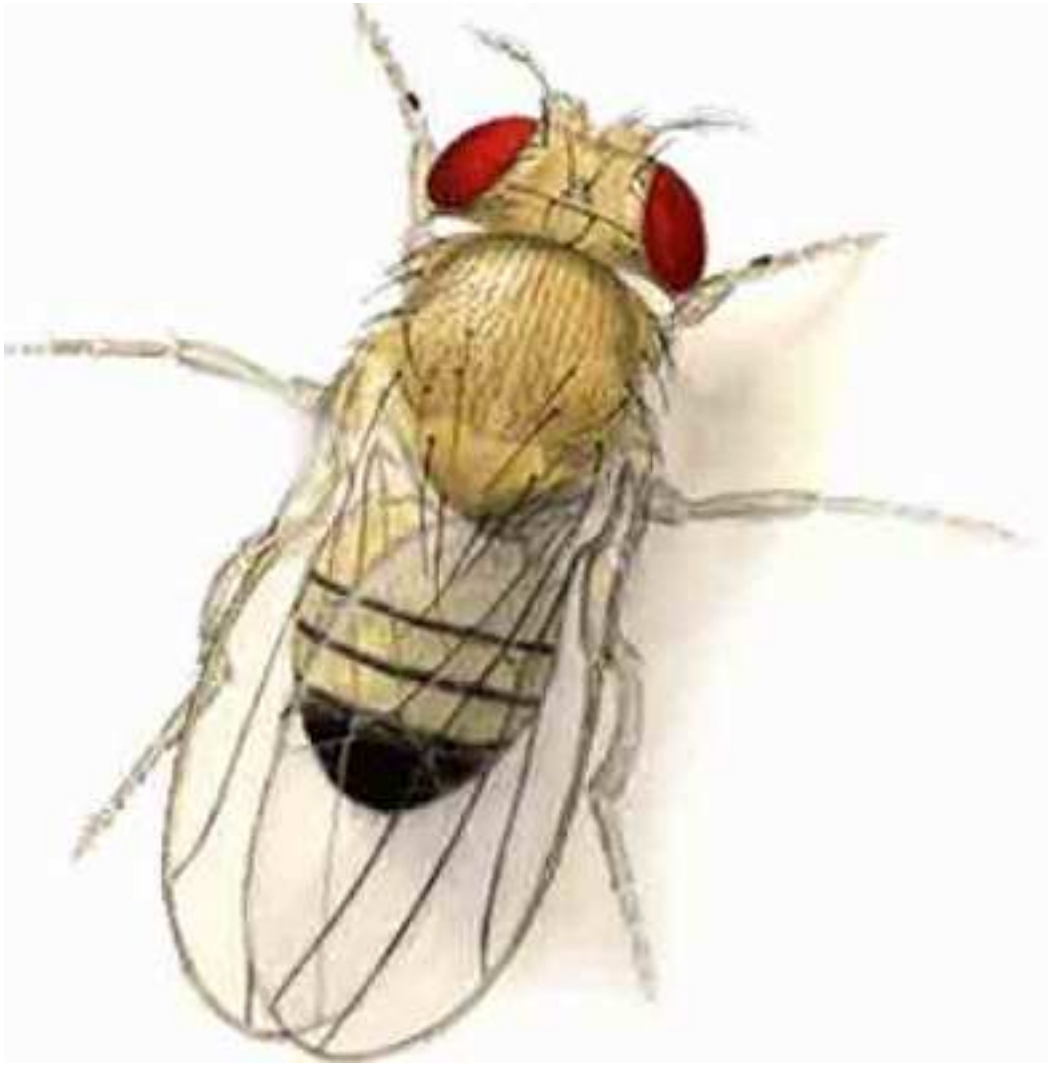
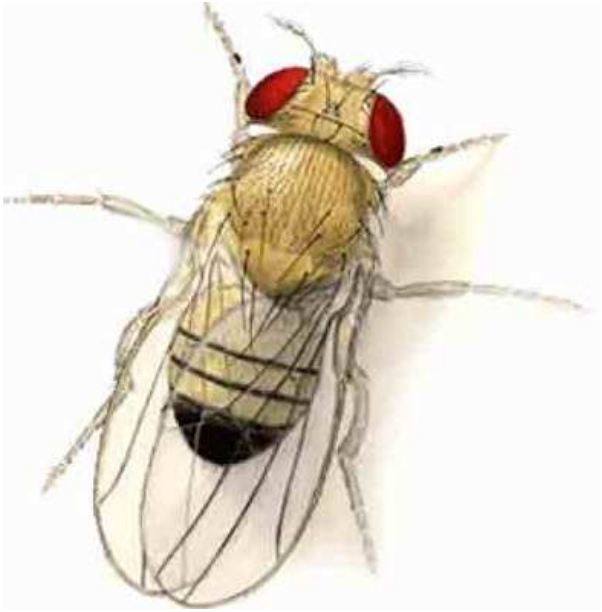
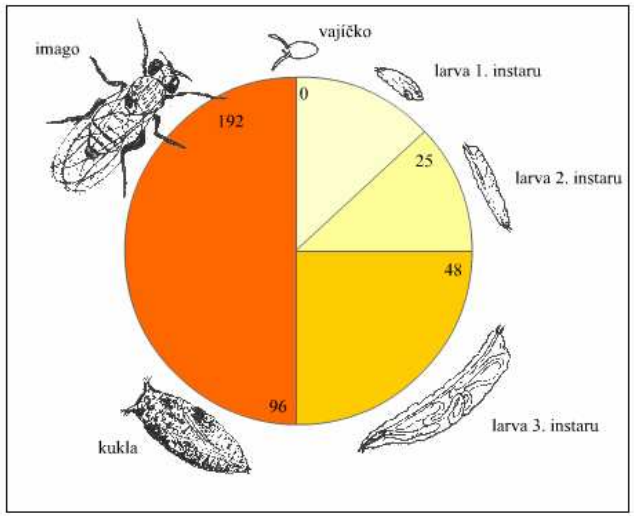


Within a few years of the rediscovery of Mendel's rules in 1900, Drosophila melanogaster (the so-called fruit fly) became a favorite "model" organism for genetics research.



Životní cyklus *Drosophila melanogaster* zahrnuje čtyři hlavní stadia vývinu: vajíčko, larva, kukla a imágo (dospělý jedinec).
Od oplození vajíčka do konce přeměny kukly v imágo uplyne asi 9–10 dní. Obrázek zachycuje délku trvání jednotlivých stadií vývoje (v hodinách).



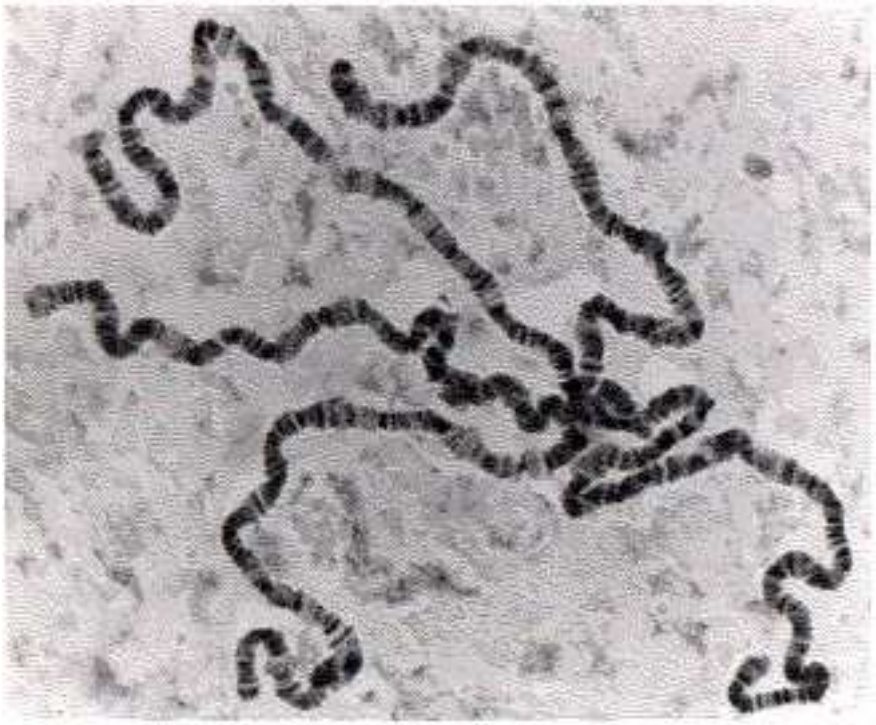
This little fly, Drosophila melanogaster, is one of the best understood animal in terms of development. The fly uses two structures for smelling, its antennae (visible at the tip of the head), and its maxillary palps (not visible in the picture). Our interests is in understanding the development of the adult olfactory system, which occurs during metamorphosis. The use of the fly for the study of genes and mutations was largely introduced by Thomas H. Morgan. Because of its short life cycle (11 days) and the ease of breeding the fly has been intensively studied at the genetic level for ~100 years. The entire fly genome has now been sequenced, and we now know that it has ~13,000 genes. The goal is to identify those genes that regulate the development of the fly olfactory system.

Thomas Hunt Morgan (1866-1945) received the Nobel Prize in Medicine or Physiology in 1933 for his discoveries concerning the role chromosomes play in heredity.

The giant ("polytene") chromosomes in the salivary (and other) glands of the mature larvae.

* These chromosomes show far more structural detail than do normal chromosomes, and
* they are present during interphase when chromosomes are normally invisible.

Function of polyteny is gene amplification leading to incresed gene expression consist of dark and light bands, separated by insulators.



Kultivační médium pro drozofily

Navážíme:

120 g kukuřičného šrotu

50 g cukru

25 g sušených kvasnic

14 g agaru

Vše smícháme v nádobě vhodné do mikrovlnné trouby, přidáme 1 l vody a důkladně promícháme. V troubě vaříme 1×4 min, 1×3 min, 2×2 min. Vždy promícháme.

Po uvaření přidáme 40 ml desinfekčního roztoku a rozléváme do připravených sterilních nádob (sterilizace 1 hod při 100 ℃). Na povrch média vložíme kole čko sterilizovaného filtračního papíru prodírkovaného jehlou.

