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Changes in DNA Methylation During Mouse Embryonic Development in Relation to X-Chromosome Activity and Imprinting

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Changes in DNA methylation during mouse embryonic development in relation to X-chromosome activity and imprinting

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Changing DNA methylation patterns during embryonic development are discussed in relation to differential gene expression, changes in X-chromosome activity and genomic imprinting. Sperm DNA is more methylated than oocyte DNA, both overall and for specific sequences. The methylation difference between the gametes could be one of the mechanisms (along with chromatin structure) regulating initial differences in expression of parental alleles in early development. There is a loss of methylation during development from the morula to the blastocyst and a marked decrease in methylase activity. *De novo* methylation becomes apparent around the time of implantation and occurs to a lesser extent in extra-embryonic tissue DNA. In embryonic DNA, *de novo* methylation begins at the time of random X-chromosome inactivation but it continues to occur after X-chromosome inactivation and may be a mechanism that irreversibly fixes specific patterns of gene expression and X-chromosome inactivity in the female. The germ line is probably delineated before extensive *de novo* methylation and hence escapes this process. The marked undermethylation of the germ line DNA may be a prerequisite for X-chromosome reactivation. The process underlying reactivation and removal of parent-specific patterns of gene expression may be changes in chromatin configuration associated with meiosis and a general reprogramming of the germ line to developmental totipotency.

1. Introduction

Recent years have seen a shift in emphasis in studies on development towards the molecular analysis of the questions concerning gene expression during differentiation. How do specific genes, or groups of genes, become activated in specific tissues at appropriate times and in appropriate amounts and what silences the genes whose expression is not required? There are many experimental approaches to these questions, one obvious approach being an investigation into the role played by DNA methylation. Methylation of the pyrimidine base, cytosine, is known to be correlated with the potential for gene expression, with the structure of active and inactive chromatin, with the activity status of the X chromosomes in the female and with the differential modification of maternal and paternal genetic complements in the individual (genomic imprinting). In this paper, I review the current data relating to the changing patterns of methylation in mouse embryonic development: in the gametes, early embryos, embryonic and extra-embryonic cell lineages and in the germ line. The implications of changing DNA methylation in development will be considered: (i) with respect to the patterns of X-chromosome activity in female development; (ii) with respect to the processes of initiation, propagation and erasure of imprinting.

2. X-chromosome activity in the developing female mouse embryo

Over the past decade or so, the biology of X-chromosome activation, inactivation and reactivation at different times and in different lineages of the developing female mouse embryo has been well documented. The activities of the two X chromosomes have been monitored by sensitive microassays for X-linked enzyme activity to investigate X-inactivation by dosage (two active X chromosomes will give twice the activity of one) and the specificity of inactivation by X-linked isozyme expression (using heterozygous female embryos with maternally and paternally inherited X chromosomes distinguishable by their expression of different isozymes). The changes in X-chromosome activity are shown in figure 1 and summarized below.

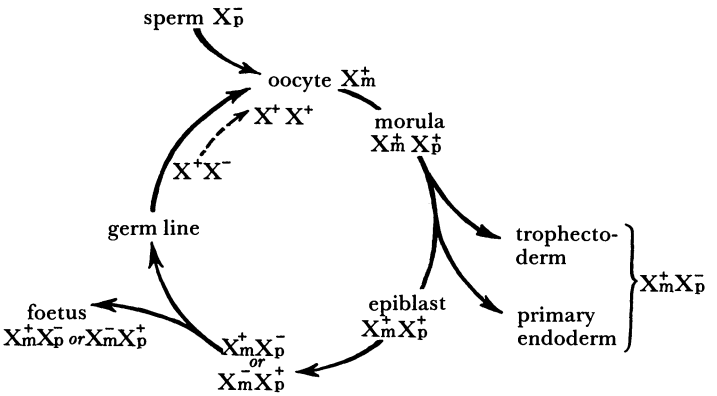

Figure 1. Diagram showing patterns of X-chromosome activity during development of the female mouse embryo (m, maternal: p, paternal; +, active; −, inactive).

1. During spermatogenesis, the single X chromosome is inactive, heterochromatic and sequestered (along with the Y chromosome) away from meiotic events in the sex vesicle. During oogenesis, however, two X chromosomes are active during meiosis and oocyte growth (Epstein 1972; Monk & Kathuria 1977).

2. Following fertilization, the paternal X chromosome is activated and two X chromosomes are active in the female morula (Monk & Harper 1978; Kratzer & Gartler 1978; Epstein *et al.* 1978).

3. When the extra-embryonic tissues delineate, however, the trophectoderm and primary endoderm, X-chromosome inactivation occurs and it is the paternal X chromosome that is preferentially inactivated (Takagi & Sasaki 1975; West *et al.* 1977; Harper *et al.* 1982). Clearly, there is some memory mechanism that distinguishes the paternal from the maternal X chromosome in these tissues. The two X chromosomes are differentially imprinted.

4. In the foetal precursor (epiblast) cells, or in embryonal stem cell lines derived from them, the two X chromosomes are active, and in this tissue, inactivation, which occurs around the time of implantation, is random: either the maternal or the paternal X chromosome is inactivated. Hence, the memory, or imprint, that distinguishes the two X chromosomes is no longer present. It has either 'worn off', or it is not 'seen' by the embryonic cells, or perhaps a subset of cells has lost the memory in the X chromosome and has 'sorted out' into the embryonic lineage.

5. The foetus, and the germ line itself, are both derived from this common pool of X-inactivated cells (McMahon *et al.* 1983) but, in the foetal somatic cells, X-chromosome

inactivation is irreversible, whereas in the female germ line the inactive X chromosome is reactivated at the time of entry into meiosis (Monk & McLaren 1981; Kratzer & Chapman 1981).

We have suggested that X-chromosome inactivation may be linked to cell differentiation (Monk & Harper 1979; see also Martin *et al.* (1978)). Conversely, X-chromosome reactivation in the germ line may be linked to a de-differentiation event, or a reprogramming of the germ line to developmental totipotency (Monk 1981).

