

A molecular view of tissue differentiation and development

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Differentiation of a tissue is characterised by expression of a specific repertoire of genes and thus the appearance of their protein products. The entire genome can be divided into two classes of genes: (a) housekeeping genes which are expressed in all tissues and encode the message for vital functions such as cell proliferation (as the products of these genes are involved in DNA, RNA and protein synthesis); (b) tissue-specific genes which are expressed in just a single or several tissues and consequently are part of the differentiation process. The present review focuses on the second type of genes whose pattern of expression raises two issues. First, what initiates transcription of specific genes in one tissue but not in others? For example, the transcription of the β -globin gene is confined to haematopoietic cells and that of the insulin gene to the pancreatic islets (Fig. 1). Second, how is a gene selectively transcribed in some tissues, even though they may have had different embryonal origins? α -Fetoprotein (AFP), for example, is expressed in three endodermal tissues (yolk sac, liver and intestine), while the gene for phosphoenolpyruvate carboxykinase (PEPCK) is expressed in tissues that derive both from endoderm (liver) and from mesoderm (kidney and adipose tissue) (Fig. 1) [1].

Development is characterised by specialisation of distinct functions in an already differentiated cell. Thus the expression of specific genes is programmed to start or cease at a certain stage of development. The transcription of the β -globin gene begins before birth and embryonic haemoglobin disappears from the blood as mature haemoglobin appears [2]. Similarly, at birth, glucose production in the liver begins with the appearance of PEPCK, the key enzyme in gluconeogenesis [3]. On the other hand, α -fetoprotein, which is actively transcribed in the fetal liver, disappears after birth [4]. It is therefore of interest to consider how a specific gene starts or ceases to be expressed during the development of a tissue already committed to a certain pathway of differentiation.

This review describes possible molecular events, occurring in the genome, which may be involved in the regulation of differentiation and development. For

the sake of simplicity, the review will be confined to aspects of differentiation involving the four genes that encode β -globin, insulin, α -fetoprotein and PEPCK.

Most genes that encode a message for the synthesis of tissue-specific proteins preserve the same sequence in all tissues. Yet they are transcribed only in some tissues but not in others. Clearly, some mechanisms are involved in the recognition of genes that need to be transcribed.

(a) Although DNA is essentially identical in all tissues, some of the nucleotides can be modified in specific tissues. The known modification for eukaryotic genes is methylation of the cytidine residue in the sequence CG (cytidine linked to guanine, in the same strand of DNA) [5].

(b) DNA is not naked in the nuclei but is covered with complex proteins—histones and non-histones. Non-histone proteins (trans-acting factors) bind to specific sequences near the structural gene (cis-regulatory elements) and may regulate transcription [6].

(c) Interaction of various nuclear proteins with DNA results in a specific conformation of the DNA in the chromatin packaged into the nucleus [7,8]. Thus the chromatin conformation of the genome may be involved in the recognition of genes that should be transcribed.

A schematic representation of the various DNA modifications is shown in Fig. 2.

DNA methylation and gene expression

In the DNA of eukaryotic cells, methylation is confined to the cytidine residues in the sequence CG, yet in each tissue only some CG residues are methylated. Moreover, the same sequence may be methylated in some tissues but remain unmethylated in others. However, in a given cell type the methylation pattern is inherited by the daughter cells [5,9,10]. The methylation status of a genomic sequence can be analysed with the help of restriction enzymes which digest a DNA sequence containing CG only if it is unmethylated [10]. Table 1 shows the methylation status of the genes encoding β -globin, insulin, α -fetoprotein and PEPCK [11–16]. Such analyses have demonstrated a reciprocal correlation between expression and methylation. Genes that are expressed in a particular tissue are undermethylated while the same genes in tissues where they are not expressed are methylated. More-

