Autosomal and X-chromosome imprinting

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Summary

Mouse genetic studies using Robertsonian and reciprocal translocations have shown that certain autosomal regions of loci are subject to a parental germ line imprint, which renders maternal and paternal copies functionally inequivalent in the embryo or later stages of development. Duplication of maternal or paternal copies with corresponding paternal/maternal deficiencies in chromosomally balanced zygotes causes various effects. These range from early embryonic lethality through to mid-fetal and neonatal lethality, and in some instances viable young with phenotypic effects are obtained. Eight to nine chromosomal regions that give such imprinting effects have been identified. Six to seven of these regions are located in only three chromosomes (2, 7 and 17). The two other regions are located in chromosomes 6 and 11. Maternal and paternal disomies for each of four other chromosomes (1, 5, 9 and 14) have been recovered with different frequencies, but the possibility that this may be due to imprinting has yet to be supported by follow-up studies on regions of the chromosomes concerned. No clear evidence of genetic-background modifications of the imprinting process have been observed in these mouse genetic experiments.

The mammalian X chromosome is also subject to imprinting, as demonstrated by the non-random, paternal X-inactivation in female mouse extra-embryonic tissues and in the somatic cells of marsupial females. There is also the opposite bias towards inactivation of the maternal X in the somatic cells of female mice. On the basis that both X-chromosome inactivation and autosomal chromosome imprinting may be concerned with gene regulation, it is suggested that evidence from X-chromosome inactivation studies may help to elucidate factors underlying the imprinting of autosomes. The relevant aspects of X-inactivation are summarized.

Key words: imprinting, parental origin effects, X-activation.

Introduction

The genetic data describing chromosome imprinting phenomena in the mouse have been the subject of a number of recent reviews (Searle and Beechey, 1985; Cattanach, 1986; Cattanach and Beechey, 1990). These have shown the development of an imprinting map which has defined regions of autosomes in which one or more genes appear to be modified as they pass through the germ lines of one, or other, or both parents, with the result that the maternal and paternal copies become functionally different in the zygote.

Differential DNA methylation has been suggested as a mechanism by which these functional differences could be brought about (Surani et al. 1988). This view has been supported by a number of studies which have demonstrated that certain transgenic insertions show differing methylation and expression levels according to parental route of transmission (Reik et al. 1987; Sapienza et al. 1987; Swain et al. 1987; Hadchouel et al. 1987). The concept implied is that the transgenes reflect the behaviour of the endogenous sequences in the region of their site of integration. However, a high proportion of the transgenes studied show such imprinting effects and none of the four that have been mapped are located in the imprinting regions defined by the genetic experiments. It has therefore been concluded that imprinting may be much more widespread throughout the genome than indicated in the imprinting map of the mouse. Moreover, a variation in expression of transgenes in cells of different tissues and organs correlated with overall changes in DNA methylation has been noted, and observed to be dependent upon genetic background (Sapienza, 1989). This, together with the recognised variability in the penetrance and expression of certain human conditions thought to be influenced by imprinting effects, has suggested that the imprinting process itself is subject to genetic modification (Sapienza, 1989). If true, this could further reduce the likelihood of imprinting effects being easily detected in the mouse genetic experiments.

This paper therefore reviews the mouse genetic data with additional emphasis given to the question of genetic background effects. An update of the imprinting map also identifies a new imprinting region, and a series of data which may also demonstrate a further consequence of imprinting is presented. In addition, certain aspects of X-chromosome inactivation that indicate imprinting effects in this chromosome are discussed. It is suggested that autosomal chromosome
and X-chromosome imprinting are manifestations of the same phenomenon, and that evidence from X-inactivation studies may help to elucidate factors underlying the imprinting of autosomes.

Methods

The genetic methods which have identified the mouse imprinting regions have been described in previous reviews (Searle and Beechey, 1985; Cattanach, 1986; Cattanach and Beechey, 1990). Suffice to say here that by intercrossing heterozygotes for Robertsonian translocations that exhibit high rates of non-disjunction, animals can be produced which have two homologues of a selected chromosome from one parent and none from the other. The maternal or paternal disomy (with corresponding paternal or maternal nullisomy, respectively) can be identified with appropriate marker genes (Fig. 1). Similarly, intercrosses of heterozygotes for reciprocal translocations can generate animals with two copies of regions proximal or distal to the translocation breakpoints from one parent, and none from the other. Again, such duplications (with corresponding deficiencies) can be identified with marker genes. All such animals are chromosomally balanced and differ genetically from normal mice only by the parental origins of their two copies of a particular chromosome or chromosome region. A failure of a disomy or duplication from one parent to complement normally a corresponding nullisomy or deficiency from the other parent constitutes the genetic evidence for the occurrence of chromosome imprinting.