Příprava desinfekčního roztoku

Navážíme

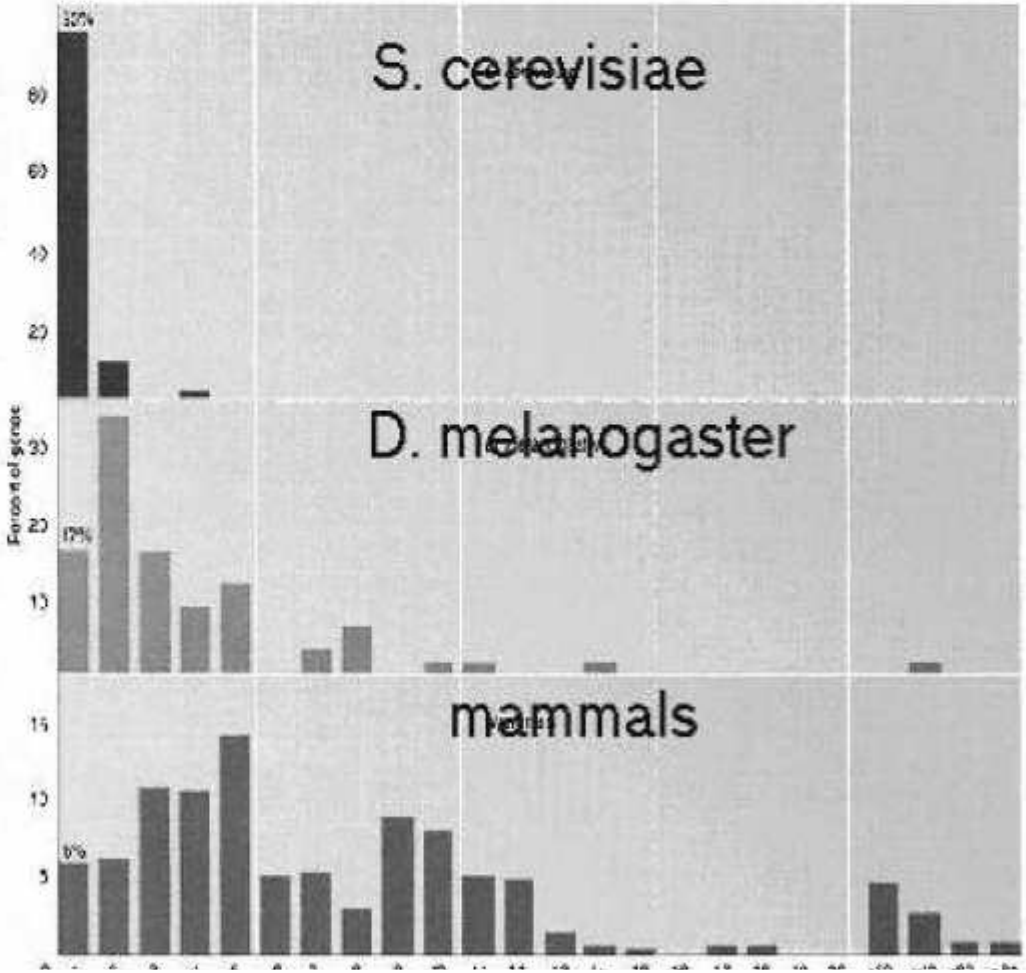
12,5 g kyseliny benzoové

2,5 g kyseliny sorbové

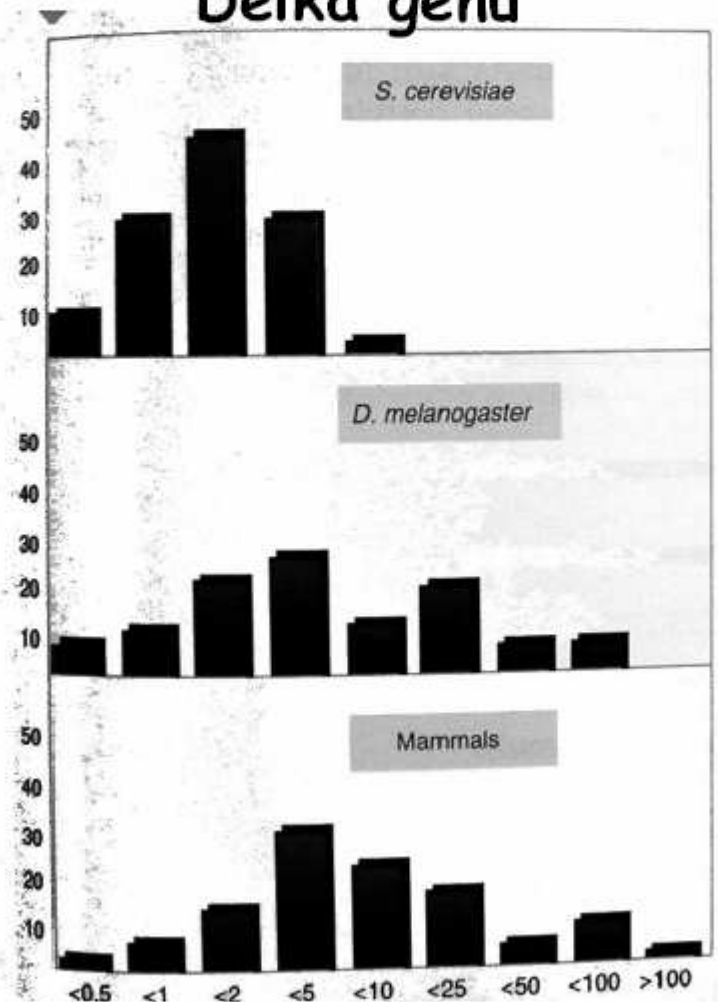
Rozpustit v 240 ml etylalkoholu.

Počty exonů jsou nejvyšší u savců

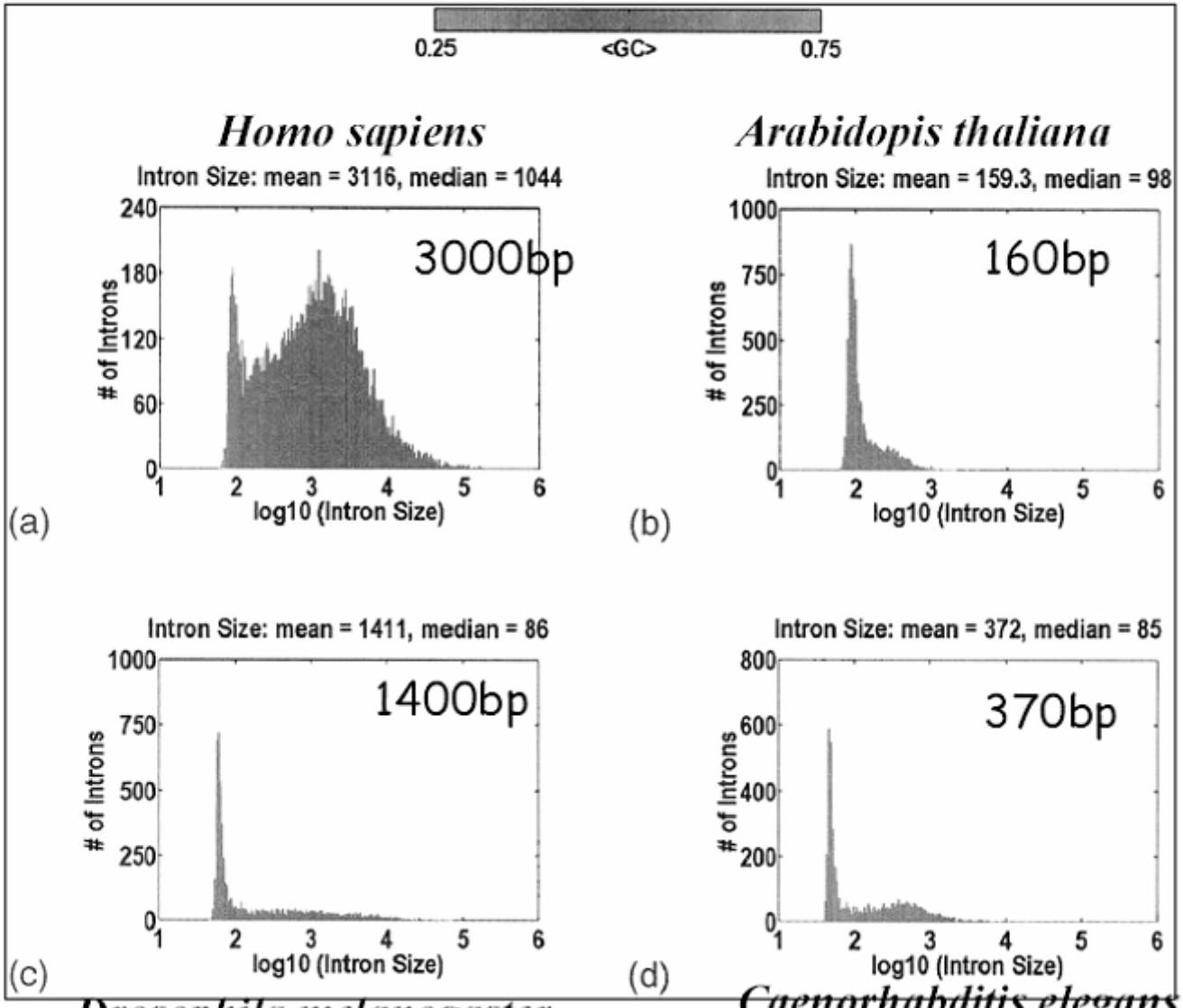
Počet exonů



Délka genu



Velikosti intronů

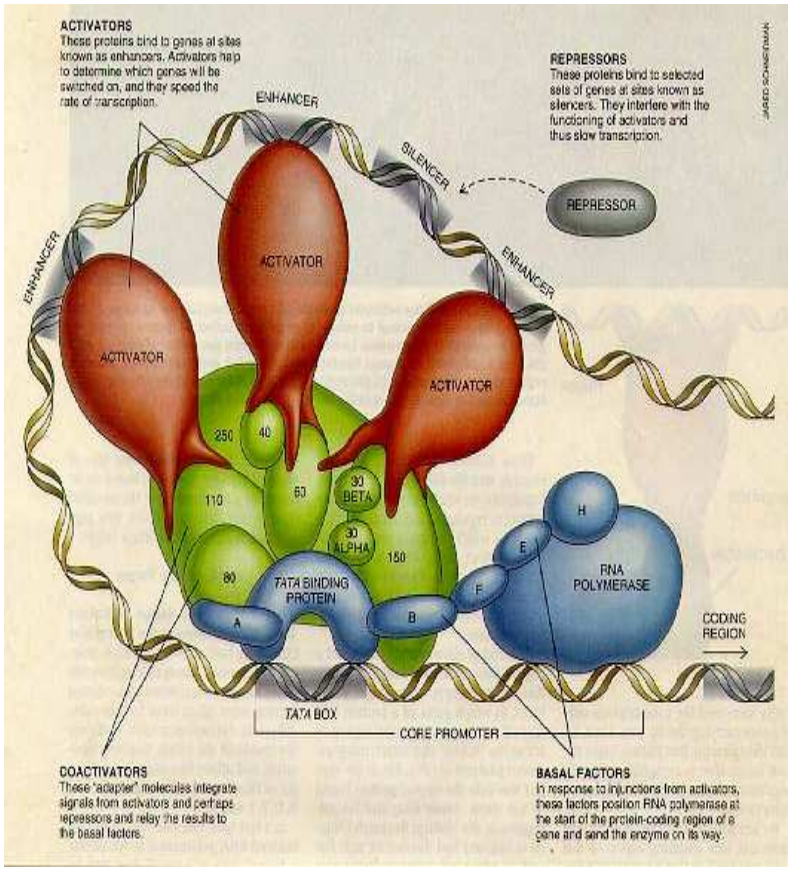


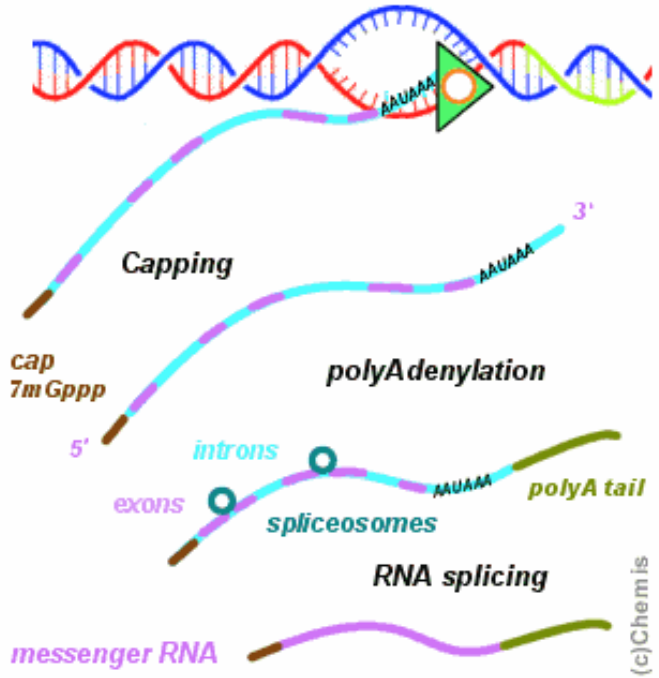
Introny byly do genů vloženy až dodatečně ("intron late")

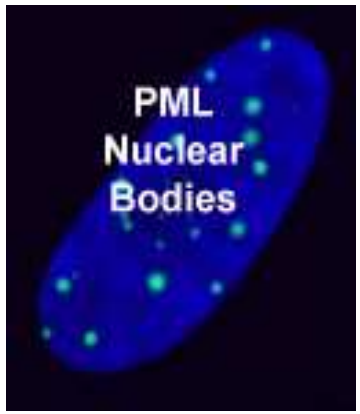
- Existuje řada různých intronů lišících se mechanizmem vystřihování z RNA – vznikaly nezávisle
- Distribuce intronů v rámci fylogenetických stromů svědčí o dodatečném vložení spíše než o opakovaném nezávislém vymizení