3. DNA methylation and X-chromosome activity

It was suggested some years ago by Holliday & Pugh (1975) and Riggs (1975), that methylation of the pyrimidine base, cytosine, in DNA might provide a molecular mechanism for X-chromosome inactivation in female cells. Since then, many correlations between DNA methylation and gene function have been reported (reviewed in Razin & Riggs (1980); Doerfler (1983)). Several workers have shown that CpG sequences in the (normally) methylation-free islands in the 5′ region of many housekeeping genes (see Bird 1986) are methylated when these genes are on the inactive X chromosome (Wolf *et al.* 1984; Yen *et al.* 1984; Toniolo *et al.* 1984; Keith *et al.* 1986; reviewed in Monk (1986)). Other CpG sequences in the body of X-linked genes, or at random sites on the inactive X chromosome are less methylated than those on the active X chromosome (Lindsay *et al.* 1985). Figure 2 shows undermethylated sites on the inactive X chromosome in the body of the human *PGK* gene. The undermethylated band at 4.1 kilo bases (kb) in the *Hpa*II *Bam*HI digests is only seen in the presence of the inactive X chromosome in DNA from females (figure 2*a*) and is relatively increased in DNA from an aneuploid female with additional inactive X chromosomes

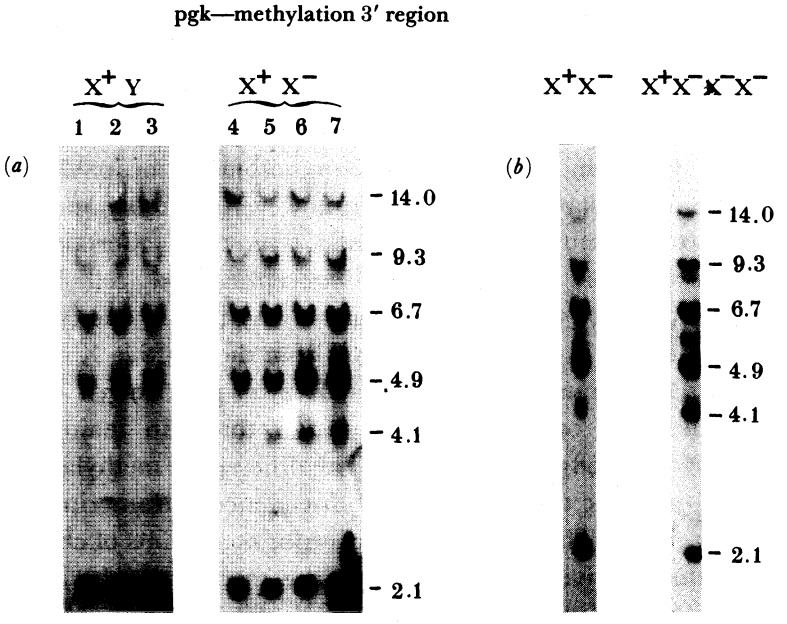

Figure 2. *Hpa*II *Bam*HI double digests of human white blood cell DNA hybridized with a DNA probe complementary to the 3′ end of the human *PGK* gene. (*a*) DNA from three male (1–3) and four female (4–7) individuals. (*b*) DNA from diploid and aneuploid females. (PGK, phosphoglycerate kinase). The data is from Lindsay *et al.* (1985).

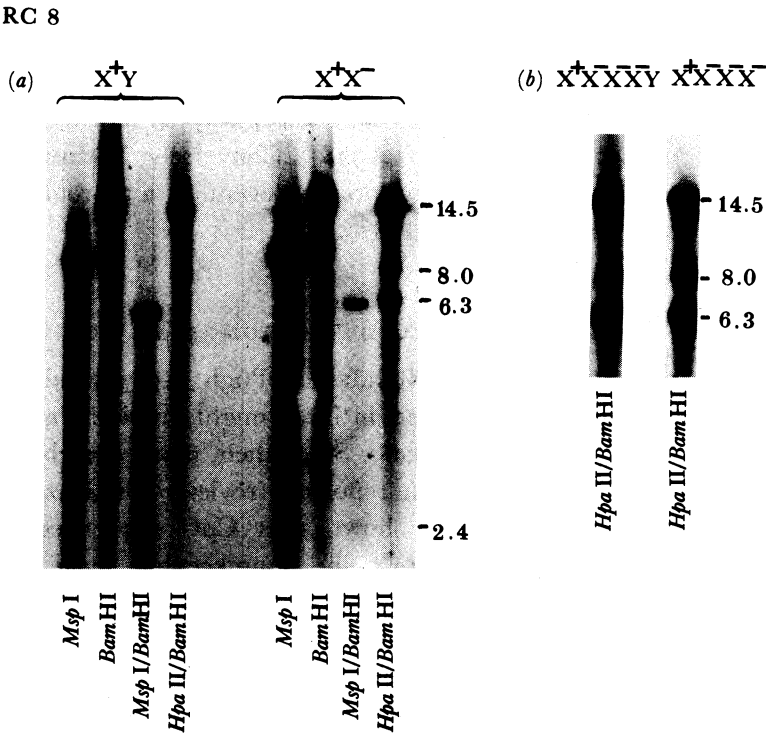

FIGURE 3. Restriction enzyme digests of human white blood-cell DNA hybridized with human X-chromosome probe RC8. (*a*) DNA from male and female. (*b*) DNA from aneuploid male and female. The data is from Lindsay *et al.* (1985).

(figure 2*b*). Figure 3 (*a*, *b*) shows undermethylated sites associated with the inactive X chromosome in the region of probe RC8. The undermethylated band at 6.3 kb in *Hpa*II *Bam*HI digests is associated with the presence of the inactive X chromosome.

It appears that the methylated sites are correlated with the inactivity of individual genes on the inactive X chromosome. For instance, the *HPRT* (hypoxanthine phosphoribosyl transferase) gene derived from the inactive X chromosome is incapable of transforming an *HPRT*-negative cell line (Liskay & Evans 1980); however, demethylation by treatment with azacytidine restores transforming activity (Venolia *et al.* 1982). Certain tissues, such as the extra-embryonic lineages of the developing conceptus and cell lines, are markedly undermethylated (see below), yet in these tissues in the female one X chromosome is still inactive. Moreover, the *HPRT* gene-DNA derived from the inactive X chromosome in extra-embryonic tissue does have transforming activity (Kratzer *et al.* 1983). Thus it would appear that there are mechanisms other than methylation that can maintain the inactive X chromosome (Gartler *et al.* 1985; Monk 1986). The differential methylation that we see in X chromosomes in adult somatic tissues may be a consequence of transcriptional inactivation or the failure to be transcribed. There is some evidence for constraints of chromatin structure on gene expression on the inactive X chromosome since reactivation by azacytidine occurs in discrete segments along the length of the inactive X chromosome (see, for example, Lester *et al.* (1982)).