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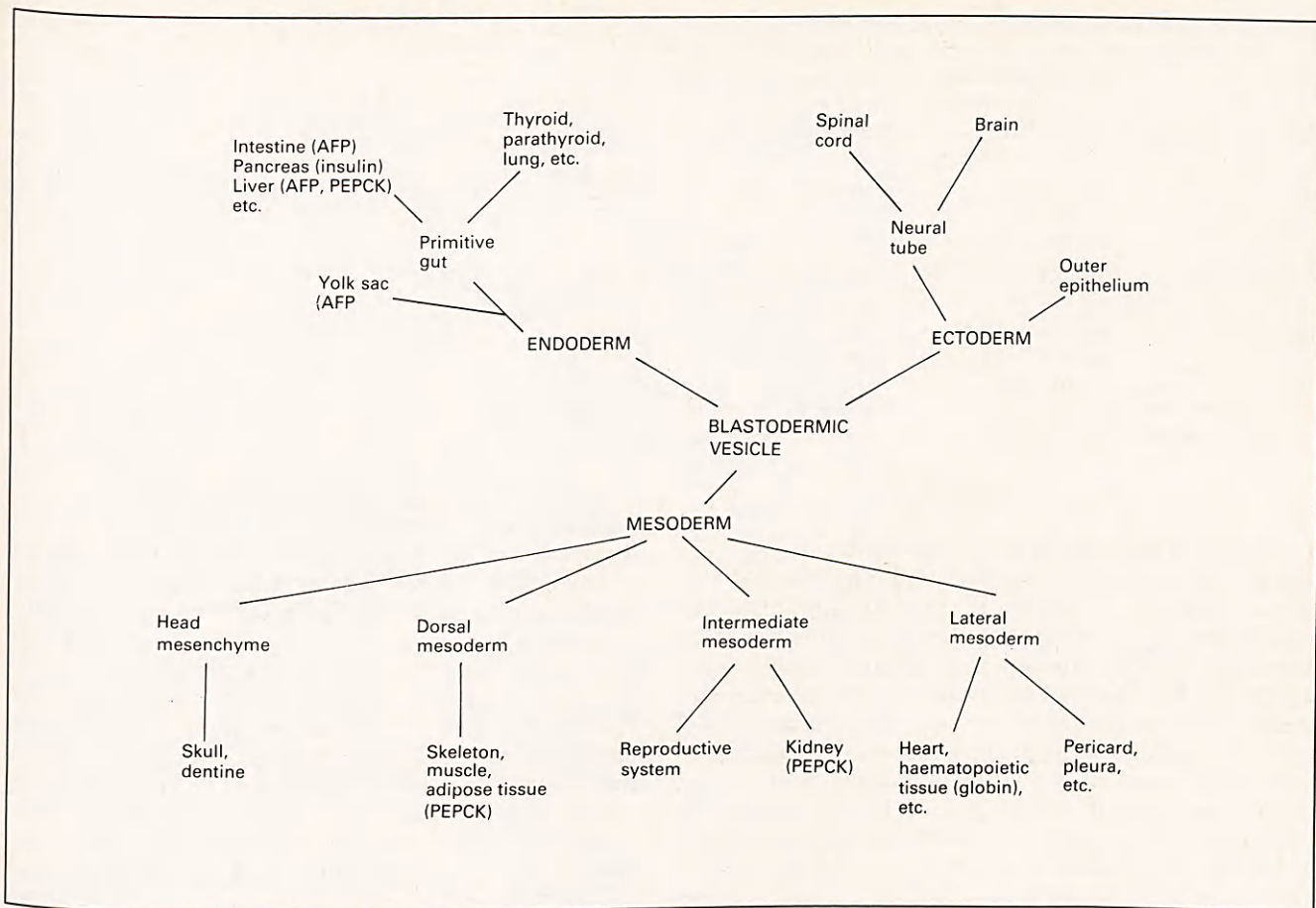


Fig. 1. Embryonic origin of tissues.

