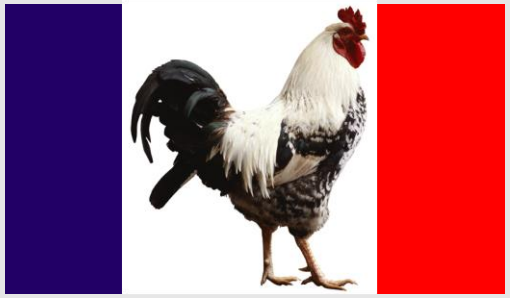
VIRUSES AND NUCLEAR ORGANIZATION IN ONCOGENESIS

Yegor VASSETZKY, CNRS UMR 8126, Institut de Cancérologie Gustave Roussy

ENGELHARDT INSTITUTE OF MOLECULAR BIOLOGY



INSTITUT JACQUES MONOD

ISREC @EPFL
INSTITUT SUISSE DE RECHERCHE EXPÉRIMENTALE SUR LE CANCER

FACULTE

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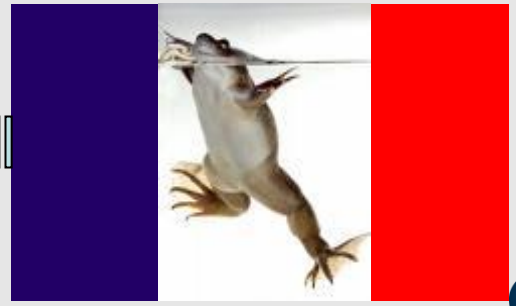

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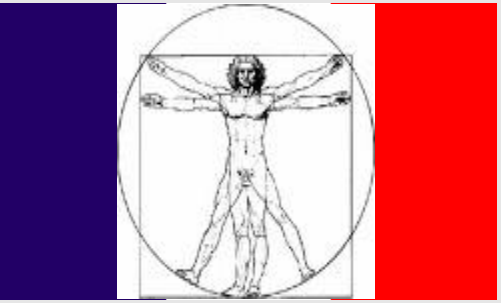
INSTITUT JACQUES MONOD

GUSTAVE ROUSSY
CANCER CAMPUS
GRAND PARIS



NUCLEAR ORGANIZATION:

A chemical reactor?

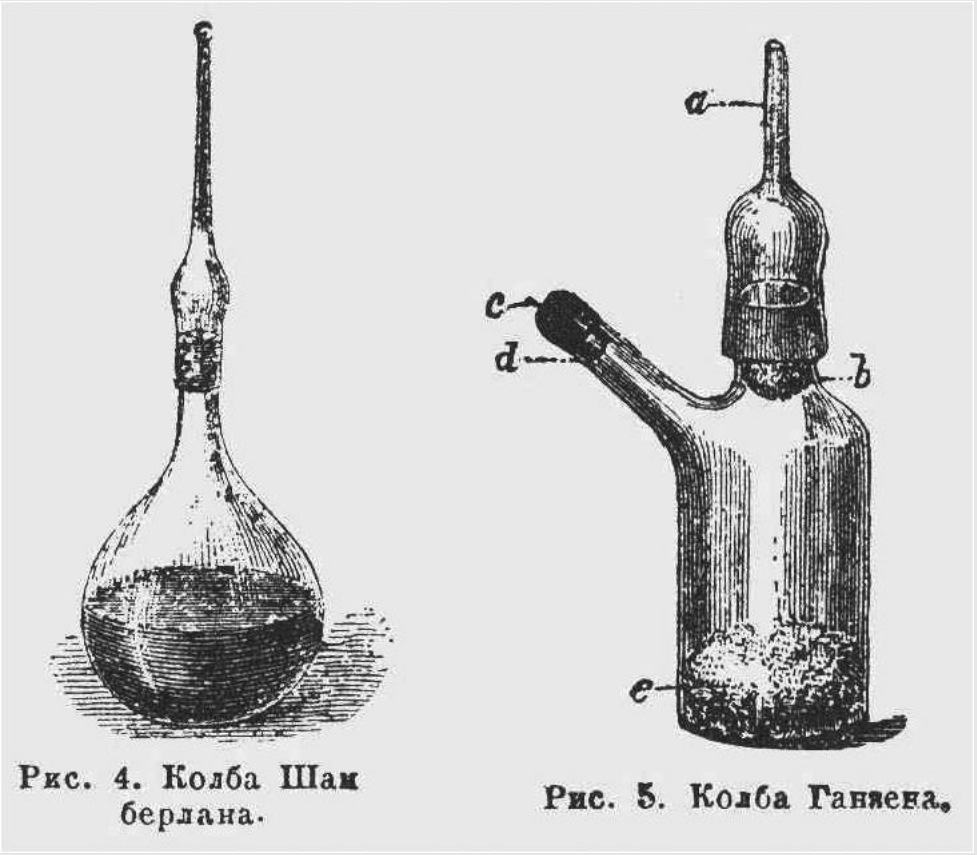


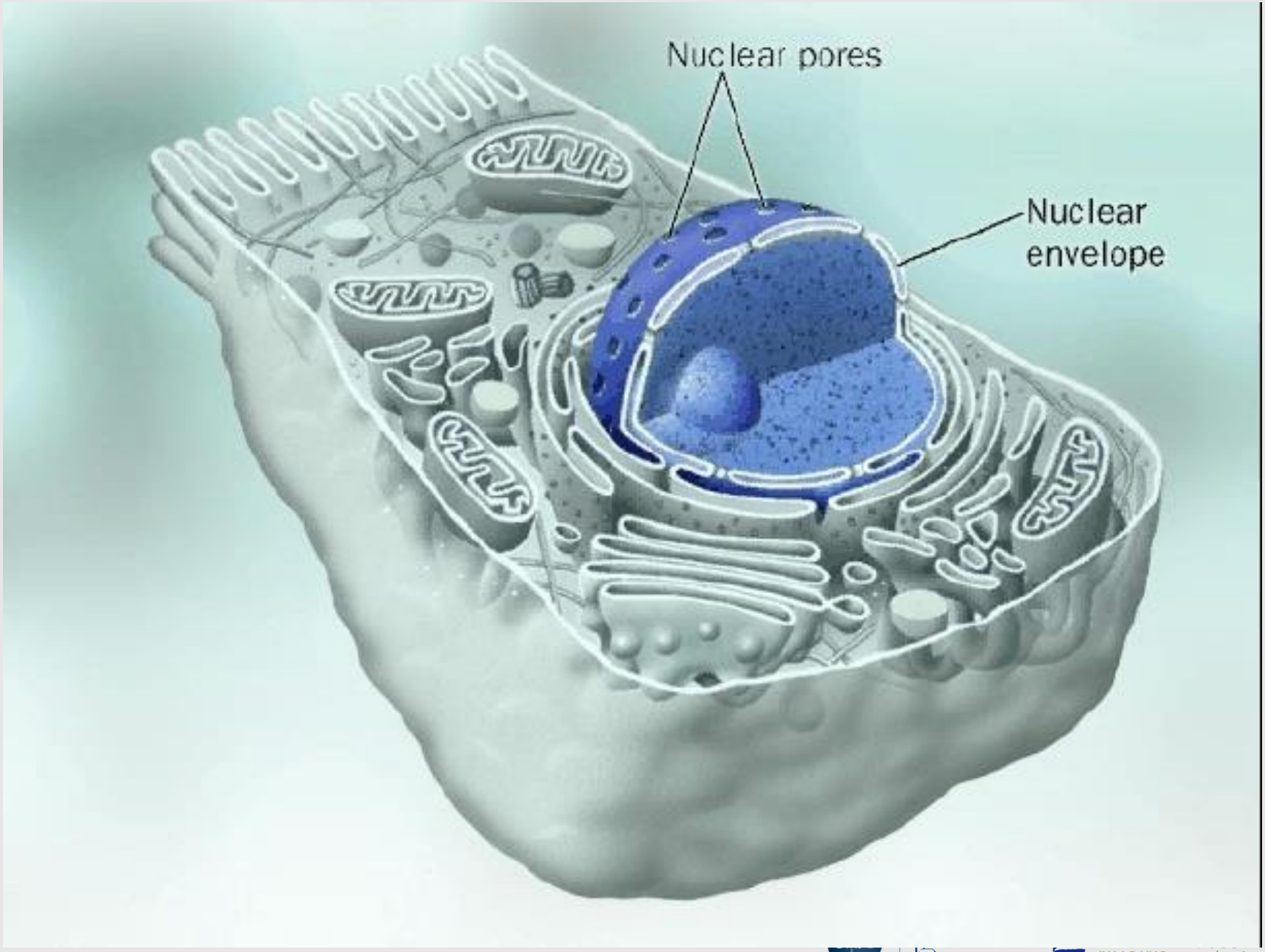
Рис. 4. Колба Шам берлана.

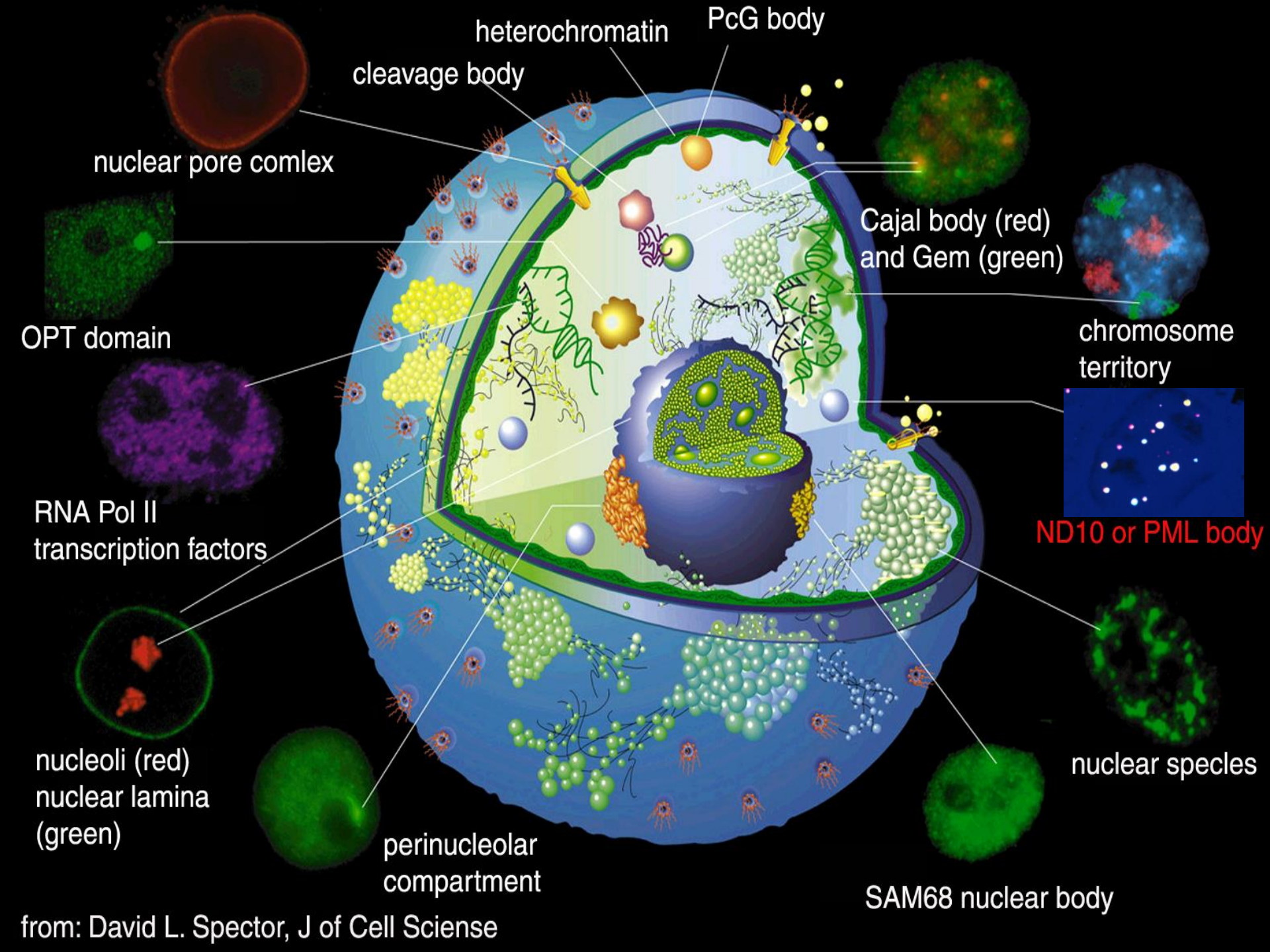
Рис. 5. Колба Ганзена.

