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On the Correlation between DNA Packaging, Replication and Transcription: Implications at the Cellular Level

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GENETICS, EVOLUTION, AND THE ORIGIN OF LIFE

LECTURE 2

ON THE CORRELATION BETWEEN DNA PACKAGING, REPLICATION AND TRANSCRIPTION: IMPLICATIONS AT THE CELLULAR LEVEL

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COOPERATION IN THE LIFE SCIENCES MAY OCCUR AT THE MOLECULAR LEVEL.

In this and the next lecture we shall be ready to discuss the coupling in terms of molecular rather than cellular data. For that purpose we shall choose the still little-understood problem of polymerase dynamics. The progress of the enzyme complex- DNA polymerase- enhances the rate of RNA synthesis in DNA replication. This is an instance of a very general phenomenon which we shall now discuss in which the hypothesis of condensation has been used.

To sum up, we now propose to discuss the analogy between

i) chromatin structural changes, that occur during gene expression (transcription and DNA replication) and

ii) certain physical phenomena -phase transitions- that normally occur in other forms of condensed matter.

THE RELATIONSHIP BETWEEN TRANSCRIPTION AND DNA REPLICATION

This relationship may be presented in physical terms as the control of the dynamics of the various polymerases involved (DNA as well as RNA). However, it is useful to highlight three aspects of this phenomenon

(i) RNA synthesis is required for the initiation of some replication origins. In fact, the concept of origin of replication is needed In eukaryotes-our main concern in Lecture 3- DNA is replicated bidirectionally from many origins (***oris***). The region itself that is served by one ori is called a **replicon**.

In humans there are some 10^4 to 10^5 replicons.(the replicon, besides having an *ori* has also a terminus at which replication stops). This strategy is needed for rapid replication, in view of the enormous length of the DNA fiber in each chromosome 7.8×10^9 bps/ $24 \approx 3 \times 10^8$ bps. For instance, in *Drosophila* one expects about one replication fork per 10 kb of DNA and with the multiple origins (some 3500) replication is completed in less than three minutes.

(ii) DNA segments used as cues for transcription can be part of the eukaryotic *oris*. These DNA segments used as part of the transcription mechanisms are of two types: promoters and enhanceres.

Promoters are nucleotide sequences in DNA at the beginning of a transcription unit (TU), rather than strictly at the beginning of a gene. A promoter is recognized by RNA pol II as the site to begin transcription. A TU is any segment of chromatin which is bounded by signals for transcriptional initiation and transcription release.

In fact, a TU is traversed as a block (which may include several genes) by RNA polymerase molecules in the process of transcription . For instance, in *Drosophila* embryo (blastoderm) TU > 40 kb (the average being 13 kb). Most promoters are composed of,

-a selector region consisting of two parts, a DNA segment located 25 bp upstream which determines the transcription start site-this is called the'TATA' box and a the second part of the selector region is a start sequence).
-an upstream regulatory element, and
-an enhancer, which in a clearer description in yeast is called an upstream activating sequence.

To sum up this aspect of transcription and replication, both promoters and their corresponding enhancers can be part of the eukaryotic origins. This may be illustrated with the *ori* of the circular tumor virus, the simian virus 40 (SV40). In this case the TATA box lies within the origin of replication. The TATA box is a sequence important for positioning RNA pol II.

(iii) The initiation of transcription and replication involve similar stages. In both processes initially a set of given enzymes is assembled at a particular site, so that synthesis starts at a certain time. Thus, the analogy between origins and promoters is persuasive. An origin can be regarded as a promoter sequence for the initiation of replication. There is a further analogy between the stage of assembly of enzymes and strand melting.

This powerful analogy suggests that in a model analysis of both processes of transcription and replication, similar physical mechanisms should be responsible for the ***polymerase dynamics.*** This is particularly plausible in the approaches of thermodynamics, to which we shall return in the third lecture.

Before leaving this introduction we may discuss another aspect of the molecular background of the coupling between transcription and replication. The relationship between DNA packaging and transcription in eukaryotic gene expression should be seen together with an intimate relationship that is observed between transcription and DNA replication. This may be illustrated with some further examples.