Schematic representation of the developmental origin of tissues during mammalian embryogenesis. A blastodermic vesicle is derived from the fertilised ovum and differentiates into three embryonic layers—ectoderm, endoderm and mesoderm—which give rise to the various body tissues. A schematic representation of tissue differentiation along each branch is given. The four genes discussed in the text (insulin, β -globin, α -fetoprotein and phosphoenolpyruvate carboxykinase) are indicated in parentheses next to the tissues that express them. (Based on Patten, B.M. and Carlson, B.M. (1974) *Foundations of embryology*, p.141. McGraw Hill. Reproduced with permission.)

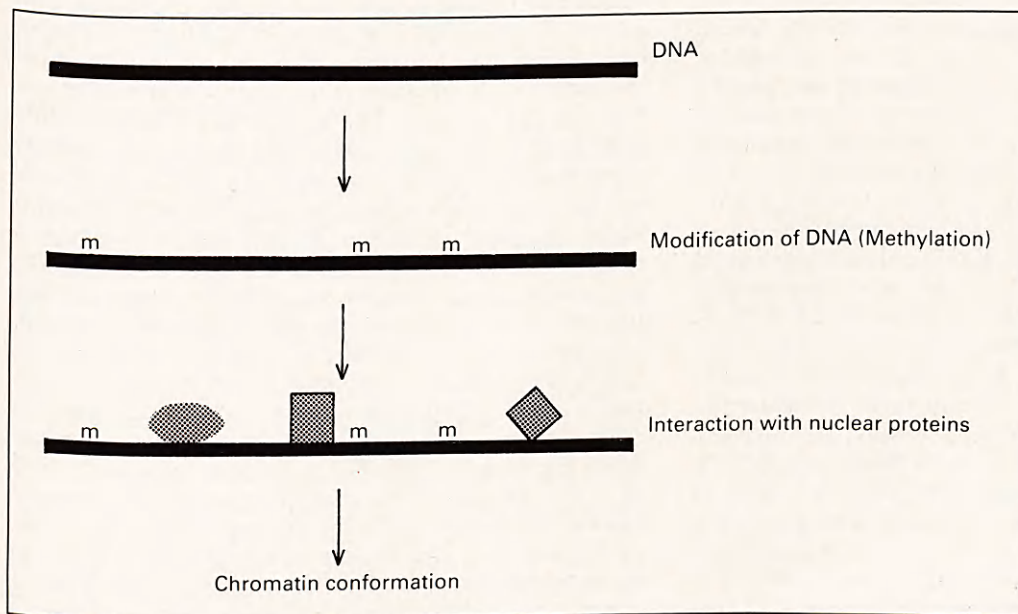


Fig. 2. Schematic representation of gene modifications.

DNA is represented by the bars. Methylation of the cytidine residues in the DNA is indicated by letters m. Geometrical figures represent nuclear proteins interacting with the DNA.

Table 1. Methylation state of four genes and their expression in various tissues

Gene	Methylated DNA	Hypomethylated DNA	Species	Ref.
β -Globin	Sperm (NE) Adult liver (NE)	Bone marrow (E)	Human	[11]
Insulin	Brain (NE) Liver (NE) Kidney (NE)	Pancreatic islets (E)	Rat	[12]
α -Fetoprotein	Kidney (NE)	Fetal liver (E) Adult liver (NE)?	Rat	[13,14]
PEPCK	Fetal liver (NE) Spleen (NE) Heart (NE)	Adult liver (E) Kidney (E)	Rat	[15,16]

E, expressed; NE, not expressed.

over, when a gene starts to be expressed during development, it undergoes demethylation. Thus the PEPCK gene is 'turned on' in the liver at the time of birth, and it undergoes substantial alteration in its methylation pattern [17]. However, in other genes, alterations in the level of their transcription may occur independently of their methylation status. For instance, the gene encoding α -fetoprotein preserves its undermethylated state in the adult liver even though it is then no longer transcribed [13,14]. Confirmation of a cause-and-effect relationship between methylation and expression is provided by experiments with the methylase inhibitor 5-azacytidine [18]. For example, active demethylation of the PEPCK gene in fetal liver can induce premature expression [16]. Even more dramatic were the successful attempts to induce expression by demethylation of the γ -globin gene in adult bone marrow which resulted in the reappearance of fetal haemoglobin. Such procedures have been exploited in the treatment of anaemic baboons [19] and of a thalassaemic patient [20].

