Genomic imprinting: normal complementation of murine chromosome 16

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Summary

Parental imprinting effects for chromosome 16 were investigated using disomic animals which were obtained by mating (*Rb32Lub* × *Rb2H*) F1 mice. Two allelic forms of the enzyme CuZn-superoxide dismutase, Sod-1[a] and Sod-1[c], were used to identify maternally or paternally disomic animals. Both types of disomic animals were found with the expected frequencies and did not visibly differ from one another or from non-disomic animals. These results indicate that the genomic imprinting mechanism either does not act on chromosome 16, or, if it does, does not do so in a manner which affects normal development.

1. Introduction

Although both parental sexes contribute equivalent genetic information to the zygote, mouse development requires the presence and expression of both the maternal and paternal genomes. It has been proposed that these genomes are imprinted in some manner during gametogenesis and that this imprinting determines how the genes on these chromosomes are expressed after fertilization. Four lines of evidence have led to this conclusion. (1) There are differences in development of maternally and paternally disomic animals (animals in which both chromosomes of an autosomal pair are derived from the same parent) (Cattanach & Kirk, 1985). (2) Androgenetic and gynogenetic embryos, either obtained with pronuclear transfer experiments or as induced parthogenotes, do not develop to term (McGrath & Solter, 1983, 1984; Barton et al. 1984; Surani 1984, 1986). (3) The methylation and expression patterns of certain transgenes are different if inherited from the mother than from the father (Hadchouel et al. 1987; Reik et al. 1987; Sapienza et al. 1987; Swain et al. 1987). (4) The development of some tumours correlates with the genetic alteration of a chromosome which is preferentially of one parental origin only (Schroeder et al. 1987; Toguchida et al. 1989).

The determination of which chromosomes or chromosome segments are imprinted has relied on the construction and analysis of either disomic animals or of animals carrying balanced combinations of re-

ciprocal translocations in which deletion of a segment of a chromosome derived from one parent is complemented by two copies of the segment on chromosomes derived from the other parent (Searle & Beechey, 1978). To date, failure of complementation has been found for parts of chromosomes 2, 6, 7, 8, 11, and 17, and normal complementation has been observed for all of chromosomes 1, 3, 4, 5, 9, 13, 14, and 15 (Beechey et al. 1988). In the case of chromosome 16, the available evidence indicates that complementation is normal for the distal tip of the maternally derived chromosome, but the status of the rest of the maternally derived chromosome and for all of the paternally derived chromosome is unknown. Because of our interest in the effects of trisomy for mouse chromosome 16 and the possible role of imprinting in the determination of the phenotype, we undertook a study of animals disomic for maternal and paternal chromosomes 16.

To determine the influence of maternally or paternally derived chromosome 16 on development, we used two different mouse strains carrying Robersonian translocations for chromosome 16: *Rb(16.17)32Lub* and *Rb(11.16)2H*. Furthermore, we made use of a chromosome 16 marker, the enzyme CuZn-superoxide dismutase (Sod-1), which exists in two allelic forms, *Sod-1[a]* and *Sod-1[c]*.

2. Materials and methods

(i) *Animals*

Mice carrying the Robertsonian translocation chromosomes (or metacentrics) *Rb(11.16)2H* (*Sod-*

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1ᵃ), *Rb(11.16)2H (Sod-1ᶜ)*, *Rb(16.17)32Lub (Sod-1ᵃ)*, and *Rb(16.17)32Lub (Sod-1ᶜ)* were bred in our animal facility. To obtain the *Rb2H* and *Rb32Lub* strains of mice carrying the allelic isozyme marker, *Sod-1ᶜ*, strain ICR (*Sod-1ᶜ*) mice had been bred with animals carrying *Sod-1ᵃ*-marked Robertsonian translocation chromosomes, and the offspring and further generations were tested for the presence of metacentric chromosomes and *Sod-1ᶜ*.

(ii) *Matings*

(*Rb32Lub* × *Rb2H*) F1 females, homozygous for *Sod-1ᵃ*, were mated with similar F1 males homozygous for *Sod-1ᶜ*, and vise versa. As a result of chromosomal non-disjunction caused by the presence of the Robertsonian translocations, a fraction of the gametes contains two chromosomes 16, while an equal proportion of the gametes is nullisomic for chromosome 16. Combination of these two types of gametes at fertilization will result in a disomic zygote which will be homozygous for either *Sod-1ᵃ* or *Sod-1ᶜ*. Other combinations of gametes will give a heterozygous Sod-1ᵃ/Sod-1ᶜ phenotype in diploid animals or will result in nonviable aneuploids.

(iii) *Characterization of superoxide dismutase*

Blood from the animals to be tested was collected in heparinized capillary tubes (Scientific Products). After the blood cells were washed in 0·5 ml phosphate buffered saline, they were pelleted and lysed in 50 μl water. The lysates were kept frozen until further use. Five μl of lysate was mixed with an equal volume of 2 × sample loading buffer and then electrophoresed on a standard nonreducing 10% polyacrylamide gel (Laemmli, 1970) without sodium dodecyl sulphate. The gel was stained in 2 mg/ml nitrobluetetrazolium (Sigma) for 15 min followed by an incubation in 100 ml 36 mM potassium phosphate buffer, pH 7·8, containing 10 μg riboflavin (Sigma) and 300 μl TEMED (Biorad), for 15 min at room temperature. The gel was illuminated under a fluorescent light source for 5 min. The regions of the gels with Sod-1 activity remained translucent while regions without activity stained

blue. Sod-1ᶜ has a slower migration rate and is therefore separable from Sod-1ᵃ.

