The albino–deletion complex in the mouse defines genes necessary for
development of embryonic and extraembryonic ectoderm*

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Summary

A detailed embryological analysis has been undertaken on embryos carrying the cFR60Hd-, cFR60Hs- or cYPSJ-albino deletions of mouse chromosome 7. Embryos homozygous for the cFR60Hd deletion are abnormal at day 7-5 of gestation. The extraembryonic ectoderm does not develop, and primitive-streak formation and mesoderm production do not occur. In contrast, extensive development of the extraembryonic ectoderm, as well as mesoderm production, are observed in the cFR60Hs- and cYPSJ-homozygous embryos. The mesoderm does not, however, organize into somites and the neural axis does not form. The embryos are grossly abnormal by day 8-5 of development. There are two other albino deletions (c6H and c1DSD) that are known to affect the embryo around the time of gastrulation (Niswander et al. 1988), and the lethal phenotype observed for the cFR60Hd-homozygous embryos is similar to that described for c6H-homozygous embryos, whereas the cFR60Hs- and cYPSJ-homozygous embryos display a phenotype that is similar to c1DSD-homozygous embryos. A detailed complementation analysis using these five deletions revealed that the cFR60Hd, cYPSJ and c1DSD deletions could partially complement the phenotype produced by the cFR60Hd and c6H deletions in any combination. Extensive development of the extraembryonic structures and production of mesoderm occurs in the compound heterozygotes. These results suggest that the distal breakpoints of the cFR60Hd, cYPSJ and c1DSD deletions lie more proximal than the distal breakpoints of the cFR60Hd and c6H deletions. This arrangement defines new functional units of chromosome 7 such that a gene(s) important for normal development of the extraembryonic ectoderm would be located in the distal region of non-overlap between the two groups of deletions, and a gene(s) important for the development of the embryonic ectoderm would be located in the region deleted by both groups of chromosomes. The cFR60Hd and c6H deletions appear to be missing both genes and belong to one complementation group; whereas the cFR60Hs, cYPSJ and c1DSD deletions are missing only the gene(s) affecting the development of the embryonic ectoderm and, therefore, belong to another complementation group.

Key words: mouse development, chromosomal deletions, homozygous-lethal mutations, early postimplantation survival, genetic complementation.

Introduction

An understanding of the complex processes involved in regulation of mammalian development depends on classical genetic, embryological and molecular studies. One model system where all three approaches are applicable is the albino–deletion complex. This complex represents an overlapping series of 37 radiation-induced chromosomal deficiencies that uncover the albino (c) coat-colour locus in mouse chromosome 7 (Russell, 1979; Russell & Raymer, 1979; Russell et al. 1979, 1982). Complementation analyses have classified these deletion chromosomes into a minimum of 12 groups with members of each group being associated with pre- or postnatal lethality when present in the homozygous state (Russell et al. 1982). One particular complementation group (Bi) has four independently generated deletion chromosomes (c1DSD, cFR60Hd, cYPSJ, cFR60Hs) assigned to it (Russell et al. 1982). These assignments were based on the fact that embryos homozygous or doubly heterozygous for any of the four deletions died sometime during the early postimplantation stages of development. A fifth deletion (c6H), while not a part of this complementation
study, was assigned to this group on the basis of published information (Lewis et al. 1976). An extensive embryological analysis, however, demonstrated distinct differences between the lethal phenotype associated with embryos homozygous for the \(c^{dh}\) and \(c^{11DSD}\) deletions (Niswander et al. 1988). The \(c^{dh}\)-homozygous embryos exhibit severe defects in development of both the extraembryonic and embryonic ectoderm, whereas the \(c^{11DSD}\)-homozygous embryos exhibit defects only in the embryonic ectoderm. The \(c^{11DSD}\) chromosome was able to complement at least partially the \(c^{dh}\) chromosome in that the former provided genetic information needed for development of the extraembryonic structures (Niswander et al. 1988). These results suggest that these deletions could be classified into two different complementation groups, defining two new functional units in this region of mouse chromosome 7. One unit contains genetic information needed for development of the extraembryonic ectoderm, whereas the second unit is needed for development of the extraembryonic ectoderm.

