

# An *In-Silico* Mammalian Whole-Cell Model Reveals the Influence of Spatial Organization on RNA Splicing Efficiency

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**Spatial organization is a characteristic of eukaryotic cells, achieved by utilizing both membrane-bound and non-bound organelles. We model the effects of this organization and of organelle heterogeneity on RNA splicing (the process of making translationally-ready messenger RNA) and on splicing particles (the building blocks of splicing machinery) in mammalian cells. We constructed a spatially-resolved whole HeLa cell model from various experimental data and developed reaction networks to describe the RNA splicing processes. We incorporated these networks into our whole-cell model and performed stochastic simulations for up to 15 minutes of biological time. We find that the number of nuclear pore complexes affects the number of assembled splicing particles; that a slight increase of splicing particle localization in nuclear speckles (non-membrane-bound organelles) leads to disproportionate enhancement in the mRNA splicing and reduction in the transcript noise; and that compartmentalization is critical for a correctly-assembled particle yield. Our model also predicts that the distance between genes and speckles has a considerable effect on effective mRNA production rate, further emphasizing the importance of genome organization around speckles. The HeLa cell model, including organelles and subcompartments, provides an adaptable foundation to study other cellular processes which are strongly modulated by spatio-temporal heterogeneity.**

Human whole-cell modeling | RNA splicing | Stochastic reaction-diffusion simulations

Cells use spatial organization to mediate the complex biochemical reaction networks. Although membranes have long been recognized as means to confine organelle-specific compounds, non-membrane-bound organelles are increasingly found to play crucial roles in cellular functions (1). These organelles can be formed as liquid-liquid phase separated regions and are therefore also known as liquid droplets (2). Cells may have numerous such liquid droplets that form either in the cytoplasm or the nucleus (3, 4). Each droplet is involved in specific cellular processes. As a prime example, nuclear speckles, or interchromatin granules, are droplets formed in the nucleus that are thought to be primarily involved in pre-mRNA splicing (5).

RNA splicing has evolved in eukaryotic cells to allow cell complexity without massively increasing gene count. Instead, the structure of genes changed such that the coding regions (exons) are interrupted by non-coding regions (introns) (6). Coding regions must then be ligated, to form functional transcripts. There are, on average, eight introns per gene (7), so coding regions can be shuffled after the removal of all or a subset of introns by a process called alternative splicing. The order of intronic removal defines the function of the protein coded by the transcript; thus, a single gene can encode a variety of functionalities (8). Spliceosome is the cellular

machinery that binds to the intron/exon sites, removes the introns and joins the exon ends. It is a multi-megadalton complex consisting of five (uridine rich) protein-RNA small nuclear ribonucleoprotein (snRNP) complexes: U1 snRNP, U2 snRNP, U4 snRNP, U5 snRNP and U6snRNP (9). The biogenesis of splicing particles (snRNP complexes) occurs in multiple steps in both nucleus and cytoplasm, and finalizes in the nucleus (6). The mature splicing particles then localize in nuclear speckles and assemble on the pre-messenger RNA (pre-mRNA) transcripts in a coordinated and step-wise fashion, and upon completion of the splicing reaction, they disassemble (10).

Klingauf et al. showed that the association of splicing particles, U4snRNP and U6snRNP in Cajal bodies (another type of phase-separated nuclear regions) is enhanced compared to the assembly reactions taking place throughout the nucleus, pointing to the importance of cellular organization even at the sub-nuclear level (11). Chang and Marshall proposed in a commentary that organelle heterogeneity can also lead to cellular phenotypic behavior, similar to the heterogeneity at the molecular level (12). Additionally, a very active effort is underway to determine the cellular organization, such as the one carried out by Johnson et al. on human induced pluripotent stem cells, from a rich set of cellular fluorescence images (13).

Although the basic utility of nuclear speckles in pre-mRNA

## Significance Statement

The spliceosome is one of the most complex cellular machineries that cuts and splices the RNA code in eukaryotic cells. It dynamically assembles, disassembles, and its components are formed in multiple compartments. The efficiency of splicing process depends on localization of its components in nuclear membrane-less organelles. Therefore, a computational model of spliceosomal function must contain a spatial model of the entire cell. However, building such a model is a challenging task, mainly due to the lack of homogeneous experimental data and a suitable computational framework. Here, we overcome these challenges and present a whole HeLa cell model, with nuclear, subnuclear, and extensive cytoplasmic structures. The three-dimensional model is supplemented by reaction-diffusion processes to shed light on the function of the spliceosome.

Z.G, J.R.P., M.G. and Z.L-S. designed research; Z.G. and J.R.P. performed research; Z.G. analyzed data; and Z.G, J.R.P., M.G. and Z.L-S. wrote the paper. All authors read and approved the manuscript.

Please declare any conflict of interest here.

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51 splicing is already appreciated, the influence of spatial lo- 111  
52 calization on splicing activity and mRNA production is not 112  
53 understood at a quantitative level. The effect of variations 113  
54 in the involved organelles has not been investigated either.  
55 Here, we construct a 18- $\mu\text{m}$  spatially-resolved model of a 114  
56 whole mammalian cell, specifically a HeLa cell, from a library 115  
57 of experimental data such as cryo-electron tomography (14), 116  
58 mass spectrometry (15), fluorescent microscopy and live-cell 117  
59 imaging (11, 16–18) and -omics data (19, 20). We simulate our 118  
60 eukaryotic cell with organelles, compartments and biomacro- 119  
61 molecules, within the framework of reaction-diffusion master 120  
62 equations with Lattice Microbes software (21, 22) for up to 15 121  
63 minutes of biological time. Our simulations explore how cellu- 122  
64 lar organization affects the efficiency of spliceosomal particle 123  
65 formation and pre-mRNA splicing. Specifically, we find that 124  
66 even a slight increase in the relative localization of splicing  
67 particles in nuclear speckles can both enhance mRNA pro-  
68 duction and reduce its noise. Additionally, we rationalize the  
69 biological selection of design parameters of nuclear speckles,  
70 specifically, their size and number. Finally, we predict that  
71 the organization of active genes around nuclear speckles can  
72 affect mRNA production.

## 73 Results and discussion

74 **Spatially-resolved model of a HeLa cell.** We used a data-driven  
75 approach to construct a representative HeLa whole-cell model  
76 that has not been available thus far. First, we gathered struc-  
77 tural and -omics information from a variety of experimental  
78 studies (11, 14–20, 23–26). Then, the assembled data was  
79 ensured to be consistent with protein composition percentages  
80 of HeLa cell organelles determined by mass spectrometry (15)  
81 (see Methods for details). On average, proteins are composed of  
82 similar C/N/O/H ratios, hence mass percentages of organelles  
83 are approximately similar to volume percentages.

84 Figure 1-A and B, show the overall HeLa cell model and  
85 a more detailed view of the nuclear region. Assuming exper-  
86 imental growth conditions resulting in spherically-shaped  
87 cells (25, 27), a volume of 3000  $\mu\text{m}^3$  (23) leads to a cellular  
88 radius of 8.9  $\mu\text{m}$ . The essential components of the cell include:  
89 plasma membrane, cytoplasm, endoplasmic reticulum (ER),  
90 mitochondria, Golgi apparatus and nucleus. The ER units  
91 were modeled by stochastic shapes using a cellular automata  
92 algorithm (see Supplementary Information for details and al-  
93 gorithm). The ER units are distributed in the cytoplasm,  
94 spanning from the nuclear envelope to the plasma membrane,  
95 and are intertwined with other cytoplasmic organelles (28, 29).  
96 The ER units make up  $\sim 4.5\%$  of the cell volume (15). About  
97 2000 rod-shaped mitochondria with dimensions of  $0.6 \mu\text{m} \times$   
98  $0.49 \mu\text{m}$  were randomly placed throughout the cytoplasm,  
99 filling  $\sim 11\%$  of the total volume (15). A Golgi apparatus  
100 consisting of five stacked sheets, each with a thickness of  $0.128$   
101  $\mu\text{m}$ , was placed close to the nucleus (30).