Established autosomal chromosome imprinting effects

Fig. 2 illustrates the current status of the imprinting map and it can be seen that most of the chromosome complement has now been investigated. Only chromosome 12 and parts of chromosomes 7, 8, 10 and 18 remain to be studied: for each of these untested regions, translocations (indicated in italics) are available that could be used for complementation analyses. The chromosomes of main interest are chromosomes 2, 6, 7, 11 and 17 and, as will be discussed, chromosomes 1, 5, 9 and 14 may also show imprinting phenomena. Three somewhat overlapping but clearly established categories of imprinting effect have been found with the main imprinting regions. First, there is the category in which a complementation type (maternal or paternal duplication) dies very early in development. Three examples of this type of effect have been observed (Fig. 2): with maternal duplication of proximal chromosome 2, with maternal duplication of proximal chromosome 6, (Beechey and Cattanach, 1989), and with paternal duplication of distal chromosome 7 (Beechey and Searle, 1987). In each case eye colour markers have been available, and this has permitted screening for complementation among the zygotes from about day 11 of gestation, when eye pigmentation is first detectable. The absence of the marked young has indicated that each of the above genotypic classes dies before or shortly after implantation (Cattanach and Beechey, 1990). Translocations (indicated in italics in Fig. 2) are available which could further define the limits of the imprinting regions of proximal chromosome 2 and distal chromosome 7. Because of the severity of these imprinting effects, their occurrence in embryonic stages...
Fig. 2. Autosomal chromosome imprinting map of the mouse. Map of the mouse autosomal chromosomes showing regions of normal and defective complementation (imprinted regions). Chromosomes showing normal complementation or giving differential recoveries of their maternal (M) or paternal (P) disomy classes are shown only once. Chromosomes showing imprinting are displayed separately, the maternal (M) and paternal (P) copies being indicated. Symbols for Robertsonian translocations (e.g. Rb1Bnr) that have been used to identify imprinted chromosomes are shown above the centromeres; symbols for reciprocal translocations (e.g. T13H) used to localise the regions are shown at their breakpoint positions. Reciprocal translocations that may be used to localise further the imprinting regions are given in italics.

that are difficult to investigate, and the rarity of recovery of maternal/paternal duplications for the proximal regions of chromosomes, they cannot be used effectively to provide information on possible genetic background effects. It is worth noting, however, that the proximal chromosome 6 lethality was observed in two separate studies with two different Robertsonian translocations (one of which is of feral origin) and with marker genes derived from unrelated stocks. The imprinting effect is not therefore dependent upon a single genetic background.

A second category of imprinting effect is observed as mid-fetal to early neonatal death. This has been seen with three regions (Fig. 2); at mid-gestation with maternal duplication for distal chromosome 7 (Beechey and Searle, 1987), at a later stage of fetal to early neonatal development with maternal duplication for proximal chromosome 7 (Beechey and Searle, 1987), and at about the time of birth with a maternal deletion (not balanced by paternal duplication) of a proximal part of chromosome 17 (Johnston, 1974, 1975). As yet, little is known about any strain-dependent variabilities in the chromosome 7 late lethality. Both, however, were detected with two different reciprocal translocations and with marker genes derived from two different stocks.

The chromosome 17 phenomenon is somewhat different from all others described so far but can be interpreted as an imprinting effect. The basic observation is that the hairpin tail (T\textsuperscript{Hp}) deletion can only be maintained by transmission through the male; when it is inherited through the female the deletion behaves as a dominant lethal (Johnston, 1974; 1975). This has been interpreted to mean that a maternal copy of a gene located in the region of the deletion is required for normal development; the same reasoning would suggest that the paternal copy is not functional. Deletion mapping has allowed the region responsible, denoted T maternal effect (T\textsuperscript{Me}), to be identified (Winking and Silver, 1984). Variability in the abnormality leading to the lethality has not been documented, but it is known that the lethality may occur before birth, or a day or two thereafter, and in some exceptional circumstances maternally-derived T\textsuperscript{Hp} young have survived to adulthood (Lyon, personal communication). It is not clear, however, whether this is due to a strain-dependent
variability in the imprinting process or only to a variation in the ability of mice of different genetic backgrounds to survive the effects.