In this report, we have extended these phenotypic observations to include the three remaining deletion chromosomes (\(4^{FR60Hd}, 2^{YPS}\) and \(5^{FR60Hg}\)) that were originally assigned to the Bi complementation group (Russell et al. 1982). The \(4^{FR60Hd}\) homozygotes were found to display a lethal phenotype that was similar to \(6H/6H\) embryos, whereas the \(2^{YPS}\) and \(5^{FR60Hg}\) homozygotes displayed a lethal phenotype similar to that described for \(11DSD/11DSD\) embryos. These results support our hypothesis that these five deletions define an area of chromosome 7 needed for development of the embryonic ectoderm, as well as a separate area needed for development of the extraembryonic ectoderm.

### Materials and methods

#### (A) Mice

The \(c^{6H}\)-albino-deletion mice used in these experiments originated at the MRC Radiobiology Unit, Harwell, UK, and were obtained from Dr Salome G. Waelsch (Albert Einstein College of Medicine, Bronx, New York). The \(c^{11DSD}, c^{4FR60Hd}, c^{2YPS}, c^{5FR60Hg}\) mice originated at the Oak Ridge National Laboratory. All lethal \(c\) deletions are maintained in closed-colony, but not strictly inbred, heterozygous mouse stocks opposite chinchilla \((c^b)\), and all mutations, when present in the heterozygous state with chinchilla, produce a dilute-chinchilla coat colour. For experimental purposes, the stocks were expanded by crossing deletion heterozygotes with random-bred CF-1 mice \((c/c)\) to produce \(c^*/c\) (albino) and \(c^b/c\) (dilute chinchilla) offspring \((c^* = c^{6H}, c^{11DSD}, c^{4FR60Hd}, c^{2YPS}\) or \(c^{5FR60Hg}\)). The albino progeny were then crossed to appropriate males (Table 1) to produce experimental embryos (see Table 1 for number of embryos examined from each cross).

#### (B) Histology

Embryos were dissected from uterine horns of naturally mated females at days 7.5, 8.5 or 9.5 of development (the day of the vaginal plug is considered to be day 0.5). The dissected embryos were fixed in 2.5% glutaraldehyde in phosphate-buffered saline (PBS) for 2-3 h at room temperature. They were then washed extensively in PBS, dehydrated and embedded in plastic. Sections of 3 μm thickness were cut and stained with Schiff’s reagent and counterstained with 0.05% toluidine blue.

### Results

#### (A) The \(c^{4FR60Hd}\) deletion

28% of the embryos dissected from \(c^{4FR60Hd}\)-hetero-

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**Table 1. Summary of embryo dissections from crosses of albino-deletion mice**

