The Albino-Deletion Complex of the Mouse: Molecular Mapping of Deletion Breakpoints That Define Regions Necessary for Development of the Embryonic and Extraembryonic Ectoderm

Shyam K. Sharan,* Bernadette Holdener-Kenny,* Siegfried Ruppert,† Andreas Schedl,† Gavin Kelsey,† Eugene M. Rinchik‡ and Terry Magnuson*

**Department of Genetics, Case Western Reserve University, Cleveland, Ohio 44106, †Institute of Cell and Tumor Biology, German Cancer Research Center, 6900 Heidelberg, Federal Republic of Germany, and ‡Biology Division, Oak Ridge National Laboratory, Oak Ridge, Tennessee 37831*

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ABSTRACT

Previous complementation analyses with five (c^{11DSD}, $c^{5FR60Hg}$, c^{2YPSj}, $c^{4FR60Hd}$, c^{6H}) of the mouse albino deletions defined at least two genes on chromosome 7, known as *eed* and *exed*, which are necessary for development of the embryonic and extraembryonic ectoderm, respectively, of early postimplantation embryos. The region of chromosome 7 containing these two genes has now been accessed at the molecular level by cloning two of the deletion breakpoint-fusion fragments. The c^{2YPSj} breakpoints were isolated by cloning an *Eco*RI fragment containing a copy of an albino region-specific repeat unique to c^{2YPSj} DNA. Similarly, the c^{11DSD} breakpoints were isolated by cloning a c^{11DSD} *Eco*RI fragment detected by a unique-sequence probe mapping proximal to the albino-coat-color locus. By mapping the cloned breakpoints relative to the remaining three deletions, the c^{11DSD} distal breakpoint was found to define the distal limit of the region containing *eed*, whereas the c^{2YPSj} and c^{6H} distal breakpoints were found to define the proximal and distal limits, respectively, of the region containing *exed*.

THE albino-deletion complex represents a region in the mouse genome where a set of specific developmental defects is associated with a series of overlapping chromosomal deletions (GLUECKSOHN-WAELSCH 1979, 1987; RINCHIK and RUSSELL 1990). More than 37 deletions exist, all of which remove varying amounts of chromosome 7 that surround and include the albino-coat-color locus (GLUECKSOHN-WAELSCH *et al.*, 1974; RUSSELL, RUSSELL and KELLY 1979; RUSSELL, MONTGOMERY and RAYMER 1982).

Embryological and complementation analyses of a subset of these deletions (c^{11DSD}, $c^{5FR60Hg}$, c^{2YPSj}, $c^{4FR60Hd}$ and c^{6H}) showed that all five remove a gene(s) (*eed*) needed for development of the embryonic ectoderm (LEWIS, TURCHIN and GLUECKSOHN-WAELSCH 1976; NISWANDER *et al.* 1988, 1989). This locus was defined primarily by the inability to establish embryonic stem cell lines from embryos homozygous for the deletions. Two of these deletions ($c^{4FR60Hd}$, c^{6H}) also appear to remove an additional gene(s) (*exed*) needed for development of the extraembryonic ectoderm (LEWIS, TURCHIN and GLUECKSOHN-WAELSCH 1976; NISWANDER *et al.* 1988, 1989). This was defined by a complete lack of extraembryonic structures in $c^{4FR60Hd}$ and c^{6H} homozygotes, and extensive development of these structures in c^{11DSD}, $c^{5FR60Hg}$ and c^{2YPSj} homozygotes. Furthermore, the c^{11DSD}, $c^{5FR60Hg}$ and c^{2YPSj} deletion chromosomes were capable of complementing the $c^{4FR60Hd}$ and c^{6H} chromosomes by allowing for development of the extraembryonic structures in the compound heterozygotes (NISWANDER *et al.* 1988, 1989). Thus, based on genetic data, we proposed that the c^{11DSD}, $c^{5FR60Hg}$ and c^{2YPSj} distal breakpoints lie more proximal than those of the $c^{4FR60Hd}$ and c^{6H} deletions (NISWANDER *et al.* 1988, 1989). The area of overlap between the two sets of deletions distal to the c^{3H} breakpoint would thus define the region containing *eed* (see Figure 1), whereas the area of nonoverlap between the two sets of deletions would define the region containing *exed* (see Figure 1). Each of these loci is defined by the phenotype exhibited by deletion homozygotes. In reality, these phenotypes could be the result of deletion of one important gene or a combinatorial effect of the loss of more than one gene.

To obtain molecular markers within the albino region, a partial genomic library of the distal region of chromosome 7 was produced using the techniques of chromosome microdissection and microcloning (NISWANDER *et al.* 1991). One microclone (palb18) was shown to define a locus, *D7Cw18*, mapping to a region of chromosome 7 proximal to *c* that is removed by c^{11DSD} deletion but not by the $c^{5FR60Hg}$, c^{2YPSj}, $c^{4FR60Hd}$ or c^{6H} deletions. Thus, *D7Cw18* and *eed/exed* map to opposites sides of the deletion chromosomes (see Figure 1), and it was clear that cloning of one or more of the deletion breakpoint-fusion fragments would be needed to provide molecular access to the region of chromosome 7 containing *eed* and *exed*.

In an attempt to walk toward the c^{11DSD} breakpoint, a genomic library was screened with palb18, and an albino-region-specific repeat sequence was identified (NISWANDER *et al.* 1991). Mapping studies localized the repeat sequence primarily to the region of chromosome 7 covered by the proximal portion of the c^{11DSD} deletion. Because the albino-region-specific repeat hybridized differentially to $c^{5FR60Hg}$, c^{2YPSj}, $c^{4FR60Hd}$ and c^{6H} deletion DNA, it was possible to define the order of the proximal breakpoints by examining Southern blot banding patterns (see Figure 1, for map) (NISWANDER *et al.* 1991). This order was subsequently confirmed with single-copy sequence probes (NISWANDER *et al.* 1991).

