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POINTS OF VIEW

DIFFERENTIATION : « KEEP THE GENOME CONSTANT BUT CHANGE OVER AND OVER AGAIN ITS IONIC AND/OR MACROMOLECULAR ENVIRONMENT » ? A CONCEPTUAL SYNTHESIS

by

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SUMMARY

The unifying concept that differentiation in animals is the stepwise formation of cells or cell clusters which differ primarily in their plasma membrane-cytoskeletal properties and which mostly become organised into a variety of epithelia, lining different compartments, is formulated. As a consequence of these differences in plasma membrane-cytoskeletal complex properties — this causal relationship being the very point — the distinct emerging cell types will be able to display a differential pattern of protein synthesis, cellular morphology and physiology, notwithstanding the fact that they have identical genomes and similar basic mechanisms of protein synthesis and processing. Differences in the plasma membrane-cytoskeletal complex, of which the structure furnishes the cells with a differential three-dimensional molecular scaffold, are usually achieved first and are of necessity followed by differential protein synthesis and pattern formation. This can be also succinctly stated as : form precedes function. In addition to this widely used principle, secondary mechanisms for controlling the expression of specific genes in specific cell types may operate. The major « strategy » used in differentiation of somatic cells seems to be to keep the genome constant (GURDON's experiments, 1962) but to change its « environment » over and over again. This environment comprises two sets of constituents, ionic and macromolecular ones, acting in complementary ways. The first one may be more appropriate for the coarse tuning of gene expression/protein synthesis and the second (especially the trans-acting factors) for the fine tuning. Consideration of animal development in terms of differential epithelium formation may make a major contribution to the unification of developmental biology of animals.

Key words : differentiation, development, gene expression, epigenetics, cytoskeleton, nuclear matrix, plasma membrane, epithelium, pattern formation.

INTRODUCTION

The question as to whether there is a universal principle underlying differentiation is fundamental to the developmental biology of animals. Although intensively sought, no such principle has yet been discovered and there is considerable doubt whether it exists at all. In view of the large number of possible mechanisms already shown to be instrumental to differentiation (GILBERT, 1991 ; GURDON, 1992), such doubt is not unwarranted.

A great number of classical and recent papers on development and control of gene expression-protein synthesis has been screened for mechanisms (*e.g.* differential protein synthesis) which generate functional asymmetry. All those that were not operational in all experimental animal species so far used were eliminated, in the hope of finding one or more universally valid asymmetry generating principles.

DATA FROM THE LITERATURE

We can make the general statement that the different cell types of a differentiated organism all have the same genome although there are a few exceptions such as antibody producing cells, and X-chromosome inactivation in mammals which may be disregarded here. There is no evidence from the literature survey of any mechanism operating directly at the level of the genome, transcription, translation or protein processing, which generates functional cellular asymmetry in all animal species. This is not incompatible with the fact that some of these mechanisms are undoubtly instrumental in differentiation in some species or cell types (GILBERT, 1991). Several sets of data, all relevant to the generation of asymmetry, and all linked in some way to the properties of the plasma membrane-cytoskeleton complex, did emerge :

