Model of DNA Dynamics and Replication

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Abstract. Before DNA replication can be initiated a definite number of adenosine triphosphate (ATP) containing pre-replication protein complexes (pre-RCs) must be assembled and bound to DNA like in a super-critical mass. A chemically driven dynamics of the Ginzburg-Landau (GL) type is derived, using the non-equilibrium equation for binding of pre-RCs to DNA and a probabilistic conformational distribution of these protein complexes. This dynamics, in which the DNA-protein system behaves like a nonlinear elastically braced string (NEBS), can control the cell cycle via conformational transitions such that G_2 cells contain exactly twice as much DNA as G_1 cells. After adjustment of previously-made derivations, the model is compared with cell growth data from the T lymphocyte MLA-144.

Key words: DNA replication, cell cycle regulation, DNA dynamics, commitment, initiation, termination, DNA condensation, DNA conformation, DNA folding, DNA compaction, DNA packing, pre-replication complex, initiator protein assembly, origin recognition, non-equilibrium dynamics, Ginzburg-Landau model, elastically braced string, reaction coordinate

1. Introduction

At a first glance the regulatory network of a living cell appears far too complex to be described by one dynamical model [1, 2]. However, at the commitment, initiation and termination of DNA replication, the DNA-protein system takes decisions as if it consisted of one coherently working unit, suggesting that a long-range, intermolecular-type interaction in the DNA-protein system might be controlling the cell cycle after the G_1 restriction point. A chemically driven, long-range DNA dynamics of the Ginzburg-Landau (GL) type was derived previously [3], using the non-equilibrium equation for binding of pre-replication protein complexes (pre-RCs) to the double helix and a probabilistic conformational distribution of these protein complexes. In the version of the model presented here, the groups of regulator proteins that control the commitment and the initiation events of DNA replication are more adequately separated than in [3]. It turns out that this model interaction is able to control the cell cycle via conformational transitions in such a way that G_2 cells contain exactly twice as much DNA as G_1 cells. After a brief recapitulation and necessary adjustments of the previously-made derivations [3], the dose-response

curve derived in this non-equilibrium model is compared with cell growth data from a population of the T lymphocyte MLA-144 [4, 5].

DNA replication is regulated by positive and negative acting proteins including the cyclins, cyclin-dependent kinases (Cdks) [2], the Cdc7 kinase, regulated by a protein Dbf4 [1], and, in mammalian cells, the family of tumor inhibiting retinoblastoma (Rb) protein [6, 7] that regulates the E2F family of transcription factors. By binding to E2Fs via a pocket-like region, the Rb proteins also recruits other factors, such as the chromatin-remodeling factor SWI/SNF, histone-deacetylases (HDACs), and histone acetyl-transferases (HATs), which, together with the E2Fs, regulate the timing and activation of many genes whose products are crucial for the DNA replication. Some of the E2F responsive gene products [6, 8] are key components of the pre-RCs [1, 9], which bind to specific origin sequences in DNA, the so-called origins of DNA replication [1]. The biological functions of the Cdks are regulated by cyclins and polypeptides such as p27 [2]. The problem is to discern which of all these factors could play a key role in the dynamics that controls the cell cycle.

The prevailing opinion has been that kinase activities more or less directly control the initiation of DNA replication [2]. However, this speaks against the fact that DNA replication is controlled by alternation between two mutually incompatible states: the assembly state (G_1), which allows formation and binding of pre-RCs to DNA but does not allow initiation of DNA replication, and the replication state (S), which allows initiation of DNA replication and the concomitant disassembly of pre-RCs but does not allow formation and binding of new pre-RCs to DNA [9].

Accordingly, protein kinases are permitted to induce origin firing *if and only if* all pre-RCs have been assembled and bound to DNA. In other words, all origins must be engaged by pre-RCs before DNA replication can be initiated, indicating that there are long-range correlations and intermolecular interactions between the pre-RCs. The DNA-protein system thus seems to be able to count the number of pre-RCs bound to DNA before it allows origin firing to start, because the number of pre-RCs attached to the origins in DNA must be equal to the total number of replicons that must be primed for DNA-replication before initiation can take place. It is assumed here that there are altogether N replicons in the genome.

The exact timing of the decision to start origin firing is thus controlled by the time it takes the DNA-protein system after the G_1 restriction point to synthesize [6, 8], assemble, and bind the N pre-RCs to DNA [1, 9], and not by the Cdks which may allow a certain spread in time. From this aspect the kinases act just like an origin firing tool after assembly of all pre-RCs, which, together with DNA and the histones, seem to function like a super-critical mass. Much like in collective phenomena of non-living condensed matter, the many identical pre-RCs in the DNA-protein system do things together that a single pre-RC cannot do *with or without kinases*. The suggestion that DNA itself, or some protein bound to it, could regulate the cyclin-Cdk activity, and not vice versa, is not new [10]. However, the proposed intermolecular interaction [3] appears to be the first model that quantitatively exploits this idea.

In contrast to yeast cells, such as *Saccharomyces cerevisiae*, in which the commitment to DNA replication takes place during an extended time interval called “start”, mammalian cells are committed at a discrete restriction point in late G_1 . The commitment is induced by D-type cyclins, which, together with their catalytic Cdk partners, Cdk4 and Cdk6, act as intracellular sensors for mitogenic stimulation [2]. The CylinD-Cdk4/6-p27 complex phosphorylates the Rb protein at phosphoacceptor sites in the carboxy-terminal region, triggering an intramolecular interaction [7] with the pocket region. This leads to conformational changes in the Rb-E2F complex and dissociation of HDAC from Rb, which is sufficient for E2F to induce expression of cyclin E. Whereas CylinD-Cdk4/6-p27 is catalytically active, cyclinE-Cdk2-p27 is not. However, after sequestration of all free p27 molecules, active CyclinE-Cdk2 can complete the phosphorylation of Rb proteins, which then change conformation and dissociate from the E2Fs, permitting a rapid transcription of all genes whose products are necessary for the DNA replication and cell cycle control [6, 8].

