Cell & Developmental Biology
School of Medicine
3181 SW Sam Jackson Park Road
Portland, OR 97239-3098
Mailcode L215
(503) 494-2493
Fax (503) 494-4253

November 1, 2005

Yves Brun
Systems Biology/Microbiology Faculty Search
Department of Biology
Indiana University
Jordan Hall 142
1001 E 3rd Street
Bloomington, IN 47405-7005

Dear Faculty Search Committee,

I am writing in response to your advertisement for a tenure-track Assistant Professor position in the Department of Biology and the Biocomplexity Institute at the Indiana University. I am a Research Assistant Professor in the Department of Cell & Developmental Biology at OHSU, specializing in RNA trafficking. I would bring to your department a strong background in multiple techniques and model systems, including *Drosophila*, *Xenopus*, and mammalian cell culture, as well as teaching experience.

My experience and education are directly relevant to your needs. During my Ph.D. studies in the Molecular Biology and Biochemistry program at the University of Connecticut Health Center, I developed new technologies for studying RNA metabolism in vivo - a novel assay for analysis of the molecular interactions of RNA using fluorescence quenching and dequenching, and a translation assay using GFP as a reporter to follow translation within a single live cell. Using these novel assays, I discovered that the RNA translation enhancer (RTE) sequence, derived from the untranslated region of the myelin basic protein mRNA, directs both the transport and the translational activation of many mRNAs (Kwon et al. *J. Cell Biol.* 147, 247-56). For this work, we received U.S. Patent No. 6,225,082.

For my postdoctoral work, which further explored RNA trafficking, I joined the lab of Dr. Bruce Schnapp in the Department of Cell Biology at Harvard Medical School. Here, in order to complement my training in protein biochemistry and cell biology, I concentrated on mechanisms of cellular development. I characterized the machinery that drives localization of Vg1 and VegT RNA, both of which are critical for primary germ layer formation, during *Xenopus* oogensis. I developed a quantitative assay for RNA localization, measuring the volume occupied by ribonucleoprotein particles in each oocyte using imaging software. With this assay, I identified a repeated sequence motif that binds the RNA binding protein Vera and functions as a cis-acting element for two different RNAs (Kwon et al. *Curr. Biol.* 12:558-564). I then used bioinformatic techniques to search for RNAs containing this element.

Moving to Oregon Health & Science University with the lab of Dr. Bruce Schnapp, I expanded my studies of RNA localization into the *Drosophila* system, in collaboration with Daniel St. Johnston's lab at the University of Cambridge, England. I found that the *Drosophila* Vera orthologue, IMP, binds *oskar* RNA at repeated motifs that are critical for translational activation and anchoring (see submitted preprint). This study established a parallel requirement for multiple copies of Vera/IMP binding motifs in the regulation of localized maternal mRNAs in the early development of *Drosophila* and *Xenopus*.

Recently, I have been expanding my research expertise to *Xenopus* embryogenesis in the lab of Dr. Jan Christian. I also have supervisory, mentoring, and teaching experience. During my Ph.D. studies, I trained a number of students and faculty members in a variety of techniques. As a postdoctoral fellow at Harvard Medical School, I helped graduate students with everything from learning microinjection techniques to scientific grant and manuscript writing. At OHSU, I have actively sought out opportunities for lecture and research mentoring as a research assistant professor. I was in charge of a current literature discussion class as a lecturer for "Mechanisms of Development", a graduate course, and am a regular presenter in the Developmental Biology Journal club. Students enjoyed my lecture and I also enjoy teaching.

Enclosed are my C.V., a statement of research and teaching interests, and representative publications. I believe my experience and enthusiasm will benefit your department. I look forward to speaking with you. Thank you for your consideration.

Sincerely,

Sunjong Kwon

Statement of research and teaching interests

Sunjong Kwon

Many mRNAs have been shown to localize to, and be translated in specific places in a single cell, thereby providing a powerful mechanism for spatial regulation of protein function. RNA localization is particularly important in the developing embryo, where it plays essential roles in diverse processes such as embryonic axis specification, formation of the primary germ layers and specification of germ cell fate. Microscopic studies have begun to define the ultrastructure of the subcellular compartments with which localized RNAs are associated, but the exact components of the RNA localization machinery remain a mystery. Cis-acting signals, often contained in the untranslated regions (UTRs) of mRNAs, are known to direct localization but in many cases the trans-acting factors that bind these sequences have not been identified. Furthermore, it is not known how these factors mediate directional movement and translation repression or depression of RNAs.

