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Intermediate filament dynamics: Disassembly regulation

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A mechanism of intermediate filament disassembly regulation is proposed in which disassembly is regulated by the amount of proteins assembled in networks. It is also hypothesized that a delay might exist between regulation and actual disassembly. Under realistic biological conditions of assembly and disassembly, it is shown that such a delay is harmless and does not destabilize the organization of intermediate filaments in networks. However, for high rates of disassembly, the model predicts that delay can destabilize the organization, with the intermediate filament material oscillating between organizations mainly in networks and in nonfilamentous particles.

Keywords: Cytoskeleton organization; intermediate filaments; time delay; global asymptotic stability; bifurcation; periodic solution.

Mathematics Subject Classification 2010: 92B05, 34Cxx

1. Introduction

Intermediate filaments are one of the three main components of the cytoskeleton. They are organized in networks spanning the cytoplasm and are involved in the stabilization and mechanical resilience of cells and tissues, cell migration and signal transduction. The organization of intermediate filaments in networks determines their functions in cells. The expression of intermediate filament proteins depends

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on the degree of differentiation of cells and the cell type. For instance, keratin is the intermediate filament protein expressed by epithelial cells and is one of the most abundant proteins in these cells. The keratin network spans the cytoplasm of epithelial cells and connects these cells through desmosomes, assuring integrity of the epithelium. The keratin network protects cells from stress [33]; defects in the keratin network organization induce tissue fragility disorders [5].

In cells, intermediate filaments exist as soluble proteins taking the form of tetramers; soluble proteins can oligomerize and form nonfilamentous particles [3, 17, 23] called filament precursors. Intermediate filament proteins also exist assembled in filaments that organize in networks, the filamentous pool. In interphasic cells, it is observed that the majority of the intermediate filament material is assembled in networks; for instance, network-assembled keratin represents at least 80% of the total keratin in interphasic epithelial cells [1, 34]. However, intermediate filament networks are highly dynamic structures capable of rapid re-organizations in response to the cellular environment [34]. The interplay between assembly, disassembly and intracellular transport allows the continuous dynamical and spatial re-organization of intermediate filament networks without network disruption and loss of structural integrity of cells [34].

Many mechanisms involved in intermediate filament dynamics are still not well understood, such as the regulation of the oligomerization of the soluble pool in filament precursors and the regulation of the disassembly of the filamentous pool. Intermediate filament protein assembly is a more favored reaction than their disassembly. For instance, intermediate filaments are highly insoluble in *in vitro* physiological conditions (no disassembly is observed) [24]. In cells, evidence shows that post-translational modifications of intermediate filament proteins, such as phosphorylation, regulate the disassembly [1, 2, 36]. Phosphorylation of intermediate filament proteins inducing filament disassembly is mediated by the activity of protein kinases [15, 31]. In a recent work combining mathematical modeling and experimental data [30], the effects of the interplay between assembly/disassembly processes and intracellular transport of the insoluble keratin on its repartition in epithelial cells were investigated by testing different scenarios. A scenario was proposed for the keratin dynamics [30] that is in good agreement with previous biological studies [21, 34]: the assembly of keratin was found to be non-compartmentalized over the cell whereas disassembly was found to be localized in the perinuclear region (at the center of the cell) as the keratin material is transported in the cell from the cell periphery to the nucleus (inward transport). Hence, disassembly was found to be localized in the perinuclear region where the network is most abundant. Furthermore, in a previous mathematical work [28], we found that disassembly is the main determinant of intermediate filament organization in cells.

It follows from these considerations that a mechanism must exist that regulates filament disassembly in cells; this is investigated here. It is hypothesized that the amount of proteins assembled in filaments plays a role in the regulation of filament disassembly. Also, it is assumed that filament disassembly occurs at time t when the

regulation is triggered at time $t - \tau$. Both cases of simultaneous and delayed regulation are investigated mathematically and illustrated with numerical simulations.

2. Model

In this work, intermediate filament material is categorized in three structural states represented by the following state variables:

- $S(t)$ soluble protein (soluble pool) concentration at time t;
- $P(t)$ filament precursor (oligomer of soluble proteins, nonfilamentous particle) concentration at time t;
- $N(t)$ filamentous state (network of filaments) concentration at time t.

The cycle of assemblies and disassemblies of intermediate filament proteins in cells is described in terms of reactions linking these three structural states.

As previously mentioned, the *in vivo* initiation of filaments is not yet well documented; however, the *in vitro* mechanism is well understood [16, 23]. *In vitro*, tetramers (the soluble proteins) aggregate laterally to form a unit-length-filament (ULF) in the first seconds of the assembly kinetics of intermediate filaments [16, 23]. These ULFs, which are the filament precursors [27, 29], can be composed of different numbers of tetramers depending on experimental conditions and protein type (e.g. four tetramers per ULF for keratin and eight tetramers per ULF for vimentin [23]). *In vivo*, similar processes are conjectured; however, the characteristics of filament precursors are not well known. *In vitro* and in the cell, the ULFs or filament precursors then longitudinally aggregate to form filaments that in turn fuse or bundle to elongate or form a network [4, 18, 25, 34, 35]. Finally, in the cell, once proteins are assembled in filaments, the activity of kinases can induce the solubilization of intermediate filaments back to soluble proteins [17, 36].

In the present model, the cell is assumed to be spatially homogeneous; events are not spatially localized and transport of the intermediate filament material within the cell is not considered. Reactions considered for the dynamics of intermediate filaments in cells are summarized in Fig. 1 and are detailed now. Soluble proteins are assumed to oligomerize to form filament precursors (Nucleation). Nucleation is described as the aggregation of two soluble proteins. Filament precursors then grow/polymerize by addition of soluble proteins to existing filament precursors (Growth). Filament precursors fuse/integrate to existing filaments (Integration) [34]. The assembly of intermediate filament proteins encompasses the nucleation, growth and integration processes. Assembly processes are formulated as in previous works [28, 32] and follow the mass action law. Filaments disassemble/solubilize, giving soluble proteins (Disassembly) [17]. The disassembly process is a consequence of the activity of kinases; however, this enzymatic activity is not explicitly modeled here. Instead, the activation/regulation of these enzymes is assumed to take $\tau \geq 0$ units of time. In previous work [30], disassembly was found to occur in regions where the filamentous pool is abundant. To translate phenomenologically this hypothesis,

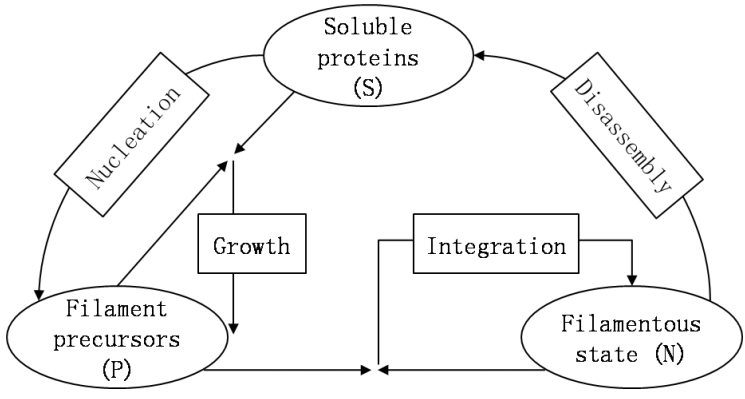

Fig. 1. Interactions between structural states of intermediate filament material (S, P and N) describing the assembly and disassembly cycle in a cell.

a negative feedback is used to describe the regulation of the disassembly; the rate of disassembly of the filamentous pool is formulated as a decreasing function of its concentration.

