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Meeting report: 11th International Mouse Genome Conference

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"And a mouse is miracle enough to stagger sextillions of infidels." So penned the American poet Walt Whitman a century ago in his epic collection *Leaves of Grass*. Perhaps not quite a sextillion, but closer to 300 scientists (both infidels and the faithful) converged upon St. Petersburg Beach, Florida, to attend the Eleventh International Mouse Genome Conference (12–16 October, 1997). And stagger they did, at the prospect and advances made in mouse genomics. If the future is function, then chemical mutagenesis is the path. Stock tip: invest in a company that sells the point mutagen ethylnitrosourea (ENU).

The 3-day conference was inaugurated with the first Verne Chapman Memorial Lecture, a series the organizers hope to continue in future conferences. Verne M. Chapman (1938–1995) was an internationally recognized leader in mouse genetics at Roswell Park Cancer Institute (Buffalo, N.Y.). He joined the laboratory in 1972, and 10 years later was appointed chairman of the Department of Molecular and Cellular Biology. He also served as the Associate Institute Director of Scientific Affairs (1985–1989). Chapman's research legacy is vast and includes, among other things, pioneering work in constructing genetic and physical mouse maps, using ENU mutagenesis to produce models of muscular dystrophy, contributing to the field of X-Chr inactivation, and resolving molecular lesions associated with cancer. He also co-founded the International Mammalian Genome Society and was instrumental in organizing the International Mouse Chromosome Committee in 1990. However, he is best remembered for his valuable assistance and generosity in sending mice from his extensive colony to other investigators around the world.

Delivering the inaugural address, National Institutes of Health (NIH) Director and Nobel laureate Harold Varmus praised Chapman for his altruism when it came to sharing mouse reagents and ideas, a welcomed relief from today's climate of technology transfer agreements and intellectual property rights. Varmus also assured the audience that the NIH is "acutely aware of how mouse science is changing science in general" by taking a whole-animal approach, and that the costs of such laboratories are being "appreciated" by those that control the flow and amount of research dollars. Towards this whole-animal approach, he reported on progress in mouse models of cancer generated with avian viral vectors that introduce suspected oncogenes into single cells of mice engineered to be susceptible to infection. Specifically, he is recapitulating glioma brain tumors by expressing the viral receptor *tva* from a glial cell-specific promoter in transgenic mice. The newborn pups are then surgically infected with avian leukemia virus expressing any gene of interest (such as b-FGF or VEGF) to test for oncogenesis. This elegant approach reflects a more realistic version of glioma models, since cancers usually develop from *single* cells gone astray, as opposed to *all* cells in a mouse being genetically altered (as results in germline transgenesis).

Throughout the meeting, the rhetorical question was asked, "Do we need more mouse mutants?" The answer, apparently, is yes. Most human diseases, especially complex traits, are probably not due to null mutations in genes but rather more subtle lesions. For this reason, chemical mutagenesis screens using the point mutagen ENU are making a huge comeback to produce better mouse models (Davis and Justice 1998). "Give me an assay, and I'll give you a gene," quipped Martin Hrabe de Angelis (GSF/Institute for Mammalian Genetics, Neuherberg, Germany), re-iterating Rudi Balling's famous boast from two years earlier. And assays they'll get. The GSF Research Center (Neuherberg) and the Gene Center of Ludwig Maximilian Univ. (Munich) are collaborating on a large-scale ENU mutagenesis project to screen 5000 F_1 mice (in a dominant hunt) and 5000 F_3 mice (a recessive hunt) to find new mutations that more accurately reflect human syndromes. This strategy represents a new wave of phenotype-driven screens that will try to narrow the "phenotype gap" (Brown and Peters 1996). In these screens, mice are examined more thoroughly than what many human patients experience with their own doctor, including complete serum and blood analysis, FACS, and ELISAs to hopefully identify new mutants in hematology and immunology. Not to be outdone, the Medical Research Council (MRC) in Harwell, England (Jo Peters) plans to examine 40,000 ENU-induced F_1 mice over the next 3 years with their SHIRPA protocol, an integrated three-step assessment for defects in muscle and lower motor neuron, spinocerebellar, sensory, neuropsychiatric, and autonomic functions (Rogers et al. 1997). A nice twist on this scheme is that 10% of the mice will be retained for one year to re-test for late-onset phenotypes. Oak Ridge National Laboratory (Monica Justice) is also weighing in with an ENU "screenotype" for behavioral and clinical mutants; they are also producing a bio-monitor chip implant that can record a mouse's heart rate, blood pressure, and body temperature for rapid physiological screening: Mighty Mouse meets the Bionic Man. Case Western Reserve (Joe Nadeau) is putting together a broad-based ENU project to identify, among other things, risk factors in complex traits, such as cardiovascular disease, schizophrenia, depression, addictive behavior, reproductive disorders, and metabolic diseases. And ENU is also big in Japan, with Yoshihide Hayashizake (RIKEN) reporting on their mutagenesis efforts. Within the next few years we should hopefully have a new wave of mouse mutants and see how successful these strategies are in producing better models of human diseases.

