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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 3

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

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1. ASPECTS OF TRANSCRIPTION AND DNA REPLICATION

The control of the rate of DNA replication is obtained by controlling the frequency with which new forks are initiated, rather than by controlling the speed r_f with which the forks themselves move relative to the DNA duplex. To illustrate this point consider the cell cycle: The duration of the S phase varies during ontogeny. Early in *Drosophila* embryogenesis the duration of the S phase is 4 minutes. In the adult fly, the same amount of DNA synthesis is completed in almost 10 hours. Similar changes may occur in chordates (***Xenopus***). This is shown quantitatively in the following table:

Stage	***Length of the S phase (hrs)***	λ_f ***(kb)***
Cleavage	0.06	7.9
Blastoderm	0.33	10.2
Larval brain	11	10.7

This remark leads us to two aspects of the r_f parameter which are worthy of our attention:

(i) r_f maintains a constant value, even in the case of early embryogenesis, when the rate of DNA synthesis is greatly magnified.

(ii) r_f represents to a large extent a continuous rate of advancement, in spite of the size of the multienzyme complex involved

In yeast the late replicating telomere region (i.e., the end of the chromosome) at the left end of chromosome III is interesting for there seems to be an ***ori*** 40 kb from the telomere, which initiates bidirectional replication early in the S phase. The fork that moves toward the telomere has a rate of advancement of about $r_f = 4$ kb / min.

As it reaches the terminal 15 kb, r_f decreases to about 1.3 kb / min. Thus the fork slows down as it enters the telomere region. Clearly our own work would not apply to such proximal regions of the telomeres.

We will assume that polymerase dynamics may be understood by means of a phase transition in the genome with the following characteristics:

(A) Once DNA synthesis is initiated, a phase transition may occur in active chromatin.
(B) We may treat our second type of phase transitions with mean field theory

Chromatin, on the other hand, may be analogous to other forms of condensed matter where thermodynamic and macroscopic quantum-mechanical concepts may be applied. This possibility may be illustrated by emphasizing a point that we made in Lecture 1, namely, that the tight packing of chromatin yields DNA concentrations ρ within localized regions of interphase nuclei comparable to that of liquid helium. This remark invites comparison between chromatin and various forms of condensed matter, in which comparable densities may occur, particularly since in metaphase chromosomes the degree of DNA packaging increases by one or two orders of magnitude, making a comparison with solid state phenomena plausible.

A thermodynamic approach to phase transitions in many forms of condensed matter may apply to changes in chromatin structure due to gene expression. Thus we are led to the question:

What happens to the inactive chromatin free energy per unit volume when it is turned into active chromatin?

An expression for changes ΔG in the Gibbs free energy (per unit volume as are all equations listed), may be anticipated from the following remarks:

(i) **ΔG may depend explicitly on temperature:**

$$\Delta G = \Delta G(T)$$

since ***normal*** transcription (i.e., transcription at the homeostatic temperature T_H) generally occurs below a certain temperature T_{hsp}, at which heat-shock proteins (hsps) are synthesized:

$$T_{hsp} < T_H$$

Therefore, we may conclude that the ΔG function may depend on temperature T

(ii) ***ΔG may depend on chromatin compaction,*** since we have seen in Lecture 1 that replication is delayed for high values of the packing ratio, for instance for heterochromatin ($\eta = 4000$). Hence,

$$\Delta G = \Delta G(\rho)$$

Since at the onset of activation of *ori*, there is a significant change in the structure of inactive chromatin, we assign a Gibbs free energy G_0 to inactive chromatin, and G to active chromatin.

We then treat this significant structural change as a phase transition. The change in the system will be denoted by:

$$\Delta G = G - G_0$$

Since the process of initiating DNA replication at *ori* must occur spontaneously, then G should be lower than G_0 :

$$\Delta G < 0$$

What we have expressed is that phase transition may occur when the polymerases and other transcription and replication factors interact with chromatin.

An effective amino acid wavefunction ψ (**x**) is identified with an order parameter for the onset of condensation. Since condensation implies that every state is given by the same ψ, then the whole polypeptide complex making up any of the polymerases is given by a wavefunction ψ(**x**). We assign the complex functions ψ_f for the replication fork, and ψ_t for the RNA polymerase associated with r_t . Then we write the complex variable ψ as a modulus R and a phase S:

$$\psi_f = R_f \exp (iS_f)$$

$$\psi_t = R_t \exp (iS_t)$$

and equate the 'superfluid' velocity with the gradient of the phase of the wavefunction:

$$r_f = | -ih\nabla S_f | / m_f$$

$$r_t = | -ih\nabla S_t | / m_t$$

Then, if the phase transition is of the second order (ie., as in the case of superconductivity), we may identify ψ_f and ψ_t with the order parameter in the free energy for either polymerase.

For instance,

$$\Delta G_f = \Delta G_f (\psi_f , \nabla\psi_f)$$

$$\Delta G_t = \Delta G_t (\psi_t , \nabla\psi_t)$$

We may also expand ΔG near T_c.

This provides expressions for the evolution of $\psi_{f,t}$, and hence of $S_{f,t}$ or r_f and r_t. The order parameters ψ_f and ψ_t may depend on:

(i) The chromatin concentration ρ , and
(ii) On whether the chromosome is transcriptionally active (i.e., *euchromatic*), or transcriptionally repressed (i.e., *heterochromatic*).

The order parameters satisfy, therefore, the following inequality:

$$|\psi_{f,t} (\rho, \textit{heterochromatin}) | \gg | \psi_{f,t} (\rho, \textit{euchromatin}) |$$

With the further assumption that the degree of compaction of the polymerases is approximately equal in both cases, we may readily obtain

$$r_f = \mu \; \mu_0 \, r_t$$

where we have:

$$\mu_0 = (m_t / m_f) \approx 1$$

as well as:

$$\mu = \lambda_f / \lambda_t$$

Hence, gathering together these equations we are led to the formula

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

since the experimental evidence does not rule out the approximate equality of the polymerase masses :

Organism or Type of Cell	m_t ***(Da)***	m_f ***(Da)***
Prokaryotic	$(4.5 - 5) \times 10^5$	8×10^5
Eukaryotic	$(5 - 6) \times 10^5$	$>(1.6\text{-}1.7) \times 10^5$
Eukaryotic: Yeast	-----	$(4.6 - 6) \times 10^5$

To sum up, chromatin structural changes may be considered as an important mechanism in gene expression from the following evidence:

(i) The time required to replicate all DNA and, hence, the duration of the S phase in somatic cells, seems to be controlled by the distribution of *oris*, rather than by any change in r_f

(ii) Origin (of DNA replication) spacings may be determined by some aspect of interphase chromosome structure (chromomeres).

(iii) The stimulatory effect of the transcriptional elements on DNA replication may be due, at least in part, to effects of chromatin structural change.

In fact, if the linear formula is valid then we must ensure that if r_f is to be considered as a constant parameter, then,:

(a) r_f and λ_f should retain their experimental values.

(b) We should understand some aspects of the diversity of values of the gene lengths (i.e., the lengths of the primary transcripts).

Keeping (a) and (b) in mind, and with the known values of the λ_f parameter we are led to conclude that:

r_f may vary according to the length of the gene being transcribed.

These statements may be verified with the values of the λ_f and λ_t parameters:shown in the following two tables:

Organism	***Type of Cell***	λ_f ***(average, kb)***
E. coli	Unicellular	4.2×10^3
S. cerevisiae	Unicellular	36 - 108
D. melanogaster	Somatic	40
D. melanogaster	Embryonic	9.7
X. laevis	Somatic	200
X. laevis	Somatic	190
T. cristatus carnifex	Somatic	500 - 600
T. vulgaris	Spermatocyte	< 150
Dipodomys ordii (kangaroo rat)	Somatic	< 25
C. griseus	Somatic	< 100
C. griseus	Ovary	< 15
Homo sapiens	Somatic	100

Organism	***Gene***	λ_t ***(kb)***
E. coli	rRNA	6
Yeast	rRNA	8-
Plant (tobacco)	rRNA	8.3-
D. melanogaster	rRNA	7.7-8.6
X. laevis	rRNA	7.9-8.3
Reptile (iguana)	rRNA	8.3
Birds	rRNA	10.5-12-
Marsupial (potoroo)	rRNA	12.6
Rodent (mouse)	rRNA	13.7
H. sapiens	rRNA	13.7
H. sapiens	β–globin	1.5
H. Sapiens	insulin	1.7
H. sapiens	PKC	11
H. sapiens	albumin	25
H. sapiens	catalase	34
H. sapiens	LDL receptor	45
H. sapiens	thyroglobulin	300
H. sapiens	dystrophin	>2000

The information in the tables implies that in a certain range of values of the λ_t parameters:

$$\lambda_t \quad >> \quad \mathbf{100\ kb}$$

and the structure of chromatin may act as a source of retardation for RNA polymerase. This may be understood as follows:

Since the chromatin repeat length (λ_{crl}), according to Lecture 1, is given by,

$$\lambda_{crl} \approx 200\ bp$$

then, for a given gene, RNA polymerase will have to proceed past some thousand nucleosomes - a factor retarding its rate of movement. This suggests that:

$$\boxed{r_t = r_t(\lambda_t)}$$

an effect which is testable since, as we have stated above, the r_f parameter may be assumed to be approximately constant.

Example:

For *H. sapiens* using the kinematic formula, we find:

$$\boxed{4r_t\ (PKC) \approx r_t\ (LDL\ receptor)}$$

This new predicted effect may be tested by measuring fluctuations in the r_t parameter for the transcription of two genes in *H. sapiens*.

The calculated values of r_f are given, according to the formula, by the following table:

Organism	**Gene**	**r_f in kb/min/f (somatic, calculated)**
Yeast	rRNA	3
Drosophila	rRNA	1
Xenopus	rRNA	5
Lower mammals	rRNA	< 1.5
Homo sapiens	rRNA	1.5
H. sapiens	β-globin	13
H. sapiens	insulin	12
H. sapiens	PKC	1.8
H. sapiens	albumin	0.8
H. Sapiens	catalase	0.6
H. sapiens	LDL receptor	2.4
H. sapiens	thyroglobulin	0.1
H. sapiens	dystrophin	< 0.01

CONCLUSIONS

For some genes in *H. sapiens* the linear formula explains the data satisfactorily, as in the case of rRNA and PKC, but it should be recalled that the expected value of r_t for eukaryotes is only intended to be an approximate result. The same reasonable agreement may be claimed for lower mammals.

