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JAX Scientist and Mice on Forefront of SMA Research

Increasing Production of Key SMN Protein is Therapeutic

Listen to an interview with Drs. MacKenzie and Farooq

www.jax.org/jaxmice/multimedia/podcasts/20110805-MacKenzie.mp3

Spinal muscular atrophy (SMA) is an autosomal recessive disease and the leading genetic cause of infant and toddler death worldwide. It is characterized by the loss of motor neurons in the spinal cord, leading to the inability to control voluntary muscle movements. Affected children are weak, cry feebly, and have trouble swallowing, sucking, and breathing. There is no cure, but two recent studies have shown that either restoring or increasing the production of the spinal motor

neuron (SMN) protein markedly alleviates or even reverses SMA in mice, offering hope to SMA-affected children and their parents.

SMA

SMA is caused by the absence of the survival motor neuron 1 (*SMN1*) gene on chromosome 5. The gene's encoded protein, SMN, is evolutionarily conserved, ubiquitously expressed, and essential for localizing and processing subcellular RNA. Due to an evolutionarily recent duplication, humans have two nearly identical copies of the *SMN* gene – *SMN1* and *SMN2*. Although people with SMA have no *SMN1* gene, they have at least one copy (sometimes several copies) of the *SMN2* gene. Generally, the more *SMN2* copies they have, the less severe is their SMA. Based on disease severity

and age of onset, SMA is subdivided into three types: type 1 (severe), type 2 (intermediate), and type 3 (mild). The absence of both the *SMN1* and *SMN2* genes is embryonically lethal. Although there is no effective therapy for SMA, scientists have speculated that increasing SMN protein production from one or more *SMN2* gene copies might compensate for the absence of the *SMN1* gene and mitigate, prevent, or even cure SMA. Indeed, *Smn1*-deficient mice that are transgenic for eight copies of the uniquely human *SMN2* gene do not develop SMA.

“Milk” hormone alleviates SMA in mice

Several molecules had been known to induce SMN2 production in mouse and human cell cultures, putatively via STAT5 signaling, but these molecules can't pass the blood-brain barrier. In contrast, the hormone prolactin, a known STAT5 inducer, can pass the barrier, and its receptors are present in motor neurons. Researchers led by Dr. Alex MacKenzie from the University of Ottawa, Canada, and Faraz Farooq from the Universidad de Granada, Granada, Spain, hypothesized that prolactin could be used to induce SMN2 production and mitigate SMA severity. The MacKenzie/Farooq team tested their hypothesis in a series of experiments in cell cultures and in SMA mouse model FVB.Cg-Tg(SMN2*delta7)4299Ahmb Tg(SMN2)89Ahmb *Smn1*^{tm1Msd/J} (SMAΔ7, 005025) (Farooq et al. 2011).

By administering prolactin to NT2 and/or MN-1 cell cultures, the MacKenzie/Farooq team demonstrated that prolactin increases SMN production, verified that it both increases STAT5 levels and activates the STAT5 pathway, and showed that it stimulates SMN production via STAT5 signaling.

In vivo experiments substantiated prolactin's role in increasing SMN levels. The MacKenzie/Farooq team demonstrated that prolactin dose-dependently stimulates



A healthy FVB/NJ mouse (001800), the background strain for the SMAΔ7 mouse. The SMAΔ7 mouse exhibits a molecular and progressive neurodegenerative phenotype similar to type 2 SMA. It is underweight, has a short, thickened tail, atrophied muscles, subcutaneous edema, degenerated or lost spinal cord motor neurons, and lives only for about 13-14 days. The severity of its phenotype correlates strongly with the estimated copy number of the transgene.

SMN production in the brain and spinal cord of CD-1 mice, significantly increases SMN2-derived full-length SMN transcripts and protein levels in the brain and spinal cord of SMAΔ7 mice, significantly increases SMN protein levels in motor neurons of the brain and spinal cord and in endothelial cells of SMAΔ7 mice, mitigates weight loss, improves motor function, significantly extends the lifespan of SMAΔ7 mice (21 vs 14 days in non-treated controls), and results in significantly higher SMN protein levels at the time of death in the brain, spinal cord, and muscle of SMAΔ7 mice compared to controls.

In summary, the MacKenzie/Farooq team demonstrated that prolactin increases SMN protein production via STAT5 signaling, has significant therapeutic effects in an SMA mouse model, and is a potentially promising treatment for human SMA.