FURTHER ASPECTS OF THE COUPLING BETWEEN DNA AND RNA SYNTHESIS.

The mechanisms for the initiation of the synthesis of DNA and RNA are analogous. This suggests that the mechanisms for regulating them should also be analogous.

Yet chromatin structure-DNA packaging- is now seen as an essential part of the transcriptional mechanism. For instance, the ability to displace histones from promoters may be critical for the action of transcriptional activators. Similarly, DNA packaging should be essential in the replication mechanism. This is seen clearly in the late replication of heterochromatin (highly packed chromatin , as, for instance, in the silenced X chromosome of female mammals).

A second related illustration is some evidence that in SV40 the binding of transcriptional activator proteins affects replication indirectly by perturbing the local distribution of nucleosomes, so that the DNA in the adjacent core region is relatively nucleosome free.

ori regions may consist of two DNA segments that separate to a certain extent the transcription process, constrained to ***a core element***; for the replication process there is a second segment consisting of ***an auxiliary component*** of promoter and enhancer relevant for both transcription and replication]. This presumably facilitates the interactions of the core origin with the initiation proteins, including the T antigen. In short, the core region is less likely to be packaged into nucleosomes than other viral genome regions.

BASIS FOR A QUANTITATIVE SUDY OF POLYMERASE DYNAMICS

Certain aspects of transcription and DNA replication data are well established:

(i) r_f *for prokaryotes is generally much larger than for eukaryotes,* where r_f denotes the rate of fork advancement.

(ii) r_t *for prokaryotes is generally much larger than for eukaryotes,* where where r_t denotes the transcription rate (or, 'turnover number').

(iii) r_f *is normally larger than* r_t.

(iv) *In prokaryotic cells the coupling of* r_f *and* r_t *is suggested by streptolydigin-induced inhibition of* r_t *which, in turn, leads to a decrease of* r_f.

A linear relationship between r_f and r_t is suggested by the following table:

Organism	*Type of cell*	r_f *(kb/min/f)*	*T(C)*
Escherchia coli	Unicellular	50	-
E. coli	Unicellular	25	37
Saccharomyce cerevisiae (yeast)	Unicellular	7 - 20	-
Drosophila melanogaster (fruit fly)	Somatic	> 2.6	-
D. melanogaster	Embryonic	2.6	25
Xenopus laevis (South African clawed toad)	Somatic	0.5	-
Triturus cristatus carnifex (Italian great-crested newt)	Somatic	1	25
Triturus vulgaris	Spermatocyte	1	25
T. vulgaris	Spermatocyte	0.6	18
Cricetulus griseus (Chinese hamster)	Somatic	< 8.3	37
HeLa(Human)	Neoplastic	1.7	37

The corresponding values for transcription are:

Type of cell	r_t *(kb/min)*	*T (C)*
Bacteriophage T7	12.0	
Escherchia coli	1.8 - 3.6	37
Eukaryotic	0.2	-

In view of this evidence we discuss in the following lecture the hypothesis that:

$$r_f \approx \mu r_t$$

The dimensionless parameter μ shall be assumed to be given by the ratio of two length parameters:

-A characteristic length the ***replicon size*** (λ_f) which corresponds to a genetic element that replicates as a whole with a unique ***origin of replication (ori).***

-A characteristic length λ_t the **pre-messenger RNA** (pre-mRNA).

The simplest hypothesis for constructing the dimensionless parameter μ in terms of the characteristic lengths λ_f and λ_t is that:

$$\mu = (\lambda_f / \lambda_t)$$

The hypothesis of the linear formula is suggested to take the form:

$$r_f \approx (\lambda_f / \lambda_t)\, r_t$$

A possible way to rationalize this formula will be left to the third lecture.

Thus, in view of the intimate relationship between DNA packaging, DNA replication, and transcription that we have attempted to establish in this lecture, we have been led to study the consequences of assuming:

Hypothesis 1

The packaging of DNA with structural proteins is correlated with the way in which DNA can interact with and, hence, respond to the strongly coupled transcriptional and replication signals.

