Genomic imprinting and allelic exclusion

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Summary

In diploid cells, allelic exclusion reduces genes to functional haploidy, because only one of two alleles is active. It is best known in cells producing immunoglobulins, but other examples also exist. X-chromosome inactivation in female mammals is related to allelic exclusion, but in this case the dosage compensation mechanism extends to the whole chromosome. Functional hemizygosity in some mammalian cell lines is probably also due to allelic exclusion, where one autosomal allele is active and the other is methylated and inactive.

In early development, it may be important to have only one functional copy of specific regulatory genes. If one considers the possible mechanisms whereby genes are switched from an active to an inactive form, or *vice versa*, complications arise if the same type of switch operates in two homologous chromosomes segregating independently at mitosis. This complication is avoided if one of the genes is totally inactive. It is therefore suggested that important regulatory genes are subject to allelic exclusion and that this provides a basis for genomic imprinting. Male or female gametes complement in the zygote, because they may have different inactive genes, and the active allele in each case is then functionally haploid in the zygote and developing embryo. These haploid genes would be those involved in critical switches of gene activity during the developmental process. Allelic exclusion imposed by imprinting might be based on the heritable DNA methylation of the regulatory regions of silent genes.

Key words: imprinting, DNA methylation, allelic exclusion, developmental switches.

Introduction

The majority of diploid organisms retain the ability to reduce their genome to haploidy by meiosis and the production of male and female gametes. There are also reasons why diploid organisms may need to haploidise parts of their genome or individual genes. In this contribution, I briefly review examples of this and also suggest that the function of genomic imprinting is to produce haploidy of individual genes or sets of genes, which is essential for normal vertebrate development.

Sex determination

In mammals the sex determination mechanism is based on haploid inheritance. A male-determining Y chromosome is present in single copy in males and absent in females. The X chromosome is also haploid in males. In females the same result is achieved by a dosage compensation mechanism based on the inactivation of one of the two X chromosomes in all cells during early development. Thus the X chromosome in females is functionally haploid. X-chromosome inactivation also demonstrates the importance of a switch acting at the DNA level, which, in this instance, results in epigenetic differences between the two homologous chromo-

somes. The inactivation process depends, at least in part, on the DNA methylation of CG-rich islands adjacent to genes on the inactive X chromosome (Monk, 1986; Toniolo *et al.* 1988; Riggs, 1989, and refs. therein). These genes can be reactivated by treatment of cells with a demethylating agent, 5-azacytidine (Ellis *et al.* 1987; Hansen *et al.* 1988; Migeon *et al.* 1988, and refs. therein). X-chromosome inactivation is analogous to allelic exclusion, but the dosage compensation mechanism extends to the whole chromosome, whereas single genes or perhaps short regions of the chromosome are inactive or silenced in the case of allelic exclusion.

Allelic exclusion

It has been known for about 25 years that diploid cells producing immunoglobulins do not use both genes on the homologous autosomal chromosomes. In cases where the products of the two genes could be identified, it was found that any given cell produces only one specific immunoglobulin molecule (Bernier and Cebra, 1964; Weiler, 1965; Pernis *et al.* 1965). The mechanism responsible for this was referred to as *allelic exclusion* (Pernis, 1967). So far, this term has been reserved almost exclusively for the immunoglobulin-producing

cells of the immune system, but as I will describe, other examples also exist.

Functional immunoglobulin genes arise from the splicing of different DNA domains (Tonegawa, 1983). Thus it seems possible that allelic exclusion could be due to the successful completion of this complex molecular process in only one of the two homologous chromosomes. If this is so, then the basic mechanism could be either stochastic or regulatory. Splicing involves several recombination reactions by the V-D-J recombinase system and it is therefore possible that the whole process is often inaccurate or incomplete. Thus, the probability of producing two functional immunoglobulin genes in any one cell could be quite low. It now seems unlikely that this interpretation is correct, and very probable that allelic exclusion is due to a regulatory mechanism. Evidence for this has recently been obtained by the use of transgenic mice that already have a functional immunoglobulin gene. It has become clear from these experiments that there is a positive feed-back process which inhibits the splicing reactions (reviewed by Kohler, 1989). Therefore, once a complete immunoglobulin gene has been assembled, the formation of a second functional gene is blocked.

