INTRODUCTION

Development in mammals is regulated by a combination of genetic and epigenetic factors. One form of epigenetic inheritance is genetic imprinting, by which maternally and paternally derived chromosomes are marked during gametogenesis to distinguish their parent-of-origin. As a consequence of genetic imprinting, maternal or paternal alleles of certain genes are specifically repressed while the opposite alleles are expressed (Barlow et al., 1991; DeChiara et al., 1991; Bartolomei et al., 1991; Hayashizaki et al., 1994; Glenn et al., 1993; Leff et al., 1992; Ozcelik et al., 1992; Cattanach et al., 1992; Kay et al., 1993). This makes both maternal and paternal genetic contributions essential for development (McGrath and Solter, 1984; Surani et al., 1984). Mono-allelic expression, however, is not determined solely by the imprint that is established during gametogenesis, but requires an additional series of post-fertilization and even post-implantation events (Latham et al., 1994), which apparently include changes in imprinted gene methylation patterns (Stöger et al., 1993; Brandeis et al., 1993).

Apart from genetic imprinting, other forms of epigenetic modifications affect gene expression in the embryo and in the adult. Modifier loci, for example, affect the expression of unlinked transgenes (Sapienza et al., 1989; McGowan et al., 1989; Allen et al., 1990; Surani et al., 1990). Constituents of the egg cytoplasm can affect transgene expression and this effect can be specific for maternally or paternally transmitted genes. The CMZ 12 transgene, for example, is highly expressed at the 2-cell stage when DBA/2 eggs are fertilized by homozygous transgenic males (Surani et al., 1990). When eggs from transgenic females are fertilized by DBA/2 males, however, expression is reduced, indicating that enhancement of expression following fertilization of DBA/2 eggs by transgenic males is mediated by the DBA/2 egg cytoplasm (Surani et al., 1990). These observations indicate that exposure of the paternally inherited CMZ 12 transgene to the egg cytoplasm of some strains can either positively or negatively influence transgene expression.

In addition to these effects of egg cytoplasm on transgene expression, other modifier loci affect transgene methylation and expression. For the TKZ 751 transgene, expression is enhanced on a DBA/2 background and this enhancement is correlated with a reduced degree of transgene methylation (Allen et al., 1990). Transgene expression is repressed in BALB/c eggs fertilized by TKZ 751 transgenic males but not when eggs of transgenic (DB3) females are fertilized by

SUMMARY

Previous experiments revealed a strain-dependent effect of egg cytoplasm on the developmental potential of androgenetic (two paternal genomes) mouse embryos. Eggs obtained from C57BL/6 mice supported androgenone development to the blastocyst stage at a much higher frequency than eggs from DBA/2 mice. Transient exposure of paternal pronuclei to DBA/2 egg cytoplasm also compromised development, indicating that the DBA/2 egg cytoplasm negatively affected the ability of paternal pronuclei to support blastocyst formation. An essential first step toward understanding the molecular mechanism by which egg modifier factors influence gene expression is to determine the number of loci that are responsible for the strain difference. To do this, (B6D2)F1 hybrid females were backcrossed to DBA/2 males and the eggs from individual female progeny assayed for their ability to support androgenetic development. Approximately one fourth of the backcross females produced eggs that failed to support androgenone development, indicating that two independently segregating genetic loci are most likely responsible for the difference between DBA/2 and C57BL/6 egg phenotypes. Comparison of DBA/2 and C57BL/6 oocytes by two-dimensional protein gel electrophoresis revealed at least 17 proteins that exhibited significant, reproducible, quantitative differences in rates of synthesis. All of these proteins were synthesized in (B6D2)F1 oocytes. These data, combined with the previous observation that the C57BL/6 egg phenotype is dominant, are consistent with a model in which a C57BL/6 allele at either locus provides a protective function, either by antagonizing the actions of the DBA/2 alleles or by providing, through partial or complete redundancy, a function not provided by the DBA/2 alleles.

