THE UNIVERSITY OF MICHIGAN
MEDICAL SCHOOL

ANN ARBOR, MICHIGAN 48109-0622

Department of Molecular & Integrative Physiology
6804 Medical Science II 0622
1301 Catherine Street

Telephone: (734) 763-9450
Fax: (734) 647-9523
E-mail: diak@umich.edu

October, 17, 2005

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

Dear Dr. Yves Brun:

I wish to apply for the Assistant Professor position in the Department of Biology, Indiana University. I believe that my experience and expertise in studying mechanisms of JAK2-dependent cell signaling and the role of the adapter protein SH2-Bβ in actin-dependent motility of bacteria *Listeria minocytogenes* qualify me for consideration for this position.

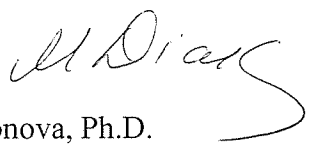
My current research is focused on the mechanisms by which JAK2 tyrosine kinase causes changes in cellular functions. Because JAK proteins are utilized by many cytokines, including interferons, most interleukins, ciliary neurotrophic factor, leptin, growth hormone, leukemia inhibitory factor, oncostatin M, erythropoietin, and granulocyte macrophage colony stimulating factor, my results may have wide applicability to cancer and other human diseases, such as obesity, diseases of the immune system, and neurological diseases. Main field of my interest is JAK2-dependent regulation of the serine-threonine kinase PAK1, a kinase implicated in multiple cellular functions, including apoptosis, MAP kinase regulation, PI3-kinase regulation and regulation of the actin cytoskeleton. Recent data suggest that PAK1 is involved in breast cancer progression. I have proposed (for consideration under the R21 mechanism) a research project that will provide insight into possible mechanism by which prolactin, JAK2 and PAK1 participate in human breast cancer. I have also submitted the grant application entitled "JAK2-PAK1 interaction in human breast cancer" to the American Cancer Society.

Another field of my interest is a link between receptor tyrosine kinase and the actin cytoskeleton. Eukaryotic cells depend on signaling to the actin assembly for establishing their asymmetrical shapes, intracellular transport and locomotion, processes essential for morphogenesis, wound healing, immune responses, and metastasis of cancer cells. Most if not all ligands that activate receptor tyrosine kinases and receptor associated tyrosine kinases initiate such signals. However, we know very little about the signaling pathways that link receptor tyrosine kinases to the actin cytoskeleton. My recent data show that the adapter protein SH2-Bβ provides such a link. I found that SH2-Bβ enhances GH-induced cell motility and binds the small GTPase Rac, one of the major actin-regulating proteins. I showed that SH2-Bβ bundles actin filaments *in vitro* and binds to an actin-binding protein VASP. I demonstrated that SH2-Bβ stimulates actin-based motility in an *in vitro* reconstitution system as well as *in vivo* using *Listeria* motility as a primary system. Intracellular movement of the pathogenic bacterium *Listeria* is a very powerful model to study the basic principles of the mammalian cytoskeleton because it depends on the formation of an actin tail using host proteins. To determine the mechanism by which SH2-Bβ increases the motility of *Listeria* will provide important insight into the fundamental mechanism by which *Listeria* spreads to infect neighboring cells.

My goal for the future is to establish an independent, extramurally funded research program, which would allow me to continue and expand my research in the areas of JAK2-dependent signal transduction and the actin cytoskeleton. Over the last several years, I have obtained financial support from the Human Growth Foundation, Michigan Diabetes Research and Training Center and the NIH. Therefore, I feel confident that I will be able to continue to obtain extramural funding.

Enclosed are my CV with names and addresses of four references, three representative publications and description of research and teaching interests. Please feel free to contact me for any additional materials or information you may need to give my application full consideration.

Sincerely,



Maria Diakonova, Ph.D.