Earlier work by Sperling and Weiss (1980) with hepatoma cells and somatic hybrids led to a weaker form of this hypothesis

Hypothesis 2

The packaging of DNA with (tissue or stage-specific) structural proteins, reflected by repeat length variations, may directly influence the way in which DNA can interact with and, hence, respond to regulatory signals.

On the basis of hypothesis 1 we have made a study of the *chromatin repeat length* (λ), which, as we have seen in Lecture 1, is the average amount of DNA wound around a core of histones.

As an example of the value of Hypothesis 1, we consider in some detail the case of migrating neurons in the rat at the time of birth. For this purpose we must insist on:

A BRIEF DESCRIPTION OF EMBRYOGENESIS.

The process of **gastrulation** occurs at an intermediate stage of development In chordates the many divisions after fertilization give rise to three layers. The outermost ('ectoderm'), the middle one ('mesoderm'), and the innermost ('endoderm'). In the first stage of the process of **neuralation** in chordates, the ectoderm gives rise to the nerve tissue, it begins as a flat expanse of tissue. which.sinks, and later rounds up to form the **neural tube**. This structure is a hollow dorsal tube, which expands in front to form the brain and a **ventricular zone** consisting of cavities called ventricles. The narrower and posterior part forms the spinal cord.

The CNS is covered by three membranes ('meninges'): The external ('dura mater'), the middle ('arachnoid mater'), and the internal ('pia mater', or '**pial surface**'), which lies in contact with the the surface of the brain and spinal cord.

Neurons may be said to have a definite time of differentiation, namely the time of the last division before the previously dividing cell goes through its last division. Once differentiated as a neuron it does not divide again; they are normally referred to as **post-mitotic neurons.**

The order of the neuron layers in the cortex corresponds to the time of their last division. The first cells to develop into neurons in the neural tube are located near the inner surface of the tube. These neurons migrate from their site of origin, towards the other side of the sheet, in an orderly way: The first one to migrate goes a smaller distance than the later ones. Evidence for this work may be found by studying the **mutant mouse *reeler*.** The mutation in the reeler gene results in the incorrect migration of cells in the developing brain. The order of the cells is reversed to some extent. The result is that the mutant mouse has an abnormal gait (it walks in a most unusual way).

The process of neurulation besides generating neurons of the central nervous system (CNS) from the epithelial cells lining the walls of the neural tube, it also generates **radial glial cells**. which in the case of the primate cortex, they extend from the ventricular zone to the pial surface, even before migration has begun.

CORTICAL NEURON MIGRATION IS PRIOR TO CEREBELLAR GRANULE CELL MIGRATION.

Typically, neuronal precursors ('neuroblasts') and neurons migrate from the sites at which they begin to differentiate. In the CNS the final position of classes of neurons is reached after a process of migration of neuroblasts from the site of their proliferation in the ventricular zone. In particular, precursors of cortical neurons and granule cells migrate at different stages:

(i) ***Cortex neurons*** arise in the rat in the 13th to 14th gestational day (E13-E14). This occurs by proliferation of primitive precursor cells. According to the classical hypothsis of Ramon y Cajal and Vignal, the precursor cells are located close to the ventricular surface. Their progeny are post-mitotic cells which migrate to the periphery of the hemispheres. On reaching the hemispheres differentiation occurs. The first neurons reach their destination in about three days, a period which is reduced for later migrations. Altogether by post-natal day 10 (P10) the cortex resembles that of a mature rat.

(ii) ***Granule cells*** in the cerebellum on the average originate later than cortex neurons. Granule cells arise from actively proliferating precursor cells, lying under the surface of the cerebellum. The process of inward migration, signalling the arrest of proliferation initiates in the rat on P3 and concludes on P20. Each individual neuron takes 2 to 3 days to reach its destination. At this particular instant the process of differentiation begins.

The work of Pasko Rakic led to the **Radial Unit Hypothesis:** In many regions of the developing brain the radial glial cells play a vital role in guiding neuronal migration.

