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Intermediate filament networks: in vitro and in vivo assembly models

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Abstract

We propose two systems of ordinary differential equations modeling the assembly of intermediate filament networks. The first one describes the in vitro intermediate filament assembly dynamics. The second one deals with the in vivo evolution of cytokeratin, which is the intermediate filament protein expressed by epithelial cells. The in vitro model is then briefly analyzed in a simplified case. ***To cite this article: S. Portet et al., C. R. Biologies 327 (2004).***

Résumé

Réseaux de filaments intermédiaires : modèles d'assemblage in vitro et in vivo. Nous proposons deux systèmes d'équations différentielles modélisant l'assemblage de réseaux de filaments intermédiaires. Le premier décrit la dynamique de l'assemblage in vitro de filaments intermédiaires. Le second traite de l'évolution in vivo de la cytokératine, qui est la protéine des filaments intermédiaires exprimée par les cellules épithéliales. Le modèle in vitro est ensuite brièvement analysé dans un cas simplifié. ***Pour citer cet article : S. Portet et al., C. R. Biologies 327 (2004).***

Keywords: Intermediate filaments; assembly dynamics

Mots-clés : Filaments intermédiaires ; dynamique d'assemblage

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1. Introduction

The cytoskeleton is a complex arrangement of proteins that are involved in many cell functions such as cell signalling, maintenance of cell integrity and of cell shape, and internal transport within the cell. These structural proteins are composed of three main types. The actin filaments, also called microfilaments (*MFs*), are the contractile elements of the cell and are involved in the cell motility. The microtubules (*MTs*), made up of tubulin, contribute to internal transport, and movement of chromosomes during cell division. Finally, intermediate filaments (*IFs*), composed of a large heterogeneous family of proteins expressed in a tissue-dependent and developmentally regulated manner, are involved in the cell architecture and provide stability against mechanical stress [1]; IFs extend across the cytoplasm, giving cells mechanical strength and rigidity. IFs also participate in cell signalling [1,2], and more specifically act as mechanotransducers [3,4].

IFs generally constitute approximately 1% of the total protein content of a cell. In some cells where IFs are especially abundant and are the major structural proteins, the proportion rises up to 85% of the total proteins. The structure of all IF proteins, that are fibrous proteins, consist of a central α-helical domain flanked by non-α-helical head and tail domains. The central domain is interrupted by three non-α-helical linker regions [5].

Epithelial cells express IF cytoplasmic proteins, called cytokeratins (*CKs*), which are divided into acidic type I and basic type II keratins. CK IFs are heteropolymers made up in a ratio 1:1 of pairs of type-I and type-II monomers, called heterodimers [6]. CK IFs span from one cell–cell junction to another to maintain structural integrity in epithelial tissues.

IF assembly is not an energy-dependent process and does not necessitate associated proteins. In vitro IFs are relatively stable and most of IF proteins are in filamentous or insoluble state. In interphase cells, more than 95% of the total of IF proteins occurs in an insoluble or filamentous form [7]. However, once assembled, IFs exhibit dynamic behavior; the occurrence of significant IF subunit exchange and dynamic assembly/disassembly of IFs has been shown [8]. IF proteins are phosphorylated by a number of kinases, and in many cases this phosphorylation attenuates or inhibits IF assembly and facilitates IF disassembly, particularly during mitosis. Thus IFs are dynamically reorganized; this reorganization is regulated by IF protein phosphorylation/dephosphorylation.

Recent research highlights the structural functions of cytoskeletal IFs in maintaining cellular integrity and the rheological properties of the cytoplasm; these functions are mediated through their mechanical as well as their dynamical properties [9]. Each IF type differs functionally and dynamically. Furthermore, IF in vitro and in vivo assembly dynamics are different in terms of stability and assembly velocity; in vivo, the occurrence of phosphorylation/dephosphorylation processes increases the dynamics of IF assembly. Another significant difference, occurring during the nucleation step (the initiation of a filament), can be mentioned; in vitro, the initiation of filaments only results from an aggregation process, whereas in vivo this event also involves a structural process.