One obstacle to understanding RNA localization lies in the limitations of the individual model systems that are normally used to study these processes. *Xenopus* oocytes, for example, enable one to rapidly assay RNA localization, and provide large volumes of material from which to isolate and characterize ribonucleoprotein complexes, but they are not suitable for imaging or for genetic analysis. The *Drosophila* system is optimal for genetic analysis, since many mutations have been identified that disrupt RNA localization, but this system is not appropriate for biochemical analysis. In my current and proposed studies, I utilize both *Xenopus* and *Drosophila* to study RNA localization in order to take advantage of the unique benefits offered by each model system.

The long-term goal of my research is to completely characterize the RNA localization machinery, including the cis-acting elements contained in localized RNA molecules, their trans-acting trafficking factors, and components that interact with the translational apparatus. In my previous work, I analyzed the cis- and trans-acting factors that are required for localization of two RNAs, Vg1 and VegT, both of which are essential for development of the primary germ layers in *Xenopus*. I identified a minimal sequence element (UUCAC), termed the E2, that is present in multiple copies dispersed throughout the 3'UTR of both Vg1 and VegT. I showed that E2 elements are essential to direct transport of these RNAs to the vegetal cortex of *Xenopus* oocytes, and that they do so by recruiting Vera protein (also known as Vg1RBP) (Kwon et al, 2002). Vera is a member of a family of highly conserved RNA-binding proteins that appear to be involved in RNA localization, stability, and/or translational control in a wide variety of cell types and organisms. These proteins are believed to play essential roles in embryonic development and carcinogenesis. In *Xenopus* embryos, loss of function studies suggest that Vera is required for the migration of cells forming the roof plate of the neural tube and, subsequently, for neural crest migration (Yaniv et al, 2003). The target RNAs that VERA binds to during neural crest migration, and the mechanisms by which it direct RNA localization, remain unknown.

To begin to investigate Vera function in a genetically tractable system, I analyzed the *Drosophila* ortholog of Vera, known as IMP, in collaboration with Daniel St. Johnston's lab in England. To identify candidate RNA targets of IMP, I generated recombinant protein for each of the RNA binding (KH) domains of IMP and asked what sequence elements each of these domains preferred to bind and whether these sequences were present in particular *Drosophila* RNAs. A preferred IMP binding element (IBE), UUUAY, was identified by SELEX (systematic evolution of ligands by exponential enrichment), using the recombinant KH domains to select RNA sequences in vitro from random pools of 25mer RNAs. The PatSearch program was then used to search the *Drosophila* cDNA database from the Berkeley *Drosophila* Genome Project in order to identify RNAs containing IBE repeats. A bioinformatic search strategy was set up to select cDNAs, among 11,203 in the database, that contained

at least five IBEs within a subregion of 250 bases. My search identified a total of 493 clones in the database, and this led to the characterization of 227 unique cDNAs.

In characterizing the pool of *Drosophila* RNAs that are candidate targets for IMP binding, *oskar (osk)* stood out as a particularly interesting candidate. Localization and restricted translation of *osk* mRNA at the posterior end of the *Drosophila* oocyte is necessary to establish the posterior pole plasm, which contains determinants that specify the primordial germ cells and abdomen of the developing embryo. Thirteen copies of the IBE are present in the 3'UTR of *osk* mRNA, and I showed, using in vitro RNA binding assays, that these bound IMP in the context of the *osk* 3'UTR. In collaboration with members of Dr. St. Johnston's lab, we then showed that IMP colocalizes with *osk* mRNA at the posterior pole of the oocyte in vivo, and that colocalization depends on the IBEs, since a single nucleotide substitution within them delocalizes IMP. Furthermore, mutations within the IBEs prevent *osk* mRNA translation and anchoring at the posterior pole, demonstrating that these binding elements are functionally important for RNA localization. Intriguingly, oocytes lacking IMP localize and translate *osk* mRNA normally, indicating that translational activation of *osk* depends on another factor that recognizes the IBEs. These findings (Munro et al, submitted) identify control sequences for *osk* translational de-repression, and establish a parallel requirement for repeated IBEs and E2s in the regulation of localized maternal mRNAs in *Drosophila* and *Xenopus.*

I have developed the following independent research plan to continue my studies of how gene expression is regulated by RNA trafficking during early development in *Xenopus* and *Drosophila*. The proposed research takes advantage of my training in biochemical, molecular, cellular, embryological and microscopic methodologies.