The following system of delay differential equations is derived from the assumptions above:

$$\frac{\mathrm{d}S(t)}{\mathrm{d}t} = \underbrace{-aS(t)^2}_{\text{Nucleation}} - \underbrace{\gamma S(t)P(t)}_{\text{Growth}} + \underbrace{\kappa N(t-\tau)N(t)}_{\text{Disassembly}}, \tag{2.1a}$$

$$\frac{\mathrm{d}P(t)}{\mathrm{d}t} = \underbrace{aS(t)^2}_{\text{Nucleation}} + \underbrace{\gamma S(t)P(t)}_{\text{Growth}} - \underbrace{iP(t)N(t)}_{\text{Integration}}, \tag{2.1b}$$

$$\frac{\mathrm{d}N(t)}{\mathrm{d}t} = \underbrace{iP(t)N(t)}_{\text{Integration}} - \underbrace{\kappa N(t-\tau)N(t)}_{\text{Disassembly}}. \tag{2.1c}$$

All parameters of system (2.1) are positive except τ which is non-negative. They are listed in Table 1. Ranges of some parameter values are roughly estimated based on previous *in vitro* and *in vivo* studies [7, 16, 27].

Table 1. Parameters in system (2.1).

Parameter	Definition	Value
c	Total concentration of intermediate filaments in a cell	100–520 μM [7]
a	Rate of filament precursor formation	20–50 $\mu\mathrm{M}^{-1}\cdot\mathrm{s}^{-1}$ [16]
γ	Rate of filament precursor growth	20–50 $\mu\mathrm{M}^{-1}\cdot\mathrm{s}^{-1}$ [16]
i	Rate of filament precursor integration to filamentous state	0.1–0.5 $\mu\mathrm{M}^{-1}\cdot\mathrm{s}^{-1}$ [16, 27]
κ	Rate of disassembly of filamentous state	—
τ	Average time of activation	—

System (2.1) is considered with initial data:

$$S(\theta) = \phi_1(\theta) \geq 0, \quad \theta \in [-\tau, 0],$$
$$P(\theta) = \phi_2(\theta) \geq 0, \quad \theta \in [-\tau, 0],$$
$$N(\theta) = \phi_3(\theta) \geq 0, \quad \theta \in [-\tau, 0],$$

where $\Phi := (\phi_1, \phi_2, \phi_3) \in C([-\tau, 0], \mathbb{R}^3_+)$. Here C is the Banach space of continuous functions mapping the interval $[-\tau, 0]$ into $\mathbb{R}^3_+$ with the topology of uniform convergence and the sup norm $\|\Phi\| = \sup_{-\tau \leq \theta \leq 0} |\Phi(\theta)|$.

3. Preliminary Results

The standard theory on delay differential equations [10, 11, 13, 20] indicates that there exists a unique local solution of system (2.1). System (2.1) obeys a mass conservation law such that $S(t)+P(t)+N(t) \equiv c$ for all t since $\frac{\mathrm{d}(S(t)+P(t)+N(t))}{\mathrm{d}t} = 0$. Hence $P(t) \equiv c - S(t) - N(t)$. System (2.1) is then attacked by studying the subsystem:

$$\frac{\mathrm{d}S(t)}{\mathrm{d}t} = -aS^2(t) - \gamma S(t)(c - S(t) - N(t)) + \kappa N(t)N(t-\tau), \tag{3.1a}$$

$$\frac{\mathrm{d}N(t)}{\mathrm{d}t} = iN(t)(c - S(t) - N(t)) - \kappa N(t)N(t-\tau), \tag{3.1b}$$

in the closed set $\Gamma := \{(S, N) \in \mathbb{R}^2_+ \,|\, 0 \leq S + N \leq c\}$.

Lemma 3.1. *The closed set* Γ *is positively invariant for system* (3.1). *That is, if* $(S(\theta), N(\theta)) \in \Gamma$ *for* $\theta \in [-\tau, 0]$, *then the solution* $(S(t), N(t))$ *of system* (3.1) *is in* Γ *for all* $t \geq 0$.

Proof. If $N(\theta) \equiv 0$ for $\theta \in [-\tau, 0]$, then $N(t) = 0$ is a solution of Eq. (3.1b) for $t \geq 0$. Moreover, if $(S(t), N(t)) \in \Gamma$, on the line $N(t) = 0$, $\frac{\mathrm{d}N}{\mathrm{d}t} = 0$, and on the line $S(t) = 0$, $\frac{\mathrm{d}S}{\mathrm{d}t} = \kappa N(t)N(t-\tau) \geq 0$. Therefore, no solution of system (3.1) can traverse or leave $S = 0$ or $N = 0$ (the boundary of Γ) when $(S(\theta), N(\theta)) \in \Gamma, \theta \in [-\tau, 0]$. On the other hand, the sum of the three structural states $S(t), P(t)$ and $N(t)$ remains constant over time, i.e. $S(t) + P(t) + N(t) \equiv c$ for $t \geq 0$. This implies all solution trajectories of system (3.1) in Γ cannot travel away from the boundary line $S + N \leq c$ if $(S(\theta), N(\theta)) \in \Gamma, \theta \in [-\tau, 0]$. We then conclude that the solution exists globally for all $t \geq 0$ and is unique and the closed set Γ is positively invariant for system (3.1). □

Next, the existence and values of equilibria for system (3.1) are considered.

Theorem 3.2. (i) *System* (3.1) *always has a trivial equilibrium* $E_0 := (0, 0)$.

(ii) *System* (3.1) *always has a unique interior equilibrium* $E_* := (c-(1+\frac{\kappa}{i})N_*, N_*)$ *with*

$$\begin{cases} N_* = \dfrac{aci}{\sqrt{\kappa^2\gamma^2 + 4ai^2\kappa}} & \text{if } \dfrac{\sqrt{\kappa^2\gamma^2 + 4ai^2\kappa} + \kappa\gamma}{2a(i+\kappa)} = 1, \\ N_* = \dfrac{-D_1 + ci\sqrt{\kappa^2\gamma^2 + 4ai^2\kappa}}{2D_2} & \text{if } \dfrac{\sqrt{\kappa^2\gamma^2 + 4ai^2\kappa} + \kappa\gamma}{2a(i+\kappa)} \neq 1, \end{cases}$$

where

$$D_1 = 2aci(i+\kappa) - ci\kappa\gamma,$$
$$D_2 = -a(i+\kappa)^2 + \kappa\gamma(i+\kappa) + i^2\kappa.$$

Proof of Theorem 3.2 is given in Appendix A. The stability of the two equilibria is now investigated.

For a given point $(\overline{S}, \overline{N})$, define $\mathbf{u}(t) = (S - \overline{S}, N - \overline{N})^T$. System (3.1) can be rewritten as $\frac{\mathrm{d}\mathbf{u}(t)}{\mathrm{d}t} = \mathbf{f}(\mathbf{u}(t), \mathbf{u}(t-\tau))$ where $\mathbf{f} : C([-\tau, 0], \mathbb{R}^2_+) \to \mathbb{R}^2_+$ is a C^k-function ($k \geq 2$). Define two matrices $M_1, M_2 \in M_{2\times 2}$, respectively, as

$$M_1 = \left[\frac{\partial \mathbf{f}}{\partial \mathbf{u}(t)}\right]_{\mathbf{u}=(0,0)} = \begin{bmatrix} 2(\gamma - a)\bar{S} + \gamma\bar{N} - \gamma c & \gamma\bar{S} + \kappa\bar{N} \\ -i\bar{N} & -i\bar{S} - (2i+\kappa)\bar{N} + ic \end{bmatrix}$$

and

$$M_2 = \left[\frac{\partial \mathbf{f}}{\partial \mathbf{u}(t-\tau)}\right]_{\mathbf{u}=(0,0)} = \begin{bmatrix} 0 & \kappa\bar{N} \\ 0 & -\kappa\bar{N} \end{bmatrix}.$$

Linearizing system (3.1) at the point $(\overline{S}, \overline{N})$ yields

$$\frac{\mathrm{d}\mathbf{u}(t)}{\mathrm{d}t} = M_1\mathbf{u}(t) + M_2\mathbf{u}(t-\tau). \tag{3.2}$$

Stability of the trivial equilibrium, E_0, is determined by using the linearization (3.2) of system (3.1). The complete proof of Theorem 3.3 dealing with the stability of E_0 is given in Appendix B.

