Long Range Force between Pre-Replication Complexes (Pre-RC) in DNA Controls Replication and Cell Cycle Progression

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Abstract. A nonstationary interaction, that controls DNA replication and the cell cycle, is derived from a manybody physics model 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 DNA, $\xi = \varphi/N$ being the relative displacement of preRCs, φ the number of pre-RCs, N the threshold for initiation, and κ the compressibility modulus in the lattice of pre-RCs which behaves like an elastically braced string. Initiation of DNA replication is induced 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 the pre-RCs, thus explaining prevention of re-replication. Replication is terminated by a switch of sign of F at $\varphi = 2N$, when all primed replicons are duplicated once, and $F(0) = 0$ corresponds to a resting cell in absence of driving force at $\varphi = 0$. The switch of sign of force at $\varphi = N$ also explains the dynamic instability in growing microtubules (MTs), as well as switch in the interleukin-2 (IL2) interaction with its receptor in late G_1 , at the restriction point. Shape, slope and scale of the response curves derived agree well with experimental data from dividing T cells and polymerizing 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).

Key words: cell cycle control, cyclin dependent kinases, DNA replication, origin recognition complex, Rb protein

1. Consensus about DNA replication?

Self-replication is often mentioned as the main property that characterizes the phenomenon of life, however, considering the ambient, nonstationary conditions for ligand-receptor interaction, the commitment to replication appears to be the most critical step. All time variation of the cell cycle is located in the G_1 phase, whereas subsequent phases follow automatically within a constant period of time, like a machine that has been switched on by a living being or a computer operating after a given software program. Initiation could thus illustrate what molecules can do together in living matter that they cannot do in inanimate condensed matter.

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

One may then ask under which conditions or in what type of collective property the phenomenon of life appears. 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 at the surface membrane, in DNA, in 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 hence also be described in terms of manybody physics relevant for the nonstationary chemically open cell system.

Initiation of DNA replication at hundreds or thousands of sites along the DNA duplex requires an exact timing and spatial coordination over long distances. Such a long range coherence could not exist without nonlocal correlations between the interacting molecular complexes. Considering the large number of reactants that participate in DNA replication another problem is to pinpoint the factor that plays the key role at the initiation. Possible answers are provided by recent experimental

findings. 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], but only ORC liganded with adenosine-triphosphate (ATP) can recognize and bind to DNA origins in the double helix [3]. However, the DNA binding by ORC does not require nucleotide hydrolysis, and ATP remains stably associated with ORC [4]. The role of ATP hydrolysis at the initiation is not yet understood. Replication of DNA starts by syntheses 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) [5], however, TFs already bound by DNA are tonically suppressed in the interphase by the Rb proteins (termed after their discovery in Retinoblastoma cells). In late G_1 the Rb proteins become phosphorylated and undergoes conformational changes which induce their dissociation from the TFs, thus allowing a rapid transcription of all genes necessary for DNA syntheses and replication.

Phosphorylation of Rb proteins in turn is induced by specific kinases, 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 , depend strictly upon a continuous interleukin-2 (IL2) exposure until a restriction point in late G_1 , the *R*-point, beyond which the cell expresses cyclin E and proceeds to initiation of replication without further exposure to IL2 [6]. However, the cyclin-CDK activity is opposed by another protein, p27, the degradation of which is also controlled by interaction of IL2 with its receptors [7].

Before initiation of DNA replication the origins must also be primed by the licensing factors (LF), which may control the completion of replication in such a way that the genome is copied once and only once per cell cycle. In budding yeast cells the Cdc6 and Mcm2-7 proteins must first bind to DNA origins before the TFs, the Mcm1 protein and others, can carry out the transcription. The pre-replication protein complexes (pre-RC) thus include those that directly select origins in the DNA duplex, the ATP-ORC units, and others such as the LFs, subsequently recruited to DNA origins [1]. 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 *S* phase [8]. All reactions and biochemical pathways activated in a T cell via the IL2 receptor (IL2R) are not entirely identified and, hence, cannot be fully described in this gross interaction model. But the important consequence is that the transport between the *R* point and the G_1 *S* interface, from the surface membrane to the nucleus, of the dynamics responsible for the molecular on-off switch like mechanism, actually takes place in a form invariant fashion. As demonstrated here, the switch-like long range dynamics has the same form irrespective, of if the carriers of this interaction are ligands and receptors at the surface membrane, or the LFs and other proteins that bind to DNA origins. Also important is that the IL2 interaction with its receptors controls both the accumulation of D-type cyclins

Table I. Control of replication and cell cycle progression by a long range interaction between protein complexes in DNA; replication is initiated when all DNA origins ($\varphi = N$) are engaged by pre-replication complexes. Doubling of mass in G_2 is not a critical event in the sense that it normally follows automatically if replication is initiated

	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, preven- tion of re-replication	Contraction of duplex about the histones	Cyto- kinesis
Force between DNA protein complexes.	Attraction (-)	Repulsion (+)	Attraction (-)	
Order-parameter intervals	$0 < \varphi < N$	$N < \varphi < 2N$		
Thresholds	$\varphi = 0$	$\varphi = N$	$\varphi = 2N$	

and the degradation of the cyclin-CDK inhibitor p27 [9]. The target for the cyclin-CDK phosphorylation is the pre-RC bound to each replicon in the DNA duplex [1].

A modelling from first principles, based on all these factors, seems to be difficult but not impossible. Experimental advances 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 disassembly of pre-RCs but inhibits the formation and binding of new such complexes (Table I) [1]. Such a 'consensus' picture, however, creates problems. Without a definite threshold for initiation the binding of arbitrary one ORC/pre-RC by a DNA origin could then imply two different reactions – continued assembly of pre-RCs, or partial disassembly of pre-RCs with release of LFs, and concomitant start of replication. Initiation under such indefinite conditions could lead to incomplete replication. This degeneracy would prevail irrespective of hydrolysis, phosphorylation, or any other factor.

Thus, to ensure a complete duplication of DNA, the following alternative interpretation is suggested. An ORC with the aid of LFs and other protein factors attached should be unable to initiate replication unless a definite number of origins/replicons in DNA have been engaged by pre-RCs. The cell must hence be able

to simultaneously count the number of pre-RCs bound to DNA along the duplex, before the irrevocable commitment to replication is made, implying the existence of long range (nonlocal) correlations. It will be demonstrated here that a nonlinear manybody physics model can explain such a counting-at-distance mechanism, as well as the transition into the *S* phase at a definite (quantal) threshold number of pre-RCs. In the model proposed the switch of interaction from assembly/attraction to partial disassembly/repulsion [1, 8], which explains the initiation of DNA replication and prevention of re-replication during the *S* phase by repulsion of the LFs, could be generated by the contribution of energy from one primed origin/replicon, above the lattice ground state. This energy, provided by hydrolysis of ATP, or an equal amount of energy provided by the pre-RCs phosphorylated by cyclin-CDK, could make the DNA lattice of pre-RCs – primed replicons – unstable.