102 The nucleus, which plays a critical role in our model, has  
103 a radius in the range of  $3.74\text{--}5.29 \mu\text{m}$  (24–26). It consists of  
104 nuclear pore complexes (NPCs) of  $0.083 \mu\text{m}$  radii (14) and  
105 a density of 7 per  $\mu\text{m}^2$  (16), 20 spherically-shaped nuclear  
106 speckles with  $0.35 \mu\text{m}$  radii (18) and 4 Cajal bodies with  $0.5$   
107  $\mu\text{m}$  radii (11). Active genes (black dots in Figure 1-B) were  
108 placed around the speckles (31–33). The nuclear components  
109 were chosen mainly among those that play a role in RNA  
110 splicing processes. Cellular components included in the *in-*

111 *silico* model are listed in Table 1 along with their dimensions.  
112 The details of the construction of each organelle are provided  
113 in the Methods section.

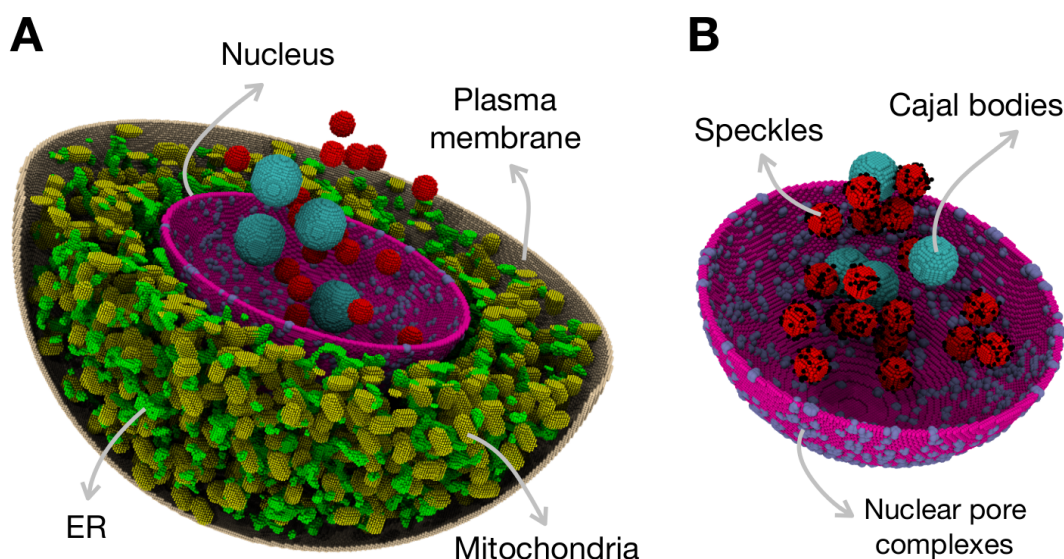
**The kinetic model for spliceosome formation and action.** We  
114 studied two processes: first, the formation of splicing particles  
115 (U1snRNP and U2snRNP) which is a multi-compartmental  
116 process and second, the spliceosome assembly, splicing reaction  
117 and generation of mRNA transcripts. Together, these capture  
118 the whole process of splicing from machinery construction  
119 to functional transcript production. After the assembly of  
120 U1snRNP and U2snRNP in our model (the first process),  
121 the pre-mRNA transcripts are spliced (the second process)  
122 according to the following reduced scheme for the spliceosome  
123 assembly:  
124

- 125 1. An active-28 Kb gene is transcribed and pre-mRNA tran-  
126 scripts are produced
- 127 2. U1snRNP and U2snRNP particles are formed and are  
128 present in the cell nucleus
- 129 3. U4/U6.U5 trisnRNP particles are also present in the  
130 nucleus; Because of the complexity in the formation of  
131 these complexes we assumed they are pre-formed in our  
132 model (34).
- 133 4. The spliceosome assembles in a stepwise manner on pre-  
134 mRNA transcripts
- 135 5. After splicing occurs, the pre-mRNA is converted to an  
136 mRNA transcript
- 137 6. The spliceosome disassembles after splicing, ready to as-  
138 semble on another transcript

139 Below, we describe in details the splicing assembly and reaction  
140 and splicing particles formation.

**Formation of splicing particles.** A splicing particle consists of a  
141 uridine-rich small nuclear RNA (U snRNA) that is bound to  
142 a heptamer ring of proteins, called Smith proteins (Sm) and  
143 variable numbers of particle-specific proteins. The formation of  
144 splicing particles happens in multiple steps and compartments.  
145 To understand the effects of geometry on the formation process,  
146 we developed a kinetic model to describe these processes and  
147 studied them in our developed spatially-resolved HeLa cell  
148 model.  
149

150 Figure 2 shows the steps associated with the formation of  
151 splicing particles and the reactions are summarized in Table 2  
152 in Methods. Upon transcription, U1(2) snRNA has to pass  
153 through nuclear pore complexes to reach cytoplasm, where  
154 by a series of complex reactions they bind to Sm proteins.  
155 Inspired by two studies (35, 36), we proposed the following  
156 mechanisms for the cytoplasmic part of the process: U1(2)  
157 snRNA transcript binds to Gemin 5 ( $G^5$ ) which is part of  
158 the survival of motor neurons (SMN)-Gemin complex that  
159 mediates the Sm proteins assembly on snRNA. The formed  
160 complex then binds to a ring of five already-assembled Sm  
161 proteins ( $Sm^5$ ) through a process called RNP exchange sug-  
162 gested by Ref. (36). This process facilitates the Sm proteins  
163 binding to the snRNA transcript and the release of  $G^5$ . In  
164 the last step, the remaining Sm proteins ( $Sm^2$ ) joins the com-  
165 plex and the  $U1(2)snRNA.Sm^7$  complex is formed. After



**Fig. 1.** A data-driven model for a 18  $\mu\text{m}$  HeLa cell: A) cytoplasmic components are: ER, mitochondria and Golgi (not shown); and B) nucleus containing nuclear pore complexes, Cajal bodies and nuclear speckles.

| Component              | Dimension ( $\mu\text{m}$ ) | Number                 | Reference   |
|------------------------|-----------------------------|------------------------|-------------|
| HeLa cell              | R = 8.9                     | 1                      | (23)        |
| Nucleus                | R = 3.74, 4.15, 4.67, 5.29  | 1                      | (15, 24–26) |
| Nuclear pore complexes | R = 0.083                   | 1230, 1515, 1918, 2461 | (14, 16)    |
| Mitochondria           | $0.6 \times 0.49$           | 2000                   | (17)        |
| Nuclear speckles       | R = 0.35                    | 20                     | (18)        |
| Cajal bodies           | R = 0.5                     | 4                      | (11)        |
| ER                     | –                           | 4.5% cell volume       | (15)        |

