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DNA methylation and epigenetic inheritance

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Classical genetics has revealed the mechanisms for the transmission of genes from generation to generation, but the strategy of the genes in unfolding the developmental programme remains obscure. Epigenetics comprises the study of the mechanisms that impart temporal and spatial control on the activities of all those genes required for the development of a complex organism from the zygote to the adult. Epigenetic changes in gene activity can be studied in relation to DNA methylation in cultured mammalian cells and it is also possible to isolate and characterize mutants with altered DNA methylase activity. Although this experimental system is quite far removed from the epigenetic controls acting during development it does provide the means to clarify the rules governing the silencing of genes by specific DNA methylation and their reactivation by demethylation. This in turn will facilitate studies on the control of gene expression in somatic cells of the developing organism or the adult.

The general principles of epigenetic mechanisms can be defined. There are extreme contrasts between instability or switches in gene expression, such as those in stem-line cells, and the stable heritability of a specialized pattern of gene activities. In some situations cell lineages are known to be important, whereas in others coordinated changes in groups of cells have been demonstrated. Control of numbers of cell divisions and the size of organisms, or parts of organisms, is also essential. The epigenetic determination of gene expression can be reversed or reprogrammed in the germ line. The extent to which methylation or demethylation of specific DNA sequences can help explain these basic epigenetic mechanisms is briefly reviewed.

1. Introduction

The diverse mechanisms for the transmission of genes from generation to generation comprise the study of genetics. These mechanisms are understood in considerable detail in a wide range of organisms. Inheritance of genes and their specialized activities also occurs in somatic cells and tissues of higher organisms, but apart from simple mitotic transmission, the mechanisms controlling the unfolding of the genetic programme for development are unknown. The study of the activities of genes in development and in specialized somatic cells was referred to as epigenetics by Waddington (1953, 1956). Epigenetic processes are those that impart temporal and spatial control on the activities of all those genes required for the development of a complex organism from the zygote to the fully formed adult. The general features of epigenetic mechanisms and the possibility that they may be controlled by specific DNA methylation will be discussed later. Initially, I will consider the question of the epigenetic control of gene activity by DNA methylation in cultured mammalian cells. This experimental system is quite far removed from the controls of gene expression in normal somatic cells in the developing or adult organism. Nevertheless, a great deal can be learned about the relation between DNA methylation and transcription in genes that produce clear cut phenotypes in cultured cells. Of

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particular importance are the rules governing the turning off of genes by *de novo* DNA methylation, their activation by loss of methylation, as well as the number of methylation sites involved and their relation to promoters and transcriptional units.

2. Epigenetic variation in mammalian cells in culture

There are two basic points of view underlying the study of the inheritance and expression of genes in cultured somatic cells. The first tends to assume that mammalian cells (or those from other higher organisms) can be studied in much the same way as microbial cells (Puck 1972; Siminovitch 1976). Mutants with defective genes can be isolated and characterized and their segregation and recombination can be studied in appropriate hybrids. The second viewpoint is that somatic cells necessarily have specialized gene functions, and that when they are grown in the foreign environment of cell culture flask or plate, the mechanisms of change of gene activity cannot be predicted and should not be assumed to be the same as those in microbial cells. In the early days of somatic cell genetics, several arguments for the second point of view were presented by Harris (1964, 1973), but in general they were disregarded. It became clear that it was possible to isolate gene mutations, arising from an alteration in base sequence of structural genes, and study these with considerable success (see Siminovitch 1976). Although this is established, in recent years Harris's point of view has also been vindicated, because it is now certain that the control of gene activities in somatic cells can also depend on epigenetic mechanisms, especially DNA methylation. Harris himself showed that strains of the hamster cell lines V79 and CHO, which lacked certain standard housekeeping enzymes and maintained a very stable phenotype, could be reverted or reactivated at very high frequency with the demethylating agent azacytidine (5-aza-CR) or azadeoxycytidine. These included strains lacking thymidine kinase (TK^-) (Harris 1982), and enzymes required for proline synthesis, glutamine synthesis or asparagine synthesis (Harris 1984*a*, *b*, 1986). CHO cells are also metallothionein deficient (cadmium sensitive) and can be reactivated by 5-aza-CR to cadmium resistance (Stallings *et al.* 1986; Gounari *et al.* 1987). Holliday (1987) lists 19 examples of inactive or silent genes, strongly reactivated by 5-aza-CR, that have been discovered in various rodent cell lines and in one human cell line. In several cases the reactivation has been shown to have been associated with the loss of methylation of *Hpa* II or *Hha* I sites. The striking feature of these non-expressed genes is the extreme stability of their phenotype. They usually revert spontaneously to wild type (i.e. the production of cells with gene activity) at a frequency of 10^{-5} to 10^{-7}, which is similar to the stability of classical base change mutation. It is therefore not surprising that cell lines with such heritable enzyme deficiencies are usually referred to as mutants (see below). Their behaviour demonstrates that there is extremely tight maintenance of DNA methylation during normal cell division.