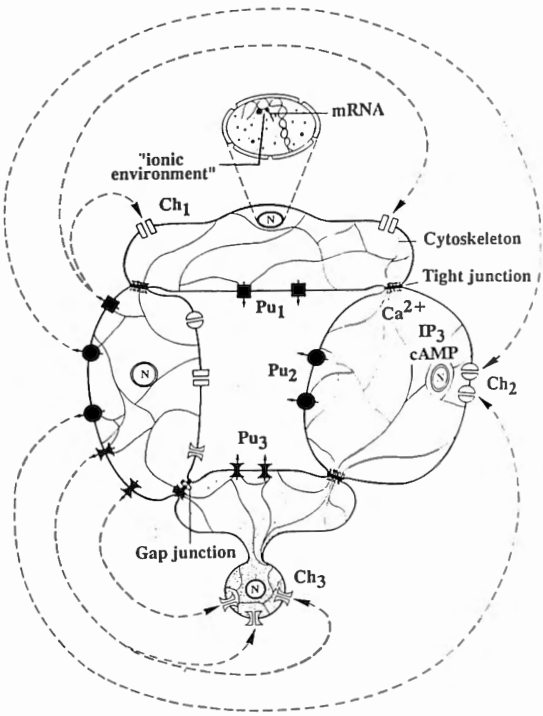
1. In contrast to the situation in unactivated fucoid eggs (QUATRANO, 1990), no description was found of an animal cell that is spherically symmetrical with respect to its membrane-cytoskeletal complex. All animal cell types have a built in asymmetry : *they all differ in their plasma membrane-cytoskeletal complex and they all have the means of segregating membrane proteins* such as receptors and ion transporting proteins, in different domains (*e.g.* by specialisations of the cytoskeleton), thereby facilitating transcellular transport and, where it occurs, self electrophoresis (Fig. 1). Non-random (asymmetrical) distribution of at least some of the membrane-bound proteins present is the rule, random distribution of all proteins present in the membranes (spherical symmetry) — if it exists at all — the exception. This is also true for zygotes.
2. The cytoskeleton, apparently never spherically symmetrical in animal cells, can serve as an anchoring site for the nucleus, for some types of RNA (SINGER, 1992), for some membrane proteins *etc.* (Fig. 1). There are types of zygote in which some maternal mRNAs seem to be associated with specific domains of the cytoskeleton. The nucleus has its own skeleton (nuclear matrix, nuclear scaffold), the form of which is flexible and probably cell- and tissue specific. It acts as an

anchoring site for DNA : genes which are actively being transcribed are associated with it (GETZENBERG *et al.*, 1991). It allows compartmentalisation of several nuclear functions (replication, transcription, RNA processing, RNA transport) for which it seems to be essential (VAN DRIEL *et al.*, 1991 ; BEREZNEY, 1991).

3. *Cleavage patterns sooner or later all yield blastomeres which differ (individually or groupwise) in at least their plasma membrane-cytoskeletal complex* (Fig. 2). This implies a causal relationship with differences in the distribution of some membrane proteins and perhaps also of some maternal mRNA's. It leads to the generalisation that all the different cell types of which differentiated organisms exist must differ in their plasma membrane-cytoskeletal complex. It follows that the « ionic environment » is likely to differ from cell type to cell type.
4. *This complex can be instrumental,* be it mostly indirectly, in a variety of ways *in controlling differential gene expression-protein synthesis* (DE LOOF *et al.*, 1992).
5. The definition of an animal as an organism that develops from a blastula refers to an important but often overlooked property of every animal : in its development it must unavoidably pass through the stage of being a closed simple (monolayer) *epithelium, the blastoderm* (WILEY *et al.*, 1990 ; DE LOOF, 1992). Furthermore, the vast majority of animal cells becomes organised in a variety of epithelia with different characteristics, sometimes highly folded, lining a number of different compartments. *Compartmentalisation by epithelia is an inherent and essential property of animal development and physiology,* just as the intracellular compartmentalisation by membrane-limited organelles is essential to eukaryotic cell physiology (DE LOOF, 1992). Cells cannot engage in epithelium formation without first generating polarity in their plasma membrane/cytoskeletal complexes. This can also be succinctly stated as : form precedes function. Epithelial organisation and *de novo* protein synthesis may be causally linked. Before blastoderm formation embryos largely depend on maternal RNAs ; substantial de novo protein synthesis starts only after epithelial organisation has become established. Such a causal link, however, has not yet been experimentally investigated, mainly because in the literature the blastula has only rarely been thought of as an epithelium.
6. The fact that proteins, for all the profound importance they undoubtedly have on the structure and biochemistry of organisms, cannot by themselves specify design and pattern (PENMAN, 1991), is frequently overlooked although this is so selfevident and has been stressed many times. This function needs the help of a three-dimensional scaffold, the cytoskeleton/nuclear matrix, itself proteinaceous in nature.