The different protein components of pre-RC bind to origins in DNA in a successive manner, such that the origin first works as receptor for a hexameric protein, the origin recognition complex (Orc) [1]. Associated with adenosine triphosphate (ATP), Orc can recognize and bind to origins in the double helix [11]. After binding to DNA the Orc-ATP complex acts as a landing pad for a helicase-loading factor, Cdc6, which in turn becomes a landing pad for a hexameric helicase, the minichromosome maintenance (Mcm) proteins. The assembled protein complex binds another protein, Cdc45, which then tethers DNA-polymerase- α /primase to the Mcm helicase [1, 9]. The hierarchic linkage between the initiation and the commitment events could be further understood from the fact that Orc1, Cdc6, polymerase- α , cyclin E, cyclin A, and Rb protein are all putative E2F-responsive gene products [6, 8], and that Cdc6, Mcm helicase, and the Orc components, Orc1, Orc4, and Orc5 are ATPases [1].

In the assembly of pre-RCs it is thus thought that the pre-RC components may work as ATP-driven conformational switches that facilitate binding of the component that comes next [1]. The proposed non-equilibrium model for initiation of DNA replication [3], which could be seen as an extension of this idea, suggests that the entire DNA-protein system, including the histones, works as a macromolecular allosteric-type switch – like a super-critical mass – for the initiation of DNA replication.

Many questions remain to be answered in more detail, for instance, concerning the switch from mitogen-dependent to mitogen-independent phosphorylation [2, 6, 7] and the role of ATP hydrolysis in origin firing [1, 9]. However, in the myriad of reactants at work in the G_1 phase, Orc has been considered to be the best candidate for an initiator protein [9]. After binding of the N th Orc-ATP to the N th origin, the completion of Orc-ATPs into ready pre-RCs takes place within a finite period of time. The rate of replication could thus be considered determined by the rate of non-equilibrium binding of Orc-ATPs to DNA; however, is it also restricted by the non-local correlations between these protein complexes.

2. Non-Equilibrium Conditions

Let r , ρ and ψ be the concentrations of origins, free, and bound Orc-ATP, and ρ_0 and r_0 their initial concentrations. Assume then that there is a sufficient concentration of Orc-ATP complexes already when their binding to DNA starts, hence $\rho_0 \geq r_0$. The non-equilibrium equation for binding of Orc-ATP to DNA then reads

$$\begin{aligned}\frac{\partial \psi}{\partial t} &= k\rho r - k'\psi = k((a - \psi)^2 - (a^2 - b^2)) \\ &= k(1 - \psi/a)^2(a^2 - (a^2 - b^2)/(1 - \psi/a)^2)\end{aligned}\quad (1)$$

where k and k' are the association and dissociation constants, $K = k'/k$, and $a^2 = (\rho_0 + r_0 + K)^2/4$ and $b^2 = \rho_0 r_0$ are obtained by inserting the initial constraints $\rho_0 = \rho + \psi$ and $r_0 = r + \psi$. The same second order rate equation can describe the binding of all other pre-RC components. With $k' \ll k$ the solution to Eq. (1) becomes $\ln[(\rho/r)(r_0/\rho_0)] = k(\rho_0 - r_0)t$, relating the time parameter to the gross turnover of reactant concentrations as if these variables were independent of space coordinates. However, in a real cell the rate of binding of initiator proteins (ligands) to DNA also depends on the spatial distribution of origins (receptors).

For simplicity it is here assumed that all pre-RCs bound to DNA are spread within a variable length along the x -axis in a one-dimensional model. It is thus assumed that the inactivated globular nucleus has zero length and the fully activated DNA-protein system a maximal length L . Since the DNA-protein system is relatively rigid, its dynamics should be dominated by a long-wavelength mode at which the lattice spacing becomes negligible. An assumption that the Orc-ATP complexes bind consecutively to the vacant origins in DNA, like in a growing polymer, should therefore not change the leading order dynamics. Since DNA is a very long polymer the conformational distribution of n Orc-ATPs bound at sites x_i ($1 \leq i \leq n$) could have been defined like in the freely joint chain (FJC) model by a probability $\varphi = c^{-n} \psi(x_1)\psi(x_2)\psi(x_3) \dots \psi(x_n)$ [12], c being a normalization parameter. In that case ψ , the random distribution of a segment at chemical equilibrium conditions, could have been normalized such that each ψ had represented one bound Orc-ATP (or pre-RC) and φ the probability to find n such complexes.

The growth of such a polymer-like DNA-protein system up to N Orc-ATPs could then have been described by a probability φ which transits between different discrete states, $c^{-1}\psi(x_1)$, $c^{-2}\psi(x_1)\psi(x_2)$ up to $c^{-N}\psi(x_1)\psi(x_2)\psi(x_3) \dots \psi(x_N)$, provided that chemical equilibrium had prevailed long enough at each state. However, ψ is a continuous variable, which at each site in space is controlled by Eq. (1) and the initial constraints at non-equilibrium conditions. It may therefore represent any number of bound Orc-ATPs between zero and N . Apart from creating a normalization problem, this also implies that a truncation at any finite order of the above series could result in less than N Orc-ATPs (pre-RCs) bound to DNA at the start of origin firing, which might lead to incomplete replication of DNA. By choosing c equal to

$a \approx (\rho_0 + r_0)/2(K \approx 0)$, in compliance with the boundary constraints, ψ becomes normalized such that it corresponds to one bound Orc-ATP or less (compare the FJC model). Therefore, since ψ can vary between zero and one, which leaves the question of total number of bound Orc-ATPs open, the conformational distribution of any finite number of such complexes must be defined by an infinite series of the form

$$\varphi = \mu \sum_{n=0}^{\infty} \left(\frac{1}{a}\right)^n \prod_{v=0}^n \psi(x_v) \approx \mu \sum_{n=0}^{\infty} \left(\frac{\psi}{a}\right)^n = \frac{\mu}{1 - \psi/a} \quad (2)$$

where $\psi(x_0) = 1$ accounts for the possibility that the Orc-ATPs could be attached before Eq. (1) started. Apart from defining the probability to find the finite number of bound Orc-ATPs, which will be determined later by normalization, $\varphi = \mu/(1 - \psi/a)$ thus also provides a measure of order in the DNA-protein system.