Theorem 3.3. *The trivial equilibrium* $E_0 := (0, 0)$ *of system* (3.1) *is an unstable saddle point for all* $\tau \geq 0$.

Linearizing system (3.1) at the interior equilibrium $E_* := (S_*, N_*)$ we get the associated transcendental characteristic equation by using the second equation in (A.1):

$$\det \begin{bmatrix} \lambda + (2a - \gamma)S_* + \dfrac{\gamma\kappa}{i}N_* & -\gamma S_* - \kappa N_* - \kappa N_* e^{-\lambda\tau} \\ iN_* & \lambda + iN_* + \kappa N_* e^{-\lambda\tau} \end{bmatrix} = 0,$$

which is equivalent to

$$\lambda^2 + A\lambda + B + (A_1\lambda + B_1)e^{-\lambda\tau} = 0 \tag{3.3}$$

with

$$A = (2a - \gamma)S_* + \left(\frac{\kappa\gamma}{i} + i\right) N_*,$$

$$B = 2aiS_*N_* + \kappa(i + \gamma)N_*^2,$$

$$A_1 = \kappa N_*,$$

$$B_1 = \kappa \left[(2a - \gamma)S_*N_* + \left(i + \frac{\gamma\kappa}{i}\right) N_*^2\right].$$

Using the characteristic equation (3.3), the local stability analysis of the interior equilibrium in system (3.1) (with delay) is carried out and presented in Sec. 5. Before pursuing the study of the system with delay ($\tau > 0$), the asymptotic behavior of the system without delay ($\tau = 0$) associated to system (3.1) is investigated in Sec. 4.

4. Mathematical Analysis in the Absence of Delay

In this section, it is assumed that disassembly and its regulation are simultaneous ($\tau = 0$). If $\tau = 0$, system (3.1) simplifies to an autonomous system of ordinary differential equations:

$$\frac{\mathrm{d}S}{\mathrm{d}t} = -aS^2 - \gamma S(c - S - N) + \kappa N^2, \tag{4.1a}$$

$$\frac{\mathrm{d}N}{\mathrm{d}t} = iN(c - S - N) - \kappa N^2. \tag{4.1b}$$

Since the time delay does not change the number and the distribution of equilibria, the conclusions in Theorem 3.2 are valid for system (4.1). Moreover, the conclusions of Theorem 3.3 about the trivial equilibrium E_0 are also valid for system (4.1). The characteristic equation of system (4.1) at the interior equilibrium E_* is derived from (3.3) and the local stability of E_* is then characterized. These results yield Theorem 4.1.

Theorem 4.1. (i) *The trivial equilibrium* $E_0 := (0, 0)$ *of system* (4.1) *is an unstable saddle point.*

(ii) *The unique interior equilibrium* $E_* := (S_*, N_*)$ *of system* (4.1) *is locally asymptotically stable.*

Proof. Theorem 3.3 establishes that the trivial equilibrium $E_0 := (0, 0)$ of system (4.1) is an unstable saddle point. From (3.3), the characteristic equation for system (4.1) at $E_* = (S_*, N_*)$ is derived as

$$\lambda^2 + (A + A_1)\lambda + (B + B_1) = 0, \tag{4.2}$$

where

$$\begin{aligned} A + A_1 &= (2a - \gamma)S_* + \left(\frac{\kappa\gamma}{i} + i + \kappa\right) N_* \\ &= iN_* - \gamma S_* + 2aS_* + \left(\frac{\kappa\gamma}{i} + \kappa\right) N_*, \end{aligned}$$

$$B + B_1 = [2ai + \kappa(2a - \gamma)]S_*N_* + \kappa\left(2i + \gamma + \frac{\kappa\gamma}{i}\right)N_*^2$$
$$= \kappa N_*(iN_* - \gamma S_*) + 2a(i + \kappa)S_*N_* + \kappa\left(i + \gamma + \frac{\kappa\gamma}{i}\right)N_*^2.$$

We derive from (A.1) that $aS_*^2 = \frac{\kappa N_*}{i}(iN_* - \gamma S_*)$, which implies $iN_* - \gamma S_* > 0$. Thus, we can infer

$$A + A_1 > 0, \quad B + B_1 > 0.$$

As a result of the Routh–Hurwitz criterion [6], all roots of the characteristic equation (4.2) at E_* have negative real parts. The interior equilibrium $E_* := (S_*, N_*)$ is then locally asymptotically stable. □

To establish the global stability of E_* the following lemma is needed.

Lemma 4.2. *System* (4.1) *does not possess any nontrivial periodic orbits in the closed set* Γ.

Proof. Methods developed by Li [22] for higher (than two)-dimensional autonomous systems are used to prove this lemma. When there is no time delay, system (2.1) reduces to:

$$\frac{\mathrm{d}S}{\mathrm{d}t} = -aS^2 - \gamma SP + \kappa N^2 =: g_1(S, P, N), \tag{4.3a}$$

$$\frac{\mathrm{d}P}{\mathrm{d}t} = aS^2 + \gamma SP - iPN =: g_2(S, P, N), \tag{4.3b}$$

$$\frac{\mathrm{d}N}{\mathrm{d}t} = iPN - \kappa N^2 =: g_3(S, P, N). \tag{4.3c}$$

From Theorem 3.2, system (4.3) has a boundary equilibrium $\bar{E}_0 := (0, c, 0)$ and a unique interior equilibrium $\bar{E}_* := (c - (1 + \frac{\kappa}{i})N_*, \frac{\kappa}{i}N_*, N_*)$.

Denote the vector field of system (4.3) as $\mathbf{g}$. Define $\Theta := \{(S, P, N) \in \mathbb{R}^3_+ \mid S + P + N \equiv c\}$. The set Θ is closed, simply connected and by direct application of Lemma 3.1, positively invariant with respect to system (4.3). Note that for system (4.3) on Θ, one can see from Theorem 4.1 that the boundary equilibrium $(0, c, 0)$ is unstable and expels nearby orbits. Also, except for orbits starting on the invariant manifold $\{(S(t), P(t), 0) \mid S(t) + P(t) \equiv c\}$, the vector field $\mathbf{g}$ traverses strictly inwards along the boundary of Θ due to its property of positive invariance. Solutions to system (4.3) are uniformly bounded on Θ. These characteristics imply that system (4.3) is uniformly persistent in Θ, and thus there is a compact absorbing set $\Upsilon \subset \Theta$.

The divergence of (4.3) is calculated as

$$\nabla \cdot \mathbf{g} = -2aS - \gamma P + \gamma S - iN + iP - 2\kappa N. \tag{4.4}$$

Transforming (4.3b) and (4.3c) respectively yields:

$$\gamma S - iN = \frac{P'}{P} - \frac{aS^2}{P}, \tag{4.5a}$$

$$iP - \kappa N = \frac{N'}{N}, \tag{4.5b}$$

where the prime "′" stands for the derivative with respect to time t. From Eqs. (4.5), the divergence of system (4.3) becomes

$$\nabla \cdot \mathbf{g} = -2aS - \gamma P - \frac{aS^2}{P} - \kappa N + \frac{P'}{P} + \frac{N'}{N}. \tag{4.6}$$

Use similar notation as in Li [22]: $n, r, \nu, Q(\mathbf{x})$ and $I_{M\times M}$, where $\mathbf{x} := (S, P, N)$, $M = \binom{n}{r+2}$ and $Q(\mathbf{x})$ is a non-singular $\binom{n}{r+2} \times \binom{n}{r+2}$ matrix-valued function. For $n = 3, r = 1, \nu = 0$, then $M = 1$. Thus $I_{1\times 1} = 1$, $Q(\mathbf{x})$ is scalar-valued and

$$\begin{aligned} Q_{\mathbf{g}}Q^{-1} + Q\frac{\partial \mathbf{g}^{[r+2]}}{\partial \mathbf{x}}Q^{-1} - r\nu I_{M\times M} &= Q_{\mathbf{g}}Q^{-1} + Q\frac{\partial \mathbf{g}^{[3]}}{\partial \mathbf{x}}Q^{-1} \\ &= \nabla \cdot \mathbf{g}, \end{aligned}$$

where $Q_{\mathbf{g}}$ is obtained by replacing each entry of Q by its derivative in the direction of $\mathbf{g}$, and $\partial \mathbf{g}^{[r+2]}/\partial \mathbf{x}$ is the $(r+2)$th-additive compound matrix of the Jacobian matrix $\partial \mathbf{g}/\partial \mathbf{x}$.

