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Developmental Consequences of Imprinting of Parental Chromosomes by DNA Methylation

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Developmental consequences of imprinting of parental chromosomes by DNA methylation

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Genomic imprinting by epigenetic modifications, such as DNA methylation, confers functional differences on parental chromosomes during development so that neither the male nor the female genome is by itself totipotential. We propose that maternal chromosomes are needed at the time when embryonic cells are totipotential or pluripotential, but paternal chromosomes are probably required for the proliferation of progenitor cells of differentiated tissues. Selective elimination or proliferation of embryonic cells may occur if there is an imbalance in the parental origin of some alleles. The inheritance of repressed and derepressed chromatin structures probably constitutes the initial germ-line-dependent 'imprints'. The subsequent modifications, such as changes in DNA methylation during early development, will be affected by the initial inheritance of epigenetic modifications and by the genotype-specific modifier genes. A significant number of transgene inserts are prone to reversible methylation imprinting so that paternally transmitted transgenes are under-methylated, whereas maternal transmission results in hypermethylation. Hence, allelic differences in epigenetic modifications can affect their potential for expression. The germ line evidently reverses the previously acquired epigenetic modifications before the introduction of new modifications. Errors in the reversal process could result in the transmission of epigenetic modifications to subsequent generation(s) with consequent cumulative phenotypic and grandparental effects.

1. Introduction

Embryonic development is influenced by the genetic constitution of zygotes as well as by epigenetic modifications, which can determine the timing and sequence of gene expression (Holliday 1987). The genetic contributions made by the mother and the father are usually equivalent. However, epigenetic modifications of some parental alleles can give rise to functional differences between certain homologous chromosomal regions (Searle & Beechey 1985; Cattanach 1986) by a process that is called genomic imprinting.

The most significant consequence of genomic imprinting is that neither the male nor the female genome is by itself totipotential; normal development in the mouse requires the presence of both a maternal and a paternal genome (Surani & Barton 1983; Surani *et al.* 1984; Barton *et al.* 1984; McGrath & Solter 1984; Mann & Lovell-Badge 1984; Renard & Babinet 1986). Specific developmental abnormalities are observed in parthenogenetic embryos (with maternal chromosomes only) and androgenetic embryos (with paternal chromosomes only) resulting in complementary phenotypes affecting the embryo and the extraembryonic tissues. Further investigations now suggest that maternal chromosomes may be more important when embryonic cells are totipotential or pluripotential whereas paternal chromosomes are

preferentially required to sustain the proliferation of progenitor cells of specific differentiated tissues, both in the embryonic and extraembryonic lineages.

2. The imprinting signals and propagation of epigenetic information

Nuclear transplantation studies provided direct evidence for heritable differences between parental genomes during early preimplantation development (Surani *et al.* 1986*a*). The differences in the inheritance of repressed and derepressed chromatin patterns from the maternal and paternal germ lines by homologous chromosomes may constitute the primary imprinting signals (Groudine & Conkin 1985; Weintraub 1985). Although these imprinting signals will change with the dynamic changes in chromatin structure during development (Monk 1988), the subsequent changes will be influenced by the initial germ-line-specific 'imprints' in the chromatin structure. The changes in chromatin structure could take a variety of forms, some of which are stable and heritable. Derepressed chromatin structure, represented by its DNAase-1 hypersensitive state, and repressed chromatin structure, associated with DNA methylation involving CpG dinucleotides (Keshet *et al.* 1986), can affect large domains encompassing at least 40–50 kilobases (kb). In addition, within these regions, site-specific changes in DNA methylation will modulate the precise timing of the expression of particular genes in specific tissues (Doerfler 1983; Cedar 1988; Yisraeli *et al.* 1986; Watt & Molloy 1988). It is particularly interesting that allele-specific DNA methylation differences have recently been detected (Chandler *et al.* 1987; Silva & White 1988), although these differences may or may not be influenced by their parental origin.

Methylation of the CpG dinucleotide is the most widely studied of all the epigenetic modifications because it is particularly amenable to experimental analysis. However, as control of gene expression is a multilevel process (Gilbert 1985), other forms of epigenetic modifications not discussed here, will undoubtedly be involved in controlling functional differences between parental chromosomes.

3. Imprinting and dynamic changes in DNA methylation

Since some of the repeat and low-copy DNA sequences are undermethylated in oocytes but hypermethylated in sperm (Monk *et al.* 1987; Sanford *et al.* 1987; Jagiello *et al.* 1989), it has been suggested that such differences between parental chromosomes could be propagated after fertilization and constitute the primary imprinting signal (Sanford *et al.* 1987; Sapienza *et al.* 1989). However, little is known about the methylation of single-copy gene sequences and it is therefore difficult to deduce whether these differences in the levels of DNA methylation have a function in gametogenesis or after fertilization or both.

It has been proposed previously that a *de novo* methylation event may occur early after fertilization with important consequences for genomic totipotency (Razin & Riggs 1980; Jähner & Jaenisch 1984). In preliminary experiments we have noticed that some sequences seem to become rapidly methylated *de novo* at an early preimplantation stage in fertilized and parthenogenetic embryos (W. Reik & S. K. Howlett, unpublished data). However, because of marked differences in chromatin structure between sperm with its highly condensed, protamine-bound DNA (Rodman *et al.* 1982) and eggs, *de novo* methylation events could have

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differential effects on parental chromosomes. It is possible that the maternal genome may be more susceptible to methylation at this early stage because imprinted transgenes show higher methylation after maternal transmission (discussed later). These differences could contribute to functional differences between parental genomes.

The precise nature of methylation differences between parental genomes during pre-implantation development is not clear at present except that there is a loss of overall DNA methylation by the blastocyst stage in the mouse (Monk 1988), as well as in the rabbit (Manes & Menzel 1981), that may be associated with the differentiation of trophectoderm and inner cell mass.

A marked *de novo* methylation event also occurs at gastrulation but it is lineage-specific since it apparently occurs primarily in epiblast cells and not in the extraembryonic tissues (Chapman *et al.* 1984; Monk *et al.* 1987; Rossant *et al.* 1986). Nevertheless, differences in epigenetic modifications of some parental alleles presumably survive this genome-wide methylation. It is particularly interesting to note that massive genome-wide demethylation to about 75% level occurs when Friend erythroleukaemia cells respond to a variety of cell effectors that result in differentiation of normoblast-like cells, concomitant with activation of a variety of specific genes (reviewed by Razin *et al.* (1985)); this is followed by *de novo* methylation to the original levels but presumably with a novel pattern of DNA methylation. Similar hypomethylation is observed in F9 embryonal carcinoma cells when induced to differentiate into visceral endoderm by retinoic acid (Young & Tilghman 1984). These results clearly demonstrate that major changes in epigenetic information can occur during development, which have to be considered in the context of imprinting signals and the role of parental chromosomes in development.