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4. DNA methylation in development

There is little information on the role played by methylation in development. There must be mechanisms for initiating and establishing differential patterns of methylation *de novo* during development and the stable inheritance of these patterns into the adult. There must also be demethylation events that might occur passively (due to protection of specific sites by steric hindrance or bound protein, or generalized demethylation due to absence of methylase; see below), or actively, by replacement of methylcytosine moieties in the DNA with cytosine (Razin *et al.* 1986).

Broadly speaking, we know that tissue-specific genes are highly methylated in sperm (Waalwijk & Flavell 1978; Mandel & Chambon 1979; Rahe & Erikson 1983; Sanford *et al.* 1987) and undermethylated in extra-embryonic tissues (van der Ploeg & Flavell 1980; Sanford *et al.* 1985; Rossant *et al.* 1986). In differentiated tissues, demethylation is correlated with specific gene expression (reviewed in Doerfler (1983); Weissbach (1987)). Therefore, in development, a methylated ground state has generally been favoured with demethylation being associated with onset of expression of specific genes in different tissues at different times. Jähner & Jaenisch (1984) argue for the presence of *de novo* methylase activity during preimplantation development to explain the methylation of Moloney murine leukaemia virus sequences in adult mice arising from preimplantation embryos infected with virus (Jähner *et al.* 1982). However, the true picture may be more complex and more flexible. In this paper I outline an hypothesis involving the dynamic interplay of changing patterns of methylation, chromatin structure and gene expression itself in the unfolding events of development. The suggested role of DNA methylation in this interplay is summarized in the following five stages.

1. Initial patterns of methylation (and chromatin configuration) in the gametes regulate initial patterns of gene expression in early development; differential patterns in sperm and egg DNA modify parental genetic input.
2. Demethylation removes these patterns following segregation of the embryonic and extra-embryonic lineages.
3. Differential *de novo* methylation programmes the different tissue lineages at gastrulation.
4. *De novo* methylation is largely absent in the germ line.
5. Specific gene demethylation occurs as differentiated cells become committed to their specialized function(s).

In considering the changes of X-chromosome activity in female embryonic development, the simplest predictions would be demethylation associated with the onset of expression of the paternal X chromosome in preimplantation development, *de novo* methylation associated with X-chromosome inactivation (at least in the foetal precursor cells) and loss (or absence) of methylation associated with the reversibility of inactivation at female meiosis.

The problem we come up against in attempts to study changes in DNA methylation in early development is the minute amounts of tissue available. To overcome this problem we, and others, have compared *Msp*I and *Hpa*II digest patterns for repetitive sequences such as the mouse L1 sequence (Bennet *et al.* 1984). A comparison of these patterns in DNA from embryonic tissue (kidney), extra-embryonic tissue (placenta, yolk sac endoderm), gonads, germ cells (at 12.5 and 14.5 days gestation) and sperm, is shown in figure 4. The presence of the two unmethylated bands in the *Hpa*II digests indicates undermethylation of the DNA. It is clear that these sequences are methylated in somatic tissue DNA (embryonic kidney) and

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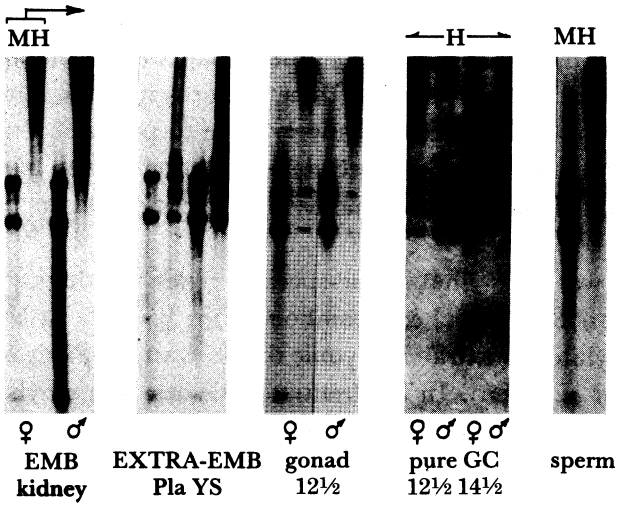

FIGURE 4. *Msp*I and *Hpa*II digests of DNA isolated from various tissues of the mouse conceptus and probed with L1 sequence. (M, *Msp*I; H, *Hpa*II; EMB, embryonic; EXTRA-EMB, extra-embryonic; Pla, placenta; YS, yolk sac endoderm; GC, germ cell). The data is from Monk *et al.* (1987).

sperm DNA and undermethylated in extra-embryonic DNA (placenta and yolk sac endoderm). Similar results were obtained by Chapman *et al.* (1984). The gonad DNAs indicate two populations of cells with methylated and unmethylated L1 sequences. The unmethylated L1 sequences are derived from the germ cells in the gonads as DNAs from samples of pure germ cells at gestation for 12.5 days (pre-meiotic) and gestation for 14.5 days (female, meiotic; male, mitotic arrest) show marked undermethylation of the L1 sequences (data from Monk *et al.* (1987)).

In another approach, we looked at overall DNA methylation in the minimal amounts of DNA available from eggs, sperm, dissected lineages from pre- and postgastrulating embryos and germ cells. This was done by comparisons of fragment size distributions, after *Msp*I and *Hpa*II digestion, end-labelling the resulting fragments and running them on a gel (Monk *et al.* 1987). In this way we could estimate overall methylation in as little as 0.1 ng of DNA (from approximately 20 cells).

