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Modelling the compartmentalization of splicing factors

G. Carrero[a], M.J. Hendzel[b], G. de Vries[a,*]

[a]Department of Mathematical and Statistical Sciences, University of Alberta, Edmonton, AB, Canada T6G 2G1
[b]Department of Oncology, University of Alberta, Cross Cancer Institute, 11560 University Avenue, Edmonton, AB, Canada T6G 1Z2

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Abstract

Splicing factor (SF) compartments, also known as speckles, are heterogeneously distributed compartments within the nucleus of eukaryotic cells that are enriched in pre-mRNA SFs. We derive a fourth-order aggregation–diffusion model that describes a possible mechanism underlying the organization of SFs into speckles. The model incorporates two hypotheses, namely (1) that self-organization of dephosphorylated SFs, 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 SFs. A linear stability analysis about homogeneous steady-state solutions of the model reveals how the self-interaction among dephosphorylated SFs 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 SFs.

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 subnuclear 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 (SFs). SFs are nuclear proteins that remove introns (non-coding 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, SFs are concentrated in approximately 25–50 clusters; during mitosis, these clusters disassemble. These membraneless clusters or aggregates of SFs, which are heterogeneously distributed in a "speckled" pattern in the nucleus (see

*Corresponding author. Tel.: +1 780 492 4561; fax: +1 780 492 6826.
E-mail address: devries@math.ualberta.ca (G. de Vries).

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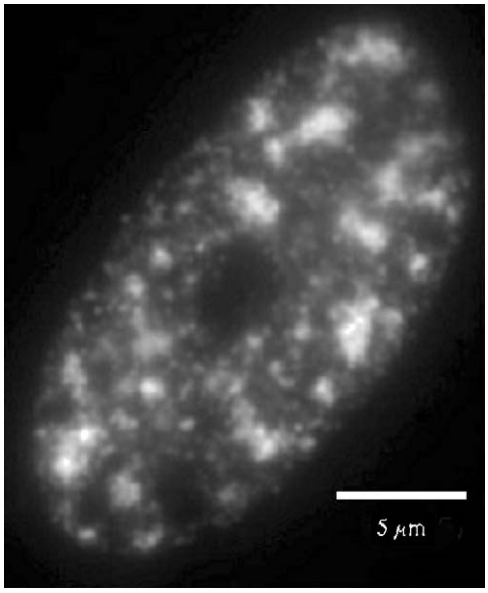

Fig. 1. An indirect immunofluorescence image of the "speckled" distribution of the splicing factor SC-35 in an Indian Muntjac Fibroblast cell nucleus. The image was obtained by staining cells with a primary antibody against SC-35 followed by a secondary antibody conjugated to the fluorophore Alexa 488.

Fig. 1), are called Splicing Factor Compartments (SFCs) 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 SFs. In other words, the organization of SFs must be highly dynamic. Indeed, fluorescence microscopy experiments have shown that (1) SFs 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) SFs move randomly throughout the cell nucleus (Pederson, 2000a; Phair and Misteli, 2000).

These dynamical aspects have brought forth two current biological hypotheses for SF compartmentalization, one relating to the role of phosphorylation and dephosphorylation in the formation and disassembly of SFCs, 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 SFs containing a carboxy-terminal domain rich in arginine–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 SFs from speckles and the disassembly of SFCs (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 SFs to SFCs requires the presence of specific phosphatases responsible for the removal of a phosphate group (Misteli and Spector, 1996, 1997). Moreover, the unphosphorylated state of SFs enhances their self-interaction (binding), whereas the phosphorylated state diminishes it (Xiao and Manley, 1998). Understanding the role of phosphorylation in the location of SFs 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 SF compartmentalization: self-organization is responsible for the formation of speckles, and phosphorylation and dephosphorylation modulate this organization.

Second, measurements of the mobility of SFs 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 SFs 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 SF compartmentalization: the existence of an underlying nuclear structure is a major determinant of the organization of SFs (Hendzel et al., 1999; Kumaran et al., 2002; Nickerson, 2001).

The dynamical aspects of SFs and their heterogeneous distribution in speckles provide strong evidence that there is more to the spatio-temporal dynamics of SFs than just simple diffusion. To unravel the mechanism underlying the organization of SFs, we incorporate the two existing biological hypotheses for SF 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 SFs (see Fig. 2). We use the model to suggest answers to a number of fundamental questions about SFCs (Lamond and Spector, 2003; Misteli, 2000, 2001a). What is the detailed mechanism of SF compartmentalization? What controls the concentration of SFs inside and outside the speckles? Is speckle formation initiated randomly? What determines the number and size of SFCs?