Don't think, however, that only large-scale laboratories are exploiting the power of ENU in mice. The chemical can also be effectively used in small genetic screens for developmental mutants. Andrew Peterson (Duke Univ.) examined E10.5 mouse embryos from only 65 mutagenized gametes and found four new mutations that affect forebrain development. Now he is testing the gene expression of prospective telencephalon markers (such as BF-1) in these mutants to determine at what developmental stage the defect is occurring.

New ENU-generated point mutations will also extend the al-

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lelic series of already known genes. Jack Favor (Neuherberg) is continuing his impressive screen for eye and lens phenotypes (at last count: 171 collected from examining 520,497 mice) and in the process is generating numerous alleles of *Pax6* that will characterize the functional regions of this developmentally important gene. Rudi Balling (GSF-Research Center, Neuherberg) wishes he had as many alleles for *Pax9,* a gene strongly expressed in the developing pharyngeal pouches. Because none previously existed, he went in and disrupted the locus by gene targeting to produce a mouse that dies immediately after birth with a cleft palate and is missing a thymus, parathyroid, and ultimobranchial bodies. The mice also lack teeth, with molar development arrested at the bud/cup transition stage.

Several strains of mice already mimic human diseases; one good example is hearing impairment (Steel 1995). This phenotype has been especially easy to identify in mice, since it usually involves circling or waltzing behavior. Over the years numerous deaf mutants have been collected, studied, and are now rapidly being cloned: bronx waltzer (M. Cheong, MRC-Institute of Hearing Research), deafwaddler (V. Street, Univ. of Washington), modifier of deafwaddler (K. Noben-Trauth, Jackson Laboratory), headbopper (D. Hughes, MRC-IHR), pirouette (D. Kohrman, Univ. of Michigan), shaker-2 (S. Camper, Univ. of Michigan), waltzer (E. Bryda, Marshall Univ.), and whirler (P. Mburu, MRC-Harwell). Karen Steel (MRC-IHR) demonstrated a 'click box,' a simple device that produces a defined pulse to directly assay for hearing and startle responses independent of circling traits, which should greatly increase the number of deaf mutants collected in mutagenesis screens. The shaker-1 mouse, caused by mutations in the myosin VIIA gene, is an interesting model for four different human deafness traits (Steve Brown, MRC-Harwell). While the seven alleles of shaker-1 produce a uniform phenotype in mice, mutations in the human population show wide phenotypic ranges including syndromic, non-syndromic, and progressive loss-of-hearing, suggesting that genetic background may strongly influence this gene (Liu et al. 1997). Breeding shaker-1 onto different mouse strains may lead to interesting insights of genetic interaction and better models for human deafness. Karen Avraham (Tel Aviv Univ., Israel) is using scanning electron microscopy to follow the developmental defects caused by a mutation in myosin VI, responsible for another deaf mutant, Snell's waltzer. Her photographs dramatically visualized the stereocilia on hair cells rapidly degenerating and fusing into large cilia by postnatal day 20.

