COMMENTARY

Genomic imprinting

Genomic imprinting is the differential modification of the maternal and paternal genetic contributions to the zygote, resulting in the differential expression of parental alleles during development and in the adult. It has been known for some time that imprinting plays a role in a diversity of biological phenomena, including sex determination and germ cell differentiation in certain insects (Brown and Nur 1964; Scarbrough et al. 1984), preferential inactivation of the paternal X chromosome in marsupials (for review, see VandeBurgh et al. 1987) and in the extraembryonic membranes of rodents (Takagi and Sasaki 1975), uniparental inheritance of chloroplast DNA (Sager and Kitchin 1975), and mating-type interconversion in fission yeast (Klar 1987). Lately, the phenomenon of imprinting is exciting considerable interest. This is due to the recent discoveries in the mouse that the differential modification of the maternal and paternal genomes is essential for successful development of the conceptus (McGrath and Solter 1984; Surani et al. 1984) and survival and normal phenotype of the adult (Cattanach and Kirk 1985). In addition, recent reports that transgenic DNA marker sequences are methylated differently, depending on the parent of origin, suggest that methylation may be involved in the mechanism of imprinting (Reik et al. 1987; Sapienza et al. 1987; Swain et al. 1987). The regions of chromatin involved are now accessible to further analysis.

The investigations that have shown a requirement for the differentially modified maternal and paternal genetic contributions for successful development and normal phenotype in the mouse have utilized duplications of maternal or paternal genomes or chromosome regions. Mouse embryos containing either two female pronuclei or two male pronuclei were created by exchange of pronuclei between eggs (Barton et al. 1984; Mann and Lovell-Badge 1984; McGrath and Solter 1984; Surani et al. 1984). In embryos with two female genetic complements, fetal development was good but development of the extraembryonic membranes and placenta was poor; in embryos with two male genetic complements, the reverse was true—the extraembryonic tissues developed well, and the fetus developed poorly. The reason for the uneven contribution to the different parts of the conceptus is not yet understood. Duplications of maternal or paternal specific chromosome regions may be achieved by matings between mice with different chromosome translocations (Searle and Beechey 1978) or carrying different metacentric chromosomes (Cattanach and Kirk 1985). The combinations may be lethal or result in different phenotypes, suggesting differential functioning of maternal and paternal gene loci within the regions concerned. Again, the loci involved and the causes of the anomalies are unknown.

In the mouse, there is a difference in the methyl cyto-

sine content in the DNA of the sperm and the egg, and this has been proposed as a possible molecular basis for imprinting (Monk 1987; Monk et al. 1987). Normally, it is not possible to distinguish the levels of methylation of different parental genomes, or specific alleles, in the individual. Therefore, transgenic marker sequences, integrated at various sites in the genome and inherited from one or the other parent, have been used to show differential methylation. The frequency of differential methylation of different transgenes has been reported in 4 out of 5 transgene loci studied by Sapienza et al. (1987), 1 out of 7 studied by Reik et al. (1987), and 1 out of 10 studied by Swain et al. (1987). In most cases, the transgene inherited from the father is less methylated than if it were inherited from the mother. So far, for any of these transgene loci showing differential imprinting in the fetus or adult, it is not known whether the difference also existed earlier between the sperm and the egg (because of the difficulty in obtaining enough oocyte DNA for analysis); but in three cases examined by the workers mentioned above, undermethylation of the paternally inherited loci in the offspring is reflected by undermethylation of these loci in the testes. This would seem to be at variance with the fact that sperm DNA is more methylated than oocyte DNA overall. However, irrespective of overall genomic methylation differences, imprinting may be due to differential modulation of methylation along particular domains of sperm and oocyte DNA. A precedent for differential modulation of methylation is seen, e.g., between the active and inactive X chromosomes in somatic tissues; clustered CpG sequences 5' to certain genes are more methylated than compared to inactive X chromosome, whereas other CpG sequences in the body of the gene are less methylated on the inactive X chromosome. The inactive X chromosome also reminds us that there are more ways to inactivate a chromosome or chromosomal domain than methylation alone; chromosome configuration must also play a part in maintaining inactivation in segmental fashion along the inactive X chromosome (for review, see Monk 1986).

