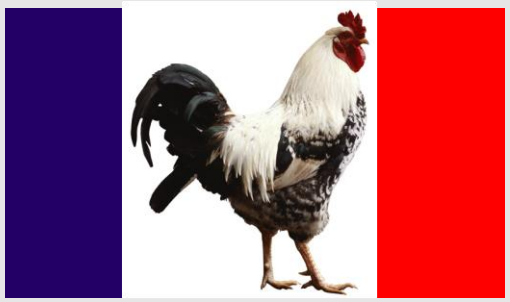
NUCLEAR ORGANIZATION AND REGULATION OF TRANSCRIPTION IN LYMPHOMAS AND NORMAL B-LYMPHOCYTES

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

Вадим Моисеевич Кавсан

1939 -- 2014

ENGELHARDT INSTITUTE OF MOLECULAR BIOLOGY



INSTITUT JACQUES MONOD

ISREC@EPFL

INSTITUT SUISSE DE RECHERCHE EXPÉRIMENTALE SUR LE CANCER

FACULTE

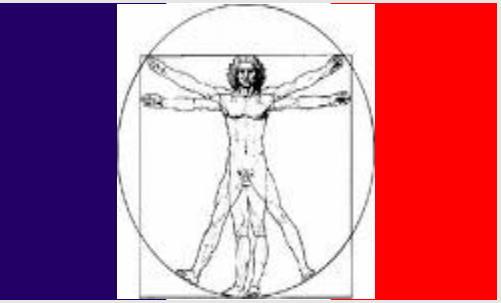
cnrs

INSTITUT JACQUES MONOD

IGH

GUSTAVE ROUSSY

CANCER CAMPUS GRAND PARIS



CHROMOSOMAL TRANSLOCATIONS

Chimeric genes

Balanced chromosomal translocations

Loss or gain of genes

C A N C E R

Theodor Boveri (1862-1915)

FACULTE DE MEDECINE PARIS-SUD

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

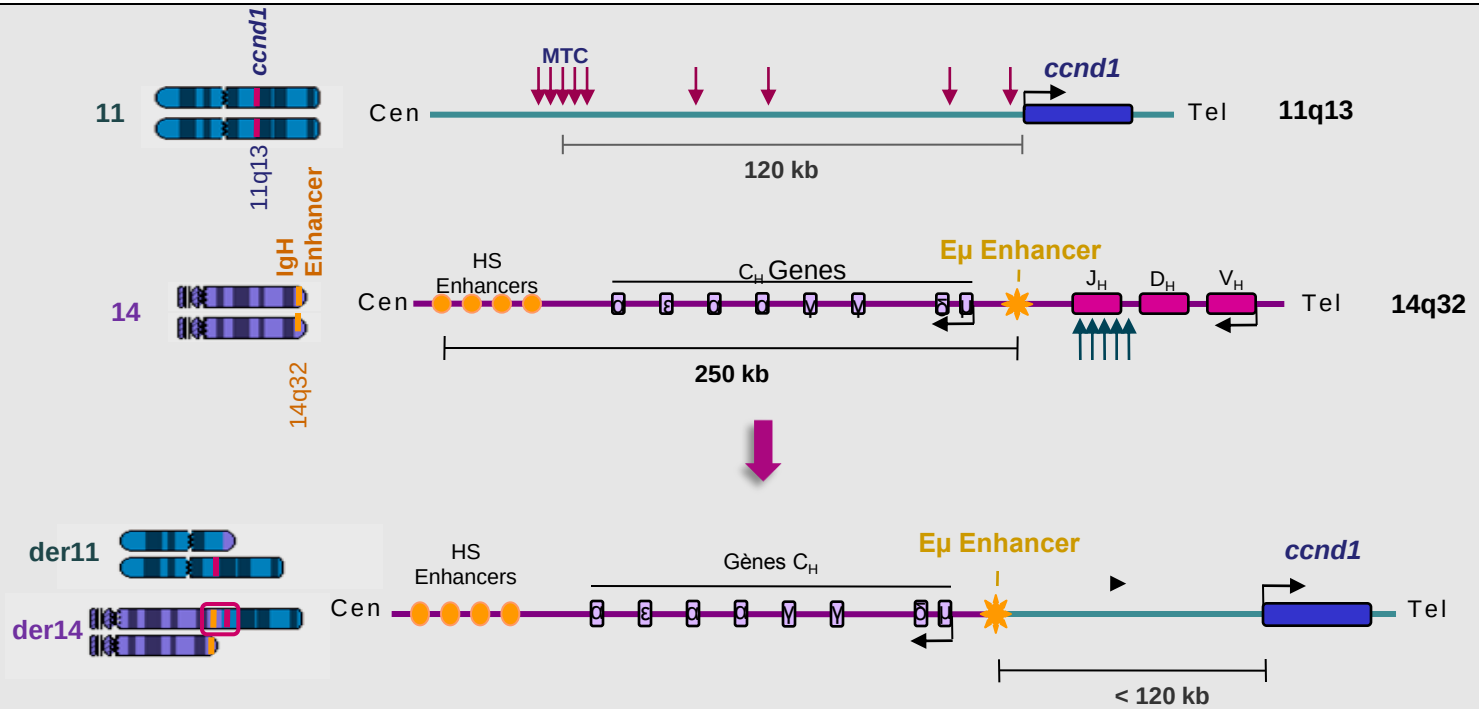
MANTLE CELL LYMPHOMA (MCL)

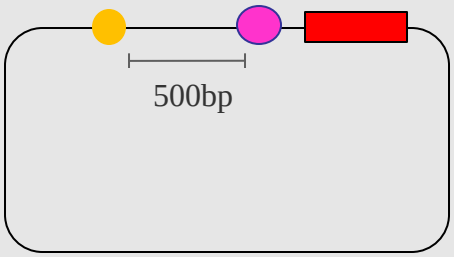
- Non- Hodgkin lymphoma.
- Highest frequency between 60 and 65 years (4% before 40 years).
- Low occurrence (1 case in 200 000).
- No curatif treatment; median survival rate is 3 to 4 years.

MANTLE CELL LYMPHOMA (MCL)

→ t(11 ; 14)(q13 ; q32) translocation is found in 100% of MCLs by FISH (Fluorescent In Situ Hybridization).

→t(11 ; 14)(q13 ; q32) translocation leads to overexpression of cyclin D1 (CCND1) in B lymphocytes



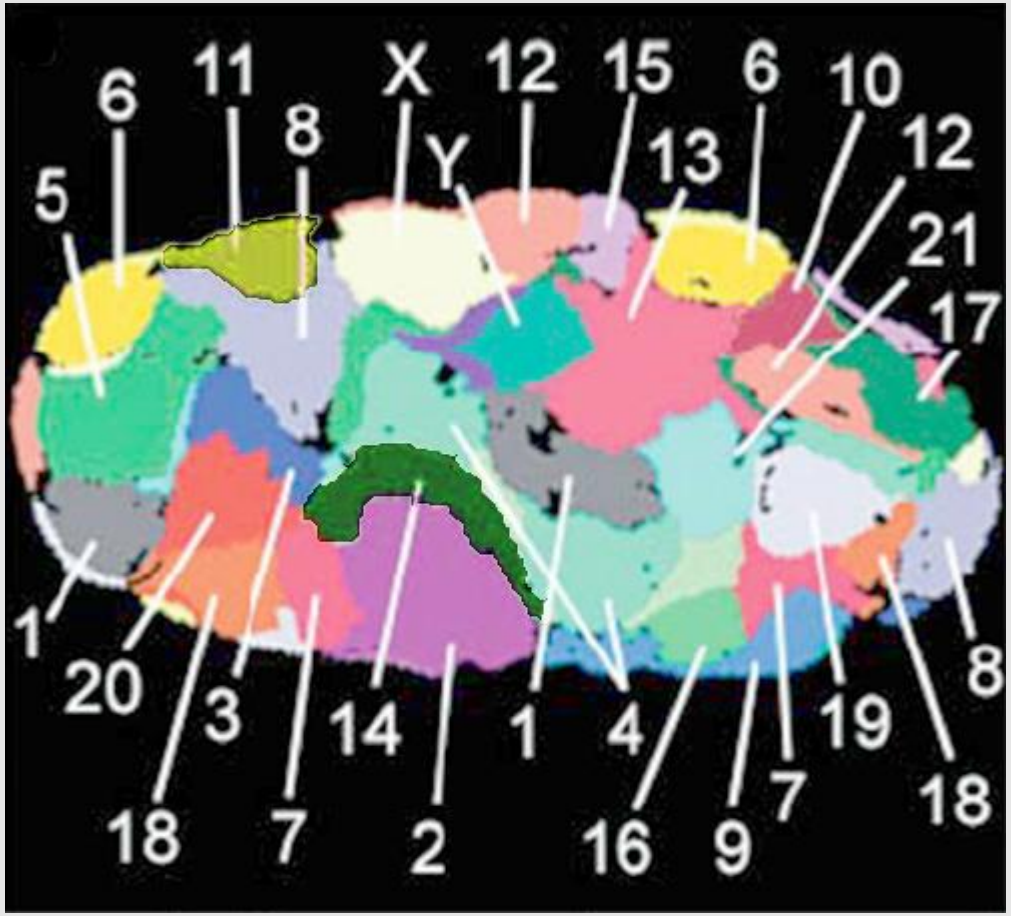


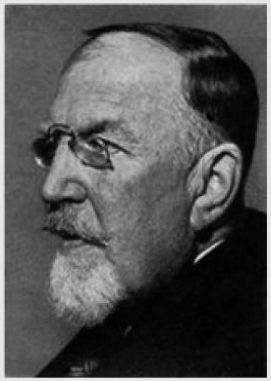
FACULTE DE MEDECINE PARIS-SUD

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

NUCLEAR ARCHITECTURE

Theodor Boveri (1862-1915)





