Single molecule dynamics of gene expression measured on single genes in living cells

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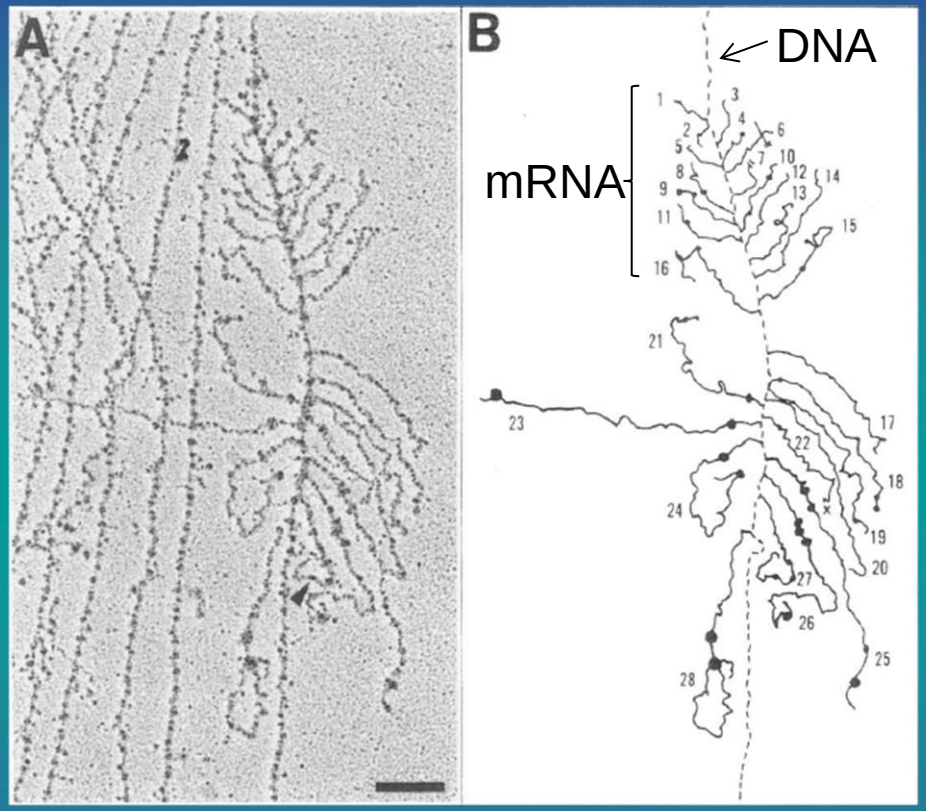
Ramat Gan, Israel

mRNA transcription in ‘real-time’

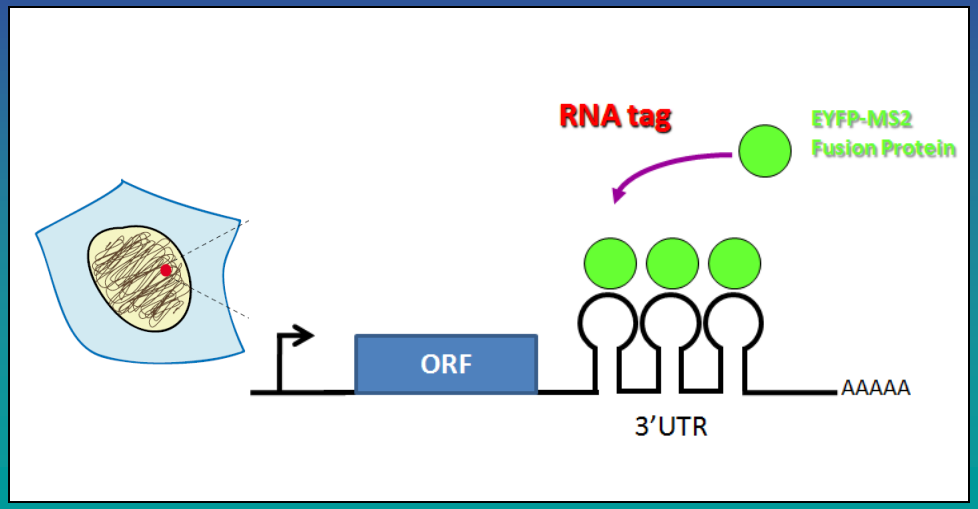


Beyer & Osheim. *Genes Dev*. 1988

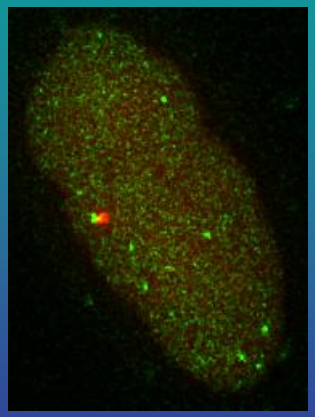
How to study gene expression in living cells?

Polymerase mRNA

Following mRNA dynamics in living cells

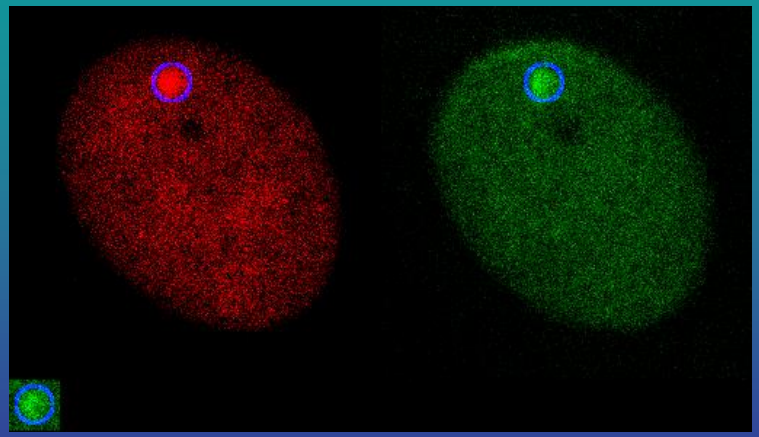


mRNP diffusion



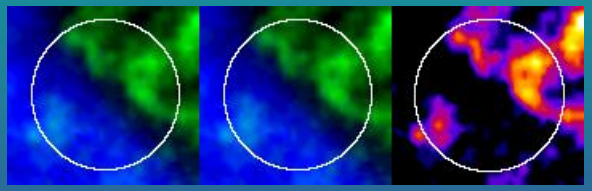
Shav-Tal et al. *Science* 2004

Transcription kinetics



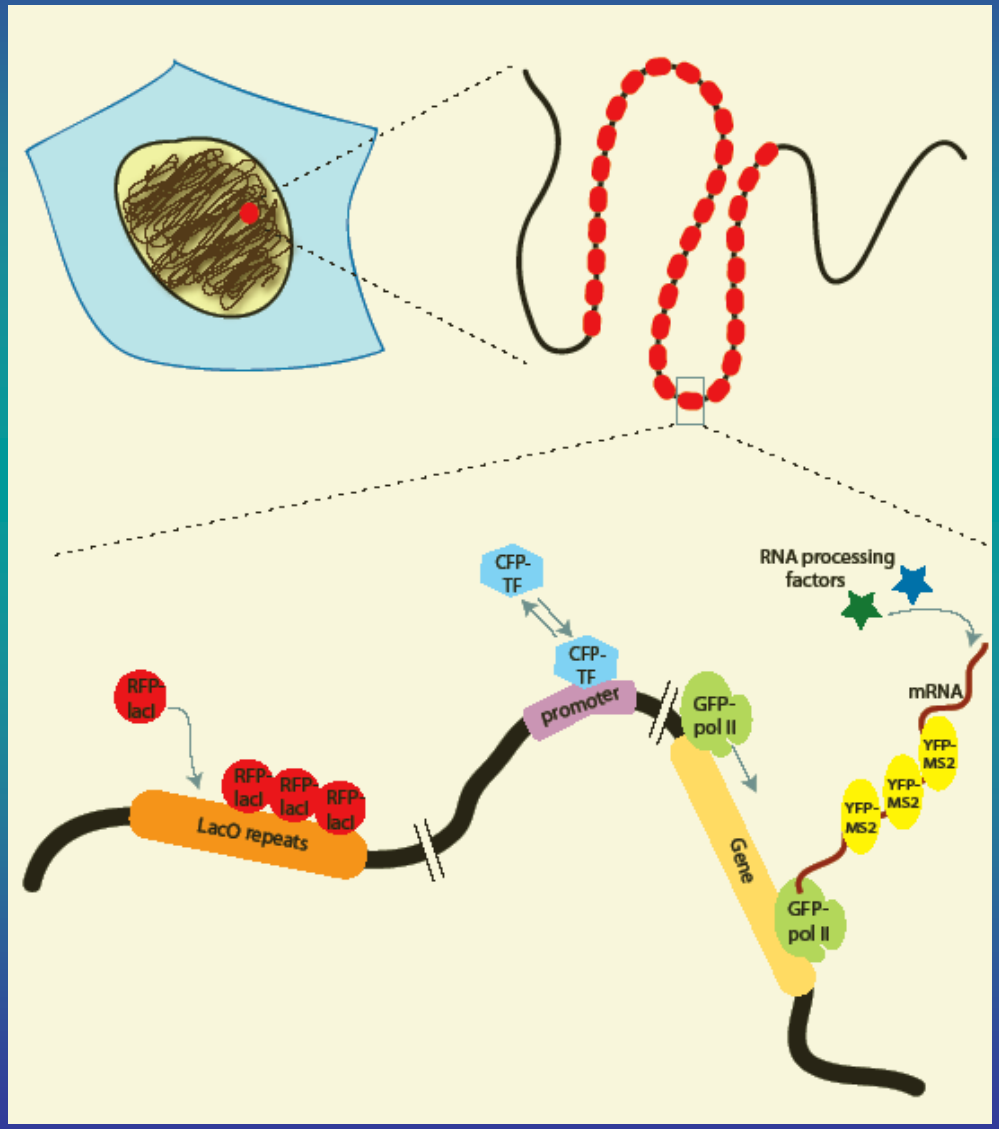
Darzacq et al. *Nat. Struct. Mol. Biol*. 2007

mRNP export



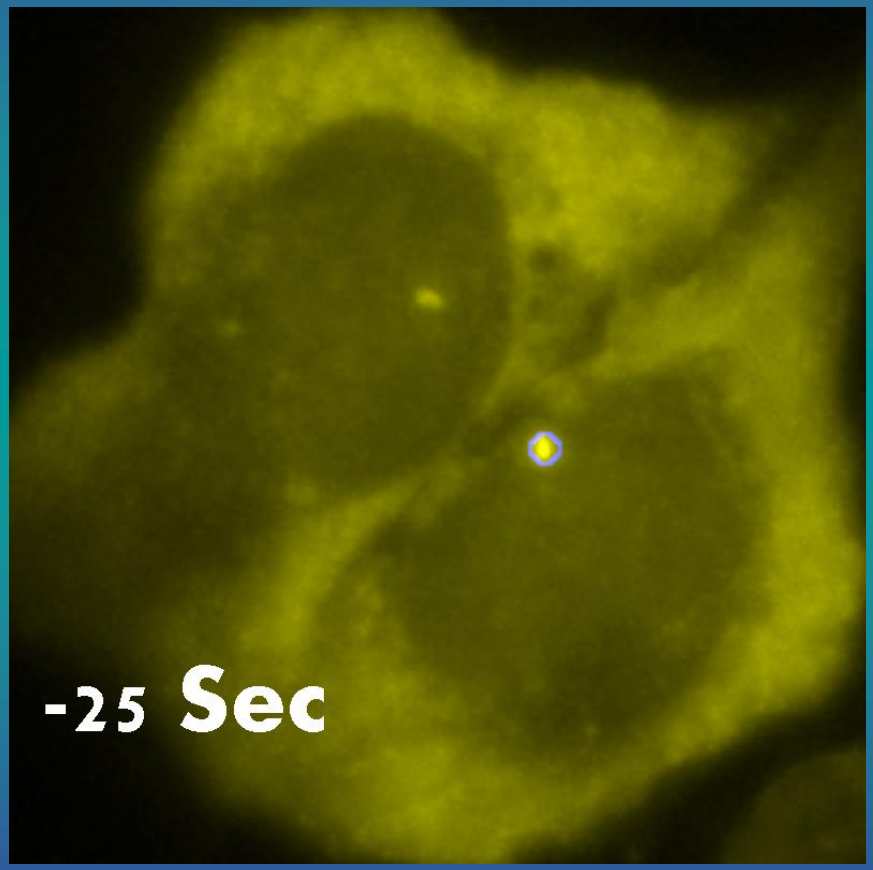
Mor et al. *Nat. Cell. Biol*. 2010

Tandem array gene systems for following gene expression in living cells



Darzacq, Singer & Shav-Tal *Curr. Opin. Cell Biol*. 2005

Tandem array gene systems for following gene expression in living cells



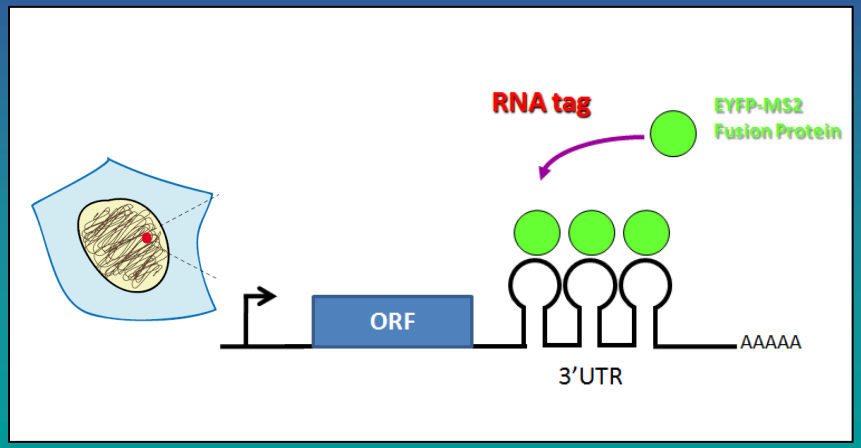
mRNA transcription (FRAP)
(mRNA = yellow)

