e-mail to: BAC_Library_Requests@mail.nih.gov

Proposal for Construction of a BAC library of the Genome of the Zebra Finch (*Taeniopygia guttata*)

Proposal submitted by:

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This proposal is submitted on behalf of the entire community of scientists using the zebra finch and related songbirds as model organisms.

1. Importance of the zebra finch to biomedical or biological research

A great deal of biological research has centered on songbirds. In the wild songbirds are highly visible and available for observation, and thus during the 20th Century a great deal of information became available about their behavior, population biology, and ecology. Because they are small, have short generation time (4 months) relative to other complex vertebrates, and can be bred in captivity, laboratory research on songbirds surged after the second World War.

By far the most studied single species is the zebra finch (*Taeniogpygia guttata*, family Estrildidae, Suborder Oscines, Order Passeriformes). This species is native to dry areas of Australia, and is the easiest songbird to breed. Zebra finch males sing a song as part of the courtship ritual. This song is learned during a critical developmental period, after which the song is relatively stable. Only males sing the courtship song. In 1976 Nottebohm and colleagues described an interconnected circuit in the brain that controls song and song learning. The discovery of this anatomically discrete neural circuit led to a rapid series of discoveries that have strongly shaped the field of neurobiology ever since. Some of the seminal discoveries are the following:

A. Large sex differences in neural structure and function

Nottebohm and Arnold (1976) found that the brain regions controlling song are highly sexually dimorphic. This was the first discovery of a large sex difference in the brain, and triggered numerous investigations that uncovered similar morphological sexual dimorphisms in the brain of mammals including humans (e.g., DeLacoste-Utamsing and Holloway, 1982). This system is a major model system for understanding sexual differentiation of the brain, and has provided evidence that challenges major dogma in this field (Arnold, 1996). Cutting edge research on sexual differentiation is being published every year (Holloway and Clayton, 2001). In 2001 the National Institute of Medicine published the report *Exploring the Biological Contributions to Human Health: Does Sex Matter?* (http://www.iom.edu/IOM/IOMHome.nsf/Pages/does+sex+matter+summary) which argues for gender-specific approaches to medicine. This report references some of the studies of the zebra finch that have contributed to an understanding of the cellular and molecular forces that shape sex-specific development.

B. The Neural Basis of Learning

Zebra finches copy their songs from their fathers during an early critical period of development. Study of the neurons in the neural circuit therefore offers tremendous advantages for understanding the cellular events that open critical periods of development and that underlie synaptic plasticity and learning. This system has become one of a very small group of model systems for the study of the cellular and molecular basis of vertebrate learning. Molecular studies of song learning have contributed to an understanding of the functional biology of molecules such as synuclein, which was discovered independently in a screen for genes regulated during song learning and subsequently found to be centrally involved in Parkinson's and other neurodegenerative diseases [1].

C. Adult Neurogenesis

In 1984 Goldman and Nottebohm showed that the adult songbird brain makes new neurons, overturning the universally held belief that neurogenesis does not occur in adults. This discovery led to studies proving that significant neurogenesis occurs daily in the adult rodent and primate brain. It is fair to say that research in songbirds led to a large shift in the field of neurology, so that a major modern goal is to control the development of stem cells so that they can be introduced into the brain to replace or repair damaged neural circuits. This sea change in neurology was foreshadowed by Nottebohm?s 1985 book, *Hope for a New Neurology,* and is highlighted in NIH Publication 01-4602 *What we learned from songbirds.* The adult songbird telencephalon remains one of the best places to study the functional significance and control of adult neurogenesis.

D. Auditory processing and sensory-motor integration

In order to copy its father's song, the young zebra finch receives sensory input and uses that neural activity to sculpt motor output via auditory feedback. The songbird is a model system for study of this complex auditory processing and sensory-motor integration. One breakthrough was the discovery that perception of novel song causes upregulation of the immediate-early gene ZENK in specific brain regions (Mello et al., 1992). Expression of ZENK has proved to be a valuable tool for studying neural processing.

E. Influences of steroid hormones on neural networks

Sex steroid hormones cause seasonal changes in the synaptic organization of the neural song circuit, a phenomenon first discovered in songbirds (Nottebohm, 1981; DeVoogd and Nottebohm, 1981) and subsequently found in other circuits in mammals (e.g., Kurz et al., 1986). The songbird brain is a model system for understanding changes in the adult brain induced by hormones.

