FRAXA UPDATE

SPRING 2002

VOLUME 9, NO. 1

A PUBLICATION OF FRAXA RESEARCH FOUNDATION

"NEVER DOUBT that a small group of thoughtful, committed citizens can change the world. INDEED, it's the only thing that ever has."

— Margaret Mead

FRAXA Funds Clinical Trial

Effects of Ampakine CX516 on Cognition and Functioning in Fragile X Syndrome and Autism

Over the past decade, several pharmaceutical companies have been developing a new class of promising medications, called "Ampakines", which seem to enhance learning. FRAXA's very first 1994 newsletter featured an article by Dr. Michael Tranfaglia about the potential that Ampakines offered for people with Fragile X. For the past year, FRAXA has been funding a study of one such compound in the Fragile X knockout mice in the laboratory of Dr. William Greenough. Now FRAXA has approved $72,000 to fund a trial of this compound in adults with Fragile X, under the direction of Dr. Elizabeth Berry-Kravis.

by Elizabeth Berry-Kravis, MD, PhD
RUSH University, Chicago

Treatment strategies in Fragile X syndrome are currently supportive treatments or treatments directed at managing specific behavioral symptoms, in order to maximize functioning. Treatments specifically directed at the underlying brain defect in Fragile X are not presently available.

In the last few years we have learned much about what FMRP does in the brain and specifically at synapses, the connections between brain cells. The relative strength or

continued on page 4

Toward Therapeutic Treatments

An overview of FRAXA Strategy

FRAXA Research Foundation's primary mission is to speed up progress towards effective treatments and a cure for Fragile X. Here we describe the research approaches that FRAXA supports.

The basic problem in Fragile X is that brain cells have a defect in a gene (FMR1) such that this gene cannot produce its normal product, the Fragile X protein (FMRP). Without FMRP, brain cells cannot communicate cleanly with each other, probably at least in part due to FMRP's roles in development. This underlies the learning and behavioral challenges seen in Fragile X syndrome.

Potential therapeutic approaches are:

1. Fix the gene so that it can make its normal protein.
2. Make and deliver the protein by some other means.
3. Substitute for the function of the protein.
4. Treat the symptoms of Fragile X.

continued on page 10

Also in this issue:

- Report from Washington
- More New Grants Funded
- Calendar of Events

FRAXA is a nonprofit, tax-exempt charity run by parents of children with fragile X syndrome. Fragile X syndrome is the most common inherited cause of mental retardation and developmental disabilities, affecting approximately 1 in 2000 males and 1 in 4000 females. FRAXA's goal is to accelerate research aimed at the treatment and cure of fragile X, by direct funding of promising research projects and by raising awareness of this disease.

Report from Washington:

by Mary Beth and David Busby

There is very good news: in the last two years, federal funding for Fragile X research has increased dramatically. The National Institutes of Health is composed of 27 separate institutes and other organizations, and several of them have recently made Fragile X a priority. Here are the numbers:

NATIONAL INSTITUTES OF HEALTH
FUNDING for FRAGILE X

	FISCAL YEAR 2000	FISCAL YEAR 2001
NICHD	$ 4,130,000	$6,247,000
NIMH	$ 1,635,000	$ 2,970,000
NINDS	$ 573,000	$ 1,666,000
NCRR	$ 464,000	$ 507,000
GMS	$ 324,000	$ 306,000
TOTAL	$7,128,000	$11,698,000

NICHD ONLY

1994: $1,860,000	1998: $4,329,000
1995: $1,894,000	1999: $4,617,000
1996: $1,808,000	2000: $4,130,000
1997: $3,628,000	2001: $6,247,000

Source:L. Kaesar, Senior Program Analyst,Office of Public Affairs, National Institute of Child Health & Development, National Institutes of Health

KEY

NICHD: National Institute of Child Health & Human Development

NIMH: National Institute of Mental Health

NINDS: National Institute of Neurological Disorders & Stroke

NCRR: National Center for Research Resources

GMS: General Medical Sciences

We think there are three reasons this funding has grown over the past few years:

1. Increasing interest in medical research in general, among members of Congress
2. Increasing interest in Fragile X among the scientific community
3. Efforts by Fragile X families and friends

In terms of Federal funding, Fragile X would have gotten lost in the crowd BUT FOR YOUR EFFORTS and those of the entire Fragile X community.

Highlights

On January 4, 2002, the NICHD announced the Fragile X research funding required by the **Children's Health Act of 2000**. You will find the announcement on the web at *http://grants.nih.gov/grants/guide/rfa-files/RFA-HD-02-009.html*

Some of the key provisions are:

- $3,375.000 is committed starting in October 2003 to fund at least three Fragile X Research Centers. These Centers are "to form networks that foster communication, innovation, and high-quality research in Fragile X syndrome."
- Annual Meetings of the Research Center Directors will be sponsored and funded by the NICHD staff.
- A Loan Repayment Program for young researchers will be instituted.

The Children's Health Act of 2000 authorized the NIH to establish a program of loan repayments to attract qualified health professionals into research on diseases, disorders, and other conditions in children. Qualified health professionals who contractually agree to conduct pediatric research for a two-year period are eligible to apply for this program. Participants can receive educational loan repayments of up to $35,000 annually. Visit *www.lrp.nih.gov* for details.