Brody et al. *PLoS Biol*. 2011

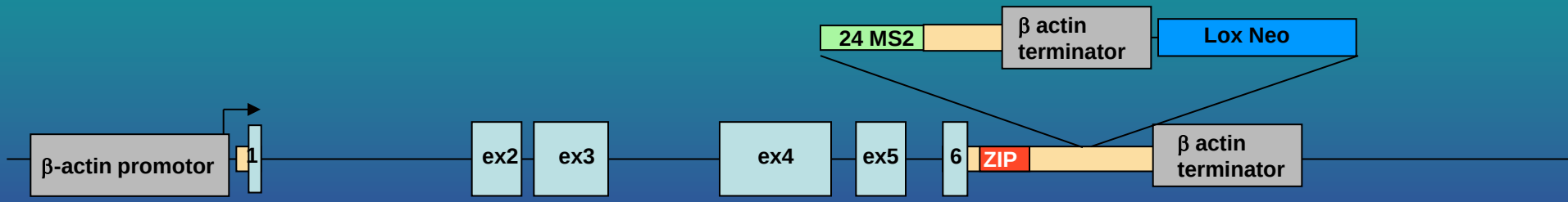
Measuring transcriptional kinetics of single genes

Approach A
Tagging of an endogenous gene

β-actin transcription observed in primary cells from a MS2-knock-in mouse



Mouse Chr 5

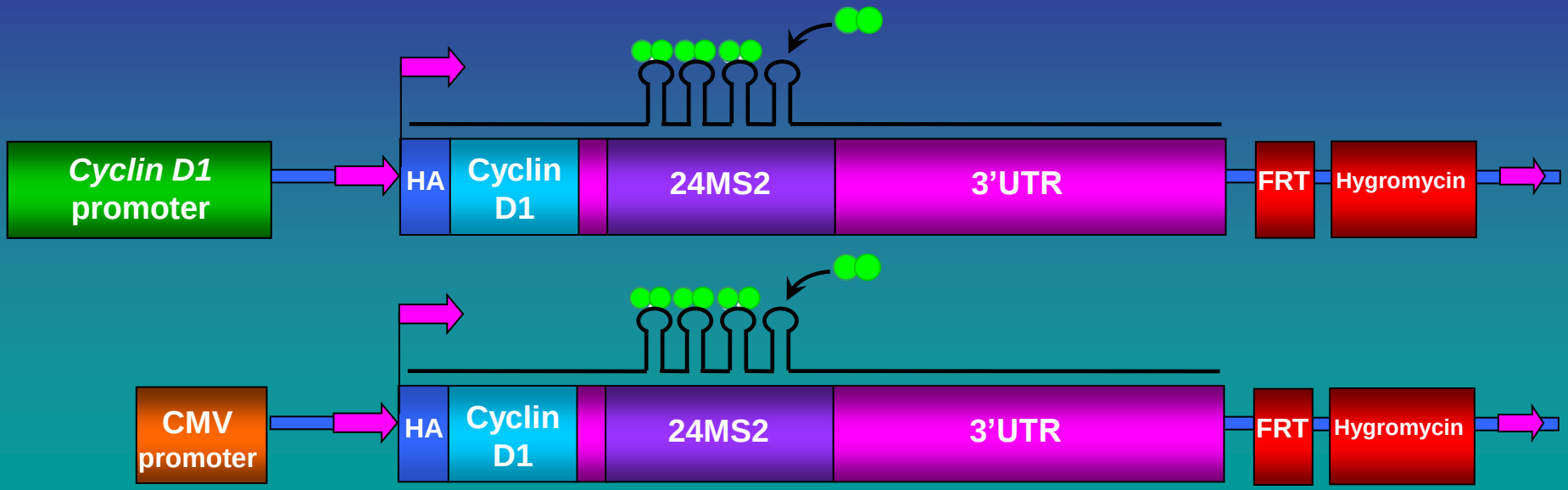


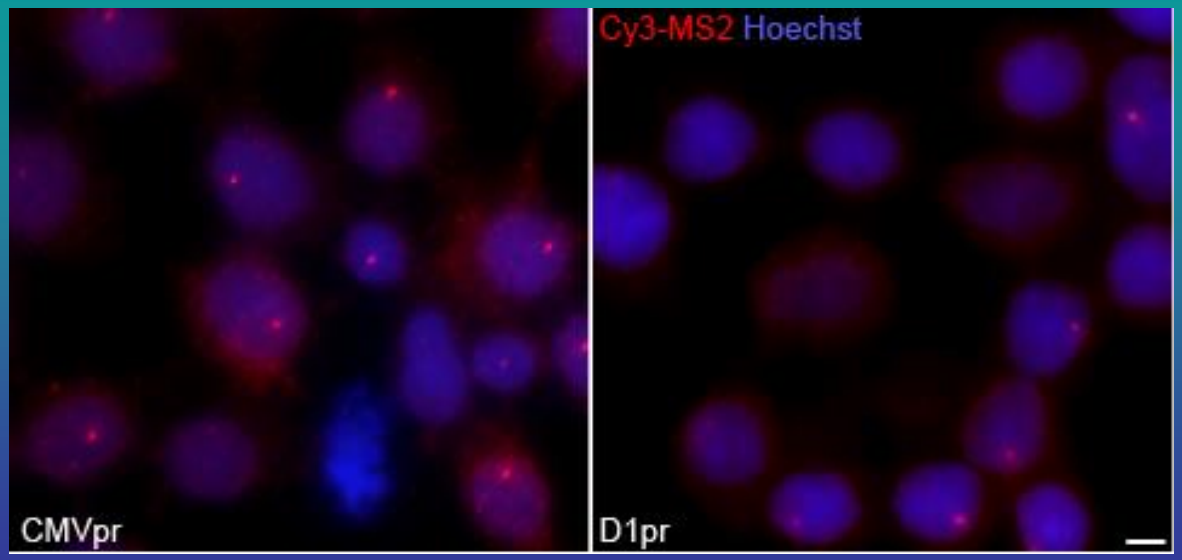
(Lionnet et al. *Nat. Methods* 2011)

Measuring transcriptional kinetics of single genes

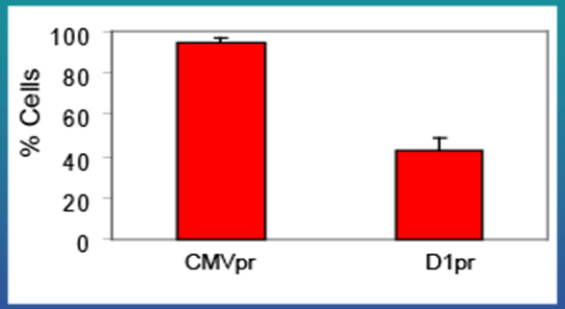
Approach B
Site-specific single-allele recombination

