

Systems Analysis Reveals Novel Activities of Male Germ Cells

Presented in the Embryo Physics Course

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By

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SYSTEMS ANALYSIS REVEALS NOVEL ACTIVITIES OF MALE GERM CELLS

The Krawetz Laboratory of Reproductive Systems Biology
Molecular – Computational – Translational

A CHILD IS BORN

150 million sperm released

20,000 lashings 2 h journey

Survival time ~48 h

1 cell



birth



10^{12} cells

~27,000 genes → 100,000 coding transcripts
To specify 210 different cell types

COMPACTING THE GENOME

Each cell contains 2 meters of DNA packaged in a
3 -20 μm diameter space

Sperm cell DNA is 13 x more compact

How is compaction determined?

How does compaction dictate phenotype?

ORGANIZING THE SPERM GENOME



H1

146 bp DNA supercoiled
~1.75 times around a histone
octamer core stabilized by H1



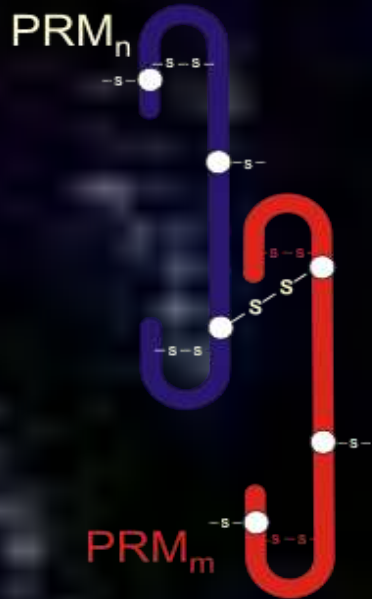
solenoid



MAR MAR MAR

nuclear matrix

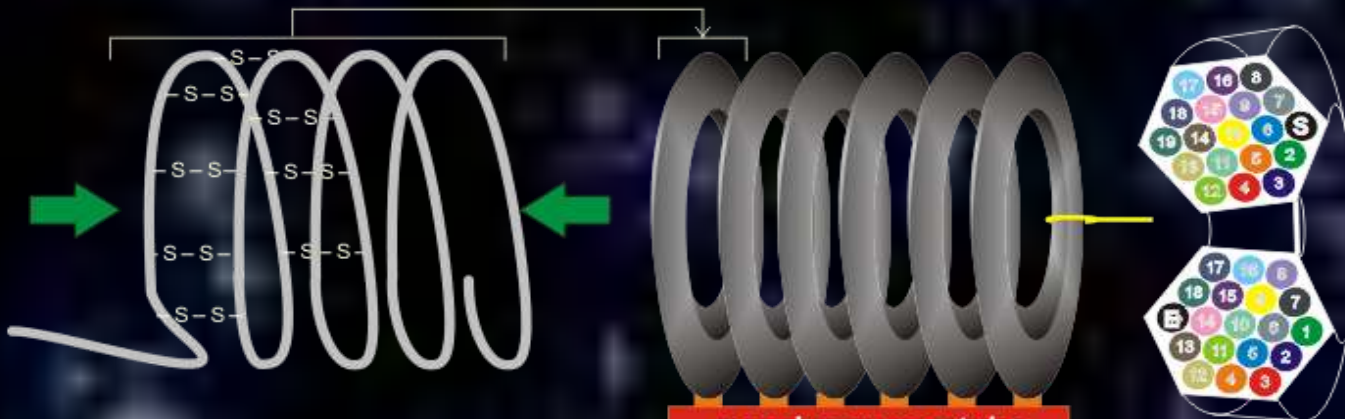
somatic DNA loop
100-200 kb



protamine dimer
stabilized by
disulfide bonds



1 PRM-dimer associates with 2 turns (~22 bp) DNA



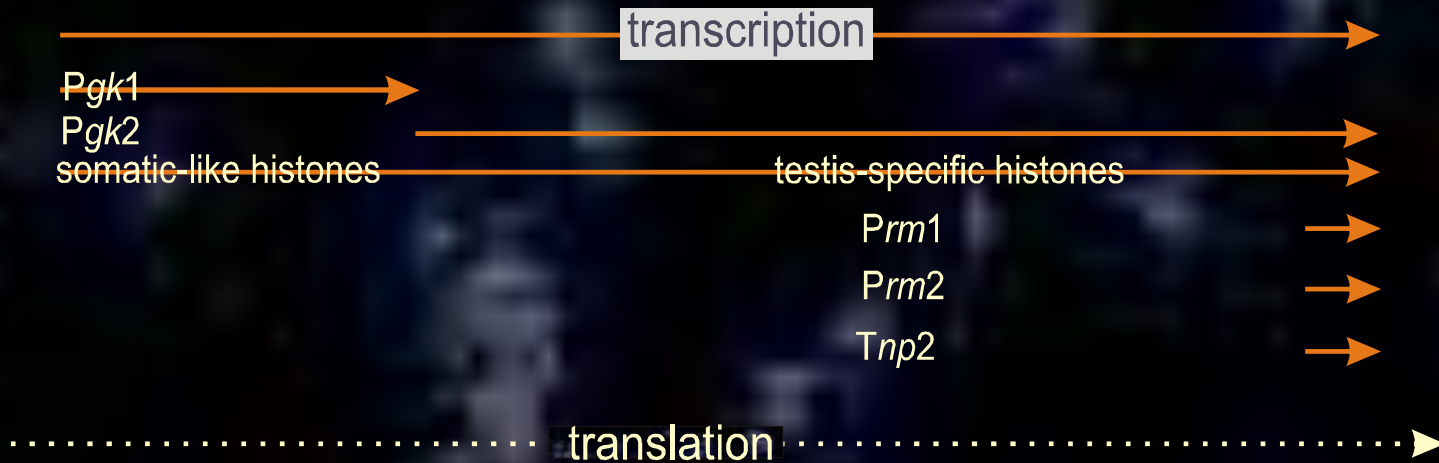
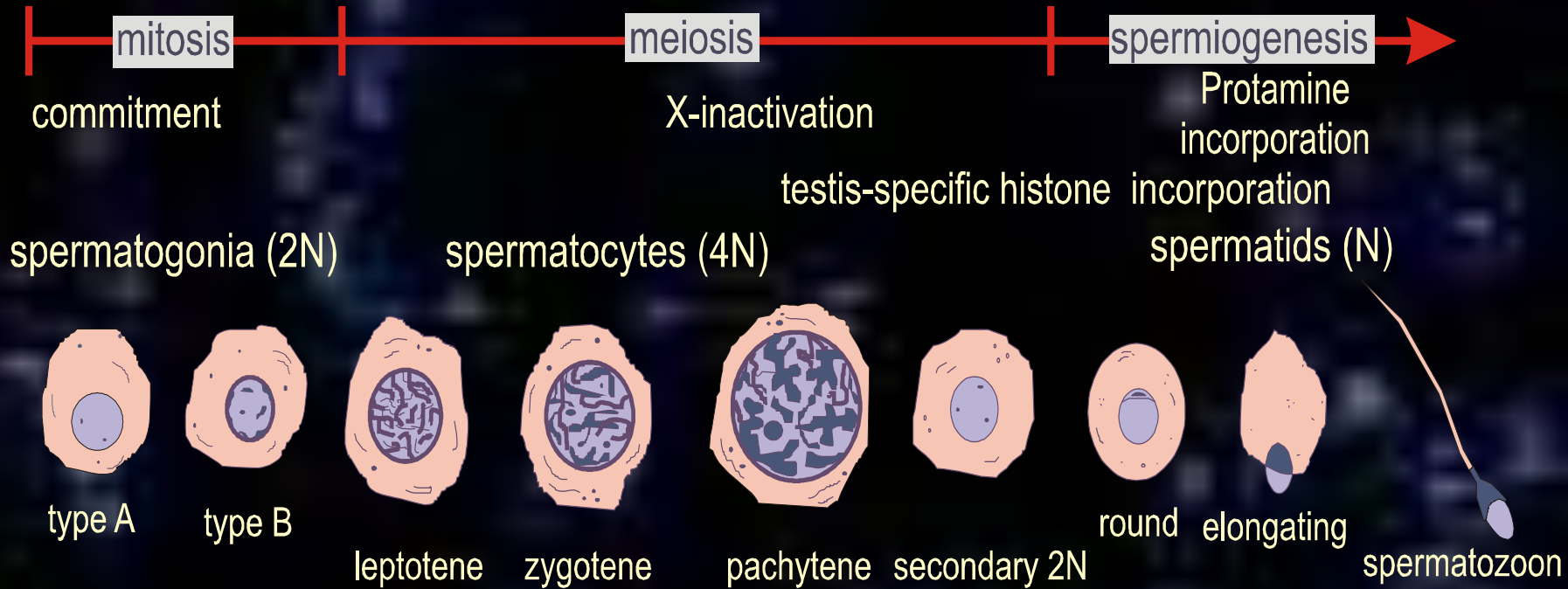
compaction of DNA loops
into toroid structure stabilized
by disulfide bonds