Like in the grand partition function, in which n represents the number of particles, n is here summed to infinite order; however, in Eq. (2) the number of bound Orc-ATPs is regulated by Eq. (1) and the initial reactant concentrations. The number of Orc-ATPs, and hence of pre-RCs, thus increases continuously, as a function of time. Accordingly, the rate of DNA replication which determines the rate of division, is regulated by the rate of priming replicons for DNA replication by pre-RCs. From studies on the T lymphocyte MLA-144 [4, 5] it is concluded that there is no net contribution from short-range cooperation between Orc-ATPs (pre-RCs), because the rate of DNA replication has a slope equal to one. This is neither in conflict with a sharper denaturation of naked DNA [13], nor does it exclude steeper responses and short range cooperation in other native cells.

The continuous form of φ , motivated already by the absence of a unique centre from which DNA replication is controlled [10], is a result of L being divided into an infinite number of intervals, hence $\Delta x \approx 0$. The spatial coherence of the dynamics is also motivated by the absence of large momentum transfer at the chemical equilibrium that should prevail when all N pre-RCs have been bound to DNA, before origin firing has started [9]. The wavelength of lattice vibrations should then become much longer than the lattice spacing, which can therefore be neglected. The dominance of such a spatially coherent dynamics implies a form of localization and enslavement of the whole DNA-protein system. The chemical equilibrium also implies a moment of coherence in time. Namely, a start of origin firing, ignited by kinases a fraction of a second before N pre-RCs are bound to DNA, could lead to incomplete replication. Therefore a factor like cyclin-Cdk cannot easily control that all replicons are primed for replication when origin firing is allowed to start. The most likely hypothesis therefore seems to be that the DNA-protein system itself [10] takes the unanimous decision to allow origin firing during the short chemical equilibrium that separates the two incompatible states of pre-RC assembly and DNA replication [9].

3. The Model

Differentiating Eq. (2) with respect to time, $\partial\varphi/\partial t = (\partial\varphi/\partial\psi)(\partial\psi/\partial t)$, and using that $\partial\psi/\partial t$ is still defined by Eq. (1), one obtains the rate of binding of Orc-ATP to DNA. All other pre-RC components obey the same law. In order to simplify derivations, the origins are assumed to be engaged by Orc-ATP in a given order, from one end of the DNA-protein system to the other, like a travelling wave that propagates along the x -axis at a velocity v . This does not change the leading order dynamics corresponding to $\varphi = \mu/(1 - \psi/a)$, which is independent of lattice spacing. Multiplying $\partial\varphi/\partial t$ by $1/v$ one can then interpret the binding rate as the first order equation of motion of a well-known non-linear potential theory [14]

$$\frac{\partial\varphi}{\partial x} = \frac{1}{v} \frac{\partial\varphi}{\partial t} = \frac{ka}{\mu v} (\mu^2 - g^2\varphi^2) \equiv \sqrt{2V(\varphi)} \quad (3)$$

where $g^2 = (a^2 - b^2)/a^2$, and V is given here below. Since Orc binds and remains bound to DNA the rest of the cell cycle, hence $K \approx 0$ and $g \approx \pm(\rho_0 - r_0)/(\rho_0 + r_0)$, the derivations are simplified considerably.

To guarantee that all N replicons have been primed for DNA replication at the origin firing the most reasonable seems to be that the solution to Eq. (3), $\varphi = (\mu/g)\tanh(kagt) \approx (\mu/g)\tanh[k(\rho_0 - r_0)t/2]$, is topologically normalized such that $N = \varphi(\infty) - \varphi(0) = \mu/g$, because at finite times there may still be vacant origins. By counting the number of bound Orc-ATPs, φ thus also plays the role as reaction coordinate. Since the lattice of Orc-ATP complexes bound to DNA increases with $ka \approx k(\rho_0 + r_0)/2$ complexes per unit time, and the lattice spacing is $d = L/N$, the lattice grows with a velocity $v = dka = Lka/N \approx Lk(\rho_0 + r_0)/(2N)$. From Eq. (3) it then follows that $V(\varphi) = 1/2(g^2/L^2)(\mu^2/g^2 - \varphi^2)^2$ and the second order equation of motion becomes

$$\frac{1}{s^2} \frac{\partial^2\varphi}{\partial t^2} - \frac{\partial^2\varphi}{\partial x^2} \equiv -\frac{\partial V}{\partial\varphi} \quad (4)$$

where s is the velocity of sound. Multiplying both sides of the static part of Eq. (4) by $\partial\varphi/\partial x$ and integrating once one reobtains Eq. (3).

Unfortunately, the symmetric double well potential V is nonzero at $\varphi = 0$, despite that no Orc-ATP has yet been attached to DNA. However, as is usually the case in Nature, the system spontaneously goes to one of its lowest energy states, $\varphi_{\text{old}} \rightarrow -N + \varphi_{\text{new}}$, breaking the symmetry of the potential, which then becomes $V(\varphi) = 1/2(g^2/L^2)\varphi^2(2N - \varphi)^2$. The corresponding asymmetric equation of motion

$$\frac{1}{s^2} \frac{\partial^2\varphi}{\partial t^2} - \frac{\partial^2\varphi}{\partial x^2} \equiv -2\frac{g^2}{L^2}\varphi(N - \varphi)(2N - \varphi) \quad (5)$$

has a static solution $\varphi = N[1 + \tanh(\mu x/L)]$, which after the replacement $x = vt$ becomes $\varphi = N[1 + \tanh(kagt)]$. Combining this with the solution to Eq. (1), $2kagt = \ln[\rho r_0/(r\rho_0)]$, one then obtains the non-equilibrium dose-response curve $\varphi = N\{1 + \tanh[1/2 \ln(\rho/\rho_{50})]\} = 2N\rho/(\rho + \rho_{50})$, in which the ligand concentration for half-maximal response, $\rho_{50} = r\rho_0/r_0$, has become a function of the reactant concentrations as could be expected in a lyotropic system.

