Published in final edited form as:

Mamm Genome. 2003 December ; 14(12): 817–829. doi:10.1007/s00335-003-2298-4.

Overlapping deletions spanning the proximal two-thirds of the mouse *t* complex

David E. Bergstrom, Rebecca A. Bergstrom, Robert J. Munroe, Barbara K. Lee, Victoria L. Browning*, Yun You**, Eva M. Eicher, and John C. Schimenti

The Jackson Laboratory, 600 Main Street, Bar Harbor, Maine 04609, USA

Abstract

Chromosome deletion complexes in model organisms serve as valuable genetic tools for the functional and physical annotation of complex genomes. Among their many roles, deletions can serve as mapping tools for simple or quantitative trait loci (QTLs), genetic reagents for regional mutagenesis experiments, and, in the case of mice, models of human contiguous gene deletion syndromes. Deletions also are uniquely suited for identifying regions of the genome containing haploinsufficient or imprinted loci. Here we describe the creation of new deletions at the proximal end of mouse Chromosome (Chr) 17 by using the technique of ES cell irradiation and the extensive molecular characterization of these and previously isolated deletions that, in total, cover much of the mouse *t* complex. The deletions are arranged in five overlapping complexes that collectively span about 25 Mbp. Furthermore, we have integrated each of the deletion complexes with physical data from public and private mouse genome sequences, and our own genetic data, to resolve some discrepancies. These deletions will be useful for characterizing several phenomena related to the *t* complex and *t* haplotypes, including transmission ratio distortion, male infertility, and the collection of *t* haplotype embryonic lethal mutations. The deletions will also be useful for mapping other loci of interest on proximal Chr 17, including T-associated sex reversal (*Tas*) and head-tilt (*het*). The new deletions have thus far been used to localize the recently identified *t* haplolethal (*Thl1*) locus to an approximately 1.3-Mbp interval.

Chromosomal deletions have long served as valuable genetic tools for geneticists. For *Drosophila melanogaster*, collections of deletion (deficiency) strains are available that collectively encompass virtually the entire genome (<http://flybase.bio.indiana.edu/aberrations>) and allow loss-of-function alleles to be mapped to specific subchromosomal intervals by crossing flies carrying such alleles to lines of flies bearing overlapping deficiencies. Likewise, in a classic set of experiments known as the “specific locus test,” mouse geneticists used large-scale, whole-body irradiation to create sets of overlapping deletions centered around several “visible” loci (Davis and Justice 1998). These experiments allowed molecular genetic analyses of phenotypes arising from the combinatorial mating of mice carrying deletions within each complex (Dhar and Johnson 1997; Rikke et al. 1997; Roix et al. 2001; Simpson et al. 2000), and from region-specific mutagenesis with the point mutagen ENU (Justice et al. 1997; Rinchik and Carpenter 1993, 1999; Rinchik et al. 1990, 1993, 2002). With the advent of advanced embryonic stem (ES) cell technologies, it is

Correspondence to: J.C. Schimenti; E-mail: jcs@jax.org.

*Present address: Department of Surgery, University of Wisconsin Medical School, CSC H5/301, 600 Highland Avenue, Madison, WI 53792-3236, USA.

**Present address: Oak Ridge National Laboratory, P.O. Box 2008, Building 1061, Bethel Valley Road, Oak Ridge, TN 37831-6445, USA.

Primer pairs defining microsatellite markers designed for this manuscript have been submitted to GenBank under Accession Numbers BV079472 to BV079564 and to the Mouse Genome Database under Accession Number J:84523.

now possible to develop deletion complexes throughout the genome and without the logistical complexities of whole-body radiation (Ramirez-Solis et al. 1995; You et al. 1997b).

We previously described a chromosomal deletion complex at the proximal end of mouse Chr 17 centered at the *D17Aus9* locus (You et al. 1997a; 1997b). In this strategy, ES cell clones containing targeted insertions of a negatively selectable Herpes simplex virus (HSV) thymidine kinase (*tk*) reporter were exposed to gamma irradiation to induce deletions, followed by selection in medium containing the drug FIAU to kill cells that retain *tk* expression. We also used this approach to generate an array of four overlapping deletion complexes spanning the rump-white (*Rw*; *Dpp6^{In(5)6H}*) inversion on mouse Chr 5 (Schimenti et al. 2000; Wilson, L. and Schimenti, J., unpublished data). Together, these Chr 17 and Chr 5 deletions demonstrated the practicality of generating comprehensive sets of deletions throughout the mouse genome, as in flies.