The DNA duplex, the nuclear envelope, and the surface membrane alone are too liquid like and soft to explain the presence of nonlocal correlations between DNA origins/replicons engaged by pre-RCs, or between surface membrane receptors engaged by IL2. However, a stabilized cytoskeletal network of microtubules (MTs) [10], the most rigid elements of the cell, can induce such correlations, within and between the membrane and nuclear subsystems. Also the histones contribute to the stabilization of DNA. The initiation of replication, for which stabilization is hence a requirement, is regulated by the concentrations of various factors – cyclins, kinases, ATP, ORC, DNA origins, TFs, LFs and others – the interaction of which is indirectly regulated by the two surface membrane reactants, IL2 and IL2R. A dynamical system like this, controlled by concentrations, is termed lyotropic [11]. The problem is that most condensed matter theories are based on thermotropic systems, hence regulated by temperature variations and, like the Hill and Langmuir equations, based on the assumption of a chemical stationary state [12, 13].

DNA replication, however, is initiated by the binding of pre-RCs to origins, an unidirectional hence nonstationary process. The ORCs/pre-RCs thus bind and remain bound to the DNA origins until replication, during which the LFs are released. The ORCs remain bound, hence, contained in the post-RCs. Also many other important biological functions are controlled by nonstationary reactions. On a T cell, for instance, the high affinity receptors engaged by IL2 internalize already 15 min after binding to IL2, while dissociation of IL2 from such receptors does not start until 30 min later [14]. Biological response may thus occur long before a stationary state has been attained, which is mainly also the reason why the threshold and scale derived in stationary models may deviate by up to three orders from the scale assessed [15] (Figure 2). In order to remedy this type of failure the scale derived is usually replaced by the ligand concentration for half-maximal response. However, then the response derived becomes a mere logistic equation. Obviously, this fits better to assessed data, but, the physical meaning and interpretation of results, in terms of molecular interaction, is then lost [16]. The discrepancy between theory and experimental data has also been ascribed to factors like transport proteins or a

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.

variable affinity [17]. Although such factors may well contribute too, they cannot compensate for the neglect of nonstationary effects in the first place.

Data from experiments on T cell proliferation show that a definite number of IL2-receptor associations are required for the cell to make the all-or-none commitment to initiate DNA replication [18]. This switch like (quantal type) response has also been observed in the T cell receptor (TCR) system [19]. Thus, the T cell is somehow able to count at a distance the number of interacting receptors [20], irrespective of their location on the surface membrane. Like for the origins in DNA, the indication of a definite threshold number of receptors engagements implies the existence of nonlocal correlations between individual receptors, hence, the existence of a long range interaction that controls the exact timing of DNA replication. However, a correlation between the R -point or the $G_1 - S$ transition and a critical mass threshold [21] has not been verified. Rather, the stable propagation of genetic information requires that the entire genome of an organism – a definite number of replicons – be faithfully replicated once and only once each cell cycle.

As stated here before, the surface membrane and DNA duplex alone are not rigid enough *per se* to explain the long range forces responsible for such correlations, neither within nor between the subsystems. A molecular complex at one end of the membrane, or at one end of the DNA duplex, would know little or nothing about another complex at the other end of these soft condensed structures, in which only adjacent complexes are correlated. A diffusive propagation of signals would be too slow to explain the exact coordination in time and space, and the simultan-

eous counting of interacting molecular complexes, and could hence not explain the initiation of DNA replication. Cell motility and division, signal transduction, and energy storage, however, depend on the dynamics of cytoskeletal MTs, the most rigid elements in the cell.

At critical points in the cell cycle, when the cytoskeleton is stabilized, a realistic cell model could hence be expected to be more solid like. However, a couple of hitherto unsolved problems have prevented a physical understanding of the dynamics and polymerization of MTs, the dynamic instability and the variable length of growing MTs [10, 22, 23], but the chemical driving reaction is well known: Only tubulin dimers liganded with two guanosine-tri-phosphate (GTP) molecules polymerize into MTs [10]. Known is that a fraction of growing MTs actually attains a stabilized state at critical points in the cell cycle. Presumably, the MTs and the histones are the only cell elements that could explain the nonlocal correlations between the membrane receptors, between DNA origins, and between the switch of interaction at the *R*-point, and at the initiation of replication. Before investigating this possibility, however, some words are in place on the energy induced by the ligand-receptor interaction. It is found that each receptor engagement contributes an equal quantum of excitation energy [24] and, hence, there is no reason to believe that this should not also be true for the pre-RC bound by DNA origins. But the total energy stored is not a linear sum of the individual excitations because the rate of cell division, as well as the dynamics that controls DNA replication and cell cycle progression, are nonlinear functions of the ligand-receptor interaction.

Studies on T cells show that the rate of division is a nonlinear function determined exclusively by the concentrations of IL2 and IL2R [14]. This may appear unexpected because all time variation of the cell cycle is normally contained in the G_1 phase, and the rate of cell division therefore approximates to the rate of cells initiating DNA replication, a function of concentrations of ORC, DNA origins, ATP, p27, Rb proteins, cyclins, CDKs and so on [1], albeit indirectly controlled by the two surface membrane reactants, IL2 and IL2R [6, 7]. However, from a physical point of view the exclusive dependence on IL2 and IL2R [14] is not a mystery. It is a form of enslavement, in which all other variables are damped at the actual time scale (hours and minutes), and this is also one of the premises for modelling the long range interaction. As demonstrated subsequently, the nonlocal correlations, corresponding to the long range interaction, have the same form in the three actual subsystems irrespective of which chemical reaction actually drives the local interaction.

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 only 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, permits nonlocal correlations. A realistic, self-consistent but nontrivial gross interaction model of a dividing cell should hence:

- i) yield a growth rate which is a nonlinear function of the two reactant concentrations only,
- ii) with the correct shape, slope, and scale,
- iii) explain the condensating type of interaction responsible for the nonstationary assembly of complexes in G_1 ,
- iv) explain the initiation of DNA replication at a definite threshold number of receptor engagements at the surface membrane and at a corresponding definite number of origine 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 the actual subsystem,
- vii) explain the disassembly of complexes and the prevention of re-replication during the S phase,
- viii) explain the dynamic instability and variable length of growing MTs,
- ix) explain physically the roles of phosphorylation, and of hydrolysis at the initiation of DNA replication and in growing MTs.