4. Imprinting and differential roles of parental chromosomes during development

The dynamic changes in DNA methylation described above may be manifested as differential roles of parental chromosomes in development. Indeed, studies show that although neither the maternal nor the paternal genome is by itself totipotential (Surani *et al.* 1984; McGrath & Solter 1984), our studies demonstrate that cells with maternal chromosomes undoubtedly display characteristics that approach totipotency. Hence, the survival of parthenogenetic cells in development becomes critical only when they are approaching final stages of cell differentiation, both in the extraembryonic and embryonic lineages (figure 1). For instance, parthenogenetic embryos develop apparently normally up to the blastocyst stage (Surani *et al.* 1986*b*). Then, the polar trophectoderm cells, which are progenitors of extraembryonic ectoderm and ectoplacental cone consisting of trophoblast cells (Gardner *et al.* 1973), do not proliferate adequately and probably fail to respond to the inductive stimulus from the inner cell mass (Barton *et al.* 1985; Surani & Barton, unpublished). This results in the general lack of extraembryonic tissues in parthenogenetic conceptuses (Surani *et al.* 1984; McGrath & Solter 1984; Clarke *et al.* 1988; Thomson & Solter 1988) as shown in figure 2, plate 1. However, in the embryonic lineage, parthenogenetic cells persist and proliferate in tissues derived from epiblast until just after the mid-gestation stage (Surani *et al.* 1988), and thereafter, in chimaeras these cells are selected against, with the establishment of differentiated

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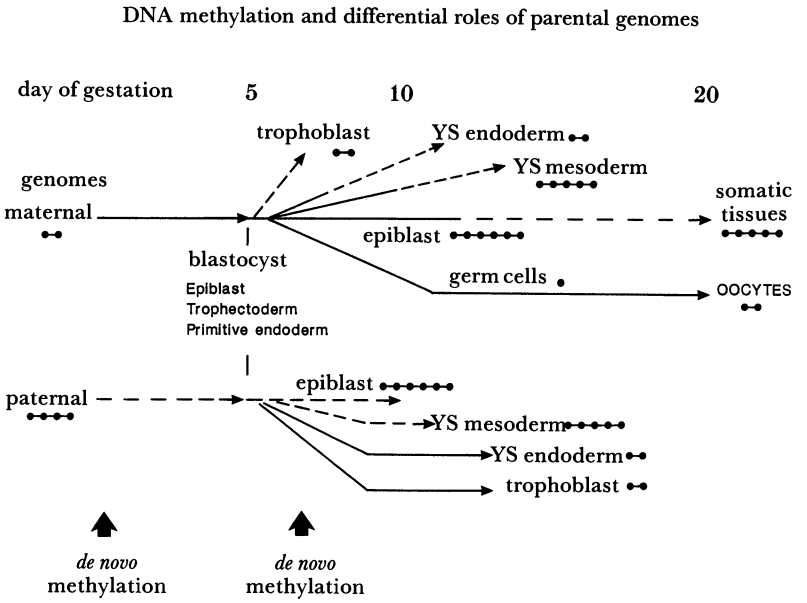

FIGURE 1. Summary of developmental potential of embryonic and extraembryonic cells with maternal or paternal chromosomes. Solid line shows normal potential; broken lines show poor developmental capacity or selective elimination in chimaeras. Note that cells with maternal chromosomes display much greater developmental capacity and are able to give rise to germ cells. Cells with paternal chromosomes have reduced developmental potential; ●–●, degree of methylation; YS, yolk sac.

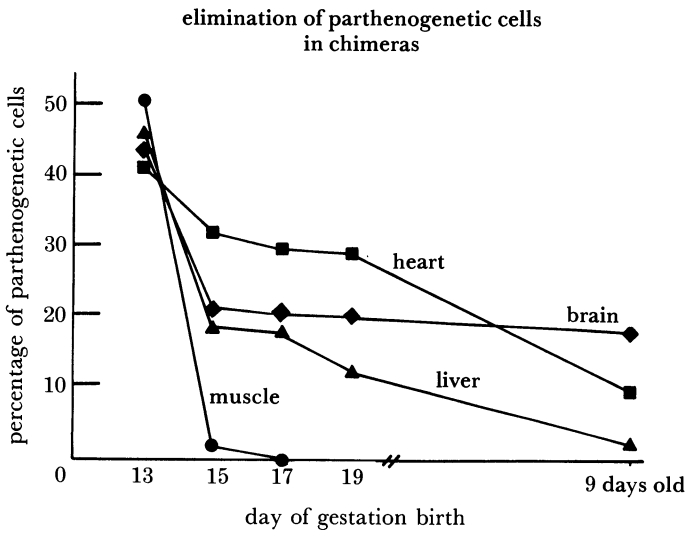

FIGURE 3. Examples of selective elimination of parthenogenetic cells from different organs after mid-gestation stage.

tissues (Nagy *et al.* 1987; Surani *et al.* 1988; Fundele *et al.* 1989; Paldi *et al.* 1989; Fundele *et al.* unpublished). The selection appears to affect different tissues at different stages of development and to a different final extent (figure 3).

Germ cells are a significant exception to the rule of selective elimination of cells as functional oocytes are produced at a high frequency from parthenogenetic cells (Stevens 1978; Anderegg & Markert 1986; Anderegg 1987; Fundele *et al.* unpublished). However, in other respects parental chromosomes must interact in some crucial way because aggregation