Restoring SMN production reverses SMA in mice

Although several researchers had examined strategies for treating SMA by increasing SMN production, none had examined the disease stages at which such a therapy would be most beneficial. To do this, a research team led by Drs. Cathleen Lutz of The Jackson Laboratory, Bar Harbor, Maine, and Umrao Monani of the Columbia University Medical Center, New York, constructed a unique SMA mouse model. The model contains an inversion of the mouse *Smn1* gene – hereafter called the “rescue allele” – that can be irreversibly flipped by orally administering tamoxifen to the mouse. Like many SMA mouse models, this mouse shows signs of weakness and lives for an average of only 17 days. By turning on the rescue allele in this mouse at successive SMA stages, the Lutz/Monani team modeled the putative effects of administering SMN therapy to people in progressive stages of SMA (Lutz et al. 2011).

The Lutz/Monani team found that if the rescue allele's expression is induced at post-natal day 4 (P4 – when SMA onset is readily apparent), the mice almost fully recover: By P17, they can right themselves better and are significantly larger than mice in which the rescue allele is not activated, express SMN levels that are 60-70% that of wild-type mice, and have considerably improved neuromuscular junctions. Approximately 75% of them survive to P28, and approximately 50% survive to at least P300. In contrast, controls – either mice in which the rescue allele is not induced or SMAΔ7 mice – do not live beyond P19. By P50-70, except for being smaller than normal, the survivors are indistinguishable from wild-type controls, do not exhibit any vascular necrosis of the extremities, perform as well as wild-type controls in grip strength, rotarod, and open field assays, exhibit no spinal motor neuron loss, and have normal muscle morphology and neuromuscular junctions.

The later the rescue allele's expression is induced, the less therapeutic are the effects: by P10, inducing SMN expression

has no benefit. Although similar amounts of SMA protein are produced whether the rescue allele is activated at P4, P6, P8 or P10, the SMA symptoms are ameliorated at the earlier ages only, highlighting the importance of early intervention.

In summary, the Lutz/Monani team demonstrated that restoring SMN production can post-symptomatically reverse SMA in mice. Restoring SMN production in later disease stages is also therapeutic but not nearly as much as when restored early in the disease process. The team's results corroborate previous observations that severe SMN deficiency does not irreversibly damage motor neurons until later SMA stages. Most importantly, they suggest a possible therapy for treating human SMA, even after symptoms become apparent.

The findings by both the MacKenzie/Farooq and the Lutz/Monani teams are encouraging signs that, finally, therapies for SMA are in sight.



Dr. Cathleen Lutz is the Associate Director of The Jackson Laboratory's Mouse Repository.

Jackson Laboratory Resources for SMA Research

- JAX® Mice Models of SMA
(www.jax.org/jaxmice/research/neurobiology/spinalmuscularatrophy)
- JAX® Compound Evaluation Services
(www.jax.org/jaxservices/compound-evaluation)
- JAX® Pathology Service
(www.jax.org/jaxservices/pathology)

References

Farooq F. 2011. et al. *J Clin Invest* 121:3042-50. PMID: 21785216.
Lutz CM. 2011. et al. *J Clin Invest* 121:3029-41. PMID: 21785219.

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www.jax.org/jaxmice/news/2011/SMA

JAX Scientists Publish Guide for Finding Mutant Mice and ES Cell Lines

How to Find Mutant Mice and ES Cell Lines

The number of mutant mice and ES cell lines available today is dizzying. Finding the ones you want can be slow and frustrating. Drs. Martin Ringwald and Janan Eppig of The Jackson Laboratory have written an excellent guide to make your search less time-consuming and more successful (Ringwald and Eppig 2011). Their guide focuses on three databases: the International Knockout Mouse Consortium Database (www.knockoutmouse.org), the Mouse Genome Informatics Database (www.informatics.jax.org), and the Cre-Portal Database (www.creportal.org). Not only can these three databases help you find what mutant mice and ES cells are available, they correlate mouse phenotypes with human disease. Below is a sampling of some of the tasks the three databases can simplify:

- Finding mutant ES cells or mice for a gene of interest
- Determining what mutations a gene of interest harbors
- Obtaining specific mutant ES cells or mice
- Finding promoter-specific recombinase driver lines
- Finding tissue-specific recombinase driver lines
- Finding mouse models for a given human disease

The three databases that Ringwald and Eppig focus on are highly curated and cross-linked and provide links to other data sources. They contain the most extensive information, the most recent developments, and the greatest promise for understanding the genetic basis of human health and disease. All three contain up-to-date online help and FAQ pages. The 15-20 minutes you spend reading this guide will be time well spent.

Reference

Ringwald M, Eppig JT. 2011. *Methods* 53:405-10. PMID: 21185380.

