Роль сателлитсвязывающих белков в высокоуровневой организации хроматина

Уровни упаковки генома

Интерфаза

?

Двойная спираль

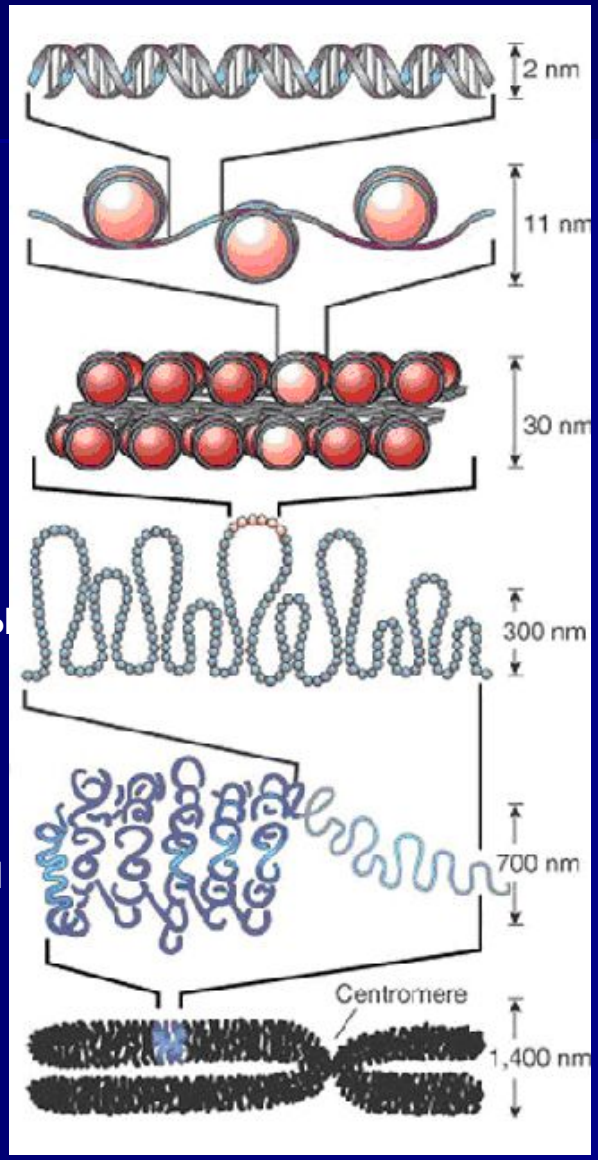
10 нм фибрилла

30 нм фибрилла

Петельные домены

Фрагмент конденсированной хромосомы

Метафазная хромосома



2 нм 1x

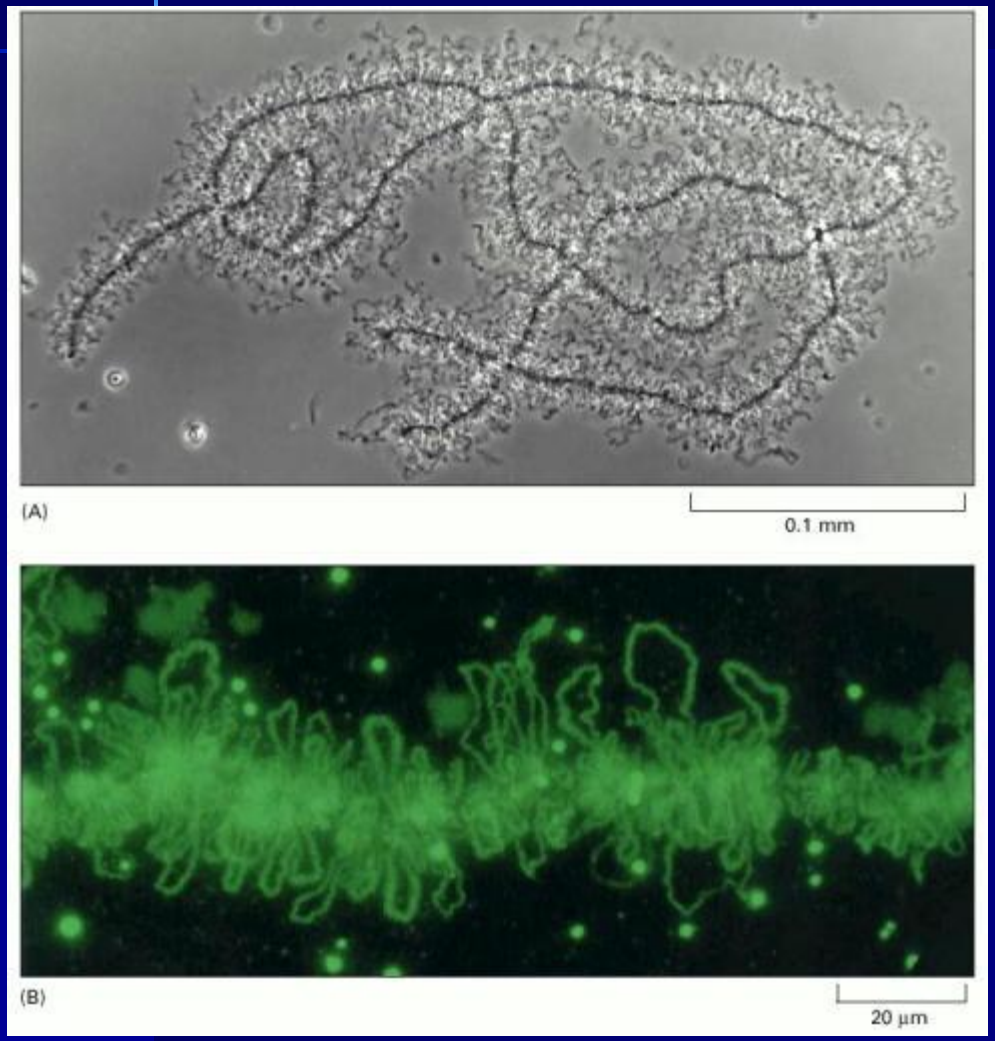
10 нм 6x

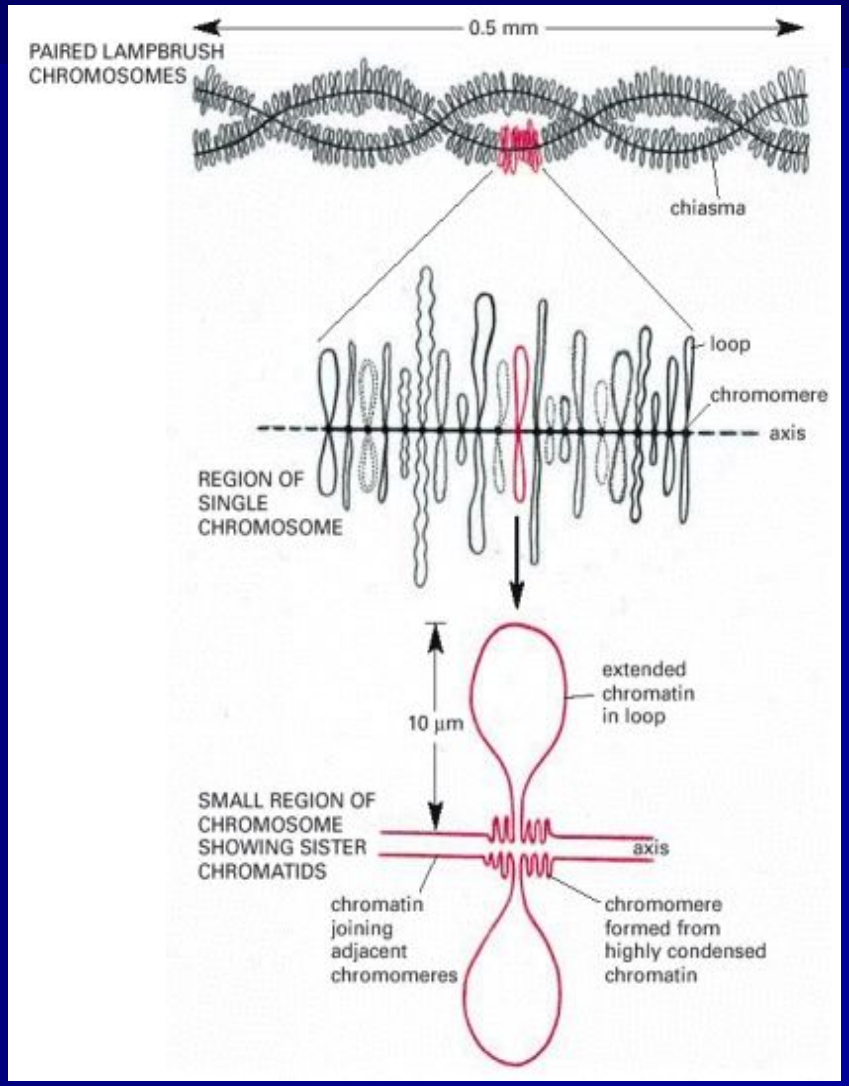
30 нм фибрилла 40x

Петельные домены 700x

Из статьи:
Controlling the double helix
Gary Felsenfeld and Mark Groudine
Nature 421, 448-453

Хромосомы типа ламповых щеток

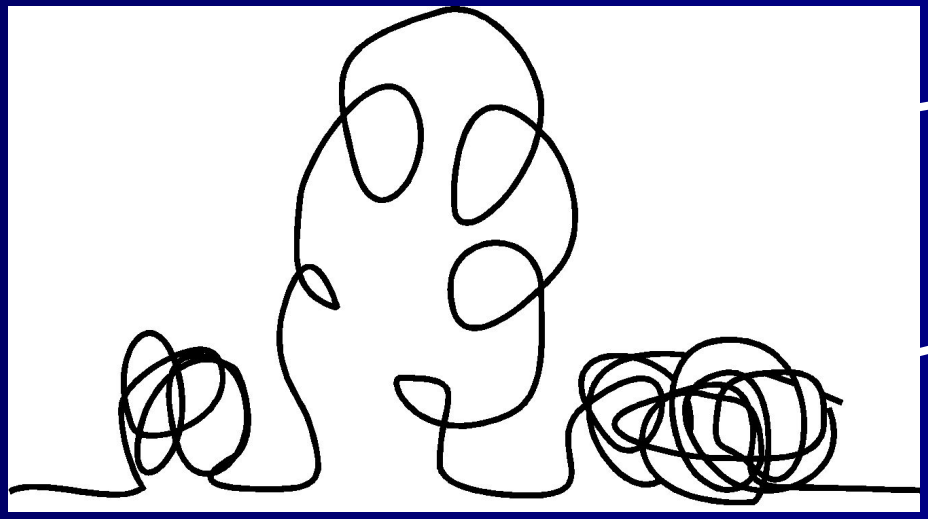




Доменная гипотеза организации эукариотического генома

Весь геном построен из однотипных блоков - доменов, которые могут включать один или несколько генов

Домены в целом находятся под контролем особых регуляторных систем, которые контролируют их транскрипционный статус на уровне упаковки (более компактной или менее компактной) всего домена в интерфазной хромосоме



Активный домен

Неактивный домен

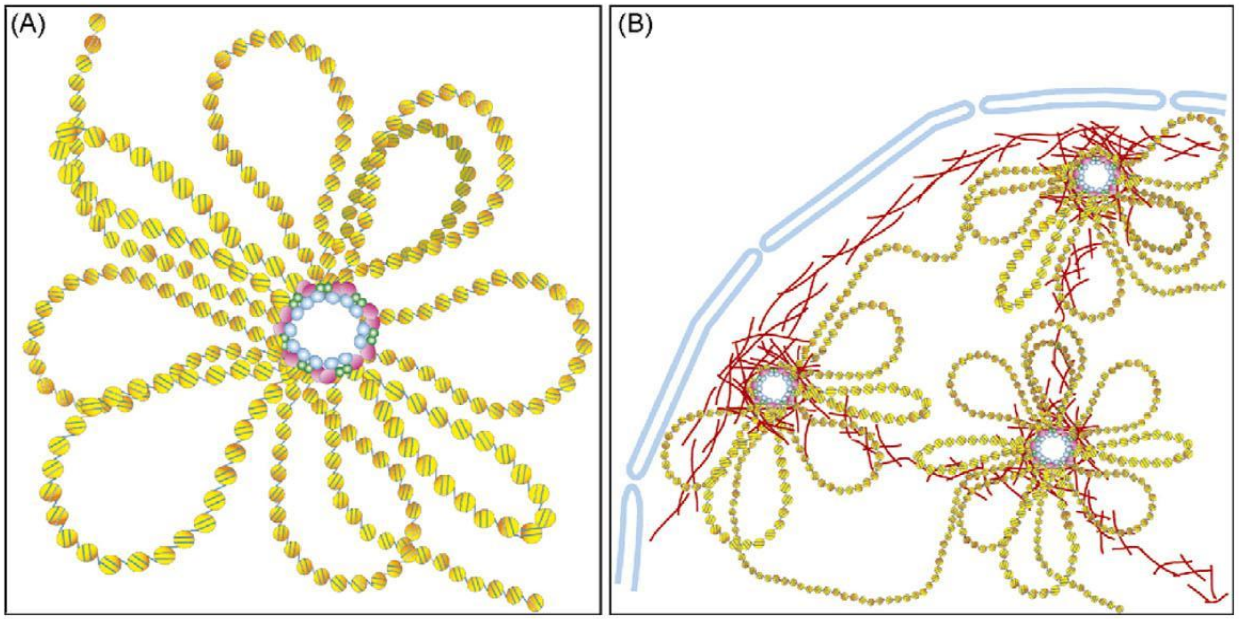


Fig. 1. Insulator elements organize the chromatin fiber in the nucleus by establishing separate compartments of higher-order chromatin structure. (A) Domains of open chromatin (yellow nucleosomes) are flanked by insulators (pink, blue and green spheres) that interact together to form a loop. (B) Diagram showing part of a nucleus with compartmentalized chromatin, anchored in part to the nuclear periphery by interactions of the insulators with the nuclear lamina (red lines).

Insulator elements organize the chromatin fiber in the nucleus by establishing separate compartments of higher-order chromatin structure. (A) Domains of open chromatin (yellow nucleosomes) are flanked by insulators (pink, blue and green spheres) that interact together to form a loop. (B) Diagram showing part of a nucleus with compartmentalized chromatin, anchored in part to the nuclear periphery by interactions of the insulators with the nuclear lamina (red lines).

Experimental approach

Nuclei

Nuclear matrix

DNase I
0,2 M $(NH_4)_2SO_4$

10mM TrisHCl pH=9,
EDTA, Triton X-100, DTT

Extraction

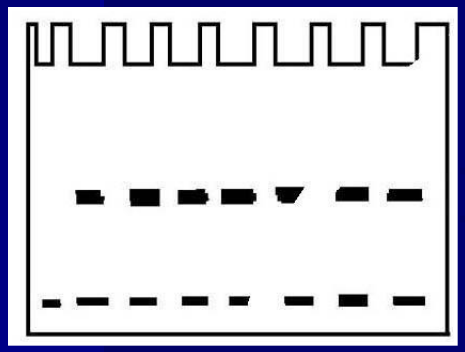
Q-sepharose
ion exchange
chromatography

SDS-

Gel mobility shift assay
(GMSA, retardation)

+

GMSA



Polyclonal
Antibodies

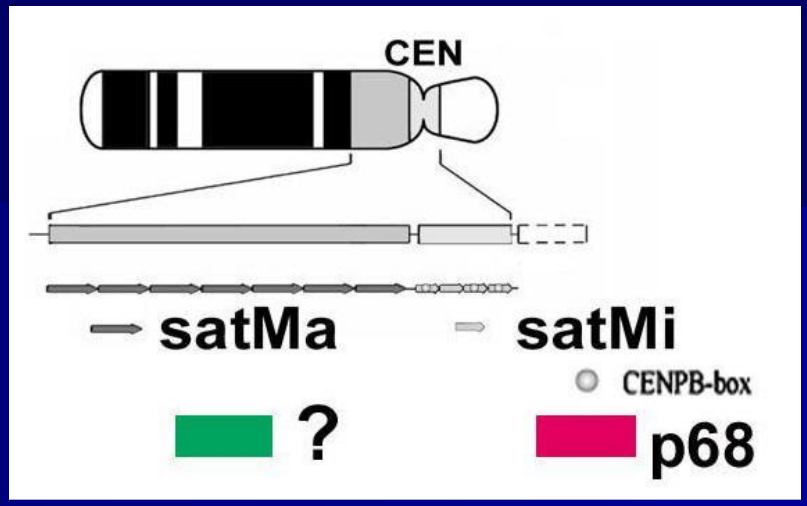
Immunoprecipitation
Immunoblotting
Hipershift in GMSA
Immunofluorescence

Affinity
Chromatography

MALDI
(mass spectrometry)