A precision mechanics?

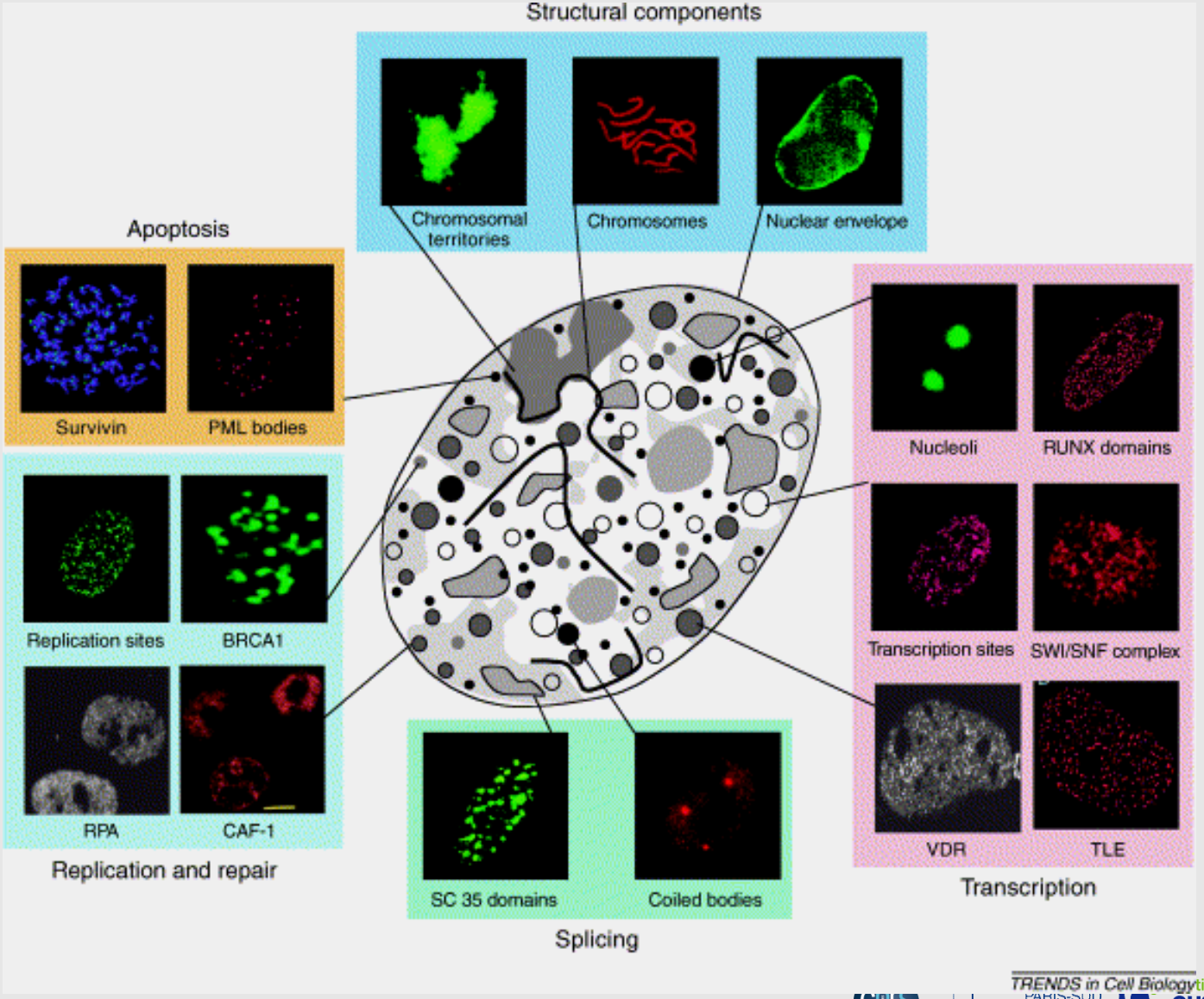
Concentration

Position


Nuclear pores
Nuclear envelope


heterochromatin
PcG body
cleavage body
nuclear pore comlex
Cajal body (red)
and Gem (green)
OPT domain
chromosome
territory
ND10 or PML body
RNA Pol II
transcription factors
nucleoli (red)
nuclear lamina
(green)
perinucleolar
compartment
nuclear specles
SAM68 nuclear body
from: David L. Spector, J of Cell Sciense

NUCLEAR STRUCTURES




AP

CHROMATIN ORGANIZATION AND EPIGENETICS

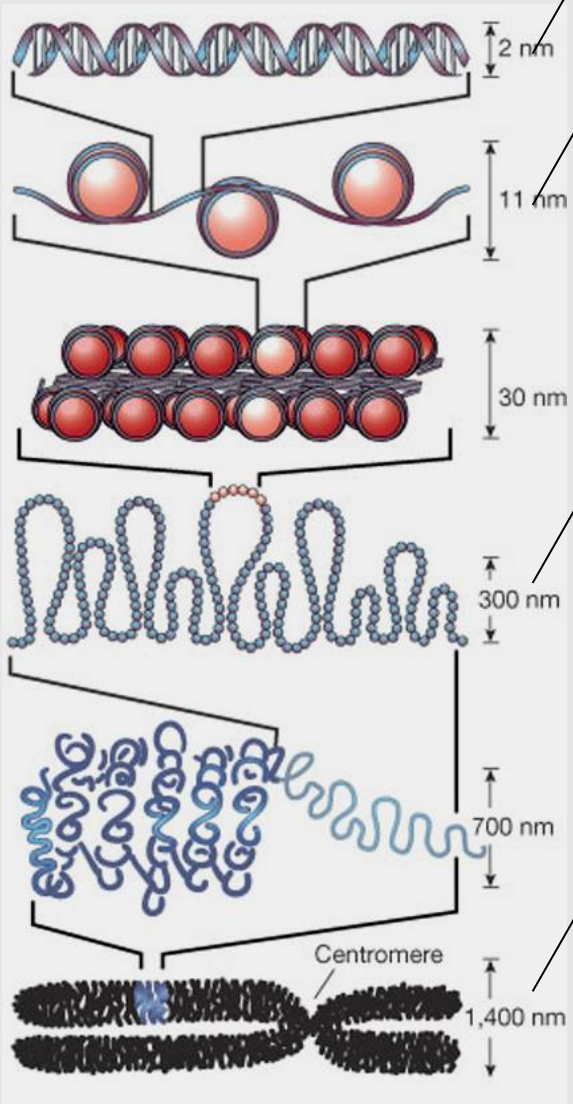
DNA

NUCLEOSOMES

FIBERS

DOMAINS

CHROMOSOMES



DNA methylation

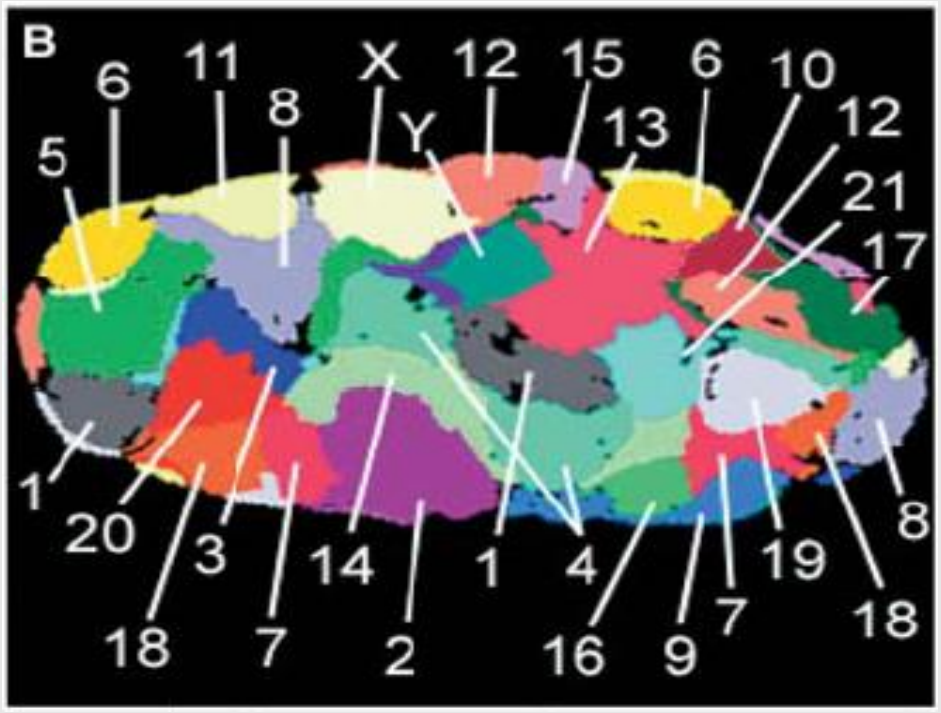
Histone modifications
Histone variants

Chromatin domains

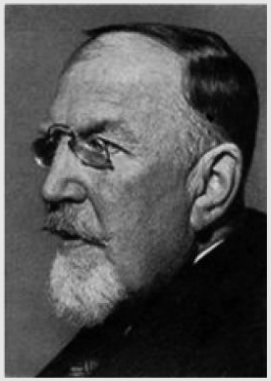
Chromosomal territories

CHROMOSOMAL TERRITORIES

Theodor Boveri (1862-1915)