Figure 5 shows the densitometer tracings of the high molecular mass regions at the top of the *Hpa*II lanes. If the distribution is skewed towards the top of the gel, the *Hpa*II fragments are large and the DNA is methylated. If the distribution is skewed away from the top of the gel, the DNA is undermethylated. It is clear that oocyte (ovulated but unfertilized) DNA is undermethylated (the peak fraction is a mitochondrial *Hpa*II band) and sperm DNA is relatively methylated. This difference in methylation between sperm and egg DNA has also been reported for specific sequences. L1 sequences, IAP (intercisternal A particle) sequences and MUP (major urinary protein) sequences are all markedly undermethylated in DNA from diplotene oocytes in female embryos (gestation for 18.5 days) and methylated in DNA from sperm (Sanford *et al.* 1987).

After this unequal input of methylation from the gametes at fertilization, the eight-cell embryo DNA appears methylated (though the methylation observed is compatible with a mixture of methylated paternal and unmethylated maternal DNA; see also

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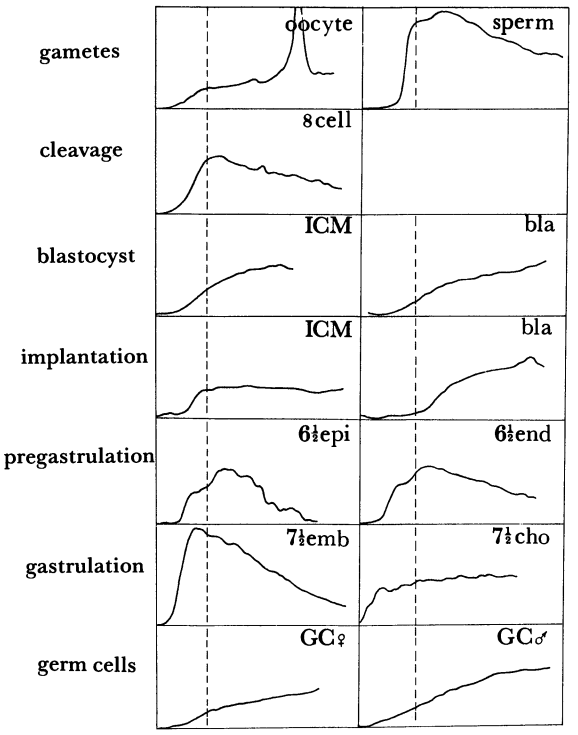

FIGURE 5. Densitometer tracings of the tops of the lanes of autoradiographed end-labelled *Hpa*II digests of DNAs isolated from various stages of preimplantation development and from dissected inner cell mass cells and embryonic and extra-embryonic tissues of postimplantation conceptuses (ICM, inner cell mass; bla, blastocyst; epi, epiblast; end, primary endoderm; emb, embryonic portion; cho, chorion; GC, germ cells). Top of gel at right.

Sanford *et al.* (1987)). However, by the blastocyst stage, the total DNA appears markedly undermethylated. This is not solely due to undermethylation of extra-embryonic trophectoderm DNA (as in the rabbit, Manes & Menzel (1981)), as the DNA from isolated ICM (inner cell mass) cells at 3.5 days' gestation is also markedly undermethylated (figure 5). Therefore, we favour a generalized demethylation by the blastocyst stage.

Around the time of implantation, overall *de novo* methylation is already apparent in the ICM cell DNA at 4.5 days' postfertilization (figure 5) and methylation continues to increase after implantation in the pre- and postgastrulation embryonic DNA. *De novo* methylation also occurs, independently, in the extra-embryonic lineage DNAs but to a lesser final extent than in the embryonic DNA (see also Razin *et al.* (1984)). *De novo* methylation appears to be slow, occurring over several days, perhaps because of inefficient function of the methylase on an unmethylated substrate. It should be noted that, because methylation is increasing in the gastrulating embryo, it is occurring independently, and therefore potentially differently, in the three germ layers, and in the extra-embryonic tissues. *De novo* methylation may therefore play a role in the differential programming of these different cell lineages. Although the onset of methylation coincides with random X-chromosome inactivation in the foetal precursor cells, methylation continues to increase after inactivation. This is in keeping with the observation that methylation of the *HPRT* gene on the inactive X-chromosome occurs after inactivation (Lock *et al.* 1987).

Much of the *de novo* methylation may serve to reinforce, and make irreversible, patterns of

gene activity established by other regulatory mechanisms (see Monk 1986). In this context, it is noteworthy that male and female primordial germ cell DNAs are markedly undermethylated (figure 5). Hence, their patterns of gene expression during gametogenesis would be reversible and subject to erasure. Such a process might be required to restore developmental totipotency to the germ line and may underly the reactivation of an X chromosome. The timing of X-chromosome reactivation at meiosis raises the intriguing question of whether meiosis itself has a role to play in reprogramming the egg genome and cytoplasm to developmental totipotency.

Although we cannot be sure that the overall methylation changes observed by the end-labelling procedure reflect DNA methylation of specific sequences, it is encouraging that the degree of methylation of those specific sequences looked at (e.g. L1, figure 4 and Sanford *et al.* (1987)) does reflect the overall changes. A hypothetical view of the overall changes in DNA methylation in development is shown in figure 6. Given this picture as a working model, we can ask what might be the basis for these changes? First, why are sperm and egg differently methylated? One could suggest that the egg, during growth and maturation, has been involved in building a large cytoplasm and an extensive cytoplasmic repertoire of stored molecules that will support development after fertilization. It is not surprising that this abundant activity is associated with marked global undermethylation. On the other hand, the methylated sperm genome is quiescent, and, in addition, methylation could be involved in the compact packaging of the DNA into the sperm head. Loss of methylation between the morula and blastocyst stages might be a result of the absence of the methylase enzyme. In keeping with other observations (cited in Harper & Monk (1983)), maternally inherited methylase enzyme could be destroyed after the eight-cell stage, and, if the embryo-coded methylase is not yet present, a loss of methylation at each DNA replication would result.

