Modelling the Compartmentalization of Splicing Factors

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Abstract

Splicing factor compartments, also known as speckles, are heterogeneously distributed compartments within the nucleus of eukaryotic cells that are enriched in pre-mRNA splicing factors. We derive a fourth-order aggregation-diffusion model that describes a possible mechanism underlying the organization of splicing factors into speckles. The model incorporates two hypotheses, namely (1) that self-organization of dephosphorylated splicing factors, modulated by a phosphorylation-dephosphorylation cycle, is responsible for the formation and disappearance of speckles, and (2) that an underlying nuclear structure plays a major role in the organization of splicing factors. A linear stability analysis about homogeneous steady-state solutions of the model reveals how the self-interaction among dephosphorylated splicing factors can result in the onset of spatial patterns. A detailed bifurcation analysis of the model describes how phosphorylation and dephosphorylation modulate the onset of the compartmentalization of splicing factors.

Keywords: Splicing factors, nuclear speckles, aggregation, pattern formation.
1 Introduction

The structure of eukaryotic cells is characterized by the presence of two intracellular compartments, namely the cytoplasm and the nucleus. While the structural and functional organization of the cytoplasm is well defined, the nucleus has been more enigmatic. This is principally due to the absence of membranes that define compartments within the nucleoplasm. For example, cellular organelles in the cytoplasm such as the endoplasmic reticulum, the Golgi apparatus, and the mitochondria have been clearly identified, whereas the nature of structures and compartments within the nucleoplasm remains poorly understood. With recent advances in fluorescence microscopy techniques, and the visualization of specific proteins within the nucleus, the structural organization of the nucleus has started to unfold. In particular, fluorescence microscopy has allowed for the identification of sub-nuclear structures or compartments (Dundr and Misteli, 2001; Lamond and Earnschaw, 1998; Matera, 1999; Spector, 1993, 2001). These compartments differ from most cytoplasmic compartments in that they lack membrane boundaries. However, the identification of nuclear domains enriched in specific proteins has led to the conclusion that the nucleus itself is highly organized and dynamically compartmentalized (Dundr and Misteli, 2001; Hendzel et al., 2001; Lamond and Earnschaw, 1998; Lewis and Tollervey, 2000; Misteli, 2001b; Phair and Misteli, 2000; Spector, 2001).

The prototypical example of a non-nucleolar compartment is found in the spatial organization of splicing factors. Splicing factors are nuclear proteins that remove introns (noncoding sequences in the genes) from precursor mRNA molecules in order to form the mature mRNA that will be transported to the cytoplasm. During the interphase of the cell cycle, splicing factors are concentrated in approximately 25 to 50 clusters; during mitosis, these clusters disassemble. These membraneless clusters or aggregates of splicing factors, which are heterogeneously distributed in a “speckled” pattern in the nucleus (see figure 1), are called Splicing Factor Compartments (SFC’s) or nuclear speckles (Kruhlak et al., 2000; Lamond and Spector, 2003; Phair and Misteli, 2000; Spector, 1993, 2001).

One might infer that co-localized processes of splicing and transcription occur within
the speckles, but this is not the case. In fact, splicing and transcription take place away from the speckles and predominantly at their periphery (Hendzel et al., 1998; Huang and Spector, 1996; Misteli, 2000; Misteli et al., 1997). This suggests the existence of a mechanism independent of interactions established during RNA splicing that is responsible for reversibly recruiting splicing factors. In other words, the organization of splicing factors must be highly dynamic. Indeed, fluorescence microscopy experiments have shown that (1) splicing factors are in continuous flux between the speckles and the nucleoplasm (Kruhlak et al., 2000; Misteli, 2001b; Misteli et al., 1997; Phair and Misteli, 2000), and (2) splicing factors move randomly throughout the cell nucleus (Pederson, 2000a; Phair and Misteli, 2000).

These dynamical aspects have brought forth two current biological hypotheses for splicing factor compartmentalization, one relating to the role of phosphorylation and dephosphorylation in the formation and disassembly of SFC’s, and the other relating to the existence of an underlying nuclear structure. First, recent experimental evidence obtained from SR proteins suggests that the flux between the speckles and the nucleoplasm is modulated by phosphorylation and dephosphorylation (Cáceres et al., 1997; Misteli and Spector, 1997, 1998; Xiao and Manley, 1998). SR proteins are a family of splicing factors containing a carboxy-terminal domain rich in argine-serine dipeptides (RS-domain) (Fu, 1995; Manley and Tacke, 1996), and the phosphorylation status (phosphorylated or unphosphorylated) of this domain plays a major role in their localization. In particular, overexpression of kinases that phosphorylate the RS-domains results in the release of splicing factors from speckles and the disassembly of SFC’s (Colwill et al., 1996; Duncan et al., 1998; Gui et al., 1994; Misteli et al., 1997; Misteli and Spector, 1997; Wang et al., 1998). In contrast, the reassociation of splicing factors to SFC’s requires the presence of specific phosphatases responsible for the removal of a phosphate group (Misteli and Spector, 1996, 1997). Moreover, the unphosphorylated state of splicing factors enhances their self-interaction (binding), whereas the phosphorylated state diminishes it (Xiao and Manley, 1998). Understanding the role of phosphorylation in the location of splicing factors and the existence of self-interacting domains (RS-domains) (Cáceres et al., 1997; Xiao and
Manley, 1997, 1998) has led to the following hypothesis for splicing factor compartmentalization: self-organization is responsible for the formation of speckles, and phosphorylation and dephosphorylation modulate this organization.

Second, measurements of the mobility of splicing factors show that they move at a rate that is two orders of magnitude lower than expected based on their molecular weight (Phair and Misteli, 2000). A possible explanation for this apparent slow mobility of splicing factors is rapid transient binding to a relatively immobile nuclear scaffold or nuclear matrix (Capco et al., 1982; He et al., 1990; Hendzel et al., 1999; Kruhlak et al., 2000; Lasky, 2000; Nalepa and Harper, 2004; Nickerson, 2001; Wasser and Chia, 2000). This idea has led to the following hypothesis for splicing factor compartmentalization: the existence of an underlying nuclear structure is a major determinant of the organization of splicing factors (Hendzel et al., 1999; Kumaran et al., 2002; Nickerson, 2001).

The dynamical aspects of splicing factors and their heterogeneous distribution in speckles provide strong evidence that there is more to the spatio-temporal dynamics of splicing factors than just simple diffusion. To unravel the mechanism underlying the organization of splicing factors, we incorporate the two existing biological hypotheses for splicing factor compartmentalization into a mathematical model. Unlike the current thinking that these two hypotheses are conflicting (Lamond and Spector, 2003), we will see that they can, indeed, complement each other in a possible mechanism responsible for the compartmentalization of splicing factors (see figure 2). We use the model to suggest answers to a number of fundamental questions about SFC’s (Lamond and Spector, 2003; Misteli, 2000, 2001a). What is the detailed mechanism of splicing factor compartmentalization? What controls the concentration of splicing factors inside and outside the speckles? Is speckle formation initiated randomly? What determines the number and size of SFC’s?