Enhancers and gene expression

Transcription of genes is also regulated by specific DNA sequences termed enhancers or cis-regulatory elements. They occur near the structural part of the gene and are able to bind various nuclear factors to the DNA which can induce gene transcription. Enhancers can be divided into two categories—those that are active in all cells and those that are activated only at a specific stage of development or in a specific type of cell [6]. From the examples illustrated in Fig. 3 it is evident that tissue-specific enhancers can be located upstream from the transcription-start site as in the insulin gene [21], or downstream from the transcription-start site as in the β -globin gene [22,23]. In genes that are only expressed in a single tissue, the regulatory sequences are recognised in that tissue only. In genes that are expressed in more than one tissue, the picture is more complicated. Some of the regulatory sequences can confer transcription in various cells while others can only be recognised in one type of cell.

It seems that, in tissues with a common origin during embryogenesis, the same regulatory sequence can be recognised by the various cells (eg elements in the α -fetoprotein gene [24]; Fig. 3), while in tissues that have totally different origins in embryogenesis some elements are unique to each tissue (eg elements in the PEPCK gene [25]; Fig. 3). Hence it seems that during differentiation various nuclear factors actively interact with the DNA to allow expression of a selective set of genes. In that sense, development is part of the differentiation process. For α -fetoprotein [24] the tissue-specific enhancers only function at a certain time during development and thus allow the temporal expression of the gene. Thus some trans-acting factors are tissue-specific or development-specific proteins involved in the commitment step of a specific cell.

Chromatin conformation and gene expression

Chromatin consists of DNA covered with histone and non-histone proteins [7,8]. The basic repeating structure in chromatin, the nucleosome, consists of a stretch of DNA, 200 base pairs long, which is associated with five major histones. However, the nucleosomal structure in the genome is not uniform and local disruptions are evident. The conformation of a specific gene in the chromatin can be assessed by its sensitivity to digestion with low concentrations of DNase I. Sensitive DNA is regarded to be in an 'open' conformation while relative resistance to DNase I digestion implies a more 'compact' conformation. There is increasing evidence that genes sensitive to DNase I ('open' conformation) occur in tissue(s) in which they are expressed, but are relatively resistant to digestion in tissues in which they are not expressed [7]. Changes in chromatin conformation during development occur in two seemingly opposite directions. Thus genes encoding adult β -globin or PEPCK, which start to be expressed at a certain stage during development, acquire DNase I sensitivity [26–28] whereas genes encoding embryonic β -globin or α -fetoprotein, which cease to be expressed after birth, become resistant to DNase I digestion [27,29]. Moreover, from studies of genes