<table>
<thead>
<tr>
<th>Cross</th>
<th>Female × Male</th>
<th>No. of embryos</th>
<th>% grossly abnormal and small</th>
<th>No. sectioned</th>
</tr>
</thead>
<tbody>
<tr>
<td>1.</td>
<td>(c/c^{4FR60Hd}) (c^*/c^{4FR60Hd})</td>
<td>74</td>
<td>28%</td>
<td>12</td>
</tr>
<tr>
<td>2.</td>
<td>(c/c) (c^*/c)</td>
<td>39</td>
<td>0%</td>
<td>-</td>
</tr>
<tr>
<td>3.</td>
<td>(c/c^{11DSD}) (c^*/c^{11DSD})</td>
<td>42</td>
<td>24%</td>
<td>7</td>
</tr>
<tr>
<td>4.</td>
<td>(c/c) (c^*/c)</td>
<td>43</td>
<td>0%</td>
<td>-</td>
</tr>
<tr>
<td>5.</td>
<td>(c/c^{11DSD}) (c^*/c^{11DSD})</td>
<td>49</td>
<td>0%</td>
<td>3</td>
</tr>
<tr>
<td>6.</td>
<td>(c/c^{4FR60Hd}) (c^*/c^{4FR60Hd})</td>
<td>50</td>
<td>2%*</td>
<td>-</td>
</tr>
<tr>
<td>7.</td>
<td>(c/c^{5FR60Hg}) (c^*/c^{5FR60Hg})</td>
<td>80</td>
<td>22%</td>
<td>6</td>
</tr>
<tr>
<td>8.</td>
<td>(c/c^{2YPS}) (c^*/c^{2YPS})</td>
<td>60</td>
<td>27%</td>
<td>7</td>
</tr>
<tr>
<td>9.</td>
<td>(c/c) (c^*/c)</td>
<td>49</td>
<td>0% (22%)</td>
<td>12</td>
</tr>
<tr>
<td>10.</td>
<td>(c/c) (c^*/c)</td>
<td>89</td>
<td>26%</td>
<td>14</td>
</tr>
<tr>
<td>11.</td>
<td>(c/c) (c^*/c)</td>
<td>70</td>
<td>0%</td>
<td>-</td>
</tr>
<tr>
<td>12.</td>
<td>(c/c^{5FR60Hg}) (c^*/c^{5FR60Hg})</td>
<td>88</td>
<td>28%</td>
<td>17</td>
</tr>
<tr>
<td>13.</td>
<td>(c/c) (c^*/c)</td>
<td>53</td>
<td>0%</td>
<td>-</td>
</tr>
<tr>
<td>14.</td>
<td>(c/c^{2YPS}) (c^*/c)</td>
<td>54</td>
<td>26%</td>
<td>10</td>
</tr>
<tr>
<td>15.</td>
<td>(c/c) (c^*/c)</td>
<td>36</td>
<td>0%</td>
<td>-</td>
</tr>
<tr>
<td>16.</td>
<td>(c/c^{2YPS}) (c^*/c)</td>
<td>53</td>
<td>26%</td>
<td>6</td>
</tr>
<tr>
<td>17.</td>
<td>(c/c^{2YPS}) (c^*/c)</td>
<td>72</td>
<td>25%</td>
<td>7</td>
</tr>
<tr>
<td>18.</td>
<td>(c/c^{11DSD}) (c^*/c^{11DSD})</td>
<td>72</td>
<td>5%</td>
<td>2</td>
</tr>
<tr>
<td>19.</td>
<td>(c/c^{5FR60Hg}) (c^*/c^{5FR60Hg})</td>
<td>38</td>
<td>19%</td>
<td>10</td>
</tr>
<tr>
<td>20.</td>
<td>(c/c^{11DSD}) (c^*/c^{11DSD})</td>
<td>68</td>
<td>22%</td>
<td>7</td>
</tr>
</tbody>
</table>

* Morphology probably unrelated to \(c\)-region genotype.

† % small but not abnormal.
zygous crosses at day 7-5 of gestation (Table 1, line 1) were small and similar in appearance to what has been described as the \( c^6H \) phenotype (Lewis et al. 1976; Niswander et al. 1988). None of the embryos dissected from control crosses showed this same phenotype (Table 1, line 2). Twelve putative homozygous mutant embryos were prepared for histological examination and sections (Fig. 1B and C) were compared to those from normal littersmates (Fig. 1A). The extraembryonic ectoderm of the \( c^{4FR60Hd} \)-homozygous embryos was completely disorganized and small in appearance when compared to that of normal littersmates. The embryonic ectoderm of homozygous mutant embryos was organized into a small egg cylinder with the beginning of proamniotic cavity formation. Neither primitive-streak formation nor mesoderm production had occurred in homozygous embryos. Both events, however, had taken place in normal littersmates.

Gross examination revealed that 48% of the abnormal embryos possessed a long extension that protruded from the antimesometrial tip into the surrounding decidual material. Histological sections from some of these embryos revealed that this extension consisted of parietal endoderm (Fig. 1C). The remaining mutant embryos, as well as their normal littermates, did not form this long extension. Instead, the parietal endoderm surrounded the visceral endoderm but was not always closely juxtaposed to it (Fig. 1A,B). It was not possible to establish a correlation between size of the mutant embryo and abnormal organization of the parietal endoderm.

