

Real-Time Visualization of ZBP1 Association with β -Actin mRNA during Transcription and Localization

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Movies

The movies corresponding to Figures 1D (Movie 1), 2E (Movie 12), 6C (Movie 13), and 6E (Movie 14) are provided.

Particle Tracking

Tracking was performed on particles manually by identifying the position of the particle in each frame (summary in Table S1; data in Table S2). The particles were observed in a deconvolved version of Movie 1, named Movie 2, which corresponds to Figure 1D. Deconvolution was performed by using Huygens II Professional (SVI). The trajectory of each tracked particle is shown in Figures S1–S9 and Movies 3–11.

Movie 1. Movement of GFP-ZBP1 in a Fibroblast Stimulated with Serum

Fibroblasts were grown overnight in serum-free conditions and were stimulated with serum while visualization was in process. Images were taken at 400-ms intervals for 310 s.

Movie 2. Deconvolved Version of Movie 1

Huygens II Professional (SVI) software was used for two-dimensional deconvolution.

Movies 3–11. Tracking of Individual Particles from Movie 2

Movie 12. GFP-ZBP1 Movement in Cells Treated with BDM

The particles do not translocate significantly.

Movie 13. Recovery from Fluorescence Photobleaching of the ZBP-GFP1 Associated with the Transcription Site

The photobleached sample was then observed in the confocal microscope at a frame rate of one per 6.25 s for 175 s. The fluorescence was quantitated by using NIH Image and was plotted as the percent recovery.

Movie 14. Movement of GFP-ZBP1 into the Nucleus

The particle jumps into the nucleus, as indicated by the arrow in Figure 6E. The frame capture rate is 800 ms.

Table S1. Tracking of Particles in Movie 2: Speed and Distance Traveled for Individual ZBP1-GFP Fusion Particles

Particle Number ($\mu\text{m/s}$)	Travel Time (s)	Net Path Length (μm)	Total Path Length (μm)	Speed, Total Path/Time	
1	5.20	1.2	1.5	0.28	
2	2.80	1.1	1.3	0.47	
3	7.60	2.3	2.9	0.38	
4	2.80	1.5	1.7	0.60	
5	3.20	1.3	2.0	0.63	
6	4.80	1.3	1.6	0.33	
7	4.80	1.8	2.2	0.46	
8	2.40	1.1	1.2	0.48	
9	5.20	1.9	2.0	0.39	
				Mean Speed ($\mu\text{m/s}$)	0.45
				SD of Speed ($\mu\text{m/s}$)	0.12

The pixel size is 0.112 μm ; the time per frame is 0.40 s.

Table S2. Raw Tracking Data of Particles in Movie 2

Frame Number	x-Centroid (Pixels)	y-Centroid (Pixels)	Distance between Frames (Pixels)	Net Distance (Pixels)
Particle 1				
68	71	65		11.0
69	71	64	1.0	
70	71	64	0.0	
71	72	63	1.4	
72	72	61	2.0	
73	72	61	0.0	
74	72	61	0.0	
75	73	60	1.4	
76	73	59	1.0	
77	72	58	1.4	
78	71	57	1.4	
79	71	56	1.0	
80	70	55	1.4	
81	70	54	1.0	
Particle 2				
165	69	70		10.2
166	70	69	1.4	
167	69	69	1.0	
168	69	68	1.0	
169	68	66	2.2	
170	68	64	2.0	
171	67	62	2.2	
172	67	60	2.0	
Particle 3				
170	65	75		20.4
171	64	73	2.2	
172	65	73	1.0	
173	65	73	0.0	
174	67	73	2.0	
175	67	72	1.0	
176	67	71	1.0	
177	68	70	1.4	
178	68	67	3.0	
179	69	66	1.4	
180	69	65	1.0	
181	69	65	0.0	
182	69	63	2.0	
183	68	63	1.0	
184	68	62	1.0	
185	69	62	1.0	
186	69	60	2.0	
187	69	59	1.0	
188	69	57	2.0	
189	69	55	2.0	
Particle 4				
185	65	69		13.3
186	67	68	2.2	
187	68	67	1.4	
188	68	65	2.0	
189	68	63	2.0	
190	67	61	2.2	
191	67	59	2.0	
192	68	56	3.2	