The *t* complex on mouse Chr 17 has several unique properties that would benefit from the availability of overlapping deletion sets. There are naturally occurring, variant forms of the *t* complex called “*t* haplotypes,” which have been studied by geneticists and developmental biologists for several decades. *t* haplotypes contain mutations resulting in male transmission ratio distortion (TRD), sterility, and embryonic lethality (Silver 1993). The TRD and sterility are classic quantitative traits involving at least four genes in *t* haplotypes (Lyon 1984, 1986). Further complicating the genetic analysis of the genes responsible for these traits, *t* haplotypes contain inversions that prevent recombination with wild-type (normal) chromosomes (Hammer et al. 1989). However, deletions can be used to circumvent these difficulties. Genes involved in TRD (*Tcd1*), sterility (*Tcs1*), and embryonic lethality (*t^{w73}*) have all been mapped to high resolution with this approach (Lyon et al. 2000; Planchart et al. 2000; Zwart et al. 2001). Furthermore, deletions in the *t* complex have revealed genes that produce phenotypes only when present in the hemizygous state, including T-associated sex reversal (*Tas*), T-associated maternal effect (*Tme*), and *t* haplolethal 1 (*Thl1*) (Browning et al. 2002; Washburn and Eicher 1983; Winking and Silver 1984).

To be useful as high-resolution mapping tools, it is important that sets of deletions have overlapping or staggered breakpoints—so-called deletion complexes. To this end, we have constructed a series of deletion complexes along the proximal end of mouse Chr 17 containing the *t* complex. Here, we present the high-resolution mapping of a compendium of deletions spanning proximal Chr 17, including previously unpublished deletions positioned around the *D17Aus9*, *Sod2*, and *Nkx2-5* loci (generated by irradiation of ES cells), together with deletions previously isolated at the *T* and *D17Leh94* loci. We have used these deletions to further map the recently identified *t* haplotype lethal 1 (*Thl1*) locus. In total, the five deletion complexes span 25 Mbp on the proximal end of Chr 17 and provide a valuable resource for the functional annotation of a region whose analysis has heretofore been refractory to traditional genetic analysis. With the recent construction of highly detailed public and private sequence assemblies (Mural et al. 2002; Waterston et al. 2002), functional maps now can be correlated with detailed physical maps and sequence annotation.

Materials and methods

Targeting the *Nkx2-5* locus

A 3.8-kb *Bam*HI genomic fragment isolated from the wild-type *Nkx2-5* locus (Lyons et al. 1995) and containing the final exon of *Nkx2-5* was subcloned into pBluescript II SK. This plasmid was linearized with *Bg*III, and a 7-kb *Bam*HI fragment containing the β -actin-Neo and HSV-*tk* genes from plasmid pBANTKcass (You et al. 1997a) was ligated into the *Bg*III site. This plasmid was then linearized at an *Xba*I site within the polylinker of the plasmid and electroporated into v6.5 ES cells. These cells were derived from (129S4/SvJae $\times$ C57BL/6)

F₁ male blastocysts and are essentially identical to v6.4 cells previously described (You et al. 1998). To confirm proper targeting, Southern blots containing *Eco*RI-digested DNA from clones resistant to Geneticin (300 µg/mL, Life Technologies, Gaithersburg, Md.) were probed with a 1.3-kb *Eco*RI/*Bam*HI *Nkx2-5* fragment located 2.5 kb upstream of the *tk*/Neo integration site (Fig. 2).

Inducing deletions

The previously described methods of You et al. were used to generate ES cell clones containing potential interstitial deletions (You et al. 1997a). Specifically, targeted clones maintained under G418 selection (300 µg/mL) on neomycin-resistant feeder cells were trypsinized and exposed to 4 Gy of radiation from a ¹³⁷Cs source. Approximately 1 × 10⁶ irradiated cells were plated onto 150-mm plates without feeders, and FIAU selection was begun after 48 h.