nuclear matrix

sperm DNA toroid
loops 20-50 kb

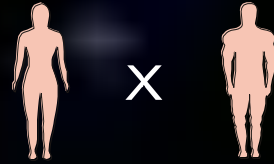


SPERMATOGENESIS



CHROMOSOME 16

HUMAN GENOME

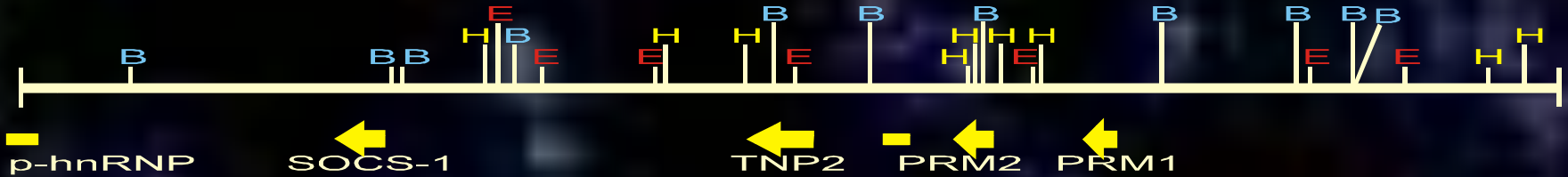


3,000,000 kb



95,000 kb

COSMID CLONE hP3.1
PRM1-PRM2-TNP2

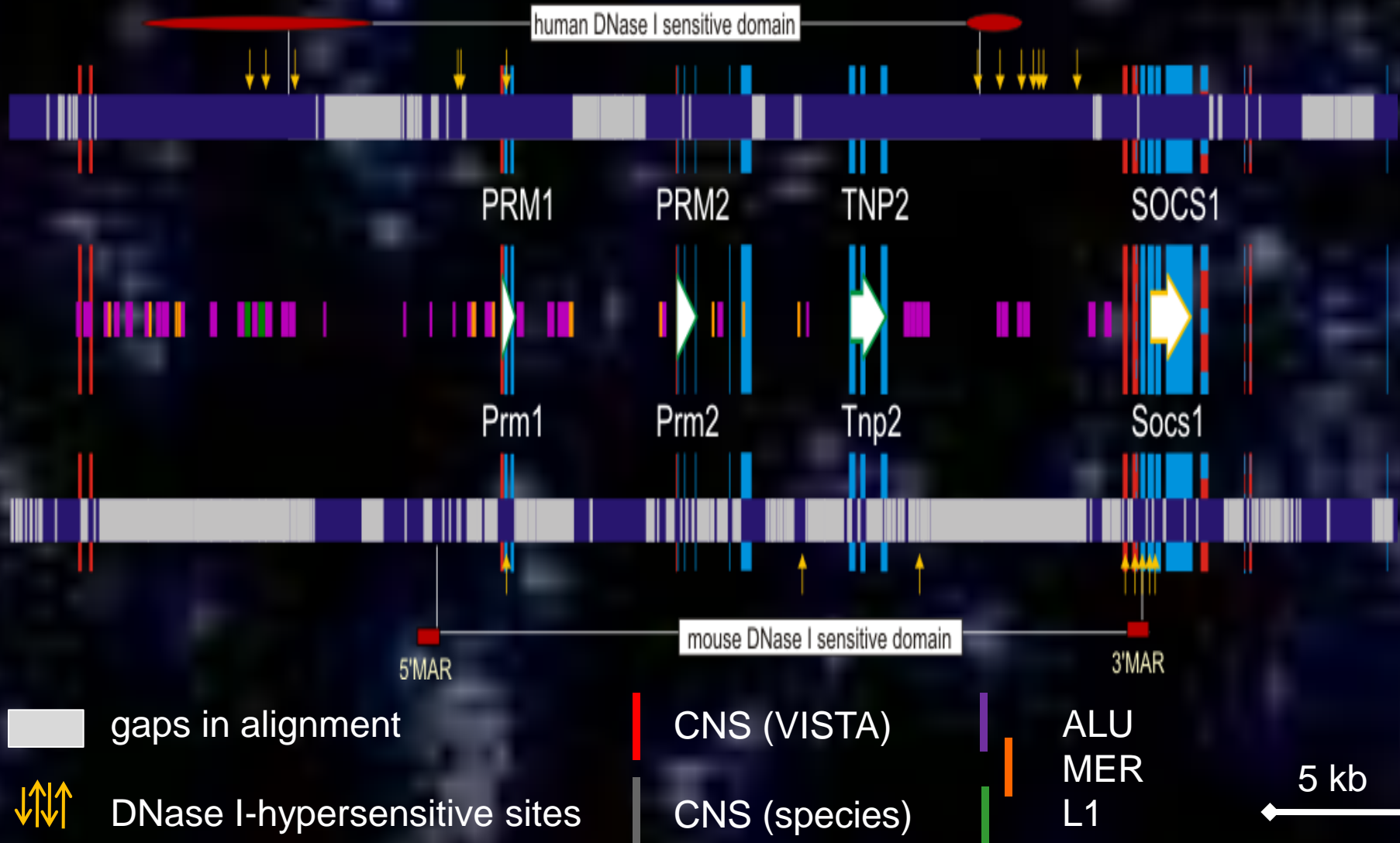


B = BamH I
E = EcoR I
H = Hind III
H = 1 kb

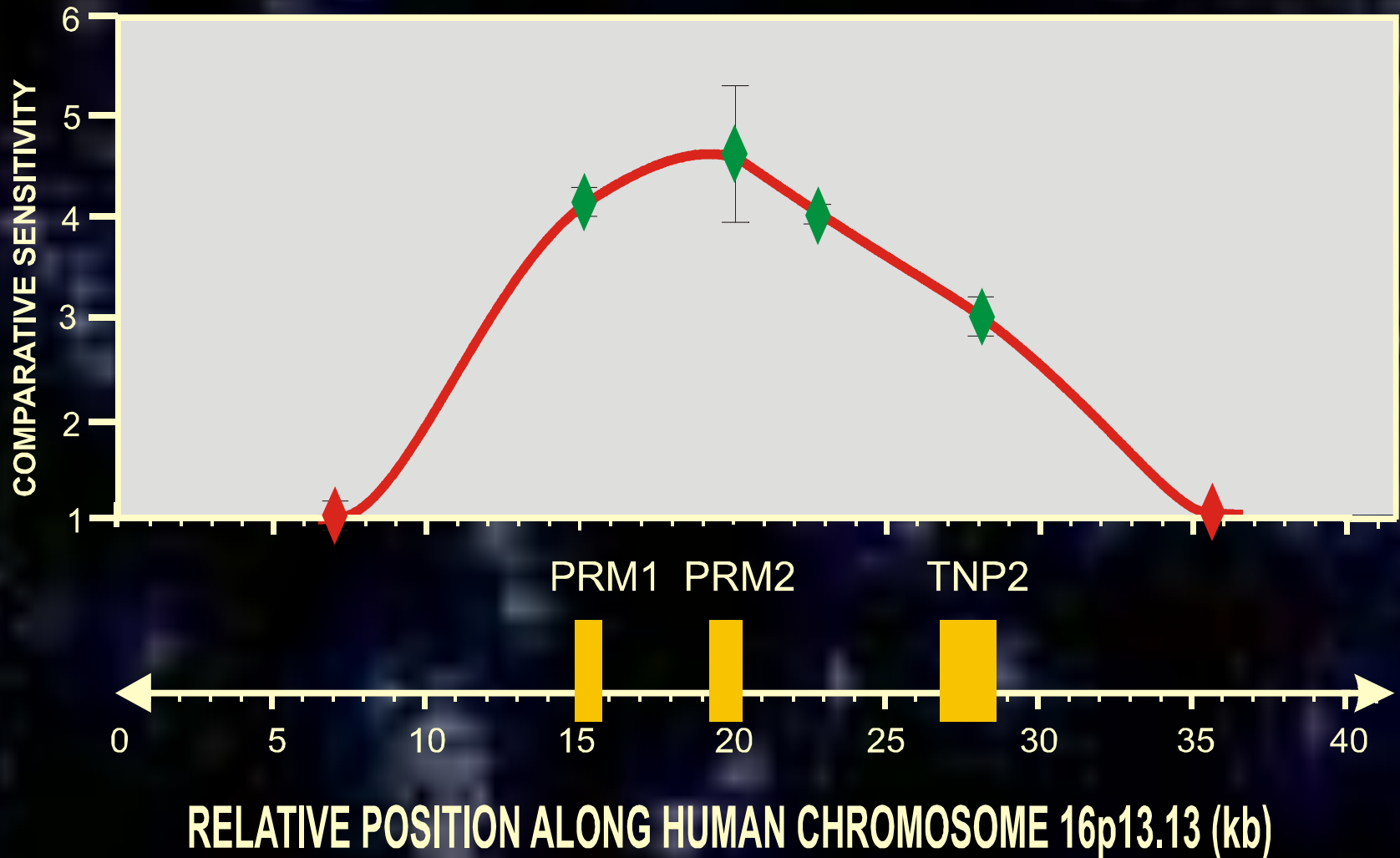
MER MLT MIR Y S J L1 HRES

Repetitive elements

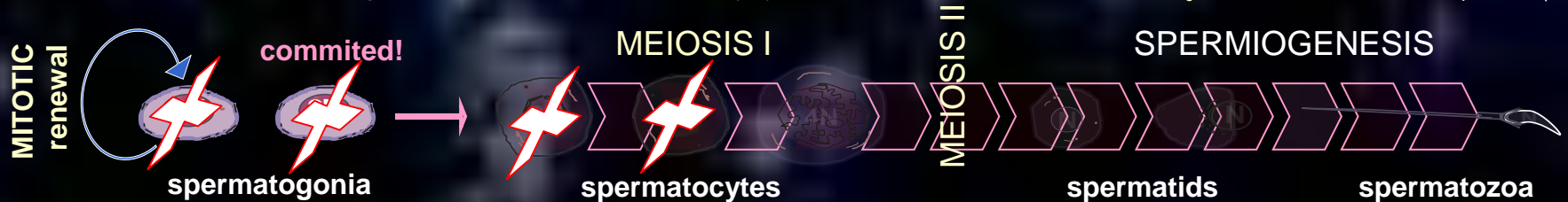
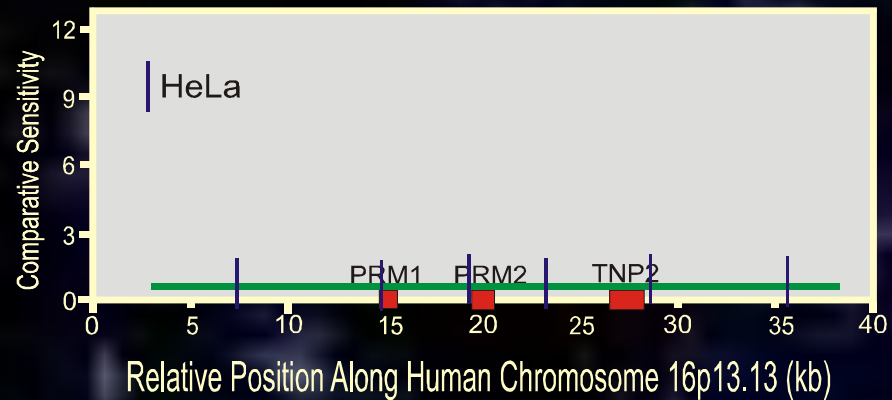
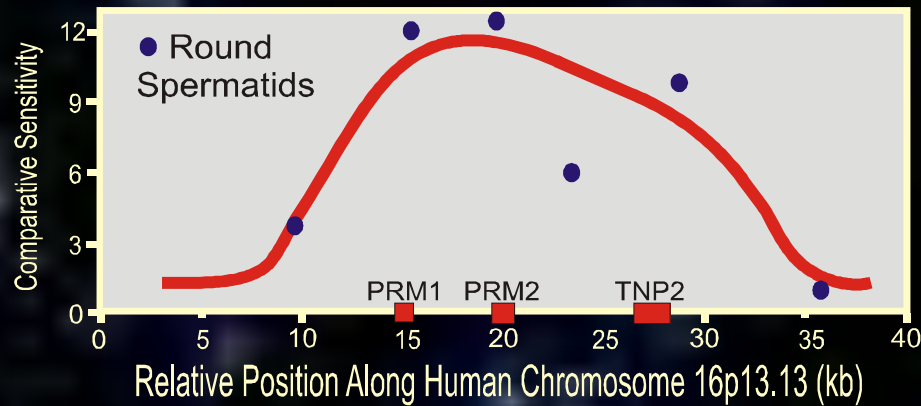
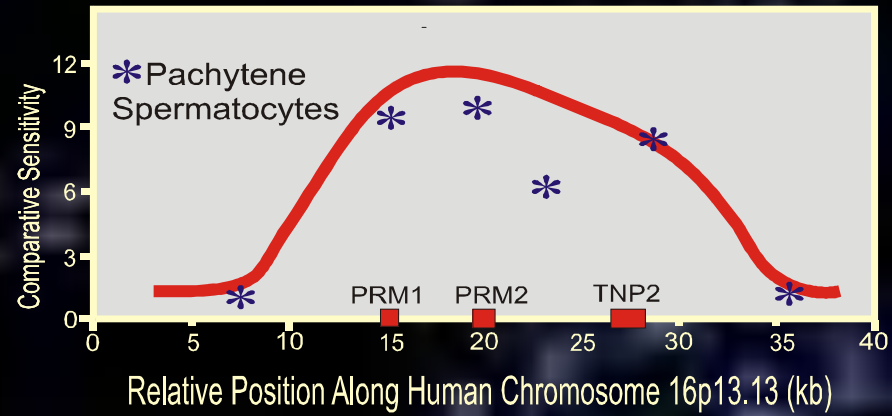
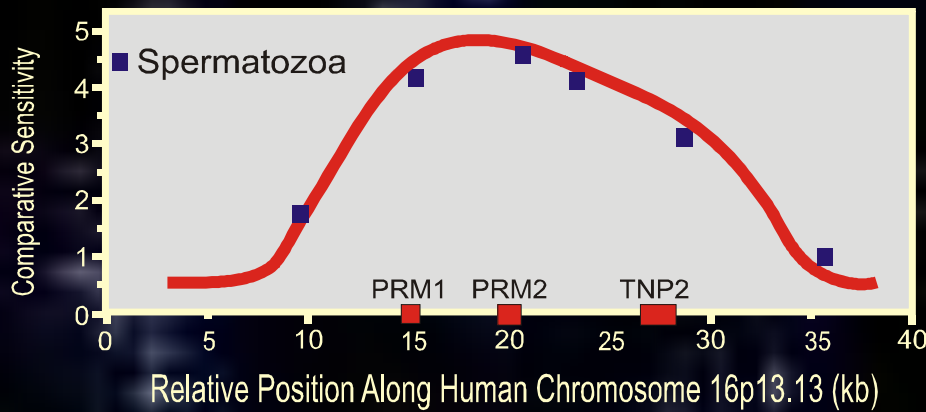
SYNTENY OF THE HUMAN AND MOUSE PROTAMINE DOMAINS



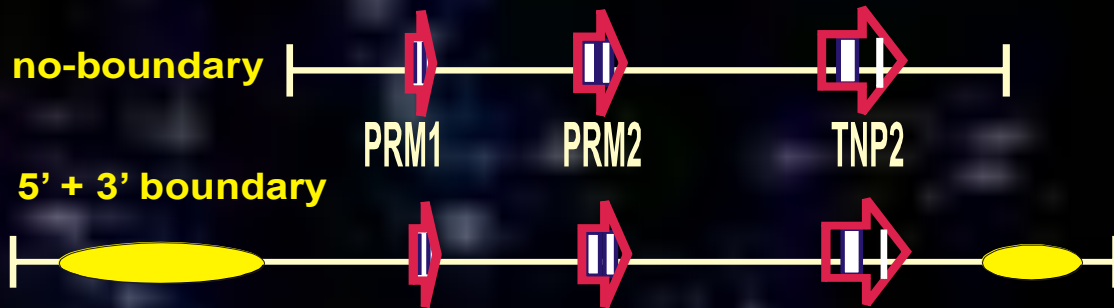
THE HUMAN PRM1- PRM2 - TNP2 MULTIGENIC DOMAIN