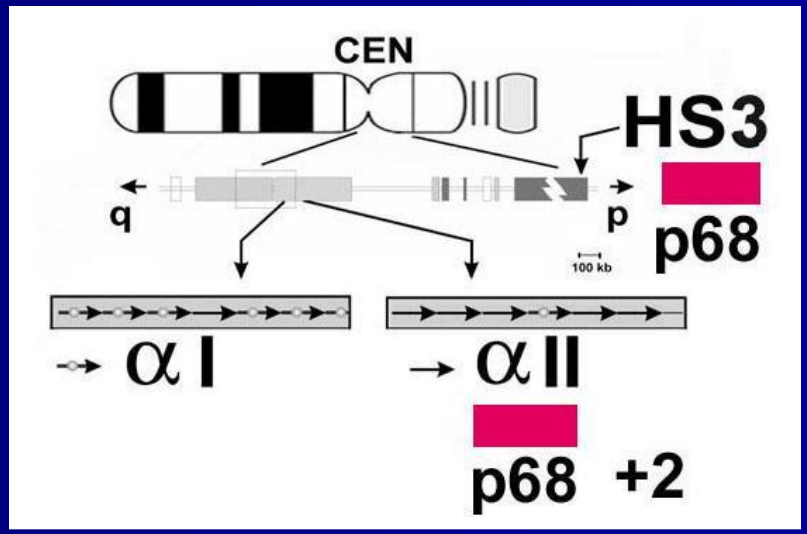
Mus

Mus musculus

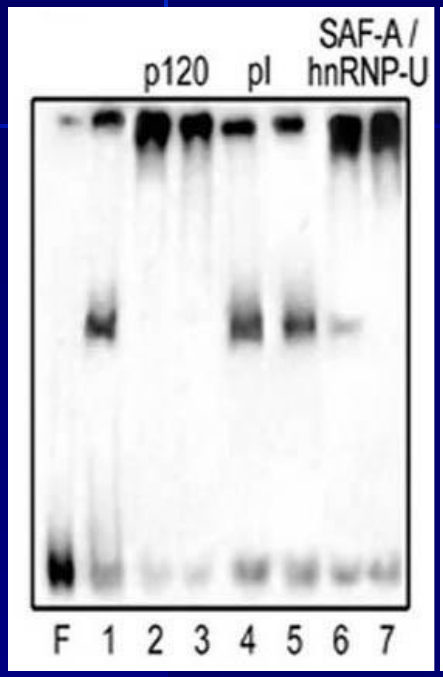


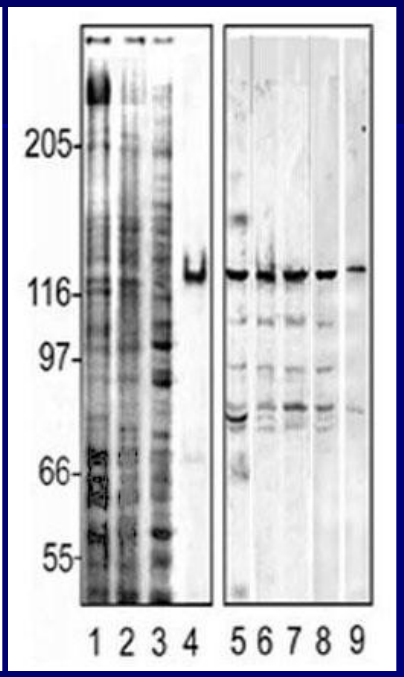
Homo

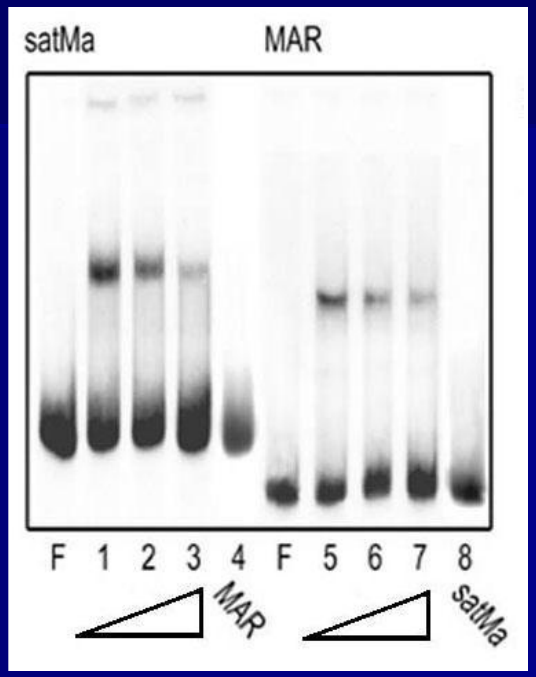
Homo sapiens

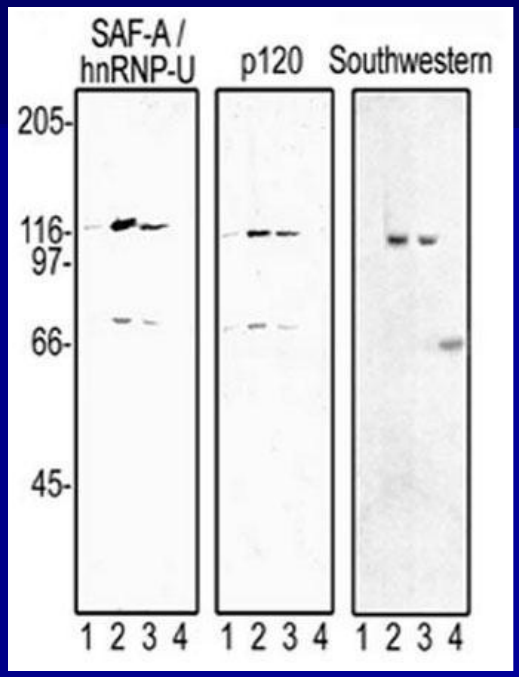


Saf-a/hnRNP U

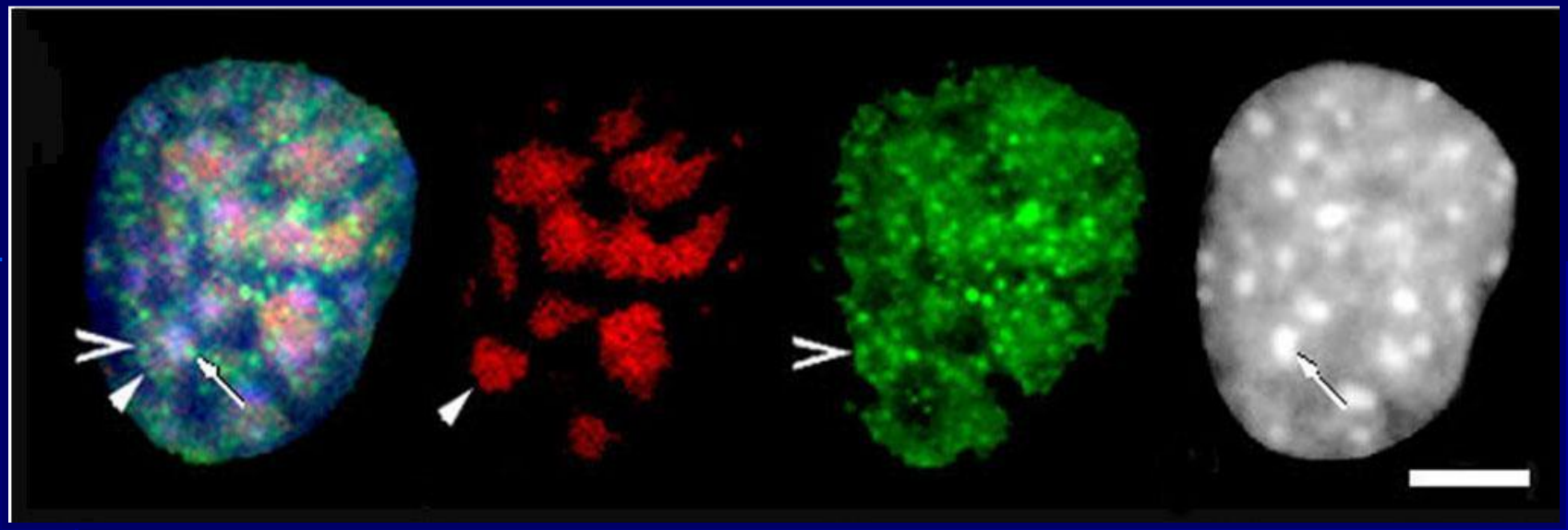




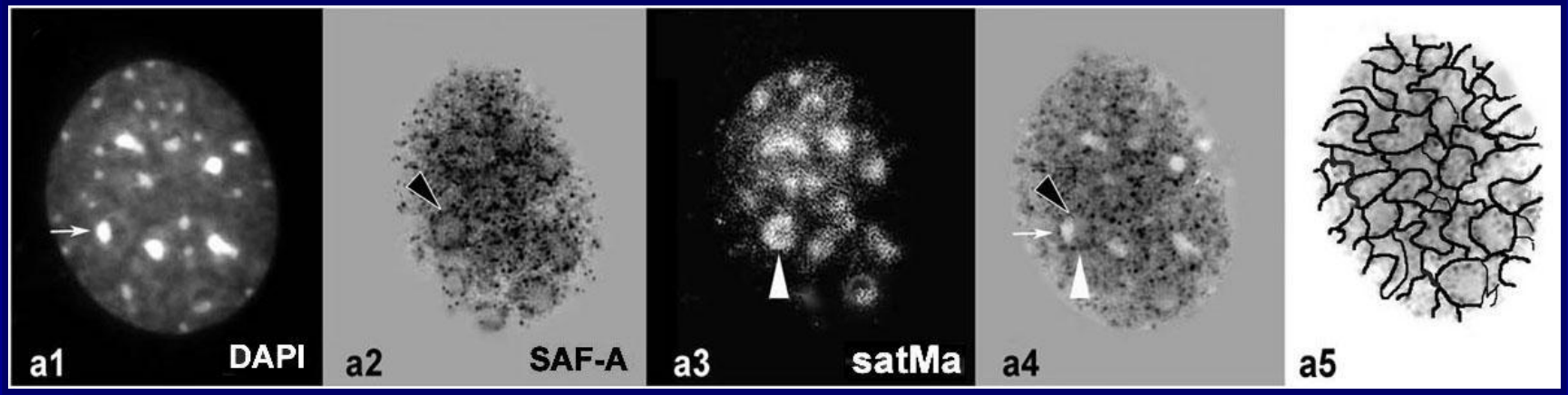




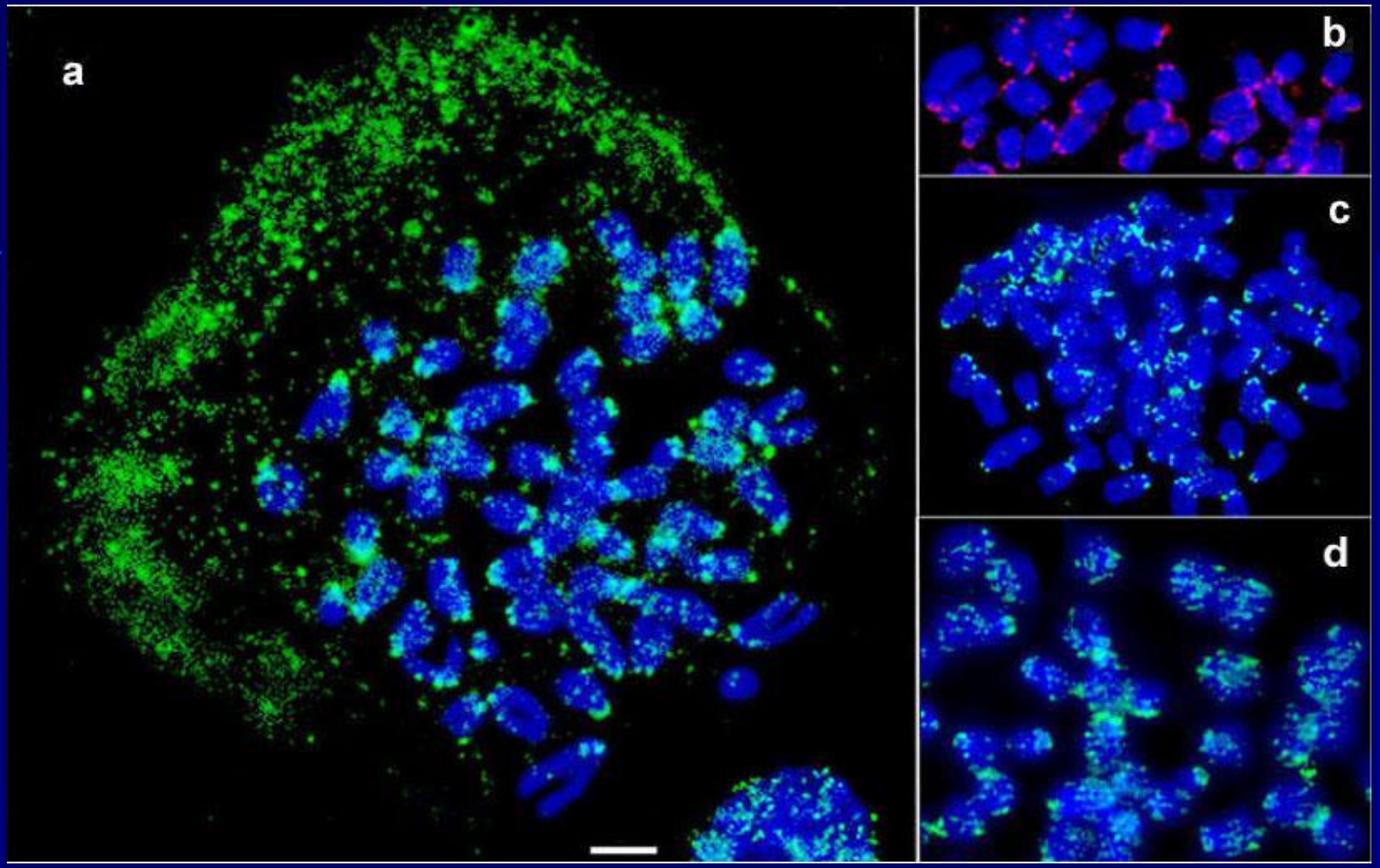
Lobov et al., 2000



satMa FISH Saf-a IF DAPI



Lobov et al., 2000


a
b
c
d
Saf-a
- CENP-B
DAPI

Saf-a/hnRNP U domain structure

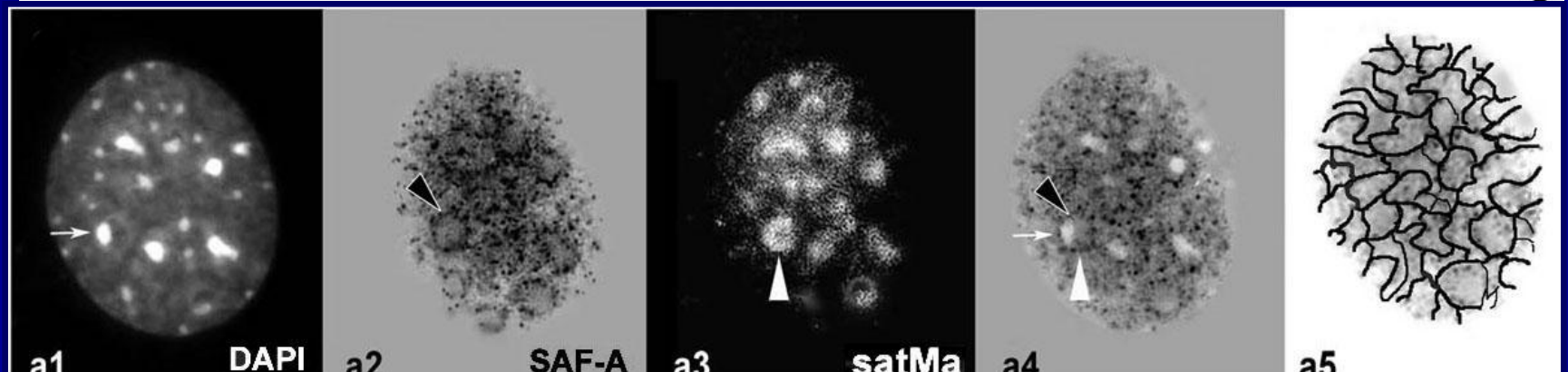
```
001 MSSSPVNVKklKVSelKEeLKKRRLSDKGLKAelMErlQA[E3]ALDDEEAGGR
051 PAMEPGNGSLDLGGDSAGRSGAGLEQEAAAGGDEEEEEEEEEEEGISALD
101 GDQMELGEENGAAGAADSGPMEEEEAASEDENGDDQGFQEGEDELGDEEE
151 GAGDENGHGEQQPQPPATQQQQPQQQRGAAKEAAGKSSGPTSLFAVTV[E3]AP
201 PGARQGQQQAGGKKKAEGGGGGGRPGAPAGDGKTEQKGGDKKRGVKRPRE
251 DHGRGYFEYIEENKYSRAKSPQPPVEEEDEHFDDTVVCLDTYNCDLHFKI
301 SRDRLSASSLTMESFAFLWAGGRASYGVSKGKVCFEMKVTEKIPVRHLYT
351 KDIDIHEVRIGWSLTTSGMLLGEEEFSYGYSLKGIKTCNCETEDYGEKFd
401 endVITCFANFESDEVELSYAKNGQDLGVAFKISKEVLAGRPLFPHVLCH
451 NCAVEFNFGQKEKPYFPIPEEYTFIQNVPLEDRVRGPKGPEEKKDCEVVM
501 MLgLPgAgkTTWVTKHAAENPGKYNILGTNTIMDKMMVAGFKKQMADTGK
551 LNTLLQRAPQCLGKFIEIAARKKRNFILDQTNVSAAAQRRKMCLFAGFQR
601 KAVWCPKDEDYKQ[Nec]RTQKKAEVEGKDLPEHAVLKMKGNFTLPEVAECF[CC]DEI
651 TYVELQKEEAQKLLEQYKEESKKAL[CC]PPEKKQNTGSKKSNKNKSGKNQFNR
701 GGGHRGRGGLN[Nec]M[NM]RGGNFRGGAPGNRGGYNRRGNMPQRGGGGGGSGGIGYP
751 YPRAPVFPGRGSYSNRGNYNRGGMPNRGNYNQNFRGRGNNRGYKNQSQGY
801 NQWQQGQFWGQKPWSQHYHQGYY[NM]
```

DNA binding

NTPase

c-coiled

RNA binding



Saf-a/hnRNP U specifically interacts with β-actin in the nucleus

Actin and hnRNP U cooperate for productive transcription by RNA Polymerase II
Kukalev A et al., Nature, 2005

Table 1. Summary of functional information for actin subfamilies

Actin subfamily	Organisms?	Localization	Essential In yeast?	Function
Actin	Human→Giardia (Ubiquitous?)	Cytoplasm^{MFY}, nucleus^{M}	Y	Cell motility/transport^{MFY}, polarity^{MFY}, chromatin remodeling^{MY}
Arp1	Human→yeast	Cytoplasm^{MFY}	N (spindle alignment)	Dynein motor function (dynactin complex^{MFY})
Arp2	Human→yeast, *Arabidopsis*	Cytoplasm^{MFY}	Y	Actin polymerization (Arp2/3 complex^{MFY})
Arp3	Human→yeast, *Arabidopsis*	Cytoplasm^{MFY}	Y	Actin polymerization (Arp2/3 complex^{MFY})
Arp4	Human→yeast, *Arabidopsis*	Nucleus^{Y}	Y	Chromatin remodeling (SWI/SNF^{M}, INO80^{Y}, NuA4^{Y}, histone acetyltransferase^{YM})
Arp5	Human→yeast, *Arabidopsis*	Nucleus^{Y}	Y	Chromatin remodeling (INO80 complex^{Y})
Arp6	Human→yeast, *Arabidopsis*	Nucleus^{FY}	N	Localized to heterochromatin^{F}
Arp8	Human→yeast, *Arabidopsis*	Nucleus^{Y}	N	Chromatin remodeling (INO80 complex^{Y})
Arp10	Likely human→yeast	Cytoplasm^{MY}	?	Dynein motor function (dynactin complex$^{M, N. crassa}$)
Orphans				
Arps 7,9	*S. cerevisiae*, *S. pombe* (Arp 9)	Nucleus^{Y}	Y/Y	Chromatin remodeling (SWI/SNF^{Y}, RSC complex^{Y})

Superscripts (^{M,F,Y}) refer to the type of organism (mammal, fly, yeast) in which the observation was made. The ARP10 subfamily is italicized to indicate that ian Arp11 is part of dynactin and that *Neurospora* : of a conserved subfamily.
ces Genome Database (http://genome- n of biochemical associations of conventional actin, et al., 1998; Galarneau et al., 2000; Harata et al., or characterization of actin, Arp1, Arp2, Arp3 and er, 1999. Our apologies to those authors we were not

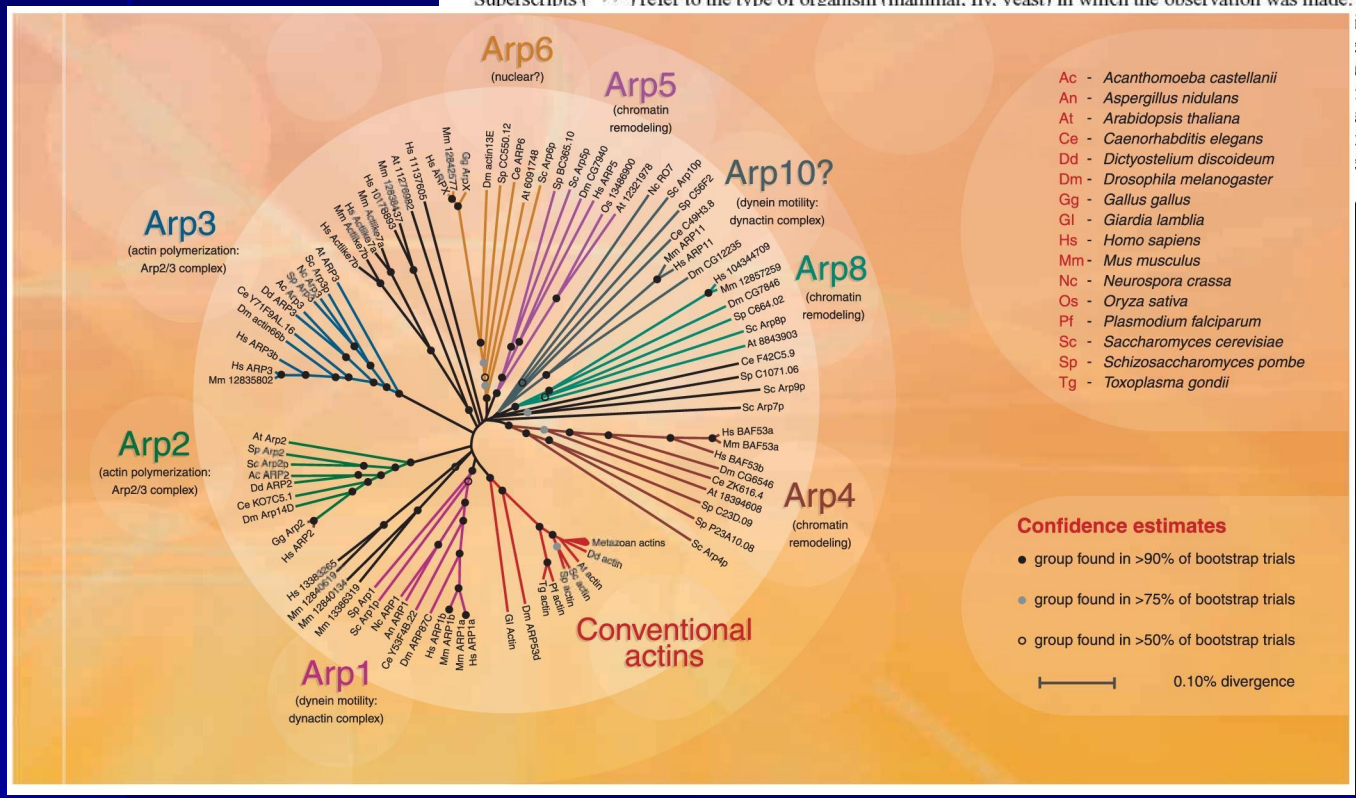
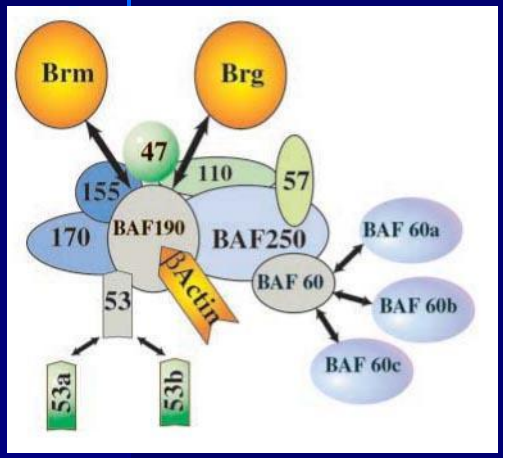
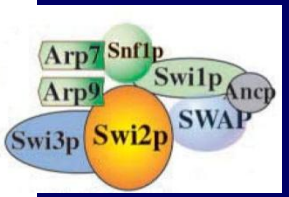


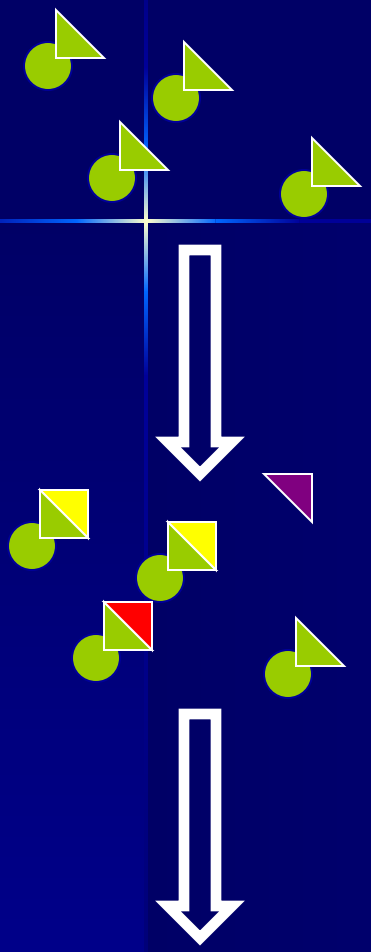
TABLE 1 Chromatin modifying complexes

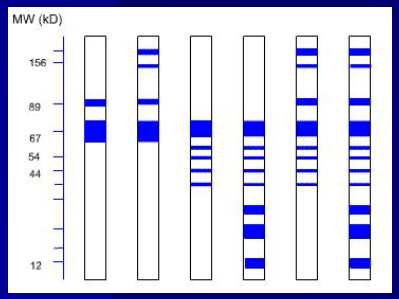
Name	Organism	Actin	Arps	ATPase	Acetylase/ deacetylase	Comments and references
SWI/SNF	*S. cerevisiae*	No	Arp7,9	SWI2/SNF2	No	(44, 45, 138, 139)
Rsc	*S. cerevisiae*	No	Arp7,9	Sth1	No	(19, 50)
INO80	*S. cerevisiae*	Act1	Arp4,5,8	Ino80	No	Phospholipid biosynthesis; helicase activity. (15, 33)
NuA4	*S. cerevisiae*	Act1	Arp4	None	Acetylase	Esa1 acetylase is essential for cell cycle progression. Arp4 binds histones in vitro and is involved in epigenetic control of transcription. (16, 38, 39, 140)
BAF	Mammals	Yes	BAF53a or b	Brg1 or hBrm	No	Heterogeneous complex. Contains BAF250. (13, 24, 25)
PBAF	Mammals	Yes	BAF53a	Brg1	No	Absence of BAF250. Only Brg1 ATPase present. (141)
p400	Mammals	Yes	BAF53	P400 TAP54α,β	N/A	ATPase and helicase activity. Shared subunits with Tip60 complex. (18)
Tip60	Mammals	Yes	BAF53	TAP54α,β	Acetylase	ATPase and helicase activity. Links HAT activity to DNA repair and apoptosis. (17)
NuRD	Mammals	No	No	Mi-2	Deacetylase	Involved in gene silencing. (142–144)
RSF	Mammals	No	No	hSNF2h	No	Facilitates transcription from chromatin templates. Has nucleosome remodeling and spacing activities. (142)
LSH	Mammals	?	?	LSH	?	Member of SNF2/RAD54 family of proteins. Lsh−/− mice die perinatally. (145, 146)
BAP	*D. melanogaster*	Yes	BAP55	Brahma	No	Maintains homeotic gene expression. (14, 29)
Domino	*D. melanogaster*	?	?	Domino	?	Identified in a genetic screen for hematopoietic disorders. (32)
ACF	*D. melanogaster*	No	No	ISWI	No	Assembles periodic nucleosome arrays. (143)
NURF	*D. melanogaster*	No	No	ISWI	No	Disrupts nucleosome arrays. Pyrophosphatase. (60, 61, 147)
CHRAC	*D. melanogaster*	No	No	ISWI	No	Remodels chromatin, displaces nucleosomes in *cis*. (62, 148)

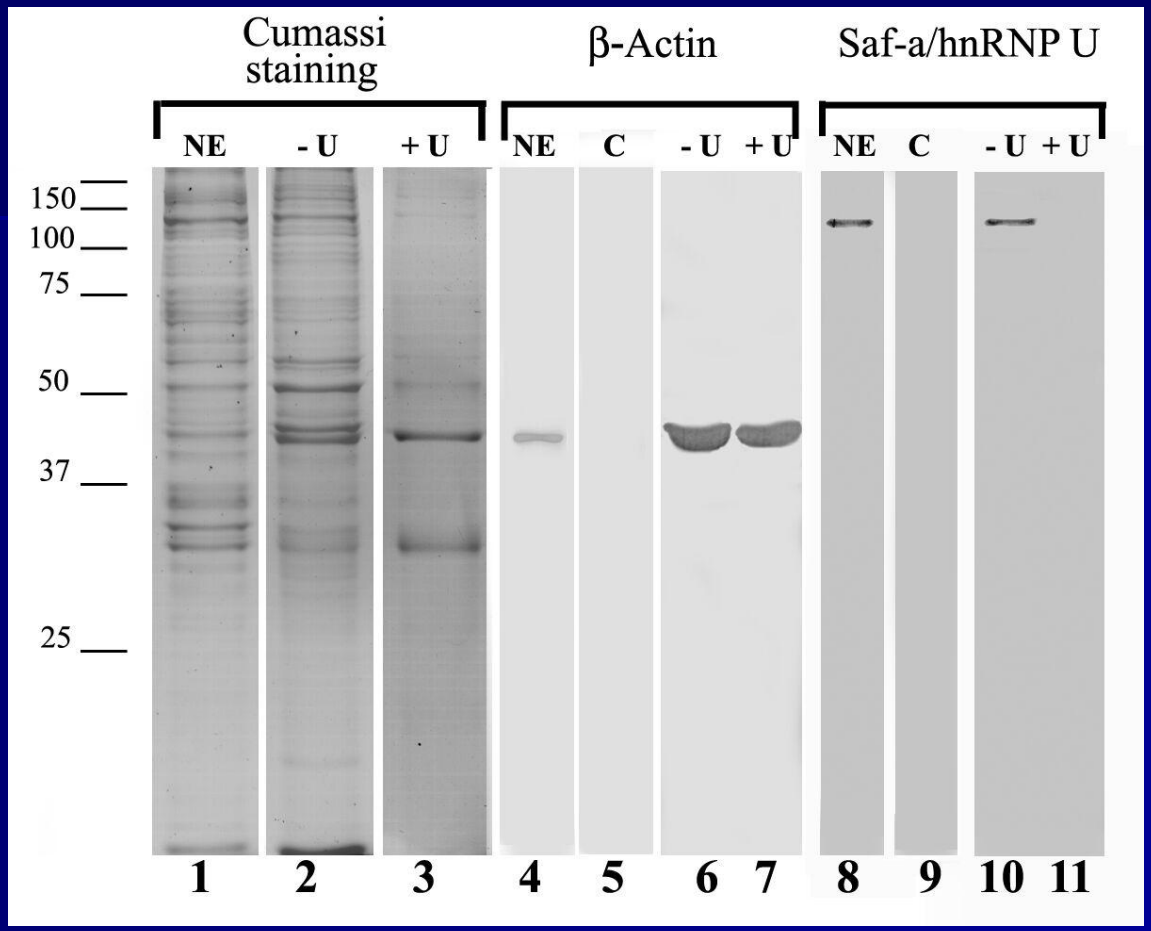




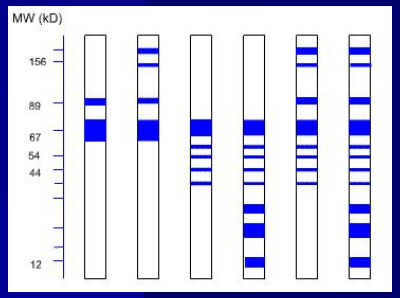
Saf-a/hnRNP U co-precipitated with b-actin



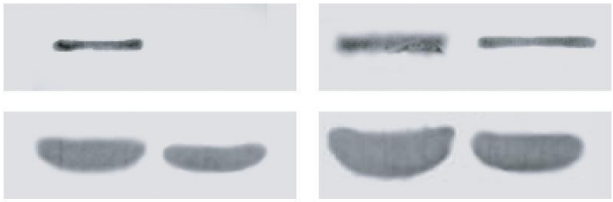




Actin binds Saf-a/hnRNP U *in vivo*

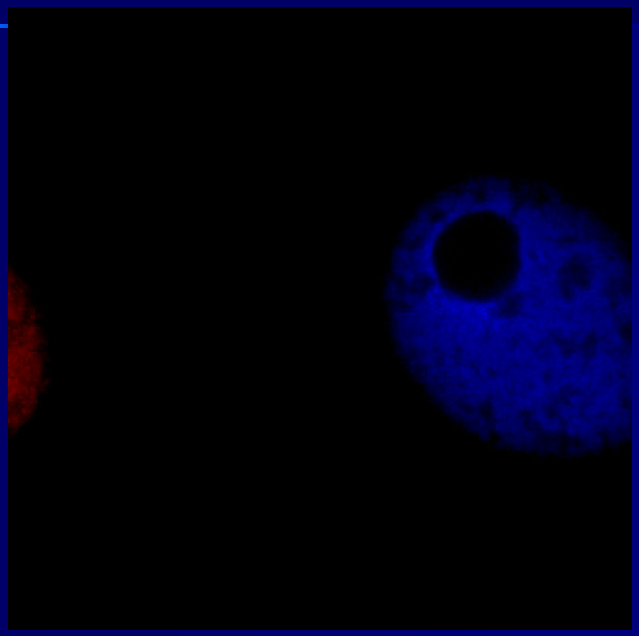


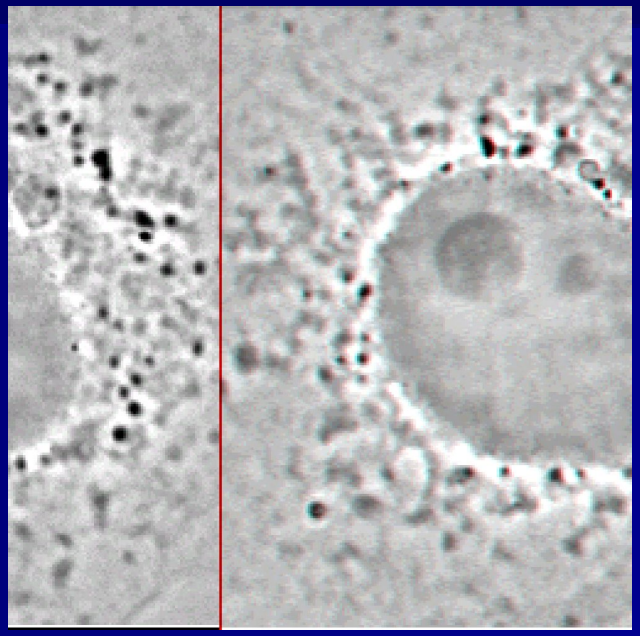
	- DSP		+ DSP	
	- U	+ U	- U	+ U
Saf-a/hnRNP U	+	-	+	+
Actin	+	+	+	+



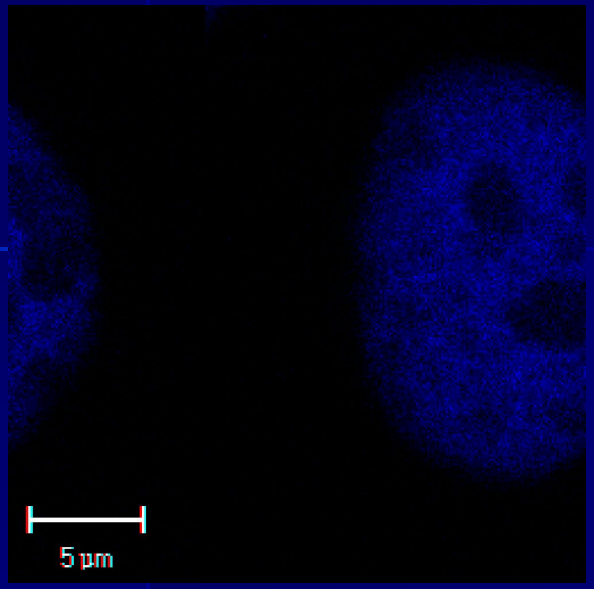
Saf-a/GFP fusion proteins

1-823 a/a

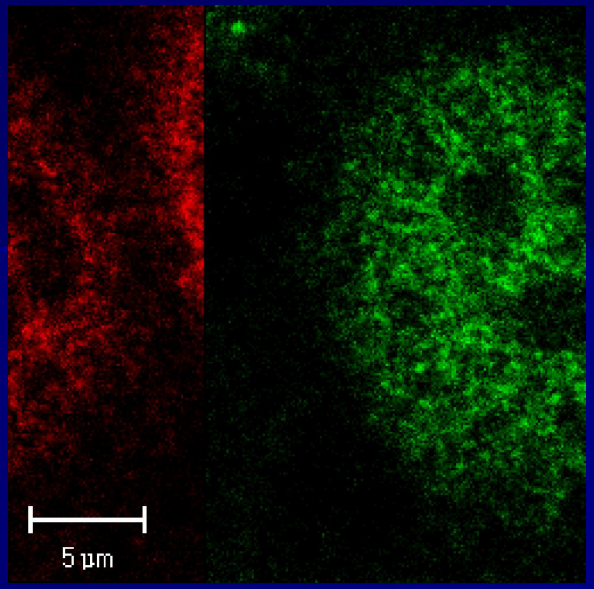




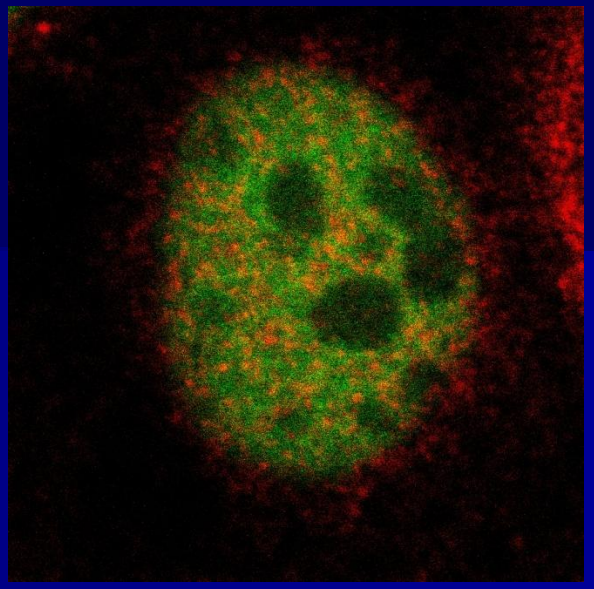
Actin co-localised with fusion Saf-a-GFP in HeLa cells