Carl Rabl (1853-1917)

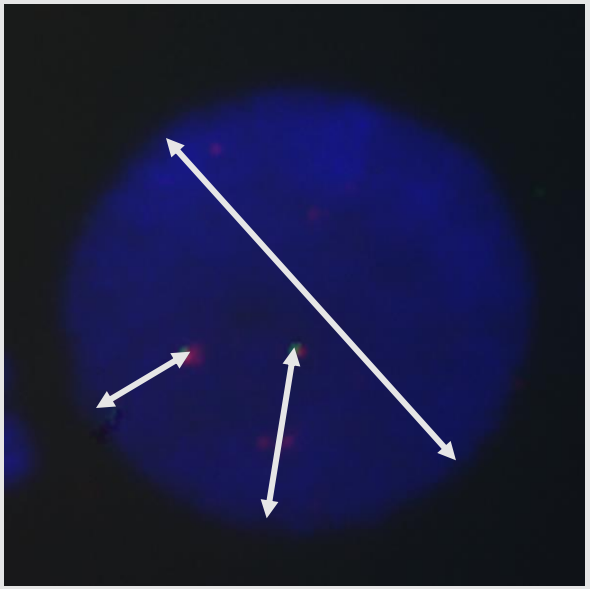
Bolzer et al., Plos Biology (2005), 3 (5) e207

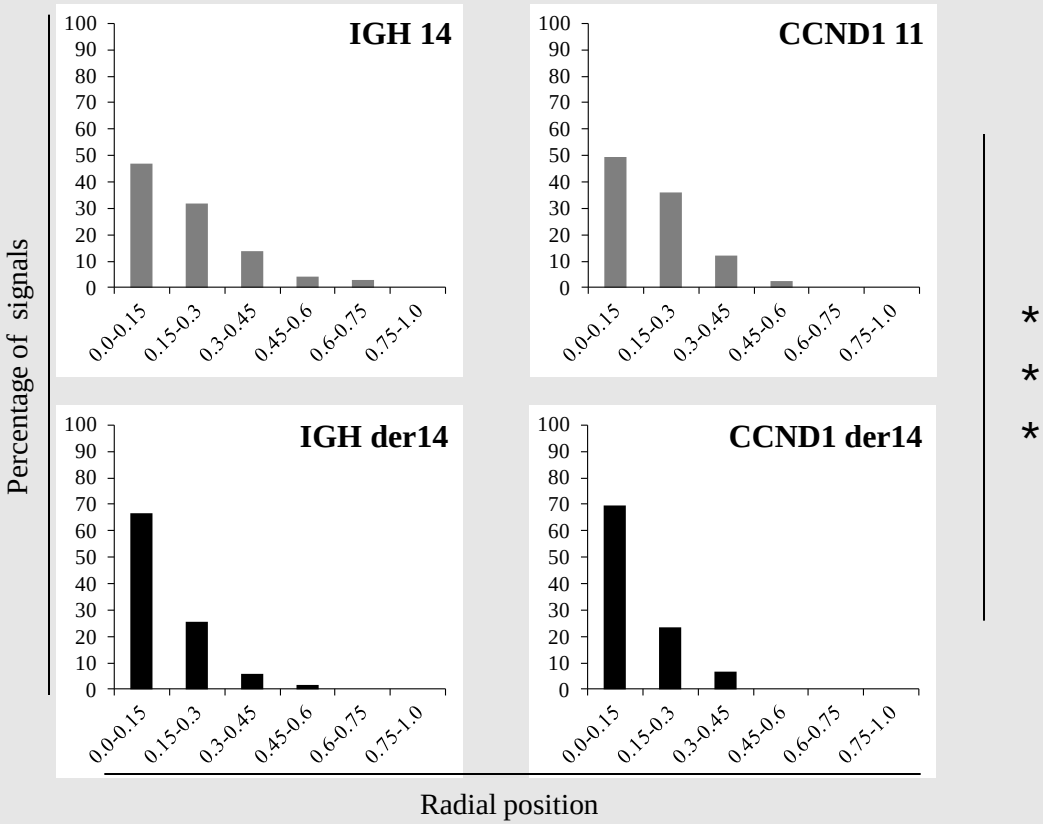
- **The chromosomes are organized in the nucleus:**
  - **In a tissue specific manner**
  - **The organization is transmitted though the cell divisions**
  - **This organization is evolutionarily conserved**
- **The gene-rich regions occupy more central position in the nucleus**

FACULTE DE MEDECINE PARIS-SUD

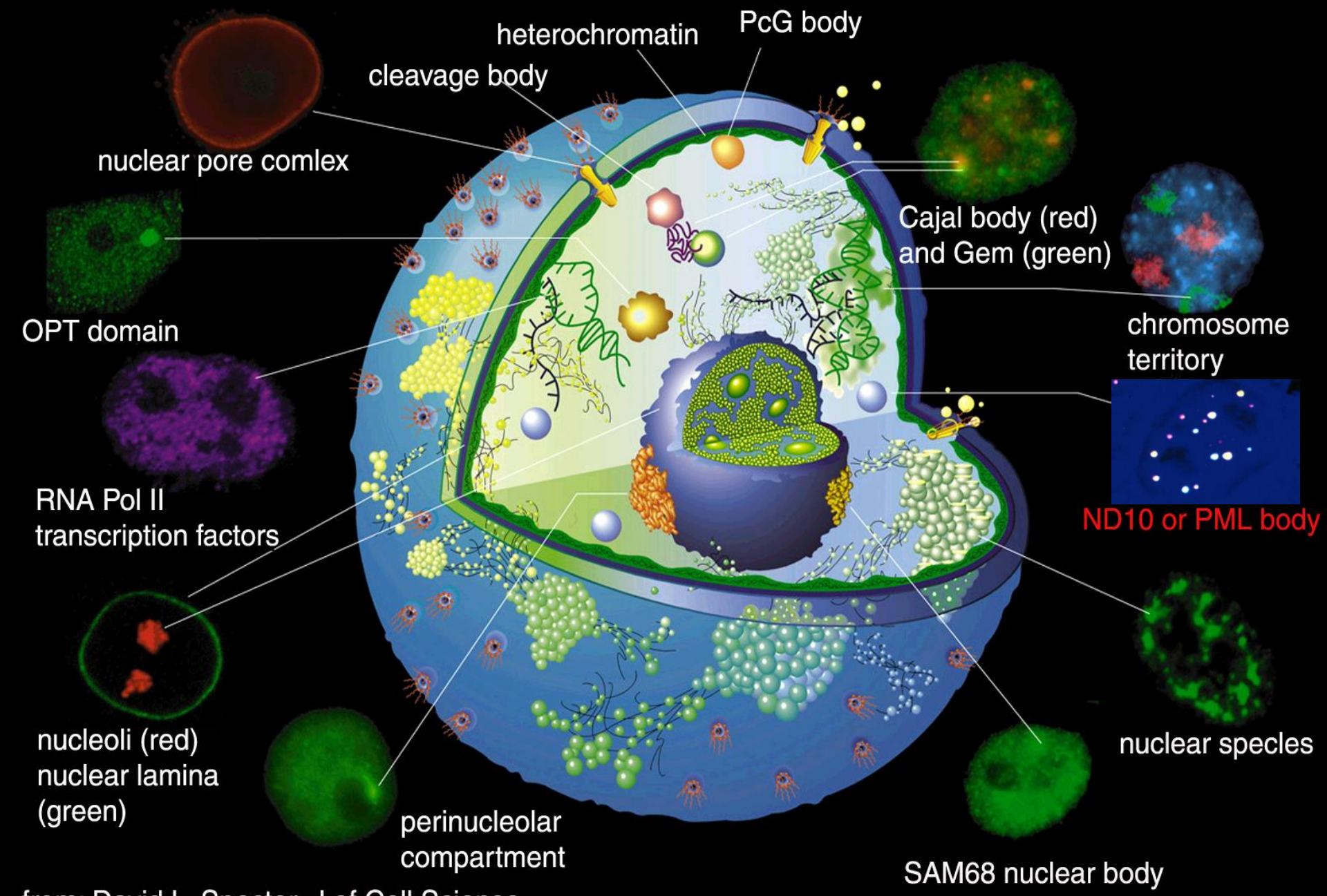
Institut de cancérologie GUSTAVE ROUSSY VILLEJUIF - www.igr.fr