THE HUMAN PRM1 - PRM2 - TNP2 DOMAIN IS A DISCRETE STRUCTURAL UNIT DURING DIFFERENTIATION

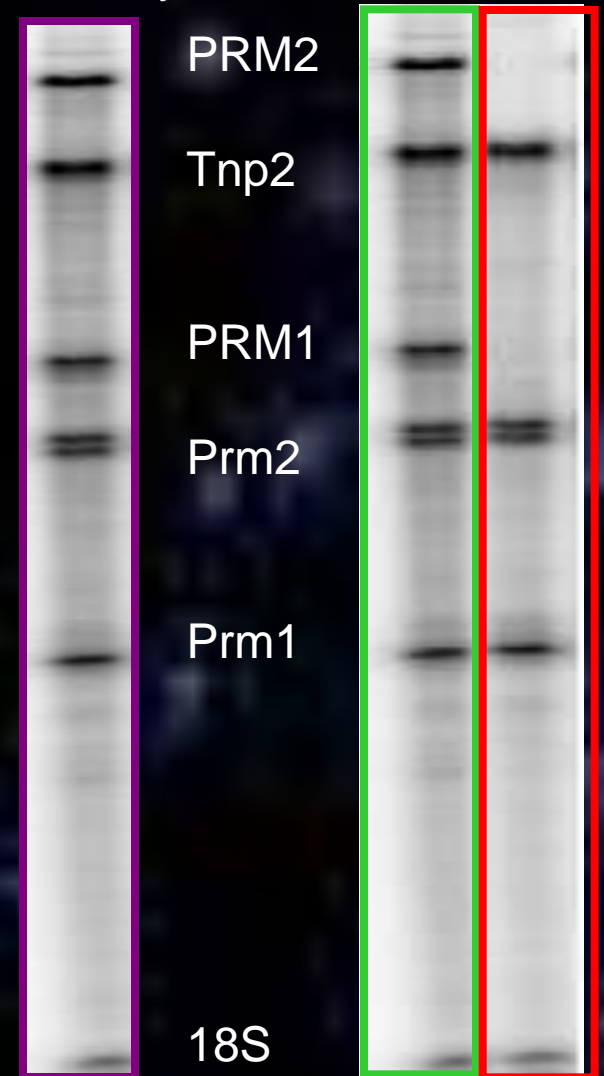


BOUNDARY ELEMENTS & POSITION EFFECTS



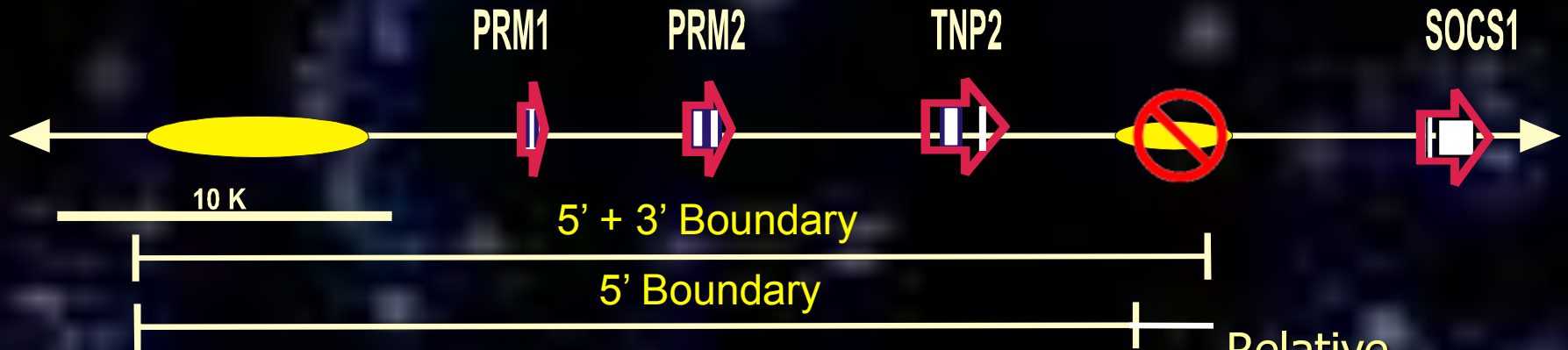
5' + 3'
Boundary

No Boundary



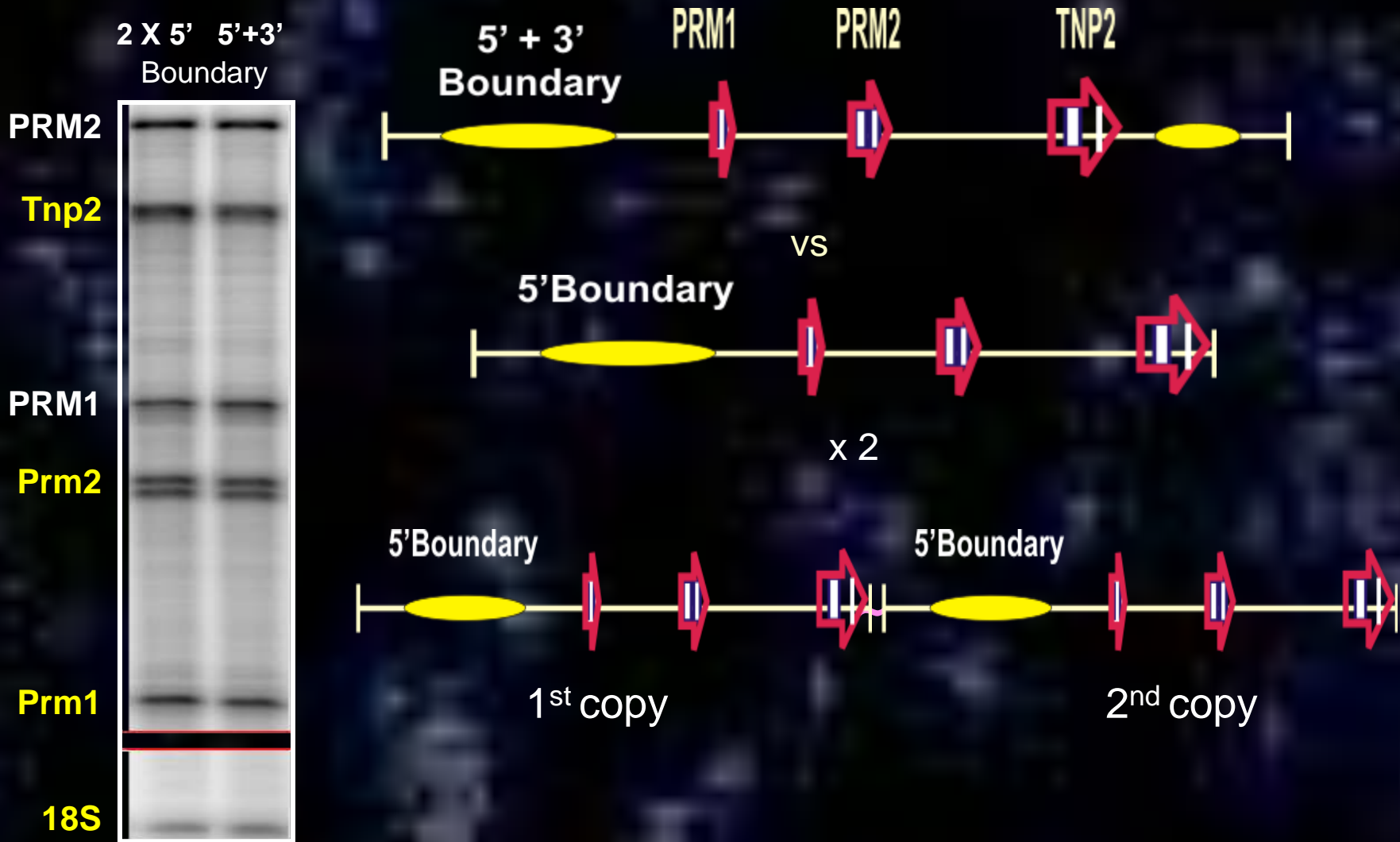
	No Boundary		5' + 3' Boundary
	F _{0,147}	F _{0,155}	
PRM1:Prm1	20.13%	---	22.65%
PRM2:Prm2	58.52%	---	65.02%

3' BOUNDARY FUNCTION & EXPRESSION



Primer	Sample	Initial Template	Relative Expression
PRM1	T	1.99E-06	100%
	Δ	2.09E-07	6.3%
PRM2	T	4.33E-05	100%
	Δ	3.1E-07	1.7%
TNP2	T	1.94E-09	100%
	Δ	0.00	0%

RECAPITULATING THE NATIVE DOMAIN



GLOBAL DISTRIBUTION OF HISTONES AND PROTAMINES IN HUMAN SPERM

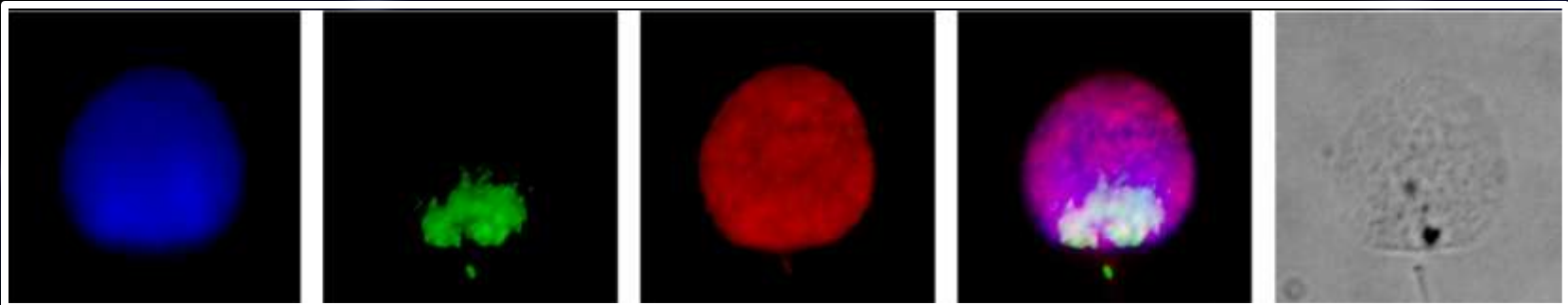
DAPI

HISTONE

PRM1

MERGE

DIC



DAPI

HISTONE

PRM2

MERGE

DIC



ISOLATION OF HISTONE AND PROTAMINE ASSOCIATED GENOMIC SEGMENTS IN MATURE HUMAN SPERMATOZOA



CTAB



0.65 M NaCl



Restriction Digestion & Separate



Protamine

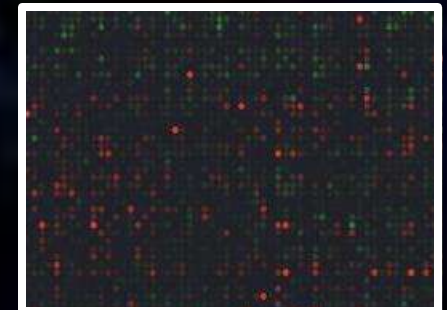
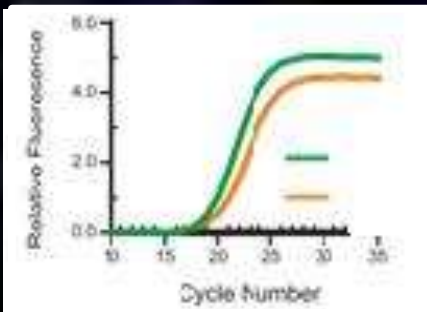