Deafness, however, is not the only disease of interest for mouse geneticists. Stacie Loftus (NIH) reported on the tri-allelic *npc* mouse which has altered intracellular cholesterol homeostasis resembling Niemann-Pick Type C disease. Using an integrated human–mouse positional candidate approach, a gene has been found (*Npc-1*) that is disrupted in the mutant mice by a retrotransposon-like element. Camilynn Brannan (Univ. of Florida) is delineating the imprinting centers (IC) thought to act as regulatory elements for Angelman syndrome (AS) and Prader-Willi syndrome (PWS). Using gene targeting to generate specific deletion sets at the hypothesized IC, she is producing mouse models that more accurately recapitulate human PWS. And if all of this is making you feel isolated or anti-social, check out the status of your dishevelled genes. Nardos Lijam (NIH) created individual disruptions for each of the three murine homologues (*Dvl1, 2,* and *3*) and found that, although mutants were viable and fertile, they have reduced social interactions, providing models for some human psychiatric disorders like autism and schizophrenia (Lijam et al. 1997). When *Dvl* double mutants are made by breeding together individual mutants, defects in neural tube closure are seen, emphasizing additional developmental roles for these genes.

Deletion stocks are also making a comeback in mouse genetics, especially since site-directed, nested deletions can now be rapidly engineered in ES cells (Ramírez-Solis et al. 1995; You et al. 1997). Maja Bucan (Univ. of Pennsylvania) in collaboration with John Schimenti (Jackson Laboratory) reported the generation and use of overlapping sets of deletions to genetically dissect the extensive Rump-white (*Rw*) inverted region on Chr 5. While *Rw* heterozygotes develop white fur on their lower body parts, homozygotes die in utero. This lethal phenotype may be due to a disruption of a dipeptidyl aminopeptidase-like protein (Andreas Lengeling, Univ. of Pennsylvania). Deletion stocks will also prove invaluable in mapping genes, modifiers, and all of these soon-to-be anticipated ENU mutants. One of the most exciting aspects will be integrating ENU mutagenesis with deletion stocks to saturate defined regions with a point-mutational analysis for fine structure-function description. This will inevitably be one of the hottest technologies for mouse centers focusing on "functional genomics" at the start of the next millennium.

Aravinda Chakravarti (Case Western Reserve) discussed Hirschsprung disease as a paradigm for complex trait analysis, whereby no one specific mouse mutant may faithfully recapitulate the human condition because the disease probably requires the accumulation of many "small phenotypic effects" in interacting biochemical pathways that may be common in the human population. While mapping such modifiers (or susceptibility alleles) may be "easy," finding the genes themselves could be tortuous. Nonetheless, quantitative trait loci (QTL) analysis is still pinpointing—more or less—chromosomal sites influencing various phenotypes, including autoimmune lupus nephritis (Ed Wakeland, Univ. of Florida), epilepsy (Wayne Frankel, Jackson Laboratory), a sex-specific autosomal factor contributing to obesity (Ben Taylor, Jackson Laboratory), and variations in brain weights (Robert Williams, Univ. of Tennessee). Amy Beebe (DNAX Research Institute) reported crossing *Leishmania*-resistant mice to *Leishmania*-sensitive BALB/c for five generations, selecting for resistence each time. At N4, 22 resistant animals were genome scanned, and those markers that were retained were then tested in the subsequent N5 generation of 139 mice to rapidly identify six candidate QTL. From the N5 generation, rapid congenics can be derived to verify resistancy.