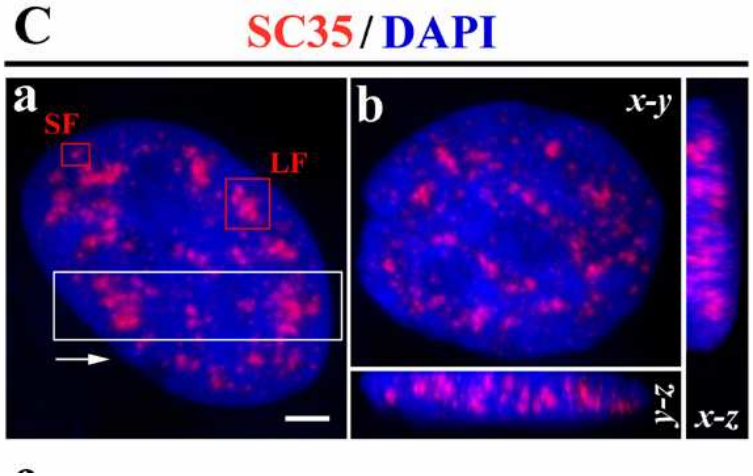
Introny jsou genomovými parazity

- Šíří se pouze v rámci genomu, vertikální přenos, aby nezabíjeli buňku, před translací se vystřihnou
- Samosestřih
- Splicesom – komplex kódovaný buňkou, původně parazitickými introny, kódují enzymy pro horizontální šíření v rámci genomu


ACTIVATORS
These proteins bind to genes at sites known as enhancers. Activators help to determine which genes will be switched on, and they speed the rate of transcription.
REPRESSORS
These proteins bind to selected sets of genes at sites known as silencers. They interfere with the functioning of activators and thus slow transcription.
ENHANCER
SILENCER
REPRESSOR
ENHANCER
ENHANCER
ACTIVATOR
ACTIVATOR
ACTIVATOR
250
40
110
60
30 BETA
30 ALPHA
150
80
TATA BINDING PROTEIN
A
B
F
E
H
RNA POLYMERASE
CODING REGION
TATA BOX
CORE PROMOTER
COACTIVATORS
These "adapter" molecules integrate signals from activators and perhaps repressors and relay the results to the basal factors.
BASAL FACTORS
In response to injunctions from activators, these factors position RNA polymerase at the start of the protein-coding region of a gene and send the enzyme on its way.


AAUAAA
3'
Capping
AAUAAA
cap
7mGppp
polyAdenylation
5'
introns
exons
AAUAAA
polyA tail
spliceosomes
RNA splicing
messenger RNA
(c)Chemis


PML
Nuclear
Bodies


C
SC35 / DAPI
a
SF
LF
b
x-y
y-z
x-z

Introny jsou užitečné pro organizmy

1. Zvyšují evoluční potenciál organizmu

- souvisí se vznikem eukaryot, v pozadí adaptivní radiace eukaryot,
- nenáhodná distribuce, oddělují funkční domény proteinů,
- stavebnicový charakter genů urychluje evoluci nových proteinů,
- snižuje pravděpodobnost rekombinace v exonech (doménách)

2. Souvisí s existencí histonů

- oblasti v kontaktu s histony nepřístupné
- introny zpřístupňují regulační oblasti

3. Umožňují detekci , případně i reparaci mutací v exonech

- detekce chyb při přenosu informace, příklad „liché parity"
- introny jako kontrolní sekvence, sekundární struktura

4. Snižují riziko nelegitimní rekombinace

- paralogy a riziko nelegitimní rekombinace, nefunkční geny
- včlenění intronů do různých míst diferencuje geny

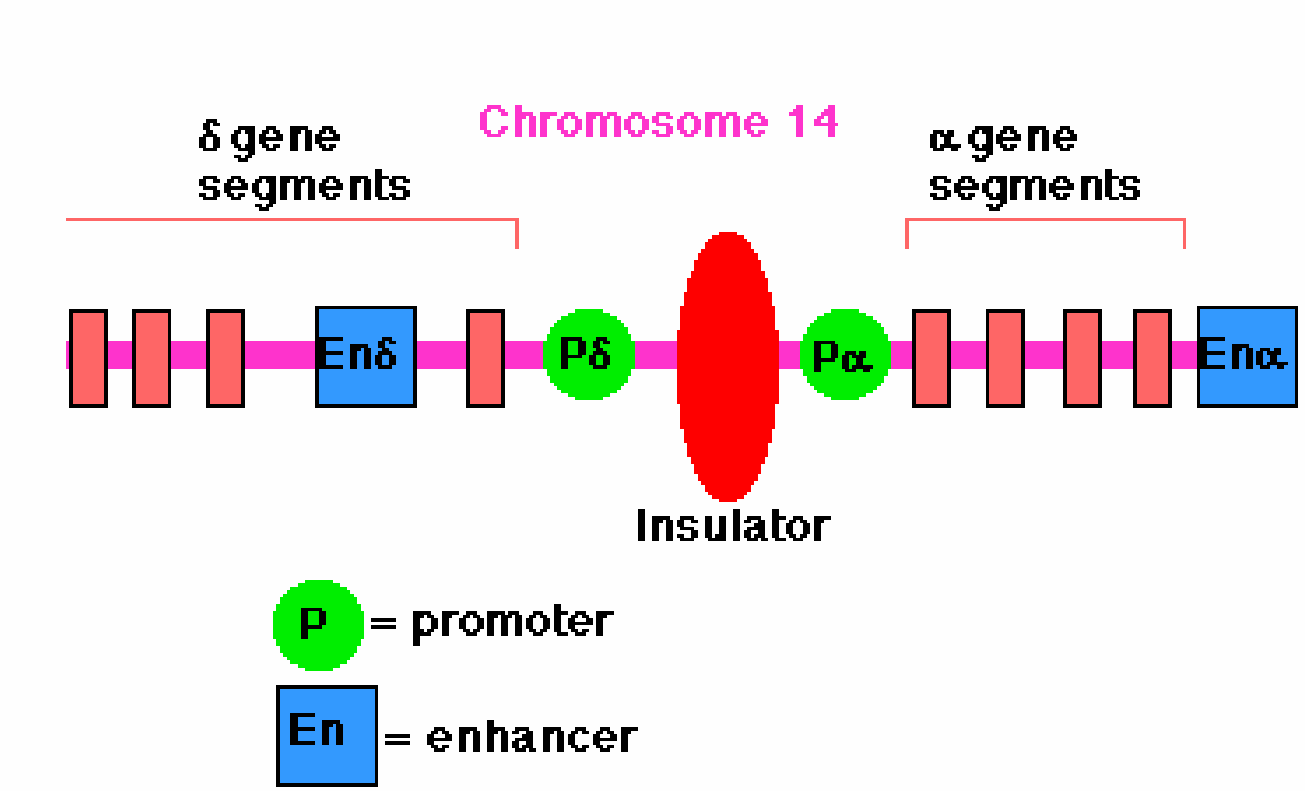
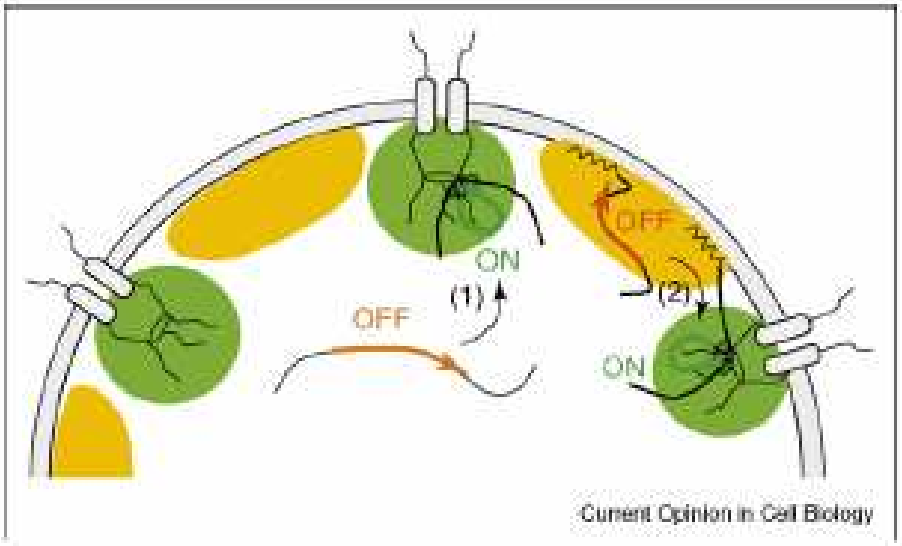
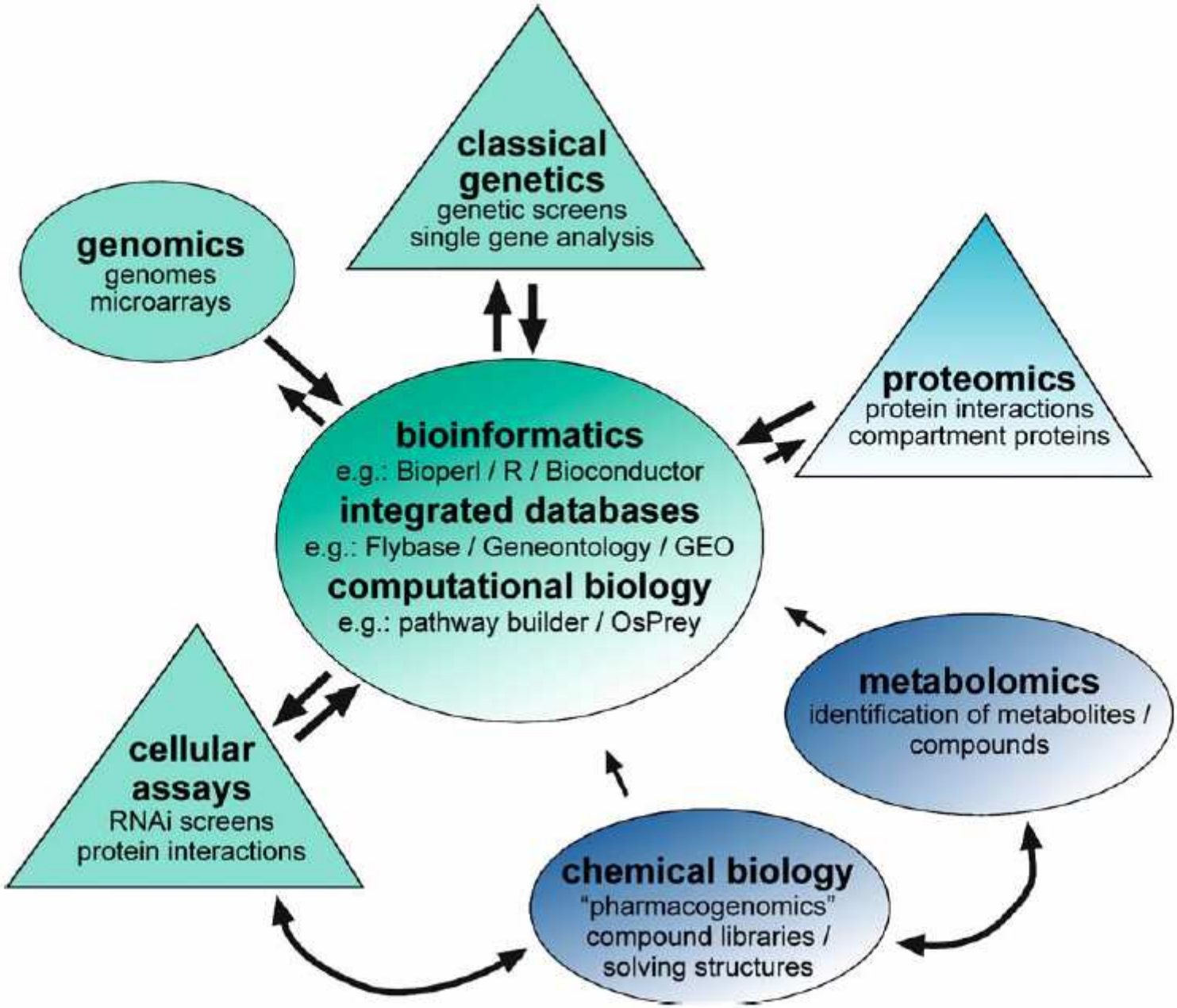
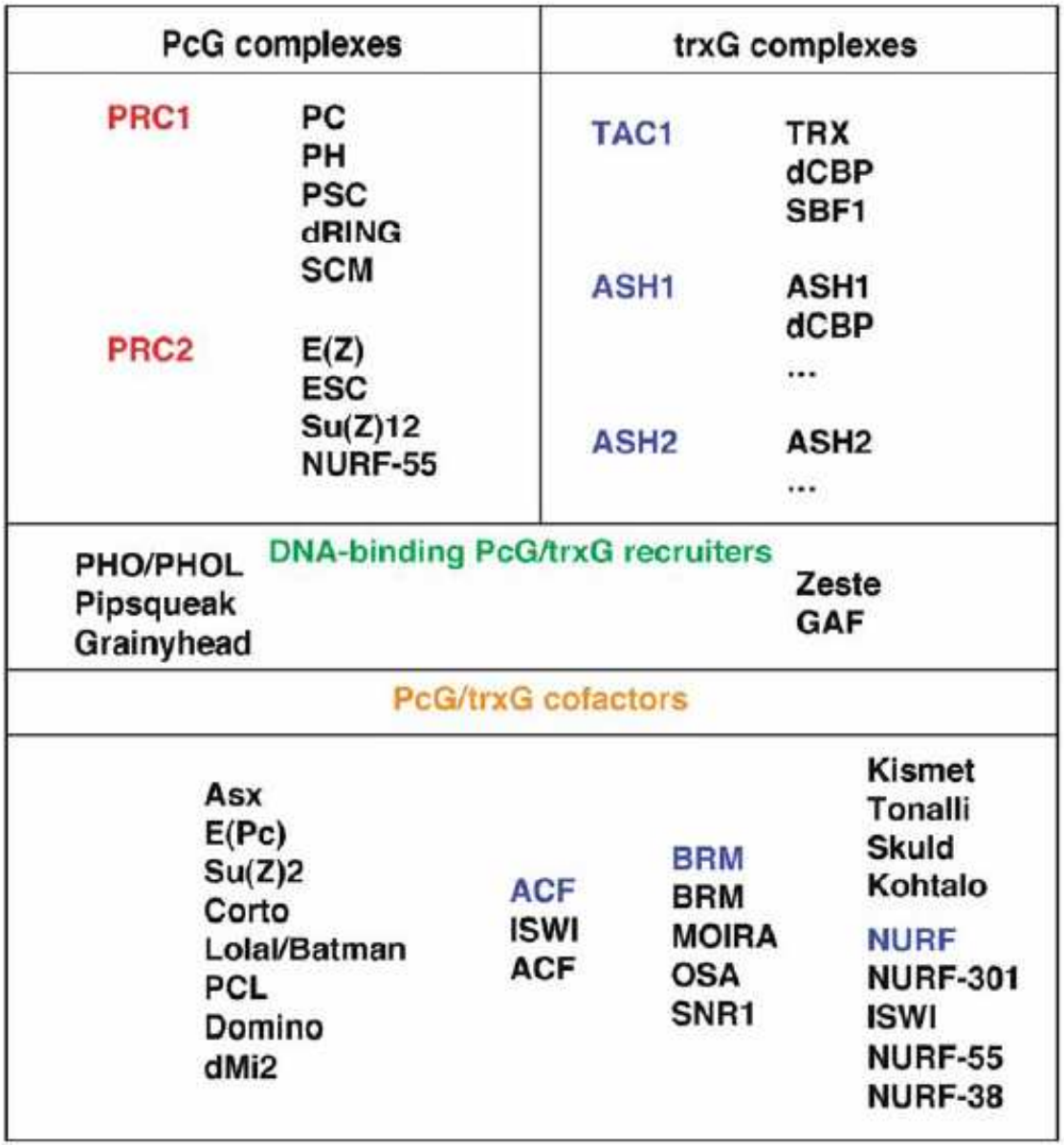