In an earlier version of the model [26] it was possible to explain the major part of these results except vi) and ix). In an improved model [27], obtained by a redefinition of the chemical potential, each molecular complex became a carrier of a constant quantum of energy. This question may appear trivial, however, restricts the system to a unique type of interaction. The improved model could also describe the gross behaviour of DNA replication and the cell cycle progression in terms of the interacting protein complexes in DNA, and also the dynamic instability and variable length of growing MTs. In the present work this model is further refined and more clearly explained.

2. Nonstationary Driving Reactions

Let ρ , r and Ψ denote the concentrations of IL2, IL2R and their bound complex. In the nucleus these variables could represent the concentrations of ATP-ORC units (ligand), DNA origine (receptor) and ATP-ORC-origine complexes (Figure 1a), or the concentrations of the last binding LF, the corresponding incomplete pre-RC, and the the complete pre-RC. The rate of receptor engagement by ligands at one specific site, or recognition-binding of an ATP-ORC at an origin, or binding of the last LF to a pre-RC, is then given by

$$\begin{aligned} \frac{\partial \psi}{\partial t} &= k\rho r - k'\psi = k((a_0 - \psi)^2 - (a_0^2 - b_0^2)) \\ &= k(1 - \psi/a_0)^2 \left(a_0^2 - \frac{(a_0^2 - b_0^2)}{(1 - \psi/a_0)^2} \right) \end{aligned} \quad (2.1)$$

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 (2.1) yields

$$\begin{aligned} \ln \left(\frac{\rho_\kappa - \psi}{r_\kappa} \frac{r_\kappa}{\rho_\kappa} \right) &= \ln \left(\frac{\rho'}{r'} \frac{r_\kappa}{\rho_\kappa} \right) \\ &= k(\rho_\kappa - r_\kappa)t = 2k\sqrt{a_0^2 - b_0^2} t \end{aligned} \quad (2.2)$$

where $\rho_\kappa = a_0 + \sqrt{a_0^2 - b_0^2}$, $r_\kappa = a_0 - \sqrt{a_0^2 - b_0^2}$, ρ' and r' play the role of dynamic concentrations, such that $\rho' + \psi = \rho_\kappa$ and $r' + \psi = r_\kappa$. In a high affinity system, where $k' \approx K \approx 0$, the dynamic concentrations thus equal the initial ones, $r_\kappa \approx r_0$, $\rho_\kappa \approx \rho_0$, $r' \approx r$, and $\rho' \approx \rho$. Unless required, no distinction will therefore be made between dynamic and initial concentrations.

In (2.2) the time parameter t may be interpreted as a ‘physiological’ time, which relates the usual time to the two continuous reactant concentrations. However, at the all-or-none transition into S phase, the dynamics suddenly becomes sharply sensitive to the number of molecular units engaged [9, 18–20]. A switch-like behaviour of this type has also been observed in progesterone induced oocyte maturation [28]. In that case individual oocytes showed a mitogen-activated protein kinase (MAPK) cascade equivalent to a cooperative enzyme with Hill coefficient of at least 35, but as has already been pointed out [29], there is no cooperativity between individual enzymes in the MAPK cascade. It should be underlined, however, that lack of local cooperativity between molecular units does not exclude presence of nonlocal correlations. As demonstrated subsequently, both the long range interaction and the threshold for response are determined by such nonlocal correlations.

3. Nonlocal Correlations due to Long Range Interaction

The discovery that a T cell counts the number of interacting receptors/origins before it takes the decision to initiate DNA replication is perplexing. However, from a many-body physics point of view it is not. It indicates the presence of nonlocal correlation between the receptors at the surface membrane, like it does for the origin in DNA. Moreover, it indicates a correlation between these two subsystems. The nonlocal correlation between n identical molecular complexes at x_i , $1 < i < n$, which equals 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 normalization constants. However, this expression is ambiguous because in nonstationary, lyotropic systems the notion of concentration could become undefined at too many or too few reactant molecules. A well-defined concentration

thus requires a large enough number of molecules in each macroscopically small but microscopically large volume element.

At high concentrations of vacant receptors on the surface membrane, however, the volume element around each one of them 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. Hence, the notion of concentration is not enough well-defined to describe the actual lyotropic interaction in which $\psi(x)$ merely denotes the probability that (2.1) generates an engaged receptor at the site x . The probability to find a definite number of ligand-receptor complexes must therefore be extended to an average of all orders

$$\varphi = \mu \sum_{n=0}^{\infty} \left(\frac{1}{a_0} \right)^n \prod_{v=0}^n \psi(x_v) \quad (3.1)$$

The surface membrane in (3.1) could be regarded as an one-dimensional aperiodic lattice, and the term $n = 0$ accounts for the possibility of having a contribution already from the start. A truncation of (3.1) 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. The infinite series (3.1) may also denote the probability to find a definite number of pre-RCs bound by origine in DNA. The first order growth of a solid type lattice (3.1) of complexes in DNA, or at the surface membrane, $\partial\psi/\partial t = k * \psi$, $k*$ being the on-rate constant, should be restricted by the rate of formation (2.1) of complexes in the actual subsystem, hence, with $k' \approx 0$ one has $\partial\psi/\partial t = k * \psi = kpr$. The lattice growth rate could then be obtained by combining (3.1) with (2.1). As demonstrated here below, this yields a unique form of interaction that controls the cell at the initiation of DNA replication.

At increasing density of receptors engaged, the membrane area per receptor becomes successively smaller and the liquid-diffusive type motion more restricted. At a certain threshold the diffusion then changes into a harmonic-displacive type interaction, because the link between receptors through the rigid cytoskeletal network converts the liquid crystal type membrane to a more solid close-packed lattice. Thus, the critical threshold is also determined by the rigidity and elasticity of cytoskeleton and the medium in which it is embedded. A transition into a harmonic-displacive type dynamics, by increasing number of protein complexes, is similarly expected also in the DNA duplex because a diffusive propagation in a liquid type DNA would be too slow to explain the exact timing and spatial coherence observed at the initiation of replication. The average distance between DNA origins engaged is then reduced to a critical limit, corresponding to a definite number of origine engaged by protein complexes. As demonstrated subsequently, with such a liquid-to-solid type transition in the membrane and DNA lattices, implying nonlocal correlations between the protein complexes, the switch of ligand-receptor interaction at the *R*-point, and the transition from assembly to replication at the initiation, follow as results of the nonstationary driving reaction (2.1).