THE 11q13 LOCUS IS RELOCALIZED WITHIN THE NUCLEUS IN MANTLE CELL LYMPHOMA





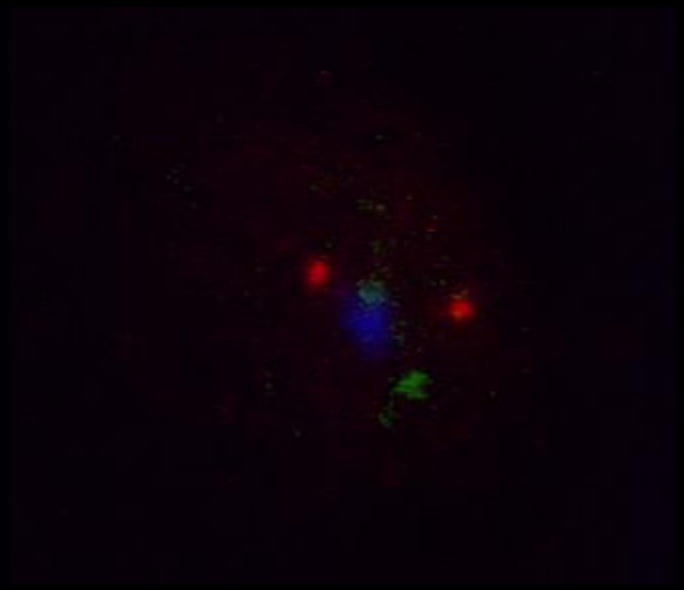
NUCLEAR SUBSTRUCTURES



from: David L. Spector, J of Cell Sciense

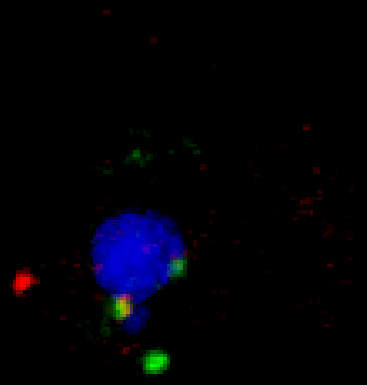
THE 11q13 LOCUS IS RELOCALIZED TOWARDS THE OUTER SURFACE OF THE NUCLEOLUS IN MCL

- 3D FISH ON INTERPHASE NUCLEI



NORMAL

LYMPHOCYTES



GRANTA

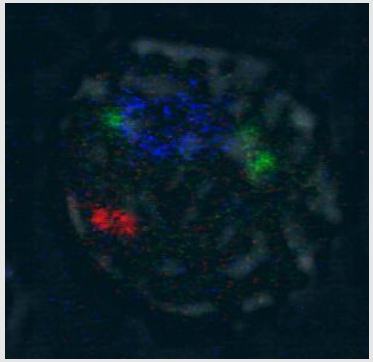
(MCL)

Cyclin D1

IgH

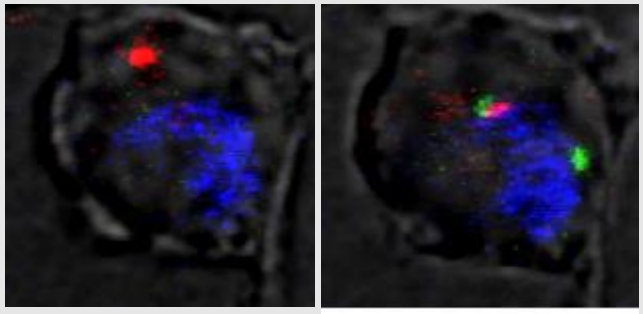
NUCLEOLUS (Anti-B23)

THE 11q13 LOCUS IS RELOCALIZED TOWARDS THE OUTER SURFACE OF THE NUCLEOLI IN MCL

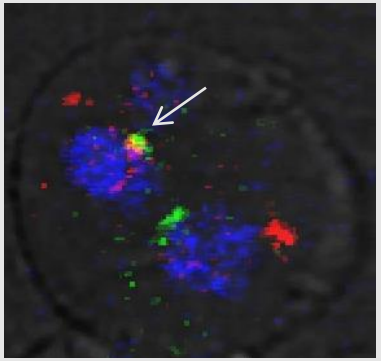


Normal B- lymphocytes

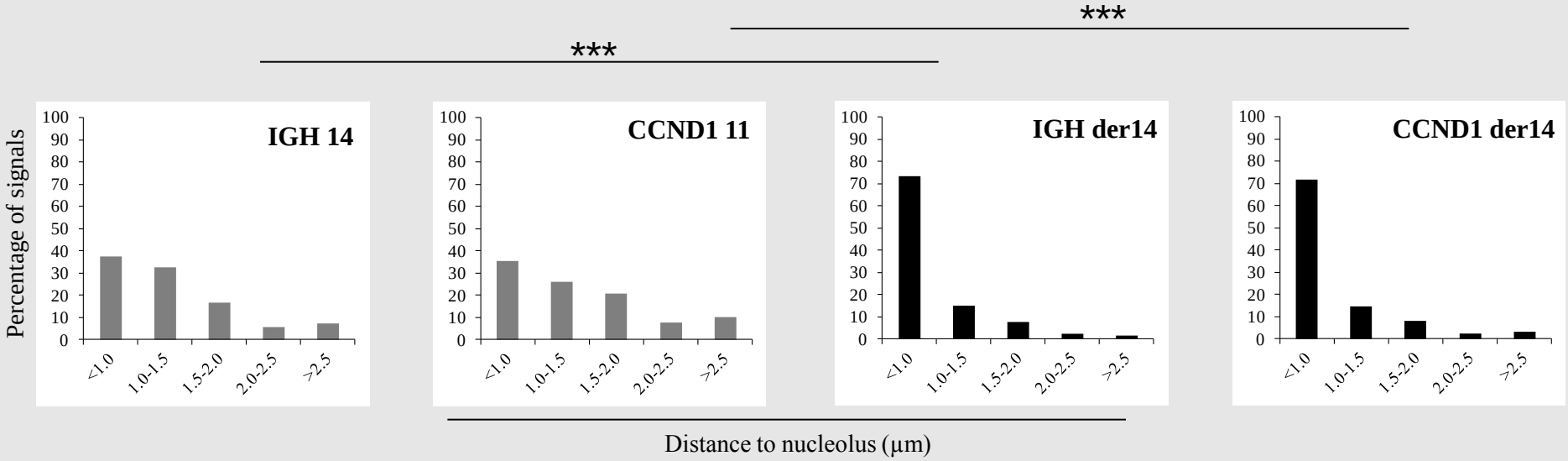
Cyclin D1 IgH Nucleolus (Anti-B23)



REC-BARC



UPN1



Statistical analysis:

- The relocalization event is highly reliable ($p<10^{-13}$)
- The relocalization is linked to translocation; non-translocated CCND1 is an excellent control
- The relocalization is observed both in MCL cell lines and patients

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

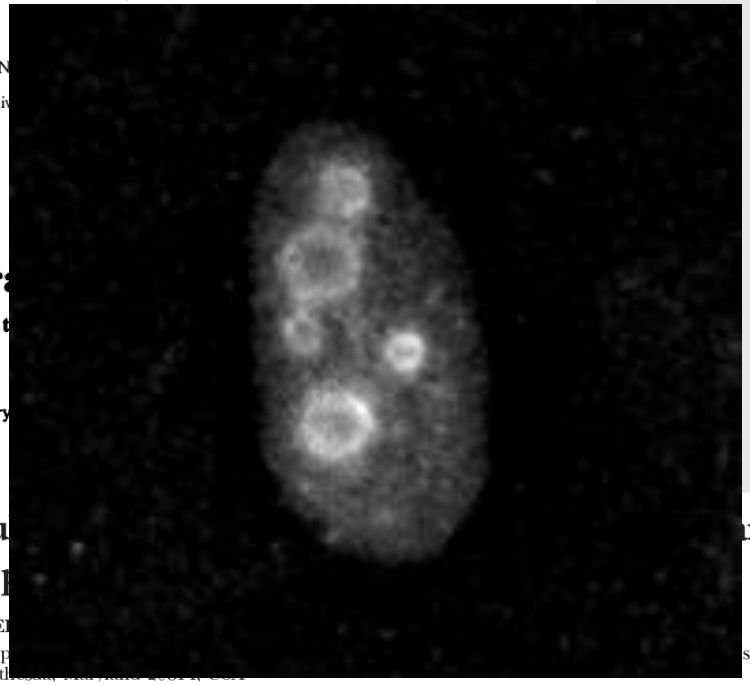
Proc. Natl. Acad. Sci. USA
Vol. 91, pp. 4915–4919, May 1994
Biochemistry

LR1 regulates c-*myc* tr

(*MYC* gene/immunoglobulin/chromosome t

APRIL BRYS† AND NANCY MAIZELS‡

Department of Molecular Biophysics and Biochemistry



Molecu nd
cell

ME
Dep s,
Bet

Vol. 13 November 1999 The FASEB Journal

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

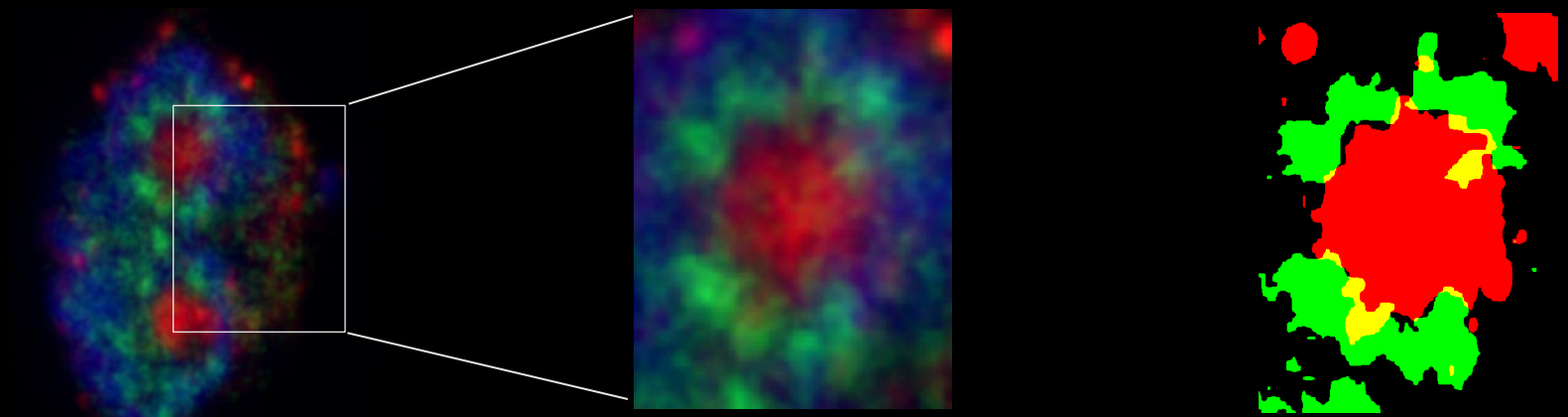
Identification of Nucleolin as an AU-rich Element Binding Protein Involved in *bcl-2* mRNA Stabilization*