(continued)

Table S2. Continued

Frame Number	x-Centroid (Pixels)	y-Centroid (Pixels)	Distance between Frames (Pixels)	Net Distance (Pixels)
Particle 5				
193	64	69		11.7
194	66	70	2.2	
195	68	70	2.0	
196	69	69	1.4	
197	69	68	1.0	
198	68	65	3.2	
199	67	61	4.1	
200	69	59	2.8	
201	68	58	1.4	
Particle 6				
284	75	76		11.7
285	74	76	1.0	
286	74	76	0.0	
287	74	75	1.0	
288	74	75	0.0	
289	72	74	2.2	
290	72	72	2.0	
291	73	71	1.4	
292	72	70	1.4	
293	72	70	0.0	
294	72	68	2.0	
295	72	67	1.0	
296	71	65	2.2	
Particle 7				
165	66	73		16.1
166	67	73	1.0	
167	67	73	0.0	
168	68	73	1.0	
169	67	73	1.0	
170	67	70	3.0	
171	67	69	1.0	
172	68	67	2.2	
173	68	66	1.0	
174	68	63	3.0	
175	68	62	1.0	
176	69	61	1.4	
177	68	57	4.1	
Particle 8				
158	67	69		10.0
159	68	68	1.4	
160	68	66	2.0	
161	68	63	3.0	
162	68	63	0.0	
163	68	61	2.0	
164	68	59	2.0	
Particle 9				
230	70	74		17.0
231	71	72	2.2	
232	71	70	2.0	
233	71	70	0.0	
234	69	68	2.8	
235	69	68	0.0	
236	69	68	0.0	
237	69	66	2.0	
238	69	64	2.0	
239	69	63	1.0	
240	69	62	1.0	
241	70	60	2.2	
242	70	58	2.0	
243	70	57	1.0	

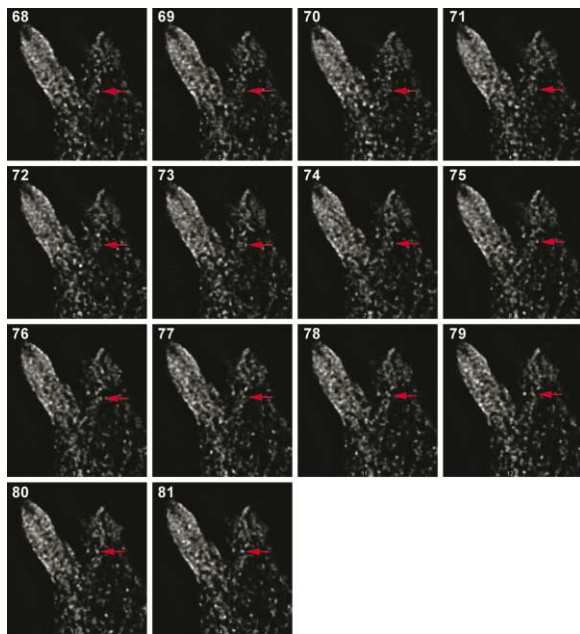


Figure S1. Paths of Individual Particles Tracked in Movie 2
Each figure corresponds to Movies 3–11.

