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Dear Search Committee,

I would like to apply for a faculty position at the Department of Biology and the Biocomplexity Institute at Indiana University, Bloomington. I am currently a postdoctoral fellow in the laboratory of Dr. Hugo Bellen at Baylor College of Medicine, Houston, TX, USA. My previous work as well as the enclosed research proposal focus on understanding the molecular and cellular basis of visual map formation and synaptic specificity in *Drosophila*.

I am attracted to the position because it clearly offers an excellent environment for research in systems biology and complexity research. The presence of numerous excellent labs with complementary interests in systems biology demonstrates the standard set and made possible by the Department of Biology and the Biocomplexity Institute. I would like to contribute my expertise in *Drosophila* neurobiology, bioinformatics and high-resolution imaging to this repertoire.

My work has always been characterized by a collaborative spirit and rewarding interactions with students, postdocs and faculty. The possibility to continue my work in a similar scientific environment is an important criterion for me and hence for my decision to apply in the Department of Biology and the Biocomplexity Institute.

This application contains my CV, research proposal, statement of teaching interests. I have arranged for four letters of reference to be sent separately (see page 12 of my application).

I am excited about the possibility to apply at the Department of Biology and the Biocomplexity Institute and hope you will find my background and research proposal equally fitting. I will be happy to provide any further information and look forward to hearing from you.

Sincerely,

P. Robin Hiesinger

Research Proposal

Scientific Background and Achievements

I am interested in understanding the mechanisms that lead to the synaptic specificity underlying the precise and reproducible wiring of neuronal networks. A prime example of such networks are the visual maps in flies and vertebrates, where large numbers of synapses must be accurately specified to represent the visual world in the brain. Two principal mechanisms govern the formation of sensory maps and the neural circuits that underlie sensory information processing and behaviour: genetically encoded, or 'hard-wired' developmental programs and activity-dependent refinement of synaptic specificity. I have recently shown that the *Drosophila* visual map is 'hard-wired', in contrast to any other visual map studied to date (Hiesinger et al., submitted). Taken together with findings of target neuron prespecification in the olfactory map (Jefferis et al., 2001) and the recent characterization of genetically encoded courtship behaviour (Demir and Dickson, 2005; Manoli et al., 2005; Kimura et al., 2005) I hypothesize that the *Drosophila* brain may be to a surprisingly large extent hard-wired prior to eclosion. However, when discussing the idea of genetically encoded neural circuits one easily underestimates the epigenetic complexity of the developmental programs involved. I propose that especially spatio-temporal control of proteins required for synapse specification plays a major role during the development of hard-wired sensory maps and neural circuits. My goal is to understand the rules and mechanisms underlying visual map formation and apply the knowledge and techniques to other neural circuits in the brain.

My research since 1996 has focused on two themes, the genetic and epigenetic basis of synaptic specificity (Hiesinger et al., 1999; Morales et al., 2002; Zhai et al., 2003; Mehta et al., 2005; Hiesinger et al., submitted) and the development and application of 3D high-resolution visualization of neuronal connectivity and synapses (Laissue et al., 1999; Rein et al., 2000; Hiesinger et al., 2001; Pennetta et al., 2002; Hiesinger et al., 2005). The *Drosophila* visual system is a well characterized and genetically superbly amenable model system to study synaptic specificity (Figure 1; Fischbach and Dittrich, 1989; Clandinin and Zipursky, 2002; Meinertzhagen and Hiesinger, 2004). I initially found that disruption of *neuronal synaptobrevin (n-syb)*, a vesicle protein crucially implicated in synaptic transmission, leads to defects in synaptic partner selection. The wiring defects only occur after axonal pathfinding and are accompanied by the mislocalization of cell adhesion molecules (CAMs; Hiesinger et al., 1999). Surprisingly, these phenotypes are independent of neuronal activity, suggesting an important developmental role of vesicle trafficking during synaptic partner selection. The developmental requirement of n-Syb is synapse-specific, as loss of *n-syb* does not affect general membrane trafficking and cell viability. I propose that loss of *n-syb* disrupts the spatiotemporally controlled localization of CAMs or other guidance cues.