More recently it has been shown that the pseudo-autosomal gene steroid sulphatase, which remains active at the tip of an inactive mouse X chromosome, can become inactive in culture and is subsequently reactivable by 5-aza-CR (Schorderet *et al.* 1988). Borrello *et al.* (1987) transfected 3T3 cells with Ha-*ras* DNA methylated at *Hpa* II and *Hha* I sites, together with a selectable marker. Clones containing inactive methylated Ha-*ras* DNA retained normal morphology, but on treatment with 5-aza-CR, transformed derivatives were obtained with a demethylated active Ha-*ras* gene. As well as a reactivation of silent autosomal housekeeping genes, there are many published examples of the reactivation of genes on the inactive X

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chromosomes in hybrids or transformed cell lines (Beggs *et al.* 1986; Ellis *et al.* 1987; Hansen *et al.* 1988, and references therein), and the reactivation of latent retroviruses or herpes virus following demethylation (Whitby *et al.* 1987; Stephanopoulos *et al.* 1988; Rascati 1988; and see Holliday (1987)). A striking example of the effects of 5-aza-CR is the induction of three types of differentiated cell in a mouse fibroblast-like cell line, the muscle cells being produced with activation of the *Myo*D1 regulatory gene (see Jones *et al.*, this symposium).

Several X-ray sensitive (*xrs*) strains of CHO cells were isolated after standard EMS mutagenesis (Jeggo & Kemp 1983), but in each case they could be reactivated to an X-ray resistant phenotype by 5-aza-CR (Jeggo & Holliday 1986). To explain this unexpected finding, it was suggested that the pseudo-diploid CHO cell has one active and one inactive *xrs* gene. The radiation sensitive phenotype would be first produced by mutation in the normal gene, and 5-aza-CR would then be able to reactivate the silent non-mutant gene, as shown in figure 1. This provides a general explanation of the functional hemizygosity of CHO genes, which was previously proposed by Siminovitch (1976). Clearly the interpretation in figure 1 can be tested by molecular procedures, and preliminary experiments have already been published (Gounari *et al.* 1987).

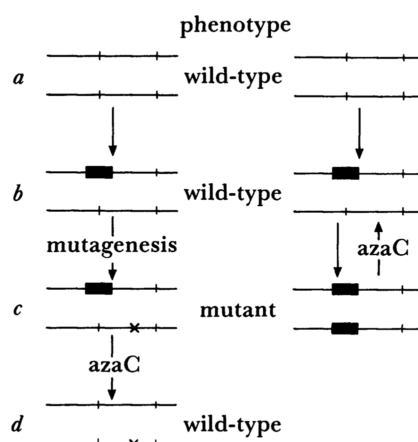

FIGURE 1. The inactivation and reactivation of genes in somatic cells. The horizontal lines represent two chromosome loci in a diploid cell. *De novo* methylation may inactivate one gene copy ($a \rightarrow b$), thus making the cell hemizygous for gene activity. The homologous gene may also be inactivated (right, $b \rightarrow c$), if there is no selection against this. Alternatively, experimental mutagenesis may destroy gene activity (left, $b \rightarrow c$). In both cases the mutant, or non-expressing phenotype, can be reversed to wild type by the demethylating agent 5 azacytidine (azaC) (left, $c \rightarrow d$; right, $c \rightarrow b$, or less commonly, $c \rightarrow a$).