The model we derive is a fourth-order aggregation-diffusion model that describes a possible mechanism underlying the organization of splicing factors in speckles (section 2). Using linear stability analysis, we show how the onset of splicing factor compartmentalization is captured by the model (section 3), and using bifurcation analysis, we explain how the compartmentalization of splicing factors is modulated by the dynamical parameters of
the model (section 4). We conclude with a discussion of the relevance of the results and possible directions of future work (section 5).

2 The Model

In order to formulate a simple model that can capture the essence of splicing factor compartmentalization, we accommodate the biological hypotheses stated in the previous section into the following general assumptions:

1. Splicing factors (SF’s) transiently bind to an immobile underlying nuclear scaffold that is assumed to be homogeneously distributed throughout the nucleus.

2. Transient binding is rapid, and results in reduced diffusive behaviour in the context of FRAP (Fluorescence Recovery After Photobleaching) experiments (Carrero et al., 2004), or instantaneous reaction in the context of chemical kinetics (Crank, 1975). This assumption allows us to characterize the motion of SF’s with an effective diffusion coefficient $D = (1 - k)D_b$, where $k$ is the proportion of SF’s dynamically bound to the underlying structure, and $D_b$ is the actual diffusion coefficient of SF’s based on their molecular weight.

3. There is no net growth of splicing factors, i.e., there is conservation of mass during the formation of speckles.

4. SF’s density is divided into two classes, namely phosphorylated SF’s, $v(x, t)$, and unphosphorylated SF’s, $u(x, t)$, where $x$ denotes space and $t$ denotes time. It is worthwhile to mention that although there are different phosphorylated states of SF’s, the simplification of considering two classes (phosphorylated and unphosphorylated) reflects the fact that net dephosphorylation promotes association with SFC’s and net phosphorylation promotes dissociation (this does not mean that associated molecules are completely unphosphorylated or that a single phosphorylation is sufficient to promote dissociation from SFC’s).
5. There is an exchange rate between phosphorylated splicing factors and unphosphorylated splicing factors given by a phosphorylation rate, $\rho$, and a dephosphorylation rate, $\delta$, caused by the activity of kinases or phosphatases, respectively.

6. Unphosphorylated splicing factors are capable of self-interaction

On the basis of these general assumptions, and considering a one-dimensional space, the resulting system of equations for the dynamical organization of splicing factors takes the following form

$$\frac{\partial v}{\partial t} = D \frac{\partial^2 v}{\partial x^2} - \delta v + \rho u ,$$

$$\frac{\partial u}{\partial t} = (\text{motion and self-interaction term}) + \delta v - \rho u ,$$

where the “motion and self-interaction term” for the density of unphosphorylated SF’s needs to be fleshed out. To find an exact expression for this term, we follow a diffusion-approximation approach (Turchin, 1998), which is based on a random walk analysis that accounts for both the motion and self-interaction effects. This type of approach has been proven to be an effective tool when describing congregative behaviour in ecological systems (Lewis, 1994; Turchin, 1989).

Let us assume that unphosphorylated splicing factor biomolecules move along a line that is discretized into small space intervals of length $\lambda$. Let us also discretize time into short intervals of length $\tau$, and assume that every time step $\tau$ a biomolecule located at position $x$ at time $t$ can move a distance of $\lambda$ either to the left with probability $L(x, t)$, to the right with probability $R(x, t)$, or remain in its current position with probability $N(x, t)$. Thus, the probability of moving is given by

$$R(x, t) + L(x, t) = 1 - N(x, t) .$$

We further assume that there is no bias in the movement, i.e., $R(x, t) = L(x, t)$. Following the procedure in (Turchin, 1998), we arrive to the following diffusion approximation model for the density of unphosphorylated SF’s

$$\frac{\partial u(x, t)}{\partial t} = D \frac{\partial^2 u}{\partial x^2} \left[ (1 - N(x, t)) u \right] = \frac{\partial^2 u}{\partial x^2} \left[ \mu(x, t) u \right] ,$$
where \( D = (1 - k)D_b = \lim_{\lambda \to \infty} \frac{\lambda^2}{2\tau} \), and \( \mu(x, t) = D(1 - N(x, t)) \) represents the motility.

As a final step, we need to develop an expression for \( N(x, t) \). For this purpose, we assume that the probability \( N(x, t) \) for an unphosphorylated biomolecule to remain in its current position is proportional to the average density of unphosphorylated SF’s bound to the underlying structure, and is given by

\[
N(x, t) = \frac{\kappa}{\omega} \int_{-\infty}^{\infty} H(s) u(x + s, t) \, ds ,
\]

(4)

where \( \omega \) represents a critical density of biomolecules dictated by space limitations, \( \kappa = k \alpha \) is called the aggregative sensitivity, \( k \) is the proportion of SF’s dynamically bound to the underlying structure, \( \alpha \) represents the binding affinity of SF’s (probability of binding to each other), and the kernel function \( H(s) \) is assumed to have the following form

\[
H(s) = \begin{cases} 
\frac{1}{2\sigma} & \text{for } |s| \leq \sigma , \\
0 & \text{for } |s| > \sigma ,
\end{cases}
\]

(5)

where \( \sigma \) denotes the range of influence of the self-interaction. Note that \( N(x, t) \leq 1 \) provided that

\[
u(x, t) < \omega ,
\]

(6)

i.e., \( u(x, t) \) does not exceed the critical density \( \omega \). We shall see that this condition is satisfied during the onset of the compartmentalization, but when aggregation is pronounced, the density may exceed \( \omega \), and the model will no longer be valid. We will return to this issue in the discussion.

The diffusion approximation model (3) falls into the class of integro-differential equations. Such equations have been shown to have rich dynamics, including potential for pattern-formation (Lewis, 1994; Mogilner and Edelstein-Keshet, 1995, 1996, 1999; Mogilner et al., 1996; Murray, 1993; Turchin, 1998). We will take the approach followed by Lewis (1994), Murray (1993), and Turchin (1998), namely to expand the integral term for the stability analysis of equation (3). However, this approach is not essential for the linear stability analysis (Mogilner and Edelstein-Keshet, 1996, 1999). Using the expansion in Taylor series

\[
u(x + s, t) = u(x, t) + \frac{\partial u}{\partial x} s + \frac{\partial^2 u}{\partial x^2} \frac{s^2}{2} + \frac{\partial^3 u}{\partial x^3} \frac{s^3}{6} + O(s^4) ,
\]

(8)
and the expression of the kernel (5), the probability of staying (4) becomes

\[ N(x, t) = \frac{\kappa u(x, t)}{\omega} + \frac{\kappa \sigma^2}{6\omega} \frac{\partial^2 u}{\partial x^2} + O(\sigma^4) . \]  

(7)