Bolzer et al., PlosBiology (2005), 3 (5) e207



Carl Rabl (1853-1917)

- **The chromosomes are organized in the nucleus:**
  - **In a tissue specific manner**
  - **The organization is transmitted though the cell divisions**
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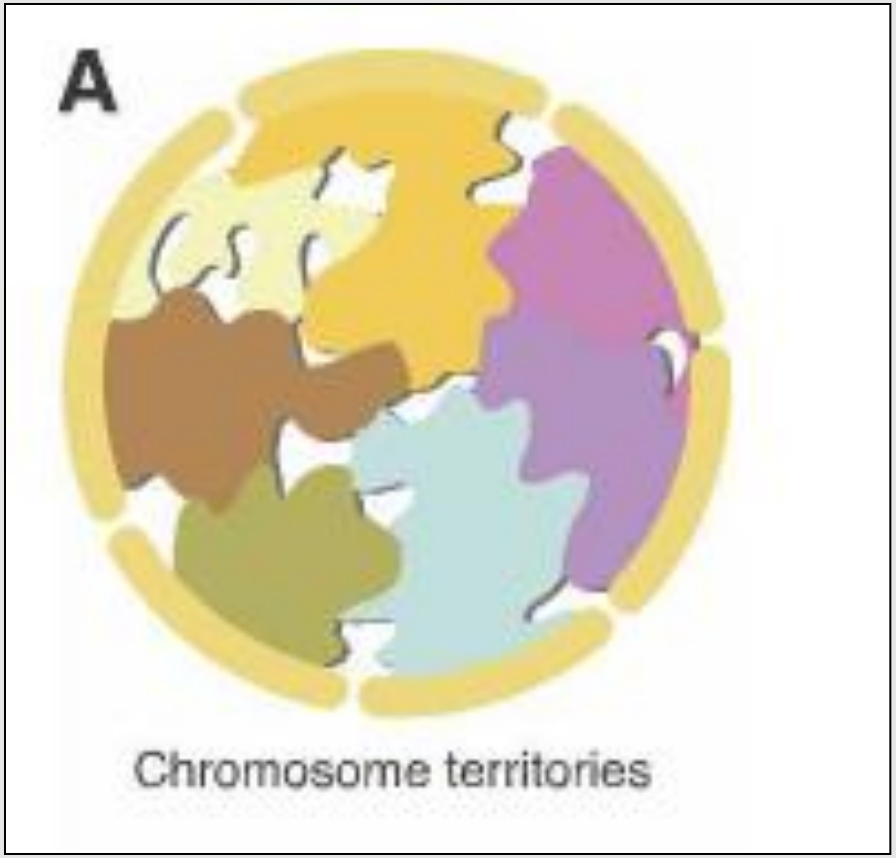
- **The gene-rich regions occupy more central position in the nucleus**

Bolzer et al., Plos Biology (2005), 3 (5) e207

CHROMOSOME DYNAMICS



Schneider et al, 2007

Spector et al, 2003

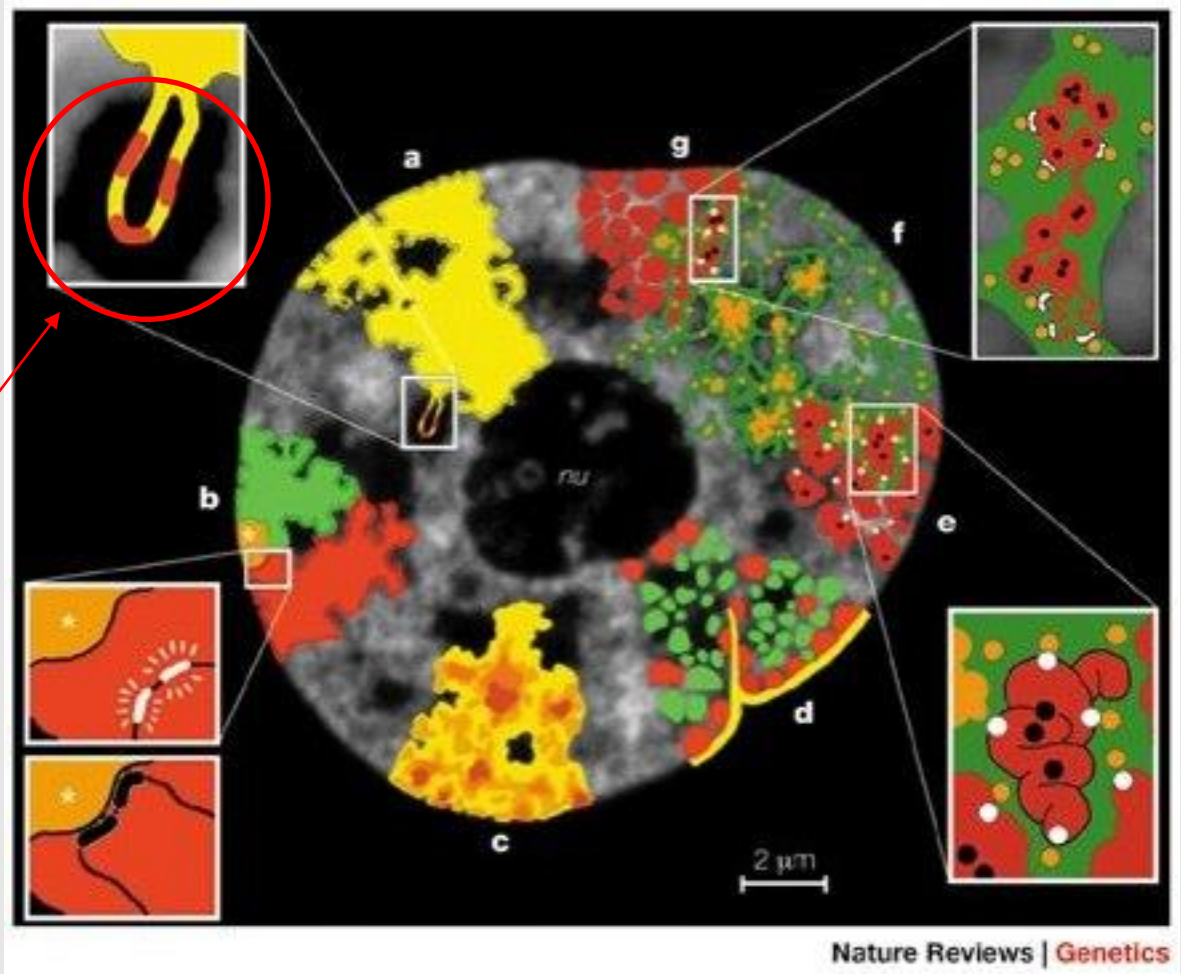
CHROMOSOME TERRITORIES: A UNIT OF NUCLEAR ORGANIZATION

- **Chromosomes have preferred position with respect to the center or periphery of the nucleus**

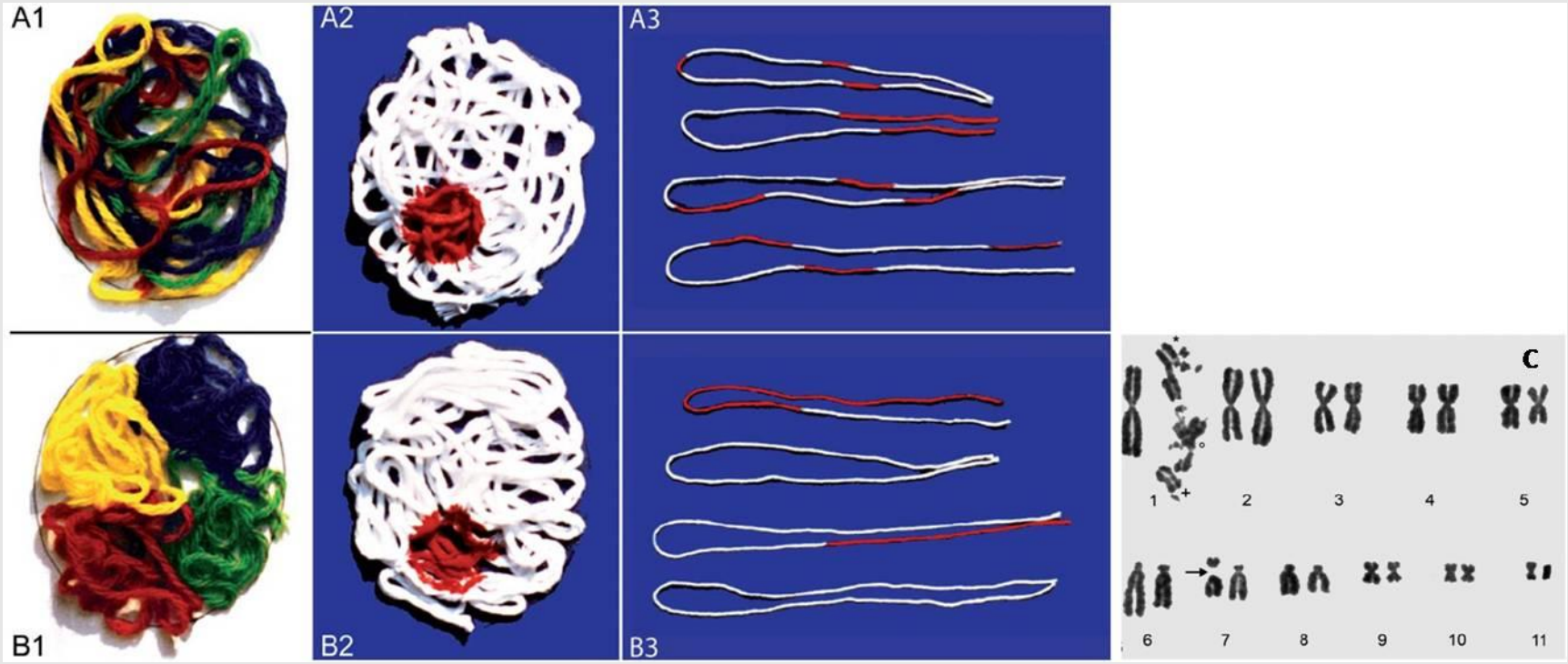
- **Variability between cell-types**

- **Non-random neighbors: purpose is to facilitate proper gene expression!**

- **Complex folded surface with active genes(red) extends (or loops) into the interchromatin space**