Although chromosome microdissection was successful in providing molecular markers localized to the proximal region, previous genetic and embryological studies positioned *eed* and *exed* on the distal side of the deletions (LEWIS, TURCHIN and GLUECKSOHN-WAELSCH 1976; NISWANDER *et al.* 1988, 1989). To gain access to the distal side of the deletions, we now report the cloning of the c^{2YPSj} and the c^{11DSD} breakpoint-fusion fragments by isolating unique restriction fragments associated with these deletions. The c^{2YPSj}- and the c^{11DSD}-specific fragments were detected, respectively, by the albino-region-specific-repeat probe and a single-copy probe (pv0.4AP) isolated after chromosomal walking and jumping from *D7Cw18*. Isolation of these breakpoints provided molecular access to the distal side of the deletions and has allowed us to define more precisely the proximal and distal limits of the region of chromosome 7 containing *exed* and *eed*.

MATERIALS AND METHODS

Mice

Deletion mice: Seven albino-deletion stocks [designated *Df(c)*] were used in these experiments. The c^{6H} and c^{3H} mice originated at the MRC Radiobiology Unit, Harwell, England, and were obtained from S. GLUECKSOHN-WAELSCH (Albert Einstein College of Medicine) (GLUECKSOHN-WAELSCH *et al.* 1974). The c^{11DSD}, $c^{5FR60Hg}$, c^{2YPSj}, $c^{4FR60Hd}$ and c^{14CoS} mice originated at the Oak Ridge National Laboratory (RUSSELL, RUSSELL and KELLY 1979). All mice have been maintained as closed colony, heterozygous stocks with chinchilla (c^{ch}). All $Df(c)/c^{ch}$ stocks are a dilute chinchilla coat color as compared to a full chinchilla color evident in c^{ch}/c^{ch} mice.

***Mus spretus/Mus musculus* interspecies cross:** Wild-type (nondeletion) *M. spretus* males were crossed with $Df(c)/c^{ch}$ *M. musculus* females. To determine which of the phenotypically wild-type F_1 progeny carried the deletion chromosome rather than the c^{ch} chromosome, progeny testing was carried out as described by JOHNSON, HAND and RINCHIK (1989). For mapping of *D7Cw2D* or *D7Cw11D*, high molecular weight DNA was prepared from spleen or liver of appropriate F_1 progeny along with their $Df(c)/c^{ch}$ dams and *M. spretus* sires as previously described (JOHNSON, HAND and RINCHIK 1989).

Genomic clones

λ14RIc2 represents a 14-kb *Eco*RI fragment isolated from a c^{2YPSj}/c^{3H} subgenomic library cloned into *lambda* Dash (Stratagene). The library was prepared from 60 μg of DNA digested to completion with *Eco*RI followed by size fractionation on a sucrose gradient (AUSUBEL *et al.* 1989). DNA in the 12–15-kb range was collected and concentrated (Centricon 30 filter, Amicon). Insert DNA (1 μg) was ligated (15° for 12 hr) to 1.5 μg *lambda* Dash vector arms using T4 DNA ligase (Boehringer Mannheim) and then packaged *in vitro* (Promega packaging extracts). *Escherichia coli* LE392 was infected with packaged phage. To identify positive clones, plaque lifts were hybridized at 65° in CHURCH (1984) buffer with ^{32}P-labeled 1.4-kb *Eco*RI-*Xba*I fragment of pTME3a containing the albino-region-specific repeat sequence (NISWANDER *et al.* 1991). Washes were done in 40 mM NaPi, pH 7.2, 1 mM EDTA, 100 mM NaCl, and 1% SDS at 63°, 4 times for 15 min each. For mapping purposes, *Rsa*I- or *Hae*III-digested phage DNA was used as template to generate ^{32}P-labeled transcripts with T3 and T7 RNA polymerases (Boehringer Mannheim), respectively, according to the procedures outlined by Stratagene.

p7.5(X/RI) represents a 7.5-kb *Xba*I-*Eco*RI fragment from λ14RIc2 subcloned into pBS +/− (Stratagene). T7 riboprobes were generated from *Rsa*I-linearized plasmid DNA.

pv0.4AP represents a 0.4-kb *Asp*718-*Pst*I fragment subcloned into Bluescript (Stratagene). The insert was derived from a λ phage (λV31) which was obtained after walking and jumping from *D7Cw18* (A. SCHEDL, S. RUPPERT, G. KELSEY, E. THIES, L. NISWANDER, T. MAGNUSON and G. SCHUTZ, manuscript in preparation).

λE10.5c11 was isolated from a c^{11DSD}/c^{3H} subgenomic library screened with pv0.4AP. The library was prepared from size selected DNA (10–12 kb range) as described for λ14RIc2.

pE10.5c11 represents a 10.5-kb *Eco*RI pBS +/− subclone from λE10.5c11.

pA4.2c11 represents a 4.2-kb *Asp*718 fragment from pE10.5c11 subcloned into pBS.

p77-2 represents a 1.0-kb *Eco*RI fragment isolated from a partial genomic library generated by microdissection of chromosome 7 (NISWANDER *et al.* 1991). This insert was shown by NISWANDER *et al.* (1991) to detect a genomic sequence that maps outside of the region of chromosome 7 removed by the albino deletions.

pH2.2H represents a 2.2-kb *Hin*dIII genomic fragment isolated from p186$^+$ (obtained from CHRISTO GORIDIS, Centre d'immunologie, de Marseille-Luminy). p186$^+$ contains a portion of the gene which encodes the neural cell adhesion molecule (NCAM). The NCAM gene has been mapped to mouse chromosome 9 (D'EUSTACHIO *et al.* 1985; N'GUYEN *et al.* 1986).

