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

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ABSTRACT

Previous complementation analyses with five (c11DSD, c5FR60Hg, c2YP5j, c6H, c5FR60Hd) of the mouse albino deletions (GLUECKSOHN-WAELEN 1979, 1987; RUSSELL, 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-WAELEN et al., 1974; RUSSELL, RUSSELL and KELLY 1979; RUSSELL, MONTGOMERY and RAYMER 1982).

Embryological and complementation analyses of a subset of these deletions (c11DSD, c5FR60Hg, c2YP5j, c6H, c5FR60Hd and c6H) showed that all five remove a gene(s) (eed) needed for development of the embryonic ectoderm (LEWIS, TURCHIN and GLUECKSOHN-WAELEN 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 (c5FR60Hd, c6H) also appear to remove an additional gene(s) (exed) needed for development of the extraembryonic ectoderm (LEWIS, TURCHIN and GLUECKSOHN-WAELEN 1976; NISWANDER et al. 1988, 1989). This was defined by a complete lack of extraembryonic structures in c5FR60Hd and c6H homozygotes, and extensive development of these structures in c11DSD, c5FR60Hg and c2YP5j homozygotes. Furthermore, the c11DSD, c5FR60Hg and c2YP5j deletion chromosomes were capable of complementing the c5FR60Hd and c6H 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 c11DSD, c5FR60Hg and c2YP5j distal breakpoints lie more proximal than those of the c5FR60Hd and c6H deletions (NISWANDER et al. 1988, 1989). The area of overlap between the two sets of deletions distal to the c6H 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 c11DSD deletion but not by the c5FR60Hg, c2YP5j, c6H or c5FR60Hd deletions. Thus, D7Cw18 and eed/exed map to opposite 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 c1IDS deletion breakpoint, a genomic library was screened with pAL18, 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 c1IDS deletion. Because the albino-region-specific repeat hybridized differentially to c1R00H7, c1R0YH4, and c1HI 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 edx 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 c1R0YH7 and the c1IDS breakpoint-fusion fragments by isolating unique restriction fragments associated with these deletions. The c1R0YH7 and the c1IDS-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 D7CuI8. 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 eed and edx.

MATERIALS AND METHODS

Mice

Deletion mice: Seven albino-deletion stocks [designated Df(c)/c"] were used in these experiments. The c1HI and c1HE 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 c1IDS, c1R00H7, c1R0YH4, and c1R0AI 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") inheritance and a single-copy probe (pv0.4AP) isolated after chromosomal walking and jumping from D7CuI8. 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 eed and edx.

Mus spretus/Mus musculus interspecies cross: Wild-type (nondeletion) M. spretus males were crossed with Df(c)/c HE M. musculus females. To determine which of the phenotypically wild-type F1 progeny carried the deletion chromosome rather than the c e chromosome, progeny testing was carried out as described by JOHNSON, HAND and RINCHIK (1989). For mapping of D7CuI2D or D7CuI1D, high molecular weight DNA was prepared from spleen or liver of appropriate F1 progeny along with their Df(c)/c HE dams and M. spretus sires as previously described (JOHNSON, HAND and RINCHIK 1989).

Genomic clones

λ14Ric2 represents a 14-kb EcoRI fragment isolated from a c1R0YH7/c1HE subgenomic library cloned into λ Dash (Stratagene). The library was prepared from 60 μg of DNA digested to completion with EcoRI followed by size fractionation on a sucrose gradient (AUSUBEL et al. 1989). DNA in the 12–15-kb range was collected and concentrated (Cen-trogen 30 filter, Amicon). Insert DNA (1 μg) was ligated (15 °C 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 °C in CHURCH (1984) buffer with α32P-labeled 1.4-kb EcoRI-XhoI 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 65 °C, four times for 15 min each. For mapping purposes, RsaI- or HaeIII-digested phage DNA was used as template to generate 32P-labeled transcripts with T3 and T7 RNA polymerases (Boehringer Mannheim), respectively, according to the procedures outlined by Stratagene.

p7.5(X/R1) represents a 7.5-kb XbaI-EcoRI fragment from λ14Ric2 subcloned into pBS +/- (Stratagene). T7 riboprobes were generated from RsaI-linearized plasmid DNA.

pv0.4AP represents a 0.4-kb Asp718-PstI fragment subcloned into Bluescript (Stratagene). The insert was derived from a λ phage (λV31) which was obtained after walking and jumping from D7CuI8 (A. SCHEIDL, S. RUPPERT, G. KELSEY, E. THIES, L. NISWANDER, T. MACNUSON and G. SCHUTZ, manuscript in preparation).