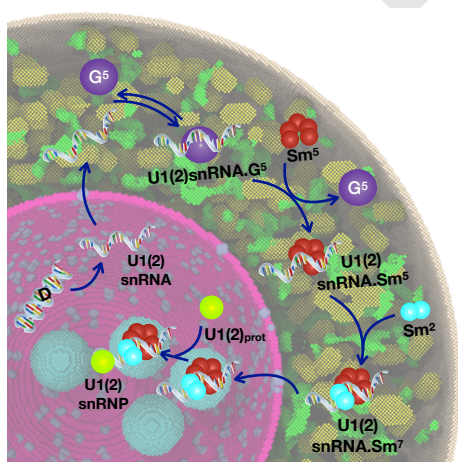
**Table 1.** The cellular components of the constructed HeLa model

166 the completion of the binding of Sm proteins on snRNA, the  
 167 complex again pass through the NPCs and make its way to  
 168 the nucleus. At nucleus, the  $U1(2)snRNA.Sm^7_{nuc}$  complex  
 169 localizes to the Cajal bodies (37) and binds to particle-specific  
 170 proteins;  $U1(2)_{prot}$ , and the mature splicing particle is formed.

the splicing particle formation reactions were mainly adopted  
 from various experimental sources and are listed in Table S2.

**Assembly of the spliceosome and splicing reaction.** The assembly  
 process of the spliceosome machinery is entangled with a  
 complex network of auxiliary and regulatory proteins that  
 detect the splice site and alternate the splice sites according  
 to cellular cues by a process called alternative splicing (8).  
 To simplify this network, we assume that a particular splice  
 site has been chosen and focus only on the assembly of the  
 spliceosomal particles on that site and the splicing reaction.

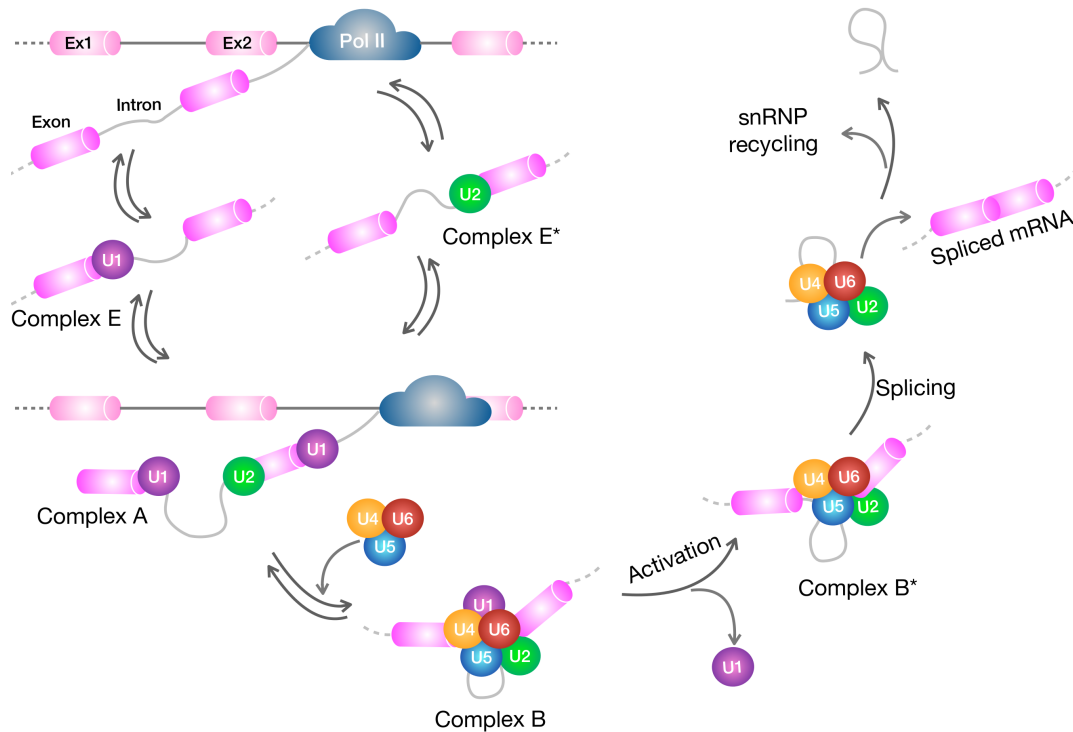
Figure 3 depicts our model for splicing reaction; and the  
 details of the reactions and their associated rates are represented  
 in Table 3 in Methods. According to the conventional spli-  
 coesome assembly model (38), the U1snRNP particle binds  
 to the 5' end of the exon ("complex E"), following by binding  
 of the U2 particle to the associate 3' end to form "complex A".  
 To make a more realistic model, we added an additional initial  
 reaction as suggested by Ref. (39): the U2snRNP can bind the  
 pre-mRNA before U1snRNP, making "complex E\*". Regard-  
 less of the binding order of these splicing particles, a viable  
 complex A is formed that can continue the remaining assem-  
 bly process. The "tri.U" (U4/U6 bound to U5) then joins the  
 complex forming "complex B". Subsequently, U1snRNP leaves  
 the complex for the catalytically active "complex B\*". The  
 intron is then removed and the splicing particles are recycled  
 for another round of assembly.



**Fig. 2.** Reaction scheme for describing the formation of U1 and U2 splicing particles mapped on a cross-section of our *in-silico* HeLa cell (35, 36). The four spherically-shaped regions in cyan color are Cajal bodies.

171 The diffusion coefficients for the species that are involved in





**Fig. 3.** Splicing reactions as implemented in our simulations. The reactions together with their corresponding rate constants are shown in Table 3. Abbreviations are: Pol II (RNA polymerase II), Ex1 and 2 (Exon 1 and 2).

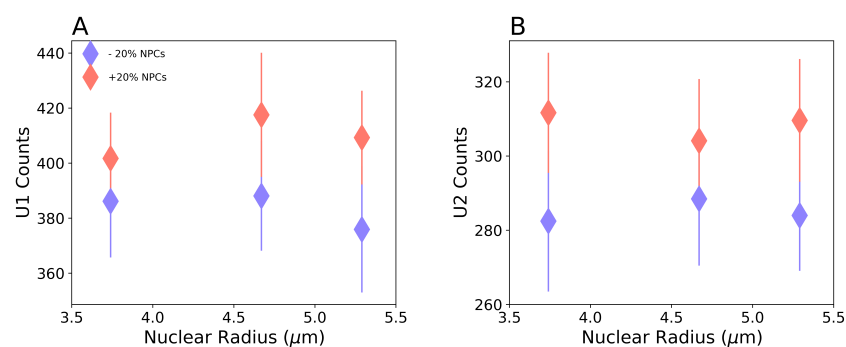
198 **Co-transcriptional splicing** Splicing is known to be overwhelmingly  
 199 co-transcriptional; meaning, as transcription is occurring,  
 200 the spliceosome assembles on the transcribed pre-mRNA and  
 201 splicing reactions begin. In our model, an average gene consist-  
 202 ing of 8 introns and an intron length of 3.4 Kbase (plus 137  
 203 base for each exon) is considered (7). After the transcription  
 204 of the first exon-intron-exon piece, the splicing reaction starts  
 205 as discussed above. Simultaneously, another intron-exon pair  
 206 is transcribed, continuing the spliced transcript. The cycle  
 207 repeats till the end of the gene.  
 208 The diffusion coefficients of spliceosomal particles are also  
 209 listed in Table S3.