RESULTS

Cloning of the c^{2YPSj} deletion breakpoint-fusion fragment: The albino-region-specific repeat probe (pTM3Ea1.4) was previously found to detect a complex pattern of bands when hybridized to *Dra*I-digested nondeleted DNA (NISWANDER *et al.* 1991). By examining the repeat pattern associated with five of the albino deletions, the proximal breakpoint order was determined to be c^{11DSD} proximal to c^{2YPSj}, which is proximal to $c^{4FR60Hd}$ and $c^{5FR60Hg}$, which are proximal

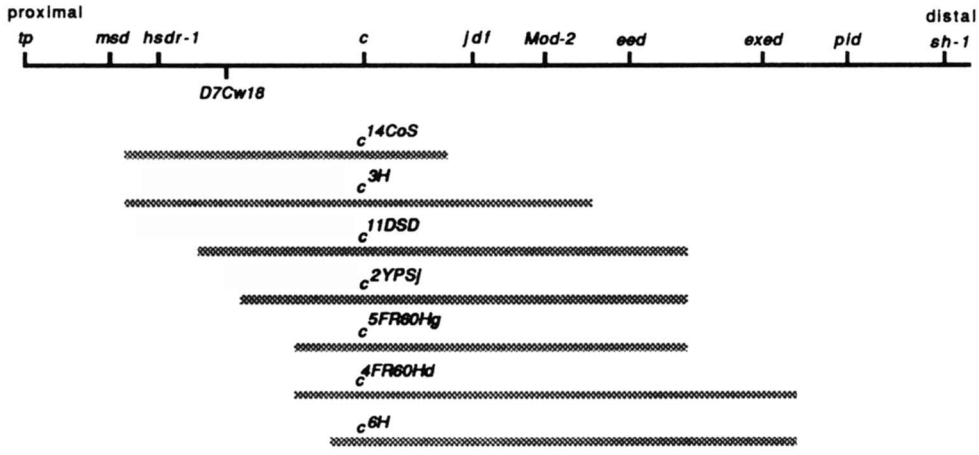

FIGURE 1.—Complementation map of the albino deletions used in this work. This map is modified from published work (NISWANDER *et al.* 1991). Deleted regions are represented by stippled lines. Although the exact positions of the chromosomal breakpoints are not known, the approximate lengths of the deletions with respect to the genetic map are based on complementation analyses (EICHER *et al.* 1978; GLUECKSOHN-WAELSCH *et al.* 1974; RUSSELL, MONTGOMERY and RAYMER 1982; NISWANDER *et al.* 1988, 1989). Marker loci include *tp* (taupe), *c* (albino), *Mod-2* (mitochondrial form of malic enzyme), *sh-1* (shaker-1). *D7Cw18* (formerly called *D7TM18*) is a locus defined by a cloned fragment (NISWANDER *et al.* 1991). The following loci are defined by complementation and embryological analyses using the various albino deletions: *msd*: mesoderm deficient, formerly designated implantation-survival region (RUSSELL, MONTGOMERY and RAYMER 1982; B. HOLDENER-KENNY and T. MAGNUSON, unpublished results), *hsdr-1*: hepatocyte-specific developmental regulator, formerly designated perinatal survival region (GLUECKSOHN-WAELSCH 1987; GLUECKSOHN-WAELSCH *et al.* 1974; MCKNIGHT, LANE and GLUECKSOHN-WAELSCH 1989; RUSSELL, MONTGOMERY and RAYMER 1982), *jdf*: juvenile development and fertility, formerly designated juvenile survival region (RUSSELL, MONTGOMERY and RAYMER 1982), *eed*: embryonic ectoderm development, formerly designated embryonic ectoderm region (NISWANDER *et al.* 1988; 1989), *exed*: extraembryonic ectoderm development, formerly designated extraembryonic ectoderm region (NISWANDER *et al.* 1988, 1989), *pid*: preimplantation development, formerly designated preimplantation survival region (GLUECKSOHN-WAELSCH *et al.* 1974; LEWIS 1978; NADIJCKA, HILLMAN and GLUECKSOHN-WAELSCH 1979; RUSSELL, MONTGOMERY and RAYMER 1982). The actual phenotype resulting from the deletion of any of these loci may be due to the removal of more than one gene. Symbols above the stippled lines represent the name of the deletion chromosomes.

to c^{6H} (Figure 1) (NISWANDER *et al.* 1991). Subsequent to this work, the albino-region-specific repeat probe was hybridized to a panel of *Eco*RI-digested deletion DNAs. A 14-kb fragment was detected in c^{2YPSj}/c^{3H} DNA (Figure 2, lane 6, band marked A). This fragment was not detected in c^{ch}/c^{ch} (lane 1), c^{3H}/c^{3H} (lane 2), c^{6H}/c^{3H} (lane 3), $c^{5FR60Hg}/c^{3H}$ (lane 4), $c^{4FR60Hd}/c^{3H}$ (lane 5), c^{11DSD}/c^{14CoS} (lane 7), or c^{14CoS}/c^{14CoS} (lane 8) DNA. This aberrant fragment is not the result of a single *Eco*RI polymorphism associated with the c^{2YPSj} chromosome since several other restriction enzymes (*Hin*dIII, *Xba*I, *Pst*I, *Sac*I) also produced an altered fragment (data not shown). Given the proposed relative order of the proximal breakpoints, it should not be possible to detect a fragment present in c^{2YPSj} DNA that is absent in $c^{4FR60Hd}$, $c^{5FR60Hg}$, c^{6H} or c^{ch} DNA, provided the deletions are linear. For this reason, we hypothesized that the altered fragment contained the c^{2YPSj} deletion breakpoint-fusion fragment.