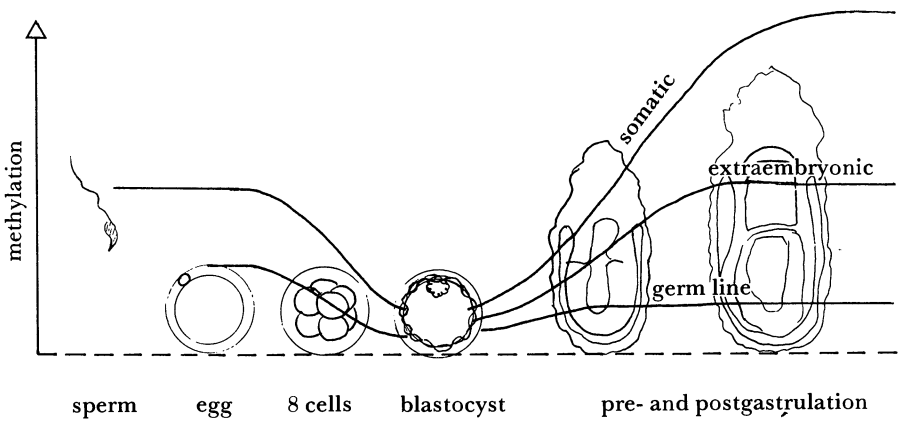

FIGURE 6. Diagram of hypothesized changes in DNA methylation during early mouse embryonic development. Although we know that sperm and egg are differently methylated at the time of fertilization, we cannot say how the parental nuclei are differently methylated after fertilization. However, it is clear that there is an overall loss of methylation by the blastocyst stage and extensive *de novo* methylation at implantation.

A direct assay of the methylase in eggs and preimplantation embryos (M. Monk & P. L. Adams, unpublished data) has shown very high levels of methylase in the egg and very little in the blastocyst that is in keeping with this view. However, another intriguing question is raised. Why are the levels of enzyme in the egg (which itself has globally undermethylated DNA) so very high? Is it to ensure the propagation of the original differential methylation patterns in sperm and egg during preimplantation development when the embryonic and extra-embryonic lineages are segregating?

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5. Genomic imprinting

Genomic imprinting is the differential modification of the maternal and paternal genetic complements, which may be essential for successful complementary development of both the embryonic and extra-embryonic lineages, respectively (see, for example, Surani *et al.* (1984); McGrath & Solter (1984); Mann & Lovell-badge (1984)) and normal expression of adult phenotype (Cattanach & Kirk 1985; Searle & Beechey 1985; reviewed in Monk (1988); Surani *et al.* (1988)). It is thought that imprinting resides initially in the differential modification of the DNA of the sperm and egg genomes as shown in figure 7. At fertilization these haploid contributions from the mother and the father come together to create the diploid nucleus of the individual and henceforth they cooperate in function. Yet, the memory of the gametic origins of each complement of genetic information persists. During development of the mouse conceptus, the female and male genomes contribute unequally in function to embryonic and extra-embryonic development, respectively (Surani *et al.* 1984; McGrath & Solter 1984). It is also known that certain chromosome segments must be represented by both maternal and paternal complements, for normal phenotype in the mouse (Cattanach & Kirk 1985;

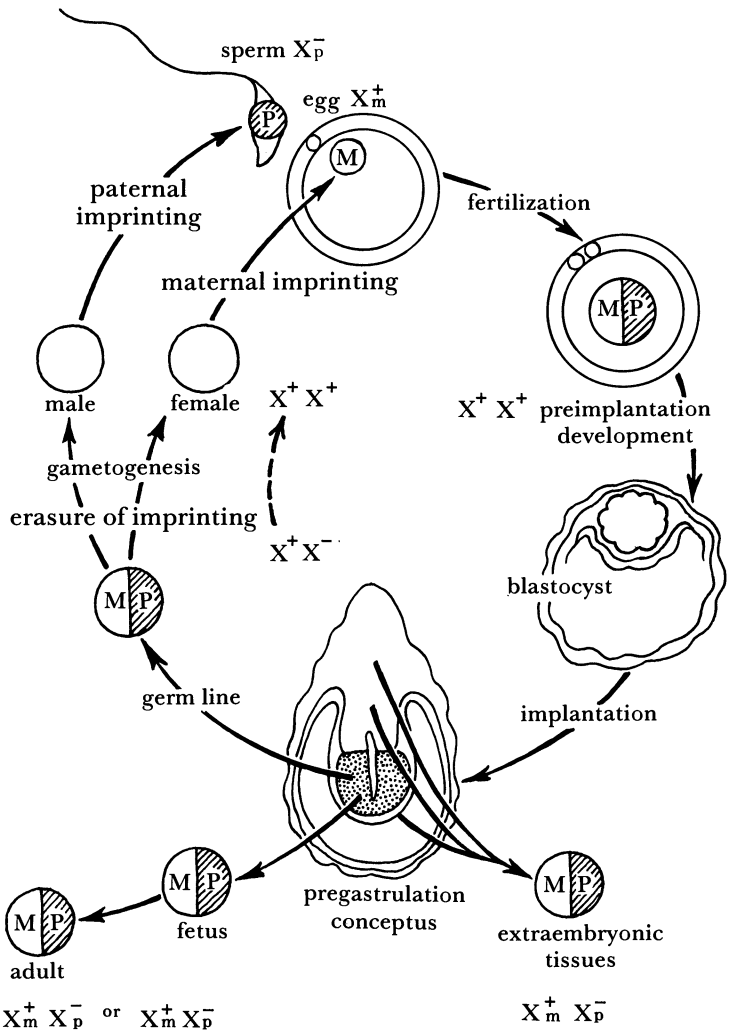

Figure 7. Diagram showing propagation and erasure of imprinting. The activity status of the two X chromosomes during female embryonic development is also shown: m, maternal; p, paternal; +, active; −, inactive.

Searle & Beechey 1985). The preferential inactivation of the paternal X chromosome in the extra-embryonic lineages of the mouse (see above and figure 7) is another example of imprinting; in marsupials, inactivation of the paternal X chromosome occurs also in somatic tissues (see VandeBurgh *et al.* 1987). Functional differential modification of parental chromosomes may be observed in diverse biological phenomena in a wide range of systems; viz, determination of maleness and germ cell differentiation in male coccids (Brown & Nur 1964), yeast mating-type interconversion (Klar 1987), uniparental chloroplast inheritance (Sager & Kitchin 1975) and gene action in maize endosperm (Kermicle 1978).