210 **Organelle heterogeneity influence the formation of splicing**  
 211 **particles.** Cellular phenotypic behavior arising from organelle  
 212 heterogeneity is a subject worthy of study (12). We investi-  
 213 gated how heterogeneities in NPC count and nuclear size  
 214 affect the formation of splicing particles. All splicing particles,  
 215 except U6, are complexes of uridine-rich small nuclear RNA  
 216 bound to a heptameric ring of Smith (Sm) proteins, along with  
 217 specific proteins that bind to each splicing complex. Among  
 218 the five particles which are required for spliceosome function,  
 219 we focus on the first two (U1snRNP and U2snRNP) that start  
 220 the spliceosome assembly. As shown in Figure 2 and described  
 221 above, these particles are formed in a multi-compartmental  
 222 process (6). Because, the components of splicing particles  
 223 have to assemble in both nucleus and cytoplasm, therefore,  
 224 translocation through the NPCs is a critical step. Live cell  
 225 imaging showed that NPC count varies (by 10%) (16), and so  
 226 does nuclear size (15, 24–26). We posited that these variations  
 227 could influence the formation of splicing particles, which we  
 228 tested by varying NPC count and nuclear size, and examining

the effect on the number of particles formed after 30 seconds  
 of biological time. Figure 4 shows that increasing (decreasing)  
 the number of NPCs by 20% results in an increase (decrease)  
 in the number of mature U1 and U2 splicing particles. This  
 effect is consistent across the tested nuclear radius with range  
 of 3.74–5.29  $\mu\text{m}$  (15, 24–26). It is found that the number of  
 splicing particles formed does not change significantly with  
 nuclear size. This can be explained by the fact that a larger  
 nucleus has a larger number of NPCs, since the density of  
 NPCs is constant. Consequently, longer diffusion times in a  
 larger nucleus are compensated by shorter translocation times  
 required when there are more NPCs.

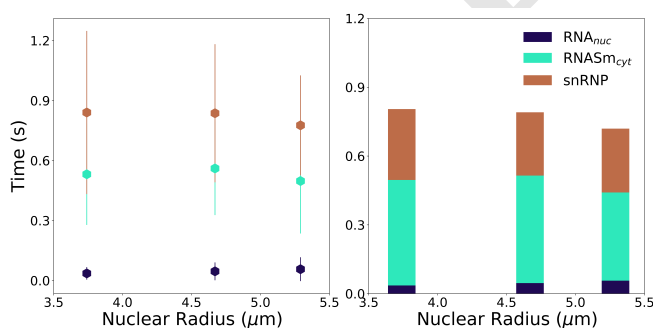
To obtain insight into the formation of splicing particles, we  
 dissected the overall kinetics of the process in terms of discrete  
 reactions occurring in each compartment, i.e., nucleus and  
 cytoplasm. These reactions include the transcription of snRNA  
 and formation of (*U1snRNA<sub>nuc</sub>*), cytoplasmic production of  
*U1snRNA* · Sm<sup>7</sup>, and finally the assembly of mature U1snRNP.  
 We determined the timescale for the formation of each of these  
 three species within the first assembled U1 particle. As shown  
 in Figure 5, the series of cytoplasmic reactions take the longest  
 to complete, irrespective of nuclear size. The timescale for  
 cytoplasmic reactions is also statistically similar regardless of  
 whether the ER or mitochondria or both are absent: for a full  
 cell the time is  $0.68 \pm 0.30$  s, as compared to  $0.63 \pm 0.35$  s,  
 $0.69 \pm 0.24$  s and  $0.59 \pm 0.21$  s, respectively for cases where  
 the cell model lacks ER, mitochondria, or both.

As mentioned above, in higher eukaryotes, different compo-  
 nents of the splicing particles join the assembly in different  
 compartments (6). This separation likely allows for higher quality  
 control and prevents mixing of the partially-assembled parti-  
 cles with their substrates, thus preventing partially formed



**Fig. 4.** Spliceosomal particle formation depends on NPC count. Increase (pink) or decrease (blue) in the number NPCs by 20% results in a corresponding change in the number of U1 (A) and U2 (B) particles formed. The effect is consistent for different nuclear sizes. P-values for U1 results are:  $1.4 \times 10^{-2}$  (3.74  $\mu\text{m}$ ),  $1 \times 10^{-4}$  (4.67  $\mu\text{m}$ ),  $9.6 \times 10^{-4}$  (5.29  $\mu\text{m}$ ); and for U2 results are:  $9.5 \times 10^{-6}$  (3.74  $\mu\text{m}$ ),  $8.3 \times 10^{-3}$  (4.67  $\mu\text{m}$ ),  $1.3 \times 10^{-5}$  (5.29  $\mu\text{m}$ ). The cellular geometry in these simulations is the same as described in Table 1, except for the ER volume, which is  $\sim 7\%$  of the cell volume. However, the slightly higher occupancy is not known to have a considerable effect. Error bars represent the standard deviations. For each condition, 20 simulation replicates were performed.

spliceosomes from deleteriously modifying pre-mRNAs. We examined the importance of multi-compartmentality by allowing all particle assembly steps to occur solely in the nucleus. We postulated that the latter modification may result in snRNA binding to proteins in an incorrect order, or, in incomplete assembly of the particle. In addition to confining assembly steps to the nucleus, we also modified the reaction network and added an extra reaction ( $U1snRNA \cdot Sm^5 + U1_{prot} \rightarrow U1RNA \cdot Sm^5$ ) to the set of reactions shown in Table 2, to account for misassembled splicing particles. The assembly of Sm-core is followed by RNA modification that triggers the nuclear import of the snRNA bound to Sm core (6). Therefore, in the multi-compartmental assembly process of splicing particles the  $U1snRNA \cdot Sm^5$  complex is not found in the nucleus. As an outcome of simulating the nuclear assembly of the splicing particles, we found that although the system can make fully assembled splicing particles, it produces significantly more misassembled particles ( $771 \pm 25$ ) as compared to mature particles ( $248 \pm 15$ ), since the former are not required to go through the full assembly cycle (see Figure 2). This simulation result demonstrates the critical need for the compartmentalization of the overall assembly of the splicing particles. Similar multi-compartmental processes have been observed for other cellular machines such as ribosomal subunits (6).



