DNA Replication and Cell Cycle Progression Regulated by Long Range Interaction between Protein Complexes bound to DNA

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Abstract. A nonstationary interaction that controls DNA replication and the cell cycle is derived from many-body physics in a chemically open T cell. The model predicts a long range force $F'(\xi) = -(\kappa/2) \xi(1-\xi)(2-\xi)$ between the pre-replication complexes (pre-RCs) bound by the origins in DNA, $\xi = \varphi/N$ being the relative displacement of pre-RCs, φ the number of pre-RCs, N the number of replicons to be replicated, and κ the compressibility modulus in the lattice of pre-RCs which behaves dynamically like an elastically braced string. Initiation of DNA replication is induced at the threshold $\varphi = N$ by a switch of sign of $F'(\xi)$, from attraction (–) and assembly in the G_1 phase ($0 < \varphi < N$), to repulsion (+) and partial disassembly in the S phase ($N < \varphi < 2N$), with release of licensing factors from pre-RCs, thus explaining prevention of re-replication. Replication is terminated by a switch of sign of force at $\varphi = 2N$, from repulsion in S phase back to attraction in G_2 , when all primed replicons have been duplicated once. $F'(0) = 0$ corresponds to a resting cell in the absence of driving force at $\varphi = 0$. The model thus ensures that the DNA content in G_2 cells is exactly twice that of G_1 cells.

The switch of interaction at the R -point, at which N pre-RCs have been assembled, starts the release of Rb protein thus also explaining the shift in the Rb phosphorylation from mitogen-dependent cyclin D to mitogen-independent cyclin E. Shape, slope and scale of the response curves derived agree well with experimental data from dividing T cells and polymerising MTs, the variable length of which is due to a nonlinear dependence of the growth amplitude on the initial concentrations of tubulin dimers and guanosine-tri-phosphate (GTP). The model also explains the dynamic instability in growing MTs.

Key words: Cell cycle, DNA replication, cyclin, kinases, DNA origin, recognition complex, microtubules, DNA duplex, Rb protein, p27, licensing factor, origin recognition complex

1. DNA Replication – Consensus or Not?

Before a cell can divide it must first duplicate its own DNA, creating a copy for each daughter cell. As a short prelude to cell division, in textbooks this may sound surprisingly simple. However, considering that the living cell with ligands and receptors is a chemically open, condensed matter system working at nonstationary conditions, and that most biological functions are collective phenomena, the whole

Figure 1. (a) Unfolded DNA helix schematically pictured as a lattice of origins liganded by ORC-ATP complexes. In reality the average distance between origins is much longer and DNA structure more complex, supercoiled and wound around histones. The pre-replication complex also consists of proteins such as licensing factors. (b) Bi-directional replication initiated at one DNA origin.

question becomes an interdisciplinary nightmare. Obviously, DNA replication cannot be initiated by the engagement of one sole receptor at the surface membrane or one origin in the DNA double helix. The cooperation of many interacting molecular units in the surface membrane, DNA, cytoskeleton and other compartments, is required for a resting cell to initiate DNA replication. Thus, living matter too is condensed and, apart from biological and chemical aspects, must also be described in terms of manybody physics relevant for the nonstationary chemically open cell system.

DNA replication is initiated simultaneously at hundreds or thousands of sites along the DNA duplex, requiring an exact timing and spatial coordination over long distances. Such a long range coherence could not be explained physically by a diffusive type interaction, hence, could not occur without non-local correlations between the interacting molecular complexes. The problem is to find the corresponding form of long range interaction. Considering the myriad of reactants

participating in DNA replication another problem is to pinpoint the factor that plays the key role at the initiation. However, recent experimental findings provide possible answers. In DNA, both from yeast and animal cells [1, 2], certain openings termed origins (Figure 1) with specific nucleotide sequences (replicators) work as receptor for an origin recognition complex (ORC). The ORC is considered to be the best candidate for an initiator protein [1]. Liganded with adenosine-triphosphate (ATP) ORC can recognize and bind to DNA origins in the double helix [3], to which other factors are then recruited. This assembly of proteins is called a pre-replication complex (pre-RC). The binding of ORC by DNA origins is a high affinity, hence, nonstationary process which does not require nucleotide hydrolysis, ATP remains stably associated with ORC [4] and the role of ATP hydrolysis at the initiation is not understood.

Replication of DNA starts by synthesis of adequate concentrations of the four nucleotide precursors and critical enzymes that carry out the replication. These substances are regulated by a family of transcription factors (TF) E2F [5], however, E2Fs already bound by DNA origins/replicons are tonically suppressed in the interphase by the Rb proteins (termed after their discovery in Retinoblastoma cells) which also recruits other repressors, such as histone deacetylases and chromosomal remodeling SWI/SNF complexes [6]. In late G_1 the Rb proteins become rapidly phosphorylated and undergoes a conformational change which induces their dissociation from the E2Fs, thus allowing a rapid transcription of all genes necessary for DNA synthesis and replication.

The phosphorylation of Rb proteins in turn is induced by specific holoenzymes, cyclin-dependent kinases (cdk), that remain inactive until complexed with proteins termed cyclins. In T cells a sequential expression of these factors, cyclin D2 in mid G_1 , cyclin D3 in late G_1 , depends strictly upon a continuous interleukin-2 (IL2) exposure until a restriction point in late G_1 , the *R*-point, beyond which the T cell expresses cyclin E and proceeds to replication without further exposure to IL2 [7]. In this sense the D type cyclins act as growth factor sensors. However, the various cyclin-cdk activities are also regulated by factors from the Cip/Kip family, such as p27, the degradation of which is indirectly controlled by the interaction of IL2 with its receptors [8]. Whereas the cyclin E-dependent cdks are inhibited by p27, the cyclin D-dependent holoenzymes, cdk4 and cdk6 complexed with p27, remain catalytically active, and their accumulation before the *R*-point rather seems to be dependent on p27 [6]. At the *R*-point, the Rb protein starts dissociating from the E2Fs, which start inducing cyclin E and then cyclin A, both of which activate their catalytic cdk partners. CyclinE-cdk2 then rapidly completes the phosphorylation of Rb proteins and also phosphorylates p27, which is converted to a form recognized by ubiquitin ligases and targeted for destruction in proteasomes. The sequestration of p27 also activates the pool of inactive cyclin E-cdk2-p27 that exists already before the *R*-point. This greatly increases the level of cyclin E-cdk2 activity which peaks at the G_1 -S interface [6]. Accordingly, apart from contributing to hyper-

phosphorylation of all Rb proteins, the activity of cyclin E should somehow also be linked to the replication origin-firing [9].

The generally accepted view is that cyclin D-cdk4/6 initiates the phosphorylation of Rb protein in mid G_1 . However, there is no explanation why the start of dissociation of Rb protein is delayed until the *R*-point. Moreover, the shift in Rb phosphorylation at the *R*-point, from mitogen-dependent cyclin D-cdk4/6 to mitogen-independent cyclin E-cdk2, only accounts in part for the loss of dependency on IL2 and concomitant release of Rb proteins [6]. A mechanistic intramolecular interaction, induced by phosphorylation at different sites of the Rb/pre-RC complex, was recently proposed to explain this sequential expression of cyclins [10]. However, the mechanistic type model presented here shows that the corresponding shift in Rb-phosphorylation at the *R*-point likely also depends on an intermolecular interaction which allows release of Rb protein if and only if a definite number of pre-RCs have first been assembled.

Before initiation of DNA replication the origins must then be primed by so called licensing factors (LF) [1], which may control the completion of replication in such a way that the genome is copied once and only once per cell cycle. For instance, in budding yeast cells the Cdc6 and Mcm2-7 proteins must first bind to the DNA origins before the TFs, such as Mcm1 and others, can regulate the transcription. Thus pre-RCs include factors that directly select origins in the DNA duplex, the ATP-ORCs, and others like the E2Fs, Rb proteins and LFs, that are subsequently recruited to DNA origins [1]. The ORCs remain bound by DNA also after initiation, hence contained in the post-RCs, but the LFs, such as Cdc6 and Mcm2-7 proteins, are released during the entire *S* phase [11]. In a T cell all reactants and biochemical pathways activated via the IL2 receptor (IL2R) are not entirely identified and, hence, cannot be fully described. However, the important consequence for the gross behaviour of the cell is that the transport between the *R* point and the G_1 -*S* interface, from the surface membrane to the nucleus, of the molecular on-off switch like mechanism actually takes place [12]. Whereas the transport dynamics is expected to be complicated, as shown subsequently, the long range gross interaction responsible has the same simple form irrespective of if the interaction carriers are IL2 and IL2R, ATP-ORC and DNA origins, or Rb proteins, LFs, or cyclin-cdk holoenzymes binding to pre-RC protein complexes. As stated already, the ligand-receptor interaction at the surface membrane controls the increase of free activated cyclin D-cdk4/6-p27 before the *R*-point [6–8], and the phosphorylation of different parts of the pre-RC which is target for this activity [1]. IL2 thus also indirectly controls the dissociation of Rb proteins, and hence the activation of E2Fs which induce the synthesis of cyclin E and degradation of p27.

