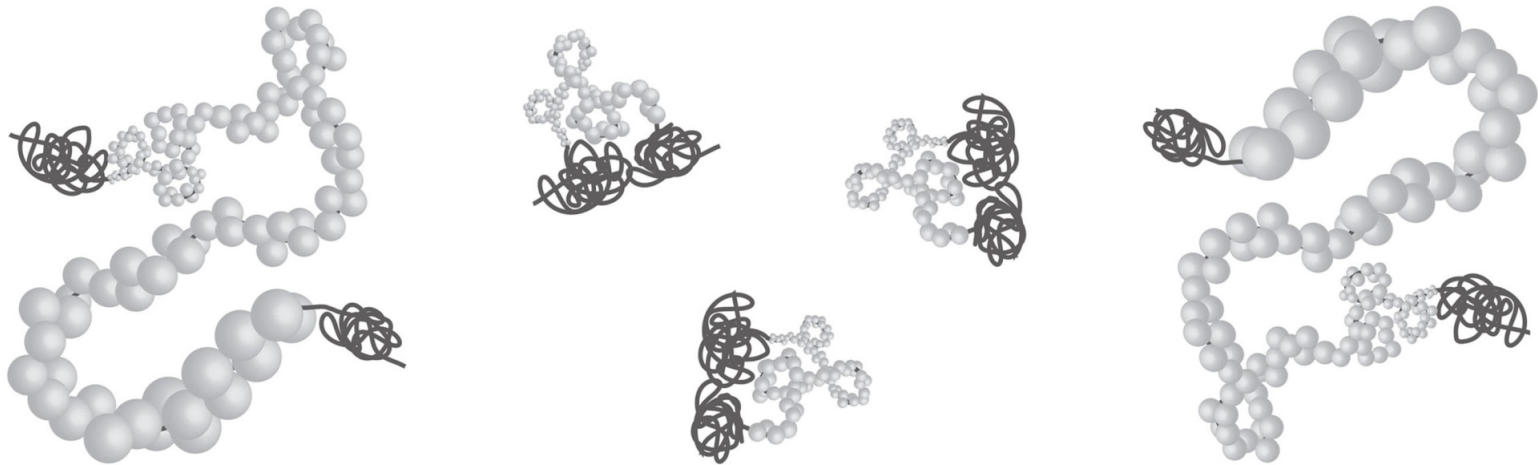


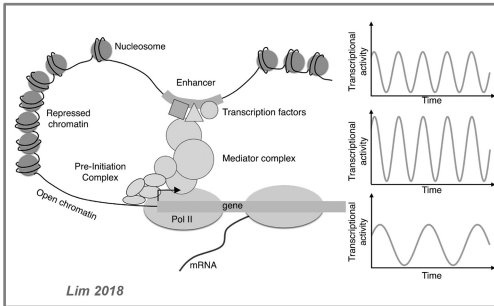
SPATIAL ORGANIZATION OF TRANSCRIBED EUKARYOTIC GENES



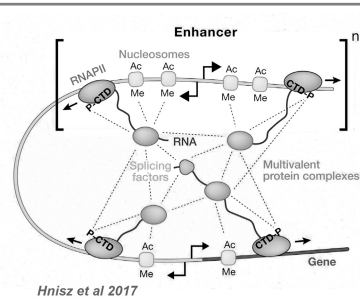
Irina Solovei
(*Biozentrum LMU*)

Biological Physics of Chromosomes
KITP program
Zoom Lecture 18.06.2020

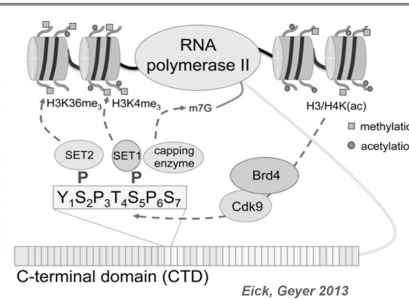
Spatial organization of transcription?



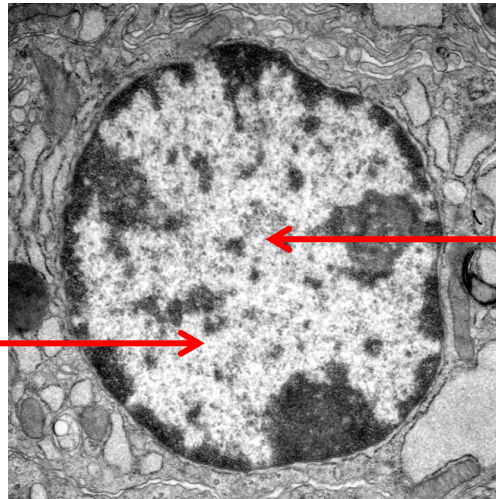
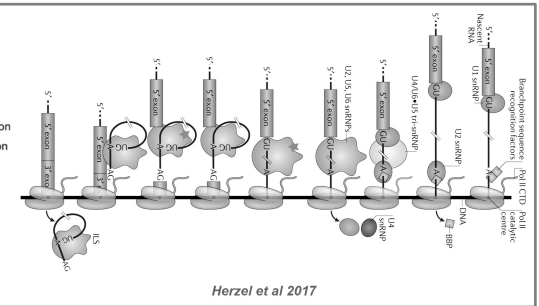
transcription regulation



mechanisms of transcription



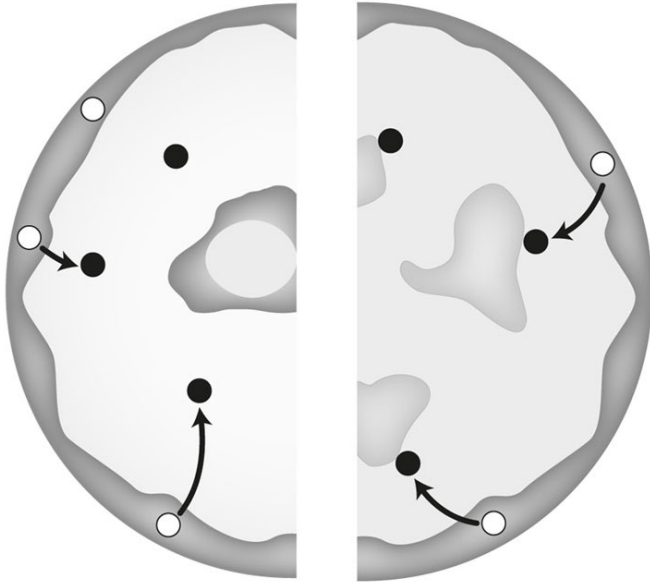
post-transcriptional modifications



euchromatin

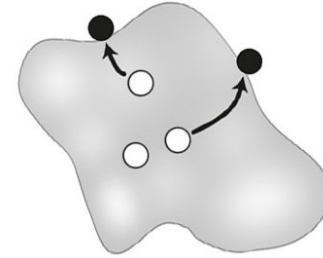
nuclear interior

What is the spatial arrangement of a single expressed gene?



active genes move in
the nuclear interior

highly expressed genes
are gathered around
nuclear speckles

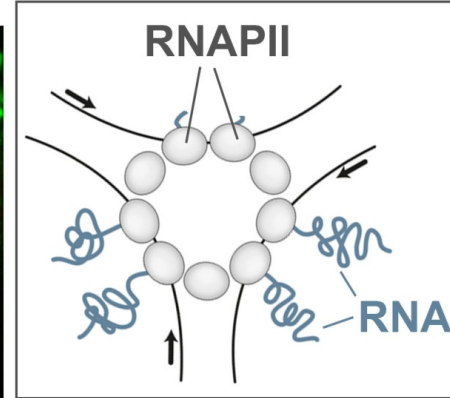
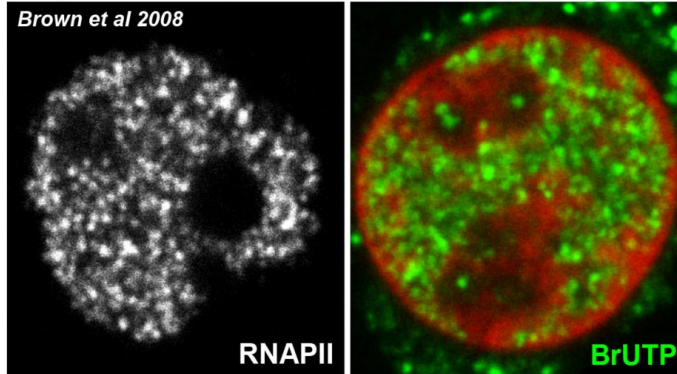


expressed genes move
to the periphery of a
chromosome territory

Light microscopy resolution is the major limiting factor

hypothesis: transcription factories

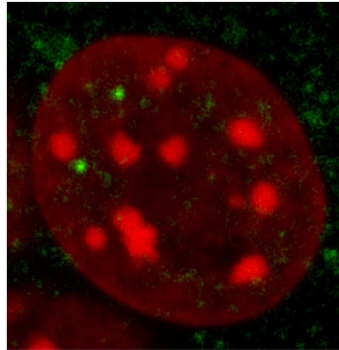
Brown et al 2008



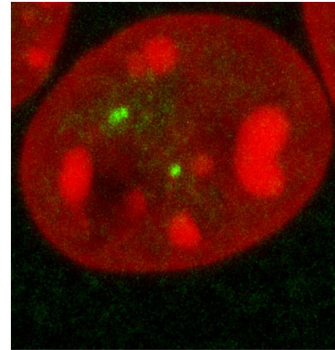
Acta1

Myl4

RNA-FISH



3 Kb



37 Kb

10 kb gene = 0.5 μ m

HBB = 1.6 kb

ACTA1 = 1.5 kb

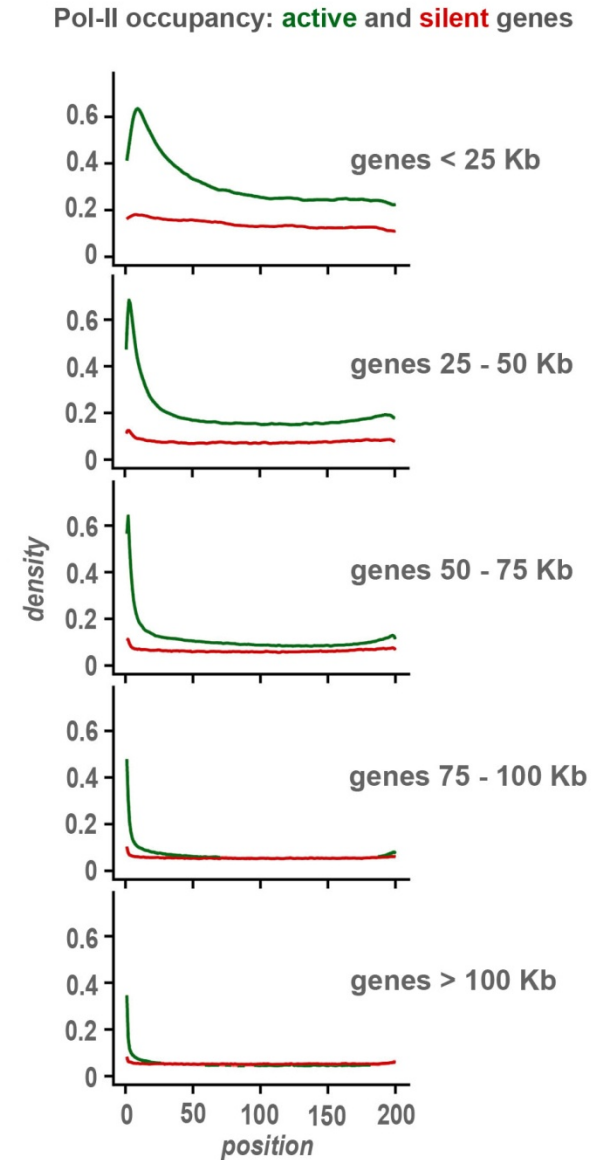
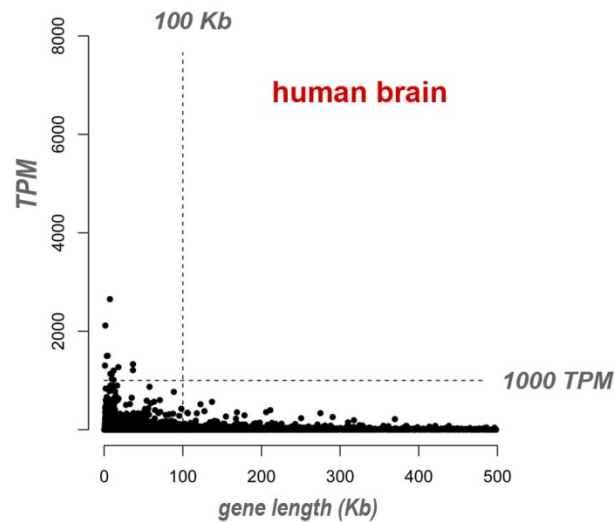
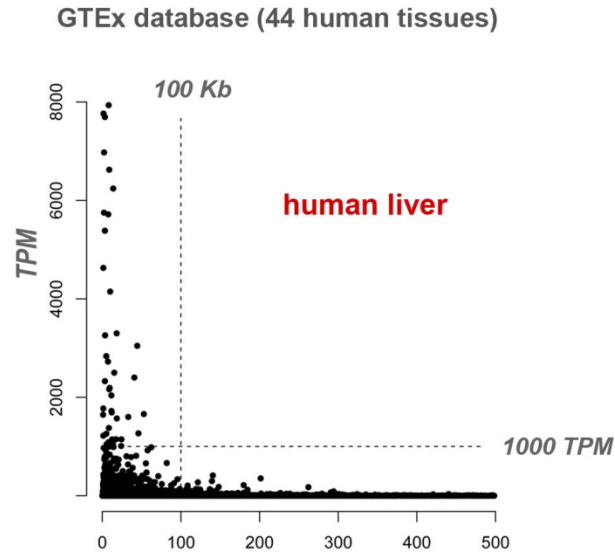
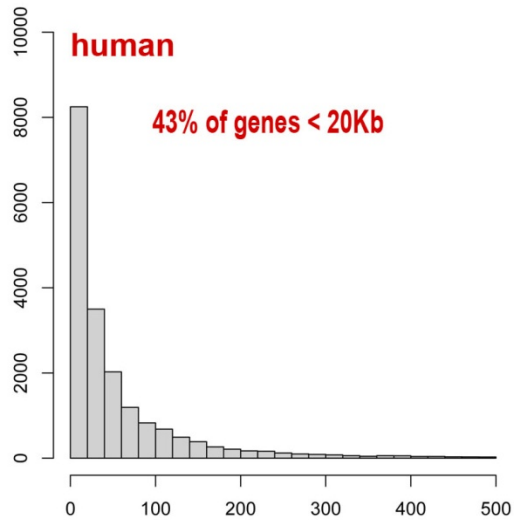
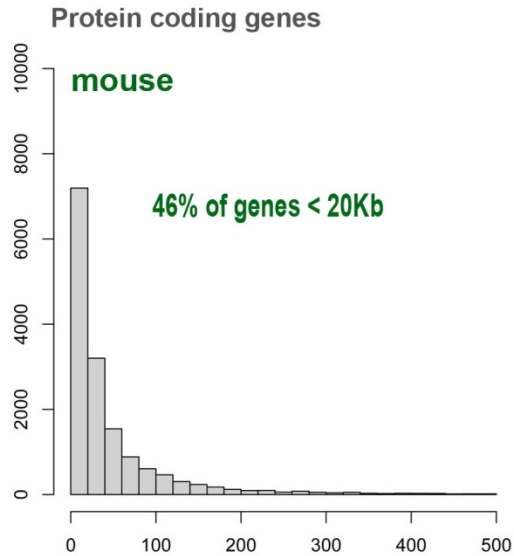
OR genes = ca. 1 kb