On day 8-5 of gestation, 24% of the embryos (Table 1, line 3) were very small and abnormal in appearance. Seven of the mutant embryos were sectioned and, of these, two were found to be composed of a disorganized array of pyknotic cells surrounded by a single layer of parietal endoderm. The remaining five resembled day-7-5 \( c^{4FR60Hd}/c^{4FR60Hd} \) embryos except that the cells were pyknotic in appearance. None of the control embryos (Table 1, line 4) exhibited a similar phenotype.

\[ \text{(B) } c^{4FR60Hd}/c^{11DSD} \text{-compound heterozygotes} \]

As previously described (Niswander et al. 1988), the \( c^{11DSD} \) chromosome can partially complement the \( c^6H \) deletion by correcting the extraembryonic ectoderm defect. To determine if the \( c^{11DSD} \) chromosome can effect a similar rescue for the \( c^{4FR60Hd} \) chromosome, crosses (Table 1, lines 5–8) were made to produce \( c^{4FR60Hd}/c^{11DSD} \)-compound heterozygotes. At day 7-5 of gestation, it was not possible to distinguish a mutant class of embryos based on gross phenotypic differences (Table 1, line 5 and 6). Although the embryos varied in size, none showed a phenotype characteristic of \( c^{4FR60Hd} \) homozygotes. Three of the slightly smaller embryos were sectioned for histological examination and were compared to their larger littersmates. Organization of extraembryonic tissues and production of mesoderm were similar in all embryos, regardless of size.

At day 8-5 of development, 22% (Table 1, line 7) and 27% (Table 1, line 8) of the dissected embryos obtained from crosses designed to produce compound heterozygotes were grossly abnormal (Fig. 1E) when compared to their littermates (Fig. 1D). All of the embryos exhibited a phenotype similar to that described for the \( c^{11DSD} \)-homozygous embryos (Niswander et al. 1988). Histological examination revealed that, although the embryonic ectoderm was severely runted compared to that of normal littersmates, primitive-streak formation and mesoderm production had occurred. The extraembryonic structures were well developed and the amniotic cavity, amnion, exocoelom, chorion and ectoplacental cavity were present (Fig. 1E). In some cases, there was extensive production of extraembryonic mesoderm. Both the parietal and visceral endoderm were normal in appearance.

\[ \text{(C) The } c^{2YPSJ} \text{ deletion} \]

22% of the day-7-5 embryos obtained from \( c^{2YPSJ} \), heterozygous crosses (Table 1, line 9) were smaller in size than their littermates but did not appear grossly abnormal. Six of the smaller embryos and six of the larger littermates were prepared for histological examination. The putative \( c^{2YPSJ}/c^{11DSD} \)-homozygous embryos displayed a slight retardation in overall growth but development at this stage was almost synchronous with that of normal littersmates. Primitive-streak formation and mesoderm production had occurred in the putative mutant embryos, and the extraembryonic structures were normally organized. Gross examination revealed that the parietal endoderm surrounding 36% of the putative homozygous embryos exhibited the same long extension protruding into the decidual tissue that was described for some of the \( c^{4FR60Hd} \) embryos. The remaining putative mutant embryos, as well as their normal littermates, did not demonstrate this abnormal morpholgy of parietal endoderm.

At day 8-5 of gestation, 26% of the embryos were grossly abnormal when compared to their littermates (Table 1, line 10). None of the control embryos (Table 1, line 11) showed this same phenotype. Examination of histological sections (Fig. 2A) revealed a morphology similar to that described for the \( c^{11DSD} \) phenotype (Niswander et al. 1988). The most striking difference from that observed for the \( c^6H \) and \( c^{4FR60Hd} \), homozygous phenotype is the extensive development of the extraembryonic structures. The amnion, chorion, exocoelom and ectoplacental cavity were well developed in the \( c^{2YPSJ} \)-homozygous embryos. The embryonic ectoderm had progressed to the stage where primitive-streak formation and mesoderm production had occurred, but the extensive morphogenetic movements characteristic of a normal day-8-5 embryo had not taken place. In many cases, there was extensive production of extraembryonic mesoderm. The visceral endoderm was normal in appearance. In 26% of the abnormal embryos, the parietal endoderm extended into the surrounding decidual material. When putative \( c^{2YPSJ}/c^{2YPSJ} \) embryos were dissected at day 9-5 of development, all that could be seen were remnants of
Fig. 1. Thick-section light micrographs of (A) day-7.5 normal embryo, (B) day-7.5 $c^{AFROH1d/cAFROH1d}$ embryo, (C) day-7.5 $c^{AFROH1d/cAFROH1d}$ embryo with abnormal morphology of parietal endoderm, (D) day-8.5 normal embryo, (E) day-8.5 $c^{AFROH1d/c11DS}$ embryo. $a$, amniotic cavity; $al$, allantois; $am$, amnion; $c$, chorion; $ec$, ectoplacental cavity; $eo$, exocoelom; $ep$, ectoplacental cone; $ee$, embryonic ectoderm; $ex$, extraembryonic ectoderm; $h$, heart; $m$, mesoderm; $o$, optic vesicle; $nt$, neural tube; $pe$, parietal endoderm; $ps$, primitive streak; $s$, somite; $tb$, trophoblast cells; $ve$, visceral endoderm; $ys$, yolk sac. $x40$. 
The albino deletions of the mouse