A modelling from first principles, based on all these factors, may seem too difficult but should not be impossible. Experiments suggest that DNA replication is regulated by a binary set of states: the assembly state which allows formation and binding of pre-RCs at the origins in DNA, but not initiation of replication; and the replication state which allows the initiation of DNA replication with disas-

Table 1. Control of replication and cell cycle progression by a long range interaction between protein complexes in DNA; replication is initiated when all DNA origins ($\varphi = N$) have been engaged by pre-RCs and primed by licensing factors.

	G_1	R	S	G_2	M
Turnover of material	assembly		disassembly	doubling of mass	chromatid separation
Characteristics	ligand-receptor association pre-RC binding to DNA ...		DNA replication, partial disassembly of pre-RCs, release of LFs, prevention of re-replication	Contraction of duplex about the histones	cytokinesis
Force between DNA protein complexes	attraction (-)		repulsion (+)	attraction (-)	
Order-parameter intervals	$0 < \varphi < N$		$N < \varphi < 2N$		
Thresholds	$\varphi = 0$		$\varphi = N$	$\varphi = 2N$	

sembly of pre-RCs but inhibits the formation and binding of new such complexes (Table I) [1]. However, without a definite threshold for initiation of replication, the binding of arbitrary one ORC/pre-RC by a DNA origin could imply two different alternatives: continued assembly of pre-RCs, or partial disassembly of pre-RCs with release of LFs, and concomitant start of DNA replication. Initiation under such indefinite conditions could lead to incomplete replication.

The complete duplication of DNA should therefore require that the attachment of arbitrary one pre-RC including Rb-protein should be able to induce the commitment at the *R*-point if and only if a definite number, N , of origins/replicons in DNA have been engaged by pre-RCs, albeit without LFs because some LFs are products of E2F responsive genes and may therefore depend on E2F for their synthesis. After dissociation of Rb proteins the lattice of incomplete pre-RCs starts binding the LFs synthesised and the so primed pre-RCs then become rapidly hyper-phosphorylated by cyclin E-cdk. Much the same as in the switch at the *R*-point, the binding of one LF should induce *S* phase entry if and only if $N-1$ pre-RCs have first been primed by LFs. Consequently, the cell is somehow able to simultaneously count the number of pre-RCs bound to DNA along the duplex, first at the *R*-point and then at the *S* phase entry, implying long range (non-local) correlations.

As demonstrated in this work, a nonlinear many-body physics model can explain such a counting-at-distance and multiple inhibitory switch mechanism at the *R*-point and G_1 -*S* interface. The model obtained provides a third switch mechanism that terminates replication at the *S*- G_2 interface, ensuring that the DNA content of G_1 cells is exactly twice that of G_2 cells. The switch of interaction from assembly/attraction to partial disassembly/repulsion [1, 11], which explains the transition to *S* phase and the prevention of re-replication during replication by release of the LFs, is induced by a 'quantum' of energy from one pre-RC above the N :th order lattice ground state energy, by hyper-phosphorylation of the same pre-RC, presumably after hydrolysis of ATP [1]. This makes the DNA lattice of pre-RCs/replicons unstable, inducing a transition into a scattering/disassembly state which allows dissociation of LFs and initiation of replication. Similarly, the energy contributed by phosphorylation of bound Rb proteins should be sufficient for dissociation of same Rb proteins and the all-or-none commitment at the *R*-point, provided that all N pre-RCs have first been assembled. Rb proteins that remain bound after the *R*-point become rapidly phosphorylated and released, after which the lattice of pre-RCs returns to the assembly state with attractive interaction, allowing binding of LFs as required for initiation.

The DNA duplex, the nuclear envelope and the surface membrane alone are too soft to explain the presence of nonlocal correlations between the DNA origins/replicons engaged by pre-RCs, between the surface membrane receptors engaged by IL2, as well as between the two subsystems. A molecular complex at one end of the membrane, or at one end of the DNA duplex, would then know very little or nothing about a complex at the other end of these soft condensed structures. Only adjacent complexes would be correlated and a diffusive propagation of signals

would be too slow to explain the exact coordination in time and space, and the simultaneous counting of interacting molecular complexes before the unanimous commitment.

2. Non-Stationary Solid Like Properties

However, the supercoiling of DNA, the winding about histones, and a stabilized cytoskeletal network of microtubules (MTs) [13], the most rigid elements of the cell, can induce nonlocal correlations within and between the membrane and nuclear subsystems. Unfortunately, a couple of hitherto unsolved problems have prevented a physical understanding of the cytoskeleton dynamics and polymerisation of MTs, the dynamic instability and variable length of growing MTs [13, 14]. But the chemical driving reaction for growth of MTs is known; only tubulin dimers liganded with two guanosine-triphosphate (GTP) molecules polymerise into MTs [13]. Known is also that a fraction of all growing MTs actually attains a stabilised state at critical points in the cell cycle, and one could therefore expect nonlocal correlations to occur.

The stabilisation of DNA is due to supercoiling of the duplex and the winding about histones. A realistic cell model is thus expected to be more solid like, however, still driven by diffusive type reactions in the liquid like boundary and intermediate regions. A complex diffusive type interaction is also expected to carry the cell between the different critical points. But according to physical unitarity principles (= sum rules) such a more complex intermediate type dynamics should sum up to one and the same gross interaction, which also controls the cell at the critical points. As explained subsequently, however, an explicit derivation of the gross interaction is much simpler at the *R*-point and the *S* phase entry. Accordingly, the rate of cell cycle progression derived from this gross interaction should be the same at all times after the *R*-point, but this also follows from the fact that all time variation of the cell cycle is normally contained in G_1 before the *R*-point.

The DNA subsystem is regulated by reactant concentrations such as cyclins, kinases, ATP, ORC, DNA origins, TFs, Rb protein, and LFs, however, in a T cell the interaction of all these reactants is directly or indirectly regulated by the two surface membrane reactants, IL2 and IL2R [7, 8, 15]. This exclusive dependence on IL2 and IL2R [15] is not a mystery from the point of view of physics. It is a form of enslavement, in which all other variables are damped at the actual time scale (hours and minutes). A dynamical system like this, controlled by concentrations, is termed lyotropic [16]. The problem is that most condensed matter theories are based on thermotropic systems – regulated by temperature variations – and like the Hill and Langmuir equations, founded on assumptions of a chemical stationary state [17, 18]. However, replication is initiated by the binding of ORC to the DNA origin, a unidirectional hence non-stationary process which is also the case for binding of TFs and subsequent factors such as Rb proteins and LFs needed to form complete pre-RCs [1, 6]. While the ORCs bind to DNA origins in G_1 and remain bound

Figure 2. (a) Dose-response curve predicted by the nonstationary lyotropic model (solid line) compared with proliferation data of a cell line MLA-144 (dots) from Gibbon ape with spontaneous lymphoma. Data taken from ref. [25] with permission. (b) Dose-response curve derived from mass-action model with a dissociation constant $K = 1.0 \pm 0.5$ nM.

also during the *S* phase, hence contained in the post-RCs, the Rb proteins start dissociating already at the *R*-point. The LFs start dissociating at the G_1 -*S* interface and then remain unbound during the *S*-phase [1, 11], thus blocking re-replication.

Also other important biological functions are controlled by nonstationary reactions. On the surface membrane of a T cell, for instance, the high affinity receptors engaged by IL2 internalize already 15 min after the engagement, while dissociation of IL2 from such receptors does not start until 30 min later [15]. Biological response may therefore occur long before a stationary state has been attained, which is mainly also the reason why the threshold and scale derived in stationary type models may deviate by up to three orders from those assessed [19] (Figure 2). In order to remedy this failure, the scale derived is usually replaced by the ligand concentration for half-maximal response, however, the response derived then becomes a mere logistic equation. Obviously, this complies better with experimental data, but the physical meaning and interpretation of the model in terms of molecular interaction is then lost [20]. The discrepancy between theory and data has also been ascribed to factors such as transport proteins and a variable affinity [21]. Although these factors may well contribute too, they cannot compensate for the neglect of nonstationary effects in the first place.

Experiments on T cell proliferation show that a definite number of IL2-receptor associations are required for a cell to make the all-or-none commitment at the *R*-point to initiate DNA replication [22]. This switch-like, quantal type response has also been observed in the T cell receptor (TCR) system [23]. Accordingly, the T

cell is somehow able to count at a distance the number of interacting receptors [24], irrespective of their location on the surface membrane. Like in the case of pre-RCs, the definite threshold number of receptor engagements at the surface membrane and the switch like behaviour at the *R*-point, indicates nonlocal correlations also between the internalised receptor complexes, which should correspond to the pre-RCs stored at the *R*-point, implying a long range interaction that controls the exact timing and spatial coordination of DNA replication. As demonstrated subsequently, the nonlocal correlations as well as the chemical driving reactions and hence also the long range interaction, have the same form in the three actual subsystems.