Replication does not start at all origine at once, however, the cell first makes sure that a definite number of DNA origins/replicons, proportional to the definite number of IL2 receptors engaged by IL2, are engaged by pre-RCs before the decision to start replication is taken. 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 (3.1) to find a lattice (condensate) with a definite number of bound complexes in the actual subsystems, in DNA corresponding to the number of replicons primed for replication, then approximates to a geometric series

$$\varphi = \mu \sum_{n=0}^{\infty} \left(\frac{\psi}{a_0} \right)^n = \frac{\mu}{1 - \psi/a_0} \quad (3.2)$$

In a field theory perturbation expansion (3.2) corresponds to a Bethe-Salpeter like summation of ladder diagrams (Figure 1a). Like in (2.1), 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 (3.2). When combining (3.2) and (2.1) the rate of binding of complexes into a whole lattice (3.2) in any of the actual subsystems becomes

$$\frac{\partial \varphi}{\partial t} = \frac{ka_0}{\mu} \left(\mu^2 \frac{(a_0^2 - b_0^2)}{a_0^2} \varphi^2 \right) \quad (3.3)$$

which apart from a constant has the solution

$$\varphi = \frac{\mu a_0}{\sqrt{a_0^2 - b_0^2}} \tanh \left(k \sqrt{a_0^2 - b_0^2} t \right) \quad (3.4)$$

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

$$N = \varphi(\infty) - \varphi(0) = \frac{\mu a_0}{\sqrt{a_0^2 - b_0^2}} \approx \mu \frac{\rho_0 + r_0}{\rho_0 - r_0} \quad (3.5)$$

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

4. A Chemical Potential Energy

No experimental signs of replication are observed until a definite number of receptors have been engaged [14]. The chemically induced energy must therefore be stored as a form of potential energy, $V(\varphi)$, during the lag time – from start of

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

exposure of the cell to ligands until start of replication. In the nucleus this storage could take place in the form of pre-RCs bound by DNA, with an average lattice spacing d between the complexes. If the number of membrane receptors engaged, corresponding to a definite number of pre-RCs bound by the DNA duplex, attains the definite threshold number observed [18], then replication is initiated. The rate of cells initiating replication thus equals the rate of cells in which the DNA lattice of bound pre-RCs attains a definite threshold length (L_0) corresponding to the definite threshold number of pre-RC. Accordingly, (3.3) could be interpreted as a travelling wave equation generated by the chemical potential $V(\varphi)$:

$$\frac{1}{v_N} \frac{\partial \varphi}{\partial t} = \frac{\partial \varphi}{\partial x} = \pm \sqrt{2V(\varphi)} = \frac{ka_0}{\mu v_N} (\mu^2 - g^2 \varphi^2) \quad (4.1)$$

v_N being the travelling wave velocity in units of lattice spacing per unit time. The binding of pre-RCs could also be assumed to induce a corresponding deformation, elongation or compression, of the DNA lattice, and the continuous variation of φ (4.1) could then also be interpreted as a result of displacive motion in the lattice due to long range interaction between the protein complexes. A subsequent comparison with experiments indicates that such an assumption is realistic.

The nonlinear coupling g is given by

$$g = \frac{\sqrt{a_0^2 - b_0^2}}{a_0} = \frac{\rho_0 - r_0}{\rho_0 + r_0} \quad (4.2)$$

and the threshold number then becomes

$$N = \mu \frac{\rho_0 + r_0}{\rho_0 - r_0} = \frac{\mu}{g} \quad (4.3)$$

With a symmetric chemical potential (Figure 3) defined by (4.1):

$$V(\varphi^2) = \frac{g^2}{2} \left(\frac{\mu^2}{g^2} - \varphi^2 \right)^2 \quad (4.4)$$

the traveling wave velocity becomes

$$v_N = k \frac{\sqrt{a_0^2 - b_0^2}}{\mu} = \frac{1}{N} v_1 \quad (4.5)$$

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

$$v_1 = k a_0 = k \frac{(\rho_0 + r_0)}{2} \quad (4.6)$$

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

However, before initiation of DNA replication by binding of an ultimate LF, in order to complete the last pre-RC, the lattice is confined in a bound state harmonic-displacive type dynamics. The second order equation of motion for small harmonic undulations and displacive motions corresponding to (4.1), in the close-packed lattice of pre-RCs at the threshold short before initiation, then takes the form [30, 31]

$$\begin{aligned} \frac{1}{s^2} \frac{\partial^2 \varphi}{\partial t^2} - \frac{\partial^2 \varphi}{\partial x^2} &= - \frac{\partial V}{\partial \varphi} \\ &= F_0(\varphi) = 2g^2 \varphi (N - \varphi)(N + \varphi) \end{aligned} \quad (4.7)$$

s being the velocity of sound, and $F_0(\varphi)$ the nonlinear driving force. Multiplying both sides of the static part of (4.7) by $\partial \varphi / \partial x$ equation (4.1) is reobtained after integration. A diffusive type equation instead of (4.7), with a first order derivative in time corresponding to a more liquid like dynamics in the two subsystems, would not be capable to explain the exact timing and spatial coherence observed at the initiation of DNA replication.

As stated here before the same dynamics (4.7) is obtained for the ligand-receptor interaction at the surface membrane, which has also been approximated by an one-dimensional lattice (3.1) in the model. All pathways responsible for the transfer of the gross interaction, from the surface membrane to the nucleus, are not entirely known, however, the important consequence is that it is transferred without any change of form, which follows from the fact that the nonlocal correlations (3.2) and the driving reaction (2.1) have the same forms in the surface membrane and DNA. Consequently, the response – the rate of cell cycle progression which is the same at the R -point and the $G_1 - S$ interface – is the same in the two subsystems, which also follows from the fact that the lag time between the R -point and the $G_1 - S$ interface is approximately constant. This form of enslavement of DNA under the surface membrane interaction, also indicated by experiments [14], crucially depends on the stabilization of cytoskeleton, without which neither the nonlocal correlations (3.2) nor this form of long range interaction, in DNA and the membrane and between the membrane and nucleus, would exist.