In this paper, we propose two ordinary differential equation models for the assembly dynamics of IF networks. The first model (Section 2) describes the in vitro assembly kinetic of IFs from the three-step process observed by Herrmann et al. [8,10]: (*i*) lateral aggregation of proteins in the so-called 'unit-length-filament', *ULF*, (*ii*) filament elongation by longitudinal aggregation of ULF into its tips, and (*iii*) radial compaction of filaments. The second model (Section 3) describes an in vivo assembly kinetic of CKs mainly based on the observations made by Windorffer and Leube [11]. In Section 4, a preliminary analysis of the in vitro model is given.

2. In vitro assembly model of IFs

During the in vitro experiments, where the material is only composed of pure protein mixture, the first step of IF assembly is the formation of a dimer from two monomers; this process is also called the dimerization. Secondly, two dimers associate, forming tetramers. Then the IF in vitro assembly consists of rapid lateral association of eight tetramers in ULF [8,10]. After a few seconds, ULFs anneal longitudinally to the filament tip. These filaments, called immature filaments, are at first loosely packed; after a while, they begin to radially compact to eventually yield mature IFs. Similar assembly pathways are followed by vimentin, desmin and the neurofilaments [12,13]. Assembly of

CKs can follow the same sequence of events, but at a faster rate [12]. It has been proposed that the nucleation of IFs necessitates the formation of an octameric intermediate as a nucleation center [7].

In order to model the above processes, we make some assumptions. We suppose that the soluble subunit of IFs takes the form of a tetramer [14,15]. Hence, the dimerization process is not taken into account in our model. Also, the exchange of IF subunits between the soluble pool and the filaments does not modify the number of subunits and filaments. Therefore, this process does not appear in our equations. The same holds true of the maturation event, i.e. the compaction of immature filaments described above. Lastly, as the IFs are non-polar polymers, the two tips of a filament are equivalent. From the biological insights and our modeling assumptions, we describe the organization process of IF networks by the series of events listed below.

Nucleation is the filament initiation phase, when a given number, n, of tetramers aggregate laterally to form a *nucleation center*. This process occurs at a rate ν. The nucleation center takes the form of an octamer, i.e. a two-tetramer aggregate. As in vitro the IFs have been shown to be insoluble under physiological conditions, the nucleation center is considered to be stable; its elimination is not possible. The nucleation phase is an irreversible reaction.

Polymerization is the process in which filaments assemble by longitudinal annealing of an ULF at their tips; it occurs at a rate π that can depend on the type of IF and on the pH [2]. An ULF is made up of the lateral aggregation of a given number, m, of tetramers. It is assumed that this lateral assembly is instantaneous.

Depolymerization is the filament shrinking phase in which filaments disassemble from their tips by releasing an ULF; this process occurs at a rate $\delta < \pi$.

Annealing models the tendency of filaments to anastomose (lengthen) and to laterally aggregate (bundle). The annealing process reflects the ability of IF polymers to self-interact. This event occurs at a rate μ that depends on the assembly conditions (e.g., salt concentration) [2].

For this model, we define two state variables, which represent the density at time t of the two populations involved. The soluble pool $C(t)$ is made up of tetramers, the subunits of IFs. The filament tips are $T(t)$. As motivated above by the underlying assembly kinetics, we postulate the following reaction terms:

$$\frac{\mathrm{d}C}{\mathrm{d}t} = -\underbrace{n\nu C^n}_{\text{nucleation}} - \underbrace{m\pi C^m T}_{\text{polymerization}} + \underbrace{m\delta T}_{\text{depolymerization}} \tag{1a}$$

$$\frac{\mathrm{d}T}{\mathrm{d}t} = \underbrace{2\nu C^n}_{\text{nucleation}} - \underbrace{\mu T^2}_{\text{annealing}} \tag{1b}$$

where n represents the number of tetramers composing the nucleation center. The number m is the number of tetramers involved in the formation of an ULF. This number can vary depending on the IF protein considered. Here ν is the rate of nucleation, π is the rate of longitudinal annealing of an ULF to the tip of filaments, δ is the rate of disassembly of an ULF from the tip of filaments, and μ represents the rate of filament annealing. The coefficient 2 appearing in the nucleation term of Eq. (1b) follows from the non-polarity property of IFs; the nucleation of one filament leads to the creation of two filaments tips. All the numbers and the rate constants are positive. Parameters of the model (1) are listed in Table 1. Model (1) is considered with the initial conditions $C(0) = c_0$, $T(0) = \tau_0$, with $c_0, \tau_0 \geqslant 0$. A brief preliminary analysis of this system is given in Section 4.