λE10.5c11 was isolated from a c1IDS/c1HE 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 EcoRI pBS +/- subclone from λE10.5c11.

pA4.2c11 represents a 4.2-kb Asp718 fragment from pE10.5c11 subcloned into pBS.

p77.2 represents a 1.0-kb EcoRI 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.

pH2.2HI represents a 2.2-kb HindIII 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 c1R0Y7 deletion breakpoint-fusion fragment: The albino-region-specific repeat probe (pTM3Es1.4) was previously found to detect a complex pattern of bands when hybridized to Dral-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 c1IDS proximal to c1R0YH7, which is proximal to c1R00H7 and c1R0YH4, which are proximal
to $c^H$ (Figure 1) (NISWANDER et al. 1991). Subsequent to this work, the albino-region-specific repeat probe was hybridized to a panel of EcoRI-digested deletion DNAs. A 14-kb fragment was detected in $c^{2PS} / c^H$ DNA (Figure 2, lane 6, band marked A). This fragment was not detected in $c^H / c^H$ (lane 1), $c^{3H} / c^H$ (lane 2), $c^H / c^H$ (lane 3), $c^{FR60Hd} / c^H$ (lane 4), $c^{FR60Hd} / c^H$ (lane 5), $c^{1DSD} / c^{4GAs}$ (lane 7), or $c^{4GAs} / c^{4GAs}$ (lane 8) DNA. This aberrant fragment is not the result of a single EcoRI polymorphism associated with the $c^{2PS}$ chromosome since several other restriction enzymes (HindIII, XbaI, PstI, SacI) 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 $c^{2PS}$ DNA that is absent in $c^{FR60Hd}$, $c^{FR60Hd}$, $c^H$ or $c^H$ DNA, provided the deletions are linear. For this reason, we hypothesized that the altered fragment contained the $c^{2PS}$ deletion breakpoint-fusion fragment.

To determine if the aberrant fragment contains the $c^{2PS}$ deletion junction, a sub-genomic library was generated from 12–15-kb size-selected $c^{2PS} / c^H$ EcoRI-digested DNA. Plaques ($1 \times 10^6$) 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, L14Rl2c2, contained a 14-kb EcoRI insert. End-specific riboprobes were hybridized to $c^H / c^H$, $c^{3H} / c^H$, and $c^{2PS} / c^H$ 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 $c^{2PS}$ DNAs and deleted from the $c^H$ 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 $c^H / c^H$ (nondeleted) EcoRI-digested DNA (Figure 3B, lane 1), and the aberrant 14-kb band when hybridized with $c^{2PS} / c^H$ EcoRI-digested DNA (Figure 3B, lane 3). No signal was detected when the T7 riboprobe was hybridized with $c^{3H} / c^H$ EcoRI-digested DNA (Figure 3B, lane 2). In contrast, the T3 riboprobe hybridized to a 9-kb band in nondeleted ($c^H / c^H$) (Figure 3D, lane 1) and $c^{3H} / c^H$ (Figure 3D, lane 2) EcoRI-digested DNAs, and to both the 9-kb and the aberrant 14-kb band in $c^{2PS} / c^H$ EcoRI-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 L14Rl2c2 contains the $c^{2PS}$ deletion breakpoint-fusion fragment. From these data, it can be concluded that the L14Rl2c2 DNA sequence included in the T7 riboprobe is homologous to a genomic locus (D7Cw2P) that lies on the proximal...
Southern blot analysis of EcoRI-digested DNA hybridized with cllDSD DNA. The relative position of molecular mass markers (kb) is designated on the left. The symbol "A" denotes the hexamer priming (FEINBERG and VOGELSTEIN 1984). Hybridization of a 1.4-kb EcoRI-XbnI fragment of pTM3Ea1.4 was isolated from low melting agarose gel and radiolabeled by random hexamer priming (FERNBERG and YOGELEIN 1984). Hybridization and wash conditions are as described in MATERIALS AND METHODS.

Figure 2.—Detection of the c^psj breakpoint-fusion fragment. Southern blot analysis of EcoRI-digested DNA hybridized with pTME5a1.4. Lanes 1–8 represent DNA from the following animals: (1) nondeleted c^a/c^a (originated from the Oak Ridge colony) (2) c^a/c^a, (3) c^a/c^a, (4) c^a/c^a, (5) c^a/c^b, (6) c^a/c^b, (7) c^b/c^a, (8) c^b/c^a. The relative position of molecular mass markers (kb) is designated on the left. The symbol "A" denotes the 14-kb EcoRI fragment detected specifically in c^psj/c^a DNA. For hybridization, a 1.4-kb EcoRI-XbaI fragment of pTME5a1.4 was isolated from low melting agarose gel and radiolabeled by random hexamer priming (FERNBERG and YOGELEIN 1984). Hybridization and wash conditions are as described in MATERIALS AND METHODS.