This year, we continue our work to ensure that the Fragile X Research Centers authorized last year are fully funded and that research funding by the NIH will continue to grow. On April 30, the day after the Mary Higgins Clark Gala, FRAXA will hold a second Lobby Day on Capitol Hill.

You can help by letting your members of Congress know of your commitment to Fragile X research. If you have not already done so, please call David Busby at (202) 824-8820 or email him at busby.david@dorseylaw.com.

FRAXA CALENDAR

THURSDAY MAY 16

Gala with Mary Higgins Clark and Master of Ceremonies, News Anchor Kim Carrigan Corinthian Yacht Club in Marblehead, MA

SATURDAY, JUNE 1

Patrick's Pals 6th Annual 3-on-3 Basketball Tournament Cambridge, MA

THURSDAY, JUNE 6

Evening Celebration in Omaha Hosted by Diane Hamsa and Megan Massey

MONDAY JULY 15

6th Annual Golf Tournament in Cleveland Ohio Hosted by Fragile X Alliance of Ohio

THURSDAY, OCTOBER 10

Anne Souder is kicking off a new chapter in Arizona, celebrating FRAXA's Fall Fling with a Wine Tasting Extravaganza. You can reach Anne at: 6111 E. Karen Drive, Scottsdale, AZ 85254. (480-483-6803) or by email at JAYSOUDER@msn.com

Debbie Stevenson's son, Taylor

FRAXA'S FALL FLING – First Annual Fling: October 2002

by Debbie Stevenson

What is it? An annual national fundraising day, to raise awareness for Fragile X and to raise much needed funds for FRAXA Research Foundation. The progress FRAXA-funded researchers are making right now is exciting, but research is expensive. In the past, most of the fundraising burden has fallen on just a few people. If everyone around the country pitches in just a little bit, WOW!

Who: We're looking for volunteers all over the country to participate in FRAXA's Fall Fling. Anyone and everyone who would be willing to do a fundraiser, no matter how small or large, is welcome. Every little bit counts.

When: Friday, October 5th (any date within a few days of October 5th is absolutely no problem)

Where: Wherever you live! We're hoping to eventually have an event in every state in the country, and more than one in each state is perfectly fine! Some day we hope FRAXA's Fall Fling will take place worldwide.

Why: First, to raise funds so that when new research proposals arrive on December 1st, FRAXA can fund every worthy project. Second, to get nation-wide publicity for Fragile X. Our goal is to make Fragile X known across the country. Reporters love the idea of families across the nation banding together for a single goal.

How: Here are some ideas–but remember, anything goes: Beef & Beer, Football Party, Golf outing, a brunch, lunch or dinner (large or small, in your home or not in your home), gala event, cocktail party, car wash,bowling, a walk or run, bake sale, collect a jar of coins, garage sale, bake sale, coin collections from local merchants or from fountains of local malls, letter writing campaign, benefit night at a local fast food restaurant, designate a Sunday to have your church or synagogue donate a percentage of the day's collections to FRAXA, having a local merchant donate a percentage of purchases to FRAXA.Some merchants will match funds raised. For example, WalMart stores will match the first $1000 you raise and provide volunteers to help with the event or with mailings. Target stores have a similar program.

We can provide you with a fundraising kit:

- *Invitations* – we will have invitations printed with blank space inside where you can fill in the details of your event – letterhead, brochures, and other materials.
- *FRAXA T-shirts or pins*
- *Ideas* – We know others who have run many kinds of events and we can help you brainstorm if you need ideas
- *Help* – We will help you with mailings, thank you letters, and whatever else you need to make your event a success!

Contact: If everyone pitches in – we'll cure Fragile X sooner than later. If you KNOW you would like to do a fundraiser, please let me know. Even if you're JUST THINKING about it, let me know that also. Just send me an email at dstevenson@pop.net with your phone number or give me a call at 212/828-1883.

continued from page 1

research

weakness of specific synapses appears to be very important in generating the "wiring" in the brain necessary for learning and higher intellectual and behavioral functions. Recent findings have identified specific neurochemical, structural and electrophysiological mechanisms at the synapse that are altered in the absence of FMRP. This new knowledge, coupled with new drug development in neuropharmacology, has allowed us to think about new medicines that might actually enhance cognitive functioning in individuals with Fragile X by targeting specific brain mechanisms deficient in the disorder.

In Fragile X syndrome, absence of FMRP leads to alteration in synaptic morphology (shape and structure) and strength and thus, deficient ability to maintain mature connections and eliminate unnecessary connections between brain cells. One specific abnormality of synaptic strength in the Fragile X mouse seems to be a decrease in AMPA-receptors in certain brain areas. Also, long-term depression (LTD), a process through which neurons regulate synaptic responsiveness and which is associated with down-regulation of AMPA receptors, is exaggerated in brain cells from the Fragile X mouse, implying that AMPA receptor activity is deficient.

AMPA receptors mediate the level of excitability of brain cells at synapses. Maintaining the proper level of excitability or "strength" of these connections is important for normal learning to occur and for promoting the structural changes in brain connections which represent the "hard wiring" of the brain's network.AMPA receptor activity is increased or decreased when synapses are activated and therefore represents one way brain cells can change the strength of selected connections, based on a specific experience.