As shown previously [3], the exact solution to Eq. (1) for $K > 0$ is $2kagt = \ln[\rho r_K/(r\rho_K)] = \ln(\rho/\rho_{50})$, in which $\rho_K = a(1+g)$, $r_K = a(1-g)$, $\rho_{50} = r\rho_K/r_K$, and where the modified constraints, $\rho_K = \rho + \psi$ and $r_K = r + \psi$, accounts for the possibility that some reactants are bound for just a short time and then dissociate. In the high affinity limit $K = 0$ the dynamical reactant concentrations ρ_K and r_K become the usual initial concentrations ρ_0 and r_0 . The form of dynamics defined by Eq. (4), the solutions and corresponding dose-response curve thus remain the same for all K -values.

4. Comparison with Experiments

The rate of DNA replication of a MLA-144 cell only controls the growth rate of that particular cell, or the proliferation of a start population in which all MLA-144 cells have the same number of IL2 receptors. In the experiments, however, the proliferation of a start population of MLA-144 cells with different numbers of IL2 receptors was assessed (Table I, column A) [4, 5]. Therefore, at low IL2

Table I. Proliferative response of MLA-144 in % of maximum as a function of IL2 concentration. Multiplying the cell growth data by the factor R one obtains the renormalized growth data, corresponding to a start population in which all cells would have approximately the same number of IL2 receptors. Data provided by K. A. Smith.

IL2 conc. (nM)	A Cell growth % of max.	Point no.	R	B Renormalized cell growth
0.0005	0.00 ± 0.05	1	2.00	0.00
0.001	0.26 ± 0.03	2	1.75	0.46
0.004	1.73 ± 0.05	3	1.50	3.00
0.01	10.30 ± 0.03	4	1.25	12.88
0.04	37.40 ± 0.05	5	1.00	37.40
0.11	67.19 ± 0.17	6	1.00	67.19
0.34	85.93 ± 0.06	7	1.00	85.93
1.00	95.71 ± 0.02	8	1.00	95.71
3.10	97.73 ± 0.04	9	1.00	97.73
9.30	93.24 ± 0.03	10	1.00	93.24
27.8	94.19 ± 0.04	11	1.00	94.19
83.0	100.00 ± 0.02	12	1.00	100.00

concentrations only cells with high IL2 receptor levels were triggered to initiate DNA synthesis. However, at high IL2 concentrations also the cells with low IL2 receptor levels were able to divide. In order to translate these results to the case in which all MLA-144 cells would have high levels of IL2 receptors, the native population is therefore divided in two equal halves, one with low and one with high levels of IL2 receptors. Similarly, the nine first response data in column A (Table I) are divided in two halves. Counting the fifth value in the upper half and treating the last three values as fluctuations about the maximum, one can then relate the values at low IL2 concentrations to the values at high concentrations of IL2. Thus, the first five values in column A (Table I) should be multiplied by a renormalization factor R that attenuates linearly from 2 to 1. The values in column B in Table I then corresponds to a population containing only cells with high levels of IL2 receptors, in which case the growth rate to a good approximation is determined by the rate of DNA replication.

With $\rho_{50} = 0.06$ nM and $K = 1$, the dose-response curve (Figure 1) derived in the NEBS model correctly reproduces the renormalized cell growth data of MLA-144 (column B, Table I) [4, 5]. It thus also correctly reproduces the rate of DNA replication because, as mentioned, the rate of DNA replication then determines the rate of proliferation. The crucial point is that the scale $\rho_{50} = r\rho_K/r_K$ in the non-equilibrium model can attain the value 0.06, both in the IL2 receptor system of MLA-144 in which $K = 1$, and in the DNA-protein system, in which Orcs bind and remain bound to DNA, hence, with $K = 0$. This would not be possible in equilibrium type models, such as, by Hill, Langmuir and Ising, in which ρ_{50} is determined essentially by K [15]. As shown in Figure 1, such an equilibrium type response derived for MLA-144 with $K = 1$ would deviate by one order of magnitude from the experimental data [4, 5]. This is but one sign of the large gap

Figure 1. The growth rate of MLA-144 as function of IL2 concentration, $\varphi/N = 2\rho/(\rho + \rho_{50})$, derived in the non-equilibrium model at $\rho_{50} = 0.06$ nM (—) and compared with experimental growth data of MLA-144 (+). The dose-response curve derived in an equilibrium model at $\rho_{50} = 1$ nM (---). Data provided by K. A. Smith.

between equilibrium and thermotropic-type models and the actual non-equilibrium system. Although the DNA-protein system takes the decision to replicate DNA at the chemical equilibrium shortly before S phase entry, this does not imply that equilibrium statistical mechanics can be applied, because this chemical equilibrium is attained from non-equilibrium conditions.

5. A Non-Linear Elastically Braced DNA String

Since φ also represents the number of replicons and each replicon should be equally elongated, the order parameter $\varphi/N = \xi = 2\rho/(\rho + \rho_{50})$, which also works as reaction coordinate, can also be interpreted as the relative elongation η/L of the DNA-protein system. In G_1 ($0 < \eta/L < 1$) the elongation is induced by the assembly of initiator proteins and in S phase ($1 < \eta/L < 2$) by the chemically driven replication. Multiplying by ε/N , $\varepsilon = ms^2$ being Young's modulus and m the density of mass of the DNA-protein string, and introducing a spring constant $k_{sp} = 4\mu^2\varepsilon/L^2$, one can write Eq. (5) on the form

$$m \frac{\partial^2 \eta}{\partial t^2} - \varepsilon \frac{\partial^2 \eta}{\partial x^2} = - \frac{\partial V_{NEBS}}{\partial \eta} = F(\eta) = - \frac{k_{sp}}{2} \eta \left(1 - \frac{\eta}{L}\right) \left(2 - \frac{\eta}{L}\right) \quad (6)$$

where $V_{NEBS}(\eta) = (k_{sp}/8)\eta^2(2 - \eta/L)^2 = (\kappa L/8)\xi^2(2 - \xi)^2$ (Figure 2) and $\kappa = k_{sp}L = 4\mu^2\varepsilon/L$ is an effective spring constant.