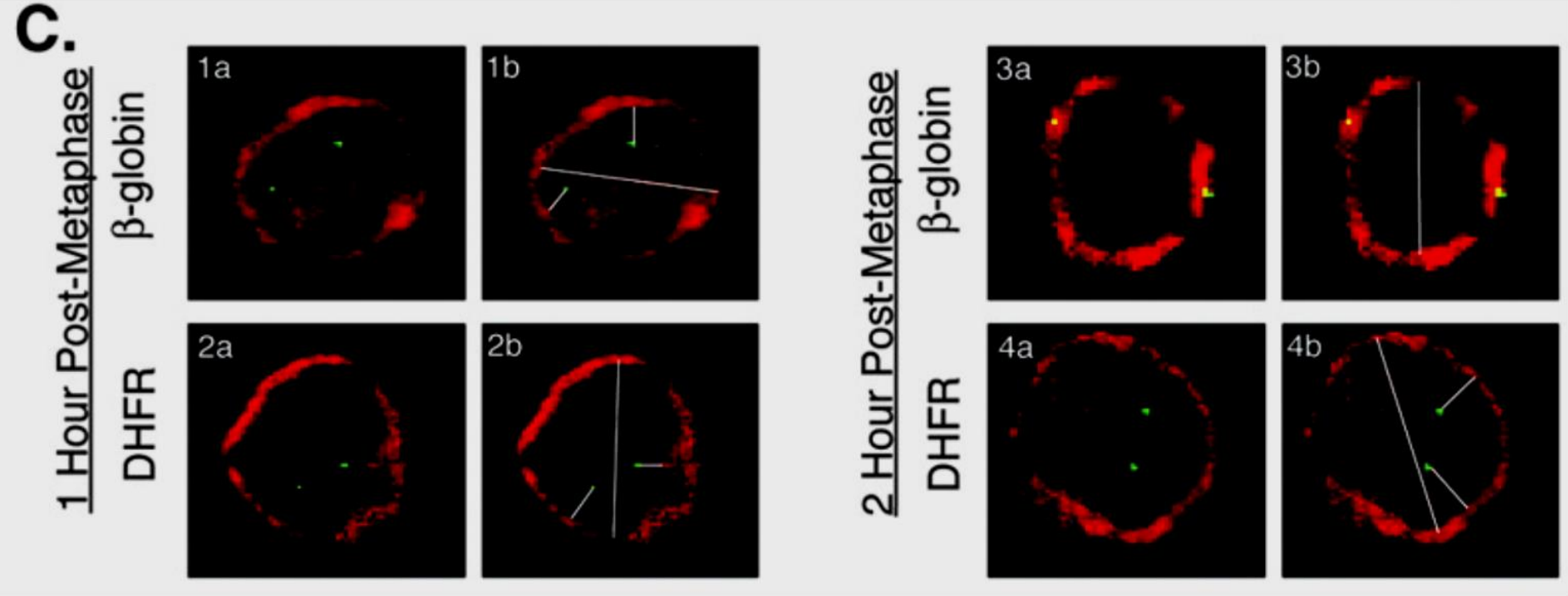


HOW THE EXISTENCE OF CHROMOSOMAL TERRITORIES WAS PROVEN?



Cremer and Cremer, 2006

GENE LOCALIZATION WITHIN CHROMOSOMAL TERRITORIES DEPENDS ON THEIR TRANSRIPTIONAL STATUS



→ Dans les cellules CHO, les gènes DHFR (actif) et beta-globine (incatif) n'ont pas de localisation preférentielle juste après le mitose, mais au début de phase S, le gène DHFR est rélocalise à l'intérieur de noyau, et le beta-globine reste périphérique

CHROMOSOMAL TRANSLOCATIONS

Chimeric genes

Balanced chromosomal translocations

Loss or gain of genes

C A N C E R

Theodor Boveri (1862-1915)

BURKITT'S LYMPHOMA

→A non-Hodgkin Lymphoma

→Three forms:

- → An endemic form in Afrtica is 100% associated with EBV
- → A sporadic form in Europe and North America, rare and non-associated with EBV
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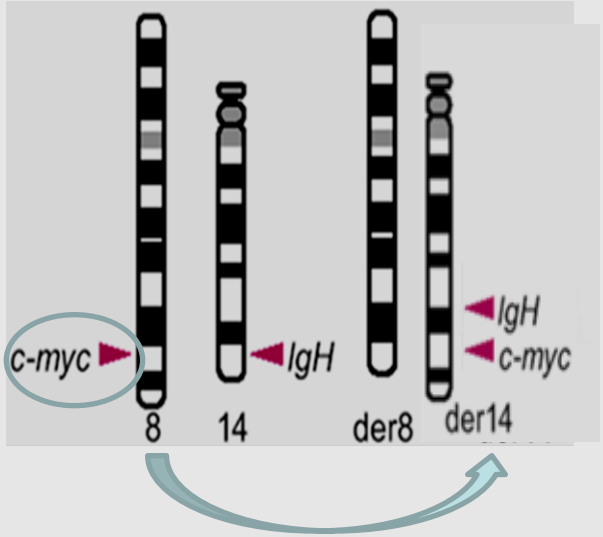
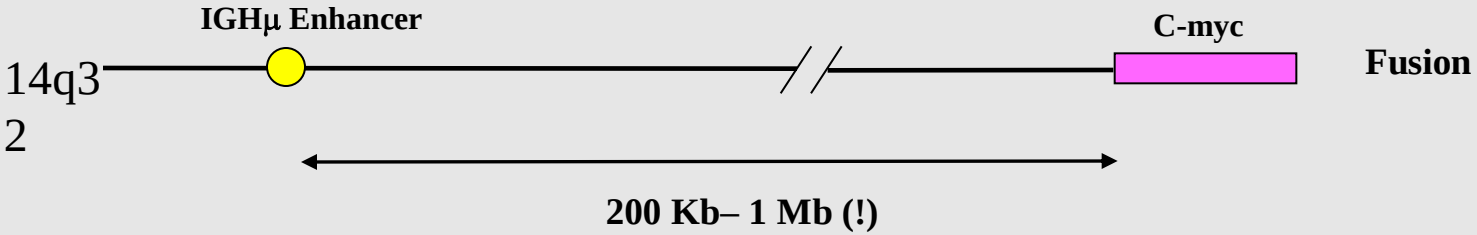


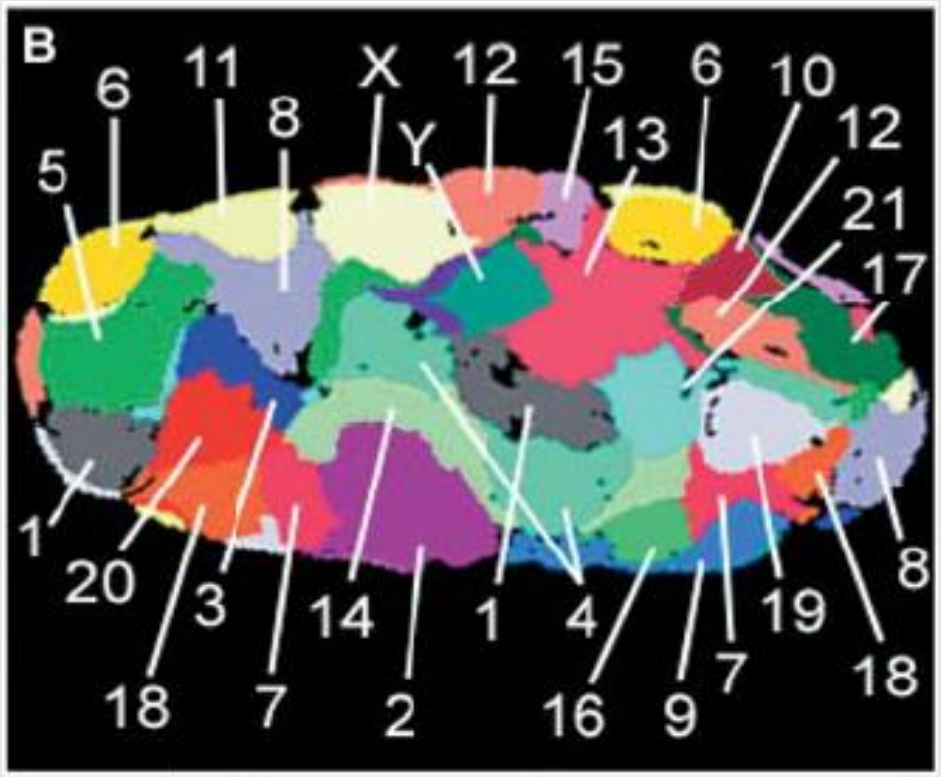
C-MYC ACTIVATION IN BURKITT LYMPHOMA: IS IGHμ ENHANCER REALLY INVOLVED?

- t(8 ; 14) translocation induces overexpression of *c-myc* in B-cells

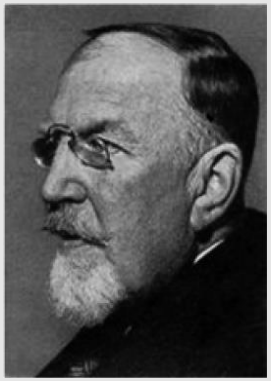


NUCLEAR ARCHITECTURE

Theodor Boveri (1862-1915)