Phenotypic abnormalities characterise the third category of imprinting effect seen in mouse genetic experiments. Three examples of this type of phenomena have been found (Fig. 2): with maternal and paternal duplication for a distal region of chromosome 2 (Cattanach and Kirk, 1985); with maternal and paternal duplication for the proximal region of chromosome 11 (Cattanach and Kirk, 1985); and, very recently, with paternal duplication for a distal region of chromosome 17. The latter finding has not been observed with all translocations with breakpoints.

Table 1. Recovery of maternal and paternal duplications for distal regions of chromosome 2

<table>
<thead>
<tr>
<th>Translocation</th>
<th>Marked parent</th>
<th>Number of young</th>
<th>% maternal duplication</th>
<th>% paternal duplication</th>
</tr>
</thead>
<tbody>
<tr>
<td>T(2:4)13H</td>
<td>-</td>
<td>124</td>
<td>12.1</td>
<td>15.3</td>
</tr>
<tr>
<td>T(2:2)24H</td>
<td>-</td>
<td>158</td>
<td>15.2</td>
<td>10.1</td>
</tr>
<tr>
<td>T(2:9)1H</td>
<td>M</td>
<td>73</td>
<td>12.3</td>
<td>9.6</td>
</tr>
<tr>
<td></td>
<td>P</td>
<td>74</td>
<td>17.6</td>
<td>13.5</td>
</tr>
<tr>
<td>T(2:11)3OH</td>
<td>a) M</td>
<td>76</td>
<td>5.3</td>
<td>6.6</td>
</tr>
<tr>
<td></td>
<td>b) P</td>
<td>147</td>
<td>8.3</td>
<td>15.2</td>
</tr>
<tr>
<td>T(2:8)26H</td>
<td>-</td>
<td>326</td>
<td>9.2</td>
<td>10.4</td>
</tr>
<tr>
<td>T(2:4)Sn</td>
<td>-</td>
<td>209</td>
<td>15.3</td>
<td>17.7</td>
</tr>
<tr>
<td>T(2:16)28H</td>
<td>-</td>
<td>90</td>
<td>-</td>
<td>-</td>
</tr>
</tbody>
</table>

M=maternally marked; P=paternally marked

between T1Sn and T28H (shown in italics in Fig. 2), and studies with these may further define the exact region subject to imprinting.

Table 1 summarizes the frequencies with which the hypo- and hyperkinetic classes have been detected in studies with a series of different chromosome 2 translocations. It can be seen that, generally, the recovery varied little among the different translocations: the biggest variation was instead found in separate studies with one translocation (T30H) carried out on different genetic backgrounds. More significant, perhaps, has been the consistent observation that whereas the hyperkinetic class invariably dies within 4–5 days of birth with T24H, T30H and T26H, it can survive for up to 10 days with T1Sn and, not infrequently, into adulthood with T11H. Clearly, survival does not correlate with the location of the translocation breakpoints in the chromosome (Fig. 2), nor, ultimately, does it appear to be dependent upon genetic background. Thus, recent work has shown that the genetic background that appeared to reduce the recovery of the hyperkinetic class with T30H (Table 1, test a cf. test b) has not been found to reduce the survival of the hyperkinetic class with T11H (15/17 cf. 12/17) (Cattanach, unpublished observations). The reason for the variability in survival of the hyperkinetic paternal duplication type within the different translocations remains an enigma.