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www.jax.org/jaxmice/news/2011/Finding_Mice_and_ES_Cells

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Podcast: Listen to an interview with Dr. Lieberman
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www.jax.org/jaxmice/news/2011/CD4_AsiCs

JAX Launches "Mouse Clique" Life Science Blog

Published online June 8, 2011
www.jax.org/jaxmice/news/2011/Mouse_Clique

Deficiency for Interleukin 15 Receptor Produces Super Athletic Mice

Published online August 24, 2011
www.jax.org/jaxmice/news/2011/Stamina

Caveolin 1 Plays a Role in Age-related Neurodegeneration

Podcast: Listen to an interview with Dr. Head
Published online July 27, 2011
www.jax.org/jaxmice/news/2011/Caveolin_1

New Insights Into Immunological Perfect Storms

Podcast: Listen to an interview with Dr. Berens
Published online July 12, 2011
www.jax.org/jaxmice/news/2011/Cytokine_Storms

Novel Tuberculosis Vaccine Works in Mice

Published online September 30, 2011
www.jax.org/jaxmice/news/2011/TB

The Genetic Sequence Variations of 17 Common Laboratory Mouse Strains Revealed

Published online September 26, 2011
www.jax.org/jaxmice/news/2011/Sanger_Sequence

Endo/lysosomes are Achilles' Heel to Ebola Virus

Published online September 19, 2011
www.jax.org/jaxmice/news/2011/Ebola

JAX® Services

JAX® Cryorecovery is Quick and Economical

Are you trying to obtain a mouse strain you've read about in a publication? Check the JAX® Mice Database first. If the strain is among the more than 5,000 JAX® Mice strains we distribute, we can deliver the mice you need quickly and economically – even if the strain is cryopreserved.

We can quickly produce live mice from cryopreserved stocks

Typically, our Cryorecovery Service (www.jax.org/jaxservices/cryopreservation/recovery) can recover a

cryopreserved line and ship live mice to you within 13-16 weeks (sometimes less) of receiving your order. Although we guarantee a minimum of two pair of mice, we frequently provide 10 or more mice. If you need a larger cohort to more quickly expand your colony, simply double or triple your order.

To maximize your value, consider having our Breeding Service (www.jax.org/jaxservices/breeding/services) establish a dedicated colony of the recovered strain and provide mice to you as you need them.

Obtaining mice from colleagues may have hidden costs

By obtaining mice directly from colleagues, you may inadvertently be adding a burden to their research, teaching, and other obligations inherent in running a research program. Though they may take on the responsibility, they may not be able to supply you with the number of mice you need when you need them. Additionally, the mice they send you will likely need to be quarantined before they enter your mouse room. Because JAX[®] Mice are specified opportunist and pathogen free (SOPF), we are an approved NIH vendor and can bypass quarantine and deliver mice directly to your mouse rooms, saving you substantial time and money.

Researchers want us to distribute their strains

Researchers have donated nearly 2,000 mouse strains to us in the last four years. Why? It's a hassle-free, quick, and economical way for investigators to make their strains readily available to you. We take care of all the details associated with distributing the mice – including publication of a Strain Data Sheet, nomenclature, genotyping, curation, ensuring health status, and genetic quality control – while investigators focus on their research (see the online article “Turn Your Mice Into Gold” at www.jax.org/jaxmice/news/2011/Rumplestiltskin). We also screen the mice for contaminating alleles (such as residual Cre or FLP1 transgenes) that may have been introduced early in a strain's development.

So, if you're wondering where to find the unique mouse strain you need, check the JAX[®] Mice Database first. Or, pick up

the phone and call The Jackson Laboratory at 1-800-422-6423. Live or cryopreserved, we can provide the mice you need when you need them.

The Jackson Laboratory on Ice

The Jackson Laboratory is a pioneer in cryopreservation technology and its applications to mouse-based biomedical research. See the following:

- JAX[®] Cryopreservation and Recovery Services (www.jax.org/jaxservices/cryopreservation)
- Cryo Cost Savings Calculator (www.jax.org/jaxservices/cryopreservation/calculator)
- “Turn Your Mice Into Gold” (www.jax.org/jaxmice/news/2011/Rumplestiltskin)
- “Mouse Cryopreservation Gaining in Popularity” (www.jax.org/jaxmice/jaxnotes/515/515f)
- “JAX Unveils new ‘Do-it-yourself’ Sperm Cryo Kit” (www.jax.org/jaxmice/jaxnotes/513/513g)
- “Cryopreservation and Recovery: Critical Components in Disaster Preparedness for Mouse-based Research” (www.jax.org/jaxmice/jaxnotes/511/511a)

Questions? Contact us at 1-800-422-6423 (US, Canada & Puerto Rico) or 1-207-288-5845 (from any location).

Published online July 11, 2011
www.jax.org/jaxmice/news/2011/Promoting_Cryo_Mice

JAX[®] Mice News

Newly Available JAX[®] Mice Strains

Below is a partial list of newly available JAX[®] Mice strains. For a complete list, see www.jax.org/jaxmice/newstrains.