Key words: genome imprinting, egg modifier, nuclear transplantation, androgeneses, nucleocytoplasmic interactions

Strain-specific differences in mouse oocytes and their contributions to epigenetic inheritance

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SUMMARY

Previous experiments revealed a strain-dependent effect of egg cytoplasm on the developmental potential of androgenetic (two paternal genomes) mouse embryos. Eggs obtained from C57BL/6 mice supported androgenone development to the blastocyst stage at a much higher frequency than eggs from DBA/2 mice. Transient exposure of paternal pronuclei to DBA/2 egg cytoplasm also compromised development, indicating that the DBA/2 egg cytoplasm negatively affected the ability of paternal pronuclei to support blastocyst formation. An essential first step toward understanding the molecular mechanism by which egg modifier factors influence gene expression is to determine the number of loci that are responsible for the strain difference. To do this, (B6D2)F1 hybrid females were backcrossed to DBA/2 males and the eggs from individual female progeny assayed for their ability to support androgenetic development. Approximately one fourth of the backcross females produced eggs that failed to support androgenone development, indicating that two independently segregating genetic loci are most likely responsible for the difference between DBA/2 and C57BL/6 egg phenotypes. Comparison of DBA/2 and C57BL/6 oocytes by two-dimensional protein gel electrophoresis revealed at least 17 proteins that exhibited significant, reproducible, quantitative differences in rates of synthesis. All of these proteins were synthesized in (B6D2)F1 oocytes. These data, combined with the previous observation that the C57BL/6 egg phenotype is dominant, are consistent with a model in which a C57BL/6 allele at either locus provides a protective function, either by antagonizing the actions of the DBA/2 alleles or by providing, through partial or complete redundancy, a function not provided by the DBA/2 alleles.

Key words: genome imprinting, egg modifier, nuclear transplantation, androgeneses, nucleocytoplasmic interactions
BALB/c males (Allen et al., 1990). Transgene expression is repressed in half of the progeny produced from matings between transgenic males and (DBA/2 × BALB/c)F1 females. Thus, the BALB/c egg modifier appears to be zygotically expressed and encoded by a single locus, and repression is associated with methylation of the transgene (Allen et al., 1990). In the reciprocal cross of transgenic females to (DBA/2 × BALB/c)F1 males, no repression was observed, indicating that the BALB/c modifier must be maternally transmitted in order to affect transgene expression (Allen et al., 1990). Thus, the BALB/c modifier may be imprinted or may participate in additional interactions with factors in the egg cytoplasm.

Nuclear transplantation experiments have revealed striking effects of egg composition on the expression of endogenous genes as well as transgenes. This was first seen as a reduction in the ability of androgenetic (two paternal genomes) mouse embryos to develop to the blastocyst stage (Latham and Solter, 1991). Androgenones produced with fertilized C57BL/6 eggs formed blastocysts at a much higher frequency than those produced with DBA/2 eggs. Androgenones produced with DBA/2 eggs arrested development at the 8- or 16-cell stage and failed to execute all of the changes in protein synthesis that normally occur between the 8-cell and blastocyst stages. Fertilized embryos and gynogenetic embryos of both strains developed to the blastocyst stage at a high frequency, indicating that strain-dependent sensitivities to in vitro culture were not responsible for the defect. Moreover, transient exposure of male pronuclei to DBA/2 cytoplasm impaired their ability to support development to the blastocyst stage even after transplantation back to C57BL/6 egg cytoplasts. By contrast, transplanting both pronuclei from an egg of one strain to an egg of another produced fully viable embryos capable of efficient blastocyst formation, indicating that developmental arrest in this instance was not the result of an incompatibility between the pronuclei of one strain and the cytoplasm of another and that the egg modifier effect was specific for the male pronucleus. In addition, the inability of paternal pronuclei exposed to DBA/2 egg cytoplasm to support development when returned to C57BL/6 cytoplasm indicated that developmental arrest was most likely the result of a stable modification of the paternal genome rather than a simple cytoplasmic insufficiency (Latham and Solter, 1991). This negative effect of DBA/2 egg cytoplasm on paternal genome function was independent of the genotype of the fertilizing males (Latham and Solter, 1991).