Recently, a new class of medications which enhance AMPA receptor activity, called AMPAKINES, or "AMPA-receptor modulators," have been developed. One of these medications, developed at Cortex Pharmaceuticals and called CX516 or Ampalex®, has been found to improve learning and memory in rats and also has produced improvements in rat models of schizophrenia and ADHD. Clinical trials have now been done in patients with early Alzheimer's disease and schizophrenia, in which CX516 was helpful. CX516 has had minimal toxicity and is now in phase II studies in humans, as an investigational new drug. CX516 activates AMPA-receptors only after the synapse has been stimulated and therefore results in experience-dependent synaptic stengthening, in a manner that would parallel learning or memory. Given the early results with CX516 in other models, and the recent data suggesting deficiency of AMPA-receptor activity in Fragile X syndrome, we thought that CX516 might partially correct a defined neurochemical defect in Fragile X, with resultant improvement in cognitive and behavioral functioning.

Our project will investigate the effects of CX516 on memory, cognition and behavior in adults (>18 years) with Fragile X syndrome (FXS). Males and females are eligible but subjects must have an IQ in the subnormal range to participate. The study will involve a 4-week placebo-controlled, double-blind trial of CX516, with behavioral, intellectual, memory, attention and speech testing performed at baseline, at 4 weeks of treatment, and 2 weeks after discontinuance of treatment. The study will involve 50 subjects (25 CX516, 25 placebo) and will be conducted at RUSH-Presbyterian-St. Luke's Medical Center in Chicago. An additional group of 50 individuals with Fragile X and autism will participate in the study at the MIND Institute at the University of California, Davis. Although some of the study monitoring can be done by a local physician and much study screening can be done by phone, participation in the study will require a minimum of three visits to a study site (Chicago or Davis). The tests of thinking and behavioral functioning will be analyzed in the entire group, to see if the individuals who are treated with CX516 show improvements when compared to the control subjects who receive placebo pills, and whether the improvement declines two weeks after stopping CX516. Blood tests, EKGs and any possible side effects will be monitored so as to help show that CX516 is safe for individuals with Fragile X.

This treatment trial with CX516 represents the first ever attempt at treatment directly aimed at reversing the intellectual disability in Fragile X. The study is a first step in an effort to see if CX516 might be useful to enhance long-term thinking and learning skills in individuals with Fragile X syndrome.

At the time of this printing, final FDA approval for this study is still pending, so there is a possibility that it could be delayed.

update:

DFXR and synaptic tagging in drosophila

JERRY YIN, PHD
Cold Spring Harbor Lab.
$50,000

by Jerry Yin

My laboratory is interested in the molecular mechanisms of memory formation. We study the Drosophila (fruit fly) and mouse model systems. All animals can form memories that persist for various lengths of time. Long-term memories, which require new proteins to be made in brain cells, enlist cell-wide processes such as gene transcription and translation. While these processes occur in the cell body, most neuroscientists agree that the specificity of neuronal circuits requires changes in the individual synapses that define the circuit. Since mammalian nerve cells can contain thousands of synapses, how are individual synapses targeted when cell-wide changes such as gene expression occur? This problem is a central issue in the field of memory formation. We are trying to test whether FMRP is involved in the molecular mechanism of synaptic specificity. We will use genetic and biochemical approaches in fruit flies to address this problem.

Analysis of FMRP ribonucleoprotein complex association with the cytoskeleton through molecular motors

GARY BASSELL, PhD, Principal Investigator
JASON DICTENBERG, PhD, FRAXA Fellow
Albert Einstein School of Medicine, $65,000

by Jason Dictenberg

Neurons are highly polarized cells that are specialized to form interneuronal connections, or synapses, that facilitate cell-to-cell communication. During brain development, neurons utilize structural elements within the cell to form extensions, or processes, that specialize in forming these connections. These structural elements are known as the cytoskelton, which supports growth of the processes and is the "superhighway" for transport of proteins, or building blocks, into these developing processes. Process formation requires that many proteins be available in high concentrations at the sites of growth, and recent studies suggest that the cell accomplishes this efficiently by synthesizing the proteins at the very site of growth. This requires that the messages (mRNAs) that encode these proteins be localized to these sites, and it is the job of mRNA binding proteins to direct their transport there.

FMRP is an mRNA-binding protein that may be important for the transport of mRNAs and local synthesis of proteins required for growth of neuronal processes. We are interested in how FMRP is transported into these processes, which cytoskeletal elements are required and which molecular motors are involved in transporting FMRP. We will also study the active transport kinetics of particular mRNAs that FMRP binds to in normal neurons and those lacking FMRP, by tagging mRNA localization sequences with fluorescent reporters in live neurons. These approaches may reveal a greater understanding of the defects that underlie neuronal growth in Fragile X syndrome.

What are mRNAs?

mRNAs are the templates that cells use to transform genetic codes (genes) into proteins. From each gene, mRNA is made, and from mRNA, protein is made. The fragile X gene, FMR1, normally produces the protein, FMRP, but in fragile X syndrome, a mutation in this gene results in a lack of FMRP protein.