To determine if the aberrant fragment contains the c^{2YPSj} deletion junction, a sub-genomic library was generated from 12–15-kb size-selected c^{2YPSj}/c^{3H} *Eco*RI-digested DNA. Plaques (1×10^5) were screened with the albino-region-specific-repeat probe and two of the recombinant clones that hybridized with the repeat probe were analyzed. The first clone was rearranged and was not analyzed further. The second clone, λ14RIc2, contained a 14-kb *Eco*RI insert. End-specific riboprobes were hybridized to c^{ch}/c^{ch}, c^{3H}/c^{3H} and c^{2YPSj}/c^{3H} DNA (Figure 3, B and D). If the 14-kb fragment carried the deletion breakpoint-fusion fragment, we expected the proximal end to be present in the nondeleted and c^{2YPSj} DNAs and deleted from the c^{3H} DNA. In contrast, the distal end should be present in all three chromosomes (Figure 3A). The T7 riboprobe detected a 6-kb band when hybridized with c^{ch}/c^{ch} (nondeleted) *Eco*RI-digested DNA (Figure 3B, lane 1), and the aberrant 14-kb band when hybridized with c^{2YPSj}/c^{3H} *Eco*RI-digested DNA (Figure 3B, lane 3). No signal was detected when the T7 riboprobe was hybridized with c^{3H}/c^{3H} *Eco*RI-digested DNA (Figure 3B, lane 2). In contrast, the T3 riboprobe hybridized to a 9-kb band in nondeleted (c^{ch}/c^{ch}) (Figure 3D, lane 1) and c^{3H}/c^{3H} (Figure 3D, lane 2) *Eco*RI-digested DNAs, and to both the 9-kb and the aberrant 14-kb band in c^{2YPSj}/c^{3H} *Eco*RI-digested DNA (Figure 3D, lane 3). Rehybridization of the blots with a probe (p77-2) located outside of the deletion complex served as a loading control (Figure 3, C and E). These results are consistent with the hypothesis that λ14RIc2 contains the c^{2YPSj} deletion breakpoint-fusion fragment. From these data, it can be concluded that the λ14RIc2 DNA sequence included in the T7 riboprobe is homologous to a genomic locus (*D7Cw2P*) that lies on the proximal

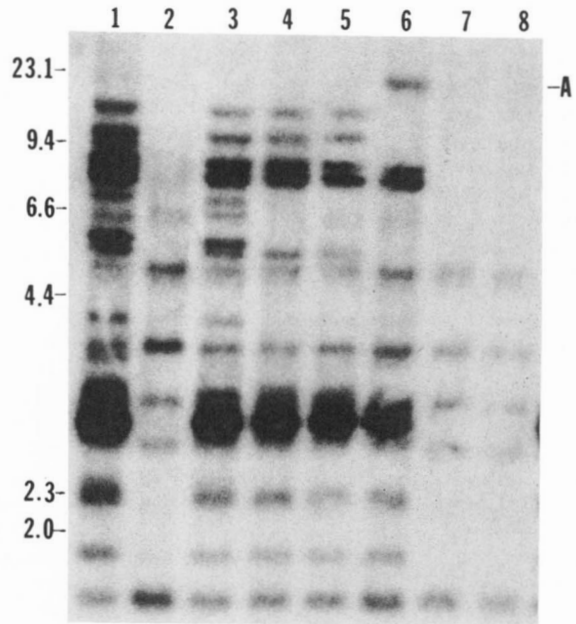

FIGURE 2.—Detection of the c^{2YPSj} breakpoint-fusion fragment. Southern blot analysis of *Eco*RI-digested DNA hybridized with pTME3a1.4. Lanes **1–8** represent DNA from the following animals: (**1**) nondeleted c^{ch}/c^{ch} (originated from the Oak Ridge colony) (**2**) c^{3H}/c^{3H}; (**3**) c^{6H}/c^{3H}; (**4**) $c^{5FR60Hg}/c^{3H}$; (**5**) $c^{4FR60Hd}/c^{3H}$; (**6**) c^{2YPSj}/c^{3H}; (**7**) c^{11DSD}/c^{14CoS}; (**8**) c^{14CoS}/c^{14CoS}. The relative position of molecular mass markers (kb) is designated on the left. The symbol "**A**" denotes the 14-kb *Eco*RI fragment detected specifically in c^{2YPSj}/c^{3H} DNA. For hybridization, a 1.4-kb *Eco*RI-*Xba*I fragment of pTM3Ea1.4 was isolated from low melting agarose gel and radiolabeled by random hexamer priming (FEINBERG and VOGELSTEIN 1984). Hybridization and wash conditions are as described in MATERIALS AND METHODS.

side of the c^{2YPSj} proximal breakpoint (Figure 3A). In contrast, the λ14RIc2 DNA sequence included in the T3 riboprobe is homologous to a genomic locus (*D7Cw2D*) located on the distal side of the c^{2YPSj} distal breakpoint (Figure 3A).

Ordering of the distal breakpoints relative to *D7Cw2D*: *D7Cw2D* provides molecular access to the general region of chromosome 7 containing *eed* and *exed*. To determine the molecular limits of the regions containing these loci, it was necessary to map the distal breakpoints of the c^{11DSD}, $c^{5FR60Hg}$, $c^{4FR60Hd}$ and c^{6H} deletions relative to *D7Cw2D*. A 7.5-kb *Xba*I-*Eco*RI fragment from the distal side of clone λ4RIc2 was subcloned into pBS +/−. A T7 riboprobe prepared from this template detects an *Eco*RI polymorphism in nondeleted DNA of different backgrounds suitable for mapping the position of the deletion breakpoints relative to *D7Cw2D*. For example, a 9-kb fragment (Figure 3D, lane 1) was detected with nondeleted DNA from c^{ch}/c^{ch} mice obtained from the Albert Einstein colony. In contrast, a 16-kb fragment was detected (Figure 4, lane 1) with non-deleted DNA from c^{ch}/c^{ch} mice obtained from the Oak Ridge colony. The probe detects a 9-kb fragment with c^{14CoS}/c^{14CoS} DNA (Figure 4, lane 2). With DNA from c^{11DSD}/c^{14CoS} compound heterozygotes, both the c^{14CoS}-associated 9-kb fragment as well as a 16-kb fragment derived from the c^{11DSD} chromosome was detected (Fig. 4, lane 3). The presence of the c^{11DSD}-associated fragment indicates that *D7Cw2D* lies distal to the c^{11DSD} deletion. Likewise, the presence of both a 16-kb c^{ch}-associated and a 9-kb $c^{5FR60Hg}$-associated fragment in $c^{ch}/c^{5FR60Hg}$ (Figure 4, lane 4), and in $c^{ch}/c^{4FR60Hd}$ (Figure 4, lane 7) DNAs, indicates that *D7Cw2D* also lies distal to the $c^{5FR60Hg}$ and $c^{4FR60Hd}$ deletions. In contrast, the presence of only the c^{ch}-associated 16-kb fragment in c^{6H}/c^{ch} DNA (Figure 4, lane 5) and the c^{14CoS}-associated 9-kb fragment in c^{6H}/c^{14CoS} DNA (Figure 4, lane 6) indicates that *D7Cw2D* is deleted from the c^{6H} chromosome. These results were confirmed with restriction fragment length variant mapping using *M. spretus*-balanced *Df(c)* chromosomes (data not shown).