Another study revealed an effect of egg cytoplasm on gene expression in the livers of adult mice (Reik et al., 1993). Two-dimensional (2D) protein gel electrophoresis revealed differences in the expression of four major urinary proteins (MUPs) between the DBA/2 and C57BL/6 strains. Although F1 hybrid mice expressed all four MUPs, the majority (25/26) of the interstrain nucleocyttoplasmic hybrids produced by exchanging maternal pronuclei between homozygous fertilized DBA/2 and C57BL/6 eggs expressed very little, if any, of the MUPs. Embryos cultured in vitro and intrastrain nucleocyttoplasmic hybrids rarely (5%) exhibited alterations in MUP expression. Thus, MUP isoform expression was impeded by an interaction between the egg cytoplasm and foreign female pronuclei (Reik et al., 1993). This result indicated that the effect of egg cytoplasm originally revealed by developmental failure of androgenones is not restricted to modification of the paternal genome and may have an impact on the expression of non-imprinted as well as imprinted genes.

An essential first step toward understanding the molecular mechanism by which egg modifier factors influence gene expression is to determine the number of loci that are responsible for the strain difference and how the products encoded by these loci might interact. I now report the results of an analysis of the progeny produced in genetic backcrosses and a 2D gel analysis of C57BL/6 and DBA/2 oocytes. The genetic backcross studies indicate that the difference in the ability of DBA/2 and C57BL/6 eggs to support androgenone development is most likely due to two independently segregating loci. The overall patterns of gene expression detected by 2D protein gel electrophoresis are quite similar between these two strains, but a number of reproducible quantitative differences are observed. These observations, combined with those derived from previous studies, can be accounted for by a model in which a C57BL/6 allele at either locus provides a protective function that allows continued expression of paternal alleles of one or more genes that are essential for blastocyst formation. Such a protective interaction might arise either through the provision of an essential function, possibly DNA methylation, as has been proposed for modifiers of transgene methylation (Chaillet, 1994), or by an antagonistic effect of either C57BL/6 allele on the action of one or both of the DBA/2 alleles.

**MATERIALS AND METHODS**

**Embryo culture and manipulation**

Adult C57BL/6, DBA/2, and (B6D2)F1 females (6-8 weeks old) and males were obtained from Harlan Sprague Dawley. Embryos were isolated at the 1-cell stage from superovulated females that had been given 5 i.u. of pregnant mare serum gonadotropin (PMSG, Calbiochem) followed 48 hours later by 5 i.u. of human chorionic gonadotropin (hCG, Sigma). Cumulus cells were removed by a brief treatment with a dilute solution (100 units/ml) of hyaluronidase (Sigma; 800 units/mg) in M2 medium. Embryos were cultured as in previous experiments (Latham and Solter, 1991) at 37°C under an atmosphere of 90% N2, 5% CO2 and 5% O2 in CZB medium (Chatot et al., 1989) until the 8-cell stage and then switched to Whitten’s medium supplemented with 100 μM EDTA (Abramczuk et al., 1977). Nuclear transplantations were performed in M2 medium (Hogan et al., 1986) as described (McGrath and Solter, 1983; Latham and Solter, 1993). Germinal vesicle-intact oocytes (GVOs) were isolated in the presence of 0.2 mM 3-isobutyl-1-methyl xanthine (IBMX) approximately 40 hours after injection of females with 5 i.u. PMSG and cultured in CZB medium containing 0.2 mM IBMX.

**Two-dimensional protein gel electrophoresis**

GVOs were labeled for 3 hours in CZB medium + IBMX containing 1 mCi/ml L-[35S]methionine (Amersham; >1000 Ci/mmol) and then lysed in a small volume (10-30 μl) of hot (100°C) lysis buffer (dSDS) as described (Latham et al., 1991, 1992, 1993; Garrels, 1983). The samples were processed and 2D gels prepared as described by Garrels (1983). After electrophoresis, the gels were imaged with a Fuji BAS 2000 phosphorimager using settings of 100 μM spatial resolution, latitude of 3, sensitivity of 10,000, and quantitative resolution of 1,024 (Patterson and Latter, 1993). The gels were exposed with calibration chips containing known amounts of 14C (Patterson and Latter, 1993). This enabled the quantitative data obtained from the phosphorimager to be expressed as decays per minute (dpm). The dpm values were divided by the number of trichloroacetic acid-precipitable dpm applied to each gel for conversion to units of parts per million (ppm).
of incorporated radiolabel. The ppm values were later normalized relative to a set of constitutively expressed proteins to correct for minor variations among calibration chips. For statistical purposes, spots falling below the level of detection in any gel were assigned values of 5 ppm for that gel. This represented the limit of reliable spot detection. Gel images were quantified and analyzed using the Quest 2 software program (Garrels and Franza, 1989; Garrels, 1989; Patterson and Latter, 1993).

