Finding Genes for Alcohol-Related Behaviors and Risk For Alcoholism

The National Institute on Alcohol Abuse and Alcoholism (NIAAA) solicits research proposals to identify and characterize genes that contribute to individual susceptibility to alcoholism and alcohol-related behaviors. This PA encourages multidisciplinary studies using advanced genetic and genomics technologies to find and characterize candidate genes in humans and animal models.

Genetic linkage and association studies have identified many chromosomal regions and quantitative trait loci (QTL) associated with complex phenotypes related to alcohol induced behaviors and/or alcoholism. However, the large number of genes residing in those previously identified chromosomal regions/QTLs remain undefined, due to the lack of power and sensitivity of previously available methodology and technology. The completion of the sequences of the human and mouse genomes, and the development of new genomics technologies, have the potential for rapidly advancing the discovery of genes associated with alcoholism and alcohol-related behaviors.

NIAAA seeks research applications that will explore and develop innovative computational, statistical, and molecular approaches to increase the power of gene discovery. Areas of emphasis include utilizing advanced technologies such as high-throughput SNP genotyping, haplotype pattern mining, admixture linkage disequilbrium mapping, and DNA pooling for fine mapping. Currently, haplotype maps for both human and mouse genomes are being developed and these maps will provide an additional resource for finding genes. Once fine mapping has further characterized a previously identified QTL or chromosomal region, candidate nucleotide variants within the locus can be identified. Some methods that could be used for fine mapping include high-throughput sequencing, RAGE, SAGE, DNA microarrays, protein arrays, and other high-throughput approaches.

Many genes are predicted to reside within a QTL, and multiple polymorphisms are likely to be located in both coding and regulatory regions. Functional tests of candidate genes are needed to determine if the polymorphism(s) or genetic variants are relevant to the alcohol-induced trait. These tests may include the development of transgenic animals, such as BAC and YAC transgenics carrying chromosomal regions that contain large regulatory sequences.

This PA specifically seeks applications that propose human or animal studies to identify and characterize genes associated with alcoholism and alcohol-related behaviors. Behaviors include alcohol consumption, tolerance/dependence, sensitivity/sedation, withdrawal, reinforcement, craving, and relapse. Genes identified may be used to develop markers for alcohol vulnerability and dependence. In addition, these genes may also be used to identify potential therapeutic targets and to predict individual responses to medications that treat alcoholism. Examples of research areas appropriate to this announcement include, but are not limited to: 1) identification and characterization of genes associated with alcoholism and alcohol-related behaviors in the chromosomal regions previously identified in humans or animal models; 2) identification of genetic variants (SNPs/haplotpes) of associated with alcoholism and alcohol-related behaviors among the different populations, which will have high potential as targets for developing pharmacotherapeutic agents; 3) discovery of new genes associated with alcoholism and alcohol-related behaviors and determination of functional relevance of the candidate genes and their polymorphic variants.

This PA will use the NIH R01 and Exploratory/Developmental Research Grant (R21) award mechanisms (see http://grants.nih.gov/grants/guide/pa-files/PA-03-107.html).

As an applicant, you will be solely responsible for planning, directing, and executing the proposed project. Applications using the R21 mechanism may request a project period of up to two years with a combined budget for direct costs of up to $275,000 for the two year period. For example, the applicant may request $100,000 in the first year and $175,000 in the second year. The request should be tailored to the needs of the project. Normally, no more than $200,000 may be requested in any single year.

This PA uses just-in-time concepts. It also uses the modular as well as the non-modular budgeting formats see http://grants.nih.gov/grants/funding/modular/modular.htm. Specifically, if you are submitting an application with direct costs in each year of $250,000 or less, use the modular format. Otherwise follow the instructions for non-modular research grant applications. This program does not require cost sharing as defined in the current NIH Grants Policy Statement at http://grants.nih.gov/grants/policy/nihgps_2001/part_i_1.htm.

Exploratory/developmental grant support is for new projects only; competing continuation applications will not be accepted. Two revisions of a previously reviewed exploratory/developmental grant application may be submitted as defined in NIH Policy at http://grants.nih.gov/grants/policy/amendedapps.htm.

Applications must be prepared using the PHS 398 research grant application instructions and forms (rev. 5/2001). The PHS 398 is available at http://grants.nih.gov/grants/funding/phs398/phs398.html in an interactive format. For further assistance contact GrantsInfo, 301-435-0714, email: GrantsInfo@nih.gov.

Applications submitted in response to this PA will be accepted at the standard application deadlines, which are available at http://grants.nih.gov/grants/dates.htm. Application deadlines are also indicated in the PHS 398 application kit.

