Perspectives

Anecdotal, Historical and Critical Commentaries on Genetics

Edited by James F. Crow and William F. Dove

An Oak Ridge Legacy: The Specific Locus Test and Its Role in Mouse Mutagenesis

Allan Peter Davis and Monica J. Justice

Life Sciences Division, Oak Ridge National Laboratory, Oak Ridge, Tennessee 37831-8080

WITHIN the last two decades, mouse genetics has undergone a revolution, an event initiated by breakthroughs in molecular biology and tissue culture techniques. Previous to this explosion, most scientists were content to puzzle over the thousand or so spontaneous mutants, deletion stocks, and specially designed strains of mice that currently existed. This analysis provided a wealth of insight into developmental biology, immunology, and mammalian genetics in general; nonetheless, the nature of the mutation and the gene that was affected often remained unknown. Today, gene targeting is in vogue, with investigators rushing to make "knock-outs" (disrupted alleles) of every cloned gene. This technique allows researchers to focus on specific genes of interest and to work backward to a phenotype, an approach opposite to studying spontaneous mutants. Gene targeting is indisputably a valuable tool for initiating a mutational analysis in the mouse (Capecchi 1989). The power of genetic functional analysis, however, lies in collecting an allelic series. A few rare examples do exist where gene targeting is used not to create a knock-out but rather a more subtle lesion, *e.g.*, Zeiher *et al.* 1995. For the most part, however, the field today does not often reflect that the early severe phenotype of a disrupted mutation can mask later functions and should only serve as a starting point—as opposed to an end-all—for gene analysis, especially when the allele results in embryonic lethality. Extrapolating from the rampant proliferation of gene targeting papers in the literature, one almost expects the 100,000 or so genes in the mouse to be mutated any day now. Ah, bliss: Every gene knocked-out and a phenotype ascribed to each mouse! In increasing numbers, however, neo-classical geneticists are stepping forward to ask everyone to rethink the analysis.

Corresponding author: Monica J. Justice, Life Sciences Division, Oak Ridge National Laboratory, P.O. Box 2009, Oak Ridge, TN 37831-8080.

Backstory: Last November represented the 50th anniversary of the arrival of mouse geneticist William (Bill) Lawson Russell to Oak Ridge, Tennessee. The move was coincident with the great fire in Bar Harbor, Maine, that destroyed most of The Jackson Laboratory, Russell's previous employer (E. S. Russell 1987). At the urging of Alexander Hollaender, Bill—and later his wife Liane (Lee) Brauch Russell, who was finishing her doctorate at the University of Chicago—joined the Biology Division at Oak Ridge National Laboratory to add to Hollaender's vision of determining the effect of radiation on genetic systems (von Borstel and Steinberg 1995). The research legacy of the Russells is vast but centers on the creation of a specially designed mouse strain called the *T* (test) stock that was used as a genetic screen for the mutagenic testing of radiation and chemicals.

The *T*-stock mouse is a unique genetic tool packed with seven recessive, viable mutations affecting easily recognizable traits. Six influence coat color: *a* (nonagouti, chromosome 2), *b* (brown, chromosome 4), c^{ch} (chinchilla at albino, chromosome 7), *d* (dilute, chromosome 9), *p* (pink-eyed dilution, chromosome 7), and *s* (piebald-spotting, chromosome 14); one controls ear morphology: *se* (short-ear, chromosome 9). Russell created the strain from a stock of NB mice that already harbored six of the recessive alleles. This strain, however, was so highly inbred that its fecundity and viability were dropping fast, and the line risked becoming extinct. To save the stock, Russell had to outcross NB to another mouse. With the recent burning of The Jackson Laboratory, however, mice were next to impossible to locate (E. S. Russell 1987). Any spare animals that existed were being shipped to Bar Harbor to reestablish the mouse colony. Persistent, Russell located a professional photographer in Florida who also dabbled as a "garage" mouse geneticist and breeder. He supplied Russell with animals that carried three of the six recessive alleles and also provided the *s* mutation.

Invigorated by this outcrossing, the seven-locus *T*-stock was created in April 1948 (Russell 1989). By packing all of these mutations into a single mouse, Russell built a valuable tool for simultaneously following the genetics of seven traits.