Fig. 2. Thick-section light micrographs of (A) day-8-5 c²YPS/c²YPS embryo, (B) day-8-5 c²YPS/c⁶H embryo, (C) day-8-5 c⁵FR/c⁵FR embryo, (D) day-8-5 c³⁵FR/c⁴⁶H embryo. Symbols as in Fig. 1. x40.

extraembryonic tissues. No embryonic derivatives were detected.

(D) The c³⁵FR/c⁴⁶H deletion
28% of the day 7-5 embryos obtained from c³⁵FR/c⁴⁶H heterozygous crosses (Table 1, line 12) were found to be grossly similar in appearance to that described for the c³⁵FR/c⁴⁶H or c⁶H homozygous phenotype except that the c³⁵FR/c³⁵FR embryos were larger in size. None of the control embryos (Table 1, line 13) showed this same phenotype. Histological examination revealed that mutant embryos could easily be distinguished from normal littermates by size difference; however, the phenotype that was displayed by the mutants varied. Two litters produced a total of five mutant embryos; three of the five abnormal embryos had undergone primitive-streak formation and produced mesoderm. The extraembryonic ectoderm was well organized but was slightly delayed in development compared to normal littermates. The amnion and chorion had formed but only a very small exocoelom and ectoplacental cavity were present. The remaining two embryos, as well as mutant embryos obtained from the other matings, appeared retarded in overall development and more closely resembled a day-6-5 to -7-0 egg-cylinder embryo. The amniotic cavity was present but primitive-streak formation and mesoderm production had not occurred. The extraembryonic ectoderm was developmentally delayed when compared to normal littermates. The parietal endoderm formed a characteristic extension that, upon dissection, could be seen to extend into the surrounding decidua of 32% of the mutant embryos. One embryo that displayed the parietal endoderm extension was an embryo that had developed to the stage where primitive-streak formation and mesoderm production occurred. The remaining abnormal embryos and all normal littermates possessed a single layer of parietal endoderm that surrounded the visceral endoderm.

At day 8-5 of development, 26% of the embryos were grossly abnormal when compared to littermates (Table 1, line 14). None of the control embryos (Table 1, line 15) displayed this same phenotype. Examination of histological sections (Fig. 2C) revealed a consistent morphology similar to that described for c³¹DDS- (Niswander et al. 1988) and c²YPS- (present report) homozygous embryos. The visceral endoderm appeared normal, and the extraembryonic structures such as the amnion, chorion and allantois were present and organized properly. Development of the embryonic ectoderm had progressed to the stage where primitive-streak formation and mesoderm production occurred. The extensive morphogenesis seen in normal day-8-5 embryos had not occurred. 21% of the abnormal embryos possessed a parietal endoderm extension. When putative c³⁵FR/c³⁵FR embryos were dissected at day 9-5 of development, all that could be seen were remnants of extraembryonic tissues. No embryonic derivatives were detected.

(E) c²YPS/c⁶H- and c³⁵FR/c³⁵FR compound heterozygotes
To determine if the c²YPS- or the c³⁵FR/c³⁵FR deletion could partially complement the c⁶H deletion by providing genetic information needed to allow for development of the extraembryonic structures, crosses were made to produce c²YPS/c⁶H (Table 1, line 16) or c³⁵FR/c³⁵FR embryos. At day 8-5 of gestation, all of the embryos dissected exhibited a gross phenotype similar to that described for the c²YPS- or c³⁵FR/c³⁵FR (present report) or c³¹DDS- (Niswander et al. 1988) homozygous embryos. Examination of histological sections revealed that primitive-streak formation and
mesoderm production had occurred, and the extraembryonic structures were well developed. The amnion, chorion, exocoelom and ectoplacental cavity were present (see Fig. 2B for c^{2YPS} / c^{EH} embryo and Fig. 2D for c^{FR0Hg} / c^{EH} embryo).