In discussion of the behaviour of silent and mutant genes, a terminological problem arises, and for this reason the term epimutation was introduced to distinguish abrupt changes in gene activity from standard classical mutations (Jeggo & Holliday 1986). Whereas mutation is a heritable change in DNA base sequence (base substitution, addition, deletion, insertion or other rearrangement), epimutation is defined as a heritable abnormality in gene expression that is not a result of a change in coded DNA base sequence. However, it should be noted that heritable changes in gene activity are a normal component of development, or in stem cells of adults, and these cannot be described as epimutations. In normal somatic cells, the term epimutation should be used to describe abnormalities that are heritable. For example, DNA

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damage followed by excision repair just before the DNA replication fork or just after replication, can lead to demethylation of DNA and possibly the ectopic expression of a previously silent gene. Such a change in gene activity would be an epimutation, which might, for example, have a role in carcinogenesis (Holliday 1979; Holliday & Jeggo 1985).

The overall evidence suggests that established cell lines frequently inactivate genes by *de novo* methylation if there is no selective disadvantage in doing so. Several examples are known of active genes, introduced by DNA transfection or following 5-aza-CR treatment, that subsequently become inactive during routine laboratory passaging, probably as a result of *de novo* methylation (Gebara *et al.* 1987; Schorderet *et al.* 1988; see Holliday (1987); Jones *et al.* and Doerfler, this symposium).

3. Search for mutants defective in DNA methylation

A classical genetic approach to the understanding of a particular cellular process involves the isolation and characterization of mutants deficient in carrying out that process. Thus to uncover the roles of DNA methylation in cellular metabolism, chromosome structure or gene activity, it is advantageous to have mutants that are unable to maintain DNA methylation, or have some other effect on methylation. Methylation defective mutants might have one or more of the following phenotypes.

1. Spontaneous reactivation of silent genes.
2. Ectopic expression of genes not usually expressed in the particular cell line in which the mutant is isolated.
3. Abnormalities or alterations in chromatin structure.
4. Finite growth, if methylation continually declines during cell division (as occurs in diploid cells with finite *in vitro* lifespan, see Holliday (1987)).
5. Mutability, if DNA methylation plays a role in the detection of base mismatches following errors in replication.

Exploitation of phenotype (1) provides the obvious means of trying to isolate such mutants, that is, to obtain strains which spontaneously reactivate at high frequency one or more silent genes already known to be artificially reactivated by 5-aza-CR. Because the failure to maintain DNA methylation might be lethal, it is necessary to use a procedure which would detect temperature-sensitive conditional lethal mutations.

The first study employed a thymidine kinase deficient (TK^-) CHO cell strain known to be strongly reactivated to TK^+ by 5-aza-CR. Cells were treated with EMS and subsequently grown in medium containing bromodeoxyuridine to remove any TK^+ cells. Individual clones were isolated in wells and grown at 34 °C and also at 39 °C for two days, followed by incubation in HAT medium at 34 °C. Reactivation induced by the high temperature treatment would be followed by the appearance of TK^+ colonies in HAT medium. Among 7000 clones screened by this method, two were found which reproducibly reactivated TK^-, but only one of these had a temperature-sensitive response. This strain was further characterized and subsequently designated *tsm* (Gounari *et al.* 1987). The phenotype can be summarized as follows.

1. Spontaneous reactivation of the TK^- and Cd^S phenotypes to TK^+ and Cd^R respectively, was increased 10^3–10^4-fold after 24 h treatment at 39 °C. (The reactivation of a third gene, *pro*$^-$, could not be easily scored in this strain).

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2. A longer period of incubation at 39 °C had no further effect, and the strain was not temperature-sensitive lethal as it could be grown continuously at 39 °C.