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

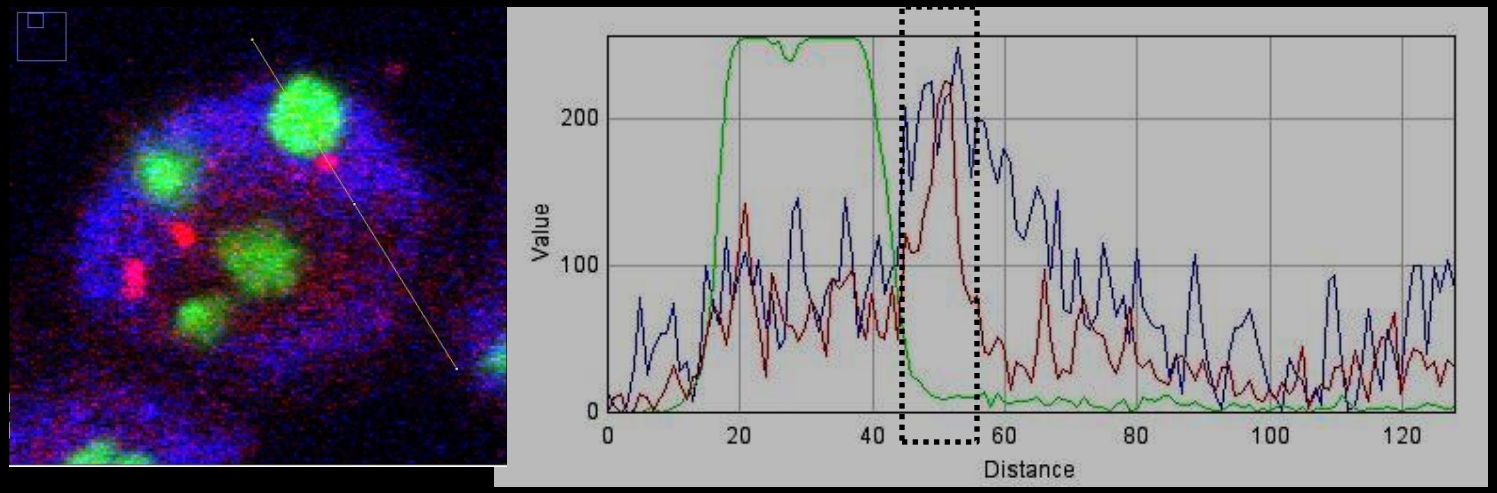
Tapas K. Sengupta‡§, Sumita Bandyopadhyay‡§, Daniel J. Fernandes‡, and Eleanor K. Spicer‡¶‖

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

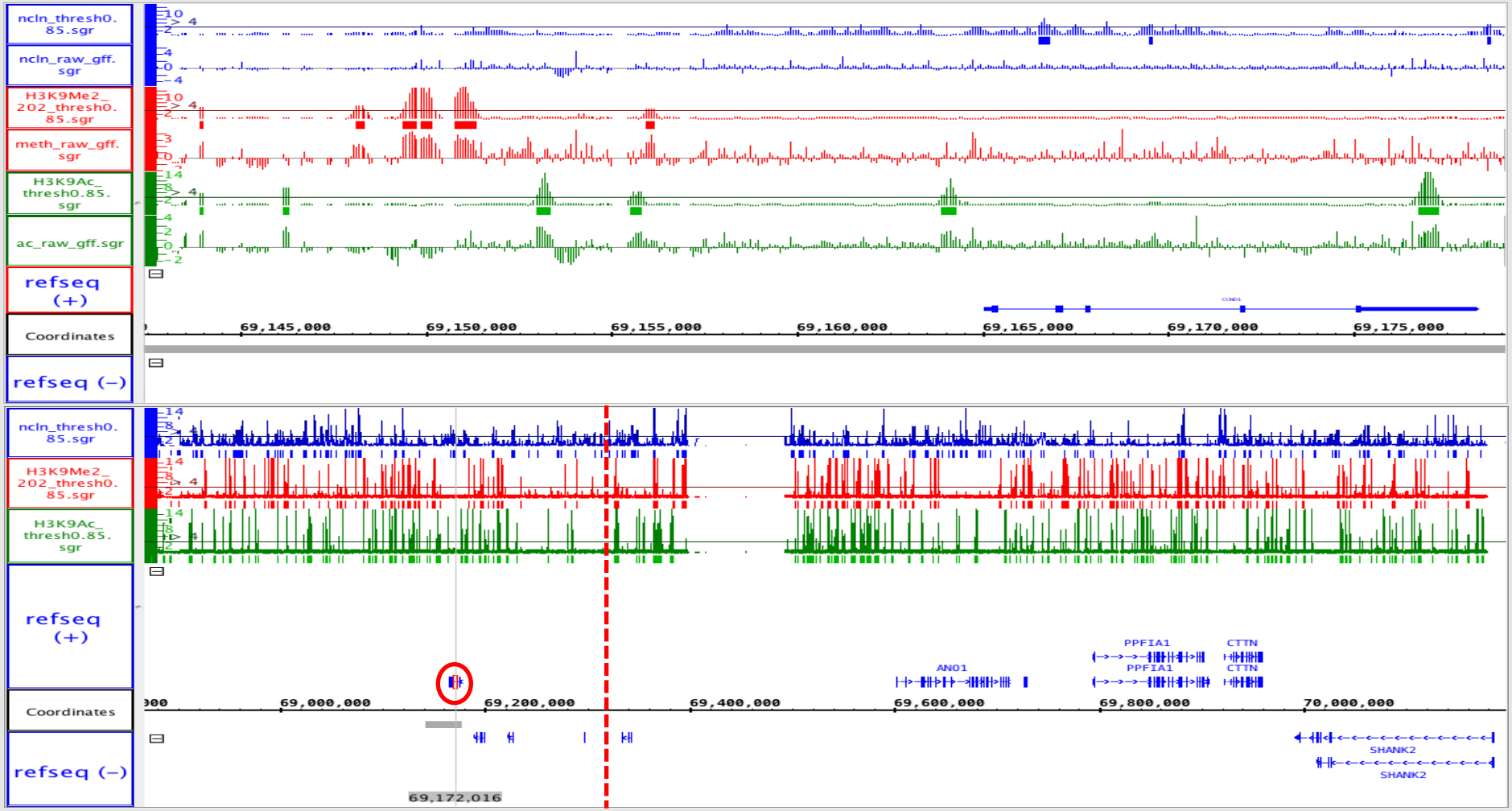


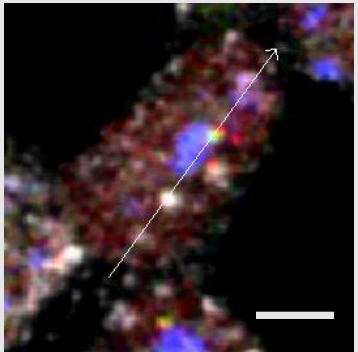
NUCLEOLUS (Anti-B23) Phosphorylated (active) RNA polymerase II NUCLEUS (DAPI)