Therefore, also the cytoskeleton dynamics must obey the same gross interaction because, only one long range interaction should control the cell cycle at a time. Cytoskeleton should thus work together with the surface membrane dynamics before and at the R -point and, after a certain lag time, with the DNA dynamics at the initiation of replication. In cytoskeleton the geometrical series (3.2) yields the probability to find an MT fragment (condensate) with a definite number of GTP-tubulin dimers. The first order rate of polymerization of MTs, $\partial\varphi/\partial t = k * \varphi$, is restricted by a second order rate of formation of complexes, $\partial\varphi/\partial t = k * \varphi = k\rho r$ (with $k' \approx 0$), and in this subsystem (2.1) represents the association of GTP with tubulin dimers, or re-phosphorylation of GDP-tubulin. (2.1). Hence, (2.1) is the driving reaction of the cytoskeleton dynamics, the attachment-detachment of energetic GTP-tubulin dimers to all orders of (3.2). Combining (2.1) with (3.2) the same equation of motion (4.7), driven by the nonlinear potential (4.4), is obtained also for the cytoskeletal network of MTs. In cytoskeleton the threshold N represents the content of bound GTP-tubulin dimers, i.e., the turbidity [10], which is determined by the initial concentrations of the two reactants in this subsystem.

5. Comparison with Experimental Data

By inspection of (4.4) 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 (4.7) turns out to be repulsive (+) for $0 < \varphi < N$, that is in the assembly state (G_2) when the system is expected to be attractive. However, this is not as bad as it may appear. The equilibrium of V in (4.4) 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 of $V(\varphi)$:

$$\varphi \rightarrow -\frac{\mu}{g} + \varphi = -\mu \frac{\rho_0 + r_0}{\rho_0 - r_0} + \varphi \quad (5.1)$$

A direct consequence of this is that the reflection symmetry of $V(\varphi)$ (Figure 3) is spontaneously broken and the total probability (3.4) becomes positive definite:

$$\varphi = \mu \frac{\rho_0 + r_0}{\rho_0 - r_0} \left\{ 1 + \tanh(k\sqrt{a_0^2 - b_0^2}t) \right\} \quad (5.2)$$

Expressing the time parameter in (5.2) in terms of the actual reactant concentrations, through (2.2), the response of the lyotropic system becomes

$$\varphi = \mu \frac{\rho_0 + r_0}{\rho_0 - r_0} \left\{ 1 + \tanh(1/2 \ln \left(\frac{\rho}{\rho_{50}} \right)) \right\} \quad (5.3)$$

The scale $\rho_{50} = r\rho_0/(Er_0)$, which is a function of the reactant concentrations, could be compared with the constant scale $\rho_{50} = K^{1/n}$ usually obtained in stationary type theories [12, 13]. The integration constant E could be related to efficacy. At suitable initial reactant concentrations the quantal number N can attain the definite number of ligand-receptor engagements observed, corresponding to a definite

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. [10] 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

threshold number of engaged DNA origine (the number of replicons primed for replication), and (5.3) then yields the rate of transition into the S phase, which approximately equals the rate of division of cells

$$R(\rho) = CN\{1 + \tanh(1/2 \ln \left(\frac{\rho}{\rho_{50}} \right))\} = 2CN \frac{\rho/\rho_{50}}{1 + \rho/\rho_{50}} \quad (5.4)$$

The constant C is proportional to the number of cells in the start population. Shape and slope ($n_H = 1$) of (5.4) agree almost exactly with data from a cell line MLA-144 [25], and contrary to stationary models [12, 13] 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 (5.4), induced by the membrane reactants at the cell surface, also controls the cytoskeleton dynamics – the assembly-disassembly of MTs. Laboratory conditions are markedly different from those of a living cell, however, on a gross interaction level (5.4) should be proportional to the average length of an MT, that is to the content of tubulin dimers [10]. Let C denote the number of MTs and N that of GTP-tubulin dimers per MT, (5.4) then yields the turbidity as a function of the concentrations of GTP and tubulin dimers, and (5.2) should hence be proportional to the variable length of a growing MT as a function of time.

At the actual concentration of 500 μM , however, the majority of reading GTP molecules do not have the same chance to bind to a tubulin dimer as those in the vicinity of the less abundant dimers. Consequently, the effective concentration of

Figure 4. The variable length of growing microtubules, as a function of time, at an effective initial GTP concentration of $32 \mu\text{M}$, and initial tubulin dimer concentrations at; (a) $19.0 \mu\text{M}$, (b) $17.0 \mu\text{M}$ and (c) $13.8 \mu\text{M}$. Data taken from ref. [10] with permission.

GTP at work in the second order rate equation (2.1), that controls the growth of MTs, is much lower, and the on and off rate constants [22, 23] are correspondingly changed by a factor f , which is also due to that the growth of MTs is markedly nonstationary. 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 here modified by a contribution from hydrolysis (h), $k' \rightarrow k' + k_h = 1.14f \text{ s}^{-1}$, where $f = 0.028/60$, hence, with $K = k'/k = 0.3 \mu\text{M}$ being left unchanged of f , a satisfactory agreement of the actual one-dimensional model (5.2) with data from growing 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) [10], provided the essential start of polymerization occurs at $t_0 = 1.85$, $t_0 = 2.1$ and $t_0 = 3.45 \text{ min}$, respectively.

At lower tubulin concentrations, the system responds much slower and laboratory conditions could therefore be expected to distort the results.

The good agreement of the derived response (5.4) with the three concentration dependent MT amplitudes assessed (Figure 4), which could explain the variable length of growing MTs, and with the growth data of dividing cells (Figure 2), lend strong support to the proposed long range gross interaction (4.7). It should be observed that not only the shape, slope and scale of the response must fit data (Figure 4); in this case the amplitude, which is as a function of the initial reactant concentrations, must also explain the variable length of growing MTs (Table II).

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

6. Long Range Force Controls DNA Replication

The shift (5.1) of the coordinate system yields an asymmetric potential (Figure 5):

$$V_A(\varphi^2) = \frac{g^2}{2} \varphi^2 \left(\varphi - \frac{2\mu}{g} \right)^2 \quad (6.1)$$

and the equation of motion becomes

$$\begin{aligned} \frac{1}{s^2} \frac{\partial^2 \varphi}{\partial t^2} - \frac{\partial^2 \varphi}{\partial x^2} &= - \frac{\partial V_A}{\partial \varphi} \\ &= F_A(\varphi) = -2g^2 \varphi \left(\frac{\mu}{g} - \varphi \right) \left(\frac{2\mu}{g} - \varphi \right) \end{aligned} \quad (6.2)$$

in which $F_A(\varphi)$ is the long range force between the interacting molecular complexes in the actual lattice. Inserting the definite number (4.3) the chemically induced driving force becomes