Side of the c^psj proximal breakpoint (Figure 3A). In contrast, the c^psj/c^a DNA sequence included in the T3 ribosome is homologous to a genomic locus (D7Cu2D) located on the distal side of the c^psj distal breakpoint (Figure 3A).

Ordering of the distal breakpoints relative to D7Cu2D: D7Cu2D provides molecular access to the general region of chromosome 7 containing c^a and c expend. To determine the molecular limits of the regions containing c^a, it was necessary to map the distal breakpoints of the c^b, c^b, c^a, c^a, c^a, c^a, and c^a deletions relative to D7Cu2D. A 7.5-kb XbaI-EcoRI fragment from the distal side of c^b was subcloned into pBS +/−. A T7 riboprobe prepared from this template detects an EcoRI polymorphism in nondeleted DNA of different backgrounds suitable for mapping the position of the deletion breakpoints relative to D7Cu2D. For example, a 9-kb fragment (Figure 3D, lane 1) was detected with nondeleted DNA from c^a/c^a 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^b/c^a mice obtained from the Oak Ridge colony. The probe detects a 9-kb fragment with c^a/c^a DNA (Figure 4, lane 2). With DNA from c^b/c^a compound heterozygotes, both the c^a-associated 9-kb fragment as well as a 16-kb fragment derived from the c^IDSD chromosome was detected (Figure 4, lane 3). The presence of the c^IDSD-associated fragment indicates that D7Cu2D lies distal to the c^IDSD deletion. Likewise, the presence of both a 16-kb c^a-associated and a 9-kb c^p^k^b^h^g^h^g^h^-associated fragment in c^a/c^p^k^b^h^g^h^g^h^- (Figure 4, lane 4), and in c^a/c^p^k^b^h^g^h^g^h^- (Figure 4, lane 7) DNAs, indicates that D7Cu2D also lies distal to the c^p^k^b^h^g^h^g^h^- and c^p^k^b^h^g^h^g^h^- deletions. In contrast, the presence of only the c^a-associated 16-kb fragment in c^a/c^a DNA (Figure 4, lane 5) and the c^a-associated 9-kb fragment in c^a/c^a DNA (Figure 4, lane 6) indicates that D7Cu2D is deleted from the c^a 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^psj distal breakpoint lies distal to c^IDSD, c^p^k^b^h^g^h^g^h^- and c^p^k^b^h^g^h^g^h^- but proximal to c^a (see Figure 7). Furthermore, they establish the c^psj and c^a distal breakpoints as the proximal and distal limits of the region of chromosome 7 containing the cextend gene. Placement of the c^psj distal breakpoint distal to that of c^p^k^b^h^g^h^g^h^- 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^p^k^b^h^g^h^g^h^- deletion is not linear.

Cloning of the c^IDSD breakpoint-fusion fragment: Based on the above results, the distal limits of the region of chromosome 7 containing cextend is defined by the distal breakpoint of either the c^IDSD, c^p^k^b^h^g^h^g^h^- or c^p^k^b^h^g^h^g^h^- deletion. To determine the proximal-to-distal order of these breakpoints relative to one another, experiments were undertaken to clone the c^IDSD breakpoint-fusion fragment. Beginning with the D7Cu18 locus, which is deleted from the c^IDSD 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 EcoRI fragment in nondeleted c^a DNA (Figure 5B, lanes 1 and 3), a 10.5-kb EcoRI fragment in c^IDSD/c^p^k^b^h^g^h^g^h^- DNA (Figure 5B, lane 2) and no fragment in c^a/c^a 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 EcoRI fragment with c^IDSD DNA and a 7.4-kb EcoRI fragment with nondeleted DNA suggests that the c^IDSD fragment spans breakpoints. This hypothesis is supported by the observation that a c^IDSD-specific fragment is also detected with XbaI and PvuII (A. SCHEDL, S. RUPPERT, G. KELSEY, E. THIES, L. NISWANDER, T. MAGNUSON, and G. SCHUTZ, manuscript in preparation).
FIGURE 3.—Mapping of the \( c^{Pys} \) breakpoint-fusion fragment. Southern blot analysis of EcoRI-digested DNA hybridized with end-specific RNA probes generated from clone \( \lambda 14Rlc2 \). (A) Complementation map of the relevant chromosomes. The names of the marker loci are indicated in legend to Figure 1. \( c^a \) represents a non deleted chromosome. \( c^{M} \) and \( c^{Pys} \) 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 \( \lambda 14Rlc2 \). (C) Rehybridization of (B) with the non-albino region probe p77-2. (D) DNA hybridized with \( T3 \) riboprobe of \( \lambda 14Rlc2 \). (E) Rehybridization of blot in (D) with p77-2. In both cases, lanes 1–3 represent DNA from the following animals: (1) nondeleted \( c^{m}/c^{m} \) (originated from the Albert Einstein colony); (2) \( c^{m}/c^{m} \) and (3) \( c^{Pys}/c^{Pys} \).

