

doi: 10.1387/ijdb.150222cl

SUPPLEMENTARY MATERIAL

corresponding to:

**Live imaging reveals spatial separation of parental chromatin
until the four-cell stage in *Caenorhabditis elegans* embryos**

JITKA BOLKOVÁ and CHRISTIAN LANCTÔT

***Address correspondence to:** Christian Lanctôt. BIOCEV- Department of Cell Biology, Faculty of Science, Charles University in Prague, Pr myslová 595, 252 42 Vestec, Czech Republic, E-mail : lanctotc@natur.cuni.cz

Full text for this paper is available at: <http://dx.doi.org/10.1387/ijdb.150222cl>

SUPPL. TABLE S1

**CELL CYCLE LENGTH IN CONTROL DENDRA2-H2B EMBRYOS
AND IN DENDRA2-H2B EMBRYOS IN WHICH CHROMATIN WAS
PHOTOCONVERTED**

Embryo	PC	Timing of division				
		AB	P1	Aba/p	EMS	P2
1	no	14.0	18.0	32.5	34.0	36.5
2*	no	12.2	15.8	28.0	31.0	34.0
3	no	13.0	15.0	26.0	28.0	32.0
4	no	13.5	15.0	28.0	30.0	34.0
5	no	13.5	15.0	27.0	29.5	32.5
6	no	13.0	15.0	27.0	30.0	34.0
7	no	13.0	15.0	27.0	29.0	32.0
8	no	13.0	15.0	25.0	29.0	32.0
	AV	13.1	15.5	27.6	30.1	33.4
	SD	0.4	0.7	1.5	1.2	1.3
9	yes	12.0	14.0	24.0	28.0	30.0
10	yes	12.5	14.5	30.0	32.0	
11	yes	14.0	16.0	28.0	31.0	34.0
12	yes	14.0	16.0	27.0	30.0	35.0
13	yes	14.0	16.0	28.0	31.0	34.5
14	yes	12.0	14.0	26.0	28.0	32.0
15	yes	16.0	18.0	32.0	34.0	40.0
16	yes	16.0	18.0	31.0	35.0	38.0
17	yes	15.0	18.0	31.0	34.0	37.0
18	yes	14.0	16.0	29.0	32.0	36.0
19	yes	14.0	16.0	30.0	32.0	36.0
	AV	14.0	16.0	28.7	31.5	35.3
	SD	1.0	1.1	1.9	1.8	2.2

When indicated, the chromatin in one of the pronuclei was photoconverted (PC) using the 405 nm laser line as described in the Materials and Methods section. The subsequent development was imaged at intervals of 1-2 minutes. Movie number 2 (*) was acquired using DIC illumination, all others using fluorescence imaging. The time at which the metaphase plate was observed in the 1-cell embryo was set at 0.0. The time to the next metaphase was measured for each of the indicated blastomeres from the imaging data. No significant differences were found between irradiated (bottom) and control (top) embryos. AV, average; SD, standard deviation.

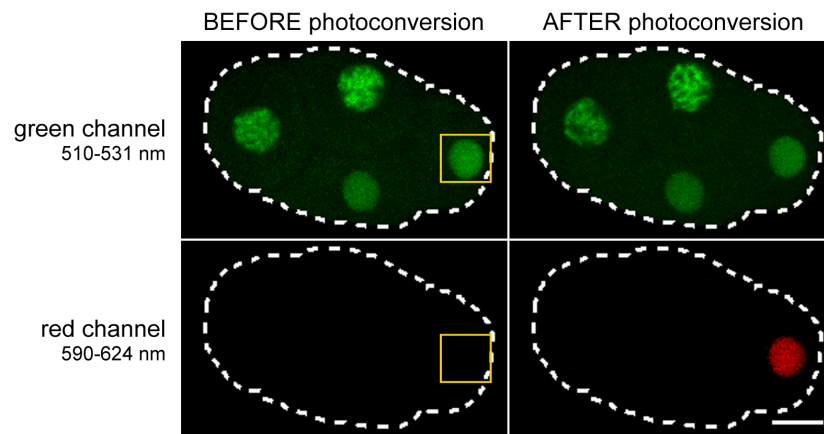


Fig. S1. Photoconversion of Dendra2-labeled chromatin in a *C. elegans* embryo. The P2 nucleus of a living 4-cell embryo expressing Dendra2-H2B (strain JBL1) was illuminated with 405 nm light. Left and right panels display embryo before and after photoconversion, respectively. The fluorescence signal at $\lambda = 510 \text{ nm}$ -531 nm (green channel) is shown on top. The fluorescence signal at $\lambda = 590 \text{ nm}$ -624 nm (red channel) is shown at bottom. The region targeted for photoconversion comprised only the P2 nucleus (boxed). Note the appearance of a strong signal in the red channel in the P2 nucleus after photoconversion. Shown are maximum projections of image stacks corresponding to an embryonic cross-section of 10 μm . The contour of the embryo is depicted by a dotted line. Scale bar, 5 μm .