1. Identify the IBE binding protein that is required for translational activation of osk mRNA. Curiously, translational activation and anchoring of *osk* mRNA require the IMP binding elements, but not the protein itself. This finding implies that in addition to IMP, IBEs interact with another critical protein, which I will call "factor X". My working hypothesis is that translational activation of *osk* mRNA requires binding of factor X to the IBEs. I will search for "factor X" using biochemical and molecular genetic strategies such as affinity purification from *Drosophila* egg chamber extracts, yeast three-hybrid assays, and Northwestern screening of *Drosophila* oocyte cDNA libraries. Alternatively, I will characterize the binding partners of IMP, assuming that IMP is a core binding protein in *osk*-containing RNA particles. I will conduct two-hybrid screens using IMP as bait to find interacting proteins in *Drosophila* oocyte expression libraries. An alternate proteomic approach would be to use recombinant IMP protein to pull down binding partners from *Drosophila* egg chamber extracts and identify candidate proteins by mass spectrometry. After identifying factors that interact with target IBEs or IMP, I will address whether these factors colocalize with *osk* RNA in vivo and will use mutational analysis to determine whether they are required for localization, anchoring and/or translation of osk mRNA. This research will shed light on the molecular mechanisms that regulate localized translational activation.

2. Identify RNA targets that bind *Drosophila* IMP and/or *Xenopus* Vera that are essential for embryogenesis. To date, the best characterized Vera and IMP targets are maternally expressed RNAs that are required during oogenesis or early embryonic patterning. Vera and IMP are also expressed in the developing nervous system, however, and it has been suggested that Vera facilitates neural cell migration by mediating the sorting of specific RNAs to the leading edge of migrating cells (Yaniv et al, 2003). These target RNAs have not been identified. My working hypothesis is that localization of specific RNAs within the developing nervous system requires IMP/Vera binding and that localization is essential for normal neural development in *Drosophila* and *Xenopus*. As a starting point to identify these RNAs, I will investigate several candidate genes which I found in my bioinformatics search for

RNAs containing IBEs in *Drosophila* that are reported to be expressed in the developing nervous system. First, I will address whether these RNAs bind IMP in vitro and are colocalized with IMP in vivo. Then, I will determine whether the subcellular localization of these RNAs is defective in *Drosophila* IMP mutant embryos. I will also create transgenic flies carrying mutations within the IBEs of these RNAs that disrupt IMP binding in order to determine whether the IBEs are essential for the localization and/or translation of specific transcripts.

To begin to examine whether regulation of RNA localization is conserved between invertebrates and vertebrates, I will analyze the role of Vera and its' target RNAs in *Xenopus* neuronal development in parallel to my studies in *Drosophila*. One approach will be to ask whether *Xenopus* orthologs of genes identified to be targets for IMP binding in flies are also regulated by Vera binding in frogs. In addition, I will search for novel target RNAs by purifying and microsequencing proteins from protein-RNA complexes immunoprecipitated from *Xenopus* embryo extracts with anti-Vera antibody. Analysis of cDNAs encoding these proteins will enable me to identify functional sequence elements (E2s or novel elements) that bind Vera. I will address whether these RNAs are co-expressed with Vera in the developing nervous system, and, as time allows, I will ask whether their subcellular localization and/or translation are disrupted in embryos in which expression of Vera has been knocked down by injection of antisense morpholinos. I am currently acquiring all of the technical skills necessary for manipulation of gene expression and phenotypic analysis of *Xenopus* embryos in Dr. Christian's lab. This research will shed light on mechanisms of early neural development, mediated by specific RNA-protein interactions.

References

Kwon, S., Abramson, T., Munro, T., John, C., Köhrmann, M., and Schnapp, B. J. (2002) UUCAC- and Vera-dependent localization of VegT RNA in *Xenopus* oocytes. *Curr. Biol.* 12:558-564

Munro, T. M*, Kwon, S.*, Schnapp, B. J., and St. Johnston, D. ***equal first authorship**. A repeated IMP-binding motif controls *oskar* mRNA translation and anchoring independently to *Drosophila* IMP. *Submitted*

Yaniv K, Fainsod A, Kalcheim C, Yisraeli JK (2003) The RNA-binding protein Vg1 RBP is required for cell migration during early neural development. *Development* 130(23):5649-5661

Teaching interests

My teaching interests stem from my research interests and accomplishments. At OHSU, I lectured on RNA localization during *Drosophila* oogenesis for the graduate course "Mechanisms of Development". In addition, I participated in Cell Biology and Developmental Biology Journal Clubs, where I presented many papers relating to RNA metabolism. I used clear diagrams from high impact review papers including papers I published in *Curr. Opin. Neurobiol (1998), J. Neurocytol.(1999),* and *Curr. Biol.(2002)* and thus provided opportunities for graduate students to learn about various aspects of RNA biology including RNA localization. I also benefited by teaching at OHSU: in discussing my work and research interests with graduate students, I often found that they provided great feedback on my work. I am eager to teach both undergraduate and graduate students. I can give lectures of Biochemistry, Molecular and Cell Biology, and Developmental Biology to undergraduate students. For graduate students level, I can give lectures on RNA-protein interactions, *Xenopus* and *Drosophila* oogenesis/embryogenesis, intracellular trafficking, cell polarity, and light microscopy techniques for cell and developmental biology.