A UNIFYING PRINCIPLE

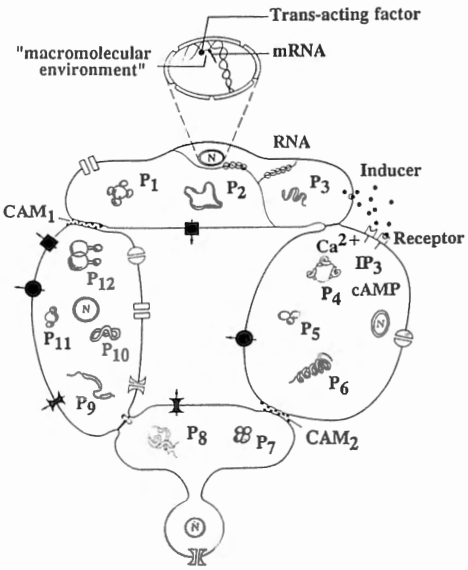
The above considerations suggest that a universal principle underlying differentiation does exist and that it is probably a simple one : it seems to be a logical consequence of some of the properties of the cytoskeleton and of biological membranes



1

Definition: Differentiation is the generation of cells which differ in their plasmamembrane-cytoskeletal complex, this being indirectly instrumental to differential protein synthesis.

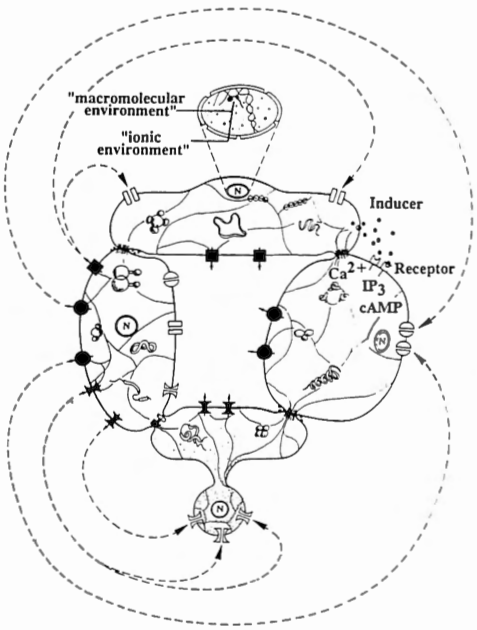
Principle: keep the genome constant but change its (ionic) environment.



2

Definition: Differentiation is the generation of cells which differ in the sets of proteins they synthesise with differential cellular physiology as a result.

Principle: keep the genome constant but change its (macromolecular) environment.



3

Principle : keep the genome constant but change again and again its macromolecular and/or ionic environment.

Figs 1-3: — Cross sections through a hypothetical 4-celled, epithelially organised animal, in which the major mechanisms instrumental to differential protein synthesis are depicted.

1. Features, definition of differentiation and underlying principle particularly relevant to membrane physiology.

The different cell types have the ability to segregate proteins such as ion pumps and channels, in their plasma membranes. As a result they can drive an (electroneutral or electrogenic) ion flux through themselves and through the organism as a whole (the dotted lines in A and C). In cell D an intracellular gradient of macromolecules is generated as the result of self electrophoresis. The cytoskeleton is instrumental in anchoring and segregating ion transporting molecules in the membrane, in forming tight junctions and in keeping the nucleus (N) in a well-defined position. Chromatin structure partially depends on the ionic « environment » in the nucleus. It is not a priori excluded that in certain circumstances the nuclear pore complexes could be closed so that the ionic composition of the nucleoplasm might be different from that of the cytoplasm. Gap junctions, when open, allow free passage of ions and small molecules. Secondary messengers are needed for transduction of some signals. The structures with an arrow through them represent ion pumps (Pu_1-Pu_3), those with a hole, ion channels (Ch_1-Ch_3). For reasons of simplicity, only one type of receptor, ion pump and channel and one direction of ion pumping is represented in cell A, B and C; in the normal situation an array of such membrane proteins is present. Molecules can evidently not be drawn to scale.

2. Features, definition of differentiation and the underlying principle particularly relevant for molecular biology.

The different cell types display differential patterns of protein synthesis, mainly as the result of differences in trans-acting factors present in the nuclei (N). As the result of different protein sets (P_1-P_{12}), the cells can have different physiological functions. There are different types of cell adhesion molecules (CAM_1-CAM_2). Intercellular communication can be realised by inducers, hormones etc. Secondary messengers are needed for transduction of some signals. The cytoskeleton can be instrumental for anchoring mRNA.