Histone

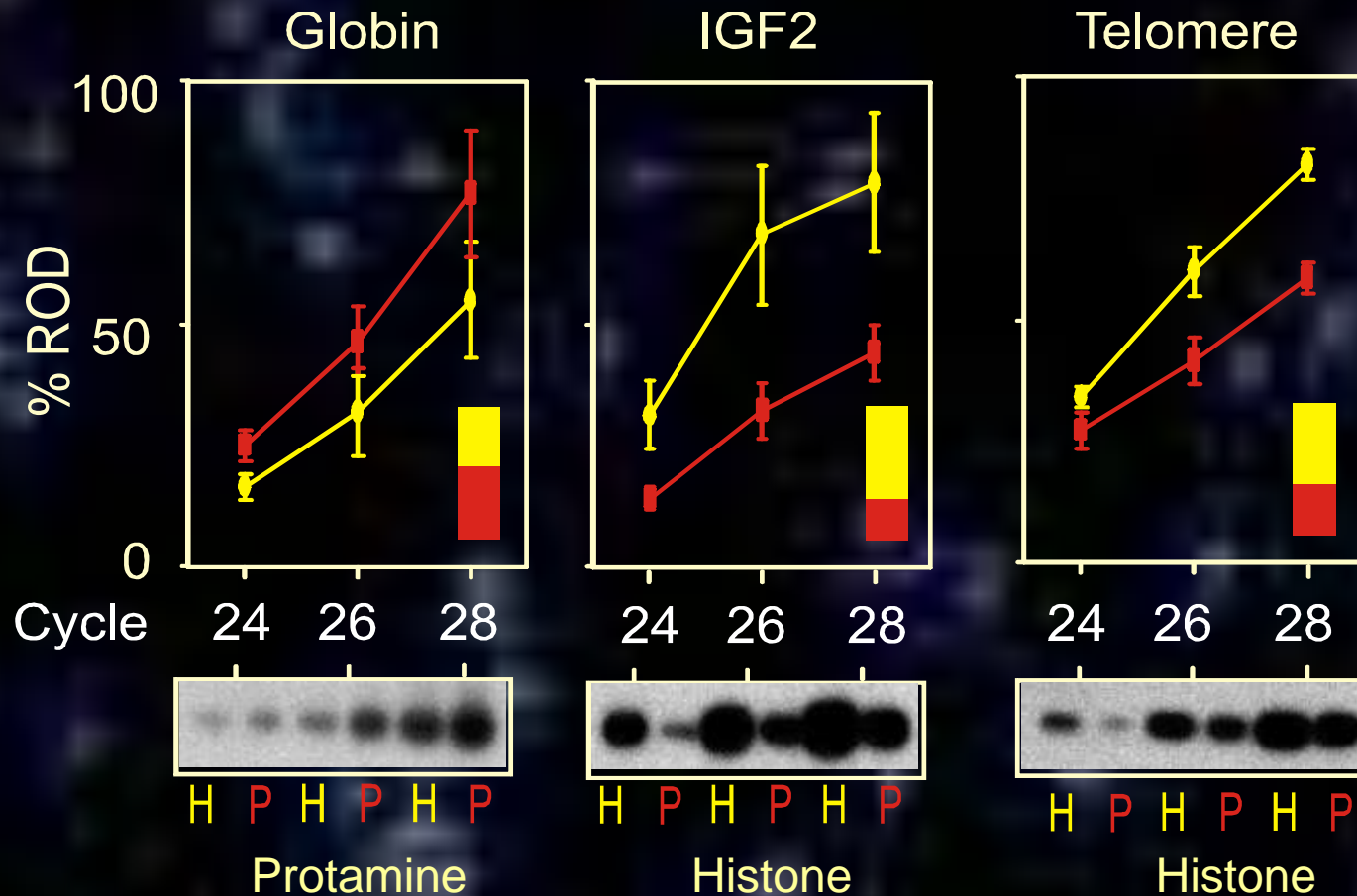
Purify & Analyze

Quantitative PCR

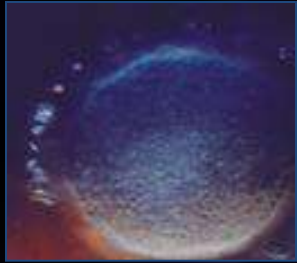
Comparative
Genomic
Hybridization



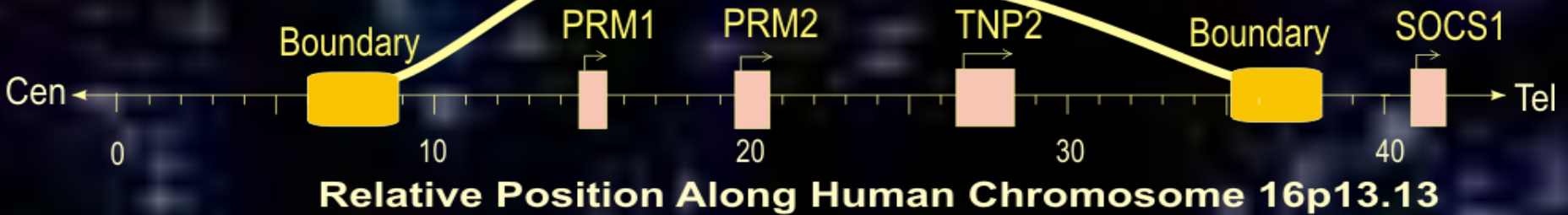
ASSAYING HISTONE AND PROTAMINE GENOMIC ASSOCIATION IN MATURE HUMAN SPERMATOZOA



HISTONES AND PROTAMINES IN HUMAN SPERMATOZOA



DNase I Sensitive Region



Histone Protamine Distribution

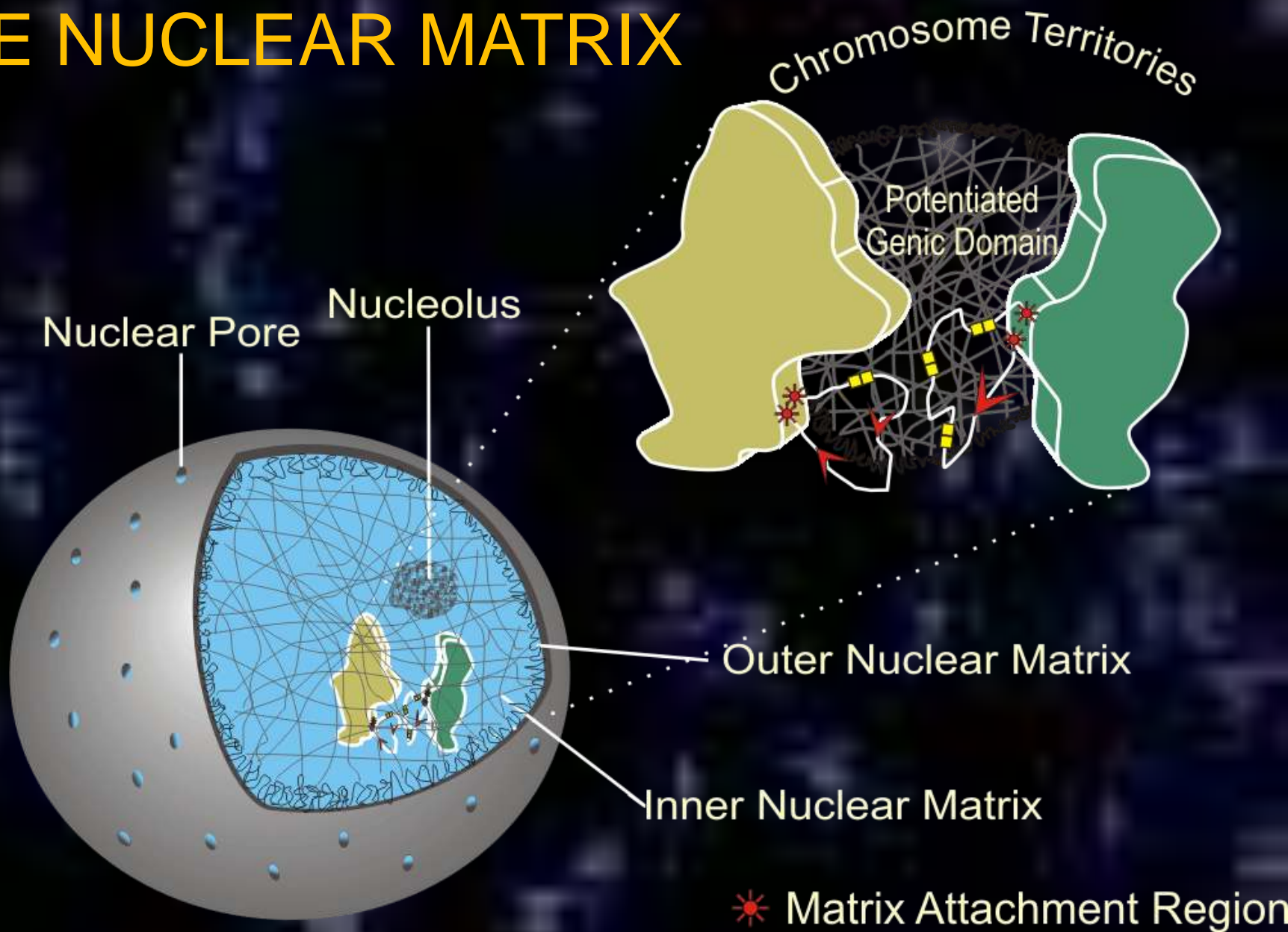
Bam HI / Eco RI



Pvu II

■ Histone ■ Mixture ■ Protamine

THE NUCLEAR MATRIX



PROPERTIES OF 74,000 S/MARS

Compartmentalize Transcription:

- Source of transcription factors
- Stability
- Functioning as boundary elements

Compartmentalize Replication:

- Origins Replication Initiation

Compartmentalize Recombination:

- breakpoint cluster regions
- guide retroviral integration

CHARACTERISTICS OF MAR SITES

Constitutive:

Exhibit cooperative mass binding

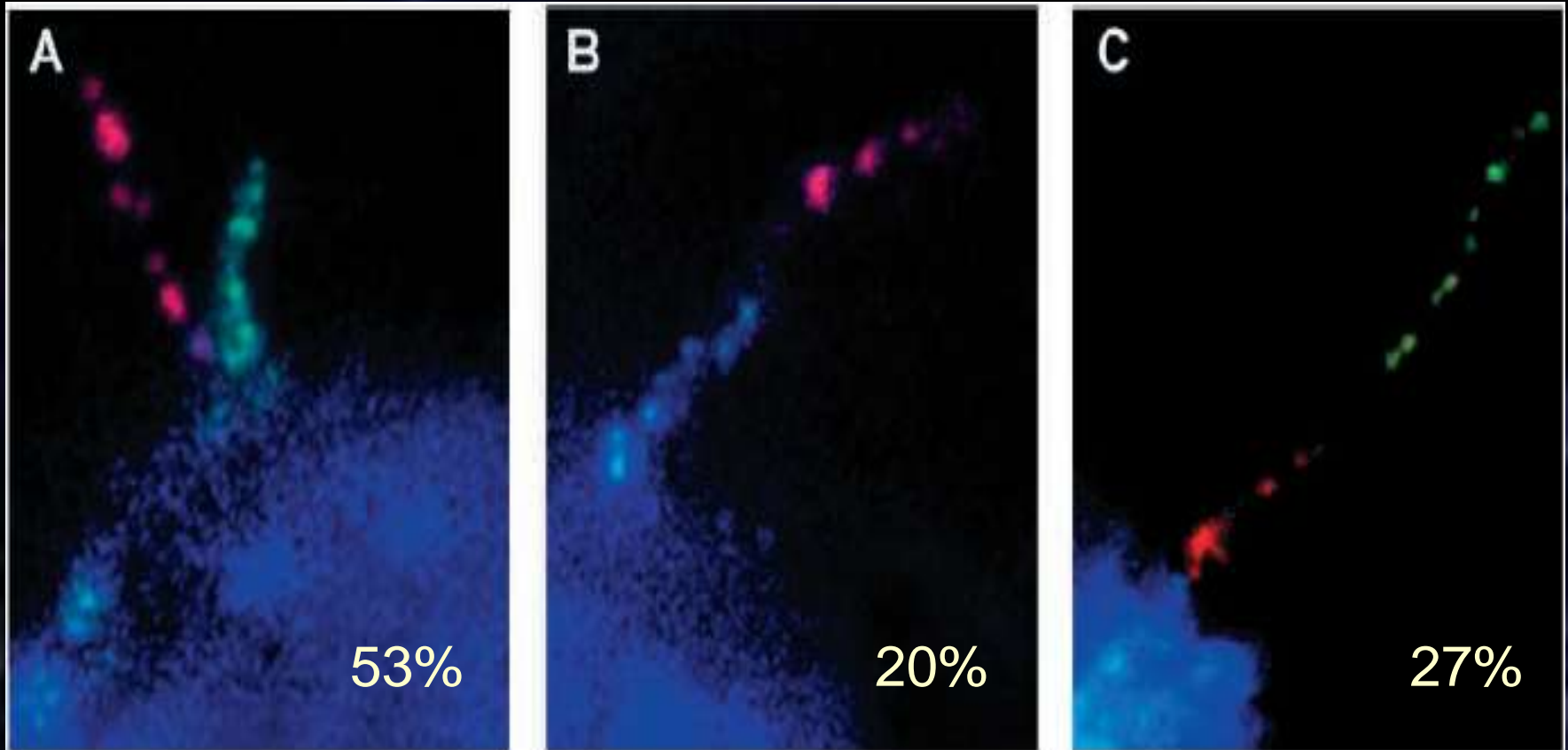
- SAF-A i.e., hnRNP-U
- Lamins
- NuMA/actin (nuclear mitotic apparatus protein)

Facultative:

Exhibit highly-specific binding

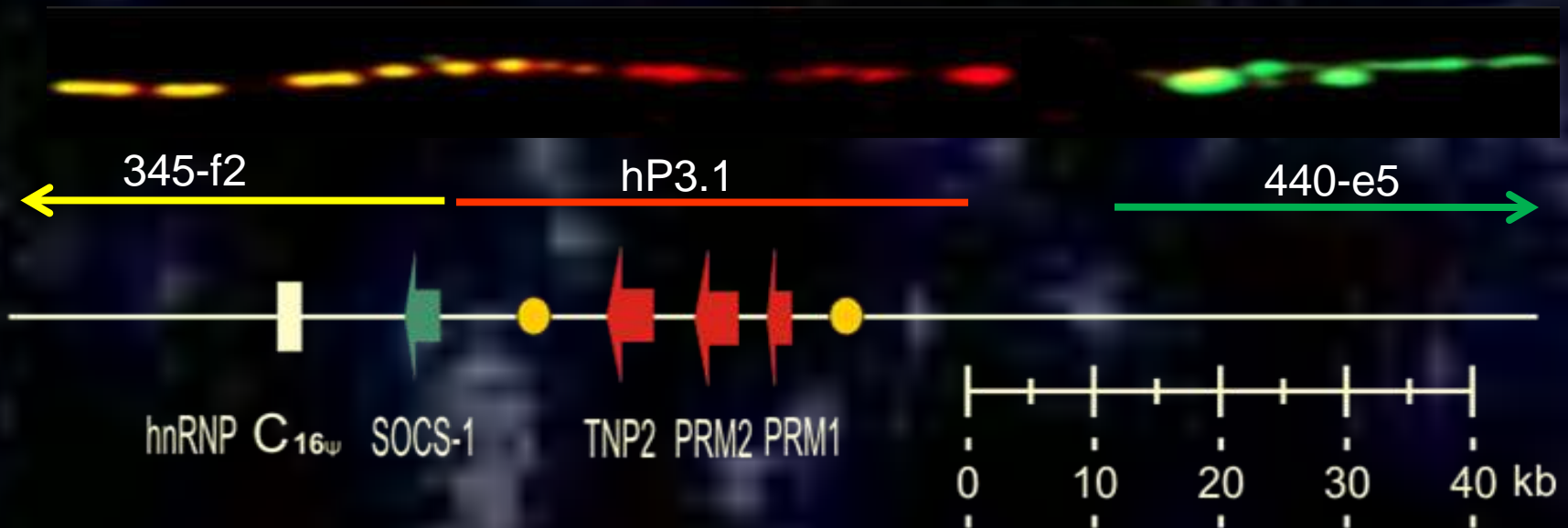
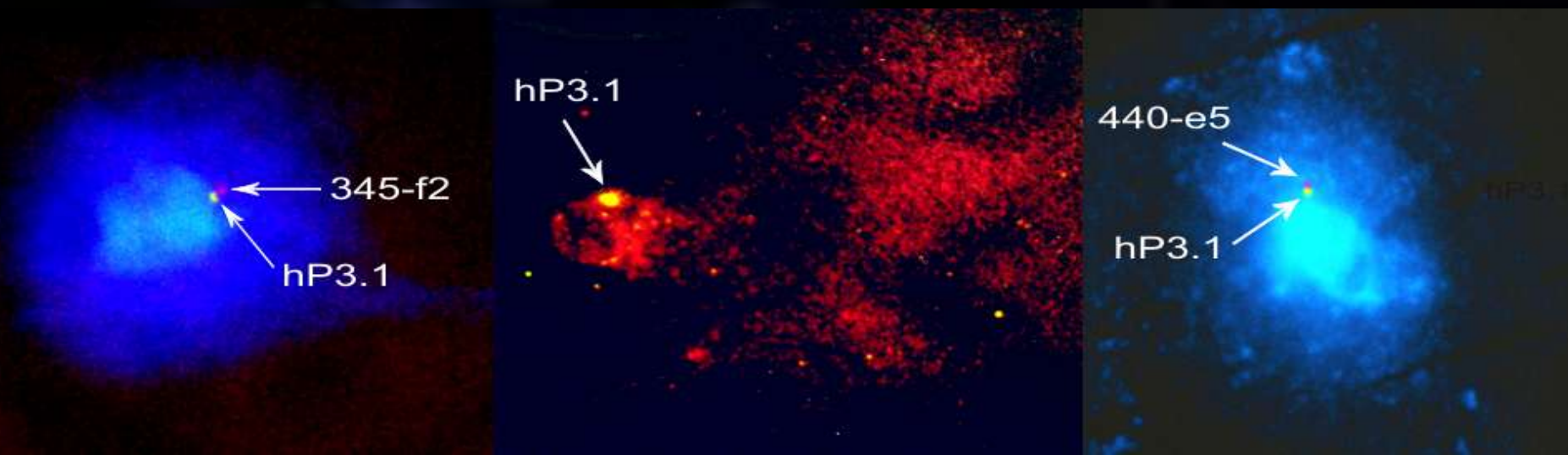
- PARP, SATB1 T-Cells, SATB2 and BRIGHT B-cells
- Topoisomerase