This regulatory mechanism is advantageous to the immune system. First, specificity in the recognition of surface immunoglobulin molecules is improved if there is only one such molecule rather than two. Second, in the evolution of cells producing antibodies with greater and greater affinity for a given antigen (Milstein, 1987; Berek and Milstein, 1988), it is advantageous to select the appropriate somatic mutations in only one gene rather than those in two different ones. The two cases of allelic exclusion so far considered, namely X-chromosome inactivation and the assembly of an immunoglobulin gene, can both be regarded as functional adaptations at the cellular level to reduce diploidy to haploidy.

There are other documented examples of allelic exclusion. Certain autosomal coat colour genes produce a mottling phenotype, which is very similar to the mosaicism of X-linked genes in females. On the basis of their properties, Searle (1968) has suggested that the mottling phenotype is due to a dosage compensation mechanism, which randomly inactivates one of two alleles in homologous autosomes.

In interspecific hybrids, it has been shown in some cases that only one of two inherited allelic genes is expressed. Thus, in some chicken–quail hybrids, the paternal allele for alcohol dehydrogenase is never expressed. (Castro-Sierra and Ohno, 1968). This has been referred to as allelic repression, and other examples have been documented in sunfish interspecific hybrids (Whitt et al. 1972, 1977) and trout hybrids (Schmidtke et al. 1976). Similarly, in hybrids between different species of Xenopus, it has been shown that the expression of one nucleolar organism is 'dominant' and the other is not expressed (Honjo and Reeder, 1973; Cassidy and Blackler, 1974). Formally, these are examples of allelic exclusion, but they probably arise as a result of some incompatibility between DNA regulat-

ory sequences of one species and regulatory proteins from the others. Such interactions are of interest in the context of gene regulation, but they are not directly related to the positive exclusion mechanisms that occur in immunoglobulin genes or in dosage compensation.

Allelic exclusion in cultured mammalian cells

Examples of allelic exclusion have recently been uncovered in mammalian cell lines, which are probably not due to a functionally adaptive mechanism. It has been known for some time that recessive autosomal mutations can be readily isolated in cultured cell lines, even though these are derived from primary diploid cells. Siminovitch (1976) coined the term 'functional hemizygosity' for genes in cells such as the Chinese hamster ovary (CHO) cell line, which has often been used to isolate recessive mutants. This line has a pseudo-diploid karyotype and he suggested that the genome has been substantially rearranged so that considerable parts of it have become haploid. This may be partially true, but recent experiments suggest an alternative explanation.

Mutations in CHO cells can be induced by standard mutagens such as EMS. Such mutations have been shown to revert to wild-type at very high frequency after treatment with the demethylating agent 5-azacytidine, which is a very weak mutagen (Jeggo and Holliday, 1986). This suggests that the cells initially contained an active and an inactive methylated gene. The former is subject to mutation induced by DNA-damaging agents, whereas the latter is still present in silent form and can be reactivated by demethylation. This interpretation is supported by further analysis using the thymidine kinase (TK) gene of CHO cells. Both spontaneous and EMS-induced TK⁻ mutations are reactivable by 5-azacytidine. An induced mutation that has been reactivated should have one mutant allele and one wild-type allele. This strain was again treated with EMS to produce secondary TK⁻ derivatives. These should now contain two mutant alleles, neither of which can be reactivated by 5-azacytidine. This prediction has been confirmed with several different isolates (Holliday and Ho, 1990).

In the case of CHO cells, and probably other cell lines, it seems that allelic exclusion arises through the de novo methylation and inactivation of one of two alleles (reviewed by Holliday, 1987, 1990a). In this regard, it resembles X-chromosome inactivation. However, it is unlikely that de novo methylation in cell lines is functionally adaptive, although it could result in a slightly increased growth rate. It is more likely that gene silencing in these cells is due to the breakdown of the normal controls of DNA methylation. Although the maintenance of the methylated state is well established, mainly from in vivo experiments, very little is known about the rules governing the acquisition and removal of methyl groups in normal or transformed cells. Cultured mammalian cells provide a model experimental system that should make it possible to understand

better the methylation mechanisms that change gene expression.