$$F_A(\varphi) = -2g^2 \varphi (N - \varphi) (2N - \varphi) \quad (6.3)$$

which vanishes at $\varphi = 0$, $\varphi = N$, and $\varphi = 2N$. Hence, F is attractive (−) in G_1 , the assembly state ($0 < \varphi < N$), which allows the formation and binding of pre-RCs by the DNA duplex but not initiation of replication (Table I). Initiation of DNA replication is induced by a switch of the interaction (6.2) at $\varphi = N$, from attraction (−) to repulsion (+). During replication, for $N < \varphi < 2N$, the force (6.2) is repulsive (+) implying a partial disassembly of pre-RCs with repulsion of the LFs, hence, preventing re-replication in the S -phase. The termination of replication is due to a vanishing and switch of sign of force at $\varphi = 2N$, at which DNA replication is completed, with all replicons at the N primed origins duplicated once. $F(0) = 0$ corresponds to a resting cell, in the absence of driving source. Without a detailed knowledge of the dependence on hydrolysis and phosphorylation, and inclusion of

polarization and electromagnetic effects, at this stage of development 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 remaining unsolved problems. Due to a vanishing force (6.3) at $\varphi = N$, however, the entire DNA system stays at rest and the lattice of pre-RCs awaits the event of excitation into the S phase [1].

7. DNA Duplex and Microtubules behave like Elastically Braced Strings

Until the ultimate growth signal transduction has actually been elicited, the DNA lattice obeys the harmonic-displacive type dynamics (4.7). Before examining the physical mechanism responsible for initiation of DNA replication, the principles of which also explain the dynamic instability of growing MTs, a useful classical interpretation of the one-dimensional lattices of molecular complexes in the DNA duplex and the MTs is made. After the spontaneous symmetry breakdown of dynamics, through (5.1), multiplying the asymmetric equation (6.2) by the linear elastic modulus $\varepsilon = \rho * s^2$, $\rho*$ being the linear (gravitational) mass density, and dividing by N , the equation of motion describes the dynamics of an elastically braced string [32]

$$\frac{\rho*}{N} \frac{\partial^2 \varphi}{\partial t^2} - \frac{\varepsilon}{N} \frac{\partial^2 \varphi}{\partial x^2} + \frac{\kappa}{N} \varphi = \rho* \frac{\partial^2 \xi}{\partial t^2} - \varepsilon \frac{\partial^2 \xi}{\partial x^2} + \kappa \xi = \frac{1}{N} F_D(\varphi) \quad (7.1)$$

where $\xi = \varphi/N$ is the relative deformation. This embedded string like lattice of molecular complexes is driven chemically by the nonlinear force $F(\varphi)/N$ defined by

$$\begin{aligned} -\frac{\varepsilon}{N} \frac{\partial V_A}{\partial \varphi} &= F(\varphi)/N = -\kappa \varphi/N + F_D(\varphi)/N \\ &= -\frac{\kappa}{2} \frac{\varphi}{N} \left(1 - \frac{\varphi}{N}\right) \left(2 - \frac{\varphi}{N}\right) = F'(\xi) = -\frac{\kappa}{2} \xi(1 - \xi)(2 - \xi) \end{aligned} \quad (7.2)$$

and $\kappa = 4\mu^2\varepsilon$ here plays the role of compressibility modulus of the medium in which the string is embedded. Hence, $-\kappa\varphi$ is the linear part of a long range restoring force $F(\varphi) = \varepsilon F_A(\varphi)$ on the string in the same medium. Thus, also the membrane system of receptors engaged by IL2 approximates to this one-dimensional elastically braced string dynamics, however, the bracing is then due to that the surface membrane, which by itself is too soft to behave like a stringlike lattice, is wrapped around the cellcytoplasm which in turn is stabilized by the cytoskeletal network.

The force (7.2), derived from the model with spontaneously broken symmetry, thus assures that replication is initiated at $\varphi = N$ when all DNA origins are primed. The attraction between pre-RCs during assembly in G_1 , inhibiting unfolding and replication by creating a tension in the DNA duplex, then switches to repulsion which leads to a partial disassembly required for replication. The repulsion between the pre-RCs elongates the DNA duplex wound around the histones

in order to allow for further opening of the origins, with unwinding and melting of the DNA duplex, for replication to be able to work. Replication is turned off at $\varphi = 2N$ when the attraction of F between protein complexes (post-RCs) again creates a tension in the duplex. The attractive force then contraas the openings around origins, thus inhibiting unwinding and continued replication. This ensures that the DNA content of G_2 cells is exactly twice that of G_1 cells.

The energy density corresponding to (7.1) is

$$H(\varphi) = \frac{\rho^*}{2N} \left(\frac{\partial \varphi}{\partial t} \right)^2 + \frac{\varepsilon}{2N} \left(\frac{\partial \varphi}{\partial x} \right)^2 + \frac{\kappa}{2N} \varphi^2 - \frac{1}{N} \Phi(\varphi) \quad (7.3)$$

where $1/2\kappa\varphi^2$ is proportional to the energy density stored in the medium. Rewriting (4.1) 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 (7.3), 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, or the energy per pre-RC (or per replicon), or per GTP-tubulin dimer [24]. In the continuum limit the one-dimensional lattice models, of ligand-receptor complexes at the surface membrane, of pre-RCs in the DNA duplex, and of GTP-tubulin dimers in the cytoskeletal MTs, thus approximate to elastically braced strings. 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 the other, depend on the elastic properties of the subsystem in which the string is embedded, and on each other via the cytoskeleton that connects the different subsystems. The interlinked parameters depend particularly on the rigidity and elasticity of the cytoskeletal network of MTs and MT associated proteins (MAPs), and the string dynamics of DNA depends mainly on the histones and supercoiling of the duplex, both of which factors provide solid like aggregations in DNA. A comparison of corresponding forms in (7.3) yields

$$\Phi(\varphi) = 2\varepsilon\mu^2\varphi^2 - \varepsilon V_A(\varphi) \quad (7.4)$$

and from (7.2) one has

$$\begin{aligned} F_D(\varphi) &= 6\mu^2\varepsilon \frac{\varphi^2}{N} - 2\mu^2\varepsilon \frac{\varphi^3}{N^2} \\ &= \frac{\kappa}{2}\varphi \left(2\frac{\varphi}{N} - \frac{\varphi^2}{N^2} \right) \end{aligned} \quad (7.5)$$