3. The reactivation of the TK and metallothionein genes was associated with a loss of methylation at specific *Hpa* and *Hha* sites in regions of the gene that were studied.

4. The measurement of total 5-methylcytosine in DNA by HPLC procedures demonstrated that there was no significant decline during growth at normal temperature or after incubation at 39 °C.

5. There was no obvious change in mutability at the HPRT locus (resistance to 6-thioguanine).

It is therefore clear that the *tsm* strain does not have a general defect in the maintenance of DNA methylation, but probably some more specific transient effect on the regulation of methyl groups that influence or control transcription.

Instead of further characterization of the *tsm* strain, it was decided to employ a selective procedure for isolating further mutants. In cases where the phenotype sought leads to a strong increase in the frequency of a particular genetic event, it is possible by double selection for two such rare events to enrich for the desired phenotype. This procedure was employed some years ago in a search for hyper-recombination mutants of yeast (R. Holliday, unpublished data), using the diploid strain isolated by Esposito (1968) that is heteroallelic for five genes. More recently Giuletto *et al.* (1987) have isolated 'amplificator' strains by selecting for simultaneous amplification at two loci, and then demonstrating an increased rate of amplification at a third locus, which was not involved in the initial selection. The same procedure is being used to isolate strains of CHO which spontaneously reactivate silent genes at high frequency, and which should therefore be defective in the maintenance or control of DNA methylation. Harris (1986) obtained a strain which has three genes reactivable by 5-aza-CR (Tk^-, pro^- and asp^-). Measurement of the 5-aza-CR reactivation of 1, 2 or 3 loci revealed an unexpectedly high frequency at triple reactivation, suggesting that a subpopulation may exist with increased reactivity, and similar observations were made by A. Holmes & P. A. Jeggo (unpublished data). However, the aim of the current experiments is to uncover strains that reactivate genes at high frequency without 5-aza-CR treatment.

The triple enzyme-deficient strain used by Harris is also sensitive to cadmium and metallothionein can be reactivated by 5-aza-CR, which provides four genes that can be experimentally manipulated. In building up other stocks with multiple enzyme deficiencies that are susceptible to 5-aza-CR reactivation, the considerable information about inhibitors of purine and pyrimidine uptake and synthesis in mammalian cells is being exploited. There are many published experimental systems where resistance to a particular purine or pyrimidine analogue leads to an enzyme deficiency and where inhibitors of endogenous synthesis can be used to back-select for isolates that have regained enzyme activity. The enzymes concerned are the well-known hypoxanthine phosphoribosyl transferase (HPRT), adenine phosphoribosyl transferase (APRT) and TK forward and reverse selection systems, as well as those for adenosine kinase (Chan *et al.* 1978), deoxycytidine deaminase (Chan *et al.* 1975), deoxycytidine kinase (Dechamps *et al.* 1974) and uridine kinase (Medrano & Green 1974).

It is already known that strains resistant to bromodeoxyuridine (TK^-) arise in a single step at a frequency of 6×10^{-5} and are revertible by 5-aza-CR (16 isolates of independent origin were all revertible). On the other hand, strains resistant to 6-thioguanine ($HPRT^-$) which have so far been tested are not reverted by 5-aza-CR. Other drug-resistant enzyme deficient lines are

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being examined (results to be published). It should be possible by sequential selection to obtain several enzymes that are known to regain activity after 5-aza-CR treatment. Such strains, as well as Harris's stock with four reactivable genes, will be used to search for derivatives that spontaneously reactivate the silent genes with high frequency, and that may be unable to maintain normal DNA methylation. The genetic, cellular and molecular characterization of such isolates should yield much new information about the epigenetic rules that govern the inactivation and reactivation of genes in cultured cells. This in turn will facilitate studies on the control of gene expression either in normal diploid cells in culture, or in the developing organism.

4. Epigenetic mechanisms of development

A discussion of the basic mechanisms which are involved in the development of an organism must include the following.