Bolzer et al., PlosBiology (2005), 3 (5) e207



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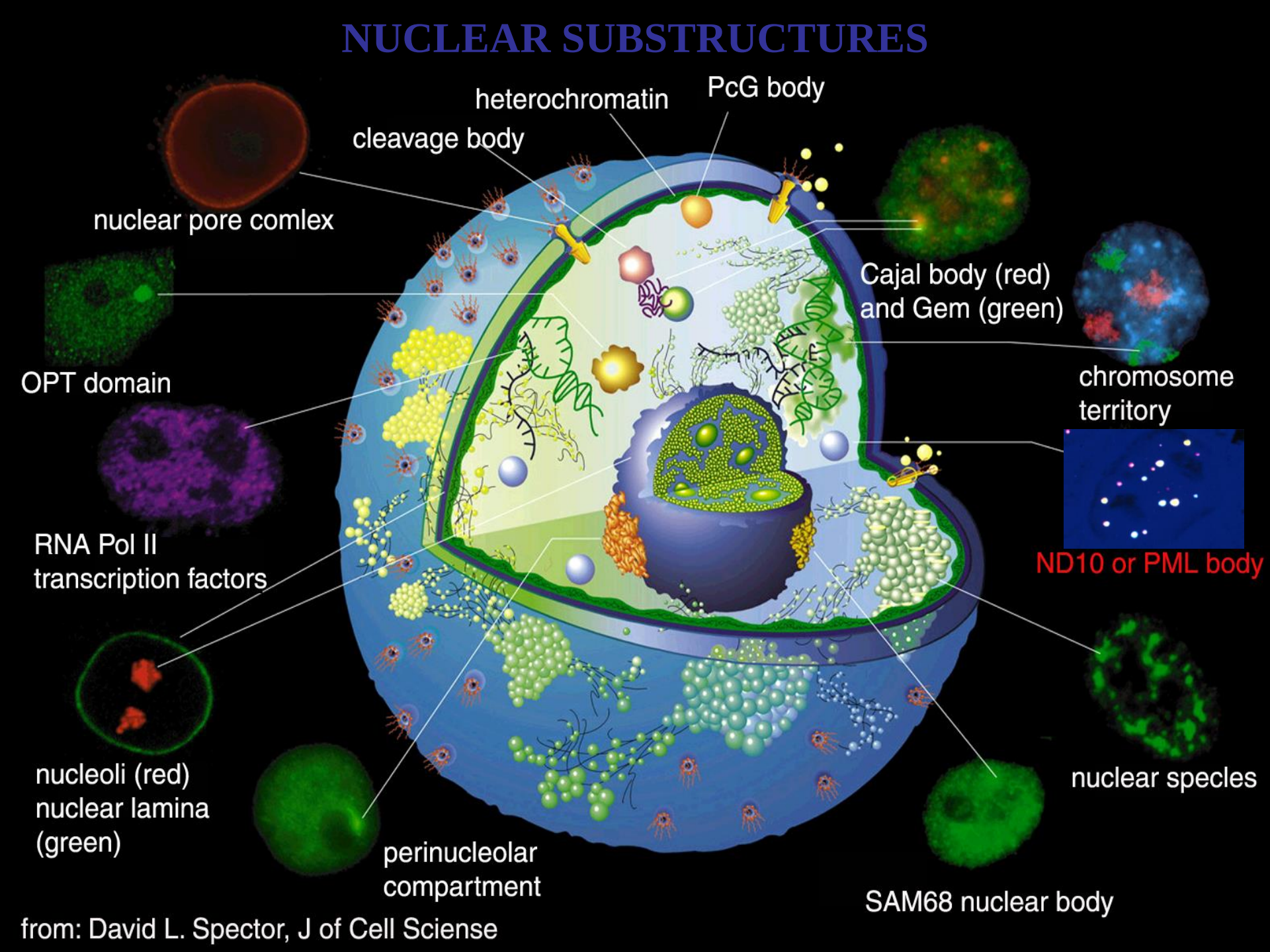
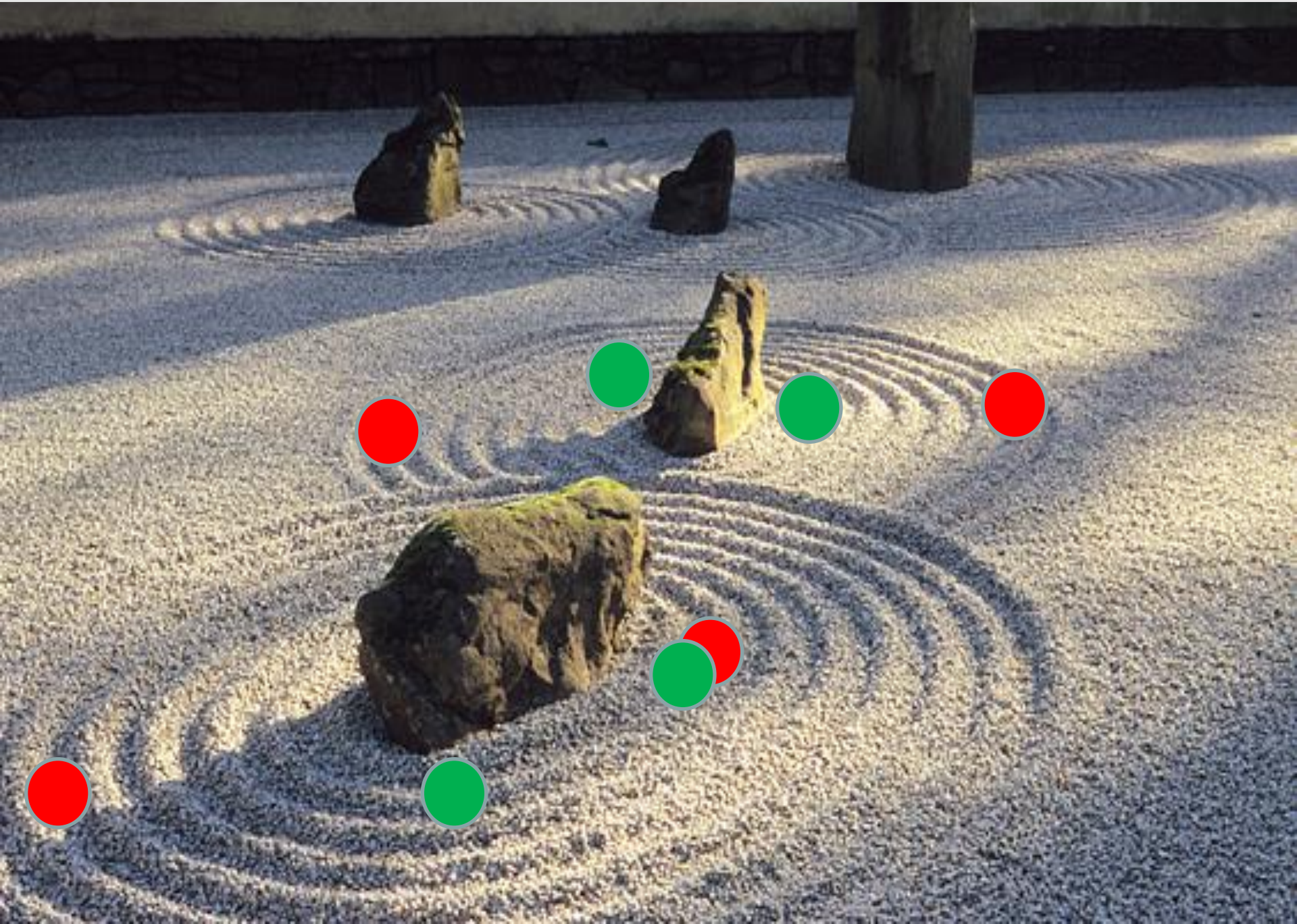
Bolzer et al., Plos Biology (2005), 3 (5) e207

NUCLEAR SUBSTRUCTURES

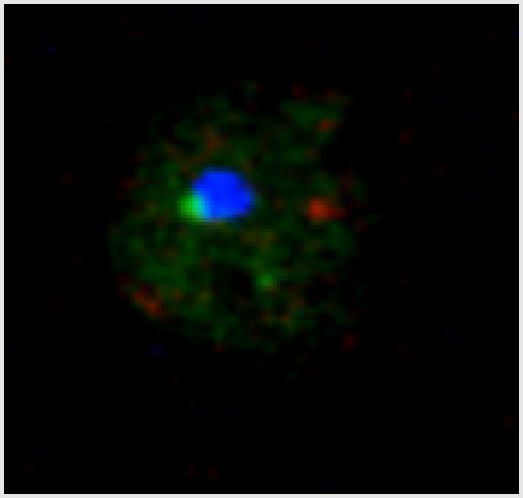


from: David L. Spector, J of Cell Sciense

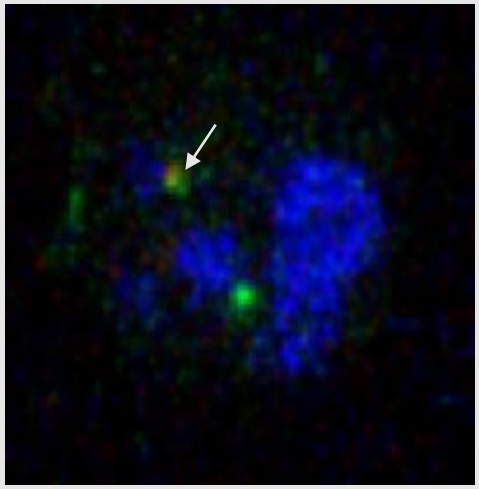
NUCLEAR ARCHITECTURE



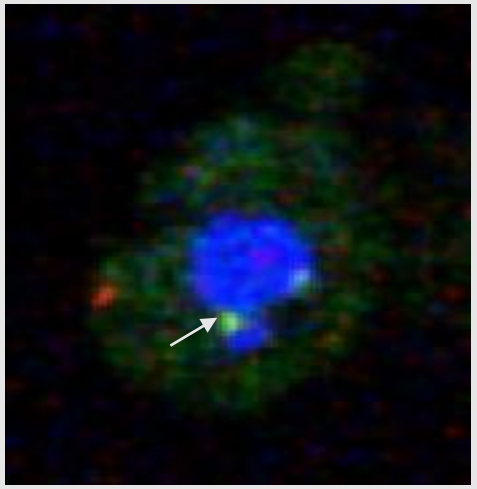
TRANSLOCATED *c-myc* LOCUS IS LOCALIZED IN THE PERINUCLEOLAR REGION IN BURKITT LYMPHOMA



Normal B-lymphocytes

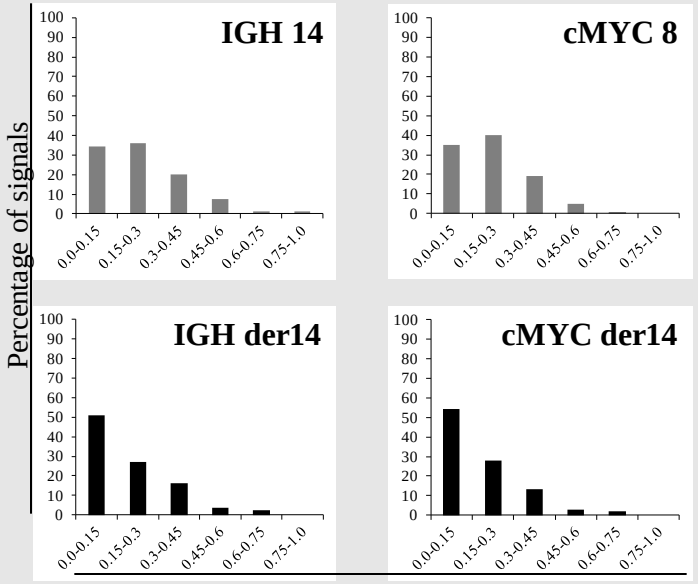


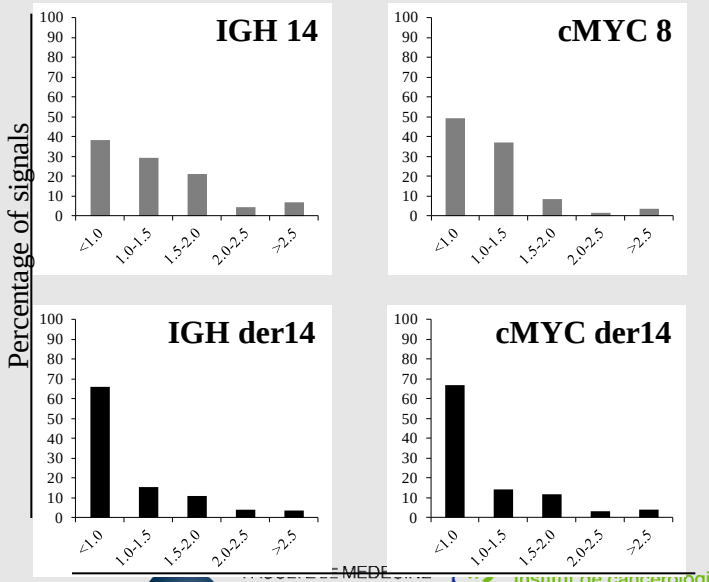
P3HR1 Burkitt cell line



RAJI Burkitt cell line

C-myc **IgH** **Nucleolus (anti-B23)**





NUCLEOLUS AND REGULATION OF TRANSCRIPTION – LR1

Proc. Natl. Acad. Sci. USA
Vol. 94, pp. 3605–3610, April 1997
Biochemistry

Nucleolin is one component of the B cell-specific transcription factor and switch region binding protein, LR1