Applications must be mailed on or before the receipt dates described at http://grants.nih.gov/grants/funding/submissionschedule.htm. The CSR will not accept any application in response to this PA that is essentially the same as one currently pending initial review unless the applicant withdraws the pending application. The CSR will not accept any application that is essentially the same as one already reviewed. This does not preclude the submission of a substantial revision of an application already reviewed, but such application must include an Introduction addressing the previous critique. Contact: Lisa A. Neuhold, Program Director for Genetics, Genetics and Proteomics Research Branch, Division of Basic Research, NIAAA, 6000 Executive Boulevard, Suite 402, MSC 7003, Bethesda, MD 20892-7003 USA, 301-594-6228, fax: 301-594-0673, email: Lneuhold@willco.niaaa.nih.gov; Zhaoxia Ren, Program Director for Human Genetics, Genetics and Proteomics Research Branch, Division of Basic Research, NIAAA, 6000 Executive Blvd, Suite 402, MSC 7003, Bethesda, MD 20892-7003 USA, 301-443-5733, fax: 301-594-0673, email: zren@mail.nih.gov; Q. Max Guo, Program Director, Genetics and Proteomics Research Branch, Division of Basic Research, National Institute on Alcohol Abuse and Alcoholism, 6000 Executive Blvd, Suite 402, MSC 7003, Bethesda, MD 20892-7003 USA, 301-443-0639, fax: 301-594-0673, email: qmguo@mail.nih.gov. Reference: PA No. PA-03-162

Neuroprotective CNS Barriers in Neurological Diseases

The goal of this Program Announcement with set-aside funds (PAS) is to invite applications for studying the neurobiological and cerebrovascular mechanisms through which the neuroprotective blood-brain and blood-csf barriers function in the healthy and diseased adult, aged and pediatric brain. Blood-Brain Barrier (BBB) research embodies the true meaning of a "translational model" of neuroscience wherein breakthroughs in basic neuroscience are delivered to the clinic and require an agent delivery strategy and/or the ability to target specific areas of the brain. This PAS encourages studies focused on improving our understanding of the neuroprotective CNS barriers and enhancing the effectiveness of drug and gene delivery strategies for treatment of neurological diseases. Chief among the challenges to be addressed is the need to increase our knowledge about the molecular and cellular biology, cells of origin, gene and protein expression, and the regional differences of brain microvascular endothelial cells and pericytes and their interactions with adjacent brain cells.

A major challenge for treatment of most brain disorders is overcoming the difficulty of delivering therapeutic agents to specific regions of the brain. In its neuroprotective

role, the blood-brain barrier (BBB) functions to hinder the delivery of many potentially important diagnostic and therapeutic agents to the brain. Therapeutic molecules and genes that might otherwise be effective in diagnosis and therapy do not cross the BBB into the brain in adequate amounts. Improving our knowledge of the molecular and cellular biology of the brain microvasculature and their interactions with surrounding brain cells, which constitutes the BBB in vivo, could lead to innovative strategies for drug and gene targeting to injured or disease tissue. Also, research is needed on the role of the brain microvasculature in protecting the brain from toxic agents and how damage to the BBB leads to long-term neurological toxicity in the development of many neurological diseases.

Understanding the basic biology of how the BBB works under normal and disease conditions across the lifespan may also provide insight on the integrative function of the brain. Research focused on cerebrovascular endothelial cell biology may provide insight into the "neurovascular unit", a conceptual model that considers brain function from the perspective of interactions among blood cells, endothelium, glia, pericytes, extracellular matrix and neurons.

This initiative was identified as the top research priority of the Brain Tumor Progress Review Group (PRG). The Stroke Progress Review Group has also identified neurovascular research and BBB biology as a high priority for advancing our understanding of stroke and brain function. Both Progress Review Groups are responsive to Congressional requests for planning in these areas. Also, the National Institute of Neurological Disorders and Stroke (NINDS) Neuroscience at the New Millennium Plan clearly identifies research on the blood-brain barrier as a scientific priority. The scientific support for this initiative can be found in these reports on the NINDS homepage at http://www.ninds.nih.gov/funding/neural_environment/index.htm#research

This PAS is intended to achieve a better understanding of the effects of neurological disorders on the blood-brain barrier (BBB), improve our knowledge of BBB biology and how it may contribute to the initiation and/or progression of neurological disease over the lifespan and develop new approaches for targeting the BBB, based on biological considerations, in order to improve drug delivery and target treatment of one or more such disorders.

Applications that address gene and protein expression for microvascular endothelial cells within normal, aging, and diseased brain such as gliomas or the ischemic penumbra are encouraged. Cerebrovascular genomics is considered a high priority. Because only very abundant BBB-specific transcripts will be detected with whole-brain gene microarrays, cerebrovascular genomics research needs to start with the initial isolation of brain capillaries from animal or human brain, both normal and perturbed. Comparison of capillaries from normal brain and perturbed tissue can help to elucidate the tissue-specific gene expression. Pattern-specific tissue expression could provide the platform for further investigations on overall brain vascular biology as it pertains to conditions such as angiogenesis, cell adhesion, antigen presentation, metastasis, cell-cell communication and local inflammation.