Defects in mRNA localization to growth cones, filopodia and spines assessed in hippocampal neurons from fmr1 knockout mice

GARY BASSELL, PhD, Principal Investigator
LAURA ANTAR, FRAXA Fellow
Albert Einstein College of Medicine, $30,000

by Gary Bassell

My laboratory has been interested in the role of mRNA binding proteins, including FMRP, in the regulation of neuronal process outgrowth and the establishment of proper connections (synapses) between axons and dendrites. The ability of these processes to grow is dependent on a specialized structure at the tips of processes, called the growth cone. We have shown that FMRP is localized to a network of actin filaments within growth cones. At a later stage of

continued on page 6

continued from page 5

neuronal development, FMRP is localized to dynamic actin structures, called dendritic filopodia, and spines, which are important for synapse formation and plasticity. Our objective is to investigate the regulation, mechanism and function of FMRP trafficking to these dynamic actin structures.

Recent advances in our laboratory have made it possible to see RNA binding proteins such as FMRP in live neurons by using fluorescent tags. Another new technology to study Fragile X is the use of fluorescent DNA probes for in situ hybridization and multi-color fluorescence microscopy. This will enable us to visualize the spatial association of FMR protein with specific mRNAs; we can then evaluate whether the localization of mRNA to dynamic actin structures is impaired in cultured neurons from FMR1 knockout mice. These innovative approaches to Fragile X should offer new insights into the normal function of FMRP and the biological basis for Fragile X Syndrome.

Axonal Elimination and Synaptic Maturation in Development and Early Adulthood in the FMR1 Knockout Mouse

IMJOO RHYU, PhD & WILLIAM GREENOUGH, PhD

Univ. of Illinois $68,000

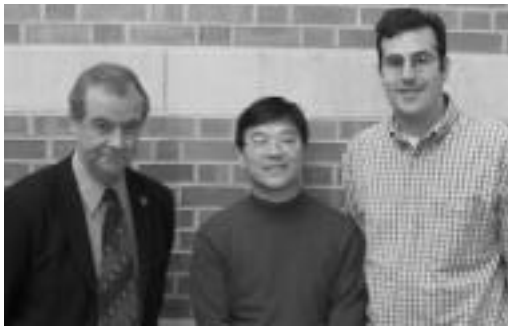
by William Greenough

There has been significant progress in understanding how nerve cell structure is changed in Fragile X syndrome (FXS). An important finding in the Fragile X affected human brain is the altered shape of dendritic spines, the projections from a neuron's dendrites that receive input from other neurons. Affected brains have more immature-appearing spines and fewer mature synapses, compared to unaffected brains. It is common during development of the brain and other parts of the body that structures created during development are removed as a part of the maturation process. In FXS, at least in the cerebral cortex, there are excess spines, and in some cases wrongly-directed dendrite branches, which are not removed during development. While one might first assume that "extra" synapses would be good things, the fact that their removal is a normal part of development suggests that they are not. In the case of FXS, extra dendrites and synapses might make the brain function in a more noisy and excitable manner.

Is this failure to mature without FMRP characteristic of all synapses? When do the unaffected and affected brains begin to differ from one another in development? Answers to these questions might provide clues to possible intervention points.

We are focusing on the dendritic branching pattern and spine shape, especially in the hippocampus, which has long been known to be associated with learning and memory.

researc



Bill Greenough, ImJoo Rhyu, and Aaron Grossman

This brain area has received very little attention in Fragile X research, despite its importance in additional cognitive processes such as attention. We will also pursue spine morphology of Purkinje cells in the adult cerebellum, a huge sensory-motor coordination center at the rear of the brain, as impairments in sensory and motor functioning are also common in FXS.

Developmental analyses of Fragile X knockout mice will be run in parallel using three morphological tools: high voltage electron microscopy (HVEM), three-dimensional reconstruction of spines using conventional electron microscopy (EM), and light microscopy (LM) for counting spine numbers. Using the three techniques in parallel will provide precise 3 dimensional spine data, refined detail regarding internal structure and location of "organelles" such as the polyribosomes that synthesize FMRP, and spine numbers and dendritic branching pattern measures.

This project is expected to provide precise information regarding the developmental course of hippocampal maturation in Fragile X syndrome. This information can be used in studies evaluating the effects of potential therapeutic intervention, ranging from behavioral treatment to gene therapy.

Alterations of GABA receptor subunit expression and the absence of FMRP in the Fragile X mouse

CARL DOBKIN, PhD

NY State Inst. for Basic Research, $15,000

by Carl Dobkin

This project will investigate the alteration in the expression of the major inhibitory system in the brain of the Fragile X mouse. Receptors for gamma amino butyric

acid (GABA) normally serve to balance the excitatory input received by neurons; however, we have found that these receptors are reduced in many regions of the brain in at least one strain of Fragile X mouse (FVB/N). This may contribute to the seizure susceptibility and other behavioral features of the Fragile X mouse.

We will examine heterozygous female Fragile X mice (which have some brain cells with FMRP, and some without) to see if neurons with a non-functional Fragile X gene express GABA receptors at a lower level than neurons with a functional gene. By comparing neurons in the same brain, we will be able to tell whether the absence of the Fragile X protein within a neuron influences its GABA receptor expression.