**RESULTS**

**Efficiency of androgenone development among eggs from individual mothers**

Previous studies (Latham and Solter, 1991) indicated that the effect of DBA/2 egg cytoplasm on androgenetic development was independent of the genotype of the fertilizing male and that the presence of a C57BL/6 genetic contribution to the maternal genome (e.g., in B6D2 F1 mothers) was sufficient to overcome this effect. These observations, which are supported by additional data shown below, provided the basis for an assay to determine the number of loci that contribute to the DBA/2 egg phenotype.

To determine whether the difference between C57BL/6 and DBA/2 eggs resulted from a single genetic difference or differences in two or more loci, individual female progeny (F1:D2) produced from matings of (B6D2):F1 hybrid females to DBA/2 males were analyzed. If the difference between C57BL/6 and DBA/2 eggs resulted from a single genetic difference, then half of the F1:D2 females were expected to produce eggs that were like DBA/2 eggs and half were expected to produce eggs that behaved like C57BL/6 and (B6D2):F1 eggs. To test this, the efficiency with which eggs from individual F1:D2 females supported androgenone development was determined. An average of 9 androgenetic embryos (mean 9.2±1.9, range 5 to 14) was obtained for all F1:D2 females included in the analysis. Most of the F1:D2 females were superovulated and mated to (B6D2):F1 males, although initially some were mated to C57BL/6 males. The use of (B6D2):F1 males greatly improved the efficiency of mating (78% versus 38% for C57BL/6 males), allowing the majority of superovulated F1:D2 females to be included in the analysis. The use of either C57BL/6 or (B6D2):F1 males is justified by the results of the previous study (Latham and Solter, 1991), in which the effect of egg cytoplasm on androgenone development was independent of the genotype of the fertilizing male. The efficiency of blastocyst formation among androgenones produced from DBA/2 eggs fertilized by (B6D2):F1 males ranged from 0 to 17%, with an average rate of 10% and a median rate of 13%. Thus, these androgenones produced with eggs fertilized by (B6D2):F1 males showed a low efficiency and poor morphology of blastocyst formation similar to that (11%) observed for eggs fertilized by C57BL/6 males (Latham and Solter, 1991).

For eggs collected from multiple C57BL/6 females, the efficiency of androgenone development to the blastocyst stage ranged from 31% to 60%, with an average rate of 44% and a median rate of 43%. For individual C57BL/6 females, this figure ranged from 30% to 43%. Thus, the efficiency of blastocyst formation was similar between C57BL/6 androgenones prepared with eggs from multiple females and those prepared with eggs from single females. In a total of 15 experiments employing C57BL/6 embryos, the lowest observed rate of androgenetic blastocyst formation was 30%. This, therefore, represents the minimum rate of blastocyst formation to be expected among androgenones produced with eggs from females with the C57BL/6 phenotype. Accordingly, F1:D2 females producing eggs that supported less than 30% androgenone development were scored as DBA/2-like while those that produced eggs supporting 30% or more androgenone development were scored as C57BL/6-like. By using the minimum rate of blastocyst formation observed for C57BL/6 androgenones, the chance of incorrectly scoring F1:D2 females as DBA/2-like was minimized. Incorrectly scoring F1:D2 females as DBA/2-like would cause the number of responsible loci to be underestimated.