Hox genes = 2 - 8 kb

Sox genes = 0.5 - 10 kb

expression \geq 1000 TPM; length \geq 100 kb

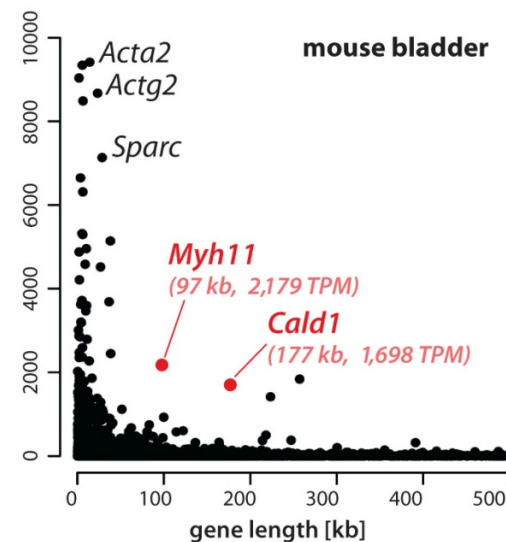
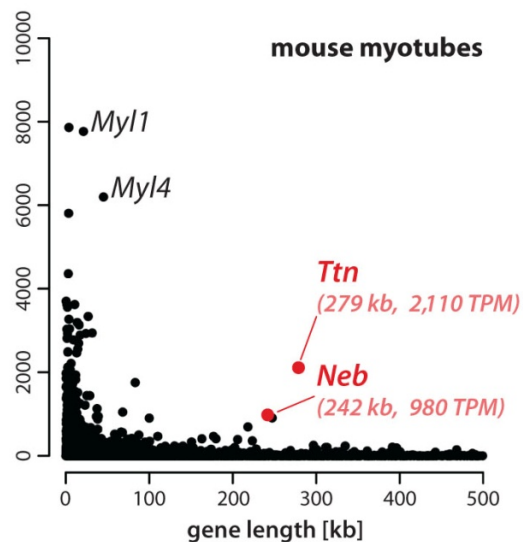
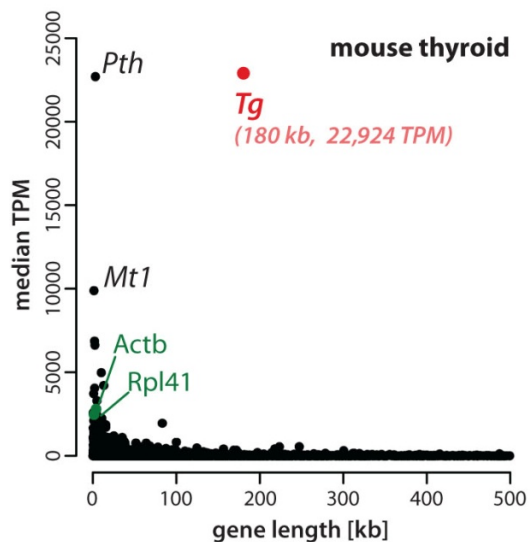
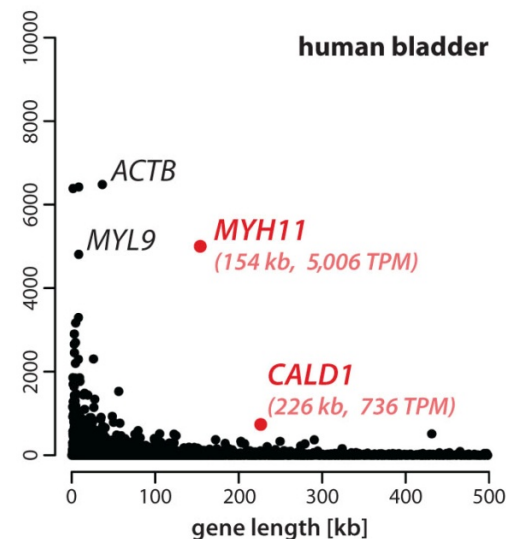
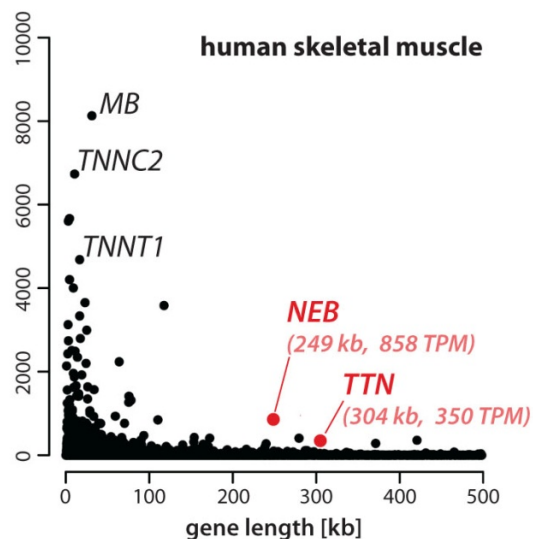
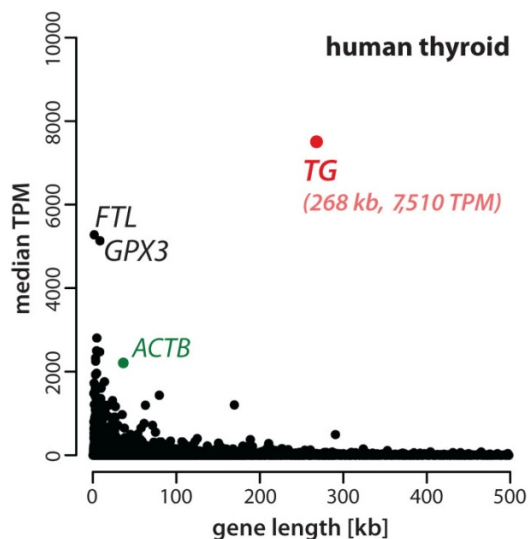
Long (> 100 kb) and highly expressed genes are uncommon



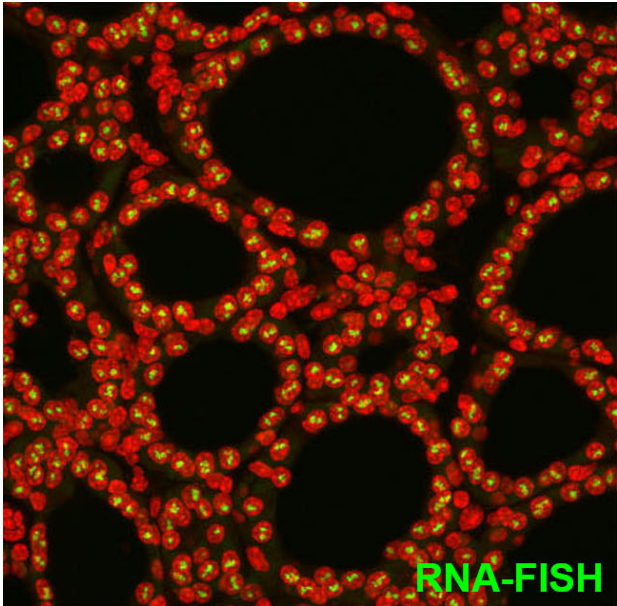
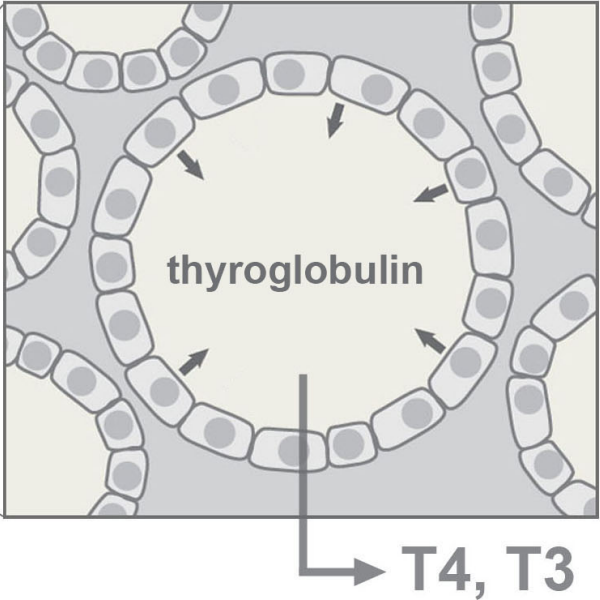
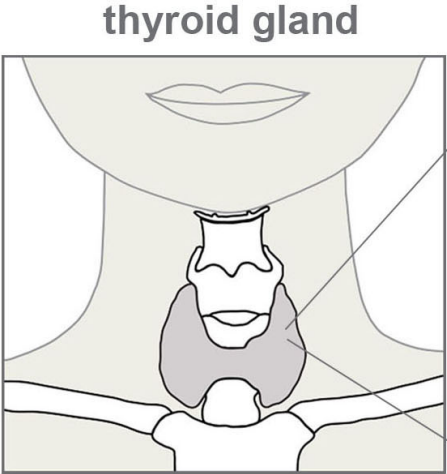
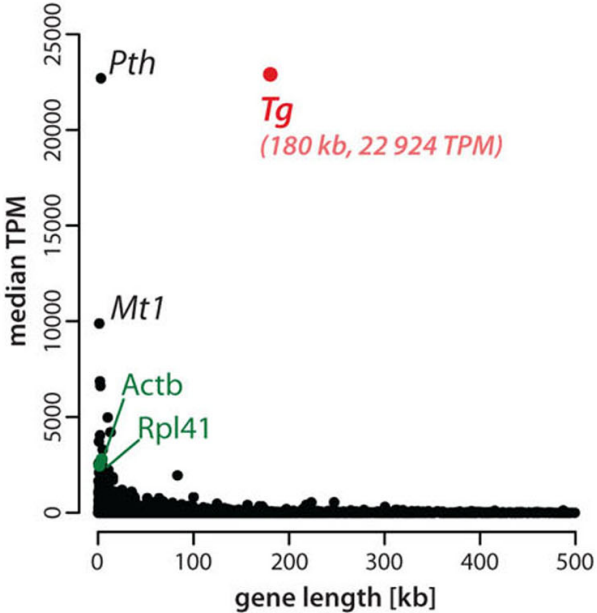
Long genes are not highly expressed in cultured cells

Selected long (> 100 kb) and highly expressed (> 1000 TPM) genes

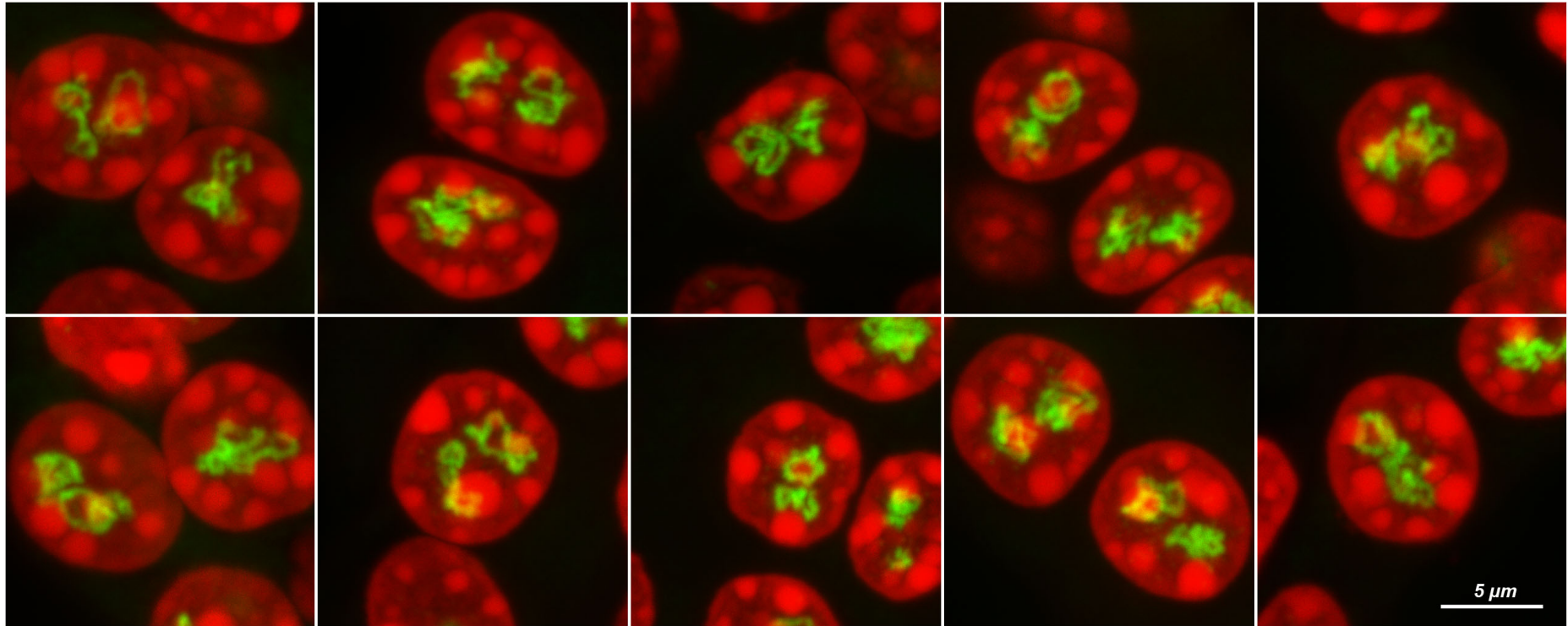
GTEX database



Thyroglobulin gene is exceptionally upregulated

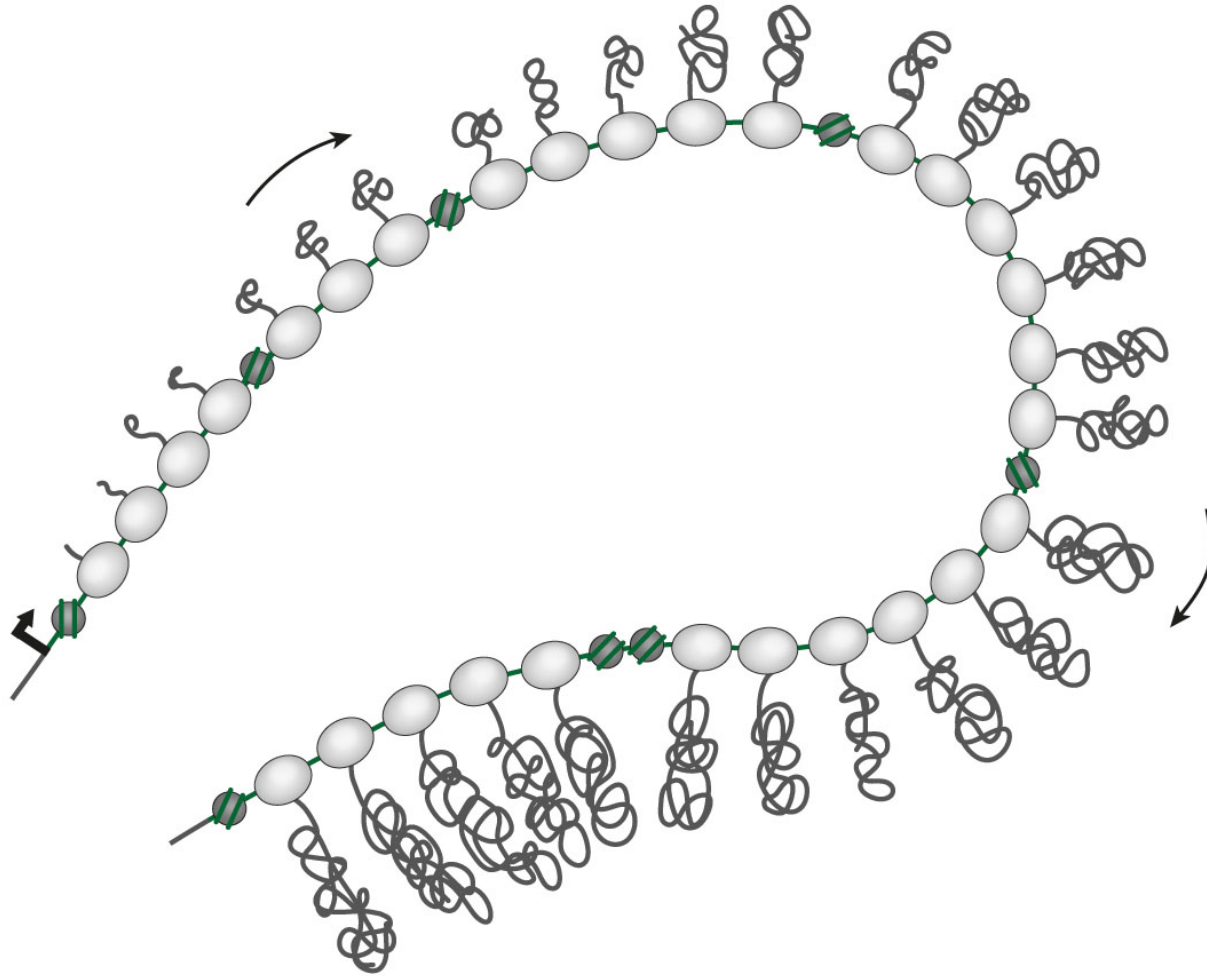


Thyroglobulin gene forms a giant loop

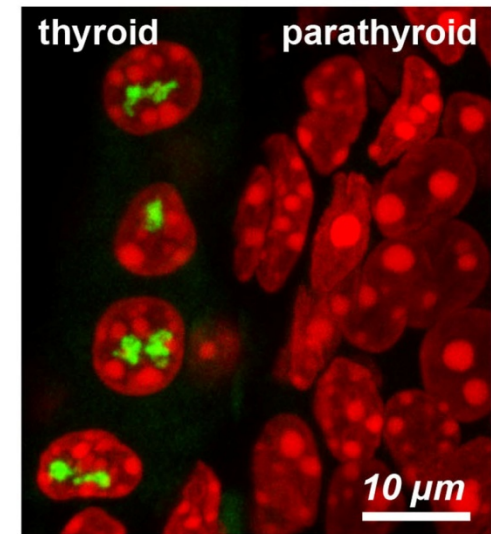
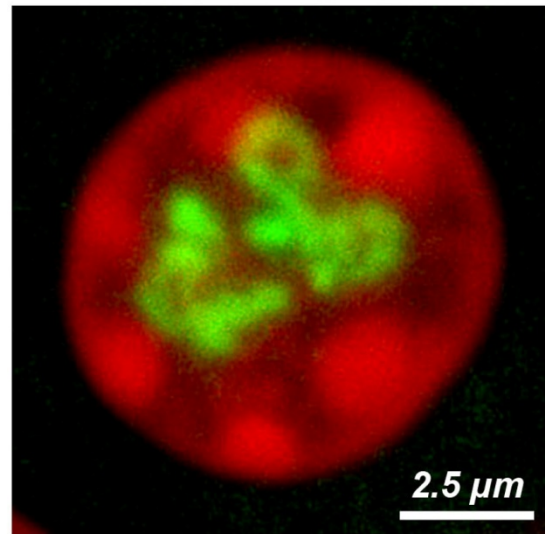
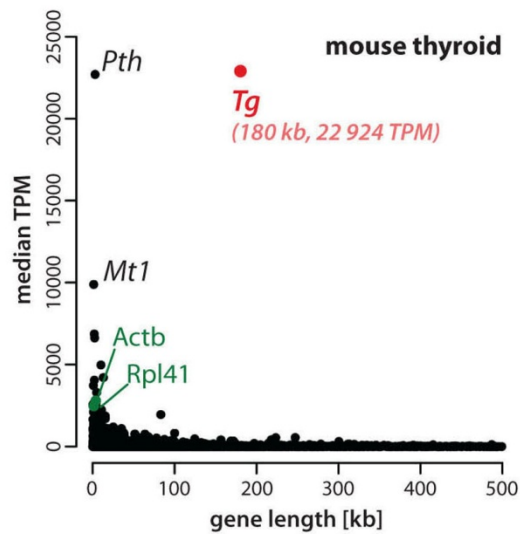