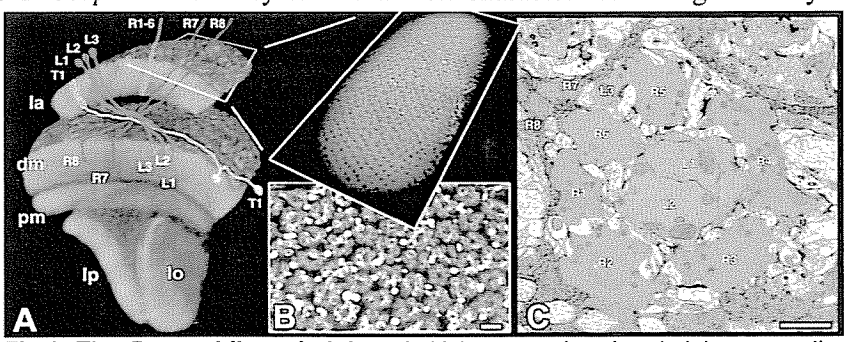

Fig.1: The *Drosophila* optic lobe. A: Volume rendered optic lobe neuropils based on anti-n-Syb staining. Some characterized cell types are depicted based on Golgi stainings (Fischbach and Dittrich, 1989). la, lamina; dm, distal medulla; pm, proximal medulla; lp, lobula plate; lo, lobula. B: Lamina cross-sections of confocal sections based on a photoreceptor-specific antibody staining. Scale bar 5μm. C: EM micrograph of a single unit (cartridge) of the sensory map in the lamina. Color code as in A. Scale bar 1μm.

To gain a better molecular and mechanistic insight into this intracellular machinery it was my goal as a postdoc in Hugo Bellen's lab to genetically identify key molecular players. I therefore focused on the isolation of synaptic specificity mutants in a large-scale genetic screen designed to isolate genes

required for synapse formation or function in the *Drosophila* visual system (Verstreken et al., 2003; Mehta et al., 2005; Hiesinger et al., submitted). I dedicated a substantial part of my postdoctoral work to a team effort to isolate and molecularly identify the lesions in more than 20 complementation groups that are implicated in synapse development and function (Zhai et al., 2003; Verstreken et al., 2003; Mehta et al., 2005).

I focused on the characterization of two genes implicated in both vesicle trafficking and synapse development. One gene, *sec15*, is a member of the exocyst, a protein complex required for secretion in yeast and vesicle trafficking in neurons. *sec15* mutants exhibit a specific disruption in a late developmental neuronal targeting step, similar to *n-syb* mutants (Mehta et al., 2005). In *sec15* mutant photoreceptors we found mislocalization of specific cell adhesion and signalling molecules that are normally required for neuronal targeting. Hence, Sec15 provides a link between vesicle trafficking and synaptic specificity. As in the case of *n-syb*, the specificity of the defects argue against a general disruption of vesicular or membrane trafficking.

The second gene I characterized was *vha100-1 (v100)*, the largest subunit of the v-ATPase V_0 complex. The V_0 complex forms the proteolipid pore of an ATPase that acidifies vesicles. In addition, an independent function in membrane fusion has been proposed largely based on yeast experiments. (Peters et al., 2001). We performed the first test of this hypothesis in neurons and found that the defects of *v100* mutants indeed suggest defective membrane fusion, a hallmark of *n-syb* mutants (Hiesinger et al., 2005). Interestingly, our work showed that *v100* and *n-syb* mutants have identical phenotypes in most assays. Like in *n-syb* mutants, loss of *v100* does not cause any discernable neuronal targeting defects in embryos, whereas clones in optic lobe neurons cause wiring defects. We found that this phenotype is due to a general trafficking defect of transmembrane molecules in specific brain cells (unpublished data). Hence, both *v100* and *n-syb* provide a link between vesicle fusion and cell-specific functions during synaptic partner selection in the visual system.

Finally, I utilized our mutant collection to understand the specification of synapses in the visual map independent of individual genes. We performed a quantitative ultrastructural screen of 60 mutants that revealed a cell-autonomous genetic program controlling synapse numbers in photoreceptors. In contrast to the activity-dependent refinement or 'postspecification' after initial excess synapse formation in vertebrates, synaptogenesis in fly photoreceptors is preceded by activity-independent prespecification of synaptic partners (Hiesinger et al., submitted).

Throughout my graduate and postdoctoral work I developed and tested novel techniques for 3D and high-resolution visualization of *Drosophila* synapses (Hiesinger et al., 2001; Pennetta et al., 2002; Hiesinger et al., 2005). In addition, I have developed programs for genome analysis and data management and implemented an integrated web database for the team-based genetic screen described above. I also provided a supportive role for the *Drosophila* Gene Disruption Project (Bellen et al., 2004; Hiesinger and Bellen, 2004; http://flypush.imgen.bcm.tmc.edu/pscreen). I am currently developing neural network simulations using Matlab/Simulink. The application of computational simulation and visualization techniques to understand the development of neural circuitry is a core aspect of my future research.