Let $(S(t), P(t), N(t))$ be any solution starting in the compact absorbing set $\Upsilon \subset \Theta$. Along $(S(t), P(t), N(t))$, we have

$$\begin{aligned} \frac{1}{t}\int_0^t \nabla \cdot \mathbf{g}\mathrm{d}t &= -\frac{1}{t}\int_0^t \left[2aS + \gamma P + \frac{aS^2}{P} + \kappa N\right]\mathrm{d}t + \frac{1}{t}\int_0^t \left[\frac{P'}{P} + \frac{N'}{N}\right]\mathrm{d}t \\ &= -\frac{1}{t}\int_0^t \left[2aS + \gamma P + \frac{aS^2}{P} + \kappa N\right]\mathrm{d}t + \frac{1}{t}\left[\ln\frac{P(t)}{P(0)} + \ln\frac{N(t)}{N(0)}\right] \\ &\le -\bar{c} < 0, \end{aligned}$$

for all sufficiently large t, where the positive $\bar{c}$ may depend on the uniformly persistent constant but is independent of the initial conditions. The conditions of [22, Theorem 2.3] are then satisfied and the proof is complete. □

Theorem 4.1(ii) and Lemma 4.2 along with the generalized Poincaré–Bendixson theorem (see e.g. [26, Theorem 2, p. 245]) guarantee the following result.

Theorem 4.3. *The unique interior equilibrium $E_* := (S_*, N_*)$ of system* (4.1) *is globally asymptotically stable in* Γ *except that orbits starting on S-axis converge to the trivial equilibrium E_0.*

Theorem 4.3 completely determines the global dynamics of system (4.1) in Γ. All solutions starting with some material assembled in network ($N(0) > 0$) will converge to the interior equilibrium E_* defined in Theorem 3.2. It is safe to assume that at the start of the experiment, there exists some intermediate filament material

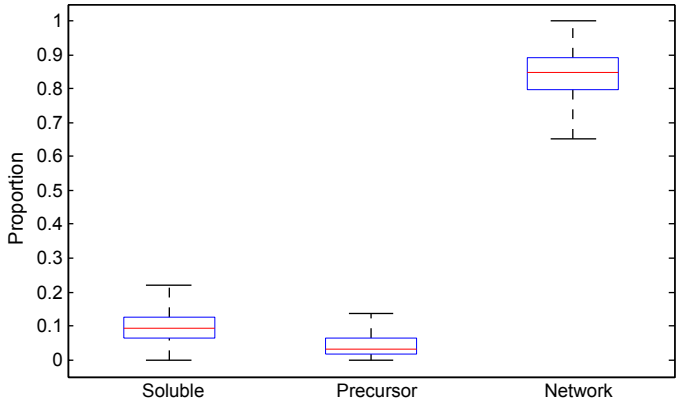

Fig. 2. Possible values of equilibrium proportions, $(S_*/c, P_*/c, N_*/c)$, for varying parameters. All parameters are made to vary in the following ranges: $a, \gamma \in [10^{-1}\,\mu\text{M}^{-1}\cdot s^{-1}, 10^{2}\,\mu\text{M}^{-1}\cdot s^{-1}]$, $i \in [10^{-3}\,\mu\text{M}^{-1}\cdot s^{-1}, 50\,\mu\text{M}^{-1}\cdot s^{-1}]$, $\kappa \in [5\times 10^{-5}\,\mu\text{M}^{-1}\cdot s^{-1}, 2\,\mu\text{M}^{-1}\cdot s^{-1}]$ and $c \in [50\,\mu\text{M}, 10^{3}\,\mu\text{M}]$. Ranges of variation of parameters are chosen according to previous works (Table 1). Points in the parameter set are sampled using Latin Hypercube Sampling with uniform distributions. The red bar indicates the median, the box shows the interquartile range and the whiskers frame the extent of values without considering outliers (not represented here).

assembled in network in the cell $(N(0) > 0)$. Indeed, even some mitotic cells that disassemble a large part of their intermediate filament network before entering into division and forming new daughter cells do not disassemble completely their networks [2, 15].

Biologically, it is important to understand what type of organization of the intermediate filaments is implied by the interior equilibrium. Components of the interior equilibrium depend on the five parameters of the model without delay. Only ranges of some parameter values, given in Table 1, can be estimated from previous *in vitro* and *in vivo* works [7, 16, 27]. For instance, values of κ in cells are unknown. Hence, to understand the effect of parameters on the intermediate filament organization, possible values of the interior equilibrium are computed by using Latin Hypercube Sampling on the parameter set. In order to interpret results, a non-dimensional version of E_*, $(S_*/c, N_*/c)$, is used. Figure 2 shows the ranges of equilibrium proportions obtained for 5×10^6 sampled parameter points, when all five parameters are made to vary and their ranges of variation are defined following previous studies; see Table 1. Note that with the proposed mechanism of disassembly regulation, the intermediate filament material is mainly assembled in networks; the median value of N_*/c is about 85%, which is in agreement with biological observations [1, 34].

5. Mathematical Analysis in the Presence of Delay

In this section, the study of the dynamic behavior of system (3.1) with delay $(\tau > 0)$ is continued. Recall that from Theorem 3.3, the trivial equilibrium E_0 of system (3.1) is an unstable saddle point. The local asymptotic stability of

the unique interior equilibrium $E_* := (S_*, N_*)$ of system (3.1) for $\tau > 0$ is now considered.

Theorem 5.1. *Assume* $\kappa \geq \frac{i(i+\gamma)}{\gamma}$. *The interior equilibrium* $E_* := (S_*, N_*)$ *of system* (3.1) *is locally asymptotically stable for* $0 < \tau < \tau_*^{(0)}$. *At* $\tau = \tau_*^{(j)}$ $(j = 0, 1, 2, \ldots)$, *the characteristic equation* (3.3) *at* $E_* := (S_*, N_*)$ *has a pair of purely imaginary eigenvalues* $\pm i\omega_*$. *The interior equilibrium* $E_* := (S_*, N_*)$ *of system* (3.1) *becomes unstable for* $\tau > \tau_*^{(0)}$, *where*:

$$\tau_*^{(j)} = \frac{1}{\omega_*}\left[\arccos\left(\frac{(B_1 - AA_1)\omega_*^2 - BB_1}{A_1^2\omega_*^2 + B_1^2}\right) + 2j\pi\right], \quad j = 0, 1, 2, \ldots,$$

$$\omega_* = \left(\frac{-p + \sqrt{p^2 - 4q}}{2}\right)^{\frac{1}{2}},$$

$$p = \left[(2a - \gamma)S_* + \left(\frac{\kappa\gamma}{i} + i + \kappa\right)N_*\right] \times \left[(2a - \gamma)S_* + \left(\frac{\kappa\gamma}{i} + i - \kappa\right)N_*\right] - 4aiS_*N_* - 2\kappa(i + \gamma)N_*^2,$$

$$q = \left\{[2ai + \kappa(2a - \gamma)]S_*N_* + \kappa\left[2i + \gamma + \frac{\gamma\kappa}{i}\right]N_*^2\right\} \times \left\{\left[2a(i - \kappa) + \frac{\kappa\gamma[i(i + \gamma) - \kappa\gamma]}{i^2}\right]S_*N_* + a\gamma\left(1 - \frac{\kappa}{i}\right)S_*^2\right\}.$$