1. The phenotype of a determined or a differentiated cell is in many cases heritable. This is well known for stem line cells which retain their own determined phenotype, while producing daughter cells that will later differentiate. Also, many fully differentiated cells are capable of prolonged division, *in vivo* or *in vitro*, without any discernible change in phenotype.

2. There is controlled segregation in gene activities during development or in the adult. This is clearly seen in the case of the stem line just mentioned, where two daughter cells from a single division have very different fates. They can be said to have different epigenotypes. It is also seen in the segregation of cell types in embryogenesis or organogenesis, or within single female cells where there is inactivation of one X chromosome. The contrast between the stability of the phenotypes of many specialized cells and the instability in gene segregation or switching mechanisms is very striking.

3. Specific cell lineages are important in many developmental situations. This is particularly well documented in the nematode *Caenorhabtitis elegans*, where the complete pedigree of cells from egg to adult has been elucidated (Sulston *et al*. 1983) and it is known that this pedigree is invariant from animal to animal. Other examples of strictly controlled cell pedigrees are also known (Davidson 1986).

4. The control of cell division and growth must be an essential feature of development. The size of an animal or part of an animal under normal environmental conditions is clearly under endogenous control. This suggests that there are developmental clocks or cell-counting mechanisms.

5. Much discussion of developmental mechanisms has been concerned with positional information, whereby cells become organized in three-dimensional space. It is generally believed that this depends on cell-signalling mechanisms, where there is a source of a diffusable inducer or morphogen and other cells which respond to a particular concentration of that effector. It is also extremely likely that there is signalling between cells that are in direct contact with each other. Such communication may depend on the interaction of proteins at the cell surface.

6. In contrast to cell lineages, it is known in many contexts that groups of cells behave in a coordinated or determined fashion during embryogenesis. A particularly clear example is the existence of 'polyclones' that give rise to compartments in *Drosophila* development (Crick & Lawrence 1975).

7. Epigenetic changes are often reversible. This is certainly true of X-chromosome

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inactivation and chromosome imprinting, both of which can be erased in the germ line (see Surani *et al.* and Monk, this symposium).

8. It is likely that the genome as a whole must be reprogrammed during or before meiosis, so that the gametes and fertilized egg can initiate a new cycle of development. The classical experiments of Gurdon *et al.* (Gurdon 1962; Gurdon & Uehlingen 1966; Gurdon & Laskey 1970) show that the nuclei of a specialized somatic cell of an amphibian could be reprogrammed by transferring them to anucleate eggs. Reversibility of the epigenetic changes in the context of normal somatic cells and tissues is more problematical, but it may well occur in the well-known regeneration of damaged tissues and amputated tail or limbs in amphibia or other species.

5. Molecular models

To what extent do molecular models, such as those based on DNA methylation, help to explain the above epigenetic features of development? For the most part, discussion must be speculative, but it nevertheless seems justified if real connections are to be made between the molecular control of gene expression (which is under intense study in many laboratories using many different experimental systems) and the study of embryology and development that, at least in the case of vertebrates, does not depend on knowledge of the activity of individual genes. When the original models for the control of gene activity by DNA modification during development were proposed (Holliday & Pugh 1975; Riggs 1975), there was no experimental evidence at all for any relation between methylation and gene expression. Justification of such models was based on the need for specific molecular mechanisms, particularly the switching of gene activities and the heritability of given patterns of gene activity. Subsequently, there has accumulated a very large body of evidence correlating presence or absence of DNA methylation with gene inactivity and activity, and also evidence for heritability of given patterns of DNA methylation (Holliday 1987). There has been little discussion of switching mechanisms, although experimental evidence for induction of gene activity following loss of methylation now exists (see, for example, Frank *et al.*; Jost *et al.*, this symposium). In its simplest form, a methylation switch must depend on at least two activities: first, a setting or switch enzyme or protein recognizes and modifies a specific sequence at one or more gene loci; second, the ability to maintain the modification in subsequent cell divisions. Such switches can, in principle, explain the behaviour of stem line cells or the heritable differences between X chromosomes in female mammalian cells (Holliday & Pugh 1975; Riggs 1975).