Studies on FMR1 Gene Delivery using Viral Vectors

DAVID C. BLOOM, PhD

Univ. MiamI,$54,000

WILLIAM GREENOUGH, PhD

Univ. of Illinois, $21,000

by David Bloom

Fragile X knockout mice, which lack the ability to produce normal FMRP, show defects in their central nervous systems which may be similar to those present in the disease in humans. The goal of this project has been to develop a gene therapy approach to deliver a functional copy of the FMR1 gene into the brains of the Fragile X knockout mice and determine if this will repair the observed defects in their central nervous system.

Viruses that have been altered to make them safe (vectors) can be used as delivery systems for gene therapy. During the past two years we have been constructing two unique delivery systems to introduce the FMR1 gene into the brain. One system we are using is a vector based on the Herpes Simplex Virus (HSV) and the second is a vector based on Adeno Associated Virus (AAV). Each vector possess different properties, which will increase the likelihood of successfully delivering the FMR1 gene to as many brain cells as possible, as well as ultimately controlling how much FMRP is made. We have now built first versions of both these vectors, and initial tests in cultured cells show that they do produce FMRP. Tests will soon begin in the knockout mice to determine if delivering the FMRP can restore function in live animals.

This study will allow us to determine if delivering the FMR1 gene to the brain is a possible therapeutic approach for the treatment of Fragile X Syndrome. This study will also allow us to learn more about how the FMR protein works, which may lead to the development of other types of therapies.

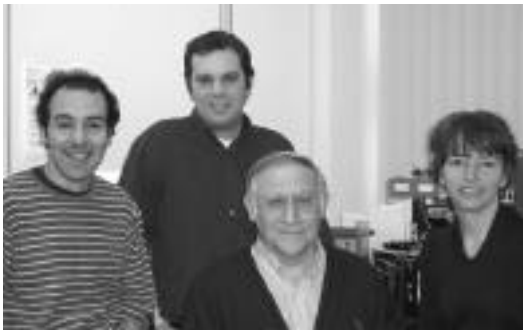
Xenopus (frog) model system for the study of Fragile X and related proteins

EDOUARD KHANDJIAN, PhD

Univ. of Quebec $24,000

by Edouard Khandjian

Animal models have been generated to study the normal functions of the FMRP protein, which is lacking in Fragile X syndrome. These models are either extremely complex---such as mouse, or very primitive---such as fish, worm and fly. Mammals have two other genes, FXR1 and FXR2, which are closely related to the Fragile X gene. Altogether, these three genes code for many different forms of proteins that have a complex pattern of expression in different tissues and organs. It is thought that the two related proteins FXR1P and FXR2P can partially compensate for the absence of FMRP, but this hypothesis needs further testing. In the more primitive fly model, these three different genes are all replaced by a single gene (dFXR), so differential tissue expression studies are not possible.



Edouard Khandjian and lab team members

We began to study an animal model that is genetically closer to man than to flies. This animal, the frog *Xenopus* laevis, contains all three genes, but the number of protein forms coded by these genes is much smaller than in mammals, which allows us to study

continued on page 9

FAMILY CORNER

In Memory of Jess Stringer

1976-2002

Jess, who had fragile X syndrome, was blessed with a warm and loving family. He died recently of a heart attack, at the age of 25. Contributions made in Jess's name are going directly to support research.

His mom, Judy, writes:

Jess, in his short life, touched more people than any of the rest of us. He made everyone smile, from the cashier at the 7-Eleven to the CEOs of huge companies; he loved everyone for themselves. He lived totally in the present. Jess loved trains, trucks, bridges, music, his pets, the waitresses at his favorite restaurant, skiing, hiking, riding his mountain bike, driving his scooter and truck. He had no driver's license, but that didn't stop him. He had over 5 acres in which to drive, run and be happy. He loved every human being and was always eager to say "hi" and "how are you."

All who knew Jess will be changed forever. He had a big smile for all of us, all the time. He would get excited and enjoy the smallest of things and have everyone in convulsive laughter many times a day. He taught us all the true meaning of loving life; we are all better people for knowing Jess.

Lorraine Morley wishes to thank the therapists of the Farmington Group for their donation to FRAXA. Lorraine writes "these are wonderful people who work with my 8 year old son, who has Fragile X."

Carol Collins wrote: Our church, Mary's Catholic Church in Sandusky, Ohio, donated part of their weekly collections to FRAXA. I'm excited that this worked out; if there is anyone out there who belongs to a church that tithes, it might be worth asking if they donate any part of the tithing to organizations.

FRAXA would like to thank the family and friends of Ricky Godorov, who chose to direct donations in her memory to FRAXA. Ricky had boundless energy; she was a loyal friend, trusted confidante, and loving mother of a daughter, Stacy Cohen, and two sons with Fragile X, Brad and Craig Godorov. She would have been thrilled to know that her memory will live on and continue to touch lives through the funds donated to FRAXA. Stacy writes, "Because of all of you, one day we will make a difference in the lives of people affected with Fragile X."

Thank you, FRAXA Volunteers!

Lynne Koltookian, thank you for giving up so many Thursdays to help in the office, and Joanie Stewart, thank you for wonderful public relations work.