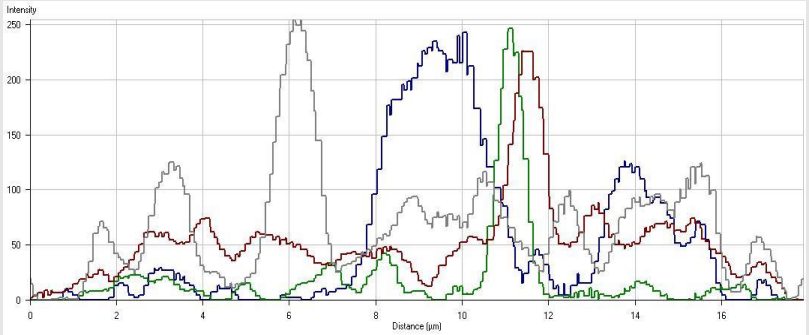


NUCLEOLUS (Anti-B23) Phosphorylated (active) RNA polymerase II Ccnd1

EPIGENETIC MARKS IN THE TRANSLOCATION REGION



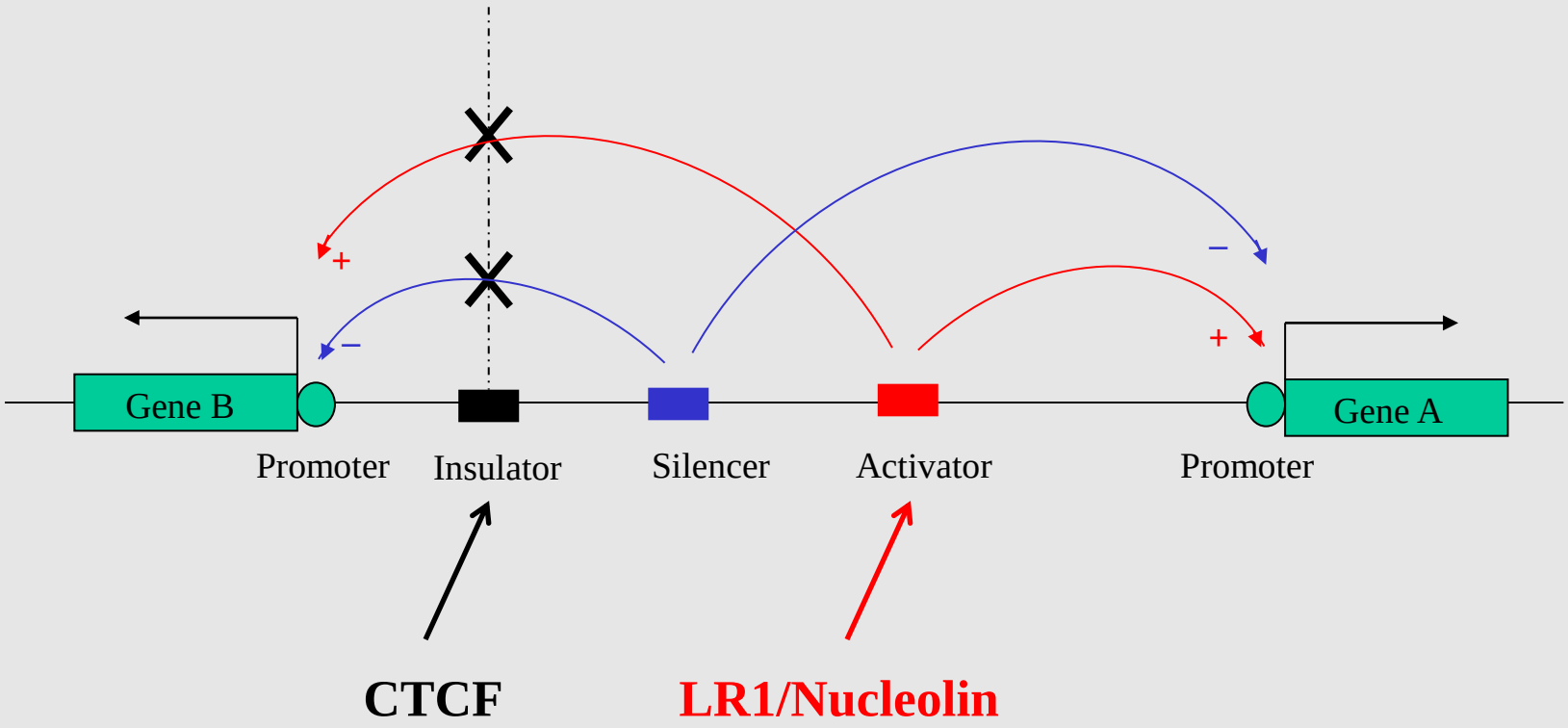




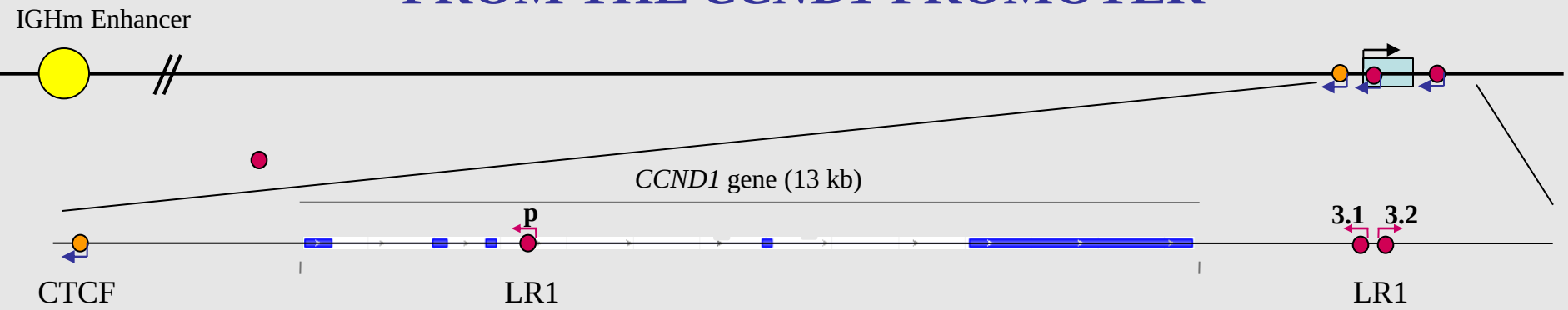
FACULTE DE MEDECINE PARIS-SUD

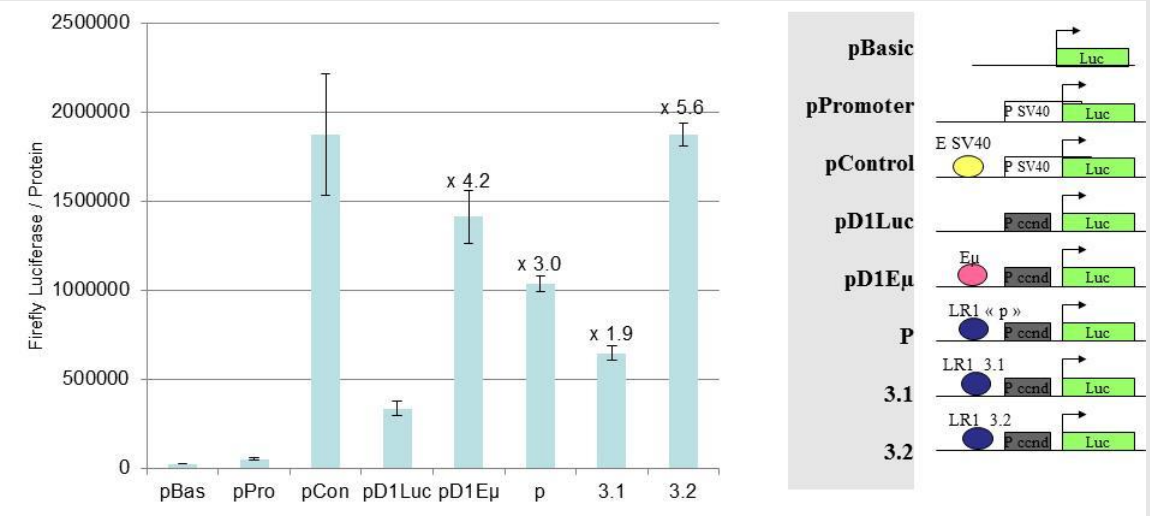
Institut de cancérologie GUSTAVE ROUSSY VILLEJUIF - www.igr.fr

REGULATION OF TRANSCRIPTION:

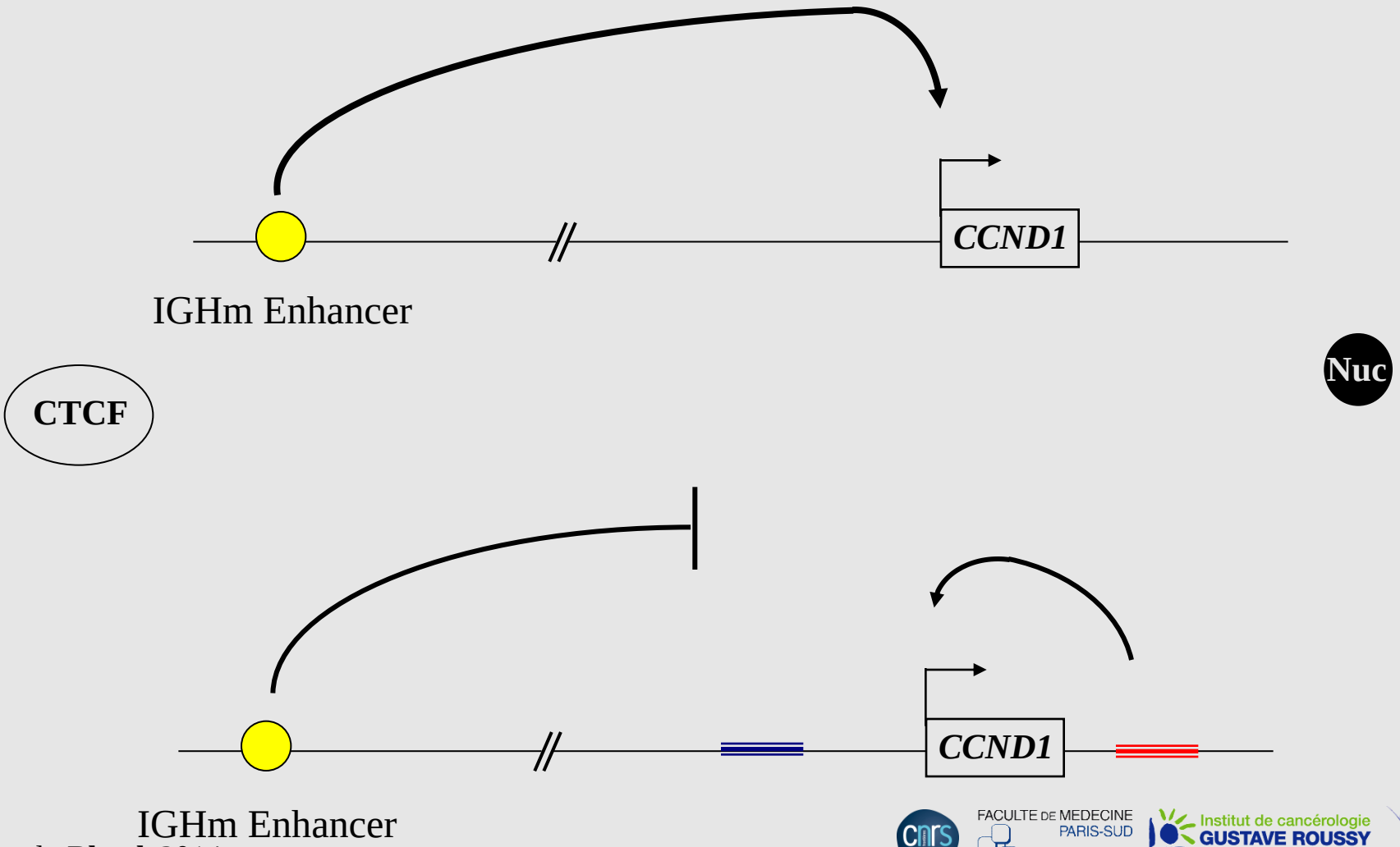


A NUCLEOLIN-BINDING SITE ACTIVATES TRANSCRPTION FROM THE *CCND1* PROMOTER





NUCLEOLIN, CTCF AND TRANSCRIPTIONAL REGULATION OF THE *CCND1* LOCUS



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

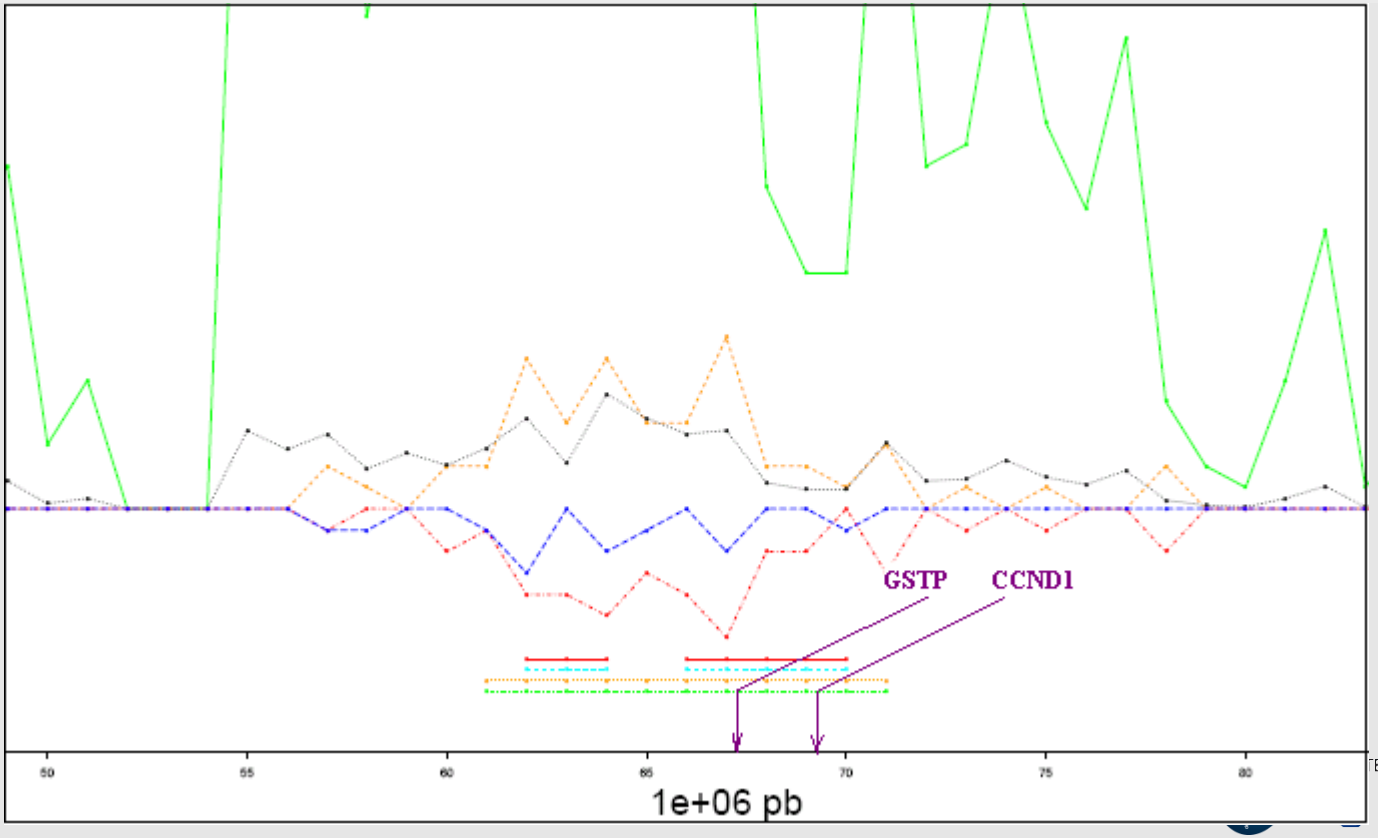
ARE OTHER 11q13 OR 14q32 GENES ACTIVATED AFTER THE TRANSLOCATION?

Curves

- Ensembl genome
- Tested list
- Hazard estimation
- Upregulated genes
- Downregulated genes

Statistucal results

- Units where Tested list > Hazard estimation
- Statistically validated ▶ **Binomial test on the unit**
- Optimized regions
- Significant ▶ **Wilcoxon test on the small region**



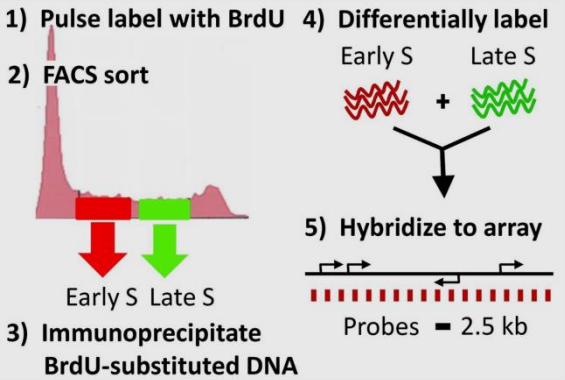
11q13 GENES ACTIVATED AFTER THE TRANSLOCATION

Start point	Gene	Expression rate	Gene name	
60739115	***CD6***	0,850392574	CD6 molecule	
60869930	***CD5***	0,959535821	CD5 molecule	
61100654	DAK	0,530690076	dihydroxyacetone kinase 2 homolog (S. cerevisiae)	
62201016	AHNAK	2,183624534	AHNAK nucleoprotein	
62360675	***MTA2***	0,511737498	metastasis associated 1 family, member 2	
62457747	BSCL2	0,771842816	Bernardinelli-Seip congenital lipodystrophy 2 (seipin)	
62623518	SLC3A2	0,806756116	solute carrier family 3	
63341934	PLA2G16	1,236042113	phospholipase A2, group XVI	
63448922	RTN3	0,941432897	reticulon 3	
64037300	***BAD***	0,607078976	BCL2-associated agonist of cell death	
64494383	RASGRP2	0,618417066	RAS guanyl releasing protein 2 (calcium and DAG-regulated)	
64513861	PYGM	0,851019014	phosphorylase, glycogen, muscle	
64692180	PPP2R5B	0,452563551	protein phosphatase 2, regulatory subunit B', beta isoform	
64794910	SNX15	0,556354468	sorting nexin 15	
65337943	SSSCA1	0,493153003	Sjogren syndrome/scleroderma autoantigen 1	
65479489	KAT5	0,478519186	K(lysine) acetyltransferase 5	
65686728	DRAP1	0,777999239	DR1-associated protein 1 (negative cofactor 2 alpha)	
66059373	TMEM151A	0,59365937	transmembrane protein 151A	
66278119	BBS1	0,546155142	Bardet-Biedl syndrome 1	
66330935	***CTSF***	0,49155074	cathepsin F	
66610883	RCE1	0,526977415	RCE1 homolog, prenyl protein peptidase (S. cerevisiae)	
67195935	RPS6KB2	0,40202797	ribosomal protein S6 kinase, 70kDa, polypeptide 2	
67205518	CORO1B	1,284980925	coronin, actin binding protein, 1B	
67259239	PITPNM1	0,401006288	phosphatidylinositol transfer protein, membrane-associated 1	
67351066	***GSTP1***	1,546682584	glutathione S-transferase pi 1	
67820326	CHKA	0,679197547	choline kinase alpha	
68080108	LRP5	0,549308072	low density lipoprotein receptor-related protein 5	
68522088	CPT1A	1,355308335	carnitine palmitoyltransferase 1A (liver)	**der11**
68671319	IGHMBP2	0,607078976	immunoglobulin mu binding protein 2	
69455873	**CCND1**	**4,651669229**	**cyclin D1**	
69480331	***ORAOV1***	0,503355776	oral cancer overexpressed 1	**der14**
70244612	CTTN	0,495420482	cortactin	
71145457	DHCR7	0,515050676	7-dehydrocholesterol reductase	
71639768	RNF121	0,386244877	ring finger protein 121	
71900602	FOLR1	1,229322176	folate receptor 1 (adult)	
75526212	***UVRAG***	0,664071631	UV radiation resistance associated gene	