Specific Aims

The discrepancy between the information required to encode complex wiring patterns in the brain and the relatively small number of genes available to program these patterns calls for sophisticated epigenetic developmental mechanisms. My recent work has shown that activity-dependent synaptic 'fine-tuning' does not occur during *Drosophila* visual map development (Hiesinger et al., submitted), which is therefore encoded by genetic and epigenetic programs ('hard-wired'). My goal is to understand these (epi-) genetic programs. I propose to use the fly visual system as a model to study molecular, cellular and computational aspects of the epigenetic programs underlying sensory map formation. Specifically I propose the following topics:

Aim 1. Vesicle trafficking and synaptic specificity: Genetic dissection of intracellular trafficking during neuronal target selection

- To analyze gene functions that link specific aspects of vesicle trafficking to synaptic partner selection in the visual system

The genetic and functional investigation of synaptic specificity, i.e. the establishment of synapses between correct partners, has largely been studied through CAMs (Dickson, 2002; Shen, 2004). However, few studies address the problem of spatio-temporally controlled transport of CAMs during synaptic partner selection, although highly dynamic regulation has been observed for most CAMs at growth cones and synapses (e.g. Keleman et al., 2005).

I propose to identify cellular pathways underlying spatio-temporal CAM regulation and characterize their contribution to synapse-specific wiring. Cell-specific *v100, n-syb* and *sec15* mutants provide excellent genetic tools to address this question: First, loss of *v100, n-syb* or *sec15* in optic lobe neurons disrupts the localization of different populations of CAMs required for visual map formation, but none of them affect protein and vesicle trafficking required for cell viability. Second, loss of *v100* only affects visual map formation when mutant in optic lobe interneurons, whereas mutant photoreceptors display no developmental defects (using different *eyeless* FLP systems, Mehta et al., 2005; Hiesinger et al., 2005). Hence, *v100* mutants separate functional vesicle trafficking in photoreceptors from developmental vesicle trafficking required for visual map formation in interneurons. I will study the mechanisms by which CAMs are aberrantly localized in optic lobe neurons of *v100, n-syb* and *sec15* mutants by analyzing candidate CAMs known to be required for neuronal targeting in fixed and live preparations (see specific aim 3). In combination with the MARCM technique (Lee and Luo, 2001) we can directly compare CAM levels in marked mutant processes in a variety of mutant and cell-specific rescue backgrounds (Figure 2). Mislocalization of specific CAMs will be further analyzed using immuno-EM, genetic experiments (e.g. challenging the mutant neuron by simultaneous overexpression of the CAM) and biochemical experiments (e.g. identification of developmentally required vesicle populations).

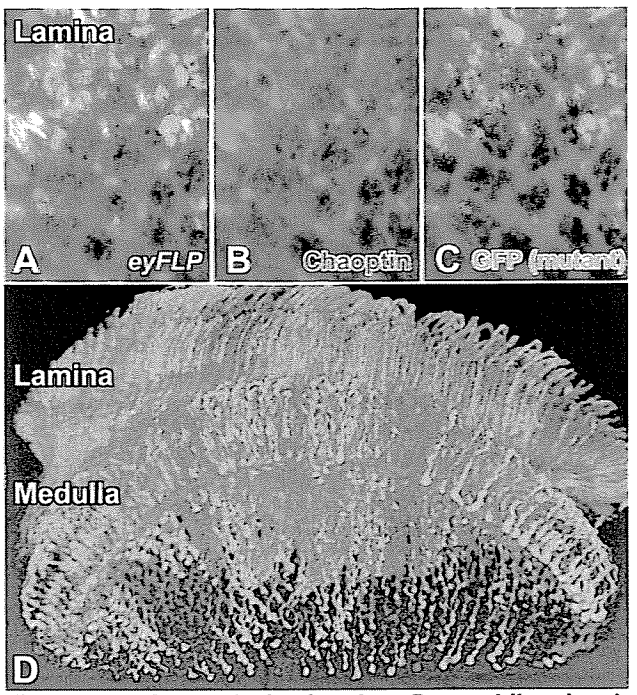

Fig.2: Mosaic analysis in the *Drosophila* visual system. A-C: Lamina cross-section showing single GFP-marked *sec15* mutant photoreceptor terminals in the lamina. D: 3D visualization of the photoreceptor terminal field viewed from inside the brain. In this mosaic 50% of the photoreceptor neurons are mutant for *shibire*^{ts1}.

Aim 2. Synaptic specificity and pattern formation: Imaging the establishment of neural superposition in the Drosophila lamina

- To analyze the formation of the visual sensory map in the first optic neuropil using high-resolution live imaging in an eye-brain culture system

The first optic neuropil, the lamina, is arguably the best characterized neuropil in the fly brain. I regard lamina development as a perfect intermediate to approach the problem of brain wiring: complex enough to address the problem of synaptic specificity in the brain, yet with a limited number of characterized cell types in a repetitive organization.