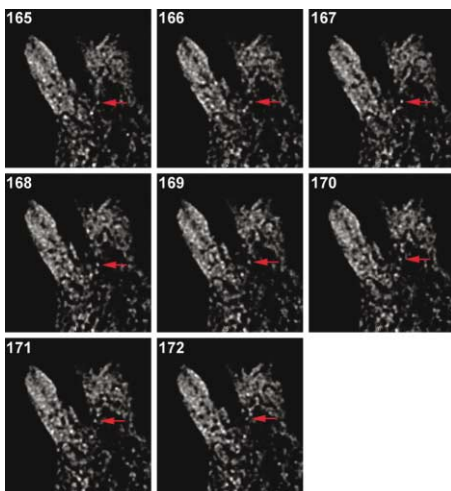


Figure S2

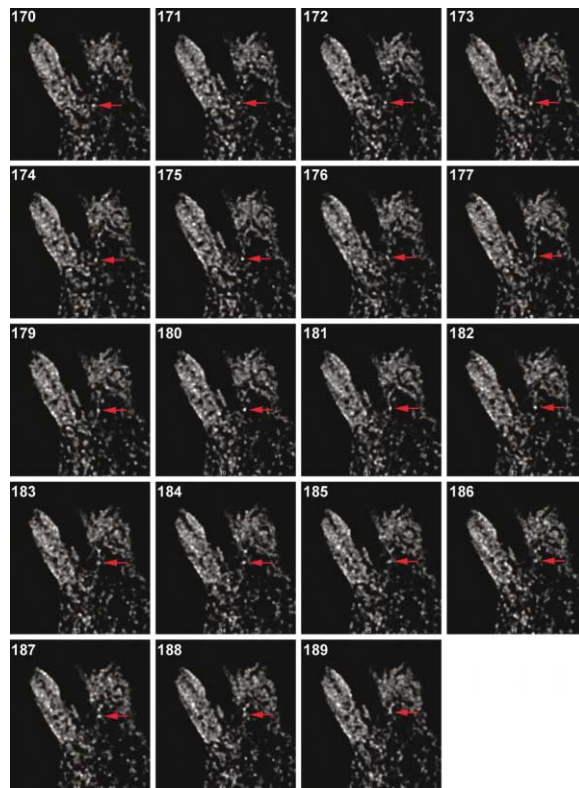


Figure S3

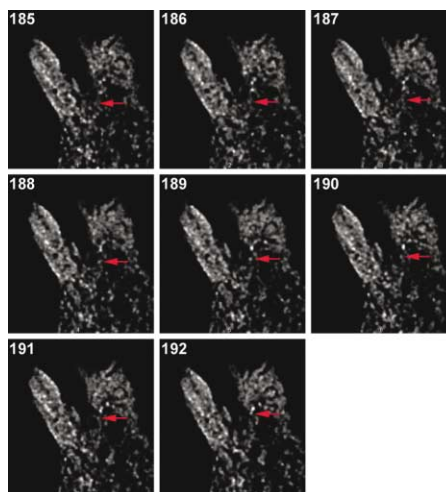


Figure S4

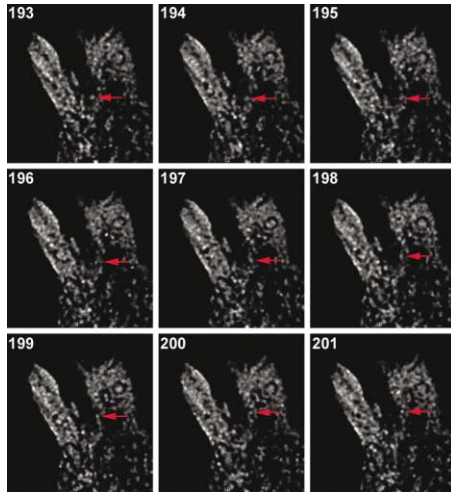


Figure S5