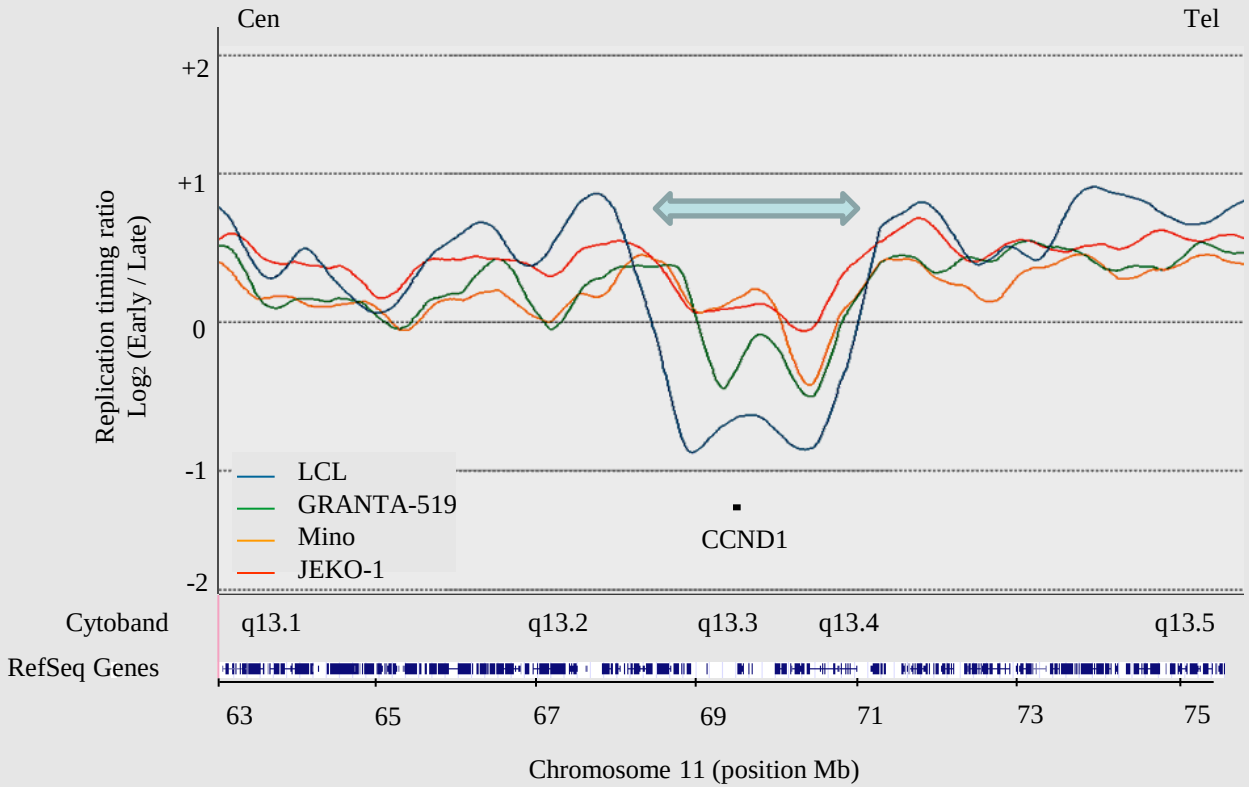
FACULTE DE MEDECINE PARIS-SUD

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

REPLICATION TIMING CHANGES BOTH ON der11 AND der14 AFTER THE TRANSLOCATION



From Ryba et al., 2012



Collaboration: MN Prioleau

OTHER CANCERS?

→A multitude of translocations involving the acrocentric chromosomes (13, 14, 15, 21, 22)

→ The most frequent variant of Burkitt lymphoma has a t(8;14)(q24;q32) translocation.

Is the nucleolus involved in this case?

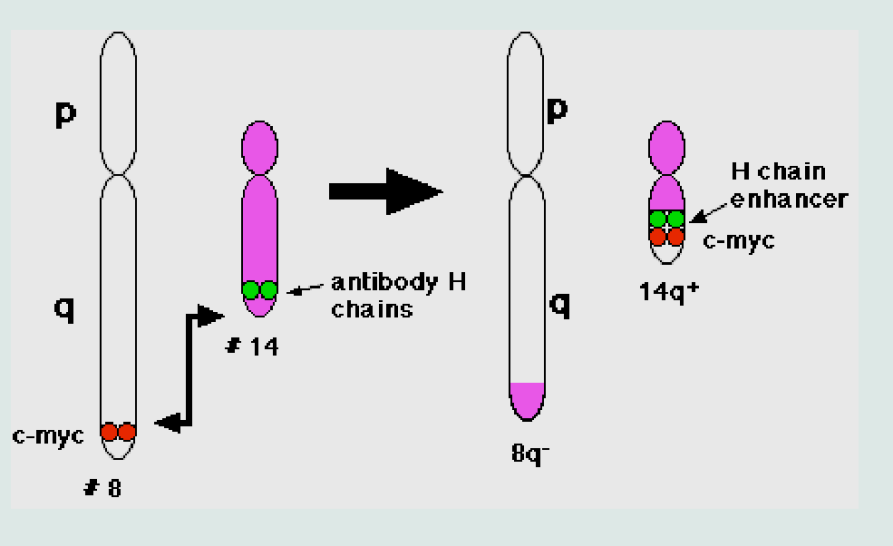
BURKITT LYMPHOMA

→ Burkitt lymphoma is a monoclonal proliferation of B lymphocytes characterized by small non-cleaved cells that are uniform in appearance and that produce a diffuse pattern of tissue involvement.

→ Most Burkitt lymphomas carry a translocation of the c-myc oncogene from chromosome 8 to either the immunoglobulin (Ig) heavy-chain region on chromosome 14 [t(8;14)] or chromosome 22 (lambda light chain) [t(8;22)].

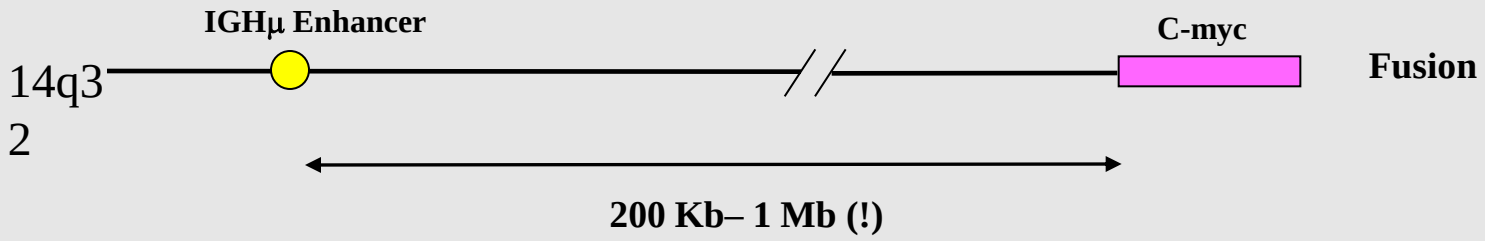
→ Burkitt lymphoma is a very rare form of cancer in France, with about 100 new cases occurring each year.

→ High occurrence in EBV-infected children in Africa and HIV patients in Europe

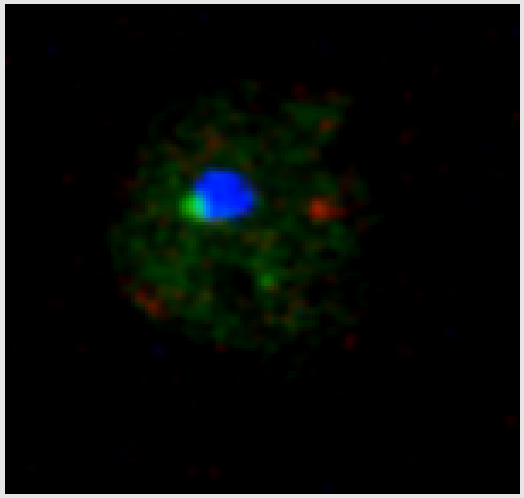


C-MYC ACTIVATION IN BURKITT LYMPHOMA: IS IGHμ ENHANCER REALLY INVOLVED?

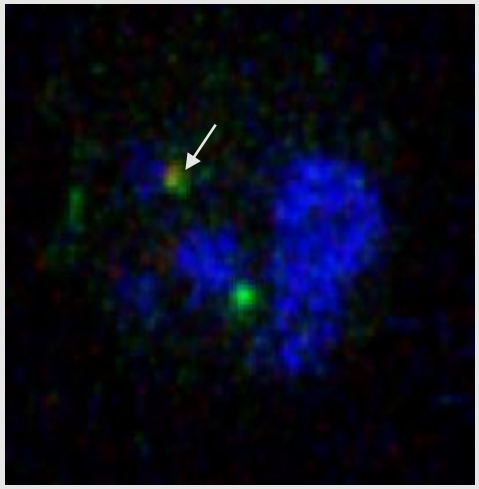
- t(8 ; 14) translocation induces overexpression of *c-myc* in B-cells



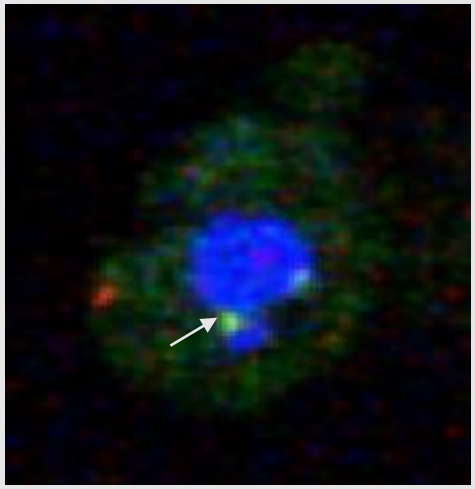
TRANSLOCATED *c-myc* LOCUS IS LOCALIZED IN THE PERINUCLEOLAR REGION IN BURKITT LYMPHOMA