According to Eq. (6) the DNA-protein system behaves like a non-linear elastically braced string (NEBS) [16] driven by the nonlinear force $F(\xi) = -\kappa/2\xi(1 - \xi)(2 - \xi)$. This form of dynamics, which is of the GL type, is also referred to as the non-linear Klein-Gordon equation, the Higgs model, or simply the φ^4 -model. The term proportional to Young's modulus in Eq. (6) describes the interaction between adjacent bound pre-RC components and $F(\xi)$ that between pre-RC components and

Figure 2. The asymmetric double-well potential $V_{NEBS}(\xi) = (\kappa L/8)\xi^2(2 - \xi)^2$ in units of κL and the reaction coordinate $\xi = \varphi/N = 2\rho/(\rho + \rho_{50})$ in multiples of whole genomes.

their origins. Apart from controlling the assembly-disassembly of pre-RC components, Eq. (6) can thus also control the compaction-decompaction transitions of the DNA-protein system.

The binding energy of the i th pre-RC component could be approximated by $E(\lambda_i) \approx [V_{NEBS}(1) - V_{NEBS}(\lambda_i)]/N = (\kappa d/8)[1 - \lambda_i^2(2 - \lambda_i)^2]$, where $\lambda_i = \delta_i/\delta_{\text{melt}}$, $0 < \delta_i < \delta_{\text{melt}}$, and δ_i and δ_{melt} are the distance and melting distance from the origin to the i th component. For simplicity only the Orc ($i = o$) and Mcm helicase complexes ($i = h$) will be discussed here. After binding to DNA Orc remains bound to the origin during the cell cycle, hence $\lambda_o = \delta_o/\delta_{\text{melt}} \approx 0$, blocking the position at the origin. Therefore the Mcm helicase must bind some distance $\lambda_h = \delta_h/\delta_{\text{melt}}$ away from the origin, hence $E(0) > E(\lambda_h)$. Perhaps this could help to explain why the Mcm helicase and, similarly, other pre-RC components are repelled from the origin while Orc remains bound also after that origin firing ($\xi \geq 1$) has started. Presumably, Orc's higher affinity to the origin compared to other pre-RC components is also due to a stronger short-range interaction.

Given that $4\mu^2\varepsilon = \kappa L$ and $N = \mu/g$, the ground state energy for N primed replicons should be given by [3, 14]

$$E_N \approx N\varepsilon \int_0^\infty \left(\frac{d\xi}{dx}\right)^2 dx = \frac{\varepsilon}{2} N \int_{-\infty}^{+\infty} \left(\frac{d\xi}{dx}\right)^2 dx = \frac{\varepsilon}{2} \frac{4}{3} N \mu = \frac{\kappa L}{6g}. \quad (7)$$

Accordingly, in order to reach the energy threshold required for initiation, all N replicons must be primed for DNA replication. Therefore, since $N = \mu/g$ and $g = (\rho_0 - r_0)/(\rho_0 + r_0)$, g must become sufficiently small and, hence, Orc-ATP attain a critical concentration almost equal to the density of vacant origins. The NEBS model thus provides an answer to the question of which of the two parameters actually controls this type of biological function, the total amount of a reactant or its concentration [10]. The nonlinear relation $N = \mu(\rho_0 + r_0)/(\rho_0 - r_0)$ shows that N and ρ_0 are equally important. As discussed below, a similar relation may hold between the total number of phosphorylated phosphoacceptor-sites on Rb proteins, corresponding to the quantal number of external IL2 receptor engagements [5], and a critical concentration of cyclinD-Cdk4/6-p27 required for commitment.

The NEBS dynamics is an effective interaction in the sense that the infinite wavelength approximation, required to obtain the order parameter $\varphi = \mu/(1 - \psi/a)$, is presumably valid only for a very rigid DNA-system that also includes the histones, i.e., for native chromatin. Since $\varphi(\psi)$ is also a non-linear mapping between the macroscopic and molecular levels, it is a prerequisite to interpret the macroscopic behavior of the DNA-protein system in terms of its molecular properties, and to understand the role of $\xi(\rho/\rho_{50}) = 2\rho/(\rho + \rho_{50})$ as a reaction coordinate. It thus becomes clear how the switching between harmonic and displative modes in the DNA-protein dynamics, which controls the assembly-disassembly and compaction-decompaction transitions in the DNA-protein system, is regulated by reactant concentrations and not by temperature as in thermotropic

systems. Such a system is lyotropic and does not obey the usual Boltzmann-type thermodynamics.

6. Chemical Excitations

However, at $\varphi = N(\xi = 1)$ the force $F(\xi)$ vanishes and without additional fuel the process would then be arrested. For pre-RC components to dissociate and DNA to melt, the DNA-protein system must therefore be chemically excited, as, for example, by phosphorylation and or ATP-hydrolysis [1, 9]. As shown previously [3, 17], a biochemical excitation can be included in the model as a small time-dependent perturbation. Thus, inserting $\varphi(x, t) = \varphi(x) + \psi_q(x) \exp(i\omega_q t)$ in Eq. (4), where $\varphi = \mu/g \tanh(\mu x/L)$, and multiplying both sides by $\varepsilon = ms^2$, one finds that the NEBS model dynamics has a discrete excitation spectrum $m\omega_\sigma^2 = (4\mu^2\varepsilon/L^2)\sigma(4-\sigma)/4 = k_{sp}\sigma(4-\sigma)$, $\sigma = \{0, 1\}$, with eigen-functions $\psi_0(x) = A_0/\cosh^2(\mu x/L)$ and $\psi_1(x) = A_1 \sinh^2(\mu x/L)/\cosh^2(\mu x/L)$, and a continuum (c) which starts at $m\omega_c^2 = 4\mu^2\varepsilon/L^2 = k_{sp}$, ($\sigma = 2$). This corresponds to an energy threshold $E_{th} \approx mL^2\omega_c^2/2 = 2\mu^2\varepsilon = k_{sp}L^2/2 = \kappa L/2$, at which the DNA-protein system becomes unstable with dissociation of pre-RC components and start of melting and replication of the DNA. By comparing $\varphi = N \tanh[\mu(x + \Delta x)/L] \approx N[\tanh(\mu x/L) + \mu \Delta x/L \partial \varphi / \partial x]$ with $\varphi(x, t)$ and $\psi_0(x)$, one finds that the zero frequency is a so-called translation mode corresponding to the wave of replication, $\varphi = N\{1 + \tanh[\mu(x \pm v_r t)/L]\}$, which travels at a velocity $\pm v_r$ in both directions of the x -axis.