In the case of X-chromosome inactivation, the requirement is for a slow reaction that modifies one chromosome and this then triggers a fast inhibitory reaction preventing modification of the second X chromosome. These events would subsequently be followed by complete inactivation of one X chromosome by a processive or spreading mechanism starting at the inactivation centre (J. E. Pugh, unpublished). It was also proposed that molecular clocks may be based on the processive methylation of short repeated DNA substrate sequences. Again, two enzymes are required, a setting enzyme and a clock enzyme, which methylates one additional sequence at each division. When all are methylated, a gene may be inactivated or activated. Clocks may control quite small numbers of cell divisions, and there could be more than one clock operating at different chromosomal locations in a single cell. If such mechanisms are tied to segregation of cell types, they can provide the basis for the behaviour of specific cell lineages. Such mechanisms can be best understood in haploid, rather than in diploid genomes,

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and this raises the possibility that genes important in developmental processes may be subject to allelic exclusion. Imprinting may serve this purpose.

Another attractive feature of the methylation model for development is that it can account for reversibility of an epigenetic programme in the germ line, perhaps at meiosis. Indeed, it is already known from studies of X-chromosome reactivation and chromosome imprinting that reversibility of a given pattern of DNA methylation must occur (see Surani *et al.* & Monk, this symposium). If this specific demethylation or remethylation can occur in these contexts, and also in specialized somatic cells, it seems reasonable to propose that developmental reprogramming before the formation of gametes might be based on the laying down of a particular pattern of methylation in specific DNA sequences, in other words, an epigenetic code or programme would be superimposed on the normal DNA code.

Finally, how can cell signalling and the controlled behaviour of groups of cells in development be accommodated within the model? No one would dispute that signal transduction from membrane to nucleus is an essential ingredient, but a crucial question is the relation of this to the inherited properties of the receiving cells. The characteristic situation in development is where a specific group of cells (or polyclone) receives a signal that alters their properties as a group. Such cells often continue to divide and may later influence, or impart a signal, to other cells. The switching of certain genes in DNA between alternative states may provide the basis for such cellular differences, if these genes also code for cell surface receptors. The interaction of such receptors with an extracellular inducer or morphogen may then mediate or modulate the switching between alternative methylation states of DNA (see figure 2). The two states of DNA produce for the most part the same proteins, but with a few very

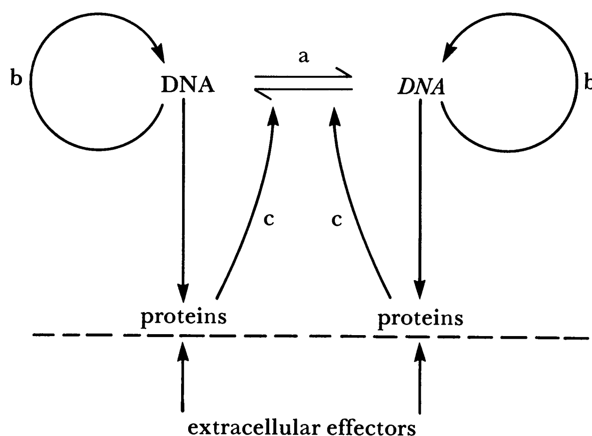

Figure 2. Extracellular effectors, such as morphogens or inducers, may induce heritable changes in groups of similar or identical receptor cells. Alternative methylated states of given genes in DNA are shown on the left and on the right (italic letters). These produce different individual receptor proteins, or families of proteins, which can respond to different signals. Signal transduction (c) influences or modulates the switching between different states of DNA (a), each of which is heritable (b). A group of cells responding to a signal can become committed to a new developmental role, including the possibility of producing its own new effector or signal.

significant differences in membrane proteins. These are the molecules that can respond to external effectors and also transmit single signals back to the nucleus. Such signals could, by altering methylation, effect changes in gene activity which are heritable.