Assuming that the range of influence \( \sigma \) is small and neglecting the fourth-order and higher-order terms with respect to \( \sigma \), the motility, \( \mu(x, t) = D(1 - N(x, t)) \), can be approximated by

\[ \mu(x, t) = D - D \frac{\kappa u(x, t)}{\omega} - D \frac{\kappa \sigma^2}{6\omega} \frac{\partial^2 u}{\partial x^2}(x, t) . \]  

(8)

Substituting (8) into (3), the diffusion approximation for the density of unphosphorylated SF’s can be written as the following fourth-order partial differential equation:

\[ \frac{\partial u}{\partial t} = D \frac{\partial^2 u}{\partial x^2} - \frac{\partial^2}{\partial x^2} \left[ \left( D - 2D \frac{\kappa u}{\omega} \right) \frac{\partial u}{\partial x} \right] - \frac{\partial^2}{\partial x^2} \left[ \left( \frac{D \kappa \sigma^2 u}{6\omega} \right) \frac{\partial^2 u}{\partial x^2} \right] , \]  

(9)

which represents the motion and self-interaction term in equation (1). We refer to equation (9) as the aggregation-diffusion equation. Thus, the system of partial differential equations describing the compartmentalization of SF’s becomes

\[ \frac{\partial v}{\partial t} = D \frac{\partial^2 v}{\partial x^2} - \delta v + \rho u , \]  

(10)

\[ \frac{\partial u}{\partial t} = D \frac{\partial^2 u}{\partial x^2} - \frac{\partial^2}{\partial x^2} \left[ \left( D - 2D \frac{\kappa u}{\omega} \right) \frac{\partial u}{\partial x} \right] - \frac{\partial^2}{\partial x^2} \left[ \left( \frac{D \kappa \sigma^2 u}{6\omega} \right) \frac{\partial^2 u}{\partial x^2} \right] + \delta v - \rho u . \]

We refer to equation (10) as the aggregation-reaction-diffusion system.

The cell nucleus is a domain bounded by a membrane. For this reason, we assume a bounded domain, \( 0 \leq x \leq L \), with no net flux of splicing factors across the boundaries. This is achieved by considering the following no-flux boundary conditions

\[ \frac{\partial v}{\partial x}(0, t) = \frac{\partial v}{\partial x}(L, t) = \frac{\partial u}{\partial x}(0, t) = \frac{\partial u}{\partial x}(L, t) = 0 , \]  

(11)

\[ \frac{\partial^3 u}{\partial x^3}(0, t) = \frac{\partial^3 u}{\partial x^3}(L, t) = 0 . \]

In order to facilitate the analysis and reduce the number of parameters, we introduce the following dimensionless variables:

\[ x^* = \frac{x}{L} , \ t^* = \frac{D}{L^2} t , \ \upsilon^* = 2\kappa \frac{\upsilon}{\omega} , \ u^* = 2\kappa \frac{u}{\omega} , \ \sigma^* = \frac{\sigma}{L} , \ \delta^* = \frac{L^2}{D} \delta , \ \rho^* = \frac{L^2}{D} \rho . \]  

(12)
After making these substitutions and dropping the asterisks, the aggregation-diffusion equation (9) becomes
\[
\frac{\partial u}{\partial t} = \frac{\partial}{\partial x} \left[ (1 - u) \frac{\partial u}{\partial x} \right] - \frac{\partial^2}{\partial x^2} \left[ \left( \frac{\sigma^2}{12} u \right) \frac{\partial^2 u}{\partial x^2} \right],
\]
(13)
subject to the no-flux boundary condition
\[
\frac{\partial u}{\partial x}(0, t) = \frac{\partial u}{\partial x}(1, t) = 0,
\]
\[
\frac{\partial^3 u}{\partial x^3}(0, t) = \frac{\partial^3 u}{\partial x^3}(1, t) = 0,
\]
(14)
and the aggregation-reaction-diffusion system (10) can be rewritten in a dimensionless form as
\[
\frac{\partial v}{\partial t} = \frac{\partial^2 v}{\partial x^2} - \delta v + \rho u,
\]
(15)
subject to the no-flux boundary conditions
\[
\frac{\partial v}{\partial x}(0, t) = \frac{\partial v}{\partial x}(1, t) = \frac{\partial u}{\partial x}(0, t) = \frac{\partial u}{\partial x}(1, t) = 0,
\]
\[
\frac{\partial^3 u}{\partial x^3}(0, t) = \frac{\partial^3 u}{\partial x^3}(1, t) = 0.
\]
(16)

Since self-organization appears to be driven by the dynamics of the unphosphorylated SF’s, described by the aggregation-diffusion equation (9), our first interest will be to demonstrate the potential of this equation for the spontaneous formation of spatial patterns (section 3). These patterns, which are a consequence of the nonhomogeneous distribution of biomolecules, can be interpreted as splicing factor compartments or speckles, where the density of biomolecules is high. In section 4, we focus on the aggregation-reaction-diffusion system (10), and study the modulating effect of phosphorylation on the compartmentalization of SF’s. From the first equation in (10), we intuitively expect the modulation to be driven by the homogenizing effect of the spatial diffusion of phosphorylated SF’s.
3 The Onset of the Compartmentalization of SF’s

The potential of equation (15) for spatial pattern formation is interpreted as the onset of splicing factor compartmentalization during early G1. We can assert intuitively that no patterns will arise if there are no unphosphorylated SF’s. For this reason, and to start the analysis as simple as possible, we will capture the essence of the onset of the compartmentalization by examining first the dynamics of just unphosphorylated SF’s in the absence of phosphorylated ones. By carrying out a linear stability analysis about the homogeneous steady-state solutions of the aggregation-diffusion equation (13) subject to the boundary conditions (14), we will examine the behaviour of perturbations, and determine whether there are wave numbers with the ability to grow.

3.1 Dispersion Relation for the Aggregation-Diffusion Equation

The uniform steady states of (13) are given by any constant density $u_{eq}$. Thus, using the assumption of conservation of mass, we can think of the uniform steady state $u_{eq}$ as a parameter that varies according to the amount of biomolecules in the system. Let us consider small perturbations of the biomolecule density $u(x, t)$ away from the spatially uniform steady state $u_{eq}$, of the form

$$u(x, t) = u_{eq} + \varepsilon \bar{u}(x, t), \quad \text{where } \varepsilon \ll 1 . \quad (17)$$

Substituting (17) into (13) yields

$$\varepsilon \frac{\partial \bar{u}}{\partial t} = \frac{\partial}{\partial x} \left[ (1 - u_{eq} - \varepsilon \bar{u}) \varepsilon \frac{\partial \bar{u}}{\partial x} \right] - \frac{\partial^2}{\partial x^2} \left[ \left( \frac{\sigma^2}{12} (u_{eq} + \varepsilon \bar{m}) \right) \varepsilon \frac{\partial^2 \bar{u}}{\partial x^2} \right].$$

Dividing this expression by $\varepsilon$, dropping the bars, and noting that $\varepsilon \ll 1$, we obtain the following linearization for the aggregation-diffusion equation (13):

$$\frac{\partial u}{\partial t} = (1 - u_{eq}) \frac{\partial^2 u}{\partial x^2} - \frac{\sigma^2}{12} u_{eq} \frac{\partial^4 u}{\partial x^4} . \quad (18)$$

To investigate the behaviour of solutions for the linearized equation (18), we study the normal mode solutions of the form

$$u(x, t) \propto \exp(\lambda t + iqx) , \quad (19)$$
where $\lambda$ is the growth rate corresponding to the wave number $q$. Thus, wavenumbers $q$ with a corresponding $\lambda > 0$ will grow with wavelength $2\pi/q$.