On the other hand, although the *Drosophila* and yeast numerical results are rather small, no definite conclusion may be inferred in these two cases due to our only approximate knowledge of the r_t parameter. The more interesting aspect of the data is the radical deviation obtained, for instance, for dystrophin (i.e., two orders of magnitude). In these extreme cases two possibilities may still arise:

(i) The linear formula is indeed approximately valid. This situation forces upon us the new predicted effect, which would then be valid in gene expression. This phenomenon, we have argued, may be due to chromatin structural changes.

The continuous and constant nature of r_f is supported by the well-established genetic control on the multiplicity of oris, rather than the control of the r_f parameter itself. The variability of r_t is hinted at the molecular level by the variability of the length of linker DNA in transcriptionally active chromatin, which displays a unique conformation.

(ii) The theory supporting the approximate validity of the kinematic formula may still be correct (i.e., condensation may be present in active chromatin structural changes, which may occur due to the coupled processes of replication and transcription); **but the predicted effect is not seen in experiments.** In this case a more rigorous solution to the equation of motion, beyond the linear approximation may be needed.

Although the theory can clearly be made more rigorous, our objective is limited only to pointing out a possible approach to the important problem of identifying the mechanisms governing polymerase dynamics:

We are suggesting that condensation in chromatin may underlie the kinetics of polymerase advancement, thus hinting at the relevance of the hypothesis of condensation in molecular genetics, supporting to some extent, the Salam hypothesis in the biochemistry of the amino acids.

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Towards the Molecular Bases of Polymerase Dynamics

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One aspect of the strong relationship that is known to exist between the processes of DNA replication and transcription is manifest in the coupling of the rates of movement of the replication fork (r_f) and RNA polymerase (r_t).

We address two issues concerning the largely unexplored area of polymerase dynamics: (i) The validity of an approximate kinematic formula linking r_f and r_t suggested by experiments in which transcription is inhibited in some prokaryotes with the antibiotic streptolydigin, and (ii) What are the molecular bases of the kinematic formula?

An analysis of the available data suggests possible molecular bases for polymerase dynamics. In particular, we are led to a hypothesis: In active chromatin r_t may depend on the length (λ_t) of the transcript of the primary messenger RNA (pre-mRNA). This new effect is subject to experimental verification. We discuss possible experiments that may be performed in order to test this prediction.

1. Introduction

In spite of the importance of DNA replication in both the duplication of the genome, as well as in its rearrangements, the dynamics of (DNA) polymerase action still remains largely unexplored (Kornberg, 1988). More surprising still is that while the most frequent mode of eukaryotic gene control is at the transcriptional level (Darnell, 1982), the factors contributing towards the underlying regulation of the rate of movement of RNA polymerases (r_t) remain elusive.

The problem brought into the foreground of molecular biology by Kornberg's remarks is clearly of deep significance but, at the same time of great complication. What we are able to learn much more easily (by inspection of the available data) concerns *polymerase kinematics* rather than *polymerase dynamics*. By scrutinizing the data certain relationships between the rate of movement of the replication fork (r_f) and r_t appear with striking regularity throughout phylogeny (cf. section 1.1 and Table 1). Our hope is that in trying to find physical phenomena underlying purely kinematic relations, some insights may be gained into polymerase dynamics. This will be the main theme of this work.

We begin the next subsection with a review of the main properties of the r_f and r_t parameters.

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TABLE 1

Values of the r_f parameter for a variety of organisms. The temperature units are °C

Organism	Type of cell	r_f (kb min^{-1}/f)	T (°C)	References
Escherichia coli	Unicellular	50	—	1
E. coli	Unicellular	25	37	2
Saccharomyce cerevisiae (yeast)	Unicellular	7–20	—	3
Drosophila melanogaster (fruit fly)	Somatic	>2·6	—	4
D. melanogaster	Embryonic	2·6	25	5
Xenopus laevis (South African clawed toad)	Somatic	0·5	—	6, 1
Triturus cristatus carnifex (Italian great-crested newt)	Somatic	1	25	6
Triturus vulgaris	Spermatocyte	1	25	6
T. vulgaris	Spermatocyte	0·6	18	6
Cricetulus griseus (Chinese hamster)	Somatic	<8·3	37	7
HeLa (human)	Neoplastic	1·7	37	7

References: 1, Lewin (1983); 2, Callan (1974); 3, Campbell (1986); 4, Blumenthal *et al.* (1974); 5, Kriegstein & Hogness (1974); 6, Callan (1972); 7, Huberman & Riggs (1968).

1.1. SOME ASPECTS OF POLYMERASE KINEMATICS MAY BE INFERRED FROM DATA OBTAINED THROUGHOUT PHYLOGENY

There are certain aspects of the data concerning transcription and DNA replication that are well-established:

(i) r_f for prokaryotes is generally much larger than for eukaryotes (cf. Table 1).

(ii) r_t for prokaryotes is generally much larger than for eukaryotes (cf. Table 2).

(iii) r_f is normally larger than r_t (cf. Tables 1 and 2, and Kriegstein & Hogness, 1974).

TABLE 2

Values of the r_t parameter for both prokaryotic and eukaryotic cells. The temperature units are °C

Type of cell	r_t (kb min^{-1})	T (°C)	References
Escherichia coli	2·4	37	1
E. coli	1·8	37	2
Eukaryotic	0·2	—	3†

References: 1, Watson *et al.* (1987); 2, Alberts *et al.* (1989); 3, Alberts & Sternglanz (1977)†.

† In eukaryotes replication forks must progress over bound nucleosomal histones. This may account for the value of r_f being about one order of magnitude smaller in eukaryotes (cf. Table 1 and Alberts & Sternglanz, 1977). Likewise we assume in this table that r_t (eukaryotes) < r_t (prokaryotes), since bound nucleosomal histones may act also as an impediment for the progress of the RNA polymerases. This point is in agreement with Brewer (1988) and Campbell (1986).

(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 (Pato, 1975).

(v) Although there is *a priori* a high probability of frequent encounters between DNA polymerases and RNA polymerases while engaged in their polymerization actions, somehow genomes have avoided this possibility by appropriate orientation of transcription units; this is clear at least in the chromosome of the bacterium *Escherchia coli* (Brewer, 1988).

Due to our present limitations, a general predictive algorithm that may encompass (i) to (v) lies beyond the scope of the present note. A more feasible line of research is preferred: We propose to search for empirical rules restricted to kinematics; then if we succeed to justify our empirical results, the physical arguments used in the justification may give us some insights into polymerase dynamics. Our present attempt is limited to only four of the above-mentioned aspects of the data [i.e. (i)–(iv)].

1.2. AN OUTLINE OF THE REMAINING PART OF THIS WORK

The rest of this note is distributed as follows:

In section 2 we present our basic hypothesis: to wit, a kinematic formula [cf. eqn (3)]. The remaining part of this section discusses possible theoretical bases that may support the validity of the kinematical formula.

In section 3 we face the problem of searching for the molecular bases of the kinematic formula. The central argument in this section is presented in terms of the possible occurrence of phase transitions in the genome after the initiation of DNA replication, since some experiments have previously suggested that phase transitions may occur as possible triggers for the initiation of DNA synthesis.

In section 4 we discuss the experimental support of the kinematic formula and, on the strength of the evidence, we suggest the existence of a new effect concerning the r_t parameter [cf. eqn (23)].

Finally, in section 5, we summarize our main conclusions. The appendices contain a more technical discussion, which is relevant to the theoretical support of the kinematic formula.

2. The First Issue in Polymerase Dynamics

2.1. IS THERE A KINEMATICAL FORMULA LINKING THE r_f AND r_t PARAMETERS!

We have referred in section 1.1 to the evidence [cf. section 1.1 (i)–(iv)] which suggests a linear relationship between r_f and r_t. We thus hypothesize that:

$$r_f \approx \mu r_t . \tag{1}$$

From the data displayed in Tables 1 and 2 we may infer that μ is expected to be greater than 1 in eukaryotic cells, and much greater than 1 in prokaryotic cells. The dimensionless parameter μ shall be assumed to be given by the ratio of two-dimensional length parameters:

(i) A characteristic length which is associated with replication, namely the replicon size λ_f. In other words (Hand, 1978), the characteristic length that concerns us here corresponds to a genetic element such as an episome, or chromosome, that replicates as a whole with a unique origin of replication (ori). It is at this particular DNA segment where the process of duplication of DNA is initiated. This process proceeds linearly until the element is copied. In Table 3 we have gathered together values of λ_f for a variety of organisms.

(ii) A characteristic length λ_t that may be associated with the transcription of a given gene. We take this length to be that of pre-messenger RNA (pre-mRNA).

TABLE 3

Values of the λ_f parameter for a variety of organisms

Organism	Type of cell	λ_f (average, kb)	References
Escherchia coli	Unicellular	$4{\cdot}2 \times 10^3$	1
Saccharomyce cerevisiae	Unicellular	36–108	2
Drosophila melanogaster	Somatic	40	1
D. melanogaster	Embryonic	9·7	3
Xenopus laevis	Somatic	200	1
X. laevis	Somatic	190	4
Triturus cristatus carnifex	Somatic	500–600	4
Triturus vulgaris	Spermatocyte	<150	4
Dipodomys ordii (kangaroo rat)	Somatic	<25	5
Cricetulus griseus	Somatic	<100	6
C. griseus	Ovary	<15	7
Homo sapiens	Somatic	100	8

References: 1, Lewin (1983); 2, Campbell (1986); 3, Kriegstein & Hogness (1974); 4, Callan (1972); 5, Hori & Lark (1976); 6, Huberman & Riggs (1968); 7, Hand (1972); 8, Falaschi *et al.* (1988).

The simplest hypothesis for constructing the dimensionless parameter μ in terms of the characteristic lengths λ_f and λ_t guided by the data in Tables 1 and 2, is that:

$$\mu = [(\lambda_f/\lambda_t]. \tag{2}$$

We consider in section 3 the physical bases that may support this conjecture. In other words, the hypothesis in eqn (1) may take the form:

$$r_f \approx [(\lambda_f/\lambda_t]r_t, \tag{3}$$

but we anticipate, in the next subsection, some possible bases for this formula.