The different characters of the long and short range interactions could be further understood as follows. A slope equal to one, such as in the division rate of the T cell line MLA-144 [25], corresponds to the Langmuir response or a Hill equation with exponent $n_H = 1$ (Figure 2). The so called Hill exponent n_H denotes the number of binding sites per receptor protein and, consequently, $n_H > 1$ is due to short range cooperativity between adjacent binding sites on the same receptor-molecule. Consequently, a slope equal to one excludes a net contribution from short range cooperativity, but, as shown subsequently, is in accordance with the nonlocal correlations indicated experimentally. As shown in this work the long range correlations are crucial for the stable propagation of genetic information, which requires that the entire genome of an organism – a definite number of replicons – must be faithfully replicated once and only once each cell cycle.

Before going into the explicit derivations of such a model, some words are in place on the energy induced by the ligand-receptor interaction. It has been found that each receptor engagement contributes an equal quantum of excitation energy [26] and, hence, there is no reason to believe that this should not be true also for the pre-RC bound by DNA origins. But the total energy stored is not a linear sum of individual excitations because the rate of cell division, as well as the dynamics that controls DNA replication and the cell cycle progression, are nonlinear functions of the ligand-receptor interaction [15]. A realistic, self-consistent but nontrivial gross interaction model of a dividing cell should thus:

- i) yield a growth rate which is a nonlinear function only of the two reactant concentrations,
- ii) with the correct shape, slope, and scale,
- iii) explain the condensating interaction responsible for assembly of complexes in G_1 ,
- iv) explain the initiation of DNA replication at a definite threshold number of receptor engagements and a corresponding definite number of DNA origins engaged by ORCs,
- v) express the definite threshold number of complexes in terms of the initial reactant concentrations,
- vi) define the constant quantum of energy induced per molecular complex engaged in each subsystem,

- vii) explain the disassembly of complexes and prevention of re-replication in the *S* phase,
- viii) explain the termination of replication at a threshold such that the DNA content in G_2 cells is exactly twice that of G_1 cells.
- ix) explain the dynamic instability and variable length of growing MTs,
- x) explain the roles of phosphorylation and hydrolysis in MTs and DNA replication.

Except for the last point, and the requirement of a constant quantum of energy, all these properties could be explained in an earlier version of the model [27]. In an improved model, by redefining the chemical potential, each molecular complex could be given a constant quantum of energy [28]. This question may look a bit formal, however, is directly crucial. Taken together with the nonlocal correlations and the non-stationary driving reaction of the actual subsystem, the requirement of a constant quantum of energy per molecular complex restricts the choice of model to a unique form of long range interaction. In the present work this model is developed further and hopefully better explained. The gross behaviour of DNA replication and cell cycle progression is described in terms of the interacting protein complexes in DNA, and the model also provides an explanation of the dynamic instability and variable length of growing MTs.

3. The Nonstationary Driving Reaction

The concentrations of IL2, IL2R and their bound complex are denoted by ρ , r and ψ . In DNA the role of ligand is played by ATP-ORC, or the Rb protein, or a LF, and the DNA origins and different forms of pre-RCs then become receptors for same ligands which are required to form complete pre-RCs at certain times in the cell cycle (Figure 1a). Also a cyclin-cdk holoenzyme could be viewed as a ligand, with the non-phosphorylated and phosphorylated pre-RCs being receptors and produced complexes, respectively. The rate of association in any of these subsystems is then defined by

$$\begin{aligned} \frac{\partial \psi}{\partial t} &= k\rho r - k'\psi = k((a_0 - \psi)^2 - (a_0^2 - b_0^2)) \\ &= k(1 - \psi/a_0)^2 \left(a_0^2 - \frac{(a_0^2 - b_0^2)}{(1 - \psi/a_0)} \right) \end{aligned} \quad (3.1)$$

The parameters $a_0 = 1/2(\rho_0 + r_0 + K)$ and $b_0 = \sqrt{\rho_0 r_0}$ are obtained by insertion of the initial constraints, $r + \psi = r_0$ and $\rho + \psi = \rho_0$, and zero indicates the start concentrations. $K = k'/k$ is the affinity constant, k' and k the dissociation and association constants in the actual subsystem. Integration of (3.1) yields

$$\begin{aligned}
\ln \left(\frac{\rho_K - \psi}{r_K - \psi} \frac{r_K}{\rho_K} \right) &= \ln \left(\frac{\rho'}{r'} \frac{r_K}{\rho_K} \right) \\
&= k(\rho_K - r_K)t = 2k\sqrt{a_0^2 - b_0^2}t
\end{aligned} \tag{3.2}$$

where $\rho_K = a_0 + \sqrt{a_0^2 - b_0^2}t$, $r_K = a_0 - \sqrt{a_0^2 - b_0^2}t$, ρ' and r' could be termed ‘dynamic concentrations’, internally related by the constraints $\rho' + \psi = \rho_K$ and $r' + \psi = r_K$. In high affinity systems, where $k' \approx K \approx 0$, the dynamic concentrations thus equal the initial concentrations, hence, $r_K \approx r_0$, $\rho_K \approx \rho_0$, $r' \approx r$, and $\rho' \approx \rho$. Therefore, no distinction will henceforth be made between dynamic and initial concentrations unless required.

The time parameter t in (3.2) could be termed ‘physiological’ time in the sense that it relates the usual time to the two continuous reactant concentrations. At the all-or-none transition to S phase, however, the dynamics suddenly becomes sharply sensitive to the number of molecular complexes engaged [12, 22–24]. The interaction then becomes switch-like, a behaviour observed also in progesterone induced oocyte maturation [29]. Individual oocytes then showed a mitogen-activated protein kinase (MAPK) cascade equivalent to a cooperative enzyme with Hill coefficient of at least 35, but as pointed out already [30], there is no cooperativity between individual enzymes in the MAPK cascade. However, it should be underlined that lack of local cooperativity between the enzymes does not exclude non-local correlations between the produced complexes. As shown here, both the long range interaction and threshold for response are determined by such non-local correlations.

4. Nonlocal Correlations Due to Long Range Force

The discovery that a T cell counts the number of receptor engagements on its surface membrane before taking the decision to initiate DNA replication might seem perplexing. However, from a many-body physics point of view it is not. Like in the case of DNA origins, it indicates the presence of non-local correlations between different receptor complexes at the surface membrane or in DNA, and also a non-local correlation between the two subsystems after a constant minimum lag time [15]. This can be interpreted such that the energy of the definite number of ligand-receptor induced complexes at the surface membrane must first be transported and stored in DNA in the form of a corresponding number of pre-RCs before the switch-like commitment at the R -point can take place. The complex transport dynamics is unknown, however, is not important for the derivation because the non-local correlations have the same form irrespective of if the complexes are internalised receptors, or pre-RCs before or after the R -point, or post-RCs. The non-local correlation between n identical molecular complexes at x_i , $1 < i < n$, which is equal to the probability to find the complexes at same sites, could be

assumed to be $\varphi \approx \mu \psi(x_1)\psi(x_2)\psi(x_3) \dots \psi(x_n)/a_0^n$, μ and a_0 being arbitrary normalisation constants. However, this expression is ambiguous because in non-stationary, lyotropic systems the notion of concentration is ill defined at sites where too many or too few reactant molecules are located. A well-defined concentration thus requires a large number of molecules in each macroscopically small but microscopically large volume element.

For instance, at high concentrations of vacant receptors on the surface membrane, the volume element around each receptor may contain less than one ligand. *Vice versa*, at the transition to S phase, where vacant receptors could become almost depleted, each volume element around a ligand may contain no receptor. The notion of concentration is therefore not well defined enough to describe the actual lyotropic interaction in which $\psi(x)$ merely denotes the probability that (3.1) generates an engaged receptor at the site x . The probability to find a definite number of internalised ligand-receptor complexes must therefore be extended to an average of all orders

$$\varphi = \mu \sum_{n=0}^{\infty} \left(\frac{1}{a_0}\right)^n \prod_{v=0}^n \psi(x_v) \quad (4.1)$$

the surface membrane subsystem being approximated by an aperiodic lattice of interacting receptors in one dimension. A term corresponding to $n = 0$ is included here to account for the possibility of having a contribution from receptors already from the start. A truncation of (4.1) at any finite order could yield a condensate containing less than the interacting IL2Rs actually observed, since each factor ψ may or may not represent one engaged receptor, and therefore a summation to infinite order is required. Similarly, (4.1) may denote the probability to find a lattice consisting of a definite number of pre-RCs bound by DNA origins. The growth of a more periodic solid like lattice, $\partial\psi/\partial t = k^*\psi$, k^* being the on-rate constant, should be restricted by the rate (3.1) of formation of complexes in the actual subsystem. With $k' \approx 0$ one then has $\partial\psi/\partial t = k^*\psi = k\rho r$. The rate of growth for a whole lattice could then be obtained by insertion of (4.1) into (3.1), which yields a unique form of long range interaction that controls the gross behaviour of the entire cell.