The differences in the egg composition of the majority of individual F1:D2 females assayed were clearly distinguishable by these criteria (Fig. 1). Embryos from a total of 39 F1:D2 females were analyzed. These females exhibited a clear bimodal distribution with respect to the percentage of androgenetic blastocyst formation (Fig. 1). Among the 39 F1:D2 progeny tested, approximately one quarter (10) produced eggs that supported less than 30% development of androgenones to the blastocyst stage. These ranged from 13% to 25%, with a mean of 19±4%. Values for those F1:D2 females scored as C57BL/6-like ranged from 33% to 70%, with a mean of 52±9%. The majority (27/29) of these females yielded values of 40% or more, which is more than twice the mean value.
obtained for those females that were scored as DBA/2-like. That 10 out of 39 F1D2 females were scored as DBA/2-like is consistent with there being two independently segregating loci that determine egg phenotype. The data cannot be explained as a single locus trait (Table 1). The data are also not explicable as involving three or more loci (Table 1).

It might be argued that some C57BL/6-like F1D2 females could have been erroneously scored as DBA/2-like due to artifacts related to embryo isolation or culture. By using the minimum rate of blastocyst formation (30%) observed in 15 independent experiments as the threshold for scoring, however, it is unlikely that this could have occurred simply because the 30% value is unreliable. With regard to the possibility that other factors may have affected androgenone development, gynogenetic blastocyst formation provides a control for satisfactory embryo isolation, manipulation, and culture in these experiments. The previously reported rates of gynogenetic blastocyst formation for eggs obtained from C57BL/6 and (B6D2)F1 females were 85% and 75%, respectively (Latham and Solter, 1991). Of the 10 F1D2 females that were scored as DBA/2-like, one produced eggs for which gynogenetic development (71%) was less than 75%. Two of those scored as C57BL/6-like, however, also produced eggs that supported less than 75% gynogenetic blastocyst formation. If all 3 of these females are excluded, the data are still explicable only by the two-locus model. If all of the F1D2 females that exhibited less than 85% gynogenone development are excluded, the data are again explicable only by the two-locus model. Thus, the simplest explanation, given the available data, is that two loci determine the egg phenotype.

**Comparison of protein synthesis patterns**

To investigate molecular-level differences in gene expression that might relate to the functional differences between C57BL/6 and DBA/2 eggs, the patterns of protein synthesis in germinal vesicle-intact oocytes (GVOs) were compared between these two strains, by 2D gel electrophoresis. A total of 5 gels were obtained for each of these strains. The overall patterns of protein synthesis were quite similar between GVOs of the two strains (Fig. 2). At the level of individual proteins, however, 17 spots differed reproducibly in their rates of synthesis between the two strains and exhibited maximum rates of synthesis of at least 40 ppm (Fig. 2). All of these proteins were synthesized at rates that differed by at least 3-fold between the two strains. Fifteen proteins were synthesized preferentially in C57BL/6 oocytes. Twelve of these were synthesized at average rates that were 4- to 300-fold (median 5-fold) greater in C57BL/6 oocytes than in DBA/2 oocytes (Table 2). Six of these 12 proteins (nos 6, 8-10, 14, 15) were minor proteins, with average rates of synthesis of less than 30

<table>
<thead>
<tr>
<th>Expected</th>
<th>Observed</th>
<th>Expected</th>
<th>Observed</th>
<th>X^2</th>
<th>P</th>
<th>Model</th>
</tr>
</thead>
<tbody>
<tr>
<td>19.5</td>
<td>29</td>
<td>19.5</td>
<td>10</td>
<td>9.26</td>
<td>&lt;0.005</td>
<td>One gene</td>
</tr>
<tr>
<td>29.25</td>
<td>29</td>
<td>9.75</td>
<td>10</td>
<td>0.009</td>
<td>&gt;0.95</td>
<td>Two genes</td>
</tr>
<tr>
<td>34.125</td>
<td>29</td>
<td>4.075</td>
<td>10</td>
<td>6.16</td>
<td>&lt;0.025</td>
<td>Three genes</td>
</tr>
</tbody>
</table>