Substitution of (19) into the linearized equation (18) gives the following dispersion relation between $\lambda$ and $q$:

$$\lambda = (u_{eq} - 1)q^2 - \frac{\sigma^2}{12} u_{eq} q^4,$$

(20)

which is shown as a function of $q^2$ in figure 3. The potential for pattern formation is determined simply by the fundamental condition $\lambda > 0$. Note that for the limit case $\sigma = 0$, the kernel (5) corresponds to a delta distribution centered at the current position of the biomolecule, and the dispersion relation increases or decreases monotonically with respect to $q$, depending on whether $u_{eq} > 1$ or $u_{eq} < 1$. Therefore, the smaller the perturbation wavelengths the faster they will grow, which means that the problem is ill-posed in the sense of Hadamard (Zauderer, 1998). For this reason, we account for the realistic biological assumption that biomolecules can be influenced by nearby conspecifics and consider only the case $\sigma > 0$, in which small wavelengths, characterized by high $q$, do not grow. The main requirement for $\lambda$ to correspond to growing wave numbers is that $u_{eq} > 1$ (see figure 3). In other words, the population of unphosphorylated SF’s has to be large enough for pattern formation to occur. Otherwise, $u_{eq}$ becomes a stable steady state.

In order to illustrate these stability scenarios, we have performed two numerical simulations, one in which $u_{eq} < 1$, where stability is obtained (figure 4-A), and another for $u_{eq} > 1$, where instability leads to a potential spatial pattern (figure 4-B). Note that as a dimensionless parameter, $u_{eq}$ has buried the dimensional parameter $\kappa$. Thus, the larger $\kappa$ is, the more likely it is that pattern formation occurs; hence $\kappa$ is called the aggregative sensitivity. Recalling that $\kappa = k \alpha$, the analysis suggests that the onset of the compartmentalization is enhanced by the affinity of the interaction, $\alpha$, as well as by a large proportion $k$ of biomolecules bound to the underlying nuclear structure.
3.2 The Onset of Spatial Patterns

In order to gain a better understanding on how the spatial patterns start to arise, we study the dispersion relation (20) in more depth and determine which is the fastest growing wavelength (or equivalently, the dominating wave number), and examine the effect of different values of the parameter $\sigma$ on the onset of the aggregation.

First, note that the only possible perturbations that satisfy the no-flux boundary conditions (14) on the domain $(0, 1)$ are those whose wavenumbers take the discrete values

$$q_n = n\pi, \quad \text{where} \quad n = 0, 1, 2, \ldots.$$  \hfill (21)

From figure 3, we know that when $u_{eq} > 1$, $\lambda$ as a function of $q^2$ has two zeroes, namely

$$q_-^2 = 0, \quad \text{and} \quad q_+^2 = \frac{12(u_{eq} - 1)}{\sigma^2 u_{eq}}.$$  \hfill (22)

Therefore, the modes with positive growth rate (modes of instability) are given by the wave numbers satisfying

$$q_-^2 < q_n^2 < q_+^2.$$  \hfill (23)

Moreover, $\lambda$ as a function of $q^2$ reaches a maximum at

$$q_{max}^2 = \frac{6(u_{eq} - 1)}{\sigma^2 u_{eq}}.$$  \hfill (24)

Thus, the dominating wave number $q_m$, with $m \in \mathbb{N}$, is a wave number of an unstable mode, such that

$$|\lambda(q_m^2) - \lambda(q_{max}^2)| = \min_{q_-^2 < q_n^2 < q_+^2} \{|\lambda(q_n^2) - \lambda(q_{max}^2)|\},$$  \hfill (25)

and the corresponding fastest growing wavelength of the spatial pattern that starts to evolve from the perturbation is

$$l_m = \frac{2\pi}{q_m} = \frac{2}{m}.$$  \hfill (26)

Note from equations (24)-(26) that $l_m \propto \sigma$, which means that initially after perturbation the longitude of the speckles or compartments is directly related to the scale of $\sigma$. This is illustrated in the numerical simulations shown in figure 4B and figure 5. These figures...
Table 1: Dominating wave numbers and wavelengths for the perturbations shown in figure 4B and figure 5 for different values of $\sigma$.

show the evolution of a random perturbation about a homogeneous steady state for various values of $\sigma$. The plot of the corresponding dispersion relations are shown in figure 6, and the numerical information gathered from them is arranged in table 1. Notice that as $\sigma$ decreases, the dominating wavelength $l_m$ decreases (or equivalently, the dominating wave number $q_m$ increases), and the growth rate increases. In other words, the larger $\sigma$ is, the fewer the speckles or compartments.

4 Modulating the Compartmentalization

In this section, we incorporate the phosphorylated population into the analysis and study its modulating effect on the compartmentalization of SF’s. We carry out a linear stability analysis about the homogeneous steady state solutions of the aggregation-reaction-diffusion system (15) and perform a bifurcation analysis to understand how the phosphorylation and dephosphorylation rates modulate the formation of speckles.

4.1 Dispersion Relation for the Aggregation-Reaction-Diffusion System

From equation (15), positive uniform steady states $(v_{eq}, u_{eq})$ are given by points in the first quadrant of the $u v$-plane satisfying

$$ u = \frac{\delta}{\rho} v. $$

(27)
Moreover, by introducing a new parameter
\[ C = \int_0^1 (u_{eq} + v_{eq}) \, dx = u_{eq} + v_{eq} , \tag{28} \]
representing the fixed amount of biomolecules in the system, the uniform steady \((v_{eq}, u_{eq})\) of (15) is determined by the intersection in the \(v u\)-plane of the straight line given by (27) and the straight line
\[ u + v = C . \tag{29} \]
Therefore,
\[ (v_{eq}, u_{eq}) = \left( \frac{\rho}{\rho + \delta}, \frac{\delta}{\rho + \delta} \right) C . \tag{30} \]