Update from the National Fragile X Foundation

A Preliminary Program Agenda is now available for the 8th International Fragile X Conference, to be held this summer, July 17-21 in Chicago. Over 125 faculty members,including many of the scientists funded by FRAXA, will be presenting the latest in scientific,medical and clinical research. Other presenters will discuss the best ways to provide for your child's education, manage behavior, evaluate the efficacy of medications, and numerous other topics of high interest to families. As in prior conferences, each time block will offer a choice of scientific and family-focused sessions. The informative and stimulating presentations, the chance to meet hundreds of families and professionals, and the special events and activities will make this a memorable experience.(And once again, childcare for ages 2-16 will be free to those staying at the conference hotel.) To view the preliminary agenda and the registration form, visit www.FragileX.org and click on the red Chicago logo. Adjustments made to the session topics,days, times or presenters will be updated online on a regular basis and color-coded in such a way that viewers can easily plan their itinerary in advance.

After six months of work, a revised and updated edition of *Fragile X Syndrome,A Handbook For Families and Professionals*, is now available. Numerous Fragile X specialists contributed to the effort to make this an exceptionally useful and very understandable publication. The Handbook is provided at no cost to all who contact the NFXF, with additional copies available for a nominal fee. Please call, email or write us for your own free copy.

See you in Chicago! Robby Miller, Executive Director, The National Fragile X Foundation, PO Box 190488 / San Francisco, California 94119 / 1-800-688-8765 / NATLFX@FragileX.org

continued from page 7

protein expression in a simpler environment. In the frog, it is possible to directly investigate the functions of each protein, either during development or in the adult animal. This can be done by introducing different molecules into the egg through micro-injection, before or after fertilization, in order to induce a phenotype. Once this model has been developed, new avenues to study the functions of the Fragile X and related proteins in the nervous system will hopefully be unveiled.

A Genetic screen for dominant modifiers of drosphila FMR overexpression in the eye

KEVIN MOSES, PhD

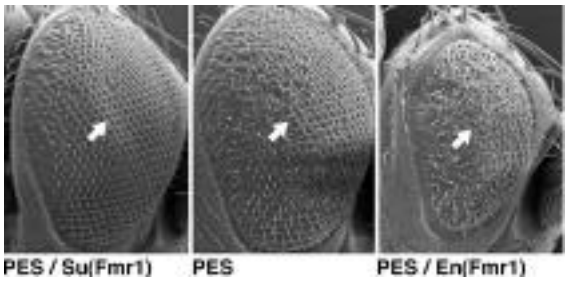
DANIELA ZARNESCU, PhD

Emory Univ., $35,000

by Daniela Zarnescu

We are employing the fruitfly, *Drosophila melanogaster*, as a model for discovery of genes in the Fragile X pathway. The past decade has seen a revolution in our understanding of the genetic homology (similarities) between flies and humans. Drosophila is an excellent system for gene discovery because of the economy of the system, the short generation time, and the available molecular and genetic technology.

The fly eye is comprised of about eight hundred facets arranged in such a precise order that it rivals a crystalline array. This precision in structure requires extreme precision in development: any additional or missing cells can be seen as external disruptions ("rough eye phenotypes"; see figure, middle panel). It is therefore easy to detect any mutation that even slightly disrupts essential cellular processes. The sensitivity to mutations of the Drosophila eye give us the means to conduct a genetic screen and identify genes that function in the Fragile X pathway.



Eye morphology of dominant modifiers of PES compared to the parent phenotype (see text). Note that the regularity of the facets is restored in the suppressor (left) and more disrupted in the enhancer (right).

We have obtained transgenic flies that overexpress the fly Fragile X gene in the eye (from our collaborator Tom Jongens; University of Pennsylvania). In these flies, eye development is disrupted and a rough eye phenotype can be easily observed (see figure, middle panel) – we call this the parent phenotype (PES). Then we generated flies that carry random mutations in addition to high levels of Fragile X protein in the eye. The principle of our quest is quite simple: if any of the random mutations affects a gene that functions in concert with the Fragile X gene, chances are that the parental rough eye phenotype will be either enhanced or suppressed, depending on the relationship between the two genes.

Recently, by generating mutations into virtually every gene in the fly, we were able to identify several genes which alter the expression of the Fragile X gene in the eye (see figure - PES/Su(Fmr1) and PES/En(Fmr1)). We are currently in the process of cloning these genes in the fly. We look forward to uncovering the relationship between these yet unknown genes and the Fragile X syndrome and hope to enhance our understanding of this disease and also of more general issues such as cognition and intelligence.

Additional grants awarded in the past few months will be described in our next newsletter. They include:

Patterns of Protein Expression in Fragile X Syndrome

WALTER KAUFMANN, PhD

Kennedy Krieger Inst.,$10,000 renewal

Towards Gene Therapy of Fragile X syndrome

MARIO RATTAZZI, MD

NY State Inst. For Basic Research, $40,000 renewal

Creation of a Model of Fragile X Syndrome: Condtional Expression of FMRP in Cells and Mice

DAVID NELSON, PhD

Baylor College of Medicine;$35,000

Generating Human Neurons carrying the Fragile X Mutation

CLIVE SVENDSEN, PhD

Waisman Center, University of Wisconsin;$50,000

continued from page 1

Currently, treating symptoms is the only option available, and the available interventions -- special education, drugs, psychological counseling -- often do not help enough. FRAXA's goal is to do more. We fund scientists who are pursuing each of these four approaches. Much of the work we fund is collaborative, as no single research team has the full range of technical skills to address all of the issues involved in developing treatments.