The parental *Sod2* ES cell line exhibited a high background of FIAU-resistant clones, which complicated the identification of a larger set of deletions. Hence, deletions induced at the *Sod2* locus were derived from a subclone of the originally targeted ES cell clone (Browning et al. 2002). We hypothesize that the high background in the parental line was due to the presence of a subset of cells that had lost *tk* expression by a spontaneous epigenetic or mutagenic event.

Generation of chimeras by microinjection

Ten to 15 cells from deletion-bearing ES cell clones were injected into 3.5-dpc C57BL/6J blastocysts. For some previously described deletions, chimeric mice, created by using v17.2 (129S4/SvJae × BALB/c) F₁ ES cells and displaying a mosaic agouti (ES cell) and black (host) coat color, were mated to C57BL/6J mice. Pups derived from ES cells were identified by their agouti coat color.

For new deletions, chimeric mice created with v6.5 (129/S4SvJae × C57BL/6J)F₁ ES cells likewise displayed agouti (ES cell) and black (host) coat color. These also were mated to C57BL/6J mice. In this case, however, only half of the pups arising from ES-derived cells could be identified on the basis of their agouti coat color. The remaining ES-descendant pups (which appeared black) were identified by the presence of 129-derived alleles flanking the deletion.

Identification of deletion-bearing mice

Transmission of the deleted allele to ES cell-derived progeny was assessed in one of three ways, depending on the nature of the deletion and the cross: (1) Presence of a short, kinked tail phenotype for deletions spanning the brachyury (*T*) locus; (2) PCR analysis of polymorphic microsatellite markers that mapped within the deleted region; and (3) PCR analysis of closely linked polymorphic markers flanking the deletion.

Recombinational mapping

To create a recombinational/meiotic map of Chr 17, an intersubspecific intercross was established between JE/Le and CAST/Ei. Three hundred seventy-one F₂ offspring (representing 742 meioses) were analyzed. Initially, 170 offspring were genotyped with each of the following polymorphic microsatellite markers: *D17Mit164*, 19, 196, 98, 171, 246, 18, 170, 25, 194, 97, 273, 112, 156, 113, 261, 213, 46, 133, 100, 226, and 145 (Dietrich et al. 1996), and *D17Tu1* (Himmelbauer and Silver 1993). For a more detailed map in the central portion of the chromosome, 201 additional mice were genotyped with *D17Mit196* and *D17Mit261*, and those mice recombinant between these two markers were genotyped with the remaining markers listed above, as well as the additional markers: *D17Mit195*, 259, 48, 58,

245, 114, 27, 131, 173, and 174 (Dietrich et al. 1996) and *D17Pri3* (Himmelbauer and Silver 1993).

Results

Overview

To establish a comprehensive collection of overlapping deletions along the 20 cM mouse *t* complex, two objectives must be met. First, the deletion complexes must be generated at multiple points, so that a set of deletions will collectively span the target region. In principle, a single set of very large deletions could span the whole *t* complex; however, this is not practical because large regions of segmental haploidy can seriously compromise viability. Dispersed sets of smaller deletions give greater flexibility for mapping and functional analysis of particular regions. Second, the breakpoints of the deletions in the collection must be well defined. This is critical for assessing the coverage of the *t* complex region and the degree of overlap between deletions, and for facilitating the selection of deletions for particular experiments.

In previous work, the methodology of You et al. (1997a) was used to generate interstitial deletions at the *D17Aus9*, *D17Leh94*, and *Sod2* loci on proximal mouse Chr 17 (Browning et al. 2002; You et al. 1997a). This method involves three steps. First, the negatively selectable HSV-*tk* gene is targeted to a locus of interest that will serve as a deletion focal point (DFP) around which all deletions of a given complex are centered. Next, cells from targeted clones are exposed to gamma radiation to induce deletions and are grown under FIAU selection to isolate clones that have lost functional *tk* activity. Third, potential deletion-bearing clones are genotyped with closely linked polymorphic markers to verify the presence and extent of each interstitial deletion. To increase polymorphism between a deletion-bearing chromosome and its homolog or to allow assessment of visible phenotypes in whole animals, deletion-bearing ES clones can be passed through the germline of chimeric mice and the progeny outcrossed to mice of appropriate strains (e.g., CAST/Ei, an inbred line derived from *Mus musculus castaneus*; or specific mutation-bearing strains, etc.).