Opposite phenotypes also characterise the chromosome 11 imprinting region (Fig. 2). Here, the effect appears to be restricted to growth and, ultimately, adult size: maternal duplication is invariably associated with a small size (approximately 70% of normal) and paternal duplication with large size (approximately 130% of normal). Growth rates from birth to adulthood have appeared normal, and prenatal the size differences of the two complementation classes have been distinguished in fetuses as early as 13.5 days of gestation, with placental sizes being correlated. A significant difference in fetal size was not detected at 12.5 days, which might suggest that the size difference may have been initiated only a little earlier. The chromosome 11 effect has been observed principally in studies using the
Table 2. Recovery of maternal and paternal disomies involving chromosomes 1, 5, 9 and 14

<table>
<thead>
<tr>
<th>Chromosome</th>
<th>Translocation</th>
<th>Test number</th>
<th>Disomy</th>
<th>Number of marked young</th>
<th>Total young</th>
<th>% marked</th>
<th>Ratio M:P</th>
<th>Reference</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>Rb(1.3)1Bnr</td>
<td>1</td>
<td>M</td>
<td>11</td>
<td>631</td>
<td>1.74</td>
<td>1:3:1</td>
<td>Tease and Cattanach, 1986</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>P</td>
<td>7</td>
<td>512</td>
<td>1.37</td>
<td></td>
<td></td>
</tr>
<tr>
<td>2</td>
<td></td>
<td>2</td>
<td>M</td>
<td>12</td>
<td>589</td>
<td>2.04</td>
<td>10:7:1</td>
<td>Cattanach. 1988</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>P</td>
<td>1</td>
<td>540</td>
<td>0.19</td>
<td></td>
<td></td>
</tr>
<tr>
<td>3</td>
<td></td>
<td>3</td>
<td>M</td>
<td>19</td>
<td>766</td>
<td>2.48</td>
<td>1:2:1</td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>P</td>
<td>14</td>
<td>788</td>
<td>1.78</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Total</td>
<td></td>
<td>42</td>
<td>M</td>
<td>42</td>
<td>1986</td>
<td>2.12</td>
<td>1:8:1</td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>P</td>
<td>22</td>
<td>1840</td>
<td>1.20</td>
<td></td>
<td></td>
</tr>
<tr>
<td>5</td>
<td>Rb(5.15)3Bnr</td>
<td>1</td>
<td>M</td>
<td>3</td>
<td>580</td>
<td>0.52</td>
<td>1:2:7</td>
<td>Tease and Cattanach, 1986</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>P</td>
<td>9</td>
<td>654</td>
<td>1.38</td>
<td></td>
<td></td>
</tr>
<tr>
<td>9</td>
<td>Rb(9.14)6Bnr</td>
<td>1</td>
<td>M</td>
<td>4</td>
<td>811</td>
<td>0.49</td>
<td>1:3:5</td>
<td>Lyon, 1983a</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>P</td>
<td>15</td>
<td>917</td>
<td>1.64</td>
<td></td>
<td></td>
</tr>
<tr>
<td>14</td>
<td>Rb(9.14)6Bnr</td>
<td>1</td>
<td>M</td>
<td>5</td>
<td>469</td>
<td>1.07</td>
<td>1:1:1</td>
<td>Lyon, 1983a</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>P</td>
<td>14</td>
<td>1229</td>
<td>1.14</td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td>2</td>
<td>M</td>
<td>2</td>
<td>811</td>
<td>0.25</td>
<td>1:6:6</td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>P</td>
<td>15</td>
<td>917</td>
<td>1.64</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Total</td>
<td></td>
<td>7</td>
<td>M</td>
<td>7</td>
<td>1280</td>
<td>0.55</td>
<td>1:2:5</td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>P</td>
<td>29</td>
<td>2146</td>
<td>1.35</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

M, maternal disomy; P, paternal disomy.

Rb4Bnr Robertsonian translocation, which is of feral origin, but it has also been detected with the T30H reciprocal translocation; and the latter finding has localised the region involved to the proximal part of the chromosome. Two different markers, derived from stocks of different genetic backgrounds were used in the former studies. Therefore, there is as yet no indication that chromosome 11 imprinting varies with genetic background.

The last example of anomalous complementation phenotypes has been detected only very recently. Paternal duplication of the region of chromosome 17 distal to T138Ca (Fig. 1) is associated with a size reduction (approximately 70% of normal); no clear effect of any kind has yet been observed with the reciprocal, maternal duplication genotype (Cattanach and Beechey, unpublished observations). Although the gene markers used have not permitted the early detection of the complementation classes, size differences among the young have not been noted at birth, in contrast to the situation found with chromosome 11 imprinting. Rather, the size difference was only noted in the third week of life after which it was maintained at about 70% of normal through to adulthood. It has not yet been established if the size effect with distal chromosome 17 imprinting is limited to the one generation, as is the case for both the distal chromosome 2 and proximal chromosome 17 imprinting effects.