Proof. The complex roots of the characteristic equation (3.3) must be in the form of conjugate pairs if they exist. Note that all roots of (3.3) have negative real parts when $\tau = 0$. The root of (3.3) is continuous with respect to the parameter τ, so all roots of (3.3) have negative real parts for very small τ, i.e. $0 < \tau \ll 1$. Suppose that there exists a positive τ_* such that (3.3) has a pair of purely imaginary roots $\lambda = \pm i\omega$, where $\omega > 0$. Substituting $\lambda = \pm i\omega$ into (3.3) and separating the real and imaginary parts yields the pairs of equations

$$\begin{cases} -B_1 \sin\omega\tau + A_1\omega\cos\omega\tau = -A\omega, \\ A_1\omega\sin\omega\tau + B_1\cos\omega\tau = -B + \omega^2. \end{cases} \tag{5.1}$$

Squaring both sides of each equation and summing the results yield

$$\omega^4 + (A^2 - 2B - A_1^2)\omega^2 + B^2 - B_1^2 = 0. \tag{5.2}$$

Define $\mu = \omega^2$ and let $p = A^2 - 2B - A_1^2$ and $q = B^2 - B_1^2$. Equation (5.2) can be written in terms of μ, p and q as

$$\mu^2 + p\mu + q = 0, \tag{5.3}$$

where

$$p = \left[(2a - \gamma)S_* + \left(\frac{\kappa\gamma}{i} + i + \kappa\right)N_*\right] \times \left[(2a - \gamma)S_* + \left(\frac{\kappa\gamma}{i} + i - \kappa\right)N_*\right] - 4aiS_*N_* - 2\kappa(i + \gamma)N_*^2,$$

$$q = \left\{ \underbrace{[2ai + \kappa(2a - \gamma)]S_*N_* + \kappa\left[2i + \gamma + \frac{\gamma\kappa}{i}\right]N_*^2}_{B+B_1} \right\}$$

$$\times \left\{ \underbrace{\left[2a(i-\kappa) + \frac{\kappa\gamma[i(i+\gamma) - \kappa\gamma]}{i^2}\right]S_*N_* + a\gamma\left(1 - \frac{\kappa}{i}\right)S_*^2}_{B-B_1} \right\}.$$

Keep in mind that $B+B_1$ is always positive (see the proof of Theorem 4.1). We then infer that $q < 0$ if

$$\kappa \geq \frac{i(i+\gamma)}{\gamma},$$

which makes $B - B_1 < 0$. Thus, (5.3) has a unique positive root, denoted by μ_*. As a result, there are critical values for delay $\tau_*^{(j)}$ corresponding to $\omega_* = \sqrt{\mu_*}$ which solve both equations in (5.1):

$$\tau_*^{(j)} = \frac{1}{\omega_*}\left[\arccos\left(\frac{(B_1 - AA_1)\omega_*^2 - BB_1}{A_1^2\omega_*^2 + B_1^2}\right) + 2j\pi\right], \quad j = 0, 1, 2, \ldots.$$

Note that all roots of (3.3) have negative real parts for $\tau = 0$ and 0 is not the root of (3.3) for all $\tau > 0$. By Butler's lemma [9], $E_* := (S_*, N_*)$ remains stable for $0 < \tau < \tau_*^{(0)}$; $E_* := (S_*, N_*)$ becomes unstable for $\tau > \tau_*^{(0)}$ [8, Proposition 1]. This completes the proof. □

Under the condition $\kappa \geq \frac{i(i+\gamma)}{\gamma}$, we find a sequence of critical delays $\tau_*^{(j)}$ and the point $\lambda = i\omega_*$ (or $\lambda = -i\omega_*$) at which a root of the characteristic equation (3.3) hits the imaginary axis. It is now necessary to check whether or not the root continues into the positive half-plane as τ increases past $\tau_*^{(j)}$.

Theorem 5.2. *Assume $\kappa \geq \frac{i(i+\gamma)}{\gamma}$. System* (3.1) *undergoes a Hopf bifurcation at $\tau = \tau_*^{(j)}$; a periodic orbit appears in a small neighborhood of the interior equilibrium $E_* := (S_*, N_*)$.*

Proof. Suppose $\lambda(\tau) = \alpha(\tau) + i\omega(\tau)$ is a root of (3.3) as $\tau \geq \tau_*^{(0)}$, with $\alpha\big(\tau_*^{(j)}\big) = 0$, $\omega\big(\tau_*^{(j)}\big) = \omega_*$. We now determine the direction of motion of λ as τ increases. Substituting $\lambda(\tau)$ into (3.3) and taking the derivative with respect to τ yields

$$\frac{\mathrm{d}\lambda}{\mathrm{d}\tau} = \frac{\lambda(A_1\lambda + B_1)e^{-\lambda\tau}}{2\lambda + A + [A_1 - \tau(A_1\lambda + B_1)]e^{-\lambda\tau}}.$$

Then

$$\left[\frac{\mathrm{d}\,\mathrm{Re}\,\lambda}{\mathrm{d}\tau}\right]^{-1}_{\tau=\tau_*^{(j)}} = \mathrm{Re}\left[\frac{(2\lambda + A)e^{\lambda\tau} + A_1}{\lambda(A_1\lambda + B_1)}\right]_{\tau=\tau_*^{(j)}}$$

$$
\begin{aligned}
&-A\omega_*\left(-B_1\sin\omega_*\tau_*^{(j)}+A_1\omega_*\cos\omega_*\tau_*^{(j)}\right)\\
&+2\omega_*^2\left(A_1\omega_*\sin\omega_*\tau_*^{(j)}+B_1\cos\omega_*\tau_*^{(j)}\right)-A_1^2\omega_*^2\\
=&\qquad\qquad A_1^2\omega_*^4+B_1^2\omega_*^2\\
=&\frac{\omega_*^2(2\omega_*^2+p)}{A_1^2\omega_*^4+B_1^2\omega_*^2}>0,
\end{aligned}
$$

since $2\omega_*^2+p=2\cdot\frac{-p+\sqrt{p^2-4q}}{2}+p=\sqrt{p^2-4q}>0$. This implies that the eigenvalue crosses the imaginary axis from left to right transversally. Thus, the transversality condition holds [11] and a Hopf bifurcation occurs for system (3.1) at $\tau=\tau_*^{(j)}$. For $\tau_*^{(j)}<\tau<\tau_*^{(j+1)}$, (3.3) has $2(j+1)$ roots with positive real parts. The proof is complete. □

The onset of oscillatory solutions can be explained by considering (2.1c):

$$\frac{dN}{dt}=(iP(t)-\kappa N(t-\tau))N(t).$$

As the activation time τ increases, the values of $N(t)$ and $N(t-\tau)$ become increasingly "uncorrelated" and an imbalance between the rate of disassembly $\kappa N(t-\tau)$ and the actual amount of assembled material $N(t)$ can be created. Because of this, the down-regulation that should occur as $N(t)$ becomes large is delayed. Assembly is still the dominating process, inducing further reinforcement of $N(t)$. After some time, the value of $N(t-\tau)$ becomes larger and disassembly kicks in, overpowering assembly. Because the value of κ is large, there follows a sharp decrease of the value of $N(t)$. Here, disassembly should stop and assembly should take over. Again, the "uncorrelation" between $N(t)$ and $N(t-\tau)$ disturbs the regulation process, $N(t)$ becomes small. Eventually, the solution is driven to and attracted in the direction of the stable manifold of the saddle equilibrium and then repulsed along the unstable manifold of the saddle point. This process repeats and finally, the solution approaches a limit cycle.