RNA FISH: *no cell DNA denaturation*

Thyroglobulin gene forms a giant Transcription Loop

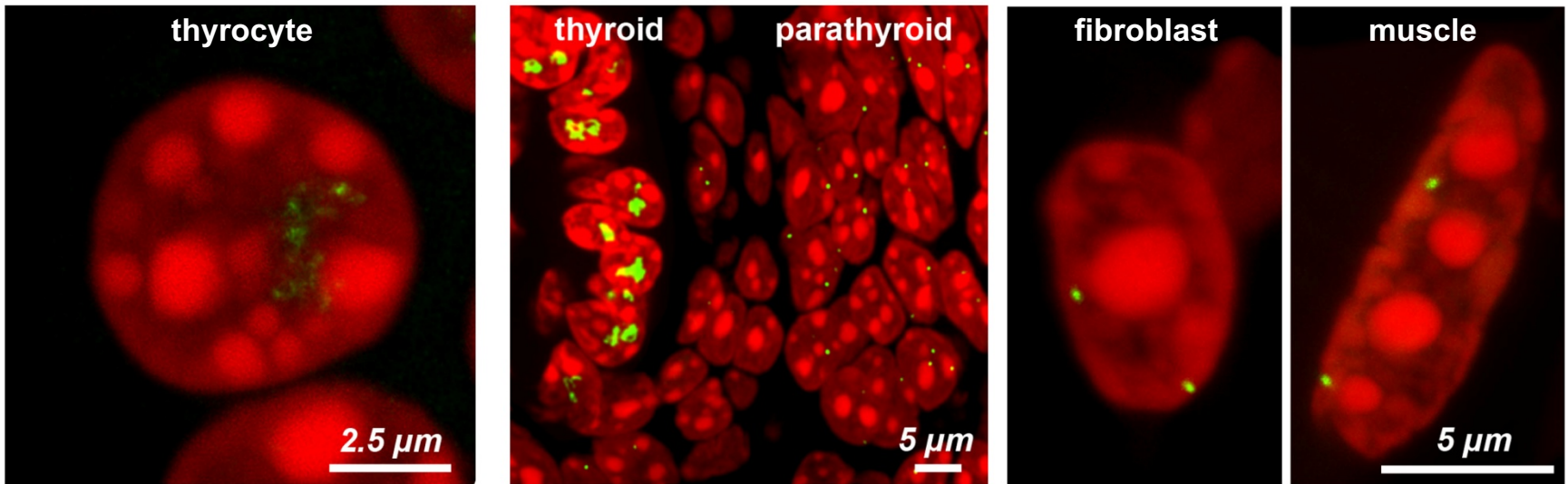


1. Only thyrocytes exhibit *Tg* Transcription Loops



RNA FISH: *no cell DNA denaturation*

2. *Tg* gene body is strongly decondensed due to high occupancy by RNAPIIs

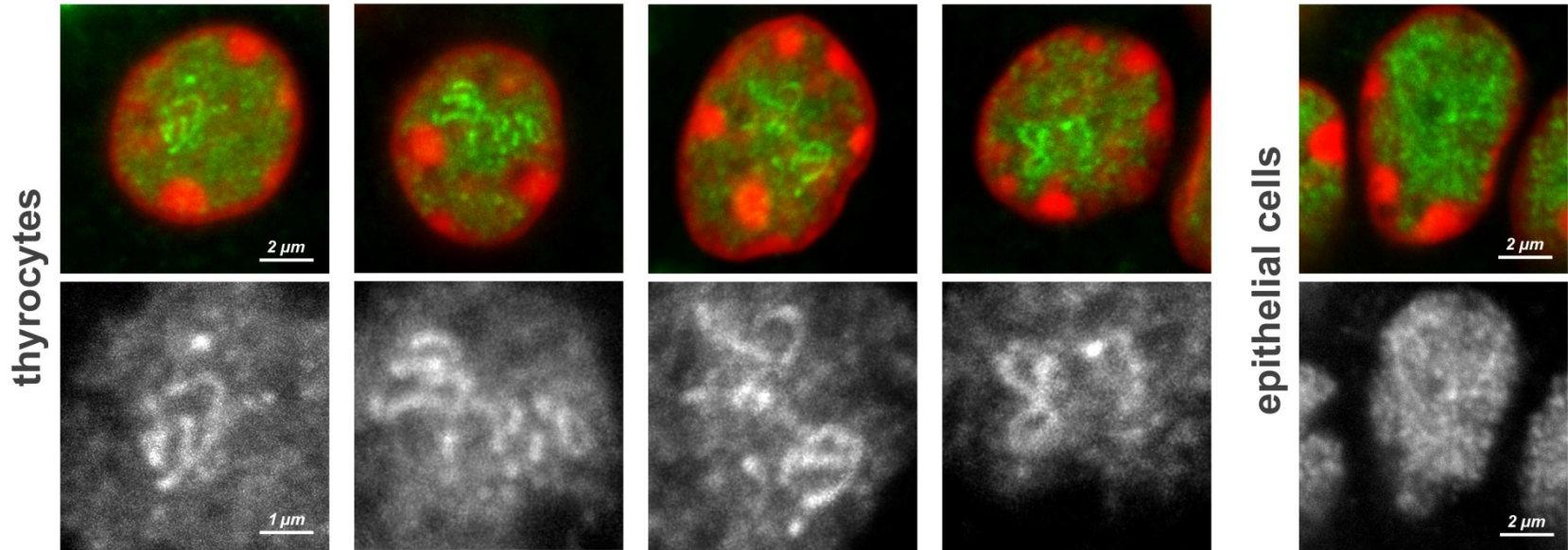


**DNA FISH: *RNasing*,
cell DNA denaturation**

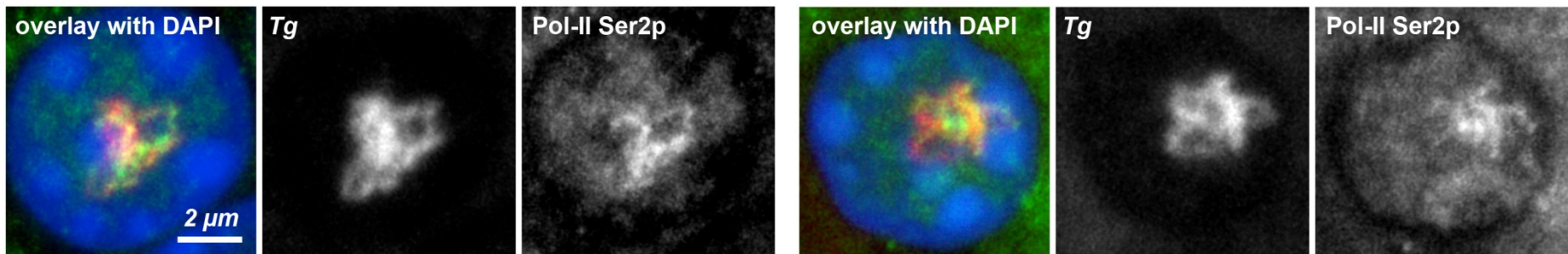
**RNA+DNA FISH: *no RNasing*,
cell DNA denaturation**

3. *Tg* TLs coincide with accumulation of Pol-II Ser5p

immunostaining

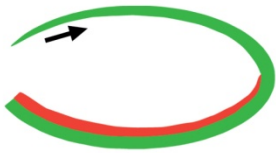
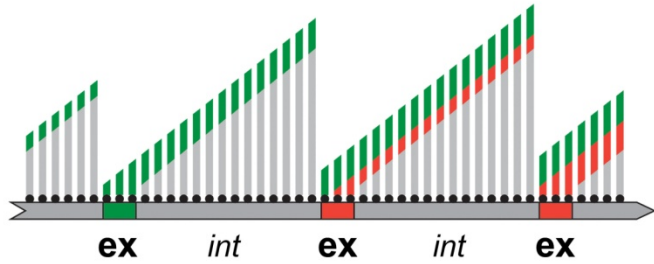
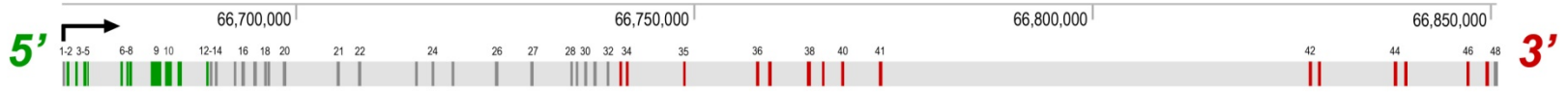


immuno-FISH

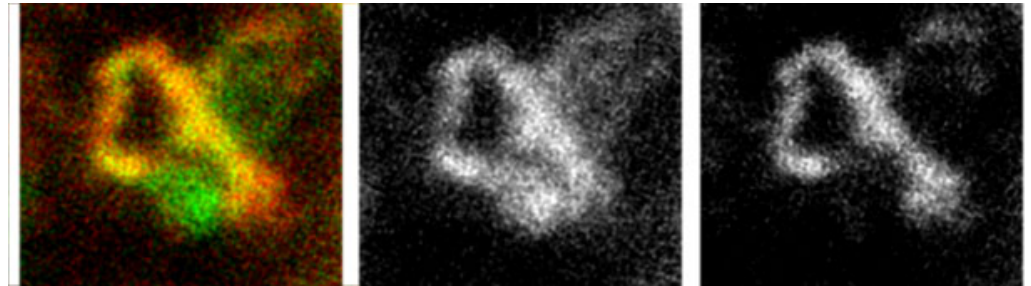
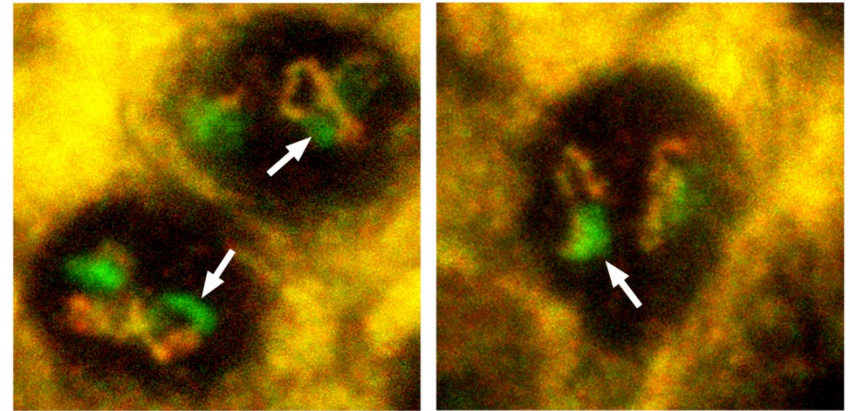


4. Progression of transcription along *Tg* TLs

labeled
exons

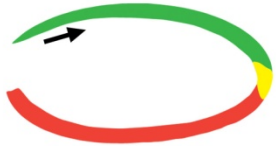
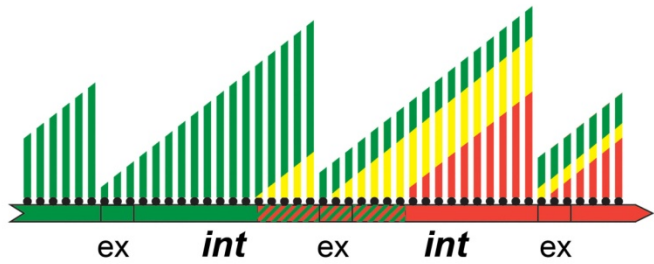
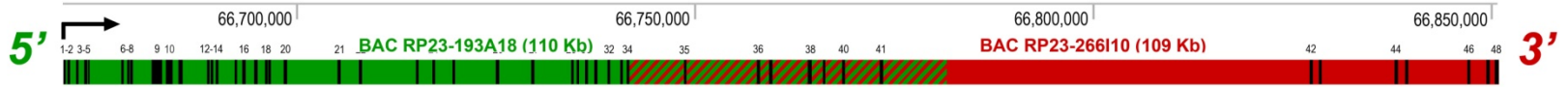


exons 2-12 33-47

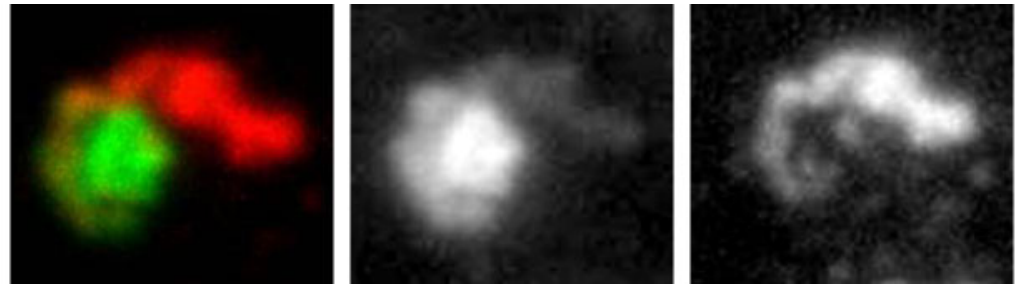
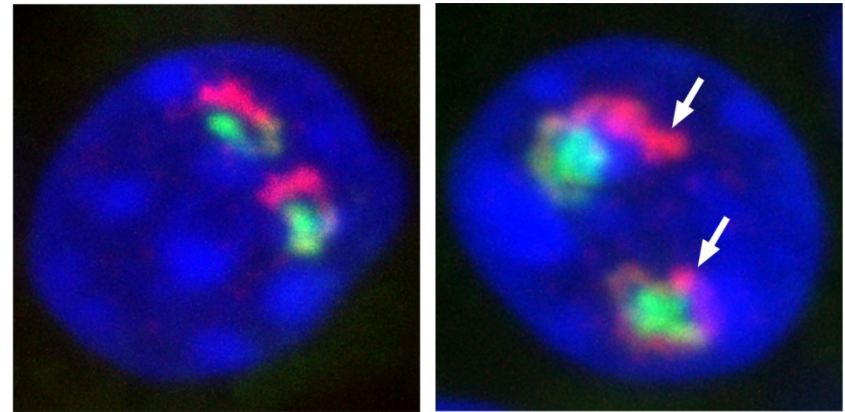


5. *Tg* TLs exhibit co-transcriptional splicing

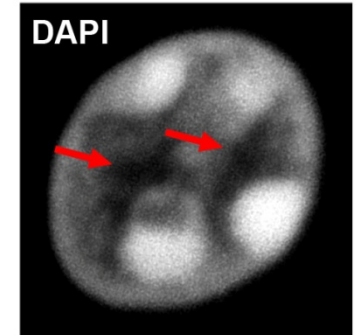
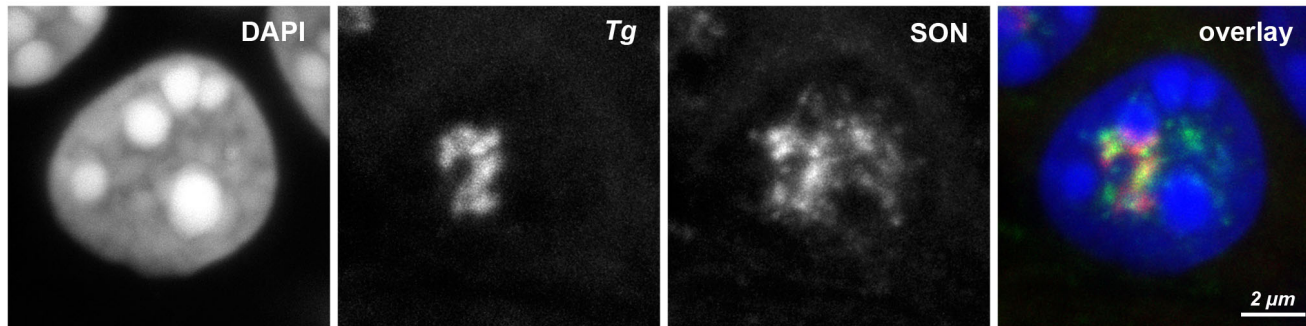
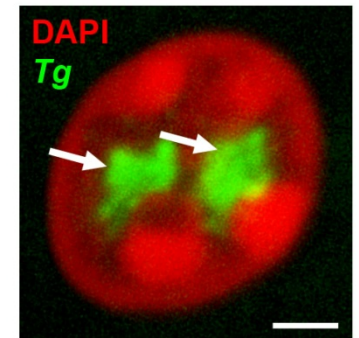
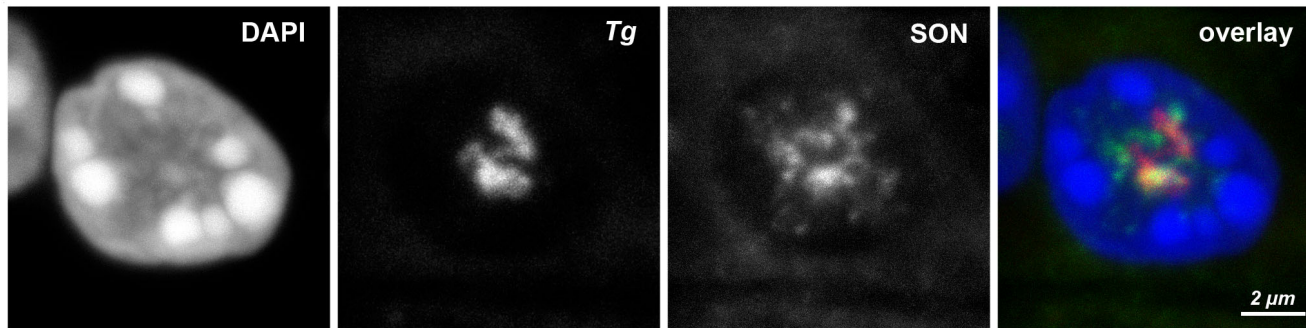
labeled
introns