At low forces ($\eta \approx 0$), with none or few proteins attached, according to Eq. (6) the DNA-protein system should behave like a spring with entropic elasticity, e.g., the worm-like chain (WLC) model [12, 18], with an effective spring constant $\kappa = 3k_B T/(2p)$, p being the persistence length. Since L is much shorter than the contour-length of DNA, and the native DNA string is braced by pre-RCs and histones, it can be assumed that $2p \approx L$ in G_1 . A simultaneous start of origin firing at N_S origins implies that the DNA-protein system is split up into N_S separate fragments, in each of which the persistence length is then reduced to $2p_S \approx L_S = L/N_S$. In order to induce the start of origin firing in $N_S = L/L_S$ fragments, the chemically induced excitation energy required for S phase entry should therefore be on the order of $\kappa L/2 = 3k_B T L/(2L_S) = N_S 3k_B T/2$ provided that $3k_B T/2 \approx 6$ pN·nm ($k_B T \approx 4$ pN·nm) is sufficient for one origin firing. Given that $L \approx 100 \mu\text{m}$, $N \approx 10,000$, $N_S = 400$ hence $d \approx 10$ nm and $L_S = L/N_S \approx 250$ nm, in G_1 , before fragmentation, the effective spring constant becomes $\kappa = 6 \times 10^{-5}$ pN and the spring constant $k_{sp} = \kappa/L \approx 6 \times 10^{-10}$ pN/nm. The corresponding values in the fragments in S phase would become $\kappa \approx 2.4 \times 10^{-2}$ pN and $k_{sp} = \kappa/L_S \approx 9.6 \times 10^{-5}$ pN/nm. However, all numbers are just taken to illustrate the model and must be treated with great caution. Apart from more exact knowledge of N , L , and N_S , it would be desirable to measure the actual energy threshold and compare it with the estimated value $E_{th} = \kappa L/2 = 600k_B T = 2400$ pN·nm, and detect the stable frequency

$\omega_1 = 3k_{sp}/(4m) = 3\kappa/(4mL)$. It also remains to be investigated if origin firing starts as a result of phosphorylation of pre-RC components or by ATP hydrolysis, or both these processes [1, 9].

7. Biological Properties of the Model in Terms of Physics

By switching sign at multiples of a whole genome, thereby controlling the compaction-unfolding transitions, the periodicity of the intermolecular force $F(\xi) = -(\kappa/2)\xi(1 - \xi)(2 - \xi)$ between bound Orcs in the DNA-protein system provides an explanation for the periodic behavior of the cell cycle. As shown in Table II, the NEBS model predicts a long-range attraction in the DNA-protein system that promotes assembly of pre-RCs in G_1 ($0 < \varphi < N$). DNA replication is initiated at $\varphi = N$ ($\eta = L$) by a long-range repulsion, which promotes dissociation of pre-RCs components such as of Mcm helicase encompassed by unzipping of DNA in the interval $N < \varphi < 2N$ [1, 9]. However, the Orcs remain bound to DNA for the rest of the cell cycle.

After a simultaneous initiation at some 400 origins [1], DNA replication is thought to propagate like a traveling wave through the DNA-protein system. By inducing condensation in the DNA-protein system at $\varphi = 2N$, the long-range attraction between Orcs can thus terminate DNA replication and thereby inhibit re-replication. However, since ξ is a continuous variable and $\xi = 2$ is attained and exceeded locally in S phase concomitantly with the propagation of DNA replication at some hundred sites at which origin firing starts, a wave of attraction and condensation should travel along with the wave of replication. The replication is also encompassed by a detachment-reattachment of histones and the attraction mediated by condensins, which bind to DNA in an ATP-dependent way [19] not unlike the binding of Orc-ATP to origins. The condensins play a critical role for the organization and higher order folding of mitotic chromosomes.

By switching sign at multiples of N , which also represents the number of replicons in the genome, the intermolecular force between Orcs can thus control the cell cycle transitions such that the DNA content in G_2 cells becomes exactly twice as much as in G_1 cells. The periodicity of $F(\xi)$ thus controls the gross turnover

Table II. Cell-cycle transitions as a function of the order parameter φ

Phase	G_1	S	G_2	M
DNA structure	Compact globular	Partially unfolded	Compact globular	
Mode/function	Assembly of pre-RCs	DNA replication, pre-RC disassembly	DNA condensation	Cell division
Forces	Attraction (−)	Repulsion (+)	Attraction (−)	
Order	$0 < \varphi < N$	$N < \varphi < 2N$	$\varphi = 2N$	

of genetic material. In contrast to the prevailing opinion, that DNA replication is more or less directly regulated by kinases, it is therefore suggested here that the cell cycle is regulated predominantly via conformational transitions in the DNA-protein system by the chemically-driven NEBS interaction defined by Eq. (6). The chemical driving of the process is described earlier as a time-dependent perturbation of Eq. (6) [3, 20]. Whereas, for the compaction-decompaction of the DNA-protein system, the intermolecular force $F(\xi) = -(\kappa/2)\xi(1 - \xi)(2 - \xi)$ works mainly between Orcs, which remain bound throughout the cell cycle, for the assembly-disassembly of pre-RCs it mainly works between an unbound pre-RC component and the pre-RC components bound to the origin. Thus, for instance, the chemically driven repulsion between Orc and Mcm helicase is a prerequisite for the unzipping of DNA and propagation of replication forks.

In contrast to diffusion-reaction based pattern formation models, which account for non-equilibrium conditions but not for correlations, and unlike Boltzmann-type statistical mechanics, which accounts for correlations at chemical equilibrium conditions, through Eqs. (1) and (2) the NEBS model is able to comply both with the increasing number of initiator protein complexes and their correlations. The NEBS model can thus also account for the rigidity and nonlinear elastic properties of the native DNA-protein system. The solid-like properties of the DNA-protein system should not come as a surprise because, as indicated already by single molecule experiments [21], the double stranded (ds) DNA molecule is itself relatively rigid also in the absence of regulator proteins and histones.