δ gene segments
Chromosome 14
α gene segments
Enδ
Pδ
Pα
Enα
Insulator
P = promoter
En = enhancer

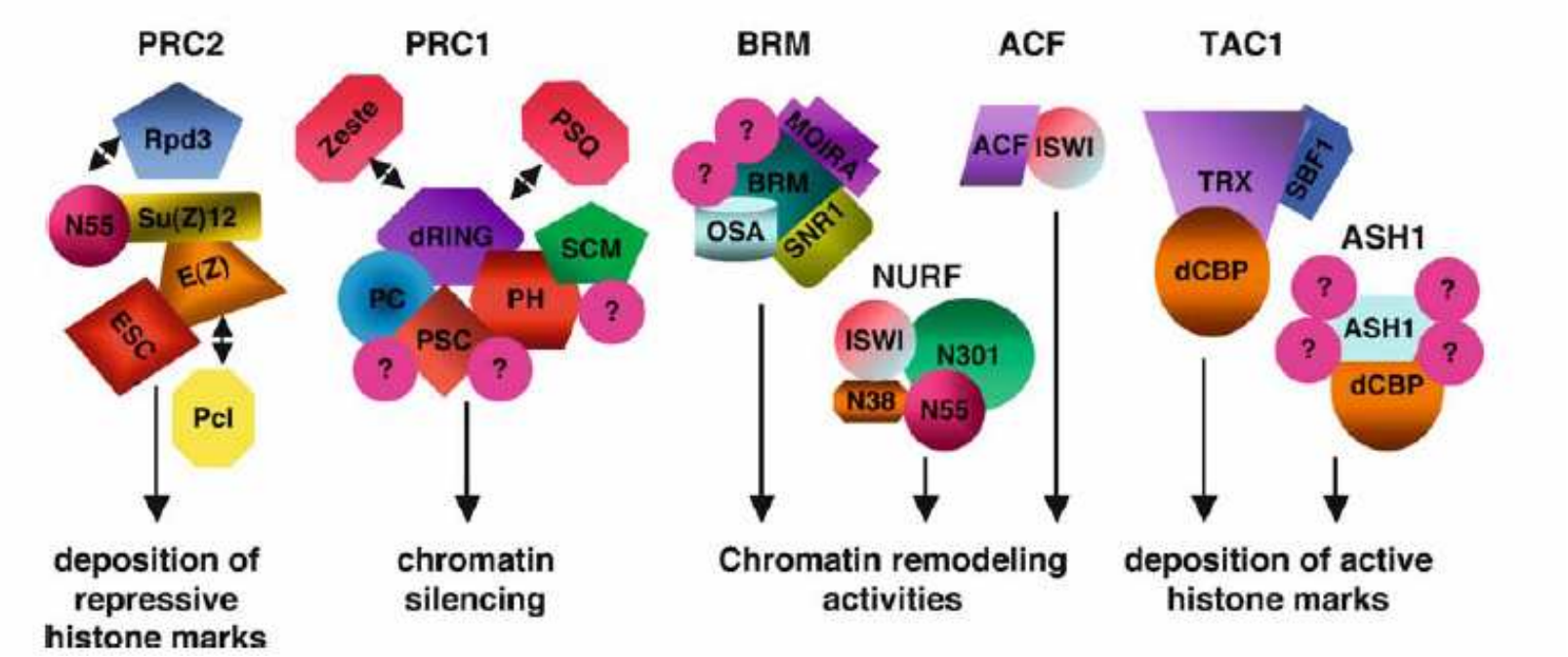
Figure 1



Scheme representing the induction of a gene and its associated movement either (1) from the nuclear interior or (2) from a repressing compartment (telomere clusters, in orange) to the NPC, where it forms an activating sub-domain (in green). Anchoring of active genes to the NPC could favor the coordination of the different processes occurring at an active gene (including transcriptional initiation, elongation, termination, mRNA processing, quality control and export) as in the original version of the 'gene gating' hypothesis proposed by G Blobel in 1985 [24]. Because of telomere anchoring, subtelomeric genes are in close proximity to the nuclear envelope [2], and may preferentially exploit mechanisms of activation that are enhanced by pore association. It is possible that activating and repressive compartments cooperate to make subtelomeric genes inducible under specific conditions.

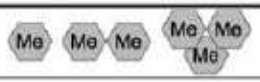

classical genetics
genetic screens
single gene analysis
genomics
genomes
microarrays
proteomics
protein interactions
compartment proteins
bioinformatics
e.g.: Bioperl / R / Bioconductor
integrated databases
e.g.: Flybase / Geneontology / GEO
computational biology
e.g.: pathway builder / OsPrey
metabolomics
identification of metabolites / compounds
cellular assays
RNAi screens
protein interactions
chemical biology
"pharmacogenomics"
compound libraries /
solving structures


PcG complexes
trxG complexes
PRC1
PC
PH
PSC
dRING
SCM
PRC2
E(Z)
ESC
Su(Z)12
NURF-55
TAC1
TRX
dCBP
SBF1
ASH1
ASH1
dCBP
...
ASH2
ASH2
...
DNA-binding PcG/trxG recruiters
PHO/PHOL
Pipsqueak
Grainyhead
Zeste
GAF
PcG/trxG cofactors
Asx
E(Pc)
Su(Z)2
Corto
Lola/Batman
PCL
Domino
dMi2
ACF
ISWI
ACF
BRM
BRM
MOIRA
OSA
SNR1
Kismet
Tonalli
Skuld
Kohtalo
NURF
NURF-301
ISWI
NURF-55
NURF-38


PRC2
Rpd3
N55
Su(Z)12
E(Z)
ESC
Pcl
deposition of repressive histone marks
PRC1
Zeste
PSO
dRING
SCM
PC
PH
PSC
chromatin silencing
BRM
MOIRA
BRM
OSA
SNR1
NURF
ISWI
N301
N38
N55
ACF
ACF
ISWI
Chromatin remodeling activities
TAC1
TRX
SBF1
dCBP
ASH1
ASH1
dCBP
deposition of active histone marks

a
H3–K9 me1
b
H3–K9 me2
c
H3–K9 me3
d
H3–K27 me1

Table 1. Differential distribution of histone modification marks in interphase nuclei of *Drosophila*, mammals and *Arabidopsis*

Position	Modification	*Drosophila*	Mammals	*Arabidopsis*
H3K4		EU (IB)	EU	EU
H3K9		EU (B)	EU	EU
		HET, [EU (B)]	EU	HET
		HET, [EU (B)]	EU, fac. HET	HET
		HET, [EU (B)]	HET	EU
H3S10		EU (IB)	EU	HET
H3K14		EU (B)	EU	EU
H3K27		HET, EU (B)	HET, EU	HET
		HET, EU (B)	EU	HET
		HET, EU (B)	EU, fac. HET	EU
H3K36		EU (IB)	unknown	EU
H4K20		HET, EU (B)	EU, fac. HET	HET
		HET, EU (B)	EU	EU
		HET, EU (B)	HET	EU

EU – euchromatin, HET – heterochromatin, B – bands, IB – interbands

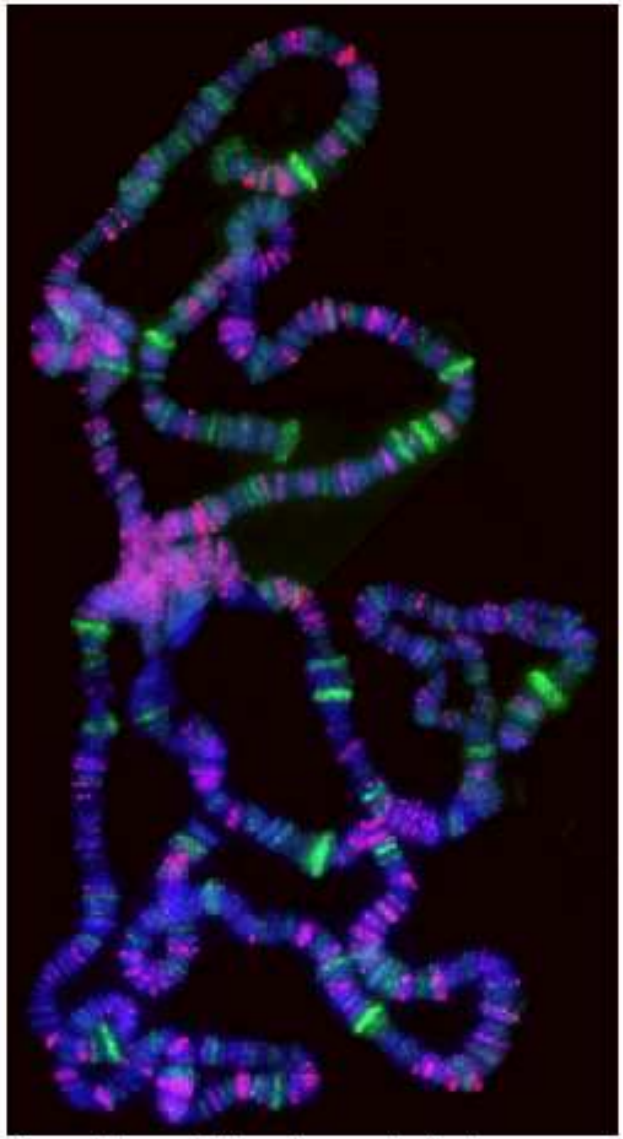


Figure: Drosophila salivary gland chromosome stained with anti-monomethyl Histone H4-K20 (red) and with antibody specific for the catalytic subunit of RNA polymerase II (green).