THE NUCLEAR MATRIX

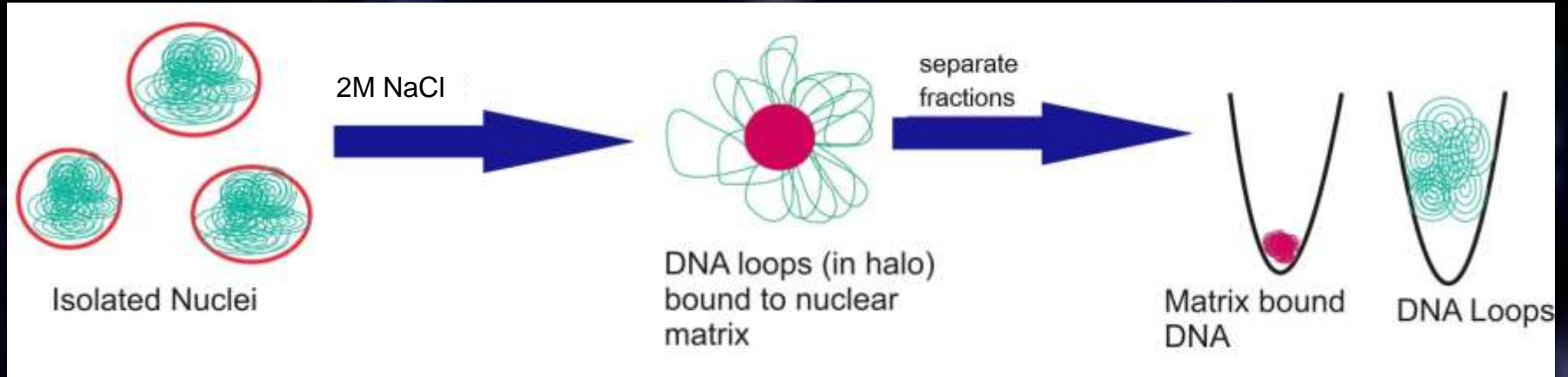


Positioning Function
A threading function: MARs are selectively utilized

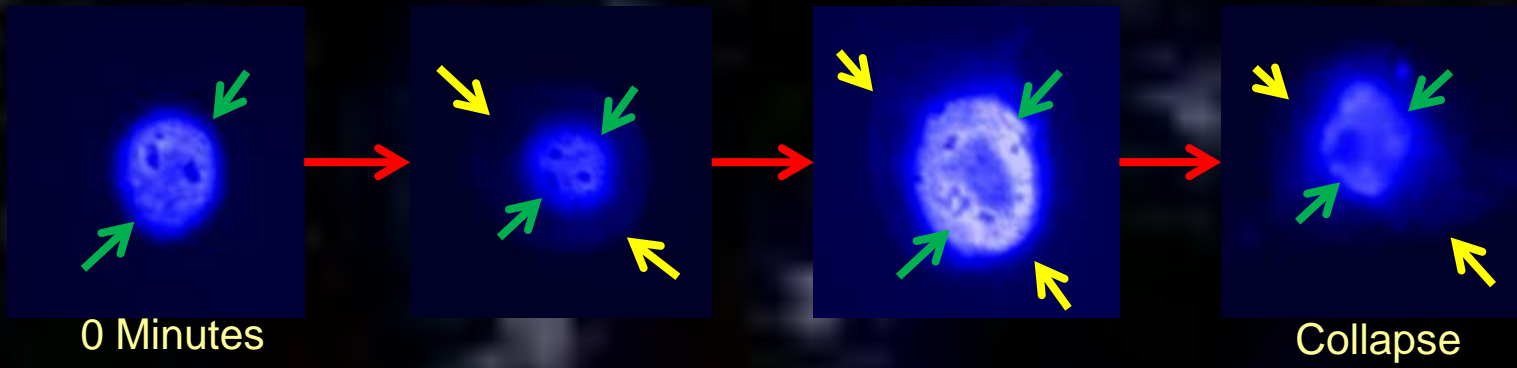
THE PROTAMINE LOCUS IS ASSOCIATED WITH THE NUCLEAR MATRIX



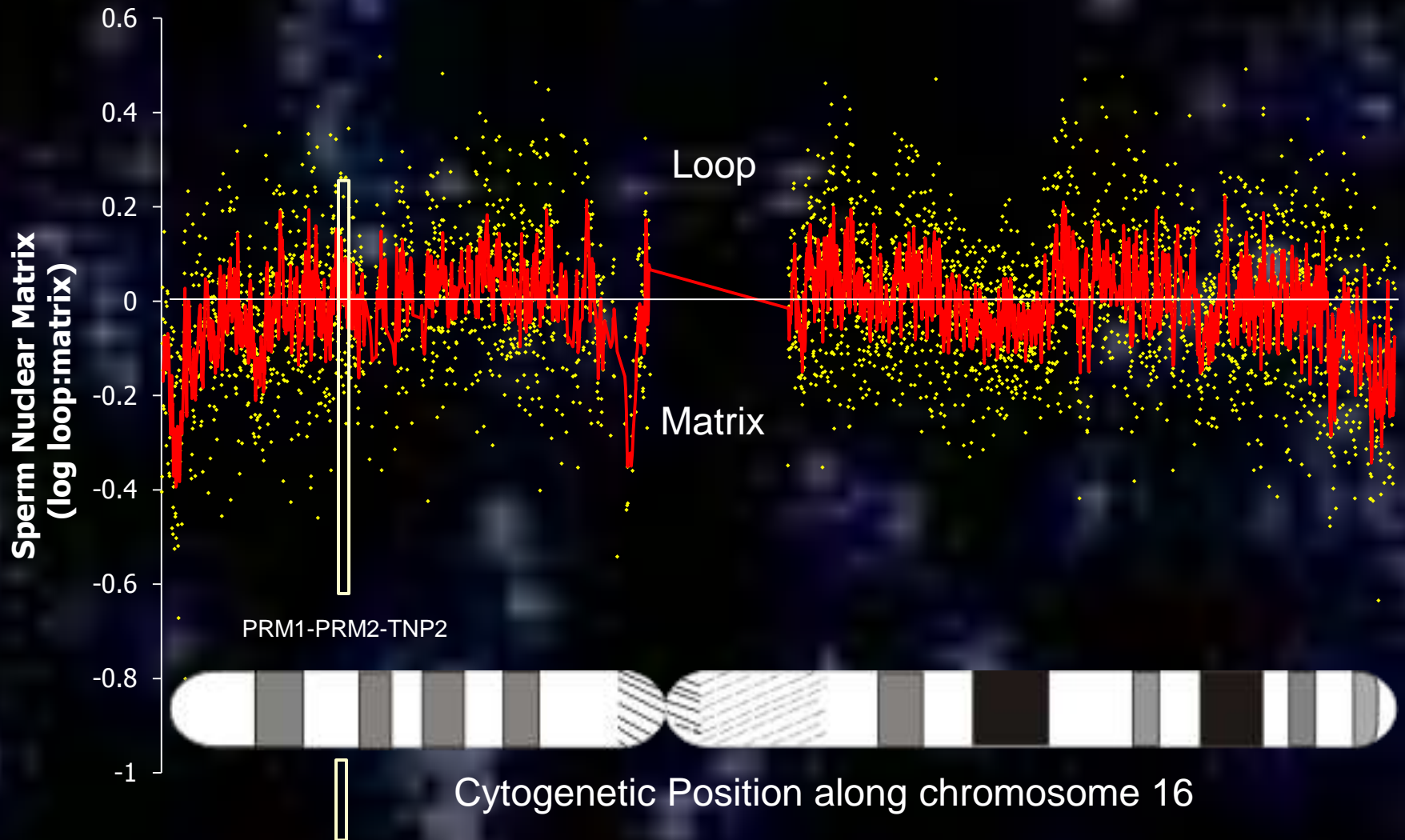
Nuclear Matrix Isolation



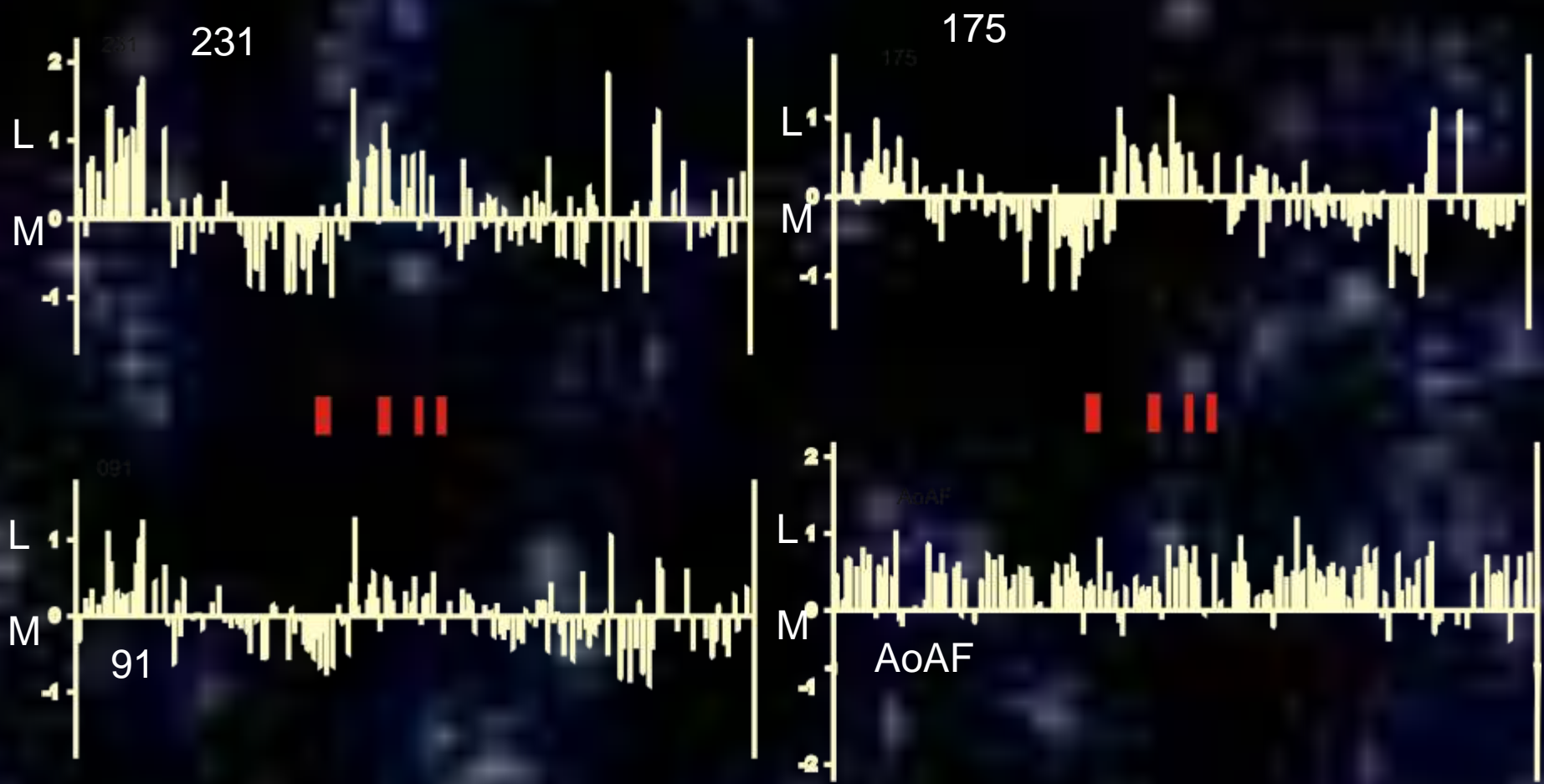
→ Nuclear Matrix
→ Nuclear Halo



THE SPERM NUCLEAR MATRIX OF CHROMOSOME 16



THE HUMAN PROTAMINE LOCUS: aCGH

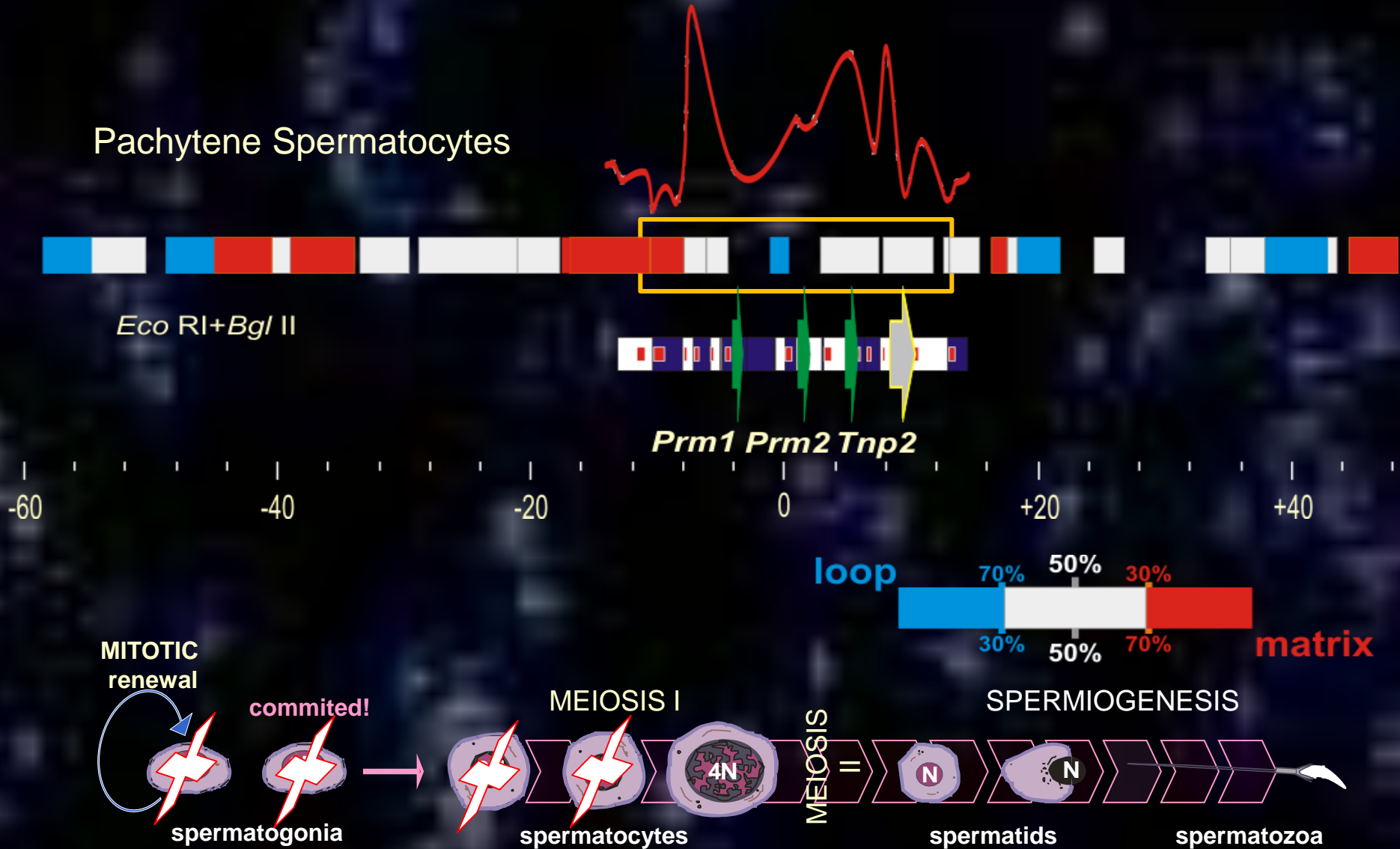


SOCS1 TNP2 PRM2 PRM1

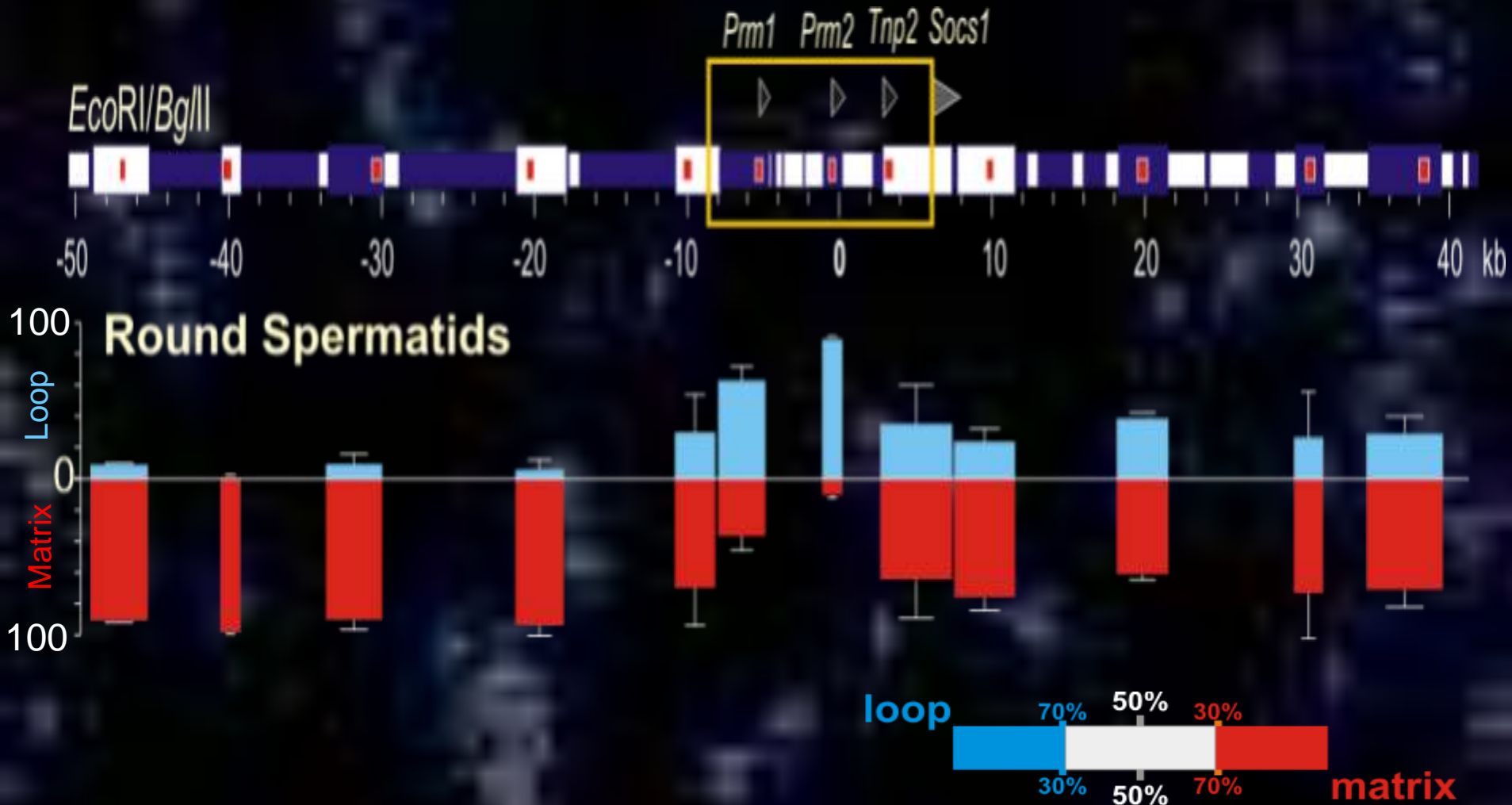


18,750 bp

NUCLEAR MATRIX ATTACHMENT DURING MOUSE SPERMATOGENESIS



RECRUITMENT TO THE NUCLEAR MATRIX DURING MOUSE SPERMATOGENESIS