(F) c^{FR0Hg}/c^{1DSD}-compound heterozygotes

Dissection of embryos at day 7-5 of gestation from crosses designed to produce c^{FR0Hg}/c^{1DSD} embryos (Table 1, lines 18 and 19) revealed that the embryos varied slightly in size. The development of the extraembryonic structures, and of the visceral and parietal endoderm was normal in all but two of the smaller embryos. In these embryos, the decidua was found to contain a disorganized ball of cells whose origin could not be distinguished. This phenotype is not consistent with what has been observed for c^{FR0Hg} - or c^{1DSD} homozygous embryos.

At day 8-5 of development, 19% (Table 1, line 20) and 22% (Table 1, line 21) of the embryos obtained from crosses designed to produce c^{FR0Hg}/c^{1DSD} compound heterozygotes were found to be grossly abnormal when compared to that of normal littermates. All of the abnormal embryos exhibited a phenotype similar to that described for day-8-5 embryos homozygous for the c^{FR0Hg} -, c^{2YPS} - (present report) or c^{1DSD} - (Niswander et al. 1988) deletions. Examination of histological sections revealed that, although primitive-streak formation and mesoderm production had occurred, the embryonic ectoderm was severely runted when compared to normal littermates. The extraembryonic structures were well developed; the amniotic cavity, amnion, exocoelom, chorion and ectoplacental cavity were present. The mesodermal layer of the amnion was often thick, and development of the allantois was extensive. Both the parietal and visceral endoderm were normal in appearance.

Discussion

A detailed analysis of the lethal phenotype associated with the homozygous (null) state of the five deletions that were originally assigned to the Bi complementation group of the albino-deletion complex has now been completed. This embryological analysis has specifically pinpointed the time at which the developmental arrest occurs in the mutant embryos and has also provided information as to the specific cell types affected. In an earlier report (Niswander et al. 1988), we examined the development of c^{EH} - and c^{1DSD} - homozygous embryos and found that the former displayed severe defects in the development of the embryonic and extraembryonic ectoderm, whereas the latter showed defects only in the embryonic ectoderm. In this report, a phenotypic analysis was done for the three remaining deletion chromosomes (c^{FR0Hd}, c^{2YPS} , and c^{FR0Hg}). The lethal phenotype associated with the homozygous state of the c^{FR0Hd} deletion closely resembled that seen for c^{EH} homozygotes, whereas the phenotype exhibited by c^{2YPS} and c^{FR0Hg} homozygotes was similar in morphology to that of c^{1DSD}/c^{1DSD} embryos. The most striking differences between the two groups are that the c^{EH} - and c^{FR0Hd} - homozygous embryos exhibit a complete lack of development of the extraembryonic ectoderm, and an absence of primitive-streak formation and mesoderm production, while embryos homozygous for the c^{1DSD}, c^{2YPS} , and c^{FR0Hg} deletions show extensive development of these tissues. Normal proliferation and differentiation of the amnion, chorion and allantois occurs in the latter group of embryos, although some degree of variability in the extent of development of the exocoelom and ectoplacental cavity was observed. In addition to development of the extraembryonic structures, these embryos also progressed to the point of primitive-streak formation and mesoderm production, events that were never seen in c^{EH} - or c^{FR0Hd} - homozygous embryos. Although the c^{FR0Hg}/c^{FR0Hg} embryos began to display abnormalities slightly earlier than did the c^{1DSD}/c^{1DSD} and c^{2YPS}/c^{2YPS} embryos, all three types were identical by day 8-5.