Table 1
List of parameters involved in the in vitro model (1) and in the in vivo model (2)

Parameter	Signification
	Specific to the in vitro model
n	Number of tetramers in a nucleation center
m	Number of tetramers in an ULF
ν	Rate of nucleation of filaments
π	Rate of longitudinal annealing of an ULF at the end of a filament
δ	Rate of disassembly of an ULF from the tip of a filament
	Common to both models
μ	Rate of annealing between two filaments
	Specific to the in vivo model
a	Number of tetramers in an aggregate
α	Rate of aggregation of tetramers in an aggregate
π_1	Rate of assembly of a tetramer to an aggregate
δ_1	Rate of disassembly of a tetramer from an aggregate
π_2	Rate of assembly of a tetramer to a filament
δ_2	Rate of disassembly of a tetramer from a filament
κ	Rate of condensation of a filament into an aggregate
ϵ	Rate of elongation of an aggregate into a filament
β	Rate of breakage of a filament

3. In vivo assembly model of CKs

Within cells (in vivo) IFs, and more specifically CKs, have a more dynamic behavior; reorganization and reversible restructuring of the CK network take place during mitosis, stress, and apoptosis [16,17]. IF assembly regulation has been supposed in the in vitro model of the previous Section to occur intrinsically via self-interactions. We now propose an in vivo model where IF assembly is also regulated extrinsically by phosphorylation, glycosylation, and ubiquitination, or associations with other cellular elements [2]. Phosphorylation is a dynamic process involved in regulating CK solubility and filament reorganization [1]. Phosphorylation of CKs favors the depolymerized state in the assembly equilibrium. This event can take place during mitosis and cell stress conditions [16,17].

The in vivo model presented here is mainly inspired by the observations and the assumptions described by Windoffer and Leube [11]. As in the in vitro model, CKs can exist in a soluble tetrameric form or a filamentous form, but also in another non-filamentous intermediate form. The latter is formed from the aggregation of a few soluble pool subunits (the tetramers). Some non-filamentous intermediate forms have been observed by Windoffer and Leube [11], taking the form of spheroid aggregates. Other authors observed these non-filamentous intermediate forms; they appeared as short fibrils, called squiggles [18]. In our model, these non-filamentous intermediate forms are called *aggregates*.

Nucleation of CK filaments is assumed to be a two-step process. First, some soluble pool subunits aggregate; secondly, this aggregate elongates to form rod-like objects. The initiation of filaments then occurs with the polymerization and the elongation of aggregates.

Polymerization and solubilization processes represent the exchange between filaments and soluble pool, and between aggregates and soluble pool. Phosphorylation may induce filament shrinking; it is associated with solubilization, a reversible process that allows remodeling of the network [16,17].

Disassembly of filaments is modeled as the recondensation of filaments into aggregates. This process describes the transition from the filamentous form to the non-filamentous form, and thus constitutes the reverse reaction to the elongation phase occurring during nucleation. In this model, this event occurs at a constant rate κ, but it could be hypothesized that this coefficient incorporates some physical properties of filaments.

Fragmentation of filaments is the process during which a filament breaks into smaller filaments; it occurs at a rate β. The restricted, localized phosphorylation of a filament by a kinase (at a given point along its length) changes the configuration and the affinity of subunits inducing the breakage of filament.

Annealing models the tendency of a filament to anastomose (lengthen) and to laterally aggregate (bundle); this process occurs at a rate μ. Annealing accounts for the self-bundling (as IF polymers can self-interact), as well as the bundling by exogeneous cross-linking activities.