AT BIRTH λ IS A DECREASING FUNCTION OF TIME IN MAMMALIAN CORTICAL NEURONS, AND AN INCREASING FUNCTION OF TIME FOR GRANULE CELLS.

In post-mitotic neurons we are in a situation in which the uncoupling of transcription and replication occurs naturally. In this case we may infer from hypothesis 1 that since in migrating neurons DNA replication has already ceased, λ alterations are mainly due to different requirements for transcription. The λ functional behaviour may then be considered in terms of the Radial Unit Hypothesis. The transcription requirements differ for migrating cells at different stages of ontogenesis:

Rakic has formulated the following related paradox:

How can one reconcile the apparent contradiction that neurons and glia show a strong affinity for each other and at the same time permit the movement of one along the other?

Amongst the several options available we prefer the hypothesis that the membranes of two cells are fixed at any one point along their interface. The migrating cell could, nevertheless, move by adding new membrane components to its growing tip and the leading process would gradually extend along the associated fiber. As a consequence the nucleus changes position within the cytoplasm. The ensuing cellular motion is estimated to be from 2-5 μm /h.

Thus while migration is occurring, new membrane proteins are transcribed, and longer λ's should be observed, as indeed it is the case, for instance in the experiments of Jaeger and Kuenzle. In fact , λ for

$$t < t_{birth}$$

in cortex neurons is high, about 200 bps. λ then drops to about 170 bps for

$$t > t_{birth} \ .$$

On the other hand, in the case of granule cells λ should be higher for

$$t > t_{birth}$$

for it is then when migration occurs requiring the synthesis of new membrane proteins. ***This is in agreement with experiment: λ is low at the time of birth and high at later times.*** This illustration confirms the relevance of our hypothesis 1.

DISCUSSION AND CONCLUSIONS

The time-dependent changes ot linker DNA in development is not limited to neurons:

In the early embryogenesis of the sea-urchin *Strongylocentrotus purpuratus* an analogous phenomenon is observed: Alterations in chromatin structure has also been observed by Ana Savic and her colleagues (PNAS 78, 3706, 1981). Indeed, during the first 90 minutes after fertilization the nucleosome spacing falls from 250 bps down to about 200bps.

All the examples we have considered in this lecture give a persuasive picture in favour of a certain consensus emerging in considering *chromatin as an essential part of the transcriptional mechanism* :

Instead of the once held opinion that the chromatin most prominent DNA-binding proteins (the histones) played mainly structural roles, the view is now emerging that nucleosomes are not merely inducing DNA bending, so as to contribute towards appropriate DNA packaging, but rather we may now say that histones form biochemically active complexes with DNA.

The above remarks imply that a relationship between chromatin structure at the nucleosome level and transcription seems possible. We have illustrated these relationships in this work in the field of developmental neuroscience.

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INFLUENCE OF CHROMATIN MOLECULAR CHANGES ON RNA SYNTHESIS DURING EMBRYONIC DEVELOPMENT

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ABSTRACT

Two aspects of the chromatin repeat length (r_t) are discussed: (i) Why is r_t longer for slowly dividing cells than in rapidly dividing cells?, and (ii) Why is the temporal evolution of r_t a decreasing function of time (t) in mammalian cortical neurons, whereas it is an increasing function of t for granule cells around the time of birth? These questions are discussed in terms of a hypothesis which assumes a correlation between deoxyribonucleic acid (DNA) packaging, transcription, and replication.

1. INTRODUCTION

1.1. DNA packaging and transcriptional levels

Some of the most important questions in molecular genetics of eukaryotic cells concern the control of gene expression. Perhaps the most intriguing unanswered question in this respect is the nature of the molecular mechanisms involved in the inactivation of whole blocks of genetic material, such as the mammalian *X* chromosome in females (Lyon, 1961; Chela-Flores, 1987), and the inactivation of a whole set of chromosomes in male mealybugs (Brown, 1966). Since the most frequent mode of gene control is at the transcriptional level (Darnell, 1982), inactivation is probably due to inhibition of ribonucleic acid (RNA) synthesis (*i.e.* transcription). Thus, the remarkable structural alterations in the heterochromatic *X* chromosome may be directly related to the regulation of transcription.