3. Superposition of 1 and 2 showing the universal principle underlying differentation.

on the one hand, and of the necessity of epithelial compartmentalisation on the other. In my opinion it can be formulated as follows :

> Differentiation in animals is the stepwise formation of cells or cell clusters which differ primarily in their plasma membrane-cytoskeletal properties and which mostly become organised into a variety of epithelia, lining different compartments. These differences in plasma membrane-cytoskeletal complex properties cause the distinct emerging cell types to display different patterns of protein synthesis, cellular morphology and physiology, notwithstanding the fact that they have an identical genome and similar mechanisms of protein synthesis and processing.

To summarize : differences in the plasma membrane-cytoskeletal complex, the structure of which confers a differential three-dimensional molecular scaffold on the cells, are first brought about ; differential protein synthesis and pattern formation follow (of necessity) : form precedes function. The major « strategy » used in differentiation of somatic cells seems to be to keep the genome constant (GURDON's experiments, 1962) but to change its « environment » over and over again. This environment has ionic and macromolecular constituents, which act in complementary fashions. The first may be more appropriate for the coarse tuning of gene expression/protein synthesis (WILDON *et al.*, 1992 ; VANDEN BROECK and DE LOOF, 1993), the second (especially the trans-acting factors) for the fine tuning.

THE IMPORTANCE OF EPITHELIUM FORMATION IN ANIMAL DEVELOPMENT

In the Five Kingdoms classification system, animals are defined as organisms developing from a blastula, while plants develop from an embryo. This definition may itself explain why the polarity in the plasma membrane/cytoskeletal complex is so important and why this has to be brought about so early. An early embryo becomes a blastula at the moment when its cells become organised into a simple *epithelium* enclosing a fluid compartment (DE LOOF, 1992). Each epithelium consists of cells which are *necessarily polarised* with respect to their plasma membrane/cytoskeletal complex. Thus, the introduction of polarity in the complex must be achieved early, otherwise neither the organisation into an epithelium nor proper functioning would be possible. An epithelium, however, is not necessarily homogeneous and such heterogeneity may also be true for a blastula. Next, the blastular epithelium starts folding, forming the archenteron. As development proceeds, more and more compartments are formed, all lined by epithelia consisting of cells with a specific plasma membrane/cytoskeletal complex, which enclose a fluid compartment with a specific ionic composition. In fact, all animals are, to a large extent, organised as a variety of more or less folded epithelia each enclosing a number of fluid compartments. Since by definition animals are to a large extent epithelially organised, the role of the plama membrane/cytoskeletal complex cannot be other than crucial. Indeed, an improper organisation of the plasma membrane/cytoskeletal complex in a given epithelium would cause malfunctioning. This may

also apply to many non-epithelially organised cell types such as muscle cells. Epithelial cells are specialised in transcellular transport of solutes, especially of ions. In my opinion, taking into consideration that function follows form, a system in which the expression of a number of epithelium-specific genes is somehow causally linked to the specific transcellular solute transport makes sense (VANDEN BROECK and DE LOOF, 1993).

WHAT COMES FIRST ?

Can different differentiated cell types of an organism engage in differential protein synthesis (which is one aspect of differential physiology) without *first* having introduced differences in their plasma membrane/cytoskeletal complexes ? A more general form of this question is : can cells having identical genomes, identical protein synthesis mechanisms and identical plasma membrane/cytoskeletal complexes engage in differential protein synthesis ? The answer is not clear cut. It might be positive if the cells were subjected to extracellular environments which differ markedly in their composition (ions, inducers *etc.*).

Imagine two cells which originated by symmetrical cytokinesis from a stem cell and which have the same number of plasma membrane- or nuclear receptors for a given inducer molecule. One cell is incubated in a medium with an inducer concentration just sufficient to ensure occupation of all the receptors for this ligand. The other cell is incubated in a medium with a much lower inducer concentration resulting in partial occupancy of the receptors by their ligand. Depending on the mode of action of the inducer, differential protein synthesis might result. This situation may occur in regions where an inducer gradient is present. Differences in ionic composition in the two media may — under certain conditions — also result in differential protein synthesis (DE LOOF *et al.*, 1992 ; VANDEN BROECK *et al.*, 1993). Differences in ionic composition of the extracellular environment can be achieved by epithelial compartmentalisation, a very commonly used mechanism in animal development (DE LOOF, 1992). There are still other possibilities. The general rule is that the further away the cells are from each other in developing organisms, the more likely it is that this strategy for generating differential protein synthesis will succeed.