In Fig. 3, the results of Theorems 5.1 and 5.2 are illustrated: when the disassembly rate is larger than the assembly rates (oligomerization in precursors and precursor integration to network), activation times exist that destabilize the intermediate filament organization. The intermediate filament material oscillates between organizations in networks and in nonfilamentous particles (filament precursors). Characterization of the direction of the Hopf bifurcation and stability of bifurcating periodic orbits from the interior equilibrium E_* of system (3.1) is left for future work.

Finally, in the last result, conditions on parameter values are found that guarantee the local asymptotic stability of the unique interior equilibrium $E_*:=(S_*,N_*)$ for system (3.1) regardless of the time delay.

Theorem 5.3. *Assume* $\gamma<2a, i^2/(2a-\gamma)\le\kappa\le i/(1+\sqrt{2})$. *The interior equilibrium* E_* *of system* (3.1) *is locally asymptotically stable for* $\tau>0$.

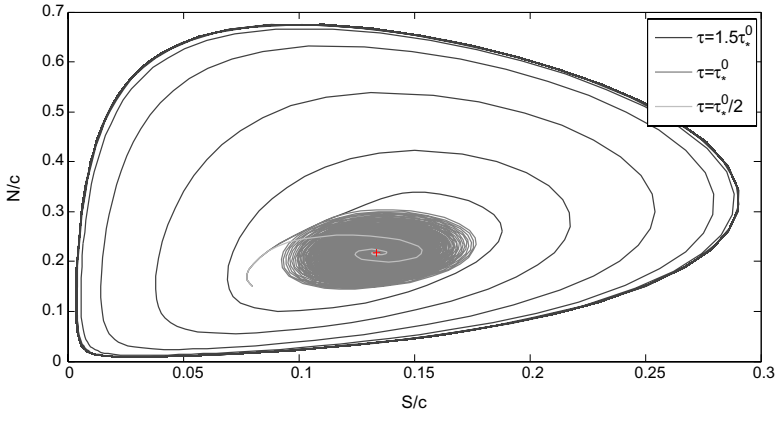

Fig. 3. Trajectories in the $(S/c - N/c)$-plane of system (3.1) under conditions of Theorems 5.1 and 5.2. Parameter values used are $a = 0.3$, $\gamma = 0.1$, $c = 100$, $\kappa = 0.3$, $i = 0.1$, satisfying $\kappa \geq i(i+\gamma)/\gamma$. Calculations give $S_* = 13.38$, $N_* = 21.65$, $A = 15.3532$, $A_1 = 6.4963$, $B = 45.5223$, $B_1 = 99.7387$, $\omega_* = 7.1579$ and $\tau_*^0 = 0.2731$. When $\tau = \tau_*^0/2$, the interior equilibrium $E_* = (S_*, N_*)$ is locally asymptotically stable. When $\tau = \tau_*^0$, the interior equilibrium E_* still remains locally asymptotically stable. When $\tau = 1.5\tau_*^0$, the interior equilibrium E_* becomes unstable and a periodic solution bifurcates from E_*.

Proof. Recall (5.3). It is easy to verify that the condition $\kappa \leq i$ guarantees $B - B_1 > 0$; so $q > 0$. Coefficient p is rewritten as follows:

$$p = (2a-\gamma)^2 S_*^2 + 2\gamma\left[(2a-\gamma)\frac{\kappa}{i} - i\right] S_* N_* + \left\{\frac{\kappa^2\gamma^2}{i^2} + [i + (\sqrt{2}-1)\kappa][i - (\sqrt{2}+1)\kappa]\right\} N_*^2.$$

In p, the coefficient of S_*^2 is always non-negative; $i^2/(2a-\gamma) \leq \kappa$ with $2a - \gamma > 0$ and $\kappa \leq i/(1+\sqrt{2})$ guarantee the non-negativity and positivity of the coefficients of $S_* N_*$ and N_*^2, respectively, which imply that $p > 0$.

The conditions, $\gamma < 2a$ and $i^2/(2a-\gamma) \leq \kappa \leq i/(1+\sqrt{2})$, guarantee the positivity of p and q. Thus, (5.3) has no positive root. Subsequently, τ_*^j $(j = 0, 1, 2, \ldots)$ does not exist. By Theorem 4.3, all roots of (5.3) have negative real parts for $\tau > 0$. The proof is complete. □

The local asymptotic stability of the unique interior equilibrium $E_* := (S_*, N_*)$ for system (3.1) holds regardless of the time delay provided parameters satisfy the relations in Theorem 5.3. Results of Theorem 5.3 are illustrated in Fig. 4: using parameter values estimated from previous studies (Table 1) and satisfying the conditions in Theorem 5.3, it is found that the intermediate filament material is mainly organized in networks for any disassembly activation times (about 80% of the intermediate filament proteins are assembled in networks, 20% form nonfilamentous particles).

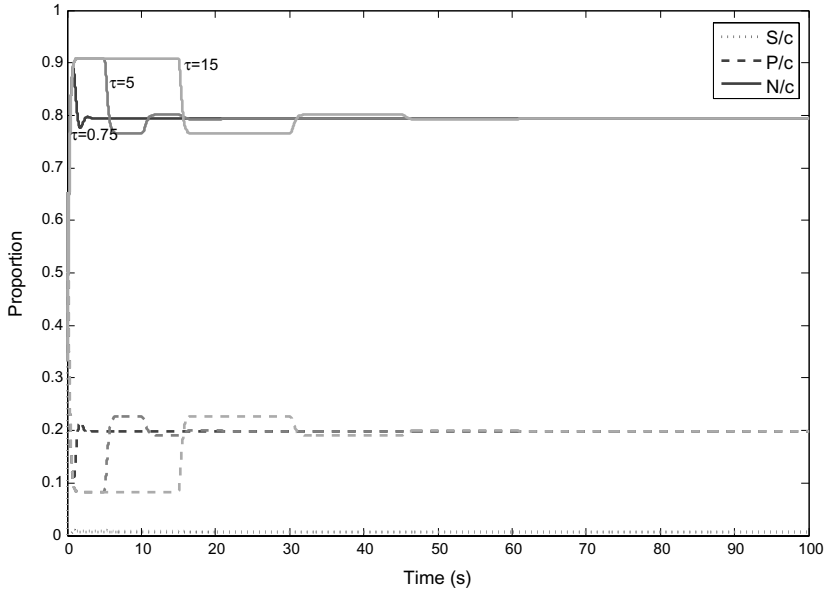

Fig. 4. Solutions $(S(t)/c, P(t)/c, N(t)/c)$ of system (3.1) and $P(t) = c - S(t) - N(t)$ under conditions of Theorem 5.3, $\gamma < 2a, i^2/(2a-\gamma) \leq \kappa \leq i/(1+\sqrt{2})$. Parameter values belonging to the ranges given in Table 1 are used: $a = 35\,\mu\mathrm{M}^{-1}\cdot\mathrm{s}^{-1}, \gamma = 20\,\mu\mathrm{M}^{-1}\cdot\mathrm{s}^{-1}, c = 300\,\mu\mathrm{M}, i = 0.2\,\mu\mathrm{M}^{-1}\cdot\mathrm{s}^{-1}, \kappa = 0.05\,\mu\mathrm{M}^{-1}\cdot\mathrm{s}^{-1}$. The interior equilibrium E_* is asymptotically stable for all three different values of τ (in black $\tau = 0.75$s, in dark gray $\tau = 5$s and in light gray $\tau = 15$s).

The dynamics of system (3.1) with delay is determined by conditions on parameters. The conditions in Theorem 5.3 that lead to a stable organization prevent the disassembly rate κ from being too large in comparison to the integration rate i. As experimentally observed, assembly is then a more favorable reaction than disassembly. Moreover, concerning assembly processes, the conditions $\gamma < 2a$ and $i(1+\sqrt{2}) \leq 2a - \gamma$ in Theorem 5.3 satisfy the biologically plausible condition $i < \gamma \leq a$ [16, 27]. Hence, conditions of Theorem 5.3 are biologically realistic and satisfied by values in Table 1. Therefore, from now on, such values will be used to define the "standard situation".