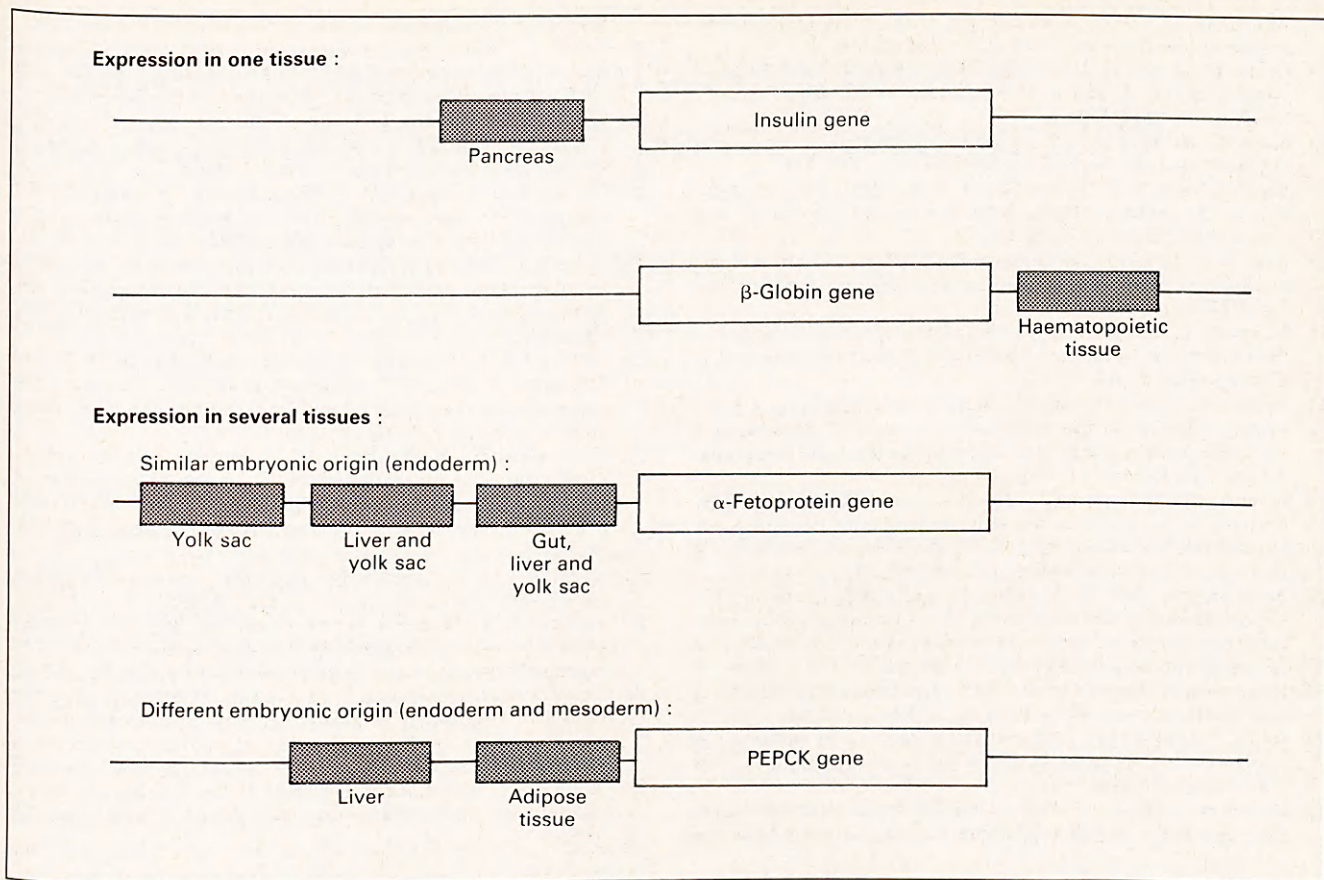


Fig. 3. Schematic representation of tissue-specific domains in the genes. Open boxes represent the genes which are transcribed from left to right. The genes are rat insulin [21], chicken β -globin [22,23], mouse α -fetoprotein [24] and rat PEPCK [25]. The shaded boxes represent the tissue-specific domain(s). Each box can represent one or various enhancers. The tissue(s) that recognise the specific domain are indicated under each box.

encoding β -globin [26] and PEPCK [28], it is evident that changes in their chromatin conformation precede the initiation of their transcription [17]. This temporal relationship suggests that a change in the chromatin conformation is needed for expression.

Concluding remarks

The evidence presented here strongly suggests that regulation of gene transcription during differentiation and development involves several DNA modifications and interactions of proteins with the genome. First, methylation of genes is inversely correlated with tissue-specific expression, ie genes are unmethylated in the tissues that express them. Second, various enhancers (cis-regulatory elements) confer cell-specific expression by interacting with specific nuclear proteins (trans-acting factors). Third, acquisition of an open chromatin conformation is necessary to assign a gene for transcription. All these modifications in the DNA and in its chromatin conformation demand involvement of tissue-specific factors. These factors, yet to be characterised, regulate the commitment and maturation steps of each tissue by controlling the modifications in the genome.

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