on *Drosophila* as a model system
2005-2013	Associate Editor, Developmental Dynamics
2014-2019	Guest Editor for multiple PLoS Biology and PLoS Genetics articles

Community Service

2012-2013	Larry Sandler Award Committee, Chair in 2013
2013-2018	*Drosophila* Board: President-elect, President, & Past President
2016	Led Revision of *Drosophila* Board Charter
2016	Led Revision of *Drosophila* White Paper
2016	Co-Organizer, *Drosophila* Research Ecosystem Meeting at Janelia Research Campus
2017	Genetics Society of America Nominating Committee

Honors and Awards:

1984-1985	John Sabian Adriance Prize in Chemistry American Chemical Society Award Elected to Sigma Xi & Phi Beta Kappa
1986-1989	NSF Graduate Fellowship
1991-1994	Helen Hay Whitney Foundation Postdoctoral Fellowship
1994-1995	New Jersey Commission on Cancer Research Postdoctoral Fellowship
1996-1997	American Cancer Society Junior Faculty Research Award
2000	Appointed as an Assistant Investigator of the Howard Hughes Medical Institute
2001	Rutgers Board of Trustees Research Fellowship for Scholarly Excellence
2016	Rutgers Board of Trustees Award for Excellence in Research
2020	Elected Fellow of the American Association for Advancement of Science

C. Contributions to Science

1. Discovery and characterization of glycosyltransferases that regulate Notch signaling

The principal focus of my lab during early years was *fringe*, a gene that I discovered as a regulator of *Drosophila* wing growth and patterning. We subsequently discovered that it functions as a modulator of Notch signaling: Fringe expression increased Notch activation by one ligand, Delta, whilst inhibiting its activation by another ligand, Serrate. These effects help to position Notch activation along boundaries between cell populations. We also, in collaboration with the Haltiwanger, Stanley, and Vogt labs, identified Fringe as a novel glycosyltransferase that acts on *O*-fucose attached to EGF domains of Notch, which provided the first demonstration that differential glycosylation could modulate receptor-ligand interactions. Additional studies identified and characterized the *O*-fucosyltransferase, demonstrating that it had both glycosyltransferase and chaperone activities, which was the first demonstration that a glycosyltransferase could act as a chaperone. We also analyzed the mechanism of Fringe effects, including characterizing glycosylation sites and glycan structures, and ultimately reconstituted the influence of Fringe glycosylation on Notch-ligand binding in vitro using purified components. Our studies provided a genetic and biochemical understanding of Notch regulation by glycosylation, establishing the best-characterized example of signal modulation by glycosylation, and a rationale for understanding the basis of genetic diseases caused by mutations in human *Fringe* genes.

a) Panin, V.M., Papayannopoulos, V., Wilson, R., and Irvine, K.D. 1997. Fringe modulates Notch-ligand interactions. **Nature** *387*, 908-913. PMID: 9202123

b) Moloney, D. J., Panin, V. M., Johnston, S. H., Chen, J., Shao, L., Wilson, R., Wang, Y., Stanley, P., Irvine, K. D. Haltiwanger, R. S. and Vogt, T. F. 2000. Fringe is a glycosyltransferase that modifies Notch. **Nature**, *406,* 369-375. PMID: 10935626

c) Okajima, T. and Irvine, K.D. 2002. Regulation of Notch signaling by *O*-linked fucose. **Cell**, *111*, 893-904. PMID: 12526814

d) Okajima, T., Xu, A., Lei, L. and Irvine, K.D. 2005. Chaperone Activity of Protein O-fucosyltransferase 1 Promotes Notch Receptor Folding. **Science** *30*, 1599-1603. PMID: 15692013