In spite of these insights in the relationships between DNA packaging and transcription in eukaryotic gene expression, many fundamental questions remain unanswered at the microscopic level, particularly associated with replication (Kornberg, 1988). A key structure related to molecular alterations in chromatin is its repeating unit known as the nucleosome. A large body of information has been available in this respect for some time (McGhee & Felsenfeld, 1980). The purpose of the present work is to analyze a variety of phenomena observed at the molecular level in chromatin in terms of

Hypothesis 1, below. For this purpose we consider the average amount of DNA packaged in each nucleosome: this is normally measured in terms of DNA length in base paris (bps), which is called the chromatin repeat length (r_t). A closely related term is the length of the DNA fibre connecting adjacent nucleosomes known as linker DNA.

1.2. Alterations of r_t

In a variety of phenomena observed in many cells, it has been pointed out that r_t is a parameter which varies, not only between different organisms, or organs, but even in the same tissue at different stages of ontogenesis (Chambon, 1978). These variations of r_t have led to apparently conflicting results, particularly in embryonic changes in r_t in the mammalian brain. We will address such data in terms of the following hypothesis:

Hypothesis 1. The packaging of DNA with structural proteins, reflected in variations of the r_t parameter, is correlated with the way in which DNA can interact with and, hence, respond to the strongly coupled transcriptional and replication signals.

Earlier, work with hepatoma cells and somatic hybrids led to a weaker form of this hypothesis (Sperling & Weiss, 1980):

Hypothesis 2. The packaging of DNA with (tissue or stage-specific) structural proteins, reflected by the repeat length variations, may directly influence the way in which DNA can interact with and, hence, respond to regulatory signals.

We have restricted our attention to the relationships between transcription and DNA replication (when they both occur simultaneously), amongst the wide variety of possible options that the genome is presented with for the regulation of gene expression. For instance, we have excluded from our consideration regulatory mechanisms at the level of RNA splicing, translational, or even posttranslational levels.

1.3. The coupling of DNA replication and transcription is of primary importance in gene expression

Over the last few years a gradual understanding has emerged of the intimate relationship between the processes of DNA replication and transcription (De Pamphilis, 1988; Falaschi *et al.*, 1988). In fact, the presence of transcriptional signals in DNA replication origins (*ori* regions) may be understood at the molecular level: Eukaryotic genomes have *ori* regions that may consist of two DNA segments that separate to a certain extent the transcription and replication processes. In other words, these two segments are:

- A core component, concerned with replication, and,
- An auxiliary component in which promoter and enhancer elements may be involved in both transcription and replication.

Although this insight into the *ori* regions is mainly based on experiments with yeast, prokaryotic, or viral genomes, more recent work suggests that initiation of mammalian chromosomal DNA replication also uses *ori* sequences (Burhans *et al.*, 1990). This

emphasizes the relevance of incorporating at the molecular level both aspects of gene regulation, namely, transcription and DNA replication, even in mammals. This seems to be particularly relevant in the case that concerns us in the present work; for arguments have been sometimes developed regarding r_t alterations during embryonic development, without taking into consideration appropriately the possible influence of the strong coupling between transcription and DNA replication.

In Sec. 2 we shall restrict our attention to gene regulation during several stages in embryogenesis, in which the genome itself *separates* the processes of transcription and DNA replication, thereby allowing Hypothesis 1 to help understand some apparently paradoxical data.

2. EMBRYOGENESIS MAY UNCOUPLE THE PROCESSES OF TRANSCRIPTION AND REPLICATION

2.1. Dynamic alterations of r_t

Dynamic, or time-dependent variations of r_t have been studied in two systems, which we shall use as illustrations that will be needed for subsequent discussion of the implications of Hypothesis 1:

(i) Early sea urchin embryogenesis. - Somewhere between fertilization and morula, changes have been observed in the average size of linker DNA in the species *Strongylocentrotus purpuratus* and *Lytechinus pictus* (Savic *et al.*, 1981).