2.2. TOWARDS THE MOLECULAR BASES OF THE KINEMATIC FORMULA

A remarkable aspect of polymerase dynamics may be appreciated by recalling the duration of the S phase in the life cycle of *Xenopus*: It is less than 25 min in early embryos compared with many hours in adult cells (Callan, 1972). Several experiments have yielded some data supporting the view that the control of the rate of DNA synthesis is accompanied by controlling the frequency with which new forks are initiated, rather than by controlling the speed r_f with which the forks move (Ward & Glaser, 1969; Blumenthal *et al.*, 1974). The remark on the apparent constant value of r_f should perhaps be seen in the context that once a leading strand starts at ori it advances continuously, while the bared opposite strand becomes the template for discontinuous synthesis of the lagging strand (Kornberg, 1978). From these remarks we would like to highlight two aspects of the r_f parameter:

(i) *r_f maintains a constant value*, even in the case of early embryogenesis, when the rate of DNA synthesis is greatly magnified.

(ii) *r_f represents a continuous rate of advancement*, in spite of the size of the multienzyme complex involved (i.e. the large mass of the replicating fork-about 1 MDa—does not seem to be a factor that may deviate r_f from keeping a continuous rate of advancement).

These two properties of the r_f parameter are remarkable, particularly if we recall that before the many polypeptides making up the replicating fork are assembled at or near ori, their effective motion may be assumed to have been random. This change in the constituent polypeptides suggests a physical phenomenon which will be introduced in the next subsection.

2.3. CAN PHASE TRANSITIONS OCCUR IN THE GENOME?

Several experiments concerning limiting factors on DNA replication may help to understand the molecular bases of the kinematic formula, eqn (3):

—Cell shape has been shown to be critical for DNA synthesis (Folkman & Moscona, 1978).

—The addition of a calcium dication ionophore to the intracellular medium has been shown to be a mitogenic factor for lymphocytes (Maino *et al.*, 1974).

—Cultures of non-transformed and transformed mammalian cells were induced to synthesize DNA by mere addition of $CaCl_2$ to the medium (Dulbecco & Elkington, 1975), even in the absence of additional growth factors.

—In rat liver-cell nuclei shape (measured in terms of volume) has been shown to display abrupt transitions as functions of ion concentration; these ion-induced abrupt transitions have also been shown to occur in chromatin structures (Nicolini *et al.*, 1984): This experiment has suggested to its authors treating the chromatin structural changes in terms of phase transitions. This proposal is in analogy with a form of condensed matter intermediate between solid and liquid, i.e. a gel, whose phase transitions were already described in terms of mean field theory (Sun *et al.*, 1980). In this context is should be pointed out that phase transitions have independently been shown to occur in biological systems other than the genome (Mizutani *et al.*, 1976). A cellular organelle, the melanosome, once isolated from human melanoma tumors, shows specific heat anomalies; the data was fitted to the usual equation:

$$C = \gamma T + \beta T^3 + \ldots .$$

A discontinuity at about 1, 9 K was observed; it is in fact the hallmark of a phase transition. To sum up, the above experiments suggest to us two important new ideas:

(i) *Phase transitions may occur in the genome*, and

(ii) *This phenomenon may be discussed in terms of mean field theory.*

2.4. POLYMERASE DYNAMICS MAY BE UNDERSTOOD BY MEANS OF A PHASE TRANSITION IN THE GENOME

In view of the two ideas mentioned in section 2.3 [i.e. (i) and (ii)], we make the following assumptions:

(i) Once DNA synthesis is initiated, a phase transition may occur in active chromatin. We should recall that inactive chromatin is complexed with various transcription and initiation factors. This particular form of condensed matter, to wit, active chromatin, is assumed to undergo a phase transition, which shall be described in detail in section 3.3 below. In other words, instead of drawing on an analogy with gels (cf. section 2.3), which helped us to understand the phase transition induced by raising ion levels in the nucleus, we now draw on an analogy with other forms of condensed matter (cf. section 3.1 below).

(ii) We treat, in the Appendices, our second type of phase transitions with mean field theory, based on an earlier work (Ginzburg & Landau, 1950).

3. The Second Issue in Polymerase Dynamics: What are the Molecular Bases Underlying the Kinematic Formula?

3.1. CHROMATIN MAY BE ANALOGOUS TO OTHER FORMS OF CONDENSED MATTER WHERE THERMODYNAMIC AND MACROSCOPIC-QUANTUM-MECHANICAL CONCEPTS MAY BE APPLIED

Two aspects of chromatin structure deserve particular attention:

(i) The packing ratio η of chromatin may be conveniently defined as:

$$\eta = L_1/L_2 \tag{4}$$

where L_1 denotes DNA length in the fully extended state, and L_2 denotes DNA length in the coiled, or folded state achieved at any state of condensation. For example, (Finch & Klug, 1976), in the nucleosome we have 166 base pairs (b.ps) of DNA rolled over a histone core; in this case:

$$L_1 \cong 600 \text{ Å}.$$

This DNA segment is compressed into a coil of height

$$L_2 \cong 55 \text{ Å}.$$

Thus, with eqn (4) we find the packing ratio:

$$\eta \text{ (nucleosome)} \cong 10. \tag{5}$$

A further example illustrates the point better; in heterochromatin (Weisbord, 1982):

$$\eta \text{ (heterochromatin)} \cong 4000. \tag{6}$$

These two examples illustrate the intimate relationship between the processes of DNA replication and packaging.

(ii) The tight packing of chromatin, as evidenced by the highest values of η yields DNA concentrations (denoted by ρ) within localized regions of interphase nuclei of (Olins & Olins, 1974),

$$\rho \cong 200 \text{ g cm}^{-3}. \tag{7}$$

The above two remarks, (i) and (ii) invite comparison between chromatin and various forms of condensed matter, in which comparable densities may occur. The disordered structure of chromatin (i.e. lacking for instance translational invariance) are reminiscent of disorder that does occur in amorphous materials. For this reason in this, and following sections, we approach the problem of polymerase dynamics in terms of thermodynamics, since this subject is well-known to apply satisfactorily to all forms of condensed matter. Thermodynamics applies particularly well to phenomena typical of macroscopic quantum mechanics, such as superconductivity (Chela-Flores *et al.*, 1988), or superfluidity (Chela-Flores & Ghassib. 1986). In the following subsections we discuss the possible relevance of macroscopic quantum mechanics in molecular genetics.

3.2. A THERMODYNAMIC APPROACH APPLICABLE TO PHASE TRANSITIONS IN MANY FORMS OF CONDENSED MATTER MAY WELL APPLY TO CHANGES IN CHROMATIN STRUCTURE DUE TO GENE EXPRESSION

We base our approach to polymerase dynamics on the following question:

What happens to the inactive chromatin free energy per unit volume when it is turned into active chromatin?

In order to answer this question we recall that according to Gibbs all systems change in such a way that the free energy G (per unit volume as are all equations listed) is minimized. An expression for changes ΔG, in the Gibbs free energy, which is given in Appendix A may, however, be anticipated from the following remarks:

(i) ΔG may depend explicitly on temperature:

$$\Delta G = \Delta G(T) \tag{8}$$

since *normal* transcription (i.e. transcription at the homeostatic temperature T_H) generally occurs below a certain temperature T_{hsp}, at which heat-shock proteins (hsps) are synthesized (Lindquist, 1986): $T_{hsp} < T_H$ (at T_{hsp} inhibition of normal genes occur, while new hsps are induced). Therefore, we may conclude that ΔG depends on temperature T.

(ii) ΔG may depend on chromatin compaction, since replication is delayed for high values of the packing ratio η, for instance for heterochromatin (Lima-de-Faria & Jaworska, 1968). Hence,

$$\Delta G = \Delta G(\rho). \tag{9}$$

In section 4.3 we will appreciate that at the onset of activation of ori, there is a significant change in the structure of inactive chromatin. We assign a Gibbs free energy G_0 to inactive chromatin, and G to active chromatin; we then treat this significant structural change as a phase transition (cf. section 2.4). The change in the system will be denoted by:

$$\Delta G = G - G_0. \tag{10}$$

On the other hand, since the process of initiating DNA replication at ori must occur spontaneously, then G should be lower than G_0:

$$\Delta G < 0. \tag{11}$$

The expression ΔG depends on the concentrations of the reactants: In the present example of DNA replication coupled to transcription, the reactants are:

—*For DNA replication*: The DNA polymerase complex (i.e. the replicating fork, or replisome), and the parental strand to be replicated.

—*For transcription*: The RNA polymerase (and the various transcription factors), and the DNA segment to be transcribed into pre-mRNA.

To sum up: ΔG in eqn (10) indicates how far the reaction is from equilibrium. It is given a very negative value [cf. eqn (11)], due to a large extent to the hydrolysis of abundant ATP molecules which, in turn, arose from efficient conversion of ADP and P_i in mitochondria. A specific form of ΔG in eqn (10) is discussed in section 3.5, and developed in the appendices.

3.3. A PHASE TRANSITION MAY OCCUR WHEN THE POLYMERASES INTERACT WITH CHROMATIN

The hypothesis we have been led to in the work above is that in chromatin there may occur a phase transition as in other forms of condensed matter, namely in the solid state (superconductivity), or in the liquid state (superfluidity). Indeed, this possibility was first raised by Delbrück, who was considering the question "whether or not something very peculiar from the quantum mechanical point of view, like superconductivity or superfluid helium, will come up. If strange cooperative phenomena can happen at room temperature in very special molecules . . . , then certainly life would have discovered this" (Delbrück, 1963).

The "strange cooperative phenomena" referred to by Delbrück is normally called a phenomenon of (Bose) condensation. However, since in genetics the term condensation sometimes is used when referring to various degrees of chromatin compaction, we prefer to reserve the term "condensation" to the well-known physical phenomena (two examples of which were already alluded to by Delbrück). Condensation was invoked again in modeling the cell membrane (Fröhlich, 1977). In the question of the origin of life, the possibility of condensation occurring in genetics was first raised a few years ago (Chela-Flores, 1985).

The most recent application of condensation in biology is in the problem of the origin of chirality in amino acids (Salam, 1991). In this work the state of an amino acid has been represented by a second quantized field: What was done more precisely was to take the wavefunction for the electronic ground state of one enantiomer of a chiral molecule (MacDermott & Tranter, 1989). In the present work we prefer to use a somewhat less microscopic concept, that of an effective amino acid wavefunction $\psi(x)$, which is later identified with an order parameter for the onset of condensation.