Genomic imprinting and allelic exclusion

In cases of genomic imprinting in insects, it is clear that whole chromosomes from one parent are totally inactivated in offspring (see Nur, this volume). Such extreme effects are not seen in mammals; instead, differences between parental chromosomes complement each other to produce a normal individual. Complementation implies that certain maternally and paternally inherited genes have differential activity. This could be some significant deviation from a 50:50 percent activity ratio, or alternatively it could be a 0:100 percent ratio of activities. In the following argument, I shall assume that, at least for some genes, there is full inactivation imposed by imprinting. It is immaterial to the argument whether for any given gene this inactivation is paternal or maternal, but the existence of complementation means that different inactive genes come from different parents. If certain genes in the haploid gametes are inactivated, then it follows that these genes are initially subject to allelic exclusion in the resulting zygote, and this will persist provided the pattern of activity and inactivity is heritable in the developing embryo. Evidence suggests that the heritable differences between paternal and maternal chromosomes are associated with differences in DNA methylation (see Jones, this volume; Reik, this volume).

It is also assumed that during development certain switch mechanisms are essential in given cell lineages. Stem line cells exemplify the basic switch mechanism. In this case, determined but undifferentiated cells divide to produce more stem cells, and also cells that will become specifically differentiated. In such a situation, there is a segregation of gene activities during cell division (Holliday and Pugh, 1975). We assumed that this segregational switch operates at the DNA level, and is presumably due to DNA–protein interactions. At some point when the DNA replicates, one daughter molecule is 'marked' and the destiny of the cell inheriting that daughter is different from that which inherits an unmarked molecule (see also Klar, this volume). Such switches may affect individual genes, but these genes may in turn have a key regulatory role in imposing changes in expression on many other genes. We suggested that the marking was due to DNA methylation of specific sequences; that changes in methylation by specific enzymes provided the basis for a molecular switch, and that methylated DNA could be maintained through subsequent cell divisions (see also Riggs, 1975). In the simplest stem line situation, one cell type is unstable in the sense that it is continually producing another cell type that is stable and committed to differentiation. In other cases, a dividing cell may produce daughters each of which have different phenotypes and developmental fates. If the same switch mechanism occurs in two homologous genes in a diploid

cell, then an obvious complication arises, since with random chromosome disjunction, the daughter cells will inherit one, two, or no marked chromosome in the ratio 2:1:1. This complication is totally avoided if the switch operates on only one active gene, the other being excluded or inactivated. In this case, the daughter cells will inherit either a marked or an unmarked gene. This is illustrated in Fig. 1.