	M	N	T	P	G	I	N	L	I	K	E	D	M	R	V	K	V	H	M	E
Dendra2	ATG	AAC	ACC	CCG	GGA	ATT	AAC	CTG	ATC	AAG	GAG	GAC	ATG	CGC	GTG	AAG	GTG	CAC	ATG	GAG
CeDendra2	ATG	AAC	ACC	CCA	GGA	ATC	AAC	CTT	ATC	AAG	GAG	GAC	ATG	CGC	GTG	AAG	GTG	CAC	ATG	GAG
	G	N	V	N	G	H	A	F	V	I	E	G	E	G	K	G	K	P	Y	E
Dendra2	GGC	AAC	GTG	AAC	GGC	CAC	GCC	TTC	GTG	ATC	GAG	GGC	GAG	GGC	AAG	GGC	AAG	CCC	TAC	GAG
CeDendra2	GGA	AAC	GTA	AAT	GGT	CAC	GCT	TTC	GTC	ATA	GAG	GGA	GAA	GGG	AAG	GGC	AAA	CCA	TAT	GAG
	G	T	Q	T	A	N	L	T	V	K	E	G	A	P	L	P	F	S	Y	D
Dendra2	GGC	ACC	CAG	ACC	GCC	AAC	CTG	ACC	GTG	AAG	GAG	GGC	GCC	CCC	CTG	CCC	TTC	AGC	TAC	GAC
CeDendra2	GGA	ACC	CAA	ACC	GCA	AAC	CTT	ACC	GTC	AAG	GAG	GGT	GCT	CCG	TTA	CCA	TTC	TCT	TAC	GAC
	I	L	T	T	A	V	H	Y	G	N	R	V	F	T	K	Y	P	E	D	I
Dendra2	ATC	CTG	ACC	ACC	GCC	GTG	CAC	TAC	GGC	AAC	CGG	GTG	TTC	ACC	AAG	TAC	CCC	GAG	GAC	ATC
CeDendra2	ATT	CTT	ACG	ACC	GCT	GTC	CAC	TAC	GGG	AAT	CGT	GTG	TTT	ACA	AAG	TAC	CCA	GAG	GAC	ATC
	P	D	Y	F	K	Q	S	F	P	E	G	Y	S	W	E	R	T	M	T	F
Dendra2	CCC	GAC	TAC	TTC	AAG	CAG	AGC	TTC	CCC	GAG	GGC	TAC	AGC	TGG	GAG	CGC	ACC	ATG	ACC	TTC
CeDendra2	CCT	GAC	TAC	TTT	AAG	CAG	TCA	TTT	CCG	GAA	GGT	TAT	TCC	TGG	GAG	CGT	ACC	ATG	ACA	TTC
	E	D	K	G	I	C	T	I	R	S	D	I	S	L	E	G	D	C	F	F
Dendra2	GAG	GAC	AAG	GGC	ATC	TGC	ACC	ATC	CGC	AGC	GAC	ATC	AGC	CTG	GAG	GGC	GAC	TGC	TTC	TTC
CeDendra2	GAA	GAC	AAA	GGA	ATC	TGC	ACC	ATT	CGT	TCC	GAC	ATC	TCC	CTT	GAG	GGC	GAT	TGT	TTC	TTT
	Q	N	V	R	F	K	G	T	N	F	P	P	N	G	P	V	M	Q	K	K
Dendra2	CAG	AAC	GTG	CGC	TTC	AAG	GGC	ACC	AAC	TTC	CCC	CCC	AAC	GGC	CCC	GTG	ATG	CAG	AAG	AAG
CeDendra2	CAA	AAT	GTT	AGA	TTC	AAG	GGA	ACC	AAT	TTC	CCA	CCC	AAC	GGA	CCA	GTC	ATG	CAG	AAG	AAG
	T	L	K	W	E	P	S	T	E	K	L	H	V	R	D	G	L	L	V	G
Dendra2	ACC	CTG	AAG	TGG	GAG	CCC	AGC	ACC	GAG	AAG	CTG	CAC	GTG	CGC	GAC	GGC	CTG	CTG	GTG	GGC
CeDendra2	ACT	CTA	AAG	TGG	GAG	CCA	TCC	ACC	GAA	AAA	CTT	CAT	GTT	CGT	GAC	GGA	CTT	CTT	GTC	GGT
	N	I	N	M	A	L	L	L	E	G	G	G	H	Y	L	C	D	F	K	T
Dendra2	AAC	ATC	AAC	ATG	GCC	CTG	CTG	CTG	GAG	GGC	GGC	GGC	CAC	TAC	CTG	TGC	GAC	TTC	AAG	ACC
CeDendra2	AAC	ATT	AAC	ATG	GCT	TTG	CTT	CTT	GAG	GGA	GGA	GGA	CAC	TAT	TTA	TGC	GAT	TTT	AAG	ACT
	T	Y	K	A	K	K	V	V	Q	L	P	D	A	H	F	V	D	H	R	I
Dendra2	ACC	TAC	AAG	GCC	AAG	AAG	GTG	GTG	CAG	CTG	CCC	GAC	GCC	CAC	TTC	GTG	GAC	CAC	CGC	ATC
CeDendra2	ACC	TAC	AAG	GCT	AAA	AAG	GTC	GTT	CAA	CTT	CCG	GAC	GCT	CAC	TTT	GTC	GAT	CAC	AGA	ATC
	E	I	L	G	N	D	S	D	Y	N	K	V	K	L	Y	E	H	A	V	A
Dendra2	GAG	ATC	CTG	GGC	AAC	GAC	AGC	GAC	TAC	AAC	AAG	GTG	AAG	CTG	TAC	GAG	CAC	GCC	GTG	GCC
CeDendra2	GAG	ATC	CTG	GGC	AAC	GAC	TCG	GAC	TAT	AAC	AAG	GTC	AAA	TTA	TAC	GAG	CAC	GCT	GTC	GCA
	R	Y	S	P	L	P	S	Q	V	W	-									
Dendra2	CGC	TAC	AGC	CCC	CTG	CCC	AGC	CAG	GTG	TGG	TAA									
CeDendra2	CGT	TAC	TCC	CCA	CTT	CCA	TCC	CAA	GTT	TGG	TAA									