Therapeutic Approach #1: Fix the Gene

In people with Fragile X, the FMR1 gene is present but switched off (methylated) by a mutation in the DNA which controls activation of the gene. The gene itself is functional and could, theoretically, be switched on. One challenge is to target the FMR1 gene selectively, without turning on other genes that are supposed to remain off. It is also possible that too much Fragile X protein or expressing FMRP in the wrong cells may be toxic. So, FRAXA funds research to evaluate these approaches in animal models, including mice and fruit flies, and in tissue culture. Recent grants have been awarded to Andre Hoogeveen, Giovanni Neri, and Paul Hagerman.

Gene therapy aims to deliver a new, working copy of the Fragile X gene to brain cells so that the gene will function, producing its normal protein. Since all the symptoms of Fragile X arise from the lack of FMRP, this in principle could "cure" the syndrome. Gene therapy has received a lot of attention in the press because although it has great potential, there are no reports of clinical success with this technique in the human brain. FRAXA is funding investigators who are working out basic details using tissue cultured nerve cells before attempting anything with intact brains, including Mario Rattazzi, David Bloom, and Robert Bauchwitz.

Therapeutic Approach #2: Make and deliver the protein by some other means

Cells use a signaling pathway (or molecular cascade) to trigger the synthesis of FMRP. If we understood how FMRP is normally made and where it needs to be made, we might find a way to bypass complications in this pathway caused by the Fragile X mutation. Knowledge of this pathway could also provide alternatives to bring about the expression of other proteins regulated by FMRP. Many teams are currently exploring this area; those with some FRAXA support include Claudia Bagni, Gary Bassell, Carl Dobkin, Justin Fallon, Bill Greenough, Kimberly Huber, Walter Kaufmann, and Robert Malinow.

Toward Therap

Therapeutic Approach #3: Substitute for the function of the protein

If we knew precisely what the Fragile X protein does in nerve cells, we might find ways to bypass the need for it. We now know that FMRP aids in the production of certain proteins, some of which appear to be necessary for communication at the synapses – the connections between the brain's nerve cells. Messenger RNA is the intermediate step between a gene and the protein that it makes. An exciting current research direction is identifying the mRNAs that are overexpressed or underexpressed when FMRP is lacking. Since mRNAs code for proteins, this may tell us which proteins depend on FMRP for their normal expression in cells. Robert and Jennifer Darnell, Jean-Louis Mandel, and Kendall Broadie have all been funded first by FRAXA and currently by a joint NIH/FRAXA program for work in this area.

Therapeutic Approach #4: Treat Symptoms

How much can we modify the brain by behavioral therapy? Can the actual shape and size of nerve cell connections be reversed by behavioral intervention? Can behavioral symptoms be reversed by behavioral intervention? Can symptoms be reversed by pharmacological intervention? FRAXA awarded grants to Don Bailey, Elizabeth Dykens, Mina Johnson-Glenberg, John Larson and Kenneth Mack to conduct studies of the human symptoms of Fragile X, and to Linda Crnic, Rich Paylor, and Frank Kooy, to pinpoint symptoms in the Fragile X mouse model. If therapies and educational strategies can be designed with the specific needs of patients in mind, they are more likely to be effective. Likewise, the more we understand specific symptoms and their causes in Fragile X syndrome, the more likely it is that drugs can be selected, or discovered, that can target these symptoms. The new class of drugs called Ampakines, is an example: FRAXA has funded a trial of Ampakines in Fragile X mice and now will fund a trial of this medication in adults with Fragile X, under the direction of Elizabeth Berry-Kravis.

Teamwork and Future Prospects

Naturally, these four areas of research overlap and most

tic Treatments

teams work in several areas (although each investigator has been listed here only once). It is critical to develop models and tools to accelerate progress in all areas. Antibodies enable scientists to detect specific proteins within cells; Alan Tartakoff has engineered a set of Fragile X antibodies that he has made available to other researchers. Fruit fly models have emerged as a fruitful avenue of study for many genetic disorders; FRAXA is funding fly studies by Kendal Broadie, Kevin Moses, David Nelson, Tom Jongens, David Nelson, Lynn Regan, and Jerry Yin. A refined Fragile X mouse model is being developed by Nobel Laureate Eric Kandel; and Edouard Khandjian will study Fragile X in frogs.

Research Meetings

Since no one research facility has the time, funding, or personnel to do all the things necessary to develop effective treatments for Fragile X, coordinating and facilitating collaborative efforts among researchers is critical. Several recent research meetings have brought together small groups of leading scientists from around the world to brainstorm aggressively about the causes and symptoms of Fragile X and possible routes to a cure. While some of these researchers have worked on Fragile X for many years, others are new to this ever-growing field and provide a critical infusion of ideas which will make this field dynamic.