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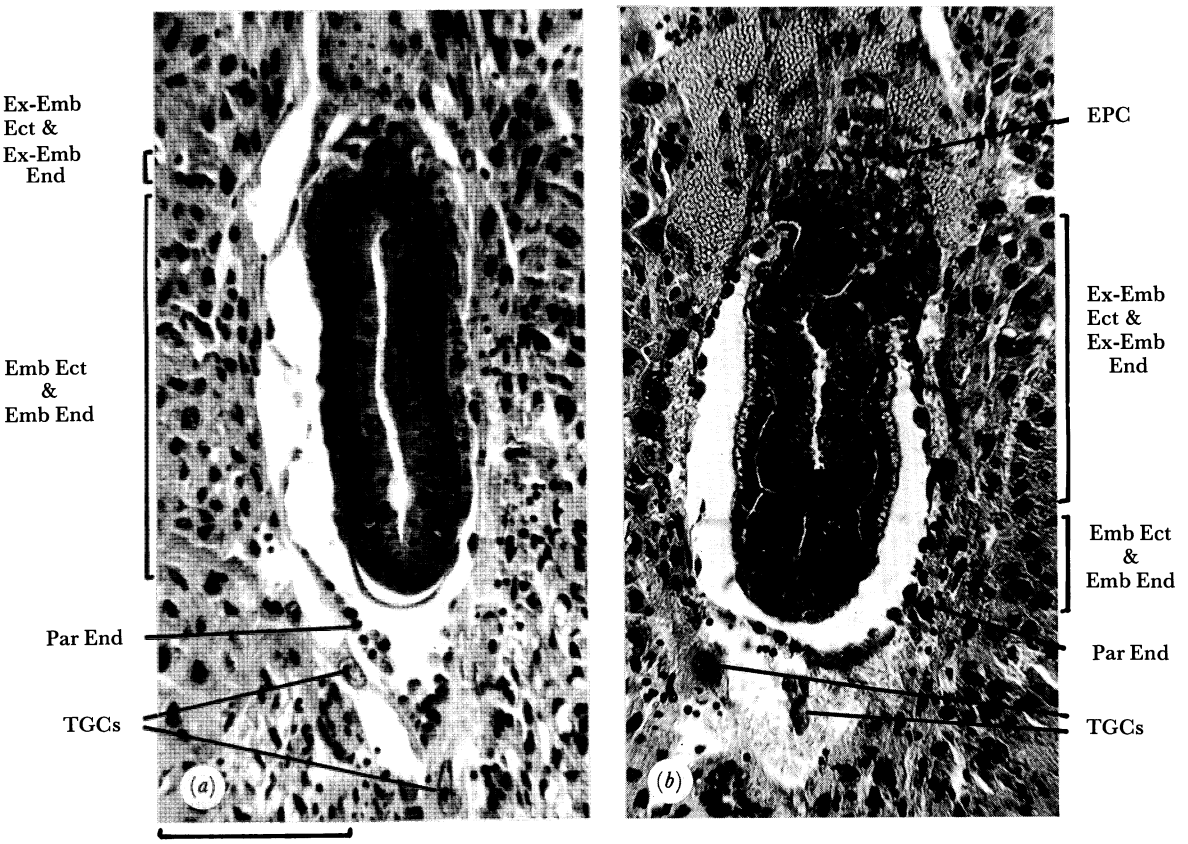

FIGURE 2. Longitudinal sections of 6.5 day embryos *in utero*. (*a*) Parthenogenetic embryo. (*b*) Normal fertilized embryo. Note the almost complete absence of ectoplacental cone and extraembryonic ectoderm and endoderm in (*a*). Abbreviations: EPC, ectoplacental cone; Emb Ect, embryonic ectoderm; Emb End, embryonic endoderm; Ex-Emb Ect, extraembryonic ectoderm; Ex-Emb End, extraembryonic endoderm; Par End, parietal endoderm; TGCs, trophoblastic giant cells. Scale bar 100 μm.

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chimaeras between androgenetic and parthenogenetic embryos fail to proceed to term (Surani *et al.* 1987). Hence, compared with androgenetic cells, parthenogenetic cells are more capable of proliferating during development when cells are pluripotential and relatively undifferentiated but are clearly less inclined to persist in differentiating tissues. Although molecular mechanisms responsible for the differential roles of parental chromosomes is not yet known, it is possible that epigenetic differences between some key parental alleles could explain how parental chromosomes could influence proliferation and differentiation of cells, even if such differences occur only transiently and affect a single or a few key methylatable sites.

No endogenous 'imprinted' genes have yet been identified unequivocally, but genes for growth factors and their receptors could be responsible, either primarily or secondarily, for producing functional differences between parental genomes. However, it is known that some transgenes are differentially modified and expressed, depending on their parent of origin, and these provide models to investigate the molecular mechanism of genomic imprinting.

5. Paradigms and paradoxes of transgene imprinting

Several studies have reported that the methylation patterns of transgenes can be influenced by their parental origin (Reik *et al.* 1987; Sapienza *et al.* 1987; Swain *et al.* 1987; Hadchouel *et al.* 1987; Surani *et al.* 1988; DeLoila *et al.* 1988). Transgenes integrate randomly in the genome and can be used as molecular probes to examine the mechanism of genomic imprinting in heterozygous animals. It might be expected that transgenes display properties compatible with imprinting behaviour if they integrate into the 'imprinted' chromosomal domains defined by genetic studies (Searle & Beechey 1985; Cattanach 1986). Alternatively, or in addition, the transgene insertion process itself may trigger a response that alters the chromatin structure at the integration site so as to make it behave as an imprinted gene. Finally, transgenes may interfere with the normally occurring transient and widespread dynamic changes and differences between homologous loci and 'lock' the integration site into a state that under normal circumstances would not retain the differences and would therefore go undetected.

Some aspects of transgene imprinting seem paradoxical but this may be because there is, at present, inadequate understanding of the phenomenon. Hence, the use of transgenes as an approach to the analysis of the molecular mechanisms of imprinting requires caution.

(*a*) *General features of imprinting of transgenes*

One of the initial observations on imprinting of a transgene is shown in figure 4 (Reik *et al.* 1987) and all the other imprinted transgenes share common features (Surani *et al.* 1988) (table 1). Various transgenes are prone to imprinting, which suggests that the process occurs irrespective of the sequence of the transgene. However, sequences flanking the transgene insertion may serve as imprinting signals in which case they would bear some common features. More importantly, imprinted transgenes are almost invariably undermethylated with a potential for expression when paternally derived while maternal transmission causes them to be hypermethylated and less likely to be expressed. In all instances, except one, the transgene methylation patterns are reversible when transmitted alternately between male and female germ lines. In the one exceptional instance, transmission through the maternal germ line caused irreversible hypermethylation (Hadchouel *et al.* 1987). Indeed, this insert remained

