BIOGRAPHICAL SKETCH

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NAME: Helmut J Krämer

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

POSITION TITLE: Professor of Neuroscience and Cell Biology

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 MM/YYYY	FIELD OF STUDY
Universität Tübingen, Germany	Diplom	08/1985	Biochemistry
Universität zu Köln, Germany	Ph.D.	03/1989	Genetics
UCLA	Postdoc	1989-1993	Developmental Neurobiology

A. Personal Statement

My qualification to participate in the proposed project is based on a 23-year experience of NIH-funded research focused on using molecular and genetic approaches in investigating the role of membrane trafficking in different aspects of developmental signaling. Running a small lab with some four to six researchers during this time, I always found it important and productive to seek and nurture collaborations. The collaboration with the lab of Dr. Orth yielded our recent discoveries at the heart of this application.

We initiated the collaboration with the Orth lab to investigate the function of Fic in Drosophila based on its proposed role in regulating the function of small GTPases and their roles in membrane trafficking. We felt that the complimentary expertise of the Kramer lab in genetics and membrane trafficking and the Orth lab in biochemistry and cell regulation was an excellent starting point for the investigation of this novel mechanism in cell regulation.

To our surprise we have discovered that *Fic* mutants display synaptic transmission defects in the visual system. Our experience in genetics and cell biology combined with the skills of the Orth lab in the biochemical analysis of AMPylation substrates places our team in an excellent position to accomplish the proposed studies.

Publications directly relevant to this proposal:

Rahman, M., Ham, H., Liu, X., Sugiura, Y. Orth, K., and **Krämer, H.,** (2012) Visual neurotransmission in *Drosophila* requires expression of Fic in glial capitate projections. **Nature Neuroscience** *15*, 871–875. PMCID: PMC3578554

Ham, H., Woolery, A. Charles T., Drew Stenesen, **Krämer, H.** and Orth, K., (2014) Unfolded protein response-regulated Fic reversibly AMPylates BiP during endoplasmic reticulum homeostasis. **J. Biol Chem** *289*, 36059-69. PMCID: PMC4276871

Stenesen D., Moehlman A.T., **Krämer H**. (2016) The carcinine transporter CarT is required in Drosophila photoreceptor neurons to sustain histamine recycling. **Elife** 4: e10972. PMCID: PMC4739767.

Casey, A.K., Moehlman, A., Zhang, J., Servage, K., **Krämer, H**. & Orth, K. (2017) Fic-mediated deAMPylation of BiP is not dependent on homo-dimerization and rescues toxic AMPylation in flies. **J. Biol Chem** jbc.M117.799296 (PMCID: in progress), *selected for Journal Cover*

B. Posit

1985 - 1989 Grad. Student, Genetics, University Cologne, Germany (Mentor: Benno Müller-Hill, Ph.D.)
1989 - 1993 Postdoc Dept Biol. Chemistry, UCLA (Mentor: Larry Zipursky, Ph.D.)
1993 - 2001 Assistant Professor Dept. Cell Biology, UT Southwestern Medical Center, Dallas
2001 - 2007 Associate Professor, Center for Basic Neuroscience, Department of Cell Biology
2007 - 2011 Associate Professor, Department of Neuroscience
2011 - Professor, Department of Neuroscience, Department of Cell Biology

Other Experience and Professional Memberships

1993 -	Member, Genetics Society of America
1996 -	Member, American Society for Cell Biology
1999	Co-Chair, Endocytosis Mini-Symposium, ASCB meeting
2003	Co-Chair Annual *Drosophila* Research Conference
2005	Chair, *Cytoskeleton & Cellular Biology*; Annual *Drosophila* Research Conference
2002-	Ad hoc reviewer for Wellcome Trust, NSF, HFSP, Deutsche Krebshilfe, Austrian Science Fund, and NIH (SYN, BDPE, MIST, IMST, NTRC, and BVS study sections)
2005-	Editorial Board, Developmental Cell
2010-	Editorial Board, Molecular Biology of the Cell
2017-	Member SYN study section

Honors and Awards

1985-1989	Predoctoral Fellowship in the Fritz-Thyssen-Graduierten-Kolleg
1987	EMBO Short Term Fellowship for a Collaboration at the Institute Pasteur
1989-1991	EMBO Long Term Fellowship
1991-1993	American Cancer Society Postdoctoral Fellowship

C. Contribution to Science

Full list of publications:
http://www.ncbi.nlm.nih.gov/sites/myncbi/helmut.kramer.1/bibliography/40433054/public/?sort=date&direction=ascending

1. DNA looping: The discovery of long-distance enhancers in the 1980s raised the - then controversial - possibility that DNA looping may contribute to the regulation of gene expression. In a set of papers, initiated during my Ph.D. work with Dr. Benno Müller-Hill, we used the *lac* Operon as the first system to unequivocally show in vitro and in vivo the formation of DNA loops and their role in regulating transcription in the *lac* operon. A sign of the endurance of this work comes from the more than 250 citations that Google Scholars notes for these papers since 2010, some 20 years after their publication.