Other possible examples of autosomal chromosome imprinting

Two other phenomena in the mouse could be regarded as possible examples of imprinting. One of these has been recognised (Cattanach and Beechey, 1990) through differential recoveries of maternal and paternal disomies, or duplications for certain chromosome regions in crosses between heterozygotes for Robertsonian or reciprocal translocations. Tables 2 and 3 summarize the pertinent data. The first example can be found in the work of Lyon and Glenister (1977) who noted a consistent shortage of marked young representing paternal, as opposed to maternal, duplication for the region of chromosome 17, proximal to the T138Ca breakpoint (Fig. 2). They suggested this represented a further manifestation of the Tme (Tlp) effect associated with this region of the chromosome, although the abnormalities associated with the Tme lethality were not directly observed. A complicating factor in these studies was the presence of r haplotypes which would have distorted the segregation ratios in one of the reciprocal crosses. However, similar observations have since been made for chromosomes 1, 5, 9 and 14 in various studies using Robertsonian translocations (Fig. 2, Table 2).

In each of the three experiments with chromosome 1, maternal disomies were more numerous than paternal disomies. This differed significantly in magnitude in the
Chromosome 6
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There was a smaller and non-significant variation also showed a significant excess of paternal disomies discussed.

Chromosome 14 there was again significant variation effect observed can be discounted as no differential recoveries of the chromosomes 1, 5, 9 and 14 are also available and these are presented in Table 3. Consistent with the observed excess of maternal disomy 1 young in the Robertsonian translocation studies (Table 2), results with T(1;13)70H intercrosses have suggested that maternal duplication for the region of chromosome 1 distal to the breakpoint may be recovered more frequently than the reciprocal class. This might localise a responsible factor to this region of the chromosome (not shown in Fig. 2) but the data are limited. That a region of chromosome 13 might be responsible for the excess of paternal disomy 5 (Table 2) is located more proximally in the chromosome but, alternatively, it could simply mean that the reciprocal translocation data do not confirm the Robertsonian translocation findings. The same conclusions could be drawn from the reciprocal translocation data for the distal regions of chromosomes 9 and 14 (Table 3). Although there was in each case an excess of the paternal duplication class over the reciprocal maternal duplication class consistent with the disomy findings (Table 2), the differences were not significant (for chromosome 9, \( P = 0.26 \); for chromosome 14, \( P = 0.54 \)).

It is difficult to evaluate the significance of the differential recoveries of the chromosomes 1, 5, 9 and 14 maternal and paternal disomies when supporting evidence is not available from the reciprocal translocation studies. Further data with T27H might establish the phenomenon for the distal region of chromosome 1, but for chromosomes 5, 9 and 14 evaluation of the disomy data must await the results of reciprocal translocation studies upon the proximal regions of the chromosomes; and, because of the rarity of the requisite meiotic (adjacent-2) disjunction, this data will be hard to obtain. Should the validity of the differential recovery phenomena be established it is likely that prenatal or neo-natal semi-lethality will be the cause. This would be consistent with the results of Lyon and Glenister (1977) with T138Ca, which showed a shortage of young with paternal duplication for the proximal region of chromosome 17, apparently due to the Time (T\( ^{dp} \)) imprinting effect. It is pertinent to note that neither in the T138Ca study, nor in the recent T\( ^{dp} \) work (Lyon, personal communication), was any impairment of post-natal development noted among the survivors of the neo-natal lethal class. The failure to detect defects in numerically deficient disomy classes is not therefore