To search for a mouse model from over 5,000 JAX[®] Mice strains, see www.jax.org/jaxmice. To order, contact Customer Service at orderquest@jax.org, 1-800-422-6423, or 1-207-288-5845.

B6.129S-Atoh1^{tm4.1Hzo}/J

013593

This knockin strain contains an enhanced green fluorescent protein fused to the 3' end of the *Atoh1* gene.

STOCK Cck^{tm1.1(cre)Zjh}/J

012706

This Cck-IRES-Cre knockin allele in this strain harbors an internal ribosome entry site and Cre recombinase in the 3' UTR of the cholecystokinin (*Cck*) locus. As such, Cre recombinase expression is directed to *Cck*-expressing cells. The donating investigator reports that Cre recombinase is active in *Cck* positive neurons.

B6;129S-Gt(ROSA)26Sor^{tm32(CAG-COP4*H134R/EYFP)Hze}/J **012569**

This strain conditionally expresses an improved channelrhodopsin-2/EYFP fusion protein (ChR2(H134R)-EYFP) from the endogenous *Gt(ROSA)26Sor* locus. Expression is enhanced by the presence of a CAG promoter. Following Cre-mediated removal of the floxed STOP cassette, this strain can be used in optogenetic studies for rapid *in vivo* activation of excitable cells by illumination with blue light (450-490 nm).

B6.Cg-Tg(Fos/EGFP)1-3Brth/J **014135**

This *Fos/EGFP* transgenic strain expresses a fusion gene consisting of the murine FBJ osteosarcoma (*Fos*) oncogene and enhanced green fluorescence protein (EGFP). It may be used to visualize *Fos* expression in stimulated neurons and to study changes in neuron excitability and synaptic efficacy.

B6.129S7-Sox9^{tm2Crm}/J **013106**

This strain carries a floxed allele of *Sox9* and may be used to produce conditional mutants for studying cell fate, including endochondral bone formation, limb development and patterning, joint formation, and hair and stem cell differentiation.

129-Wls^{tm1.1Lan}/J **012888**

This *Wls* floxed mutant strain possesses *loxP* sites before the ATG start site in the 5' untranslated region of exon 1 and upstream of exon 2 of the *wntless* homolog (*Drosophila*) (*Wls*) gene. It may be used to study *Wnt* signaling in any organ or tissue that can be targeted with Cre recombinase.

JAX Research News

JAX Cre Strains More Comprehensively Characterized

New Slide Scanner Also Added

Planning a new experiment involving Cre-lox mouse strains? Our Cre Repository makes over 275 unique Cre-expressing mouse strains (www.jax.org/jaxmice/list/xprs_creRT.html#xprs1801) available to the scientific community. We regularly receive new strains from donating scientists and the NIH Neuroscience Blueprint Cre Driver Network (www.credrivermice.org). Recently the Repository significantly expanded the characterization data (cre.jax.org/data) available for some strains and added a new slide scanner so you can better visualize Cre expression in various tissues.

Although Cre-expressing strains imported into the Repository are well characterized for the Cre-expressing tissues of interest, many express Cre in other tissues at various developmental stages. Therefore, we are using a new characterization pipeline to more comprehensively characterize Cre expression in subsets of these strains. Recently, we put the following 15 strains through the pipeline: *Lyz2-cre*, *Alb-cre*, *Camk2a-cre*, *Cd19-cre*, *Cdh5-cre*, *Chat-cre*, *Emx1-cre*, *Gfap-cre*, *Grik4-cre*, *Krt14-cre*, *Mnx1-cre*, *Nr5a1-cre*, *Omp-cre*, *Sim1-cre*, and *Osr2-cre* (see characterization data at cre.jax.org/data). Using anatomical terms congruent with the mouse anatomical dictionary, the Cre activity in these strains is now annotated and

extensively characterized for four developmental stages (E10.5, E15.5, P7, and P56) in 12 organ systems and 83 tissue types. See a sample page at cre.jax.org/Emx1/Emx1_creNano, showing characterization data for *Emx1-cre* mice.

In addition to adding new characterization data, the Cre Repository recently implemented the Hamamatsu NanoZoomer 2.0-HT slide scanner, a virtual microscope that allows you to visualize tissue-specific Cre-expression online at 1.25x to 20x magnification. See a sample scan (go to the link on the online article at: www.jax.org/jaxmice/news/2011/Cre_Repository_Expands), showing β -galactosidase labeling indicative of Cre recombinase expression in adult ovaries of *Nr5a1-cre* mice. (choose "Sign in as Guest" option)

Check the Cre Repository site (<http://cre.jax.org/data>) frequently, as new Cre expression data are added monthly.

To donate Cre expressing or other genetically modified mouse models to the Jackson Laboratory Repository, see www.jax.org/grc.