In order to assess the influence of the dynamical parameters of system (15) on the formation of speckles, we will consider small perturbations from the spatially homogeneous steady state \((v_{eq}, u_{eq})\) of the form
\[ v = v_{eq} + \varepsilon \overline{v}(x, t) , \]
\[ u = u_{eq} + \varepsilon \overline{u}(x, t) , \tag{31} \]
where \(\varepsilon \ll 1\). Substitution of these perturbations into (15) yields
\[ \varepsilon \frac{\partial \overline{v}}{\partial t} = \varepsilon \frac{\partial^2 \overline{v}}{\partial x^2} - \delta \varepsilon \overline{v} + \rho \varepsilon \overline{u} , \]
\[ \varepsilon \frac{\partial \overline{u}}{\partial t} = \frac{\partial}{\partial x} \left[ (1 - u_{eq} - \varepsilon \overline{u}) \frac{\partial \overline{u}}{\partial x} \right] - \frac{\partial^2}{\partial x^2} \left[ \left( \frac{\sigma^2}{12} (u_{eq} + \varepsilon \overline{u}) \right) \varepsilon \frac{\partial^2 \overline{u}}{\partial x^2} \right] + \delta \varepsilon \overline{v} - \rho \varepsilon \overline{u} . \tag{32} \]
Equating first-order terms with respect to \(\varepsilon\), neglecting higher-order terms, and dropping the bars, we obtain the following linearized system for (15):
\[ \frac{\partial v}{\partial t} = \frac{\partial^2 v}{\partial x^2} - \delta v + \rho u , \tag{33} \]
\[ \frac{\partial u}{\partial t} = (1 - u_{eq}) \frac{\partial^2 u}{\partial x^2} - \frac{\sigma^2}{12} u_{eq} \frac{\partial^4 u}{\partial x^4} + \delta v - \rho u . \]

To find the dispersion relation between the growth rate \(\lambda\) and the wave number \(q\) and draw conclusions about the stability of uniform steady states of (15), we study the following normal mode solutions of the linear system (33):
\[ v(x, t) = A \exp(\lambda t + i qx) , \]
\[ u(x, t) = B \exp(\lambda t + i qx) , \tag{34} \]
where $A$ and $B$ are constants. Substitution of (34) into the linear system (33), cancellation of the factor $\exp(\lambda t + i q x)$, and proper rearrangement leads to

$$
\begin{bmatrix}
\lambda + \delta + q^2 \\
-\delta 
\end{bmatrix}
\begin{bmatrix}
-\rho \\
\lambda + \rho - (u_{eq} - 1)q^2 + \frac{\sigma^2}{12}u_{eq}q^4
\end{bmatrix}
= L
\begin{bmatrix}
A \\
B
\end{bmatrix}
= \begin{bmatrix}
0 \\
0
\end{bmatrix}.
$$

(35)

For nonzero solutions, it is required that $A$ and $B \neq 0$. Therefore, we require $\det(L) = 0$, giving the following quadratic equation for the growth rate:

$$
\lambda^2 + \beta(q)\lambda + \gamma(q) = 0,
$$

(36)

where

$$
\beta(q) = \frac{\sigma^2}{12}u_{eq}q^4 - (u_{eq} - 2)q^2 + \rho + \delta
$$

and

$$
\gamma(q) = \frac{\sigma^2}{12}u_{eq}q^6 + \left(\delta\frac{\sigma^2}{12}u_{eq} - u_{eq} + 1\right)q^4 + (\rho - \delta u_{eq} + \delta)q^2.
$$

(37)

The two roots of (36) are given by

$$
\lambda_{1,2} = \frac{-\beta \pm \sqrt{\beta^2 - 4\gamma}}{2}.
$$

(38)

The necessary and sufficient condition for the growth rate $\lambda$ of the perturbations (34) to have positive real part is that $\beta < 0$ or $\gamma < 0$. Note that if $\beta \leq 0$ then $u_{eq} > 2$. In terms of the dimensional variables (see variables (12)), this implies that the density of unphosphorylated SF’s exceeds the critical density $\omega$, i.e., the restriction given by (6) is violated. For this reason we assume $\beta > 0$. Therefore, one of the roots in (38) always has negative real part and the dispersion relation for possible growing modes is determined by the other root

$$
\lambda = \frac{-\beta + \sqrt{\beta^2 - 4\gamma}}{2},
$$

(39)

which will have positive real part if and only if $\gamma < 0$, where $\gamma$ is as in (37).

For the purpose of simplifying the analysis of the dispersion relation, we will consider all the parameters in system (15) fixed except for the dephosphorylation rate $\delta$. An analogous analysis could be carried out if the free parameter were the phosphorylation rate $\rho$. Note that varying $\delta$ affects the slope of (27), and consequently the value of the steady state
Thus, the natural question that arises is whether or not this variation affects the stability of the uniform steady state. In other words, we want to understand the influence of the dynamical parameter $\delta$ on the pattern forming potential of system (15).

The dispersion relation is visualized in figure 7 for two particular values of the dephosphorylation rate, namely $\delta = 1.1$, for which we obtain a dispersion relation defined by wave numbers with positive growth rates (modes of instabilities), and $\delta = 1$, for which the dispersion relation is defined by negative growth rates for all wave numbers. In other words, the uniform steady state $(v_{eq}, u_{eq})$ obtained with $\delta = 1.1$ is unstable and the evolving dynamics of its perturbation is characterized by the onset of spatial patterns, whereas the uniform steady state $(v_{eq}, u_{eq})$ obtained with $\delta = 1$ is stable and no spatial patterns will evolve. These stability results are corroborated by the numerical simulations shown in figures 8 and 9, respectively.

It is important to note that although self-interaction occurs between unphosphorylated splicing factors, the model predicts the aggregation for both phosphorylated and unphosphorylated populations. In fact, these populations aggregate in the same place. This results from the fact that the wave numbers corresponding to unstable modes have an effect on both the normal mode solutions (34) for the phosphorylated and unphosphorylated splicing factors.

In the case of the aggregation-diffusion equation (13), we were able to use the dispersion relation (20) in order to find the dominating wave number $q_m$ dictated by (24) and the fastest growing wavelength given by (26). But in the case of the aggregation-reaction-diffusion system (15), the complicated expression for the dispersion relation (39) has made the task of finding the dominating wave number very difficult. In spite of this, Hadeler and Hillen (2005) were able to estimate the dominating wave number for system (15). Under the assumption that the turnover rate of phosphorylated and unphosphorylated splicing factors is fast, the authors approximated system (15) with a limiting model that was linearized in order to obtain an estimate of the dominating wave number for system (15).