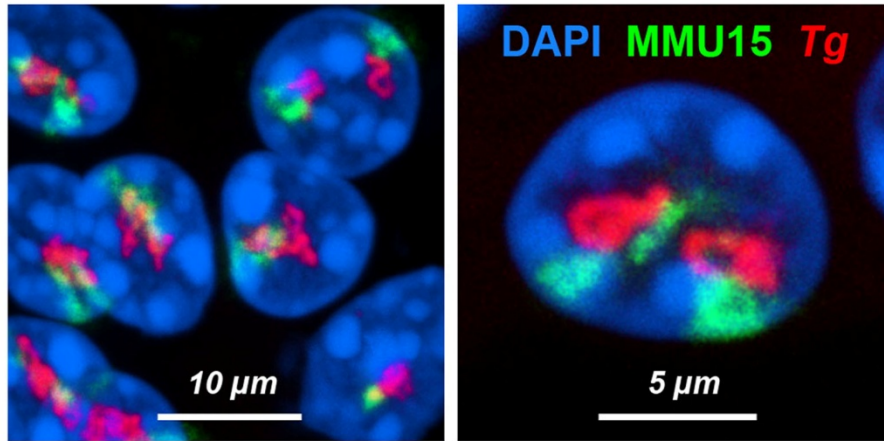
first 110 Kb last 109 Kb



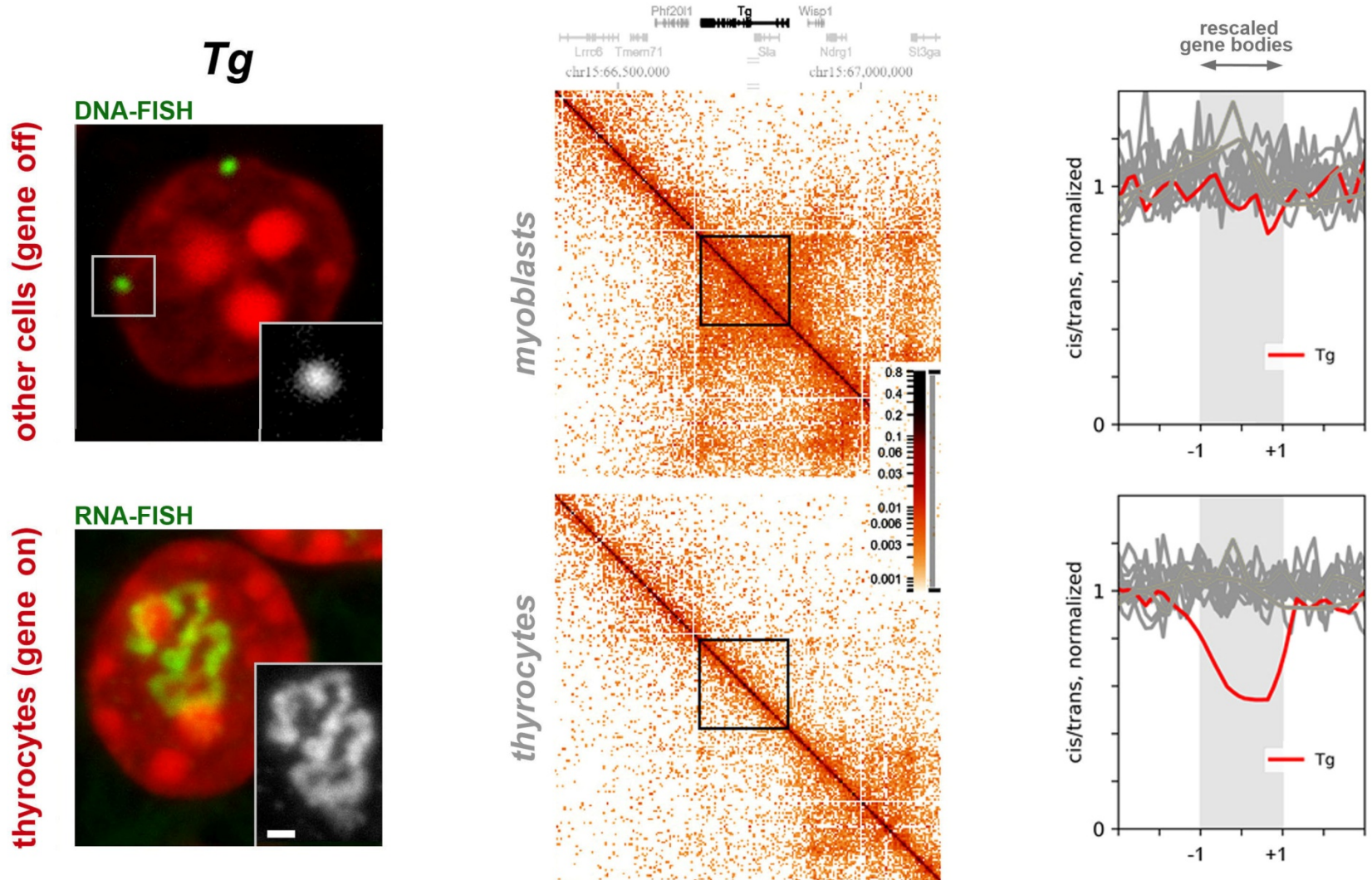
5. *Tg* TLs are adjacent to or co-localize with speckles



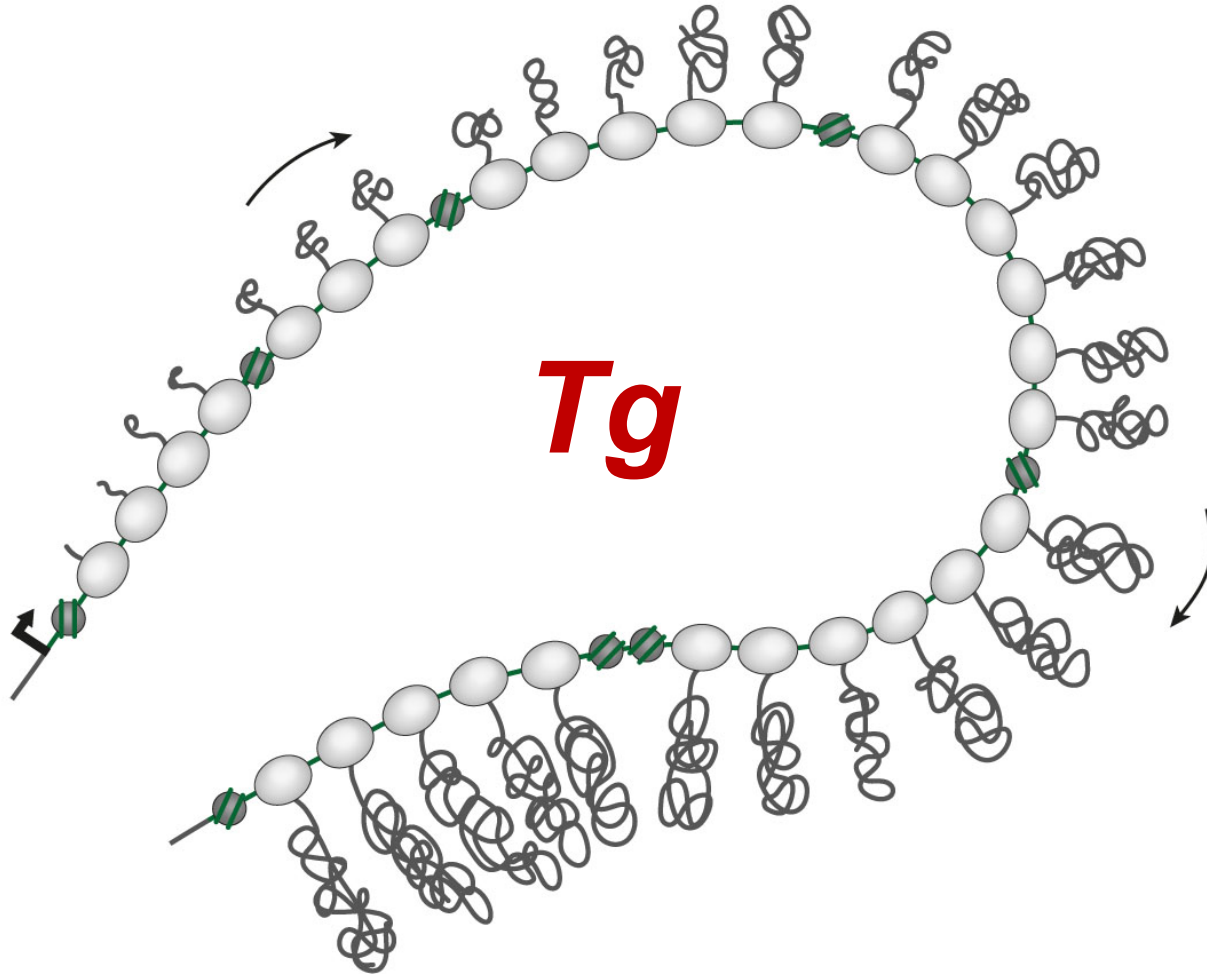
6. *Tg* gene loops out from the harboring chromosome when expressed



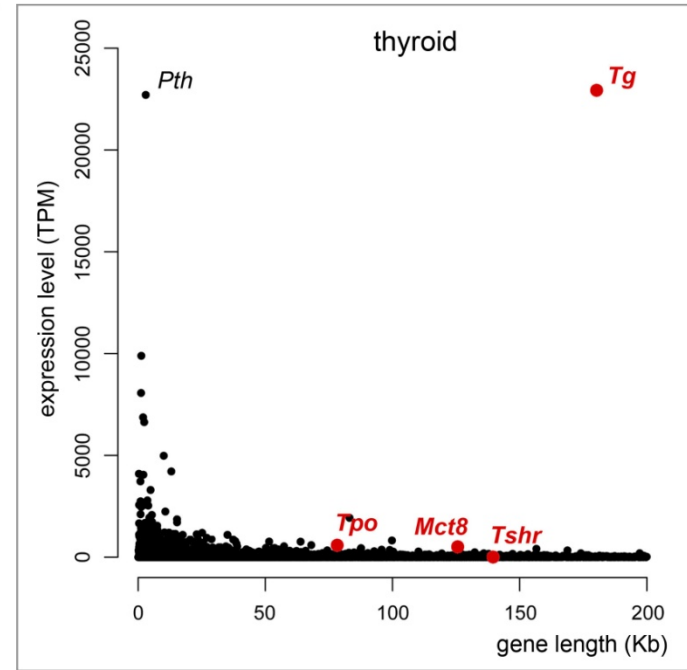
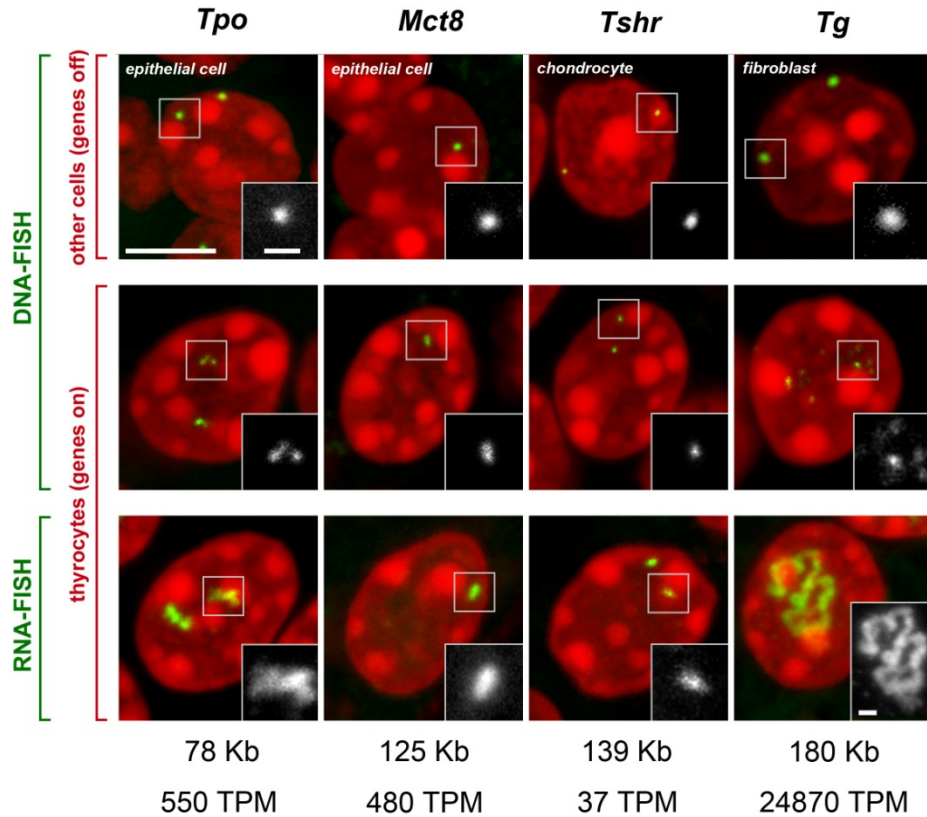
6. *Tg* gene loops out from the harboring chromosome when expressed



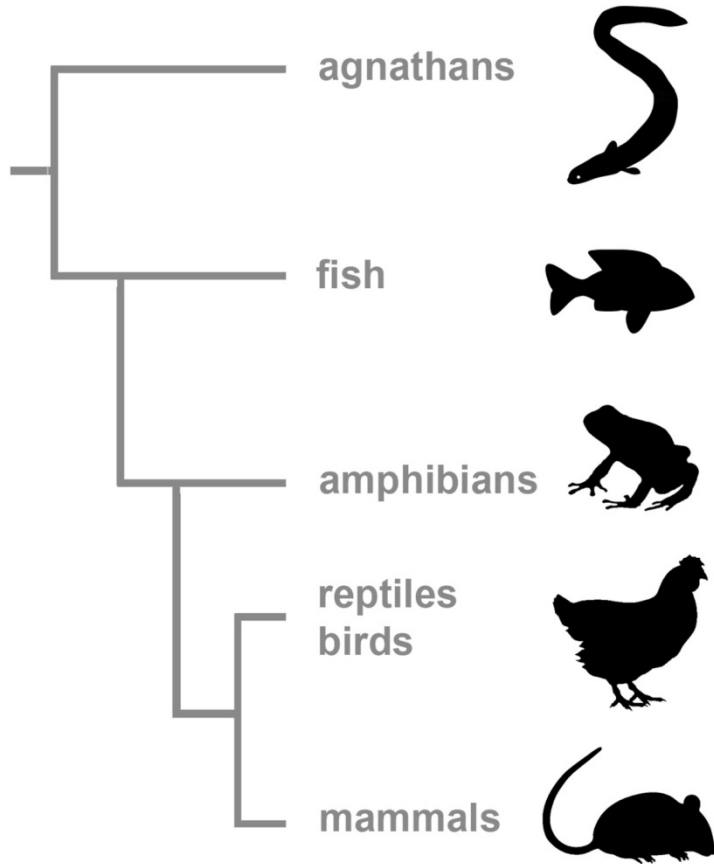
Transcription loop



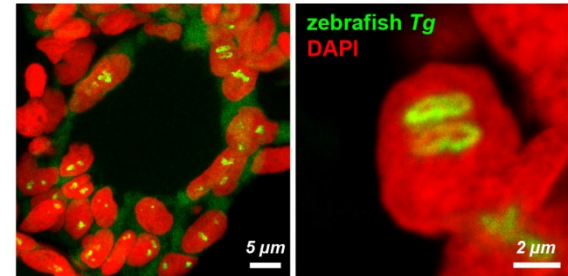
Thyroglobulin gene is remarkably highly upregulated



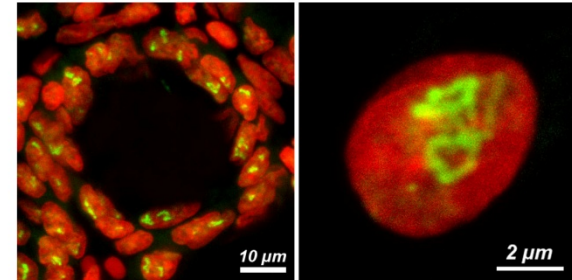
Thyroglobulin gene is highly upregulated and forms transcription loop in thyrocytes of all vertebrate classes