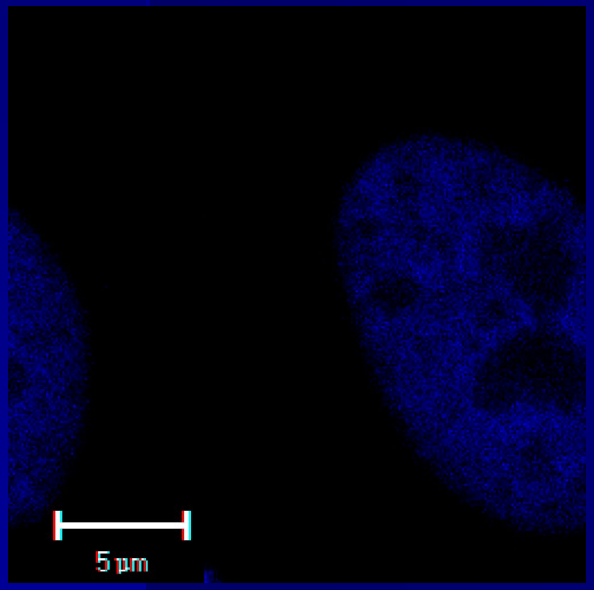
Saf-a FL/pEGFP-N1

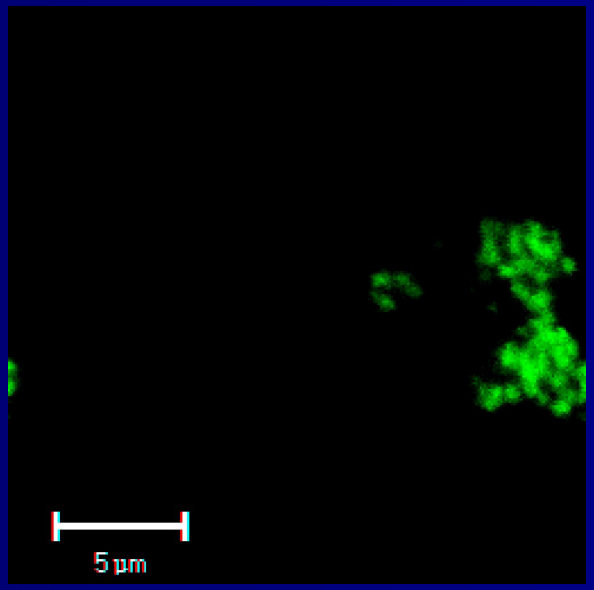


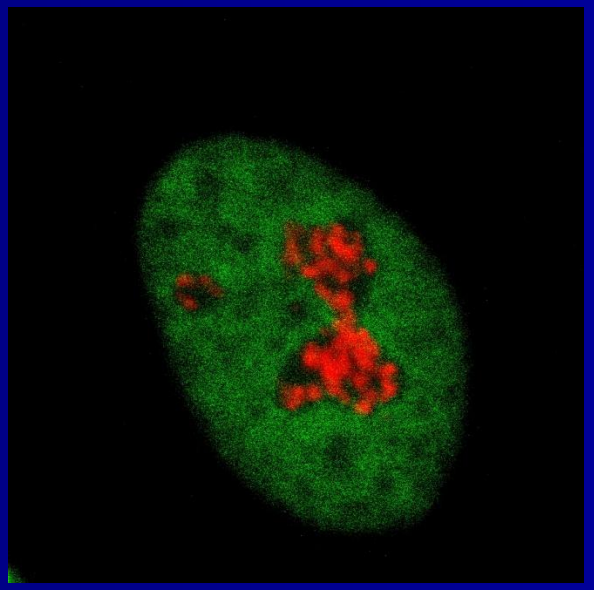
β-Actin/Fibrillarin



Merged



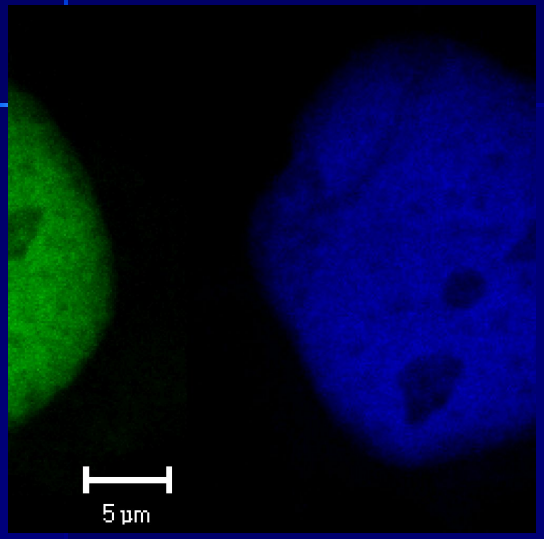




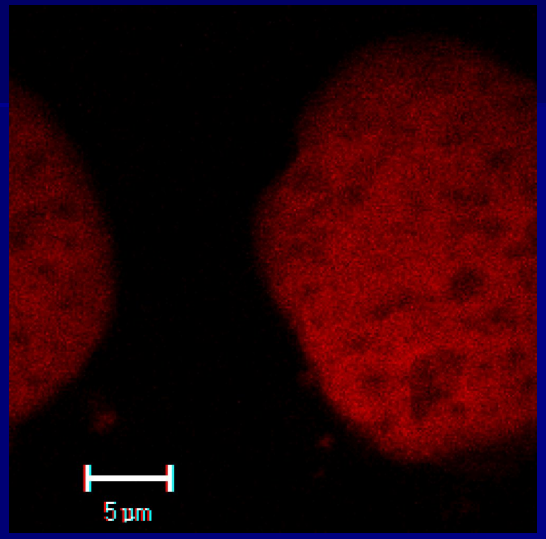
Saf-a N1-12/GFP fusion protein

Saf-a N1-12

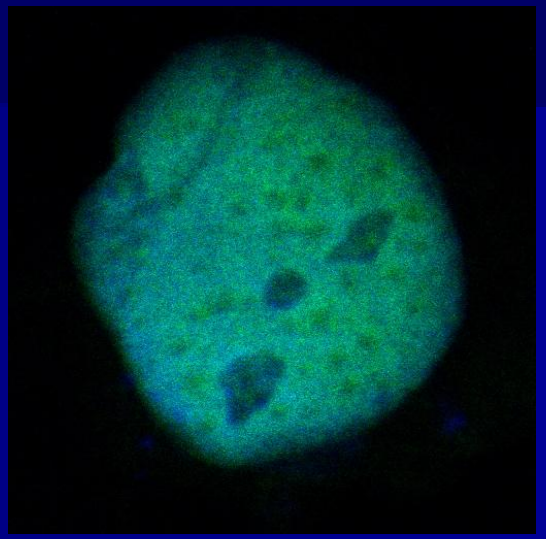
1-250 a/a - DNA-binding region



Saf-a 21-22/pEGFP-N1



DAPI



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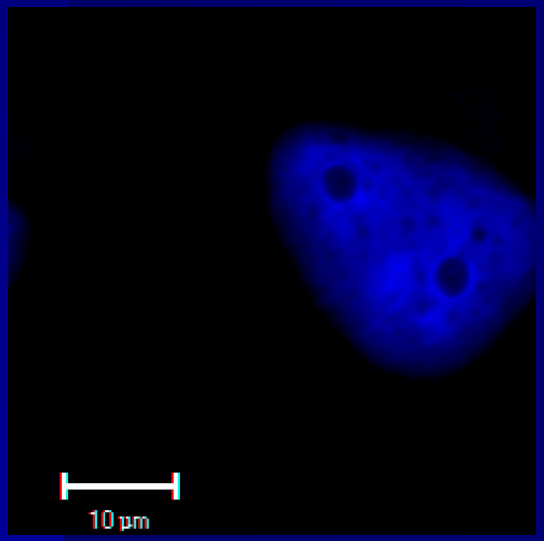
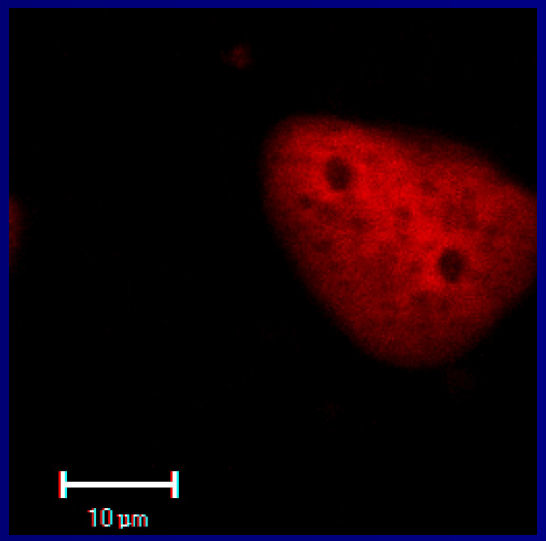
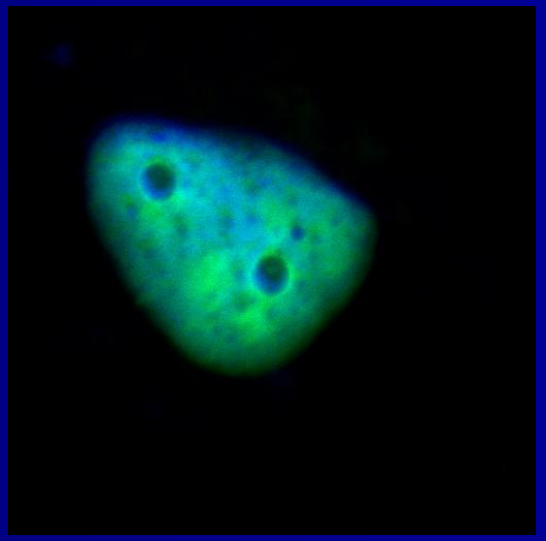


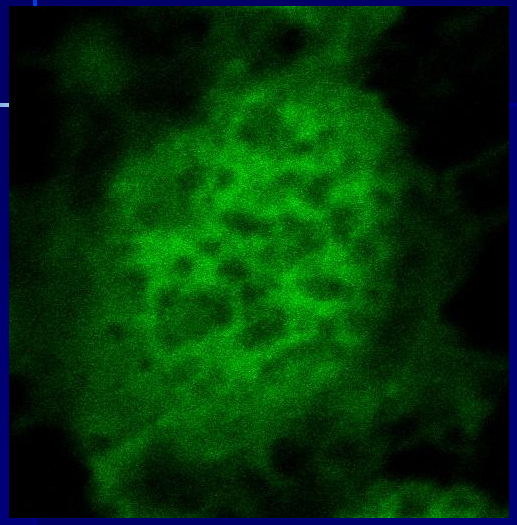




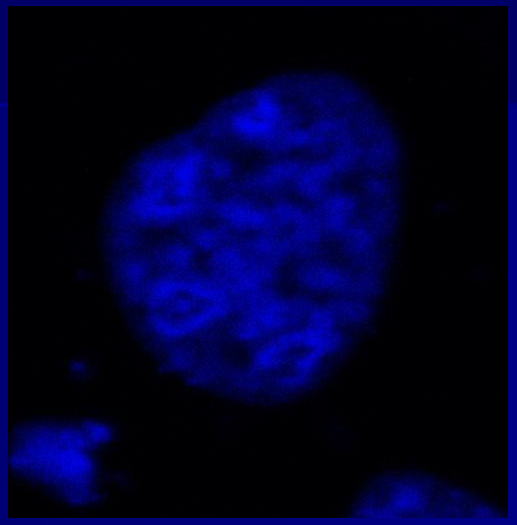
Saf-a 21-22/GFP fusion protein

Saf-a 21-22

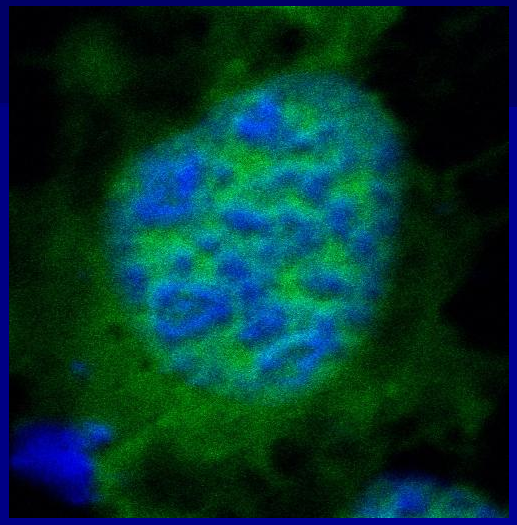
250-550 a/a - middle part, potential NTP binding domain



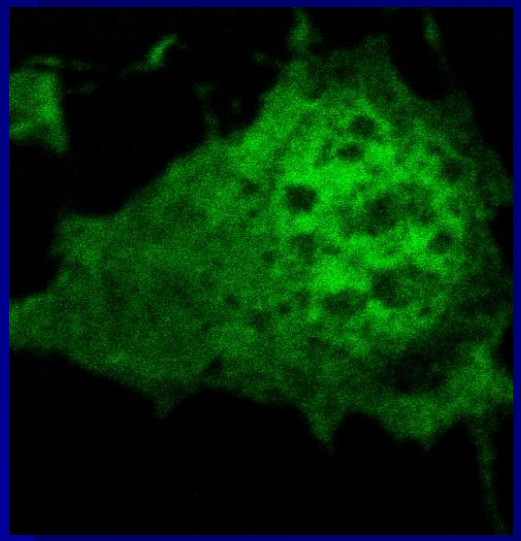
Saf-a 21-22/pEGFP-N1

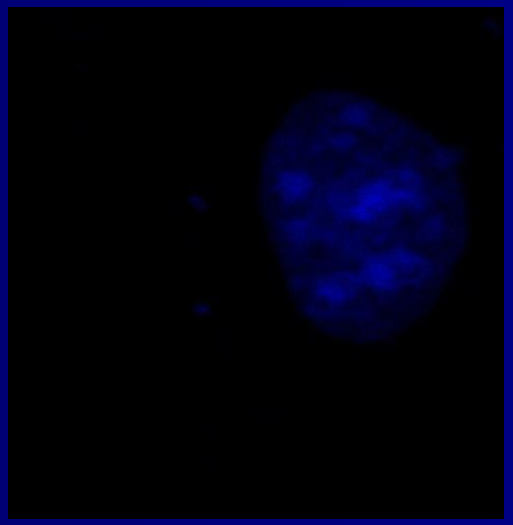


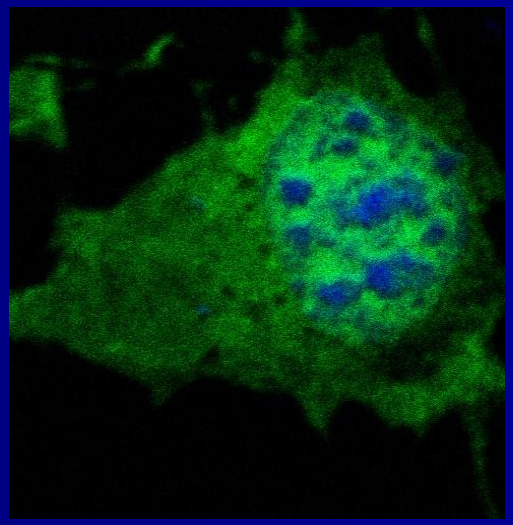
DAPI



Merged



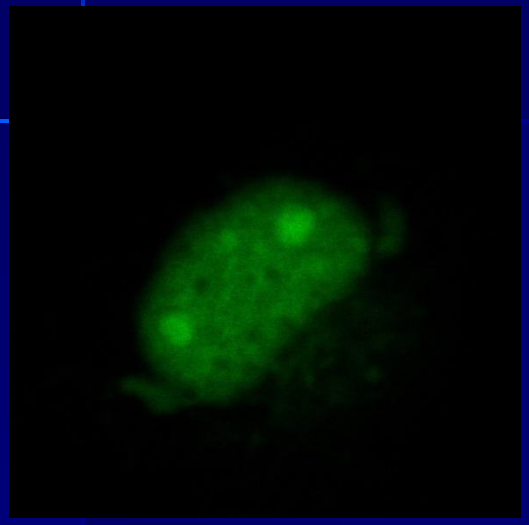




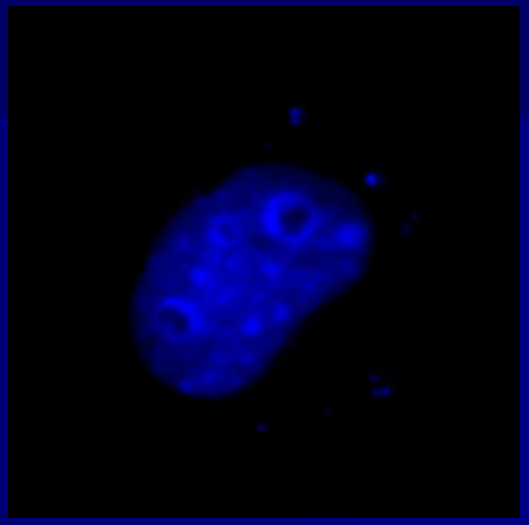
Saf-a 31-N3/GFP fusion protein

Saf-a 31-N3 

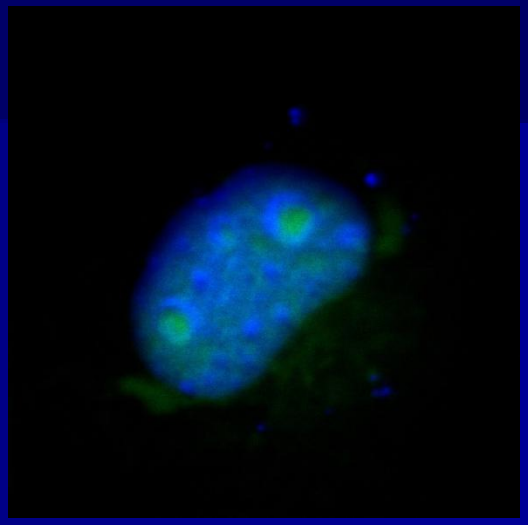
550-823 a/a - RNA-binding region



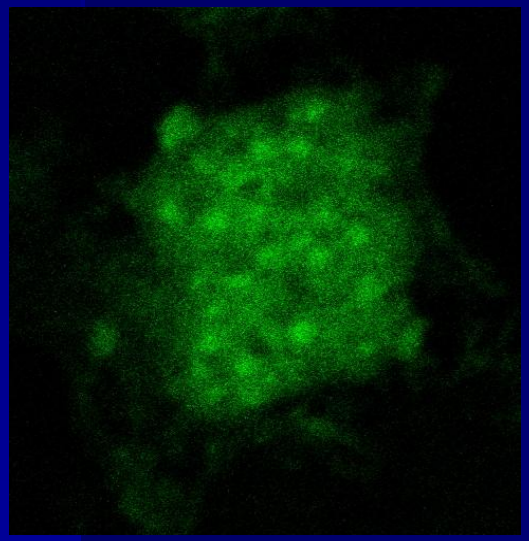
Saf-a 31-N3/pEGFP-N1

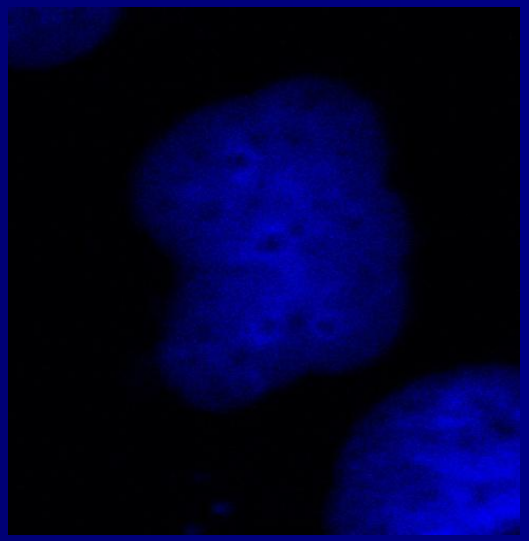


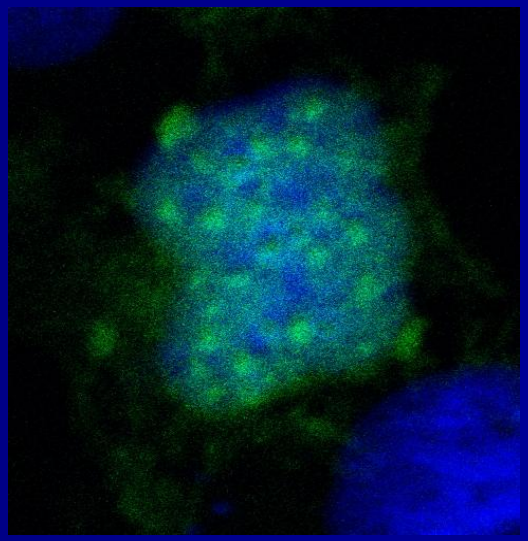
DAPI



Merged





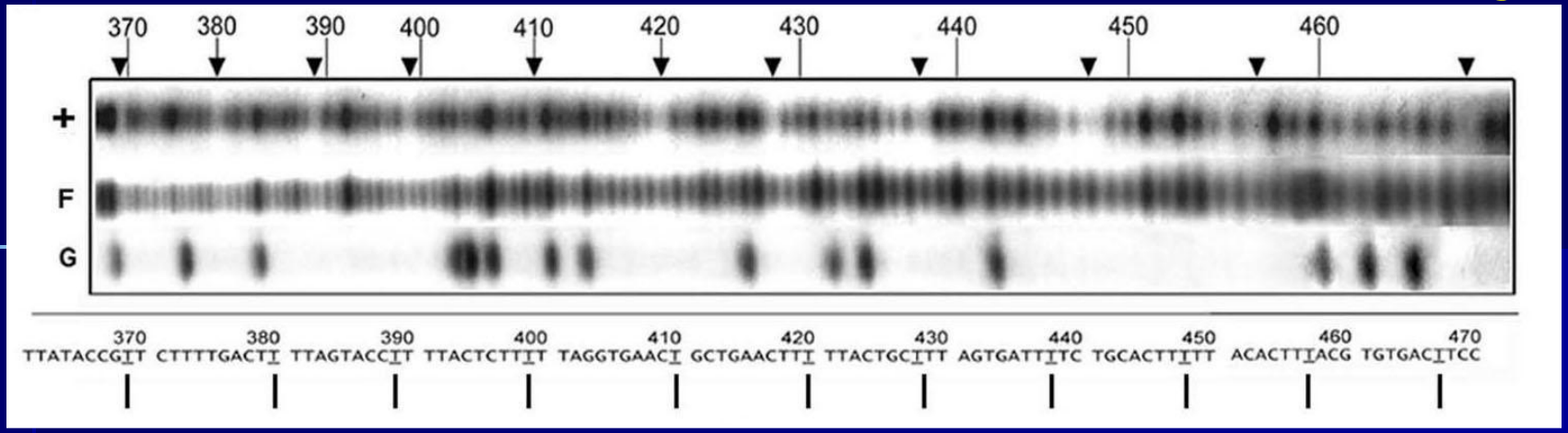


QRTQK motif presents in other nuclear and cytoplasmic proteins

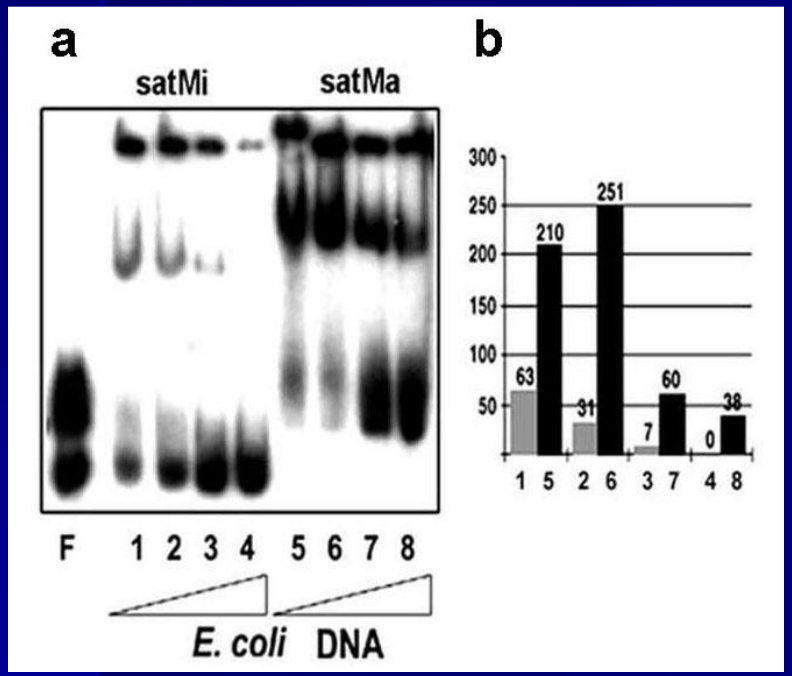
		QRTQK	
Hrp65-2	507	NQRPQKAA	Chironomus tentants
SAF-A Human	611	KQRTQKKA	Homo sapiens
SAF-A Xenopus	557	KERTQKKS	Xenopus laevis
RNA polymerase	56	KERTQKKK	Plasmodium falciparum
Matrin CYP Danio	363	MQRTQKAK	Danio rerio
Matrin CYP Rat	363	MQRAQRMR	Rattus norvegicus
S6 ribosomal protein	195	KQRTQKNK	Homo sapiens
Slac2c	784	KQRTQVQT	Homo sapiens
Desmoplakin	1608	KQRTQEEL	Homo sapiens

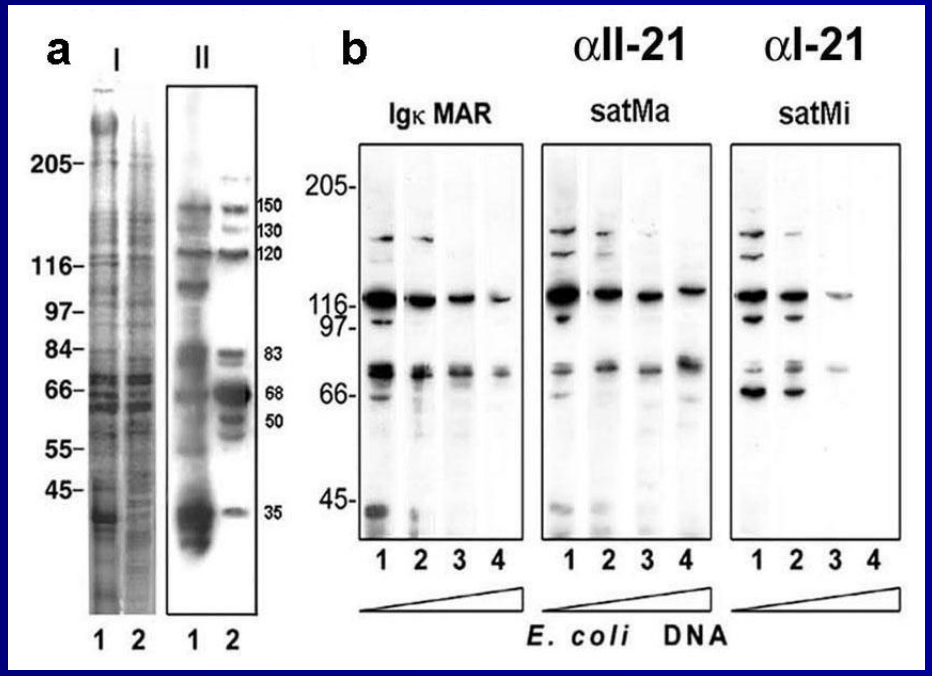
Hrp65-2 (CAC42828 - C. tentants), SAF-A Human (NM_031844 - Homo sapiens, NP_058085 – Mus musculus), SAF-a Xenopus (AAD02820 – Xenopus laevis), DNA-dependent RNA polymerase (NP_701124 - Plasmodium falciparum), Matrin cyclophilin Danio (AAH44189 - Danio rerio), Matrin Cyclophilin Rat (AAC00191 - Rattus norvegicus), S6 ribosomal protein (Q9YGF2 - Oncorhynchus mykiss, NP_989152 - Xenopus tropicalis, NP_990556 - Gallus gallus, CAC69540 - Elaphe sp., AAH27620 – Homo sapiens, AAH10604 – Mus musculus), Desmoplakin (XP_225259 - Rattus norvegicus, NP_004406 – Homo sapiens, BC033467 – Mus musculus), Slac2c (BAC15555 - Homo sapiens, AAP94626 – Rattus norvegicus, Q8K3I4 – Mus musculus).