Assuming an increasing density of internalised receptors at the surface membrane, the area per receptor complex becomes successively smaller and the liquid-diffusive type motion in the membrane more restricted. At a certain threshold this dynamics changes into a harmonic-displacive type interaction, because the cytoskeletal link between complexes and the shrinking area per complex converts the liquid crystal type membrane to a more close-packed solid like lattice. Accordingly, the critical threshold is also determined by the rigidity and elasticity of the cytoskeletal network and embedding medium. Irrespective of if the internalised complexes are piled up near the surface membrane or stored in DNA in the form of pre-RCs before the R -point the result will be the same. A transition into a harmonic-displacive type dynamics is similarly expected in DNA because a diffusive type propagation in a liquid like DNA would be too slow to explain the exact timing

and spatial coherence observed at the initiation of replication. With increasing number of pre-RCs bound by DNA origins, the average distance between pre-RCs is reduced to a critical limit, corresponding to the definite number of replicons primed for replication.

The liquid-to-solid type transition in the actual lattices, makes the non-local correlations between molecular complexes less complicated to handle. As demonstrated here, the non-local correlations (4.1) combined with the driving reaction (3.1), then yields a non-stationary, long range interaction that controls the cell. The switch of ligand-receptor interaction at the *R*-point, and the transition from assembly to disassembly at the initiation of DNA replication, then follow as direct results of the non-stationary driving reaction (3.1). This does not exclude a diffusive type propagation of signals and more complex transport dynamics between the actual subsystems, for instance between the surface membrane and the nucleus, and between the *R*-point and the *G*₁-*S* interface, at a time scale much longer than the switches at the *R*-point and the transition to *S* phase.

Although replication is simultaneously initiated at only a fraction η of all origins, the important point is that the cell first makes sure that the definite number of origins/replicons, proportional to the definite number of IL2 receptors engaged by IL2, are engaged by pre-RCs before it takes the decision to start replication. In the slow interaction, long wave-length limit, that characterizes the actual bound state, the average lattice spacing d between surface receptors engaged, or between DNA origins engaged, becomes negligible compared to the wavelength. The probability (4.1) to find a lattice (condensate) with a definite number of bound complexes, in DNA corresponding to the number of replicons primed for replication (Figure 1), then approximates to a geometrical series

$$\varphi = \mu \sum_{n=0}^{\infty} \left(\frac{\psi}{a_0} \right)^n = \frac{\mu}{1 - \psi/a_0} \quad (4.2)$$

Like in (3.1), a_0 is a function of the initial reactant concentrations which, as shown subsequently, determine the number of molecular complexes contained in the actual subsystem (4.2). Combining (4.2) and (3.1), the rate of binding of complexes into a whole lattice (4.2) in any of the actual subsystems becomes

$$\frac{\partial \varphi}{\partial t} = \frac{ka_0}{\mu} \left(\mu^2 - \frac{(a_0^2 - b_0^2)}{a_0^2} \varphi^2 \right) \quad (4.3)$$

which apart from a constant has the solution

$$\varphi = \frac{\mu a_0}{\sqrt{a_0^2 - b_0^2}} \tanh \left(k \sqrt{a_0^2 - b_0^2} t \right) \quad (4.4)$$

The total number of interacting membrane receptors, or DNA origins/replicons engaged, is hence given by

$$N = \varphi(\infty) - \varphi(0) = \frac{\mu a_0}{\sqrt{a_0^2 - b_0^2}} \approx \mu \frac{\rho_0 + r_0}{\rho_0 - r_0} \quad (4.5)$$

Depending on μ and the initial reactant concentrations, N can thus assume different values in the various subsystems. If N does not attain the threshold number observed, replication does not start. The cell then undergoes so called premature abortion and returns to a quiescent state.

5. The Chemical Potential Energy

No sign of replication has been observed in proliferation studies on T cells, until a definite number of receptors have been engaged [22]. During the lag time, from start of exposure of the cell to ligands until start of replication, one could therefore assume that the chemically induced energy is stored as a potential energy $V(\varphi)$. For instance, in DNA this storage could take place in the form of pre-RCs bound by origins, albeit with different content at the *R*-point and *S* phase entry. Experiments indicate that replication is initiated if and only if the number of membrane receptors engaged attains the definite threshold number observed [22], corresponding to the definite number of pre-RCs bound by the entire DNA duplex. With an average lattice spacing d between the protein complexes, the rate of cells initiating replication thus equals the rate of cells in which the DNA lattice of pre-RCs attains a definite length (L_0) corresponding to the number of replicons to be replicated. Accordingly, (4.3) could also be interpreted as a travelling wave equation

$$\frac{1}{v_N} \frac{\partial \varphi}{\partial t} = \frac{\partial \varphi}{\partial x} = \frac{ka_0}{\mu v_N} (\mu^2 - g^2 \varphi^2) = \sqrt{2V(\varphi)} \quad (5.1)$$

with a travelling wave velocity v_N in units of lattice spacing per unit time, $V(\varphi)$ being the chemically induced potential energy. The binding of pre-RCs and subsequent phosphorylation of different components are also assumed to induce a corresponding deformation, elongation or compression, in the DNA lattice and the continuous variation of φ could then be interpreted as displacive motion in the lattice, generated by $V(\varphi)$. A subsequent comparison with experiments shows that such an interpretation is realistic.

The non-linear coupling g is given by

$$g = \frac{\sqrt{a_0^2 - b_0^2}}{a_0} = \frac{\rho_0 - r_0}{\rho_0 + r_0} \quad (5.2)$$

and the threshold number then becomes

$$N = \mu \frac{\rho_0 + r_0}{\rho_0 - r_0} = \frac{\mu}{g} \quad (5.3)$$

With a symmetric chemical potential (Figure 3) defined by (5.1):

$$V(\varphi^2) = \frac{g^2}{2} \left(\frac{\mu^2}{g^2} - \varphi^2 \right)^2 \quad (5.4)$$

the travelling wave velocity becomes

$$v_N = k \frac{\sqrt{a_0^2 - b_0^2}}{\mu} = \frac{1}{N} v_1 \quad (5.5)$$

v_1 being the wave velocity when the lattice contains just one complex:

$$v_1 = k a_0 = k \frac{(\rho_0 + r_0)}{2} \quad (5.6)$$

This relation trivially states that the time required for the lattice to grow one unit equals the average time it takes to form a molecular complex.

As argued previously, before replication the lattice could be expected to be confined in a bound state harmonic-displacive type dynamics. The leading order equation of motion for small harmonic undulations and displacive motion corresponding to (5.1), in the close-packed lattice of pre-RCs, then takes the form [31, 32]

$$\begin{aligned} \frac{1}{s^2} \frac{\rho^2 \varphi}{\partial t^2} - \frac{\partial^2 \varphi}{\partial x^2} &= - \frac{\partial V}{\partial \varphi} \\ &= F_0(\varphi) = 2g^2 \varphi (N - \varphi)(N + \varphi) \end{aligned} \quad (5.7)$$

s being the velocity of sound and $F_0(\varphi)$ the nonlinear driving force. The proof of this leading order dynamics is simple. Multiplying both sides of the static part of (5.7) by $\partial \varphi / \partial x$ equation (5.1) is reobtained after integration. On the contrary, a diffusive type equation instead of (5.7), with a first order derivative in time corresponding to a more liquid like dynamics in the two subsystems, would not be able to explain the exact timing and spatial coherence observed at the initiation of DNA replication.

All pathways for signal transduction from the surface membrane to the nucleus are not entirely known, however, the important consequence is that the dynamics (5.7) has the same form, irrespective of if internalised receptor complexes are stored at the surface membrane or in DNA as pre-RCs. This follows from the fact that the driving reactions (3.1) are equal in both subsystems, and the non-local correlations (4.2) of ligand induced complexes are equal too irrespective of where and in which form they are stored. Therefore the same gross interaction (5.7) controls the cell at the R -point and the G_1 - S interface. In both cases a cyclin-cdk holoenzyme and a pre-RC could play the roles of ligand and receptor, and (4.2) then represents nonlocal correlations between the phosphorylated pre-RCs. Consequently, also the rates of cell cycle progression at these two points are equal, however, which is also due to that all time variation in the cell cycle normally occurs before the R -point.

Figure 3. The symmetric, lyotropic double-well potential.