Initial characterization of deletion breakpoints was conducted mainly with the use of microsatellite markers from the MIT F₂ mapping cross (Dietrich et al. 1996). However, the recent availability of public and private sequence assemblies (Marshall 2001; Mural et al. 2002; Waterston et al. 2002) has simplified the development of additional polymorphic markers that afford higher resolution break-point mapping and often circumvent the need for outcrossing to wild-derived strains. These new markers have been used to characterize both previously described deletions and the new ones reported here. The sum of these studies is presented in Fig. 1.

A new deletion complex centered on the *Nkx2-5* locus of mouse Chr 17

A set of deletions was generated at the *Nkx2-5* locus (Lyons et al. 1995), which lies 20 Mbp from the centromere (proximal end) of Chr 17. A *tk*/Neo cassette was fitted into an *Nkx2-5* genomic clone (Fig. 2) and targeted into the *Nkx2-5* locus of (129S4/SvJae × C57BL/6)F₁ ES cells. A properly targeted, germline-competent clone, designated B11, was expanded and exposed to gamma radiation to induce interstitial deletions.

Fifteen *tk*-negative lines were initially tested for the absence of closely linked *D17Mit* markers. Cel- era genome assemblies corresponding to the *Nkx2-5* region were aligned with the deletion complex. Deletion breakpoint regions were refined by designing and analyzing additional polymorphic markers in the *Nkx2-5* region.

Collectively, the *Nkx2-5* deletions span at least 5.7 Mbp and are present in mouse stocks here designated *Nkx2-5^{df1J}*, *2J*, *4J*, and *5J*, and ES cell lines C3B, C3F, C3LL, C3MM, C3PP, C3R, C3X, D4JJ, D4U, D4W, and E1. These lines are shown in yellow in Fig. 1. The deletions in the ES cell lines C3F, C3LL, and C3R extend most proximally and delete *D17Mit133* and the closely linked but slightly more proximal marker *D17Jcs50*. Each of these lines retains the more proximal marker *D17Jcs14*. The deletions in the ES cell lines C3MM, C3PP, D4U, and D4W extend most distally and delete *D17Jcs77* located in the Celera genome assembly (release 2) contig GA_x6KO2T2PRF8.

Additional deletions at the *D17Aus9* and *Sod2* loci

In addition to the set of deletions originally described at the *D17Aus9* locus (You et al. 1997a), Fig. 1 includes four additional deletions (*D17Aus9^{df25J}*, *26J*, *27J*, and *33J*). Each of these deletions has been recovered in live mice (those in other ES cell lines that were found not to be germline competent, or which were not tested for germline transmissibility, are not shown). Collectively, the *D17Aus9* deletion set spans at least 9.8 Mbp (shown in green in Fig. 1). Many of these lines have lost the *T* locus, causing a short tail (brachyury) phenotype in hemizygous mice. Several of these deletions have been used to localize the vestibular mutant head-tilt, *het* (a simple Mendelian trait) (Bergstrom et al. 1998); the *t* complex sterility factor, *tcs1* (a quantitative trait locus) (Planchart and Schimenti 2001; Planchart et al. 2000); and the *T*-associated sex reversal locus, *Tas* (Eicher et al., unpublished).

A deletion complex at the *Sod2* locus consisting of seven deletion lines (six germline) was previously described (Browning et al. 2002). To supplement this collection, we created additional deletions centered on the same locus, 17 of which were examined in detail (shown in violet in Fig. 1). Nine of these 17 have been shown to transmit through the germline as well (although the resulting mice are frail). Proximally, lines *Sod2^{df4J}* and 2B10 extend the furthest, deleting at least as far as *D17Br6*. These lines, in and of themselves, encompass the entire *D17Aus9* deletion complex. Distally, one line (*Sod2* F4) deletes *D17Jcs48* that is closely linked to *D17Mit173*. Thus, distally, the *Sod2* deletion complex has significant overlap with the *Nkx2-5* deletion complex. In total, the *Sod2* deletion complex spans some 18 Mbp.