EPIGENETIC MODIFICATION OF THE DOMAIN DURING SPERMATOGENESIS

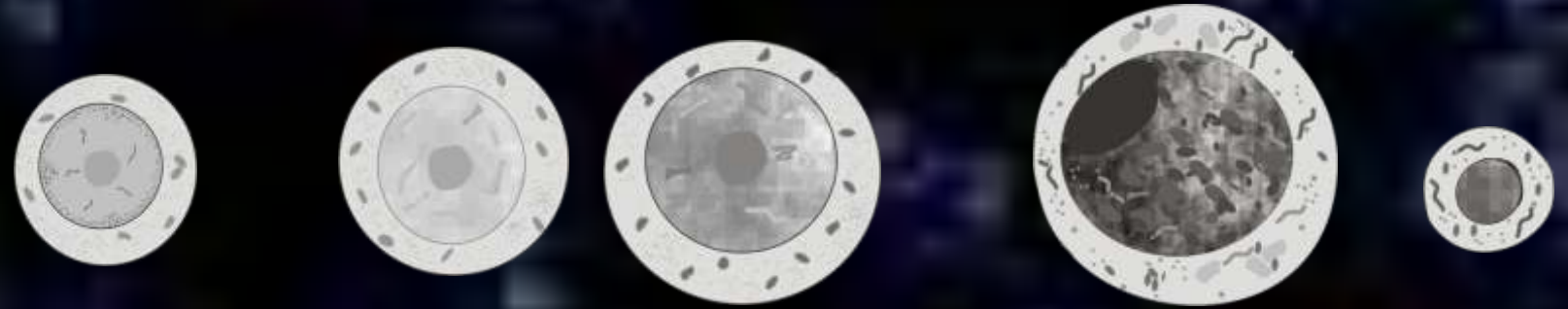
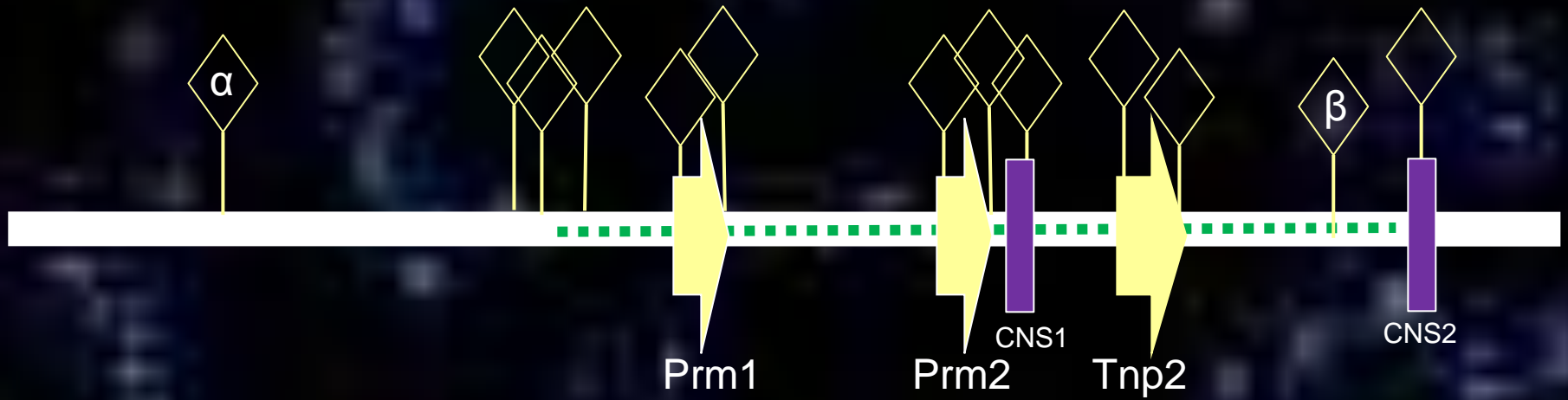
acH3K9/14	<input checked="" type="checkbox"/>	acH3K9/14	<input checked="" type="checkbox"/>		
acH4K16	<input checked="" type="checkbox"/>	acH4K16	<input checked="" type="checkbox"/>		
me2H3K9	<input checked="" type="checkbox"/>	me2H3K9	<input checked="" type="checkbox"/>	Top 2 α β	<input checked="" type="checkbox"/> <input checked="" type="checkbox"/>
HP1	<input checked="" type="checkbox"/>	HP1	<input checked="" type="checkbox"/>		
SATB1	<input checked="" type="checkbox"/>	SATB1	<input checked="" type="checkbox"/>		