The methylation model for development is therefore not only concerned with switches of

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gene activity in stem lines or cell lineages, but can also accommodate the all important changes in groups of cells during development. This general concept is entirely different from classical genetics, as this is necessarily concerned with the germ line which produces gametes and the zygote. The external influence of somatic cells or the environment on this type of inheritance, although still sometimes debated, has never been shown to be significant. On the other hand, the development of an organism is itself mediated by somatic cells, and may be subject to completely different rules, whereby the external environment of cells specifically changes a pre-existing epigenotype to a new one, with a different pattern of gene activities and a different fate in later development.

References

Beggs, A. H., Axelman, J. & Migeon, B. R. 1986 Reactivation of X-linked genes in human fibroblasts transformed by origin-defective SV40. *Som. Cell molec. Genet.* **12**, 585–594.

Borrello, M. G., Pierotti, M. A., Bongarzone, I., Donghi, R., Mondellini, P. & Della Porta, G. 1987 DNA methylation affecting the transforming activity of the human Ha-*ras* oncogene. *Cancer Res.* **47**, 75–79.

Chan, T., Creagan, R. P. & Rearden, M. P. 1978 Adenosine kinase as a new selective marker in somatic cell genetics: isolation of adenosine kinase-deficient mouse cell lines and human–mouse hybrid cell lines containing adenosine kinase. *Som. Cell Genet.* **4**, 1–12.

Chan, T., Long, C. & Green, H. 1975 A human–mouse somatic hybrid line selected for human deoxycytidine deaminase. *Som. Cell Genet.* **1**, 81–90.

Crick, F. H. C. & Lawrence, P. A. 1975 Compartments and polyclones in insect development. *Science, Wash.* **189**, 340–347.

Davidson, E. H. 1986 *Gene activity in early development*, 3rd edn. New York: Academic Press.

Dechamps, M., Robert de Saint Vincent, B., Evrard, C., Sassi, M. & Buttin, G. 1974 Studies on 1-β-D-arabinofuranosylcytosine (araC) resistant mutants of Chinese hamster fibroblasts. *Expl Cell Res.* **86**, 269–279.

Ellis, N., Keitges, E., Gartler, S. M. & Rocchi, M. 1987 High-frequency reactivation of X-linked genes in Chinese hamster X human hybrid cells. *Som. Cell molec. Genet.* **13**, 191–204.

Esposito, R. E. 1968 Genetic recombination in synchronized cultures of *Saccharomyces cereviseae*. *Genetics* **59**, 191–210.

Gebara, M. M., Drevon, C., Harcourt, S. A., Steingrimsdottir, H., James, M. R., Burke, J. F., Arlett, C. F. & Lehmann, A. R. 1987 Inactivation of a transfected gene in human fibroblasts can occur by deletion, amplification, phenotypic switching or methylation. *Molec. Cell. Biol.* **7**, 1459–1464.

Giuletto, E., Knights, C. & Stark, G. R. 1987 Hamster cells with increased rates of DNA amplification, a new phenotype. *Cell* **48**, 837–845.

Gurdon, J. B. 1962 The developmental capacity of nuclei taken from intestinal epithelium cells of feeding tadpoles. *J. Embryol. exp. Morph.* **10**, 622–640.

Gurdon, J. B. & Laskey, R. A. 1970 The transplantation of nuclei from single cultured cells into anucleate frogs eggs. *J. Embryol. exp. Morph.* **24**, 227–248.

Gurdon, J. B. & Uehlingen, V. 1966 'Fertile' intestine nuclei. *Nature, Lond.* **210**, 1240–1241.

Gounari, F., Banks, G. R., Khazaie, K., Jeggo, P. A. & Holliday, R. 1987 Gene reactivation: a tool for the isolation of mammalian DNA methylation mutants. *Genes Devel*, **1**, 899–912.

Hansen, R. S., Ellis, N. A. & Gartler, S. M. 1988 Demethylation of specific sites in the 5′ region of the inactive X-linked human phosphoglycerate kinase gene correlates with the appearance of nuclease sensitivity and gene expression. *Molec. Cell. Biol.* **8**, 4692–4699.

Harris, M. 1964 *Cell culture and somatic variation.* New York: Holt, Rinehart & Winston.