The model is based on the assumption that endogenous switches are important in the unfolding of the developmental programme, and that there is strict determination of events in time and space. It is unlikely that stochastic events, such as those produced by random chromosome segregation, are an integral part of this process. Also, it seems improbable that
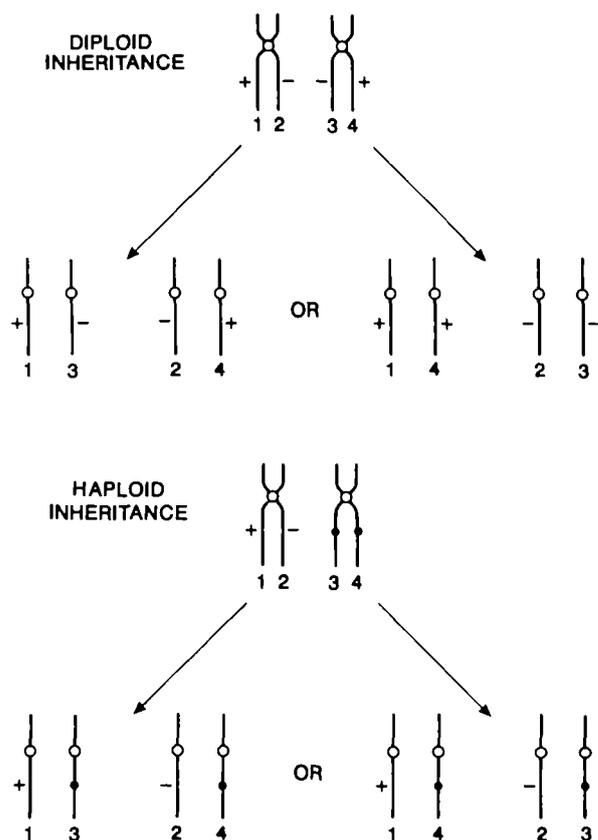
Fig. 1. The consequences of a chromosomal switch mechanism in a diploid situation, compared with the haploidy brought about by allelic exclusion. Top: it is assumed +/− switches occur at the same time in homologous chromosomes. With random segregation of chromatids, 50% of the pairs of daughters will inherit +/− switches, and 50% will have +/+ and −/−. In some developmental situations, this degree of randomness may be acceptable. Below: the closed circles indicate inactive genes in the chromatids of one of the chromosomes whilst the other homologue undergoes switching. In this case, one daughter will always be + and the other −, which can provide a much more precisely ordered change in gene activity during development. The model proposed is that the function of imprinting is to inactivate either a paternal or a maternal allele to produce the situation shown below in the zygote and developing embryo.
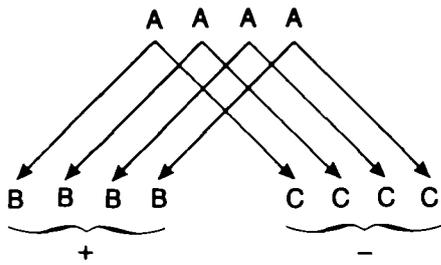
Fig. 2. Switching mechanisms may operate in groups of cells (A), possibly as the result of an external signal. Following division each cell produces two progeny (B and C) with distinct + or − phenotypes. The B and C cells sort out by self-recognition into two populations with different developmental fates.

developmental decisions are determined solely by positional information; in other words, that all important signals emanate from outside the cell (for a contrary view, see Wolpert, 1989). If it is accepted that controlled changes in gene expression are an integral component of developmental processes, there is still much disagreement about the relative importance of cell lineages *versus* ordered changes in groups of cells during development (see Holliday, 1990b). The pedigree of cell lineages implies that events are intrinsic to the cells, whereas groups of cells may change in response to an external signal. The model proposed may be largely independent of this controversy. Although it can be most easily understood in the context of a cell pedigree, where different daughter cells have different developmental fates, it could also occur in groups of cells (see Fig. 2). Imagine several cells of type A that all undergo a switch in a regulatory gene subject to allelic exclusion, perhaps in response to an external stimulus. Following division, such cells will produce equal numbers of type B and type C cells (or alternatively types A and B cells). Assuming the cell population is reasonably small, the mixture of the two types of cell could easily sort out by self cell recognition into two groups, each with its own phenotype and developmental fate (for a full discussion, see Holliday, 1990c).

Summary and the prediction of the model

It is proposed that the function of genomic imprinting is to provide in the zygote a set or limited population of genes that are functionally haploid, through the silencing or exclusion of one parental copy in each case. Allelic exclusion could be due to the heritable methylation of DNA sequences in CG islands or other sequences. The active genes concerned have an essential role in development, since they comprise the machinery for switch mechanisms acting at the DNA level. Such switches may affect single genes, but these may have a master regulatory role and control many other genes in the given developmental context. It follows that the genes subject to allelic exclusion in early development are also likely to be extremely

important in unfolding the developmental programme itself. If the studies of the mechanisms of imprinting can focus down onto individual genes, the prediction is that these same genes will also be found to be vital tools in unravelling this developmental programme. The reverse prediction is perhaps less powerful, namely, that genes suspected of having important roles in development, such as those containing homeobox domains or certain oncogenes, may be subject to allelic exclusion imposed by the imprinting mechanism.

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