A change in the chemical potential, through (7.2), due to a compression of MTs could thus be quantitatively related through (4.3) to a lowering of the critical tubulin concentration, at the threshold for depolymerization, as proposed in the tensegrity model [33]. By the use of $2V(\varphi) = (\partial\varphi/\partial x)^2$, $\partial\varphi/\partial t = 0$, and the static wave solution (Figure 6a)

$$\varphi = \frac{\mu}{g} \tanh(\mu x) \quad (7.6)$$

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

the localized energy density (7.3) becomes (Figure 6b).

$$H(\varphi) = \frac{\varepsilon}{N} \left(\frac{\partial \varphi}{\partial x} \right)^2 = \frac{\mu^3}{g} \frac{\varepsilon}{\cosh^4(\mu x)} \quad (7.7)$$

The ground state energy in the membrane, in the MTs, or in the DNA helix, is proportional to [30]

$$E \approx \frac{\varepsilon}{N} \int_0^{+\infty} \left(\frac{\partial \varphi}{\partial x} \right)^2 dx = \frac{\varepsilon}{2N} \int_{-\infty}^{+\infty} \left(\frac{\partial \varphi}{\partial x} \right)^2 dx = \frac{2}{3} \varepsilon \frac{\mu^2}{g} = \frac{2}{3} \varepsilon \mu N = \frac{1}{6} \frac{\kappa}{\mu} N \quad (7.8)$$

Hence, the total energy is not a direct sum of the energies from the N complexes, each of which contributes an equal quantum of energy κ . The energy, induced by the ligand-receptor interaction, can thus be stored as quanta in the form of pre-RCs in DNA, until the cell takes the unanimous, irrevocable decision to initiate DNA replication. Interestingly, the mechanism responsible for this all-or-none type transition into the S phase – the switch of interaction from attraction to repulsion which moreover explains the prevention of re-replication during the S phase by repulsion of the LFs – also explains the dynamic instability in growing MTs. As shown subsequently by analysis of a time-dependent perturbation, the initiation of DNA replication and the dynamic instability in growing MTs are both due to the energy contributed by hydrolysis of one molecular complex in the actual subsystem, above the lattice ground state.

8. Initiation of DNA Replication and Dynamic Instability in Growing MTs

Inserting small time-dependent excitations in the dynamics (4.7) of the surface membrane, of the DNA, or the MT lattices

$$\varphi(x, t) = \varphi(x) + e^{i\omega_q t} \psi(x) \quad (8.1)$$

and keeping only the linear terms, a Schrödinger like exactly soluble equation is obtained [32]:

$$\left(\frac{d^2}{dx^2} + v''(\varphi) \right) \psi_v(x) = \quad (8.2)$$

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

This equation has two discrete eigenmodes

$$\frac{\omega_v^2}{s^2} = \mu^2 v(4 - v); v = 0.1 \quad (8.3)$$

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

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

$$\frac{\omega_v^2}{s^2} = 4\mu^2 + q^2 \quad (8.4)$$

$$\psi_q(x) = e^{i\omega_q x} (3 \tanh(\mu x)) = 3iq \frac{\tanh(\mu x)}{\mu} - 1 - \frac{q^2}{\mu^2}$$

Discrete eigenvibrations

When comparing the expansion $\varphi(x + \Delta x) \approx \varphi(x) + \Delta x d\varphi/dx$ with (8.1), which shows that $d\varphi/dx$ equals ψ_0 (8.3), one finds that $\omega_0 = 0$ is a so called translation mode of the unperturbed wave (7.6) of the N molecular complexes. The translation mode implies that all the N membrane receptor engagements, and similarly the corresponding definite number of pre-RCs in DNA, can respond coherently as one unit in the all-or-none type transition into the S phase. This coherence corresponds to the infinite wavelength approximation of (3.2) and is a result of the rigidity of the system, a prerequisite for the long range correlations and the cell's ability to count the number of molecular complexes irrespective of their localization. The cell first counts, becomes 'aware', and then takes the unanimous all-or-none type decision to initiate DNA replication. The nonzero mode $\omega_1 = \sqrt{3}\mu s$ (at $v = 1$) is a stable eigen-vibration determined by the values of μ and s in the different subsystems.

The model thus automatically provides a frequency selective mechanism [34] that could play an essential role in the storage of energy and signal transduction.

Scattering threshold

At zero momentum, $q = 0$, the scattering (continuum) energy threshold (at $v = 2$) is given by $\omega_c^2 = 4\mu^2 s^2$, or $\omega_c^2 = 4\mu s^2/d^2$ 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 $d_m \approx \sqrt{2}d$. An energy contribution, κ_{DNA} by hydrolysis of ATP or other phosphorylated component from the pre-RC-replicon aggregation, above the ground state energy of the lattice of N pre-RCs should hence be sufficient to excite the DNA system to the scattering threshold. The system then switches from a condensating state (8.3) with attractive force to a scattering state (8.4) with repulsive force. This could be a first step to explain the role of hydrolysis at the initiation of DNA replication.

In the DNA duplex this switch of interaction could explain the transition from assembly in G_1 to partial disassembly in the S phase, with initiation of replication and release of LFs from the pre-RCs, after degradation of the cyclin-CDK inhibitors, phosphorylation of Rb proteins and a myriad of other preceding steps at different time scales. The planar bi-directional replication of DNA (Figure 1b) starting at η of the N primed origins, the hedgehog like cytoskeleton and the formation of mitotic spindle, however, require a three-dimensional extension of the actual model including electromagnetic effects. The energy per origin/replicon engaged in the DNA lattice, κ_{DNA} should be associated with the complete pre-RC/replicon aggregate. Consequently, after being engaged by the ultimate ATP-ORC, binding of TFs and Rb proteins, phosphorylation of same Rb proteins, a complete set of LFs must bind to the pre-RC before initiation of DNA replication could finally take place. The switch of interaction, from condensation to repulsion, at the energy contributed by hydrolysis of one ultimate GTP-tubulin dimer, κ_{TD} , above the threshold at $\varphi = N$, can similarly explain the dynamic instability in a growing MT. The MT filament then suddenly starts to depolymerise. Given the different (gravitational) mass densities (ρ^*) and the velocities of sound (s), of the hydrolyzed (h) and nonhydrolyzed (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 traveling waves before and after symmetry breakdown of the dynamics.