When the cells are very close to each other, they would be expected to experience the same or at least very similar extracellular environments. Can cells with an identical genome, identical mechanisms of protein synthesis and an identical plasma membrane/cytoskeletal complex and furthermore experiencing the same extracellular environment engage in differential protein synthesis ? The answer is probably negative as long as none of the above parameters changes. If the genome, the mechanisms of protein synthesis and the extracellular environment are kept unchanged as is very often the case in developing embryos, the plasma membrane/cytoskeletal complex becomes the preferential parameter for introducing differential

protein synthesis. There are, indeed, several ways in which this complex can be instrumental in differential protein synthesis (DE LOOF *et al.*, 1992).
The generation of asymmetry during early development would not be easy if the cytoskeleton could not function as an anchoring site for some plasma membrane proteins and/or some maternal mRNAs or if it were spherically symmetrical. Furthermore, the organisation into epithelia would not be possible without specialisations and polarisation of the cytoskeleton.

THE GENERATION OF DIFFERENCES IN PLASMA MEMBRANE CYTOSKELETAL COMPLEXES

At the moment of oviposition or upon fertilisation, almost all animal eggs already display some form of polarity, that of a non-spherically symmetrical plasma membrane-cytoskeletal complex. This in combination with cleavage in planes other than from that of bilateral symmetry (the double asymmetry principle, DE LOOF, 1986 ; GURDON, 1992 ; HORVITZ and HERSKOWITZ, 1992) results in blastomeres which differ in their plasma membrane/cytoskeletal complexes without the necessity of first engaging in differential protein synthesis (Fig. 2). This is a very common mechanism in the Animal Kingdom.

It is striking that in all animal organisms studied in this respect, differences in plasma membrane/cytoskeletal complex are introduced very early in development, not later than the third cleavage, thus while the cells are still very close to each other. This suggests -but does not prove- that this is an important mechanism instrumental to differentiation. Some maternal mRNAs, on being translated, could also play a role, directly (when they code for membrane proteins) or indirectly (when they code for factors that influence the activity of membrane proteins already present) (Fig. 2).

A very nice example of the possibility of inducing polarity in the plasma membrane of a spherically symmetrical egg comes from outside the Animal Kingdom, namely from fucoid eggs. Initially « fresh » eggs are not polarised. This can be deduced from the observation that the thallus/rhizoid axis can develop in any direction following an appropriate physical stimulus such as light. Illumination causes directed rearrangemants in the plasma membrane properties resulting in the segregation of ion pump/channel activity : the activated egg then starts to drive an ion flux, especially of Ca^{+2}, through itself. Soon thereafter polarity becomes morphologically visible (JAFFE, 1966 ; QUATRANO, 1990). There are no indications that this requires protein synthesis first. Changes in the cytoskeleton can under some conditions be achieved by polymerisation from a pool of preformed monomers or by depolymerisation. This proves that differences in plasma membrane/cytoskeletal complex can be induced without prior differential protein synthesis.

Once *de novo* protein synthesis starts, there are evidently many ways of influencing membrane properties and physiological processes, which in their turn may again influence gene expression and so on : the system becomes « self-propelling » and