Since condensation implies that every state is given by the same ψ (Chela-Flores, 1975), then the whole polypeptide complex making up any of the polymerases involved in the r_f and r_t parameters is given by an ψ wavefunction. We assign the complex functions ψ_f for the replication fork, and ψ_t for the RNA polymerase associated with r_t (i.e. RNA polymerase for prokaryotes and RNA polymerase II for eukaryotes). Then, following a standard procedure (Ginzburg & Landau, 1950), we write the complex variable ψ as a modulus R and a phase S:

$$\psi_f = R_f \exp(iS_f) \tag{12}$$

$$\psi_t = R_t \exp(iS_t) \tag{13}$$

and equate the "superfluid" velocity with the gradient of the phase of the wavefunction:

$$r_f = |-ih\nabla S_f|/m_f \tag{14}$$

$$r_t = |-ih\nabla S_t|/m_t. \tag{15}$$

Then, if the phase transition is of the second-order (i.e. as in the case of superconductivity), we may identify ψ_f and ψ_t with the order parameter in the expansion of eqn (10), corresponding to the free energy for either polymerase. For instance,

$$\Delta G_f = \Delta G_f(\psi_f, \nabla\psi_f) \tag{16}$$

$$\Delta G_t = \Delta G_t(\psi_t, \nabla\psi_t). \tag{17}$$

Assuming that the homeostatic temperature of a given organism (T_H) is beneath the critical temperature (T_c) for the phase transition into the condensed mode, we may expand ΔG near T_c, as we have done in Appendix A [cf. eqn (A.1)]. This work provides us with convenient expressions for the evolution of $\psi_{f,t}$, where we have used an abbreviated expression for both order parameters. This, in turn implies that we can study the evolution of the $S_{f,t}$. Finally, in view of the expressions in eqns (14) and (15), we are able to obtain expressions for the genetically relevant parameters, to wit, r_f and r_t.

3.4. A CONSEQUENCE OF THE HYPOTHESIS OF POLYMERASE ACTIVITY TRIGGERING A PHASE TRANSITION FROM INACTIVE INTO ACTIVE CHROMATIN

We have assigned order parameters ψ_f and ψ_t to the replication fork and RNA polymerase respectively. According to the arguments given in section 3.1 these functions may depend on:

(i) The chromatin concentration ρ, and

(ii) On whether the chromosome is transcriptionally active (i.e. euchromatic), or transcriptionally repressed (i.e. heterochromatic). The order parameters satisfy, therefore, the following inequality:

$$|\psi_{f,t}(\rho, \text{ heterochromatin})| \gg |\psi_{f,t}(\rho, \text{ euchromatin})|. \tag{18}$$

3.5. A POSSIBLE DERIVATION OF THE KINEMATIC FORMULA

The evolution of the order parameters $\psi_{f,t}$ may be studied as functions of the chromatin concentration ρ and the position variable x (cf. Appendices A and B). With the further assumption that the degree of compaction of the polymerases is approximately equal in both cases [cf. Appendix C, eqn (C.3)], we may readily obtain:

$$r_f = \mu_0 \mu r_t \tag{19}$$

where, according to eqn (C.8), we have:

$$\mu_0 = (m_t/m_f) \approx 1. \tag{20}$$

On the other hand, according to eqn (C.5), we have:

$$\mu = \lambda_f/\lambda_t. \tag{21}$$

Hence, eqns (20) and (21) show that eqn (19) corresponds to the kinematic formula [cf. eqn (3)]. The approximate validity of eqn (19) will be further discussed in section 4, and confronted with some data that has been obtained from eukaryotes.

4. Discussion

4.1. LIMITATIONS TO THE APPLICATION OF THE KINEMATIC FORMULA

Since prokaryotes do not have histones playing a major role in gene expression, we omit their discussion in terms of the kinetic formula; the theoretical arguments sketched in the appendices are based on chromatin structure, and may not apply to these single cell organisms.

The interesting alterations of λ_f observed in *Drosophila* in embryonic cells (cf. Table 3), as compared with somatic cells (Kriegstein & Hogness, 1974) are not discussed in detail in terms of the kinematic formula; for it is clear that the particular embryonic stage in which the experiments were performed was prior to the mid-blastula transition: This particular stage of ontogenesis represents an abrupt physiological change, in which the onset of appreciable transcription is initiated by RNA

TABLE 4

Values of the gene length (i.e. the length of the primary transcript λ_t), for a selection of different genes and organisms

Organism	Gene	(1) λ_t (kb)	(2) λ_t (kb)	(3) λ_t (kb)	(4) λ_t (kb)
Escherchia coli	rRNA	6	—	—	—
Yeast	rRNA	—	8	—	—
Plant (tobacco)	rRNA	—	8·3	—	—
Drosophila melanogaster	rRNA	7·7	8·6	—	—
Xenopus laevis	rRNA	7·9	8·3	—	—
Reptile (iguana)	rRNA	—	8·3	—	—
Birds	rRNA	—	12	10·5	—
Marsupial (potoroo)	rRNA	—	12·6	—	—
Rodent (mouse)	rRNA	13·7	—	—	—
Homo sapiens	rRNA	13·7	—	—	—
H. sapiens	β-globin	—	—	—	1·5
H. sapiens	insulin	—	—	—	1·7
H. sapiens	PKC	—	—	—	11
H. sapiens	Albumin	—	—	—	25
H. sapiens	Catalase	—	—	—	34
H. sapiens	LDL receptor	—	—	—	45
H. sapiens	Thyroglobulin	—	—	—	300
H. sapiens	Dystrophin	—	—	—	>2000

References: 1, Lewin (1980); 2, Perry *et al.* (1970). These authors give λ_t (rRNA) in terms of molecular weight; we have expressed the data in kb (1 bp $\approx 3{\cdot}26 \times 10^2$ Da); 3, Long & Dawid (1980); 4, Alberts *et al.* (1989: 486).

Abbreviations: LDL, low-density lipoprotein; PKC, protein kinase C; rRNA, ribosomal RNA.

polymerase II and III; RNA polymerase I begins slightly later, at least for *Xenopus* (Watson *et al.*, 1987: 756), thereby rendering a discussion of the *Drosophila* developmental data futile, since the formula applies to the *coupled* processes of transcription and DNA replication. For the below we need the values of gene length (λ_t), given in Table 4.

4.2. EXPERIMENTAL SUPPORT OF THE KINEMATIC FORMULA

In this subsection we consider the experimental evidence that, to a certain extent, may support the kinematic formula [cf. eqn (3)]. In Table 5 we have gathered some values of the polymerase masses for both prokaryotes and eukaryotes. This evidence does not rule out the approximate validity of eqn (19), for the following reasons:

(i) In prokaryotes the molecular weight of DNA polymerase III is about 8×10^5 Da (Darnell *et al.*, 1990: 461); thus, we may take:

$$m_f^{(\mathrm{prok})} \approx 8 \times 10^5 \text{ Da},$$

whereas in yeast (Watson *et al.*, 1987: 566):

$$4 \text{ kDa} < m_f^{(\mathrm{euk})} < 6 \text{ kDa}.$$

TABLE 5

Values of the masses (molecular weights, in daltons) of the RNA polymerases for both prokaryotic and eukaryotic cells; in particular, values for the eukaryotic yeast cells are also tabulated

Organism or type of cell	m_t (Da)	m_f (Da)
Prokaryotic	$(4{\cdot}5–5) \times 10^{5\,(1,2)}$	$8 \times 10^{5\,(3)}$
Eukaryotic	$(5–6) \times 10^{5\,(4)}$	$\delta_1 > (1{\cdot}6–1{\cdot}7) \times 10^{5\,(5)}$
Eukaryotic: yeast	—	$(4{\cdot}6–6) \times 10^{5\,(6)}$

References: 1, Watson *et al.* (1987: 366); 2, Darnell *et al.* (1990: 231); 3, Darnell *et al.* (1990: 461); 4, Sawadago & Sentenac (1990); 5, Sitney *et al.* (1989). (The value reported in this table applies only for the core catalytic subunit); 6, Watson *et al.* (1988: 566) (This range of values corresponds to each of the three DNA polymerases.)

(ii) In prokaryotes the mass of RNA polymerase is given by (Watson *et al.*, 1987: 366; Darnell *et al.*, 1990: 231):

$$4{\cdot}5 \text{ kDa} < m_t^{(\text{prok})} < 5 \text{ kDa}$$

whereas for the yeast cell (Watson *et al.*, 1987: 566):

$$4 \text{ kDa} < m_t^{(\text{euk})} < 6 \text{ kDa}.$$

Therefore, from (i) and (ii) we may assume the approximate equality $m_f \approx m_t$ and we may then infer then, from eqn (19) that,

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

thus justifying the approximate validity of the kinematic formula, eqn (3).

4.3. CHROMATIN STRUCTURAL CHANGES MAY BE AN IMPORTANT MECHANISM IN THE REGULATION OF DNA REPLICATION AND TRANSCRIPTION

There are some general considerations on DNA replication and transcription in chromosomes that may help to clarify the preliminary understanding of polymerase dynamics, which we have initiated in this note:

(i) The time required to replicate all DNA and hence, the duration of the S phase in somatic cells, seems to be controlled by the distribution of oris, rather than by any change in r_f (Blumenthal *et al.*, 1974; Ward & Glaser, 1969).

(ii) Origin (of DNA replication) spacings may be determined by some aspect of chromomeric structure in the interphase chromosomes (Blumenthal *et al.*, 1974), a view which is reinforced by the fact that heterochromatization of the regions next to the centromere- observed in somatic cells- does not appear in embryonic cells. This is in agreement with the observed disappearance of centromeric heterochromatization after the onset of *Drosophila* embryonic cellularization as the blastoderm stage is initiated.

To sum up, the relative invariance of r_f may be controlled by specific chromatin structural changes.

(iii) It also seems likely that the stimulatory effect of the transcriptional elements on DNA replication may be due, at least in part, to effects of chromatin structural changes. Some evidence in this respect is the following: In Simian Virus 40 (SV40) genomes the binding of transcriptional activator proteins may perturb the local distribution of nucleosomes, so that the DNA in the adjacent core region is relatively nucleosome free, thereby allowing initiation proteins, such as the T antigen, to interact with the core origin (Challberg & Kelly, 1989); in fact, it may be that even when there is no concurrent DNA replication, the nucleosome structure may be transiently and locally disrupted during the act of transcription (Keller *et al.*, 1977).