FIGURE 4.—Deletion breakpoint order relative to the \( c^{Pys} \) distal breakpoint. Southern blot analysis of EcoRI-digested DNA hybridized with \( T7 \) end-specific riboprobe generated from p7.5(X/R1). Lanes 1–8 represent DNA from the following animals: (1) nondeleted \( c^{m}/c^{m} \) (originated from the Oak Ridge Colony); (2) \( c^{14Gos}/c^{14Gos} \); (3) \( c^{14Gos}/c^{14Gos} \); (4) \( c^{14Gos}/c^{14Gos} \); (5) \( c^{14Gos}/c^{14Gos} \); (6) \( c^{14Gos}/c^{14Gos} \); (7) \( c^{14Gos}/c^{14Gos} \).

To determine if pv0.4AP is detecting a DNA fragment containing the \( c^{1DSD} \) deletion breakpoint, a subgenomic library was generated from 10–12-b size-selected \( c^{1DSD}/c^{M} \) EcoRI-digested DNA. Plaques (3 × 10⁵) were screened, and one recombinant clone that hybridized with pv0.4AP was analyzed further. This lambda clone, \( \lambda E10.5c11 \), contained a 10.5-kb EcoRI fragment from which a 4.2-kb \( Asp718 \) fragment was subcloned (pA4.2c11). End-specific riboprobes from this subclone were hybridized to \( Asp718 \)-digested \( c^{A}/c^{A} \), \( c^{14Gos}/c^{14Gos} \) and \( c^{1DSD}/c^{14Gos} \) DNA. A 7.2-kb band was detected when the \( T3 \) riboprobe was hybridized to \( c^{A}/c^{A} \) DNA (Figure 5D, lanes 1 and 4) and \( c^{14Gos}/c^{14Gos} \) DNA (Figure 5D, lane 2). When hybridized to \( c^{1DSD}/c^{14Gos} \) 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 \( c^{1DSD} \) chromosome and the 7.2-kb band being generated by the \( c^{14Gos} \) 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 \( c^{1DSD} \) 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 \( c^{1DSD} \) 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
map the distal breakpoints of the \( c^{FPR60Hg} \) and \( c^{FPR60Hd} \) deletions relative to \( D7Cw11D \). To do so, pA4.2c11 was hybridized to \( TaqI \)-digested DNA from an \( F1 \) hybrid that carried a \( M.\ spreitus \) nondeleted chromosome 7 and either a \( M.\ musculus \) \( c^{FPR60Hg} \) or a \( M.\ musculus \) \( c^{FPR60Hd} \) deletion chromosome. A \( TaqI \) polymorphism was detected between the two species. A 9.5-kb fragment was present in \( M.\ spreitus \) DNA (Figure 6, lane 1). The hybridization pattern for the \( M.\ musculus \) parental DNAs is shown in Figure 6 for \( c^{A4}/c^{FPR60Hh} \) (lane 3) and \( c^{A4}/c^{FPR60Hd} \) (lane 5). The \( M.\ spreitus/M.\ musculus \) \( F1 \) DNAs showed only the \( M.\ spreitus \) 9.5-kb fragment (Figure 6, lanes 2 and 4). The absence of any \( M.\ musculus \)-specific fragment from either \( F1 \) DNA indicates that genomic sequence homologous to the pA4.2c11 insert is removed both by the \( c^{FPR60Hg} \) and \( c^{FPR60Hd} \) deletions. Thus, the \( c^{11DSD} \) breakpoint, and therefore the \( D7Cw11D \) locus, lie proximal to the \( c^{FPR60Hg} \) and \( c^{FPR60Hd} \) distal breakpoints.