We investigate the modulating effect of the phosphorylation and dephosphorylation rates on the onset of the compartmentalization by addressing the question of how the transition from stability to instability takes place when the dephosphorylation rate is taken as the bifurcation parameter. We already know that this transition occurs when the real part of the dispersion relation \( \lambda \), given by (39), becomes positive. Equivalently, the transition occurs when \( \gamma \), given by (37), becomes negative. Therefore, this transition is described by a real bifurcation (Lewis, 1994). To simplify the analysis of this bifurcation problem, we let both \( \lambda \) and \( \gamma \) be functions of \( z = q^2 \). From (30) and (37), it then follows that the roots of \( \lambda(z) \) are determined by the positive roots of

\[
\gamma(z) = z p(z) = z \left[ a(\delta) z^2 + b(\delta) z + c(\delta) \right],
\]

where

\[
a(\delta) = C \frac{\sigma^2 \delta}{12 \, \delta + \rho},
\]
\[
b(\delta) = C \frac{\delta}{\delta + \rho} \left( \frac{\delta \sigma^2}{12} - 1 \right) + 1,
\]
\[
c(\delta) = \rho - C \frac{\delta^2}{\delta + \rho} + \delta,
\]

and \( C \) is as in equation (28). Thus, one of the roots of \( \gamma(z) \) is zero, and the other roots are given by the roots of \( p(z) \):

\[
r_{1,2} = \frac{-b(\delta) \pm \sqrt{b^2(\delta) - 4a(\delta) c(\delta)}}{2a(\delta)}.
\]

Since the coefficient \( a(\delta) > 0 \), the transition from a stable to an unstable steady state can only occur when \( f(\delta) = 0 \) or \( c(\delta) = 0 \), where

\[
f(\delta) = b^2(\delta) - 4a(\delta) c(\delta).
\]

To distinguish these two cases, we note first that the coefficient \( b(\delta) = 0 \) if and only if \( \rho = l(\delta) \), where

\[
l(\delta) = -\frac{C \sigma^2}{12} \delta^2 + (C - 1) \delta.
\]
Note as well that \( c(\delta) = 0 \) if and only if \( \rho = m_{\pm}(\delta) \), where

\[
m_{\pm}(\delta) = \frac{C - 1}{(1 \pm \sqrt{C})} \delta. \tag{45}
\]

For \( 0 < C \leq 1 \), \((v_{eq}, u_{eq})\) is stable. To see this, we note that the values of both (44) and (45) are negative. On the one hand, \( l(\delta) < 0 \) implies that \( b(\delta) > 0 \). The bifurcation cannot take place at any root of \( f(\delta) \). If it did, the only root of \( p(z) \) at the bifurcation point, given by (42), would be negative. On the other hand, the bifurcation cannot occur when \( c(\delta) = 0 \), because \( \rho > 0 \) and \( m_{\pm}(\delta) < 0 \). Therefore, \((v_{eq}, u_{eq})\) is stable for \( 0 < C \leq 1 \).

For \( C > 1 \), the stability of \((v_{eq}, u_{eq})\) depends on the value of \( \delta \). We let \( \delta_b \) be the bifurcation value at which the stability changes. For \( \delta < \delta_b \), \((v_{eq}, u_{eq})\) is stable, and for \( \delta > \delta_b \), \((v_{eq}, u_{eq})\) is unstable. The value of \( \delta_b \) depends on the choice of other model parameters. We give the details of \( \delta_b \) below. First, we note that the parabola \( l(\delta) \) reaches a maximum value \( \rho_m = \frac{3(C-1)^2}{C\sigma^2} \) at \( \delta_m = \frac{6(C-1)}{C\sigma^2} \) (see figure 10) and \( c(\delta) = 0 \) if \( \rho = m(\delta) \), where

\[
m(\delta) = \frac{C - 1}{(1 + \sqrt{C})} \delta. \tag{46}
\]

Since the slope of \( m(\delta) \) is smaller than the slope of \( l(\delta) \) at \( \delta = 0 \) and \( m(\delta_m) < \rho_m \), the straight line \( m(\delta) \) is as shown in figure 10.

With figure 10 in mind, we conclude that if \( \rho > \frac{3(C-1)^2}{C\sigma^2} \), then \( b(\delta) > 0 \) for all \( \delta \), and the bifurcation cannot occur at any root of \( f(\delta) \). Thus, the bifurcation takes place when \( c(\delta) = 0 \), i.e., at \( \delta_b = \frac{(1 + \sqrt{C})}{C - 1} \rho \).

To study the case \( \rho \leq \frac{3(C-1)^2}{C\sigma^2} \), we observe that the roots of \( p(z) \), given by (42), are both negative when \( \delta \ll 1 \). As \( \delta \) increases, we note from figure 10 that there is at least one value \( \overline{\delta} \) such that \( b(\overline{\delta}) = 0 \) and \( c(\delta) \neq 0 \) for \( \delta \leq \overline{\delta} \). This means that at \( \overline{\delta} \), the roots (42) of \( p(z) \) have distinct signs or are conjugate complex numbers. Therefore, the only possible way for this transition in the roots of \( p(z) \) to happen as \( \delta \) increases is that there exists a \( \tilde{\delta} < \overline{\delta} \) such that \( f(\tilde{\delta}) = 0 \), i.e., \( f(\delta) \) has at least one root. Moreover, it is not difficult to see that all the roots of \( f(\delta) \) are smaller than those of \( c(\delta) \). Since we are interested in the bifurcation from a stable to an unstable steady state, and \( f(\delta) \to \infty \) as
\( \delta \to \infty \), we let \( \delta^* \) denote the largest root of \( f(\delta) \). Thus, if \( b(\delta^*) < 0 \), then the bifurcation occurs at \( \delta_b = \delta^* \), and if \( b(\delta^*) \geq 0 \), then the root of \( p(z) \) is negative and the bifurcation occurs when \( c(\delta) = 0 \), i.e., at \( \delta_b = \frac{(1 + \sqrt{C})}{C - 1} \).

We have provided not only details on the real bifurcation, but also an algorithm to determine the bifurcation value. To illustrate this, we find the bifurcation value \( \delta_b \) for the example shown in figures 7-9, in which the dispersion relation becomes positive as the dephosphorylation rate \( \delta \) increases. For the particular choice of the parameter values in the example (\( \sigma = 0.05, \rho = 1, C = 2 \)), we note that \( \rho = 1 < \frac{3(C - 1)^2}{C \sigma^2} = 600 \). The largest root of \( f(\delta) \), defined in (43), is \( \delta^* \approx 1.06 \). Since \( b(\delta^*) \approx -0.03 < 0 \), the bifurcation value is given by \( \delta_b = \delta^* \approx 1.06 \). The evolution of the dispersion relation (39) as \( \delta \) passes through \( \delta_b \) is shown in figure 11A, which is consistent with the results in figures 7-9.

Figure 11B shows the bifurcation diagram for the steady state \((v_{eq}, u_{eq})\) obtained from the intersection of line (27), \( u = \frac{\delta}{\rho} v \), and (29), \( v + u = 2 \). When \( \delta \) is small, the steady state is stable (denoted by the solid portion of the line \( v + u = 2 \)). The slope of line (27) increases as \( \delta \) increases (or equivalently, decreases as \( \rho \) increases), and as it passes through the value \( \delta_b/\rho \), the resulting steady state becomes unstable (denoted by the dotted portion of the line \( v + u = 2 \)). The outcome is consistent with the biological fact that dephosphorylation (increased \( \delta \), or decreased \( \rho \)) enhances the self-organization of splicing factors, and that phosphorylation (increased \( \rho \), or decreased \( \delta \)) enhances the disassembly of speckles.