There are several modalities for drug and gene delivery through the BBB. They include: BBB disruption; the use of endogenous transport systems, including carrier-mediated transporters such as glucose and amino acid carriers; receptor-mediated transcytosis, systems such as the insulin or transferrin receptor; and active efflux transporters such as p-glycoprotein and the associated anti-porters. Studies to determine which strategies are most effective and how they can be improved for patients with neurological diseases are encouraged. Research areas appropriate for this PAS include, but are not limited by the following examples: 1) Develop and characterize in vivo and in vitro models that reflect the unique features of the BBB as translational models of neurological disease. Existing *in vivo* models, such as those for stroke or lysosomal storage diseases, may also prove useful for studying the structure and dynamics of the BBB. Studies that validate *in vitro* results with in vivo models are encouraged; 2) Examination of the genes and proteins that are uniquely expressed by the intact BBB and mechanisms by which brain cells regulate endothelial cell gene expression. This includes changes that occur in response to neurological disease or the aging process, for example, characterization of molecular signatures for disease diagnosis and targeting; 3) Explore the genesis and regulation of the BBB, its stem cell origins and remodeling of the (diseased/damaged/aged) brain microvasculature; 4) Identify signal transduction pathways of brain capillary endothelial transcytosis and tight junction regulation under normal and disease conditions; 5) Characterize endogenous influx and efflux properties of the barriers including transporters in luminal and abluminal membranes of brain endothelium and epithelium; 6) Identify regional diversity of barrier properties within the brain and spinal cord microvasculature; 7) Characterize brain endothelial tight junction proteins in normal and disease states; 8) Investigate the various enzymatic barrier mechanisms; 9)Characterize membrane protein expression by cells of the BBB; 10) Development of novel brain drug and gene delivery methods based on unique properties of the BBB including gene therapy via vectors or via modified autologous cell transfer; 11) Explore the molecular basis of microbial interactions with brain endothelium; 12) Develop neuroimaging tools to identify changes in BBB permeability *in vivo*; 13) Examination of the molecular and cellular mechanisms of leukocyte migration at the BBB throughout the lifespan; 14) Comparison of transport systems in brain endothelia with choroids plexus epithelium as well as systemic epithelial cells; 15) Explore the interactions among the cellular and matrix elements of the BBB, for example, the microvascular basement membrane; 16) Examine the plasticity of the blood-brain and blood-CSF interfaces throughout the lifespan.

Applications should focus on neurological disorders relevant to the research missions of NINDS, the National Institue of Mental Health (NIMH) and/or the National Institute on Aging (NIA). A partial list of diseases of interest to NINDS is given in Appendix A of the planning document Neuroscience at the New Millennium; see http://www.ninds.nih.gov/about_ninds/strategic_plan.htm.

These include neurological disorders (e.g. stroke, brain tumors, Parkinson's disease, brain and spinal cord trauma, epilepsy, multiple sclerosis, brain lysosomal storage disorders, neuro-AIDS and Alzheimer's disease). The NIMH is interested in mechanistic studies of trafficking of cells, immune molecules and drugs across the blood-brain and blood-csf barriers during development and adulthood and how these processes impact the pathogenesis of neuroAIDS and mental disorders; see http://www.nimh.nih.gov/research/nimh-webs.cfm.

NIA is interested in age-related neurodegenerative disorders, such as Alzheimer's disease, brain injury, and impairments in cognitive, motor and sensory functions. Research areas relevant to the mission of the NIA can be found at: http://www.nia.nih.gov/research/extramural/neuroscience/programs.htm.

A large amount of basic research is needed to significantly change how we translate neuroscience research bidirectionally. This PAS is focused on stimulating new concepts in the BBB field through the exploratory/developmental grant (R21) and the R01 mechanisms. The current workforce in the BBB field is small relative to the scientific and clinical needs for improved understanding of the BBB and progress will require collaboration among current investigators and scientists from disciplines not currently working in this area. Therefore, training and early career development will be encouraged (please contact Program Staff for additional information). This PAS will remain active for 3 years to address the many gaps in our knowledge of how the neuroprotective barriers function and the time needed to increase the workforce in this critically important translational research area.

As an applicant, you will be solely responsible for planning, directing, and executing the proposed project. Applicants are encouraged to contact program staff for advice about choosing the appropriate grant mechanism.

The R21 mechanism (see http://grants.nih.gov/grants/guide/pa-files/PA-03-107.html) is intended to encourage new exploratory/developmental research projects by providing support for the early stages of their development. For example, such projects could assess the feasibility of a novel area of

investigation or a new experimental system that has the potential to enhance health-related research. These studies may involve considerable risk but may lead to a breakthrough in a particular area, or to the development of novel techniques, agents, methodologies, models or applications that could have major impact on a field of biomedical, behavioral, or clinical research.