4.4. STRUCTURAL CHANGES DUE TO CHROMATIN ACTIVITY SUGGESTS A NEW EFFECT

Replication over nucleosomes demands major chromatin structural changes (Alberts & Sternglanz, 1977), which may be considered as an impediment for RNA polymerase movement. This phenomenon hints at possible variations in r_f, which shall be discussed below [cf. eqn (23)]. The changes in chromatin are not only bound to the replicating process of DNA, but there also seems to be a difference related to transcription in the manner of spacing the nucleosomes:

(i) The transcriptionally active chromatins (e.g. yeast and HeLa cells) may have linker DNA of variable length, while the transcriptionally inactive chromatins (for instance, chicken erythrocyte) have more regular linker DNA (Lohr *et al.*, 1977).

(ii) The presence of highly-mobile group proteins in the spacer region of chromatin might determine the position and length of the spacer regions themselves, thus contributing to the unique structure of transcriptionally active regions of chromatin. This phenomenon has been confirmed by experiments with cells from rainbow trout testis (Levy *et al.*, 1977).

4.5. SIGNIFICANT ALTERATIONS IN r_f MAY OCCUR IN ACTIVE CHROMATIN

When a sample of various genes from a single organism is analyzed, for instance, in the case of *Homo sapiens* (cf. Table 4), we have to recall that experiments impose upon us at least the following set of criteria:

(i) The r_f parameter is given approximately by the values reported in Table 1.

(ii) The replicon size, for organisms at a definite stage of ontogenesis (for instance, in the adult form) is given by the values reported in Table 3.

In order to retain (i) and (ii), and understand some aspects of the diversity of values in Table 6, we are led to conclude that r_t may vary according to the length of the gene being transcribed. In Table 2 we have given a reasonable value of r_t, which is expected to be valid in eukaryotes.

TABLE 6

A sample of values of the r_f *parameter. Calculations were done using the kinematic formula* [*eqn* (3)] *in the following cases*: (i) *For a single gene* (*rDNA*) *in various organisms*. (ii) *For a sample of genes in a single organism* (Homo sapiens). *The standard replicon size is taken here to be about* 100 *kb* (*Falaschi* et al., 1988). *In the calculation for lower mammals* λ_t *was taken for Chinese hamster and* λ_t *for marsupial*

Organism	Gene	r_f in kb/min/f (somatic calculated)	Data from tables:
Yeast	rRNA	2·7	2, 3, 4
Drosophila	rRNA	1	2, 3, 4
Xenopus	rRNA	4·6	2, 3, 4
Lower mammals	rRNA	<1·6	2, 3, 4
Homo sapiens	rRNA	1·5	2, 3, 4
H. sapiens	β-globin	13	2, 3, 4
H. sapiens	Insulin	12	2, 3, 4
H. sapiens	PKC	1·8	2, 3, 4
H. sapiens	Albumin	0·8	2, 3, 4
H. sapiens	Catalase	0·6	2, 3, 4
H. sapiens	LDL receptor	0·4	2, 3, 4
H. sapiens	Thyroglobulin	0·1	2, 3, 4
H. sapiens	Dystrophin	<0·01	2, 3, 4

On the other hand, the data of Tables 1 to 6 implies that in a certain range of values of the λ_t parameters, for instance, for

$$\lambda_t \gg 100\ \text{kb} \tag{22}$$

the structure of chromatin may act as a source of retardation for RNA polymerase; this may be understood as follows: Since the chromatin repeat length r_l is such that

$$r_l \approx 200\ \text{bp}$$

then for a gene such as dystrophin (cf. Table 4), RNA polymerase will have to proceed past some thousand nucleosomes, which will be a factor retarding its rate of movement. Therefore, we are led to consider the possibility that:

$$r_t = r_t(\lambda_t). \tag{23}$$

This effect may be tested, by recalling that the r_f parameter may generally be assumed to be constant (cf. section 2.2); thus, for instance, for *H. sapiens* we may use the data in Table 4, and infer [using eqn (3)]:

$$4r_t\,(\text{PKC}) \approx r_t\,(\text{LDL receptor}). \tag{24}$$

5. Conclusions

In Table 6 we have gathered together the numerical implications of the kinematic formula [eqn (3)]. Some aspects of these results deserve some comments:

For some genes in *H. sapiens* the kinematic formula explains the data satisfactorily (e.g. for RNA and PKC); but it should be recalled that the expected value of r_t for eukaryotes (cf. Table 2) is only intended to be an approximate result. The same reasonable agreement may be claimed for lower mammals. On the other hand, although the *Drosophila* and yeast numerical results are rather small, no definite conclusion may be inferred in these two cases due to our only approximate knowledge of the r_t parameter. The more interesting aspect of the data is the radical deviation obtained, for instance, for dystrophin (i.e. two orders of mágnitude). In these extreme cases two possibilities may still arise:

(i) The kinematic formula is indeed approximately valid; this situation forces upon us the new effect predicted in eqn (23), which would then be valid in gene expression. This phenomenon, we have argued, may be due to chromatin structural changes. The continuous and constant nature of r_f is supported by the well-established genetic control on the multiplicity of oris, rather than the control of the r_f parameter itself (cf. section 2.2). The variability of r_t [cf. eqn (23)] is hinted at the molecular level by the variability of the length of linker DNA in transcriptionally active chromatin, which displays a unique conformation [cf. section 4.4].

(ii) The theory supporting the approximate validity of the kinematic formula may still be correct (i.e. condensation may be present in active chromatin structural changes, which may occur due to the coupled processes of replication and transcription); but the effect predicted in eqn (23) is not seen in experiments. In this case a more rigorous solution to the equation of motion, beyond the linear approximation, may be needed [cf. eqn (B.5)].

Although the theory sketched in the appendices can clearly be made more rigorous, the objective of the present note is limited only to pointing out a possible approach to the important problem of identifying the mechanisms governing polymerase dynamics: We are suggesting that condensation in chromatin (in the sense of sections 3.1 and 3.3) may underlie the kinetics of polymerase advancement.

Finally, we would like to mention that the new effect predicted in eqn (23) may be tested by measuring fluctuations in the r_t parameter for the transcription of two genes in *H. sapiens*, as pointed out in eqn (24).

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REFERENCES

Alberts, B., Bray, D., Lewis, J., Raff, M., Roberts, K. & Watson, J. D. (1989). *Molecular Biology of the Cell* 2nd edn. p. 202. New York: Garland.

Alberts, B. & Sternglanz, R. (1977). *Nature, Lond.* **269**, 655–661.

Blumenthal, A. B., Kriegstein, H. J. & Hogness, D. S. (1974). *Cold Spring Harbor Symp. quant. Biol.* **38**, 205–223.

Brewer, B. (1988). *Cell* **53**, 679–686.

Callan, H. G. (1972). *Proc. R. Soc. Lond.* **B181**, 19–41.

Callan, H. G. (1974). *Cold Spring Harbor Symp. quant. Biol.* **38**, 195–203.

Campbell, J. (1986). *Ann. Rev. Biochem.* **55**, 733–771.

Challberg, M. D. & Kelly, T. J. (1989). *Ann. Rev. Biochem.* **58**, 671–717.

Chela-Flores, J. (1975). *J. Low Temp. Phys.* **21**, 307–319.

Chela-Flores, J. (1985). *J. theor. Biol.* **117**, 107–118.

Chela-Flores, J. & Ghassib, H. B. (1986). *Int. J. Theor. Phys.* **25**, 273–291.

Chela-Flores, J., Saif, A. & Shehata, L. N. (1988). *J. Low Temp. Phys.* **71**, 295–310.

Darnell, J. E. (1982). *Nature, Lond.* **297**, 365–371.

Darnell, J., Lodish, H. & Baltimore, D. (1990). *Molecular Cell Biology*. 2nd edn. New York: W. H. Freeman & Co.

Delbrück, M. (1963). In: *Commemoration of the 50th Anniversary of Niels Bohr's Papers on Atomic Constitution*, Session on Cosmos and Life. Copenhagen: Institute for Theoretical Physics. pp. 41–67.

Dulbecco, R. & Elkington, J. (1975). *Proc. natn. Acad. Sci. U.S.A.* **72**, 1584–1588.

Falaschi, A., Biamonti, G., Cobianshi, F., Csordas-Toth, E., Faulkner, G., Giacca, M., Pedacchia, D., Perini, G., Riva, S. & Tribioli, C. (1988). *Biochim. Biophys. Acta* **951**, 430–442.

Finch, J. T. & Klug, A. (1976). *Proc. natn. Acad. Sci. U.S.A.* 73, 1897–1901.

Folkman, J. & Moscana, A. (1978). *Nature, Lond.* **273**, 345–349.

Fröhlich, H. (1977). *Rivista del Nuovo Cimento* **7**, 399–418.

Ginzburg, V. L. & Landau, L. D. (1950). *Soviet Phys. JETP* **20**, 1064. (Reprinted in Ter Haar (1965). pp. 546–568.)

Hand, R. (1978). *Cell* **15**, 317–325.

Hori, T. I. & Lark, K. G. (1976). *Nature, Lond.* **259**, 504–505.

Huberman, J. A. & Riggs, A. D. (1968). *J. molec. Biol.* **32**, 327–341.

Keller, W., Müller, U., Eiken, I., Wendel, I. & Zentgraf, H. (1977). *Cold Spring Harbor Symp. quant. Biol.* **42**, 227–244.

Kornberg, A. (1978). *Cold Spring Harbor Symp. quant. Biol.* **43**, 1–9.

Kornberg, A. (1988). *J. biol. Chem.* **263**, 1–4.

Kriegstein, H. J. & Hogness, D. S. (1974). *Proc. natn. Acad. Sci. U.S.A.* **71**, 135–139.

Laskey, R. A., Fairman, M. P. & Blow, J. J. (1989). *Science* **246**, 609–614.

Levy, B., Wong, N. C. W. & Dixon, G. H. (1977). *Proc. natn. Acad. Sci. U.S.A.* **74**, 2810–2814.