Imprinting must be established during (or before) gametogenesis, persist stably throughout DNA replication and cell division in the soma, and be erased in the germ line to be differentially established once more in the sperm and egg genomes. Stable, heritable, differential modification of chromatin is required. Differential patterns of methylation are stable, heritable (Holliday and Pugh 1975), and, moreover, have been implicated in the regulation of gene expression, chromatin configuration, and X-chromosome expression. The configurational differences between repressed and derepressed chromosome domains are also heritable (Weintraub 1985) and correlated with methylation (Keshet et al. 1986). Sperm and oocyte are methylated differently; they are also sub-

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ject to different configurational constraints in the packaging of their genomes. Therefore, I would like to propose a model of establishment, propagation, and erasure of imprinting that works on at least two different levels—differential methylation and differential chromatin configuration. Three important aspects of this model follow: (1) Initial imprinting differences between the gametes influence the timing of onset of parental allele expression. Thereafter, 'function determines form' in the sense that recruitment of specific maternal or paternal domains of chromatin into active gene expression during development leads to 'open' chromatin configuration in these regions and undermethylation. Conversely, the failure of a specific maternal or paternal chromosome domain to enter active expression during certain interval of time in development may lead to condensation and subsequent methylation of that domain. (2) The germ line escapes extensive *de novo* DNA methylation, and this hypomethylation is a prerequisite or a precondition for erasure of the configurational constraints of imprinting and for reprogramming of the germ line to developmental totipotency; this process may be connected to meiosis. (3) Some apparent differential imprinting phenomena (preferential allele expression, abnormal phenotype, or nonreciprocal lethality) may be a consequence of DNA sequence differences between the maternal and paternal chromosome regions concerned.

Further considerations related to this model are outlined below.

We have studied the temporal and regional changes in overall DNA methylation in the embryonic, extraembryonic, and germ cell lineages during development of the mouse (Monk et al. 1987; Sanford et al. 1987). A summary of the results is given in Figure 1 in the form of densitometer tracings of the distributions of the larger fragment sizes resulting from *HpaII* digestion of DNA from eggs, sperm, eight-cell embryos, blastocysts and implanting blastocysts (and their isolated inner cell masses), embryonic and extraembryonic regions of postimplantation embryos (pre- and postgastrulation), and premeiotic germ cells of male and female embryos. Increased genomic methylation (e.g., sperm and gastrulating embryo DNAs; Fig. 1) is correlated with higher-molecular-weight *HpaII* fragments to the left of the distributions (top of the lanes on the gel) and decreased genomic methylation, with a skewing of the distribution away from the top of the gel (e.g., oocyte, blastocysts, fetal germ cell DNAs; Fig. 1). It is clear that the egg genome is strikingly undermethylated (the peak fraction is a *HpaII* fragment of mitochondrial DNA) and the sperm genome is relatively methylated. There is a loss of methylation during preimplantation development (perhaps due to a delay in synthesis of the embryo-coded methylase) and the blastocyst is markedly undermethylated. Thereafter, embryonic and extraembryonic genomes are methylated progressively *de novo*, independently (and therefore potentially differently), and to different final extents. *De novo* methylation also continues postgastrulation and, hence, could be a mechanism initi-

Figure 1. Densitometer tracings of DNA isolated from different tissues of the mouse conceptus and digested with *HpaII*. Fragments resulting from *HpaII* digestion were end-labeled, electrophoresed, blotted to a nitrocellulose filter, and autoradiographed. The discrete band in the oocyte track is one of the mitochondrial *HpaII* fragments. (ICM) Inner cell mass; (bla) blastocyst; (epi) epiblast; (end) primary endoderm; (emb) embryo; (cho) chorion; (GC) germ cell; 6.5 and 7.5, days of gestation. (For details, see Monk et al. 1987.)

ating or confirming differential programming of the definitive germ layers. We proposed that much of the *de novo* methylation observed in somatic tissues acts to stabilize and reinforce prior events regulating the activity of specific genes, chromosome domains, or the X chromosome (in females). A similar conclusion—that methylation may be a secondary event involved in maintenance of the inactive state—was reached by Lock et al. (1987), who showed that methylation of gene sequences on the inactive X chromosome occurred after X-chromosome inactivation in development.

These observations must be taken into account when considering the propagation of differential methylation as a mechanism for the propagation of imprinted information in development (see Fig. 2). If there is a delay in the onset of expression of the gene for maintenance methylase, we expect that each cell doubling will exponentially decrease the methylated DNA strands remaining. If the methylation difference between the ga-

metes is the sole source of imprinting information, how is this information propagated if methylation is being lost? It could be argued that certain 'key sites' remain differentially methylated in the maternal and paternal genetic complements and that these key sites direct the subsequent patterns of de novo methylation (although

not in the germ line). Alternatively, or in addition, a second mechanism of propagating imprinted information may rely on heritable differential chromatin structure. The process could be dynamic (albeit circular) in that these initial imprinting mechanisms, both methylation and structural modification, may distinguish the

Figure 2. A diagrammatic model showing two levels of imprinting differences in the gametes, configurational (shown as different outlines of nuclei) and methylation (shown as dots within nuclei), the propagation of imprinting in the embryonic and extraembryonic regions of the conceptus, and erasure of imprinting in the germ line. The changes in overall genomic methylation during development are indicated. In the female conceptus, preferential inactivation of the paternal X chromosome (X_p) occurs in extraembryonic tissues, random X-chromosome inactivation (maternal, X_m , or X_p) occurs in embryonic tissue, and X-chromosome reactivation (X^+X^- to X^+X^+) occurs at meiosis in the germ line.

timing of onset of maternal or paternal gene activity, and differential gene activity itself may lead to further differences in methylation and structural modification of the maternal and paternal loci as development proceeds.