Detection of transcription activity on single alleles of *Cyclin D1* alleles





HEK293 cells

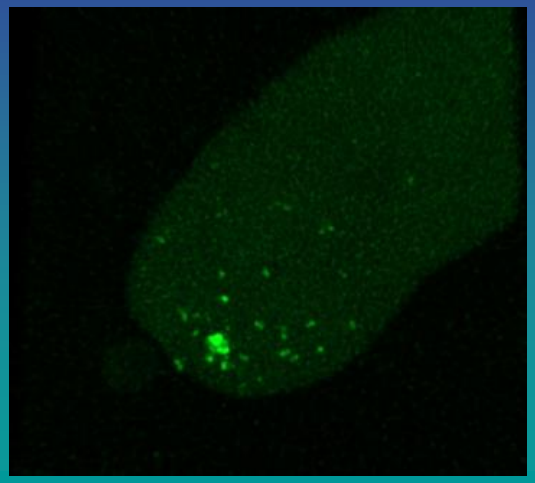


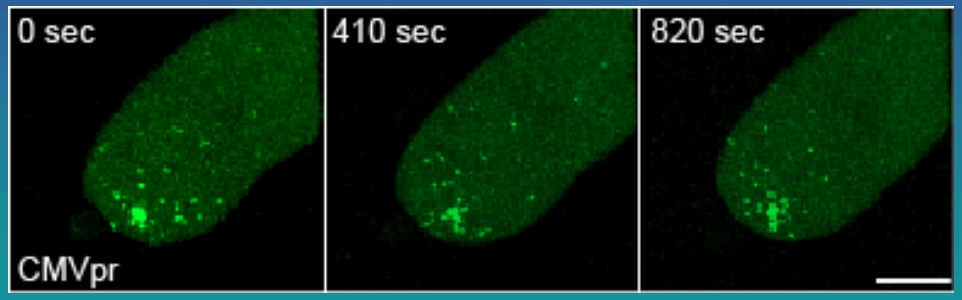
Yunger et al. *Nat. Methods* 2010

Yunger et al. *Nat. Protocols* 2013

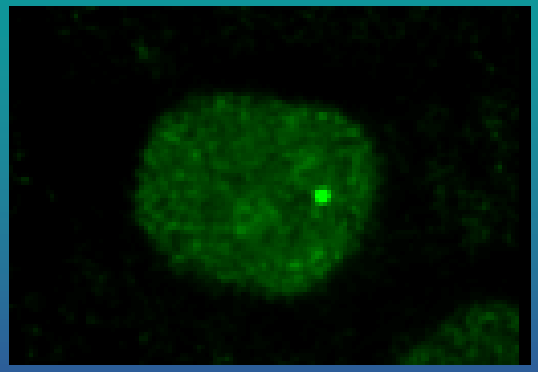
Detection of active *cyclin D1* alleles

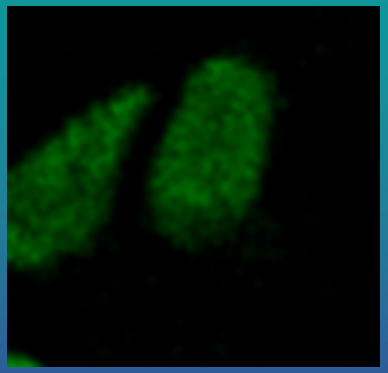
CMV promoter

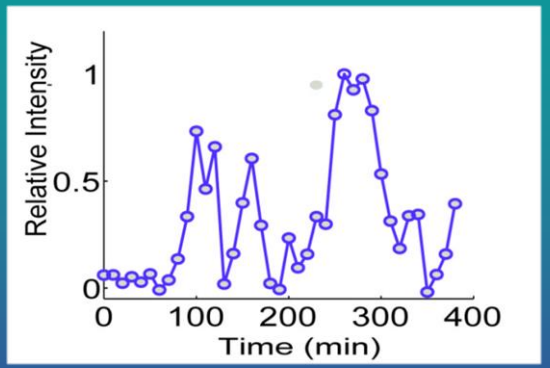




Endogenous *CCND1* promoter

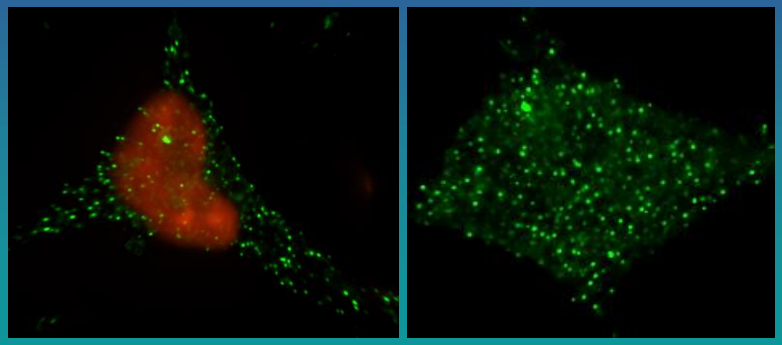




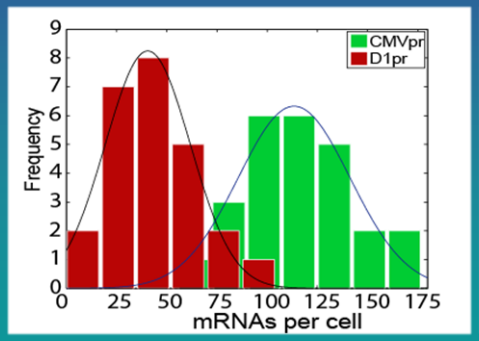


The kinetic difference between the CMV and *CCND1* promoters

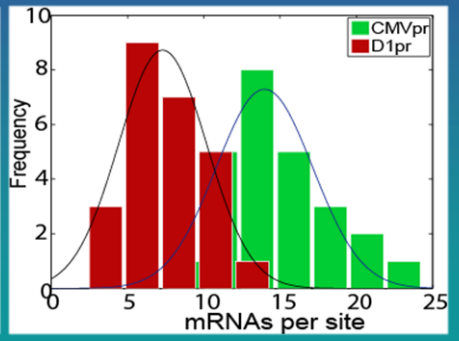
Single mRNA quantification



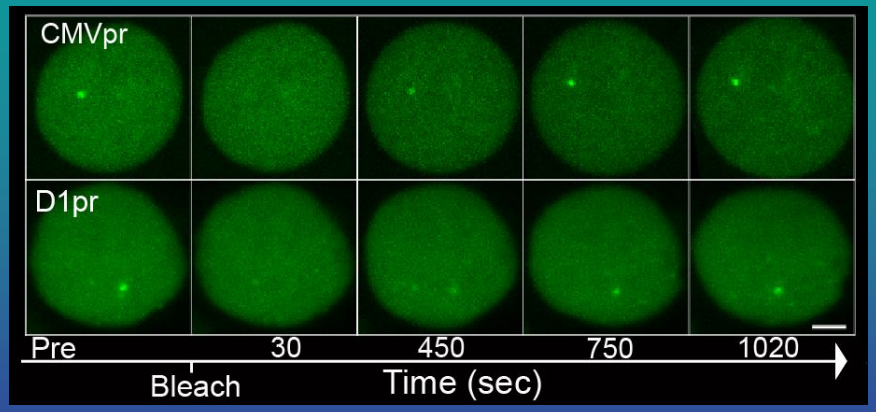
Total cell mRNA

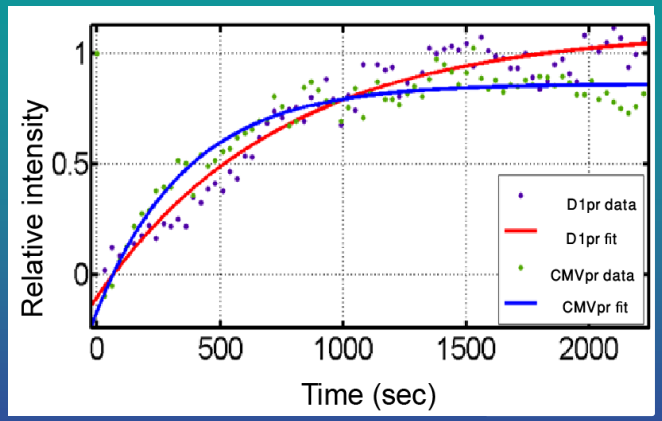


Nascent mRNA



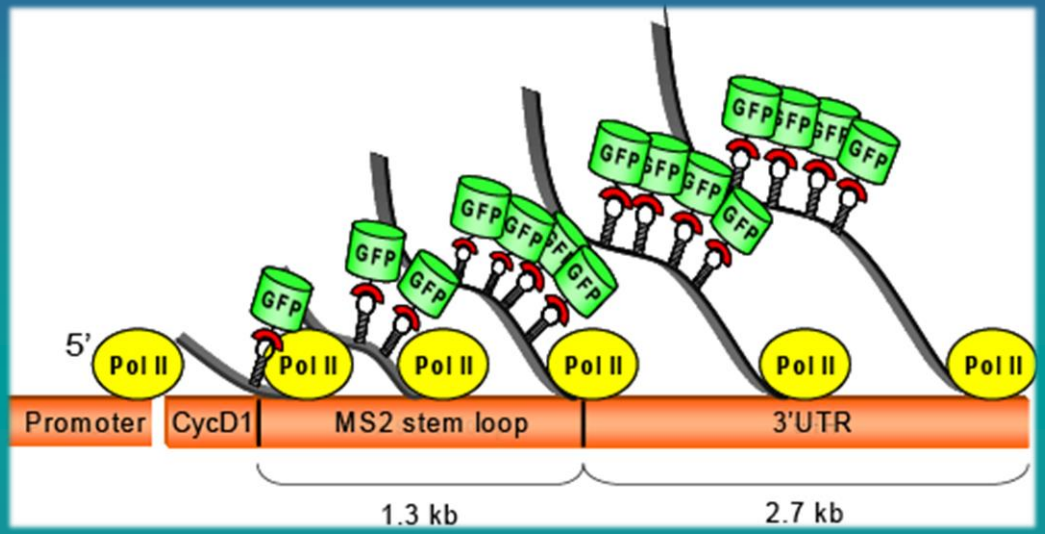
FRAP – transcription kinetics





The kinetic difference between the CMV and *CCND1* promoters

Model: FRAP analysis + RNA quantification



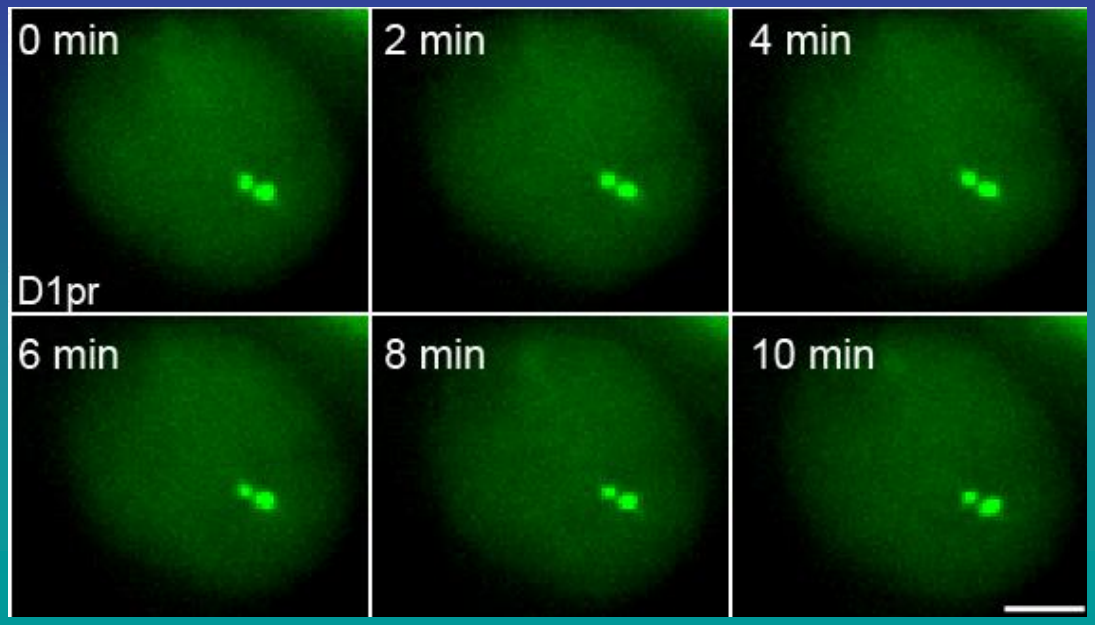
	CMVpr	D1pr
RNAs per cell	114±40	41+30
RNA per allele	14+4	7+4
Polymerase spacing (nt)	237	335
Promoter firing (sec)	22	52
Transcription rate	~0.3-0.8kb/min	

Yuval Garini, Liat Altman

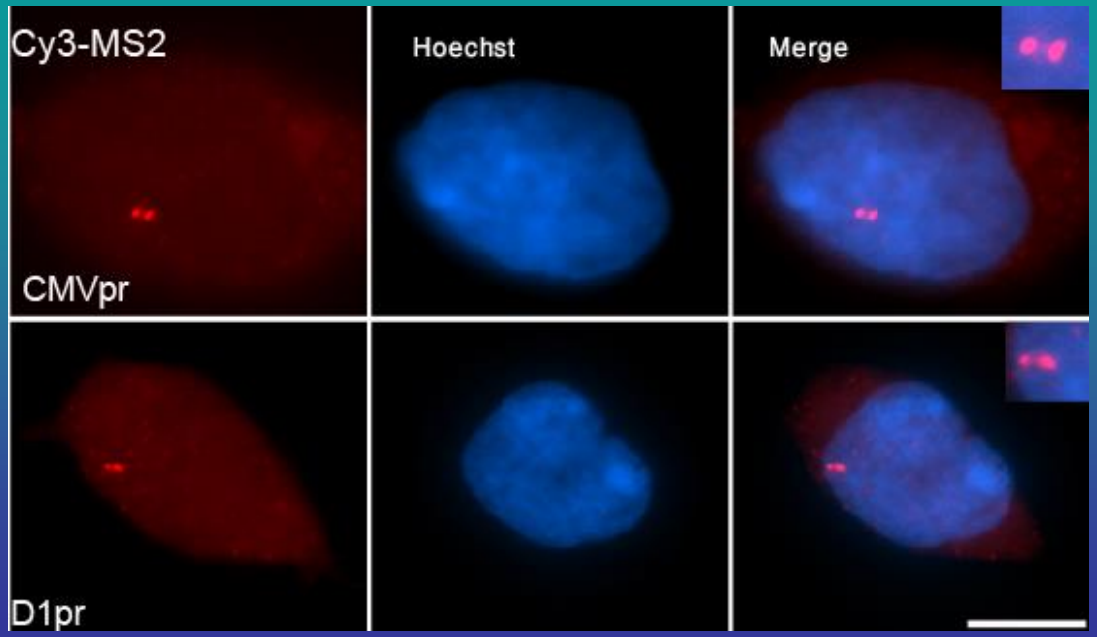
Transcription kinetics throughout the cell cycle