AN EPIGENETIC MODIFICATION OF THE DOMAIN DURING SPERMATOGENESIS



preleptotene spermatocyte leptotene/zygotene spermatocyte pachytene spermatocyte spermatid

POTENTIATING THE Prm1 → Prm2 → Tnp2 DOMAIN DURING SPERMATOGENESIS



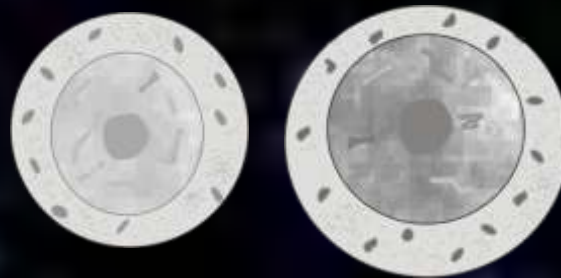
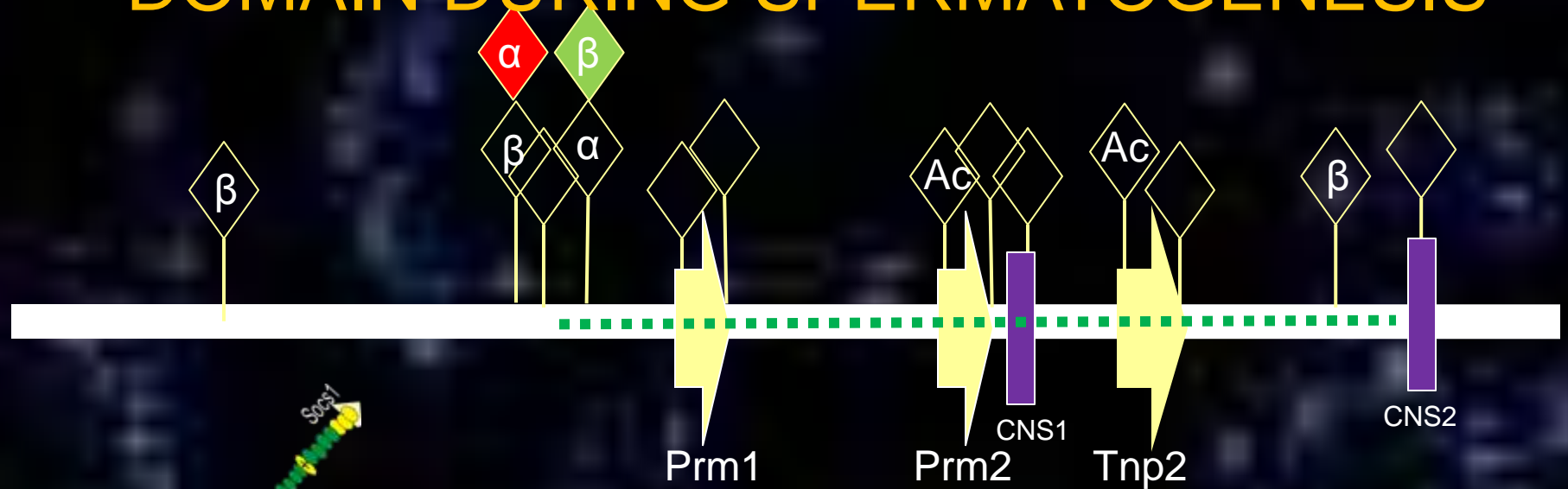
preleptotene spermatocyte leptotene /zygotene spermatocyte pachytene spermatocyte spermatid

→

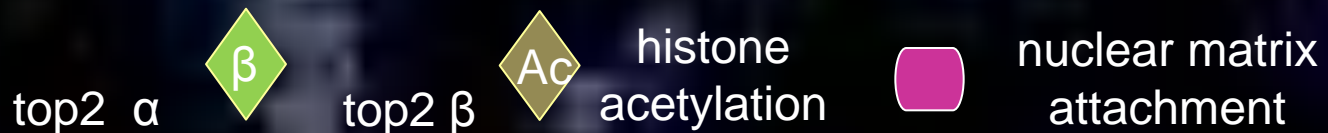
◆ α top2 α ◆ β top2 β ◆ Ac histone acetylation ▭ nuclear matrix attachment