We come now to a consideration of the erasure of the biparental imprinting in the germ line and reestablishment of either the male or female uniparental imprint in spermatogenesis or oogenesis, respectively. Here, a consideration of cycles of X-chromosome activation, inactivation, and reactivation in different lineages of the female mouse embryo may be relevant (for review, see Monk 1981, 1986). The paternally inherited X chromosome, previously inactive during spermatogenesis, becomes active during cleavage stages of development (perhaps as a consequence of loss of methylation and/or of decondensation of paternal chromosomes) and is then preferentially inactivated in the extraembryonic lineages and randomly inactivated in the fetal and germ cell lineages (see Fig. 2). Whatever the imprint is that distinguishes the paternal X chromosome for inactivation in the extraembryonic lineages, this imprint is either preferentially inherited by the extraembryonic lineages (perhaps those cells retaining methylated DNA 'sort out' into extraembryonic lineages) or it is lost or 'not seen' when the time comes to inactivate an X-chromosome in the fetal and germ line precursor cells. The reasons for preferential paternal X chromosome inactivation in extraembryonic tissues of rodents and somatic tissues of marsupials is not known. Although the inactive X chromosome is inactivated irreversibly in somatic cells, it is reactivated just prior to meiosis in the female germ cells (see Fig. 2). It has been suggested previously (Monk 1981) that X-chromosome inactivation is linked to cell differentiation and, conversely, that X-chromosome reactivation in the female germ line is linked to cell dedifferentiation or a reprogramming of the germ line to developmental totipotency. It is tempting to equate erasure of imprinting with this latter process (see Fig. 2). If, as seems likely, the germ line of both female and male embryos escapes *de novo* methylation, erasure of imprinting would require erasure of chromatin structure constraints—a process that could conceivably be a part of the unraveling of chromosomes in preparation for synapsis at meiosis. If erasure were not complete, imprinting could pass through more than one generation as grandparental memory or even a permanent imprint. Indeed, a permanent imprint has been observed (as a methylation change) for one particular transgene marker following transmission through the female (Hadchouel et al. 1987) and proposed as a mechanism to explain the pattern of inheritance of the fragile X syndrome in humans (Laird 1987). In other cases, what appears as a permanent imprint, or indeed as a mutation, may arise as a consequence of DNA sequence differences between the parental genomes in the chromatin regions concerned. This DNA sequence difference could lead to a difference in timing of activation of these regions during development. Inactivation would then be due to the fact that the region failed to be expressed. This leads to the

final aspect of the model to be considered.

Some apparent imprinting phenomena observed will be a consequence of DNA sequence divergence between the maternal and paternal chromosome regions concerned. This has been well established in interspecific hybrids of fish, where differential timing of expression of the two parental loci, preferential allele expression, developmental abnormalities and lethality are correlated with the degree of genetic divergence between the parents (Whitt et al. 1973, 1977; Whitt 1981). The nonreciprocal lethality of some crosses in fish is reminiscent of the DDK strain of mice, where matings of DDK females with males of other strains of mice are sterile, whereas reciprocal crosses are fertile. The DDK egg cytoplasm is rendered incapable of supporting development by an interaction with 'foreign' sperm (Renard and Babinet 1986). A similar difference, perhaps in timing of sex-determining functions, may underlie the aberrant sexual differentiation involving a foreign Y chromosome on a C57B6 genetic background (Eicher and Washburn 1986). Restriction maps for a number of loci for C57B6 DNA differ from that of other inbred strains of laboratory mice (P. Martin and M. Monk, unpubl.). Hence, the use of the C57B6 strain as one parent in investigations of imprinting may show more modifications than would be observed within an inbred strain.

In summary, the role of imprinting in a number of essential biological processes determining sex, germ cell differentiation, development, and normal adult phenotype is now well established. It is suggested that tissue- and timing-specific functional haploidy in early development occurs as a consequence of differential heritable chromatin configuration imposed by packaging constraints in sperm, as well as differential DNA methylation of sperm and egg genomes. Later in development, the configurational and methylation differences observed in the fetus or in the adult may be correlated with the different patterns of gene expression occurring earlier. Some phenomena reminiscent of genomic imprinting may occur as a consequence of genetic distance (DNA sequence differences) between the gametic genomes, and the greater the distance is, the more pronounced the effects. In this respect, differential expression of parental alleles may result in lethality and hence play a role in speciation, or 'protection against foreign DNA.' Finally, erasure of imprinting (and reactivation of the inactive X chromosome) may be a function of meiosis.

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Marilyn Monk

MRC Mammalian Development Unit
4 Stephenson Way
London NW1 2HE, UK

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M Monk

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