Harris, M. 1973 Anomalous patterns of mutation in cultured mammalian cells. *Genetics* **73** (suppl.), 181–185.

Harris, M. 1982 Induction of thymidine kinase in enzyme deficient Chinese hamster cells. *Cell.* **29**, 483–492.

Harris, M. 1984*a* Variants inducible for glutamine synthesis in V79-56 cells. *Som. Cell molec. Genet.* **10**, 275–281.

Harris, M. 1984*b* High frequency induction by 5-azacytidine of proline independence in CHO-K1 cells. *Som. Cell molec. Genet.* **10**, 615–624.

Harris, M. 1986 Simultaneous induction of epigenetic variants. *Som. Cell molec. Genet.* **12**, 567–573.

Holliday, R. 1979 A new theory of carcinogenesis. *Br. J. Cancer* **40**, 513–522.

Holliday, R. 1987 The inheritance of epigenetic defects. *Science, Wash.* **238**, 163–170.

Holliday, R. & Jeggo, P. A. 1985 Mechanisms for changing gene expression and their possible relationship to carcinogenesis. *Cancer Surveys* **4**, 557–581.

Holliday, R. & Pugh, J. E. 1975 DNA modification mechanisms and gene activity during development. *Science, Wash.* **187**, 226–232.

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Jeggo, P. A. & Holliday, R. 1986 Azacytidine-induced reactivation of a DNA repair gene in Chinese hamster ovary cells. *Molec. Cell. Biol.* **6**, 2944–2949.

Jeggo, P. A. & Kemp, L. M. 1983 X-ray sensitive mutants of Chinese hamster ovary cell line: isolation and cross sensitivity to other DNA-damaging agents. *Mutat. Res.* **112**, 313–327.

Medrano, L. & Green, H. 1974 A uridine kinase-deficient mutant of 3T3 and a selective method for cells containing the enzyme. *Cell* **1**, 23–26.

Puck, T. T. 1972 *The mammalian cell as a microorganism.* San Francisco: Holden-Day.

Rascati, R. J. 1988 Effects of cytidine analogues on methylation of DNA and retrovirus induction. *Mutat. Res.* **208**, 21–25.

Riggs, A. D. 1975 X inactivation, differentiation and DNA methylation. *Cytogenet. Cell Genet.* **14**, 9–25.

Schorderet, D. F., Keitges, E. A., Dubois, P. M. & Gartler, S. M. 1988 Inactivation and reactivation of sex-linked steroid sulphatase gene in murine cell culture. *Som. Cell molec. Genet.* **14**, 113–121.

Siminovitch, L. 1976 On the nature of heritable variation in cultured somatic cells. *Cell* **7**, 1–11.

Stallings, R. L., Crawford, B. D., Tobey, R. A., Tesmer, J. & Hildebrand, C. E. 1986 5-azacytidine-induced conversion to cadmium resistance correlates with early S phase replication of inactive metallothionein genes in synchronized CHO cells. *Som. Cell molec. Genet.* **12**, 423–432.

Stephanopoulos, D. E., Kappes, J. C. & Bernstein, D. I. 1988 Enhanced *in vitro* reactivation of Herpes Simplex virus type 2 from latently infected guinea pig neural tissues by 5-azacytidine. *J. gen. Virol.* **69**, 1079–1083.

Sulston, J. E., Schierenberg, E., White, J. G. & Thomson, J. N. 1983 The embryonic cell lineage of the nematode *Caenorhabditis elegans*. *Devl Biol.* **100**, 64–119.

Waddington, C. H. 1953 Epigenetics and evolution. *Symp. Soc. exp. Biol.* **7**, 186–199.

Waddington, C. H. 1956 *Principles of embryology*, London: Allen and Unwin.

Whitby, A. J., Blyth, W. A. & Hill, T. J. 1987 The effect of DNA hypomethylating agents on the reactivation of Herpes Simplex virus from latently infected mouse ganglia *in vitro*. *Archs Virol.* **97**, 137–144.

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