Applications for R21 awards should describe projects distinct from those supported through the traditional R01 mechanism. For example, long-term projects, or projects designed to increase knowledge in a well-established area will not be considered for R21 awards. Applications submitted under this mechanism should be exploratory and novel. These studies should break new ground or extend previous discoveries toward new directions or applications. R21 applications may request a project period of up to two years with a combined budget for direct costs of up $275,000 for the two-year period. For example, you may request $100,000 in the first year and $175,000 in the second year. The request should be tailored to the needs of your project. Normally, no more than $200,000 may be requested in any single year.

This PAS uses just-in-time concepts. It also uses the modular budgeting as well as the non-modular budgeting formats (see http://grants.nih.gov/grants/funding/modular/modular.htm). Specifically, if you are submitting an application with direct costs in each year of $250,000 or less, use the modular budget format. Otherwise follow the instructions for non-modular budget research grant applications. This program does not require cost sharing as defined in the current NIH Grants Policy Statement at http://grants.nih.gov/grants/policy/nihgps_2001/part_i_1.htm.

Competing continuation applications submitted in response to this PAS will compete with all investigator-initiated applications and be referred and reviewed according to the customary peer review procedures. Responsibility for the planning, direction, and execution of the proposed project will be solely that of the applicant. The earliest anticipated award date is June 1, 2004.

NINDS, NIMH, and NIA have set aside $2,000,000 in total costs per year, in addition to funds available for applications sent in response to this PA that score within the NINDS payline (see NINDS Funding Strategy at http://www.ninds.nih.gov/funding/ninds_funding_strategy.htm), depending on the overall scientific merit of the applications and the availability of funds throughout the duration of this solicitation (3 years). Applications submitted in response to this PAS will compete with all investigator-initiated applications for funding.

The total project period for an application submitted in response to this PAS may not exceed 5 years. Because the nature and scope of the research proposed may vary, it is anticipated that the size of each award will also vary. Although the financial plans of the Institute provide support for this program, awards pursuant to this PAS are contingent upon the availability of funds and the receipt of a sufficient number of meritorious applications.

Applications must be prepared using the PHS 398 research grant application instructions and forms (rev. 5/2001). Applications must have a Dun and Bradstreet (D&B) Data Universal Numbering System (DUNS) number as the Universal Identifier when applying for Federal grants or cooperative agreements. The DUNS number can be obtained by calling (866) 705-5711 or through the web site at http://www.dunandbradstreet.com. The DUNS number should be entered on line 11 of the face page of the PHS 398 form. The PHS 398 is available at http://grants.nih.gov/grants/funding/phs398/phs398.html in an interactive format. For further assistance contact GrantsInfo, 301-435-0714, email: GrantsInfo@nih.gov.

Applications submitted in response to this PAS will be accepted at the standard application deadlines, which are available at http://grants.nih.gov/grants/dates.htm. Application deadlines are also indicated in the PHS 398 application kit. Please note that AIDS related applications have separate receipt dates.

Supplemental Instructions: All instructions for the PHS 398 (rev. 5/2001) must be followed, with these exceptions: 1) Research Plan. For R21 applications only, items a – d of the Research Plan (Specific Aims, Background and Significance, Preliminary Studies, and Research Design and Methods) may not exceed a total of 15 pages. No preliminary data is required for R21 proposals, but may be included if it is available. Please note that a Progress Report is not needed for R21 awards; competing continuation applications for an exploratory/developmental grant will not be accepted. 2) Appendix. Use the instructions for the appendix detailed in the PHS 398 except that for R21 applications, no more than 5 manuscripts, previously accepted for publication, may be included.

Applications must be received by or mailed on or before the receipt dates described at http://grants.nih.gov/grants/funding/submissionschedule.htm. The CSR will not accept any application in response to this PAS that is essentially the same as one currently pending initial review unless the applicant withdraws the pending application. The CSR will not accept any application that is essentially the same as one already reviewed. This does not preclude the submission of a substantial revision of an unfunded version of an application already reviewed, but such application must include an Introduction addressing the previous critique.