If you can't find the Cre strain you're looking for, contact our Technical Information Scientists at www.jax.org/jaxmice/micetech.

Published online June 24, 2011
www.jax.org/jaxmice/news/2011/Cre_Repository_Expands

Vitamin D, a Monkey Wrench in the MS Biochemical Machinery

Vitamin D Inhibits IL17 Transcription

Listen to an interview with Dr. Christakos (10:17)

www.jax.org/jaxmice/multimedia/podcasts/20110831-Christakos.mp3

Multiple sclerosis (MS) is the most common cause of acquired disability in adults. It typically affects people between the ages of 20 and 50, but it can strike at any age. It affects two to three times as many women as men and is more common among people with northern European ancestry. Approximately 400,000 Americans and as many as 2.5 million people worldwide have MS (National Multiple Sclerosis Society, www.nationalmssociety.org). There is no cure for MS. Although interleukin 17 (IL17) mediates and vitamin D mitigates its pathogenesis, the biochemical mechanisms by which they do so were relatively unknown - until recently. A research team led by co-principal investigators Dr. Sylvia Christakos at New Jersey Medical School and Dr. Lawrence Steinman at Stanford University has provided new insight on how vitamin D interferes with the complex MS biochemical pathways (Joshi et al. 2011). As a result of their findings, novel MS therapies may be on the horizon.

Interleukin 17 and vitamin D

IL17 is an inflammatory cytokine produced by several kinds of T cells, including a recently identified CD4⁺ T cell subset called Th17 cells. IL17 is implicated in numerous autoimmune diseases, including MS, which is modeled in mice as experimental autoimmune encephalomyelitis (EAE). In contrast, vitamin D, more commonly known for its role in maintaining calcium and phosphate homeostasis, down-regulates autoimmunity and offers some protection against both MS and EAE. To determine the action mechanisms of both IL17 and vitamin D in the context of MS and EAE, the Christakos-Steinman team conducted a series of experiments using human and mouse cells (some of them genetically altered) and several mouse models of MS.

Experimental autoimmune encephalomyelitis

Laboratory mice are not susceptible to MS. However, a disorder similar to MS, EAE, can be induced in mice via injections of myelin peptides, such as myelin basic protein, proteolipid protein, or myelin oligodendrocyte glycoprotein, in complete Freund's adjuvant. Among the several EAE-susceptible mouse strains are C57BL/6J (B6J, 000664) and SJL/J (SJL, 000686).

Vitamin D mitigates EAE by down-regulating IL17A expression

To determine vitamin D's effects on MS and EAE, the Christakos-Steinman team conducted several *in vitro* and *in vivo* experiments. They found that the active form of vitamin D – 1,25(OH)₂D₃ – down-regulates the expression of IL17A in cultures of CD4⁺ T cells isolated from healthy human donors and in cultures of various splenocyte and lymph node T cell subsets isolated from naïve “myelin oligodendrocyte glycoprotein (MOG) T cell receptor transgenic” (2D2) mice. The active form of the vitamin significantly reduces IL17A expression in cultures of splenocytes and draining lymph node cells isolated from EAE-induced SJL mice seven days after EAE is induced (before clinical symptoms appear).

Substantiating the findings of previous researchers, the Christakos-Steinman team found that administering the active form of vitamin D to EAE-induced SJL mice on the day of EAE induction and every other day thereafter markedly mitigates disease progression. Administering the active form of vitamin D to SJL mice paralyzed by EAE or to B6J mice with ongoing EAE slows disease progression and reverses paralysis and EAE symptoms, especially if the active form of the vitamin is administered repeatedly during the course of the disease. Eighteen days after EAE induction, IL17A expression from the spinal cord and brain CD4⁺ mononuclear cells of the diseased SJL and B6J mice are down-regulated, indicating that the improvement of EAE symptoms is associated with reduced IL17A expression. B6J mice inoculated with Th17 cells differentiated from CD4⁺ cells isolated from EAE-induced 2D2 mice and cultured with the active form of vitamin D develop a milder EAE, have fewer IL17A-expressing cells, and express less splenic and central nervous system IL17A than do B6J mice inoculated with Th17 cells cultured without the active form of vitamin D.

These findings indicated that the active form of vitamin D mitigates EAE by down-regulating IL17A expression.

Vitamin D mitigates EAE by repressing IL17A transcription

To determine the biochemical pathways through which the active form of vitamin D down-regulates IL17A expression, the Christakos-Steinman team conducted a series of cell culture experiments involving several kinds of genetically engineered cells. They identified several molecules that play key roles in vitamin D-mediated IL17A down-regulation:

- The ability of the active form of vitamin D to inhibit human IL17A expression is considerably less if the vitamin D receptor's (VDR's) IL17A promoter binding domain is absent.
- The “nuclear factor for activated T” cells (NFAT), an essential regulator of T cell-mediated IL17A transcription, dose dependently mediates IL17A's down-regulation by the active form of vitamin D.