In the in vivo model, we only observe the dynamics of different states of the IF material. The concentrations of other molecule populations that could be involved in the IF assembly are assumed to be constant and taken into account implicitly in the rate coefficients. Thus, for the in vivo model, we define the following state variables: $S(t)$ is the soluble pool density at time t, $G(t)$ is the aggregate density at time t, and $F(t)$ is the filament density at time t. Using the above assumptions, we obtain the following system:

$$\frac{\mathrm{d}S}{\mathrm{d}t} = \underbrace{-\underbrace{a\alpha S^a}_{\text{aggregation}} - \underbrace{\pi_1 SG}_{\text{polymerization}} + \underbrace{\delta_1 G}_{\text{solubilization}}}_{\text{aggregate-soluble pool exchange}} \underbrace{- \underbrace{\pi_2 SF}_{\text{polymerization}} + \underbrace{\delta_2 F}_{\text{solubilization}}}_{\text{filament-soluble pool exchange}} \tag{2a}$$

$$\frac{\mathrm{d}G}{\mathrm{d}t} = \underbrace{\underbrace{\alpha S^a}_{\text{aggregation}} - \underbrace{\epsilon G}_{\text{elongation}}}_{\text{nucleation}} + \underbrace{\kappa F}_{\text{condensation}} \tag{2b}$$

$$\frac{\mathrm{d}F}{\mathrm{d}t} = \underbrace{\underbrace{\epsilon G}_{\text{elongation}}}_{\text{nucleation}} - \underbrace{\underbrace{\kappa F}_{\text{condensation}}}_{\text{disassembly}} \underbrace{- \underbrace{\mu F^2}_{\text{annealing}} + \underbrace{2\beta F}_{\text{fragmentation}}}_{\text{network formation}} \tag{2c}$$

The constant a represents the number of tetramers involved in an aggregate; it can likened to the in vitro parameter n, the number of tetramers involved in the nucleation center. The aggregation rate of tetramers to

form aggregate, α, can be likened to the in vitro nucleation rate ν. The constants ϵ and κ represent the rates of elongation into filament and condensation into aggregate, respectively. The constants π_1 and π_2 are involved in the polymerization process. The constants δ_1 and δ_2 are involved in the solubilization process. As in the in vitro model (1), μ represents the rate of filament annealing. The constant β is the rate of filament fragmentation. As for the in vitro model, all the numbers and the rate constants are positive; they are listed in Table 1. The in vivo model (2) is considered with the initial conditions $S(0) = s_0$, $G(0) = g_0$, and $F(0) = f_0$, with $s_0 \gg g_0 \geqslant 0$ and $f_0 \geqslant 0$.

4. Some analysis

As this paper is mainly concerned with the statement of the models, we postpone to further work a more thorough analysis. The in vitro system (1) is quite complicated for general n and m. Indeed, the existence of nontrivial equilibria is governed by a quasi-polynomial equation. In order to simplify this preliminary analysis, we consider the biologically motivated case where the nucleation center consists of an octamer ($n = 2$) [7] and the ULF is a 32-mer ($m = 8$) [8,10].

Under these assumptions, equilibria of the system (1) are the origin $C = T = 0$ and an interior equilibrium (C^*, T^*), where $C^* = \sqrt{\frac{\mu}{2\nu}}T^*$, with T^* the positive zeros of the polynomial

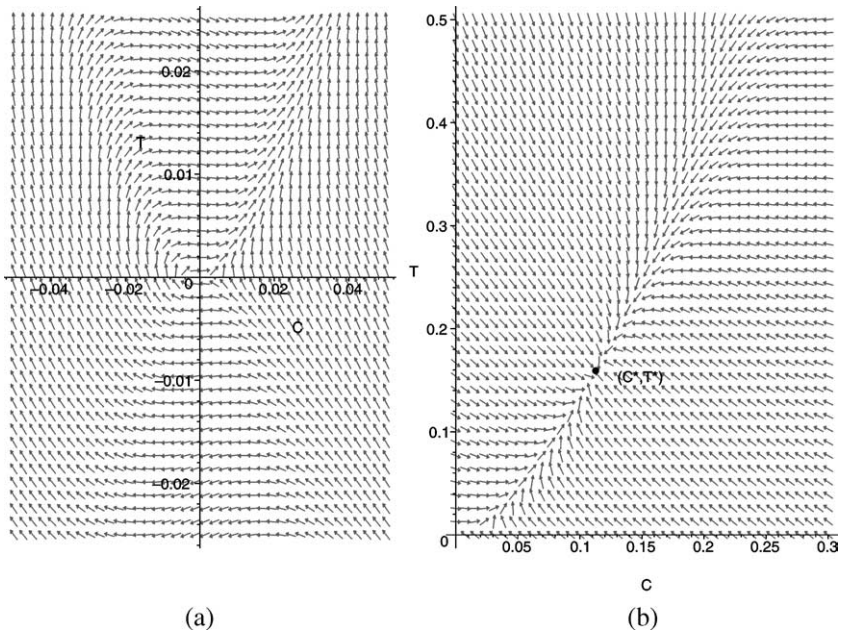
$$P(T) = 8\delta - \frac{\pi\mu^4}{2\nu^4}T^8 - \mu T \tag{3}$$