On the other hand, the condition in Theorems 5.1 and 5.2 that leads to a destabilization of the organization requires the disassembly rate to be larger than the assembly rates. The condition in Theorems 5.1 and 5.2 will be referred to as the "atypical situation".

Results on the dynamics of the system (2.1) considered with delay are summarized in Fig. 5. Thus, delay is harmless in the standard situation: the material remains assembled mainly in networks (see "Delay" part of the left column in Fig. 5), which is in agreement with experimental observations [1, 34]. In the atypical situation, there exist some activation times that will cause the material to oscillate between an organization in nonfilamentous particles and in networks (see "Delay" part of the right column in Fig. 5).

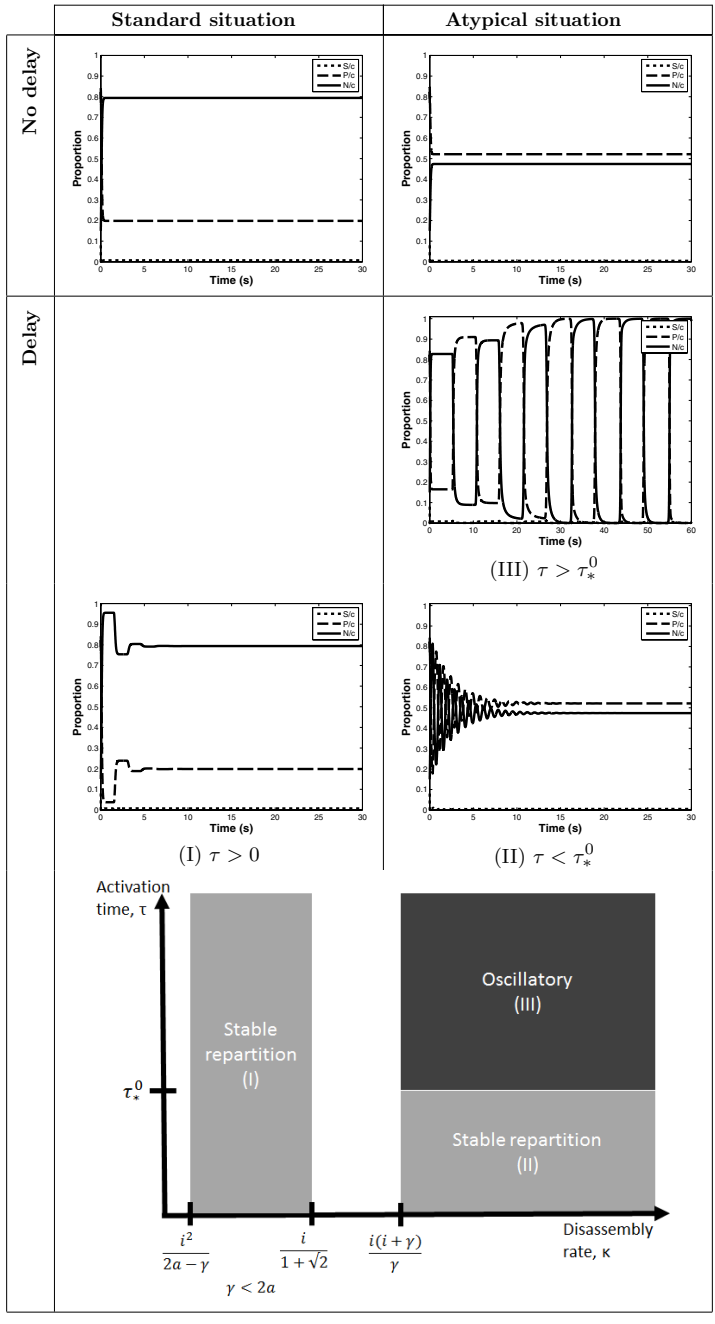

Fig. 5. Summary of the dynamics of system (2.1) considered without and with delay. Parameter values used for simulations belong to ranges given in Table 1: $a = 35\,\mu\text{M}^{-1}\cdot\,\text{s}^{-1}$, $\gamma = 20\,\mu\text{M}^{-1}\cdot\,\text{s}^{-1}$, $i = 0.2\,\mu\text{M}^{-1}\cdot\,\text{s}^{-1}$, $c = 100\,\mu\text{M}$. For the standard situation (respectively, atypical situation), the rate of disassembly used is $\kappa = 0.05\,\mu\text{M}^{-1}\cdot\,\text{s}^{-1}$ (respectively, $\kappa = 0.22\,\mu\text{M}^{-1}\cdot\,\text{s}^{-1}$) satisfying the condition of Theorem 5.3 (respectively, Theorems 5.1 and 5.2).

6. Discussion and Conclusion

This paper is focused on the regulation of the intermediate filament disassembly process in cells. Previous studies of keratin networks in epithelial cells [21, 30, 34] suggest a localization of the disassembly in the perinuclear region, which is characterized by an abundance of intermediate filament material. Based on these observations, a mechanism for the regulation of filament disassembly is proposed, namely, that the amount of proteins assembled in filaments regulates the disassembly process. Furthermore, since filament disassembly is triggered by kinase activity, the effect of the regulation time is also considered. Thus, the rate of disassembly is expressed as $\kappa N(t-\tau)$. This rate implicitly represents the activity of kinases. This begs the question: how can the kinase activity be regulated by the amount of assembled intermediate filament proteins? Experimental evidence shows that kinases bind/associate with intermediate filaments; intermediate filament networks and kinases co-localize [15]. The more filaments there are in the network, the more kinases there are. Moreover, as the network becomes more dense, filaments get closer, more packed and bundled; the proximity between filaments might favor the cross-activation or autophosphorylation of kinases, resulting in increased kinase activity. Furthermore, the absence of delay in disassembly regulation might represent the baseline activity of some intermediate filament protein kinases in interphasic cells, whereas a delay might describe the activity of specific kinases, which are activated in response to stresses such as JNK and p38 [14, 19] or for mitosis [2]. Hence, the delay corresponds to the activation time of some signaling pathways regulating kinases responsible for the intermediate filament disassembly.

The dynamics of system (2.1) in absence of delay is completely characterized by Theorem 4.3. It is found that the system has always a unique globally asymptotically stable interior equilibrium E_* (top of Fig. 5). On the other hand, the dynamics of system (2.1) in presence of delay is not yet fully mathematically characterized. In particular, the behavior has not been established for some values of κ. The present results show that the dynamics of the system depends on conditions on parameters (see Theorems 5.1–5.3). It is found that the value of the disassembly rate κ is the main factor determining the stability of the unique interior equilibrium E_*. Interestingly, it is found that with biologically realistic parameter values requiring, *inter alia*, κ to be small in comparison to i (standard situation defined from Theorem 5.3), no regulation time can destabilize the stable repartition of intermediate filament material. The interior equilibrium is locally asymptotically stable regardless of the time delay (Figs. 4 and 5). However, when the rate of disassembly κ is large enough in comparison to the assembly rates (atypical situation defined from Theorems 5.1 and 5.2), there exist disassembly activation times that can destabilize the intermediate filament material repartition; periodic solutions can arise from the interior equilibrium by Hopf bifurcation at critical periods (Figs. 3 and 5).

It appears that the value of κ does not only determine the stability of E_* but also its value. Numerical investigations of the values of E_* as function of κ were

carried out (results not shown). As the disassembly rate increases, the proportion of material organized in networks decreases in favor of nonfilamentous particles. This observation holds both in absence and presence of delay. Similarly to previous mathematical work [28], it is found that disassembly is the main determinant of intermediate filament dynamics in cells; high disassembly rates favor nonfilamentous particle formation.