### Table 3. Recovery of maternal and paternal duplications for the distal regions of chromosomes 1, 5, 9 and 14

<table>
<thead>
<tr>
<th>Chromosome</th>
<th>Translocation</th>
<th>Test number</th>
<th>Duplication</th>
<th>Number of marked young</th>
<th>Total young</th>
<th>% marked</th>
<th>Ratio</th>
<th>Reference</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>T(1:13)70H</td>
<td>1</td>
<td>M</td>
<td>6</td>
<td>14</td>
<td>45.0</td>
<td>4.5:1</td>
<td>Searle et al. 1971</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>P</td>
<td>4</td>
<td>42</td>
<td>9.5</td>
<td></td>
<td>Beechev, unpublished data</td>
</tr>
<tr>
<td>5</td>
<td>T(5:13)264Ca</td>
<td>1</td>
<td>M</td>
<td>1</td>
<td>16</td>
<td>6.3</td>
<td>1.18</td>
<td>Searle and Beechey, 1978</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>P</td>
<td>8</td>
<td>72</td>
<td>11</td>
<td></td>
<td></td>
</tr>
<tr>
<td>2</td>
<td></td>
<td>2</td>
<td>M</td>
<td>12</td>
<td>67</td>
<td>12.9</td>
<td>1.81</td>
<td>Searle et al. 1971</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>P</td>
<td>6</td>
<td>60</td>
<td>10</td>
<td></td>
<td></td>
</tr>
<tr>
<td>3</td>
<td></td>
<td>3</td>
<td>M</td>
<td>15</td>
<td>120</td>
<td>12.5</td>
<td>1:1.3</td>
<td>Beechev, unpublished data</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>P</td>
<td>34</td>
<td>208</td>
<td>16.4</td>
<td></td>
<td>(unpublished data)</td>
</tr>
<tr>
<td>9</td>
<td>T(9:17)138Ca</td>
<td>1</td>
<td>M</td>
<td>12</td>
<td>148</td>
<td>8.1</td>
<td>1:1.6</td>
<td>Cattanach and Beechey</td>
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<td></td>
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<td>P</td>
<td>11</td>
<td>87</td>
<td>12.6</td>
<td></td>
<td>(unpublished data)</td>
</tr>
<tr>
<td>14</td>
<td>T(14.15)6Ca</td>
<td>1</td>
<td>M</td>
<td>10</td>
<td>124</td>
<td>8.1</td>
<td>1:1.3</td>
<td>Cattanach and Beechey</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>P</td>
<td>5</td>
<td>44</td>
<td>11.4</td>
<td></td>
<td>(unpublished data)</td>
</tr>
</tbody>
</table>

M. maternal; P. paternal.

Three studies (\( \chi^2 = 6.04; P = 0.049 \)), but overall there was a significant difference in the frequency of the two disomic classes (\( \chi^2 = 5.29; P = 0.011 \)). With chromosome 5 there was a smaller and non-significant variation between the results of repeat experiments (\( \chi^2 = 0.25; P = 0.62 \)) and, here, there was a significant excess of paternal disomies over maternal disomies (\( \chi^2 = 7.41; P = 0.33 \times 10^{-2} \)). The one result involving chromosome 9 also showed a significant excess of paternal disomies over maternal disomies (\( P = 0.035 \)). And, finally, with chromosome 14 there was again significant variation between the results of repeat experiments (\( \chi^2 = 4.47; P = 0.035 \)) but, overall, a significant excess of paternal disomies over maternal disomies (\( \chi^2 = 5.35; P = 0.01 \)). No phenotypic abnormalities or indications of impaired development were noted for any of the disomic classes discussed.

Some reciprocal translocation data pertaining to the distal regions of chromosomes 1, 5, 9 and 14 are also available and these are presented in Table 3. Consistent with the observed excess of maternal disomy 1 young in the Robertsonian translocation studies (Table 2), results with T(1:13)70H intercrosses have suggested that maternal duplication for the region of chromosome 1 distal to the breakpoint may be recovered more frequently than the reciprocal class. This might localise a responsible factor to this region of the chromosome (not shown in Fig. 2) but the data are limited. That a region of chromosome 13 might be responsible for the excess of paternal disomy 5 (Table 2) is located more proximally in the chromosome but, alternatively, it could simply mean that the reciprocal translocation data do not confirm the Robertsonian translocation findings. The same conclusions could be drawn from the reciprocal translocation data for the distal regions of chromosomes 9 and 14 (Table 3). Although there was in each case an excess of the paternal duplication class over the reciprocal maternal duplication class consistent with the disomy findings (Table 2), the differences were not significant (for chromosome 9, \( P = 0.26 \); for chromosome 14, \( P = 0.54 \)).
7) of the 8 (or 9) regions locate to only 3 chromosomes. There are now at least 8 autosomal regions in which genetic experiments. This contrasts with the findings etic background effects has been found for any of the only crudely defined by the breakpoints of the somatic chromosome complement is non-random; 6 (or 9 if the two distal chromosome 7 lethalities clearly-established imprinting effects have been ident-
ized in the mouse is that based on the observation that the semi-dominant gene, fused, (Fu) shows a differential expression according to parental origin. Thus, the penetrance and expression is lower when the gene is transmitted by the mother than by the father, and this is influenced by genetic background (Reed, 1937). A recent brief report has further suggested that it is the penetrance of the maternally-derived gene that is subject to genetic background modification (Agulnik and Ruvinsky, 1988), but the results are not consistent with the earlier work. Significantly, the Fu gene is located proximally on chromosome 17 near to Tme and within the region of differential recovery defined by the T138Ca studies (Lyon and Glenister, 1977). The phenomenon, even if attributable to imprinting, does not therefore extend the range of imprinting regions in the mouse beyond those demonstrated by the various genetic studies. In this connection, it might be noted that in man, numerous examples of differential expression of semi-dominant genes according to parental origin have been cited as possible examples of imprinting, and most of these tend to locate in or near regions of the human chromosome map homologous to the mouse imprinting regions (Reik, 1989; Cattanach and Beechey, 1990).