8. Discussion

A chemically driven non-equilibrium dynamics of the native DNA-protein system is derived. The model, which is of the Ginzburg-Landau type, is obtained through a combination of Eq. (1), that describes the non-equilibrium binding of pre-RCs to DNA, and Eq. (2) that defines the probabilistic conformational distribution of these pre-RCs. In this nonlinear dynamics, i.e., the NEBS model, the DNA-protein system behaves like an elastically braced string driven by the nonlinear potential $V_{NEBS}(\xi) = (\kappa L/8)\xi^2(2 - \xi)^2$. By switching sign the corresponding force between the Orc-containing pre-RCs, $F(\xi) = -\partial V/\partial \xi = -(\kappa/2)\xi(1 - \xi)(2 - \xi)$, can thus control the cell cycle via conformational transitions such that G_2 cells will contain exactly twice as much DNA as G_1 cells. The dose-response curve derived in this non-equilibrium model correctly reproduces the growth-data of the T lymphocyte MLA-144, with the correct slope and scale (Figure 1). The NEBS model dynamics is thus able to control the chemically driven melting, i. e., fragmentation, and replication of the DNA-protein system in compliance with the periodicity of the cell cycle. It also exhibits a frequency selective mechanism that can be experimentally tested. Detection of a dominant acoustic mode, in the NEBS dynamics predicted at $\omega_\sigma^2 = 3k_{sp}/(4m)$, would lend further support to the model. However, in order to test the model, one should like to know the values of N , L , N_S and the reduced

mass m more exactly, as well as the mass of the protein complex repelled from the origin to the replication fork, M_{RF} , and the mass of the fragment protein complex associated with the origin, M_F . If $M_{RF} \ll M_F$ one could then approximate the reduced mass $m = M_{RF} \cdot M_F / (M_{RF} + M_F)$ by $m \approx M_{RF}$, the mass of the complex that contains proteins such as Mcm helicase and Polymerase- α . Otherwise one would have to recalculate the acoustic mode and then account for both masses.

It is understood that the native DNA-protein system is much more complex than the NEBS model system. As already mentioned, the pre-RC complexes contain more than two protein complexes, which also interact with the histones. However, at the transient chemical equilibrium that prevails shortly after all the N pre-RCs have been bound and before the origin firing [9], the momentum transfer between pre-RCs, between pre-RC components, and between pre-RC components and histones, should become vanishingly small. The wavelength of mechanical vibrations should then become large compared with the spacing between origins as well as between all interacting components. Such spacing distances can then be neglected, the number of effectively interacting components becomes reduced, and the non-equilibrium conformational distribution of occupied origins in Eq. (2) reduces to an order parameter of the form $\varphi = \mu / (1 - \psi/a)$. Alternatively, this form of order parameter could be obtained through a path integral representation of the DNA-protein string [22], or corresponding Bethe-Salpeter expansion, but this would not change the leading order dynamics. It is important, however, that the transient equilibrium, i.e., the long wavelength limit, is approached via the non-equilibrium conformational distribution defined by Eq. (2). If chemical equilibrium had been taken for granted already from start, as in thermotropic models, and the origins had been distributed like the segments in a finite order polymer [12], the order parameter in the long wavelength limit would have been given by $\varphi = \mu(\psi/a)^n$, which would not have been consistent with our hypotheses.

The origins are short openings in the DNA duplex, small denaturation loops from which melting and replication of DNA is initiated by chemical excitations. The denaturation and melting transition of naked DNA has been extensively studied within a class of thermotropic statistical mechanical models for almost four decades [13, 23–26]. These models exhibit first-order or second-order phase transitions depending on whether one or another form of excluded volume effects between single and double stranded DNA are included or not. A first order transition may well be verified for naked DNA but has not been indicated in native chromatin, like that of MLA-144, at the initiation of DNA replication.

The chemical non-equilibrium and lyotropic properties of the DNA-protein dynamics comes to direct expression in the reaction coordinate, i. e., the order parameter $\xi = \varphi/N = 2\rho/(\rho + \rho_{50})$, showing that the fraction of occupied to non-occupied origins is controlled via Eq. (1) by the reactant concentrations and not by the temperature as in thermotropic theories. Although the two incompatible states, the pre-replication assembly state and the replication state, are separated by

a short chemical equilibrium [9], the usual Boltzmann-type statistical mechanics does not apply because this equilibrium is attained through the non-equilibrium assembly of the N pre-RCs at the origins. It is immediately thereafter perturbed by chemical excitations, e.g., by kinases and ATP hydrolysis, inducing some hundred origins to start firing. The fact that this chemically-induced start of origin firing may be spread out within a short interval of time does not imply that the two incompatible states are not separated by an exact time, at which the DNA-protein system can take the decision to allow origin firing.

As described in the model presented here, if the DNA-protein system can take decisions or not is rather a dynamical problem as to which energy states are attainable and preferred by the system or not. The chemical excitation energy $E_{th} = \kappa L/2$ defined here above should thus be sufficient to initiate melting and replication of DNA and disassembly of pre-RCs if the ground state energy of the spatially coherent DNA-protein system is increased to E_N , i.e., if all N ATP-containing pre-RCs are assembled ($\varphi/N = \xi = 1$). The force $F(\xi)$ between pre-RCs then switches from attraction to repulsion via zero and the energy gap, $E_s - E_N = E_{th}$, then becomes small enough for the chemical excitation to lift the DNA-protein system up to the scattering energy level E_s . At $\varphi/N = \xi < 1$ the intermolecular force is still attractive, inhibiting disassembly of the pre-RCs and initiation of DNA replication. Both in the assembly state and the replications state one may well say that the DNA-protein system is enslaved the surroundings [27], i. e., the reactions that drive the dynamics.

Conformational transitions can also control the activation of genes, such as those responsive to E2F. A mechanistic type of intramolecular interaction, mediated by phosphorylation at phosphoacceptor sites in the carboxy-terminal region of the Rb protein, has been suggested [7] to explain the rheostat-like conformational changes which first lead to dissociation of HDAC from Rb protein and synthesis of cyclin E. The fact that a definite number of external receptor engagements are required for commitment to DNA replication [4, 5, 28] seems to indicate that a corresponding definite critical threshold of mitogen-dependent phosphorylation of Rb protein molecules is required. The generally accepted view is that cyclin D-Cdk4/6 starts to phosphorylate Rb protein in mid G_1 [2]. However, dissociation of Rb proteins starts only later at the G_1 restriction point, at which probably a definite number of Rb proteins (probably all) have been phosphorylated at all phosphoacceptor sites in the carboxy-terminal region, and at least one but not all Rb proteins has been phosphorylated at all phosphoacceptor sites [2, 7], because then the dissociation of Rb proteins starts.