How has imprinting come about and why? The first thing to note is that differential imprinting implies complementary gains and losses of function contributed by the gametes and hence renders sexual reproduction essential. The evolutionary initiation of such a process could be accidental; chance loss of function in one hermaphroditic individual would require complementation by reproduction with another. Thus interdependent, further losses of function could be accumulated (so long as they were complemented by sexual reproduction) hence, ensuring obligatory interdependence and introducing certain advantages over self-fertilizing systems by this sharing of genetic contributions and, of course, the enhancement of genetic variation. As to why the paternal genome contributes preferentially to the extra-embryonic membranes, we need to search for some reason as to why the evolution of viviparity might involve an unequal contribution from one sexual partner.

6. Imprinting and methylation

Whatever the molecular mechanism of imprinting, it must be initially established in the gametes, be propagated in some form throughout cell division in the soma, be erased in the germ line and be differentially re-established, depending on the sex of the individual, in the sperm or the egg genome once more (see figure 7). Stable, heritable, differential modification of DNA is required and differential patterns of DNA methylation seem a likely mechanism. Certainly, transgenes inherited from the mother or the father, may be methylated differently in the individual (Reik *et al.* 1987; Sapienza *et al.* 1987; Swain *et al.* 1987; Hadchouel *et al.* 1987) but whether these methylation differences pre-existed in the gametes is not known.

The sex-specific methylation of the sperm and egg genomes (see above) could be the basis for differential imprinting. However, a simple quantitative difference in methylation between the gametes is not likely to be the basis, but rather a differential modulation of patterns of methylation as already described above for the active and inactive X chromosomes. Note, for instance, that in most cases where differential methylation of a transgene is observed, the transgene is less methylated if it is inherited from the globally methylated sperm (reviewed by Surani *et al.* (1988); Monk (1988)). If imprinting is propagated in development by the inheritance of specific methylation patterns, how are we to incorporate the large changes in overall DNA methylation observed; the marked decrease by the blastocyst stage (see above) and the new patterns of *de novo* methylation that begin around the time of implantation?

It is unlikely that the original imprinted information in sperm and egg genomes survives throughout development and adult life in the paternal and maternal genetic complements. At fertilization, differences in chromatin configuration (related to packaging), as well as differences in DNA methylation, in the gametes will influence the onset of expression of different parental alleles in preimplantation development. Thereafter, the initial patterns of

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gene activity in early development (including the differential activity in imprinted regions of parental chromosomes) may influence whether these genes, or chromatin regions encompassing them, remain as active chromatin (by virtue of their current activation) or are rendered inactive chromatin with consequent effects on subsequent *de novo* methylation patterns. In addition, the embryonic and extra-embryonic lineages segregating in preimplantation development and the definitive germ layers and germ line segregating at gastrulation will be subject to developmental cues and position effects that will induce tissue-specific patterns of gene expression. Thus the true picture will be a dynamic one with differences in the parental genomes accumulating due to 'knock-on' effects of the original starting differences. Implicit in this scenario, is that different patterns of methylation can cause different patterns of gene expression, and, conversely, that different patterns of gene expression can cause different patterns of methylation.

When does erasure of imprinting occur in the formation of the germ line? In terms of methylation differences, erasure might well occur due to loss of methylation by the blastocyst stage as well as absence of further *de novo* methylation in the germ line. Certainly, erasure of the methylation imprint on endogenous L1 sequences in sperm has already occurred in the primordial germ cells at 11.5 days' gestation (Monk *et al.* 1987). If there are differential programmes of gene expression regulated by mechanisms other than methylation in the germ line, these in turn might be erased during the unravelling of chromosome structure required for synapsis and genetic exchange at meiosis.

As to when the methylation differences are re-established in the gametes, little is known. Methylation of L1 sequences are already observed in pachytene spermatocytes (Sanford *et al.* 1987). It is probable that methylation of the L1 sequences in the sperm genome occurs at resumption of mitosis around the time of birth. The earlier suggestion that methylation may be occurring in male germ cells by 16.5 days gestation (Monk *et al.* 1987) has not been born out by further experiments.

7. Conclusions

Some implications of the dynamic roles of changing patterns of methylation, chromatin configuration and gene expression during development are as follows.

1. Methylation may be the cause and the effect of gene inactivation. There have been many investigations into whether methylation is the cause or the effect of gene inactivation. There is considerable evidence that prior *in vitro* methylation can inhibit gene activity (Bussingler *et al.* 1983; Vardimon *et al.* 1982; Fradin *et al.* 1982; Stein *et al.* 1982; Keshet *et al.* 1985). There is also evidence that methylation may occur after gene inactivation (Niwa *et al.* 1983; Gautsch & Wilson 1983; Lock *et al.* 1987). Likewise, in development, the original methylation patterns in the gametes may influence early differential gene expression (including differential parental allele expression) and then *de novo* methylation may 'set' new specific patterns of gene expression determined by other mechanisms. The processes of methylation and demethylation in development will be intimately linked to changes in chromatin structure and changing patterns of gene expression (see Keshet *et al.* 1986).

2. Imprinting. Parent-specific methylation differences observed during development or in the somatic tissues of the adult could occur after fertilization and the methylation observed at any point of time could be due to the interplay of a number of factors set in motion by the original gametic methylation patterns. Whether parent-specific or not, the methylation status

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of a particular site need not be the same as it was in the gametes, nor the same in different tissues.

3. Erasure of the imprint – incomplete erasure of parent–specific methylation patterns will modify inheritance ranging from 'grandparental' inheritance to a permanent change (a parent-independent imprint) which would appear as a mutation.

4. In general, an alteration of methylation patterns in the germ line could appear as a 'modification of inheritance patterns' and even as 'inheritance of acquired characters' – a popular hypothesis in the time of Lamarck. Could the memory mechanism represented by the methylation patterns be altered or adapted by experience or environmental change from generation to generation?

I thank my collaborators Mary Harper and Andy McMahon in studies on patterns of X-chromosome activity in development; Susan Lindsay in studies of methylation differences on the active and inactive X chromosomes, and Michael Boubelik, Sigrid Lehnert and Peta Maidens in studies on methylation in development.