Abnormal organization of the parietal endoderm was seen in some, but not all, of the c^{FR0Hd} -, c^{2YPS} - and c^{FR0Hg}-homozygous embryos. In those embryos that showed this abnormality, the parietal endoderm was observed to protrude from the antimesometrial tip to form a long extension that could be seen, upon dissection, to extend into the surrounding decidual material. Lewis et al. (1976) originally reported this abnormality as being consistent with the lethal phenotype associated with the c^{EH} - homozygous embryos, and we also observed this phenotype in the limited number of c^{EH}/c^{EH} embryos that were examined in our earlier work (Niswander et al. 1988). Because of the variability in morphology of the parietal endoderm in c^{FR0Hd} -, c^{FR0Hg} - and c^{2YPS} - homozygous embryos, we examined additional c^{EH}/c^{EH} embryos and found a similar variability. Only twenty of the c^{EH} - homozygous embryos (67% of the total examined) showed the phenotype. It was not possible to establish a correlation between size of the mutant embryo and abnormal organization of the parietal endoderm. Interestingly, none of the c^{1DSD} - homozygotes ever exhibited this phenotype nor did embryos that were doubly heterozygous for c^{1DSD} and one of the other four deletions.

In mutant embryos not displaying this abnormality, as well as in normal littermates, the parietal endoderm formed a single layer of cells that surrounded the visceral endoderm but was not always closely juxtaposed to it (demonstrated in Fig. 1A). Thus, although variability in morphology of parietal endoderm was observed even in normal embryos, the range in shape or form was not as extreme as that observed in the homozygous embryos discussed above. The simplest explanation for these results is that parietal-endoderm morphology may be influenced by segregating background genes and that the extreme morphology observed in some of the mutant embryos may only be a secondary effect associated with embryos in more advanced stages of death.

The embryological evidence reported here supports our original hypothesis (Niswander et al. 1988) that the...
Fig. 3. Complementation map of the albino deletions. This map is modified from that published by Russell et al. (1982) and Niswander et al. (1988) in that the original Bi complementation group has been separated into two complementation groups (Bex and Bem) defining two new functional units (embryonic ectoderm survival and extraembryonic ectoderm survival) in this region of chromosome 7. Deleted regions are represented by dark lines. The exact positions of the chromosomal breakpoints are not yet known. Postulated functional units of the chromosome are indicated below the genetic map, and no correlation with physical distance is implied. Marker loci include *tp* (taupe), *c* (albino), *Mod-2* (mitochondrial form of malic enzyme), and *sh-1* (shaker-1). cM = centimorgan. Symbols on the right represent the name of each complementation group and the number in parentheses represents the number of individual deletion chromosomes assigned to each group. The number for complementation group E and for groups Dp or Dq includes deletions *c*6H and *c*25H (Gluecksohn-Waelsch, 1979), respectively, which were not part of the complementation analysis involving the other 35 deletions but were tentatively assigned to these groups on the basis of published information (Russell et al. 1982); *c*25H is assignable to either Dp or Dq (denoted by the ?). The dotted lines for the deletion chromosomes within the Di and Dj complementation groups indicate that nothing can be said about whether these deletions uncover both the embryonic-ectoderm- and extraembryonic-ectoderm-survival regions or just the embryonic-ectoderm-survival region. The phenotype associated with the Di- or Dj-deletion homozygotes, which die shortly postimplantation, has not yet been examined. 'V' indicates viable albino mutants; the number of V mutants listed represents mutations derived from radiation (or control) mutagenesis experiments.

A gastrulation-survival region can be subdivided into at least two genetic regions. All five deletions define gene(s) needed for development of the embryonic ectoderm, as evidenced by the poor development of this cell type in all Bi deletion homozygotes (present report and Niswander et al. 1988), and also by the fact that it was not possible to establish embryo-derived stem-cell lines either from *c*6H- or *c*11DSD-homozygous embryos (Niswander et al. 1988). This indicates that the deleted gene(s) is needed for the viability of the embryonic ectoderm. In addition to deleting these gene(s), *c*6H and *c*4FR60Hd also remove a gene(s) needed for development of the extraembryonic ectoderm. Evidence for the existence of these genes is given by the complete lack of development of these structures in *c*6H- and *c*4FR60Hd-homozygous embryos, whereas extensive development of the extraembryonic structures is observed in *c*11DSD, *c*2YPS1 and *c*4FR60Hs homozygotes. Furthermore, the *c*11DSD, *c*2YPS1 and *c*5FR60Hs deletions are capable of complementing, in any combination, the *c*6H and *c*4FR60Hd chromosomes for development of extraembryonic structures.