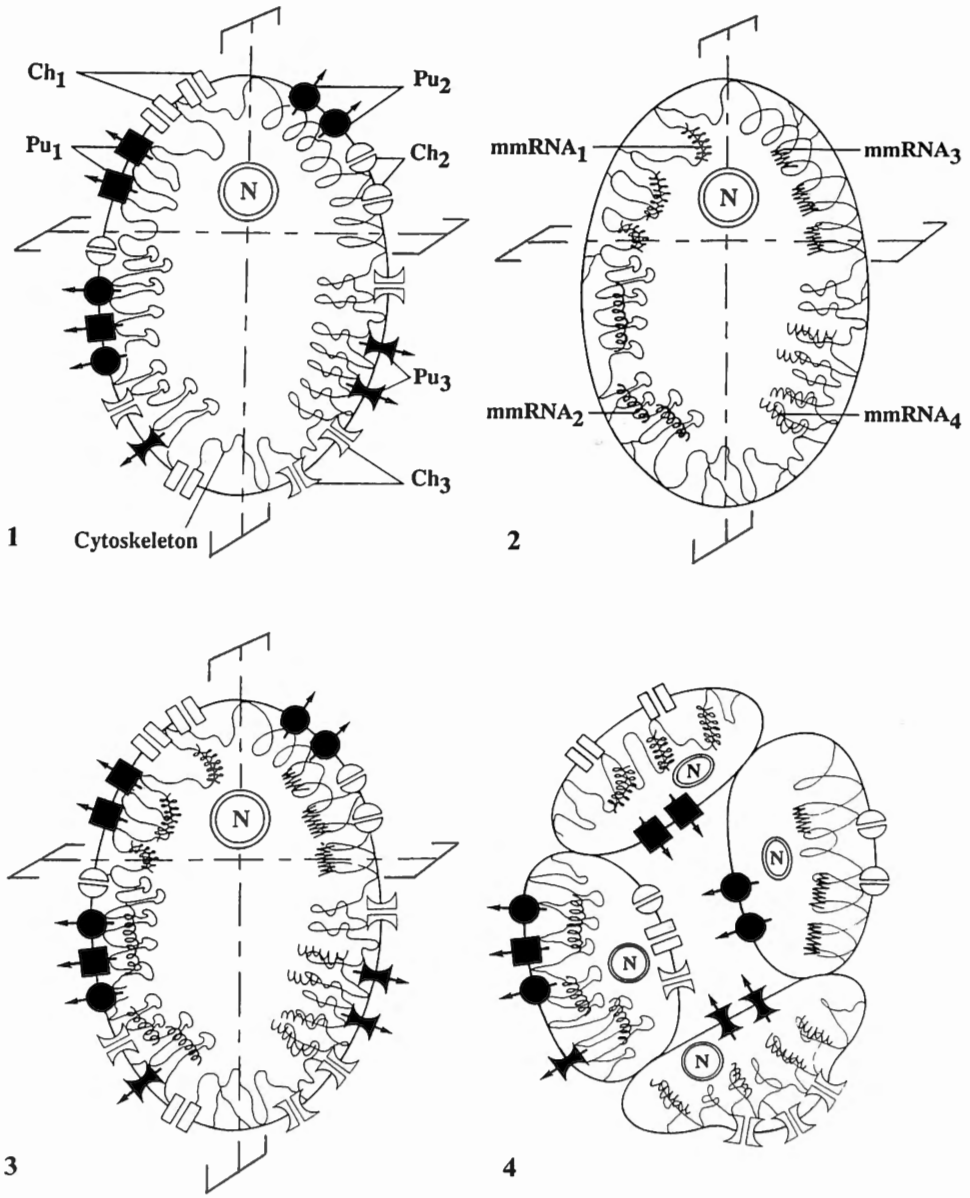


Fig. 2. — Mechanisms instrumental in the generation of functional asymmetry in a hypothetical 4-celled, epithelially organised animal. Cross sections through a « stem cell » (or more generally, a zygote), illustrating the possible role of the cytoskeleton in anchoring membrane proteins and/or maternal mRNAs.

1. Features particularly relevant to membrane physiology. Membrane proteins, here depicted as ion pumps (Pu_1-Pu_3) and channels (Ch_1-Ch_3), are asymmetrically distributed over the plasma membrane and held in place by some means, *e.g.* the the cytoskeleton. If some of the

cause and result become difficult to distinguish between (membrane impression and gene expression : BRUNNER, 1977).

There is no contradiction between the fact that the differences in the plasma membrane/cytoskeletal complex in differentiated cells are a result of this differentiation and the fact that these differences are themselves instrumental in differential protein synthesis.