The Rb phosphorylation occurs through transfer by a kinase of a phosphate moiety from ATP to the Rb-E2F complex, implying a non-equilibrium binding like that defined in Eq. (1). Moreover, the probabilistic conformational distribution of phosphorylated Rb-E2F complexes should be given by Eq. (2). Perhaps the NEBS model could thus also describe the dynamics that controls the commitment to DNA replication, and help to explain the ambivalent functional roles of Cdk

during the cell cycle [1, 29]. Questions related to this problem are, for instance, exactly when all p27 molecules have been sequestered, how the mitogen dependent phosphorylation of Rb proteins becomes quantified at the G_1 restriction point, and how this is related to the quantal external IL2 receptor enagagement [4, 5, 28], need to be further investigated.

As shown in the NEBS model, via Eq. (1) the DNA-protein dynamics depends on all the reactant concentrations, such as, of ligands, spare receptors and their initial values. As explained previously [15], it is therefore not sufficient to modify equilibrium statistical mechanics based models by adding a term that depends logarithmically on the concentration of free ligands in solution to the free energy. The wrong scale of the dose-response curve (Figure 1) derived in equilibrium type models is but one sign of the large gap between such theories and the actual non-equilibrium DNA-protein system. The contributions from local cooperativity, such as, between vacant and occupied receptors, also depend heavily on the non-equilibrium conditions because of the drastic turnover of reactants. Thus, even though slope, scale and form of a response derived in an equilibrium type model may agree well with experimental data from a non-equilibrium living system, both the physical and biological properties obtained in such models remain obscure.

The proposed non-equilibrium model applies in cases such as MLA-144 [4, 5], in which the slope of response is approximately one. However, this does not necessarily exclude contributions from local cooperation in the DNA-protein system. For instance, positive and negative short range correlations may cancel out, or be removed by initiator proteins and histones that attaches to DNA. In other native cells local cooperation may actually be indicated. Such effects could then perhaps be described in a generalized non-equilibrium Ising-like system, using the concepts employed in the NEBS model [3, 15].

More detailed information about the native DNA-protein system and its biological functions could perhaps be obtained by stretching a single DNA molecule [21]. However, beside the initiator proteins and histones there are many other important differences between naked DNA and native chromatin. As already mentioned, in the NEBS model the traveling wave of disassembly and unfolding that precedes DNA replication is induced at $\xi = 1$ by a switch of interaction from attraction to repulsion, via a vanishing force. By contrast, in single molecule experiments the unfolding of DNA is enforced by a nonzero external force. It would therefore be interesting to know if a relation between the force-extension formula obtained by single DNA molecule experiments and the intermolecular force $F(\xi) = -(\kappa/2)\xi(1 - \xi)(2 - \xi)$ of the native DNA-protein system exists. Such a relation could then contribute to a better understanding of the dynamics of native chromatin, and perhaps also confirm the validity of the NEBS model.

By controlling the DNA replication, and thereby the wave of DNA melting that travels as a precursor for DNA replication, the NEBS dynamics also seems to apply to much smaller scales, such as of a base-pair. Assuming for instance that

the interaction between bases in the DNA could be described by a Morse potential $V_M(\xi) = (\kappa L/2)[\exp(-\xi) - 1]^2$, which for small ξ -values becomes $V_M(\xi) \approx (\kappa L/8)\xi^2(2 - \xi + \xi^2/3)^2$, one would obtain an intermolecular force of the form $F_M(\xi) = -(\kappa/2)\xi(1 - \xi + \xi^2/2)(2 - \xi + \xi^2/3)$, which deviates from that of the NEBS model and, hence, from the periodicity of the cell cycle. However, it may be speculated that Orc, the most closely bound pre-RC component, could interfere between the long range NEBS dynamics and a Morse-type short range interaction. In such a combined type of interaction working at different scales Orc would be attracted by the origin also at and beyond $\xi = 1$. One might therefore guess that a Morse-type component of the interaction could explain the higher affinity of Orc to DNA compared to other pre-RC components, which are being repelled from the origin at the *S* phase entry.

As demonstrated earlier [17, 30] it can be shown that the model is dual in the sense of Kramers and Wannier [31]. However, by contrast to their form of duality for thermotropic theories, in this lyotropic theory the duality transformation relates dynamical observables at low concentrations to their dual counterparts at high concentrations. This involutory property of the dual transformation is important for the interpretation of the experimental dose-response curve and for the comparison with the dose-response curve derived in the model. Another important property of the suggested non-equilibrium model, as has earlier been explained in principle [30], is that the topological quantum number corresponds to a conserved current. In the actual case the conservation of the topological quantum number, which here designates the number of replicons, is a guarantee for the exact one-to-one mapping required for a faithful replication of the genome.

A deeper insight in the DNA protein dynamics, for instance, in order to include the twisting of DNA and the interaction between bases in a base-pair, however, also requires a generalization of the NEBS model into three spatial dimensions. As described previously [15], a combination of different methods could then turn out to be useful. However, the aim in this work was not to describe the cell and its biological functions in detail, but to find the simplest possible nontrivial model for the DNA-protein dynamics in the case of MLA-144, for which short range cooperation and excluded volume effects do not seem to be indicated. It is the hope that knowledge of the NEBS dynamics of the native DNA-protein system, and the machinery that controls the cell cycle and gross turnover of genetic material in normal cells, could lead to a better understanding of abnormal DNA replication, such as in proliferation of cancer cells, and also how cancer emerges. By explaining what molecules can do together in living condensed matter that they cannot do together in inanimate condensed matter, the proposed model could also provide a better physical understanding of what life is and how it once emerged. Perhaps it could also contribute to a better understanding of chemical non-equilibrium systems involving particle-particle interactions and reactions [32], however, in condensed phases like the elastic DNA-protein system.

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