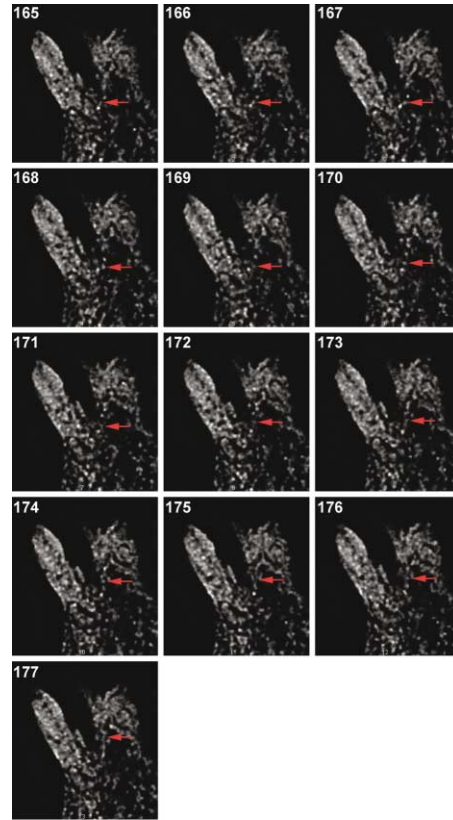


Figure S7

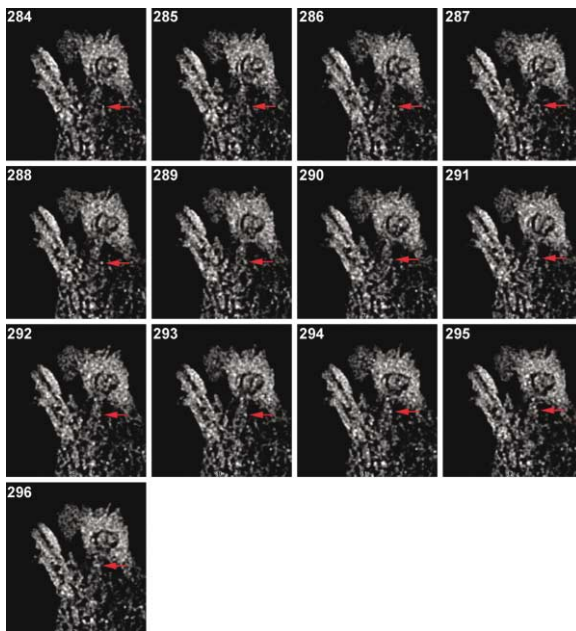


Figure S6

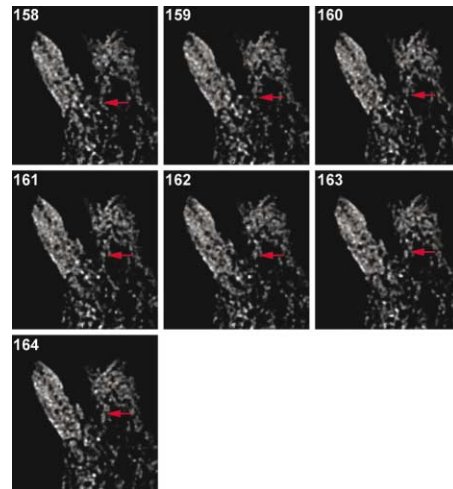


Figure S8

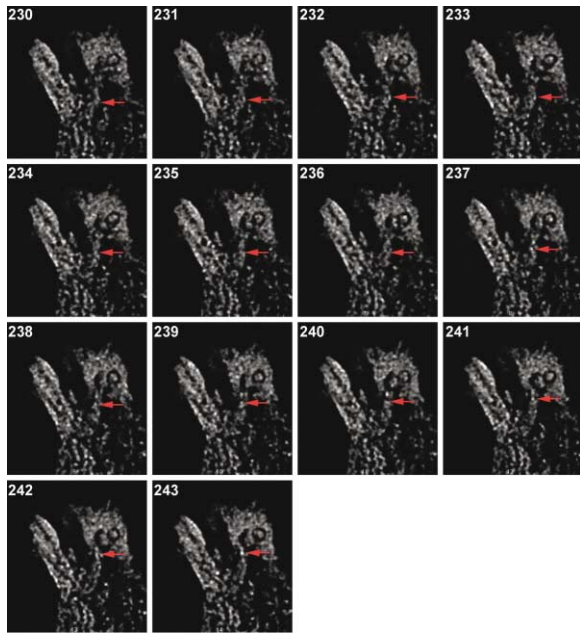


Figure S9