Furthermore, differences in macromolecular trans-acting factors among the differentiating cells will as well play an important role. There are several possible ways to generate such differences. Some trans-acting factors could be coded for by maternal mRNAs and anchored to specific parts of an asymmetrical cytoskeleton. If cleavage does not occur along a plane of bilateral symmetry, blastomeres will be formed with different populations of maternal mRNAs (here again the double asymmetry principle). Another possibility is that a given maternal mRNA, or the protein it codes for, is not anchored but freely diffusible. If the molecule is charged and if the zygote drives an electrogenic ionic flux through itself, as is the case in *e.g.* the *Drosophila* egg (OVERALL and JAFFE, 1985), preferential localisation could result. There is so far no experimental evidence in favour of such a mechanism. Another possibility is that no such maternal mRNAs are present in the zygote but that as the result of differences arising in ionic environments, specific trans-acting factors or complexes of such factors are formed in some cells as the result of *de novo* protein synthesis.

DISCUSSION

The concept I propose here is based mainly on the following observations. Firstly, animal development and epithelial compartimentalisation are intrinsically connected. Secondly, not only epithelial cells but probably many other cell types as well, must elaborate their own specific plasma membrane-cytoskeletal complex before they can start functioning properly. Thirdly, all the differentiated cell types of any animal probably differ in their plasma membrane/cytoskeletal complex. It follows that the intracellular « ionic environment » is likely to differ from cell type

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early cleavages occur elsewhere than along the plane of bilateral symmetry, progeny cells will be formed which differ in their plasma membrane properties. N : nucleus.

2. Cross section through the same « stem cell » or zygote, showing features relevant for direct macromolecular control of gene expression/differentiation.
Maternal mRNA's ($mmRNA_1$-$mmRNA_4$), here depicted as strands of different form, are asymmetrically distributed in the cytoplasm and are held in place by some means such as the cytoskeleton. If some of the early cleavages occur elsewhere than along the plane of bilateral symmetry, progeny cells will be formed which differ in their « stem cell » mRNA's properties.

3. Superposition of 1 and 2.

4. As the result of the double asymmetry principle, 4 different cell types are formed.

to cell type. In the past, differences in the plasma membrane/cytoskeletal complex have usually but not always been considered to be a result of differentiation, which is evidently correct. In my opinion, the possibilities of the plasma membrane/cytoskeletal complex operating as a powerful driving force for differential physiology/protein synthesis, although well documented from physiological studies, have been largely undervalued in developmental biology.

The variability in properties of membranes (plasma membrane and perhaps also membranes of some cell organelles) is primarily, but not exclusively, due to differences in the nature and distribution of membrane-associated proteins, the relevant ones for development being receptors, ion transporting proteins, cell adhesion molecules/receptors and some cytoskeletal proteins which extend into the plasma membrane. Each cell type has its own set of membrane proteins and its own specific ionic environment. Although only relatively few (mostly inorganic) ionic species (the major ones being H^+, Na^+, K^+, Ca^{2+}, Mg^{2+}, Cl^-, HCO_3^- and organic ions) participate in the ionic environment in all species, the complexity of this environment should not be underestimated. Indeed, it comprises ionic concentrations/activities, ionic/voltage gradients, gradients of charged macromolecules which can arise as the result of selfelectrophoresis (WOODRUFF and TELFER, 1980) in some systems, and ionic/electrical compartmentalisation of at least some membrane-limited cell organelles. The number of different ionic environments which can be generated with these universally present inorganic ions is very large and is sufficient to allow each of the several hundred different cell types of which complex higher animals exist, to have its own specific ionic environment. Changes in ionic environment or electrical activity are potentially instrumental in the « coarse » control of gene expession by changing the conformation and/or activity of some proteins (*e.g.* enzymes), the form of the cytoskeleton/nuclear scaffold, the interaction between the cytoskeleton and membrane proteins or RNA, between DNA and DNA binding proteins in general (chromatin structure, LEZZI, 1970) or between some other macromolecules *etc.* (DE LOOF, 1986, 1992 ; DE LOOF *et al.*, 1992 ; VANDEN BROECK *et al.*, 1993). For the fine tuning of specific gene expression, macromolecular transacting factors are evidently more appropriate (BIGGIN and TJAN, 1989 ; KARIN, 1990) although electrical control is also a possibility for some genes (VANDEN BROECK *et al.*, 1993). One should not overlook the fact that almost all membrane-limited cell organelles have been reported to be able to establish their own specific intraorganelle ionic environment (DE LOOF *et al.*, 1992). Whether or not this is also true for the nucleus is still controversial (DINGWALL, 1991 ; DE LOOF, 1992). For the moment it is not known whether the properties of some cell organelles, such as the nuclear envelope and the Golgi complex, might also differ slightly from cell type to cell type or whether they are subject to change in a given cell type and contribute to differential gene expression.