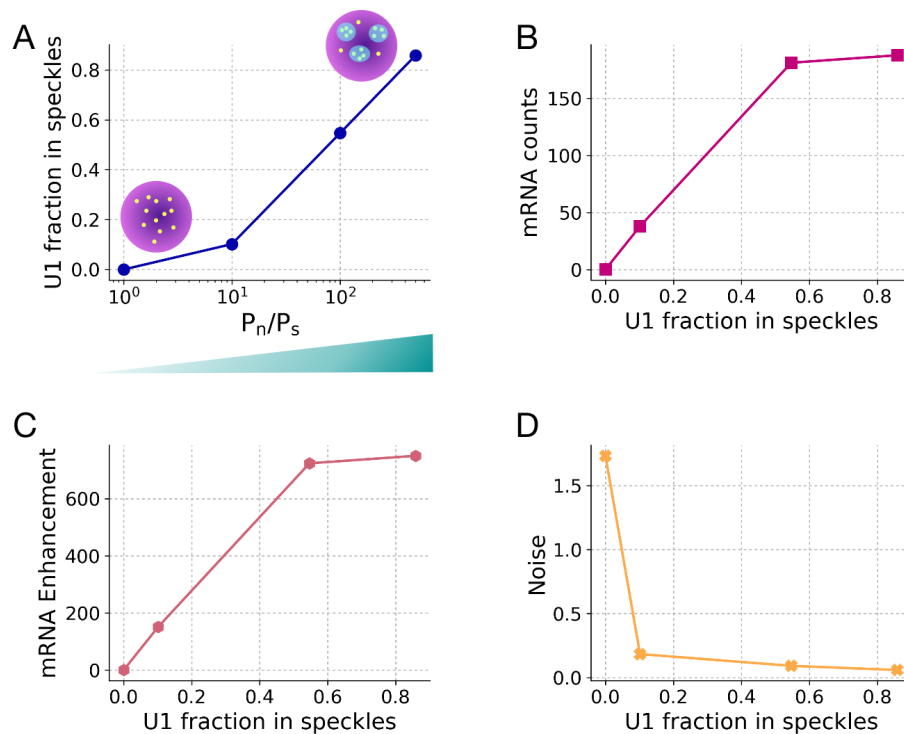
**Fig. 5.** The time required to form the first splicing particle dissected by the set of discrete reactions occurring in the nucleus and cytoplasm. Error bars represent the standard deviations. For each condition, 20 simulation replicates were performed.

285

**Nuclear speckles enhance effective splicing rate and control noise in mRNA.** Nuclear speckles are self-organized liquid droplets that are known to act as stores for splicing particles in addition to other processes such as DNA repair and RNA modifications (5, 18). There is evidence that a subset of splicing

occurs within or at the periphery of these nuclear bodies (40). Nuclear speckles, being liquid-liquid phase-separated regions, promote certain biochemical reactions, which is suggested to be due to an enhanced concentration of the reactants (2). To examine this phenomenon, we developed a reaction network to account for spliceosome assembly on pre-mRNA transcripts and splicing reaction described in details previously (see Figure 3). This network was included in stochastic simulations containing speckles in the HeLa cell and we determined the resulting effect on mRNA production and noise. The speckles were comprised of a concentrated store of splicing particles produced in the following manner (see Methods section). Briefly, we set the probability,  $P_n$ , for splicing particles to transit from the nucleus into the speckles higher than the probability,  $P_s$ , for the reverse transition. We found that the higher this imbalance ( $P_n/P_s$ ) is, the greater the degree of localization of splicing particles in the speckles (see Figure 6-A). We compared mRNA production in cells with different degree of splicing particle localization in the speckles and also that in a control cell containing no speckles but with splicing particles randomly distributed throughout the nucleus. Relative to the case of no speckles (U1 fraction = 0 in Figure 6), a cell with about 10% of U1 located in speckles showed a large enhancement in the number of spliced mRNA transcripts from 0.25 to 40, which is effectively a  $\approx 150$ -fold amplification (Figure 6-B,C). Thus, even a slight increase in the localization of splicing particles enhances mRNA production. The mRNA production continues to grow with further increase in the splicing particle localization up to an enrichment level of  $\approx 55\%$  U1 in speckles. Beyond this point, little increase in average mRNA count is observed (Figure 6-C). Alongside these trends, we examined the effect of speckles on the noise associated with mRNA production, estimated in terms of the coefficient of variation,  $\eta$ , which is the ratio of average mRNA counts to the standard deviation. As the percentage of splicing particles in speckles increases (Figure 6-D), the noise decreases. Thus, nuclear speckles not only enhance splicing activity, but they also help limit the noise that splicing introduces into the whole gene expression process. Using green fluorescent protein labels, Rino et al. determined the ratio of splicing protein U2AF in speckles to that in the nucleus to be  $1.27 \pm 0.07$  (41). Strikingly, this experimentally determined ratio corresponds in our model to a  $P_n/P_s = 100$  (Figure 6-A), which is effectively a  $\sim 55\%$  localization of splicing particles in speckles, the point at which the mRNA production has maximized.

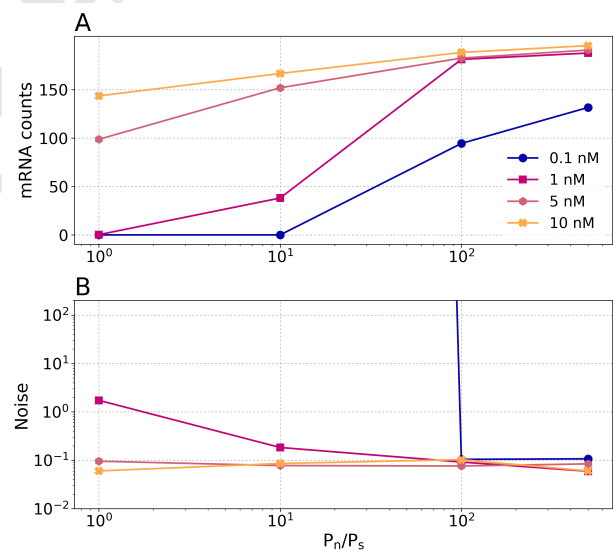
**Speckle-enhanced splicing is concentration-dependent.** The number of splicing particles required per pre-mRNA transcript



**Fig. 6.** Splicing efficiency increases in the presence of speckles in the cells: A) The higher the probability for the splicing particles to transition from the cell nucleus to the speckles, relative to the reverse transition, the higher is the localization of splicing particles in speckles. Schematically, the randomly distributed splicing particles (yellow dots) in the cell nucleus (colored in purple), localize in nucleus speckles (blue shaded regions) as the probability imbalance increases. B) As the percentage of splicing particles located in speckles increases, the number of spliced mRNA also increases. C) This enhancement in mRNA production is highly sensitive to the localization of splicing particles in speckles: with only a 10% localization of splicing particles in speckles, the splicing reaction is enhanced  $\sim 150$ -fold relative to the case with no speckles. D) Noise (average of mRNA counts/the standard deviation) decreases as a greater percentage of splicing particles are localized in speckles. For each condition, 20 simulation replicates were performed.