Saf-a/hnRNP U DNA binding

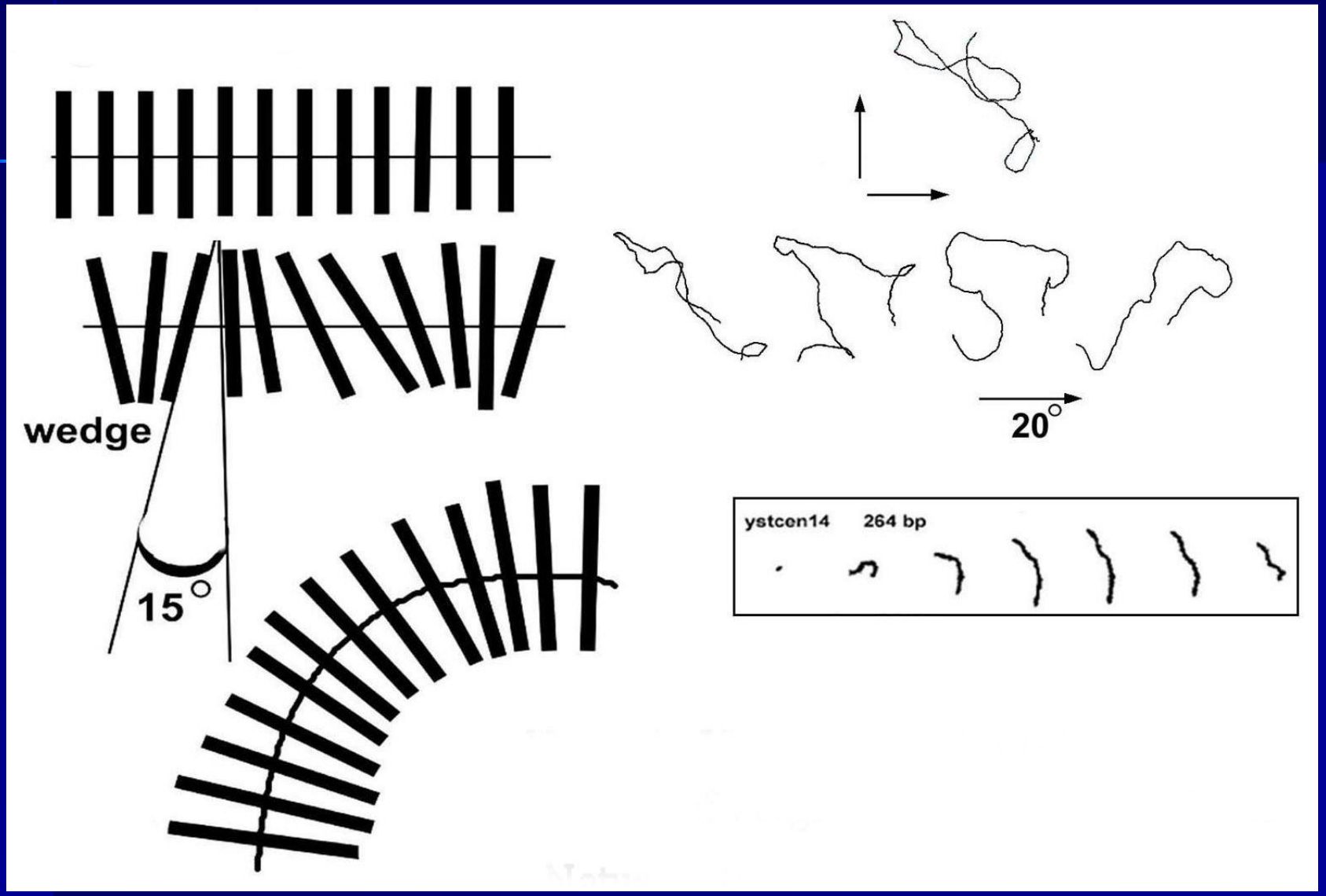


GMS Southwestern blot

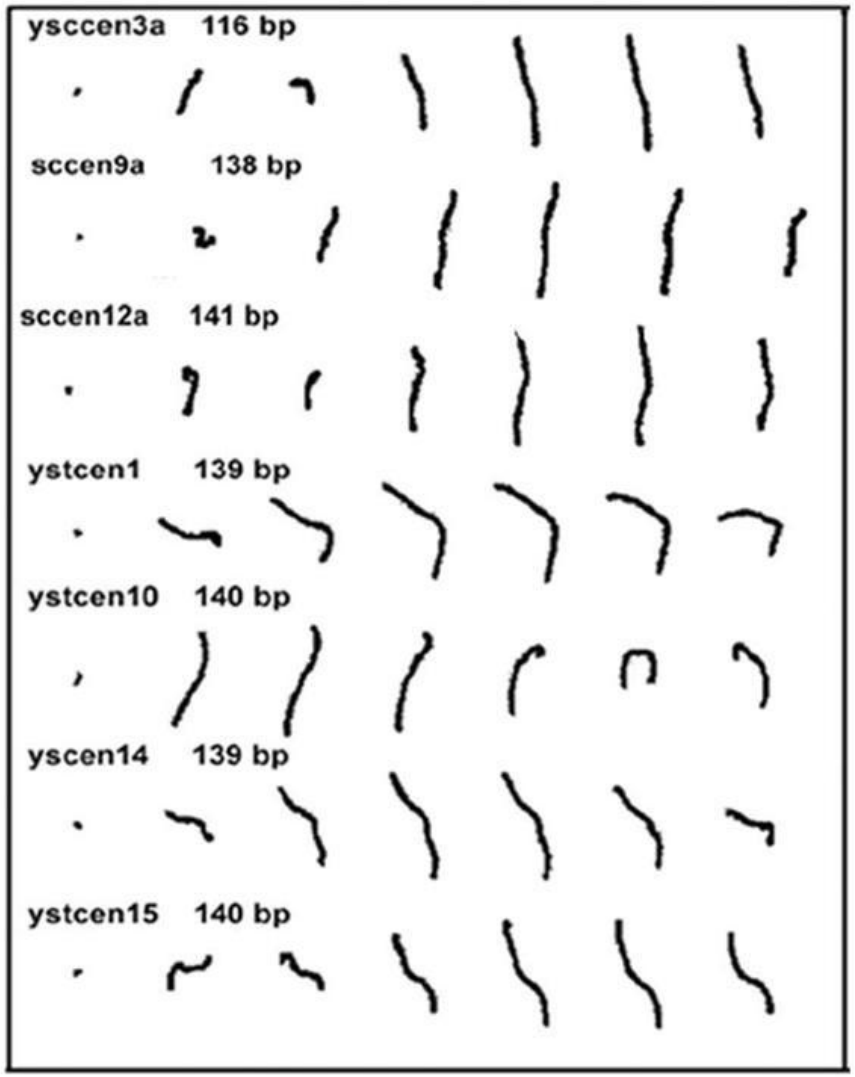




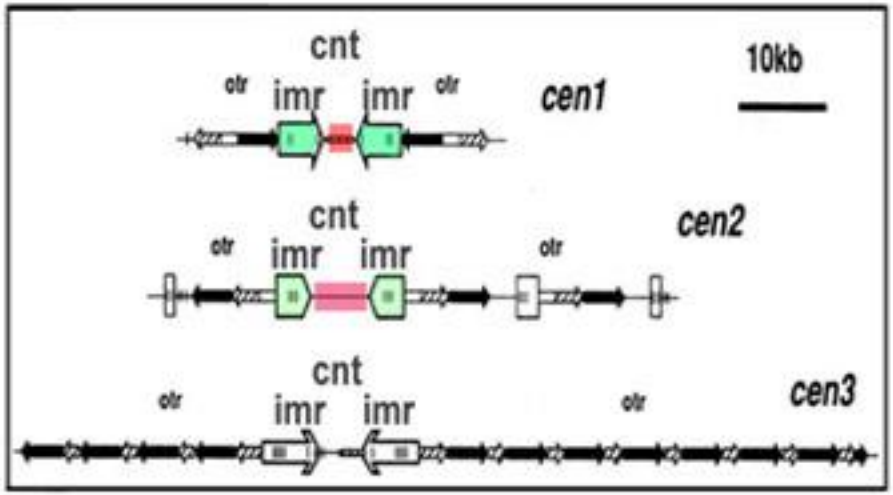
Lobov et al., 2001

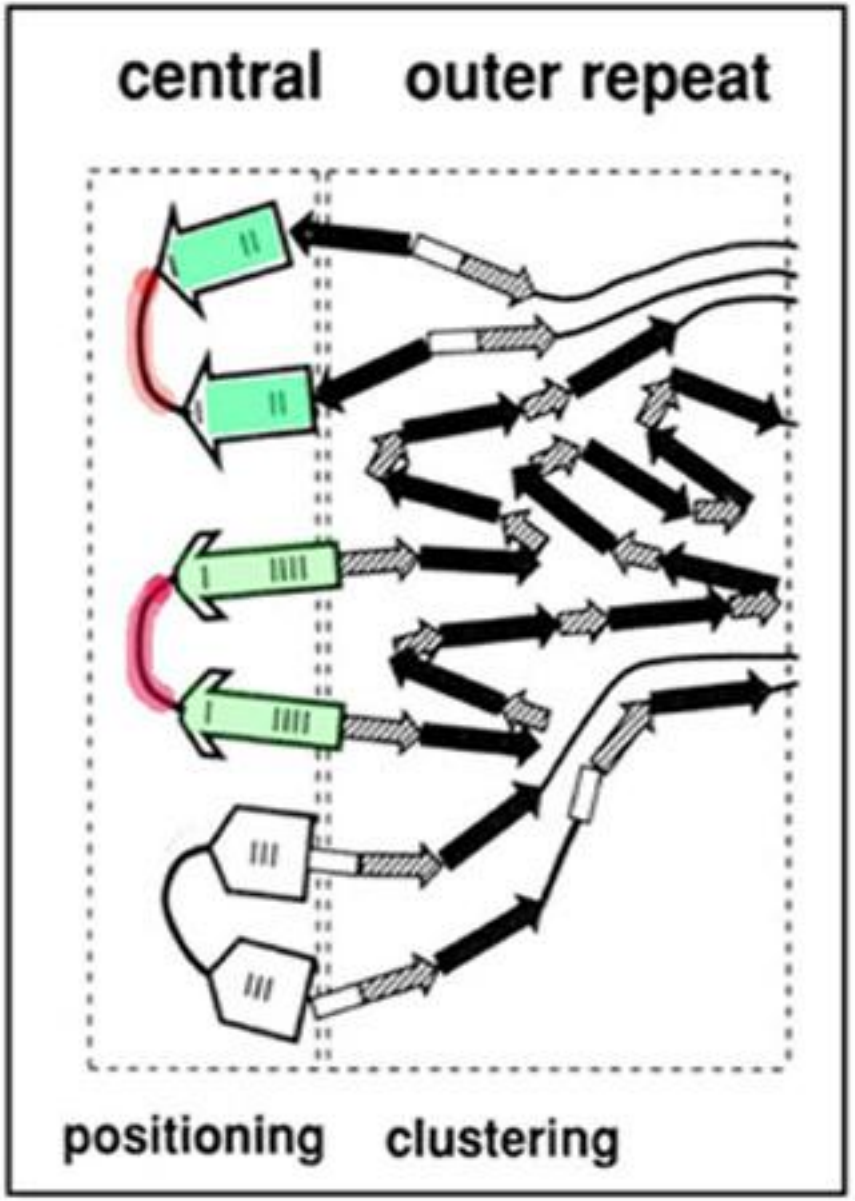

wedge
15°
20°
ystcen14 264 bp

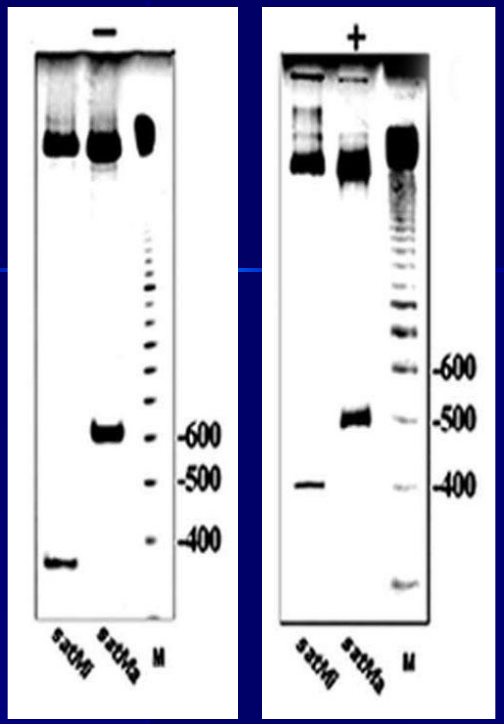
S.cerevisiae

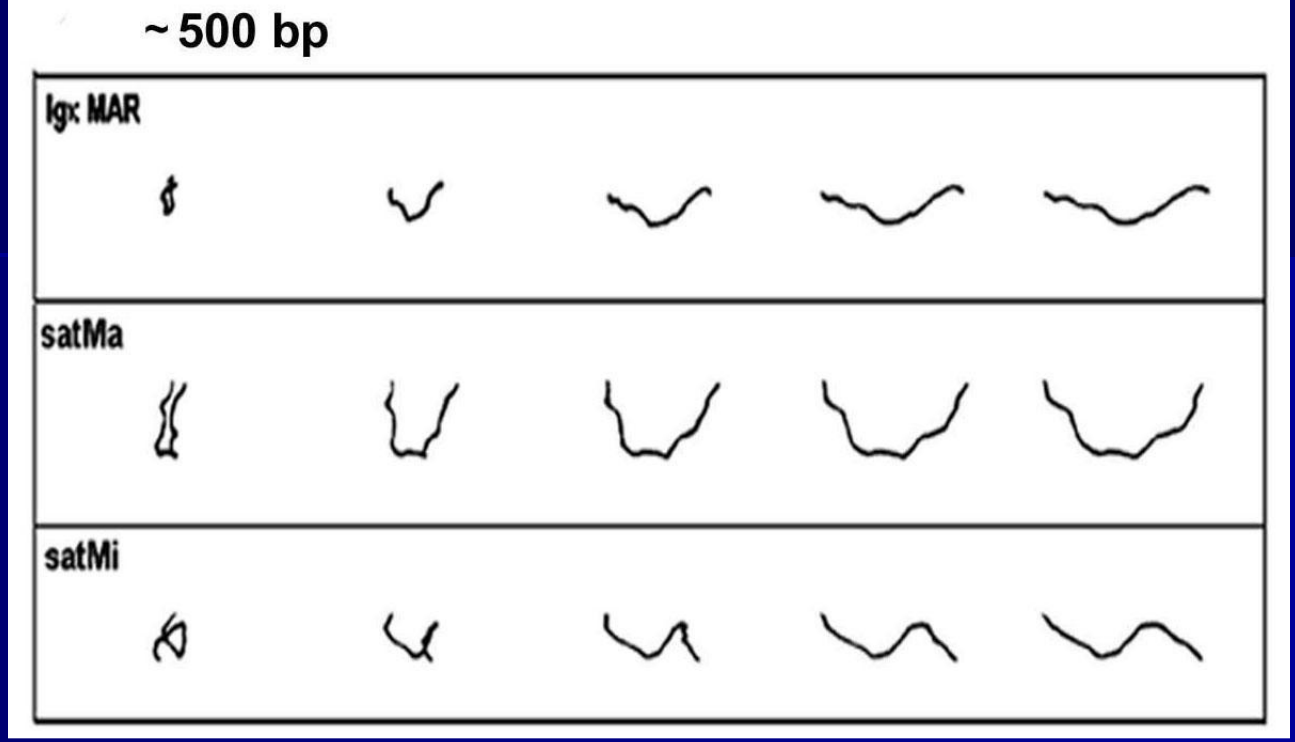


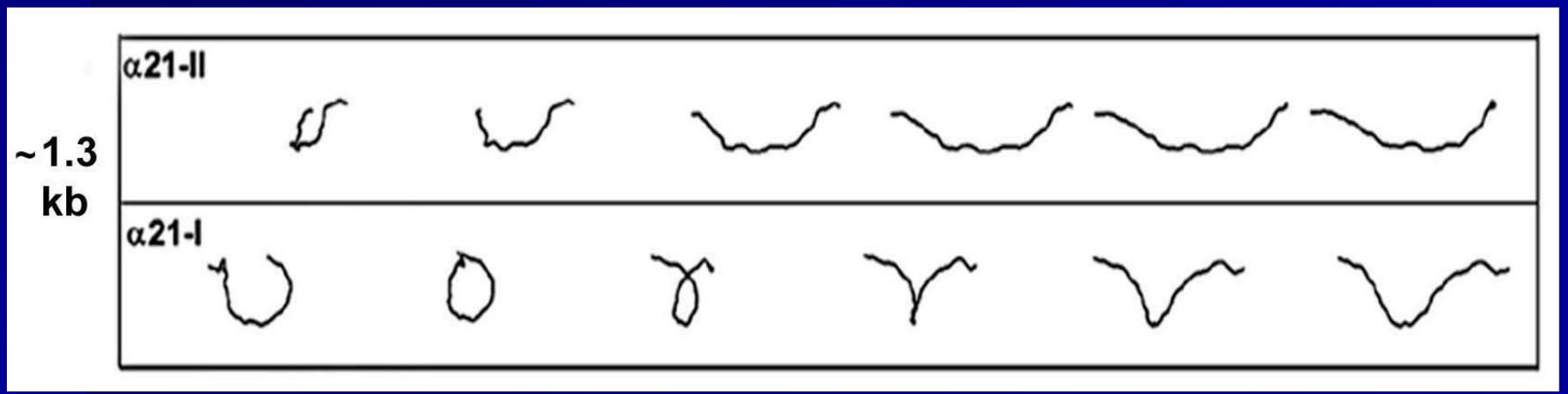
S.pombe


cnt
otr
imr
imr
otr
cen1
10kb
cnt
otr
imr
imr
otr
cen2
cnt
otr
imr
imr
otr
cen3

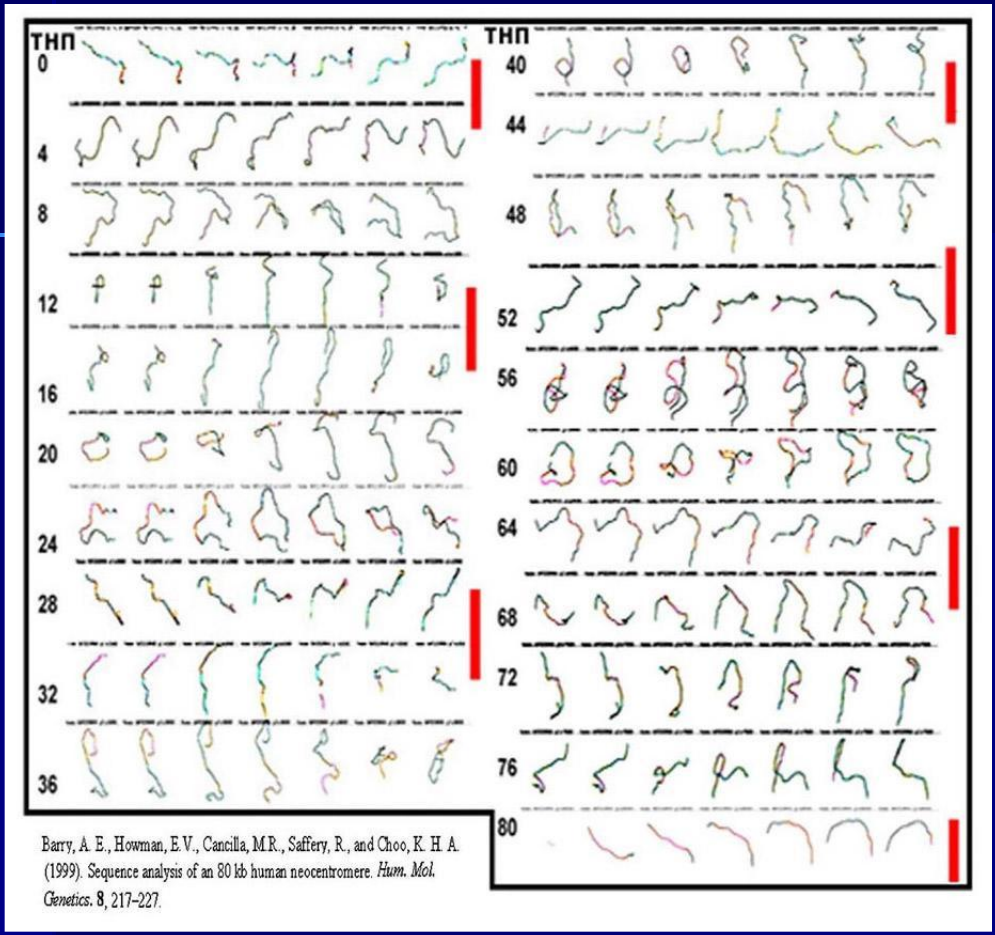

central
outer repeat
positioning
clustering



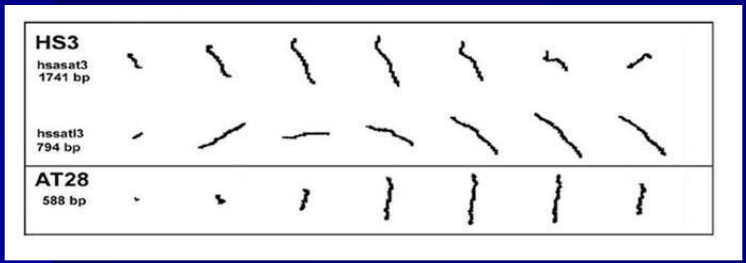


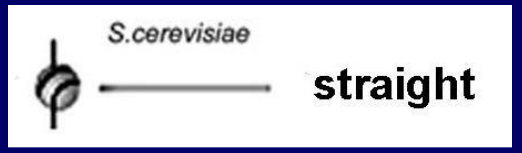


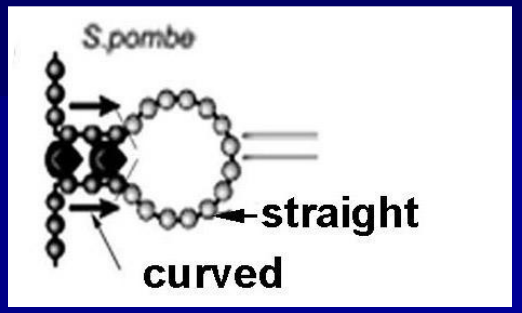
Lobov et al., 2001

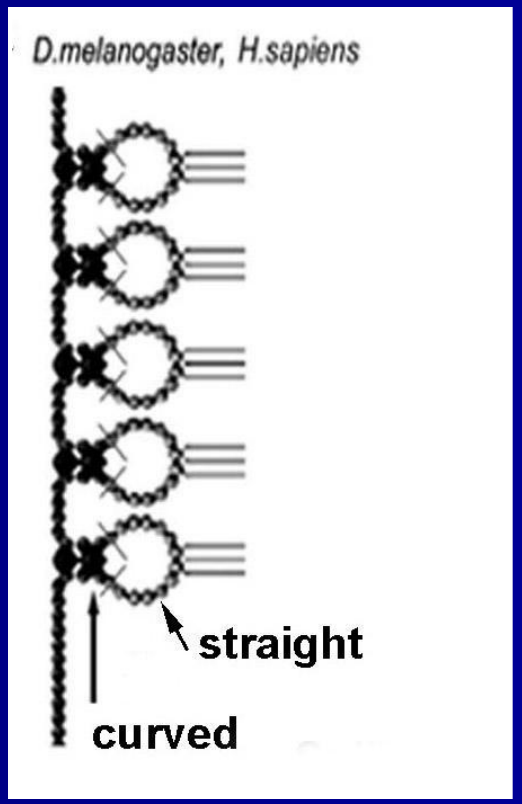


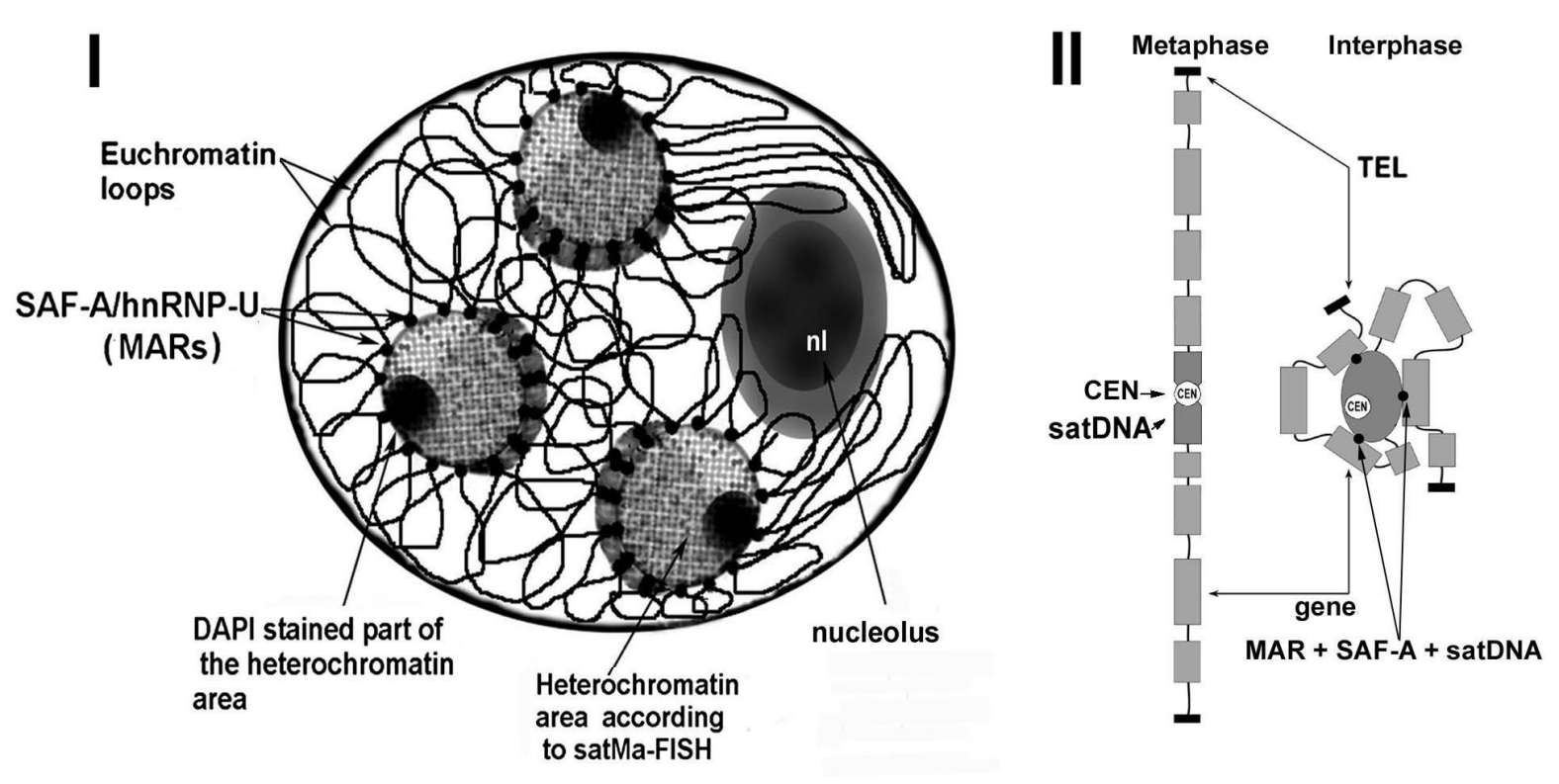
Barry, A. E., Howman, E. V., Cancilla, M. R., Saffery, R., and Choo, K. H. A. (1999). Sequence analysis of an 80 kb human neocentromere. *Hum. Mol. Genetics.* **8**, 217–227.