(Ig/rDNA/recombination)

L. A. HANAKAHI*, LAURIE A. DEMPSEY*, MING-JIE LI*, AN

Departments of *Molecular Biophysics and Biochemistry and †Genetics, Yale Univ

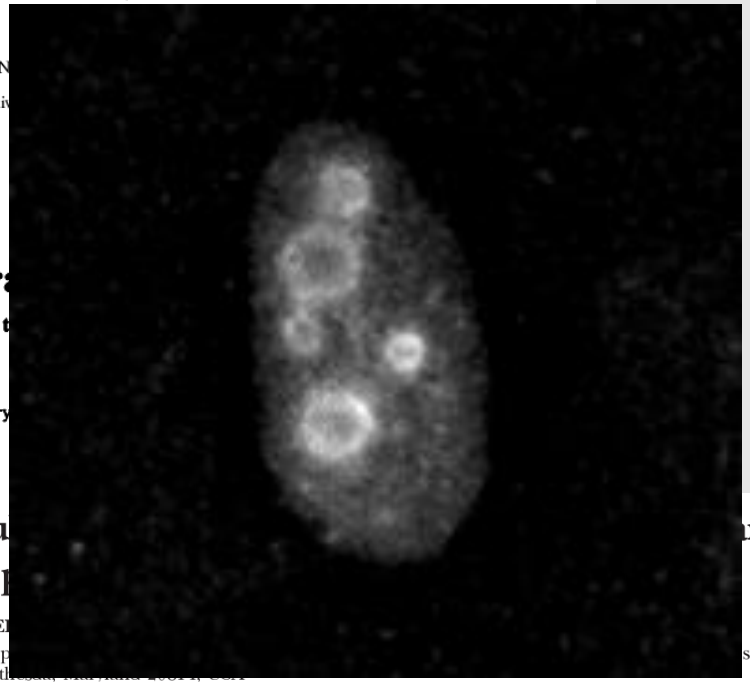
Proc. Natl. Acad. Sci. USA
Vol. 91, pp. 4915–4919, May 1994
Biochemistry

LR1 regulates c-*myc* tra

(*MYC* gene/immunoglobulin/chromosome t

APRIL BRYS† AND NANCY MAIZELS‡

Department of Molecular Biophysics and Biochemistry



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THE JOURNAL OF BIOLOGICAL CHEMISTRY

Vol. 279, No. 12, Issue of March 19, pp. 10855–10863, 2004
Printed in U.S.A.

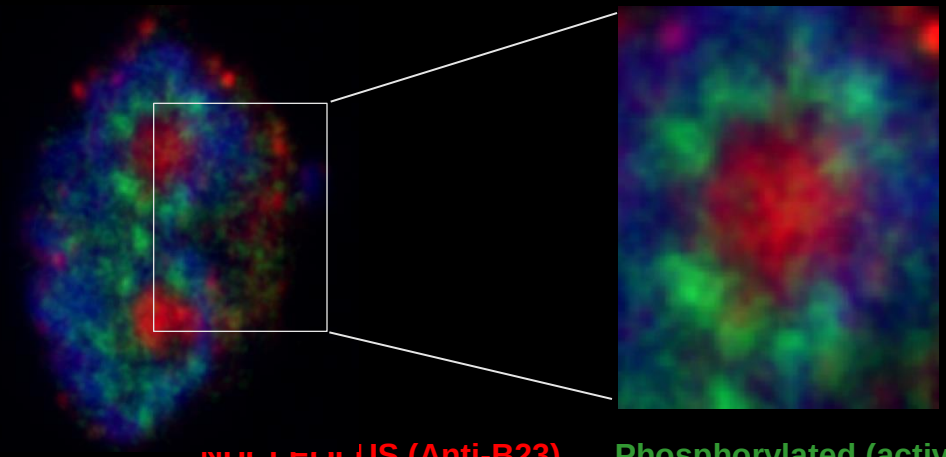
Identification of Nucleolin as an AU-rich Element Binding Protein Involved in *bcl-2* mRNA Stabilization*

Received for publication, August 18, 2003, and in revised form, December 4, 2003
Published, JBC Papers in Press, December 16, 2003, DOI 10.1074/jbc.M309111200

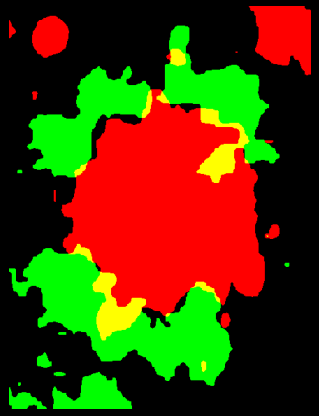
Tapas K. Sengupta‡§, Sumita Bandyopadhyay‡§, Daniel J. Fernandes‡, and Eleanor K. Spicer‡¶‖

From the ‡Department of Biochemistry and Molecular Biology and ¶Department of Pharmaceutical Sciences, Medical University of South Carolina, Charleston, South Carolina 29425

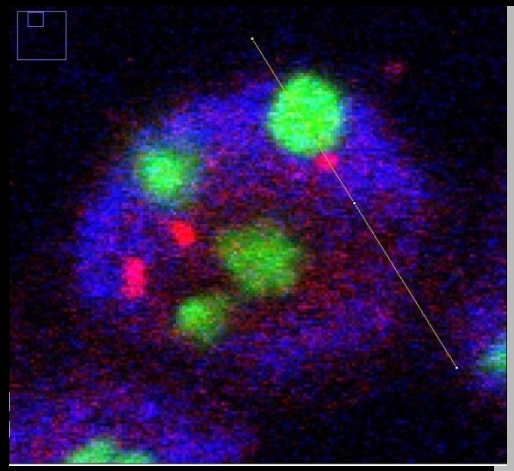
TRANSCRIPTION FACTORIES ARE LOCATED IN THE PERINUCLEOLAR REGION

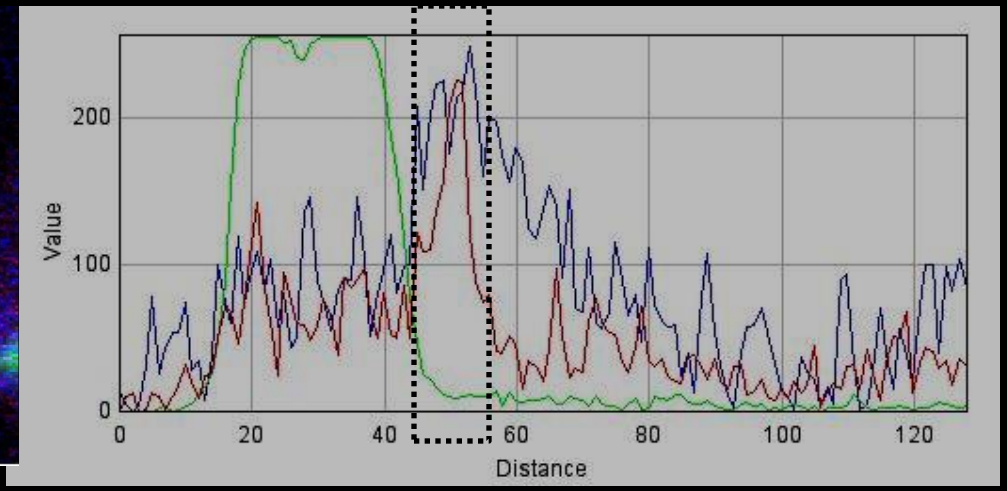


NUCLEOLUS (Anti-B23) Phosphorylated (active) RNA polymerase II



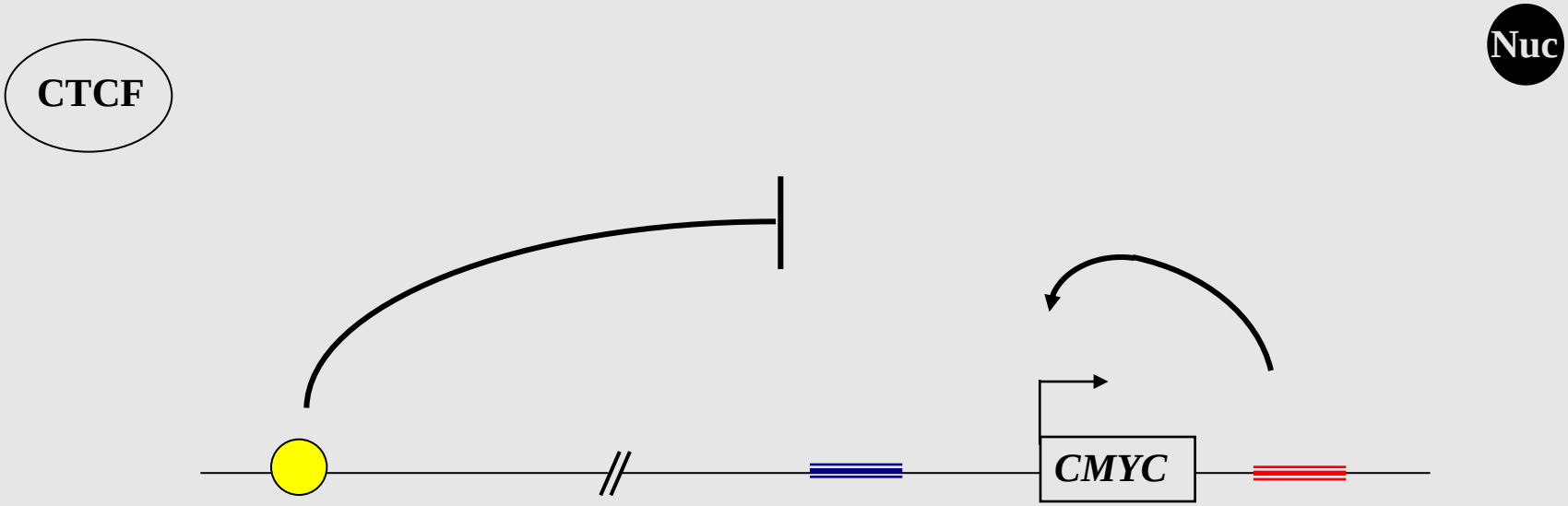
NUCLEUS (DAPI)