(ii) Mammalian fetal neurogenesis. - In the rat, cortex neurons and cerebellar neurons (granule cells) show a distinct time evolution of the r_t parameter during early post-natal development. In this experimental animal the following remarkable result has been obtained (Jaeger & Kuenzle, 1982):

In both cortex and cerebellar neurons r_t changes in temporal coincidence with terminal differentiation; however, whereas r_t shortens in the cortex, it lengthens in the cerebellum.

Such a diverse behaviour of r_t as a function of time in the same organ shall be considered in the next (and following) sections.

2.2. Alterations in r_t cannot always be discussed entirely in terms of altered transcriptional levels

Before we attempt to understand the time evolution of r_t in differentiated neurons, (c.f., the examples we mentioned in Sec. 2.1), a certain difficulty must be discussed first: The only general rule that can be inferred from experiments in which alterations in r_t have been measured seems to be that r_t is different in different tissues (McGhee & Felsenfeld, 1980; Chambon, 1978). In other words, we may say that we are facing a *paradox*:

A correlation sometimes seems to exist between a short r_t and a high level of transcriptional activity (and conversely, between a long r_t and transcriptional inactivity); but, nevertheless, transcriptionally inert micronuclei in some protozoa have shorter r_t than active macronuclei.

Although methylation of the DNA of the transcriptionally active macronucleus of *Tetrahymena thermophila* may occur (Weisbrod, 1982), thereby altering gene expression, a solution to the above paradox cannot rely entirely on an argument that refers only to modification of chromatin structure (cf. Sec. 1.3). As stated elsewhere (Chela-Flores & Migoni, 1990) although an inverse proportionality has been observed between the extent of methylation of a given base and its transcriptional activity (Bird, 1986), it has not been possible to give a definite answer to this question. Therefore, we may conclude that more general arguments must be invoked in order to understand the mechanisms underlying r_t alterations.

2.3. Some examples of the uncoupling of DNA replication and transcription

There are some particular stages in ontogenesis in which the molecular changes in chromatin may be understood more readily, thus facilitating the study of the dynamic alterations of r_t. We illustrate this point with the following cases, in which either transcription or DNA replication are turned off:

(i) *DNA replication occurs in early* Drosophila *development in the absence of transcription.* We know that events initiated in mitotic cycle 10 render all classes of genes competent for transcriptional activation. This suggests the hypothesis that the proteins necessary for transcription are first synthesized at cycle 10 Edgar & Schubinger, 1986).

(ii) *DNA replication occurs in early* Oncopeltus *development in the absence of rDNA transcription.* During the blastoderm stage of the milkweed bug *O. fasciatus* (Dallas) there is no evidence of transcription (Foe, 1978).

(iii) *Transcription in mammalian neurons occurs in the later stages of embryogenesis in the absence of DNA replication.*

Our third example is well known in cellular ontogeny. It has been selected from vertebrate development, specifically in mammals partly because of the extensive work existing on post-mitotic neuronal migration in the neocortex of fetal monkey (Rakic, 1972, 1981), and partly because it will help us understand the dual behaviour of r_t in two parts of the rat brain (cf. Sec. 2.1(ii)). This particular example shall be developed in Sec. 3, which is needed to understand the main conclusions of this work, presented in Sec. 4.

3. TEMPORAL EVOLUTION OF r_t IN THE MAMMALIAN BRAIN DURING THE LATER STAGES OF EMBRYOGENESIS

3.1. The radial unit hypothesis

Some classes of neurons, such as those involved in the developing mammalian cortex, as well as in the cerebellar cortex, appear to move along the surface of rectilinear vertically-oriented glial fibres; these are called the radial glia (Rakic, 1972; Rakic & Sidman, 1973). The hypothesis itself is known as the radial unit hypothesis, in terms of which we shall discuss in turn both types of behaviour of r_t, as observed in the mammalian fetal brain (cf. Sec. 2.1(ii)).

3.2. Neuronal migration of granule cells

Cerebellar neurons are most abundant as granule cells, and on the average they originate later than cortex neurons (Jacobson, 1978). Granule cells arise from actively proliferating precursor cells, lying under the surface of the cerebellum. The process of inward migration, signalling the arrest of proliferation initiates on post-natal day 3 (P3) and concludes on P20. Each individual neuron takes 2 to 3 days to reach its destination. At this particular instant the process of differentiation starts.