Krämer, H., Niemoller, M., Amouyal, M., Revet, B., v Wilcken-Bergmann, B. and Müller-Hill, B. (1987). Lac repressor forms loops with linear DNA carrying two suitably spaced *lac* operators. ***EMBO J.*** *6*:1481-1491.

Krämer, H., Amouyal, M., Nordheim, A. and Müller-Hill, B. (1988). DNA supercoiling changes the spacing requirement of two *lac* operators for DNA loop formation with Lac repressor. ***EMBO J****. 7*:547-556.

Oehler, S., Eismann, E.R., **Krämer, H.** and Müller-Hill, B. (1990). The three operators of the *lac* operon cooperate in repression. ***EMBO J****. 9*:973-9.

Alberti, S., Oehler, S., v. Wilcken-Bergmann, B., **Krämer, H.** and Müller-Hill, B., (1991). Dimer-to-tetramer assembly of Lac repressor involves a leucine heptad repeat. ***New Biol.*** *3*:57-62.

2. Neuronal cell fate determination: The fly eye is one of the leading model systems for the dissection of cell-cell interactions during neuronal cell fate determination. During my postdoctoral work with Dr. Larry Zipurksy we used genetic and molecular approaches to identify Bride-of-Sevenless as the first molecularly identified ligand that induces a specific neuronal cell fate, that of R7 photoreceptor neurons in the fly eye. The interactions we described between the Boss ligand and the Sevenless receptor became a textbook example of neuronal induction through the interaction of transmembrane proteins.

Krämer, H., Cagan, R.L. and Zipursky, S.L., (1991). Interaction of bride of sevenless membrane-bound ligand and the sevenless tyrosine-kinase receptor. ***Nature*** *352*:207-212.

Van Vactor, D.L.J., Cagan, R.L., **Krämer, H.** and Zipursky, S.L., (1991). Induction in the developing compound eye of *Drosophila*: multiple mechanisms restrict R7 induction to a single retinal precursor cell. ***Cell*** *67*:1145-1155.

Cagan, R.L., **Krämer, H.**, Hart, A.C. and Zipursky, S.L. (1992). The bride of sevenless and sevenless interaction: internalization of a transmembrane ligand. ***Cell*** *69*:393-399.

Hart, A.C., **Krämer, H**. and Zipursky, S.L., (1993). Extracellular domain of the Boss transmembrane ligand acts as an antagonist of the sev receptor. ***Nature*** *361*:732-736.

3. Genetics of endosomal trafficking: Our discovery of the unexpected trans-endocytosis of the Boss transmembrane ligand triggered my long-standing interest in genetic approaches to dissect endocytic

trafficking and its roles in cell-cell interactions. As part of this effort, we described several new mutations interfering with endocytic trafficking since I started my lab in 1993. The papers below focused on our investigations of the *hook* gene that we first described based on its effect on trafficking of the Boss and Delta transmembrane ligands. Later work, by others and us, revealed participation of fly and mammalian Hook proteins in the regulation of microtubule-based vesicle movement by Kinesin and Dynein motor proteins.

Krämer, H. and Phistry, M., (1996). Mutations in the *Drosophila hook* gene inhibit endocytosis of the Boss transmembrane ligand into multivesicular bodies. **J. Cell Biol.** *133*:1205-1216. PMCID: PMC2120908

Walenta, J., Didier, A., Liu, X. and **Krämer, H**., (2001) The Golgi-Associated Hook3 Protein is a Member of a Novel Family of Microtubule-Binding Proteins. **J. Cell Biol**. *152*:923-934. PMCID: PMC2198811

Haberman, A.S., Akbar, M.A., Ray, S., and **Krämer, H.** (2010) The *Drosophila Acinus* gene encodes a nuclear factor regulating endocytic and autophagic trafficking. **Development** *137*:2157-2166. PMCID: PMC2882135

Maldonado-Báez, L., Cole, N.B., **Krämer, H.** and Donaldson J.G. (2013) Microtubule-dependent Endosomal Sorting of Clathrin-independent Cargo by Hook1. **J. Cell Biol** *201*:233-247. PMCID: PMC3628520

4. Genetics of lysosomal delivery: An important aspect of endocytic trafficking is its final step, the delivery of cargo to lysosomes. Our initial discovery that several classic eye color genes in *Drosophila*, including *carnation* and *deep orange*, encode components of the HOPS complex and other regulators of endo/lysosomal fusions triggered my enduring interest. Our observation that these genes are critical for neural maintenance due their function in autophagy later reinforced this interest. Our current work in this area, as highlighted by the two recent papers on Acinus, focuses on the lysosomal delivery route provided by autophagosomes and their regulation in response to different cellular stress situations.