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Interactions Between Stem Cells and the Microenvironment *In Vivo*

National Institute of Neurological Disorders and Stroke (NINDS), the National Institute on Drug Abuse (NIDA), the National Institute of Deafness and Other Communication Disorders (NIDCD) the National Institute of Alcohol Abuse and Alcoholism (NIAAA), and the National Institute on Aging (NIA) invite applications for studies on the cellular and molecular signaling between the local environment within organisms and stem and progenitor cells that are either introduced as transplants or are normally resident within host tissues and organs. The objective of this initiative is to promote a thorough exploration and characterization of the bi-directional communication between multipotent cells and the three-dimensional local milieu or niche that they encounter *in vivo* under normal and compromised states, such as with aging or following injury, disease or drug exposure. Of particular interest is the rigorous characterization of how interactions with localized cues in space and time regulate stem cell survival, migration, replication and 'plasticity' in the nervous system and other parts of the body. Projects that address comparisons between the responses of stem cells within niches in the developing and mature or aging nervous system *in vivo*, or in host microenvironments modified by injury, disease, or by exposure to drugs and alcohol would also be directly relevant to this Program Announcement with Set-aside (PAS), as are studies to compare different classes of stem cells or progeny at progressively more advanced stages of differentiation when placed in the same sites *in vivo*.

Unlike organs such as the skin and the gut that self-renew throughout life, the nervous system in adult mammals is restricted in its ability to replace neurons and glia that have been lost through injury, disease, alcohol and drug abuse or even advancing age. Stem cell research offers enormous potential for treating many congenital, developmental, psychiatric or degenerative diseases of the nervous system for which there are no treatments or cures. Under the appropriate tissue culture conditions, a variety of multipotent cells appear to acquire many properties of neurons and glia – a first step toward developing cell replacement therapies for neurological dysfunction. The discovery of endogenous stem cells, residing either within the nervous system or in other tissues raises the possibility that these intrinsic systems may be harnessed to

restore defective cells and functions. In both cases the expectation is that, when exposed to the optimal microenvironment *in vivo*, endogenous or transplanted stem cells will differentiate in a manner appropriate to the local brain region, and integrate with the existing circuits in the nervous system. The past decade has seen enormous progress in our understanding of the specific requirements of stem cells to proliferate and differentiate along specified lineages. This progress has been made possible by the discovery of a myriad of growth factors and substrate conditions followed by careful testing in culture. Unfortunately, the behavior of cells in tissue culture does not adequately predict how these same cells will behave when transplanted into the living host where multiple known and unknown factors converge to influence the biological process. We do not know the whole spectrum of factors present *in vivo* that influence cell fate. Effective use of stem and progenitor cells for therapeutic purposes hinges on their ability to thrive, integrate, and function in a biologically meaningful manner *in vivo* without causing adverse events. Therefore the next stage in developing cell restoration therapy requires understanding how the newly generated cells will behave within the host.

Recent reports indicate that the "niche" or local microenvironment that a stem cell encounters governs its behavior and fate. For example, adult neural stem cells produced neurons when transplanted into the neurogenic zone of the hippocampus, but produced astrocytes in the environment of the spinal cord. Further investigation showed that a specific component of the local environment, the regional astrocytes from the hippocampus were capable of instructing these stem cells to adopt a neuronal fate *in vitro*. In addition to regional differences within the nervous system, the microenvironment encountered by a stem cell may vary as a function of age of the host organism. Similarly, alteration of the niche by injury, drugs or other circumstances is likely to affect the ability of transplanted stem cells to survive, differentiate and integrate into existing neural circuitry. Understanding these changes will be important in making decisions about the use of cell replacement therapies in very young or elderly patients, in patients with a history of alcohol or drug usage, or suffering from injury or other neurological conditions.

Transplanted cells can act to influence and change host cells in their vicinity. Stem cells may release agents that alter the activity or resiliency of damaged host cells. These dynamic interactions are inevitable as living cells and tissue contact, react and respond to each other in time and space. Teasing out and understanding these interactions poses a major challenge that must be faced in order to develop realistic cell replacement therapies and enhance normal tissue regeneration.

This PAS is intended to promote studies that establish and identify the nature and action of microenvironmental cues in the nervous system that regulate stem cell fate. It specifically targets cellular, molecular and genetic mechanisms that act *in vivo* to influence stem cell survival, homing/migration, adhesion, differentiation, plasticity and tumorigenicity in both the central and peripheral nervous systems. Applications that only propose *in vitro* studies will not be responsive to this initiative.

The following examples illustrate areas that are of high interest; other innovative projects are also encouraged. These examples of research approaches are not meant to be all-inclusive or restrictive. Plans for data and/or reagent sharing and promulgation of results will be integral to the applications. 1) Identification, localization and comparison of known or novel cues within the developing, adult and aging nervous system that influence the mitotic potential, cell cycle and differentiation of stem and progenitor cells along specific lineages; 2) Characterization of the cell-extrinsic and cell-intrinsic signaling pathways and components involved in transducing the action of local cues on stem and progenitor cells *in vivo*; 3) Investigation of the causal relationship between site-specific changes of endogenous cues resulting from injury, disease, exposure to alcohol, drugs of treatment or abuse, and any resulting alterations of stem cell activity; 4) Evaluation of the effects of external factors such as stress, exercise, or an enriched versus impoverished living conditions on the microenvironment within the host organism, and how these changes in microenvironment influence the behavior of stem cells at different periods throughout the life span of the organism; 5) Investigation of local cellular interactions that determine and maintain the structural and functional integration of progenitor cells into the host nervous system and existing circuitry; 6) Development of assays facilitating the discovery of novel endogenous signals that modulate stem cell behavior and fate, as well as signals generated by stem cells that regulate components of the local host tissue. These may include the development of measures (physiological, behavioral, neurochemical, imaging) to evaluate the integration and function of progenitor cells in the developing, adult and aging nervous system; 7) Assessment of the short and long-term local effects of the interactions between the immune system and glial reactions gendered in response to the infiltration of stem cells and their progeny in the host.