From these data, it can be concluded that the c^{2YPSj} distal breakpoint lies distal to c^{11DSD}, $c^{5FR60Hg}$ and $c^{4FR60Hd}$ but proximal to c^{6H} (see Figure 7). Furthermore, they establish the c^{2YPSj} and c^{6H} distal breakpoints as the proximal and distal limits of the region of chromosome 7 containing the *exed* gene. Placement of the c^{2YPSj} distal breakpoint distal to that of $c^{4FR60Hd}$ is in direct contrast to the order suggested by our genetic data (NISWANDER *et al.* 1988, 1989). One possible explanation to explain the discrepancy is that the $c^{4FR60Hd}$ deletion is not linear.

Cloning of the c^{11DSD} breakpoint-fusion fragment: Based on the above results, the distal limits of the region of chromosome 7 containing *eed* is defined by the distal breakpoint of either the c^{11DSD}, $c^{5FR60Hg}$ or $c^{4FR60Hd}$ deletion. To determine the proximal-to-distal order of these breakpoints relative to one another, experiments were undertaken to clone the c^{11DSD} breakpoint-fusion fragment. Beginning with the *D7Cw18* locus, which is deleted from the c^{11DSD} chromosome (Figure 1), a combination of walking and jumping procedures were used to isolate the probe pv0.4AP (A. SCHEDL, S. RUPPERT, G. KELSEY, E. THIES, L. NISWANDER, T. MAGNUSON, and G. SCHUTZ, manuscript in preparation). The latter clone was found to contain an insert that detects a 7.4-kb *Eco*RI fragment in nondeleted c^{ch} DNA (Figure 5B, lanes 1 and 3), a 10.5-kb *Eco*RI fragment in c^{11DSD}/c^{14CoS} DNA (Figure 5B, lane 2) and no fragment in c^{14CoS}/c^{14CoS} DNA (Figure 5B, lane 4). Rehybridization of the same blot with a probe outside of the deletion is shown in Figure 5C as a loading control. The fact that pv0.4AP detects a 10.5-kb *Eco*RI fragment with c^{11DSD} DNA and a 7.4-kb *Eco*RI fragment with nondeleted DNA suggests that the c^{11DSD} fragment spans breakpoints. This hypothesis is supported by the observation that a c^{11DSD}-specific fragment is also detected with *Xba*I and *Pvu*II (A. SCHEDL, S. RUPPERT, G. KELSEY, E. THIES, L. NISWANDER, T. MAGNUSON, and G. SCHUTZ, manuscript in preparation).

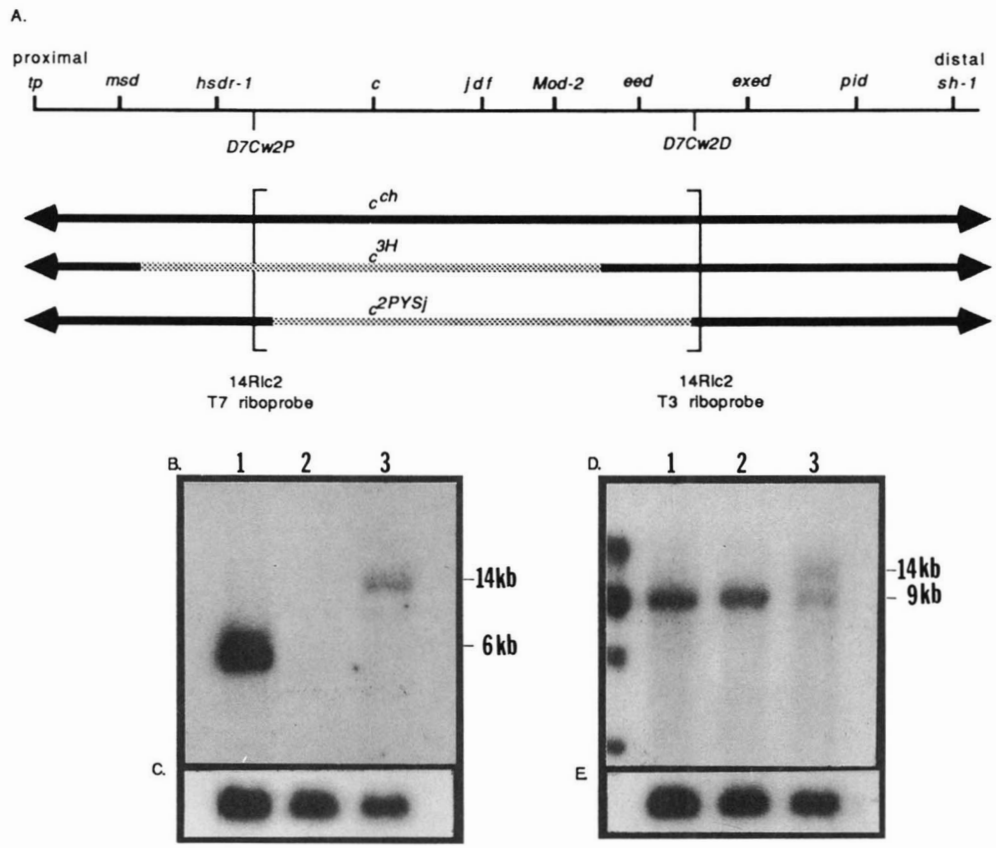

FIGURE 3.—Mapping of the c^{2YPSj} breakpoint-fusion fragment. Southern blot analysis of *Eco*RI-digested DNA hybridized with end-specific RNA probes generated from clone λ14RIc2. (**A**) Complementation map of the relevant chromosomes. The names of the marker loci are indicated in legend to Figure 1. c^{ch} represents a nondeleted chromosome. c^{3H} and c^{2YPSj} represent deletion chromosomes. In each case, the portion of the chromosome deleted is indicated by the stippled portion of the line. The vertical lines are schematic representations of the regions from each chromosome to which the T7 or T3 riboprobe would hybridize. (**B**) DNA hybridized with T7 riboprobe of λ14RIc2. (**C**) Rehybridization of (B) with the non-albino region probe p77-2. (**D**) DNA hybridized with T3 riboprobe of λ14RIc2. (**E**) Rehybridization of blot in (D) with p77-2. In both cases, lanes **1–3** represent DNA from the following animals; (**1**) nondeleted c^{ch}/c^{ch} (originated from the Albert Einstein colony) (**2**) c^{3H}/c^{3H}; (**3**) c^{2YPSj}/c^{3H}.