Transcription site doublets

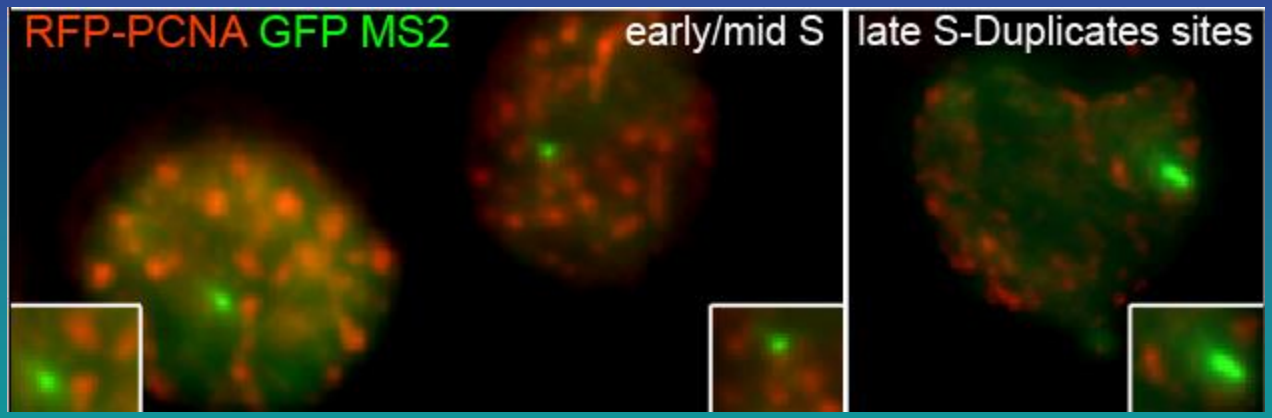
Living cells

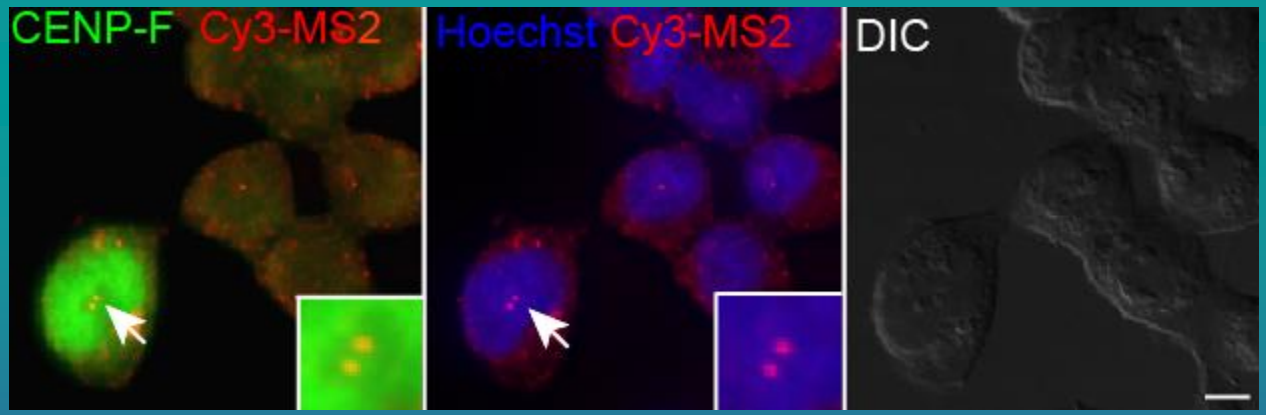


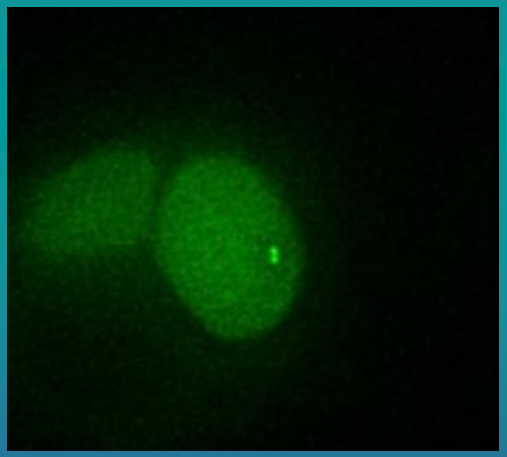
RNA FISH

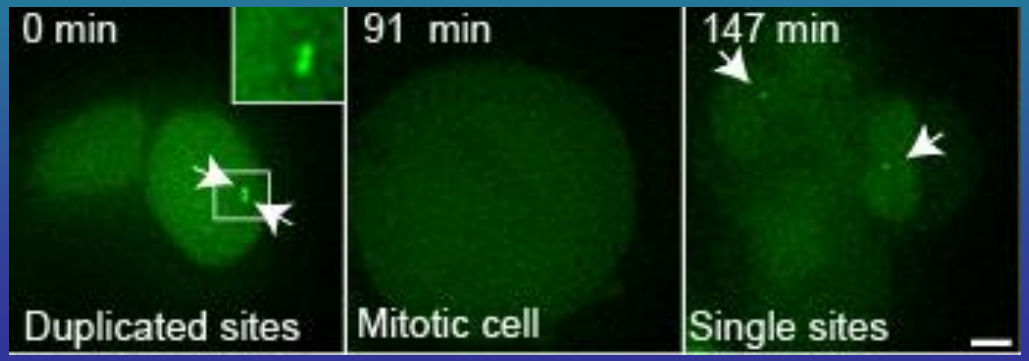


Two transcription sites are prominent from late S phase and onwards

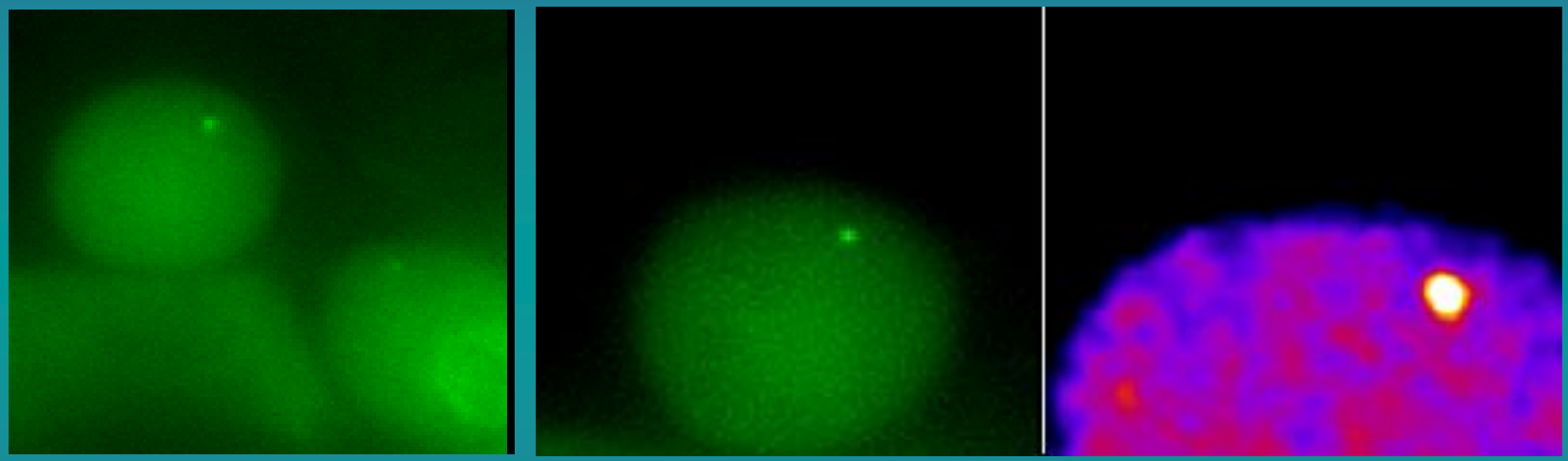








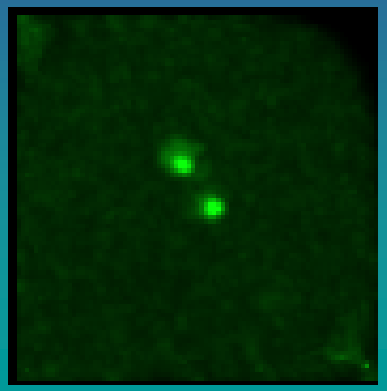
The formation of a second transcription site



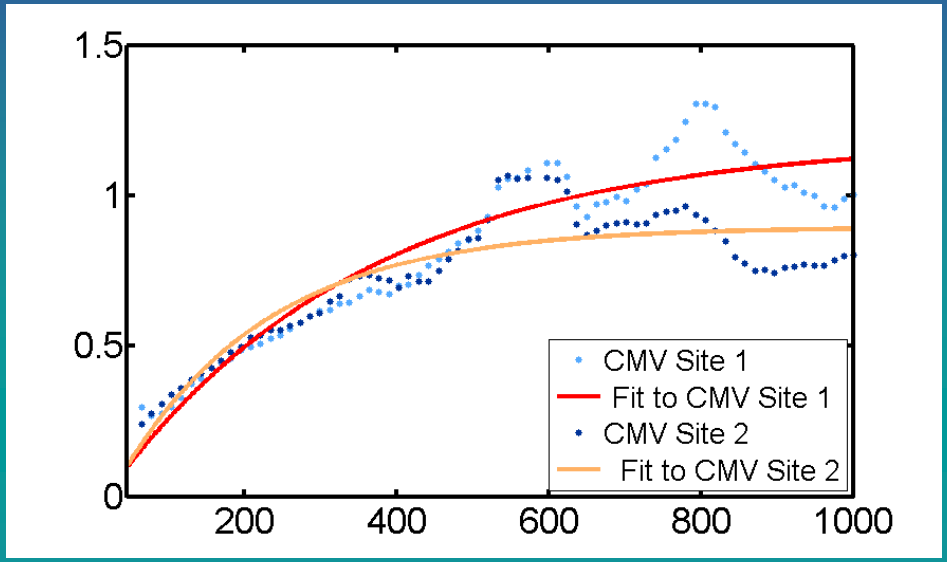
65 min

44 min

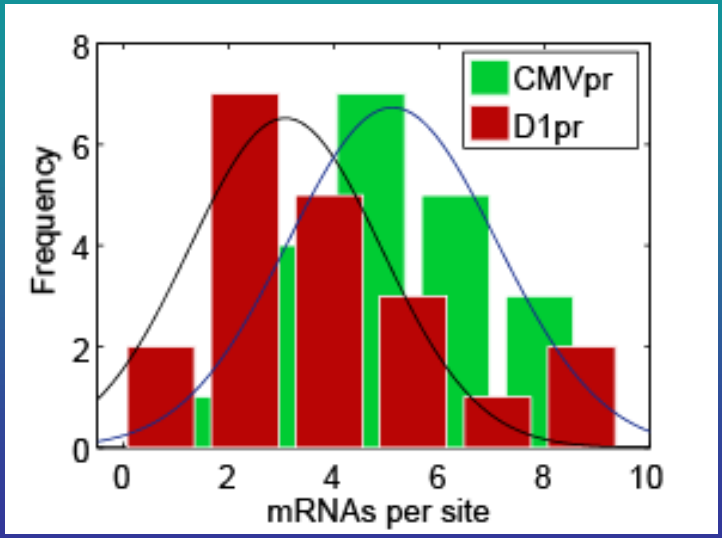
Duplicated transcription sites are less potent



FRAP on 2 sites



Nascent mRNA quantification



Transcription kinetics

mRNA

Translation

Protein

mRNA processing:

*5'-end capping

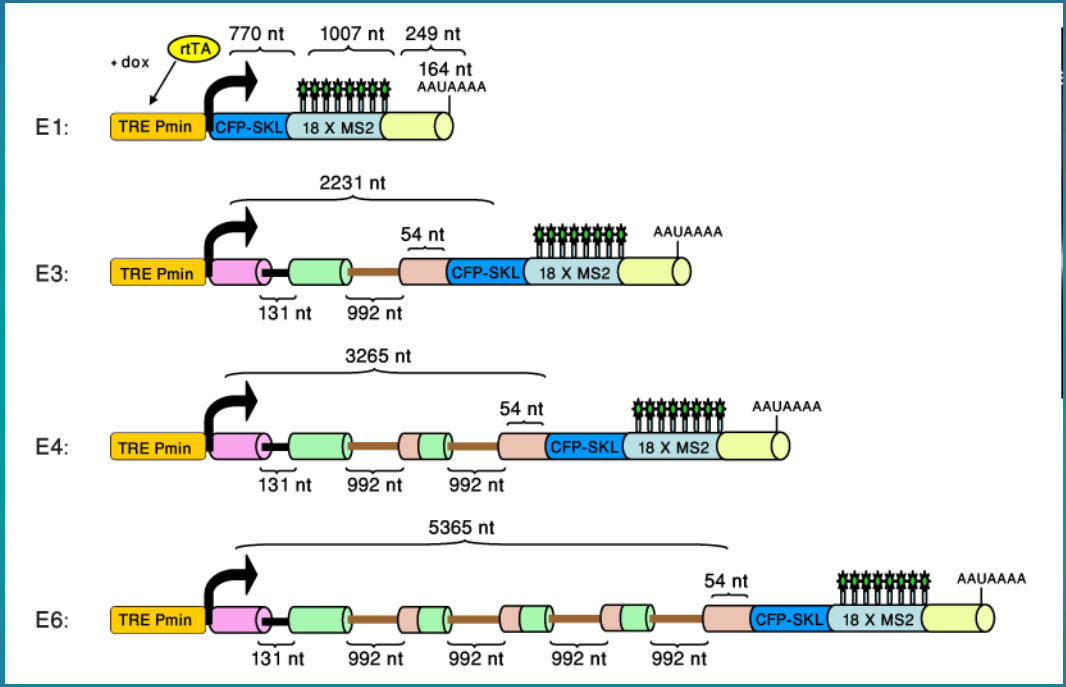
*Editing

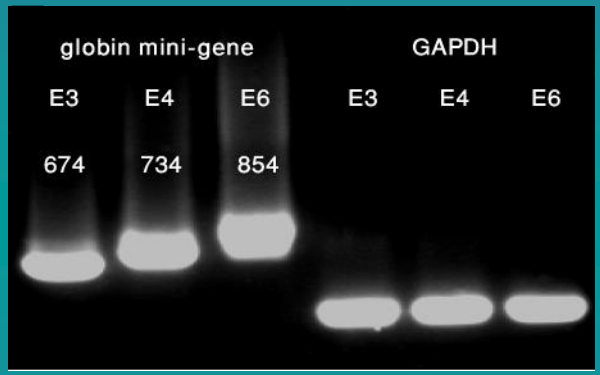
*Splicing

*3'-end processing

mRNA export

Studying the effect of splicing on transcription kinetics





(Brody et al. *PLoS Biology 2011*)

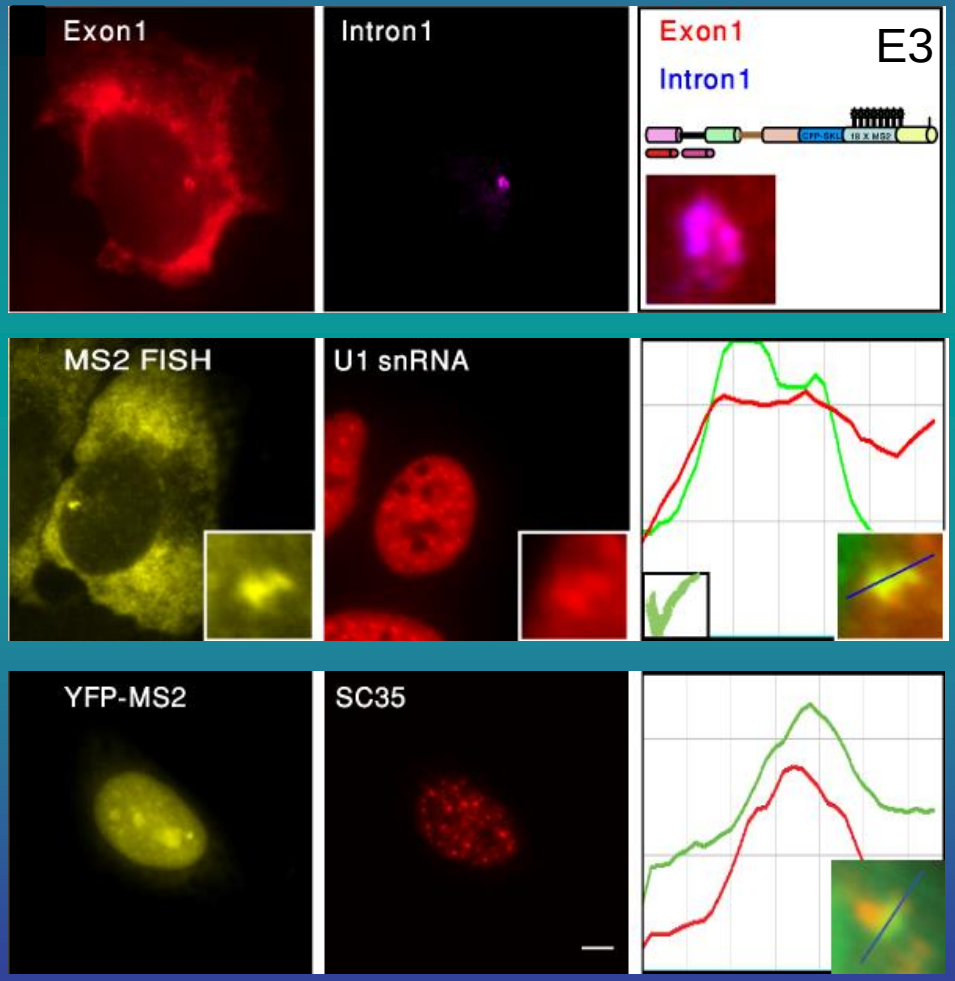
Splicing is co-transcriptional

Detected at transcription sites

Exons and introns

Core splicing machinery (U snRNAs)

Splicing factors



Are splicing factors recruited to an intronless gene?