Without the histones and a stable cytoskeleton neither the non-local correlations (4.2) nor this form of long range interaction, in the membrane and DNA lattices, would exist. Accordingly, also the cytoskeleton must obey (5.7) because, only one long range gross interaction should possess the control of the cell cycle at a time. Cytoskeleton thus first works together with the surface membrane dynamics before the R -point, at which the DNA dynamics takes over. In cytoskeleton the geometrical series (4.2) yields the probability to find an MT fragment (condensate) with a definite number of GTP-tubulin dimers. The first order rate of polymerisation of MTs, $\partial\psi/\partial t = k^*\psi$, is restricted by a second order rate of formation of complexes, $\partial\psi/\partial t = k^*\psi = k\rho r$ (with $k' \approx 0$), and in this subsystem (3.1) represents the association of GTP with tubulin dimers, or the rate of re-phosphorylation of GDP-tubulin. Equation (3.1) could thus also represent the driving reaction for cytoskeleton dynamics, with attachment-detachment of energetic GTP-tubulin dimers to all orders in (4.2). Combining (3.1) with (4.2) the same leading order equation of motion (5.7), driven by the non-linear potential (5.4), is obtained also for the cytoskeletal network of MTs. In cytoskeleton the threshold N represents the content of bound GTP-tubulin dimers, the turbidity [13], which in this subsystem is determined by the initial concentrations of tubulin dimers and GTP.

6. Comparison with Data

By inspection of (5.4) one finds that the potential energy stored at $\varphi = 0$, at which no pre-RCs are bound to DNA, is nonzero. In addition the long range intermolecular force F_0 in (5.7) turns out to be repulsive (+) for $0 < \varphi < N$, that is in the assembly state (G_1) when the interaction is expected to be attractive. However, this is not as bad as it looks. The equilibrium of $V(\varphi)$ in (5.4) at $\varphi = 0$ (Figure 3) is unstable, and as is usually the case in Nature, the system then spontaneously transfer to one of its lowest energy states:

$$\varphi \rightarrow -\frac{\mu}{g} + \varphi = -\mu \frac{\rho_0 + r_0}{\rho_0 - r_0} + \varphi \quad (6.1)$$

This leads to a breakdown of symmetry of $V(\varphi)$ (Figure 3), and the total probability (4.4) becomes positive definite for all times:

$$\varphi = \mu \frac{\rho_0 + r_0}{\rho_0 - r_0} \left\{ 1 + \tanh \left(k \sqrt{a_0^2 - b_0^2 t} \right) \right\} \quad (6.2)$$

Expressing the time parameter in (6.2) in terms of the actual reactant concentrations, through (3.2), the response of the lyotropic system becomes

$$\varphi = \mu \frac{\rho_0 + r_0}{\rho_0 - r_0} \left\{ 1 + \tanh \left(1/2 \ln \left(\frac{\rho}{\rho_{50}} \right) \right) \right\} \quad (6.3)$$

Contrary to stationary models [17, 18], where the scale parameter is related directly to the affinity, $\rho_{50} = K^{1/n}$, the scale in the actual nonstationary, lyotropic model, $\rho_{50} = r\rho_0/(Er_0)$, is a function of the reactant concentrations. The integration constant E could be related to efficacy. At suitable initial reactant concentrations the quantal number N can thus attain the definite number of ligand-receptor engagements observed, corresponding to the definite threshold number of engaged DNA origins (= the number of replicons primed for replication), and (6.3) then yields the rate of transition into the S phase, which is approximately equal to the rate of cell division

$$R(\rho) = CN \left\{ 1 + \tanh \left(1/2 \ln \left(\frac{\rho}{\rho_{50}} \right) \right) \right\} = 2CN \frac{\rho/\rho_{50}}{1 + \rho/\rho_{50}} \quad (6.4)$$

The constant C is proportional to the number of cells in the start population. Shape and slope ($n_H = 1$) of (6.4) agree almost exactly with data from a cell line MLA-144 [25], and contrary to stationary models [17, 18] an exact agreement between the derived scale and that assessed could be obtained for realistic values of the initial and vacant receptor concentrations (Figure 2).

As already stated the same enslaving interaction that yields the rate of transition into the S phase (6.4), induced by the membrane reactants at the cell surface, should also control the leading order cytoskeleton dynamics, and the assembly-disassembly of MTs. Although laboratory conditions are markedly different from those of a living cell, on a gross interaction level (6.4) should therefore be proportional to the average length of an MT which is proportional to the content of tubulin dimers [13]. With C denoting the number of MTs and N that of GTP-tubulin dimers per MT, (6.4) should then yield the turbidity as a function of concentrations of GTP and tubulin dimers, and (6.2) be proportional to the variable length of a growing MT as a function of time.

However, at a concentration of $500 \mu\text{M}$ [13], the majority of reacting GTP molecules do not have the same chance to bind to a tubulin dimer as those in the vicinity of one of the less abundant dimers. Consequently, the effective concentration of GTP at work in the second order rate equation (3.1), that controls

Table II. Turbidity time data at different tubulin dimer concentrations (see Figure 6);(a) 19.0 μM , (b) 17.0 μM and (c) 13.8 μM . Data taken from ref. [13] with permission

Time (min)	Turbidity (a)	Time (min)	Turbidity (b)	Time (min)	Turbidity (c)
0.90	2.10	1.15	1.70	0.70	0.10
1.05	2.50	1.30	2.40	1.40	0.20
1.15	3.40	1.50	3.40	2.00	0.90
1.55	5.50	1.80	4.80	2.50	2.00
2.00	9.00	2.00	6.50	3.00	3.90
2.60	12.40	2.50	9.60	3.50	5.80
3.00	13.75	3.00	11.50	4.10	7.70
4.00	14.65	4.00	13.00	4.70	8.90
4.90	15.10	5.00	13.50	5.20	9.50
5.80	15.40	6.00	13.85	6.20	10.40
6.60	15.50	7.00	14.00	7.00	10.60
7.50	15.65	8.00	14.00	7.40	10.70

Figure 4. The variable length of growing microtubules, as a function of time, at an effective initial GTP concentration of 32 μM , and initial tubulin dimer concentrations at; (a) 19.0 μM , (b) 17.0 μM and (c) 13.8 μM . Data taken from ref. [13] with permission.

the growth of MTs, is much lower, and the on and off rate constants [14, 33] are correspondingly changed by a factor f , also because the growth of MTs is markedly non-stationary. With an effective GTP concentration of $\rho_0 = 32 \mu\text{M}$, an association constant $k = 3.80f (\mu\text{Ms})^{-1}$, a dissociation constant modified by hydrolysis (h), $k' \rightarrow k' + k_h = 1.14f \text{ s}^{-1}$, hence $K = k'/k = 0.3 \mu\text{m}$ left unchanged of f , and $f = 0.028/60$ a satisfactory agreement of the derived growth rate (6.2) with data from polymerising MTs (Table II) is obtained at the three highest tubulin concentrations, $r_0 = 19.0, 17.0$, and $13.8 \mu\text{m}$ (Figure 4) [13], provided the essential start of polymerisation occurs at $t_0 = 1.85, t_0 = 2.1$ and $t_0 = 3.45$ min, respectively. At lower tubulin concentrations, the system responds much slower and laboratory conditions could therefore be expected to distort the results.

The good agreement between the derived proliferation rate (6.4) and the data assessed in dividing MLA-144 cells (Figure 2), and of the time dependent response (6.2) with polymerising MTs (Figure 4), lend strong support to the proposed long range gross interaction (5.7). In the case of growing MTs not only the shape, slope and scale of the derived response (6.2) must agree with growth data (Figure 4), but also the concentration dependent amplitude with the variable length of MT filaments (Table II).

After the spontaneous symmetry breakdown the time-dependent rate of replication (6.2) automatically implies termination of replication at $\varphi = 2N$, when all replicons have been duplicated once because

$$\varphi = N \left\{ 1 + \tanh \left(k \sqrt{a_0^2 - b_0^2 t} \right) \right\} \leq 2N \quad (6.5)$$

By insertion of the displaced field (6.1) into (5.1) the principle for termination becomes obvious also from

$$\frac{\partial \varphi}{\partial t} = k a_0 g \varphi \left(2 - \frac{\varphi}{N} \right) \quad (6.6)$$

which vanishes at $\varphi = 2N$. The molecular dynamics responsible for this mechanism is further analysed subsequently. Due to nutritional and other variable constraints, however, a doubling of all replicons, through (6.5) and (6.6), does not automatically implies a doubling of the cell mass. Therefore, a formula like (6.6) with φ/N replaced by m/m_{th} , m_{th} being a critical mass threshold, would not ensure a faithful propagation of the genetic information in cell division. However, with φ being the number of replicons (= number of origins engaged) and N the total number of replicons of the genome, (6.6) ensures an exact copying of the genome.