With the bifurcation diagram from figure 11B in mind, we can also obtain the bifurcation values for any value of \( C \). In particular, we consider values of \( 0 < C \leq 2 \) to avoid breaking the restriction on the density of splicing factors given by (6). For each value of \( C \), we obtain a bifurcation value \( \delta_b(C) \), yielding the stability and instability regions for the steady state \((v_{eq}, u_{eq})\) shown in figure 12A. The uniform steady states that have the potential for pattern formation after perturbation lie in the instability region. The bifurcation curve

\[
(v_{eq}(C), u_{eq}(C)) = \left( C \frac{\rho}{\rho + \delta_b}, C \frac{\delta_b}{\rho + \delta_b} \right)
\]  

(47)

that separates the regions of stability and instability in figure 12A lies slightly above
the curve $u_{eq} = 1$. Thus, for the onset of the compartmentalization to take place, it is necessary to have enough biomolecules in the system ($C > 1$), and to have enough unphosphorylated biomolecules, which is consistent with the results obtained in the analysis of the aggregation-diffusion equation (13), where it was required that $u_{eq} > 1$ for instability to occur. The natural question that arises now is how the bifurcation curve changes as the phosphorylation rate increases. As expected, the stability region will increase, but only up to a certain point. Considering that the bifurcation curve is given by equation (47), we conclude that the curve that separates the regions when $\rho > \frac{3(C - 1)^2}{2\sigma^2}$ is given by the intersection of the line $v_{eq} + u_{eq} = C$ and the line $u_{eq} = r(C)v_{eq}$, where

$$r(C) = \frac{1 + \sqrt{C}}{C - 1};$$  \hspace{1cm} (48)

i.e., by the parameterized curve

$$(v_{eq}(C), u_{eq}(C)) = \left(\frac{C(C - 1)}{C + \sqrt{C}}, \frac{C(1 + \sqrt{C})}{C + \sqrt{C}}\right).$$ \hspace{1cm} (49)

Therefore, the largest region of stability, reached when $\rho > \frac{3(C - 1)^2}{2\sigma^2}$, will be determined by (49) (see figure 12B). This implies that no matter how big the phosphorylation rate is, there will always be a region of instability, i.e., there exists a dephosphorylation rate that is high enough such that the resulting uniform steady state $(v_{eq}, u_{eq})$ becomes unstable.

If one wants to obtain information on the modulating effect of both the phosphorylation and dephosphorylation rates at the same time, one can portray the regions of stability in the $\rho\delta$-plane. By keeping all the parameters fixed except $\rho$ and $\delta$, we obtain the following bifurcation curve:

$$\delta = \delta_\rho(\rho) = \begin{cases} 
1 + \sqrt{C} & ; \quad \rho > \frac{3(C - 1)^2}{C\sigma^2}, \\
\frac{C(C - 1)}{C + \sqrt{C}} & ; \quad \rho \leq \frac{3(C - 1)^2}{C\sigma^2} \text{ and } b(\delta^*) < 0, \\
\frac{C(1 + \sqrt{C})}{C + \sqrt{C}} & ; \quad \rho \leq \frac{3(C - 1)^2}{C\sigma^2} \text{ and } b(\delta^*) \geq 0, \\
1 + \sqrt{C} & ; \quad \rho > \frac{3(C - 1)^2}{C\sigma^2}, \\
\frac{C(C - 1)}{C + \sqrt{C}} & ; \quad \rho \leq \frac{3(C - 1)^2}{C\sigma^2} \text{ and } b(\delta^*) < 0, \\
\frac{C(1 + \sqrt{C})}{C + \sqrt{C}} & ; \quad \rho \leq \frac{3(C - 1)^2}{C\sigma^2} \text{ and } b(\delta^*) \geq 0, \\
\end{cases}$$ \hspace{1cm} (50)
where $\delta^*$ denotes the largest root of $f(\delta)$ (equation (43)), and $b(\delta)$ is as in equation (41). Figures 13A and 13B show this bifurcation curve and the resulting stability regions in the $\rho \delta$-plane when $\sigma = 0.05$, for $C = 2$ and $C = 1.5$, respectively. In figure 13B, note that decreasing the amount of biomolecules in the system, represented by $C$, has caused the region of instability to be reduced, i.e., there is a lower chance for the onset of compartmentalization of splicing factors.

In this section, we have demonstrated the existence of unstable uniform steady states of system (15), whose perturbation can result in the onset of the compartmentalization of splicing factors. Through the bifurcation analysis, we have concluded that the potential for the formation of compartments can be eliminated by increasing the phosphorylation rate or decreasing the amount of splicing factors in the system.

## 5 Discussion

Understanding the dynamical organization of splicing factors is an important step towards understanding the architecture of eukaryotic cell nuclei and the changes that take place under different physiological conditions or stresses. In this work, we have approached understanding nuclear compartmentalization using a mathematical model. In particular, we have proposed a model that describes a possible mechanism for the onset of splicing factors compartmentalization, i.e., for the formation of speckles. The model, namely the aggregation-reaction-diffusion equation (10), is based on the current biological hypotheses that self-organization of splicing factors is modulated by phosphorylation and dephosphorylation (Cáceres et al., 1997; Misteli and Spector, 1997, 1998; Xiao and Manley, 1998), and that the existence of an underlying nuclear structure plays a major role in the formation of speckles (Hendzel et al., 1999; Kumaran et al., 2002; Nickerson, 2001).

A linear stability analysis of the model and a study of its dispersion relation has brought to light a possible mechanism for the formation of speckles, in which slight perturbations of a uniform steady state of the system may lead to potential spatial patterns. This is possible so long as there are sufficient biomolecules in the cell nucleus. This result
mimics the onset of the compartmentalization of splicing factors during the early stage of the cell interphase, when the distribution of splicing factors undergoes a transition from spatially homogeneous to heterogeneous (Lamond and Spector, 2003), and suggests that speckle formation can be initiated randomly. Moreover, the model illustrates how the ratio between the size of the domain (cell nucleus) and the size of the range of influence of the self-interaction, defined by the dimensionless parameter $\sigma$, can determine the number and size of SFC’s. The larger $\sigma$ is, the fewer the speckles or compartments.

The bifurcation analysis illustrates that an appropriate ratio of phosphorylation-dephosphorylation rates is needed for speckles to form. An increase in the dephosphorylation rate leads to a larger region of instability, and therefore enhances the compartmentalization of splicing factors, whereas an increase in the phosphorylation rate leads to a small region of instability, and therefore has a homogenizing effect on the spatial distribution of splicing factors. This is consistent with naturally occurring processes that have been shown to lead to speckle disassembly, caused right before mitosis by an increase in kinases activity or induced experimentally by increasing the activity of specific kinases (Colwill et al., 1996; Duncan et al., 1998; Gui et al., 1994; Misteli et al., 1997; Misteli and Spector, 1997; Wang et al., 1998).