3.3. The migration of neocortex neurons is not synchronized with the migration of cerebellar cortex neurons

Neocortex neurons arise in the rat in the 13th to 14th gestational day (E13-E14). This occurs by proliferation of primitive precursor cells. According to the classical hypothesis (Ramón y Cajal, 1891; Vignal, 1888), the precursor cells are located close to the ventricular surface. Their progeny are post-mitotic cells which migrate to the periphery of the hemispheres (according to the Radial Unit Hypothesis). On reaching the hemispheres differentiation occurs. The first neurons reach their destination in a temporal period extending up to 3 days. This period is somewhat longer for later migration. By P10 the cortex resembles that of the mature rat.

In other words, the proliferation of precursor cells in the cortex is limited to the *pre-natal period*. By P10 the last cohort of cells has reached its destination in the cortex (Jacobson, 1978). In Sec. 3.2 we described a neuronal migration pattern which is in sharp temporal contrast with the present discussion of cortical neurons. For example, the onset of the post-mitotic migratory stage of cerebellar ontogenesis occurs as late as P3.

Is it possible to understand both sets of experiments (*i.e.* those described in Secs. 3.2 and 3.3. in terms of r_t alterations?

We attempt to address this question in the concluding section.

4. DISCUSSION AND CONCLUSIONS

4.1. Hypothesis 1 may help to understand the experimental data on r_t alterations

Our interest in the r_t parameter derives mainly from the fact that it reflects an important aspect of protein-DNA interaction in chromatin and the data discussed in this work indicates that there appears to be some functional correlates of the transcriptional activity.

The general trend of the data seems certain, the r_t of slowly-dividing inactive cells is generally *longer* than that of rapidly dividing cells. Our Hypothesis 1 provides a basis for understanding the main trend of the data, as we shall proceed to consider.

4.2. Why is r_t of slowly dividing cells longer than r_t of rapidly dividing cells?

First of all we make two comments on particular aspects of the processes of DNA replication and transcription during early development:

(i) the number of replication origins is known to increase during early development (Callan, 1972). Substantial evidence supports the fact that shorter *S* phases of embryos are achieved mainly by decreasing replicon size, *i.e.* by initiating replication at closer intervals on DNA.

(ii) The duration of the *S* phase of the cell cycle is not constant throughout ontogenesis. For instance, in *Xenopus* embryos the *S* phase lasts some 25 mins. compared with many hours in adult cells (Laskey *et al.*, 1989).

From (i) and (ii) we can get some insight into the data pertaining to dividing cells at different stages of ontogenesis. For we know that the total metabolic energy available for cellular processes is finite (mainly arising from the hydrolysis of the high energy bonds in adenosine triphosphate (ATP)). Thus when the *S* phase is very brief and the number of replicons increases, more origins of replication will require a very high level of consumption of basal metabolic energy. This situation may be maintained if the energy required for transcription is inhibited, so as to make it available for replication. Transcription inhibition will be achieved by increasing the degree of DNA compaction, which in turn means that r_t would be shortened, thus providing a rationale for the question posed at the title of this subsection.

4.3. An approach to the paradox

In Sec. 2.2 we faced a paradox when a correlation between the length of r_t and transcriptional activity does not appear to be valid.

In the later stages of embryogenesis (and in adult cells) the replicon size increases and the demand for basal metabolic energy is therefore not so severe. Therefore, both processes of transcription and replication will compete for the available energy. In this case we are not in a clear cut situation as when transcription was severely inhibited at earlier stages of embryogenesis. We are then not able to distinguish a clear trend in this case of r_t alterations, since cellular requirements on the coupled processes of transcription and replication will vary.

4.4. Why is $r_t(t)$ in cortical neurons a decreasing function of time t, and an increasing function of t for granule cells?