F. Functional importance of steroid synthesis in brain

The zebra finch is unusual because estrogen is synthesized in brain at a rate high enough to contribute to circulating levels of estrogen in the blood. Recently estrogen of neural origin has been implicated in causing masculine patterns of neural development (Holloway and Clayton, 2001), the first time that sex steroids of neural origin have been directly related to sex differences in neural development.

Most studies of songbirds to date come from the fields of neuroscience, behavior, and ecology. The work on songbirds has been held back because of a dearth of genetic information which could be used as a foundation for studies of molecular and cell biology. Studies by neurobiologists and behaviorists have uncovered a series of incredible phenomena (neurogenesis, learning, sex differences, steroid synthesis in brain) that raise numerous questions about the underlying molecular and cell biology. The field has attracted a generation of young cell/molecular biologists who are beginning to characterize the molecular basis of these phenomena. Severely lacking are genetic reagents important for understanding the structure of the genome and the genes it encodes. If we had a zebra finch genomic BAC library, it would provide us with a powerful set of new reagents for functional biological studies.

It is important to emphasize that the functional biology research on songbirds has had dramatic impact on concepts of brain development and function in humans because the concepts developed first from discoveries on songbirds have been subsequently found to be generally true also in mammals.

2. Uses to which the zebra finch BAC library would be put

THEME 1: Obtain full coding and regulatory sequences for genes with already identified functional significance

Specific gene products have already been identified that have roles in learning, adult neurogenesis, sexual differentiation, and steroid synthesis in brain. One goal is to begin direct analysis of the DNA elements that regulate their expression, and to reintroduce altered forms of these gene products into the nervous system to test for functional consequences. Examples of known genes include: zenk, synuclein, aromatase, estrogen and androgen receptors, cannabinoid receptor, retinoic acid binding protein, and sexually dimorphic genes such as protein L7 and neurocalcin. Although any one of these projects could be pursued independently with conventional phage-based genomic cloning approaches, having a BAC library available would greatly expedite the overall effort. Identification of a single BAC clone by specific cDNA screening has a reasonable chance of providing not only the entire coding sequence, but also the majority of associated gene regulatory elements.

THEME 2: Platform for development of gene transfer approaches to analyze functional consequences of specific mutations, and to define control elements that underlie normal patterns of regulation in vivo.

In many ways the zebra finch is an ideal vehicle for developing and applying targeted gene transfer methods. The song system is composed of a distributed set of discrete, large anatomical nuclei with defined interconnections, making it quite suitable for microinjection approaches. A rich set of behavioral assays is available for study of the functional consequences of specific gene transfer. Pilot studies in the Clayton lab have demonstrated that zebra finch neurons in the brain are readily infectable with both HSV and AAV gene vectors. Wade-Martins et al (2001) have shown that BACs can be readily ported into an HSV amplicon system for efficient introduction of sequences >100 KB into cells. The profound advantage of BACs is that a larger "normal" chromosomal context is preserved for the transferred gene, making it more likely that normal patterns of gene regulation will occur (Heintz, 2000). This could be critical for functional studies, and also may facilitate the identification of critical gene regulatory regions by mutational approaches.

THEME 3: Gene discovery and genome sequencing

The BAC library will facilitate discovery of new genes, for example by exon trapping of sex chromosome BACs related to studies of sexual differentiation.

THEME 4: Use of BACS to study highly informative mutations in individual birds

We (Arnold lab) have been studying individual birds that have unusual sex reversal (e.g., true gynandromorphs) to understand the genetic abnormalities that interfere with sexual differentiation. The lack of a BAC library has severely hampered our effects to understand the genetic and chromosomal linkage of these critical abnormalities.

THEME 4: Other applications

a) facilitate sequencing of parts (and eventually all) of the songbird genome

b) map genes and ESTs to specific chromosomes

c) studies of populations genetics and ecology, genetic polymorphisms

d) studies of the structure and organization of the zebra finch genome in evolutionary relation to chicken, human, and other vertebrates

We are strongly convinced that the existence of a BAC library will catalyze numerous research projects that could not be contemplated in the absence of this resource.

3. Size of the research community that could potentially use the BAC library and the community's interest in and support for having a BAC library

A search of the NIH CRISP database indicates that the following currently funded grants list ?zebra finch? or ?canary? in the title or abstract: 13 R01, 2R03, 3F31/F32. Search further for ?songbird? identifies an additional 16 R01, 10 F31/F32, and 7 others including one P01. A conservative estimate of the amount of funding these grants provide in a single year is in excess of $10 million. The numbers of years of support previously awarded just for these *currently funded* grants is 250 grant-years (61 grants total). A publicly available BAC library will have wide impact on the success of these already funded projects.