Obviously, congenic mouse strains are a powerful genetic tool for analyzing complex disease traits, and the technique of 'speed congenics' will only heighten their use (Markel et al. 1997). Using such mice, several teams localized modifying QTLs affecting a variety of traits, including polycystic kidney disease (D. Beier, Harvard; L. Guay-Woodford, Univ. of Alabama), susceptibility to Theiler's virus (F. Bihl, Institut Pasteur), plasmacytomagenesis (B. Mock, NIH), and diabetes (E. Melanitou, Institut Pasteur; S. Wakana, National Institutes of Genetics, Japan). Angabin Matin (Case Western Reserve) used a special type of a 129/Sv congenic mouse (properly called a consomic) which had its chromosome 19 replaced by the one from MOLF/Ei to localize factors influencing the incidence of testicular tumors. An entire set of such consomics—where each mouse contains a different individual chromosome replaced by one from an unrelated strain—may prove valuable as a new genetic reagent. Other traits being analyzed by standard interbreeding and genome scanning include pneumococcal infection (E. Hopes, MRC-Harwell), resistance to *Salmonella* (G. Sebastiani, McGill Univ.), energy expenditure (D. Moody, Univ. of Nebraska), and suppressor genes for mammary tumor metastasis (K. Hunter, Fox Chase Cancer Center, PA) and thymic lymphoma (R. Kominami, Niigata Univ., Japan). By intercrossing susceptible A/J mice with resistant B6 animals, Anny Fortin (McGill Univ.) has identified a locus on Chr 8 that influences susceptibility to malaria, the endemic global—and often underreported—disease that kills two million people each year. The genetic interval of susceptibility contains some interesting candidate genes coding for erythrocyte proteins, interleukin-15, and a scavenger receptor expressed on phagocytes (Fortin et al. 1997).

As QTL analysis zeroes in on genes of interest, positional cloners are exploiting interspecific crosses, YACs, BACs, and PACs to clone out classical mouse mutations. It is rewarding to see

so many of these becoming resolved at the molecular level and is a scientific tribute to the early pioneers of the field who maintained and perpetuated these strains for decades. Liping Huang (Univ. of California, San Francisco) has cloned the mutation responsible for *lm* (lethal milk). The gene *Znt4* encodes a predicted six transmembrane domain protein that is abundantly expressed in breast tissue, is homologous to other genes controlling zinc-sequestration, and the wild-type copy can confer zinc-resistance to a zinc-sensitive yeast strain, all consistent with the *lm* phenotype of milk from mutant females being lethal to their nursing pups owing to zinc deficiency (Huang and Gitschier 1997). Stargazer, a spontaneous mutation that results in long seizures, is apparently due to an early transposon insertion into a novel gene that surprisingly does not appear to be related to any known ion channel protein (Verity Letts, Jackson Laboratory). Two coat color mutations, mocha (Margit Burmeister, Univ. of Michigan) and pearl (Lijun Feng, Roswell Park Cancer Institute) turn out to be defects in the genes for the delta and beta subunits, respectively, of the AP-3 heterotetrameric adaptor complex involved in membrane trafficking and protein sorting at the *trans*-Golgi network. In addition to producing diluted coat colors (from disrupting the melanosomes), mocha and pearl result in platelet storage pool defects that mimic aspects of Hermansky-Pudlak syndrome. A new allele of pearl, called *rim2* (T. Sagai, National Institutes of Genetics, Japan), should help confirm the molecular lesion. Aamir Zuberi (Jackson Laboratory) has positionally cloned the minor histocompatibility antigen *H-3a* (Roopenian and Davis 1989). The large gene responsible for eliciting a strong CTL response during tissue rejection contains three zinc-finger motifs but has no significant homology to any other known gene.