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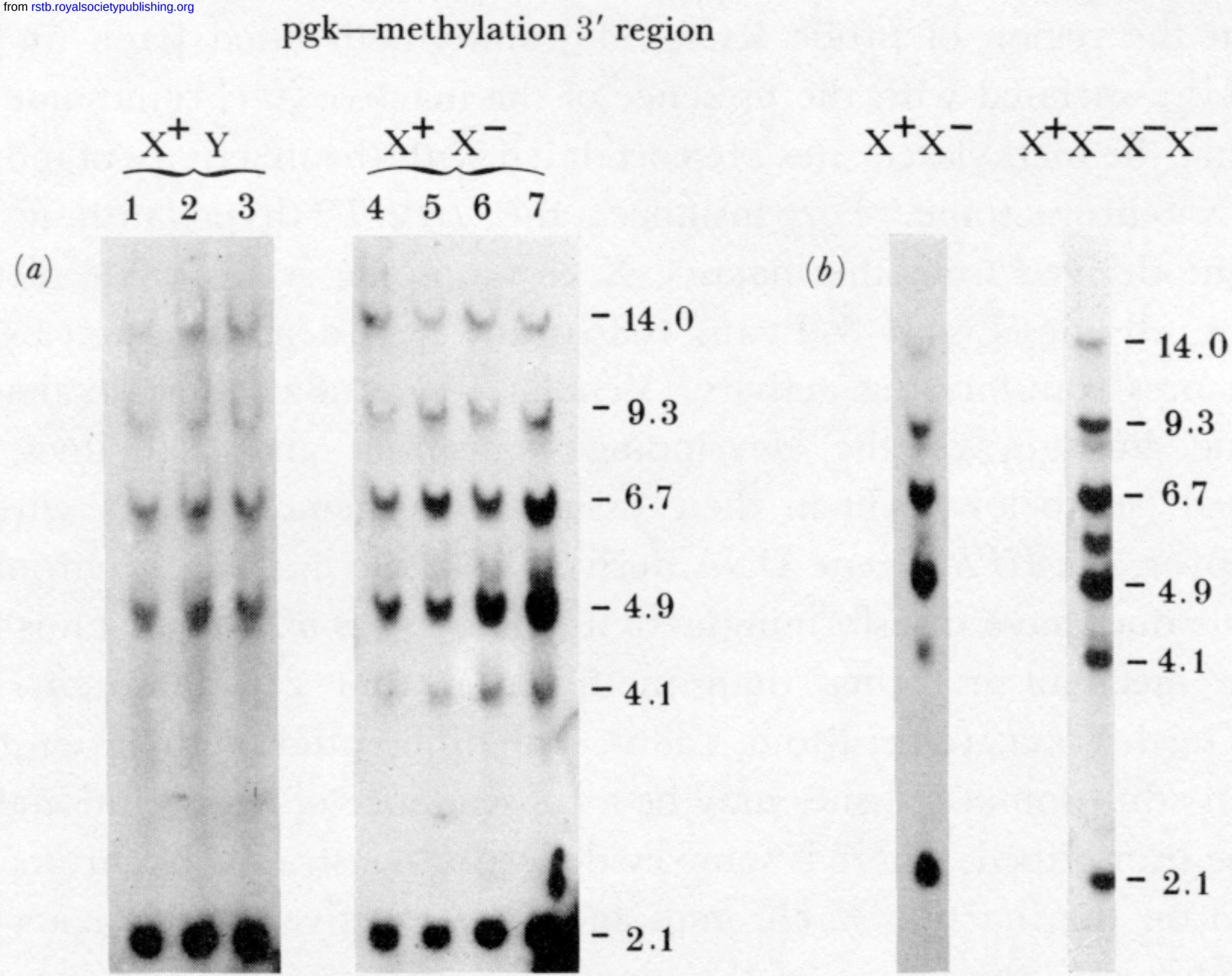

GURE 2. *Hpa*II *Bam*HI double digests of human white blood cell DNA hybridized with a DNA probe complementary to the 3′ end of the human *PGK* gene. (*a*) DNA from three male (1–3) and four female (4–7) individuals. (*b*) DNA from diploid and aneuploid females. (PGK, phosphoglycerate kinase). The data is from Lindsay *et al.* (1985).