Sevrioukov, E., He, J.-P., Sunio, A, Moghrabi N. and **Krämer H.,** (1999) A role for the *deep orange* and *carnation* eye-color genes in lysosomal delivery in *Drosophila.* **Molecular Cell** *4*:479-486.

Akbar, M.A., Ray, S., and **Krämer, H**. (2009) The SM Protein Car/Vps33A is Necessary for SNARE-Mediated Trafficking to Lysosomes and Lysosome-Related Organelles. **Mol. Biol. Cell** *20*:1705-1714. PMCID: PMC2655250

Nandi, N., Tyra, L.T., Stenesen, D., and **Krämer, H.,** (2014) Acinus integrates AKT1 and sub-apoptotic caspase activities to regulate basal autophagy. **J. Cell Biol** *207*:253-268. PMCID: PMC4210446

Nandi, N., Tyra, L.T., Stenesen, D., and **Krämer, H.,** (in press) Stress-Induced Cdk5 Activity Enhances Cytoprotective Basal Autophagy by Phosphorylating Acinus at Serine^{437}. **Elife** PMCID: in progress

5. *Drosophila* Models of Disease: Our early work on work on *carnation* revealed that partial loss-of-function mutants in HOPS subunits cause cellular phenotypes typical for **H**ermansky-**P**udlak **S**yndrome (HPS). At that time, interactions with HPS researchers, such Dr. Bill Gahl at the NIH, convinced me that *Drosophila* is a useful system for the molecular dissection of other genes that cause such lysosome-related disorders. Our continued interest in such diseases led to the characterization of Mauve, the *Drosophila* homolog of the Chediak-Higashi syndrome protein. Through characterization of the *Drosophila fob* mutant we gained unexpected insights into the role of the immune system in ARC syndrome. We followed up on this discovery in collaborations with the Kahr and Pasare labs to define an unexpected role of the Vps16B/Vos33B complex in phagosomal maturation and regulation of pro-inflammatory signaling.

Akbar, M.A., Tracy, C., Kahr, W. and **Krämer, H**., (2011) The *full-of-bacteria* gene is required for phago-some maturation during immune defense. **J. Cell Biol** *192*:383-390. PMCID: PMC3101095

Urban D., Li L., Christensen H., Pluthero F.G., Chen S.Z., Puhacz M., Garg P.M., Lanka K.K., Cummings J.J., **Krämer H**., Wasmuth J.D., Parkinson J., Kahr W.H. (2012) The VPS33B binding protein VPS16B is required in megakaryocyte and platelet alpha-granule biogenesis. **Blood**. *120*, 5032-40. PMCID: PMC3538988

Rahman, M., Haberman, A.S., Tracy, C., Ray, S., H., and **Krämer, H**., (2012) *Drosophila mauve* mutants reveal a role of LYST homologs late in the maturation of phagosomes and autophagosomes. **Traffic** *13*:1680-1692. PMCID: PMC3528838

Akbar, M.A., Mandraju R., Tracy C., Hu W., Pasare C., **Krämer H.** (2016) ARC Syndrome-Linked Vps33B Protein Is Required for Inflammatory Endosomal Maturation and Signal Termination. **Immunity** *4*, 267-79. PMCID: PMC4988897.

D. Research Support

Ongoing Research Support

RO1 EY10199-21 Krämer 12/1/14 – 11/30/18
NIH/NEI Role: PI
Title: Genetics of Endocytic Trafficking in the Drosophila Eye

- *Current Specific Aims:*
  - Aim 1: Define the mechanisms that regulate Acn levels.
  - Aim 2: Dissect the interaction of Acn with the Hippo pathway.
  - Aim 3: Define the mechanisms by which Acn regulates autophagy.

R01 GM120196-01 Krämer 4/1/17 – 11/30/21
NIH/NIGMS Role: PI (C. Pasare Co-PI)
Title: Endocytic Trafficking and Cell Signaling in Models of ARC syndrome

- *Specific Aims:*
  - Aim 1: Define the signaling elements critical for hypersensitivity of ARC mutants
  - Aim 2: Define the inducible endocytic pathway that depends on ARC proteins for lysosomal delivery

Note that this grant is shared 50/50 with Dr. Pasare's lab.

Past Research Support (within the last three years)

R21HD075361-01 Krämer 9/5/12 – 8/31/14
NIH/NICHD Role: PI
Title: Proteomics of a neurotransmitter recycling domain in glia of the visual system

- *The goal of this study was to develop HRP-mediated biotin tagging of proteins as a tool to define functional subdomains in the plasma membrane of glia cells in the fly visual system.*

RO1 EY021922-5 Krämer 9/1/11 – 8/31/16
NIH/NEI Role: PI Co-PI: Dr. Kim Orth
Title: AMPylation, a novel mechanism regulating visual neurotransmission

- *The goal of this study was to establish a physiological role for Fic-mediated AMPylation and the identification of possible targets that mediate this function of Fic.*