Throughout the paper, we have emphasized a qualitative mathematical description of the onset of the compartmentalization. For this purpose, we introduced the following dynamical parameters with biological significance that are of interest for the experimentalists: effective diffusion coefficient ($D$), phosphorylation and dephosphorylation rates ($\rho$ and $\delta$), aggregative sensitivity ($\kappa$), and range of self-interaction ($\sigma$). Except for the effective diffusion coefficient of splicing factors, experimental estimates for these parameters are currently lacking. Although the estimation of parameters such as the phosphorylation and dephosphorylation rates, and the aggregative sensitivity represents a real challenge that may require the design of new experiments, the current biological information on splicing factors can be used to provide an idea of the magnitude of the range of self-interaction $\sigma$. In particular, we use the information on the dimensions of the cell nucleus and the number of speckles to estimate $\sigma$, as follows. We notice from table 1 that on a one-dimensional
space, a value of \( \sigma = 0.012 \) gives rise to ten speckles. If we were to introduce real dimensions, for example a longitude of 10 \( \mu \text{m} \) for the cell nucleus, this would mean that in order to obtain ten speckles, the biological range of self-interaction is 0.12 \( \mu \text{m} \), which is approximately a tenth to a third of the actual diameter of a speckle.

Although the model illustrates successfully a mechanism for the onset of the compartmentalization, the restriction given by (6) limits it from being able to describe the long-term behaviour for the aggregation of splicing factors. This has left us with an interesting modelling problem that is currently under investigation.

One of the basic assumptions of the aggregation-reaction-diffusion model is the existence of an underlying nuclear structure. Despite the existence of strong experimental evidence justifying this assumption (Capco et al., 1982; Hendzel et al., 1999; Kumaran et al., 2002; Nalepa and Harper, 2004; Nickerson, 2001), it remains a topic of controversy (Pederson, 2000b). For this reason, it would be worthwhile to develop alternative models for splicing factors compartmentalization, for example, models in which no underlying nuclear structure is considered. It is hoped that testing the predictions of a variety of models against experimental results ultimately will contribute towards determining the mechanism underlying the formation of nuclear speckles.

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References


Figure 1: An indirect immunofluorescence image of the "speckled" distribution of the splicing factor SC-35 in an Indian Muntjac Fibroblast cell nucleus. The image was obtained by staining cells with a primary antibody against SC-35 followed by a secondary antibody conjugated to the fluorophore Alexa 488.
Figure 2: Mechanism for the compartmentalization of splicing factors. The thin arrows describe the transient binding of splicing factors to a nuclear scaffold or matrix, and the thick arrows represent the phosphorylation and dephosphorylation that modulate the flux between the speckles and the nucleoplasm. The self-interaction among dephosphorylated splicing factors lead to their self-organization into speckles, whereas phosphorylated splicing factors don’t self-interact. Adapted from figure 3A in (Misteli, 2000).
Figure 3: Dispersion relation $\lambda(q^2)$ as a function of $q^2$ (equation (20)).
Figure 4: Evolution of the solution $u(x, t)$ of equation (13) for $\sigma = 0.04$ after a random perturbation of the steady state $u_{eq}$ (represented by the dots). In figure (A), the simulation describes the stable steady state $u_{eq} = 0.9 < 1$, and the solid curve represents the solution $u(x, t)$ for $t = 3$. In figure (B), the simulation describes the unstable steady state $u_{eq} = 1.1 > 1$, and the solid line represents the solution $u(x, t)$ for $t = 0.25$. 
Figure 5: Evolution of the solution $u(x,t)$ of equation (13) after a random perturbation (represented by the dots) of the steady state $u_{eq} = 1.1$. In figure (A), the solid curve represents the solution $u(x,t)$ for $\sigma = 0.02$ at $t = 0.05$. In figure (B), the solid line represents the solution $u(x,t)$ for $\sigma = 0.012$ at $t = 0.01$. 
Figure 6: Dispersion relation (20) for \( u_{eq} = 1.1 \) and three values of \( \sigma \): \( \sigma_1 = 0.04 \), \( \sigma_2 = 0.02 \), and \( \sigma_3 = 0.012 \).
Figure 7: Dispersion relation (39), with $\delta = 1$ and $\delta = 1.1$, when $\sigma = 0.05$, $\rho = 1$, and $v_{eq} + u_{eq} = 2$. For the case $\delta = 1.1$, the modes of instability are determined by wave numbers $q$ such that $q_{-}^{2} < q^{2} < q_{+}^{2}$, the dominating wave number is $q_{3} = 3\pi$ with a growth rate $\lambda(q_{3}^{2}) \approx 1.5$, and the fastest growing wave length is $l_{3} = 2/3$. 
Figure 8: Evolution of the solution \((v(x,t), u(x,t))\) of system (15), with \(\sigma = 0.05\), \(\rho = 1\), and \(\delta = 1.1\), from a random perturbation (represented by the dots) of the unstable steady state \((v_{eq}, u_{eq}) = (0.95, 1.05)\). The solid curves represent the solutions \(v(x,t)\) and \(u(x,t)\) at \(t = 4\).
Figure 9: Evolution of the solution \((v(x,t), u(x,t))\) of system (15), with \(\sigma = 0.05\), \(\rho = 1\), and \(\delta = 1\), from a random perturbation (represented by the dots) of the stable steady state \((v_{eq}, u_{eq}) = (1,1)\). The solid curves represent the solutions \(v(x,t)\) and \(u(x,t)\) at \(t = 4\).
Figure 10: Graphs of the curves $l(\delta)$ and $m(\delta)$ given by (44) and (46), respectively.
Figure 11: A: Evolution of the dispersion relation (39), with $\sigma = 0.05, \rho = 1, C = 2$, as $\delta$ passes through the bifurcation value $\delta_b \approx 1.06$. B: Bifurcation diagram for the stability of the steady state $(v_{eq}, u_{eq})$ as the dephosphorylation rate $\delta$ varies. The dotted portion of the line $v + u = 2$ represents unstable uniform steady states, the solid portion represents stable ones, and the filled circle represents the steady state at the bifurcation value $\delta_b$. 
Figure 12: Stability and instability regions for the resulting steady state \((v_{eq}, u_{eq})\), with \(\sigma = 0.05\). In figure A, \(\rho = 1\) and the curve that separates the stability regions is given by equation (47). In figure B, \(\rho > \frac{3(2 - 1)^2}{2\sigma^2}\) and the curve that separates the largest possible stability region to the instability region is given by equation (49).
Figure 13: Stability and instability regions in the $\rho \delta$-plane when $\sigma = 0.05$, $C = 2$ (figure A), and $C = 1.5$ (figure B). The solid curve $\delta = \delta_b(\rho)$ that separates the stable region to the unstable region is obtained according to equation (50).