Workshop: Treatment Research Perspectives

In November, the National Institute of Mental Health (NIMH) held a workshop, Mental Health Aspects of Fragile X Syndrome: Treatment Research Perspectives. The inspiration for this meeting was provided by Dr. Steven Hyman, formerly Director of NIMH and now Provost of Harvard University and FRAXA's newest Scientific Advisor. The goal was to determine if there are specific mental health aspects of Fragile X which merit (and even require) targeted research approaches and treatments and, if so, to pinpoint what those are. A group of leading investigators was convened, resulting in creative and productive discussions.

A report on the meeting, including recommendations for future research directions, has been written by Dr. Linda Crnic and Dr. Bill Greenough, with input from other participants. It is available at the NIMH and FRAXA websites and printed copies are available from FRAXA.

We sincerely thank the organizers, Dr. Edgardo Menvielle and Dr. Benedetto Vitiello of NIMH, and the participants, Don Bailey, Mark Bear, Elizabeth Berry-Kravis, Paul Hagerman, Walter Kaufmann, MaryLou Oster-Granite, Jaswinder Ghuman, Richard Paylor, and Allan Reiss. Also participating from FRAXA were Katie Clapp, Michael Tranfaglia, and Debbie and Jeffrey Stevenson.

Fragile X Investigators' Meeting

In 2000, a five year, seven million dollar, research initiative was established by NICHD, FRAXA, and NIMH. Nine teams, currently funded under this program, are investigating topics ranging from math abilities of carrier women to fruit fly models of Fragile X. On

March 25-26th, these investigators and others funded by NICHD were invited to come together to present and discuss their latest findings. The discussions were a testament to the rapid progress being made in this field. After each presentation, other scientists in the room asked questions and shared their own perspectives. It was striking to see how very different lines of research are converging to confirm conclusions. We are grateful to Mary Lou Oster-Granite at NICHD for organizing this productive meeting.

Banbury Meetings

The third annual Banbury meeting on Fragile X is taking place as this newsletter goes to print. The Banbury Center at Cold Spring Harbor Laboratory in New York accommodates about 35 people, far away from city distractions; even phones are hard to find. This year's meeting is being organized by Robert Darnell, Steve Warren, and David Nelson, and will focus on RNA metabolism and Fragile X. Topics vary from year to year, depending on the hottest emerging research trends. Fragile X Banbury meetings are funded by the National Institute of Mental Health (NIMH) with additional funding from NICHD and FRAXA.

The Future

Clearly there is more to do, but we think the Fragile X field is on the right path!

FRAXA POSTDOCTORAL FELLOWSHIPS REQUEST FOR GRANT APPLICATIONS

Upcoming Deadlines: May 1, 2002 and December 1, 2002

FRAXA offers fellowships and grants to encourage research aimed at finding a specific treatment and ultimate cure for fragile X syndrome:

- Postdoctoral fellowships of up to $35,000 each per year
- Investigator-initiated grants for innovative pilot studies aimed at developing and characterizing new therapeutic approaches (no funding limit)

FRAXA is particularly interested in preclinical studies of potential pharmacological and genetic treatments for fragile X and studies aimed at understanding the function of the FMR1 gene. Applications are accepted twice each year. Information is available at www.fraxa.org or by contacting FRAXA.

FRAXA UPDATE

EDITOR: Katherine Clapp, M.S.
CONTRIBUTORS: Elizabeth Berry-Kravis, MD, PhD
David and Mary Beth Busby
Jen Nardo
Debbie Stevenson
Michael Tranfaglia, MD
Judy Stringer
and many others
DESIGN: Mary Lou Supple

This newsletter is published regularly and sent to all supporters of FRAXA Research Foundation. Permission is granted to reproduce and distribute this newsletter for non-commercial purposes.

FRAXA would like to thank Networx of Newburyport, MA for hosting, at no charge, the FRAXA website and email.

Delaware Benefit

Delaware's First Annual Fragile X Benefit was held on Saturday, February 9th in Hockessin. More than 75 people attended, some from as far away as Fairfax, Virgina, and Hamden, Connecticut. A disc jockey, courtesy of The Stone Balloon nightclub in Newark, Delaware, kept everyone dancing all night! Area businesses donated food, beverages, and items for the silent auction.

The auction was a huge success. Bidding wars throughout the night added a lot of excitement. There were original works of art, with the featured painting by Donna Crescenzo-Nardo, "Jake's Flight". This piece centers on a butterfly with bright colors exploding under it as it soars upward. The painting was named after Nardo's nephew, Jake, whose mother, Jennifer, coordinated the event.

After all was counted, the benefit raised over $6,600 as well as a higher level of understanding of Fragile X. Plans are already in the work for next year's event!

PLEASE HELP FRAXA

in supporting research aimed at treatment for fragile X

FRAXA is a national 501(c)(3) tax-exempt organization. Every penny you donate goes to research: FRAXA has specific grants to cover all overhead. Supporters receive this newsletter and are welcome to participate as active volunteers.

Yes, I would like to help FRAXA

- ☐ Member ($25+)
- ☐ Donor ($50+)
- ☐ Sponsor ($100+)
- ☐ Benefactor ($500+)
- ☐ Research Underwriter ($1000+)
- ☐ Named Research Fund ($5000+)
- ☐ Named Research Chair ($25,000+)

FRAXA

NONPROFIT ORG.
U.S. POSTAGE
PAID
W. NEWBURY, MA
PERMIT NO. 59