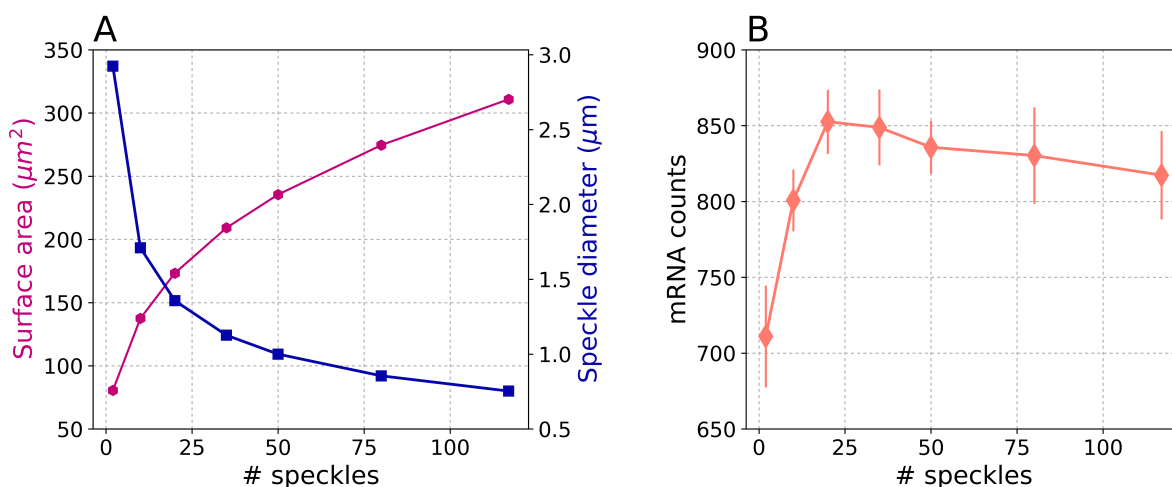
338 is a function of many variables including the rate of transcription  
 339 (7) and therefore this number may vary from one  
 340 gene to another. We investigated how variation in the ratio  
 341 of splicing particles to pre-mRNA transcripts affects overall  
 342 mRNA transcript production and noise in a cell with nuclear  
 343 speckles. Specifically, for 20 constitutively transcribing genes,  
 344 we changed the number of particles available for pre-mRNA  
 345 binding and splicing from 16 to 1600 corresponding to a con-  
 346 centration range of 0.1–10 nM. The remainder of the total  $10^5$   
 347 splicing particles (42) were bound to pre-mRNA transcripts  
 348 and actively splicing. Figure 7-A summarizes how the con-  
 349 centration of U1 splicing particles affects the ability of speckles  
 350 to enhance splicing. At 1 nM U1, mRNA production in a cell  
 351 with speckles (with  $P_n/P_s = 500$ ) is  $\approx 750$ -fold that for a cell  
 352 with no speckles. At 10 nM, this enhancement factor reduces  
 353 to 1.4-fold. Thus, at lower concentrations of U1, speckles  
 354 enhance splicing much more strongly. Consistently, as figure 7-B  
 355 shows, the noise of mRNA production is also influenced. At 1  
 356 nM U1, the noise in the presence of speckles is  $\approx 30$ -fold  
 357 lower as compared to a cell with no speckles; whereas, at 10 nM,  
 358 the noise is unaffected by whether speckles are present or not.

359 **Speckle size and number have been fine-tuned by cells to opti-**  
 360 **mize mRNA production.** We investigated how a cell decides  
 361 the number and the size of nuclear speckles, after the ded-  
 362 ication of certain percentage of its nuclear volume to these  
 363 organelles. We hypothesize that the experimentally observed  
 364 anatomy of the speckles is optimized by the cells. To test  
 365 this hypothesis, we assigned about 10% of the nuclear vol-  
 366 ume to speckle (20) and keeping the total volume of speckles  
 367 constant, we increased the number of speckles and reduced  
 368 their sizes, as shown in Figure 8-A. Increasing the number of  
 369 speckles results in increasing the surface area (Figure 8-A),  
 370 which in turn enhances the pre-mRNA splicing, as shown in  
 371 Figure 8-B. This is because, the higher surface area, increases



**Fig. 7.** Splicing particle concentration affects the functional advantage of speckles: A) Enhancement in mRNA production due to the presence of speckles, depends on the U1 splicing particle concentration. B) Effect of the U1 splicing particle concentration on the mRNA production noise. For each condition, 20 simulation replicates were performed.

372 the probability of splicing particles to diffuse into the nuclear  
 373 speckles resulting in increased localization. However, beyond  
 374  $\sim 50$  speckles the number of produced mRNA plateaus which  
 375 could be due to the compensation of splicing particle localiza-  
 376 tion by relatively smaller-sized nuclear speckles. Production  
 377 of mRNA was maximized when there were between 20 and  
 378 50 speckles, which coincides directly with the experimentally  
 379 determined values (18). In addition, the size of the nuclear  
 380 speckles corresponding to the maximum mRNA production



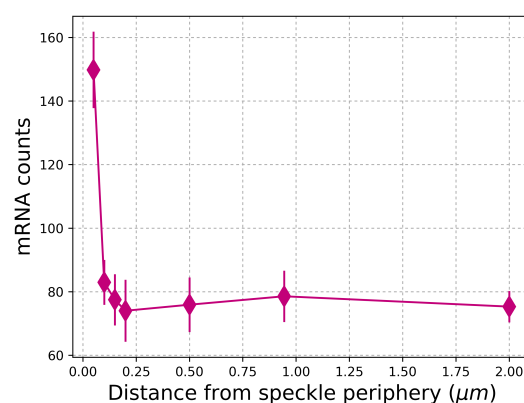
**Fig. 8.** A) Increasing the number of nuclear speckles, results in an increase of the surface area (magenta curve) and decrease of the speckles diameter (blue curve); B) mRNA production increases as the number of speckles increases till about 50 speckles beyond which the production plateaus. Error bars represent the standard deviations. For each condition, 20 simulation replicates were performed.

381 falls between 1.4 to 1  $\mu\text{m}$ , which is also compatible to the  
 382 known nuclear speckles diameters of one to a few microns (18).  
 383 Therefore, our results suggest that it is plausible that the cells  
 384 optimize the design parameters of speckles (the number and  
 385 size) to maximize the mRNA production.

386 **Gene distribution around speckles affect transcript splicing  
 and mRNA production.** It is known that genes are organized  
 387 nonrandomly around nuclear speckles (31, 32). In a recent  
 388 study, Chen et al. investigated the organization of whole  
 389 genome using TSA-seq method (33). They showed that the  
 390 most highly expressed genes are located between  $\approx 0.05$  and  
 391  $0.4 \mu\text{m}$  from the periphery of a speckle. They also speculated  
 392 that the genome movement of several hundreds of nano meter  
 393 from nuclear periphery towards speckles could have functional  
 394 significance. To test their hypothesis, we investigated the  
 395 effects of active genes distribution around speckles' periphery.  
 396 We varied the gene distance from  $0.054$  to  $2 \mu\text{m}$  and observed  
 397 the effects on the number of spliced mRNA transcripts found in  
 398 cytoplasm. As Figure 9 demonstrates, increasing the distance  
 400 of the genes to speckle periphery from  $\approx 0.05$  to  $0.2 \mu\text{m}$   
 401 sharply decreases the mRNA counts by a factor of 2, with  
 402 no further significant decrease at larger distances. Thus, the  
 403 effect can be even more pronounced over a short distance range  
 404 than they were able to resolve. Considering the fact that our  
 405 speckle model does not involve any active recruiting of the  
 406 pre-mRNA transcripts, nor do our speckles move toward an  
 407 active transcription site, the observed effect is mainly due  
 408 to the diffusion of the transcripts in the nucleus before they  
 409 become associated with the speckles. Our model predicts that  
 410 the gene distribution around speckles has an effect on mRNA  
 411 splicing. It is plausible that this effect might be regulated  
 412 by speckle movement towards transcriptionally active genes,  
 413 consistent with the fluid nature of these nuclear bodies.

## 414 Conclusions

415 Spatial organization is one of the key features of eukaryotic  
 416 cells that brings order to complex biochemical reactions. We  
 417 studied two aspects of this organization both in connection



**Fig. 9.** Our model predicts that the mRNA production decreases by a factor of two, above  $0.054 \mu\text{m}$ . Error bars represent the standard deviations. For each condition, 20 simulation replicates were performed.