3. Results

If there is a significant imprinting effect for chromosome 16, the number of liveborn mice carrying two maternally derived chromosomes 16 should be significantly different from the number carrying two paternally derived chromosomes, or the two types of disomics should be phenotypically different. Table 1 shows the summary of the *Sod-1* alleles detected in the tested offspring from the F1 × F1 matings described above. Both types of disomic animals, maternal and paternal, were readily obtained and occurred with the expected rates.

Two previous studies (Magnuson *et al.* 1985, Debrot & Epstein, 1986) have shown that non-disjunction during meiosis in *Rb32Lub/Rb2H* double heterozygotes results in 0·175 of the gametes having both metacentric chromosomes and 0·175 having neither. At fertilization, 0·031 each (0·175 × 0·175) of the zygotes will be maternal and paternal disomics and 0·423 (0·65 × 0·65) will be heterozygous diploids; the other gametic combinations will lead to nonviable aneuploids. Non-disjunction of chromosomes 11 and 17 occurs only in very rare instances. Therefore, if imprinting effects do not occur, 12·7% of the living progeny should be disomic, half each maternal and paternal. For 205 tested animals, this corresponds to 26 disomics, 13 of each type. As can be seen in Table 1, we observed a total of 24 disomics, 10 maternal and 14 paternal. Furthermore, there was no indication from body weight measurements or external examination of any non-lethal imprinting effects. The observed number of disomic mice does not significantly differ from the expected values ($\chi^2 = 0·67$). We can, therefore, reject the hypothesis for a significant chromosome 16 imprinting effect.

4. Discussion

Maternally and paternally disomic animals were produced to determine whether there are any detectable effects of genomic imprinting of chromosome

Table 1. *Production of disomic animals*

Parental Sod-1 phenotype	Number of offspring analysed	Sod-1 phenotype of offspring			Number of mice disomic for chromosome 16	
		a/c	a/a	c/c	Maternal type	Paternal type
aa × cc	93	86	5	2	—	—
cc × aa	112	95	12	5	—	—
Total	205	181	24		10 (4·9%)	14 (6·8%)
Expected numbers		179	26		13	13

16. The prenatal development of the two types of disomic mice was found to be the same and to be indistinguishable from that of normal diploid littermates. We conclude, therefore, that either the genomic imprinting mechanism does not affect genes on chromosome 16 or, if it does, does not do so in a manner that leads to lethality or to gross differences in development. Previous studies had shown that there is no evidence of an imprinting effect for maternally derived genes in the region of chromosome 16 distal to the *T17H* breakpoint (Searle & Beechey, 1978).

These findings now add chromosome 16 to the list of chromosomes for which imprinting effects are not grossly recognizable. It is, therefore, of considerable interest that at least one DNA sequence, situated on a chromosome 16 in a transgenic mouse strain, does show evidence of imprinting as indicated by differential methylation and expression between paternally and maternally transmitted transgenes. Animals of this strain carry a transgene containing the immunoglobulin heavy chain gene and a truncated *c-myc* gene placed next to a Rous sarcoma virus LTR and a region of plasmid pBR322. The immunoglobulin gene was shown to be methylated and not to be expressed if inherited from the mother, but methylation was eliminated and expression was observed if the gene was inherited from the father (Swain *et al.* 1987). Recently, this transgene has been found to be linked to the *App* locus, which codes for the amyloid precursor protein (Richard Chaillet, personal communication). The latter gene, which is located in the distal part of chromosome 16 (Lovett *et al.* 1987), does not show any differences in the pattern of methylation or expression between chromosomes of maternal and paternal origin (Richard Chaillet, personal communication). Similarly, a transgene consisting of hepatitis B virus and plasmid pBR322 sequences has been found to be differentially methylated and expressed depending on its parental origin. This transgene has been found to be integrated into chromosome 13 (Hadchouel *et al.* 1987). However, normal complementation of gametes nullisomic for chromosome 13 by maternal or paternal chromosome 13 disomics has been observed (Searle *et al.* 1971; Searle & Beechey, 1978).

Thus, in both cases a transgene integrated into a chromosome whose disomic zygotes develop normally has been found to be imprinted, with differential methylation and expression depending upon its parental origin. These observations are compatible with the hypothesis (Solter, 1988) that the behaviour of transgenes is essentially irrelevant to the phenomenon of endogenous gene imprinting and does not necessarily reflect the local state of the chromosome. Perhaps their behaviour is more a reflection of their chromosome of origin than of the chromosome into which they become inserted. However, it is also possible that the imprinting phenomenon does not uniformly affect all of the loci on a chromosome and,

further, that imprinting itself, by whatever mechanism it occurs, does not necessarily result in a deleterious outcome in cases of abnormal parental origin. Only a close examination of the state and expression of a variety of loci on chromosomes that do not have visible imprinting effects will make it possible to determine whether and in what manner imprinting as a molecular process, differential gene expression as a result of imprinting, and altered survival and development are correlated with one another.

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