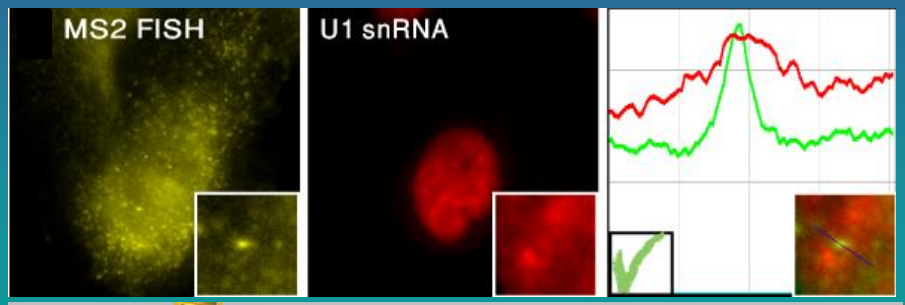
(de Almeida and Carmo-Fonseca 2008)

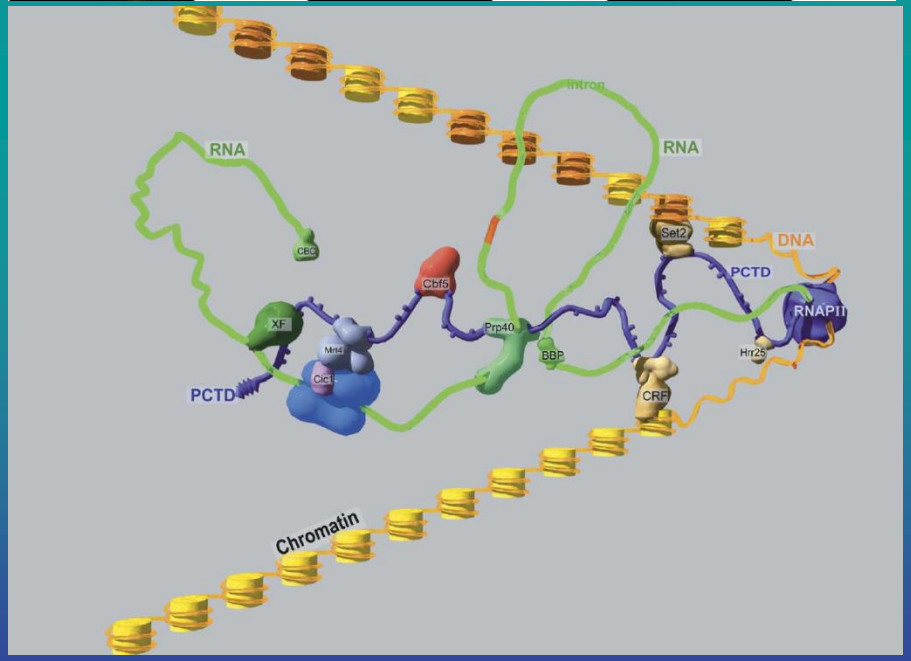
U1 snRNP is recruited to an intronless gene

U1 snRNA

U2 snRNA

U4 snRNA

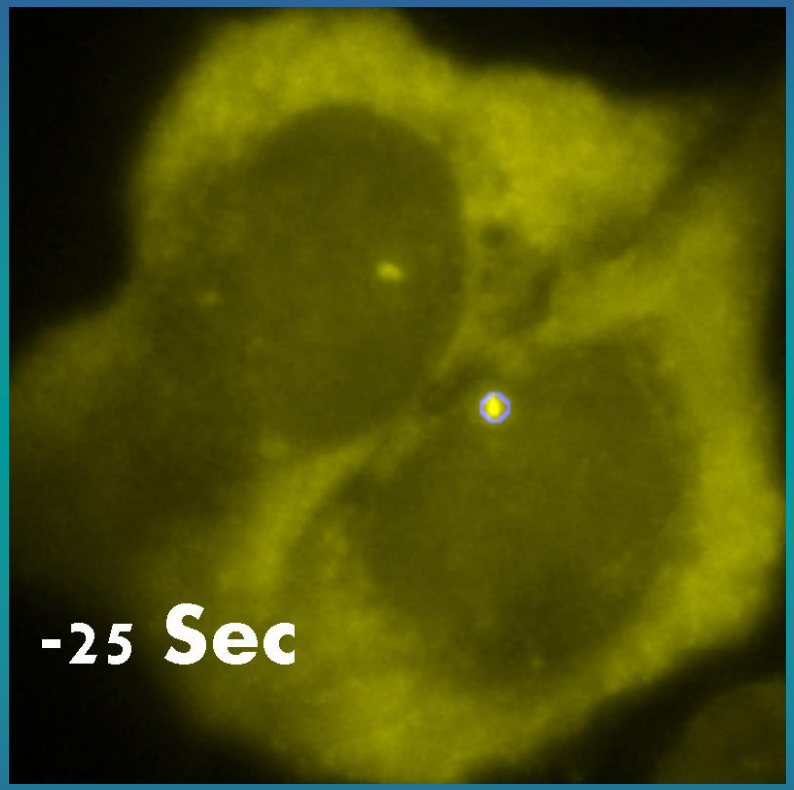


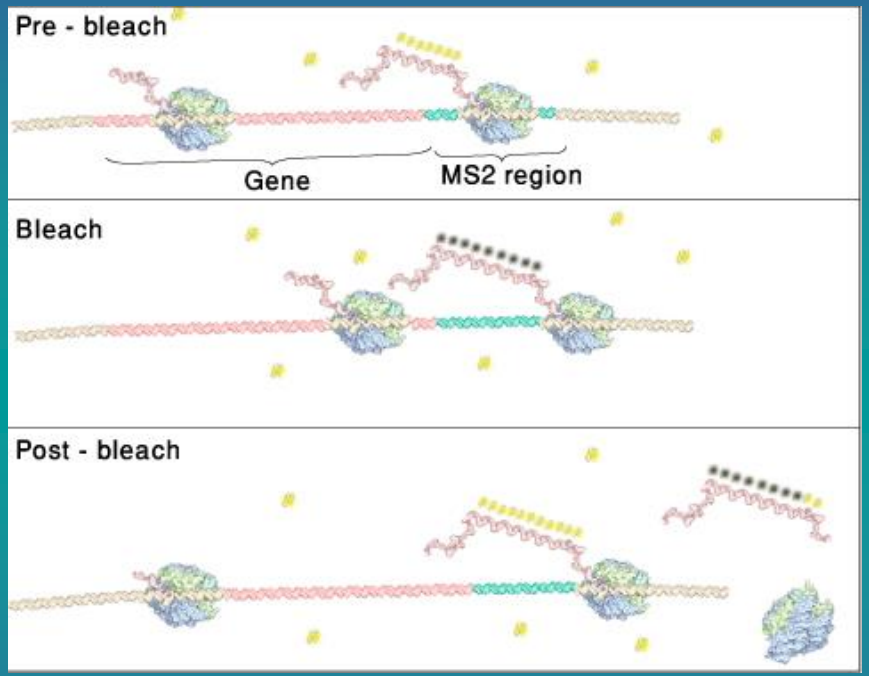


(Phatnani and Greenleaf, *Genes Dev* 2006)

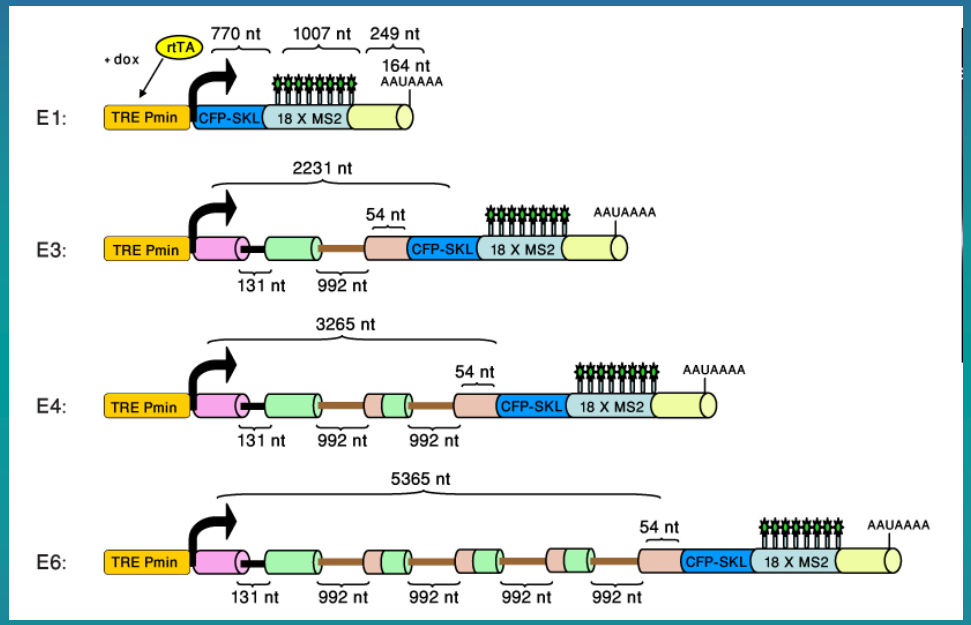
Measuring elongation kinetics using FRAP

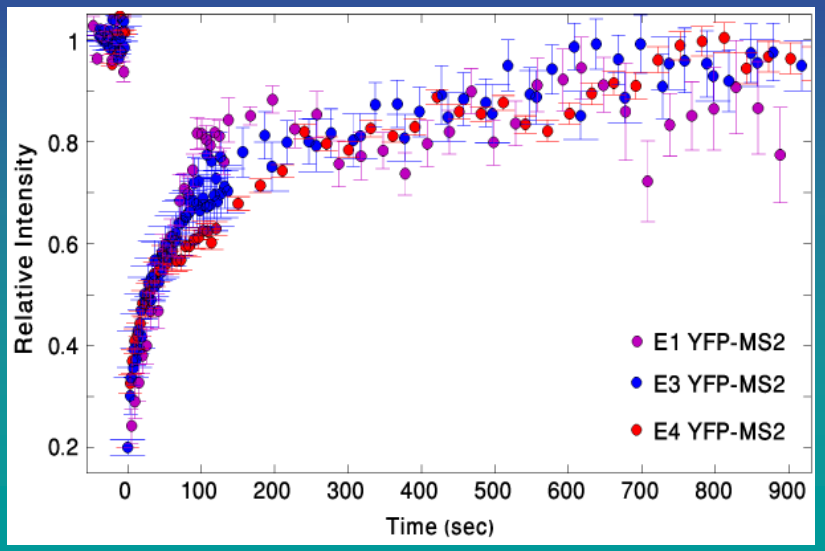
FRAP (mRNA)