In post-mitotic neurons we are, once again, in a simple situation in which uncoupling occurs naturally. For this reason we infer from our Hypothesis 1 that the experimental results on r_t (shortly before and shortly after birth) in the rat brain neurons are not necessarily incompatible with either tendency shown by r_t in the case of the paradox discussed in Sec. 4.3.

In other words, our Hypothesis 1 implies that since in migrating neurons, DNA replication has already ceased, then r_t alterations are due mainly to different cellular

requirements for transcription. The $r_t(t)$ functional behaviour may then be understood in terms of the Radial Unit Hypothesis: The transcription requirements differ for migrating cells at different stages of ontogenesis (cortical neuron migration is prior to cerebellar granule cell migration). The migrating cell moves by adding *new membrane components to its growing tip* (Rakic, 1981). Thus, while migration is proceeding, transcription of new membrane proteins occurs, and longer r_t's should be observed, as indeed it is the case (Jaeger & Kuenzle, 1982). In fact, $r_t(t)$ for cortex neurons (and for $t < t_{Birth}$, *i.e.* for times before the time of birth, t_{Birth}) is high (about 200 bps); it then drops to about 170 bps (cf. Fig. 1 in the Jaeger-Kuenzle paper) for $t > t_{Birth}$ (*i.e.* for t after t_{Birth}). On the other hand, in the case of granule cells $r_t(t)$ should be high for $t > t_{Birth}$ for it is then when migration occurs (and the need for new membrane components is then called for). This is in fact what is observed in fig. 4 of the Jaeger-Kuenzle paper: $r_t(t)$ is low at $t = t_{Birth}$ (about 165 bps), and high for $t > t_{Birth}$ (about 220 bps).

4.5. Final comments and conclusion

Finally, we would like to summarize our main results, in view of the wide variety of data we have considered from the field of genetics and, in particular, in neurogenetics. This should help to clarify the logical steps we have followed.

First of all, the work has taught us that Hypothesis 1 has some heuristic value. This was illustrated by emphasizing (with special reference to experimental data) the importance of maintaining the coupling of transcription and DNA replication in discussing the relationship between DNA packaging and transcriptional levels.

The arguments for and against such a viewpoint should be seen in the light of a recent analytic model (Chela Flores, 1992) that implements the various aspects of gene expression which were suggested in this paper.

It may be useful to refer to some key underlying questions that were considered in the present work under the viewpoint of Hypothesis 1:

(A) Does fast replication take up a significant amount of energy during early embryogenesis, entirely at the expense of transcription?

(B) Is the phenomenon of transcription suppression related to chromatin compaction?

(C) Is the chromatin repeat length related to transcription?

It would be fair to state that a definitive answer to these three relevant questions requires further research, both experimental, as well as theoretical. The discussion of the present paper is a preliminary effort to address these questions.

On the other hand, a certain consensus is now emerging in the sense that *chromatin is an essential part of the transcriptional mechanism*: Instead of the once held opinion that the chromatin most prominent DNA-binding proteins (the histones) played mainly structural roles, the view is now emerging that nucleosomes are not merely inducing DNA bending, but rather they are biochemically active complexes (Felsenfeld, 1992).

Likewise, the chromatin repeat length, being a reflexion of the DNA winding around a histone core plus the linker segment of DNA joining adjacent histone-cores, should be envisaged as we have done in this work, as a dynamic parameter to be understood in its intimate relationship with the transcription process.

In this context, it should not be forgotten that the nucleosomal repeat structure does

not distinguish between inactive and active genes (Weisbrod, 1982), thus although the chromatin repeat length is typically an average parameter dominated by chromatin structure (which is to a large extent transcriptionally inert) the repeat structure itself should still play a key role. This implies that a relationship between chromatin structure at the nucleosome level and transcription can no longer seem an extremely unlikely relationship. On the contrary, it seems possible that changes in the chromatin repeat length and transcription are related, as suggested by the main hypothesis of this work (Hypothesis 1).

To sum up, it is now becoming clear that chromatin structure has an intimate connection with the transcriptional mechanism. This includes, at the molecular level, the nucleosome which is characterized by the chromatin repeat length, a point which was emphasized throughout this work.

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