418 with RNA splicing (the process of removal of non-coding re-  
 419 gions from pre-mRNA transcripts). We investigate, firstly,  
 420 the effects of cellular organelles on assembly of the building  
 421 blocks of splicing; and secondly, mRNA production at nu-  
 422 clear speckles which are liquid organelles (droplets) that have  
 423 higher concentrations of reactants involved in splicing. We  
 424 constructed a spatially-resolved mammalian whole-cell (HeLa  
 425 cell) model from a variety of experimental data. Using this  
 426 model, we simulate the splicing event with a kinetic model  
 427 we developed, as stochastic reaction-diffusion processes. We  
 428 show that number of nuclear pore complexes—semblies of  
 429 proteins embedded in the nuclear envelope that control the  
 430 traffic between nucleus and cytoplasm—affects the splicing  
 431 particles formation rate for different nuclear sizes. The phase-  
 432 separated nature of speckles enhances the mRNA production  
 433 and reduces its associated noise. We suggest a rationale for  
 434 the number and size of speckles based on optimal resulting  
 435 mRNA. By demonstrating the effects of the genes distribution  
 436 around our non-fluid speckles on mRNA counts, we propose



437 that the movement of nuclear speckles toward active chromatin  
438 regions could be regulated by cells to control the transcripts  
439 production. Overall, our reaction-diffusion model of spliceo-  
440 some assembly and function in a realistic mammalian cell  
441 environment allowed us to meaningfully connect the cellular  
442 geometry to the underlying biological processes. At the same  
443 time, we expect the presented whole-cell model to provide  
444 a versatile platform for studying processes beyond mRNA  
445 splicing.

## 446 **Methods**

### 447 **Construction of a representative HeLa whole-cell model.**

448 Spliceosomal assembly and activity consists of multi-  
449 compartmental, reaction-diffusion processes, necessitating a  
450 spatial representation of the cellular geometry. HeLa cells are  
451 an optimal model system as they have been the subject of  
452 extensive investigations exploring cell geometry and cellular  
453 composition. Additionally, data from individual measurements  
454 of specific components (e.g., size, morphology, relative mass  
455 fraction) were used to inform the construction of a represen-  
456 tative cell model (11, 14–20, 23–26). A constructive solid  
457 geometry (CSG) approach, wherein basic geometric objects  
458 are combined programmatically via set operations (e.g. unions,  
459 differences, intersections), was used to build the HeLa cell.  
460 Since Lattice Microbes software (v 2.3) (21, 22) requires that  
461 each location within the space be defined as a single site-type,  
462 the various CSG objects were stenciled onto the simulation  
463 lattice in “depth order” (also called the “Painter’s algorithm”).  
464 Overall, our model consists of 11 different site-types including:  
465 1) extracellular space, 2) plasma membrane, 3) cytoplasm, 4)  
466 nuclear membrane, 5) nucleoplasm, 6) Cajal bodies, 7) nuclear  
467 speckles, 8) nuclear pore complexes, 9) mitochondria, 10) Golgi  
468 apparatus and 11) endoplasmic reticulum (ER). The overall  
469 simulation volume was constructed as a cubic box with 18.432  
470  $\mu\text{m}$  side-length. The space was discretized into a cubic lattice  
471 of points spaced 64 nm apart. HeLa cell volumes have been  
472 measured at 2600, 3000 and 4400–5000  $\mu\text{m}^3$  (24, 43, 44); we  
473 chose the mid- size cell as a template for our model. HeLa cells  
474 that are grown in suspension appear spherically-shaped, so we  
475 chose to design the overall cell architecture as a sphere with  
476 radius 8.9  $\mu\text{m}$ . Nuclei have measured volumes of, 220 and 374  
477  $\mu\text{m}^3$  (25, 26). Refs. (24) and (15) suggested nuclear volumes  
478 corresponding to 10% and 21.1% of the total cell volume. As  
479 we wanted to test the importance of nuclei size on splicing,  
480 multiple nuclear radii were investigated, including 3.74, 4.15,  
481 4.67 and 5.29  $\mu\text{m}$  corresponding to all the above-motio-  
482 ned volumes and volume-fractions. Plasma and nuclear membranes  
483 were implemented as a thin sheet of lattice points (128nm  
484 thick) separating the extracellular space, the cytoplasm and  
485 the nucleus. The Golgi apparatus was constructed as an in-  
486 tersection of a cone with several spherical shells of various  
487 radii placed successively from the edge of the nucleus into  
488 the cytoplasm. The apex of the cone was centered in the cell  
489 with the based positioned deep in the cytoplasm. In this way,  
490 the Golgi roughly approximates what is seen in experiments.  
491 Nuclear speckles and Cajal bodies were modeled as spheres  
492 placed within the nucleus. Mitochondria were modeled as ran-  
493 domly oriented spherocylinders placed within the cytoplasm.  
494 Nuclear pore complexes were embedded in the nuclear mem-  
495 brane. NPCs were constructed as a set of spheres of radii  
496 approximately equal to that of the experimentally-measured

497 NPC. Sizes for these organelles can be found in Table 1. Total  
498 counts for these organelles were based on either direct exper-  
499 imental quantification or based on relative volume fraction  
500 measured for the overall cell. The ER was also constructed  
501 in a randomized fashion with the details and construction  
502 algorithm presented in Supplementary Information. The endo-  
503 somes, lysosomes, actin-cytoskeleton, peroxisomes in the  
504 cytoplasm; nucleolus and chromatin have not been included  
505 into the present version of the model. According to Ref. (15),  
506 each of the cytoplasmic organelles contribute less than 1% of  
507 to the total cell composition, and therefore, were not mod-  
508 elled. The nuclear components were chosen mainly among  
509 those that play a role in RNA splicing processes. A repre-  
510 sentative HeLa cell geometry resulting from this procedure is  
511 shown in Figure 1. The associated code for setting up a HeLa  
512 cell model for Lattice Microbes software (21, 22) is available  
513 upon request.

514 In addition to structural features, the abundance of the pro-  
515 teins participating in the processes we studied were derived  
516 from proteomics data of HeLa cells (19). The number of active  
517 snRNA genes have been determined to be 30 (45). These abun-  
518 dances were used as the initial condition for the simulations.  
519 Particles are randomly distributed throughout their parent  
520 region with locations sampled from a uniform distribution; for  
521 instance, genes are distributed throughout the nucleus. For  
522 each separate simulation replicate, a different initial particle  
523 placement was used.