Russell's initial goal at Oak Ridge was not to determine whether radiation caused hereditary changes in mice because George Snell and others had shown a decade earlier that chromosomal changes induced by X rays had phenotypic consequences (reviewed in Russell 1954). Rather, he wanted to calculate a rate for heritable gene mutations induced by radiation in germ cells. By using the *T*-stock, he proposed to study a defined set of loci to see how often they mutated. His approach was simple: Wild-type males were divided into two groups, one set irradiated with various doses of X rays and the second set used as controls. All of these males were crossed to his *T*-stock females. Because of the recessive nature of the mutations, the progeny would appear wild type ($a/+$; $b/+$; $p\ c^{ch}/++$; $d\ se/++$; $s/+$). However, a mutation at any one of the specific loci would be immediately recognized in the progeny. This approach, called the specific locus test (SLT), allowed Russell to score the number of specific mutations and to calculate a radiation-induced mutation rate in mammals.

It is important to stress the significance of the *T*-stock mouse. The experiment could have taken several different approaches that did not employ a specially designed tester mouse. In fact, alternative ideas were suggested by H. J. Muller and Russell's thesis advisor Sewall Wright during a closed-door meeting in Hollaender's office (Russell 1989). Wright thought it would be more appropriate to measure the vital statistics, *e.g.*, weight, longevity, and fertility, of the offspring of irradiated males. Muller feared an SLT in mice would be too difficult to conduct and suggested examining recessive lethals over a larger segment of the genome. Russell argued that was disadvantageous because it would require three generations of breeding. The SLT with the *T*-stock mouse, Russell boasted, would allow an individual to rapidly score in the first generation 2000 loci per hour by focusing on the coat color and ear shape. More importantly, however, the SLT would provide better data on gene mutation rate for comparison with Drosophila. This was critical to him. The human risk-estimates of radiation at that time were almost exclusively based on fly studies, and Russell wanted to make as precise a correlation between Drosophila and mouse as possible. He reasoned that it would be too difficult to compare mutation rates for all dominant visibles in a fly with those in a mouse owing to the "the virtual impossibility of equating morphological and physiological levels of detectability in the two species" (Russell 1951). Instead, the SLT in mice would provide a specific mutation rate for defined loci, a feature especially important because no one had any idea how comparable the two genomes of a mouse and fly would be. Finally, the SLT allowed the capture and propagation of all of these new mutations in one generation, even those that were lethal when homozygous, allowing many different alleles to be acquired for each locus. By examining each allele individually and in comparison with others, Russell hoped to gain information on the nature of the mutations, initiating a detailed functional genetic analysis in mice.

Russell examined over 85,875 offspring (data rarely matched by today's mouse geneticists) for his first paper on the subject (Russell 1951). In the experiment, he collected 53 new alleles for the seven loci and two spontaneous mutations in the control group, allowing him to calculate a radiation-induced mutation rate per locus for mammals that was 10 times higher than that for Drosophila. In his results, however, Russell discovered a wide range in mutation yield among the seven loci and realized that the data could not be quantitatively extrapolated to the entire genome: Some genes just appeared to be more mutable than others. Because most of the seven loci in Russell's SLT were originally discovered by mouse fanciers and pet owners, they might have an intrinsically higher mutation rate, which would explain why they were easily isolated by amateur breeders (like the photographer in Florida). To test this, a team in Harwell, England, constructed a new *T*-stock, using different loci: *bp* (brachypodism), *fz* (fuzzy), *ln* (leaden), *pa* (pallid), and *pe* (pearl). None of these are known to have been originally collected by mouse fanciers, even though some of them produce coat colors similar to those in Russell's loci. This Harwell-test stock was subjected to the same X-ray treatments that Russell employed. While examining a smaller data set (about 26,000 offspring), the researchers calculated an averaged radiation-induced mutation rate that was four to five times lower than Russell's but still much higher than that of Drosophila (Lyon and Morris 1966). The value of the SLT approach, however, was not that it could extrapolate to whole-genome mutation rates but that it provided a rapid and defined assay to address the parameters affecting mutagenesis.