The zebra finch has emerged as an important model avian species, probably second only to the chicken in the number of papers published in recent years. A Medline search for ?zebra finch? retrieves about 260 papers published since 1990. In comparison, a search for ?Meleagris?, the genus of the turkey, retrieves about 140. Roughly 60 laboratories around the world study the neural circuit for song,, and we know of approximately 40 in the USA. Many more investigators study songbirds in the context of field biology and other behaviors. Three members of the US National Academy of Sciences (Peter Marler, Fernando Nottebohm, and Masakazu ?Mark? Konishi) have built their careers on the study of song behavior and the neural circuit controlling song and vocal learning.

We recently sent an email request to virtually all of the 40 lab heads in the USA asking for expressions of interest in a zebra finch BAC library. Twenty laboratory heads responded on short notice with enthusiastic support for this application, including some who study songbirds as models of autism, mental retardation, and other human diseases. Below are copies of two email messages from Drs. Nottebohm and Konishi supporting this application:

Date: Mon, 5 Nov 2001 14:48:03 -0500
To: arnold@protos.lifesci.ucla.edu From:
Fernando Nottebohm <nottebo@mail.rockefeller.edu>
Subject: Your plan for constructing a BAC library.

Dear Art:

I am delighted to learn that you are interested in constructing a zebra finch Bacterial Artificial Chromosome library and that there is a chance that NIH might support this effort. Zebra finches have become a most useful model system to study a variety of basic processes -- vocal learning, sexual differentiation and adult neuronal replacement, among others. As you well know, approximately 50 laboratories around the world now work with this material, and that number is bound to grow as we learn more about the basic biology of this species. Because of their short reproductive cycle and special behavioral and circuitry features, they have become the mouse, or Drosophila of the avian world. There is a great need to bring to bear on this species the techniques of molecular biology. Several laboratories have started to do this, but no one to my knowledge has yet taken the bold step of starting to construct a BAC library. I and all others in our field will be much indebted to you and to NIH if this project gets under way and the BAC library gets constructed.

All best,
Fernando Nottebohm

Dr. Fernando Nottebohm, Professor
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Date Tue, 23 Oct 2001 105021 -0700
From Mark Konishi <konishim@its.caltech.edu>
Subject BAC library
To Art Arnold <arnold@ucla.edu>

I strongly endorse the proposal by Prof. Arthur P. Arnold for the development of a zebra finch BAC library. The zebra finch is an excellent model for the study of auditory memory and vocal learning. A chain of discrete brain areas controls the production of song. Cellular and molecular studies of these areas have already yielded very interesting findings such as neurogenesis in the adult brain, estrogen synthesis, sexual differentiation of the brain, an antigen unique to the forebrain song nuclei, and expression of immediate early genes after singing or hearing song. Some laboratories have already begun to use DNA chips to study gene expression associated with song learning. The main missing link in the molecular approach to the song system is genomic information. The development of a BAC library will fill this gap.

Mark Konishi, Ph.D.
Bing Professor of Behavioral Biology.
California Institute of Technology
http://www.its.caltech.edu/~biology/brochure/faculty/konishi.html

4. Has the zebra finch been proposed to NHGRI or another publicly funded agency for BAC-based genomic sequencing?

This is the first application for construction of a zebra finch BAC library, to our knowledge.

5. Other genomic resources that are available that will complement this resource

To date, the only resources are cDNA and genomic libraries made in individual laboratories. These are traded within the community.

6. The strain of the organism proposed and rationale for its selection

There are no inbred strains available. We would select a single individual from domesticated stock.

7. The size of the genome

Stevens (1996) states that the size of an avian genome is approximately 2.45×10^9 bp.

8. The availability of a source of DNA for construction of the BAC library

We propose to isolate high quality genomic DNA from a single female zebra finch from our colony of domesticated zebra finches. A female is chosen because its genome includes both sex chromosomes (ZW) in contrast to the homogametic male (ZZ).

9. Specifications for the library (e.g., library depth, BAC insert size)

The library depth, vector, and insert size would be selected based on the recommendation of the laboratory producing the BAC library. A BAC library with conventional properties (insert size 100kB, ~5 or greater fold coverage) would be extremely useful to us.

10. The time frame in which the library is needed

We have no deadline. The library will be useful as soon as it is produced.

11. Other support

We have not previously requested support for BAC library construction from other sources. We are currently planning an application to NIMH to construct a zebra finch brain cDNA microarray with 20,000 sequenced cDNAs, for public use, which would greatly complement the BAC library resource.

12. The need for an additional BAC library if one or more already exists

None exists.

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