Additional deletions

Previously described deletions at the *T* (shown in orange in Fig. 1; Bilinski et al. 1997) and *D17Leh94* (shown in blue in Fig. 1; Browning et al. 2002) loci also are shown. The deletions at *T* overlap extensively with those at *D17Aus9* and *Sod2*, while those at *D17Leh94* provide additional coverage at the distal end of the *Sod2* complex.

Ordering and orientation of physical map features

To better characterize the chromosomal content spanned by the deletions, we integrated data from a variety of sources to construct a composite map across the proximal portion of Chr 17 (Fig. 3). These sources include the recombination- and deletion-based maps described here, the private sequencing efforts of Celera, and two public sequence assemblies (UCSC/February 2002 and Ensembl/MGSCv3).

Although a few unresolved discrepancies remain between the genetic and physical maps (*D17Mit25*, *100*, *133*, *170*, *195*, and *198*, data not shown), the vast majority of markers are concordant in order, orientation, and spacing across all five maps. One notable exception involves sequences at the extreme proximal end of Chr 17 encompassing the genetic markers *D17Mit19*, *164*, *196*, and *D17Tu1*. In both the Ensembl and UCSC maps, *D17Mit19* is absent from the assembly, and the three remaining markers are depicted in the order: proximal—*D17Mit196*, *D17Tu1*, *D17Mit164*—distal. This orientation is not substantiated by the placement of BAC end sequences on the UCSC sequence assembly, as there is a discontinuity in the BAC

tiling map between *D17Mit164* and the more distally situated *D17Mit171* (data not shown). However, the opposite orientation (proximal–*D17Mit164*, *D17Mit19*, *D17Tu1*, *D17Mit196*–distal) is consistent with several informative deletions (including *D17Aus9^{df11J}*, *Sod2^{df1J}*, *Sod2^{df3J}*, *D17Aus9^{df26J}*, and *T^{30H}*), multiple recombinants on the genetic map, and the Celera assembly. In summary, our data favor placement of the markers in the latter order and indicate that the surrounding sequences are improperly inverted in the current Ensembl and UCSC genome maps.

Refined mapping of t haplolethal 1 (*Thl1*)

The *Thl1* gene was discovered during the course of breeding chimeric mice carrying ES cells containing *Sod2* and *D17Leh94* deletions (Browning et al. 2002). Germline chimeras derived from a subset of these cell lines never produced deletion-bearing progeny in matings to C57BL/6J (B6) females, but could transmit the deletions when mated to females of other strains such as C3H (albeit at lower than 50% ratios). The basis for failed transmission through B6 females was late embryonic/neonatal lethality, in which affected animals displayed exencephaly or edema. The results indicate that *THL1* levels are critical for certain aspects of fetal development, on certain genetic backgrounds more than others. A molecular analysis of the deletion breakpoints identified an approximately 3-Mbp region, located between markers *D17Mit131* and *D17Jcs80*, that contains the haplolethal locus *Thl1*. The key deletions defining this interval are *Sod2^{df5J}* and *D17Leh94^{df10J}*, neither of which causes the phenotype. Mice carrying deletions that removed this interval showed the *Thl1* phenotype.

Several of the new *Sod2* deletions reported here have breakpoints within the *Thl1*-critical region and thus enable further dissection of the *Thl1*-critical region. Accordingly, chimeric mice for each of these new deletions (*Sod2^{df7J}*, *Sod2^{df8J}*, *Sod2^{df10J}*) plus two deletions whose breakpoints extend beyond the distal end of the *Thl1* locus (*Sod2^{df9J}* and *Sod2^{df12J}*) and one (*Sod2^{df11J}*) that breaks proximal to *Thl1* were evaluated for the *Thl1* phenotype. The data are shown in Table 1.