**Table 1. Expected and observed results for phenotypes of F1D2 females**

![Fig. 2. Two-dimensional gel comparison of protein synthesis patterns of germinal vesicle-intact oocytes from different strains. (A) C57BL/6 oocytes, (B) DBA/2 oocytes. Each panel shows a synthetic gel image produced by phosphorimaging and subsequent background subtraction using the Quest 2 image analysis software. The positions of 17 proteins that exhibit reproducible differences in rates of synthesis are indicated.](image-url)
ppm in C57BL/6 oocytes, while the other six were synthesized strongly (median 200 ppm) in C57BL/6 oocytes. Three proteins (nos. 1, 7, and 13) were synthesized at rates >10-fold greater in C57BL/6 oocytes. Two proteins (nos. 2 and 5) were synthesized at rates 20-fold greater in DBA/2 oocytes than in C57BL/6 oocytes (Table 2). Both of these were prominent proteins, with maximum rates of synthesis of 174 and 148 ppm, respectively.

**Protein synthesis patterns in F1 hybrids**

Previous studies with (B6D2)F1 hybrid females indicated that the C57BL/6 egg phenotype is dominant. Eggs from (B6D2)F1 females supported androgenone development to the same degree as C57BL/6 eggs (Latham and Solter, 1991). The results described above for the F1 D2 females confirmed the dominance of the C57BL/6 egg phenotype. If any of the strain-specific differences in protein synthesis described above were related to the ability to support androgenone development, then this might be reflected in the protein synthesis pattern of (B6D2)F1 oocytes, either as a lack of expression of those proteins synthesized at greater rates in DBA/2 oocytes or as a continued expression of those proteins showing enhanced expression in C57BL/6 oocytes (Table 2). Both of these were prominent proteins, with maximum rates of synthesis of 174 and 148 ppm, respectively.

### Table 2. Major differences in protein synthesis between C57BL/6 and DBA/2 oocytes

<table>
<thead>
<tr>
<th>Spot</th>
<th>ppm* C57BL/6</th>
<th>ppm* DBA/2</th>
<th>ratio (B6D2)/F1</th>
<th>ratio B6/D2</th>
<th>ratio D2/B6</th>
<th>ratio B6/B6D2</th>
<th>ratio D2/B6D2</th>
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<tbody>
<tr>
<td>1</td>
<td>158±22</td>
<td>5±1</td>
<td>131±1</td>
<td>32</td>
<td>20</td>
<td>1.2</td>
<td>1.14</td>
</tr>
<tr>
<td>2</td>
<td>5±0±4</td>
<td>104±14</td>
<td>91±16</td>
<td>11.35</td>
<td>2.8</td>
<td>0.93</td>
<td>0.72</td>
</tr>
<tr>
<td>3</td>
<td>295±51</td>
<td>54±11</td>
<td>249±55</td>
<td>5.5</td>
<td>1.18</td>
<td>20</td>
<td>0.93</td>
</tr>
<tr>
<td>5</td>
<td>5±1</td>
<td>110±30</td>
<td>119±15</td>
<td>4.8</td>
<td>0.72</td>
<td>20</td>
<td>0.93</td>
</tr>
<tr>
<td>6</td>
<td>26±3</td>
<td>6±1</td>
<td>36±2</td>
<td>11.35</td>
<td>2.8</td>
<td>0.93</td>
<td>0.72</td>
</tr>
<tr>
<td>7</td>
<td>122±15</td>
<td>11±4</td>
<td>43±11</td>
<td>4.9</td>
<td>0.63</td>
<td>20</td>
<td>0.93</td>
</tr>
<tr>
<td>8</td>
<td>27±7</td>
<td>5±1</td>
<td>24±14</td>
<td>4.7</td>
<td>0.47</td>
<td>20</td>
<td>0.93</td>
</tr>
<tr>
<td>11</td>
<td>273±31</td>
<td>68±24</td>
<td>163±19</td>
<td>4.0</td>
<td>1.67</td>
<td>20</td>
<td>0.93</td>
</tr>
<tr>
<td>13</td>
<td>1519±279</td>
<td>5±1</td>
<td>2022±469</td>
<td>4.7</td>
<td>0.47</td>
<td>20</td>
<td>0.93</td>
</tr>
</tbody>
</table>

*Values reflect the mean ± s.e.m. of 5 gels for C57BL/6, 5 gels for DBA/2, and 2 gels for (B6D2)F1 germinal vesicle-intact oocytes. Undetected spots were assigned a minimum value of 5 ppm.