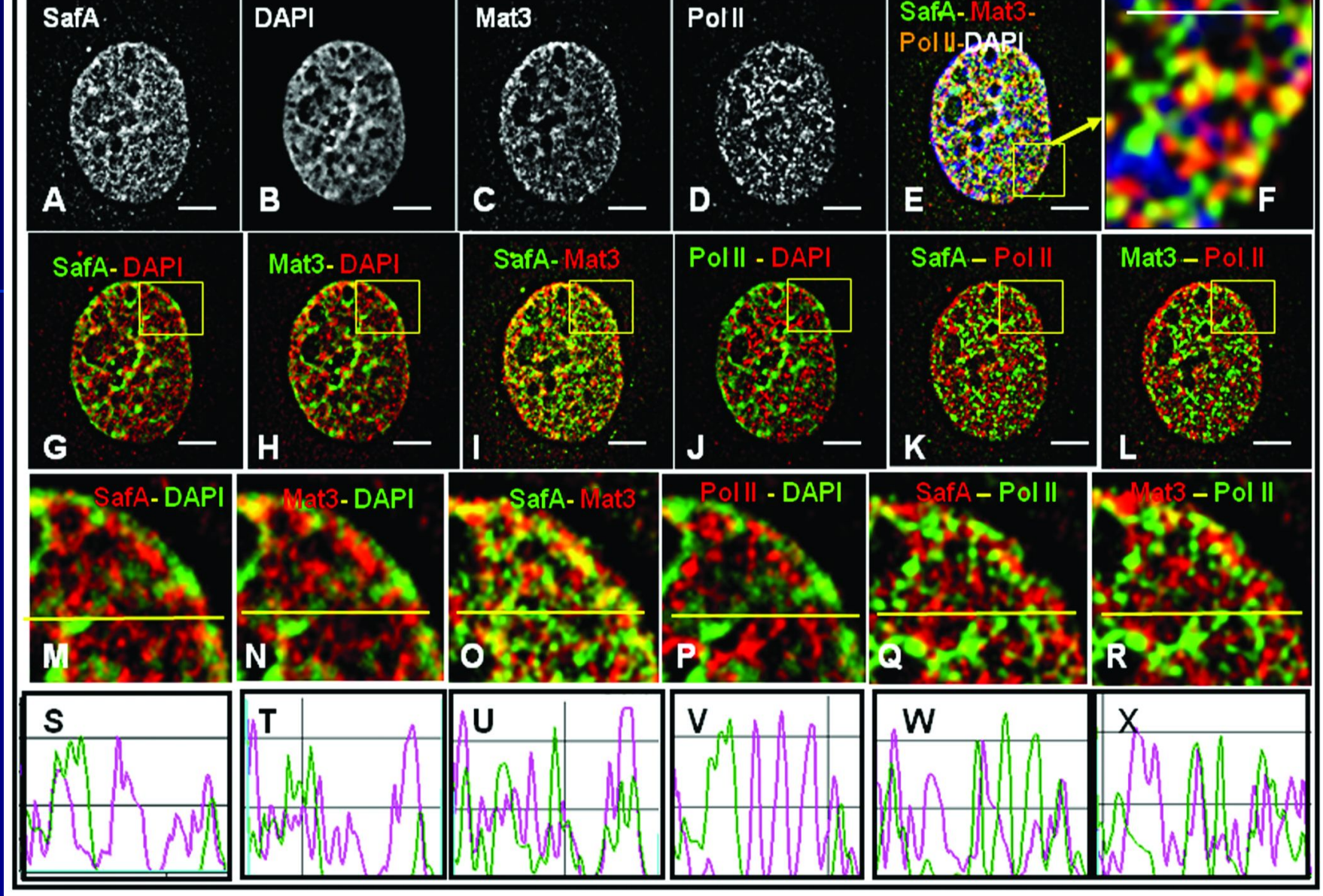




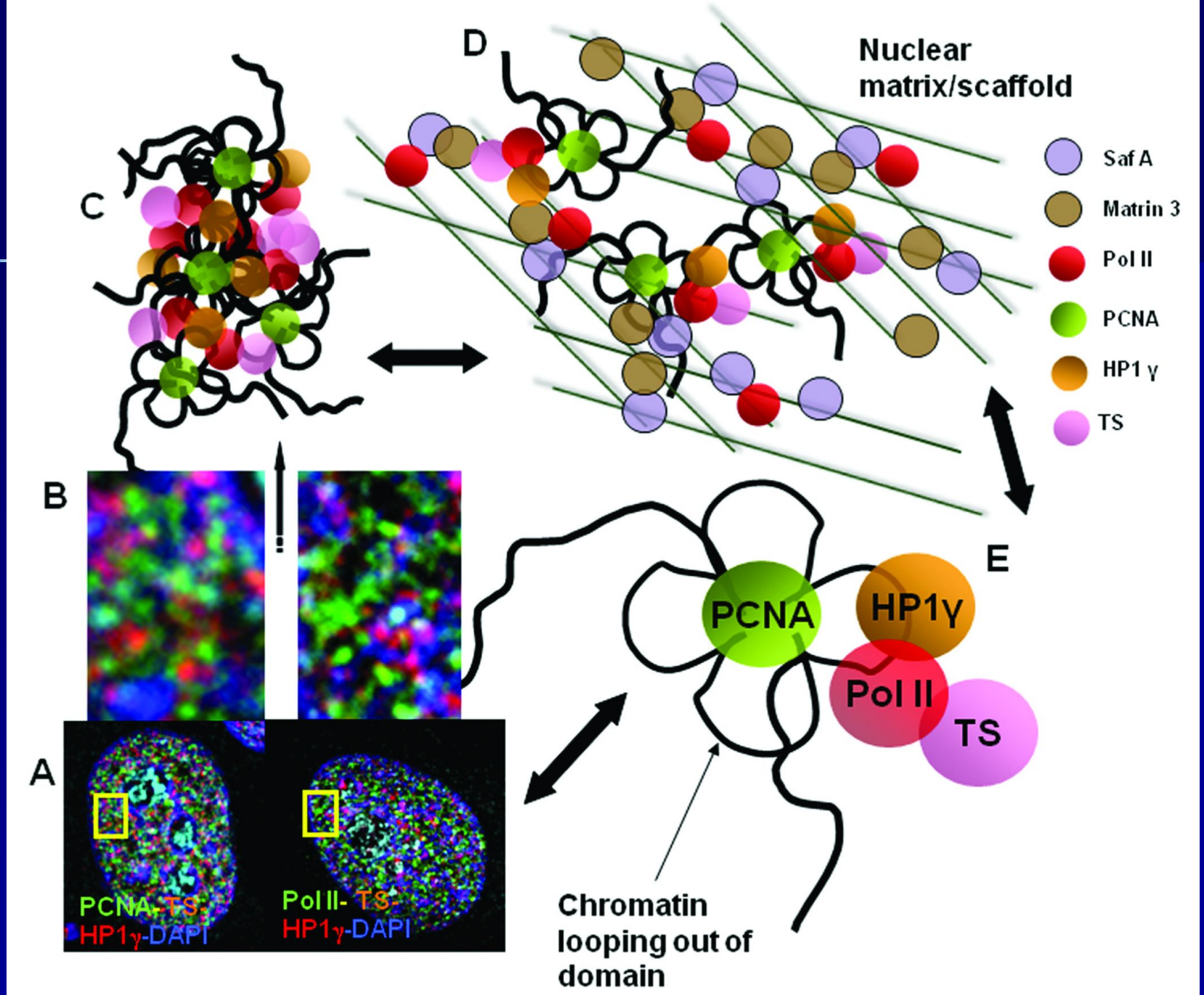




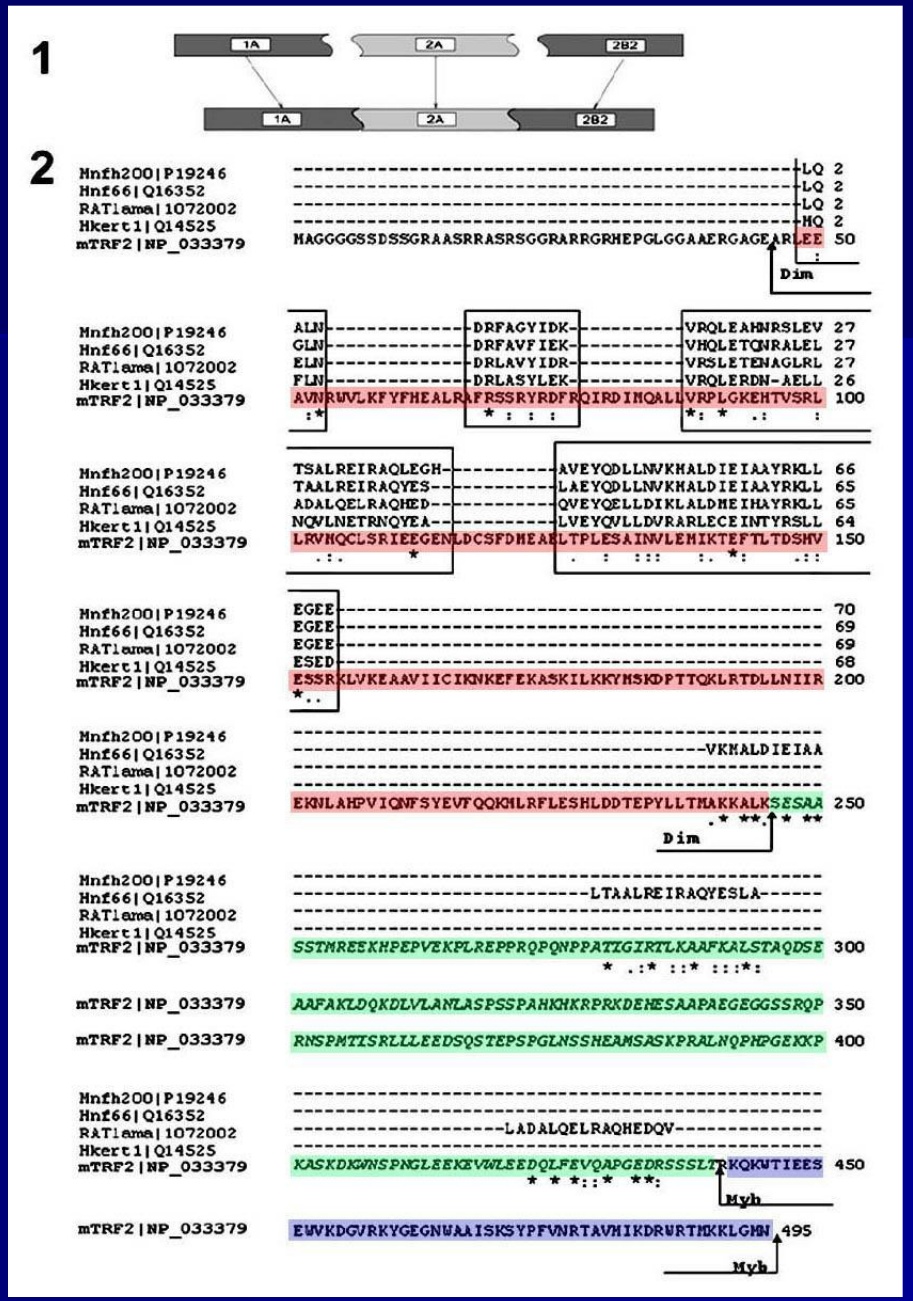
Podgornaya et al., 2003



Identifying Functional Neighborhoods within the Cell Nucleus: Proximity Analysis of Early S-Phase Replicating Chromatin Domains to Sites of Transcription, RNA Polymerase II, HP1γ, Matrin 3 and SAF-A Malyavantham ……Berezney, J Cell Biochem. 2008

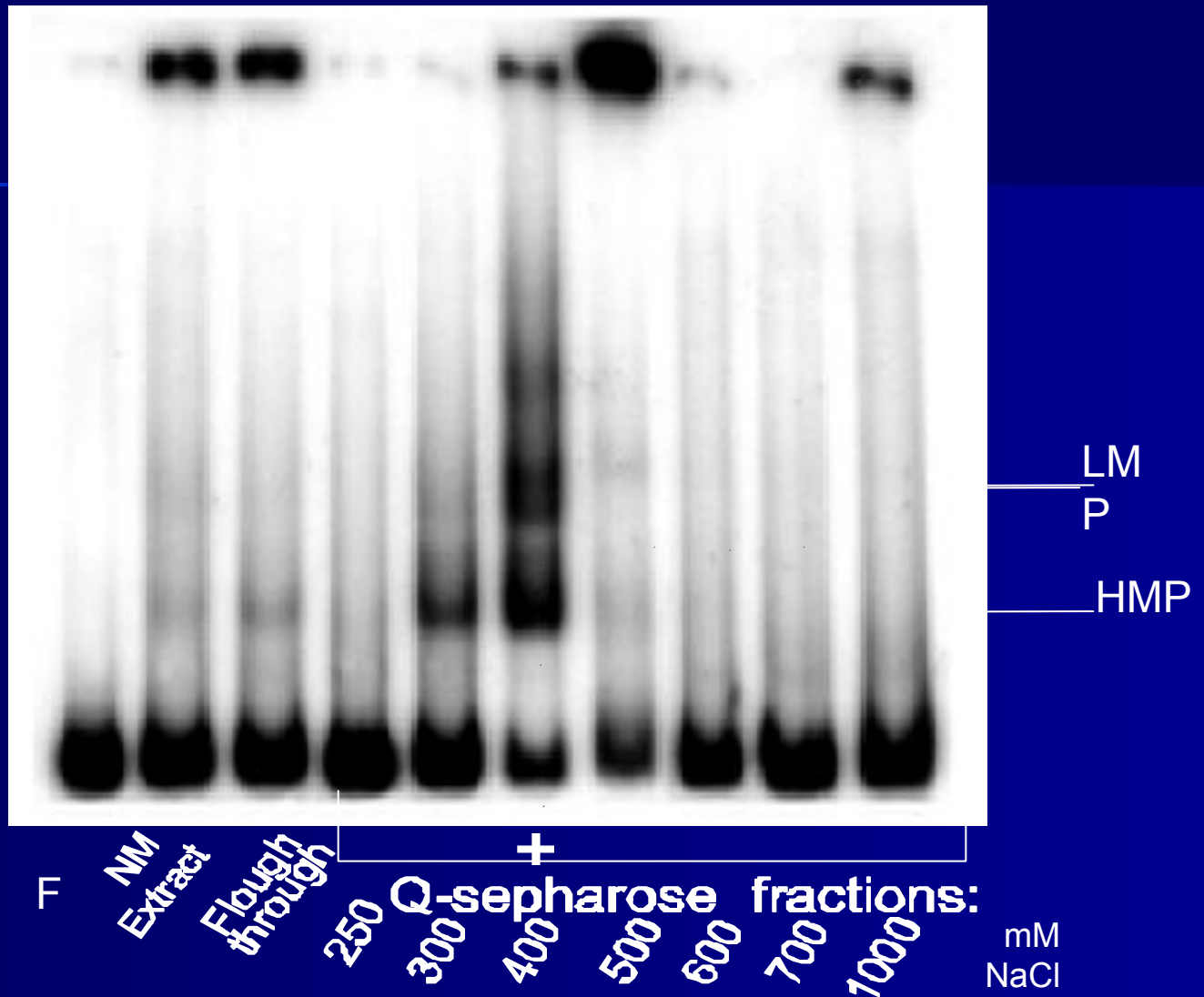

A
B
C
D
E
Nuclear matrix/scaffold
Saf A
Matrin 3
Pol II
PCNA
HP1 γ
TS
PCNA- TS- HP1γ-DAPI
Pol II- TS- HP1γ-DAPI
PCNA
HP1γ
Pol II
TS
Chromatin looping out of domain

TRF2 sp domen



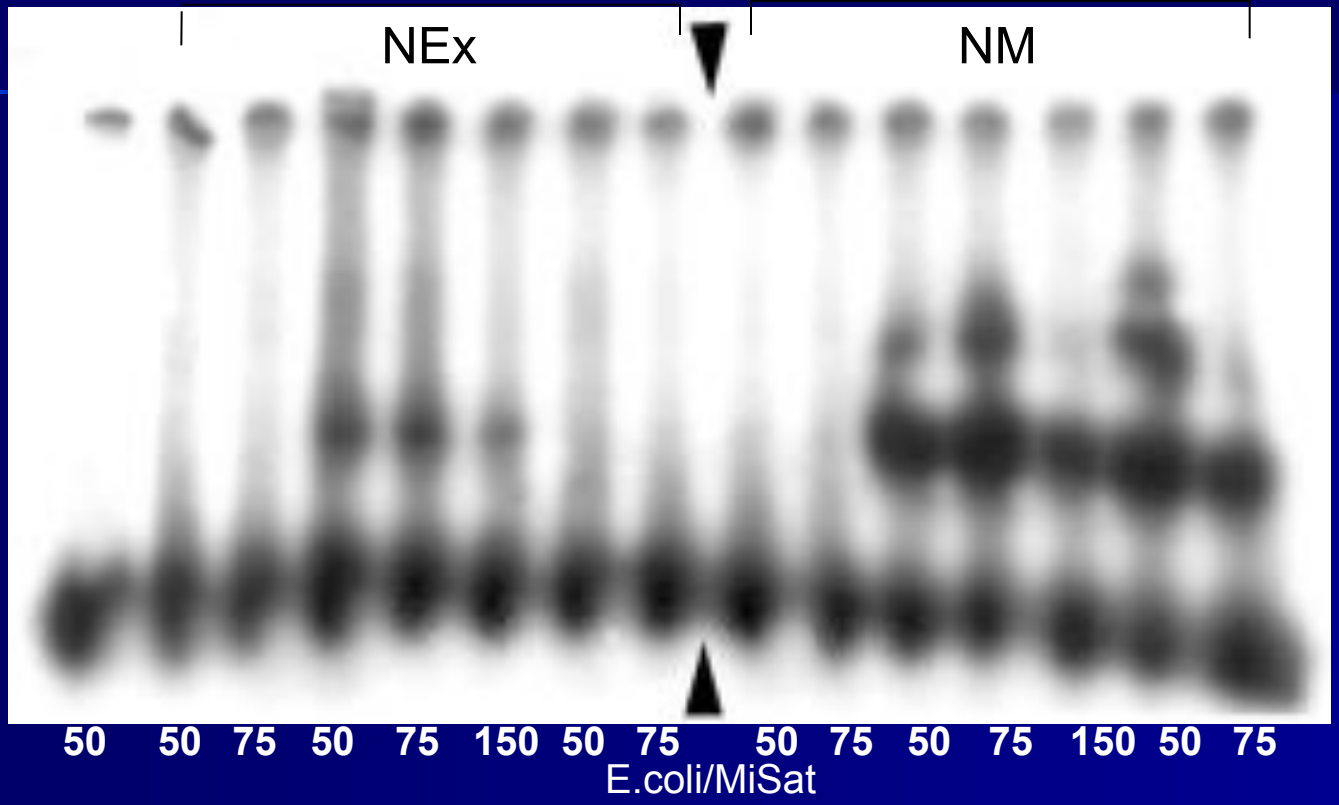
Voronin et al.,

Homo sapiens



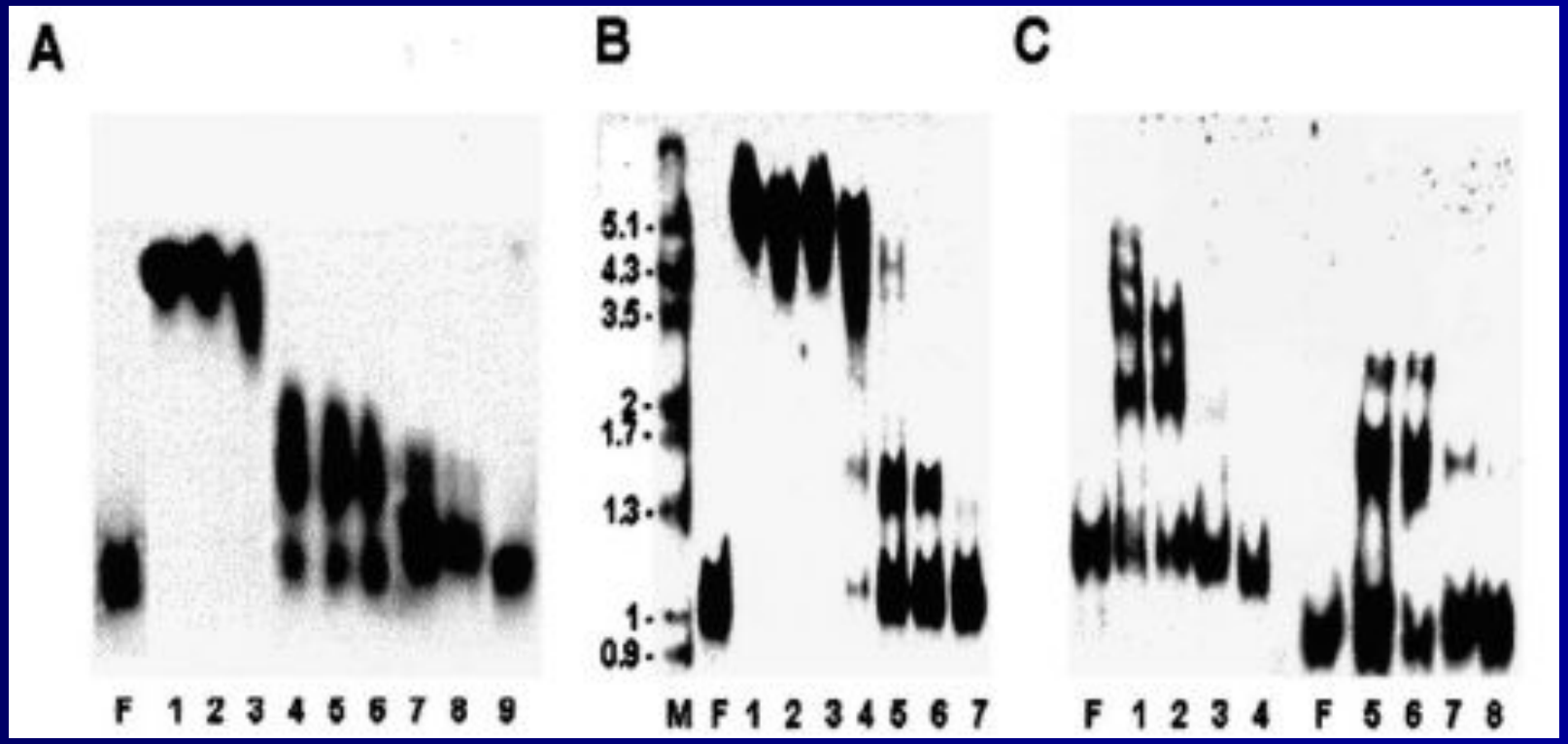
- ЯМ человека связывает центромерную альфа-сателлитную ДНК

Mus musculus



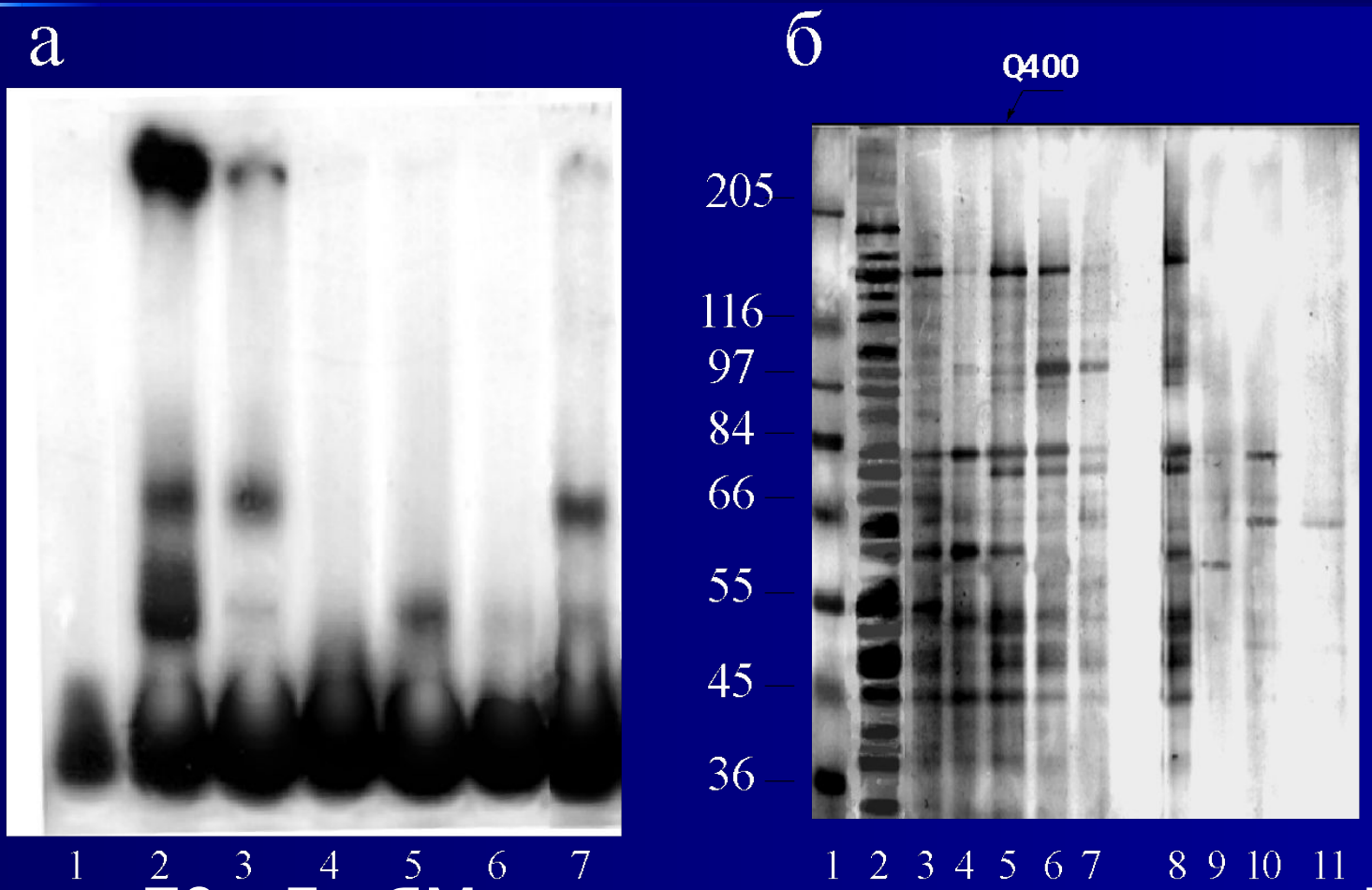
- ЯМ мыши связывает ДНК минорного сателлита