RESEARCH INTERESTS

My research interests bridge the fields of signal transduction and actin-dependent cellular functions. The complexity of actin-dependent cellular functions (e.g. determination and maintenance of cell shape, transport of intracellular constituents and cell motility) and their regulation by many hormones, cytokines and growth factors suggest that multiple signaling mechanisms exist to regulate these processes. During the recent few years, I have worked on understanding the molecular mechanism by which growth hormone (GH) via JAK2 tyrosine kinases causes physiological responses. JAK proteins are utilized by all these ligands that bind to members of the cytokine family of receptor, including interferons, most interleukins, ciliary neurotrophic factor, leptin, growth hormone, prolactin, leukemia inhibitory factor, oncostatin M, erythropoietin, and granulocyte macrophage colony stimulating factor. Binding of ligands to their receptor and activation of one or more JAK tyrosine kinase initiates a variety of downstream signaling events that lead to the diverse physiological responses to cytokines (Fig.1).

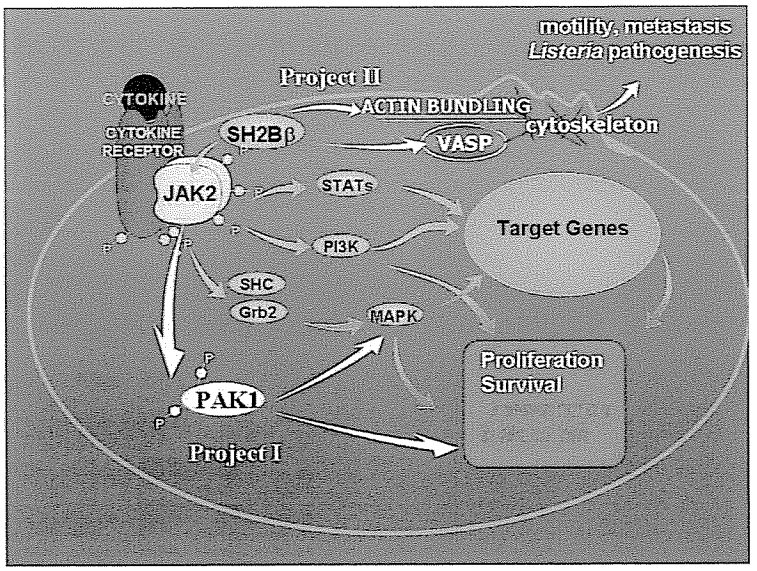


Fig.1 Schematic representation of signaling pathways for cytokines

The widely-expressed SH2 domain-containing protein SH2-Bβ was initially identified as a binding partner and substrate of JAK2, which is utilized by roughly two thirds of cytokine receptor family members, including GH receptor. SH2-Bβ contains multiple potential protein and/or lipid interacting domains suggesting that SH2-Bβ may serve as an adapter/scaffolding protein. In response to GH, SH2-Bβ associates with and is tyrosyl phosphorylated by JAK2. SH2-Bβ also binds to the activated forms of the receptor tyrosine kinases for insulin, insulin-like growth factor-1, nerve growth factor, platelet-derived growth factor (PDGF) and fibroblast growth factor. Recently, my colleagues and I implicated SH2-Bβ in the regulation of the actin cytoskeleton by GH and PDGF. I have shown that SH2-Bβ is involved in the regulation of GH-dependent cell motility. The region of SH2-Bβ required for this effect is also required for SH2-Bβ binding to the small GTPase Rac1, suggesting that SH2-Bβ may regulate cell motility at least in part by recruiting Rac1 to membrane localized GHR-JAK2 complex (Diakonova et al., 2000; 2002).

Although several important molecular players have been identified that link receptor tyrosine kinases to changes in the actin cytoskeleton, the precise molecular pathways are poorly characterized. The investigation of the signal transduction pathways

triggered by cytokines, growth factors, and hormones that lead to changes in cellular function represents the major direction of my future research.