NUCLEOLUS (Anti-B23) Phosphorylated (active) RNA polymerase II

NUCLEOLIN, CTCF AND TRANSCRIPTIONAL REGULATION OF THE *CMYC* LOCUS IN BURKITT'S LYMPHOMA

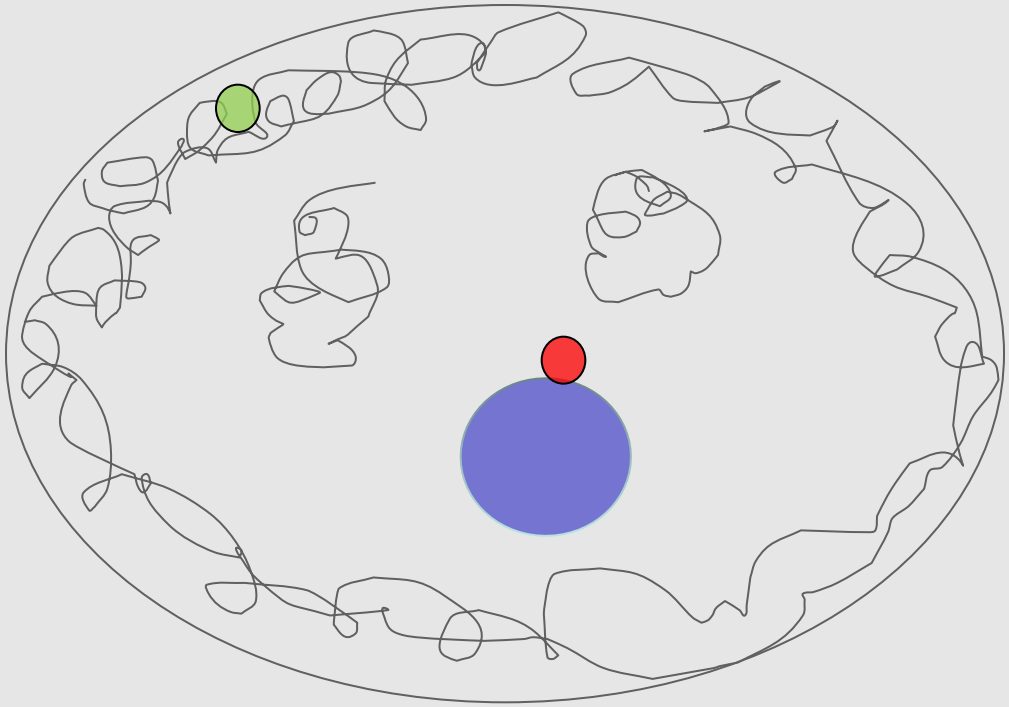


Allinne et al., **Blood,** 2014

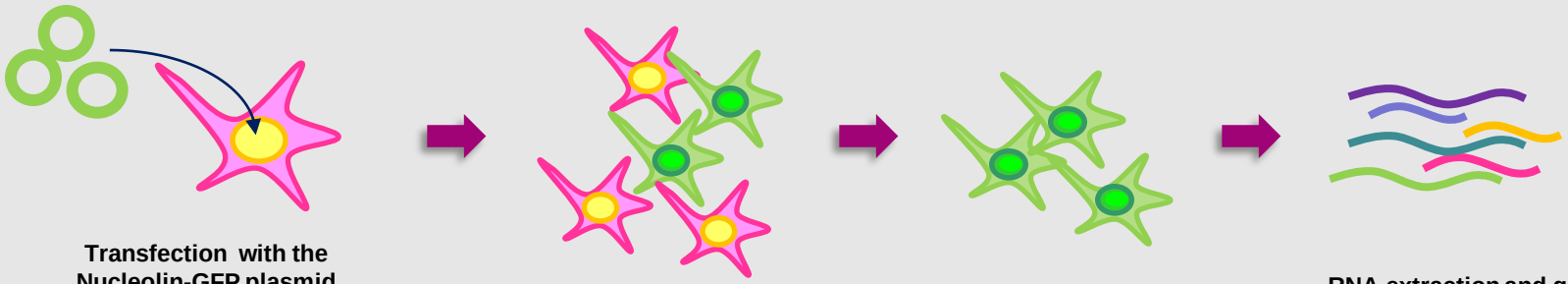
FACULTE DE MEDECINE PARIS-SUD

Institut de cancérologie GUSTAVE ROUSSY VILLEJUIF - www.igr.fr

INTRANUCLEAR RELOCALIZATION AND GENE ACTIVATION IN CANCER



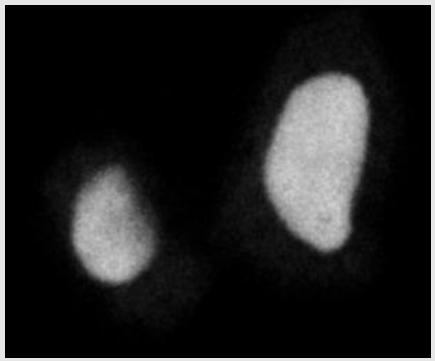
IF THE MOUNTAIN WILL NOT COME TO MAHOMET, MAHOMET MUST GO TO THE MOUNTAIN



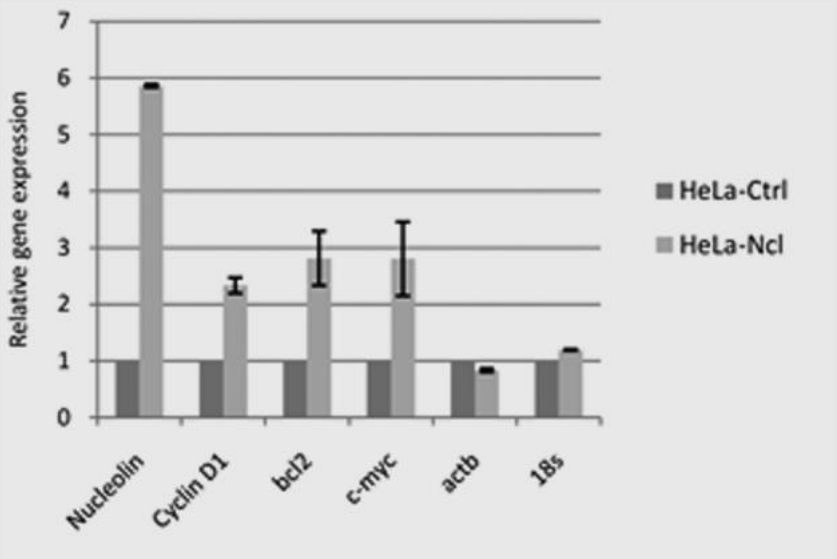
Transfection with the Nucleolin-GFP plasmid

Cell sorter

RNA extraction and qPCR



Nucleolin-GFP



→**Ectopic overexpression of nucleolin leads to its delocalization in the nucleus and overexpression of endogenous *CCND1, c-myc and bcl-2* oncogenes**

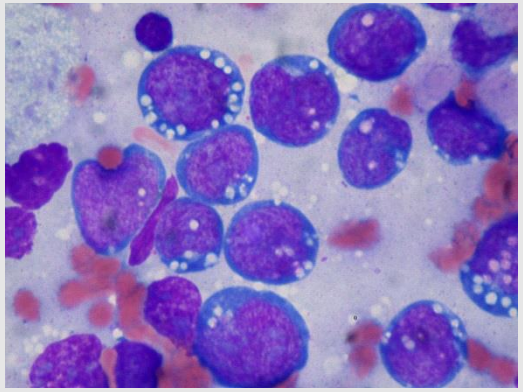
VIRAL THEORY OF CANCER: UPS AND DOWNS

Peyton Rouss
1911: discovery of RSV

1966: Nobel Prize

Denis Burkitt
1957: discovery of Burkitt's lymphoma

Anthony Epstein **Yvonne Barr**
1964: discovery of EBV in Burkitt's lymphoma samples



BURKITT'S LYMPHOMA

→A non-Hodgkin Lymphoma

→Three forms:

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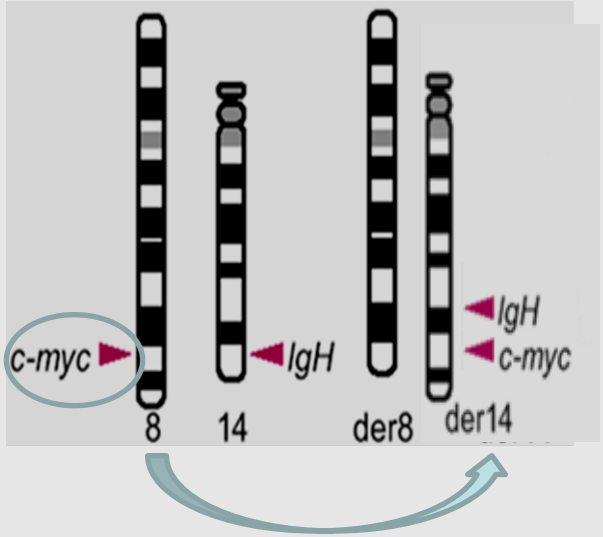


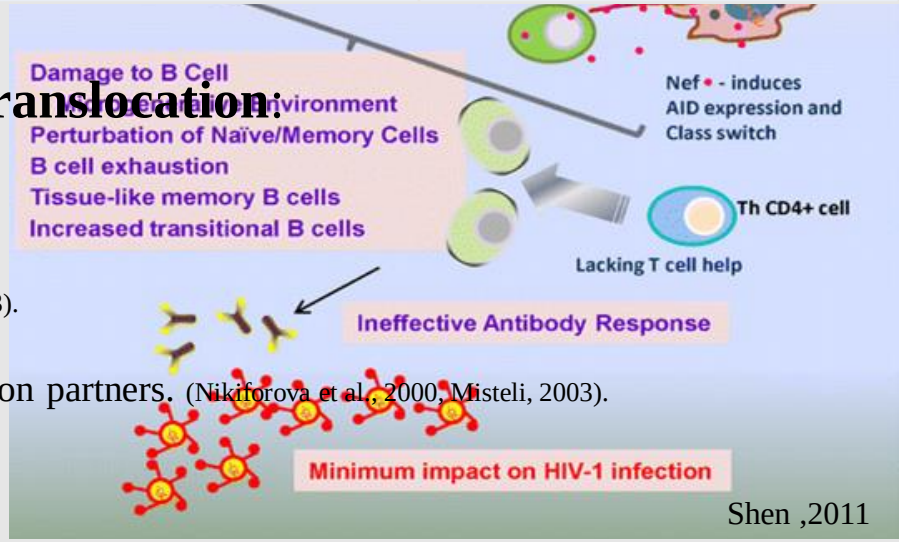
HIGH OCCURRENCE OF BURKITT'S LYMHOMA IN HIV PATIENTS: WHY?