Homo sapiens



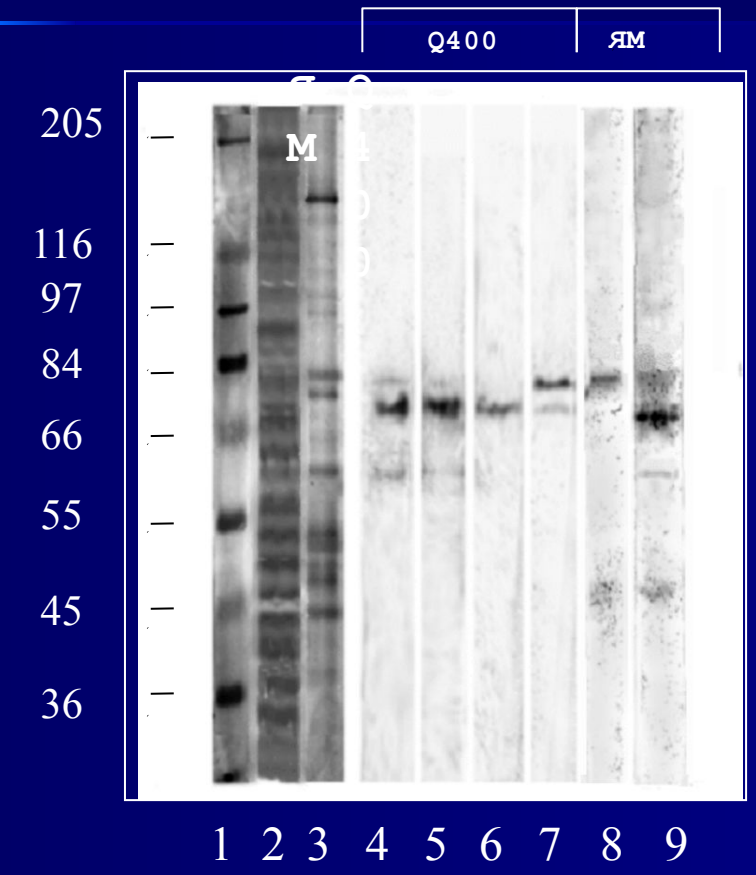
- ЯМ человека связывает прицентромерный сателлит 3

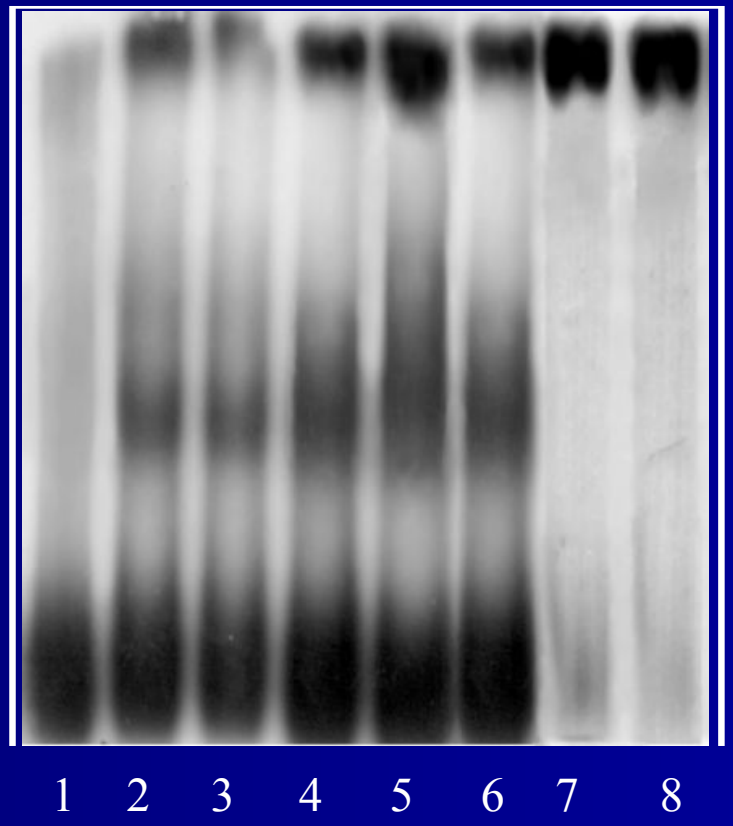
Аффинная хроматография



- Белок 70 кДа ЯМ человека связывает а-сат ДНК

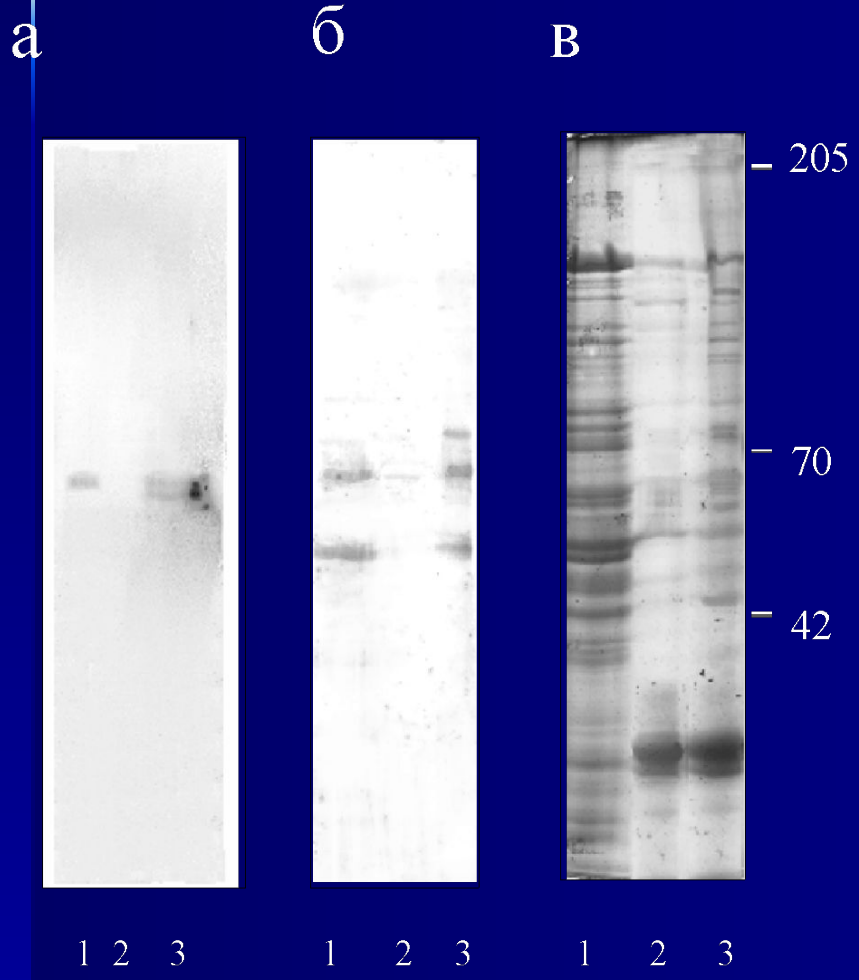
Получение АТ





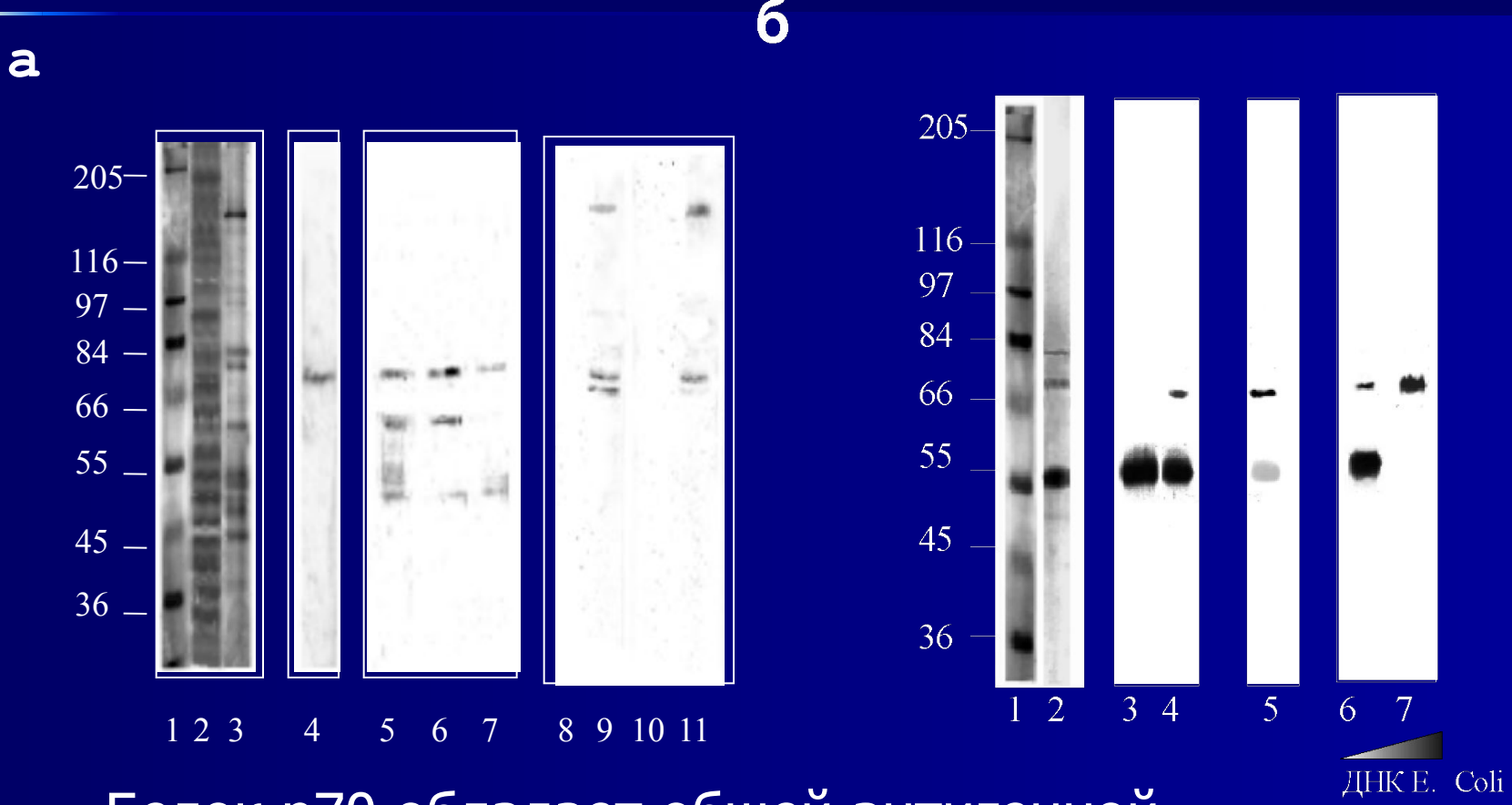
- Поликлональные АТ распознают белки массой p80, p70 и p57

Саузвестерн гибридизация



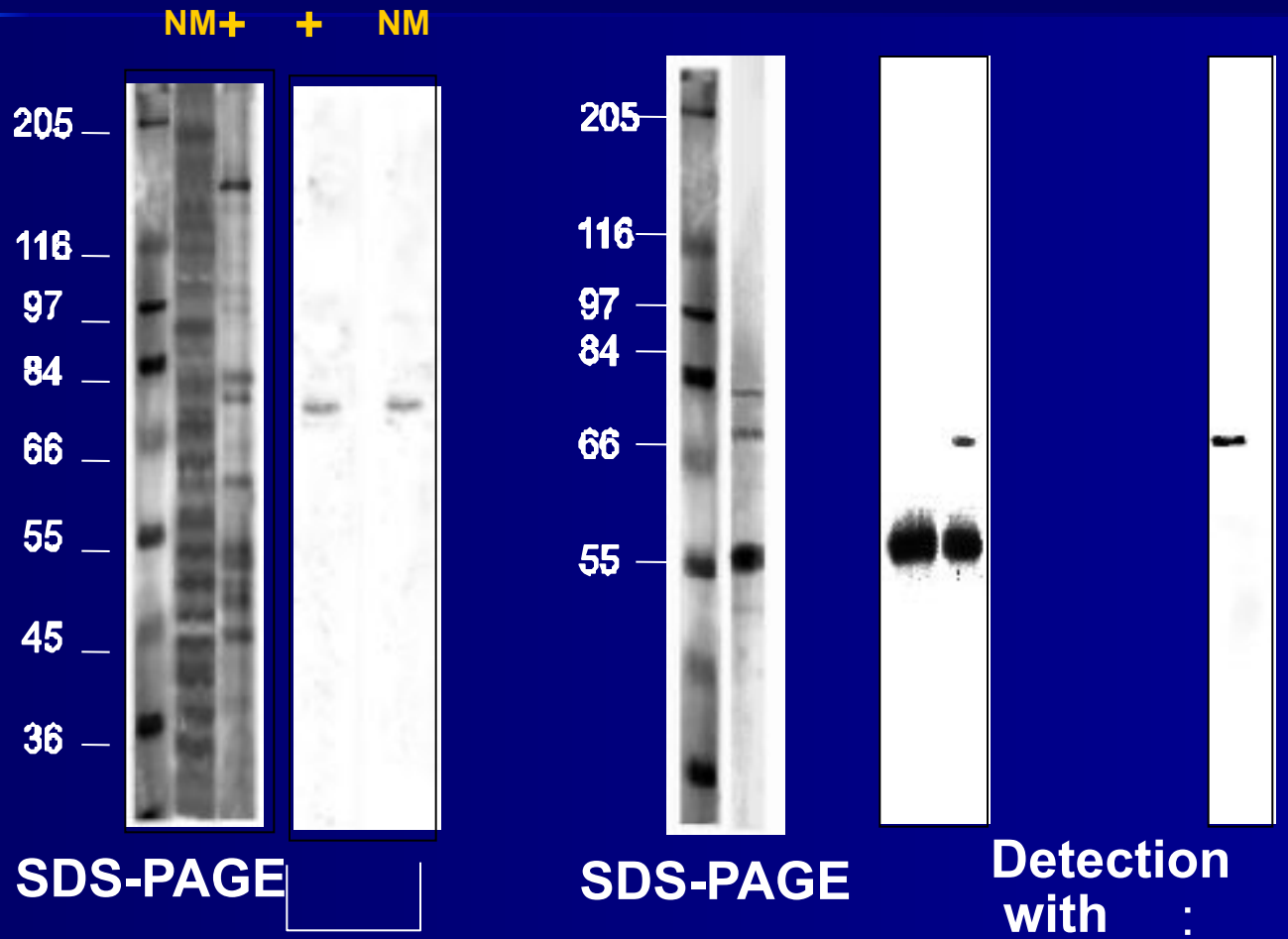
- Среди белков, распознаваемых сывороткой именно p70 отвечает за связывание с ДНК

Выявление IFA детерминанты



- Белок p70 обладает общей антигенной детерминантой с белками промежуточных филаментов

P70 связывает а-сат ДНК и ДНК сателлита 3



A
B
C
G/A F +
+ F
+ F
TCCCTTTCGA GTCCATTCAA TGATTCCATT CCAGTCCATT CGATGATTCC ATCTGATTCC ATTCAATGAA TCCATTCTAT
TAGTTGT AGCTTACTTA GCTTACCTTT GTGGTAGCTT AACTTTGCTT ACCTTAATAG TACTTTAACT TTACCTACCT GAGTAGTAGC TTACCTA
TCCCTTT-CGAG-
TCCATTCAATG-ATTCCATT-CCAGTC
CATTCGATGATTCCATCTGATT-CCA---
TTCAATGAATCCATTCGATT-CCATTC
TATGACGATTCCATTC-ATTTCCATCT
GATGATGATTCCATTCGATT-CCATTTC
---AATGATACCATTCGATT-CCATTG
GATGATGATTTCAATCAATTTT-ATTC
---GATGATTCCATTCGAAT-CCATTC
GATGATGAGTCCA TGGATTTCAATTT
CATGATAATTCCATTCG-TTTC ATTC
GATGGTGTTTCCATTCGATT-C-ATTC
GATGTTGATTCCATTAGCTT-CCGTTG
GATGATGATTCCATTCGGGT-CCATTC
GATGATGATC
* - GATGATGATTCCATTCGATT-CCATTC

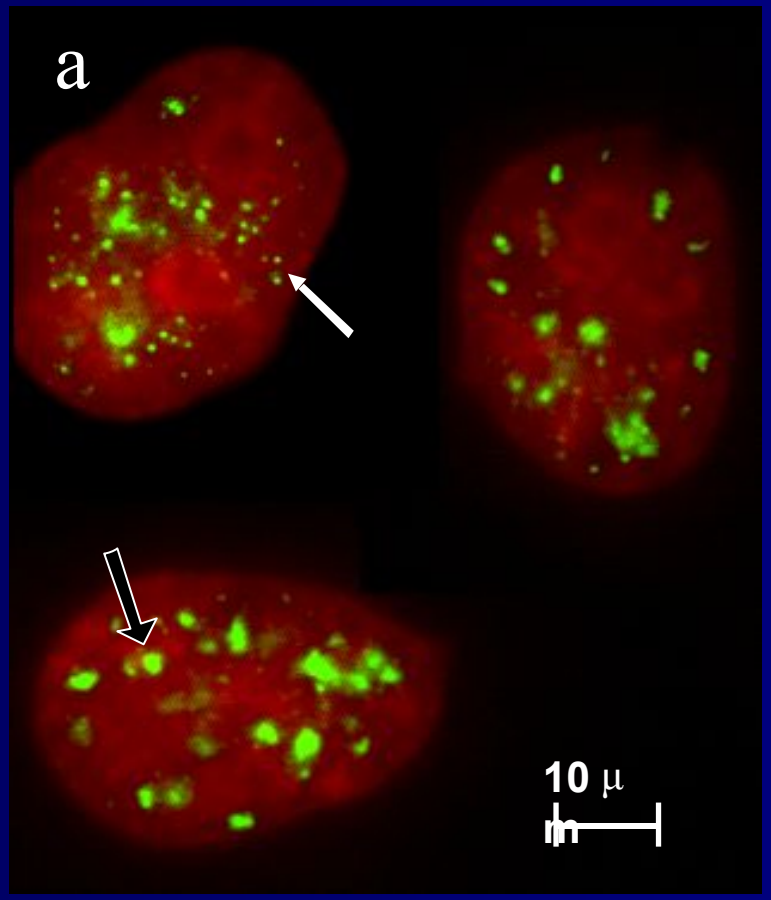
Mass-spectrometry analysis:

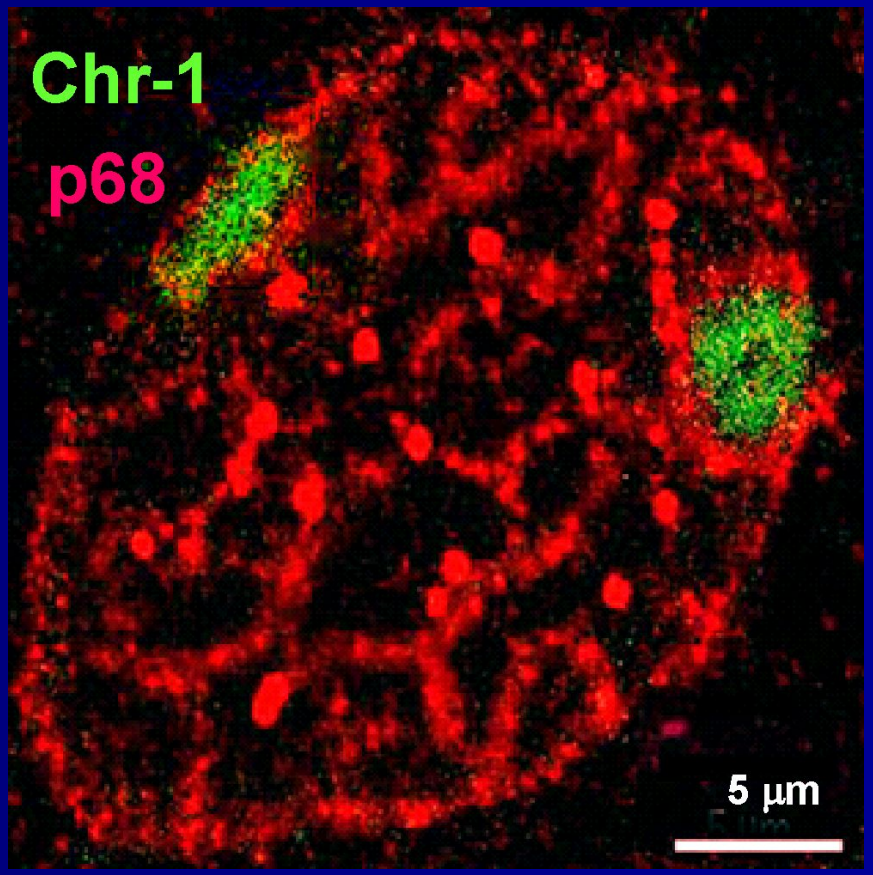
p70=RNA-helicase p68= DEAD/H box polypeptide 5

Распределение p70 в ядре

HeLa

MRC5 (сульфат аммония)

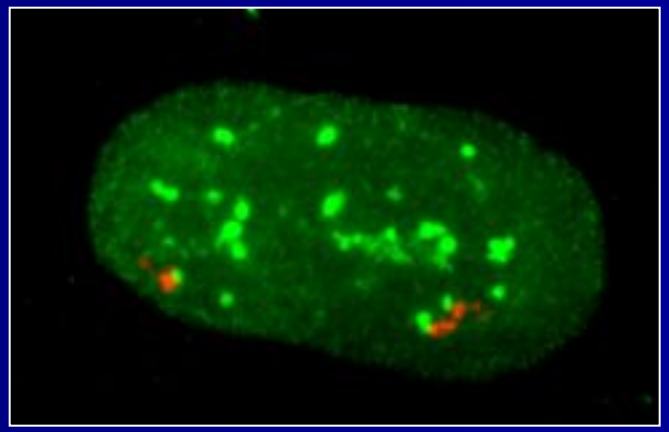




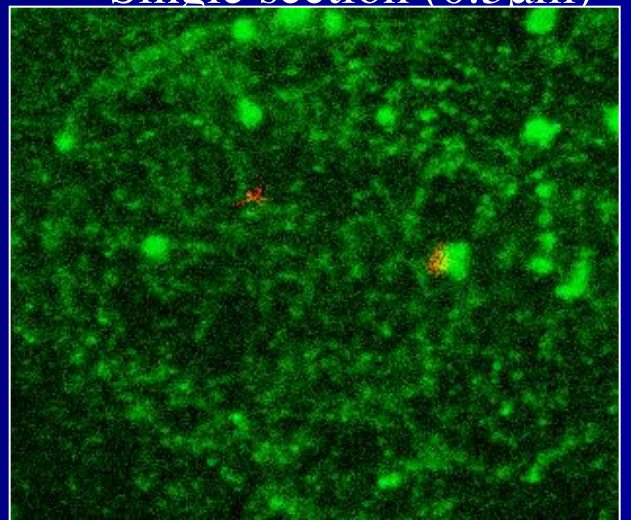
P70 по отношению к а-сат ДНК

MRC5,
Upregulated

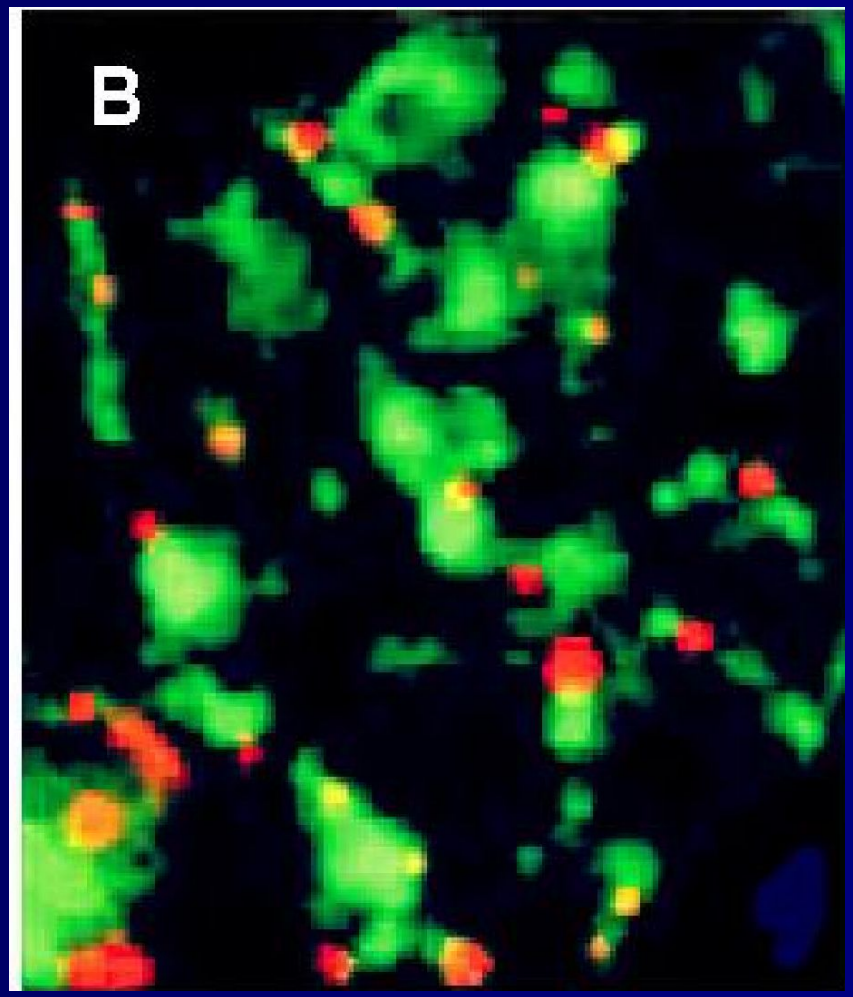
epifluorescent



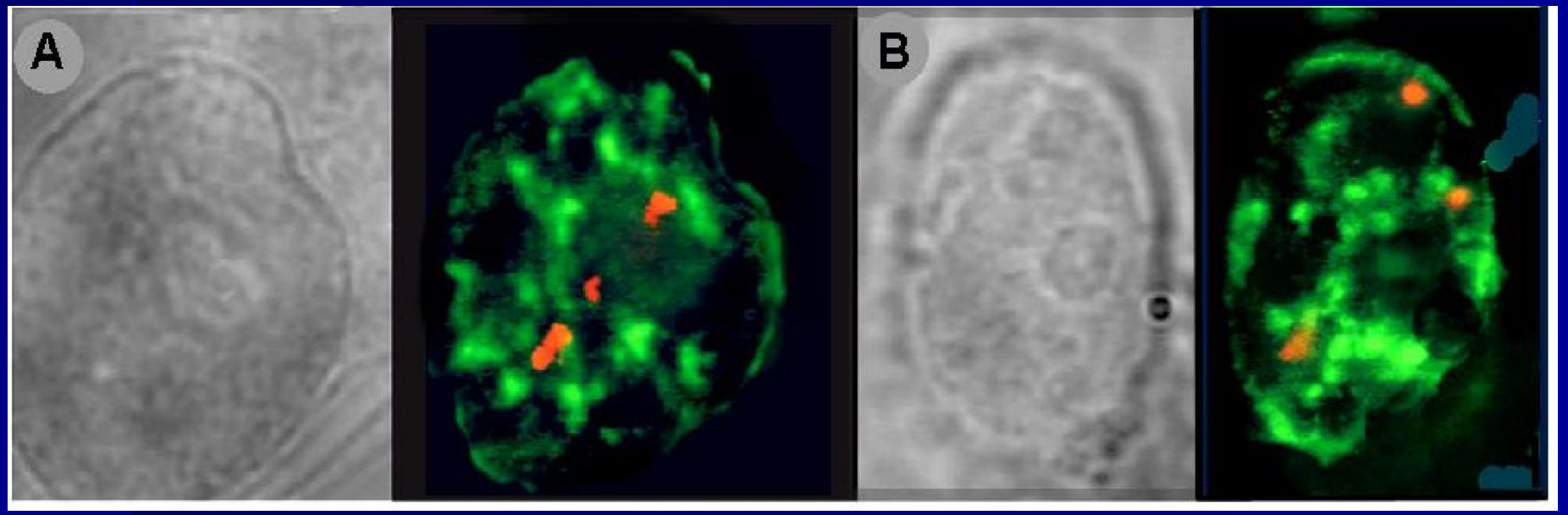
Single section (0.3µm)