2. Characterization of Dachsous-Fat signaling
The *Drosophila* genes *fat, four-jointed,* and *dachsous* were known as mutations that influenced planar cell polarity and the shape of *Drosophila* appendages. We discovered in 2004 that these genes functioned, together with *dachs*, in a novel intercellular signaling pathway that regulates gene expression and growth. We then discovered that this Fat signaling pathway regulated the recently discovered Hippo signaling pathway, and identified a molecular mechanism by which it does so. Dachsous and Fat were the first ligand and receptor identified for Hippo signaling. We also discovered and characterized a novel form of pathway regulation, by showing that Fat signaling responds to the gradients of Four-jointed and Dachsous expression, not simply their absolute level, with the vector of the gradient influencing PCP but the slope of the gradient influencing Hippo signaling. The discovery of this novel form of gene regulation suggested a basis for understanding classic experiments in developmental biology that implied that growth could be regulated by gradients of positional values. Additional studies have included genetic, cellular and biochemical identification and characterization of key genes in Ds-Fat signaling, characterization of molecular mechanisms that link Ds-Fat signaling to the regulation of PCP, and characterization of the homologues of Dachsous and Fat (Dchs1 and Fat4) in mammals, where they are required for the development of multiple organs and associated with human genetic diseases including Van Maldergem syndrome, Hennekam syndrome, and Mitral valve prolapse.

a) Cho, E., Feng, Y., Rauskolb, C., Maitra, S., Fehon, R., and Irvine, K.D. 2006. Delineation of a Fat tumor suppressor pathway. **Nature Genetics** *38*, 1142-1150. PMID: 16980976
b) Ambegaonkar, A.A. and Irvine, K.D. 2015. Coordination of planar cell polarity pathways through Spiny legs. **eLife** *4*, e09946. PMCID: PMC4764577
c) Misra, J.R. and Irvine, K.D. 2016. Vamana couples Fat signaling to the Hippo pathway. **Dev. Cell** *39*, 254-266. PMCID: PMC5102026
d) Misra, J.R. and Irvine, K.D. 2019. Early girl is a novel component of the Fat signaling pathway. **PLoS Genetics**, *15*, e1007955.

3. Discovery and characterization of Golgi-localized protein kinases
The vast majority of protein phosphorylation occurs in the cytoplasm and nucleus. It was known that some secreted proteins could also be phosphorylated, but the responsible enzymes had not been identified. In the course of studies we undertook to determine the molecular mechanism by which the *four-jointed* gene influences Dachsous-Fat signaling, we discovered that it encodes a novel, Golgi-localized protein kinase that phosphorylates cadherin domains, and characterized its biochemical properties. We characterized the molecular basis for its influence on planar cell polarity, and together with Mike Simon, we showed that Four-jointed phosphorylation of Fat increases its binding to Dachsous, whereas Four-jointed phosphorylation of Dachsous decreases its binding to Fat. These opposing effects enable it to contribute to Dachsous-Fat polarization in response graded Four-jointed expression. Our studies further established a basis for using Four-jointed as a gene discovery tool to identify an additional Golgi-localized protein kinases, including the long-sought casein kinase. We discovered that the FAM20C/DMP4 gene, previously known for its linkage to a human genetic disease (Raine syndrome), encodes a Golgi-localized casein kinase that phosphorylates proteins involved in bone mineralization.

a) Ishikawa, H.O., Takeuchi, H., Haltiwanger, R.S. and Irvine, K.D. 2008. Four-jointed is a Golgi kinase that phosphorylates a subset of cadherin domains. **Science** *321*, 401-404. PMCID: PMC2562711
b) Simon, M.A., Xu, A., Ishikawa, H.O. and Irvine, K.D. 2010. Modulation of Fat-Dachsous binding by the cadherin domain kinase Four-jointed. **Current Biology** *20,* 811-817. PMCID: PMC2884055
c) Ambegaonkar, A.A., Pan, G., Mani, M., Feng, Y. and Irvine, K.D. Propagation of Dachsous-Fat Planar Cell Polarity. 2012. **Current Biology** *22*, 1302-1308. PMCID: PMC3418676
d) Ishikawa, H.O., Xu, A., Ogura, E., Manning, G., and Irvine, K.D. 2012. The Raine syndrome protein FAM20C is a Golgi kinase that phosphorylates bio-mineralization proteins. **PLoS ONE** *7*, p. e42988. PMCID: PMC3416761