The atypical situation is characterized by larger rates of disassembly than in the standard situation. To the best of our knowledge, values of the disassembly rate for intermediate filaments in cells are not known. Large disassembly rates could be interpreted as the existence of a fragility of filaments or an up-regulation of kinases. Fragility of filaments could result from mutations on intermediate filament genes, which are linked, for instance, to skin fragility diseases (see, e.g. epidermolysis bullosa simplex [5, 12]). In cells with mutant intermediate filaments, it is observed that the action of mechanical stimuli triggers the re-organization of the intermediate filament material in granules (nonfilamentous particles) instead of in networks [12]. Furthermore, it has been shown that the up-regulation of the stress-activated kinase p38 induces re-organizations of the keratin material and promotes the granule formation in epithelial cells [36].

In summary, in the standard situation, delay in disassembly regulation is harmless for intermediate filament dynamics; the model predicts that the intermediate filament proteins are mainly assembled in networks. This conclusion is in agreement with experimental observations [1, 34]. In the atypical situation and for long activation times, the model predicts that cells exhibit a steady and continuous cycling of re-organizations of the intermediate filament material switching between an organization in networks and in particles. With the ranges of parameters considered, only very short oscillatory periods have been found. This behavior seems to be biologically unrealistic or has not been observed yet. This suggests that either those parameter values are unrealistic or the proposed mechanism for disassembly regulation needs to be refined. Further mathematical work needs to be done to completely characterize the dynamics of the system in presence of delay. Biological experiments will need to be conducted to check if the disassembly of filaments in cells is actually regulated by a negative feedback. To confirm that disassembly is regulated by the amount of proteins assembled in filaments, experimental data will have to show that the disassembly rate varies according to the amount of intermediate filament proteins assembled in networks in cells. The spatial distribution of the level of phosphorylation of intermediate filament networks could also be used to investigate the relevance of the hypothesized regulation mechanism.

Acknowledgments

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Appendix A. Proof of Theorem 3.2

Proof of Theorem 3.2. It is easy to verify that system (3.1) always has the trivial equilibrium $E_0 := (0, 0)$; it has no boundary equilibrium such as $(S, 0)$ or $(0, N)$.

Suppose that $E_* := (S_*, N_*)$ is an interior equilibrium of system (3.1), which can be obtained by setting $\frac{\mathrm{d}S_*}{\mathrm{d}t} = 0 = \frac{\mathrm{d}N_*}{\mathrm{d}t}$. This leads to

$$\begin{cases} -aS_*^2 - \gamma S_*(c - S_* - N_*) + \kappa N_*^2 = 0, \\ i(c - S_* - N_*) - \kappa N_* = 0. \end{cases} \tag{A.1}$$

From the second equation of (A.1), we have

$$S_* = c - \left(1 + \frac{\kappa}{i}\right) N_*. \tag{A.2}$$

Substituting (A.2) into the first equation of (A.1) yields

$$D_2 N_*^2 + D_1 N_* + D_0 = 0, \tag{A.3}$$

where

$$\begin{aligned} D_0 &= -ac^2 i^2, \\ D_1 &= 2aci(i + \kappa) - ci\kappa\gamma, \\ D_2 &= -a(i + \kappa)^2 + \kappa\gamma(i + \kappa) + i^2\kappa. \end{aligned}$$

(i) If $i + \kappa = \frac{\kappa\gamma + \sqrt{\kappa^2\gamma^2 + 4ai^2\kappa}}{2a}$, then $D_1 > 0$, $D_2 = 0$ and (A.3) has only one positive root, $N_* = \frac{aci}{\sqrt{\kappa^2\gamma^2 + 4ai^2\kappa}}$. From (A.2), $S_* = c - (1 + \frac{\kappa}{i}) \cdot \frac{aci}{\sqrt{\kappa^2\gamma^2 + 4ai^2\kappa}}$; it is easy to verify that S_* is positive. Thus, system (3.1) has a unique interior equilibrium E_* when $i + \kappa = \frac{\kappa\gamma + \sqrt{\kappa^2\gamma^2 + 4ai^2\kappa}}{2a}$.

(ii) If $i + \kappa < \frac{\kappa\gamma + \sqrt{\kappa^2\gamma^2 + 4ai^2\kappa}}{2a}$, then $D_2 > 0$ and (A.3) has only one positive root with $N_* = \frac{-D_1 + ci\sqrt{\kappa^2\gamma^2 + 4ai^2\kappa}}{2D_2}$. From (A.2), $S_* = c - (1 + \frac{\kappa}{i}) \cdot \frac{-D_1 + ci\sqrt{\kappa^2\gamma^2 + 4ai^2\kappa}}{2D_2}$, it is easy to verify that S_* is positive. System (3.1) has a unique interior equilibrium E_* when $i + \kappa < \frac{\kappa\gamma + \sqrt{\kappa^2\gamma^2 + 4ai^2\kappa}}{2a}$.

(iii) If $i + \kappa > \frac{\kappa\gamma + \sqrt{\kappa^2\gamma^2 + 4ai^2\kappa}}{2a}$, then $D_1 > 0$, $D_2 < 0$ and (A.3) has two positive roots with $N_*^{(j)} = \frac{-D_1 \pm ci\sqrt{\kappa^2\gamma^2 + 4ai^2\kappa}}{2D_2}$ $(j = 1, 2)$, where $N_*^{(1)} < N_*^{(2)}$. Using (A.2) and since $S_*^{(j)} > 0$ $(j = 1, 2)$,

$$\begin{cases} S_*^{(1)} = c - \left(1 + \frac{\kappa}{i}\right) \cdot \frac{-D_1 + ci\sqrt{\kappa^2\gamma^2 + 4ai^2\kappa}}{2D_2} > 0, \\ S_*^{(2)} = c - \left(1 + \frac{\kappa}{i}\right) \cdot \frac{-D_1 - ci\sqrt{\kappa^2\gamma^2 + 4ai^2\kappa}}{2D_2} > 0, \end{cases}$$

$$\Rightarrow \begin{cases} 2ciD_2 + (i + \kappa)(D_1 - ci\sqrt{\kappa^2\gamma^2 + 4ai^2\kappa}) < 0, \\ 2ciD_2 + (i + \kappa)(D_1 + ci\sqrt{\kappa^2\gamma^2 + 4ai^2\kappa}) < 0, \end{cases}$$

$$\Rightarrow \begin{cases} i\kappa\gamma(i+\kappa) + 2i^3\kappa - i(i+\kappa)\sqrt{\kappa^2\gamma^2 + 4ai^2\kappa} < 0, \\ i\kappa\gamma(i+\kappa) + 2i^3\kappa + i(i+\kappa)\sqrt{\kappa^2\gamma^2 + 4ai^2\kappa} < 0, \end{cases}$$

$$\Rightarrow \begin{cases} i + \kappa > \dfrac{\kappa\gamma + \sqrt{\kappa^2\gamma^2 + 4ai^2\kappa}}{2a}, \\ \text{Contradiction.} \end{cases}$$

This implies that system (3.1) has a unique interior equilibrium E_* when $i + \kappa > \frac{\kappa\gamma + \sqrt{\kappa^2\gamma^2 + 4ai^2\kappa}}{2a}$.

The interior equilibrium of (3.1) corresponds to the unique positive root of (A.3). □

Appendix B. Proof of Theorem 3.3

Proof of Theorem 3.3. By Eq. (3.2), the linearization of system (3.1) at the trivial equilibrium $E_0 := (0, 0)$ is computed and the associated characteristic equation is obtained:

$$\det \begin{bmatrix} \lambda + \gamma c & 0 \\ 0 & \lambda - ic \end{bmatrix} = 0. \tag{B.1}$$

It is obvious that $\lambda_1 = -\gamma c$ and $\lambda_2 = ic$ are two eigenvalues of (B.1). The proof is complete. □

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