The NIDA is interested in how drugs of abuse and factors such as stress andenvironment affect the behavior of stem cells and the functional consequences of such alterations, which might be related to the cognitive impairments, developmental deficits, neuroadaption and addictive behaviors seen in drug abuse. The NIDCD is particularly interested in stem cell research targeting the various peripheral components of the auditory (hearing), olfaction (smell) and gustatory (taste) systems. The NIAAA is interested in how alcohol exposure alters the biochemical environment of tissues, thus interfering with the capacity of stem cells to establish contact, differentiate and function in target tissue. The NIA is interested in stem cell research and neurogenesis in the aging nervous system with emphasis on basic neurobiology, motor and sensory systems, integrative neurobiology, cognition and the dementias of aging, particularly Alzheimer's disease.

This PAS will use the NIH Exploratory/Developmental Grant (R21) and the Research Project Grant (R01) award mechanisms. As an applicant, you will be solely responsible for planning, directing, and executing the proposed project. The proposed project period during which the research will be conducted should adequately reflect the time required to accomplish the stated goals and should be no more than 5 years for R01 grants. The R21 grants are one-time awards to support innovative, high impact research projects that would either 1) generate pilot data to assess the feasibility of a novel avenue of investigation, 2) involve high risk experiments that could lead to a breakthrough in a particular field, or 3) demonstrate the feasibility of new technologies that could have major impact in a specific area. Support for the R21 grants is limited to two years with a cumulative maximum of $275,000 direct costs requested for both years. This program is appropriate both for new investigators seeking to establish independent research careers and for established investigators wishing to explore new areas of neuroscience or develop novel technologies. For further information on the R21 mechanism, including Institute-specific information, see http://grants.nih.gov/grants/guide/pa-files/PA-03-107.html.

This PAS uses just-in-time concepts. It also uses the modular as well as the non-modular budgeting formats; see http://grants.nih.gov/grants/funding/modular/modular.htm. Specifically, if you are submitting an application with direct costs in each year of $250,000 or less, use the modular format. Otherwise follow the instructions for non-modular research grant applications. This program does not require cost sharing as defined in the current NIH Grants Policy Statement at http://grants.nih.gov/grants/policy/nihgps_2001/part_i_1.htm.

The participating ICs have set aside a total of $2 million dollars per year to support this initiative. The amount and timing of awards paid from set aside funds will depend on the overall scientific merit of the applications and the availability of funds throughout the duration of this solicitation (2 years). Because the nature and scope of the proposed research will vary from application to application, it is anticipated that the size and duration of each award will also vary. Although the financial plans of the IC(s) provide support for this program, awards pursuant to this PAS are contingent upon the availability of funds and the receipt of a sufficient number of meritorious applications.

Upon initiation of the program, the participating institutes will sponsor an annual meeting to encourage the exchange of information among investigators who participate in this program. In the preparation of the budget for the grant application, applicants should REQUEST ADDITIONAL TRAVEL FUNDS for one meeting each year to be held in Bethesda, Maryland. Applicants should also include a statement in the applications indicating their willingness to participate in such meetings. Applicants are also strongly encouraged to include plans for data and/or reagent sharing and promulgation of results.

Applications must be prepared using the PHS 398 research grant application instructions and forms (rev. 5/2001). Applications must have a Dun and Bradstreet (D&B) Data Universal Numbering System (DUNS) number as the Universal Identifier when applying for Federal grants or cooperative agreements. The DUNS number can be obtained by calling (866) 705-5711 or through the web site at http://www.dunandbradstreet.com/. The DUNS number should be entered on line 11 of the face page of the PHS 398 form. The PHS 398 is available at http://grants.nih.gov/grants/funding/phs398/phs398.html in an interactive format. For further assistance contact GrantsInfo, 301-435-0714, email: GrantsInfo@nih.gov.