As expected, germline male chimeras bearing the *Sod2^{df9J}* and *Sod2^{df12J}* deletions, which entirely removed the *Thl1* interval, never transmitted these deletions when mated to C57BL/6J females. When these males were mated to C3H females, however, the deletions were inherited. This is the behavior previously observed for deletions that remove *Thl1*. In contrast, chimeras readily transmitted the *Sod2^{df7J}*, *Sod2^{df8J}*, *Sod2^{df10J}*, and *Sod2^{df11J}* deletions when mated to B6 females, indicating that none of these removed *Thl1*. The most distally extending of these four, *Sod2^{df7J}*, thus delimits the *Thl1*-critical region to the interval between *D17Jcs23* and *D17Jcs80*. The physical distance, according to both the Celera mouse genome sequence (assembly 2) and public mouse assembly (February 2002 freeze) presented by the UCSC browser (<http://genome.ucsc.edu/>) is approximately 1.3 Mbp.

Discussion

Deletions have long served as valuable experimental tools for geneticists. Deletions can be used as gene mapping reagents, as substrates for mutagenesis screens, and as a means to ablate genetic functions known only by position. In mice, deletions can serve as models of human contiguous gene deletion syndromes (Kimber et al. 1999; Lindsay et al. 1999; Naf et al. 2001; Paylor et al. 2001). In *Drosophila*, where the logistics of large-scale animal rearing are relatively simple, deletion analyses have played important roles in the functional analysis of the genome. In contrast, analysis of deletions in mice has generally been limited to a relatively small number of spontaneous deletions and to deletion complexes arising from logistically cumbersome, specific locus tests.

With the advent of technologies to induce and select chromosomal deletions in mouse ES cells, many of the positional and logistical limitations of deletion analysis have been surmounted. We implemented ES cell deletional mutagenesis technology to construct four overlapping deletion complexes in the proximal region of Chr 17 that collectively span some 25 Mbp. These are augmented by a collection of *T* locus deletions recovered spontaneously [T^{34H} , T^{hp} , and $Del(17)T^{7J}$] and in germ cell mutagenesis experiments at Harwell (T^{22H} , T^{29H} , T^{30H} , T^{32H} , and T^{33H}). Together, this collection of deletions provides an extensive array of resources for further detailed analysis of mouse Chr 17. As described here and previously, we have begun to exploit this resource to uncover novel genetic functions (Browning et al. 2002), as well as to fine map and then clone previously known genes (Bergstrom et al. 1998). Given the unusual nature of *t* haplotypes in which a variety of fascinating mutations have co-evolved (but are semi-refractory to traditional genetic analysis as a consequence of the chromosomal inversions) (Silver 1993), a collection of segmental deficiencies such as those described here are particularly valuable.

Most *t* haplotypes present in mice from around the world possess recessive lethal mutations that act early in development, many of which were characterized genetically and phenotypically decades ago (Bennett 1975). It has been hypothesized that such mutations were selectively maintained to counteract the deleterious population effects of homozygous male sterility (Lyon 1986). Unfortunately, the inversions that cause recombination suppression in *t*/+ mice have prevented cloning of any of these lethal mutations [*T*, a non-*t* haplotype mutation, was cloned many years ago (Herrmann et al. 1990)]. However, the rough location of some lethal mutations was determined by exploiting the fact that normal recombination occurs in fertile female mice carrying two different lethals, and that there is some (low) degree of polymorphism among *t* haplotypes (Artzt 1984).

Some of the better studied lethal alleles are t^{l2} , which blocks development at the morula stage; t^{w73} , resulting in faulty trophectoderm development; t^0 , which acts before mesoderm formation; t^{w5} , which affects embryonic ectoderm; and t^{w18} , which affects growth of the primitive streak cells and their transition to mesoderm, due to a failure to migrate and form proper interactions typical of a gastrulating embryo (Spiegelman and Bennett 1974). The collection of deletions described here should permit higher resolution mapping of these mutations, possibly leading to identification of the underlying genes. On the basis of existing mapping data, these *t* lethals lie within the regions covered by the *Sod2*, *D17Leh94*, or *Nkx2-5* deletions (Artzt 1984; Zwart et al. 2001).