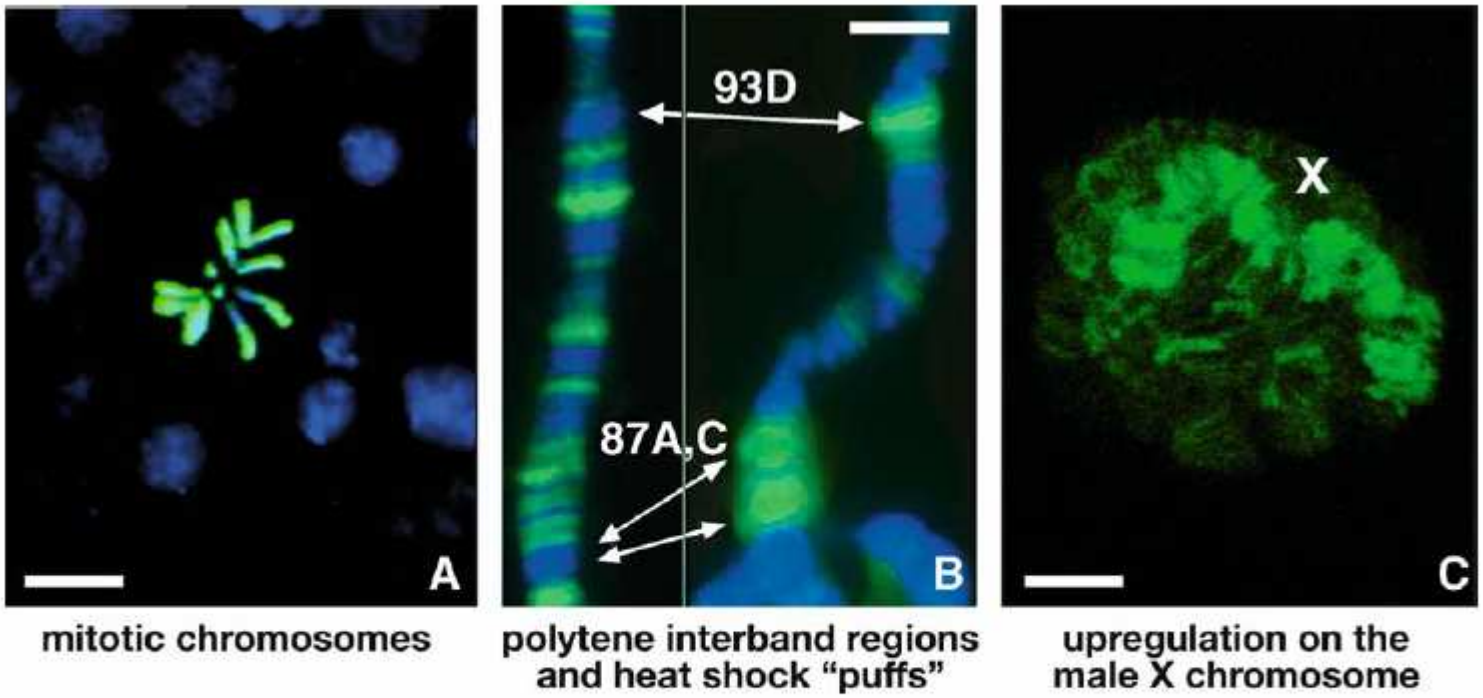
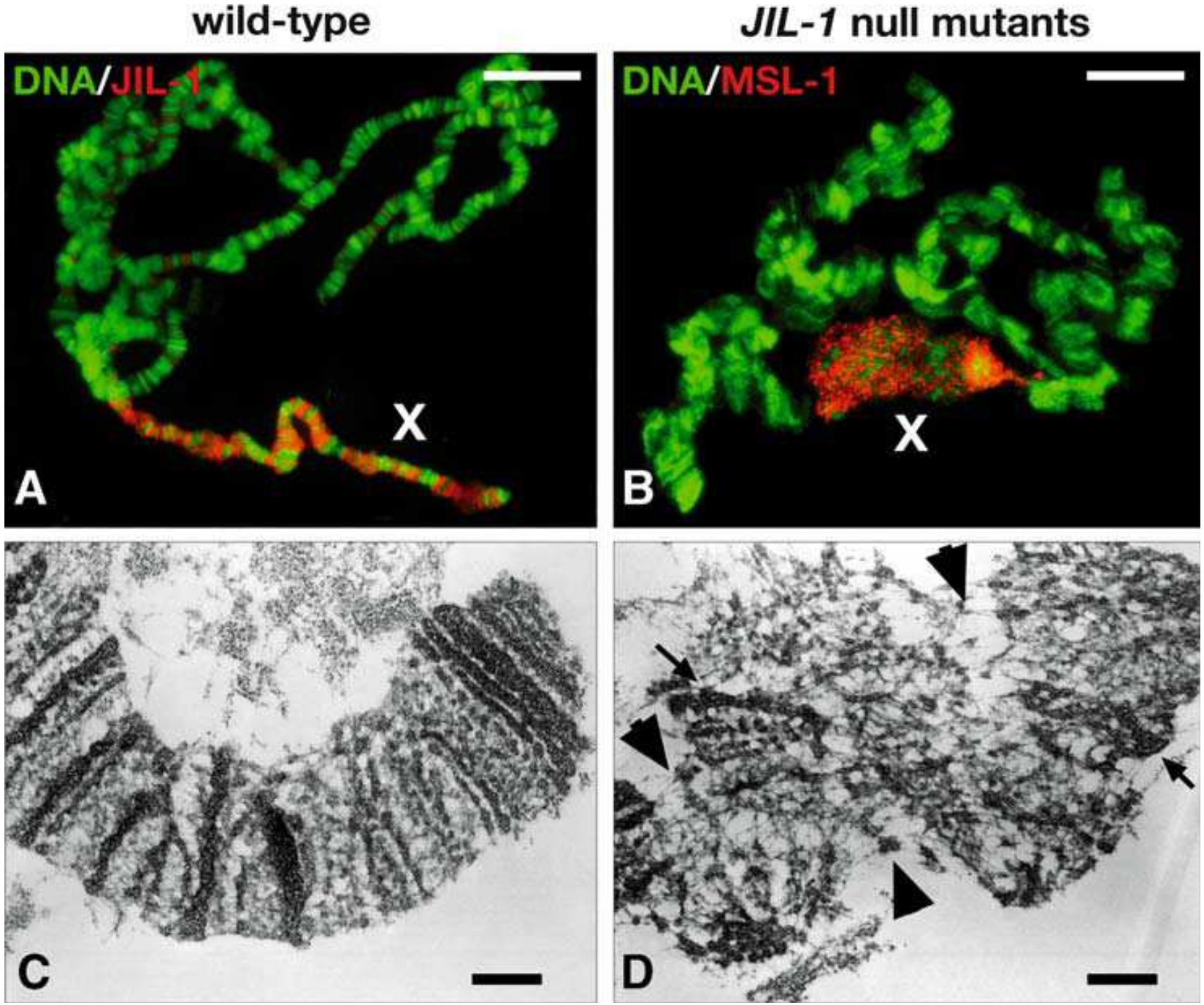
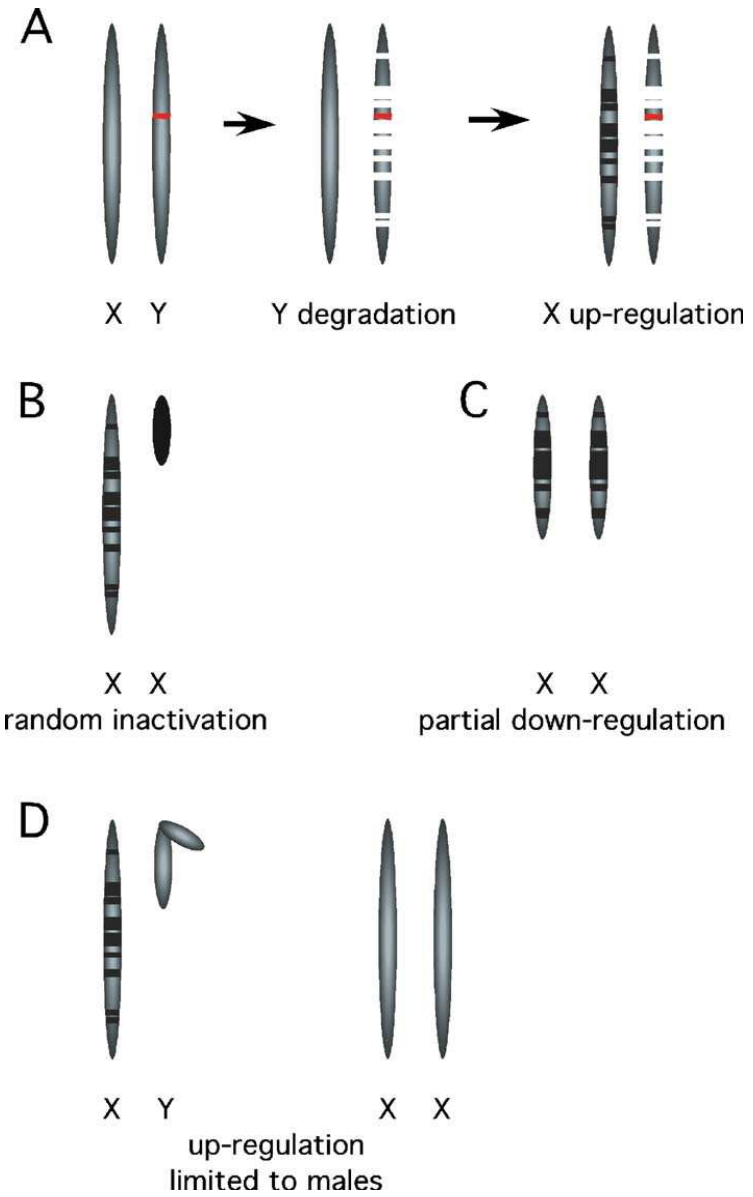


Figure 1. Histone H3S10 phosphorylation in *Drosophila*. **A:** H3S10ph antibody labeling (in green) of mitotic chromosomes in a larval neuroblast. Labeling of DNA by Hoechst is shown in blue. **B:** Distribution of phosphorylated histone H3S10 in polytene chromosomes before and after heat shock. The preparations were double-labeled with H3S10ph antibody (in green) and with Hoechst (in blue). The images show the change in staining of three heat shock loci (87A, 87C, and 93D) on a section of chromosome 3R. The heat shocked chromosome is to the right. The figure is modified from Nowak & Corces (2000). **C:** Confocal image from a whole-mount preparation of a salivary gland polytene nuclei from a male third-instar larvae labeled with H3S10ph antibody. The labeling of phosphorylated histone H3S10 is up-regulated on the male X chromosome (X). Scale bar equals 5 μm in (**A**) and (**C**) and 2 μm in (**B**).