It is easy to see that for $T \geqslant 0$, (3) is monotone decreasing; since $P(0) = 8\delta$, it follows that the positive equilibrium point (C^*, T^*) is unique. Note that both equilibria always exist.

At the origin, the Jacobian matrix J has two zero eigenvalues, with $\operatorname{tr} J_{(0,0)} = 0$, $\det J_{(0,0)} = 0$, and $J_{(0,0)} \neq 0$. Using the method in Andronov et al. [19], we put system (1) in normal form:

$$\frac{dC}{dt} = T \tag{4a}$$

$$\frac{dT}{dt} = \frac{\nu}{4\delta}C^2 - \frac{\nu\pi}{4\delta^2}C^{10} + \left(-\frac{\nu}{2\delta}C\right)T + f(C,T)T^2 \tag{4b}$$



(a) (b)

Fig. 1. (a) Vector field of system (1) in the vicinity of the origin, illustrating the topological structure of cusp of the latter. (b) Vector field of system (1) with the stable steady state (C^*, T^*).

where $f(C,T) = \frac{1}{\delta(\delta-\pi C^8)T^2}(4\delta T - \nu C^2)[\frac{-\mu}{128\delta}(4\delta T - \nu C^2) - 2\pi C^7 T]$. From [19 (Th. 67, p. 362)] the origin is a cusp; see Fig. 1a. As the set along which solutions approach the origin lies entirely in $\mathbb{R}_+ \times \mathbb{R}_-$ and that a straightforward calculation shows that the positive quadrant $\mathbb{R}^2_+$ is positively invariant under the flow of (1), it follows that the origin is repelling for solutions of system (1) starting in $\mathbb{R}^2_+ \setminus \{(0,0)\}$.

At any point in the positive quadrant except the origin, the Jacobian matrix J has a negative trace. Thus, from Bendixson's criterion, there are no non-constant periodic solutions in the positive quadrant. It can also be shown that solutions of (1) are bounded. As the interior equilibrium (C^*, T^*) is unique and the origin is unstable, it follows from the Poincaré–Bendixson theorem that all trajectories limit to the interior equilibrium, i.e. that the interior equilibrium (C^*, T^*) is globally asymptotically stable with respect to $\mathbb{R}^2_+$ (see an example in Fig. 1b).

Analysis of the in vivo model (2) is beyond the scope of this introductory paper, and will be the object of further work.

5. Discussion

To the best of our knowledge, there are very few models that focus on the IF organization dynamics. In an earlier work [20], some of the authors had considered a model of IF network organization in the cell. However, this model made a phenomenological description of the processus involved. Recently, there has been a surge in the number of biological publications dealing with the dynamical behavior of IFs as well as with their physical properties. It is therefore now possible to envision a model that would give a more appropriate description of the biological mechanisms. This paper constitutes the first stage of this project. We have focused here on the assembly kinetics of the IF network. Two models were introduced that describe the behavior of the in vitro and in vivo IFs. In this paper, we have mainly concentrated on the biological justifications of the models. For this reason, we have only given a preliminary analysis of the in vitro model, in a simplified case (which is, however, biologically relevant). Analysis of the in vitro model in the general case, and of the in vivo model, is a difficult task because of the nature of the nonlinearity, which leads to quasi-polynomial (or transcendental) equations even for the determination of the nontrivial equilibria.

The dynamical assembly properties of IFs as well as their mechanical properties mediate their structural role in the cell. In order to improve our description of the organization processes of IF networks, the in vivo model is going to be extended first to account for the spatiality of the events, and secondly to depict the physical properties of the IFs.

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