Fig. S2. Alignment of the Dendra2 coding sequence and the Dendra2 coding sequence optimized for expression in *C. elegans*. The identity is 79% (125/230 codons were optimized). Nucleotide changes are highlighted in gray. The percentage of AT is 53% in the optimized sequence (up from 37% in the starting sequence). The positions of the 3 short introns that were inserted in the expression cassette are indicated by arrowheads. The predicted protein sequence is shown.

Video S1. Expression of Dendra2-H2B in the *C. elegans* strain JBL1 visualized by single plane illumination microscopy. The Dendra2-H2B signal is shown in green. The autofluorescence signal from gut granules (obtained through excitation with green light) was pseudo-colored in red and overlaid on the Dendra2-H2B signal. The fusion protein is expressed in the gonads and in the embryos inside the uterus. Note the more intense expression of the transgene starting in pachytene nuclei. Dendra2-H2B is expressed in spermatozoa in this strain.

Video S2. Spatial distribution of parental chromatin in the early *C. elegans* embryo. The male pronucleus was photoconverted in the early zygote of the Dendra2-H2B-expressing strain (JBL1), after which imaging of the chromatin from the photoconverted pronucleus (red) and from the other pronucleus (green) was performed for 22 minutes, almost until the 4-cell stage. Shown are maximum projections of image stacks (~30 optical sections, ~15 μm in total thickness) acquired at 30-second intervals. In order to account for the variations in signal intensity that result from varying levels of chromatin condensation during the cell cycle, the brightness was adjusted independently for each image. The P0, AB and P1 nuclei are labeled. The contour of the embryo is depicted by a dotted line. The breakdown of the pronuclear envelopes occurred at time 0:00. Note the clear segregation of the photoconverted chromatin in the 2-cell embryo. Note also that since the division of the AB blastomere occurs along the z-axis of the imaging volume, the separation of paternally- and maternally-derived chromatin in the daughter nuclei is not faithfully represented on the maximum z-projections that are shown here (time points 13:00 to 15:00). At the beginning of the movie (time points 0:00 to 2:30), the arrows point to the photoconverted chromatin in order to highlight the 180° rotation of the nascent nuclei. Time is indicated in minutes:seconds. Scale bar, 5 μm .

Video S3. Rotational movement of cytoplasmic P granules during division of the *C. elegans* zygote. A worm strain expressing Dendra2-H2B and GFP-labeled PGL-1, a marker of P granules, was imaged at 10-second intervals starting at zygotic metaphase. The Dendra2-H2B signal, which is much weaker than the GFP-PGL-1 one, was mostly detected during mitosis (the first 2.5 minutes). The asymmetric cytokinetic furrow (arrowhead) is clearly visible starting at time point 1:40. Three individual P granules are color-labeled to visualize their position throughout the movie. These underwent a rotational movement during cytokinesis, as did the second polar body (arrow), which ended up being closer to the objective as a result (and therefore displayed a stronger signal at later time points). Time is indicated in minutes:seconds. Scale bar, 5 μm .