Lewin, B. (1980). *Gene Expression* **3**, p. 867. New York: Wiley.

Lewin, B. (1983). *Genes* III. 3rd edn. p. 302. New York: Wiley.

Lima-De-Faria, A. & Jaworska, H. (1968). *Nature, Lond.* **217**, 138–142.

Lindquist, S. (1986). *Ann. Rev. Biochem.* **55**, 1151–1191.

Lohr, D., Corden, J., Tatchell, K., Kovacic, R. T. & Van Holde, K. E. (1977). *Proc. natn. Acad. Sci. U.S.A.* **74**, 79–83.

Long, E. O. & Dawid, I. B. (1980). *Ann. Rev. Biochem.* **49**, 727–764.

MacDermott, A. J. & Tranter, G. E. (1989). *Croatica Chem. Acta* **62** (2A) 165–187.

Maino, V. C., Green, N. M. & Crumpton, M. J. (1974). *Nature, Lond.* **251**, 324–327.

Mizutani, U., Massalski, T. B., McGinnes, J. E. & Corry, P. M. (1976). *Nature, Lond.* **259**, 505–507.

Nicolini, C., Carlo, P., Finollo, R., Vigo, F., Cavazza, B., Ledda, A., Ricci, E. & Brambilla, G. (1984). *Cell Biophys.* **6**, 183–196.

Olins, A. L. & Olins, D. E. (1974). *Science* **183**, 330–332.

Pato, M. L. (1975). *J. Bact.* **123**, 272–277.

Perry, R. P., Cheng, T.-Y., Freed, J. J., Greenberg, J. R., Kelly, D. E. & Tartof, K. D. (1970). *Proc. natn. Acad. Sci. U.S.A.* **65**, 609–616.

Salam, A. (1991). *J. molec. Evol.* **33**, 105–113.

Sawadogo, M. & Sentenac, A. (1990). *Ann. Rev. Biochem.* **59**, 711–754.

Sitney, K. C., Budd, M. E. & Campbell, J. L. (1989). *Cell* **56**, 599–605.

Sun, S.-T., Nishio, I., Swislow, G. & Tanaka, T. (1980). *J. Chem. Phys.* **73**, 5971–5975.

Ter Haar, D. (1965). *Collected Papers of L. D. Landau*. Oxford: Pergamon.

Ward, C. B. & Glaser, D. A. (1969). *Proc. natn. Acad. Sci. U.S.A.* **64**, 905–912.

Watson, J. D., Hopkins, N. H., Roberts, J. W., Steitz, J. A. & Weiner, A. M. (1987). *Molecular Biology of the Gene* 4th edn. p. 754. Menlo Park, CA: Benjamin/Cummings.

Weisbord, S. (1982). *Nature, Lond.* **297**, 289–295.

On the other hand, at the onset of replication (transcription) we may consider the Gibbs free energy expressions per unit volume; since the order parameters vanish, we may write:

$$g_{f,t} = g_{f,t}(0), \qquad g_{f,t}^{\text{kin}} = 0. \tag{A.7}$$

We let the further contributions to the free energies (arising from factors other than the polymerases themselves) be denoted by $E_{f,t}$. Therefore, the general form of the Gibbs free energy ($\tilde{G}$) may be written as:

$$\tilde{G} = g_{f,t} + E_{f,t} \tag{A.8}$$

where $g_{f,t}$ is given by eqn (A.3). Once again, at the ori and at the starting point of transcription we are led to a second pair of constraints:

$$\alpha_{f,t}|\psi_{f,t}(\rho, 0)|^2 + (\beta_{f,t}/2)|\psi_{f,t}(\rho, 0)|^4 + E_{f,t} = 0. \tag{A.9}$$

Therefore, from eqns (A.6–A.9) we obtain:

$$E_{f,t} = \alpha_{f,t}^2/2\beta_{f,t}. \tag{A.10}$$

Using the first pair of constraints in the equations of motion (A.4), we obtain

$$\begin{aligned}(h^2/2m_{f,t})\nabla^2\psi_{f,t}(\rho, \mathbf{x}) = {} & -\beta_{f,t}|\psi_{f,t}(\rho, 0)|\psi_{f,t}(\rho, \mathbf{x}) \\ & + \beta_{f,t}|\psi_{f,t}(\rho, \mathbf{x})|^2\psi_{f,t}(\rho, \mathbf{x})\end{aligned} \tag{A.11}$$

which may be conveniently written as

$$\begin{aligned}(h^2/2m_{f,t})\nabla^2\psi_{f,t}(\rho, \mathbf{x}) = {} & \beta_{f,t}[-|\psi_{f,t}(\rho, 0)|^2 \\ & + |\psi_{f,t}(\rho, \mathbf{x})|^2]\psi_{f,t}(\rho, \mathbf{x}).\end{aligned} \tag{A.12}$$

Using a new rescaled parameter:

$$\Psi_{f,t}(\rho, \mathbf{x}) = \psi_{f,t}(\rho, \mathbf{x})/\psi_{f,t}(\rho, 0) \tag{A.13}$$

we are led to a convenient form of the equations of motion:

$$\nabla^2\Psi_{f,t} = \kappa_{f,t}^2[-1 + |\Psi_{f,t}|^2]\Psi_{f,t} \tag{A.14}$$

where,

$$\kappa_{f,t}^2 = (2m_{f,t}\beta_{f,t}/h^2)|\psi_{f,t}(\rho, 0)|^2. \tag{A.15}$$

However, the conditions $\alpha_{f,t} < 0$ must be taken into account because at the minimum of the Gibbs function (i.e. $\delta\tilde{G}_{f,t} = 0$):

$$\Delta G < 0 \tag{A.16}$$

Hence, writing $|\alpha_{f,t}| = a_{f,t}$, in eqn (A.6), we obtain:

$$a_{f,t} = \beta_{f,t}|\psi_{f,t}(\rho, 0)|^2. \tag{A.17}$$

From eqns (A.17) and (A.10) we may solve for $\beta_{f,t}$:

$$\beta_{f,t} = 2E_{f,t}/|\psi_{f,t}(\rho, 0)|^4. \tag{A.18}$$

Then, introducing eqn (A.18) in eqn (A.15), we obtain:

$$\kappa_{f,t}^2 = 4m_{f,t}E_{f,t}/h^2|\psi_{f,t}(\rho, 0)|^2. \tag{A.19}$$

APPENDIX B

An Approximate Solution of the Equations of Motion

We are now in a position to search for solutions of eqn (A.14) close to the initiation of genetic activity (i.e. DNA replication at ori, or initiation of transcription).

For this purpose we write the $\Psi_{f,t}$ parameters in terms of their modulii and phases:

$$\Psi_{f,t} = R_{f,t} \exp(iS_{f,t}). \tag{B.1}$$

Since the problems of both DNA replication and transcription are essentially unidimensional, we may replace the $\mathbf{x}$ variable by the unidimensional x variable, but instead it is more convenient to work with a dimensionless variable z, where

$$z_{f,t} = x/\lambda_{f,t}, \tag{B.2}$$

In the one-dimensional approximation the equations of motion (A.14) may be rewritten keeping in mind eqns (B.1) and (B.2):

$$\lambda_{f,t}^{-2}\Psi''_{f,t} \equiv \lambda_{f,t}^{-2}\{R''_{f,t} + 2iR'_{f,t}S'_{f,t} + iR_{f,t}S''_{f,t} - R_{f,t}(S'_{f,t})^2\} \exp(iS_{f,t}) \tag{B.3}$$

where the prime symbol means differentiation with respect to the z-variables. This result represents the left-hand side of the equation of motion (A.14), since clearly:

$$d^2/dx^2 = \lambda_{f,t}^{-2}(d^2/dz_{f,t}^2). \tag{B.4}$$

We may linearize eqn (A.14) sufficiently close to the phase transition; thus, the right-hand side of the linearized equation of motion is:

$$-\kappa_{f,t}^2 R_{f,t} \exp(iS_{f,t}). \tag{B.5}$$

Equating both sides of the equations of motion [i.e. eqns (B.3) and (B.5)], we obtain four real equations by equating real and imginary parts:

$$R''_{f,t} - R_{f,t}(S'_{f,t})^2 = -\kappa_{f,t}^2\lambda_{f,t}^2 R_{f,t} \tag{B.6}$$

as well as:

$$2R'_{f,t}S'_{f,t} + R_{f,t}S''_{f,t} = 0. \tag{B.7}$$

We study a particular integral of this pair of coupled differential equations:

$$R_{f,t} = \text{constant} \tag{B.8a}$$

$$S'_{f,t} = \kappa_{f,t}\lambda_{f,t}. \tag{B.8b}$$

From the definition of the velocity of propagation of the replicating fork [cf. eqn (14)] we find:

$$r_{f,t}=(h\kappa_{f,t}/m_{f,t})\lambda_{f,t}. \qquad \text{(B.9)}$$

Finally, eqns (B.9) and (A.19) imply:

$$r_{f,t}=2|\psi_{f,t}(\rho,0)|^{-1}(E_{f,t}/m_{f,t})^{1/2}\lambda_{f,t}. \qquad \text{(B.10)}$$

Alterations of the r_f Parameter

Under the hypothesis that the degree of compaction of the polymerases is approximately invariant, we have:

$$|\psi_f(\rho,0)|^2\approx|\psi_t(\rho,0)|^2. \qquad \text{(B.11)}$$

Then we may estimate the quotient r_t/r_f from eqns (B.10) and (B.11):

$$r_t/r_f=(\lambda_t/\lambda_f)(E_t m_f/E_f m_t)^{1/2}. \qquad \text{(B.12)}$$

Defining the dimensionless parameter μ_0 as:

$$\mu_0=(\lambda_f/\lambda_t)(E_f m_t/E_t m_f)^{1/2}. \qquad \text{(B.13)}$$

We remark that for a given event of gene expression all the parameters appearing in the formula in eqn (B.13) are invariant. For illustrative purposes we may consider gene expression in the early (cleavage stage) embryo of *Drosophila*:

$$r_t\ll r_f \qquad \text{(B.14)}$$

but the total activation energy (E_{TOT}) required for this particular process is entirely devoted to replication (since transcription has not yet started):

$$E_{\mathrm{TOT}}\approx E_f. \qquad \text{(B.15)}$$

On the other hand, the activation energy required for transcription, which only begins after cycle 10, is negligible:

$$E_t\approx\varepsilon \qquad \text{(B.16)}$$

where ε is a very small parameter. In fact, no appreciable mRNA is detected at this early stage of embryogenesis (Laskey *et al.*, 1989). Thus, combining eqns (B.12) and (B.13) we are led to eqn (1). Equation (2) will be derived in Appendix C, under the assumption of approximately equal masses for DNA and RNA polymerase.