7. DNA replication controlled by long range force

The shift of coordinate system (6.1) inserted in (5.4) yields an asymmetric potential (Figure 5):

$$V_A(\varphi^2) = \frac{g^2}{2} \varphi^2 \left(\varphi - \frac{2\mu}{g} \right)^2 \quad (7.1)$$

and the equation of motion then becomes

$$\begin{aligned} \frac{1}{s^2} \frac{\partial^2 \varphi}{\partial t^2} - \frac{\partial^2 \varphi}{\partial x^2} &= -\frac{\partial V_A}{\partial \varphi} \\ &= F_A(\varphi) = -2g^2 \varphi \left(\frac{\mu}{g} - \varphi \right) \left(\frac{2\mu}{g} - \varphi \right) \end{aligned} \quad (7.2)$$

$F_A(\varphi)$ being the long range force between interacting molecular complexes in the actual lattices. Inserting the definite number (5.3) the chemically induced driving force becomes

$$F_A(\varphi) = 2g^2 \varphi (N - \varphi) (2N - \varphi) \quad (7.3)$$

which vanishes at $\varphi = 0$, $\varphi = N$, and $\varphi = 2N$. Accordingly, F is attractive (–) in G_1 , the assembly state ($0 < \varphi < N$), which in DNA allows formation and binding of pre-RCs by the duplex but not initiation of replication (Table I). DNA replication is initiated at the G_1 - S interface, by a switch of sign of the interaction (7.2) at $\varphi = N$, from attraction (–) to repulsion (+). However, the switching of the force (7.3) starts already at the R -point.

As demonstrated subsequently, the energy of a phosphorylated Rb protein added to the ground state energy of N pre-RCs is sufficient for same Rb protein to attain the threshold for dissociation, a step that likely also involves hydrolysis of ATP. However, if all N pre-RCs are not assembled the force (7.3) remains attractive and promotes further binding of pre-RCs. Therefore, Rb proteins phosphorylated after mid G_1 by cyclinD-cdk4/6 first remain bound to E2Fs in pre-RCs and then dissociate simultaneously at the R -point [6]. The activation of E2Fs at this point, with expression of cyclin E, phosphorylation, ubiquitination and degradation of p27 which activates the pool of p27-cyclinE-cdk2, greatly increases the rate of phosphorylation and dissociation of Rb proteins that remain bound after the R -point. With all Rb proteins released the lattice of pre-RCs returns to a non-repulsive state. The increased cyclin E-cdk2 activity, responsible for hyper-phosphorylation of different parts of pre-RCs probably also including various ORC components [1,34,35], attains a maximum at the G_1 - S transition [6]. One therefore assumes that it should somehow also be linked to replication origin firing [9].

Apart from inducing cyclin E, the E2Fs are expected to promote synthesis such as of LFs [6,34], which are also necessary for the S phase entry. Once assembled, with all N pre-RCs/replicons primed by LFs, the complete DNA lattice of pre-RCs stand poised to initiate replication but does not do so until the final excitation by cyclin E-cdk2, the activity of which is taken over by cyclin A-cdk2 at the S phase entry. Experiments suggest that phosphorylation of different parts of pre-RCs including the LFs, such as Cdc6 and Mcm2-7, induce a disassembly of pre-RCs, origin unwinding, with concomitant initiation of daughter strand synthesis [1,9,34,35], in accordance with the predictions of the model (7.3). Not unlike the release of Rb proteins at the R -point, the switch of interaction (7.3) explains the release of LFs and the transition to S phase.

Figure 5. The asymmetric, lyotropic double-well potential.

However, this switch of interaction is more stable. The repulsive force (7.2) between $N < \varphi < 2N$, with partial disassembly and release of LFs, is maintained during the entire *S* phase by the greatly increased level of phosphorylation, a hyperphosphorylation of different parts of the pre-RC, implying a form of prevention of re-replication. Termination of replication is similarly explained by a switch of sign of forces at $\varphi = 2N$, at which DNA replication is completed, with all N replicons duplicated once. $F(0) = 0$ corresponds to a resting cell, in the absence of driving force. However, without a detailed knowledge of the dependence on hydrolysis, inclusion of polarization and electromagnetic effects in a three-dimensional extension, at this stage the model is unable to describe the bidirectional character of DNA replication (Figure 1b) and to predict the value of η , to mention just a couple of all problems that remain unsolved.

8. Microtubules and DNA as Elastically Braced Strings

Before examining the mechanism responsible for initiation a useful classical interpretation of the one-dimensional lattices of molecular complexes in DNA and MTs is made. Dividing by N and multiplying by the linear elastic modulus $\varepsilon = \rho^* s^2$, ρ^* being the linear (gravitational) mass density, the asymmetric equation of motion (7.2) of the lattice takes the form of an elastically braced string [36]

$$\frac{\rho^*}{N} \frac{\partial^2 \varphi}{\partial t^2} - \frac{\varepsilon}{N} \frac{\partial^2 \varphi}{\partial x^2} + \frac{\kappa}{N} \varphi = \rho^* \frac{\partial^2 \xi}{\partial t^2} - \varepsilon \frac{\partial^2 \xi}{\partial x^2} + \kappa \xi = \frac{1}{N} F_D(\varphi) \quad (8.1)$$

where $\xi = \varphi/N$ is the relative displacement in the lattice, and $\kappa = 4\mu^2\varepsilon$ the compressibility modulus of the medium in which the string like lattice is embedded. If internalised receptors first accumulate at the surface membrane before the *R*-point, also this subsystem could be approximated by an one-dimensional elastically braced string.

The dynamics of the string-like lattice of molecular complexes is driven chemically by the nonlinear restoring force $F(\varphi) = \varepsilon F_A(\varphi)$ defined by

$$\begin{aligned}
-\frac{\varepsilon}{N} \frac{\partial V_A}{\partial \varphi} &= F(\varphi)/N = -\kappa\varphi/N + F_C(\varphi)/N \\
&= -\frac{\kappa}{2} \frac{\varphi}{N} \left(1 - \frac{\varphi}{N}\right) \left(2 - \frac{\varphi}{N}\right) = F'(\xi) = -\frac{\kappa}{2} \xi(1 - \xi)(2 - \xi)
\end{aligned} \tag{8.2}$$

$-\kappa\varphi$ being the linear part of this force. The force (8.2), derived from the asymmetric potential (7.1), ensures that replication is initiated at $\varphi = N$ when all DNA origins are engaged by pre-RCs. During the assembly in G_1 , unwinding and replication is inhibited by the attraction which creates a tension in the DNA duplex. At the initiation threshold $\varphi = N$, the interaction switches to repulsion which leads to a partial disassembly, a prerequisite for replication. The repulsion between pre-RCs prolongs the DNA duplex wound about the histones, allowing unwinding and further opening of the origins – local melting of the duplex – such that replication can take place, as suggested by experiments [1,9,34,35]. Termination of replication occurs at $\varphi = 2N$ by a switch back to attraction between the protein complexes (post-RCs), creating a tension in the DNA duplex with contraction of the origin openings, thus inhibiting unwinding and replication. This ensures that the DNA content of G_2 cells is exactly twice that of G_1 cells.

The energy density corresponding to (8.1) is

$$\begin{aligned}
H(\varphi) &= \frac{\rho^*}{2N} \left(\frac{\partial \varphi}{\partial t}\right)^2 + \frac{\varepsilon}{2N} \left(\frac{\partial \varphi}{\partial x}\right)^2 + \frac{\kappa}{2N} \varphi^2 - \frac{1}{N} \Phi(\varphi) \\
&= \frac{\varepsilon}{N} \left\{ \frac{1}{2s^2} \left(\frac{\partial \varphi}{\partial t}\right)^2 + \left(\frac{\partial \varphi}{\partial x}\right)^2 + V_A(\varphi) \right\}
\end{aligned} \tag{8.3}$$

where $1/2\kappa\varphi^2$ is proportional to the energy density per complex stored in the medium. Rewriting (5.1) on the form $1/2(\partial\varphi/\partial x)^2 = V(\varphi)$, which yields a factor 2 to the potential in the static part of (8.3), for small φ -values, $\varphi^2 \approx 1 \ll N^2$, κ can thus be interpreted as the quantum of energy stored per ligand-receptor engagement [26], or the energy per pre-RC, or per GTP-tubulin dimer, depending on which subsystem is actually studied. The parameters κ , ε and the coefficients of $F(\varphi)$, which like N , s , ρ^* , μ and the initial reactant concentrations may vary from one subsystem to another, depend on the elastic properties of the medium in which the string is embedded and on each other via the cytoskeleton that connects different subsystems. The interlinked parameters thus depend particularly on the rigidity and elasticity of the cytoskeletal network of MTs and MT associated proteins. In DNA these parameters depend on the histones and the supercoiling of the duplex. A direct comparison of terms in (8.3) yields

$$\Phi(\varphi) = 2\varepsilon\mu^2\varphi^2 - \varepsilon V_A(\varphi) \tag{8.4}$$

and from (8.2) one has

$$F_D(\varphi) = 6\mu^2\varepsilon \frac{\varphi^2}{N} - 2\mu^2\varepsilon \frac{\varphi^3}{N^2}$$

$$= \frac{\kappa}{2} \varphi \left(3 \frac{\varphi}{N} - \frac{\varphi^2}{N^2} \right) \quad (8.5)$$