wild-type
JIL-1 null mutants
DNA/JIL-1
DNA/MSL-1
X
X
A
B
C
D



human

C. elegans

Drosophila
H4K16Ac
H3S10 phosphorylation

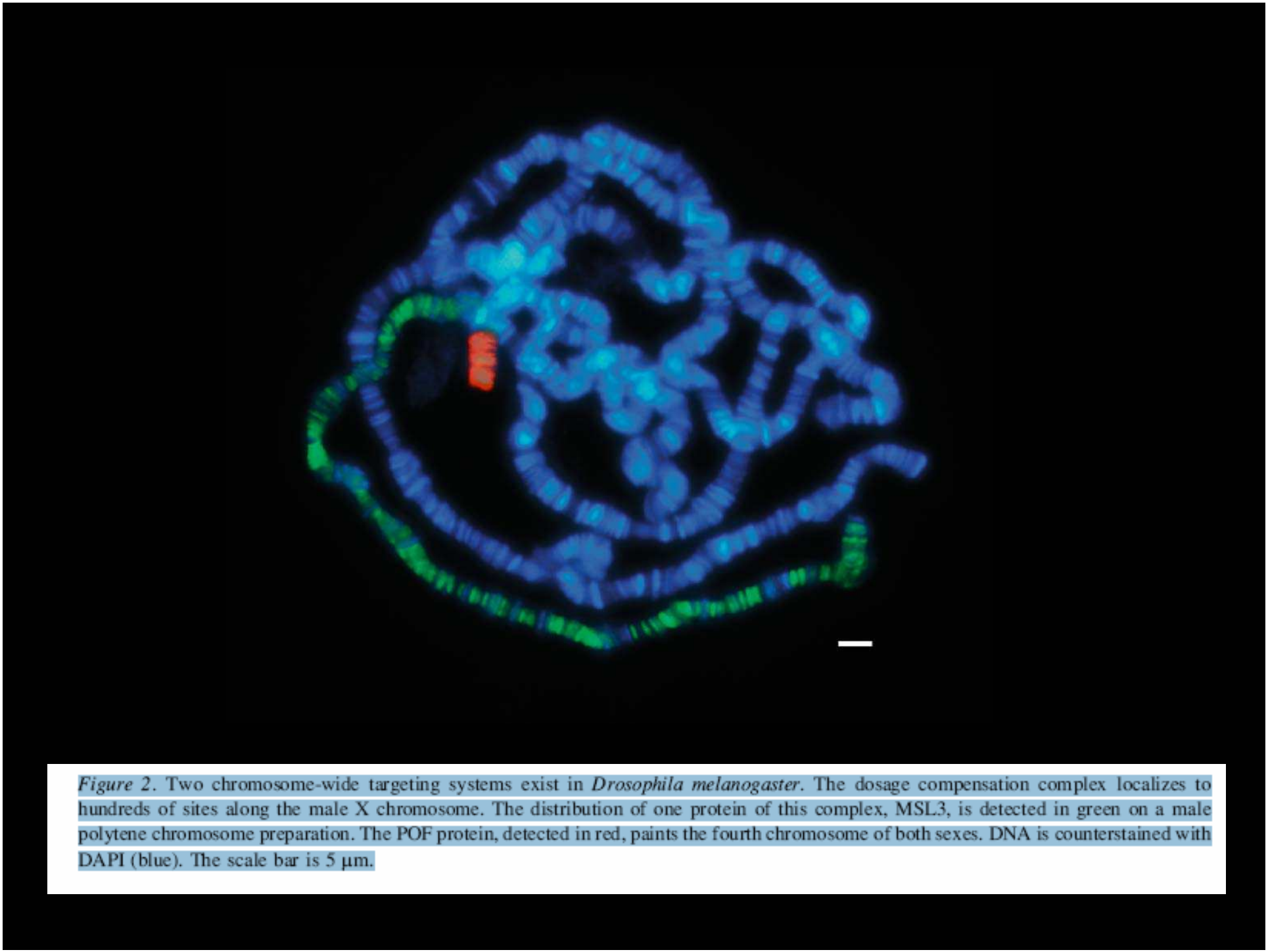


Figure 2. Two chromosome-wide targeting systems exist in *Drosophila melanogaster*. The dosage compensation complex localizes to hundreds of sites along the male X chromosome. The distribution of one protein of this complex, MSL3, is detected in green on a male polytene chromosome preparation. The POF protein, detected in red, paints the fourth chromosome of both sexes. DNA is counterstained with DAPI (blue). The scale bar is 5 μm.