APPENDIX C

The Velocities of Propagation of the Polymerases may be Expressed in Terms of Measurable Parameters

One difficulty in applying the formula in eqn (B.12) with the μ_0 parameter given by eqn (B.13) is that molecular biology is concerned mainly with given enzymatic activity during the various processes occurring in gene expression; yet, our modeling of DNA replication and transcription has retained the E_f and E_t energies, which are not easily measurable.

A possible remedy of this situation is to eliminate these variables: From eqns (A.19) and (B.11), we find:

$$\kappa_t^2\kappa_f^{-2} = m_tE_t(m_fE_f)^{-1}. \tag{C.1}$$

Then, recalling eqn (B.12) we may obtain from eqn (C.1) the following relation:

$$r_t/r_f = \lambda_t\kappa_t m_f(\lambda_f\kappa_f m_t)^{-1} \tag{C.2}$$

which may be conveniently written as:

$$r_f = \mu_0\mu r_t. \tag{C.3}$$

Here the μ parameters are given by:

$$\mu_0 = (\kappa_f/m_f)(\kappa_t/m_t)^{-1} \tag{C.4}$$

$$\mu = \lambda_f/\lambda_t. \tag{C.5}$$

The expansions of the Gibbs free energies (cf. Appendix A) are valid for temperatures T_H close to the transition temperature T_c; here we have denoted the physiological homeostatic temperature of the given organisms by T_H. Thus, for

$$T_H \leq T_c \tag{C.6}$$

we may assume that the order parameters $|\Psi|$ are small in the domain of validity of the equations. We may approximate the κ^2 parameters .

$$\kappa_f^2 \approx k_t^2 \tag{C.7}$$

since such relation is suggested in a Ginzburg-Landau type of phenomenology by considering the κ parameters to represent the same phenomenon of condensation in biology. With the approximation (C.7), the μ_0 parameter is given by:

$$\mu_0 = (m_t/m_f) \approx 1, \tag{C.8}$$

under the assumption of approximate equality of m_t and m_f (cf. Table 5); we may now achieve our objective of deriving eqn (3) by combining eqns (C.8), (C.5), and (C.3).

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Erratum

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The expression for the ρ parameter in eqn (7) is incorrect as printed, and should read:

$$\rho \cong 200 \text{ mg ml}^{-1}. \tag{7}$$

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GENETICS, EVOLUTION, AND THE ORIGIN OF LIFE

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Alberts, B. & Sternglanz, R. (1977). Recent excitment in the DNA replication problem. Nature 269, 655-661.

Bada, J.L. (1985) Amino acid racemization dating of fossil bones. Ann. Rev. Earth Planet. Sci. 13, 241-268.

Bada, J.L. and Miller, S.L. (1987) Racemization and the origin of optically active organic compounds in living organisms. BioSystems 20, 21-26.

Bonner, W.A., Blair, N.E., and Dirbas, F.M. (1981) Experiments on the abiotic amplification of optical activity. Origins of Life 11, 119-134.

Blumenthal, A.B., Kriegstein, H.J. & Hogness, D.S. (1974). The units of DNA replication in *Drosophila melanogaster chromosomes*. Cold Spring Harbor Symp. Quant. Biol. 38, 205-223.

Brown,C.J., Ballabio, A., Rupert, J.L., Lafreniere, R.G., Grompe, M., Tonlorenzi, R., and Willard, H.F. (1991). A gene from the region of the human X inactivation centre is expressed exclusively from the inactive X chromosome. Nature 349, 38-44.

Brewer, B.J. (1988). When polymerases collide: Replication and transcriptional organization in the *E. coli* chromosome. Cell 53, 679-686.

Callan, H. G. (1972). Replication of DNA in the chromosomes of eukaryotes. Proc. R. Soc. Lond. B181, 19-41.

Callan, H. G. (1974). DNA replication in the chromosomes of eukaryotes. Cold Spring Harbor Symp. Quant. Biol. 38, 195-203.

Challberg, M.D. & Kelly, T.J. (1989). Animal virus DNA replication. Ann. Rev. Biochem. 58, 671-717.

Chambon, P. (1978) The Molecular Biology of the Eukaryotic Genome is Coming of Age. Cold Spring Harbor Symp. Quant. Biol. 42, 1209-1234.

Chela-Flores, J. (1985). Evolution as a collective phenomenon. J. Theor. Biol. 117, 107-118.

Chela-Flores, J. (1987a). Towards a collective biology of the gene. J. Theor. Biol. 126, 127-136.

Chela-Flores, J. (1987b). Collective biology of neoplastic disease in dicotyledonous plants. Acta Biotheorica (Leiden) 36, 241-246.

Chela-Flores, J. (1988). Gene expression as a collective phenomenon. In: Leite Lopes Festschrift-A pioneer physicist in the Third World. Eds. N. Fleury, J.A. Martin Simoes, and A. Troper. Singapore: World Scientific. pp. 252-265.

Chela-Flores, J. (1990). Evolution and epigenesis in a qualitative description of molecular genetics. In: J.J. Giambiagi Festschrift. Eds. H. Falomir, R.E. Gamboa Saravi, P. Leal Ferreira, and F.A. Schaposnik. Singapore: World Scientific Publishers. pp. 107-124.

Chela-Flores, J. (1991). Comments on a novel approach to the role of chirality in the origin of life. Chirality 3, 389-392

Chela-Flores, J. (1992a). Influence of Chromatin Molecular Changes on RNA Synthesis during Embryonic Development. Acta Biotheoretica 40, 41-49.

Chela-Flores, J. (1992b). Towards the Molecular Bases of Polymerase Dynamics. Journal of Theoretical Biology 154, 519-539.

Chela-Flores, J. (1992c). Erratum. J. Theor. Biol. 157, 269.

Chela-Flores, J. (1993). Spontaneous regulating mechanisms that may have led to the origin of life. In Proc.Conf. Chemical Evolution and the Origin of Life, Trieste, Italy October 26-30, 1992. Eds. C. Ponnamperuma and J. Chela-Flores. Hampton, Virginia, USA: A. Deepak Publishing. To be published.

Chela-Flores, J. and Espejo Acuña, C. (1990). On the possible effects of homeostatic shifts in human embryonic development. Acta Biotheoretica (Leiden) 38, 135-142.

Chela-Flores, J., Liquori, A.M., and Florio, A. (1988). A kinetic thermodynamic approach to genetic expression in heat-shock proteins. J. Theor. Biol. 134, 319-325.

Chyba, C.F. (1990). Extraterrestial amino acids and terrestial life. Nature 348, 113-114.

Crick, F. (1981). Life Itself. London: MacDonald & Co.

Darnell, J.E. (1982). Variety in the level of gene control in eukaryotic cells. Nature 297, 365-371.

Darnell, J., Lodish, H. & Baltimore, D. (1990). Molecular Cell Biology. 2nd ed. New York: W.H. Freeman & Co.

Darwin, C. (1859). The origin of species by means of natural selection or the preservation of favoured races in the struggle for life. London: John Murray.

Delbrück, M. (1963). In: Conmemoration of the 50th. Anniversary of Niels Bohr's Papers on Atomic Constitution, Session on Cosmos and Life. Copenhaguen: Institute for Theoretical Physics. pp. 41-67.

Diener, T.O. (1979). Viroids: Structure and function. Science 205, 859-866.

Dyson, F. (1985). Origins of life. p.12. Cambridge: Cambridge University Press.

Eigen, M., Lindemann, B.F., Tietze, M., Winkler-Oswatitsch, R., Dress, A. and von Haeseler, A. (1989). How old is the genetic code? Statistical geometry of tRNA provides an answer. Science 244, 673-679.

Ernst, W.B. (1983). The early Earth and the archaean rock record. In: Schopf, (1983).

Falaschi, A., Biamonti, G., Cobianchi, F., Csordas-Toth, E., Faulkner, G., Giacca, M., Pedacchia, D., Perini, G., Riva, S. & Tribioli, C. (1988). Presence of transcription signals in two putative DNA replication origins of human cells. Biochim. Biophys. Acta 951, 430-442.

Finch, J.T. & Klug, A. (1976). Solenoidal model for superstructure in chromatin. Proc. Natl. Acad. Sci. USA 73, 1897-1901.

Fröhlich, H. (1977). Long-range coherence in biological systems. Rivista Nuovo Cimento 7, 399-418.

Gilbert, W. (1986). The RNA world. Nature 319, 618.

Ginzburg, V.L. & Landau, L.D. (1950). On the theory of superconductivity. Soviet Phys. JETP 20, 1064. Reprinted in: Ter Haar (1965). pp. 546-568.

Haldane, J. (1965). In: The origins of prebiological systems. Ed. S.W. Fox. New York: Academic Press. p. 11.

Hand, R. (1978). Eukaryotic DNA: Organization of the genome for repliccation. Cell 15, 317-325.

Hannay, N.B., Geballe, T.H., Matthias, B.T., Anders, K., Schmidt, P., and MacNair, D. (1965). Superconductivity in graphitic compounds. Phys. Rev, Letters 14, 225-226.

Hebard, A.P., Rosseinsky, M.J., Haddon, R.C., Murphy, D.W., Glarum, S.H., Palstra, T.T.M., Ramirez, A.P., and Kortan, A.R. (1991). Superconductivity at 18 K in potassium doped fullerene K_xC_{60}. Nature 350, 600-603.

Holczer, K., Klein, O., Huang, S.-M., Kaner, R.B., Fu, K.-J., Whetten, R.L., and Diederich, F. (1991). Alkali-Fullerides Superconductors: Synthesis, Composition and Diamagnetic Shielding. Science 252, 1154-1157.

Hunter, T. (1991). Cooperation between oncogenes. Cell 64, 249-270.

Huxley, A.F. (1982). How far will Darwin take us? In: Evolution from molecules to men. Ed. D.S. Bendall. p.6. Cambridge: Cambridge University Press.

Jacob, F. (1977). Evolution and tinkering. Science 196, 1161-1166.