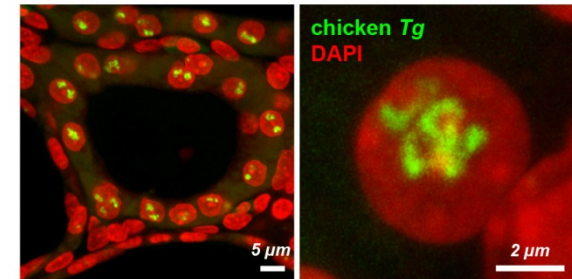
zebrafish
Tg 68 Kb



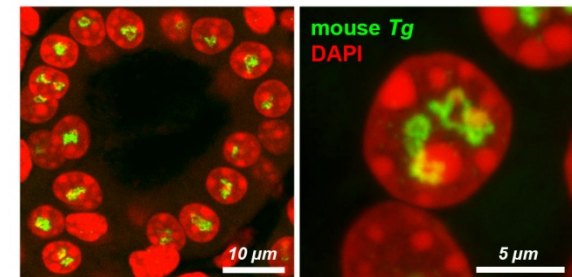
frog
Tg 122 Kb



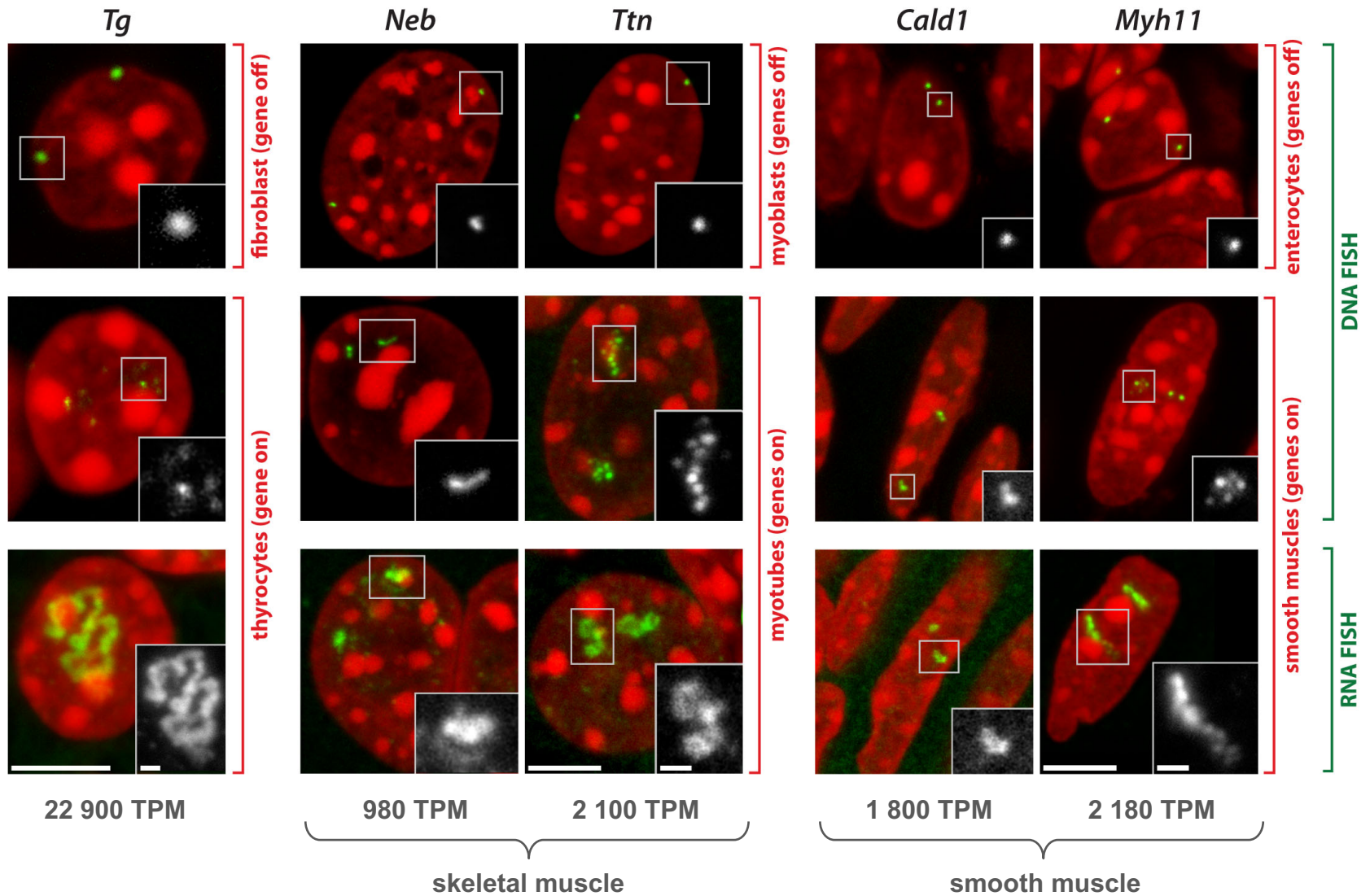
chicken
Tg 139 Kb



mouse
Tg 180 Kb

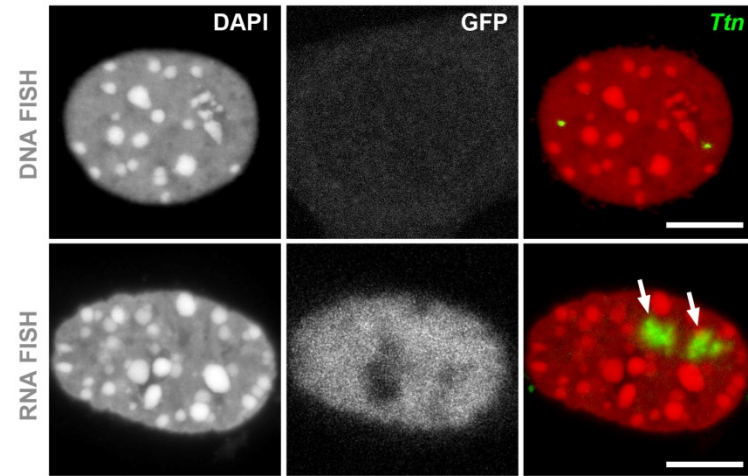
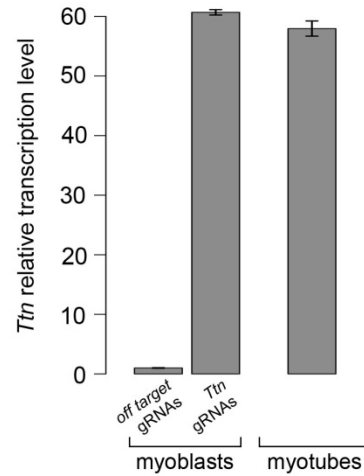
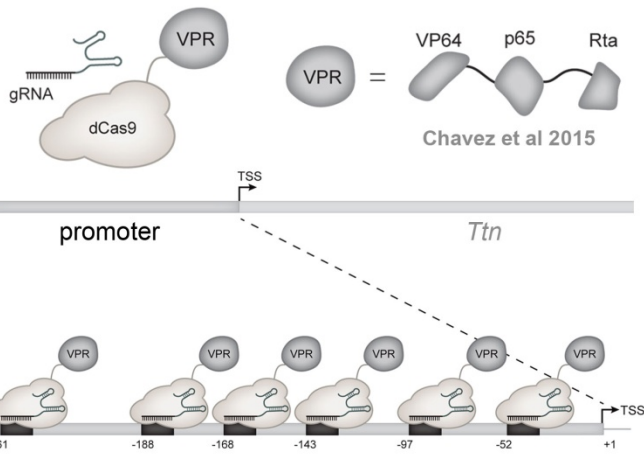


Other highly expressed genes form TLs



Ttn transcription loops can be induced via dCas9 mediated induction in myoblasts

myoblasts expressing dCas9-VPR



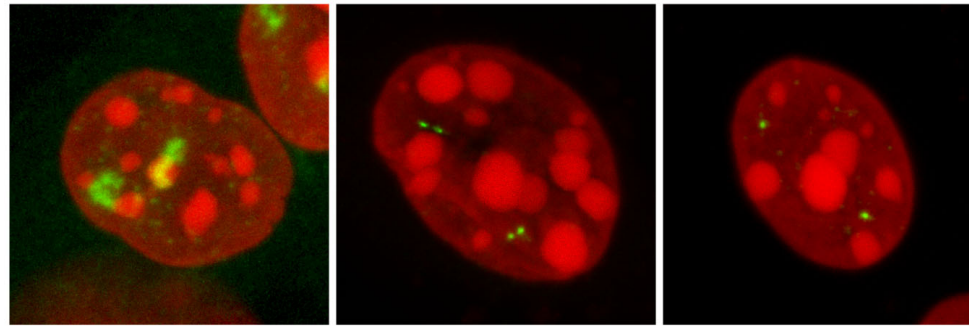
co-transfection with gRNAs and H2B-GFP

Ttn transcription loops are obliterated by transcription inhibition

no treatment

Actinomycin D

alpha-Amanitin



signals:

RNA

DNA

DNA

+ DRB →

- DRB →

+ DRB, 2 min

+ DRB, 25 min

+ DRB, 50 min

+ DRB, 75 min

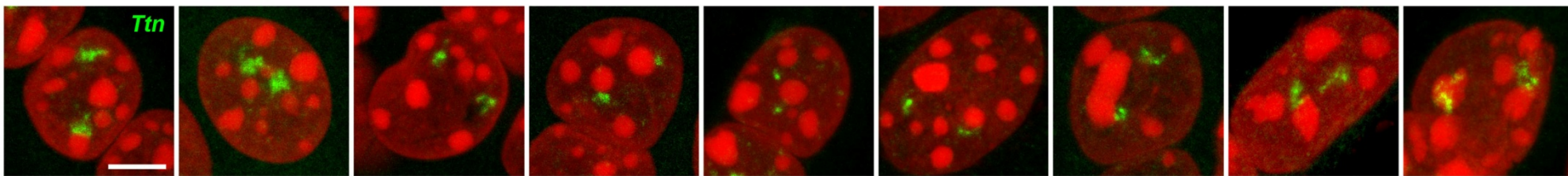
+ DRB, 3 h

- DRB, 25 min

- DRB, 50 min

- DRB, 75 min

- DRB, 3 h



signals:

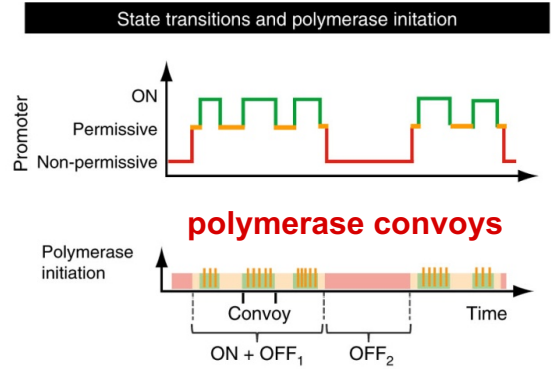
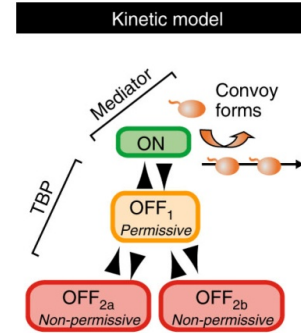
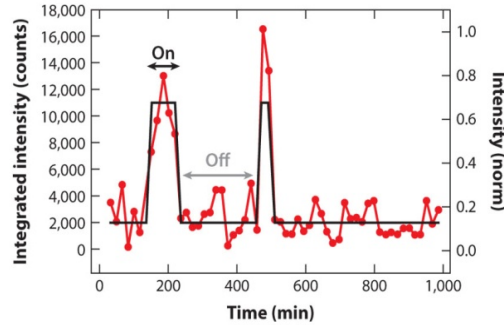
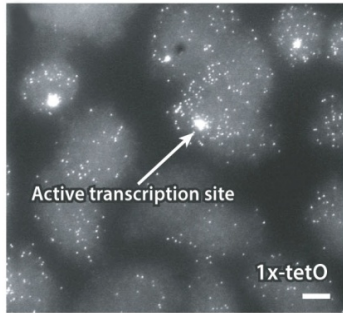
RNA

DNA

RNA

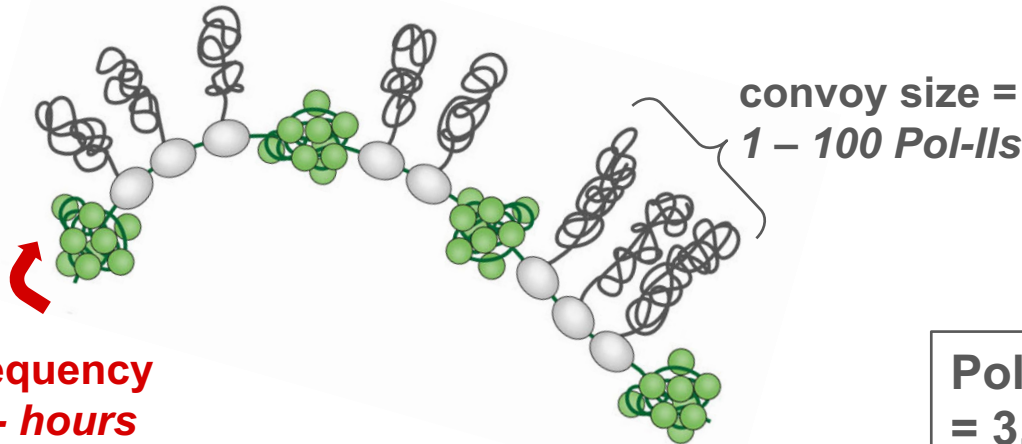
Transcription occurs in bursts

Chubb et al., 2006
Nicolas et al., 2017
Li et al., 2019



Tineke et al, 2016, Ann Rev Biophys

Tantale et al, 2016, Nat Comm



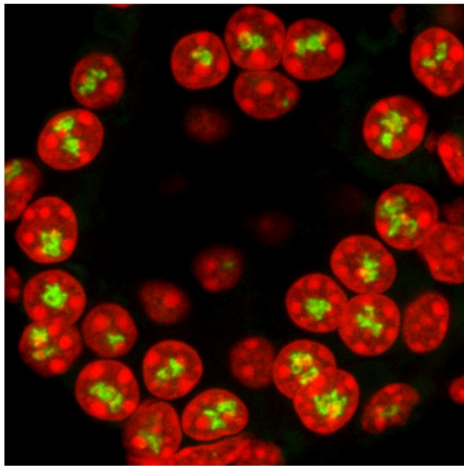
burst frequency
= 1 min - hours

Pol-II elongation speed
= 3.8 kb/min

Singh & Padgett, 2009

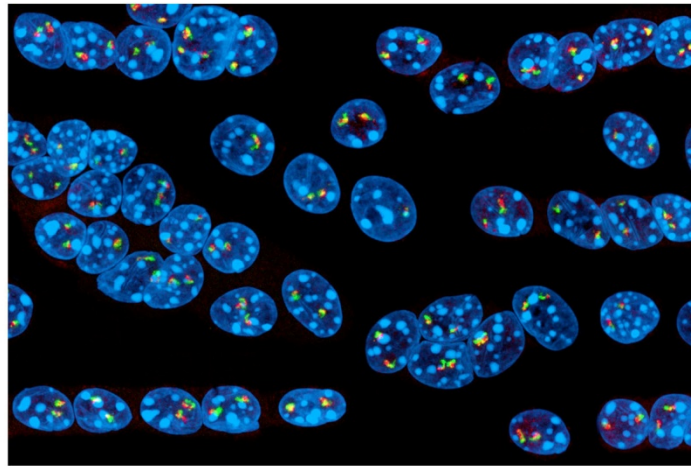
Highly upregulated long genes are expressed biallelically

thyroid gland: *Tg*



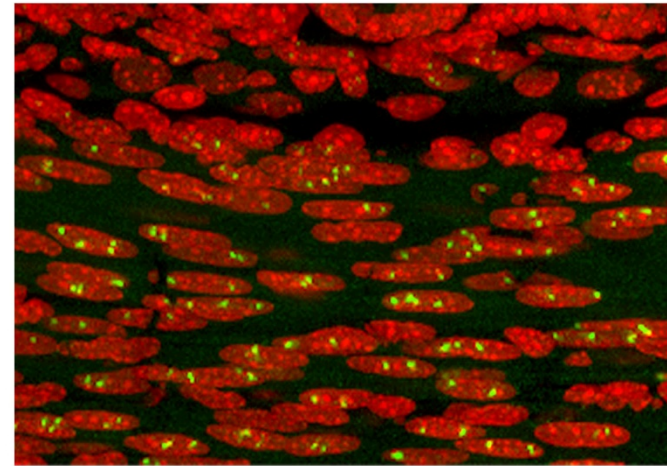
5.3 %

cultured myotubes: *Ttn3'* & *Ttn5'*



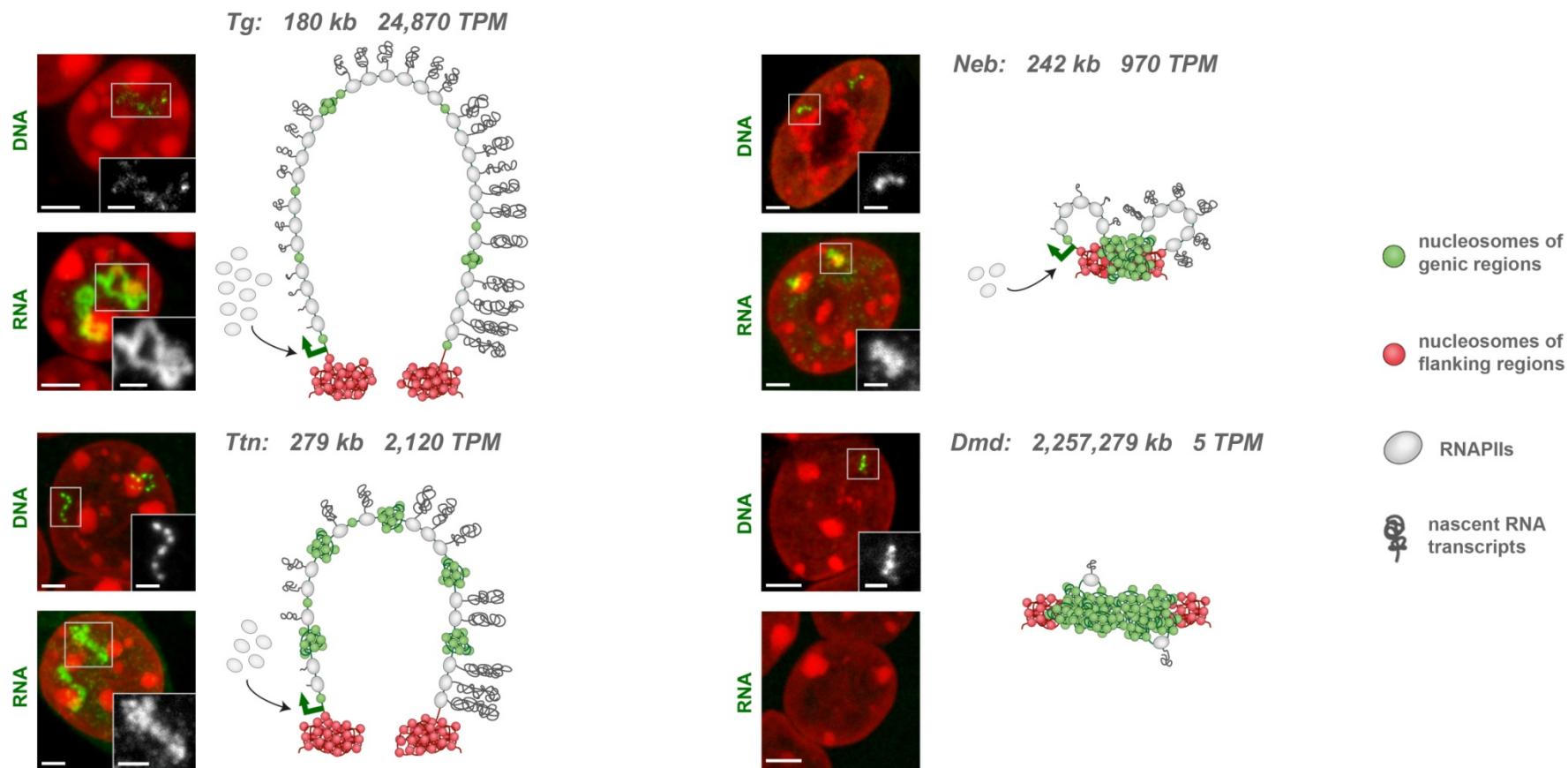
5.4 %

smooth muscle, intestine: *Myh11*

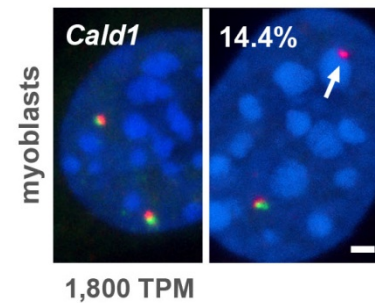
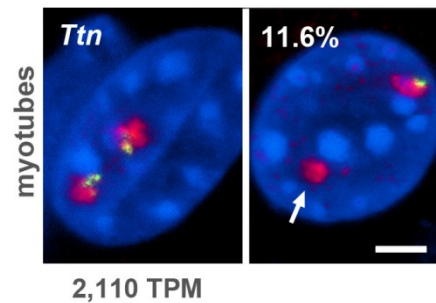
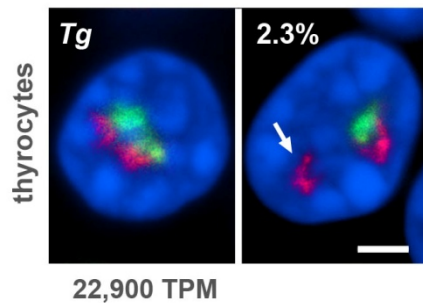