Mechanisms of making cells aggregate in specific ways (adherins, specialisations of the cytoskeleton etc., EDELMAN, 1986 ; TAKEICHI, 1991 ; HYNES and LANDER, 1992) are essential especially in an epithelium-type organisation (GUMBINER, 1990 ; RODRIGUEZ-BOULAN and NELSON, 1989). Transembryonic ionic (electric) currents and selfelectrophoresis, which have been observed in several developing systems

(*e.g.* JAFFE and STERN, 1979 ; ROBINSON and STUMP, 1984) are also a logical consequence of epithelial organisation and inherent to that of the segregation (*e.g.* by anchoring them to some proteins of the cytoskeleton) of ion pumps and channels. They are thought to be more than just epiphenomena (JAFFE and NUCCITELLI, 1977 ; NUCCITELLI, 1986).

Intercellular communication by gap junctions, another type of specialisation of the plasma membrane, allows not only exchange of small organic molecules but also adjustments in ionic environments. Disruption of this flexible system can lead to disruption of the normal differentiation patterning (GUTHRIE, 1987 ; FRASER *et al.*, 1987).

The principle formulated here may appear unrealistically simplistic, especially if it is confronted with the array of mechanisms known to be instrumental in controlling the expression of specific genes in specific cell types. Many workers in the field of contemporary developmental biology take the view that there is no need for a universal principle underlying differentiation since so many mechanisms can lead to differential protein synthesis in different cell types. I am very reluctant to accept this defeatist way of thinking, which certainly looks plausible at first sight. My thesis is that, because of the universal epithelial organisation of animals, the generation of differences in plasma membrane/cytoskeletal complex is *necessarily* used by almost all differentiating animal cell types (at least the epithelially organised ones), but that in *addition* some cell types may use one, or perhaps more, *secondary* specialised mechanism(s) for controlling the expression of some of its genes. In my opinion this is rather the rule than the exception for many cell types. Indeed, each differentiated cell type expresses so many genes that the use of combinations of control mechanisms is more likely to ensure a correct expression pattern than would a single mechanism. The more cell types and genes an organism has, the more *superimposed* control mechanisms may be needed. All these mechanisms lead to generating differences in the ionic and/or macromolecular environment of the genome.

This simple holistic principle -keep the genome constant but repeatedly change its macromolecular and/or ionic environment- can reconcile and integrate all established mechanisms of epigenetic (LOVTRUP, 1974) and of genetic control of differentiatiation as well as a number of postulated ones : environmental cues can be interpreted by the plasma membrane's making use of different secondary/tertiary signal transducing systems, while the membrane proteins are coded for by the genome. Of basic importance in the principle is that there are two complementary tuning mechanisms controlling gene expression : one in which the inorganic ions are instrumental (organic ions like polyamines are also important, FEUERSTEIN *et al.*, 1991) and the other with macromolecular trans-acting factors. Both allow a very large variability and number of different combinations. The principle also stresses the importance of taking the whole cellular infrastructure into account in order to understand cellular functioning (CLEGG, 1984 ; 1992) and development.

Because of its very nature (changes in membrane properties, ion permeability etc., usually cause electrophysiological and biochemical cascade effects ; the symmetry or asymmetry of the fragile cytoskeleton/nuclear scaffold cannot be easily

demonstrated), the principle can at present hardly be fully proven or disproven experimentally : there are too many variables. However, it gains plausibility from the vast number of data which fit into it. It may stimulate the design of new lines of research both in animal systems and in plant and fungal ones, where the concept is probably applicable as well. It may also contribute to a better understanding of the mechanisms of epigenetic-, tissue specific- and multifactorial regulation of gene expression. It certainly will require the study of the activity of genes in their natural nuclear environment : this is a challenge for the future. Thinking about animal development in terms of differential epithelium formation may bring more unity in developmental biology.

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