Inaktivace X chromosomu ve vztahu k epigenetickým modifikacím in mammals

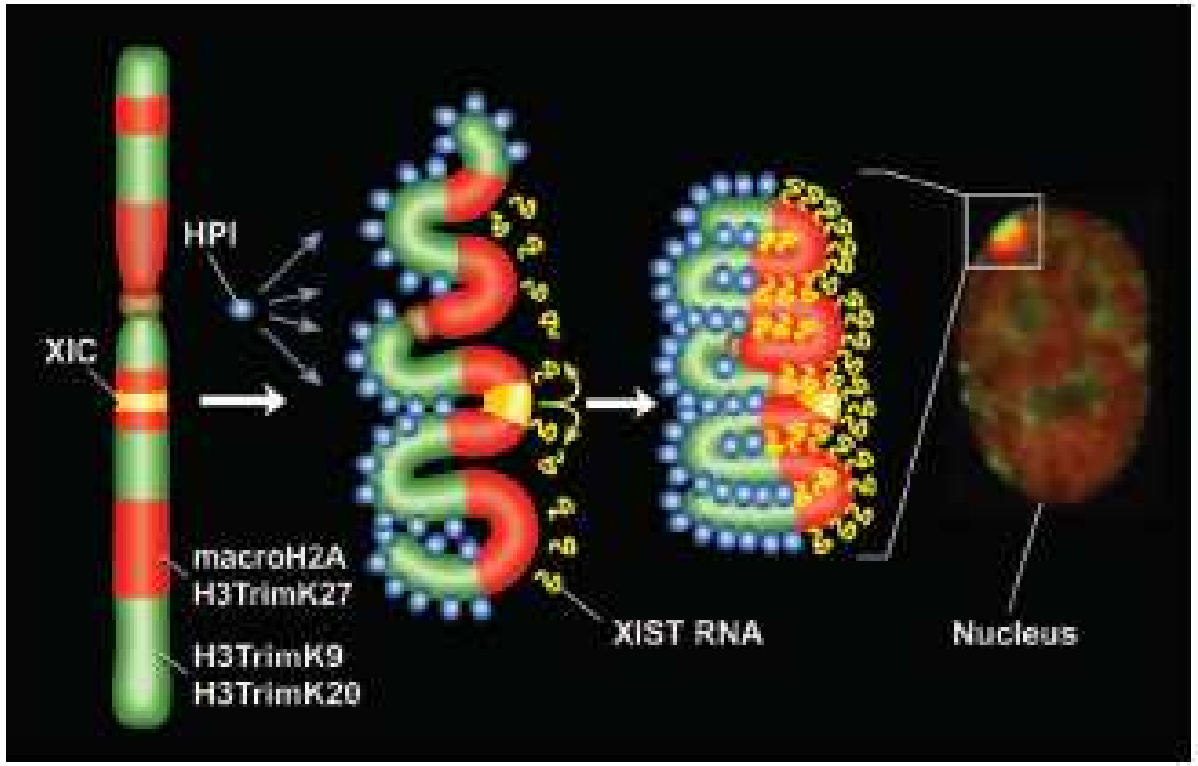
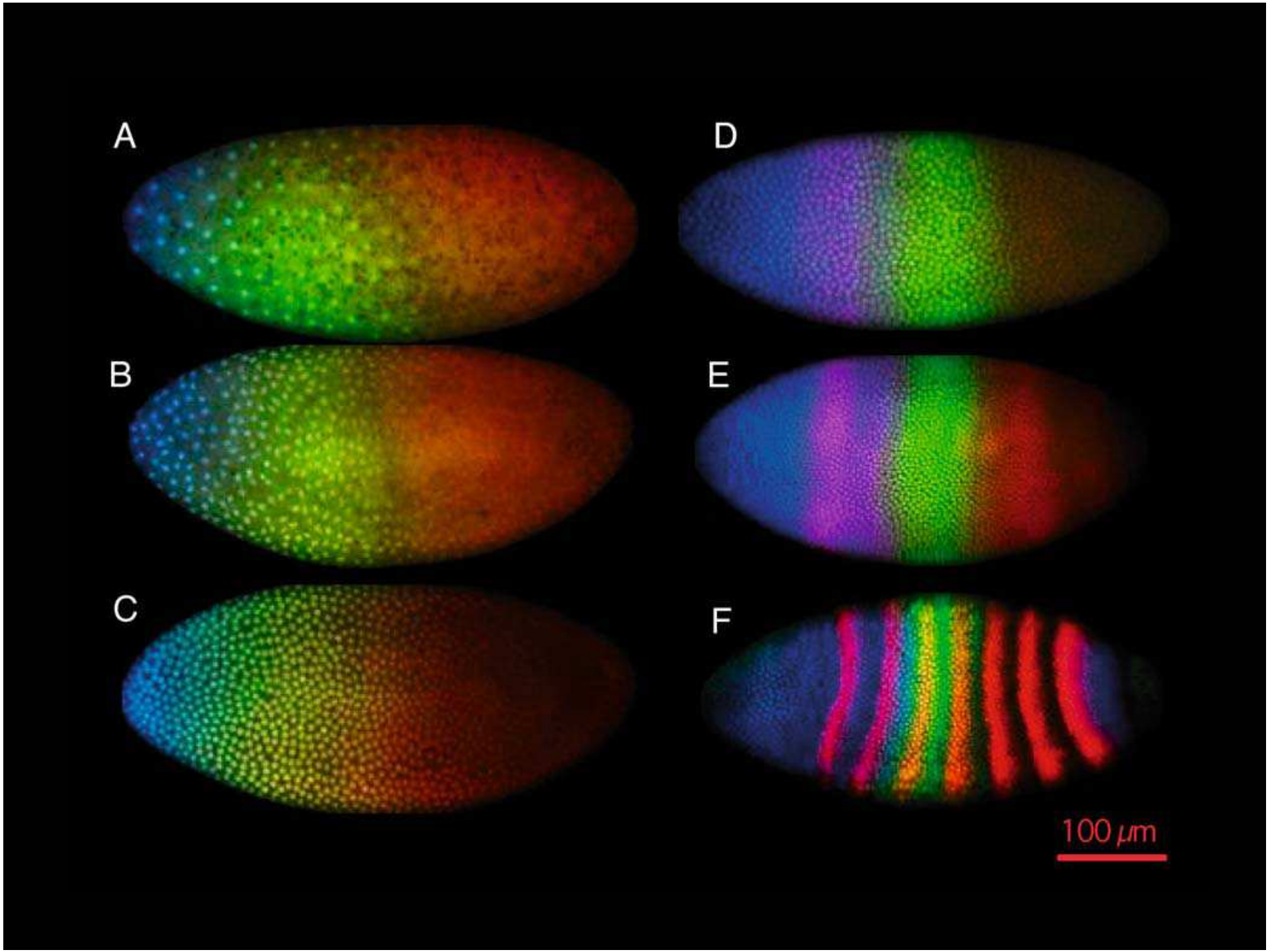
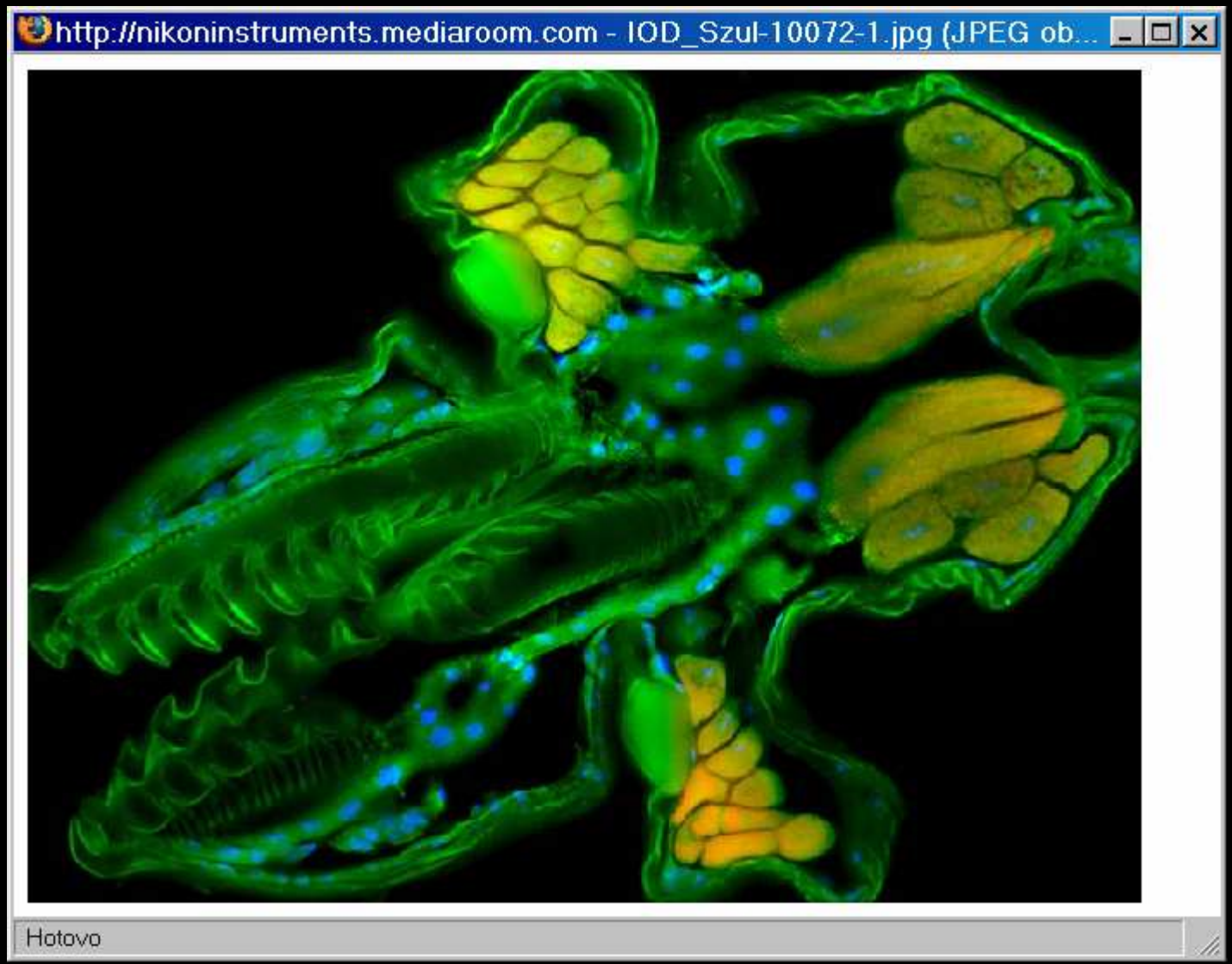
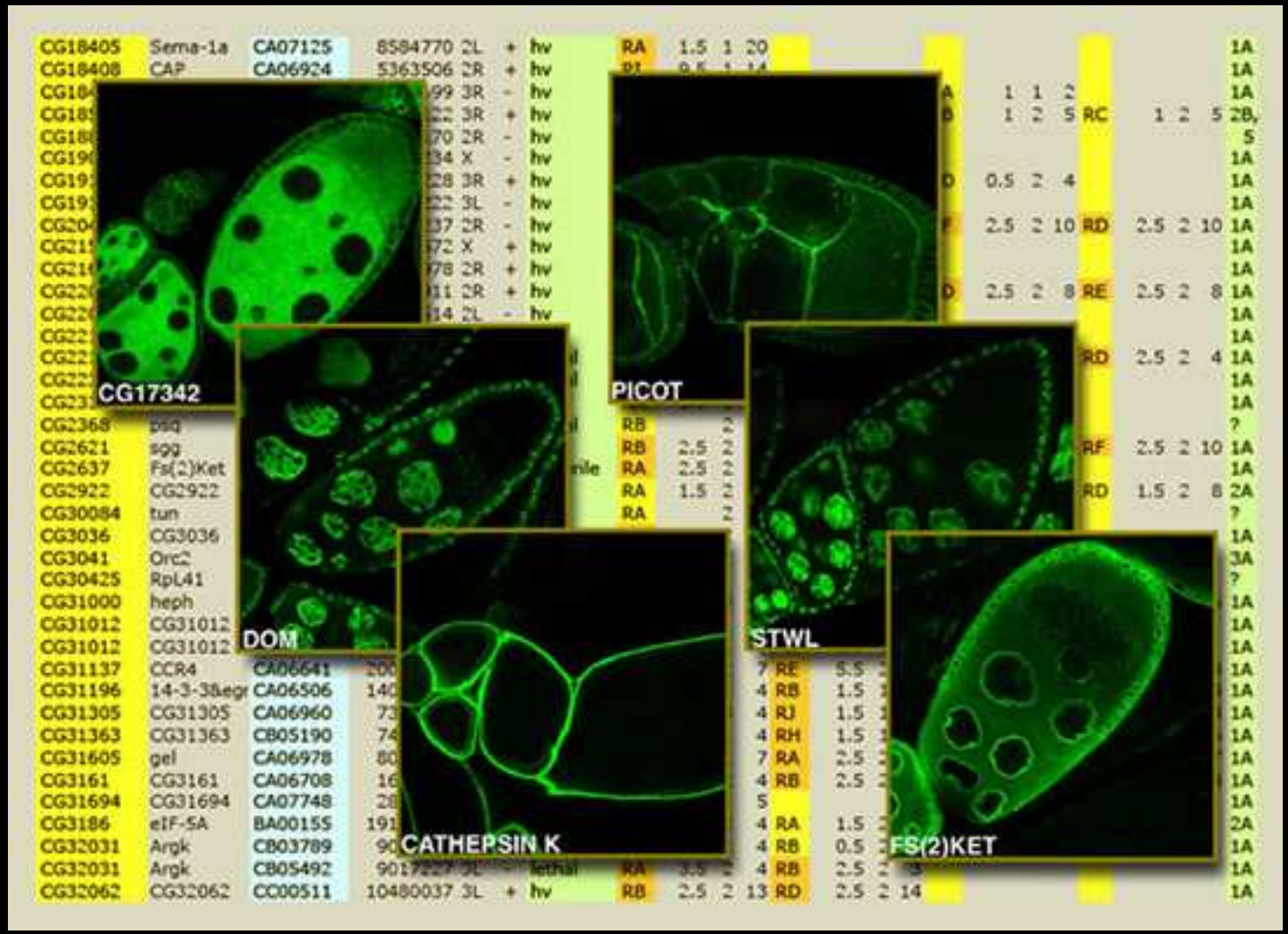
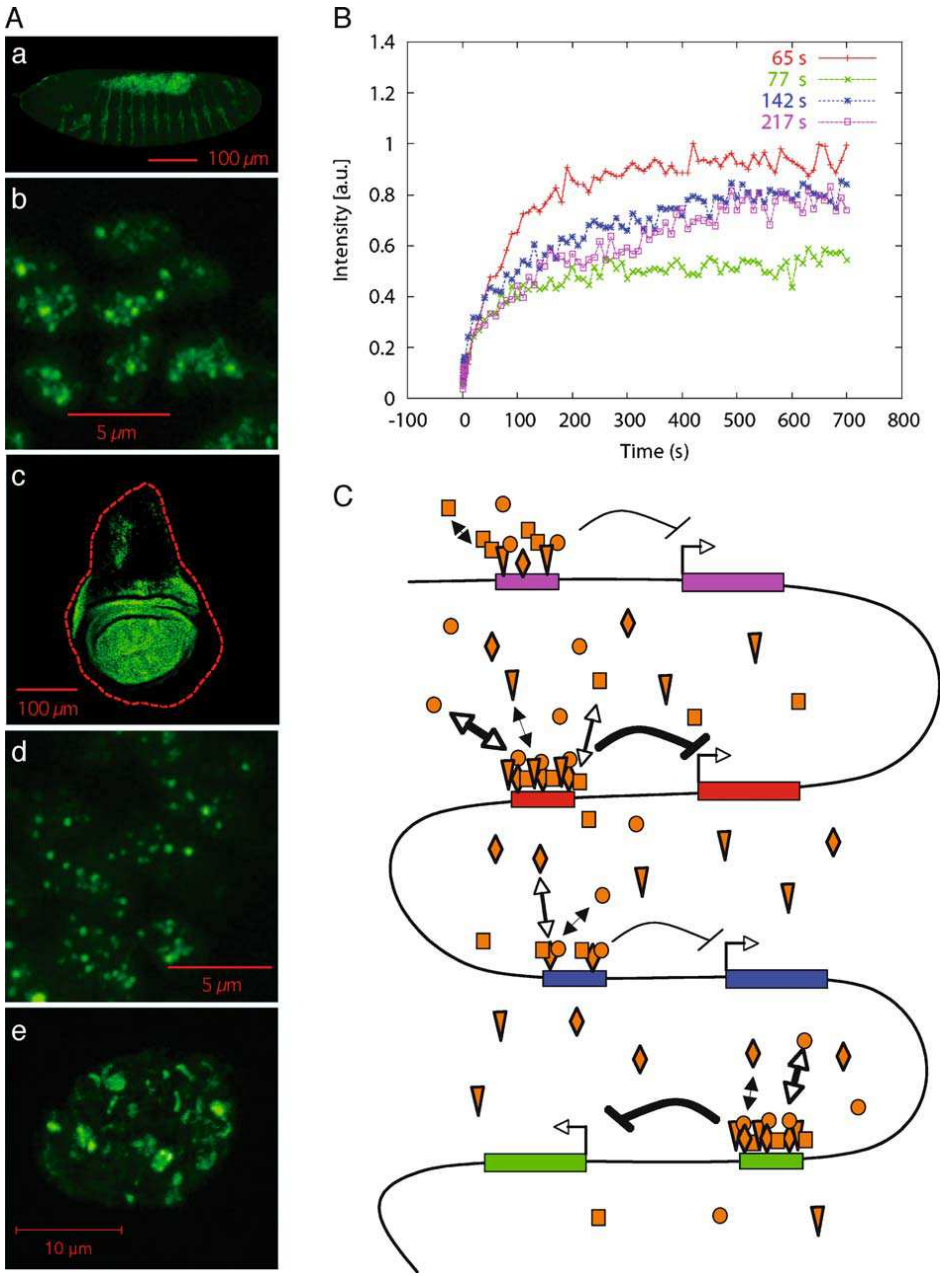


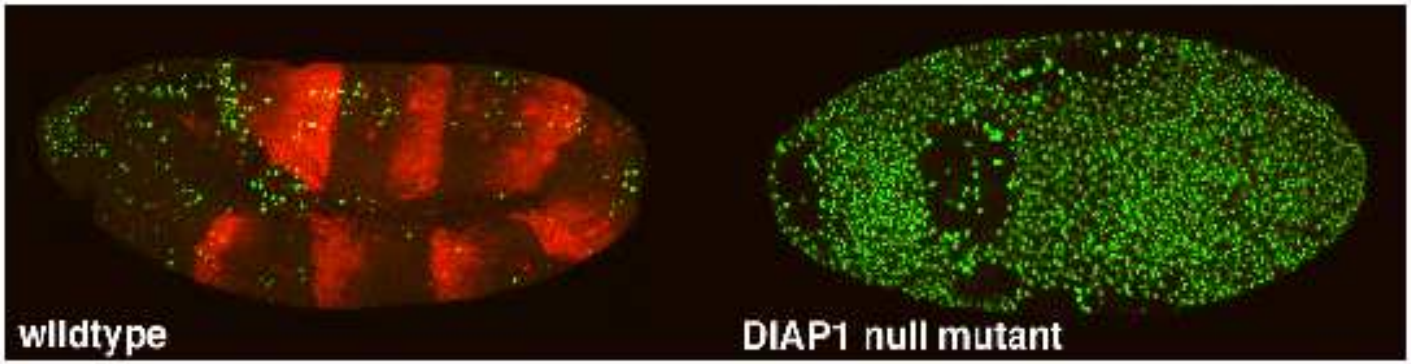
Fig. 4. Schematic model showing how heterochromatin of the Xi could transition between metaphase and interphase to be organized into the two nonoverlapping heterochromatin territories and to explain how XIST RNA could rapidly spread in cis outward from the X inactivation center (XIC) along only part of the Xi. See main text for details.