Conclusions on autosomal imprinting

There are now at least 8 autosomal regions in which clearly-established imprinting effects have been identified, and these act upon at least three different stages of development. The number of such regions could be extended to 9 if the two distal chromosome 7 lethals are regarded as distinct, separate phenomena. Irrespective of the exact number, it is evident that the distribution of the imprinting regions over the autosomal chromosome complement is non-random; 6 (or 7) of the 8 (or 9) regions locate to only 3 chromosomes (chromosomes 2, 7 and 17), the others being on chromosomes 6 and 11. This situation could be modified somewhat if the differential recovery effects noted with the disomies for chromosomes 1, 5, 9 and 14 were established as imprinting phenomena, but as yet the evidence is not convincing. The fact that the closely linked Tm and Fu loci show evidence of imprinting, suggests that more than one locus is subject to imprinting in each of the imprinting regions currently only crudely defined by the breakpoints of the translocation studies. No convincing evidence of genetic background effects has been found for any of the imprinting phenomena distinguished in the mouse genetic experiments. This contrasts with the findings made in the transgenic work (Sapienza, 1989). Further investigation with a broader range of mouse strains may be needed to determine if the imprinting process is subject to genetic background modification.

X chromosome Imprinting

In addition to the imprinting phenomena seen with the mouse autosomal chromosome translocations there is also the earlier evidence of X chromosome imprinting (Lyon and Rastan, 1984). The best known example is the preferential inactivation of the paternal X in the extra-embryonic membranes of female mice (Takagi and Sasakl, 1975; West et al. 1977; Harper et al. 1982), which indicates that the maternal and paternal X chromosomes have received some imprint to permit their distinction in the zygote. The non-randomness is not rigidly fixed, however. Patroclinous XO females do not have their single (paternal) X inactivated in these tissues (Frels and Chapman, 1979; Papaionnou and West, 1981); parthenogenetic diploid embryos may have one X inactivated in both embryonic (Kaufman et al. 1978) and extra-embryonic tissues (Rastan et al. 1980) even though both their X chromosomes are of maternal origin; androgenic diploid moles with two paternal X chromosomes inactivate only one X (Tsukahara and Kajii, 1985). The imprint to distinguish maternal and paternal X chromosomes may therefore only confer an enhanced probability of inactivation of the paternal X, rather than the maternal X, in extra-embryonic tissues (Lyon 1983a).

A further example of non-random X inactivation is found in marsupials (Vandeberg et al. 1987). Rather than the random X inactivation that occurs in the somatic cells of eutherians, paternal X inactivation is the rule in the somatic cells of marsupials. Less well known, however, is the small but consistent non-random element in the inactivation of the X in the somatic cells of female mice. This was initially observed in a large series of studies using an X-autosome translocation that caused autosomal gene variegation attributable to X inactivation (Cattanach and Perez, 1970). The level of variegation differed between females derived from reciprocal crosses in a way which would suggest that the maternal X is more likely to become inactivated. An equivalent difference in expression of the X-linked gene Ta in reciprocal crosses (Dun and Fraser, 1959; Fraser and Kindred, 1960) can be similarly interpreted. The same type of finding has since been made with the X-linked genes, brindled (Mo<sup>br</sup>) (Falcooran et al. 1982), phosphoglycerate kinase (P<sub>gk</sub>-1) (Bucher and Krietsch, 1988; Cattanach and Bucher, unpublished observations) and then very recently with glucose-6-phosphate dehydrogenase (Gpdx) (Peters, personal communication). This phenomenon in somatic cells is therefore similar to that in the extra-embryonic membranes and to that in the somatic cells of marsupials, but the bias is in the opposite direction, and because of the substantial random element it is relatively hard to detect. Another possible example of X-chromosome imprinting is the earlier time in development at which the maternal P<sub>gk</sub>-
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1 allele is thought to be expressed, relative to that of the paternal allele (Krietsch et al. 1982). In all such situations, an imprinting of the maternal or paternal chromosomes to influence their behaviour in the zygote is indicated.