5 kb

POTENTIATING THE Prm1 → Prm2 → Tnp2 DOMAIN DURING SPERMATOGENESIS

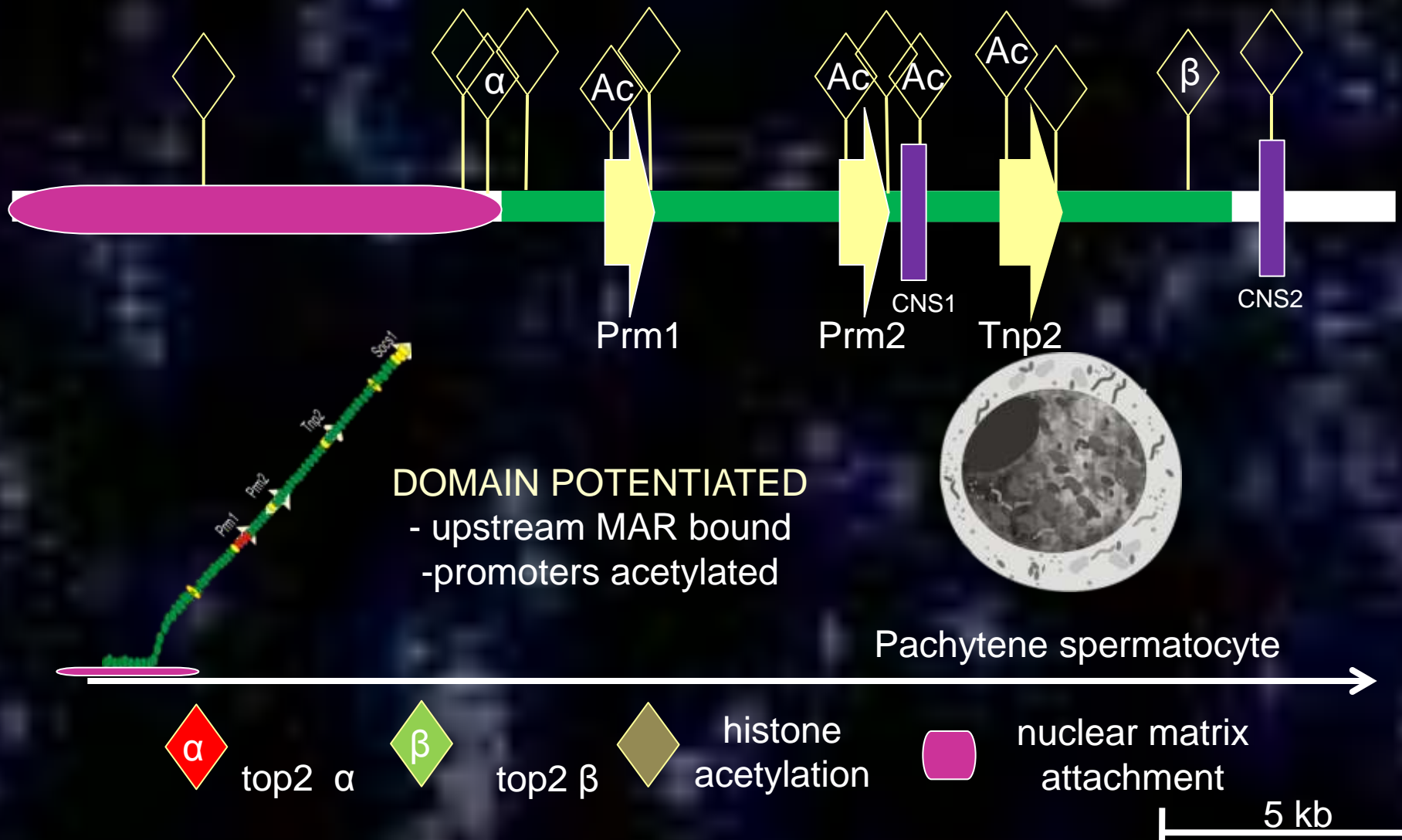


leptotene /zygotene spermatocyte

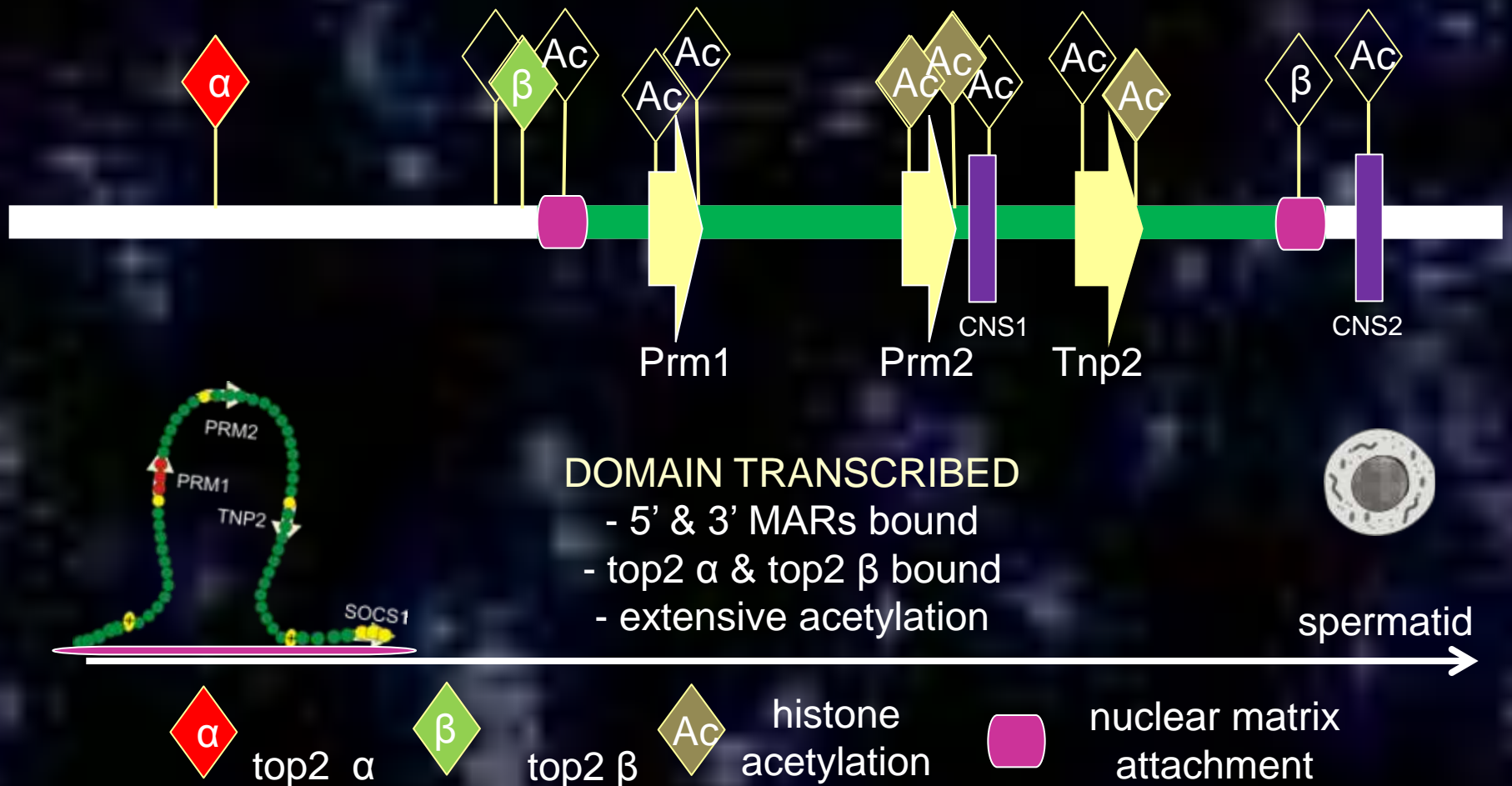


5 kb

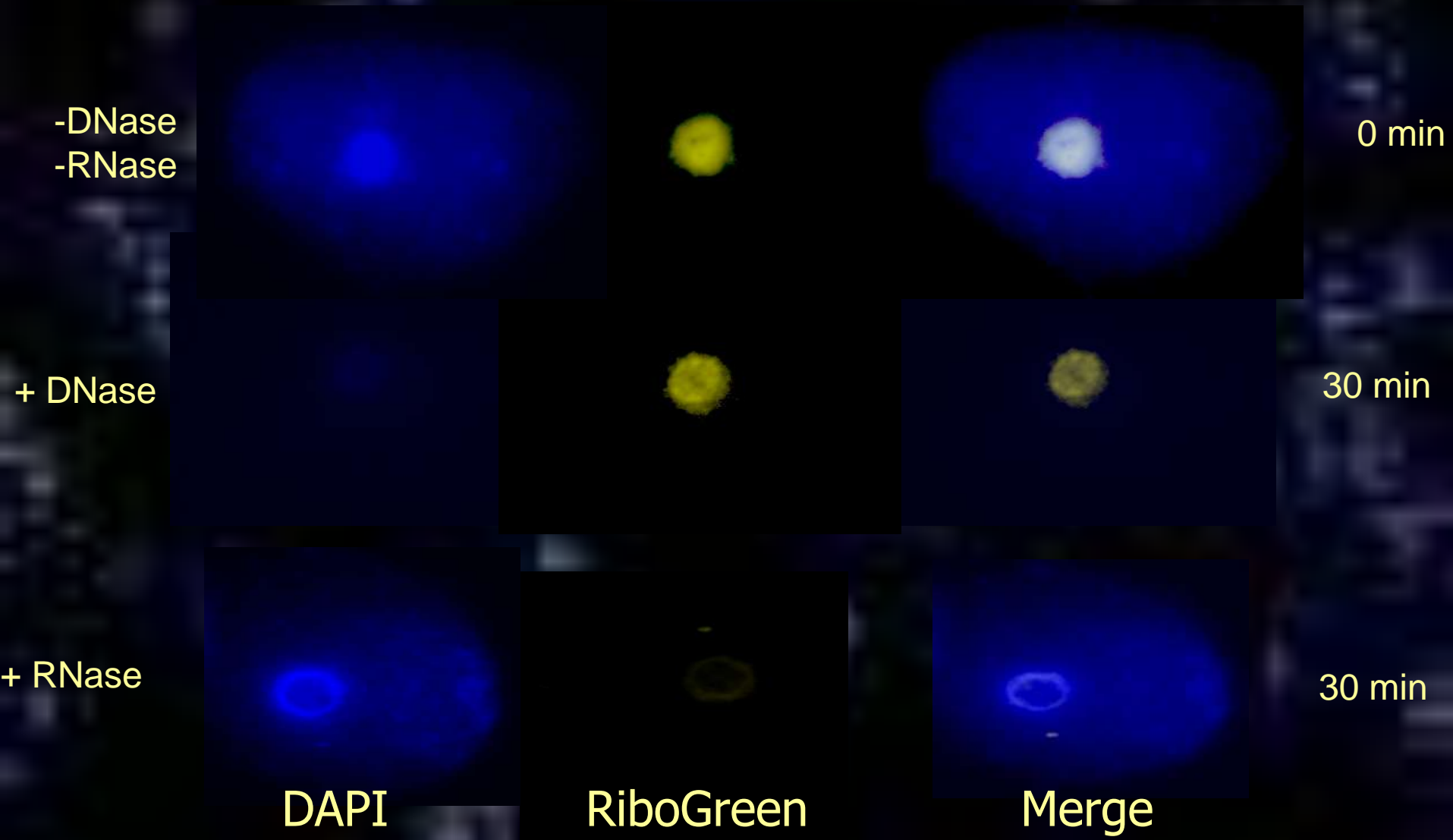
POTENTIATING THE Prm1 → Prm2 → Tnp2 DOMAIN DURING SPERMATOGENESIS



POTENTIATING THE Prm1 → Prm2 → Tnp2 DOMAIN DURING SPERMATOGENESIS



LOCALIZATION OF RNA WITHIN THE SPERM NUCLEAR MATRIX

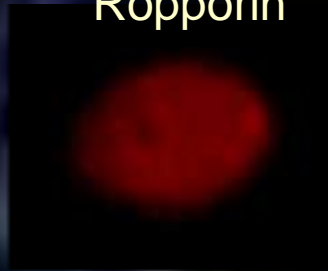


LOCALIZATION OF SPERM RNAs TO THE NULCEAR MATRIX

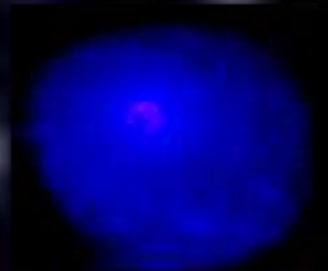
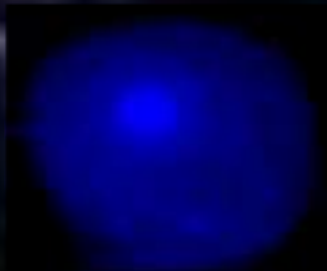
DAPI

Ropporin

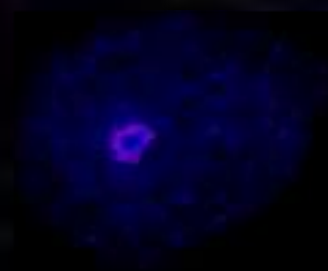
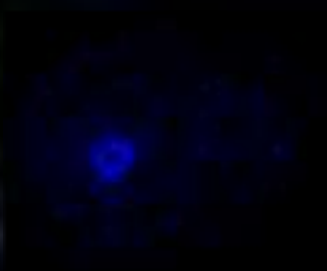
Merge



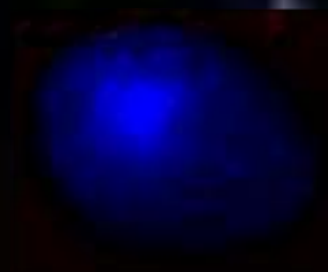
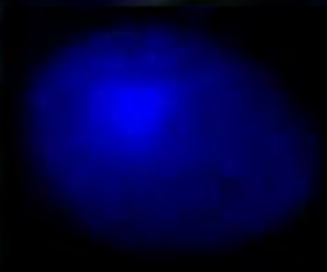
Decondensed nucleus



Nuclear Matrix & Halo

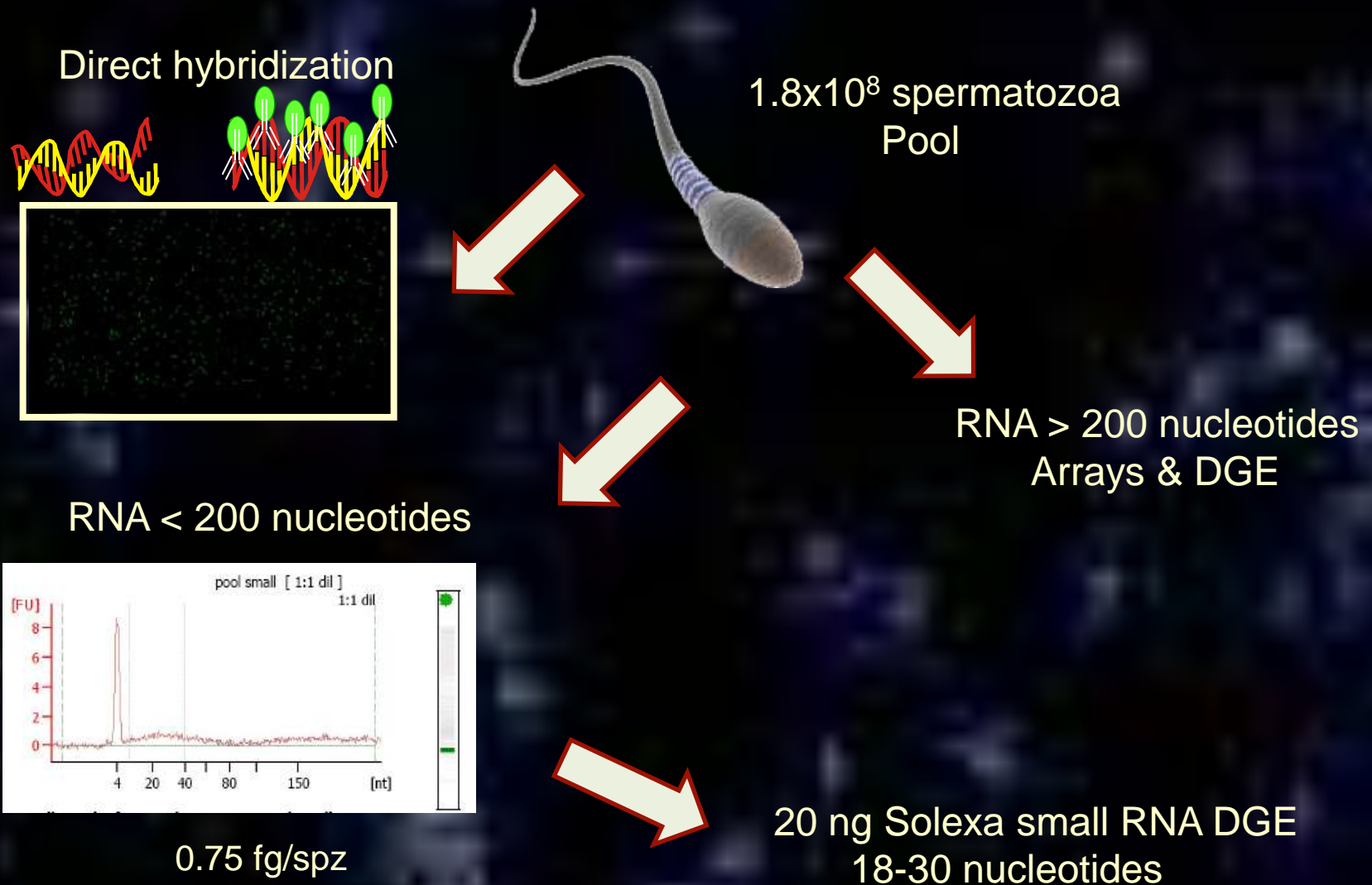


DNase treated
Nuclear Matrix & Halo

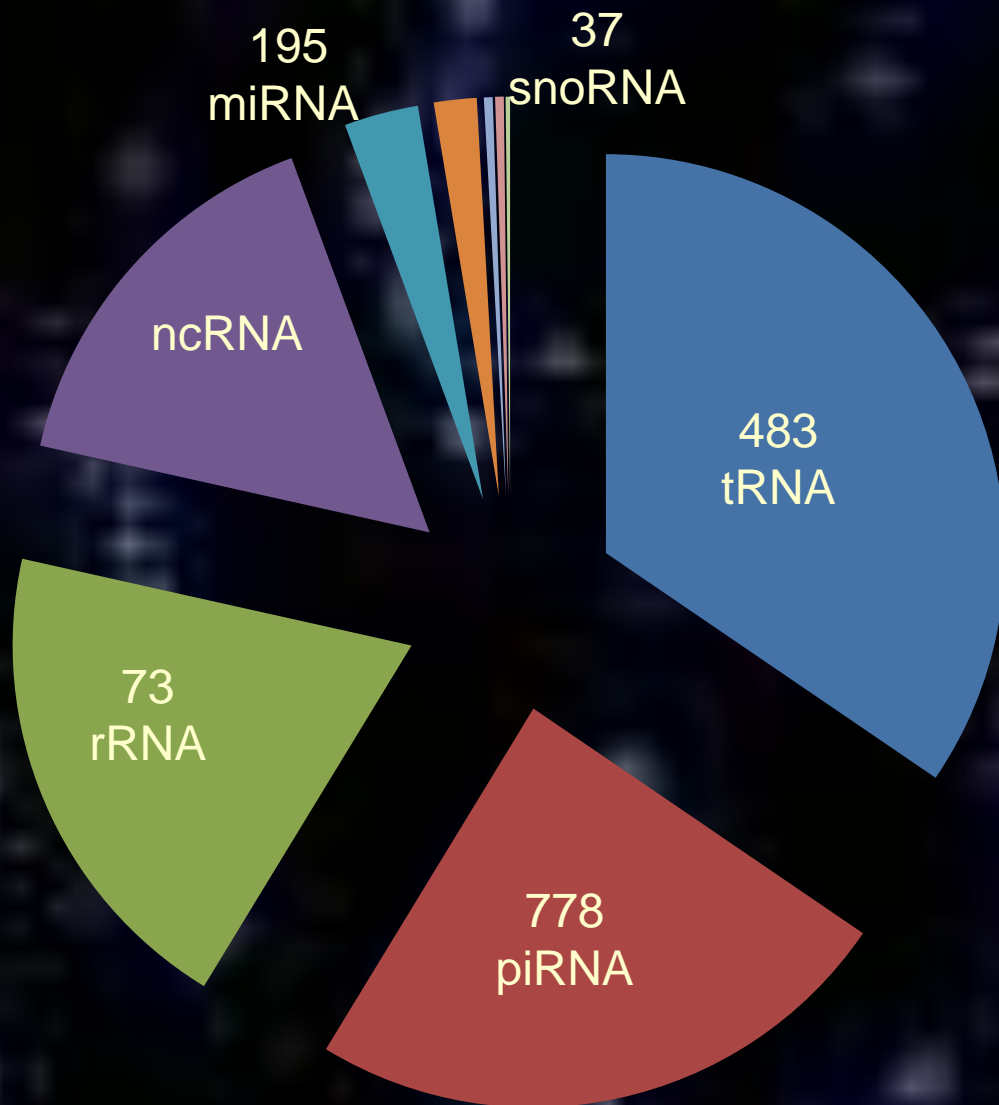


RNase treated
Nuclear Matrix & Halo

Sperm RNAomics



Distribution of small RNAs



Class	Reads
tRNA	107196
piRNA	75103
rRNA	61423
ncRNA	49324
miRNA	9344
scRNA	5372
snRNA	1102
mt tRNA	1087
snoRNA	557

SUMMARY

- ❖ The potentiated protamine domain is maintained in an open chromatin conformation independent of histone association.
- ❖ Potentiation appears to initiate through a topoisomerase mediated mechanism.
- ❖ Organization of chromatin by the sperm nuclear matrix appears markedly different than somatic cells.
- ❖ Regions of strong attachment with a background of attachment to the nuclear matrix across the chromosome.

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LANL: N. Doggett

Univ. Utah:
D. Carrell

GBSF: J. Bode

Oakland University:
G. Singh