**DISCUSSION**

Male pronuclei are negatively influenced by DBA/2 egg cytoplasm and rendered incapable of supporting development...
of androgenetic embryos to the blastocyst stage (Latham and Solter, 1991). The most likely explanation for this is that the DBA/2 egg expresses one or more factors that negatively affect the abilities of certain genes to be expressed at later stages. The results reported here indicate that this does not involve a large number of loci (Table 1). Since approximately one quarter of the F1/D2 females produced eggs that failed to support androgenone development, two independently segregating loci probably determine this phenotype (Table 1). This contrasts with the BALB/c modifier effect (Allen et al., 1990) and the effect of maternal genotype of methylation and expression of the pHRD transgene (Engler et al., 1991), both of which are attributable to single genetic loci, the products of which can act zygotically. It should be noted that even if the two F1/D2 females whose eggs supported 30-35% androgenone development (Fig. 1) were scored as DBA/2-like, the data would still not be explicable as a single gene effect. Although it is formally possible that multiple loci exhibiting incomplete dominance contribute to this phenotype, there is no evidence to indicate that this is the case and the results of Allen et al. (1990) and Engler et al. (1991) support the conclusion that such a phenotype can be attributed to a small number of genes. The simplest explanation for the data, therefore, is that two loci are responsible for the effect of DBA/2 egg cytoplasm on male pronuclear function.

The factors encoded by these two loci probably interact directly or indirectly. Previous results indicated that the C57BL/6 phenotype is dominant, since eggs from (B6D2)/F1 females supported androgenone development to the same extent as C57BL/6 eggs (Latham and Solter, 1991). Concurrently, the DBA/2 egg cytoplasm appears to contain at least one factor that is able to negatively modify male pronuclei (Latham and Solter, 1991) and enhance transgene expression (Surani et al., 1990). The dominance of the C57BL/6 phenotype and the requirement for heterozygosity at two loci indicates that the presence of a C57BL/6 allele at either locus is able to provide a protective function and rescue development. This could occur through either of two mechanisms. First, the two loci may encode factors that operate in a redundant or additive manner, such that a C57BL/6 allele of either locus is sufficient to provide an essential activity to allow expression of critical genes from the paternal genome. The DBA/2 alleles of these loci may not provide the necessary activity. Although a number of possible functions for such factors can be envisioned, one interesting possibility is that these factors direct the establishment of an appropriate pattern of methylation, either through removal or establishment of parent-specific methylation sites. It has been suggested that strain-dependent differences in methylation and expression of transgenes (Sapienza et al., 1989; Allen et al., 1990; Engler et al., 1991) result from differences in methylating activities of modifier alleles (Chaillet, 1994). Thus, methylation or demethylation mediated by either of the C57BL/6 alleles may allow expression of certain genes from the paternal genome.

An alternative possibility is that a C57BL/6 allele of either locus encodes a factor that counteracts the DBA/2 encoded factors. Thus, only females that are homozygous for the DBA/2 allele at both loci would produce eggs that behave like DBA/2 eggs and negatively modify male pronuclei. The DBA/2 phenotype would then require homozygosity at both loci and absence of the C57BL/6-encoded factors. A system in which two mouse egg modifiers operate in an antagonistic manner to modify male pronuclei, and perhaps female pronuclei as well (Reik et al., 1993), and thereby affect the ability of specific genes to be expressed during later development, would be reminiscent of modifiers of position-effect variegation (PEV) in Drosophila. These Drosophila proteins influence the abilities of certain genes to be expressed. As many as 22 different loci encoding PEV modifiers may promote or inhibit heterochromatin spreading and gene inactivation (Tartof and Bremer, 1990). Alterations in the gene dosage for any one of these modifiers can affect the degree of PEV. It has been suggested that mammalian egg modifiers might resemble, both molecularly and with regard to their mechanisms of action, Drosophila PEV modifiers (Surani et al., 1990; Latham and Solter, 1991). This hypothesis has recently received increased attention following the identification of mammalian genes that contain a specialized DNA motif, the chromobox, which is homologous to a DNA binding domain of the Drosophila Polycomb and (Su[z])2 genes. These include the M31, M32 and M33 genes, and the proto-oncogene bmi-1 (van Lohuizen et al., 1991; Singh et al., 1991; Pearce et al., 1992). The results reported here lend additional support to the notion that mouse egg modifiers might function in a manner that resembles closely Drosophila PEV modifiers.