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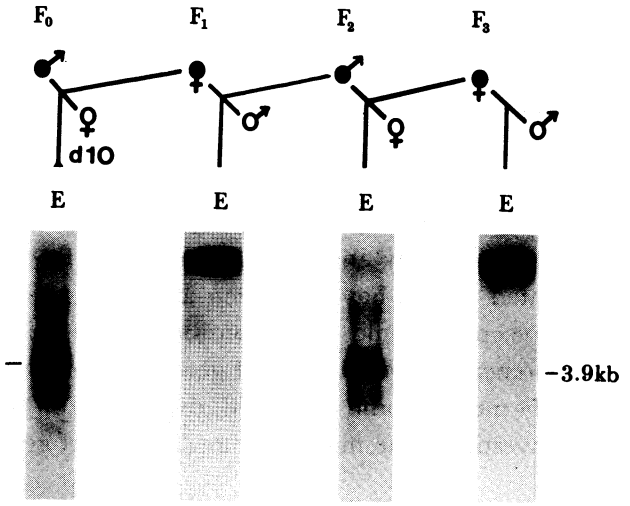

FIGURE 4. Reversible DNA methylation of imprinted transgenes. Transgene insertion (CAT 17) is undermethylated when transmitted through the paternal germ line (3.9 kb band) but methylated when transmitted through the female germ line. Note that methylation is reversible upon transmission of transgene through the germ line of the opposite sex (from Reik *et al.* 1987).

active and capable of being expressed, provided it was repeatedly transmitted through the paternal germ line. It is not known if the maternally derived transgene could ultimately become demethylated and resume activity if it was repeatedly transmitted through the male germ line or if tested in mice of different strains. It also appears that the size of transgene insertion does not influence the capacity to be imprinted even though large tandem repeats of 50–200 kb will disrupt the chromatin structure more severely. Finally, all somatic tissues show a similar overall pattern of DNA methylation even though expression, when it occurs, is confined to the appropriate tissues. It is, however, possible that certain specific sites within the imprinted transgene undergo demethylation in the appropriate tissues. This would allow promoter specificity to be retained even when a transgene displayed the behaviour of an imprinted gene. Finally, imprinted transgenes do not always show the same degree of penetrance and expressivity because of the influence of strain-specific modifier genes (Sapienza *et al.* 1989), as discussed later.

(*b*) *Paradoxes of transgene imprinting*

The first intriguing feature is the very high frequency of imprinting, approaching nearly 20% of the transgene inserts (Surani *et al.* 1988). Given that genetic studies estimate about 10% of the mouse genome being subject to imprinting (Cattanach 1986), the incidence of transgene imprinting is too high (assuming random insertions). Secondly, the paternally derived imprinted transgenes are invariably undermethylated. Although this agrees with genetic studies where maternal disomy leads to deficiency symptoms, whereas paternal disomy leads to excess symptoms (Searle & Beechey 1985; Cattanach 1986), it was expected that at least some maternally derived transgenes could become undermethylated and expressed, while the paternally derived ones become hypermethylated. Finally, there is some evidence to show that transgenes can show imprinting behaviour even if they integrate outside the imprinted domains as defined by genetic studies.

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TABLE 1. INFLUENCE OF PARENTAL ORIGIN OF TRANSGENES ON DNA MODIFICATION AND EXPRESSION

transgene construct (size)	number of copies integrated (total size of insert)	number of transgenic strains showing imprinting influence	observations on transgenes: paternally inherited DNA methylation	paternally inherited DNA expression	maternally inherited DNA methylation	maternally inherited DNA expression	reference
SV CAT IgH (3 kb)	3 (9 kb)	1	+	None	+++	none	a
Troponin 1 (7 kb)	10 (70 kb)	3	+	fast muscle fibre	+++	fast muscle fibre	b
		1	+++	fast muscle fibre	+	fast muscle fibre	
RSV Ig c-myc (20 kb)	10 (200 kb)	1	+	myocardium	+++	none	c
HBsAG (3 kb)	1 (3 kb) (on chromosome 13)	1	+	liver	+++ (irreversible)	none (irreversible)	d

[a] Reik *et al.* (1987); [b] Sapienza *et al.* (1987); [c] Swain *et al.* (1987); [d] Hadchouel *et al.* (1987). See Surani *et al.* (1988) for further details. Symbols used: +, undermethylation; +++, hypermethylation.

(c) *Possible resolutions of paradoxes*

If the integration of transgenes occurred in preferred sites in the open chromatin regions and imprinted domains, it would be possible to explain why paternally derived transgenes are undermethylated, as well as the high frequency with which transgenes are observed to display the phenomenon of imprinting. This is because in most instances, transgenic mice are obtained by microinjecting the DNA into the male pronucleus and therefore integration of the transgenes could occur preferentially in paternally imprinted chromatin regions. We do not believe that this explanation is very likely. However, if this is the case then introduction of DNA into the female pronucleus would show the opposite trend.

Alternatively, transgene imprinting is of one type simply as a result of the design of experiments used to detect them. For example, examination of foetuses at earlier stages than mid-gestation could reveal transgenes that are undermethylated after transmission through the female germ line. Furthermore, careful analysis of individual cell types and tissues in foetuses and adults and of potential methylation sites in transgenes may show a greater variety of methylation imprinting differences in transgenes.

The apparent conflict in the overall size of the imprinted regions deduced from genetic and transgene studies, could be resolved in several ways. The genetic complementation studies could underestimate the size because such tests rely on effects with obvious phenotypes (Cattanach 1986). More subtle phenotypic effects and those occurring only transiently during development may not be readily recognized. Indeed, it is possible that a large proportion of the mouse genome may be imprinted by molecular criteria without resulting in obvious phenotypic consequences. Furthermore, the genetic map of imprinted regions may also vary significantly if tests were done on different genetic backgrounds.

Even if the above explanation is correct, it still fails to explain the apparent difficulties in detecting DNA methylation differences between endogenous parental alleles (Reik *et al.* unpublished). This apparent paradox may have several explanations. First, these differences may only be revealed by careful site-by-site examination of important methylatable sites in parental alleles of particular genes. Furthermore, even if DNA methylation differences occur between homologous parental loci, they may only be transient. Finally, these studies may require examination of specific cells and tissues at different stages of development.

Even if we assume transient differences in epigenetic modifications in endogenous parental loci, the problem remains that transgenes are relatively prone to imprinting. One possible reason for the observations is that transgenes behave as insertional mutations. Under normal circumstances, transient waves of chromatin modifications may pass through specific loci uninterruptedly. However, the presence of a transgene may interrupt such dynamic chromatin modifications and these insertions will tend to 'lock' the loci and those beyond in a methylated or undermethylated state. As methylation differences are detected in flanking sequences outside the transgenes (Hadchouel *et al.* 1987; W. Reik *et al.* unpublished; N. D. Allen *et al.* unpublished), these effects on endogenous flanking sequences could be due to the influence of the insertion itself because they may not exist (or persist?) in the absence of the transgene. One would expect that specific deletions may also perturb the normal progression of changes in chromatin structure during development. Although no such experimentally induced deletion is known, a naturally occurring deletion, T^{hp} (hairpin-tail), is lethal when maternally derived

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but it is without effect when paternally derived (Johnson 1974). However, the influence of this deletion on DNA methylation remains to be analysed in detail. There is also an experimentally induced translocation in one line of our transgenic mice that causes abnormal development, but only when transmitted from the paternal germ line (W. Reik *et al.* unpublished).