5.0 %

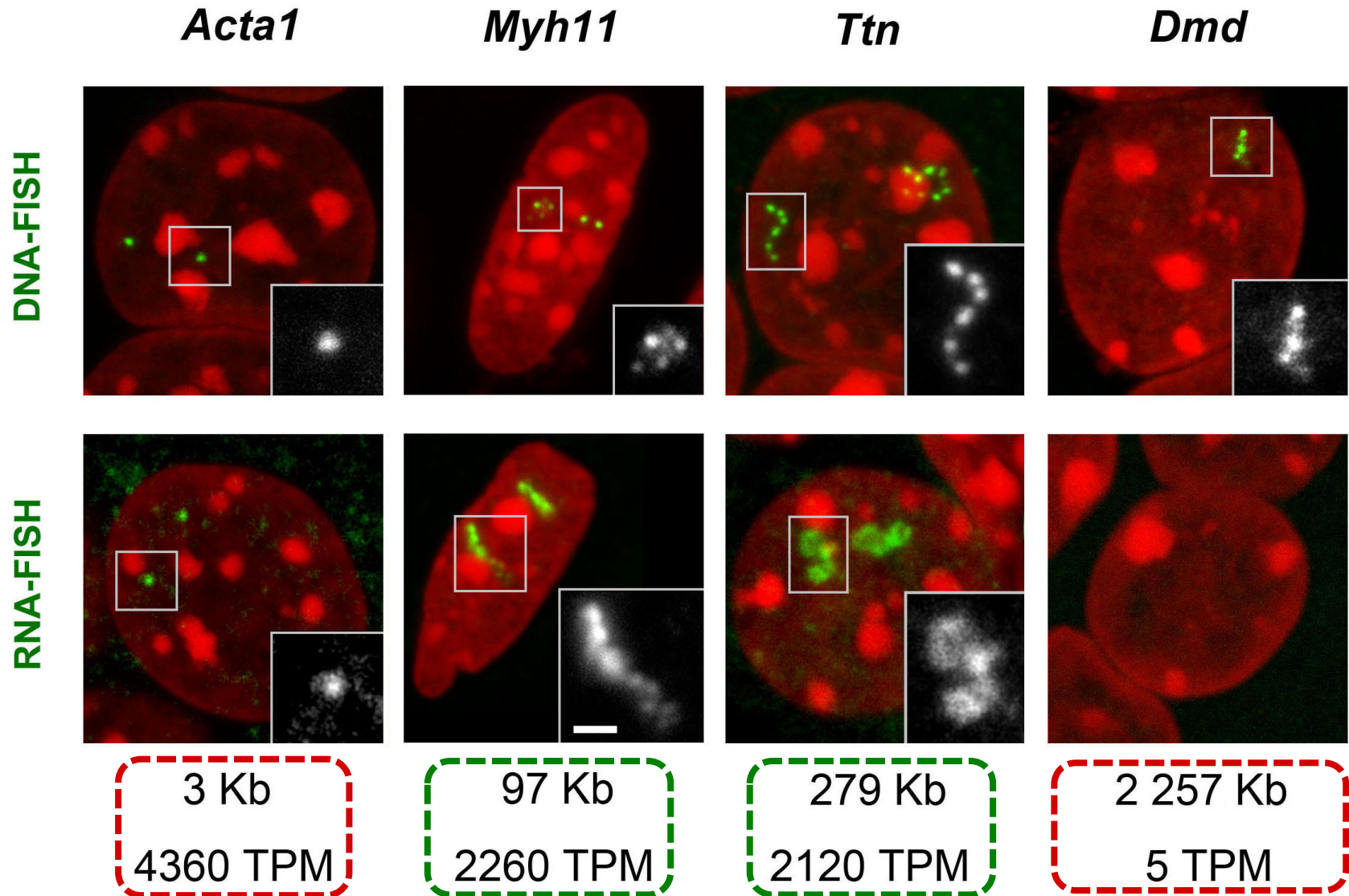
TLs exhibit transcription bursting



RNA-FISH
30 kb probe

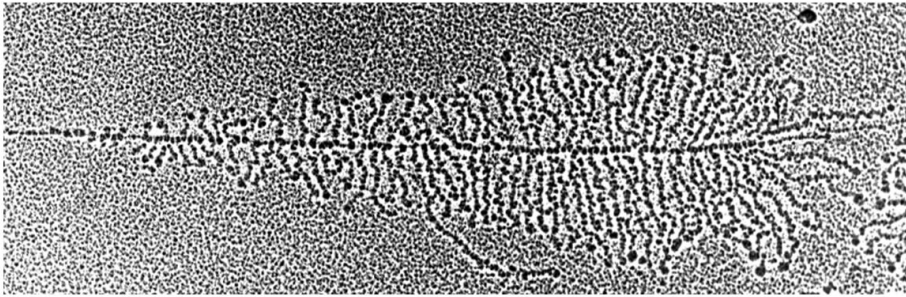


A gene has to be both long and highly expressed to form a resolvable TL

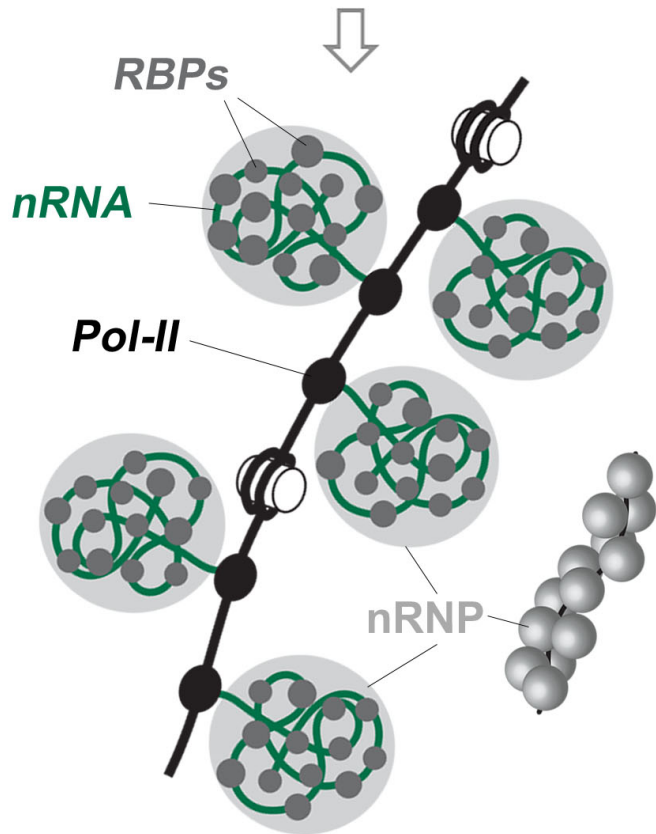
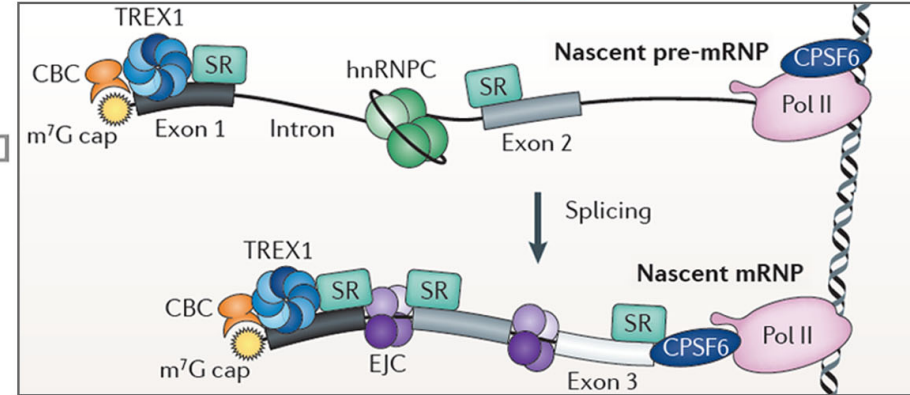


RNPs densely decorate the gene axis rigidifying and expanding the locus

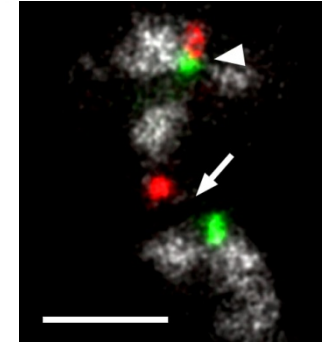
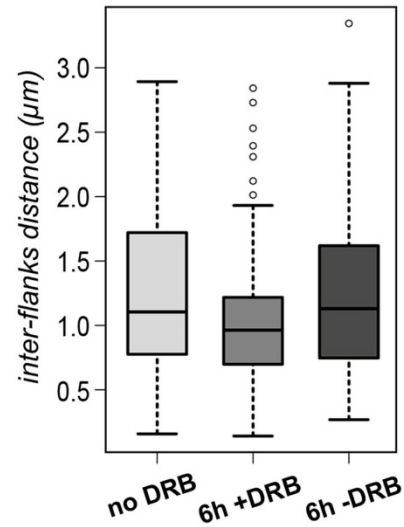
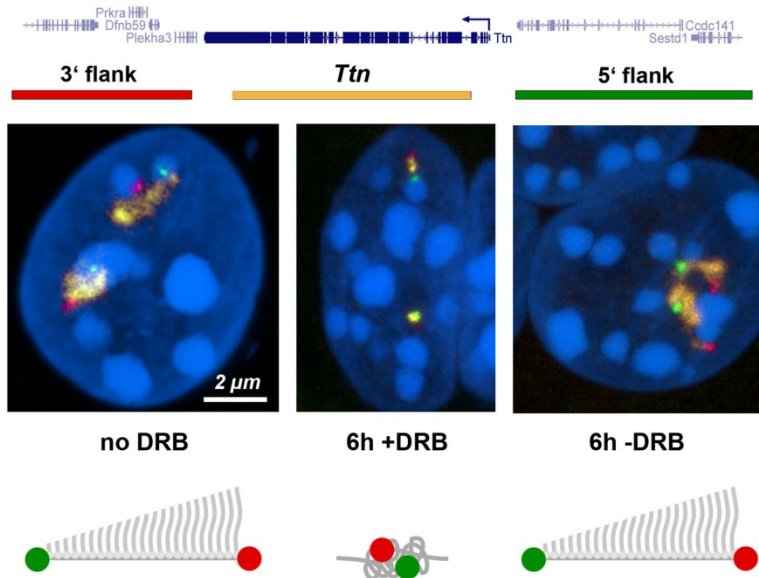
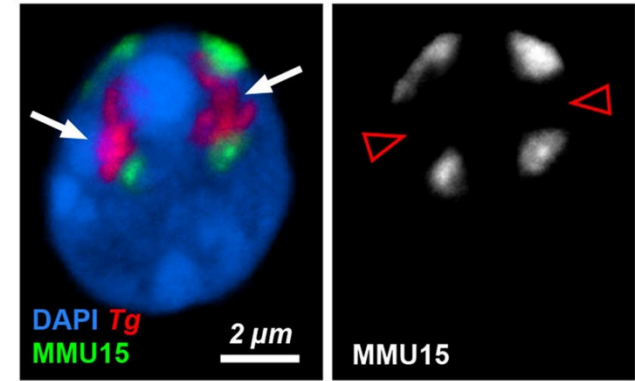
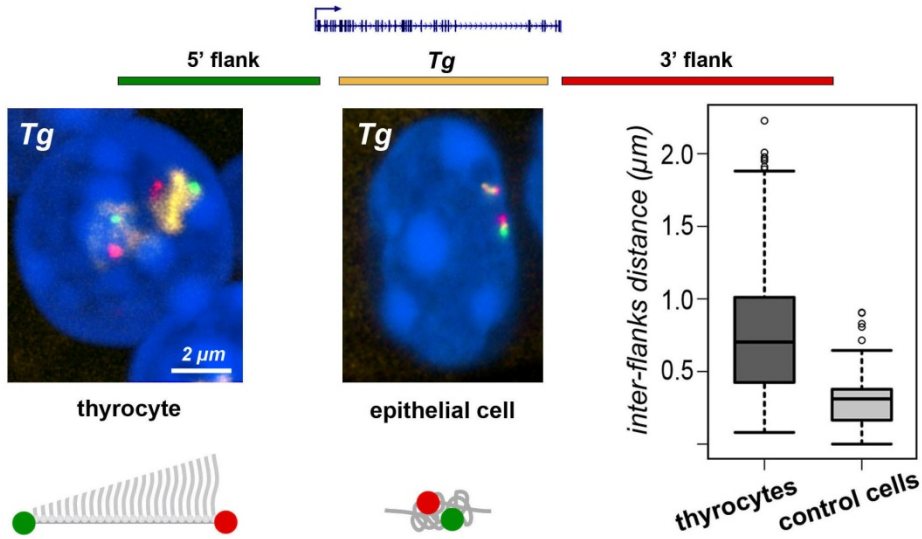
Miller & Beatty 1969



mod. from Müller-McNicoll & Neugebauer 2013



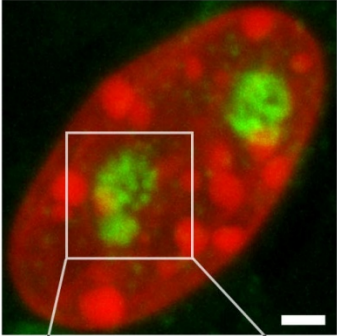
TLs are open loops with separated 5' and 3' ends



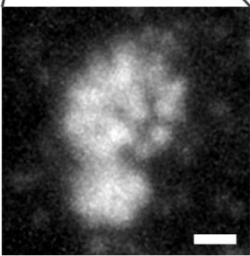
Large nRNPs are formed on long genes



Ttn

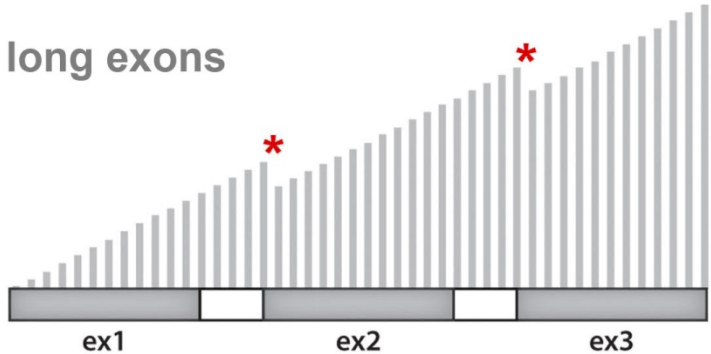


279 kb

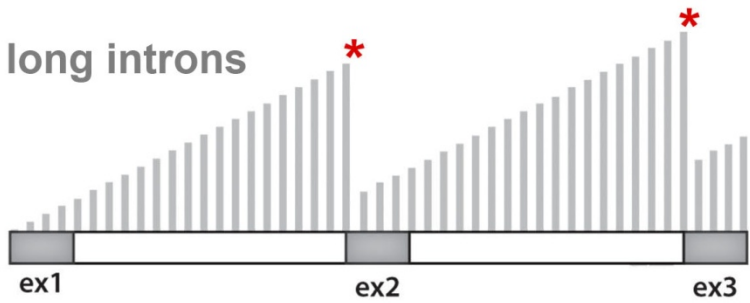


2,120 TPM

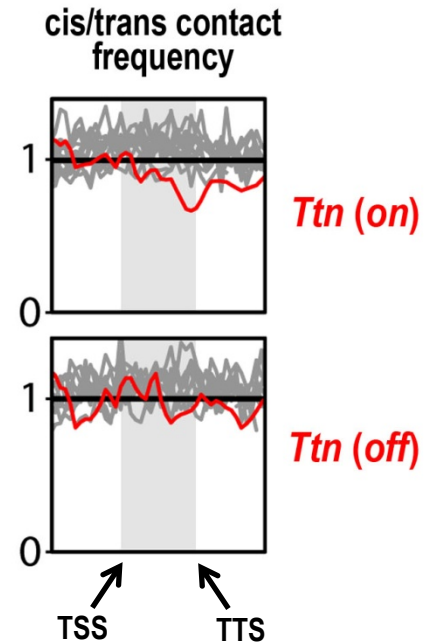
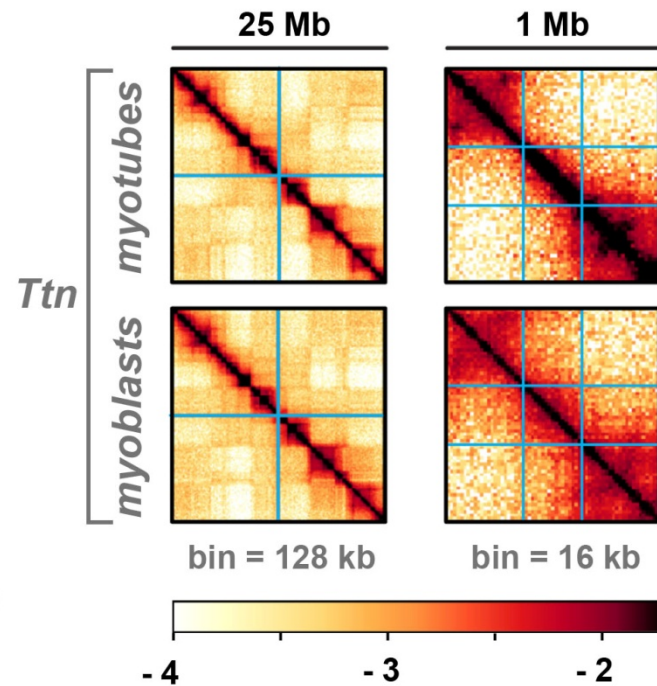
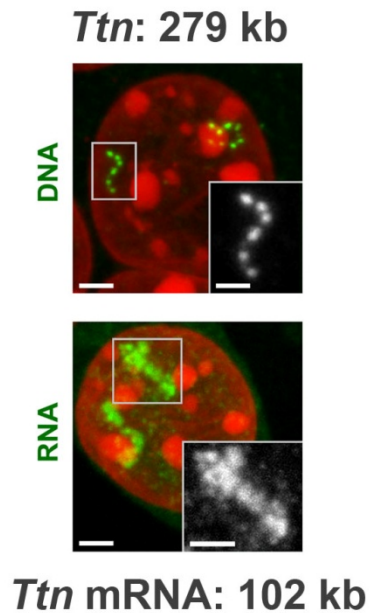
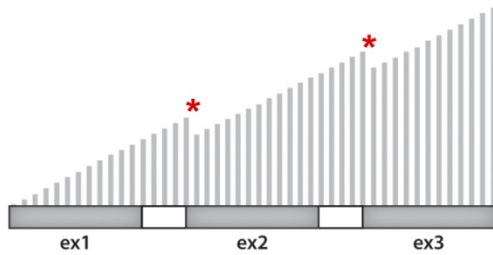
long exons