4. Characterization of Hippo signaling
The Hippo signaling pathway was first identified based on mutations that cause overgrowth phenotypes in *Drosophila* imaginal discs. Our research has expanded understanding of Hippo signaling in several directions. We identified and characterized cross-talk between other signaling pathways and Hippo signaling, including distinct mechanisms of cross-talk involving Dpp, Jnk, and EGFR signaling with Hippo signaling. Our investigations of Jnk cross-talk with Hippo signaling established its importance for regenerative growth after tissue damage in multiple tissues, and defined a new input into the pathway. Our investigations of EGFR

cross-talk identified regulation that is both developmentally important, and also contributed to our understanding of YAP activation in cancer. We also identified multiple novel roles for Hippo signaling in different *Drosophila* tissues, including discovery of its roles in neuroepithelial cells, glial cells, and in controlling intestinal stem cell behavior during regeneration. We have also made fundamental contributions to understanding the cell biology of Hippo signaling, including the first report on localization of endogenous Warts in vivo, and the first identification of active Warts in vivo. We have also made fundamental contributions to understanding the regulation of Yki, including the role of phosphorylation in influencing its localization, identification of multiple phosphorylation sites on Yki, and identification of multiple co-factors that act with Yki to activate transcription of target genes.

a) Staley, B.K. and Irvine, K.D. 2010. Warts and Yorkie mediate intestinal regeneration by influencing stem cell proliferation. **Current Biology** *20*, 1580-1587. PMCID: PMC2955330
b) Sun, G. and Irvine, K.D. 2013. Ajuba family proteins link JNK to Hippo signaling. **Science Signaling** *6*, ra81. PMCID: PMC3830546
c) Reddy, B.V.V.G. and Irvine, K.D. 2013. Regulation of Hippo signaling by EGFR-MAPK signaling through Ajuba family proteins. **Dev. Cell** *24*, 459-471. PMCID: PMC3624988
d) Sun, S., Reddy, B.V.V.G., and Irvine, K.D. 2015. Localization of Hippo Signaling complexes and Warts activation in vivo. **Nature Communications** *6*, 8402. PMCID: PMC4598633

5. Discovery and characterization of a mechanism for regulation of Hippo signaling by cytoskeletal tension

Observations that mechanical stress can influence cell proliferation had been made as early as the 1960s, and in 2011 the Piccolo lab reported studies implicating transcription factors of the Hippo pathway (YAP and TAZ) in mechanotransduction. However, the molecular mechanisms responsible were unknown. We identified the first biomechanical pathway that could link cytoskeletal tension to Hippo signaling by discovering that the localization and activity of the *Drosophila* Ajuba LIM protein (Jub), and the Warts kinase, are modulated by cytoskeletal tension, providing a direct link between myosin activity and organ growth. We have since demonstrated that this mechanism contributes to feedback regulation of growth in compressed cells, and that it contributes to density-dependent regulation of cell proliferation. We have characterized links between mechanical forces and Hippo signaling both in *Drosophila* and in cultured mammalian cells, and discovered both conservation of this Jub pathway and its role for it in cell density-dependent regulation of Hippo signaling. We also identified a contribution of cyclic stretch to regulation of Hippo signaling that is mediated through Ajuba family proteins. More recently, we have demonstrated that the spectrin cytoskeleton regulates Hippo signaling through Jub in the *Drosophila* wing. Our studies have provided a molecular understanding of how tissue mechanics can influence Hippo signaling, while also emphasizing that there are multiple mechanisms by which mechanical forces regulate this pathway.

a) Rauskolb, C., Sun, S., Sun, G., Pan, Y. and Irvine, K.D. 2014. Cytoskeletal Tension inhibits Hippo signaling through an Ajuba-Warts complex. **Cell**, *158*, 143-156. PMCID: PMC4082802
b) Ibar, C., Kirichenko, E., Keepers, B., Enners, E., Fleisch K. and Irvine, K.D. 2018. Tension-dependent regulation of mammalian Hippo signaling through LIMD1. **J Cell Science**, *131*, jcs214700. PMCID: PMC5897721
c) Alégot, H., Markosian, C., Rauskolb, C. Yang, J., Kirichenko, E., Wang, Y.-C., and Irvine, K.D. 2019. Recruitment of Jub by α-catenin promotes Yki activity and *Drosophila* wing growth. **J Cell Science**, *132*, jcs222018. PMCID: PMC6432719
d) Rauskolb, C., Han, A., Kirichenko, E., Ibar, C. and Irvine, K.D. 2022. Analysis of the *Drosophila* Ajuba LIM protein defines functions for distinct LIM domains. **PLoS One**, *17*, e0269208.

Complete List of Published Work in MyBibliography:
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