6. The relative influences of germ line and soma on imprinting

The most significant effect of the germ line on imprinting of genes is to reverse previously acquired epigenetic modifications and introduce an 'imprint' to establish the new parental origin of chromosomes. However, segregation of genotype-specific modifier genes will also influence gene expression after fertilization. Studies on the penetrance of the fused (*Fu*) gene show that in some inbred strains of mice, there is a suppressor gene not linked to the mutation that dramatically decreases penetrance of maternal *Fu* to 12–18%, while reducing that of the paternal *Fu* only slightly to 70% (Agulnik & Ruvinsky 1988). The suppressor itself has this effect, regardless of its parental origin. In theory, the functioning of suppressor or modifier genes themselves could be affected by imprinting, in which case such genes could have a significant influence on the expression of a number of responding genes at unrelated loci. Such responder genes would display all the attributes of being imprinted, but as a secondary consequence of the parental origin of the regulatory suppressor or modifier genes. However, androgenetic and parthenogenetic phenotypes are consistent when examined in a number of strains which confirms the fundamental nature of the process of imprinting.

(*a*) *Epigenetic modifications in the germ line*

The primordial germ cells, both male and female, display remarkably low levels of methylation (Monk 1988), perhaps because they are set aside from epiblast cells before *de novo* methylation commences at gastrulation. Preliminary evidence from some transgene insertions suggests that they tend to become highly undermethylated in male germ cells. This occurs regardless of whether the transgene is subsequently expressed, X-linked, homozygous lethal or behaves as if imprinted (Collick *et al.*, unpublished results). Little is yet known about the epigenetic modifications of transgenes in the female germ line.

The epigenetic modifications responsible for re-setting the genome in the germ line may not be infallable. Grandparental phenotypic effects may result if previously acquired modifications are not reversed.

(*b*) *Influence of modifier genes on epigenetic modifications and gene expression*

Several observations show that expression of transgenes can be modulated by strain differences. It appears that different sets of modifier genes may modulate expression of different genes at different stages of development (Surani *et al.* unpublished).

In one particular transgenic line with the thymidine kinase promoter linked to a β-galactosidase reporter gene (TKZ751), expression was detected in postimplantation embryos (Allen *et al.* 1988; Kothary *et al.* 1989; Allen *et al.* unpublished). The founder male 751 was made on a mixed genetic background (C57BL/6♀ × CBA♂) F_1 × CFLP♂, and transgenic embryos were analysed after backcrossing onto the (C57BL/6♀ × CBA♂) F_1 hybrid strain. Two types of foetuses were detected after paternal transmission, those with high expression (Hi) and

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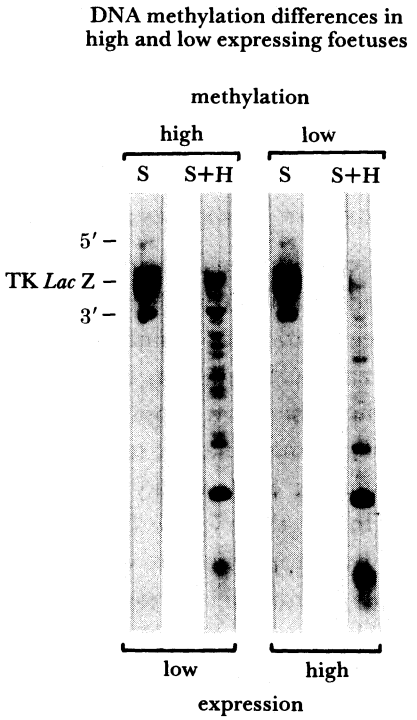

FIGURE 5. Southern blots of DNA from Hi- and Lo-expressing TKZ751 transgenic lines. The DNA was restricted with *Sac*I (S) alone or combined with *Hpa*II (S+H); *Hpa*II is a methylation-sensitive enzyme and requires undermethylated CpG dinucleotides. Hence, Lo-expressing lines reveal limited digestion compared with the Hi-expressing lines (compare S+H tracks in the two cases).

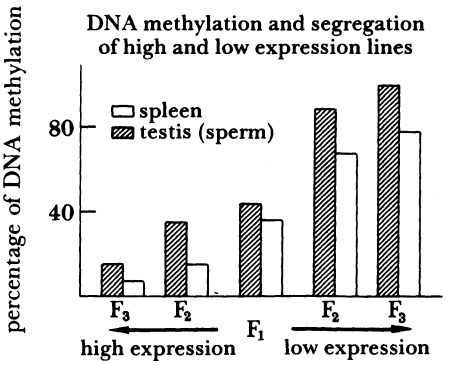

FIGURE 6. Cumulative effects of DNA methylation with segregation of Hi and Lo expression. Note especially that demethylation in testis (sperm) is proportional to the level of methylation in somatic (spleen) tissue.

those with low expression (Lo). The ratio of Hi and Lo foetuses was approximately 1:1. Furthermore, the level of expression correlated with the methylation of the transgene (figure 5). These lines could be segregated by breeding through to the F_3 generation so that both the Hi and Lo expression differences were enhanced and also correlated with DNA methylation levels. It is possible that the segregation of Hi and Lo expressing transgenic lines is accompanied by the segregation of strain-specific modifier or suppressor genes.

Perhaps more interestingly, preliminary studies reveal that in the TKZ751 mice, the degree of demethylation in testis (sperm) is proportional to the levels in spleen (soma) (figure 6). This

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produces cumulative DNA methylation differences from one generation to the next in both the Hi and the Lo expressing lines. This may suggest a failure of the germ line to neutralize the previously acquired epigenetic modifications. Such cumulative effects of epigenetic modifications could form the basis of extended phenotypes (Dawkins 1981) and for grandparental effects observed for certain mutations in man (see later). Furthermore, expression of the Hi transgene was suppressed on the Balb/c background, perhaps as a consequence of the strain-specific modifier genes. The influence of the parental origin of modifier genes themselves on expression of this transgene has not yet been tested.