A
B
C
D
E
F
100 μm

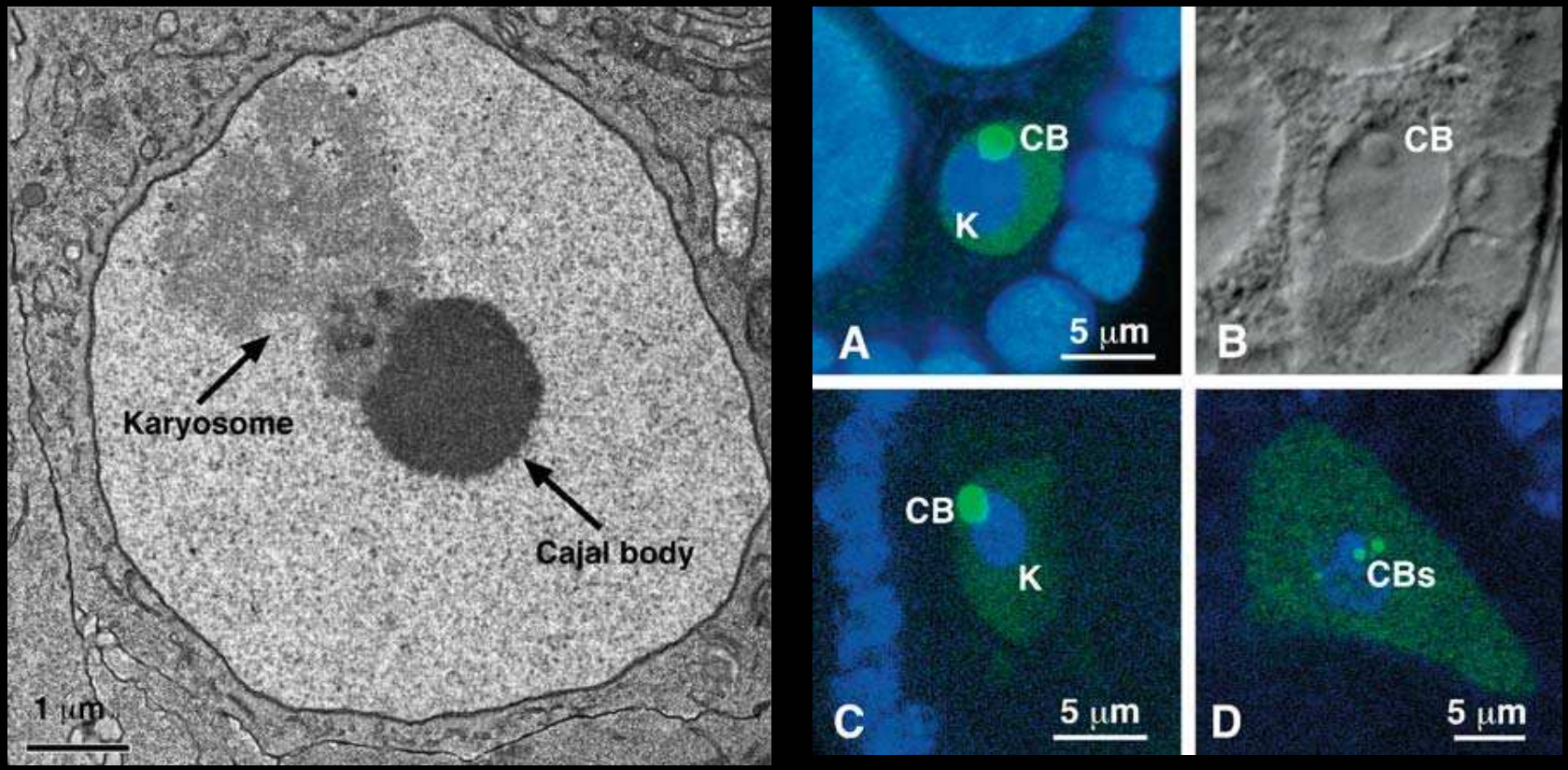

http://nikoninstruments.mediaroom.com - IOD_Szul-10072-1.jpg (JPEG ob...
Hotovo

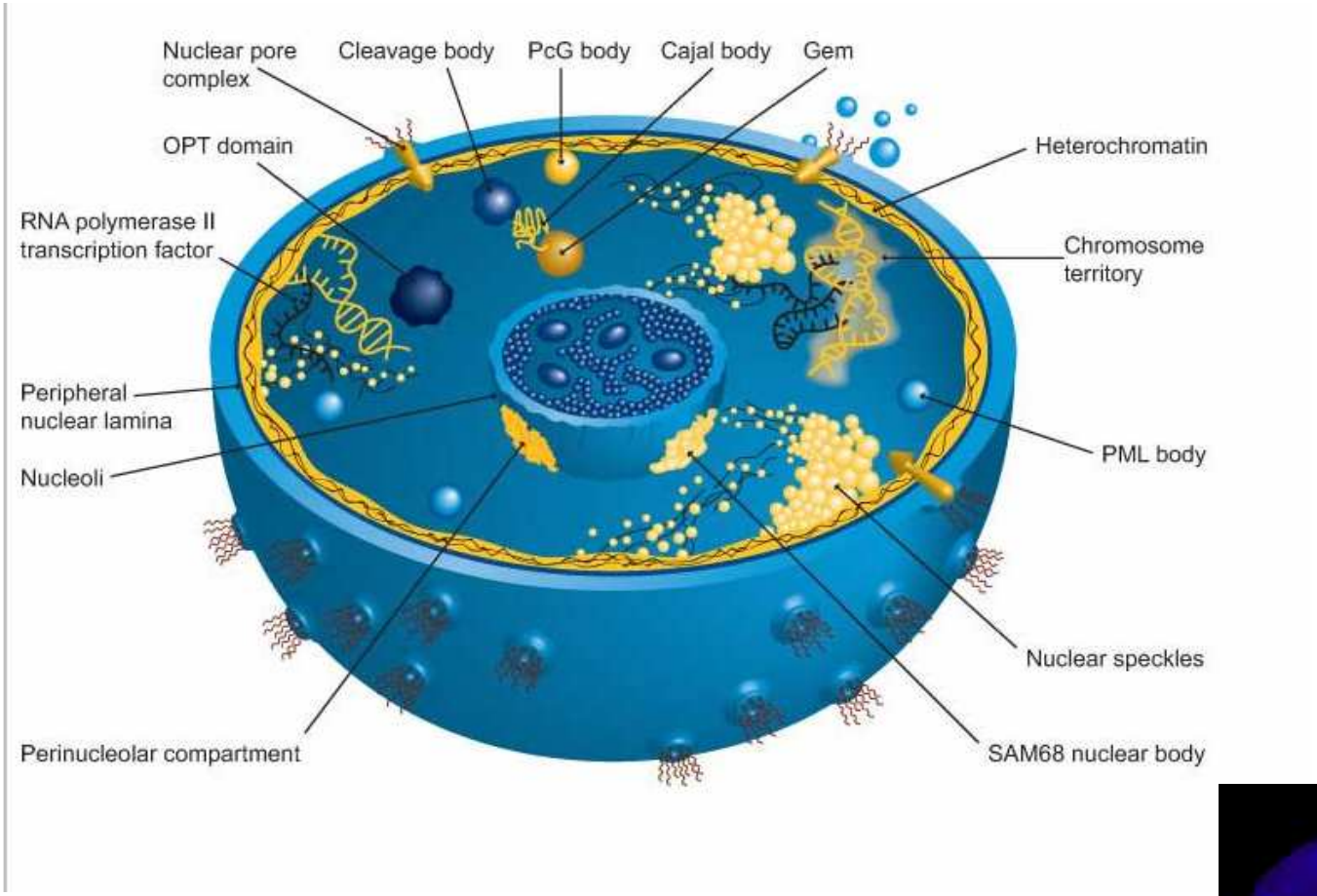

CG17342
PICOT
DOM
STWL
CATHEPSIN K
FS(2)KET

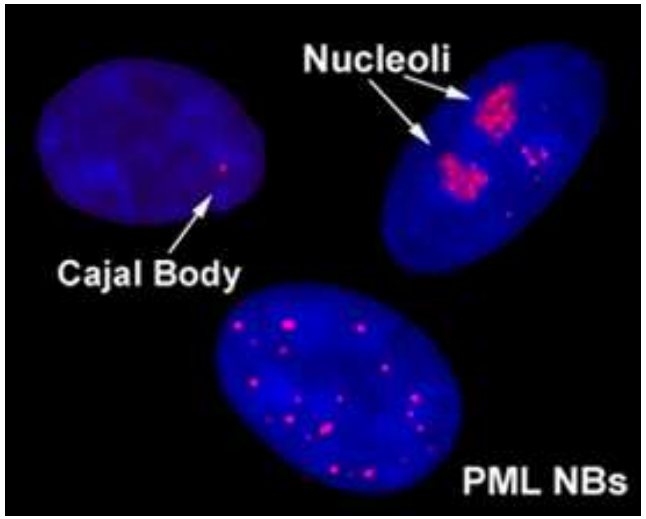

A
a
100 μm
b
5 μm
c
100 μm
d
5 μm
e
10 μm
B
65 s
77 s
142 s
217 s
Intensity [a.u.]
Time (s)
C

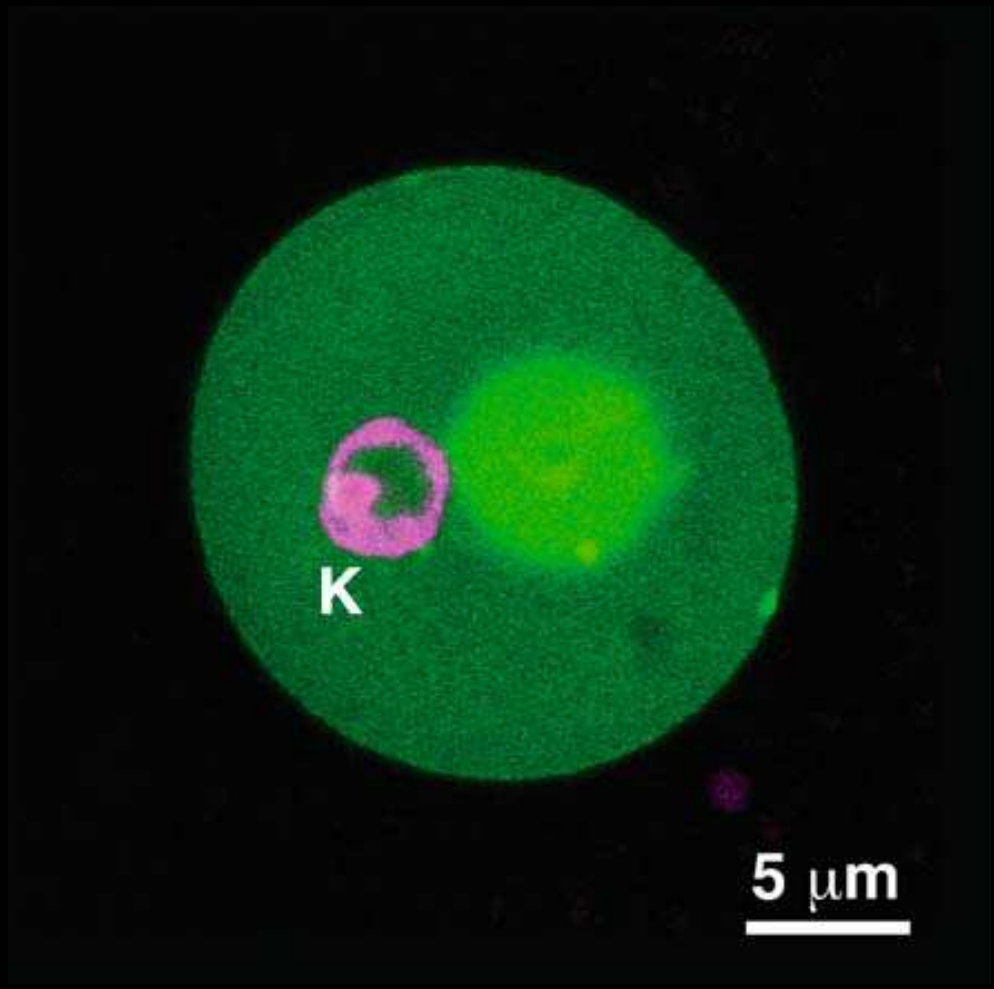


TUNEL staining (green) marks cells that have undergone apoptosis. In wildtype embryos only little apoptosis occurs at this particular developmental stage. In DIAP1 null mutant embryos of the same developmental age, all cells are TUNEL positive, indicating that DIAP1 is essential for cell survival and protects cells in the normal embryos from apoptotic cell death.


Karyosome
Cajal body
1 μm
CB
K
5 μm
A
CB
B
CB
K
5 μm
C
CBs
5 μm
D


Nuclear pore complex
Cleavage body
PcG body
Cajal body
Gem
OPT domain
Heterochromatin
RNA polymerase II transcription factor
Chromosome territory
Peripheral nuclear lamina
PML body
Nucleoli
Nuclear speckles
Perinucleolar compartment
SAM68 nuclear body


Nucleoli
Cajal Body
PML NBs



Origin of figures: Chromosome Research, Special Issue – Drosophila – 100 years after Morgan