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Metabolomics Technology Development

The Institutes and Centers of the National Institutes of Health (NIH) invite applications for development and application of new technologies in metabolomics to enable research aimed at elucidating biological pathways and networks. The purpose of this initiative is to encourage the development of highly innovative and sensitive tools for identifying and quantifying cellular metabolites and their fluxes at high anatomical, spatial, and temporal resolution. The general aim of metabolomics is to identify, measure and interpret the complex time-related concentration, activity and flux of endogenous metabolites in cells, tissues, and other biosamples such as blood, urine, and saliva. For the purposes of this solicitation, metabolites include small molecules that are the products and intermediates of metabolism, but also carbohydrates, peptides, and lipids. The need for innovative technologies for measuring and quantifying metabolites involved in cellular pathways and networks was articulated in the 2003 NIH Roadmapping Initiative. It is expected that the technologies developed under this initiative will play a major role in transferring capabilities to laboratories and research institutes that are investigating the underlying pathways involved in cellular homeostasis, perturbation, development, and aging. Many ongoing research programs focus on development of new genomics and proteomics tools and utilization of those approaches for studying cellular function. In contrast, relatively few research programs focus on metabolomics technology development and application. This initiative is to encourage the development of highly innovative and sensitive tools for identifying and quantifying cellular metabolites and their fluxes at high anatomical resolution—extending to subcellular—and at a temporal resolution that would be appropriate to understanding cellular processes at biologically relevant timescales. The scope of projects that would be appropriate ranges from techniques for improving and refining the process of sample separation and processing; to new methods, reagents or instrumentation for identifying and measuring metabolites and their fluxes; to the development and utilization of data reduction, management, and analysis tools needed to establish proof of principle for the technology. New technologies that, if successful, have the potential to be scalable, either as high-throughput applications or as advances that would be used in a large number of laboratories, are especially encouraged. While it is also important to develop data storage, data mining, and pathway modeling capabilities for metabolomics, these issues are explicitly not included in this particular solicitation.

Metabolomics presents unique challenges for sample collection and extraction and for determining analyte identity, concentration, structure, activity and flux in cells. The cellular metabolome is complex, involving several compound classes of small molecules (peptides, lipids, amino acids, carbohydrates) that vary in subunit concentration, size, structure, polarity, and functional groups. Technologies currently in use for metabolomic analysis include NMR, chromatography and mass spectrometry, each of which has significant limitations in quantification, scope, and/or throughput. No one technology can effectively measure, identify and quantify, with sufficient sensitivity and precision, the diverse range of metabolites and their dynamic fluctuations in cells. An integrated set of technologies is needed to address the entire spectrum of challenges for metabolomics. Ideally, new technologies should yield quantitative, comprehensive data and be applicable to achieving anatomical resolution at the cellular and subcellular level.

This initiative seeks to encourage technology developments to address three interrelated components of metabolomics: (1) sample collection, extraction, recovery and validation for specific classes of metabolites; (2) analyte detection, identification, quantification, and structure elucidation; and (3) and data management, reduction and analysis. Specific areas of research emphasis include approaches to address the large dynamic range of metabolite concentration in biological samples, the complexity of metabolite mixtures, the inherent noise of the metabolite profile, the vast number of unidentified compounds present within single samples, and the rapidly changing temporal and spatial variability (flux) of the cell's metabolite complement. It is imperative that new technology incorporate approaches for data management, reduction and analysis to support the technology development. This initiative encourages applications that seek to improve existing technologies, including scaling up to high throughput application, as well as those that seek to develop new approaches that have the potential for measuring entire cellular metabolomes or subsets (e.g., amino acid derivatives, peptide derivatives) whose analysis provides enabling technologies, including appropriate tools for data reduction and analysis.

Applications will be evaluated for the potential of the proposed activities to address all three components of metabolomics that are listed above. However, it is anticipated that these components, collectively, might be too broad for a single application to address all

three comprehensively. Accordingly, applications may focus on any of the components of metabolomics listed above and may propose single or multiple technologies. Investigators will be expected to clearly define the scope of their activities, and to justify why the specific type(s) of data that the technologies will address are likely to be important to understanding cellular pathways and networks. This initiative encourages applications involving multi-disciplinary teams representing self-assembled groups of collaborating investigators, at one or several sites, with specific expertise in metabolomics technology as well as data management and statistical approaches relevant to the proposed technology. Partnerships between academia and industry are encouraged, to facilitate technology transfer and capacity building at academic institutions. This solicitation seeks to encourage highly innovative and potentially risky approaches. However, it is likely that some proposed technologies will be more mature, at the onset, than others. Accordingly, this RFA will use the NIH Phased Innovation (R21/R33) and Exploratory/Development Research Grant Phase 2 (R33) award mechanisms. Applicants may submit a combined R21/R33 application, or a stand-alone R33 application if technological feasibility can be documented at the time of submission. Applicants may request up to three years of funding, either using the R33 mechanism for the entire time, or via a phased format that begins as an R21 award and transitions to an R33 award. The duration of the R21 phase may be either one year or two years. A grant may be considered for renewal or supplementation after the three year period of support if it is obvious that a newly developed technology will have exceptionally high impact on the field, but additional time is required to optimize it. Applicants for a phased award cannot request more than $800,000 in direct costs per year of the R21 phase. Applicants may not request more than $1.5 million in direct costs per year of the R33 phase, or per year of the entire award if it is solely R33. It is emphasized that the figures above are a maximum. We envisage that a range of activities could be appropriate for this solicitation, from an individual well-focused goal, to a set of closely related or well-integrated technology development aims. The R21 award mechanism supports innovative, high-risk/high-impact research requiring preliminary testing or development; exploration of the use of approaches and concepts new to a specific substantive area; or research and development of new technologies, techniques, or methods.