7. Mutations, methylation and imprinting

Mutations could interfere with epigenetic modifications and germ line specific imprinting. In this sense, transgenes are insertional mutations that mimic imprinted genes. Similarly, somatic mutations could also affect imprinted genes in specific cells leaving active only an allele of one or other parental origin. Considerable interest has been aroused recently with respect to some genetic disorders in the human in which genomic imprinting may have a role to play.

(*a*) *Influence of parental origin of genetic disorders*

One interesting example of the effect of parental origin on genetic disorders is Huntington's disease (HD) (Ridley *et al.* 1988; Reik 1988). Relatively little variation in the timing of onset of the disease is observed when HD is inherited from the mother, but the age of onset of the disease in a small group occurs much earlier if inherited from the father, on average 24 years younger than in the affected father (Ridley *et al.* 1988).

Furthermore, HD transmitted continuously through the paternal line from grandfather via father (GF→F) results in early onset of the disease. Comparing the effects of GF→F and GF→M (mother) transmission indicates that the passage of HD from male to female germ line reverses the trend observed with repeated paternal transmission, which resembles the reversibility of DNA methylation and expression of the transgene insertions discussed earlier.

Extrapolating from the study on transgenic mice TKZ751 discussed above, we can propose that cumulative epigenetic modifications with each passage of HD through the paternal germ line, cause the locus to become more modified resulting in either an increase or decrease in methylation. This effect is obviously more severe in a small proportion of cases which may be due to the segregation of particular modifier genes acting on the HD locus. The female germ line is apparently more efficient at restoring the original epigenetic modifications.

(*b*) *Imprinting and embryonal tumours*

Imprinting may have an important role in maintaining a balance between proliferation and differentiation of embryonic cells. If such balance is altered, it could result in recessive tumour syndromes, such as embryonal tumours (Reik & Surani 1989). In recessive tumours, both alleles of a tumour suppressor gene are lost in succession, the first one by mutation and the second one frequently by somatic recombination or nondisjunction (Green 1988), or both. The consequence is duplication of the mutant allele together with a large part of the chromosome. Accordingly, a frequent observation in these tumours is the loss of heterozygosity for regions of the chromosome where the suppressor locus is thought to lie. Many tumours in the human are now suspected to arise by this mechanism, including rare embryonal tumours, such as

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Wilm's tumour and retinoblastoma, and very common types, such as colon cancer (Schroeder *et al.* 1987; Mannens *et al.* 1988). Surprisingly, in some of these tumours such as in Wilm's tumour and in osterosarcoma, where loss occurs in chromosome 11p and 13q respectively, the maternal chromosome is much more frequently lost than the paternal one (Toguchida *et al.* 1989). In the case of osteosarcoma, it has been suggested that the maternal and paternal alleles are differentially susceptible to somatic mutation because of imprinting (Toguchida *et al.* 1989).

It should be borne in mind however, that paternal duplication of a large region of the chromosome could have additional effects arising from imprinting of other loci on that chromosome. This would lead to dosage effects whereby some imprinted alleles could be expressed at a higher level than normal. Such accompanying effects could involve growth factors, as for example, in the case of Wilm's tumour where the insulin-like growth factor II (IGFII) is localized close to the putative Wilm's locus (Scott *et al.* 1985). Incidentally, IGFII is an embryonic mitogen and could thus contribute to the phenotype in Wilm's tumour (Scott *et al.* 1985). A similar model has been proposed for the Wilm's locus and imprinting of a target (transforming) gene by DNA methylation on the same chromosome (Wilkins 1988).

8. Evolutionary implications of genomic imprinting

An imprinting process, which can distinguish between homologous chromosomal regions, is widespread in plants, insects and animals (see Monk 1988; Solter 1988). Perhaps this process has been significant for the evolution of reproductive strategies, viviparity, speciation and development. More importantly, genomic imprinting could have ensured that the asexual mode of reproduction is suppressed in favour of the more advantageous sexual mode of reproduction (Maynard Smith 1976). It certainly appears that the potential for asexual or parthenogenetic mode of reproduction does not exist in mammals (Markert 1982). Once

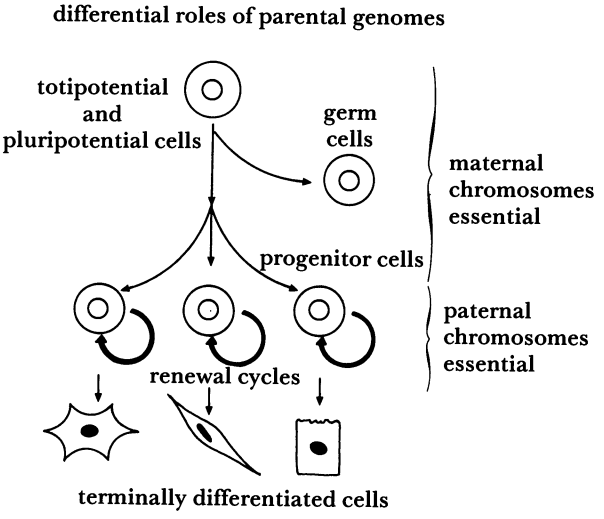

Figure 7. A summary of the proposed role of parental chromosomes in the control of embryonic cell growth and differentiation. Maternal chromosomes are essential for embryonic cells which are totipotential, pluripotential, or both Germ cells can give rise to viable oocytes in the absence of paternally derived chromosomes. However, the proliferation of progenitor cells for differentiated tissues needs paternal chromosomes, in the absence of which cells are subjected to selective elimination.

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genomic imprinting became established, it could subsequently be used during development through its influence on the expression of parental alleles. Epigenetic modifications such as DNA methylation could then have a significant role in conferring functional differences on parental chromosomes.

Genomic imprinting suggests that there has been a shift from strict allelic equivalence towards independence of functions. We must assume that allelic differences provide a better control over events during development. Mammalian development is highly prone to deleterious effects of aneuploidy, which suggests a sensitivity to the levels of gene products (Baranov 1983; Epstein 1985; Solter 1988). It is even more remarkable that even in genetically balanced genotypes, aberrant development occurs if some chromosomal regions are derived exclusively from one parent (Cattanach 1986). The apparent differences in the role of parental chromosomes during development suggest the possibility that there is indeed a tight control over cell proliferation and differentiation conferred by such functional differences (figure 7). Epigenetic modifications such as DNA methylation may be utilized to achieve a precise control of gene expression and ensure differential functions for parental chromosomes.