The mapping and cloning of *t* haplotype sterility and transmission ratio distortion loci has been hampered similarly by the *t* haplotype inversions. This is particularly true for the large distal inversion, which contains the *t* complex sterility 2 (*tcs2*) and *t* complex distorter 2 (*Tcd2*) genes. Given that the proximally located *tcs1* and *Tcd1* are loss-of-function alleles (Bennett and Artzt 1990; Lyon 1992) and that this fact was exploited to fine map them by using deletions centered at the *D17Aus9* and *T* loci (Lyon et al. 2000; Planchart and Schimenti 2001; Planchart et al. 2000), it is possible that *tcs2* and *Tcd2* can be further localized by using the *Sod2*, *D17Leh94*, and *Nkx2-5* deletion complexes.

Another unusual locus in the *t* complex that should benefit from the deletions described here is T-associated sex reversal (*Tas*). The *Tas* phenotype is manifested in mice carrying a Y Chr from the strain AKR (Y^{AKR}) on an otherwise predominantly C57BL/6J genetic background with one copy of the *Tas* locus deleted (Washburn et al. 1990, 2001; Washburn and Eicher 1983). The gonads of these genetic males become feminized (partly or entirely converted to ovarian tissue). The phenomenon was discovered during the course of studies involving the deletion Chr T^{hp} , but is also caused by the T^{Orl} deletion. Thus haploinsufficiency is at the root

of the sex reversal. Accordingly, the collection of proximal *t* complex deletions with nested breakpoints are uniquely suited for better localizing *Tas*.

The subset of newly created *Sod2* deletions was exploited to reduce considerably the critical region containing the *Thl1* locus to approximately 1.3 Mbp from approximately 3 Mbp. This contains only three known genes—TATA box binding protein (*Tbp*, encoding TFIID), programmed cell death 2 (*Pdcd2*), and chromodomain helicase DNA binding protein 1 (*Chd1*)—plus two spliced mouse ESTs (according to the UCSC mouse genome browser). There are also several Genescan gene predictions. A targeted disruption of one of these genes (*Tbp*) has been created that shows no haploinsufficient phenotype (Martianov et al. 2002). These observations have two implications. First, the likelihood has increased that the haplolethality may, in fact, be attributable to a single gene, rather than from concurrent loss of multiple genes, i.e., a contiguous gene syndrome. Second, the low gene density of the critical region may simplify identification of *Thl1*.

The ES cell irradiation approach was used previously to create nested sets of deletion complexes on proximal Chr 5 (Schimenti et al. 2000). This report further confirms the utility and power of this approach for creating comprehensive deletion sets across the mouse genome. Given the availability of the Delbank resource of “irradiation ready” ES clones (Goodwin et al. 2001), and likely creation of gene trap libraries containing *loxP* sites useful for deletion induction by chromosome engineering (Su et al. 2000), it is now feasible and practical to consider constructing a mouse equivalent of the *Drosophila* deficiency kits.

Additional comments

Primer pairs defining microsatellite markers designed for this manuscript have been submitted to GenBank under Accession Numbers BV079472 to BV079564 and to the Mouse Genome Database under Accession Number J:84523. Extant clones and mouse strains can be obtained by emailing J. Schimenti at jcs@jax.org. This manuscript represents the integration of large amounts of data from our laboratory and from other sources. Although this manuscript represents a “snapshot” of our current efforts at integrating these data, some errors may exist. Maps are subject to change in light of new evidence.

Barbara Lee, whose contribution to this paper was the genetic map of Chr 17, passed away unexpectedly on December 6, 2000. Barbara also was a significant contributor to many other efforts in the Eicher laboratory. Her ready smile and ever-present wit made her a friend to all. She is dearly missed.

Acknowledgements

The authors thank Dr. Richard P. Harvey (Victoria, Australia) for the *Mx2-5* genomic clone. The authors also thank Leona Gagnon for assistance with genetic crosses and Drs. Greg Cox, Tim O'Brien, and Beverly Richards-Smith of The Jackson Laboratory for critical review of the manuscript. This work was supported by NIH grants HD24374 to J.C. Schimenti, GM20919 and RR01183 to E.M. Eicher and CA34196 to The Jackson Laboratory. Private sequence data were obtained through a subscription to the Celera Discovery System purchased by The Jackson Laboratory.