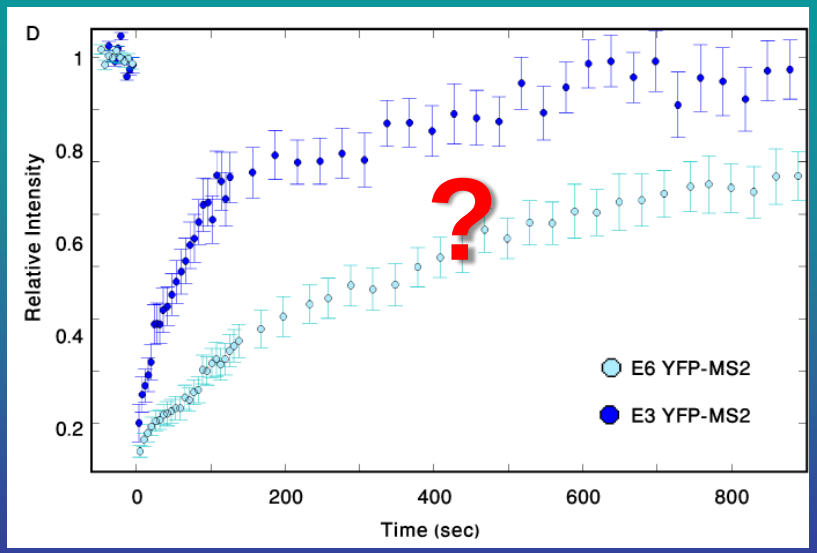




Measuring elongation kinetics using FRAP

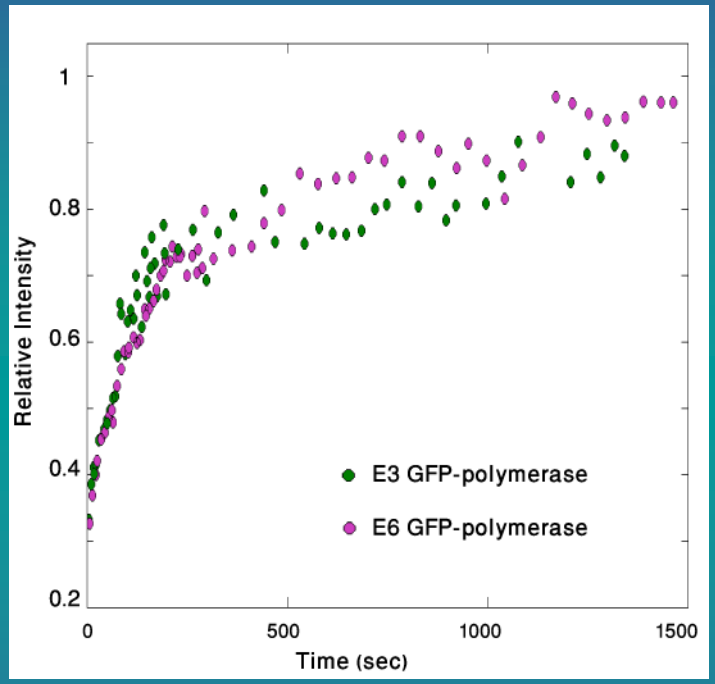




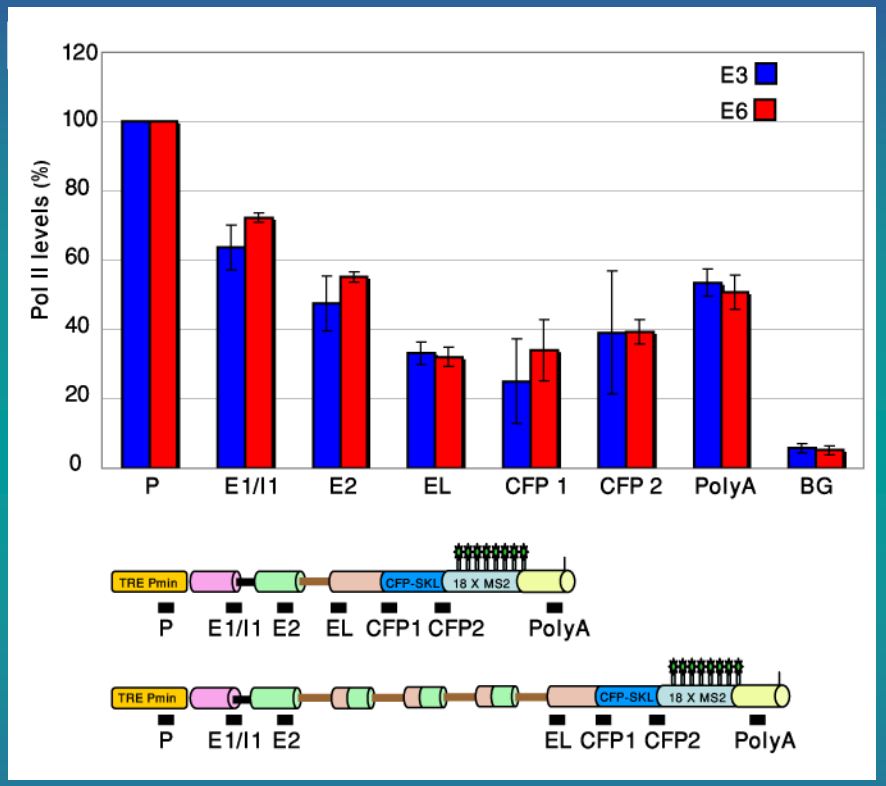


Total polymerase kinetics remain unchanged

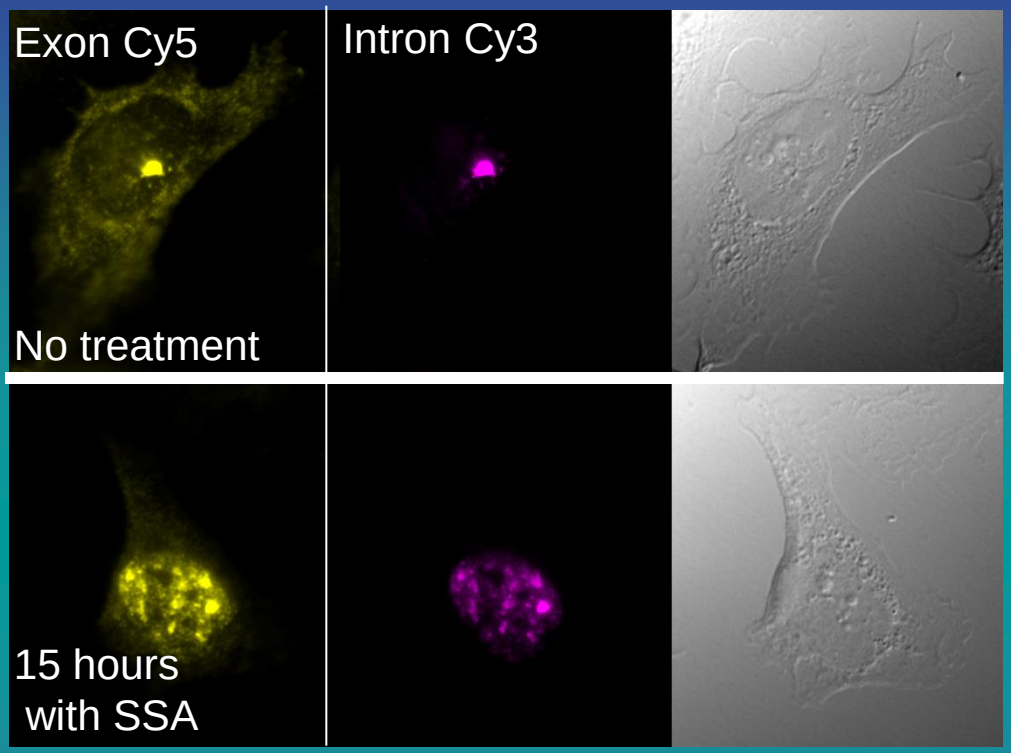
FRAP (GFP- Pol II)



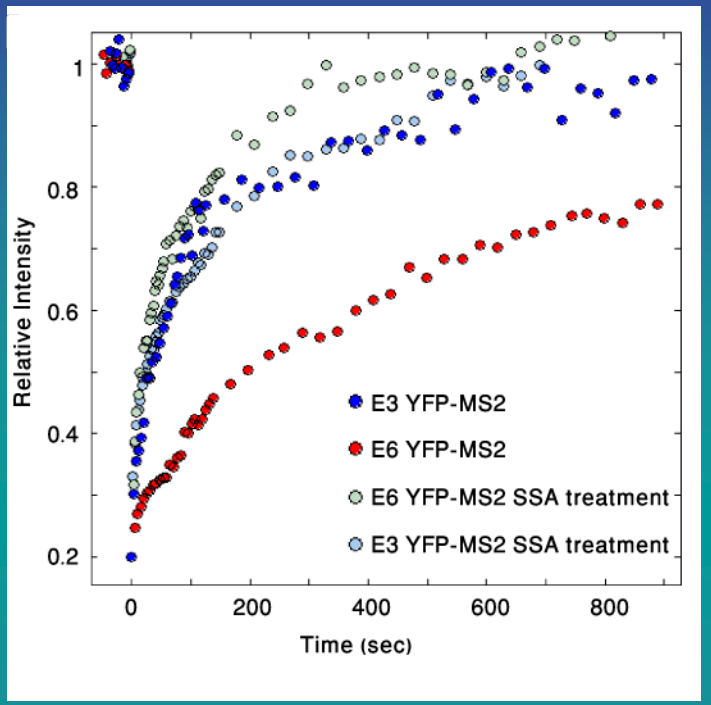
DNA ChIP (Pol II)



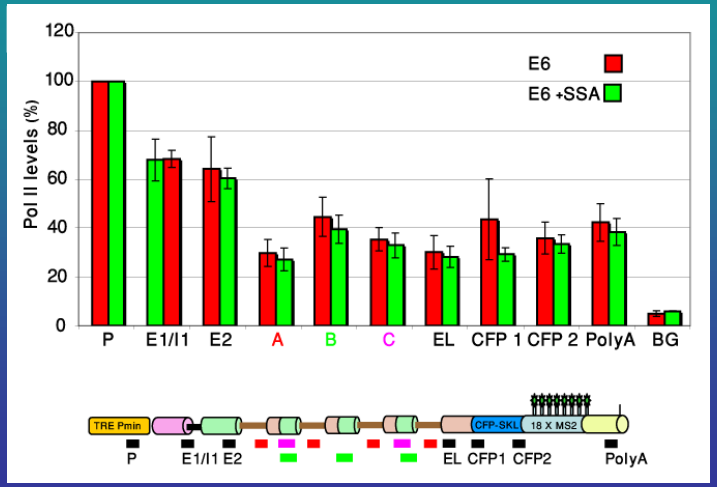
Splicing inhibition affects E6 kinetics



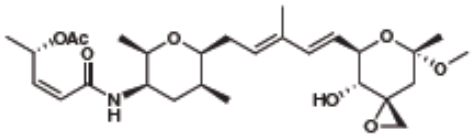
FRAP (mRNA)



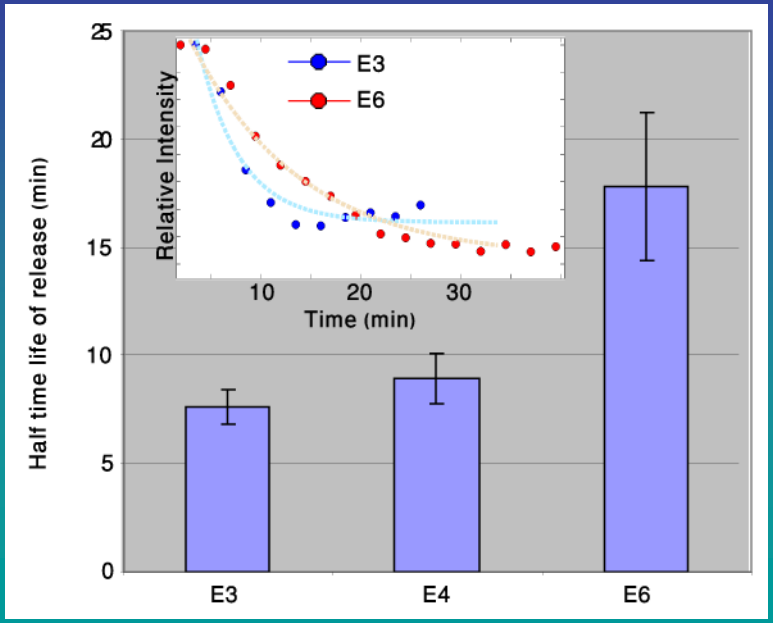
DNA ChIP (Pol II)

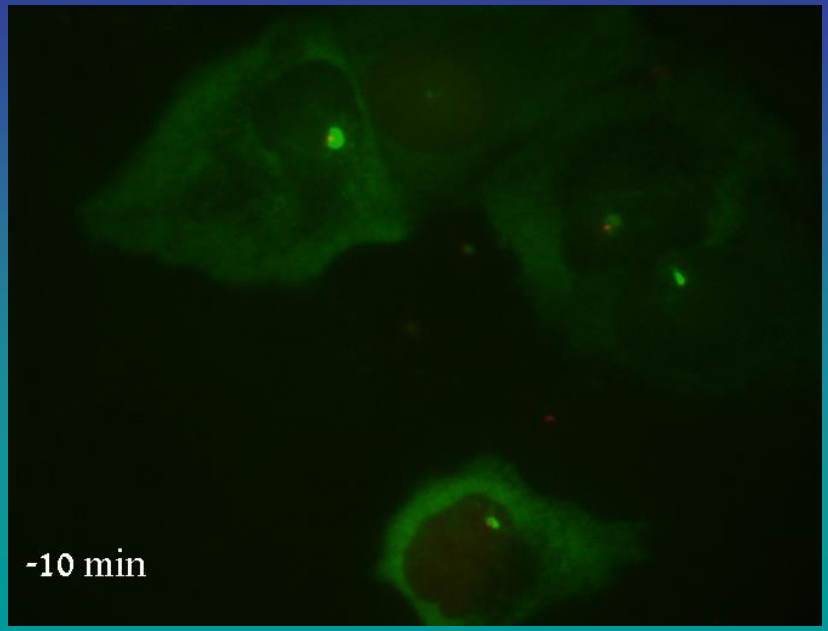


Spliceostatin A (SSA)