Other mutants being physically mapped, some with candidate genes under analysis, include: *amn* (amnionless, a transgene insertion that disrupts gastrulation; E. Lacy, Sloan-Kettering Institute), *bl* and *my* (blebbed and myelencephalic blebs; E. Bently, Univ. College London), *bt* (belted; D. Foernzler, Harvard), *dan* (digitation anormale, a transgene insertion that produces short, fused digits on all four limbs; D. Simon-Chazottes, Institut Pasteur), *Ds* (Disorganization; N. Abbadi, Case Western Reserve), *du* (ducky; J. Barclay, Univ. College London), *Er* (repeated epilation; R. Liddell, Thomas Jefferson Univ.), *gm* (gunmetal; C. Detter, Univ. of Florida), *ky* (kyphoscoliosis; S. Brown, MRC), *Loa* (Legs at odd angles, a new mutation that induces progressive motor neuron degeneration; E. Fisher, Imperial College, London), *Lp* (Loop-tail; D.A. Underhill, McGill Univ.), *mnd2* (motor neuron degeneration-2; W. Jang, Univ. of Michigan), *mu* and *sdy* (muted and sandy; V. Mishra, Univ. of Florida), *sf* (scurfy; M. Brunkow, Darwin Molecular Corp.), *Ter* (Teratoma; A. Matin, Case Western Reserve), and *sgl* (scraggly, a new mutation that causes hair loss; B. Herron, David Axelrod Institute, Albany, NY). Three additional mutants that were all transgene-induced (but have not yet been named) result in phenotypes of male-specific postnatal lethality (A. Buchberg, Jefferson Medical College, Philadelphia, PA), cranial developmental defects reminiscent of the Twirler mutation (J. Jones, Univ. of Michigan), and an induced deletion that removes the promoter region of two collagen genes, recapitulating Alport syndrome (W. Lu, Univ. of Michigan). To aid positional cloning projects in identifying coding regions, Gail Herman (Ohio State Univ.) and Paul Denny (MRC-Harwell) demonstrated the power of large-scale sequence comparison between mouse and human DNA to pull out new genes, especially when applied to the disease gene-rich region of the X-Chr. With such enthusiasm and technical progress, it should not be much longer before many more of the classical mutants are resolved.

The future is function, so goes the mantra (Fields 1997), and yeast geneticists are skillfully applying chip technology to lead the way. For now, we can only marvel at the oligo arrays designed by photolithography that can screen for fluctuations in genome expression of yeast cells grown under various conditions or decipher the expression discrepancies between a wild-type cell and a virulent strain (Elizabeth Winzeler, Stanford). Such analysis is attempting to be mimicked in the mouse by RT-PCR to assay the expression profiles of over 500 genes from 46 individual mouse tissues (Alistair Dixon, Sanger Center, UK). Steve Kingsmore (CuraGen, New Haven, CT) compared profiles from two different rat strains—one normal and the other prone to strokes—to detect subtle differences in heart tissue gene expression and identified an atrial natriuretic factor (*ANF*) that maps to a major stroke influencing locus on rat Chr 5. Not only is the expression pattern of *ANF* different in these two strains, but sequence analysis also shows a substitution in a highly conserved amino acid.

Table 1. It's a whole new world-wide-web for mouse genomics.

Sites (http://)	Database
www.informatics.jax.org	Jackson Laboratory
www.gsf.de/isg/ENU.index.html	GSF Research Center ENU program
www.mgu.har.mrc.ac.uk/handbook/handbook.html	MRC SHIRPA protocol
www.hgmp.mrc.ac.uk/MBx/MBxPhysicalChrom XStatus.html	X-Chromosome physical map
www.hgmp.mrc.ac.uk/MBx/MBxHomepage.html	European Collaborative Interspecific Mouse Backcross
ratmap.gen.gu.se	Rat map
bacpac.med.buffalo.edu	C57Bl/6 BAC library
mcbio.med.buffalo.edu/mmQT.html	Map Manager QT
www.ncbi.nlm.nih.gov/Omim/Homology/	Human–mouse homology map
www-genome.wi.mit.edu/cgi-bin/mouse/index	MIT/Whitehead physical maps

To keep all of this information manageable, The Jackson Laboratory (Janan Eppig, Joel Richardson, Lois Maltais, Lucy Rowe) is still providing some of the most comprehensive web sites for mouse genetics and informatics. Together with the MRC, they are compiling a "Universal Stocklist" for the web, hopefully by the end of January 1998. Until then, check out some of the other on-line services pertinent to mouse genomics mentioned at the conference (Table 1).