There are also a number of other points regarding X-inactivation that may be pertinent to the imprinting of the chromosome.

First, X-inactivation appears to be dependent upon a single locus, or inactivation centre. A variety of data has established this in the mouse (reviewed by Cattanach, 1975); and in man (Therman et al. 1974, 1979; Tabor et al. 1983). In the mouse the putative inactivation centre, \( X_{ce} \), has different allelic forms which govern the probability of the X in which they are located becoming the inactive one in somatic cells (Johnston and Cattanach, 1981). The evidence on whether they modify the non-random X-inactivation in extra-embryonic tissues is, unfortunately, conflicting (Rastan and Cattanach, 1983; Bücher et al. 1985).

Second, inactivation of the X is achieved by a spread of inactivation from the centre to contiguous regions of the X, and into attached autosomal regions (Lyon, 1968; Cattanach, 1975; Russell, 1983).

Third, the spread of inactivation is reversible such that with age, or time, inactivated loci may be reactivated (Cattanach, 1974; Wareham et al. 1987; Brown and Rastan, 1988). Reactivation of certain loci also typifies the paternal X inactivation of marsupials (Cooper et al. 1977; Johnston et al. 1978).

As a final point, it may be noted that experiments to search for genetic control of X-inactivation and position effect variegation in the mouse identified only the X-chromosomal \( X_{ce} \) system of control (Cattanach and Isaacson, 1965, 1967; Falconer and Isaacson, 1972; Falconer et al. 1982). As yet, no evidence of genetic background modifications have been found.

**X-chromosome imprinting as a model for autosome imprinting**

The term 'chromosome imprinting' was originally coined by Crouse (1960) to define the mechanism that led to the selective paternal chromosome eliminations that occur in the soma and germ line of the fly, *Sciara*. It has also been used in relation to the genetic inactivation and heterochromatic behaviour of the paternal chromosome set in coccids (Brown and Nur, 1964) and, more recently, it has been applied to differential DNA strand modification in yeasts and allelic exclusion in higher plants (this volume). It is a moot point as to whether all imprinting effects, including the autosomal and X chromosome examples in the mouse, represent diverse manifestations of the same basic phenomenon. However, it would be surprising if more than one mechanism for identifying maternal and paternal chromosomes and, thence, regulating gene dosage, were to have evolved within a species (mouse), or within a class (mammals). As such, information on imprinting and inactivation of the X chromosome could help to elucidate factors underlying autosomal chromosome imprinting effects (see Fig. 3). Thus: (1) in each imprinting region there may be a controlling locus which receives the maternal/paternal germ line imprint; (2) according to the imprint the controlling locus may modify adjacent gene loci in the zygote to render them inactive; (3) the modification may be achieved by a spread of inactivation such that only one or a series of adjacent loci may be inactivated; (4) the inactivation may not be permanent and could therefore last for only a short period in development and be absent at later ages; (5) the imprinting may only influence the probability with which one or other parental copy may be active, and therefore not all maternal/paternal disomies or duplications may give major, easily detectable effects; (6) there could be
different alleles at each controlling locus which have different sensitivities to imprinting and therefore different probabilities of inactivating adjacent loci.

Resolution of how autosomal imprinting effects are brought about must await identification of loci subject to the imprinting processes. It is remarkable, however, that so many of the established and putative examples of imprinting appear to concern growth (Cattanach and Beechey, 1990); in mammals, there may be a specific need for the regulation of genes for growth factors and this may be achieved by chromosome imprinting.

References