It remains to be established that functional differences between endogenous alleles exist in normal embryos at some stages of development. When such evidence is available, together with an understanding of the underlying molecular mechanism, it will be possible to judge accurately and with a greater degree of conviction the real purpose of genomic imprinting in mammalian development.

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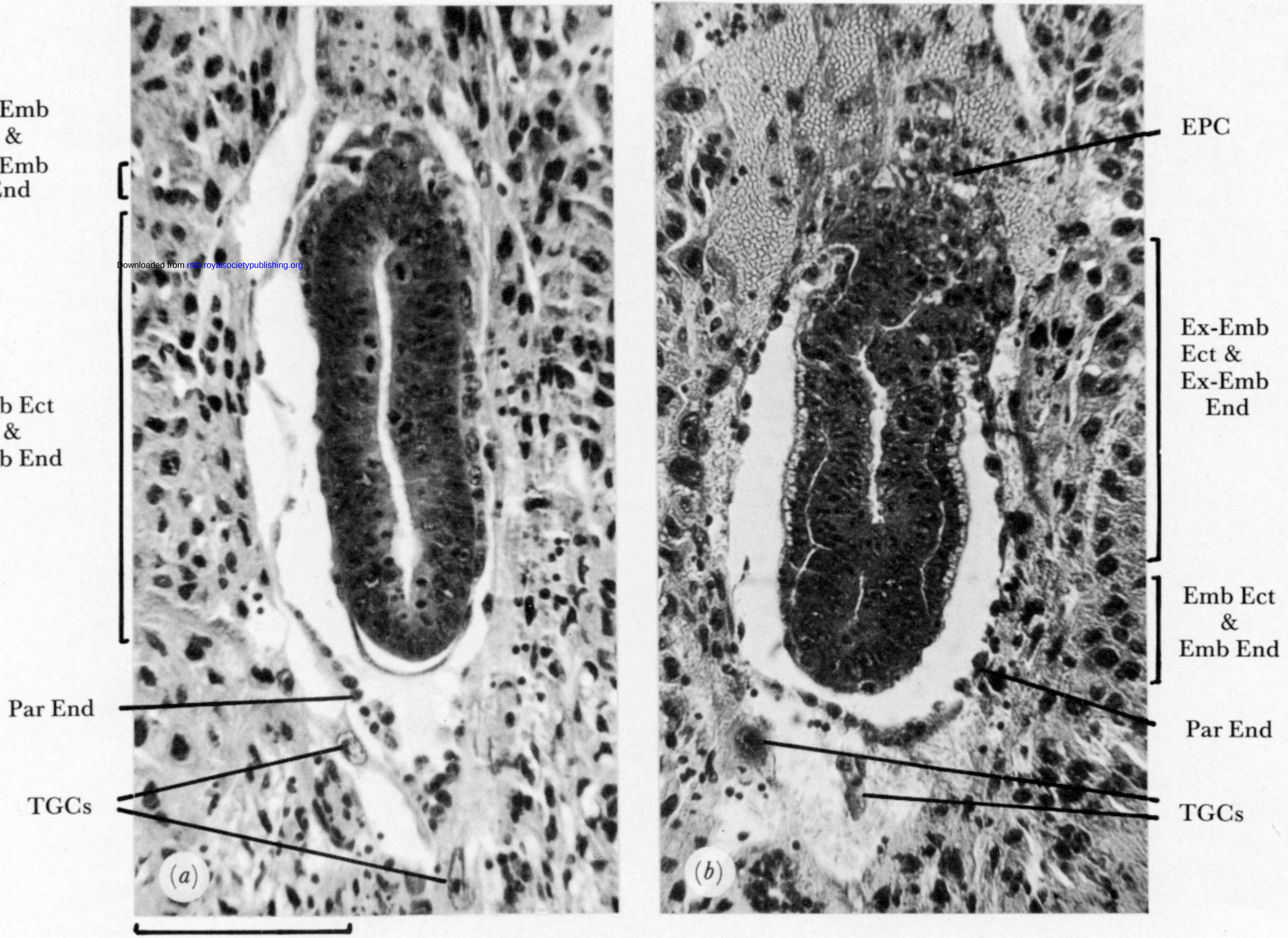
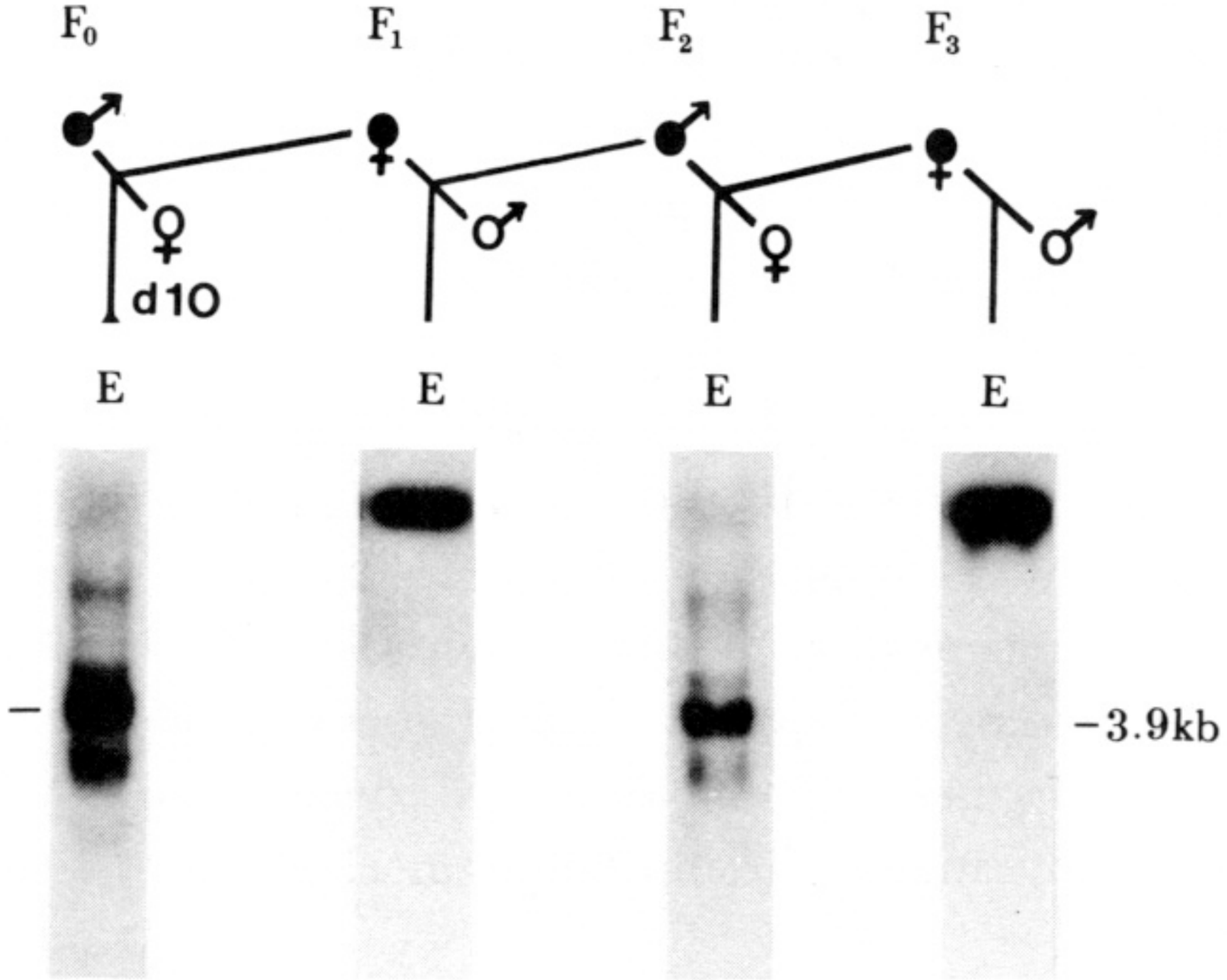