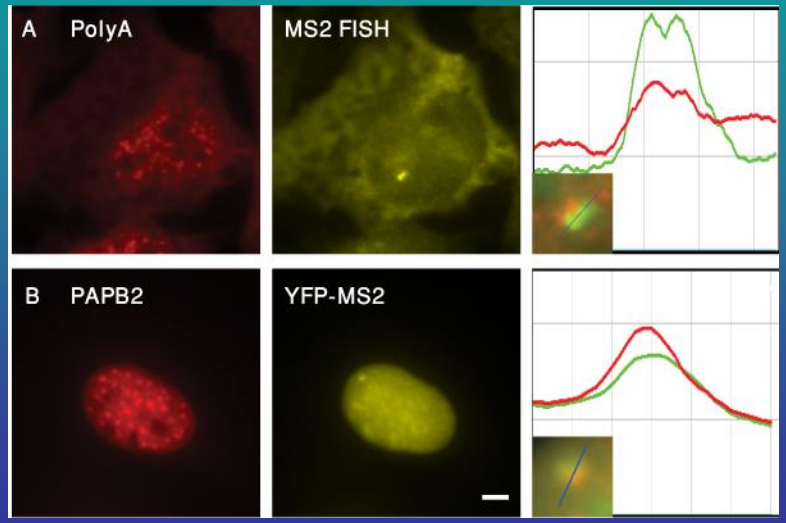


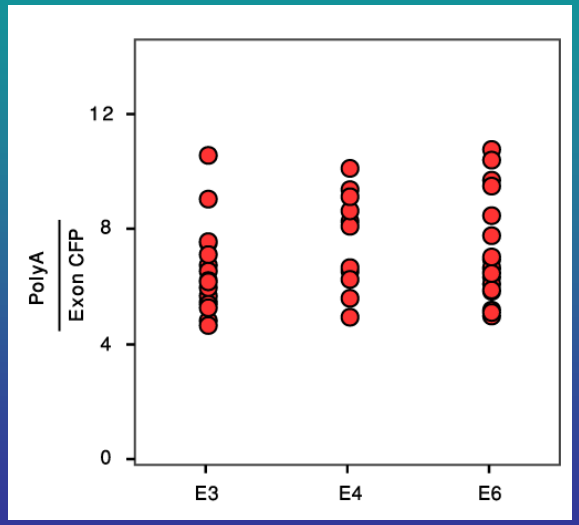
mRNAs are stalled on the E6 gene



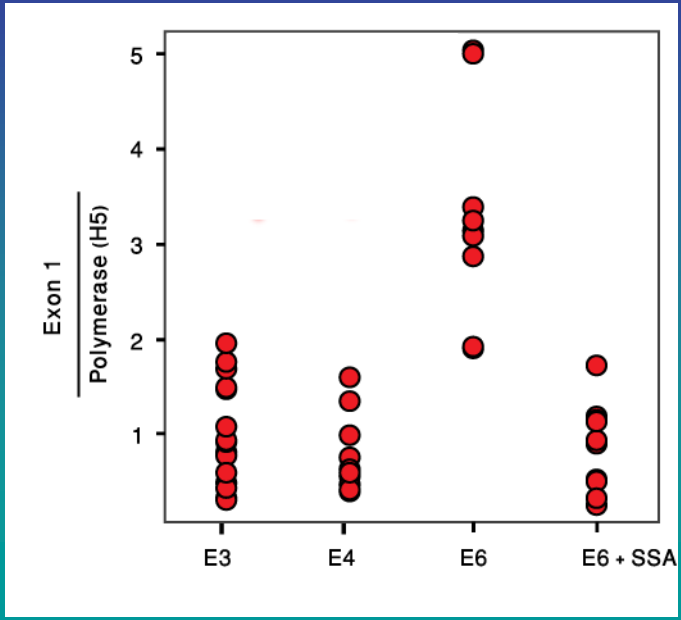


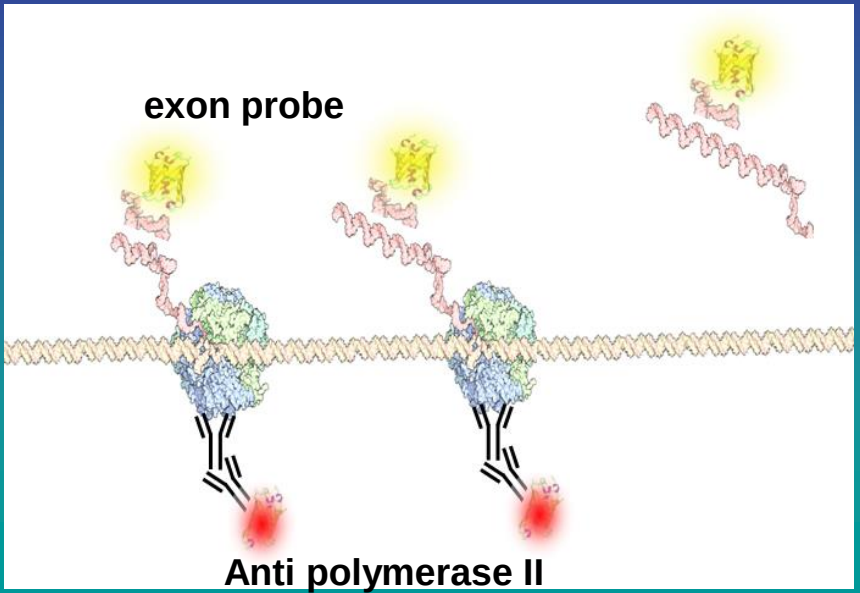
This is not accumulation of aberrant transcripts

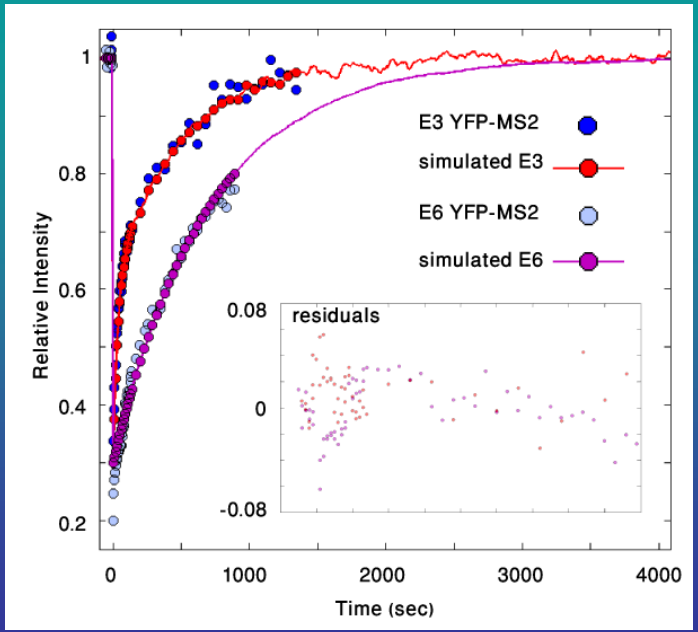


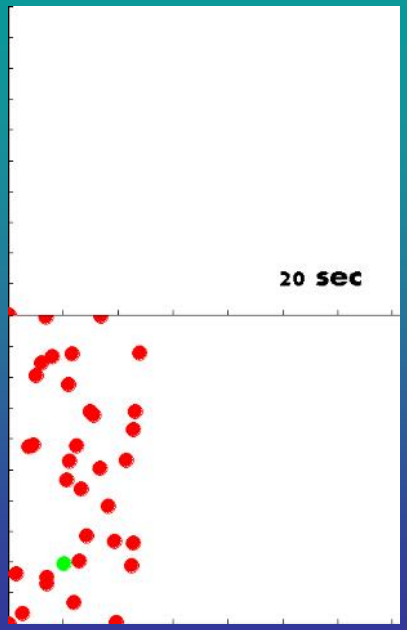


mRNAs are retained although the polymerase has left







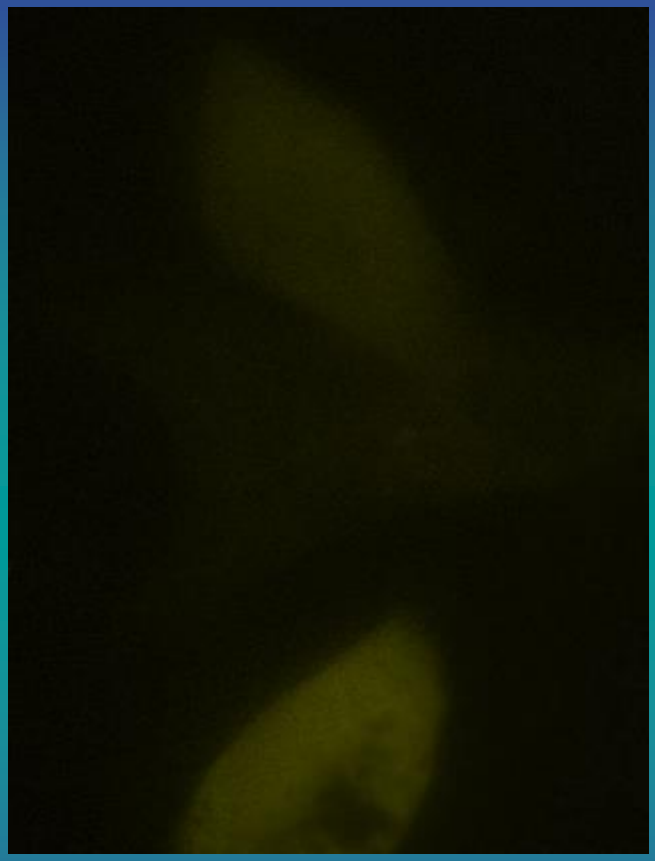


Elongation rate: 3.6 kb/min

E3 gene: 50 sec retention time

E6 gene: 10 min retention time

Kinetics of mRNP nucleoplasmic transport



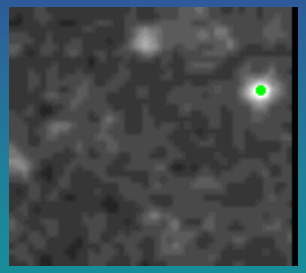
Every 6 min for 6:45 hrs

mRNPS appeared in the nucleoplasm 5-10 min after transcription induction. After another ~10 min the mRNPs could be detected in the cytoplasm.

(Mor et al. *Nat. Cell Biol*. 2010)

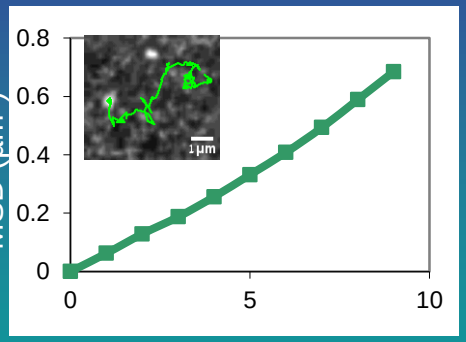
mRNP transport kinetics

2D and 3D tracking

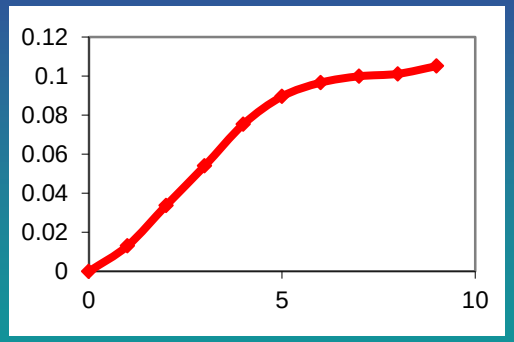


X10 original speed

Diffusive

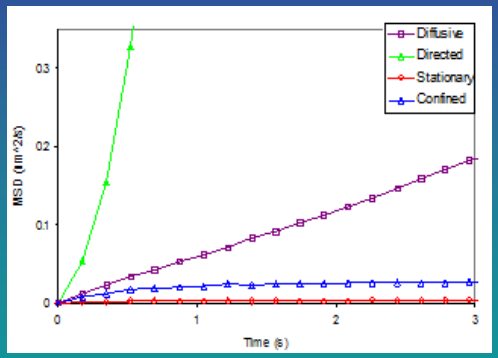


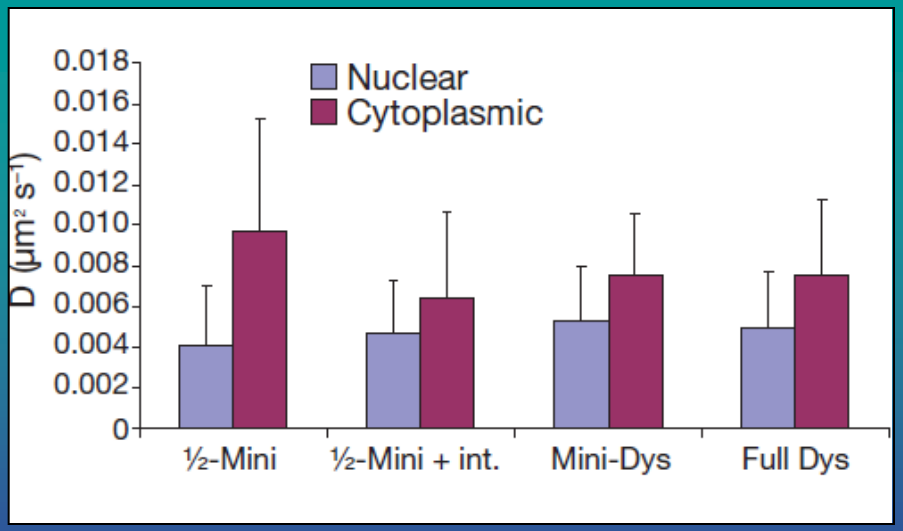
Corralled

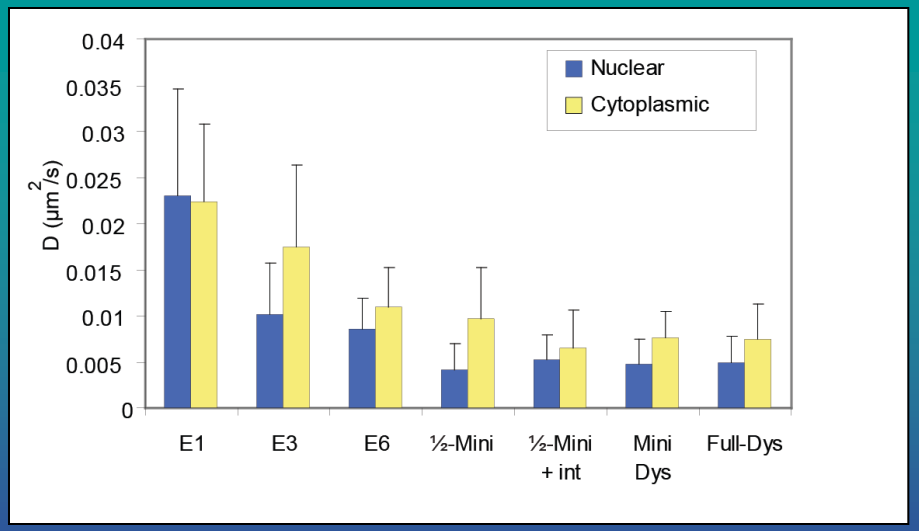


time (sec)