**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. \( c^{11DSD} \) 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 \( EcoRI \)-digested DNA hybridized with \( T3 \) riboprobe generated from \( RamHI \)-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) \( c^{11DSD}/c^{ch} \) (3) nondeleted \( c^{ch}/c^{ch} \) (originated from the Albert Einstein colony) (4) \( c^{11DSD}/c^{11DSD} \). (C) Rehybridization of the same blot shown in B with \( the chromosome 9-specific probe \( pH2.2H \). (D) Southern blot analysis of \( Asp718 \)-digested DNA hybridized with \( T3 \) end-specific riboprobe generated from pA4.2c11. Lanes 1–4 represent DNA from the following animals: (1) non-deleted \( c^{ch}/c^{ch} \) (originated from the Oak Ridge colony) (2) \( c^{ch}/c^{ch} \) (3) \( c^{ch}/c^{ch} \) (4) nondeleted \( c^{ch}/c^{ch} \) (originated from the Albert Einstein colony).
proximal distal
[Image 0x0 to 590x808]
[44x726]proximal distal
[46x234]D7Cw1lD
[46x530]D7Cw1lD
[46x482]deletions is
[46x127]traembryonic-ectoderm defect. In contrast,
predicted by our earlier genetic analyses
by the distal side of the
show the embryonic-ectoderm defect but not the ex-
to that of
and embryological data are consistent with the
C4FR60Hd
[46x262]c5FR60Hg
[46x274]c2YPSJ
[46x310]cSFR60Hg c4FR60Hd
[46x378]et
[46x402]breakpoints. Based on the distal breakpoint order
from the
breakpoints as being
mal-to-distal breakpoint order of the distal side of the
ure 7).
From these data, it can be concluded that the proxi-
placement of the
Placement of the
C5FR60Hg C4FR60Hd c2YPSJ
[74x414]7
[76x305], ,
[78x716]mSd
[80x274]or
[82x716]r
[88x330]eed
[88x518]7
[90x342]breakpoint) should map to positions that lie
between the genetic and molecular data is that the
c4FR60Hd
deletion on the proximal side and the
deletion could juxtapose some heterochromatin
silencing region next to
Another possibility is that the
distal breakpoint 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
c4FR60Hd
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 c110SD, c4FR60Hd, c7PSJ and c6H 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 c7H distal breakpoint and on the distal side by D7Cw11D (the c110SD distal breakpoint). The region containing the exed gene is delimited by the c7PSJ deletion on the proximal side and the c6H deletion on

**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.

**DISCUSSION**

Molecular access to the eed/exed region of chromosome 7 was provided by cloning the c7PSJ and c110SD 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 c7PSJ breakpoint) and D7Cw11D (genomic locus defined by the distal side of the c110SD breakpoint) should map to positions that lie between eed and exed (Figure 7). To confirm these predictions, the distal breakpoints of the c110SD, c4FR60Hd, c7PSJ and c6H deletions were mapped relative to D7Cw2D and D7Cw11D. D7Cw2D was found to be deleted from c6H but not from c110SD, c4FR60Hd or c7PSJ. In contrast, D7Cw11D was deleted from c4FR60Hd, c4FR60Hd, c7PSJ and c6H. 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 c110SD–(c4FR60Hd and c4FR60Hd)–c7PSJ–c6H.

Placement of the c4FR60Hd distal breakpoint proximal to that of c7PSJ is in direct analysis to the order suggested by our genetic data (NISWANDER et al. 1988, 1989). Embryos homozygous for the c7PSJ deletion show the embryonic-ectoderm defect but not the extraembryonic-ectoderm defect. In contrast, c4FR60Hd homozygotes show a phenotype consistent with both eed and exed being deleted or inactivated. Furthermore, c7PSJ can complement the extraembryonic defect by providing the wild-type copy of exed in c7PSJ/c4FR60Hd compound heterozygotes. All of the genetic and embryological data are consistent with the c4FR60Hd distal breakpoint lying distal to the c7PSJ distal breakpoint. Yet, our molecular data indicate that the c4FR60Hd breakpoint lies proximal to that of c7PSJ.

One possible explanation for the discrepancy between the genetic and molecular data is that the c4FR60Hd 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 c4FR60Hd deletion. For example, the large chromosomal region between hsd1-1 and c which is proximal to the c4FR60Hd deletion is known to be devoid of CpG islands and, therefore, might represent particularly inactive DNA (G. KELSEY, A. SCHEID, 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 c4FR60Hd 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 c4FR60Hd 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 c110SD, c4FR60Hd, c7PSJ and c6H 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 c7H distal breakpoint and on the distal side by D7Cw11D (the c110SD distal breakpoint). The region containing the exed gene is delimited by the c7PSJ deletion on the proximal side and the c6H 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 c60 and c3M 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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