FIGURE 2. Longitudinal sections of 6.5 day embryos *in utero*. (*a*) Parthenogenetic embryo. (*b*) Normal fertilized embryo. Note the almost complete absence of ectoplacental cone and extraembryonic ectoderm and endoderm in (*a*). Abbreviations: EPC, ectoplacental cone; Emb Ect, embryonic ectoderm; Emb End, embryonic endoderm; Ex-Emb Ect, extraembryonic ectoderm; Ex-Emb End, extraembryonic endoderm; Par End, parietal endoderm; TGCs, trophoblastic giant cells. Scale bar 100 μm.



GURE 4. Reversible DNA methylation of imprinted transgenes. Transgene insertion (CAT 17) is undermethylated when transmitted through the paternal germ line (3.9 kb band) but methylated when transmitted through the female germ line. Note that methylation is reversible upon transmission of transgene through the germ line of the opposite sex (from Reik *et al.* 1987).

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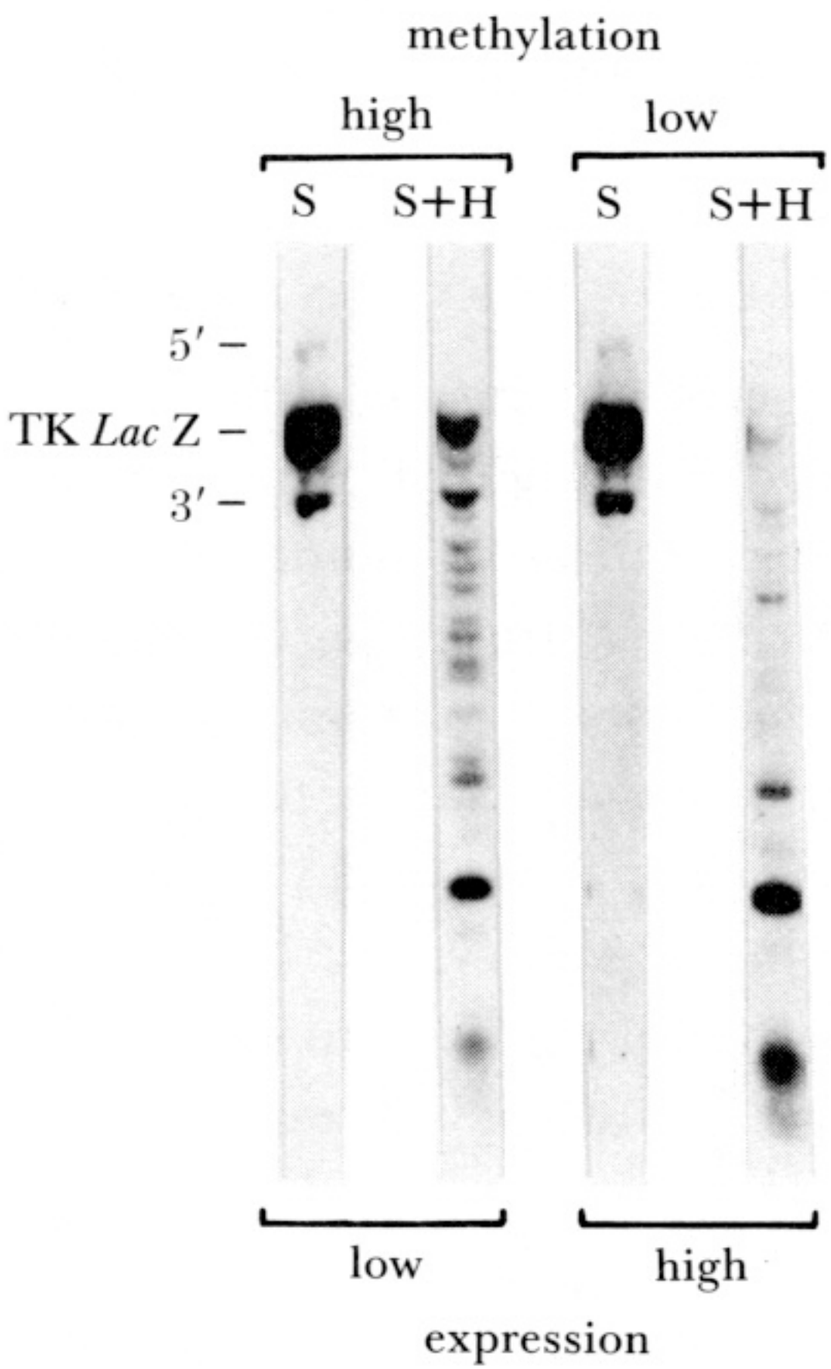

IGURE 5. Southern blots of DNA from Hi- and Lo-expressing TKZ751 transgenic lines. The DNA was restricted with *Sac*I (S) alone or combined with *Hpa*II (S+H); *Hpa*II is a methylation-sensitive enzyme and requires undermethylated CpG dinucleotides. Hence, Lo-expressing lines reveal limited digestion compared with the Hi-expressing lines (compare S+H tracks in the two cases).