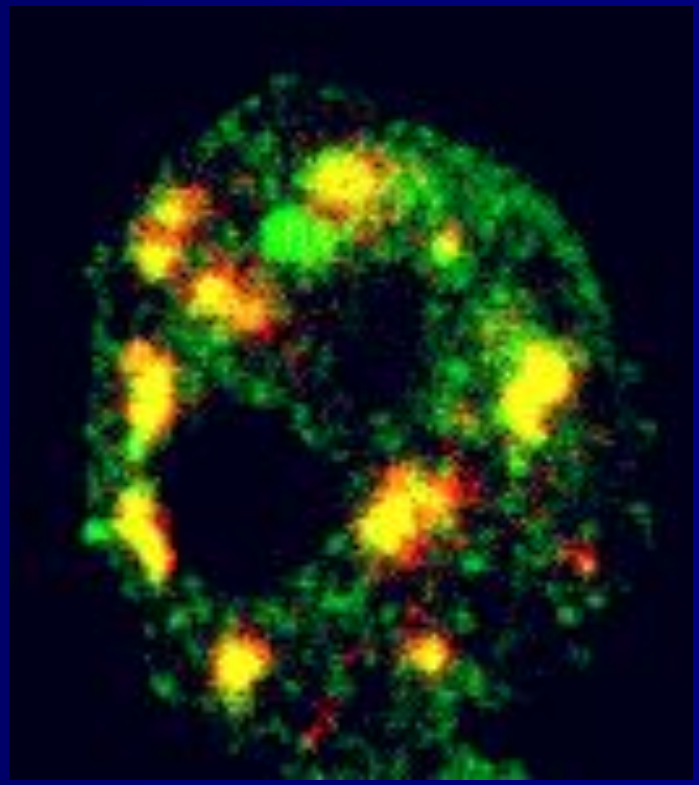
HeLa

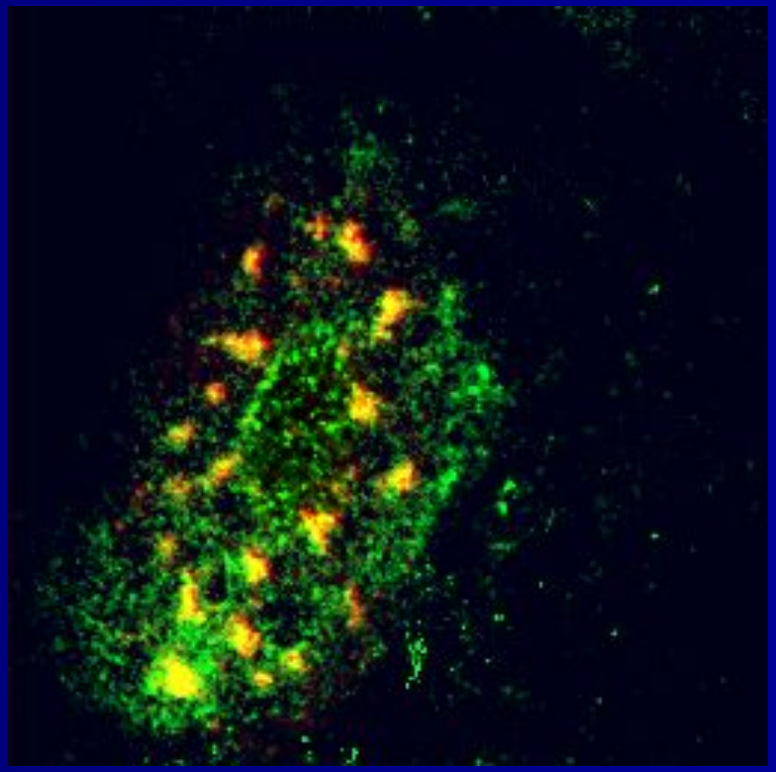


P70 по отношению к сат3



Определение домена локализации p70



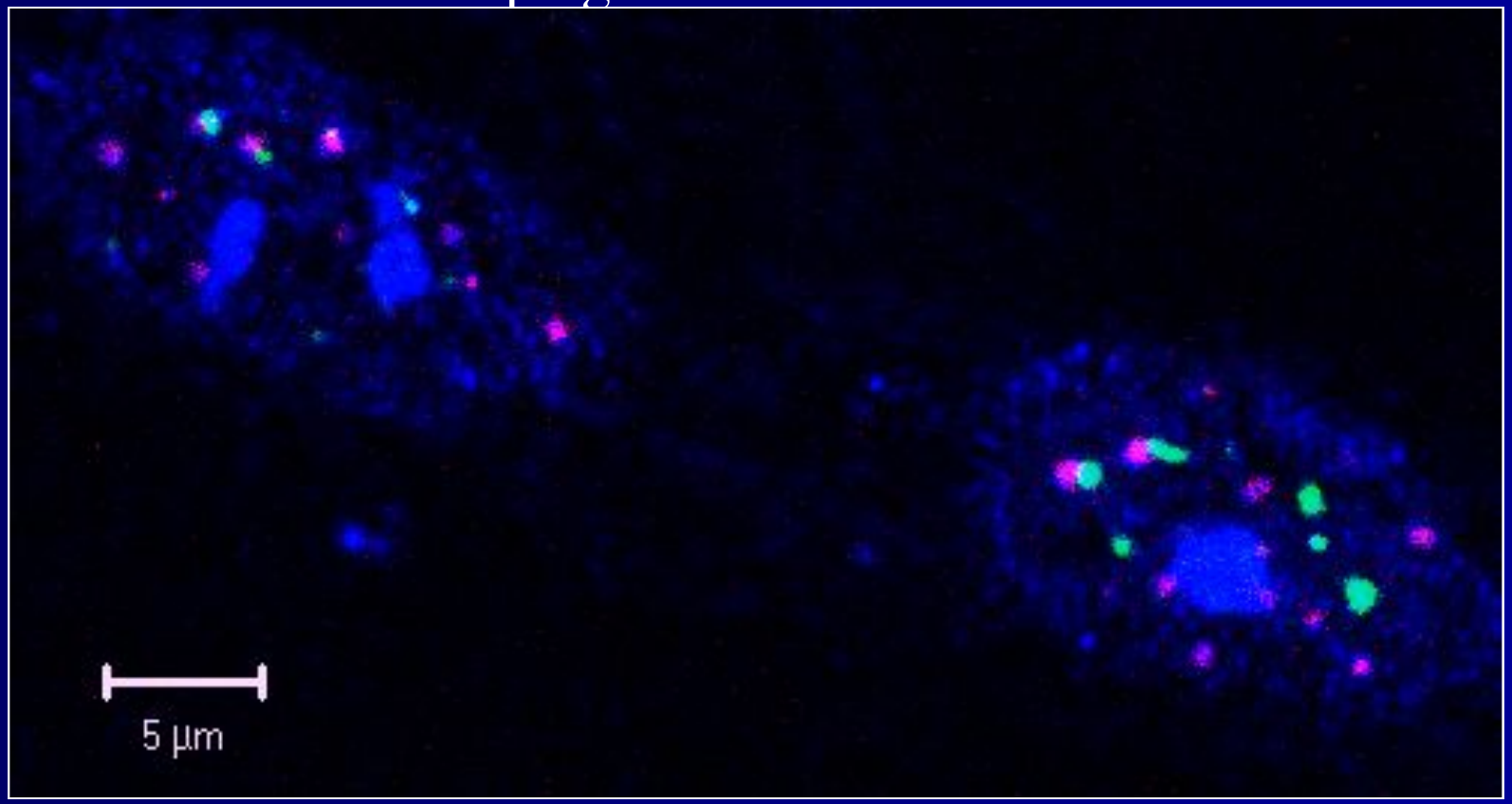


- В фибробластах и HeLa p70 находится в SC35 доменах и фибрилах между ними

In MRC5, p70 has been found in PML and SC35 domains

Upregulated

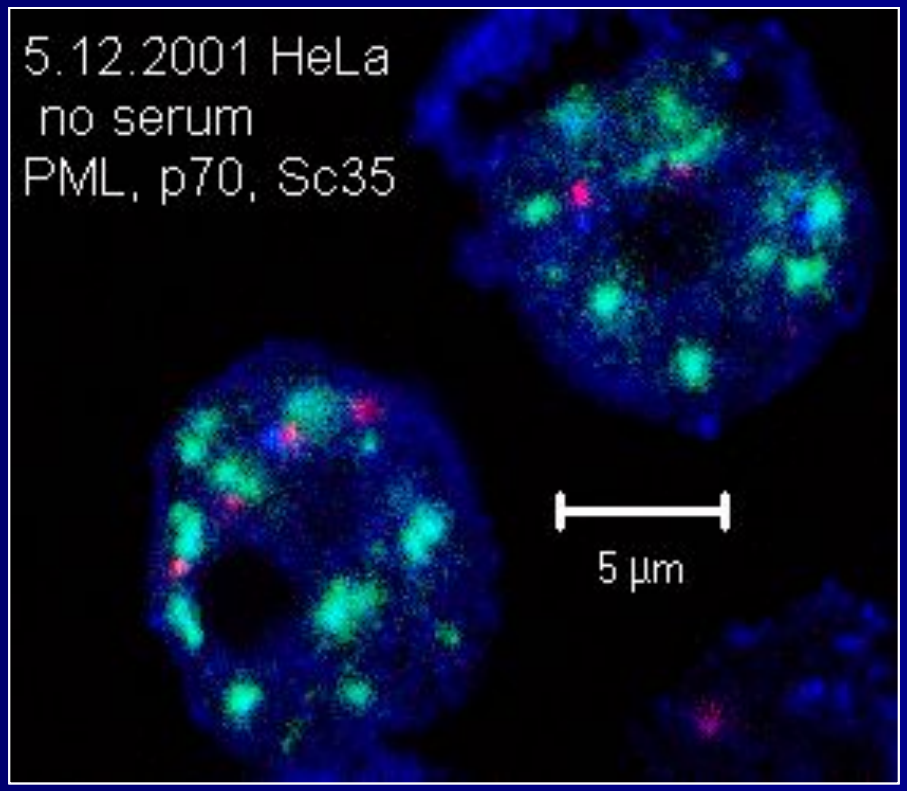
P70
Sc35
PML

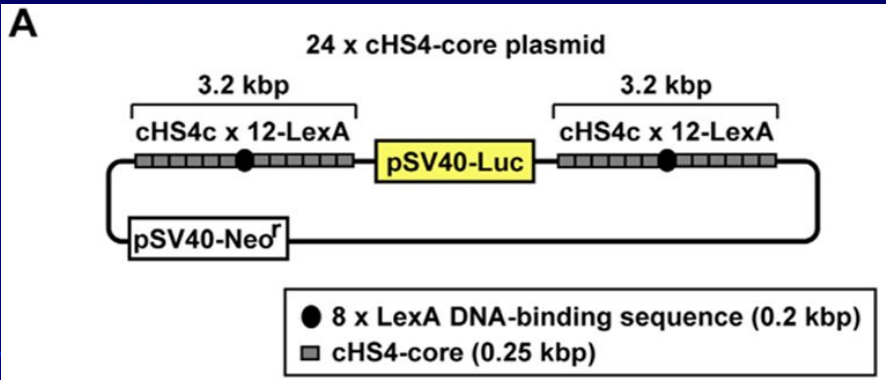


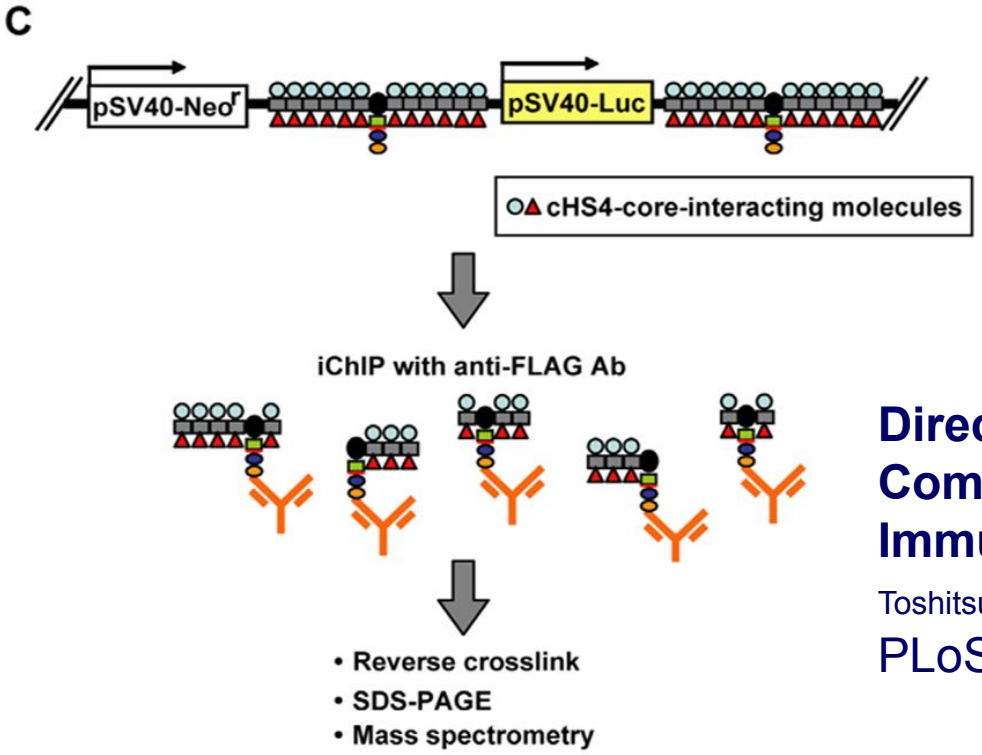
In HeLa S (SD), p70 has been found in SC 35 but not in PML

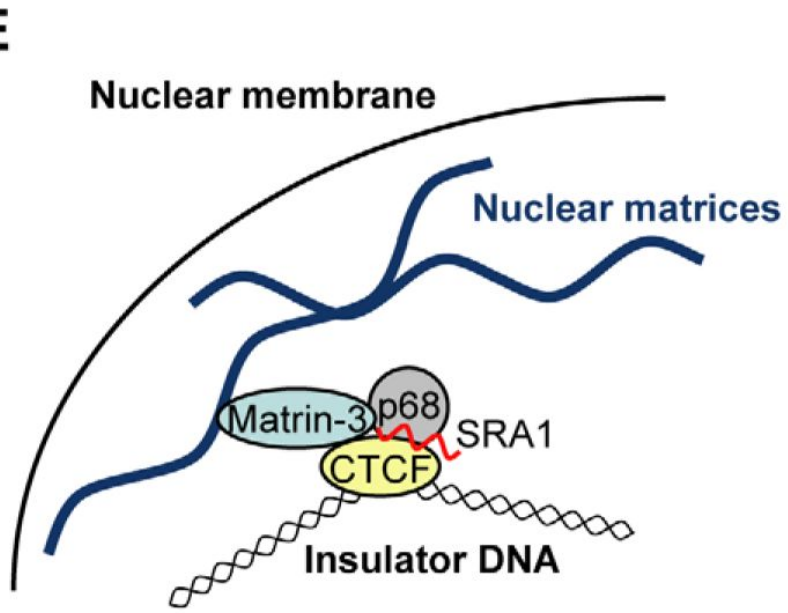
P70
Sc35
PML

HeLa S, grown in serum deprived medium









CCCTC-binding factor (CTCF)

Direct Identification of Insulator Components by Insertional Chromatin Immunoprecipitation

Toshitsugu Fujita, Hodaka Fujii*

PLoS ONE, 2011

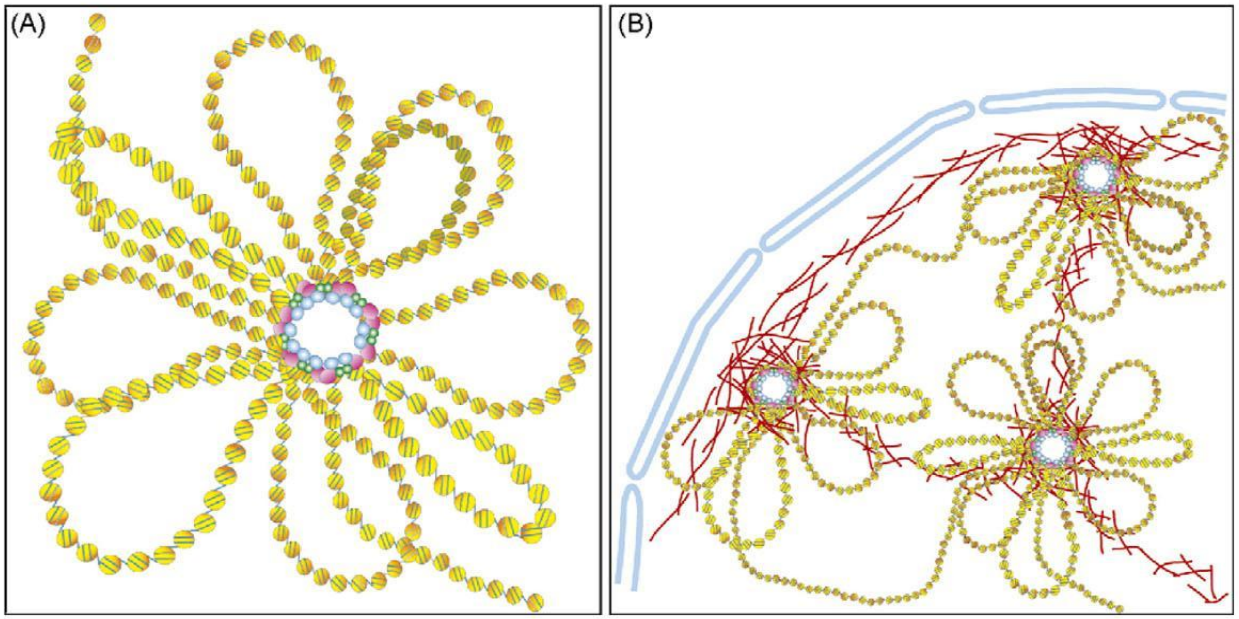
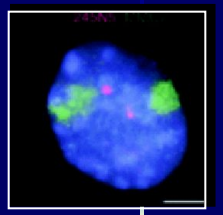


Fig. 1. Insulator elements organize the chromatin fiber in the nucleus by establishing separate compartments of higher-order chromatin structure. (A) Domains of open chromatin (yellow nucleosomes) are flanked by insulators (pink, blue and green spheres) that interact together to form a loop. (B) Diagram showing part of a nucleus with compartmentalized chromatin, anchored in part to the nuclear periphery by interactions of the insulators with the nuclear lamina (red lines).

Insulator elements organize the chromatin fiber in the nucleus by establishing separate compartments of higher-order chromatin structure. (A) Domains of open chromatin (yellow nucleosomes) are flanked by insulators (pink, blue and green spheres) that interact together to form a loop. (B) Diagram showing part of a nucleus with compartmentalized chromatin, anchored in part to the nuclear periphery by interactions of the insulators with the nuclear lamina (red lines).

Взаимодействие хромосом в интерфазе и в митозе

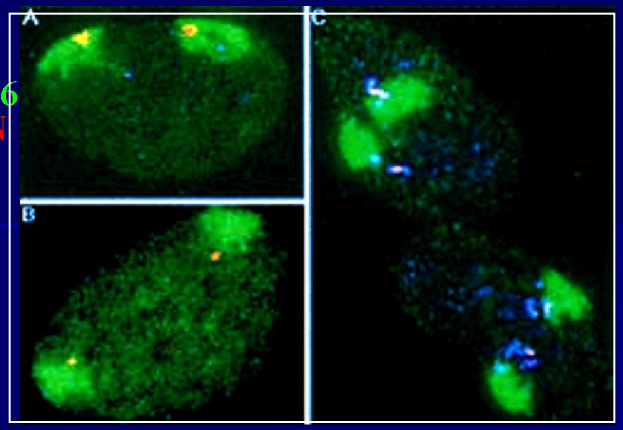
Mahy et al., 2002



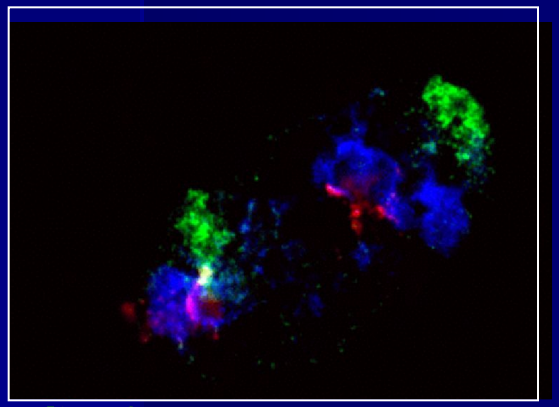
MMU 7
245N5

Chevret et al., 2000

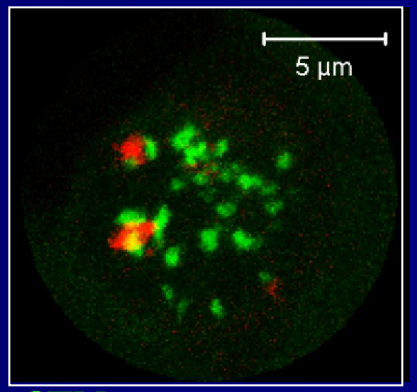
Chr 6
CEN



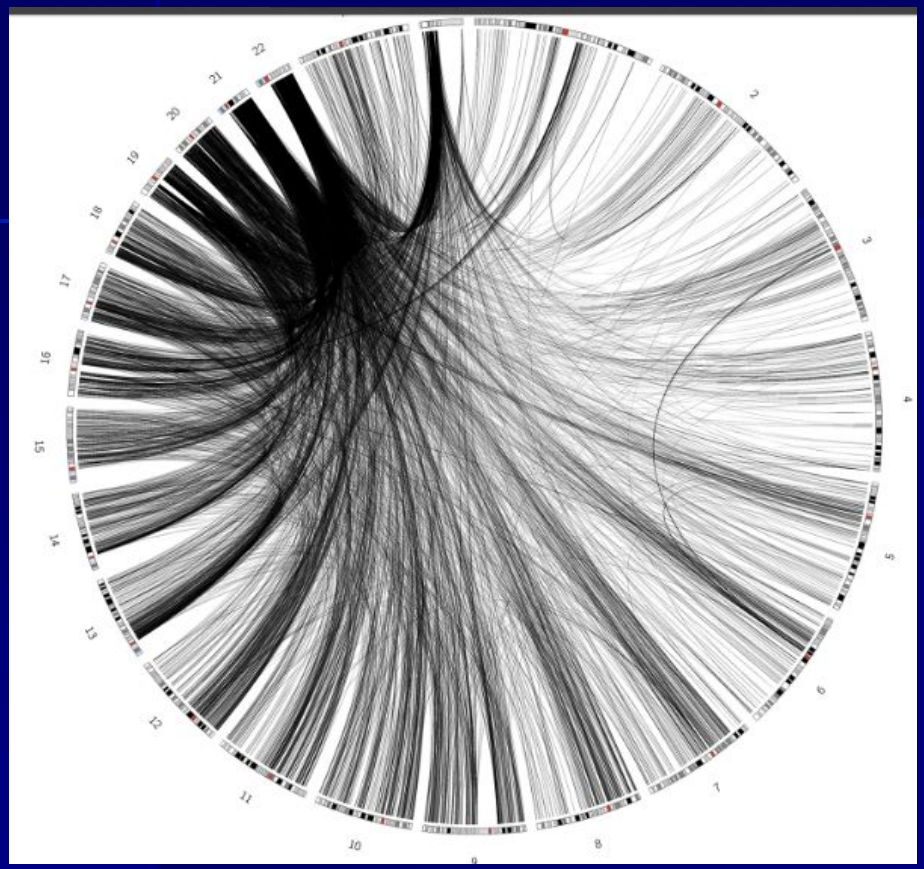
Chr 6
MHC
MHC



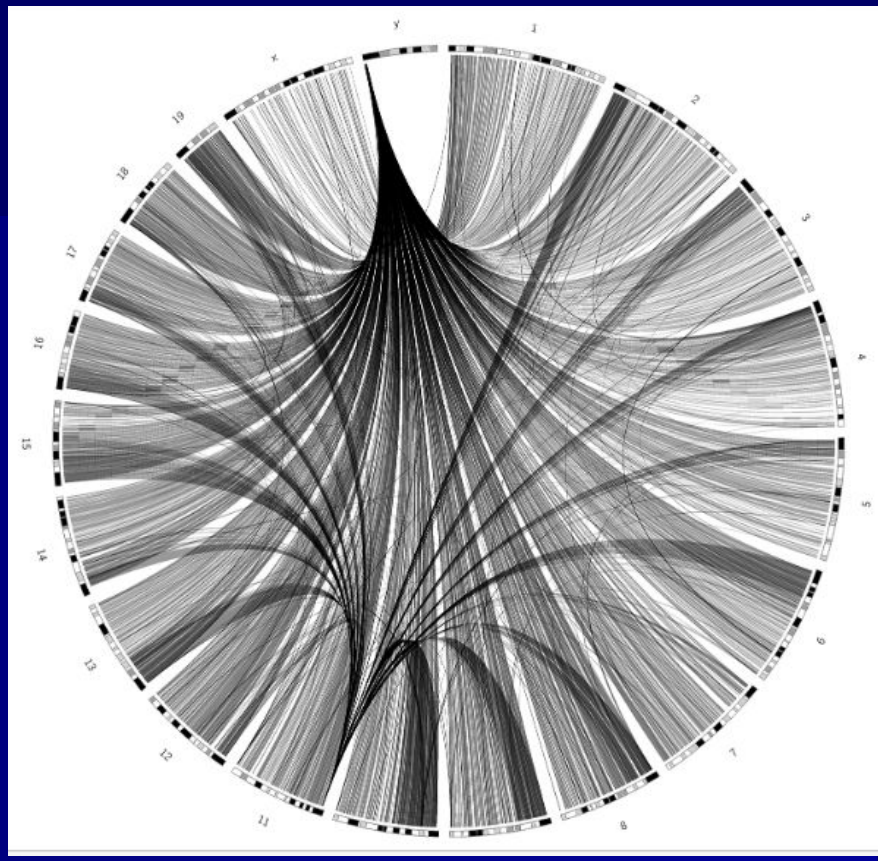
Chr 6
Chr 1
HS3



CEN
HS3



Контакты в области центромера и активного хроматина



Inter-Chromosomal Contact Networks Provide Insights into Mammalian Chromatin Organization

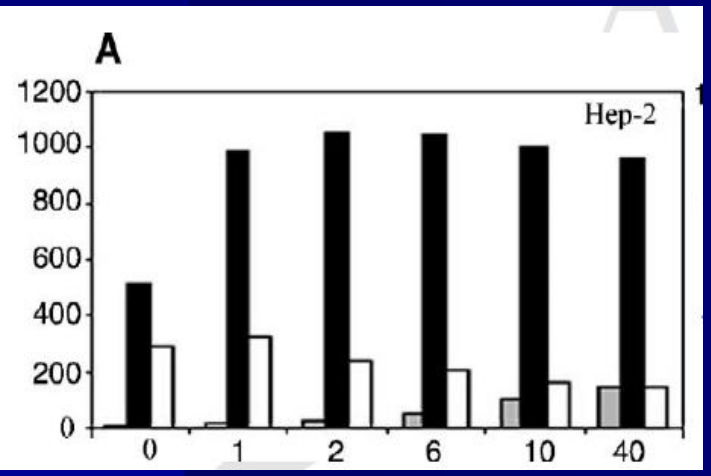
Stefanie Kaufmann, Christiane Fuchs, Mariya Gonik, Ekaterina E. Khrameeva, Andrey A. Mironov, Dmitrij Frishman