Thus, the future bodes well for mouse geneticists. Positional cloners are resolving the classical mutants, and we stand on the cusp of a new wave of mouse mutants to be generated by point mutagens, induced deletions, and QTL analysis. As these methodologies become easier and more commonplace, the next emphasis will be on detailed functional analysis of genes and their role in the biology of mammals. Collating molecular lesions with comprehensive phenotypic analysis and refining the technique of gene expression profiling will start to resolve mouse genetics into descriptive, dynamic, integrated systems that will play a pivotal role in advancing our knowledge of human biology and the diseased condition. And Whitman's poetic line will ring even truer.

This year's meeting was a success not only because of the quality of work presented, but also because of the location and organization. The Tradewinds Resort (St. Petersburg Beach, Fla) offered beautiful facilities, complete with a poolside bar (try the Rum Runners) next to the lounge chairs for watching dolphins frolick under spectacular sunsets. The meeting was coordinated by Ed Wakeland (Univ. of Florida) and Miriam Meisler (Univ. of Michigan) with the invaluable administrative assistance of Darla Miller and Nancy Holdsworth (Roswell Park Cancer Institute). Rudi Balling will host next year's Twelfth International Mouse Genome Conference from September 29 to October 3, 1998 in Garmisch-Partenkirchen, Bavaria, Germany. Pack your lederhosen and book your room and flight early because of the crowded Oktoberfest held at that time of the year. Membership in the International Mammalian Genome Society (IMGS), which sponsors these meetings, is open to all scientists. Special membership rates are available for conference registration and subscription to *Mammalian Genome*. To become a member, contact Darla Miller, IMGS Administrative Manager, Department of Molecular Biology,

Roswell Park Cancer Institute, Buffalo, New York 14263, USA (email: dmiller@mcbio.med.buffalo.edu).

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References

Brown SDM, Peters J (1996) Combining mutagenesis and genomics in the mouse—closing the phenotype gap. Trends Genet 12, 433–435

Davis AP, Justice MJ (1998) An Oak Ridge legacy: the specific locus test and its role in mouse mutagenesis. Genetics 148, 1–6

Fields S (1997) The future is function. Nat Genet 15, 325–327

Fortin A, Belouchi A, Tam MF, Cardon L, Skamene E, Stevenson MM, Gros P (1997) Genetic control of blood parasitaemia in mouse malaria maps to chromosome 8. Nat Genet 17, 382–383

Huang L, Gitschier J (1997) A novel gene involved in zinc transport is deficient in the lethal milk mouse. Nat Genet 17, 292–297

Lijam N, Paylor R, McDonald MP, Crawley JN, Deng C-X, Herrup K, Stevens KE, Maccaferri G, McBain CJ, Sussman DJ, Wynshaw-Boris A (1997) Social interaction and sensorimotor gating abnormalities in mice lacking *Dvl1*. Cell 90, 895–905

Liu X-Z, Walsh J, Tamagawa Y, Kitamure K, Nishizawa M, Steel KP, Brown SDM (1997) Autosomal dominant non-syndromic deafness caused by a mutation in the myosin VIIA gene. Nat Genet 17, 268–269

Markel P, Shu P, Ebeling C, Carlson GA, Nagle DL, Smutko J, Moore KJ (1997) Theoretical and empirical issues for marker-assisted breeding of congenic mouse strains. Nat Genet 17, 280–284

Ramírez-Solis R, Liu P, Bradley A (1995) Chromosome engineering in mice. Nature 378, 720–724

Rogers DC, Fisher EMC, Brown SDM, Peters J, Hunter AJ, Martin JE (1997) Behavioral and functional analysis of mouse phenotypes: SHIRPA, a proposed protocol for comprehensive phenotype assessment. Mamm Genome 8, 711–713

Roopenian DC, Davis AP (1989) Responses against antigens encoded by the H-3 histocompatibility locus: antigens stimulating class I MHC and class II MHC-restricted T cells are encoded by separate genes. Immunogenetics 30, 335–343

Steel KP (1995) Inherited hearing defects in mice. Annu Rev Genet 29, 675–701

You Y, Bergstrom R, Klemm M, Lederman B, Nelson H, Ticknor C, Jaenisch R, Schimenti J (1997) Chromosomal deletion complexes in mice by radiation of embryonic stem cells. Nat Genet 15, 285–288