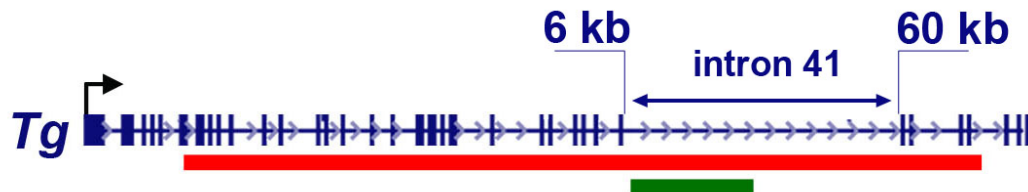
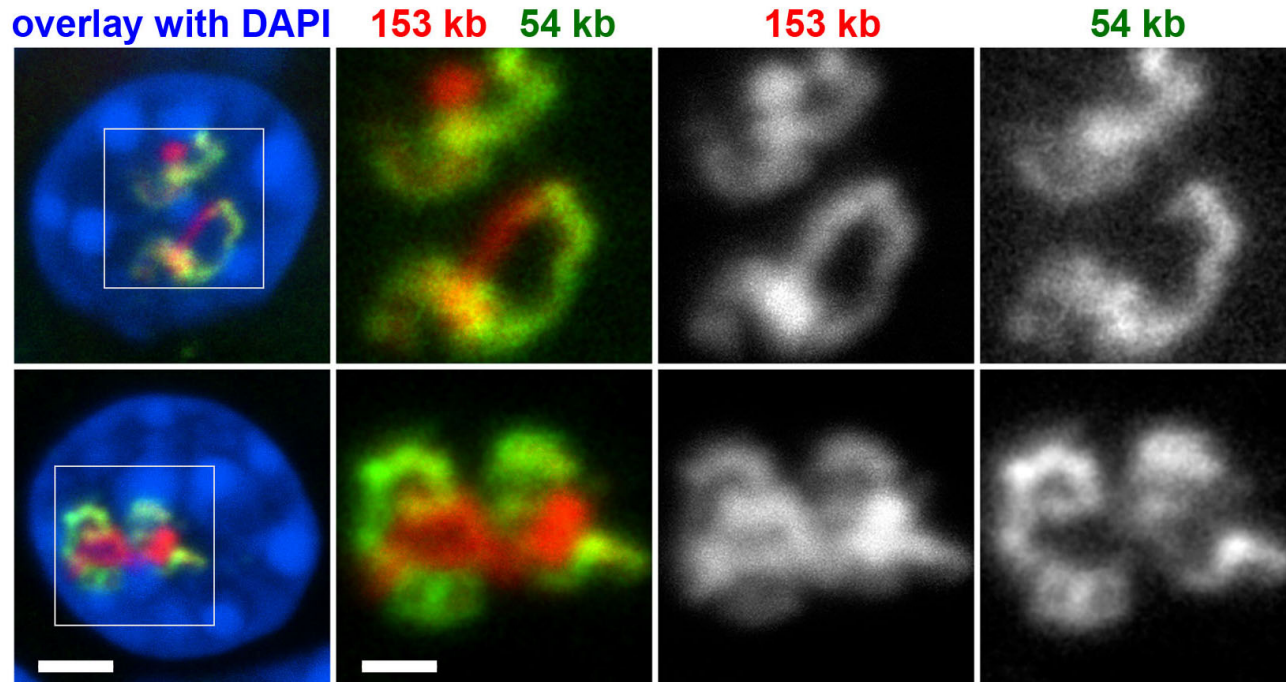
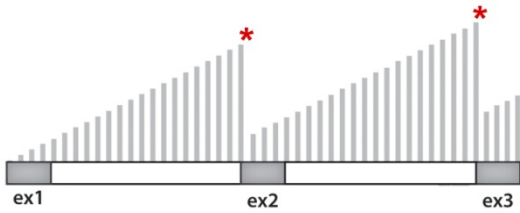
long introns



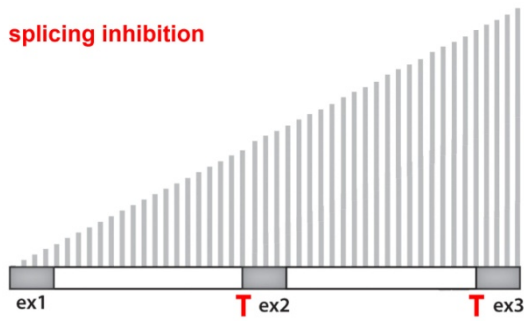
Extension of TLs is increasing towards the 3' end



Extension of TLs is increasing over long introns



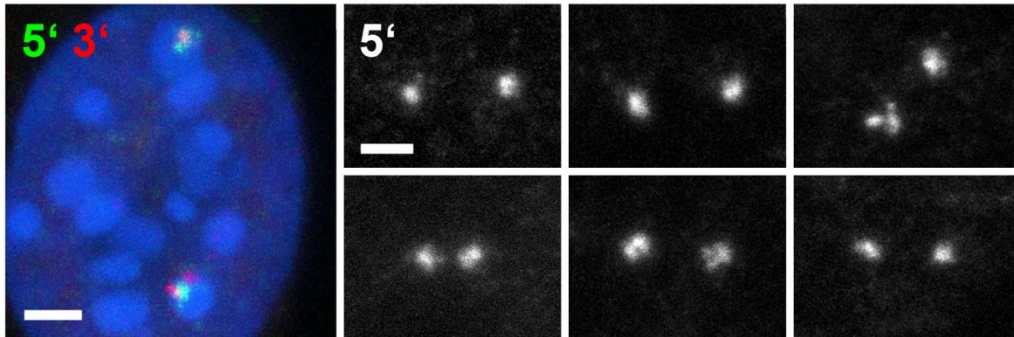
splicing inhibition



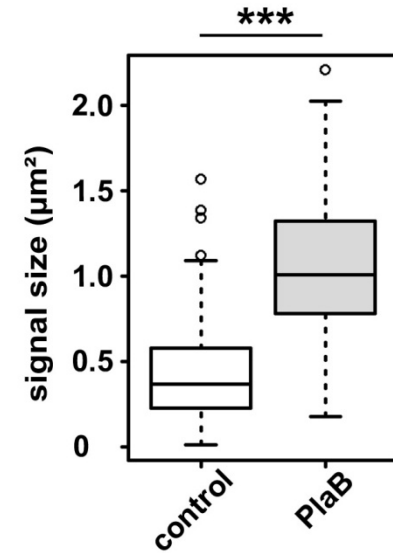
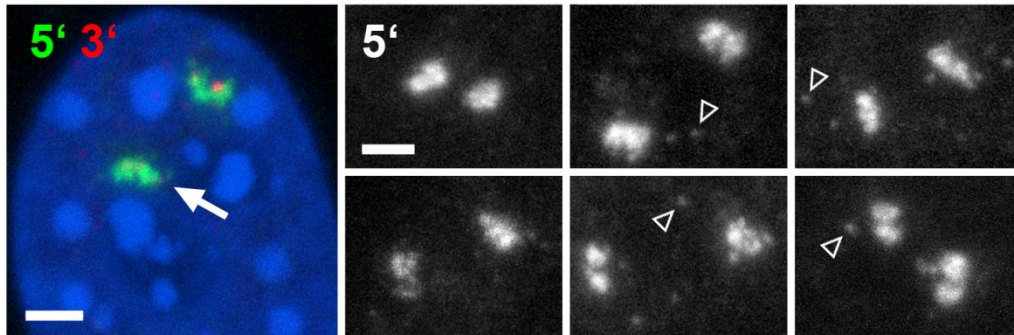
Extension of TLs is increasing upon splicing inhibition



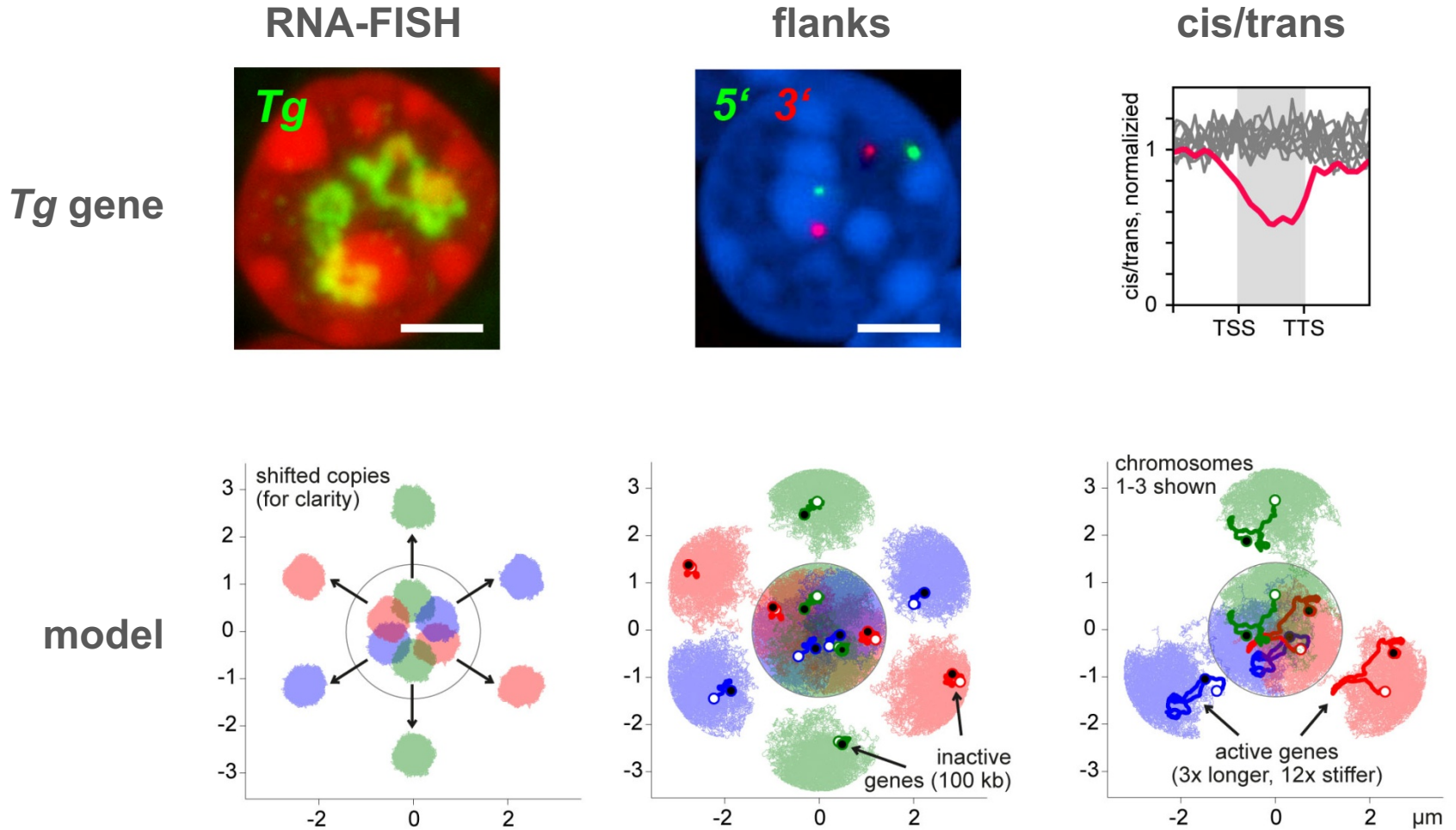
control (0.1% DMSO)



Pladienolide B treatment



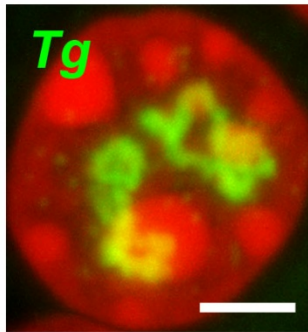
Polymer modeling confirms transcription loop stiffness



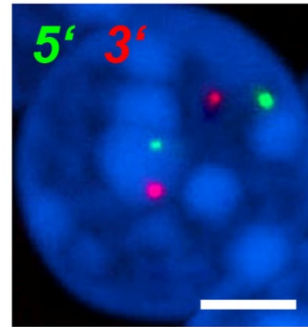
Polymer modeling confirms transcription loop stiffness

RNA-FISH

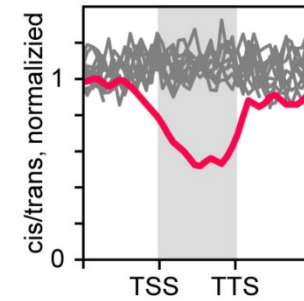
Tg gene



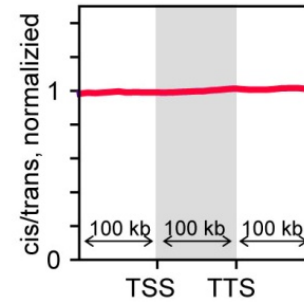
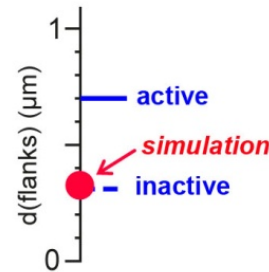
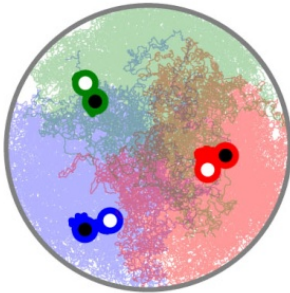
flanks



cis/trans



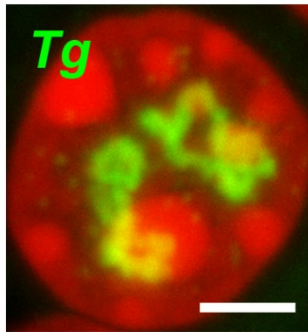
inactive gene, 100 kb



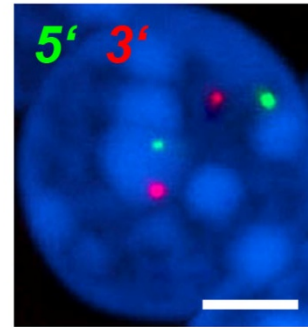
Polymer modeling confirms transcription loop stiffness

RNA-FISH

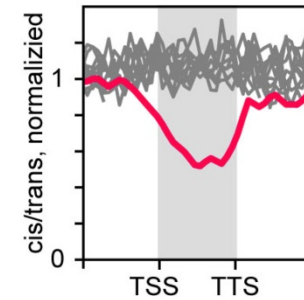
Tg gene



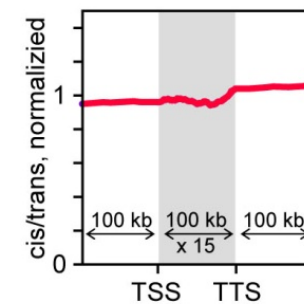
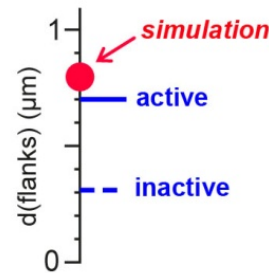
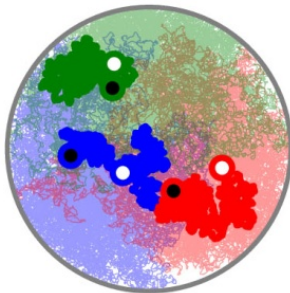
flanks



cis/trans



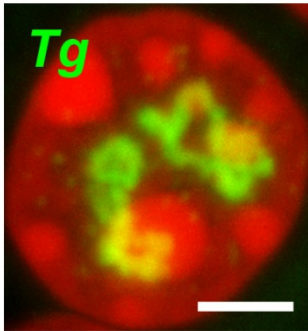
increased length (15x)