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Fig. 1. Deletion map of Mouse Chromosome 17. Figure 1 summarizes our collection of Chr 17 deletions. Megabase positions from the centromeric end of the chromosome are shown across the top of the figure. Celera scaffolds are shown as alternating light and dark gray rectangles with truncated scaffold names (GA_x6K02T2...). Scaffold axis positions are shown within yellow boxes below each scaffold name. Genetic markers (*D17*...) and genes are shown within columns (newly designed microsatellite markers, black; MIT markers, blue; genes, green; deletion focal points, red). When two (or more) loci are shown in one column, the more proximal locus appears lower in the column. Deletion sets are shown as groups of horizontal lines (those centered on *D17Aus9*, green; *T*, orange; *Sod2*, purple; *D17Leh94*, blue; *Nkx2-5*,

yellow). The regions of uncertainty for each deletion breakpoint are shown as gray rectangles. All deletions are named along the left side of the figure with the exception of the *Nkx2-5* deletions, which are labeled on the right. Deletions that have been recovered in mice (✓) are given a stock designation. Those in which transmission has not been confirmed (U) are labeled by ES cell designation. Genotyping results are shown for each deletion (heterozygosity, O; hemizyosity, X). With the exception of *T* (a dominant, visible phenotype), defined genes were not scored for presence/absence). The thick vertical line in the center of the figure (near the 17-Mbp mark) separates genotyping done on *D17Aus9* cell lines from genotyping performed on *Nkx2-5* cell lines. The exact location of some loci along the Celera axis could not be determined. The approximate location of these loci (designated •) was determined by other means (*i.e.*, consistency with the deletion and recombinational maps). The extent of the *Thl1*-critical region is shown as a light blue rectangle at the bottom of the figure.

Fig. 2.

Targeting of the *Nkx2-5* genomic locus. The uppermost horizontal line shows a schematic representation of the *Nkx2-5* genomic locus. The center horizontal line shows the structure of the targeting construct with the inserted *tk* and *Neo* genes. Homologous recombination at the left and right arms is shown by two $\times$ s. The lowest horizontal line shows the properly targeted locus. A 1-kilobase scale bar and the location of a 5' probe are shown. B, *Bam*HI; Bg, *Bgl*II; E, *Eco*RI; H, *Hin*DIII; Xb, *Xba*I.

Fig. 3.

Composite map of mouse Chromosome 17. Shown are genetic, deletion, and three sequence (Celera, Ensembl, and the similar UCSC) maps of the proximal region of Chr 17. The intersecting lines near the proximal (top) end of the chromosome between the Celera map and the Ensembl map highlight an apparently artifactual inversion in the Ensembl/UCSC assembly. Scale bars for each map are shown at the bottom of the figure.

Table 1

Deletion transmission from chimeras *Strain of dams*

Deletion	Germline chimeras (N)	Strain of dams				
		C57BL/6J		C3H/HeOlaJ		
		Agouti pups (%)	# Del progeny/total	Transmission (%)	# Del progeny/total	Transmission (%)
<i>Sod2</i> ^{del1J}	5	50.0	12/57	21.1	—	—
<i>Sod2</i> ^{del8J}	2	54.8	11/31	35.5	—	—
<i>Sod2</i> ^{del10J}	5	70.0	21/40	52.5	—	—
<i>Sod2</i> ^{del11J}	5	33.3	25/60	41.7	—	—
<i>Sod2</i> ^{del9J}	6	52.0	0/75	0	10/20	50
<i>Sod2</i> ^{del12J}	2	59.1	0/22	0	3/9	33

“Del” = deletion. Males carrying those deletions that could be transmitted to C57BL/6J were not mated to C3H females. The % agouti progeny reflects the number of progeny that are ES cell derived; since the parental ES cells are heterozygous for the recessive agouti mutation and the host blastocyst is C57BL/6J, maximally half of offspring can be expected to be agouti; thus, 50% agouti would indicate 100% ES cell-derived progeny. Note the possibility that not all chimeras transmitted the ES cell genome to all offspring.