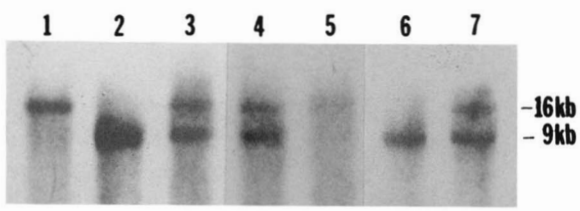

FIGURE 4.—Deletion breakpoint order relative to the c^{2YPSj} distal breakpoint. Southern Blot analysis of *Eco*RI-digested DNA hybridized with T7 end-specific riboprobe generated from p7.5(X/RI). Lanes **1–8** represent DNA from the following animals: (**1**) nondeleted c^{ch}/c^{ch} (originated from the Oak Ridge Colony) (**2**) c^{14CoS}/c^{14CoS}, (**3**) c^{11DSD}/c^{14CoS}, (**4**) $c^{5FR60Hg}/c^{ch}$, (**5**) c^{6H}/c^{ch}, (**6**) c^{6H}/c^{14CoS}, (**7**) $c^{4FR60Hd}/c^{ch}$.

To determine if pv0.4AP is detecting a DNA fragment containing the c^{11DSD} deletion breakpoint, a subgenomic library was generated from 10–12-b size-selected c^{11DSD}/c^{3H} *Eco*RI-digested DNA. Plaques (3×10^5) were screened, and one recombinant clone that hybridized with pv0.4AP was analyzed further. This lambda clone, λE10.5c11, contained a 10.5-kb *Eco*RI fragment from which a 4.2-kb *Asp*718 fragment was subcloned (pA4.2c11). End-specific riboprobes from this subclone were hybridized to *Asp*718-digested c^{ch}/c^{ch}, c^{14CoS}/c^{14CoS} and c^{11DSD}/c^{14CoS} DNA. A 7.2-kb band was detected when the T3 riboprobe was hybridized to c^{ch}/c^{ch} DNA (Figure 5D, lanes 1 and 4) and c^{14CoS}/c^{14CoS} DNA (Figure 5D, lane 2). When hybridized to c^{11DSD}/c^{14CoS} DNA, 4.2- and 7.2-kb bands were detected (Figure 5D, lane 3). These results are consistent with the 4.2-kb band being associated with the c^{11DSD} chromosome and the 7.2-kb band being generated by the c^{14CoS} chromosome.

From the mapping data, it can be concluded that pv0.4AP defines a genomic locus (*D7Cw11P*) that lies on the proximal side of the c^{11DSD} breakpoint (Figure 5A). In contrast, the pA4.2c11 sequence generating the T3 riboprobe is homologous to a genomic locus (*D7Cw11D*) that lies on the distal side of the c^{11DSD} distal breakpoint (Figure 5A).

Ordering of the distal breakpoints relative to *D7Cw11D*: To determine the distal limits of the region of chromosome 7 containing *eed*, it was necessary to