Accordingly, a change in the chemical potential, through (8.2), due to compression of MTs could be quantitatively related by (5.3) to a lowering of the critical tubulin concentration at the threshold for depolymerisation, as proposed in the tensegrity model [37]. By insertion of $\partial\varphi/\partial t = 0$ in (8.3), the use of $2V(\varphi) = (\partial\varphi/\partial x)^2$ and the static wave solution (Figure 6a)

$$\varphi = \frac{\mu}{g} \tanh(\mu x) \quad (8.6)$$

the localized energy density (8.3) becomes (Figure 6b)

$$H(\varphi) = \frac{\varepsilon}{N} \left(\frac{\partial\varphi}{\partial x} \right)^2 = \frac{\mu^3}{g} \frac{\varepsilon}{\cosh^4(\mu x)} \quad (8.7)$$

The ground state energy in the DNA helix, or in the MTs, or in the surface membrane (if ligand induced quanta should be temporarily stored there), is proportional to [31]

$$E \approx \frac{\varepsilon}{N} \int_0^{+\infty} \left(\frac{\partial\varphi}{\partial x} \right)^2 dx = \frac{\varepsilon}{2N} \int_{-\infty}^{+\infty} \left(\frac{\partial\varphi}{\partial x} \right)^2 dx = \frac{2}{3} \varepsilon \frac{\mu^2}{g} = \frac{2}{3} \varepsilon \mu N = \frac{1}{6} \frac{\kappa}{\mu} N \quad (8.8)$$

In any case the ligand induced energy is ultimately stored in a form accessible to DNA, such as pre-RCs in DNA, until the cell makes the unanimous, irrevocable commitment at the *R*-point to initiate DNA replication at the *G*₁-*S* interface. However, the total energy is not a direct sum of energies from the *N* complexes, each of which contributes an equal quantum of energy κ . As shown subsequently, by analysis of a time-dependent perturbation, the initiation of DNA replication and the dynamic instability of growing MTs are both due to the energy contributed by a phosphorylated molecular complex, likely also including hydrolysis of ATP, with the energy threshold counted from the ground state energy level of the actual subsystem.

9. Initiation of DNA Replication and Dynamic Instability in MTs

Inserting small time-dependent excitations in the dynamics (5.7) of the DNA, MT, or surface membrane lattices

$$\varphi(x, t) = \varphi(x) + e^{i\omega_q t} \psi(x) \quad (9.1)$$

and keeping only linear terms, a Schrödinger like exactly soluble equation is obtained [31]:

Figure 6. (a) The symmetric kink-solution $(\mu/g) \tanh(\mu x)$. (b) The energy density $\varepsilon \mu^4/[g^2 \cosh^4(\mu x)]$; $\Delta = 1/\mu$.

$$\left(\frac{d^2}{dx^2} + V''(\varphi) \right) \psi_v(x) =$$

$$\left(\frac{d^2}{dx^2} + 4\mu^2 - \frac{6\mu^2}{\cosh^2(\mu x)} \right) \psi_v(x) = \frac{\omega_v^2}{s^2} \psi_v(x) \quad (9.2)$$

This equation has two discrete eigenmodes

$$\frac{\omega_v^2}{s^2} = \mu^2 v(4 - v); \quad v = 0, 1 \quad (9.3)$$

$$\psi_0(x) = A_0 \frac{1}{\cosh^2(\mu x)}; \quad \psi_1(x) = A_1 \frac{\sinh(\mu x)}{\cosh^2(\mu x)}$$

and a continuum ($q \geq 0$) starting at 2μ ($v = 2$):

$$\frac{\omega_v^2}{s^2} = 4\mu^2 + q^2 \quad (9.4)$$

$$\psi_0(x) = e^{i\omega q x} \left(3 \tanh(\mu x) - 3iq \frac{\tanh(\mu x)}{\mu} - 1 - \frac{q^2}{\mu^2} \right)$$

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Comparing the expansion $\varphi(x + \Delta x) \approx \varphi(x) + \Delta x d\varphi/dx$ with (9.1), which shows that $d\varphi/dx$ equals ψ_0 (9.3), one finds that $\omega_0 = 0$ is a so called translation mode of the unperturbed wave (8.6) of the N molecular complexes. This translation mode implies that all N pre-RCs/replicons in DNA, corresponding to the threshold number of membrane receptor engagements [22], can respond coherently as one unit in the all-or-none commitment at the R -point to subsequently replicate the entire genome. This coherence in time and space is due to the non-local correlations, a prerequisite for the counting at a distance of molecular complexes. The cell first counts, becomes ‘aware’, and then takes the unanimous decision to initiate DNA replication. Although consistent with a simultaneous commitment at the R -point of all N replicons to be replicated, as discussed subsequently, the model still allows a simultaneous initiation in a fraction η of all origins at the S phase entry, not unlike the situation at the R -point where only a fraction of all Rb proteins are released from pre-RCs. The nonzero mode $\omega_1 = \mu s$ (at $\nu = 1$) is a stable eigen-vibration determined by the values of μ and s in the different subsystems. Without additional assumptions the model thus automatically provides a frequency selective mechanism [38] that might play an essential role in the storage of energy as well as in signal transduction between the various subsystems.

SCATTERING THRESHOLD

At zero momentum, $q = 0$, the scattering (continuum) energy threshold (at $\nu = 2$) is given by $\omega_c^2 = 4\mu^2 s^2$, or $\omega_c^2 = 4\mu^2 s^2/d^2$ if the usual length scale d of the lattice unit is used. Multiplying by $\varepsilon = \rho^* s^2$ and inserting $4\mu^2 = \kappa/\varepsilon$, this threshold energy equals $\rho^* \omega_c^2 d^2 = \kappa = 1/2 \rho^* \omega_c^2 d_m^2$, which corresponds to a ‘melting’ displacement $d_m \approx d$. Accordingly, the energy carried by one molecular complex, or part of it, is sufficient for the same complex or part to dissociate from the lattice. It should be recalled, however, that the quantum of energy, κ , as well as the ground state energy (8.8), are different in the various subsystems and a system can also change during the cell cycle. For instance, the system defined by the DNA lattice at the R -point, with pre-RCs that contain Rb protein, is different from the DNA lattice at the G_1 - S interface, with pre-RCs that contain LFs but not Rb protein [6]. Moreover, the cyclin-cdk activity has a maximum at the S phase entry [6], but is rather low at the R -point. After dissociation of all Rb proteins, the DNA system returns to a non-repulsive interaction, a prerequisite for the subsequent binding of LFs and the assembly of a complete lattice of pre-RCs containing LFs. Once again the system then counts the number of pre-RCs in the sense that the N pre-RCs must be primed by LFs before the DNA lattice can switch to repulsive interaction (8.2) required for disassembly and initiation of DNA replication. Driven by the cyclin E-cdk2 activity, however replication is initiated only at the η pre-RCs that carry a phosphorylated LF, presumably after ATP hydrolysis. Replication then propagates like a wave through the system, with individual origin ‘firing’ tempor-

ally distributed during the *S* phase, in compliance with experimental observations [1]. The Cdc6 and Mcm licensing factors are similarly removed progressively as DNA replication proceeds [1]. However, contrary to the *R*-point switch, after which the system switches back to the assembly state, due to the subsequently increased cyclin E-cdk2 activity with hyper-phosphorylation of different parts of pre-RCs and factors like Histone 1 [39], after initiation of DNA replication the system remains in the repulsive state during the entire *S* phase (Table I), which also follows from (8.2) for $1 < \xi < 2$.

The switch of interaction from condensation to repulsion, by contribution of energy from hydrolysis of GTP-tubulin dimers counted from the ground state energy of the MT lattice, can similarly explain the dynamic instability in a growing MT. At a definite length, corresponding to N tubulin dimers, the MT filament suddenly switches from growth to depolymerisation. Given the different (gravitational) mass densities (ρ^*) and the velocities of sound (s), of the hydrolysed (h) and non-hydrolysed (n) parts of a MT or DNA string, the acoustic impedance, $Z = \rho^*s$, becomes known like the amplitude ratios for reflection, $A_r/A_{in} = \tanh(1/2 \ln(Z_n/Z_h))$, and transmission, $A_t/A_{in} = 1 + \tanh(1/2 \ln(Z_n/Z_h))$. These ratios could then be related to the travelling waves before and after symmetry breakdown of the dynamics. If such a comparison applies to DNA this may well indicate that hydrolysis of ATP is required for growth signal transduction and initiation of DNA replication to occur.