The Russells continued to use the *T*-stock in numerous applications to estimate the genetic hazards of radiation to humans. A seminal paper demonstrated that radiation-induced mutations were dependent on the dose rate, a result in stark contrast to Drosophila studies. In a Herculean task of raising over half a million mice in an SLT, the Russells found that animals exposed to a chronic dose of radiation produced markedly lower numbers of mutations than mice given the same radiation as an acute dose (Russell *et al.* 1958). To reconcile this effect with Drosophilia, they proposed that in mammals some mutations were "reparable" by an as yet unknown mechanism. Having earlier shown that mouse genes were over 10 times more mutable than those of Drosophila, the Russells had now demonstrated that "the genetic hazards at least under some

radiation conditions may not be as great as those estimated from the mutation rates obtained with acute radiation" (Russell *et al.* 1958).

Several hundred specific locus mutations were scored and collected in a few decades of radiation mutagenesis. Thanks to the foresight of the Russells, many of these were propagated and maintained for analysis. With dozens of independently induced alleles at each locus, Lee Russell conducted complementation tests that identified sets of overlapping, nested deletions (Russell 1971). This organized the alleles into complementation groups and localized functional units, pioneering a new mapping strategy in the mouse (reviewed in Rinchik and Russell 1990).

The SLT had other valuable spin-offs. First, radiation-induced translocations between autosomes and the *X*-chromosome were made visible when coat color markers showed variegated patterning owing to the influence of *X*-inactivation. Such mutants helped propel the single-active *X*-chromosome hypothesis (Lyon 1961; Russell 1961). Second, specific locus markers allowed Lee to develop the spot test, an assay that quickly scores somatic genetic events such as point mutations, deletions, recombination, and chromosome loss. In the spot test, embryos heterozygous for four of the coat color loci from the SLT were mutagenized and upon birth screened for somatic mutations at these loci by looking for spots of colored fur patches in the pups (Russell and Major 1957). Today, the spot test is still the only general primary *in vivo* screen for mitotic recombination in the mouse and is likely to be revived for today's interest in DNA repair and recombination. Finally, mosaics recovered from the SLT are providing surprising insights into the timing of spontaneous mutations that arise in the germline (Russell and Russell 1996). The analysis of such mice will be invaluable in understanding the basis of human mosaic disease syndromes (reviewed in Kent-First 1997).

Chemical mutagenesis: In addition to study of radiation, the SLT could also be used to assay for harmful effects caused by chemicals. It was already known that certain compounds injected into mice had genetic consequences, *e.g.*, Falconer *et al.* (1952), and concerns for human safety were raised by J. B. S. Haldane's "plea" to examine "the mutagenic effect of substances which are frequently added to human food as preservatives" (Haldane 1956). However, most of the early experiments testing a variety of compounds (from caffeine to diethyl sulfate) provided ineffective at inducing mutations in spermatogonia (reviewed in Ehling 1978). Procarbazine, a drug used in the treatment of Hodgkin's disease, was the most effective chemical found to cause any type of significant spermatogonial mutagenesis in an SLT, yet even this rate was still only one third of the maximum Russell attained with X rays (Ehling and Neuhauser 1979). It was almost beginning to appear as if mouse spermatogonia were strongly protected against chemical insults or were highly efficient at repairing such lesions. Even diethylnitrosamine (DEN), a compound known to be strongly mutagenic in Drosophila, was completely ineffective in mice (Russell and Kelly 1979). To be mutagenic, however, DEN is enzymatically converted into an alkylating agent, and it was possible either that this activation process was not occurring in mammals or, if it was, that the short-lived metabolite was not capable of reaching the testis in time to be effective. To circumvent this complication, Ekkehart Vogel suggested that Russell try the experiment again using ethylnitrosourea (ENU), a chemical that forms the same alkylating species as DEN but does not require metabolism. Russell, still disappointed by the DEN results, thought ENU was going to be another long shot, but he set up a small pilot experiment.