### 524 **Kinetic models.**



| Reaction   | Rate                 | Units          | Reference | Compartment |
|--|----------------------|----------------|-----------|-------------|
| <b>In Nucleus</b>  |                      |                |           |             |
| $D \rightarrow D + U1snRNA_{nuc}$                                | 0.285                | $s^{-1}$       | (46)      | N           |
| $D \rightarrow D + U2snRNA_{nuc}$                                | 0.224                | $s^{-1}$       | (46)      | N           |
| <b>Nucleus to Cytoplasm</b>                                      |                      |                |           |             |
| $U1(2)snRNA_{nuc} \rightarrow U1(2)snRNA_{cyt}$                  | $2 \times 10^4$      | $s^{-1}$       | M         | P           |
| <b>Cytoplasmic Assembly</b>                                      |                      |                |           |             |
| $U1(2)snRNA_{cyt} + G^5 \rightarrow U1(2)snRNA \cdot G^5$        | $1.02 \times 10^8$   | $M^{-1}s^{-1}$ | D.L.      | C           |
| $U1(2)snRNA \cdot G^5 \rightarrow U1(2)snRNA_{cyt} + G^5$        | 3.05                 | $s^{-1}$       | (47)      | C           |
| $U1snRNA \cdot G^5 + Sm^5 \rightarrow U1snRNA \cdot Sm^5 + G^5$  | $5.9 \times 10^7$    | $M^{-1}s^{-1}$ | D.L.      | C           |
| $U2snRNA \cdot G^5 + Sm^5 \rightarrow U2snRNA \cdot Sm^5 + G^5$  | $1.18 \times 10^7$   | $M^{-1}s^{-1}$ | D.L.      | C           |
| $U1(2)snRNA \cdot Sm^5 + Sm^2 \rightarrow U1(2)snRNA \cdot Sm^7$ | $1.39 \times 10^8$   | $M^{-1}s^{-1}$ | D.L.      | C           |
| $U1(2)snRNA \cdot Sm^7 \rightarrow U1(2)snRNA \cdot Sm^5 + Sm^2$ | 2.78                 | $s^{-1}$       | (47)      | C           |
| <b>Cytoplasm to Nucleus</b>                                      |                      |                |           |             |
| $U1(2)snRNA \cdot Sm^7 \rightarrow U1(2)snRNA \cdot Sm^7_{nuc}$  | $2 \times 10^4$      | $s^{-1}$       | M         | P           |
| <b>Nuclear Maturation</b>  |                      |                |           |             |
| $U1snRNA \cdot Sm^7_{nuc} + U1_{prot} \rightarrow U1snRNP$       | $1.22 \times 10^7$   | $M^{-1}s^{-1}$ | (48)      | J-N         |
| $U1snRNP \rightarrow U1snRNA \cdot Sm^7_{nuc} + U1_{prot}$       | $4.8 \times 10^{-4}$ | $s^{-1}$       | (48)      | J-N         |
| $U2snRNA \cdot Sm^7_{nuc} + U2_{prot} \rightarrow U2snRNP$       | $0.24 \times 10^7$   | $M^{-1}s^{-1}$ | (48)      | J-N         |
| $U2snRNP \rightarrow U2snRNA \cdot Sm^7_{nuc} + U2_{prot}$       | $4.8 \times 10^{-4}$ | $s^{-1}$       | (48)      | J-N         |

**Table 2. Reactions describing U1snRNP and U2snRNP splicing particles formation together with their associated rates. Abbreviations are: DNA(D), Gemin 5( $G^5$ ), five already-assembled Sm proteins ( $Sm^5$ ), the remaining Sm proteins ( $Sm^2$ ), diffusion-limited (D.L.), model assumption (M), nucleus (N), NPC (P), Cajal bodies (J) and cytoplasm (C).**

| Reaction   | Rate                 | Units          | Reference | Compartment |
|--|----------------------|----------------|-----------|-------------|
| $D \rightarrow D + pre - mRNA$                   | $4.7 \times 10^{-3}$ | $s^{-1}$       | (7)       | S-N         |
| $U1 + pre - mRNA \rightarrow complexE$           | $4.66 \times 10^7$   | $M^{-1}s^{-1}$ | D.L. (11) | S-N         |
| $complexE \rightarrow U1 + pre - mRNA$           | 1.57                 | $s^{-1}$       | (49)      | S-N         |
| $U2 + pre - mRNA \rightarrow complexE^*$         | $1.4 \times 10^7$    | $M^{-1}s^{-1}$ | D.L. (11) | N           |
| $U2 + pre - mRNA \rightarrow complexE^*$         | $0.93 \times 10^7$   | $M^{-1}s^{-1}$ | D.L. (11) | S           |
| $complexE^* \rightarrow U2 + pre - mRNA$         | 1.57                 | $s^{-1}$       | (49)      | S-N         |
| $complexE + U2 \rightarrow complexA$             | $8.8 \times 10^7$    | $M^{-1}s^{-1}$ | D.L. (11) | S-N         |
| $complexA \rightarrow complexE + U2$             | 0.062                | $s^{-1}$       | (49)      | S-N         |
| $complexE^* + U1 \rightarrow complexA$           | $8.7 \times 10^7$    | $M^{-1}s^{-1}$ | D.L. (11) | S-N         |
| $complexA \rightarrow complexE^* + U1$           | 1.57                 | $s^{-1}$       | (49)      | S-N         |
| $complexA + tri \cdot U \rightarrow complexB$    | $4.66 \times 10^7$   | $M^{-1}s^{-1}$ | D.L. (11) | S-N         |
| $complexB \rightarrow complexA + tri \cdot U$    | 1.55                 | $s^{-1}$       | (49)      | S-N         |
| $complexB \rightarrow complexB^* + U1$           | $6 \times 10^4$      | $M^{-1}s^{-1}$ | M         | S-N         |
| $complexB^* \rightarrow mRNA + tri \cdot U + U2$ | 0.067                | $s^{-1}$       | (50)      | S-N         |

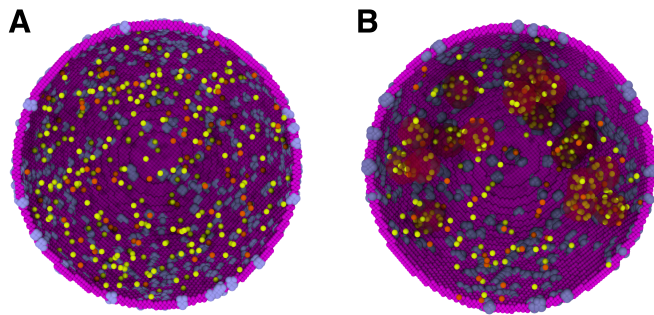
**Table 3. Spliceosome assembly and splicing reaction. Abbreviations are: DNA (D), U1snRNP (U1), U2snRNP (U2), U4/U6- U5snRNP (tri · U), model assumption (M), diffusion-limited (D.L.), nuclear speckles (S) and nucleus (N).**

525 **Implementation of nuclear speckles.** Nuclear speckles were  
 526 modelled as spherical regions in the nucleus with radii of  
 527  $0.35 \mu\text{m}$ . Previously, the splicing particles localization in  
 528 speckles has been implemented by assuming a higher affinity  
 529 for splicing particles to bind to unknown binding partners in  
 530 speckles with respect to binding to pre-mRNA transcripts in  
 531 the nucleoplasm (41). We imposed an imbalance on the tran-  
 532 sition probabilities for the splicing particles and pre-mRNA  
 533 transcripts to move from the nuclear speckles to the nucleo-  
 534 plasm, and vice-versa. This approach, as shown in the Results  
 535 section, will reproduce experimentally observed concentration  
 536 ratio of splicing particles between the speckles and the nucleo-  
 537 plasm (41), additionally, the presence of dummy particles in  
 538 the speckles are not required. Specifically, the probability of  
 539 the splicing particles to move from the nucleoplasm regions to  
 540 the speckles ( $P_n$ ) was higher than the reverse direction ( $P_s$ ).  
 541 To examine the effect of the bias, the  $P_n/P_s$  values were varied.  
 542 With increasing  $P_n/P_s$  values, more particles accumulate in  
 543 the speckles and the nucleus becomes more diluted. Figure 10

shows the localization of splicing particles in speckles upon  
 application of this bias in our model.

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**Fig. 10.** Formation of speckles in our simulations: A) splicing particles (U1 and U2 colored in yellow and orange, respectively) diffusing freely in the nucleus without speckle, B) Introduction of an imbalance on transition probabilities of splicing particles from the nucleus to speckles results in the localization of the splicing particles in the speckles shown as red-shaded regions.

562 code.

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