Based on these phenotypic results, we propose that the distal breakpoints for the *c*11DSD, *c*2YPS1 and
ectoderm. Alternatively, there could be additional information that is needed for primitive-streak formation and extensive mesoderm production, and this gene(s), along with the gene(s) for extraembryonic ectoderm, can be assigned to the new complementation group Bern (the symbol 'B' derived from the original Bi designation, and 'em' to indicate deletion of genetic information needed for embryonic ectoderm survival). Thus, the original Bi group has been subdivided into two new groups, Bern and Bex (summarized in Table 2).

<table>
<thead>
<tr>
<th>Member deletions</th>
<th>Bex</th>
<th>Bem</th>
</tr>
</thead>
<tbody>
<tr>
<td>Stage gross abnormalities are detected</td>
<td>$c^{6H}$, $c^{FR60Hd}$</td>
<td>$c^{1DSD}$, $c^{FR60Hd}$, $c^{VPS}$</td>
</tr>
<tr>
<td>Panel endoderm</td>
<td>Day 7-5</td>
<td>Day 8-5</td>
</tr>
<tr>
<td>Extraembryonic ectoderm</td>
<td>Affected in some embryos</td>
<td>Affected in some embryos</td>
</tr>
<tr>
<td>Embryonic ectoderm</td>
<td>Severely affected</td>
<td>Extensive development</td>
</tr>
<tr>
<td>Primitive streak formation</td>
<td>No</td>
<td>Affected</td>
</tr>
<tr>
<td>Mesoderm production</td>
<td>No</td>
<td>Yes, extensive production but no somites</td>
</tr>
<tr>
<td>Embryo-derived stem-cell lines</td>
<td>No ($c^{6H}$)</td>
<td>No ($c^{1DSD}$)</td>
</tr>
</tbody>
</table>

*See text for detailed description of lethal phenotypes.

c$^{5FR60Hg}$ deletions are more proximal than the distal breakpoints for the $c^{6H}$ and $c^{FR60Hd}$ deletions (Fig. 3). If our model is correct, new functional units of chromosome 7 would be defined such that a gene(s) important for normal development of the extraembryonic ectoderm would be located in the distal region of overlap between the two groups of deletions and a second gene(s) important for the development of the embryonic ectoderm would be located in the region deleted by both groups of chromosomes. The $c^{6H}$ and $c^{5FR60Hd}$ deletions are missing both genes and can be assigned to a new supplementation group designated Bex (the symbol 'B' derived from the Bi designation, and 'ex' to indicate that they display both the extra-embryonic as well as embryonic ectoderm defect). $c^{1DSD}$, $c^{VPS}$ and $c^{FR60Hg}$, which are deleted for gene(s) affecting development of the embryonic but not extraembryonic ectoderm, can be assigned to the new supplementation group Bem (the symbol 'B' derived from the original Bi designation, and 'em' to indicate deletion of genetic information needed for embryonic ectoderm survival). Thus, the original Bi group has been subdivided into two new groups, Bem and Bex (summarized in Table 2).

What remains to be explained is why the embryonic ectoderm of the $c^{1DSD}$, $c^{VPS}$ and $c^{5FR60Hg}$ homozygous embryos progresses to the point of primitive-streak formation and extensive mesoderm production whereas there was no indication of these events occurring in embryos homozygous for the $c^{6H}$ and $c^{FR60Hd}$ deletions. It is possible that extensive development of the extraembryonic structures in embryos homozygous for the $c^{1DSD}$, $c^{VPS}$ or $c^{FR60Hg}$ deletions can support further development of the embryonic ectoderm, while the complete lack of development of the extraembryonic structures in $c^{6H}$, $c^{FR60Hd}$ embryos inhibits further development of the embryonic ectoderm. Alternatively, there could be additional genetic information that is needed for primitive-streak formation and mesoderm production, and this gene(s), along with the gene(s) for extraembryonic ectoderm, are deleted from the $c^{6H}$ and $c^{FR60Hd}$ deletions but not from the $c^{1DSD}$, $c^{VPS}$ and $c^{FR60Hg}$ deletions. A test of this latter hypothesis may become possible as single-gene, non-deletion, N-ethyl-N-nitrosourea-induced lethal mutations are recovered from this chromosomal region (Rinchik & Carpenter, 1988a,b). In addition, the definitive order of the five deletion breakpoints will be ascertained as molecular probes become available for this particular segment of the albino–deletion complex.

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References


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