9. Summary and Outlook

A nonstationary interaction, which controls the gross behaviour of DNA replication and cell cycle progression is derived from manybody physics. The model, which is previously published in somewhat different form [27], predicts a long range force

(7.2), $F'(\xi) = -(\kappa/2)\xi(1-\xi)(2-\xi)$, between the ORCs/pre-RCs, $\xi = \varphi/N$ being the relative deformation (elongation or contraction) of the string like assembly of these protein complexes, φ the number of same molecular complexes, N the threshold number at which DNA replication is initiated, and κ the compressibility modulus in the lattice of protein complexes which behaves like an elastically braced string. The so obtained force possesses all the desired properties needed to explain the gross regulation of DNA replication and cell cycle progression in terms of the basic molecular interactions (Table I). Initiation of DNA 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 thus also providing a mechanism which prevents re-replication. The attraction between protein complexes (pre-RCs) in G_1 creates a contraction and tension in the DNA string, thus inhibiting unwinding and replication. In the S phase the repulsive force between protein complexes induce an elongation and unfolding of DNA around the histones, thus allowing unwinding of the duplex and replication. The termination of DNA replication is due to a switch of sign of the long range force $F'(\xi)$ for $\xi = 2(\varphi = 2N)$, at which all primed replicons in DNA have been duplicated once. Like in G_1 the attraction in G_2 between protein complexes (postRCs) bound to DNA creates contraction and tension in the duplex, thus inhibiting continued unwinding and replication. *The model thus assures that the DNA content of G_2 cells is exactly twice that of G_1 cells.*

The switch of sign of F' at $\varphi = N$ similarly explains the dynamic instability in growing MTs. It could also explain the switch of the interaction of IL2 with its receptor in late G_1 , at the R -point, after which the T cell proceeds to DNA replication without further exposure to IL2. 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 polymerizing 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 (4.3) of molecular complexes engaged, $N = \mu(\rho_0 + r_0)(\rho_0 - r_0)$, at which DNA replication is initiated, and a corresponding number at which also the dynamic instability in growing MTs occurs, ρ_0 and r_0 being the initial reactant concentrations in the actual subsystem. Given the explicit forms of the threshold and scale, in terms of initial reactant concentrations, it should then be possible to examine certain forms of cancer, with abnormal expression of vacant receptors at the surface membrane. In addition the model defines positive definite and constant quanta of energy, $\kappa = 4\mu^2\varepsilon$, per ligand-receptor association [24], per pre-RC, and 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 (4.7) instead of the assymmetric one (6.2), then μ^2 and hence κ would have carried the wrong sign.

The response (5.4) is derived as if DNA replication is initiated simultaneously with the switch of IL2-receptor interaction at the R -point, beyond which the cell proceeds to replication without further IL2-exposure. However, also with a nonzero but constant lag time between the R -point and the $G_1 - S$ interface (Table I), the rates of cell cycle progression derived at these two critical points should be equal since all time variation is expected to occur before the R -point. Also the switch-like gross interaction transferred between these points remains invariant because, the nonlocal correlations (3.2), and the driving reactions (2.1), have the same approximate forms in each of these subsystem irrespective of if the reactants are IL2 and IL2R, ATP-ORCs and DNA origins, or LFs and almost complete pre-RCs. This provides a clue to understand how the cell cycle and DNA replication could be explained in terms of the long range gross interaction of pre-RCs, without going into detail of the more complex reactions of the cyclin-CDK activity that may still induce the switch-like events. The target for cyclin-CDK phosphorylation is the pre-RC, but these objects must first be assembled, ordered and close-packed – according to (3.1) and the nonstationary reaction (2.1) – before the switch-like all-or-none type commitment to replication is made. The role as primer for assembly and ordering is another reason why ORC could be considered the best candidate for an initiator protein [1]. The first reason for this is that ORC complexed with ATP is the only factor that directly selects DNA origins [3]. Other factors can be subsequently recruited to the origin to form a complete pre-RCs, but only after binding of ATP-ORC by the DNA origin.

The cyclin-CDK activity undoubtedly plays important key roles in the regulation of DNA replication and the cell cycle, however, its biological functions in the G_1 and S phases seem to occur on terms dictated by the nonstationary assembly and interaction of pre-RCs, and the sequential expression of D type cyclins, which in turn depends strictly on the IL2 exposure [6]. Accordingly, the cyclin-CDK activity is also under the spell of the nonstationary ligand-receptor interaction at the surface membrane, which equals the long range gross interaction of pre-RCs in DNA. It should be underlined that the proposed model does not exclude the more complex intermediate diffusive type interaction, which propagates the cell system between the actual switch-like events in the cell cycle, with kinase cascades and complicated sequential reactions. However, physical dispersion laws implies that the form of gross interaction summed up from these more complex intermediate steps should equal the less complex dynamics derived at the switch-like events, at the R -point and $G_1 - S$ interface.

Much about DNA replication is obtained from yeast, and the question is if 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 [36]. 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 (6.3), crucial for the phase-transition between the assembly and replication states, is determined solely by the number of origins engaged by ORCs. In the actual one-dimensional version the model cannot yet account for spatio-temporal dependence of the initial reactant concentrations. Cytoskeletal MTs therefore grow identically in all directions. A more realistic response theory, that also explains the hydrolysis of GTP and ATP, the phosphorylation of Rb proteins and other factors, and that predicts the value of η , should obviously be derived in three spatial dimensions and include electromagnetic interaction and polarization. In order to analyse the anharmonic corrections and emergence of the elastically braced string dynamics in three dimensions, a preliminary version of such an extension has been made [35]. Interestingly, a superconduction like dynamics was obtained, with DNA and MTs appearing as string-like vortex solutions. The elastically braced string dynamics (7.1) only accounts for the leading order interaction, and the remaining dissipative effects (8.1) could then be treated as time-dependent corrections [32].

However, the counting of receptors and origins engaged, the mechanisms for initiation and termination of DNA replication and for the assembly and disassembly of pre-RCs with prevention of re-replication during the *S* phase, the good agreement with growth data of dividing cells and polymerising MTs, the explanations of their variable length and the dynamic instability, are results obtained through one and the same model. These results encourage me to suggest that the one-dimensional model proposed could work as platform for generalizations into three spatial dimensions with inclusion of electromagnetic interaction. Through charge conservation, which implies a symmetry termed gauge invariance that links the electromagnetic field to the matter field, the lyotropic one-dimensional model also predicts a concentration dependent relation between the coefficients that regulate the interpolation between harmonic and displacive modes, and the electric charge [35]. The observed spatio-temporal coherence, induced by stabilization of cytoskeleton, and by the histones, may be taken as a classical definition of a form of consciousness of the cell, a state of 'mind' developed prior to its unanimous decision to initiate replication. In the infinite wavelength approximation such a definition of consciousness could eventually also work for a network of cells, connected through the cytoskeletal networks. The model could also help to interpret experimental findings from transformed and normal cells, and further explain their biological functions and dysfunctions in the organism from a more realistic condensed matter aspect.

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