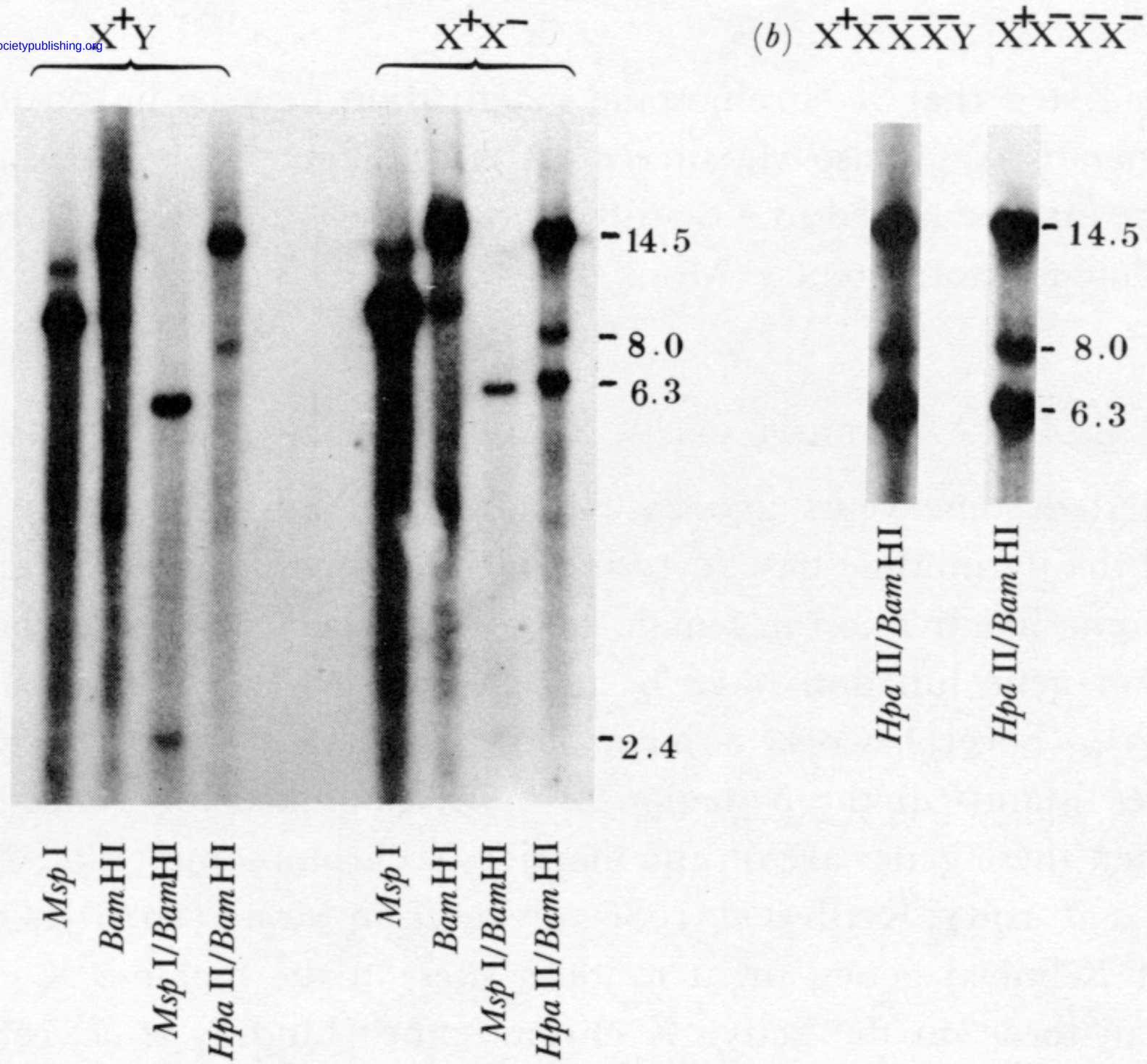

FIGURE 3. Restriction enzyme digests of human white blood-cell DNA hybridized with human X-chromosome probe RC8. (*a*) DNA from male and female. (*b*) DNA from aneuploid male and female. The data is from Lindsay *et al.* (1985).

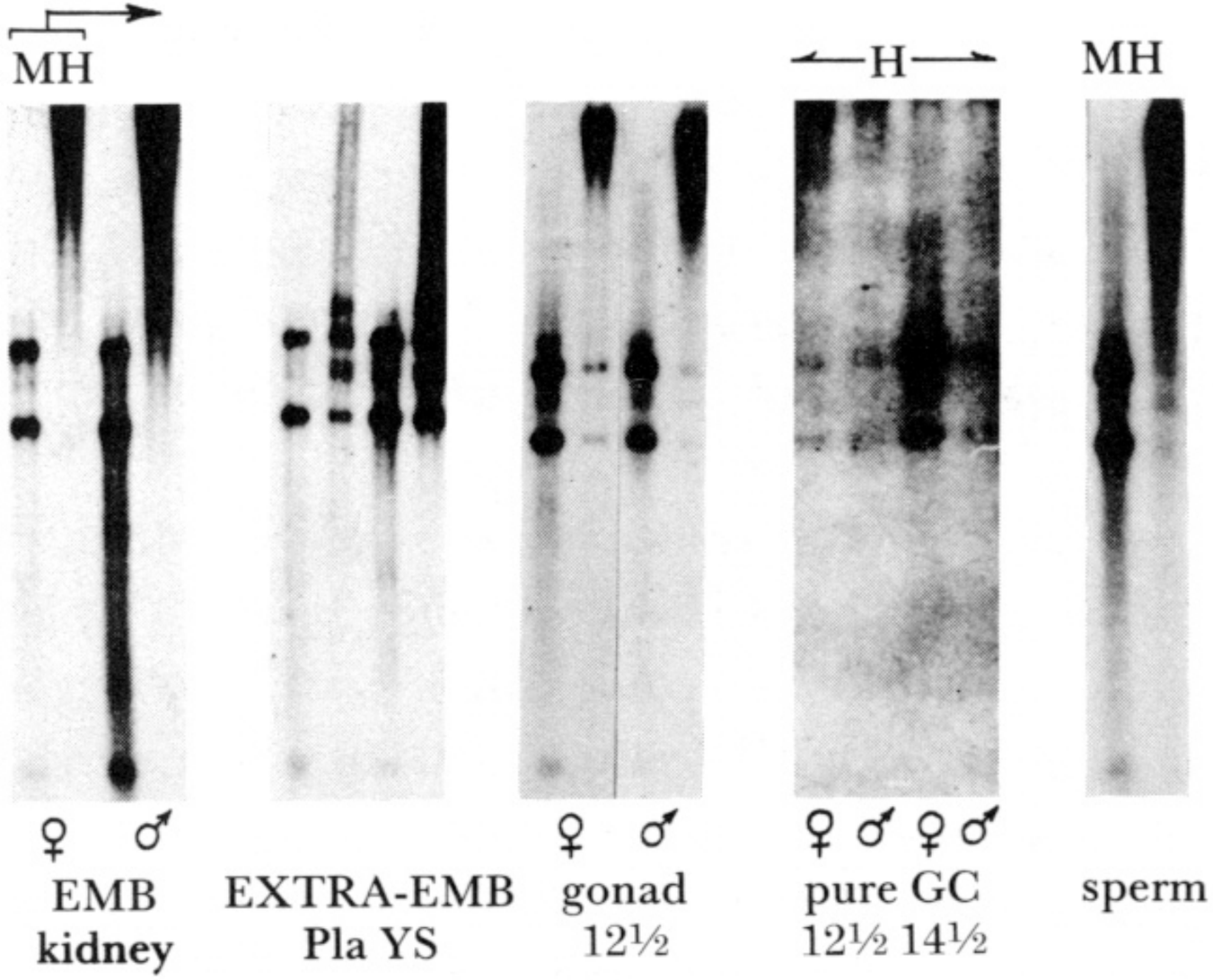

FIGURE 4. *Msp*I and *Hpa*II digests of DNA isolated from various tissues of the mouse conceptus and probed with L1 sequence. (M, *Msp*I; H, *Hpa*II; EMB, embryonic; EXTRA-EMB, extra-embryonic; Pla, placenta; YS, yolk sac endoderm; GC, germ cell). The data is from Monk *et al.* (1987).

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