JAK2 kinase regulates serine-threonine kinase PAK1

My current research interest is the regulation of PAK1 protein by JAK2 kinase. Serine-threonine protein kinases PAKs 1-6 are activated by binding of the small GTPases Cdc42 and Rac, sphingosine or in the case of PAK2, caspase-catalyzed proteolytic cleavage. Activated PAK1 regulates (via serine/threonine phosphorylation) MAP kinases pathway, apoptosis, the cell cycle, oxidant generation in phagocytic leukocytes, and cytoskeletal dynamics. PAK1 is activated by growth factors (PDGF and EGF) and by insulin via activated Rac1 and Cdc42. My preliminary data indicate that JAK2 phosphorylates PAK1 on tyrosines. Two-dimensional peptide mapping, phosphoamino acid analysis and *in vitro* kinase assays were used to identify three tyrosines which are targets for JAK2. I am interested in determining the role of PAK1 in cytokine signaling and the role of these three tyrosines in that signaling. Known PAK1 functions that PAK1 might regulate include PI3-kinase and MAP kinase pathways, apoptosis and cell motility.

In normal mammary development, the JAK2 activating hormone prolactin (PRL) is critical for alveolar proliferation and differentiation. Increasing evidence supports the involvement of PRL in breast cancer, the leading type of cancer in women and the second leading cause of cancer death among women. JAK2 was identified as a PRL-receptor (PRLR)-bound signaling molecule. Although the significance of both PRL and PAK1 in breast cancer is widely acknowledged, the mechanism by which PRL and PAK1 contribute to breast cancer remains poorly understood. There is a gap between upstream PRL-PRLR-JAK2 events and downstream PAK1 and PAK1-dependent functions in our understanding of the mechanism of breast cancer progression. My preliminary data suggest that tyrosyl phosphorylation of PAK1 by JAK2 protects cells from apoptosis. I will examine the hypothesis that PAK1 is a substrate for JAK2 and that in response to PRL, PAK1 is activated by JAK2-dependent tyrosyl phosphorylation and enhances PRL-dependent cell survival. My project will provide needed insight into the possible mechanism by which PRL, JAK2 and PAK1 participate in breast cancer. Tyrosyl phosphorylation of PAK1 by JAK2 is likely to represent a novel molecular target in the search for the etiology and treatment of human breast cancer.

Another possible target mechanism for PAK1 in human breast cancer may be MAPK cascades. PAK1 was identified as a key kinase in the regulation of Raf 1, MEK1, p38 and C-Jun N-terminal kinasse. PAK1 directly phosphorylates serine- 338 of Raf 1, which is critical for Raf 1 activation. I will determine whether PAK1 plays a role in activation of the MAPK cascades, and whether tyrosyl phosphorylation of PAK1 by JAK2 is required for or regulates that cascades.

PAK is a known regulator of the actin cytoskeleton. PAKs play an important role in promoting turnover of focal complexes and actin stress fibers. These effects are in part mediated by PAK inhibition of myosin light chain kinase and activation of LIM kinase.

PAK activity is regulated by Rac and CDC42. I will examine the role of PAK1 as a regulator of the actin cytoskeleton (together with Rac and SH2-Bβ) in ligand-dependent cell motility.

I will examine the role of SH2-Bβ in ligand-dependent regulation of PAK function. SH2-Bβ is a known activator of JAK2. My preliminary data strongly suggest that in addition to JAK2, SH2-Bβ is a binding partner for PAK1. I have shown that PAK1 directly binds to SH2-Bβ *in vitro* and *in vivo* and that SH2-Bβ changes the intracellular localization of PAK1 in response to GH treatment. It seems likely that PRL will also change the intracellular localization of PAK1. I will determine a role of SH2-Bβ -PAK1 interaction in GH and/or PRL -dependent intracellular processes.