10. Summary

A nonstationary interaction, which controls the gross behaviour of DNA replication and the cell cycle progression is derived from manybody physics. The model, which is previously published in a preliminary form [28], predicts a long range force (8.2), $F'(\xi) = -(\kappa/2)\xi(1-\xi)(2-\xi)$ between the protein complexes bound to DNA, $\xi = \varphi/N$ being the induced relative deformation of the DNA lattice (elongation or contraction), φ the number of protein complexes, N the threshold number at which replication is initiated, and κ the compressibility modulus in the lattice of complexes which behaves like an elastically braced string. The so obtained dynamics possesses all desired properties needed to describe the gross regulation of DNA replication and cell cycle progression in terms of the basic molecular interactions (Table I). Initiation of replication is explained by a switch of sign at $\varphi = N$ of the long range force $F'(\xi)$, from attraction (−) and assembly in the G_1 phase ($0 < \varphi < N$), to repulsion (+) and partial disassembly in the *S* phase ($N < \varphi < 2N$), with concomitant release of LFs from pre-RCs and prevention of re-replication. The attraction between pre-RCs in G_1 creates a contraction and tension in the DNA lattice, inhibiting unfolding, origin unwinding and replication. In the *S* phase the repulsive force between protein complexes induces an elongation and unfolding of DNA around the histones and origin unwinding, allowing the replication machinery to work. The termination of DNA replication is due to a switch of the long range

force $F'(\xi)$ back to attraction at $\varphi = 2N$ ($\xi = 2$), when all primed replicons in DNA have been duplicated once. Like in G_1 the attraction in G_2 between protein complexes bound to DNA creates a tension and contraction in the duplex, inhibiting continued unfolding and replication. The model thus ensures that the DNA content of G_2 cells is exactly twice that of G_1 cells. The switch of sign of force at $\varphi = N$ similarly explains the dynamic instability in growing MTs.

The model also explains the switch of interaction at the R -point, with dissociation of Rb proteins and shift from IL2-dependent cyclin D-cdk4/6 to IL2-independent cyclin E-cdk2, after which the T cell proceeds to DNA replication without further exposure to IL2. Although phosphorylation of Rb proteins starts already at mid G_1 , the attractive interaction (8.2) prevails and the assembly of pre-RCs continues until the R -point at which N such complexes are assembled. The long range interaction (8.2) then switches to repulsion by which phosphorylated Rb proteins are being released. Rb proteins that remain bound after the R -point become rapidly phosphorylated and dissociate, after which the lattice returns to a non-repulsive state with assembly of LFs induced by the E2Fs. The induction of cyclin E and sequestration of p27, which activates the pool of p27-cyclin E-cdk2 after the R -point, greatly increases the cyclin E-cdk2 activity that has a maximum at the S phase entry. This leads to a rapid phosphorylation of the LFs, however, to start dissociation of LFs and initiate replication through (8.2), the DNA lattice first ensures that N pre-RCs are primed by LFs. Contrary to the switch of interaction at the R -point, after the switch at the G_1 - S interface the interaction (8.2) maintains the repulsive state during the entire S phase, by phosphorylation of LFs and different inner parts of the pre-RC that are not being released. It could therefore be assumed that the excitation of these inner parts of the pre-RC corresponds to the non-zero eigenvibrations (9.3).

Note that in the actual system only the cyclin-cdk holoenzymes (ligands) are in substrate form, whereas the proteins (receptors) to be phosphorylated are bound to the DNA lattice, a condensed matter system controlled by the non-local correlations (4.2), and to less extent by Michaelis-Menten type equations [40]. Shape, slope and scale, $\rho_{50} = r\rho_0/(Er_0)$, of the response curves derived agree well with experimental data from dividing T cells (Figure 2a) and polymerising MTs, the variable length of which is explained by the model through a nonlinear dependence of the growth amplitude on the initial concentrations of GTP and tubulin dimers (Figure 4). The model also provides a nonlinear expression for the definite threshold number (5.3) of molecular complexes engaged, $N = \mu(\rho_0 + r_0)/(\rho_0 - r_0)$, at which DNA replication is initiated, and at which the dynamic instability in growing MTs occurs, ρ_0 and r_0 being the initial reactant concentrations in the actual subsystem. More exact results could be obtained by replacing the initial reactant concentrations by the so called 'dynamic concentrations' in (3.1), with k' and K different from zero, because the driving reactants are the actual holoenzymes. Given the explicit forms of the threshold and scale, it should then in principle be possible to examine certain forms of cancer, with abnormal expression of regulator

proteins. The actual form of long range interaction (8.1) depends crucially on the formulation of (4.3) which permits definition of a dimensionless coupling constant (5.2), and a constant term μ^2 that yields the positive definite quantum of energy, $\kappa = 4\mu^2\varepsilon$, per ligand-receptor association [26], per pre-RC, or per GTP-tubulin dimer. This property may look formal, however, is nontrivial too because, had one tried to define $\kappa = 4\mu^2\varepsilon$ through the symmetric equation (5.7) instead of the asymmetric one (7.2), then μ^2 and hence κ would have carried the wrong sign. The elastically braced string dynamics (8.1) thus accounts for the leading order interaction only, and the remaining dissipative effects (9.1) could be treated as time-dependent corrections [36].

Much about DNA replication is obtained from yeast, and it can therefore be asked whether such information could apply to T lymphocytes from mammals. There is no general answer but analogues of yeast ORC genes and proteins have been described in a wide range of eukaryotic organisms, suggesting that ORC is a conserved component of eukaryotic DNA replication [1]. Experiments [2] also provide solid evidence for replication origins, with specific DNA sequences (replicators), in animal cells [41]. Other components of eukaryotic DNA replication may turn out to be non-conserved, however, this should have little influence on the model proposed as long as ORC is considered to be the best candidate for an initiator protein [1]. Moreover the switch of sign of the long range interaction (7.3), crucial for the transition between assembly and replication states, is essentially determined by the number of origins/replicons engaged by ORCs complexed with various regulators.

The model, which is here formulated for the inhibitory factor p27 and the Rb protein, should in principle work similarly also when cyclin-cdk is regulated by Cip/Kip proteins such as p21 and p57, or members of the so called INK4 family, p15, p16, p18 and p19. The exact implementation and relative importance of various mechanisms may differ in different organisms and at different times in the cell cycle. However, the model proposed provides an explanation for the multiple inhibitory machinery that must function in order to have a complete replication and prevention of re-replication in normal cell division. Disruption of function at different levels of regulatory factors, in particular Rb protein, frequently occurs in many forms of human cancer [39]. Hopefully, the model proposed could be used to further analyse such forms of cancer, as well as certain forms due to centrosome malfunction [42]. Experiments indicate that cyclin E-cdk2 activity is required for centrosome duplication [43]. A possible explanation of the separation of centrioles could be provided by the switch to repulsive interaction at the *S* phase entry, and through the preceding shift from cyclin D to cyclin E dependent cdk activity, the model interaction also indirectly controls the centrosome duplication.

Normal cell division crucially depends on the cells ability to take a unanimous decision to initiate replication, if and only if a definite number of replicons are primed for replication. The similarity with decision taking in a human brain suggests that the spatio-temporal coherence in the system, required for the all-or-

none transition into the *S* phase, could be taken as a form of classical definition of consciousness of the individual cell, a state of 'mind' developed prior to its unanimous decision to initiate replication. In the infinite wavelength approximation such a definition could also work for a system of cells connected through a rigid cytoskeletal network.

The proposed model only accounts for the leading order interaction. A more realistic response theory, that could also explain the value of η , the bidirectional character of replication, the role of hydrolysis of ATP and GTP such as in tubulin dimers with mobile electrons [44], requires a description in three spatial dimensions, inclusion of polarisation and electromagnetic interaction. A preliminary version of such an extension has been made to analyse the unharmonic corrections and emergence of the elastically braced string dynamics in three spatial dimensions [45]. Interestingly, a superconductor like dynamics is obtained, with DNA and MTs appearing as string-like vortex solutions. A three-dimensional version of that model, with spherical symmetry and an additional requirement of electric charge conservation in the cell, also provided particular 'hedgehog' solutions that could be interpreted as a three-dimensional cytoskeleton structure with MTs pointing in different spatial directions. Another problem that remains to be solved is to account for spatio-temporal dependence of the initial reactant concentrations. Without such corrections, cytoskeletal MTs in the model would grow identically in all directions.

However, the counting of receptors and DNA origins engaged, the interaction that promotes assembly and disassembly of pre-RCs with prevention of re-replication during the *S* phase, the mechanisms responsible for initiation and termination of DNA replication, such that the DNA content is exactly duplicated during replication, and the good agreement with growth data of dividing cells and polymerising MTs with variable length and dynamic instability, are results obtained through one and the same model. This has encouraged me to propose that already the one-dimensional model presented here could serve as a guide for further studies of both normal and transformed cells, as well as their functions and dysfunctions in the organism. Through charge conservation, which implies a symmetry termed gauge invariance that links the electromagnetic field to the matter field, the lyotropic one-dimensional model obtained could then work as platform for further generalizations into three spatial dimensions.

Acknowledgements

I thank Hans Frauenfelder and Anders Hamberger for useful comments and Charles Sherr and Kendall Smith for explaining the experiments.

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