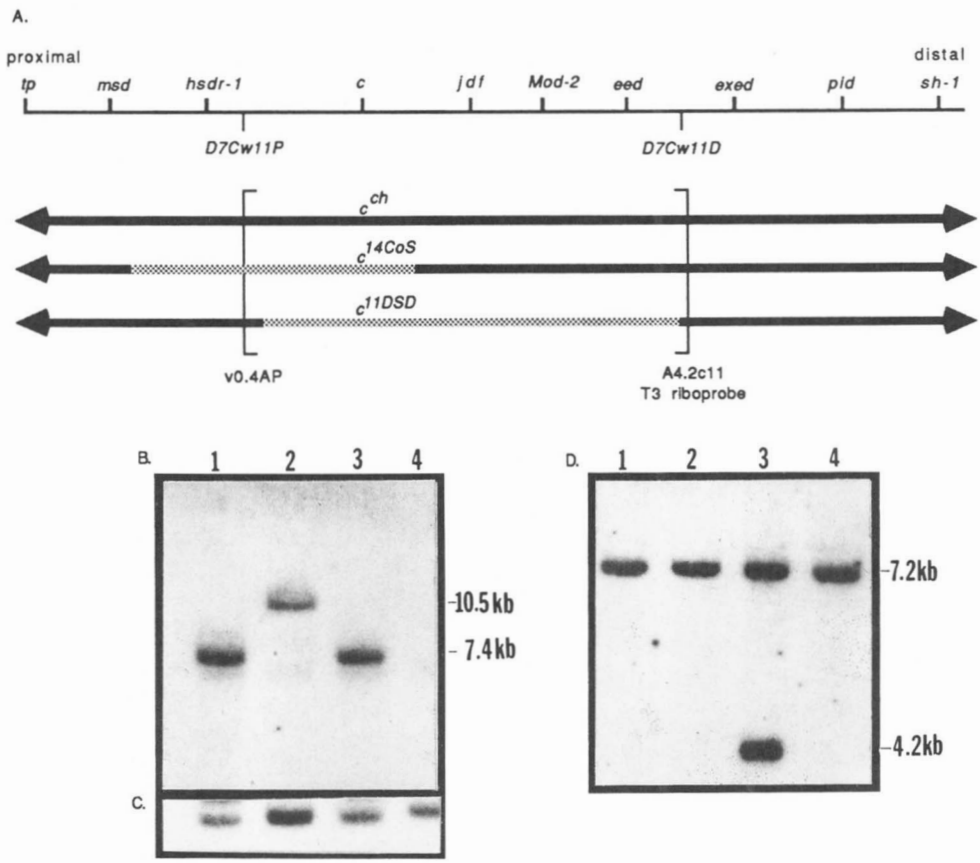

FIGURE 5.—Identification and mapping of the c^{11DSD} breakpoint-fusion fragment. (**A**) Complementation map of the chromosomes used in mapping the c^{11DSD} breakpoint-fusion fragment. The names of the marker loci are indicated in legend to Figure 1. c^{ch} represents a nondeleted chromosome. c^{14CoS} and c^{11DSD} represent deletion chromosomes. In each case, the portion of the chromosome deleted is indicated by the stippled portion of the line. The vertical lines are schematic representations of the regions from each chromosome to which the respective probes would hybridize. (**B**) Southern blot analysis of *Eco*RI-digested DNA hybridized with T3 riboprobe generated from *Bam*HI-digested pv0.4AP. Lanes **1–4** represent DNA from the following animals: (**1**) nondeleted c^{ch}/c^{ch} (originated from the Oak Ridge colony) (**2**) c^{11DSD}/c^{14CoS}, (**3**) nondeleted c^{ch}/c^{ch} (originated from the Albert Einstein colony) (**4**) c^{14CoS}/c^{14CoS}. (**C**) Rehybridization of the same blot shown in B with the chromosome 9-specific probe pH2.2H. (**D**) Southern blot analysis of *Asp*718-digested DNA hybridized with T3 end-specific riboprobe generated from pA4.2c11. Lanes **1–4** represent DNA from the following animals; (**1**) non-deleted c^{ch}/c^{ch} (originitated from the Oak Ridge colony) (**2**) c^{14CoS}/c^{14CoS}, (**3**) c^{11DSD}/c^{14CoS}, (**4**) nondeleted c^{ch}/c^{ch} (originated from the Albert Einstein colony).

map the distal breakpoints of the $c^{5FR60Hg}$ and $c^{4FR60Hd}$ deletions relative to *D7Cw11D*. To do so, pA4.2c11 was hybridized to *Taq*I-digested DNA from an F_1 hybrid that carried a *M. spretus* nondeleted chromosome 7 and either a *M. musculus* $c^{5FR60Hg}$ or a *M. musculus* $c^{4FR60Hd}$ deletion chromosome. A *Taq*I polymorphism was detected between the two species. A 9.5-kb fragment was present in *M. spretus* DNA (Figure 6, lane 1). The hybridization pattern for the *M. musculus* parental DNAs is shown in Figure 6 for $c^{ch}/c^{5FR60Hg}$ (lane 3) and $c^{ch}/c^{4FR60Hd}$ (lane 5). The *M. spretus/M. musculus* F_1 DNAs showed only the *M. spretus* 9.5-kb fragment (Figure 6, lanes 2 and 4). The absence of any *M. musculus*-specific fragment from either F_1 DNA indicates that genomic sequence homologous to the pA4.2c11 insert is removed both by the $c^{5FR60Hg}$ and $c^{4FR60Hd}$ deletions. Thus, the c^{11DSD} breakpoint, and therefore the *D7Cw11D* locus, lie proximal to the $c^{5FR60Hg}$ and $c^{4FR60Hd}$ distal breakpoints

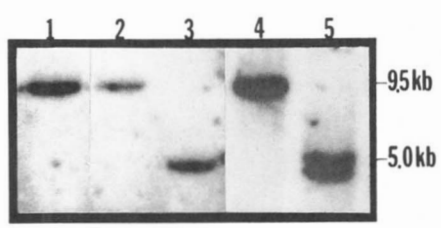

FIGURE 6.—Mapping of *D7Cw11D* relative to the $c^{5FR60Hg}$ and $c^{4FR60Hd}$ deletions. Southern blot analysis of *Taq*I-digested DNA. Lanes **1–5** represent DNA from the following animals: (**1**) *M. spretus/M. spretus*, (**2**) *M. spretus/M. musculus* ($c^{5FR60Hg}$), (**3**) *M. musculus* (c^{ch})/*M. musculus* ($c^{5FR60Hg}$), (**4**) *M. spretus/M. musculus* ($c^{4FR60Hd}$), (**5**) *M. musculus* (c^{ch})/*M. musculus* ($c^{4FR60Hd}$). Lanes **1-3** were hybridized with T3 end-specific riboprobe generated from pA4.2c11 linearized with *Rsa*I or *Hae*III. Lanes **4-5** were from a separate blot that was hybridized with random-primer ^{32}P-labeled *Asp*718 fragment from pA4.2c11. The Asp-718 fragment contains a *Taq*I site and therefore hybridizes to a 4.5- as well as 5.0-kb genomic fragment.

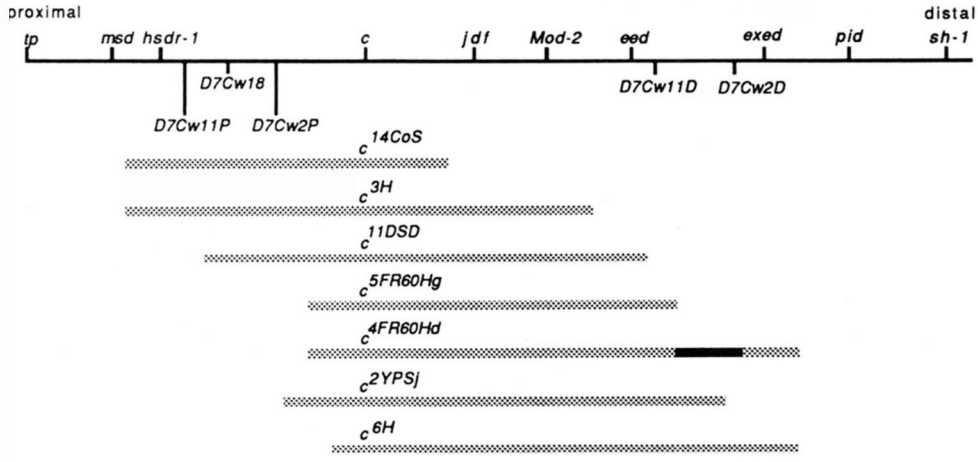

FIGURE 7.—Proximal and distal breakpoint order of the deletions used in this work. Deleted regions are represented by stippled lines and nondeleted regions are represented by solid lines. See legend of Figure 1 for details. Loci defined by complementation and embryological analyses are indicated above the chromosomal line, whereas loci defined by cloned DNA probes are indicated below the chromosomal line.