- A complex interaction between the retinoid X receptor (RXR), vitamin D receptor (VDR), and other co-regulatory proteins enhances vitamin D repression of IL17A transcription.
- At least in part, the active form of vitamin D down-regulates IL17A expression by recruiting histone deacetylase (HDAC) to the IL17A promoter.
- The active form of vitamin D decreases the EAE-induced recruitment of “runt-related transcription factor 1” (RUNX1) (an IL17A transcription regulator) to its binding sites on the IL17A promoter.
- The active form of vitamin D significantly increases the number of immunosuppressive FOXP3⁺ regulatory T (T_{reg}) cells in the spleen, brain, and spinal cord of EAE-induced B6J mice. The IL17-repression domain in the *Foxp3* gene is highly conserved in mice and humans. FOXP3 inhibits RUNX1 and NFAT, which in turn results in vitamin D repression of IL17A transcription.

In summary, the Christakos-Steinman team is the first to identify the molecular mechanisms by which vitamin D down-regulates autoimmune phenotypes characteristic of MS and EAE. They demonstrated that the active form of vitamin D represses IL17A transcription by blocking NFAT, recruiting HDAC, complexing with the VDR and sequestering RUNX1, and inducing FOXP3. Their work provides a much needed framework for initiating clinical trials to test the efficacy of vitamin D or its analogs in fighting MS and other autoimmune diseases.

Resources for MS Research

- Selected EAE-susceptible JAX® Mice
 - B10.RIII-*H2^r H2-T18^b*/(71NS)SnJ (000457)
 - BALB/cByJ (001026)
 - C57BL/6J (000664)
 - SJL/J (000686)
 - SWR/J (000689)
- Compound Evaluation Services for multiple sclerosis (www.jax.org/jaxservices/invivo/ms)
- Mouse models and services for immunology research (www.jax.org/jaxmice/research/immunology)
- Pathways to Discovery: Autoimmune Diseases (www.jax.org/jaxmice/pathways/autoimmune)
- Pathology Services (www.jax.org/jaxservices/pathology)

Reference

Joshi S. 2011. et al. *Mol Cell Biol* 31:3653-69. PMID: 21746882.

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www.jax.org/jaxmice/news/2011/MS_vitD

Fragile X – a Faulty Molecular Braking System

Missing Protein Leads to Gene Over-expression

Fragile X is the most common cause of genetically-inherited mental disorders – even more common than Down syndrome. Its symptoms range from mild learning disabilities to severe mental retardation and include autistic behaviors, seizures, and physical abnormalities (The Fragile X Foundation, www.fragilex.org). Although scientists knew that fragile X is due to mutations in the fragile X mental retardation 1 (FMR1) gene, which encodes the fragile X mental retardation protein (FMRP), FMRP's role was a mystery. That mystery is slowly being unraveled. A team of scientists led by Drs. Robert and Jennifer Darnell of the Rockefeller University, New York, recently reported that FMRP stalls translation, preventing the over-expression of proteins that appear to cause fragile X and other neurological disorders (Darnell et al. 2011). The team's research may lead to novel therapies for these disorders.

Before the Darnell team's findings, some broad mRNA translation-regulating mechanisms were known, but the specific proteins that regulate the translation of the transcripts involved in synaptic remodeling were not. Whatever those regulators were, their roles were likely pretty important because the synthesis of new neuronal proteins is critical to synaptic plasticity (Wikipedia, http://en.wikipedia.org/wiki/Synaptic_plasticity) – a phenomenon thought to play a key role in forming and maintaining memory. Because FMRP is expressed in neuronal cells and binds to mRNA that is being translated on polyribosomes, it was a likely candidate. Moreover, exogenous FMRP had been shown to repress the translation of a variety of mRNA transcripts *in vitro*.

To better understand FMRP's functions, its RNA targets needed to be identified. Previous attempts to identify them had met with limited success. The Darnell team was undaunted. They developed an ultraviolet radiation-mediated technique called “crosslinking IP” (CLIP), which, when combined with high-throughput sequencing (HITS-CLIP), can identify specific mRNA-protein interactions. They used this and other biochemical assays to compare mRNA-FMRP interactions in the brains of two *Fmr1*-deficient mouse models – the *Fmr1* knockout mouse FVB.129P2-*Fmr1*^{tm1Cgr/J} (004624) and an I304N point mutant knockin mouse B6.129-*Fmr1*^{tm1Rbd/J} (010504) – to mRNA-FMRP interactions in the brains of FVB/NJ (001800) and

C57BL/6J (000664) wild-type littermate controls. Following are their key findings:

- *In vivo*, FMRP binds to at least 842 neuronal mRNA target transcripts
- Those transcripts encode proteins from many gene families, most of which function in synaptic signaling
- A large percentage of both pre- and post-synaptic proteins are targets of FMRP regulation
- 28 of the 842 FMRP target transcripts are implicated in autism spectrum disorders
- Whereas ribosomes are stalled in an FMRP dependent manner in the brains of wild-type mice, they are not stalled in the brains of *Fmr1*- knockout mice
- FMRP appears to stall translation by physically associating and forming large complexes with polyribosomes and target mRNA transcripts

Significantly, the results obtained by the Darnell team are consistent in the two different fragile X mouse models – two different *Fmr1* mutations on two different genetic backgrounds – indicating that genetic background effects did not influence their results. The mRNAs that FMRP binds to indicate that it directly regulates translation of synaptic proteins and synaptic plasticity. The fact that many FMRP target transcripts are linked to autism spectrum disorders may explain why people with fragile X exhibit autistic behaviors. That FMRP binds to and stalls the translation of synaptic protein-encoding mRNA transcripts suggests that neurological disorders such as fragile X are caused by the over-expression of genes normally repressed by FMRP. This revelation may lead to the development of novel therapies for fragile X and related disorders.

*“It is significant that we used two independent fragile X mouse models to validate our findings. In addition to the *Fmr1* knockout, we used the I304N point mutant knockin mouse that we made (Zang et al. 2009) and bred into two genetic backgrounds (FVB and C57BL/6) to match the conventional *Fmr1* KO. I think the use of both models adds significance to the findings, and I’d love to see more people use multiple models, especially given the occasional problems inherent in genetically engineered models. For example, the conventional *Fmr1* KO still has the neo cassette, and there can be neighboring gene effects from genes originating from the 129 strain of ES cells still present near the engineered gene, even after multiple generations of backcrossing into C57 or FVB.”* Dr. Jennifer Darnell.

Jackson Laboratory Resources for Fragile X Research

- JAX® Mice Models for Fragile X (www.jax.org/jaxmice/research/neurobiology/fragile_x)
- JAX® Compound Evaluation Services (www.jax.org/jaxservices/compound-evaluation)
- JAX® Speed Congenic Service (www.jax.org/jaxservices/breeding/speed-congenic)

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www.jax.org/jaxmice/news/2011/Fragile_X

Your Lungs Are Listening to Your Ears

Researchers Substantiate Suspected New Inner Ear Function

Listen to an interview with Dr. Rubens

www.jax.org/jaxmice/multimedia/podcasts/20110714-Rubens.mp3



Inducing inner ear injury in the CBA/CaJ mouse severely limits its ability to adapt its breathing rate to changes in ambient CO₂ levels.

When we think of the inner ear, we think of its role in our sense of hearing and balance. However, within the last decade, numerous studies have produced compelling evidence that the inner ear has another important function – the regulation of our breathing rate. These studies have established that inner ear dysfunction correlates with low breathing rates and sudden infant death syndrome (SIDS). A new study led by Dr. Daniel Rubens of the Seattle Children’s Hospital, Seattle, Washington, further substantiates this correlation.

The study reports that mice with inner ear injuries do not adapt their breathing rate to changes in CO₂ levels and offers insights into the causes of SIDS and other diseases linked to inner ear dysfunction (Allen et al. 2011; USA Today 2011).

Dr. Rubens and his team undertook their study because people with inner ear diseases often suffer from “compensated respiratory acidosis,” a condition in which decreased respiration lowers blood pH (acidosis) and raises blood CO₂ (hypercapnia). Normally, hypercapnia induces a reflex – such as arousal or head turning during sleep – that increases breathing rate and access to oxygen. Evidence suggests that this reflex fails in SIDS. The Rubens team hypothesized that the inner ear helps regulate the respiratory response to CO₂ levels. To test this hypothesis, they studied the effects of induced inner ear injury on the respiratory response of CBA/CaJ (CBA, 000654) mice to abnormally high levels of CO₂. Because of their acute sense of hearing at younger ages, CBA mice are commonly used in hearing research.

To induce inner ear injury in young CBA mice, Rubens and his team injected gentamicin into their inner ears. This procedure is an established method for damaging inner ear cochlear and vestibular hair cells. One week later, they compared the respiratory response of these and control mice to air containing high levels of CO₂. They found that, compared to controls, mice with injured inner ears do not significantly increase their breathing rate.

The Rubens team’s study substantiates that, in addition to its role in hearing and balance, the inner ear regulates breathing rate. The study may lead to novel ways of treating respiratory irregularities in people with an inner ear dysfunction.