A single dose of ENU was injected into a group of male mice, which then underwent temporary sterility owing to massive killing of spermatogonia. Upon recovery about 10 wk later, however, 90 males were crossed to *T*-stock females and sired 7584 pups. Among this small set of offspring, 35 were mutant for one of the seven loci, yielding an induced mutation rate five times higher than the maximal rate obtained with X rays (Russell *et al.* 1979). Encouraged by these findings, Russell's group showed that if, instead of one large dose, the ENU was fractionated and injected on a weekly schedule to permit a higher total dose to be tolerated, then the mutation frequency jumped to 12 times that of X rays, 36 times higher than procarbazine, and over 200 times the spontaneous rate. When averaged across all seven loci, ENU was now inducing mutations at a frequency of one per locus in every 700 gametes (Russell *et al.* 1982a, 1982b; Hitotsumachi *et al.* 1985). Because the spermatogonial stem cells were being affected, the genetic lesions were not restricted to transient stages but could be recovered indefinitely (at least as long as the mutagenized male survived). Additionally, the ENU mutants were slightly different from the ones induced by X rays. First, the phenotypes sometimes appeared intermediate between the wild-type and *T*-stock alleles. Second, there were never any mutations simultaneously affecting the two closely linked *d* and *se* loci. Third, the number of mutations for the *T*-stock loci that were lethal when homozygous was very low (Russell 1982). ENU apparently caused subtle intragenic lesions (instead of the X-ray-generated deletions) and was heralded a "supermutagen," deigned the "mutagen of choice for the production of any kind of desired new gene mutations in the mouse" (Russell *et al.* 1979).

ENU as a genetic tool: The earliest application of ENU to create new mouse mutations was in detecting electrophoretic mobility variants of blood proteins, an efficient screen that could easily assay 21 different loci from a single preparation (Johnson and Lewis 1981).

Because the primary structure of many of these proteins had already been biochemically determined, it was of interest to characterize the structure of the ENU-induced variants to identify the molecular basis of mutagenesis. In the first analysis of a hemoglobin variant, a single amino acid substitution was discovered, and it was proposed that ENU had induced an A to T transversion in a histidine codon (Popp *et al.* 1983), supporting the idea that ENU acts as a point mutagen in mice.

Vernon Bode at Kansas State University and William Dove and Alexandra Shedlovsky at the University of Wisconsin used ENU to dissect the properties of the mouse *t-region*, a bizarre genetic locus with many distinctive traits including interaction with *T* (Brachyury) to produce tailless mice, transmission ratio distortion, and male sterility in compound heterozygotes. The analysis of *t* was complicated by the fact that recombination at *t-region* was strongly suppressed, disallowing the locus to be genetically dissected by crossovers. Thus, to study individual functional units, ENU mutagenesis was used to saturate the area and make discrete intragenic lesions (Bode 1984; Justice and Bode 1986, 1988; Shedlovsky *et al.* 1986, 1988).

Realizing the value of the mouse as a model for human diseases, it was now feasible to mutagenize an animal with ENU and screen for phenotypes resembling clinical disorders. Phenylketonuria, one of the first inborn errors of metabolism characterized in humans, was chose by Bode as a disease to reproduce in the mouse with chemical mutagenesis (McDonald *et al.* 1990). Besides creating new mouse models, one of the interesting results from this study was the dramatic frequency at which mutations in the phenylketonuria pathway were collected: an astounding one mutant for every 175 gametes examined (Shedlovsky *et al.* 1993). This value, close to 10 times better than the frequency of other loci, may mean that different genes could have very different induced mutation rates, as Russell's group had noticed in the SLT (Hitotsumachi *et al.* 1985).

The value of ENU alleles and the different types of screens used to capture them are diverse:

1. In the positional cloning of complex genetic lesions, ENU-induced mutations can confirm the functional identity of candidate genes, as was done for the *kreisler*, *quaking*, *eed*, and *Clock* loci (Cordes and Barsh 1994; Ebersole *et al.* 1996; Schumacher *et al.* 1996; King *et al.* 1997b).
2. The easiest screen is a hunt for dominants. These will inherently fall out of any ENU experiment and can yield diverse phenotypes from circling behavior to neoplasia disposition (Moser *et al.* 1990). *Clock*, probably the most famous example, is a dominant, antimorphic, ENU-induced allele captured by carefully assaying mice for abnormal well-running activity, resulting in the first cloned mouse mutation to disrupt circadian rhythm (Vitaterna *et al.* 1994; Antoch *et al.* 1997; King *et al.* 1997a, 1997b).
3. Alleles of an already known mutation can be recovered by conducting an SLT similar to Russell's, where a mutagenized male is crossed to a female homozygous for the test locus (*m*/*m*). ENU mutations specific to the locus (*) will be uncovered and recognized in the F_1 generation (*/*m*). To fully characterize any one mouse gene, this technique should be applied to any disrupted allele made by gene targeting because a functional analysis can be appreciated only by examining an allelic series. While null mutations are necessary, subsequent alleles generated by point mutations including hypermorphs, hypomorphs, antimorphs, and neomorphs can yield vastly different phenotypes. For example, *eed* is a mouse mutation that causes early embryonic lethality. A hypomorphic allele of *eed* induced by ENU, however, allows the mouse to survive embryogenesis. The hypomorph shows skeletal transformations along the vertebral column and provides insight into *eed* as a regulator of homeotic genes (Schumacher *et al.* 1996). Thus, a knock-out database for the mouse genome should be considered only as a starting point; additional alleles are mandatory to complete the functional analysis.
4. Besides structural mutations, ENU will also induce lesions in regulatory elements, a feature not considered in most gene targeting studies.
5. By exploiting nonallelic noncomplementation, it may be possible to conduct sensitized screens in mice. An induced mutation at another locus that happens to interact with the specific locus of interest might fail to complement (*+; +/*m*) yet still yield a phenotype reminiscent of the original homozygous mutant (*m*/*m*). This approach, reiterated with each new mutation captured, might generate an extensive functional map of genetic interactions.
6. Another application of ENU is in saturation mutagenesis at defined deletions, yielding discrete functional units at any chromosomal site. Eugene Rinchik designed elegant screens exploiting coat color genetics to provide a fine-structure functional analysis for the *c* and *p* loci deletions originally produced in Russell's X-ray treatments in the SLT (Rinchik *et al.* 1990, 1995). Because deletions in the mouse can now be quickly generated in any part of the genome (Ramirez-Solis *et al.* 1995; You *et al.* 1997), the merging of this technology with chemical mutagenesis will undoubtedly be one of the most productive phases of functional genomics starting off the next millennium.

We now stand at an exciting crossroad in mouse genetics. The field has exploded with an infusion of molecular biologists applying their "tricks-of-the-trade" to manipulate the genome. For a while, chemical mu-

tagenesis fell out of favor. It seemed as if knowing the nucleotide lesions in a mutation would be necessary to produce any value to understanding the biology of genetics. Though ENU mutagenesis may produce interesting variants, the nature of its own power, that being a point mutagen, frightened many people who were obsessed by the fear of never being able to clone the affected gene. Instead, pushes were made to sequence genomes, and the concern over function and phenotype would come later. Well, folks, it's later. The tremendous sequencing projects are starting to pay off by now, providing molecular landmarks throughout the mouse genome that can serve as launching points to sequence new mutations. The pendulum has started to swing back to ENU mutagenesis to generate and collect those interesting mice in phenotype-driven screens that will allow an in-depth study of any gene, chromosomal region, or biological system, thanks to the legacy of the Russells on their 50th Anniversary in Oak Ridge.

LITERATURE CITED

Antoch, M. P., E-J. Song, A.-M. Chang, M. H. Vitaterna, Y. Zhao *et al.* 1997 Functional identification of the mouse circadian *Clock* gene by transgenic BAC rescue. Cell **89**: 655–667.

Bode, V. C., 1984 Ethylnitrosourea mutagenesis and the isolation of mutant alleles for specific genes located in the *t* region of mouse chromosome 17. Genetics **108**: 457–470.

Capecchi, M. R., 1989 Altering the genome by homologous recombination. Science **244**: 1288–1292.

Cordes, S. P., and G. S. Barsh, 1994 The mouse segmentation gene *kr* encodes a novel basic domain-leucine zipper transcription factor. Cell **79**: 1025–1034.

Ebersole, T. A., Q. Ghen, M. J. Justice and K. Artzt, 1996 The quaking gene product necessary in embryogenesis and myelination combines features of RNA binding and signal transduction proteins. Nat. Genet **12**: 260–265.

Ehling, U. H., 1978 Specific-locus mutations in mice, pp. 233–256 in *Chemical Mutagens*, edited by A. Hollaender and F. J. deSerres. Plenum Press, New York.

Ehling, U. H., and A. Neuhauser, 1979 Procarbazine induced specific-locus mutations in male mice. Mutat. Res. **59**: 245–256.