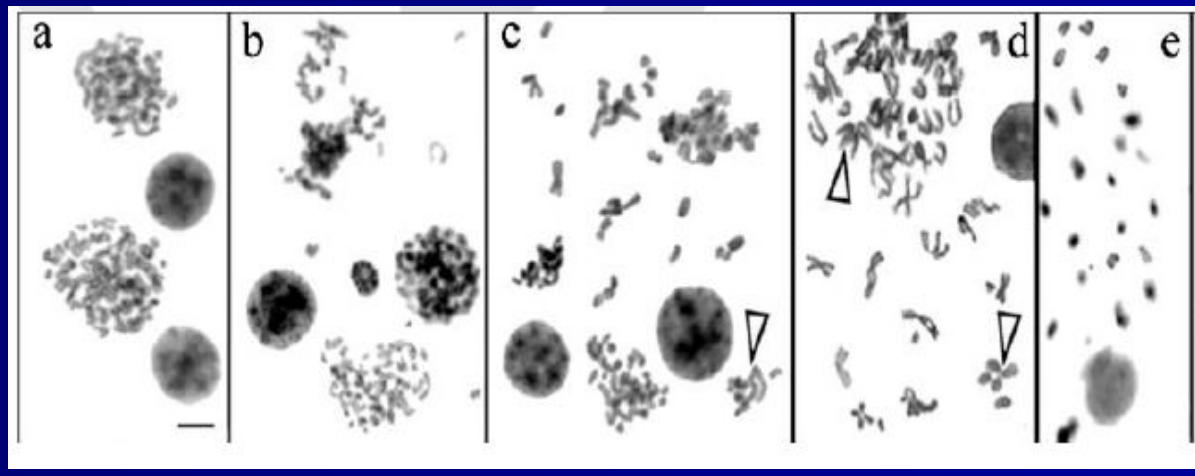
Аггрегация хромосом

TABLE I. The Amount of the Aggregates and Aggregate/Chromosome Ratio in Mitosis Progression of GM-130 Cell Line

The time of incubation (hours)	0	0.3	0.7	1	2	4
The amount of the aggregates (μm^3)	166	165	118	34	3	5
Aggregates/chromosomes ratio	0.181	0.165	0.186	0.185	0.132	0.169

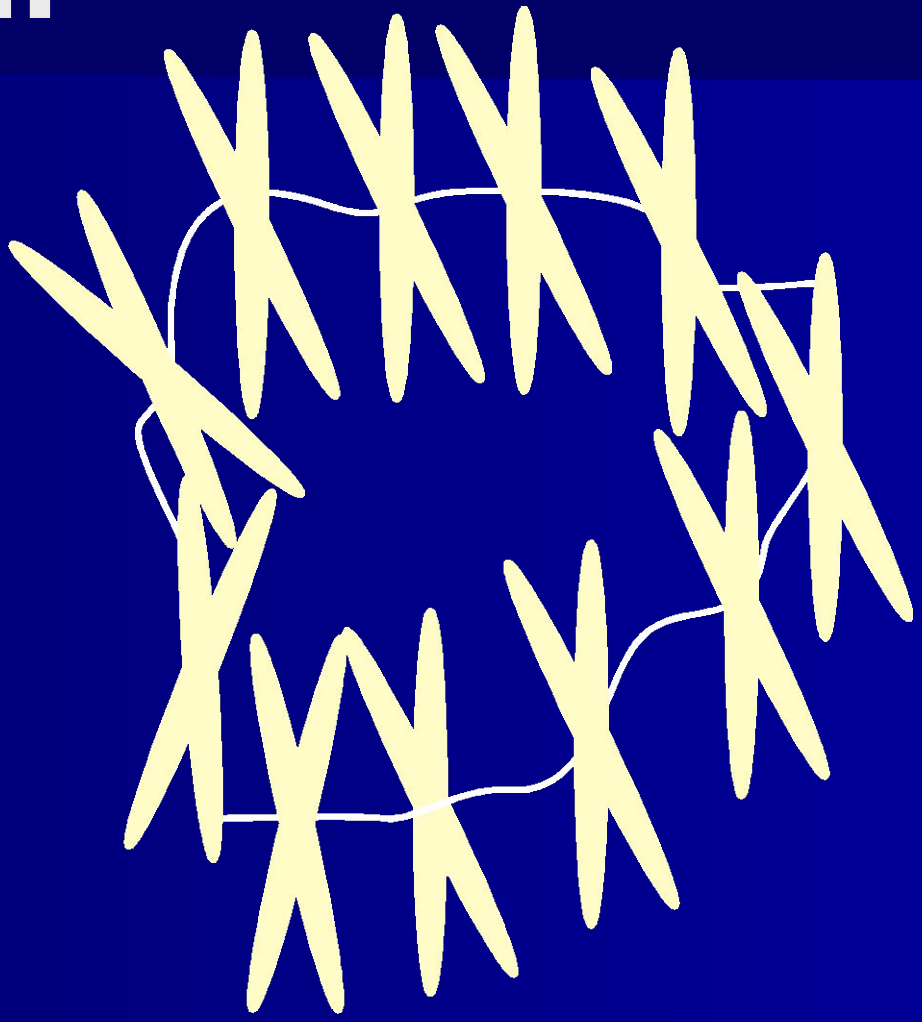
The time of the cells incubation after nocodazole removal in hours (see Fig. 8B). The amount of the aggregates was counted in μm^3. The ratio of aggregates to chromosomes is in relative units.



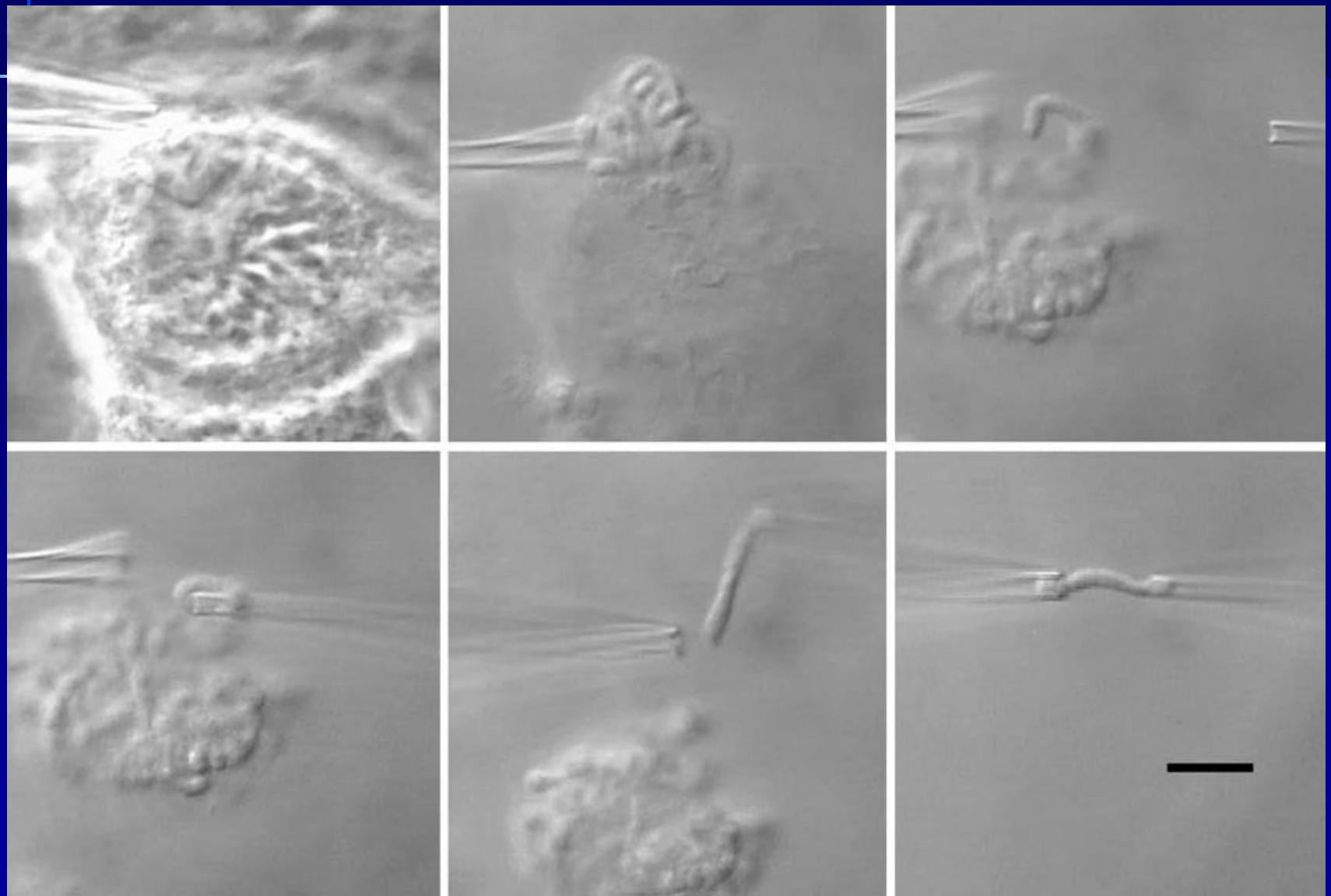


Межхромосомную нить наблюдали:

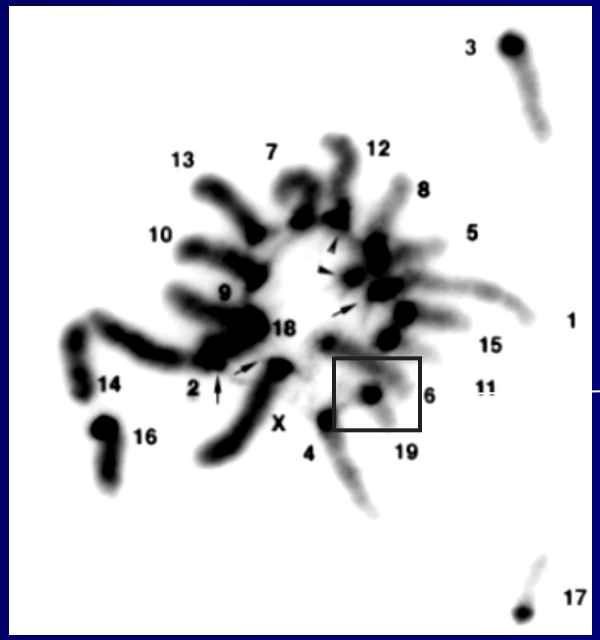
- Wilson, 1925;
- Hsu et al.,1967;
- Hoskins, 1968;
- Henderson et al.; 1973;
- Schneider 1973;
- Takayama, 1976;
- Chiarelli et al. 1977;
- Bennet et al., 1983;
- Lavany et al., 1984
- Radic et al., 1987;
- Maniotis et al., 1997;
- Nagele et al., 1998;
- Dozortsev et al., 2000;
- Saifitdinova et al., 2001
- Enukashvily et al., 2005
- Kuznetsova et al., 2007
- Marco et al., 2008

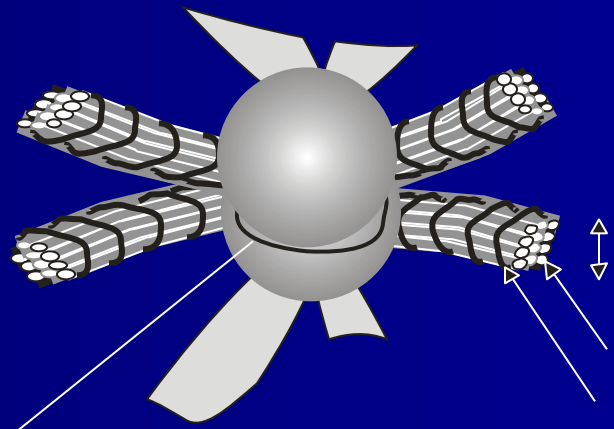


Межхромосомная нить



Состав нити





600 А
(200 по
другим данным)

20 А

10 А

кинетохор

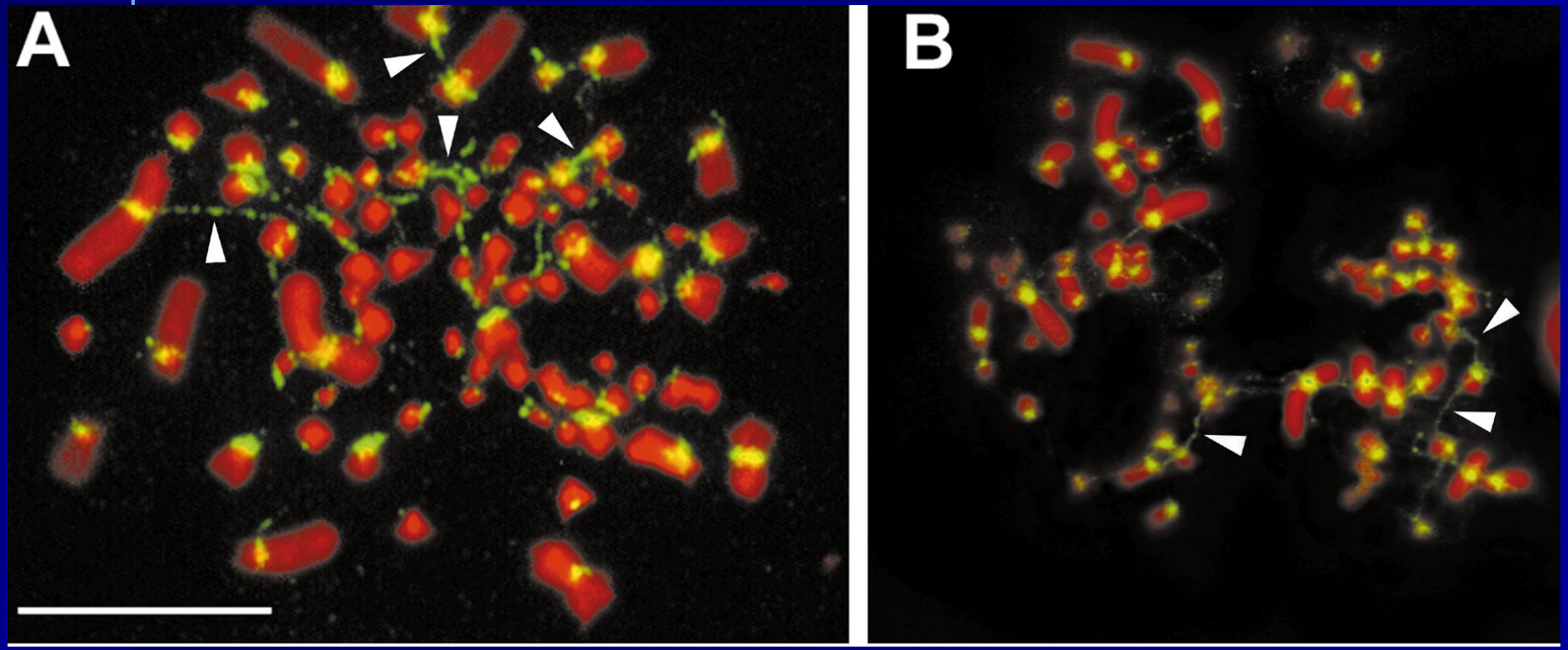
ДНК-аза – утрата связи

РНКаза и пепсин –
утрата эластичности

Состав нити

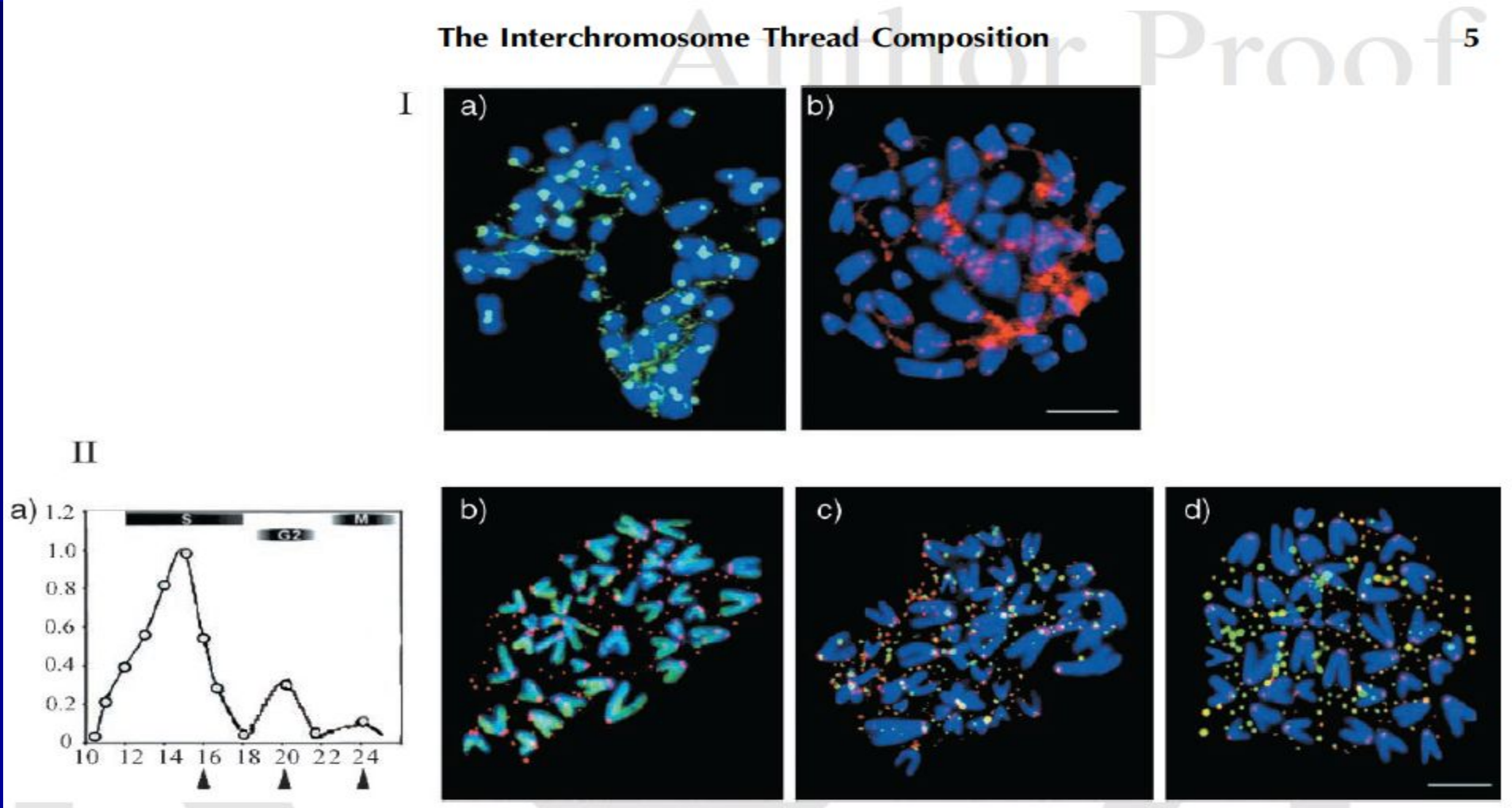
- ДНК (теломерная, интерстициальная, сателлитная)
- РНК (рибосомная, мРНК)
- Белки, в т.ч. чувствительные к меркаптоэтанолу и малорастворимые

ДНК – FCP повтор зяблика

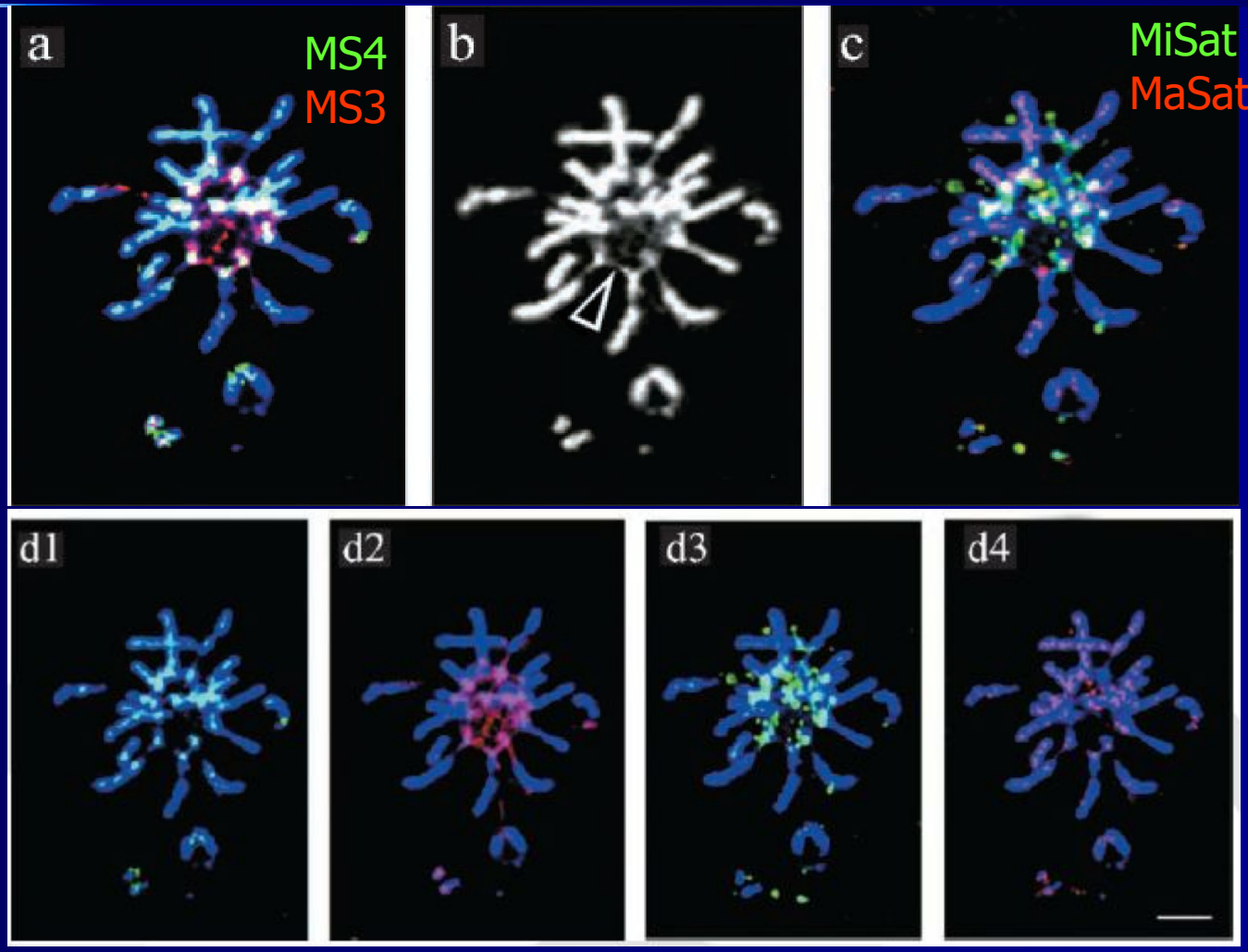


Saifitdinova et al., 2001

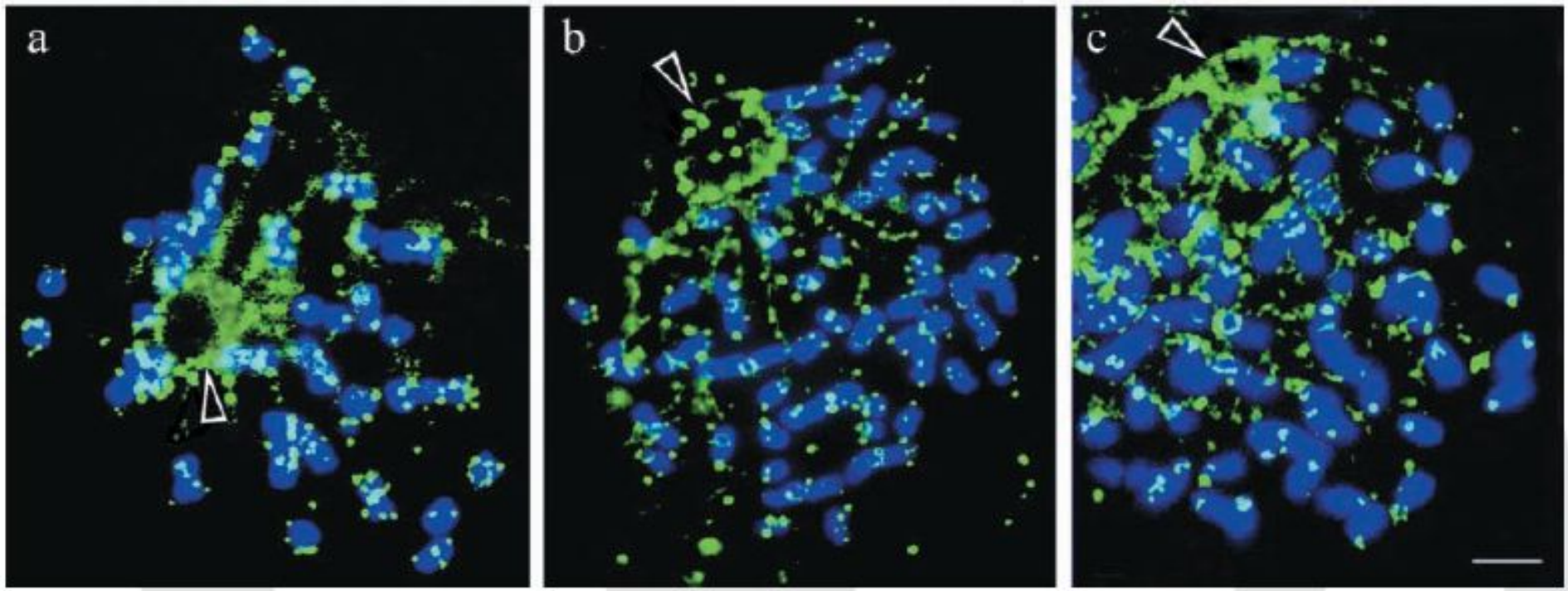
M. Musculus – позднореплицирующаяся ДНК



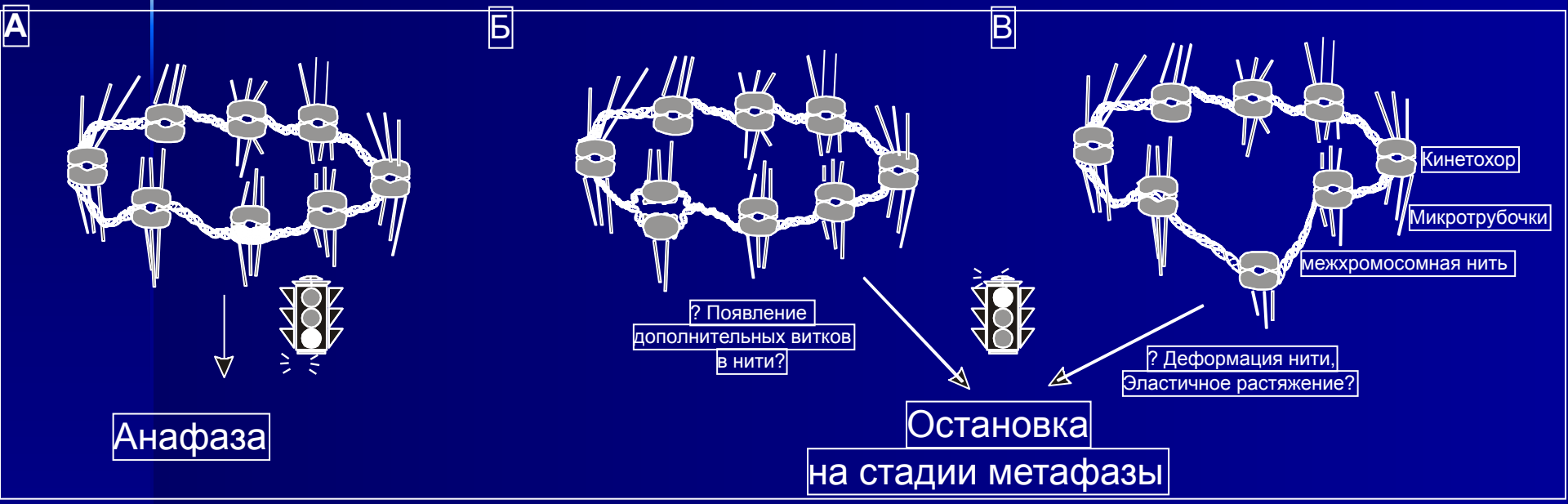
M. Musculus – сатДНК межхромосомной нити



P68 в межхромосомной нити



Межхромосомная нить



Енукашвили и др., 2002