Role of SH2-Bβ in the regulation of actin cytoskeleton

I initially implicated SH2-Bβ in the regulation of cell motility and the actin cytoskeleton by showing it binds the small GTPase Rac, one of the major actin-regulating proteins (Diakonova et al., 2000, 2002). Recently I have shown a novel role for SH2-Bβ in actin-based motility using *Listeria* as a primary model system. *Listeria* is an intracellular, pathogenic bacterium that uses components of the host's actin cytoskeleton to make an actin tail to propel itself through the cytoplasm. Intracellular *Listeria* motility provides a very powerful model to study the basic principles of the mammalian cytoskeleton. I showed that SH2-Bβ dramatically increases the speed of *Listeria* in infected cells and cytoplasmic extracts. I demonstrated that SH2-Bβ bundles actin filaments *in vitro* and is in a complex with VASP. Ena/VASP proteins constitute a family of proteins implicated in a variety of actin-based processes including cell migration, axon guidance, T cell polarization and the motility of *Listeria*. In collaboration with Dr. M.-F. Carlier (CNRS, Gif-sur-Yvette, France), we showed that in motility medium reconstituted *in vitro* from six pure proteins, SH2-Bβ greatly enhances actin-based motility, but only in the presence of VASP. I have just identified the region of SH2-Bβ which binds actin and VASP. We propose a novel mechanism of SH2-Bβ -dependent regulation of *Listeria* motility (submitted to Mol. Cell. Biol). I am currently testing the hypothesis that SH2-Bβ plays an essential role in *Listeria* motility and distribution by recruiting actin-regulating proteins from the host cell's cytoplasm to sites of active actin polymerization. I plan to continue to determine the mechanism by which SH2-Bβ regulates the actin cytoskeleton. I test whether binding of SH2-Bβ to actin and VASP is necessary for actin-dependent cellular functions, first of all, *Listeria* motility.

Based on these results, I will be in a position to address the issue of whether SH2-Bβ is required for spreading of *Listeria* infection. I shall test the ability of *Listeria* to spread from cell to cell, an action that requires actin-dependent motility, using a dominant negative SH2-Bβ mutant deficient for binding to actin and VASP.

To study further the mechanism of VASP-dependent regulation of actin rearrangement by SH2-Bβ, I shall overexpress cDNAs encoding fluorescent SH2-Bβ, VASP and actin to follow their interaction using FRET-based stoichiometric analyses by

video microscopy. This analysis allows one to assay interactions between fluorescent proteins inside of cells.

My preliminary data demonstrated that SH2-Bβ binds to N-WASP, a protein whose function is crucial for actin regulation. I plan to continue to determine the mechanism by which SH2-Bβ regulates actin assembly via N-WASP protein and N-WASP substrates.

Finally, having very powerful working systems to measure *in vitro* and *in vivo* *Listeria* motility, I will expand my studies to the role of other known members of the SH2-B family of adapter proteins, APS and Link in the regulation of actin-dependent motility.

Overall, my future studies will provide new insights into JAK2-dependent signal transduction events. Elucidating the mechanism by which JAK2 and the signaling events downstream of JAK2 are regulated is critical for our understanding of cytokine signaling. A cell's ability to move is essential for morphogenesis, wound healing, immune response, and metastasis of cancer cells. Therefore, it is also of great importance to unravel the signaling pathways that regulate the actin cytoskeleton at the molecular level.

STATEMENT OF TEACHING INTERESTS

During my scientific career, I accumulated significant amount of knowledge and experience which I would be happy to share with those who are just entering the scientific world. From my own experience, I know exactly that having a good teacher at the beginning of someone's career is critical for subsequent accomplishments. The major task of a teacher is not to pour a lot of information at students (it can be found in books after all), but to uncover the underlying logic within the flood of information, which exists in the contemporary science, to educate students how to organize their own thinking, how to set questions and get answers, and how to digest and critically evaluate scientific data, including their own results.

Based on my previous experience of giving research and educational lectures and seminars, and mentoring students and postdocs, I feel well prepared for academic teaching at undergraduate and graduate levels. I am most qualified and interested to teach basic and advanced cell and molecular biology, which represent the field of my research interests and experience.