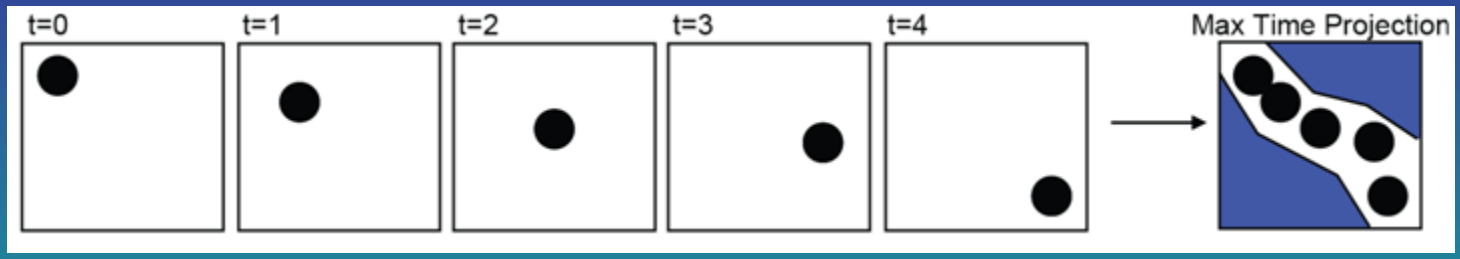
Mean square displacement analysis

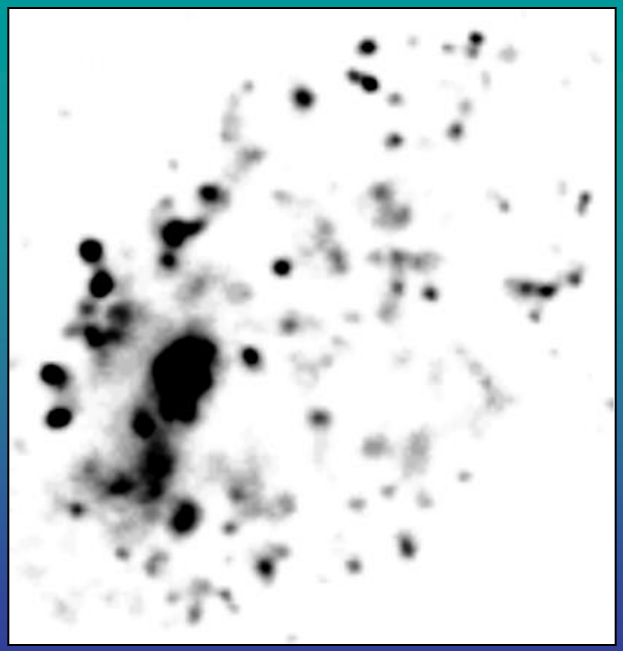


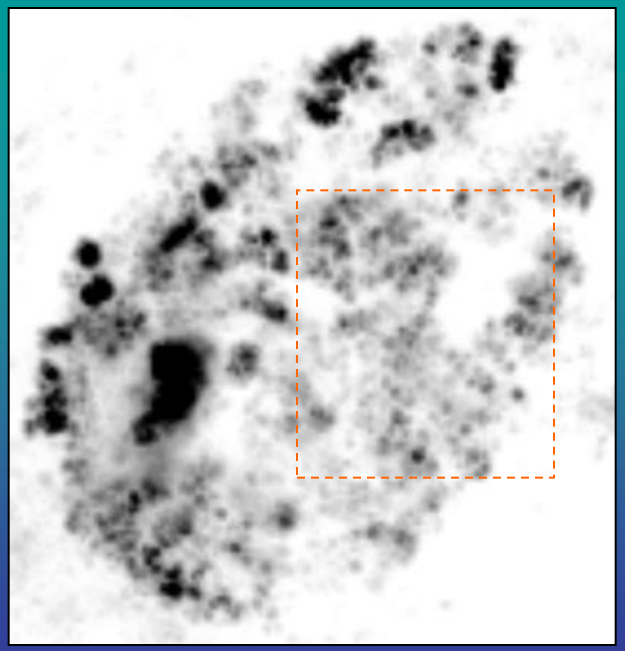


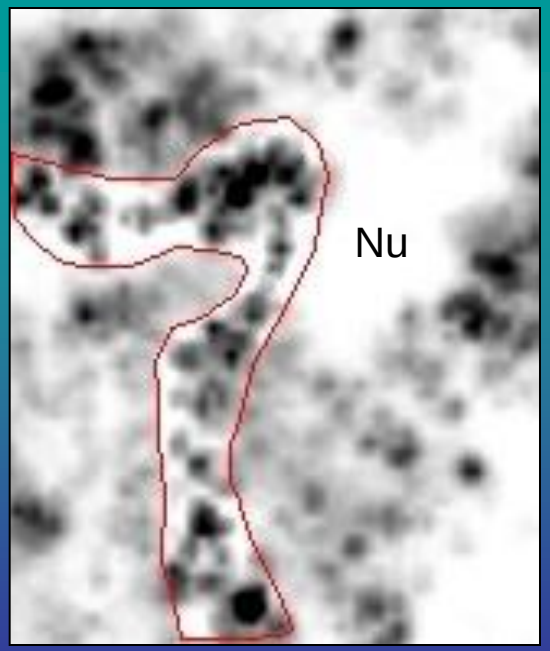


mRNP tracks

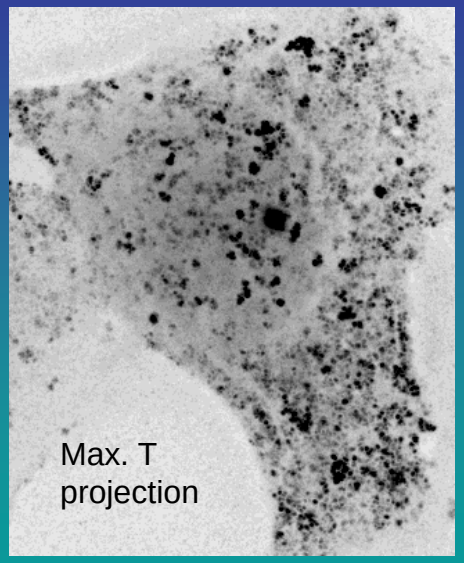


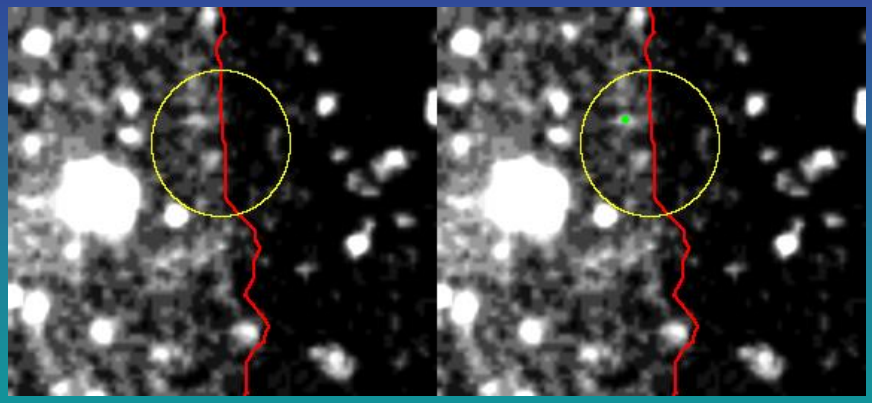




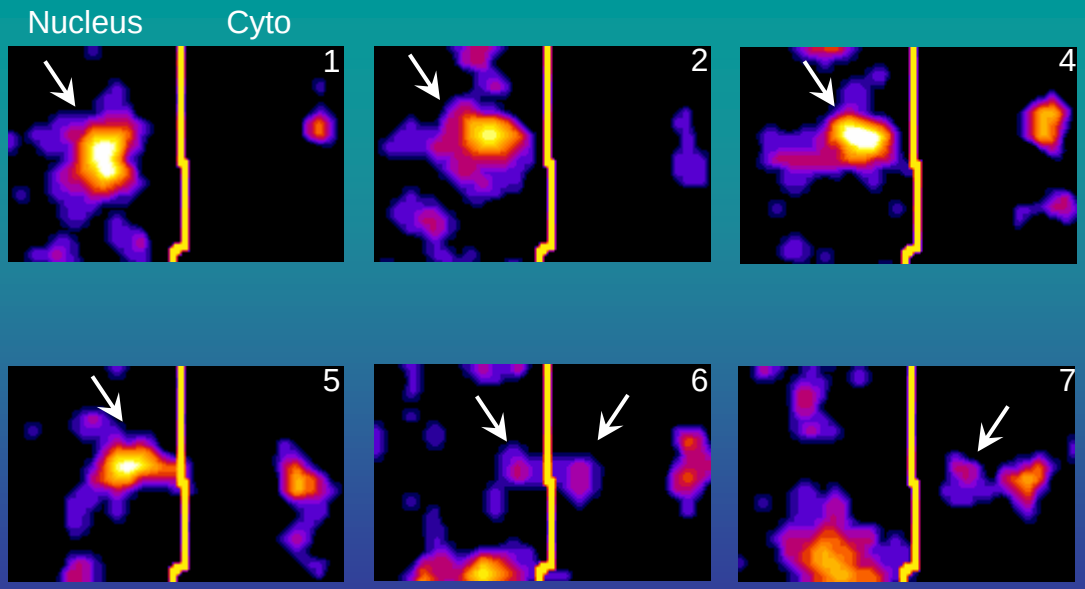


mRNP export





7 sec

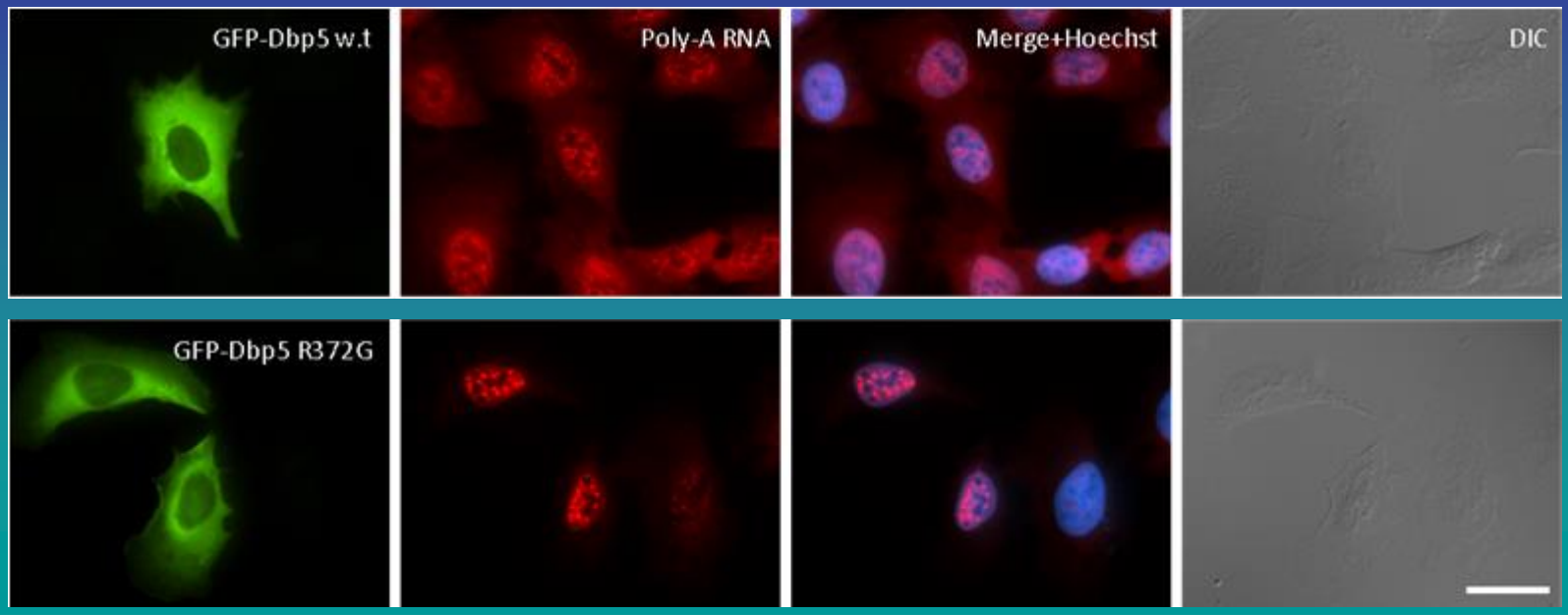


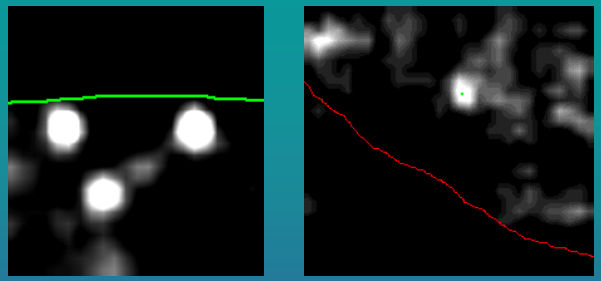
Dbp5 is required for mRNA export in human cells

RNA FISH

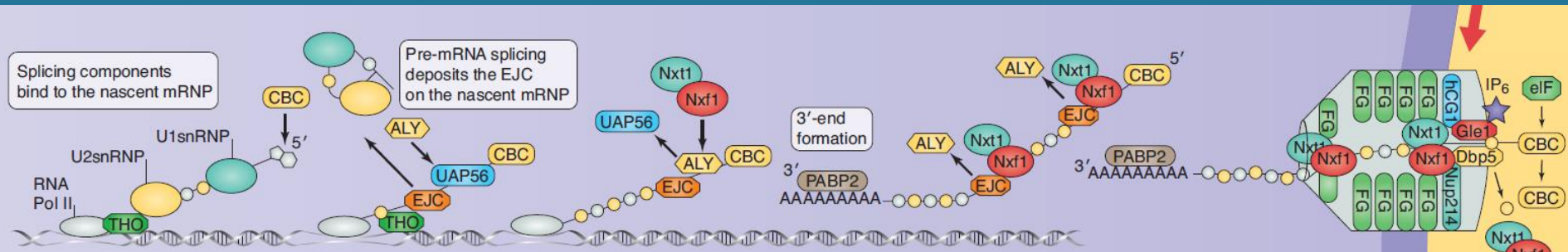
W.T

Dominant Negative R372G





(Hodge et al. *Genes Dev* 2011) (Noble et al. *Genes Dev* 2011)



Carmody & Wente *JCS* 2009

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Ministry of Science, Technology and Space
وزارة العلوم, التكنولوجيا والفضاء

Oliver Vugrek

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