The overall patterns of protein synthesis were quite similar between germlinal vesicle-intact oocytes of the two strains. Two prominent proteins, however, were synthesized almost exclusively in DBA/2 oocytes and several were synthesized almost exclusively in C57BL/6 oocytes. Oocytes of (B6D2)/F1 mice expressed all of the proteins that were synthesized differentially between these two strains. Of the 12 proteins that were synthesized at ≥4-fold greater rates in C57BL/6 oocytes, 9 were synthesized in (B6D2)/F1 oocytes at rates that were similar or greater than the rates in C57BL/6 oocytes. Two of the three proteins that were synthesized at ≥10-fold greater rates in the C57BL/6 oocyte were synthesized at approximately the same rates in (B6D2)/F1 oocytes as in C57BL/6 oocytes. These latter two proteins, and the two DBA/2 encoded proteins may represent good candidates for the egg modifier factors. Future experiments using recombinant inbred strains may reveal whether particular combinations of these proteins correlate with the ability of an egg to affect paternal genome function in androgenones.

It will be of interest to determine where the two loci that are involved in the strain-dependent modification of paternal pronuclei map within the genome, and to eventually isolate and characterize the molecules encoded by them. One possible location would be on chromosome 4, to which one locus responsible for strain-specific differences in transgene methylation and recombination has been mapped (Engler et al., 1991). A second possibility would be chromosome 17, for which a locus has recently been implicated in inter-strain differences between C57BL/6 and BALB/c mice (J. Walter, N. Allen, and W. Reik, personal communication). A third possibility would be the X chromosome, for which results of experiments using hybrids between C57BL/6 and DDA mice (C. Sapienza, personal communication) indicate the possible existence of an imprintor locus.

The overall contribution of these egg modifiers to normal development is unclear. The negative effect of DBA/2 egg cytoplasm on male pronuclear function is only observed in the
absence of a maternal genetic contribution. The alterations in adult phenotype that arise in interstrain nucleocytoplasmic hybrids constructed by exchanging female pronuclei between DBA/2 and C57BL/6 eggs (Reik et al., 1993) indicate that the egg cytoplasm also modifies female pronuclei. It is possible that, within the egg of any given strain, modifications of both maternal and paternal pronuclei are coordinated to allow expression of the full range of genes required during development and in the adult. This possibility is supported by the finding that transplantation of both pronuclei from the cytoplasm of one strain to the cytoplasm of another produces embryos that develop to the blastocyst stage at a high efficiency (Latham and Solter, 1991). Androgenetic embryos and interstrain nucleocytoplasmic hybrids may, therefore, lack the essential complementarity of pronuclear programming to allow expression of one or more genes normally expressed during development or in the adult. This explanation requires that pronuclear modifications occur immediately after fertilization, as appears to be the case (Latham and Solter, 1991). It will be of interest to determine whether the factors that mediate these modifications persist in the cytoplasm beyond the early 1-cell stage. It will also be of interest to determine whether the pronuclear programming is fully determined during the 1-cell stage or whether the pronuclei themselves contribute to further programing events as development proceeds. Last, it will be of great interest to determine whether these modifications affect imprinted or non-imprinted genes, or both. If the pronuclear programming mediated by these egg modifiers differs between strains, it is possible that the array of genes regulated by genetic imprinting is strain-dependent. Some genes may be uniformly imprinted in all strains, some may be non-imprinted in some strains, and for some the direction of imprinting may be strain specific. Such differences have, of course, been observed for transgenes. If such differences are eventually found to exist for endogenous genes, it would be of interest to know whether the imprints that are established during gametogenesis regulate such modifications or whether these modifications comprise a gamete-independent set of imprints, owing their genesis to other differences in maternal and paternal pronuclear structure. Resolution of these questions will require identification of the affected genes.

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