Falconer, D. S., B. M. Slizynski and C. Auerbach, 1952 Genetical effects of nitrogen mustard in the house mouse. J. Genet. **51**: 81–88.

Haldane, J. B. S., 1956 The detection of autosomal lethals in mice induced by mutagenic agents. J. Genet. **54**: 327–342.

Hitotsumachi, S., D. A. Carpenter and W. L. Russell, 1985 Dose-repetition increases the mutagenic effectiveness of N-ethyl-N-nitrosourea in mouse spermatogonia. Proc. Natl. Acad. Sci. USA **82**: 6619–6621.

Johnson, F. M., and S. E. Lewis, 1981 Electrophoretically detected germinal mutations induced in the mouse by ethylnitrosourea. Proc. Natl. Acad. Sci. USA **78**: 3138–3141.

Justice, M. J., and V. C. Bode, 1986 Induction of new mutations in a mouse t-haplotype using ethylnitrosourea mutagenesis. Genet. Res. **47**: 187–192.

Justice, M. J., and V. C. Bode, 1988 Three ENU-induced alleles of the murine quaking locus are recessive embryonic lethal mutations. Genet. Res. **51**: 95–102.

Kent-First, M., 1997 A lesson from mosaics: don't leave the genetics out of molecular genetics. J. NIH Res. **9**: 29–33.

King, D. P., M. H. Vitaterna, A-M. Chang, W. F. Dove, L. H. Pinto *et al.*, 1997a The mouse *Clock* mutation behaves as an antimorph and maps within the W^{19H} deletion, distal of *Kit*. Genetics **146**: 1049–1060.

King, D. P., Y. Zhao, A. M. Sangoram, L. D. Wilsbacher, M. Tanaka *et al.*, 1997b Positional cloning of the mouse circadian *Clock* gene. Cell **89**: 641–653.

Lyon, M. F., 1961 Gene action in the X chromosome of the mouse (*Mus musculus* L.) Nature **190**: 372–373.

Lyon, M. F., and T. Morris, 1966 Mutation rates at a new set of specific loci in the mouse. Genet. Res. **7**: 12–17.

McDonald, J. D., V. C. Bode, W. F. Dove and A. Shedlovsky 1990 Pah^{hph-5}: a mouse mutant deficient in phenylalanine hydroxylase. Proc. Natl. Acad. Sci. USA **87**: 1965–1967.

Moser, A. R., H. C. Pitot and W. F. Dove 1990 A dominant mutation that predisposes to multiple intestinal neoplasia in the mouse. Science **247**: 322–324.

Popp, R. A., E. G. Bailiff, L. C. Skow, F. M. Johnson and S. E. Lewis, 1983 Analysis of a mouse alpha-globin gene mutation induced by ethylnitrosourea. Genetics **105**: 157–167.

Ramirez-Solis, R., P. Liu, and A. Bradley, 1995 Chromosome engineering in mice. Nature **378**: 720–724.

Rinchik, E. M., and L. B. Russell, 1990 Germ-line deletion mutations in the mouse: tools for intensive functional and physical mapping of regions of the mammalian genome, pp. 121–158 in *Genome Analysis, Volume 1: Genetic and Physical Mapping* edited by K. Davies and S. Tilghman. Cold Spring Harbor Laboratory Press, Cold Spring Harbor, NY.

Rinchik, E. M., D. A. Carpenter and P. B. Selby, 1990 A strategy for fine-structure functional analysis of a 6- to 11-centimorgan region of mouse chromosome 7 by high-efficiency mutagenesis. Proc. Natl. Acad. Sci. USA **87**: 896–900.

Rinchik, E. M., D. A. Carpenter and M. A. Handel, 1995 Pleiotropy in microdeletion syndromes: neurologic and spermatogenic abnormalities in mice homozygous for the p^{6H} deletion are likely due to dysfunction of a single gene. Proc. Natl. Acad. Sci. USA **92**: 6394–6398.

Russell, E. S., 1987 A mouse phoenix rose from the ashes. Genetics **117**: 155–156.

Russell, L. B., 1961 Genetics of mammalian sex chromosomes. Science **133**: 1795–1803.