I propose to carry out a detailed characterization of axonal and dendritic pattern formation during the crucial step of target refinement using live imaging. I have established the culture of eye-brain complexes during visual map formation according to the protocols of Gibbs and Truman (1998). By using photoreceptor subtype-specifically driven GFP and RFP (e.g. mΔ0.5-Gal4; Gaengel and Mlodzik, 2003). I envisage a concerted ballet of filopodial rearrangements in the lamina due to the repetitive organization of the developing cartridges. Individual lamina 3D datasets acquired hourly will be realigned using warping algorithms. For visualization of neuronal processes at the resolution limit of light, we will use our well established 3D deconvolution technique (Hiesinger et al., 2001).

An understanding of the sequence of photoreceptor-subtype filopodial interactions as well as their interactions with epithelial glia and other interneurons in the lamina will allow the determination of a series of interactive decisions required to establish neural superposition. I have recently started modelling lamina development *in silico* using Matlab/Simulink. The simulations are designed to allow predictions about the number and nature of recognition events (most prominently mediated by CAMs). Hence, experimental data feeds forward into the development of a model that in turn feeds back into predictions of recognition molecules between certain filopodia/growth cone extension, thus providing a means to test the model.

Aim 3. Pattern formation and vesicle trafficking: Live imaging of intracellular, spatiotemporal dynamics of molecules required during sensory map formation

- To analyze live vesicle trafficking in photoreceptors during map formation in comparison with NMJ development using fluorescently labelled vesicle markers in wild type and mutants

The most prominent family of molecules conferring vesicular trafficking specificity are Rab GTPases. Matthew Scott's and Hugo Bellen's laboratories are presently generating a collection of UAS-Rab-YFP strains for the 32 known wild type Rab proteins as well as the corresponding dominant negative and constitutively active Rab-YFP transgenic fly strains. We have immediate access to these lines, and Hugo and Matt will eventually make these strains available to the *Drosophila* community. I propose to characterize photoreceptor-specific expression of these Rab-YFP fusion proteins in combination with live imaging in our *in vivo* culture system. Comparison of dominant negative, constitutively active and wild type Rab-YFP fusion proteins will allow us to understand their compartment-specific trafficking behavior. I expect these studies to elucidate vesicle trafficking behavior of specific vesicle populations and to provide tools for mutant analyses - especially in conjunction with the characterization of target refinement as proposed in specific aim 2.

In summary, I propose to utilize both the power of *Drosophila* as a genetic model organism as well as computational and imaging approaches to study synaptic partner selection and sensory map formation. In particular, I will focus on the role of spatiotemporally controlled vesicle and protein trafficking required for such synaptic partner choices, as it may provide an under-appreciated key to the epigenetic complexity of brain wiring. Finally, I intend to apply the techniques presented here to other neural circuits to investigate the extent to which the *Drosophila* brain is (epi-) genetically 'hard-wired'.

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Statement of Teaching Interests

The ability to communicate science to nonscientists, students and colleagues has always been an integral part of my scientific life. My application to the Department of Biology and the Biocomplexity Institute is driven by my career choice to combine research with science communication and teaching in an interdisciplinary environment.

I have enjoyed communicating my own science as well as its 'textbook' foundations throughout my career. As a graduate student I organized and taught two courses called *'The Cockpit of the Fly'* - basic, hands-on courses to understand the complexity of the fly's brain. During my postdoctoral work I gained experience in training graduate students and presenting departmental lectures. For the training of a summer student who successfully presented our work in the national *Siemens Westinghouse Competition* I won a mentor recognition award. Furthermore, I have been invited numerous times to prepare and present educational lectures, such as a seminar on imaging techniques in a Zeiss symposium held last year. I particularly enjoy the teaching of fundamental concepts in biology and favor associative learning methods rather than disconnected data presentation.

I am looking forward to my teaching responsibilities as a faculty member of the Department of Biology and the Biocomplexity Institute at Indiana University. I am particularly interested in organizing undergraduate courses in genetics and computational neuroscience. It is my own experience that an understanding of the basic experiments that lead to what we know today are not only of historical value, but greatly help in the appreciation and understanding of the scientific process. At the graduate level my advanced training in molecular and computational neurobiology will allow me to provide courses for graduate student. *Drosophila* is an excellent, inexpensive model organism for hands-on experiments for classical as well as molecular neurosciece. Finally I am interested in organizing seminars and discussion groups on interdisciplinary subjects. In summary, I am looking forward to collaborative efforts in both research and teaching endeavors with students and colleagues.

References (Contact Information)

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