Cancer	Frequency in the general poplation	Frequency in AIDS patients	Ratio
Burkitt's Lymhoma	1:200 000	1: 4000	50
Mantle Cell Lymphoma	1:200 000	1:200 000	1

- ✓ gp-120 can interact with CD21 expressed on B cells *(Moir et al 2000)*
- ✓ HIV-1 causes B-cell hyperactivation *(Schnittan et al,1984)*
- ✓ Elevated class switch in B lymphocytes
- ✓ Induces B cell to proliferate (Nair MPN and al 1988)
- ✓ Causes B cell abnormal response
- ✓ The production of autoantibodies
- ✓ Aberrant B-cell surface markers:

→Three events are necessary to produce a translocation:

- ✓DNA **double strand breaks** (Vilenchik et Knudson, 2003).
- ✓Double strand breaks repair *via* **NHEJ** (Abeysinghe et al., 2003).
- ✓Spatial proximity (**colocalization**) of the two translocation partners. (Nikiforova et al., 2000, Misteli, 2003).



Shen ,2011

EBV AND BURKITT'S LYMPHOMA

→ EBV is 100% associated with the endemic form in Afrtica
→Malaria and the use of latex-producing plants are additional risc factors in Africa

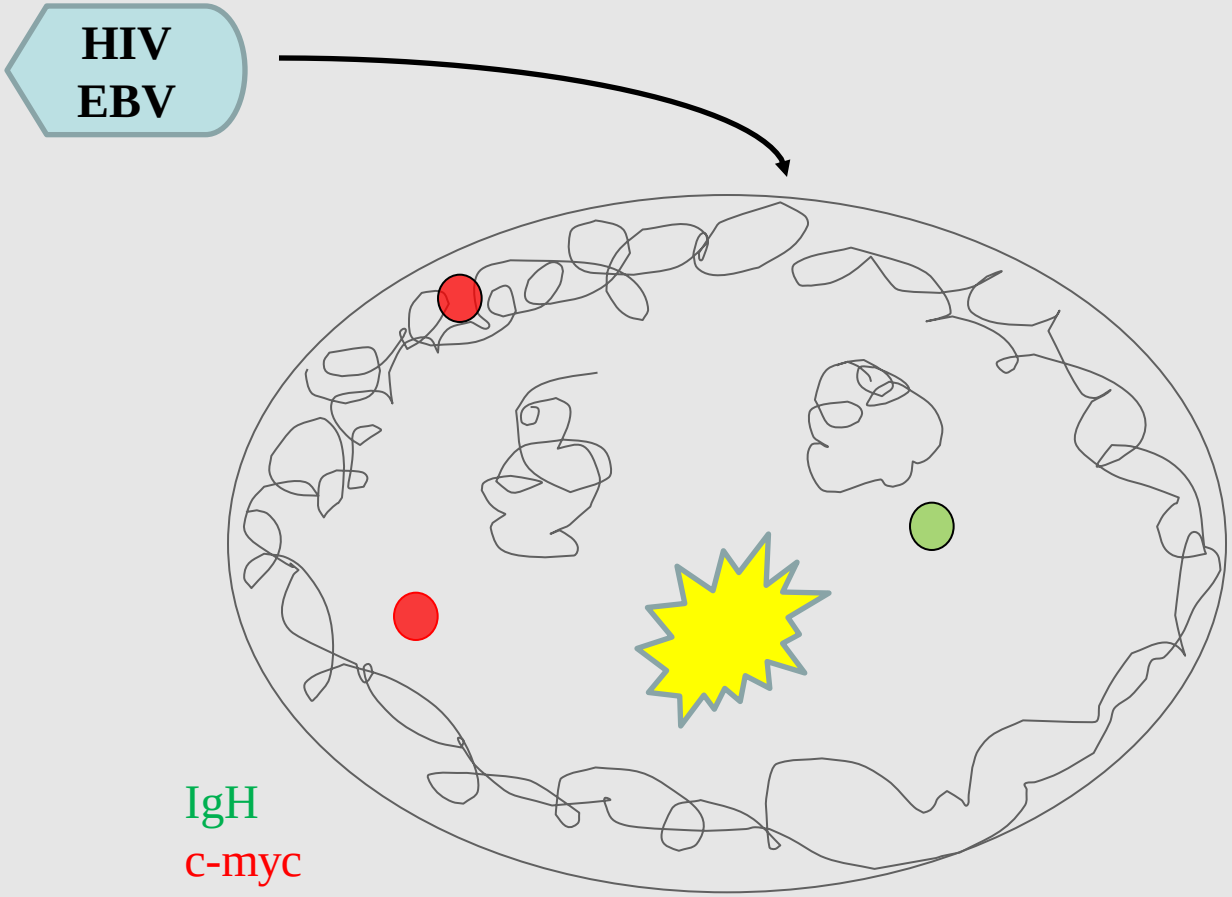
→EBV is an innocent passenger in tumour cells?

→EBV plays a role in initial transformation?
→ EBNA1 Stabilizes B-lymphocytes

→A role or EBV in sustenance of the tumour?
→A role of non-coding RNAs (EBER)?

→Tumour formation due to other cellular changes
→**Does EBV infection affect the nuclear architecture?**

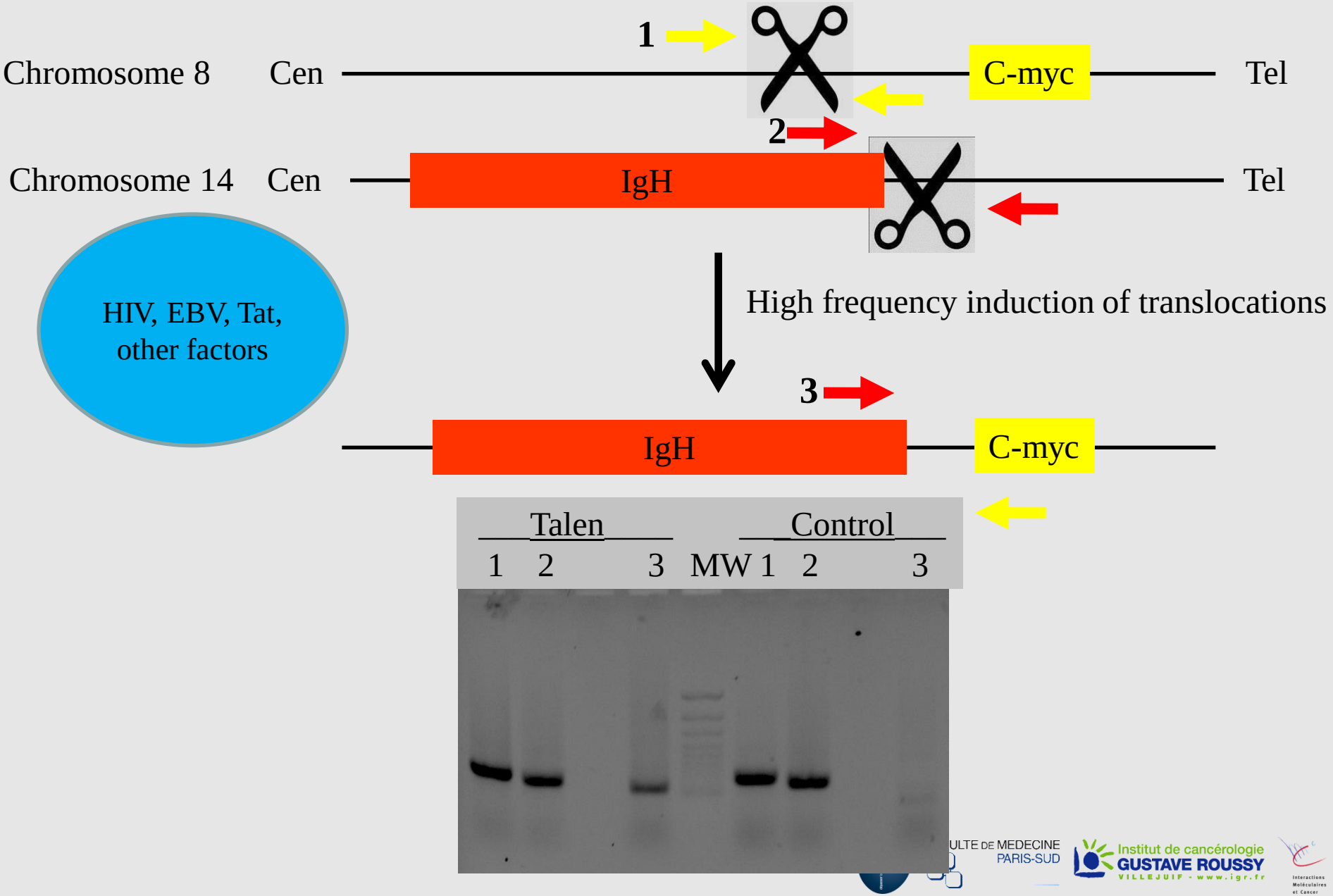
HIV: A ROLE IN INTRANUCLEAR REORGANIZATION AND IN GENERATION OF SPECIFIC TRANSLOCATIONS



HIV Tat → NFkB → RAG → DSB → NHEJ → CMYC relocalization

Tat C22 Transription — **RAGi** — **Mirin NU7026**

AN EXPERIMENTAL SYSTEM FOR INDUCTION OF TRANSLOCATIONS IN LCL USING TALEN/CRISPRs



Nuclear Organization and Pathologies Lab (UMR-8126, IGR, Villejuif, France):

- **Marc Lipinski,** DR CNRS
- **Chrystèle Bilhou-Nabera**, MCU-PH
- **Diego Germini**, postdoctorant
- **Tatiana Tsfasman**, postdoctorante
- **Yara Bou Saada,** doctorante UPS
- **Shirmoné Botha,** doctorante
- **Anatasia Sukhanova,** M1
- **Rawan El-Amine**, doctorante (cotutelle Liban)
- **Carla Dib**, doctorante

- **Eric Oksenhendler**, HSL, Paris
- **Sergey Razin,** IBG, Moscow
- **Olga Iarovaya,** IBG, Moscow
- **Evgeny Sheval,** MSU, Moscow
- **S. Bury-Moné,** ENS Cachan