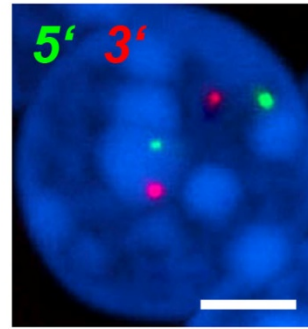
Polymer modeling confirms transcription loop stiffness

RNA-FISH

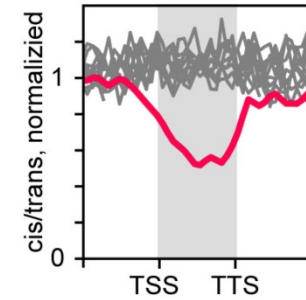
Tg gene



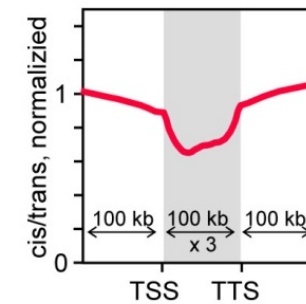
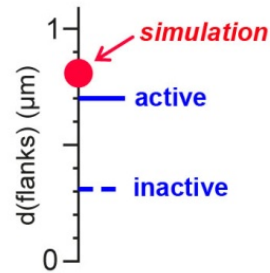
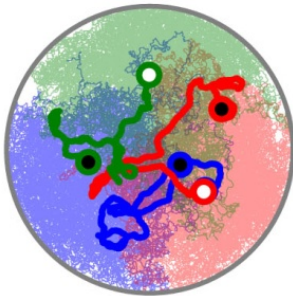
flanks



cis/trans



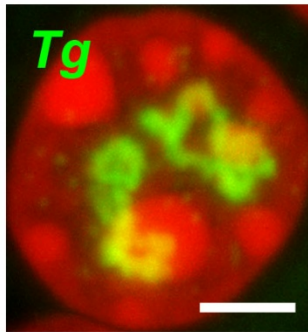
increased stiffness (12x)



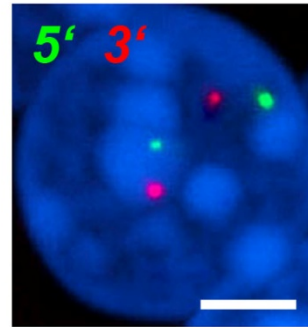
Polymer modeling confirms transcription loop stiffness

RNA-FISH

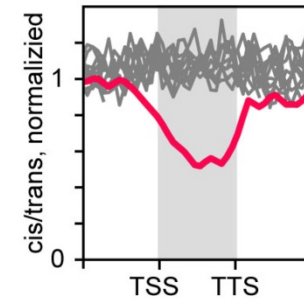
Tg gene



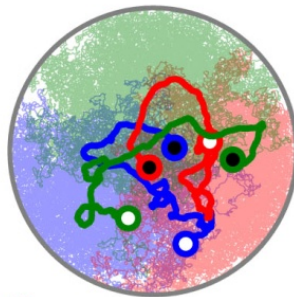
flanks



cis/trans

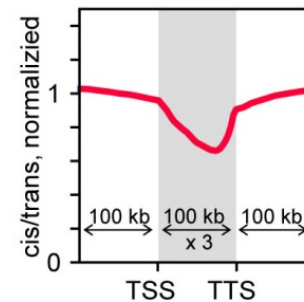
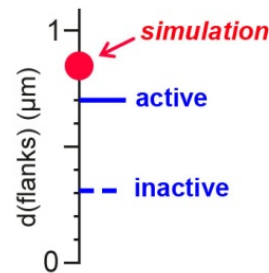


tapered stiffness (1-24)

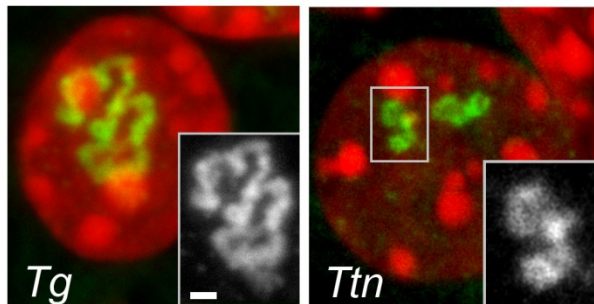
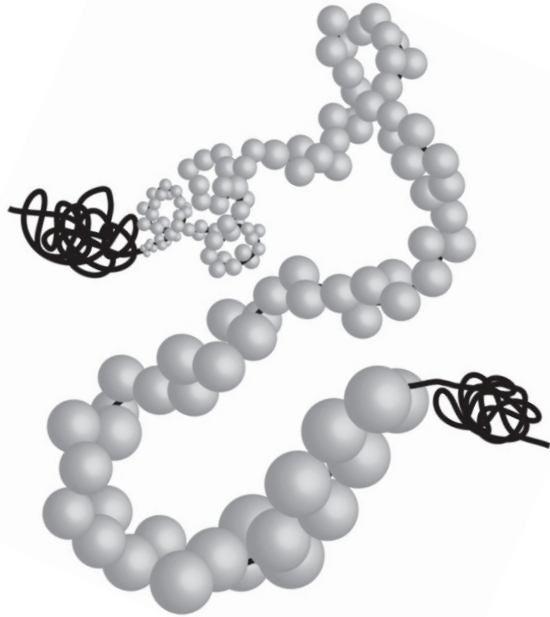


○ TSS
● TTS

1 μ m



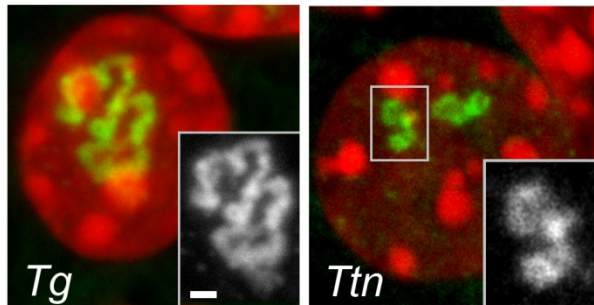
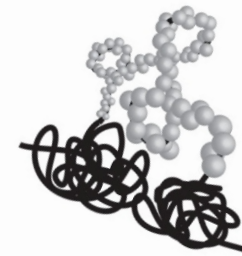
RNPs densely decorate the gene axis rigidifying and expanding the locus



180 Kb
24870 TPM
long introns

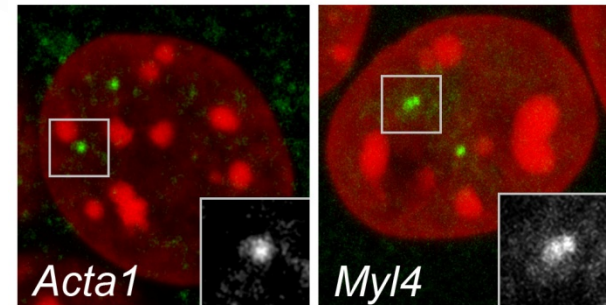
279 Kb
2120 TPM
long mRNA

Transcription loops of small genes cannot be resolved microscopically because they are not stiff and do not expand



180 Kb
24870 TPM
long introns

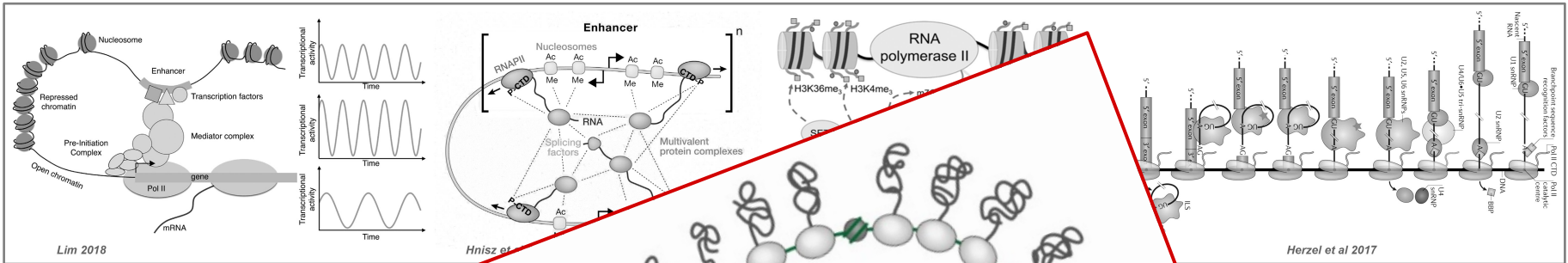
279 Kb
2120 TPM
long mRNA



3 Kb
4360 TPM
short introns and short mRNA

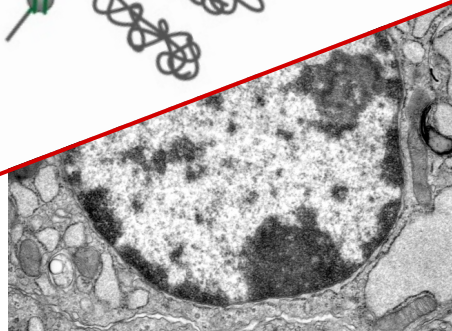
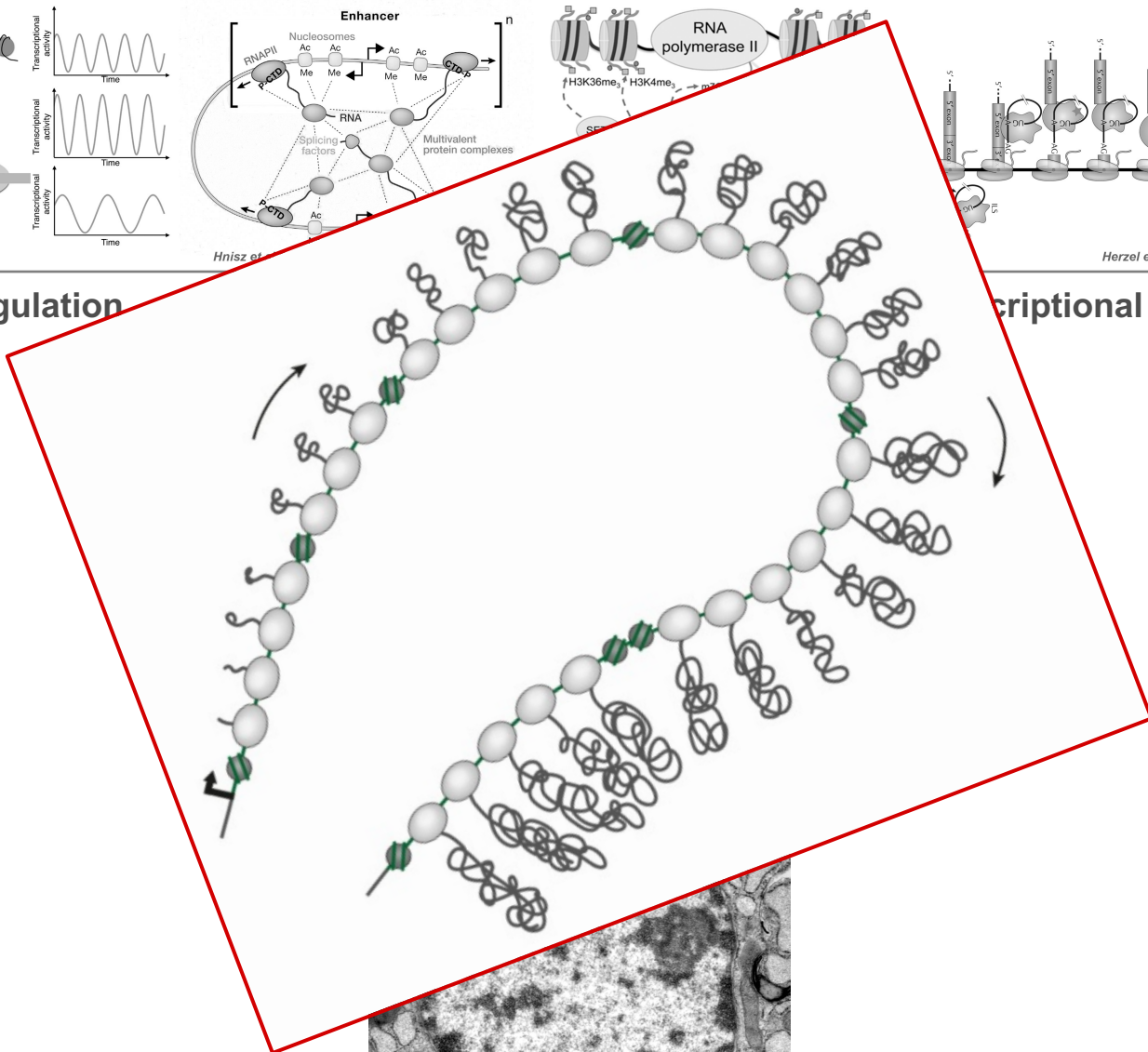
37 Kb
6180 TPM
short introns and short mRNA

Spatial organization of transcription

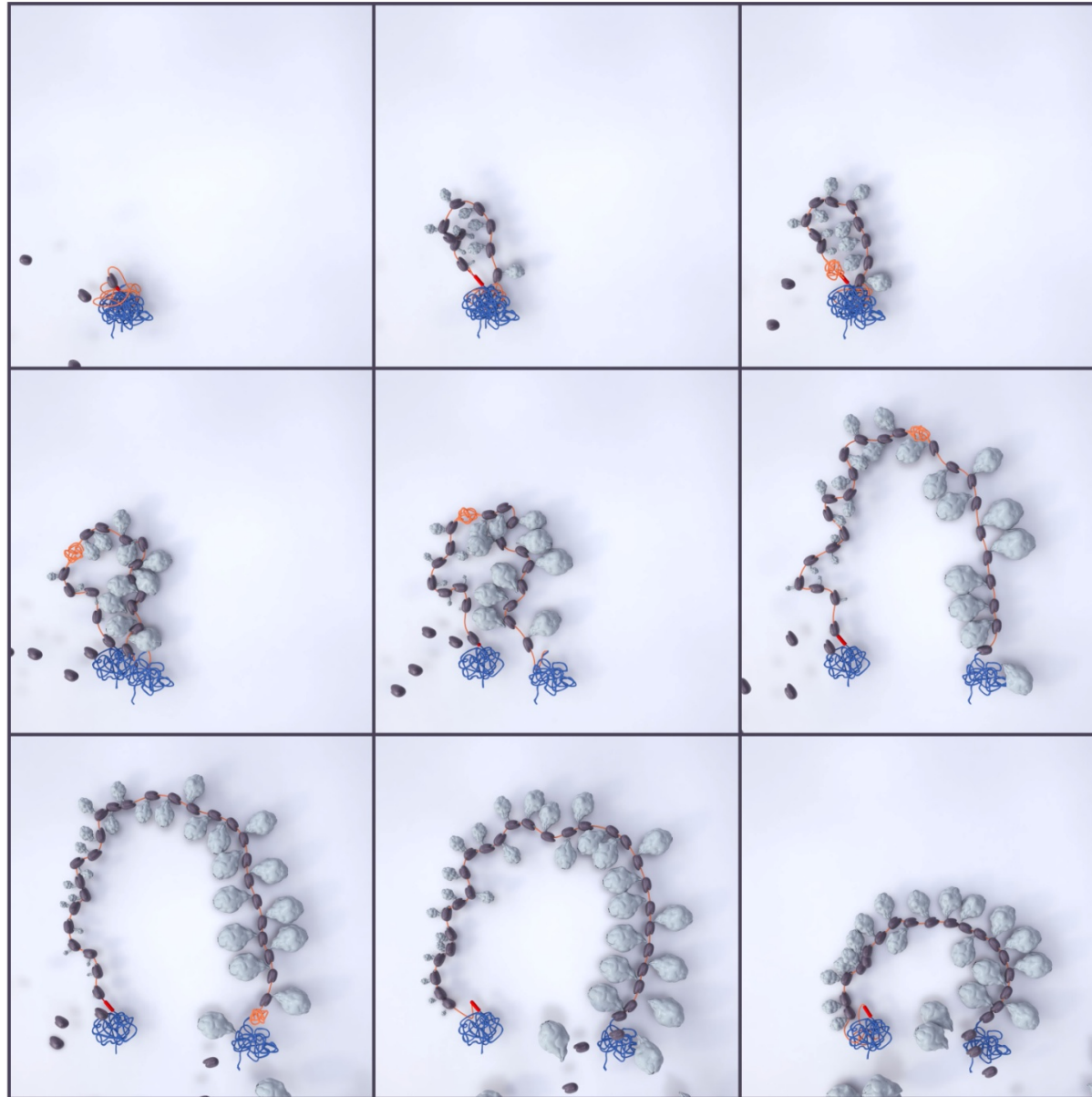


transcription regulation

transcriptional modifications



The rise and fall of a transcription loop



Irina Solovei

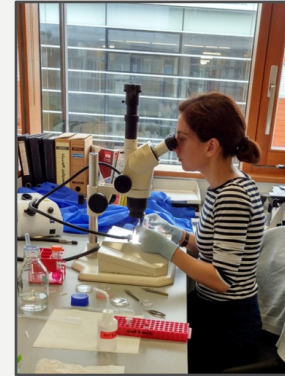
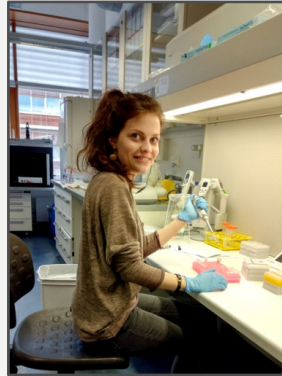
Susanne Leidescher

Simon Ullrich

Yana Feodorova

Katharina Thanisch

Sebastian Bultmann *LMU*



Job Dekker *UMass Med*

Erica Hildebrand

Leonid Mirny *MIT IMES*

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Animation

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