Russell, L. B., 1971 Definition of functional units in a small chromosomal segment of the mouse and its use in interpreting the nature of radiation-induced mutations. Mutat. Res. **11**: 107–123.

Russell, L. B., and M. H. Major, 1957 Radiation-induced presumed somatic mutations in the house mouse. Genetics **42**: 161–175.

Russell, L. B., and W. L. Russell, 1996 Spontaneous mutations recovered as mosaics in the mouse specific-locus test. Proc. Natl. Acad. Sci. USA **93**: 13072–13077.

Russell, W. L., 1951 X-ray-induced mutations in mice. Cold Spring Harbor Symp. Quant. Biol. **16**: 327–335.

Russell, W. L., 1954 Genetic effects of radiation in mammals, pp. 825–859 in *Radiation Biology*, edited by A. Hollaender. McGraw-Hill, New York.

Russell, W. L., 1982 Factors affecting mutagenicity of ethylnitrosourea in the mouse specific-locus test and their bearing on risk estimation, pp. 59–70 in *Environmental Mutagens and Carcinogens*, edited by T. Sugimura, S. Kondo and H. Takebe. Alan R. Liss, New York.

Russell, W. L., 1989 Reminiscences of a mouse specific-locus test addict. Environ. Mol. Mutagen **14** (Suppl. 16): 16–22.

Russell, W. L., and E. M. Kelly, 1979 Ineffectiveness of diethylnitrosamine in the induction of specific-locus mutations in mice. Genetics **91** (Suppl.): s109–s110.

Russell, W. L., L. B. Russell and E. M. Kelly 1958 Radiation dose rate and mutation frequency. Science **128**: 1546–1550.

Russell, W. L., E. M. Kelly, P. R. Hunsicker, J. W. Bangham, S. C. Maddux *et al.*, 1979 Specific-locus test shows ethylnitrosourea to be the most potent mutagen in the mouse. Proc. Natl. Acad. Sci. USA **76**: 5818–5819.

Russell, W. L., P. R. Hunsicker, G. D. Raymer, M. H. Steele, K. F. Stelzner *et al.*, 1982a Dose-response curve for ethylnitrosourea-induced specific-locus mutations in mouse spermatogonia. Proc. Natl. Acad. Sci. USA **79**: 3589–3591.

Russell, W. L., P. R. Hunsicker, D. A. Carpenter, C. V. Cornett and G. M. Guinn, 1982b Effect of dose fractionation on the ethylnitrosourea induction of specific-locus mutations in mouse spermatogonia. Proc. Natl. Acad. Sci. USA **79**: 3592–3593.

Schumacher, A., C. Faust and T. Magnuson, 1996 Positional cloning of a global regulator of anterior-posterior patterning in mice. Nature **383**: 250–253.

Shedlovsky, A., J-L. Guénet, L. J. Johnson and W. F. Dove

1986 Induction of recessive lethal mutations in the *T/t-H-2* region of the mouse genome by a point mutagen. Genet. Res. **47**: 135–142.

Shedlovsky, A., T. R. King, and W. F. Dove, 1988 Saturation germ line mutagenesis of the murine *t* region including a lethal allele at the quaking locus. Proc. Natl. Acad. Sci. USA **85**: 180–184.

Shedlovsky, A., J. D. McDonald, D. Symula and W. F. Dove, 1993 Mouse models of human phenylketonuria. Genetics **134**: 1205–1210.

Vitaterna, M. H., D. P. King, A-M. Chang, J. M. Kornhauser, P. L. Lowrey *et al.* 1994 Mutagenesis and mapping of a mouse gene, *Clock*, essential for circadian behavior. Science **264**: 719–725.

von Borstel, R. C., and C. M. Steinberg, 1995 Alexander Hollaender: myth and mensch. Genetics **143**: 1054–1056.

You, Y., R. Bergstrom, M. Klemm, B. Lederman, H. Nelson *et al.*, 1997 Chromosomal deletion complexes in mice by radiation of embryonic stem cells. Nat. Genet. **15**: 285–288.

Zeiher, B. G., E. Eichwald, J. Zabner, J. J. Smith, A. P. Puga *et al.*, 1995 A mouse model for the delta F508 allele of cystic fibrosis. J. Clin. Invest. **96**: 2051–2064.