Applications will be considered high-impact if they demonstrate the potential for ground-breaking significance, and high-risk because they either lack sufficient preliminary data to ensure their feasibility, or propose use of a new model or a unique system. Eligibility for transition to the R33 phase will be based on successful completion of the negotiated milestones, which must be specified in the application, and programmatic review. The objective of the R33 phase is continuation of innovative exploratory and developmental research initiated during the R21 mechanism. Research conducted in the R33 phase will focus on demonstrating proof of principle for application of the technology to elucidate functional components and interactions of metabolites within biological pathways and networks. While the intent of the R21 award is to encourage the development of highly innovative technologies, the potential for the proposed technology to advance our understanding of biological pathways and networks will be an important criterion for evaluating the R33 phase of an application or the entire application, if it is for a stand—alone R33 award. Development of complex, integrated technologies for metabolomics problems will require a context within which methods development can proceed. Accordingly, investigators should select a model system, defined at the cellular or subcellular level, to serve as a framework for demonstrating the technological capabilities of the resource.

This RFA will use the NIH Phased Innovation (R21/R33) and Exploratory/Development Research Grant Phase 2 (R33) award mechanisms. As an applicant you will be solely responsible for planning, directing, and executing the proposed project. This RFA is a one-time solicitation. Future unsolicited, competing-continuation applications based on this project will compete with all investigator-initiated applications and will be reviewed according to the customary peer review procedures. The anticipated award date is September 30, 2004. Applications that are not funded in the competition described in this RFA may be resubmitted as NEW investigator-initiated applications using the standard receipt dates for NEW applications described in the instructions to the PHS 398 application.

This RFA uses just-in-time concepts. It also uses the non-modular budgeting format. Please follow the instructions for non-modular budget research grant applications. This program does not require cost sharing as defined in the current NIH Grants Policy Statement at http://grants.nih.gov/grants/policy/nihgps_2001/part_i_1.htm.

Prospective applicants are asked to submit a letter of intent that includes the following information: 1) Descriptive title of the proposed research, 2) Name, address, and telephone number of the Principal Investigator, 3) Names of other key personnel, 4) Participating institutions, 5) Number and title of this RFA.

Applications must be prepared using the PHS 398 research grant application instructions and forms (rev. 5/2001). Applications must have a DUN and Bradstreet (D&B) Data Universal Numbering System (DUNS) number as the Universal Identifier when applying for Federal grants or cooperative agreements. The DUNS number can be obtained by calling (866) 705-5711 or through the web site at http://www.dunandbradstreet.com/. The DUNS number should be entered on line 11 of the face page of the PHS 398 form. The PHS 398 document is available at http://grants.nih.gov/grants/funding/phs398/phs398.html in an interactive format. For further assistance contact GrantsInfo, 301-435-0714, email: GrantsInfo@nih.gov.

The RFA label available in the PHS 398 (rev. 5/2001) application form must be affixed to the bottom of the face page of the application. Type the RFA number on the label. Failure to use this label could result in delayed processing of the application such that it may not reach the review committee in time for review. In addition, the RFA title and number must be typed on line 2 of the face page of the application form and the YES box must be marked. The RFA label is also available at: http://grants.nih.gov/grants/funding/phs398/labels.pdf.

Applications must be received on or before the application receipt date listed in the heading of this RFA. If an application is received after that date, it will be returned to the applicant without review.

The Center for Scientific Review (CSR) will not accept any application in response to this RFA that is essentially the same as one currently pending initial review, unless the applicant withdraws the pending application. However, when a previously unfunded application, originally submitted as an investigator-initiated application, is to be submitted in response to an RFA, it is to be prepared as a NEW application. That is, the application for the RFA must not include an Introduction describing the changes and improvements made, and the text must not be marked to indicate the changes from the previous unfunded version of the application.

Letter of Intent Receipt Date: February 24, 2004; Application Receipt Date: March 24, 2004; Peer Review Date: June/July 2004; Council Review: September 2004; Earliest Anticipated Start Date: September 30, 2004.

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