(Figure 7). Using similar mapping procedures, *D7Cw11D* also was found, as predicted, to be deleted from the c^{2YPSj} and c^{6H} deletions (data not shown). From these data, it can be concluded that the proximal-to-distal breakpoint order of the distal side of the deletions is c^{11DSD}–($c^{5FR60Hg}$ and $c^{4FR60Hd}$)–c^{2YPSj}–c^{6H} (Figure 7).

DISCUSSION

Molecular access to the *eed/exed* region of chromosome 7 was provided by cloning the c^{2YPSj} and c^{11DSD} breakpoints. Based on the distal breakpoint order predicted by our earlier genetic analyses (NISWANDER *et al.* 1988, 1989), *D7Cw2D* (genomic locus defined by the distal side of the c^{2YPSj} breakpoint) and *D7Cw11D* (genomic locus defined by the distal side of the c^{11DSD} breakpoint) should map to positions that lie between *eed* and *exed* (Figure 7). To confirm these predictions, the distal breakpoints of the c^{11DSD}, $c^{5FR60Hg}$, $c^{4FR60Hd}$, c^{2YPSj} and c^{6H} deletions were mapped relative to *D7Cw2D* and *D7Cw11D*. *D7Cw2D* was found to be deleted from c^{6H} but not from c^{11DSD}, $c^{5FR60Hg}$ or $c^{4FR60Hd}$. In contrast, *D7Cw11D* was deleted from $c^{5FR60Hg}$, $c^{4FR60Hd}$, c^{2YPSj} and c^{6H}. These results are in agreement with the prediction that *D7Cw2D* and *D7Cw11D* map between *eed* and *exed*; they also establish the relative proximal-to-distal order of the distal breakpoints as being c^{11DSD}–($c^{5FR60Hg}$ and $c^{4FR60Hd}$)–c^{2YPSj}–c^{6H}.

Placement of the $c^{4FR60Hd}$ distal breakpoint proximal to that of c^{2YPSj} is in direct contrast to the order suggested by our genetic data (NISWANDER *et al.* 1988, 1989). Embryos homozygous for the c^{2YPSj} deletion show the embryonic-ectoderm defect but not the extraembryonic-ectoderm defect. In contrast, $c^{4FR60Hd}$ homozygotes show a phenotype consistent with both *eed* and *exed* being deleted or inactivated. Furthermore, c^{2YPSj} can complement the extraembryonic defect by providing the wild-type copy of *exed* in c^{2YPSj}/$c^{4FR60Hd}$ compound heterozygotes. All of the genetic and embryological data are consistent with the $c^{4FR60Hd}$ distal breakpoint lying distal to the c^{2YPSj} distal breakpoint. Yet, our molecular data indicate that the $c^{4FR60Hd}$ breakpoint lies proximal to that of c^{2YPSj}.

One possible explanation for the discrepancy between the genetic and molecular data is that the $c^{4FR60Hd}$ deletion is discontinuous, thereby "skipping" and not deleting the region containing *D7Cw2D*. Precedent for radiation-induced, noncontinuous or "skipping" mutations can be found within the dilute-short ear-deletion complex of mouse chromosome *9* (RUSSELL 1971; RINCHIK *et al.* 1986). At least five of the dilute-short ear deletions appear to "skip" and inactivate genes on both sides of an active functional group. One possibility to explain the "skipping" is that *exed* is deleted. Alternatively, *exed* could be inactivated because of a position effect imposed upon the locus by the $c^{4FR60Hd}$ deletion. For example, the large chromosomal region between *hsdr-1* and *c* which is proximal to the $c^{4FR60Hd}$ deletion is known to be devoid of CpG islands and, therefore, might represent particularly inactive DNA (G. KELSEY, A. SCHEDL, S. RUPPERT, L. NISWANDER, T. MAGNUSON and G. SCHUTZ, manuscript in preparation). Thus, it is possible that the deletion could juxtapose some heterochromatin or silencing region next to *exed*. Another possibility is that the distal side of the $c^{4FR60Hd}$ deletion is associated with some sort of chromosomal rearrangement such as an inversion or translocation which breaks within *exed*, and thereby inactivates the locus. In any case, the molecular basis for the inactivation of *exed* by the $c^{4FR60Hd}$ deletion will only be resolved when the breakpoint-fusion fragment has been cloned and a physical map of the region is completed.

Ordering of the c^{11DSD}, $c^{5FR60Hg}$, c^{2YPSj} and c^{6H} distal breakpoints established the molecular limits of the *eed* and *exed* genes. The region of chromosome 7 containing the *eed* gene is bounded on the proximal side by the c^{3H} distal breakpoint and on the distal side by *D7Cw11D* (the c^{11DSD} distal breakpoint). The region containing the *exed* gene is delimited by the c^{2YPSj} deletion on the proximal side and the c^{6H} deletion on

the distal side. We have cloned two of the molecular markers delimiting the regions containing *eed* and *exed*. Future work will concentrate on the isolation of the c^{6H} and c^{3H} breakpoint-fusion fragments, establishment of a long-range physical map of the regions containing *eed* and *exed*, and jumping and/or walking through both regions using mouse jumping and YAC libraries. In addition, for the purpose of determining the number of genes present in these regions as well as isolating point mutations in *eed* and *exed*, saturation mutagenesis is underway (RINCHIK 1991).

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