Keller, W., Müller, U., Eiken, I., Wendel, I. & Zentgraf, H. (1977). Biochemical and ultrastructural analysis of SV40 chromatin. Cold Spring Harbor Symp. Quant. Biol. 42, 227-244.

Kimura, M. (1968). Evolutionary rate at the molecular level. Nature 217, 624-626.

King, M.-C. and Wilson, A.C. (1975). Evolution at two levels in humans and chimpanzees. Science 188, 107-116.

Kornberg, A. (1978). Aspects of DNA replication. Cold Spring Harbor Symp. Quant. Biol. 43, 1-9.

Kornberg, A. (1988). DNA replication. J. Biol. Chem. 263, 1-4.

Kriegstein, H.J. & Hogness, D.S. (1974). Mechanism of DNA replication in *Drosophila* chromosomes: Structure of replication forks and evidence of bidirectionality. Proc. Natl. Acas. Sci. USA 71, 135-139.

Land, H., Parada, L.F., and Weinberg, R.A. (1983). Tumorigenic conversion of primary embryo fibroblasts requires at least two cooperating oncogenes. Nature 304, 596-602.

Levy, B., Wong, N.C.W. & Dixon, G.H. (1977). Selective association of the trout-specific H6 protein with chromatin regions susceptible to DNase I and DNase II: Possible location of HMG-T in the spacer region between core nucleosomes. Proc. Natl. Acad. Sci. USA 74, 2810-2814.

Lima-De-Faria, A. & Jaworska, H. (1968). Late DNA synthesis in heterochromatin. Nature 217, 138-142.

Lindquist, S. (1986). The heat-shock response. Ann. Rev. Biochem. 55, 1151-1191.

Lohr, D., Corden, J., Tatchell, K., Kovacic, R.T. & Van Holde, K.E. (1977). Comparative subunit structure of HeLa, yeast, and chicken erythrocyte chromatin. Proc. Natl. Acad. Sci. USA 74, 79-83.

London, F. (1954). Superfluids, 2. New York: Dover.

Lyon, M.F. (1961). Gene action in the X-chromosome of the mouse (*Mus musculus L*). Nature 190, 372-373.

Lyon, M.F. (1990). Evolution of the X chromosome. Nature 348, 585-586.

MacDermott, A.J. & Tranter, G.E. (1989). Electroweak bioenantioselection. Croatica Chem. Acta 62 (2A) 165-187.

Margulis, L. (1968). Evolutionary criteria in thallophytes: A radical alternative. Science 161, 1020-1022.

Margulis, L. (1981). Symbiosis in Cell Evolution. San Francisco: Freeman & Co.

Margulis, L. and Fester, R. (Eds.) (1991) Symbiosis as a source of Evolutionary Innovation, The MIT Press, Cambridge, Mass, 178-204.

Margulis, L. and Sagan, D. (1987). Microcosm. London: Allen & Unwin.

Mason, S.F. (1984). Origins of biomolecular handedness. Nature 311, 19-23.

Mayr, E. (1982). The growth of biological thought. p. 598. Cambridge, Mass.: Harvard University Press.

Mayr, E. (1982a). Darwin intellectual revolutionary. In: Evolution from molecules to men. Ed. D.S. Bendall, Cambridge: Cambridge University Press. pp. 23-41.

Mayr, E. (1982b). The growth of biological thought. Diversity, evolution, and inheritance. Cambridge,Mass.: Harvard University Press.

Miller, D.A. (1991). Useful perspective on the relation between biological and physical descriptions of phenomena. J. Theor. Biol. 152, 341-355.

Mizutani, U., Massalski, T.B., McGinnes, J.E., and Corry, P.M. (1976). Low temperature specific heat anomalies in melanins and tumor melanosomes. Nature 259, 505-507.

Moorbath, S., O'Nions, R.K., Pankhurst, R.J., and Gale, N.H. (1972). Further rubidium-strontium age determinations on the very early Precambrian rocks of the Godthaab District, West Greenland. Nature 240, 78-82.

Nicolini, C., Carlo, P., Finollo, R., Vigo, F., Cavazza, B., Ledda, A., Ricci, E., and Brambilla, G., (1984) Phase transitions in nuclei and chromatin. Cell Biophys 6, 183-196.

Ohno, S. (1971). Simplicity of mammalian regulatory systems inferred by single gene determination of sex phenotypes. Nature 234, 134-137.

Ohno, S. (1974). Ancient linkage groups and frozen accidents. Nature 244, 259-262.

Olins, A.L. & Olins, D.E. (1974). Spheroid chromatin units (v bodies). Science 183, 330-332.

Oparin, A.L. (1953). Origin of Life. New York: Dover.

Pace, N.R. (1991). Origin of life-Facing up to the physical setting. Cell 65, 531-533.

Pace, N.R., Olsen, G.J., and Woese, C.R. (1986). Ribosomal RNA phylogeny and the primary lines of evolutionary descent. Cell 45, 325-326.

Pato, M.L. (1975). Alteration of the rate of movement of deoxyribonucleic acid replication forks. J. Bacteriol. 123, 272-277.

Ponnamperuma, C. (1989). Experimental studies in the origin of life. J. British Interplanetary Soc. 42, 397-400.

Rassoulzadegan, M., Cowie, A., Carr, A., Glaichenhaus, N., Kamen, R., and Cuzin, F. (1982). The role of individual polyomavirus early proteins in oncogenic transformation. Nature 300, 713-718.

Ruley, H.E. (1983). Adenovirus early region 1A enables viral and cellular transforming genes to transform primary cells in culture. Nature 304, 602-606.

Sagan, C. (1965). The origins of prebiological systems. Ed. S.W. Fox. New York: Academic Press. p. 207.

Sagan, L. (1967). On the origin of mitosing cells. J. theor. Biol. 14, 225-274.

Salam, A. (1990). Unification of the fundamental forces. Cambridge: Cambridge University Press. pp. 56-58.

Salam, A. (1991). The role of chirality in the origin of life. J. Mol. Evol. 33: 105-113.

Salam, A. (1992). Chirality, phase transitions and their induction in amino acids. Phys. Lett. B (In press).

Schopf, J.W. (1983). Earth's earliest biosphere its origin and evolution. Princeton: Princeton University Press.

Schopf, J.W., J.M. Hayes, and M.R. Walter, 1983: Evolution of Earth's Earliest Ecosystems: Recent Progress and Unsolved Problems. In J.W.Schopf (ed.), 1983: Earth's earliest biosphere its origin and evolution. Princeton University Press, Princeton, 361-384.

Schwemmler, W. (1991) Symbiogenesis in Insects as a Model for Morphogenesis, Cell Differentiation, and Speciation. In Margulis, L. and Fester, R. (Eds.) (1991) Symbiosis as a source of Evolutionary Innovation, The MIT Press, Cambridge, Mass, 178-204.

Sperling, L. and M.C. Weiss. (1980). Chromatin Repeat Length Correlates With Phenotypic Expression In Hepatoma Cells, their Dididifferentiated Variants, and Somatic Hybrids. Proc. Natl. Sci. Acad. USA , 77, 3412-3416.

Struhl, K. (1989) Molecular mechanisms of transcriptional regulation in yeast. Ann. Rev. Biochem., 58, 1051-1077.

Stryer, L. (1988) Biochemistry. 3rd ed. New York: W.H. Freeman and Co. p. 794.

Ulbricht, T.L.V. (1981). Reflections on the origin of optical asymmetry on Earth. Origins of Life 11, 55-70.

Vijayan, M. (1980). Prebiotic evolution: An approach to polymerization based on crystal structures. FEBS Letters 112, 135-137.

Vijayan, M. (1987). Crystalline complexes involving amino acids and peptides. Acta Cryst. 43C, 64.

Yagubskii, E.B., Shchegdev, I.F., Laukhin, V.N., Konovich, P.A., Karatsovnik, M.V., Zvarykina, A.V., and Buravov, L.I. (1984). Normal pressure superconductivity in an organic metal $(BEDT\text{-}TTF)_2 I_3$ [bis(ethylene dithiolo)tetrathiofulvalene triiodide]. JETP Lett. 39, 12-16.

Wald, G. (1957). The origin of optical activity. Ann. NY Acad. Sci. 69, 352-368.

Ward, C.B. & Glaser, D.A. (1969). Analysis of the chloramphenicol-sensitive and chloramphenicol-resistant steps in the initiation of the DNA synthesis in *E. coli* B r.. Proc. Natl. Acad. Sci. USA 64, 905-912.

Watson, J.D., Hopkins, N.H., Roberts, J.W., Steitz, J.A. & Weiner, A.M (1987a). Molecular Biology of the Gene. 4th. ed. Menlo Park, California: The Benjamin Cummings Publishing Co.

Weisbord, S. (1982). Active chromatin. Nature 297, 289-295.

Weyl, P.K. (1968). Precambrian marine environment and the development of life. Science 161, 158-160.

Wilson, A.C., Maxson, L.R., and Sarich, V.M. (1974). Two types of molecular evolution. Evidence from studies of interspecific hybridization. Proc. Natl. Acad. Sci. USA 71: 2843-2847.

Zubay, G. (1983). Biochemistry. Reading, Mass.: Addison-Wesley. pp. 1186-1193.

Zuckerkandl, E. (1963). Perspectives in Molecular Anthropology. In: Classification and Human Evolution. Ed. S.L. Washburn. Chicago: Aldine Publishing Co. p. 243.

Zuckerkandl, E. (1964). Controller-gene diseases: The Operon model as applied to β−thalassemia, familial fetal hemoglobinemia and the normal switch from the production of fetal hemoglobin to that of adult hemoglobin. J. Mol. Biol. 8: 128-147.

Zuckerkandl, E. (1981). A general function of noncoding polynucleotide sequences: Mass binding of transconformational proteins. Molec. Biol. Rep. 7: 149-158.

Zuckerkandl, E. (1983). Topological and Quantitative Relationships in Evolving Genomes. In: Structure, Dynamics, Interactions and Evolution of Biological Macromolecules. Ed. C. Hélène. Dordrecht-Holland: D. Reidl Publishing Co. pp. 395-412.

Zuckerkandl, E. and Villet, R. (1988). Generation of High-Specificity of Effect through Low-Specificity Binding of Proteins to DNA. FEBS Letters 231: 291-298.