JAX® Timed Pregnant Mice

Because Dr. Rubens needed CBA/CaJ mice to be 17 days old on Mondays (when his team was available to perform the gentamicin injections), he ordered timed pregnant mice (www.jax.org/jaxmice/preconditioned/timedpregnant) from us. Although the standard service applies to only five strains, we can customize it (for minimum orders of 30 mice) to apply to many more strains. For more information, contact JAX® Services at 1-800-422-6423 (US, Canada & Puerto Rico) or 1-207-288-5845 (from any location).

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Allen T, et al. 2011. *Neuroscience* 175:262-72. PMID: 21130842.

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Caveolin 1 Plays Role in Age-related Neurodegeneration

Caveolin 1-deficient Mouse May be Novel Model of Alzheimer’s

Listen to an interview with Dr. Brian Head

www.jax.org/jaxmice/multimedia/podcasts/20110721-Head.mp3

How healthy are your brain’s synapses – those inter-neuronal junctions that allow electrical and chemical signals to be transmitted from one neuron to another? Evidence indicates that they degrade with age and make us more susceptible to neurodegenerative diseases, such as Alzheimer’s and Parkinson’s. Understanding why this happens could help scientists develop therapies to prevent, stop, or even reverse it. A research team led by Drs. Brian Head and Hemal Patel at the University of California San Diego (UCSD), La Jolla, California and VA San Diego, has shown that, in mice, one of the reasons for age-related synapse degradation is the age-related decline in the expression of the cholesterol-binding protein called caveolin 1 (CAV1) (Head et al. 2010).

Membrane/Lipid Rafts, Cholesterol, and Caveolin 1

To develop, stabilize and function properly, synapses depend on neuronal plasma membrane units called membrane/lipid rafts (MLRs). One of the major components of MLRs is cholesterol, which, along with other lipids, is essential for effective neurotransmission. In MLRs, cholesterol binds to CAV1, which organizes synaptic components of the neurotransmitter and neurotrophic receptor signaling pathways. MLRs and cholesterol are known to mitigate the damage caused by the amyloid-beta (AB) toxicity in Alzheimer’s disease, and age-related defects in the biosynthesis, transport, or uptake of cholesterol are thought to mediate neurodegeneration.

Age-related Caveolin 1 Loss is Tied to Age-related Synapse Loss

The UCSD team wanted to know if age-related synapse loss is due to age-related CAV1 loss. To determine this, they compared the levels of CAV1 and synaptic signaling molecules in MLRs, the number of synapses, and the degree of neurodegeneration in the brains of young, middle age, and old C57BL/6J (B6J, 000664) controls and *Cav1*-deficient B6.Cg-*Cav1*^{tm1Mls}/J mice (007083, available at The Jackson Laboratory), which develop Alzheimer’s-like pathologies. They found the following:

- The amount of synaptic signaling molecules and CAV1 in the hippocampal MLRs and synaptosomes of B6J mice decreases with age

- This decrease is more severe in *Cav1*-deficient mice
- The brains of old *Cav1*-deficient mice have significantly fewer synapses than either young *Cav1*-deficient mice or B6J controls
- Young *Cav1*-deficient mice show signs of premature neuronal aging and degeneration
- Young *Cav1*-deficient mice are more susceptible to cerebral ischemia-reperfusion injury than are young B6J mice
- Young *Cav1*-deficient mice have higher levels of A β , P-Tau, and astrogliosis, and a lower cerebrovascular volume than young B6J controls
- Neuron-targeted re-expression of CAV1 in *Cav1*-deficient neurons *in vitro* decreases A β expression

These results demonstrated that the loss of caveolin 1 accelerates neurodegeneration and aging in mice.

This is the first study to demonstrate that, in mice, the cholesterol-binding CAV1 protein in MLRs and the synaptic signaling molecules in the hippocampus form complexes that facilitate neurite outgrowth and axonal branching and guidance, form and stabilize synapses, protect neurons against ischemic injury, and possibly mitigate the A β toxicity associated with Alzheimer's. They show that these CAV1/MLR complexes are disrupted with age. They also demonstrate that *Cav1*-deficient

mice develop an Alzheimer-like neuropathology (A β production, elevated astrogliosis, hippocampal neuronal loss, and reduced cerebrovasculature), making them an Alzheimer's mouse model that doesn't harbor any of the three commonly known Alzheimer-associated gene mutations – amyloid precursor protein (APP), presenilin 1 (PSEN1), and presenilin 2 (PSEN2). The findings by Head and Petal may help researchers develop therapies for preserving neuronal function or repair neuronal damage due to injury or neurodegenerative diseases such as Alzheimer's.

Aged JAX® Mice

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Reference

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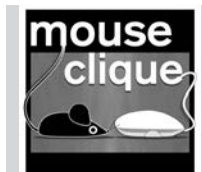
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