Normal B-lymphocytes

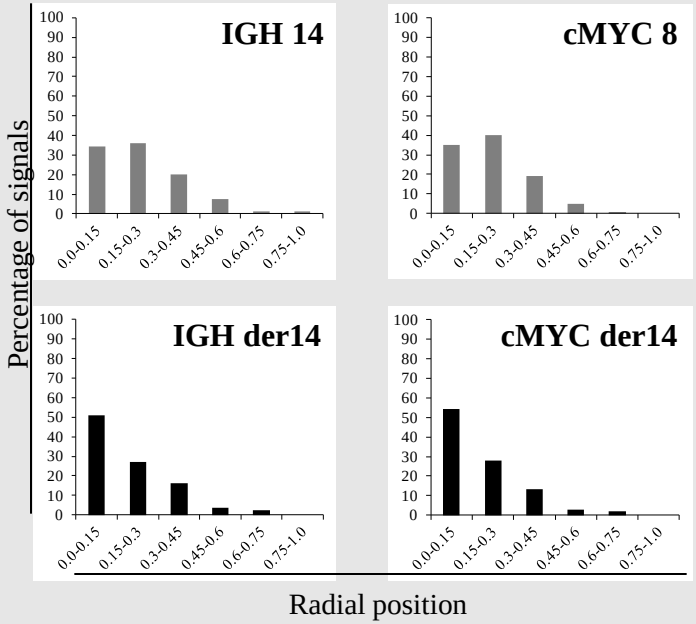


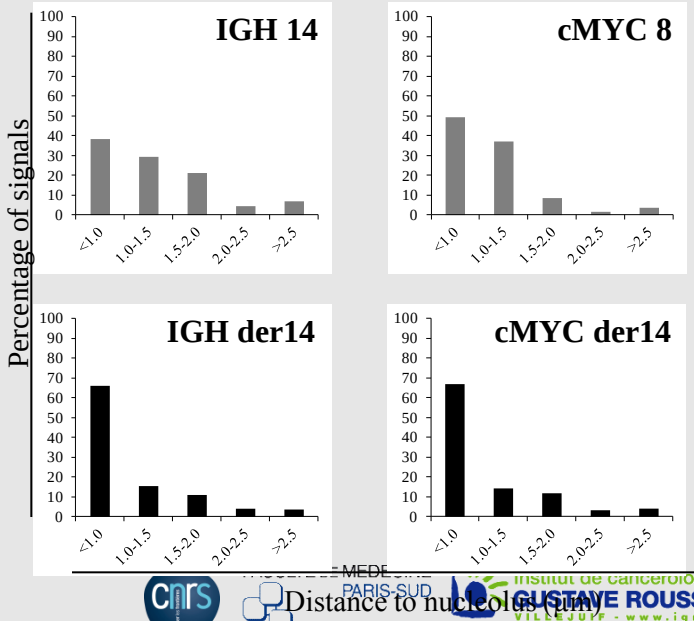
P3HR1 Burkitt cell line



RAJI Burkitt cell line

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





NUCLEOLIN IS A FACTOR OF TRANSCRIPTION THAT REGULATES *C-MYC*

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*, and Nancy Maizels*†‡

Departments of *Molecular Biophysics and Biochemistry and †Genetics, Yale University, 266 Whitney Avenue, New Haven, CT 06520-8114

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

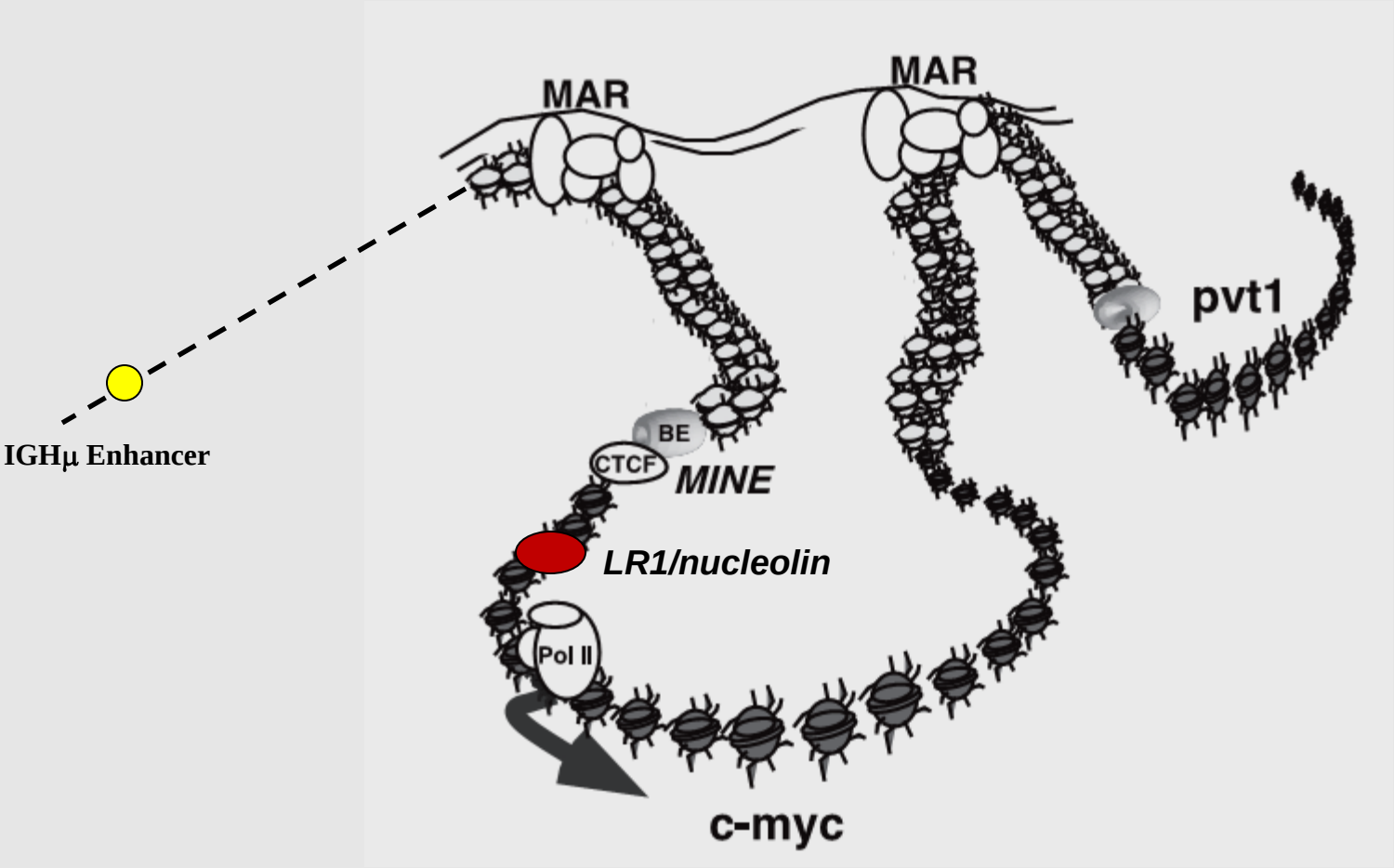
LR1 regulates c-*myc* transcription in B-cell lymphomas

(*MYC* gene/immunoglobulin/chromosome translocation/Burkitt lymphoma)

April Brys† and Nancy Maizels‡

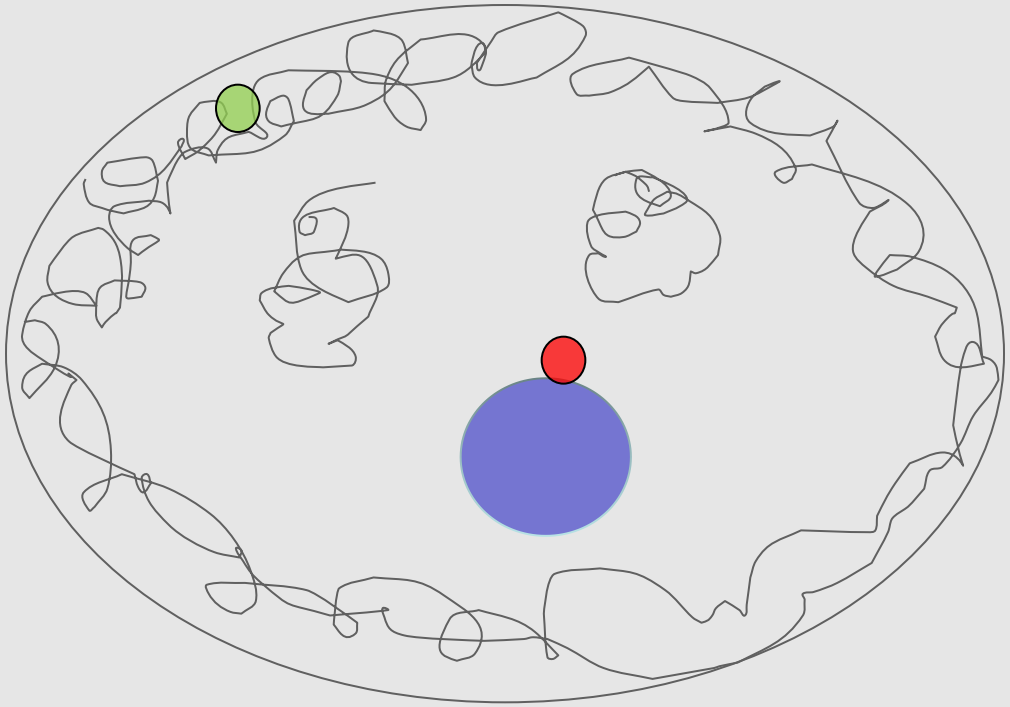
Department of Molecular Biophysics and Biochemistry, Yale Medical School, 333 Cedar Street, New Haven, CT 06510

AN INSULATOR AND A NUCLEAR MATRIX ATTACHMENT SITE SEPARATE *c-myc* FROM THE IGHμ ENHANCER



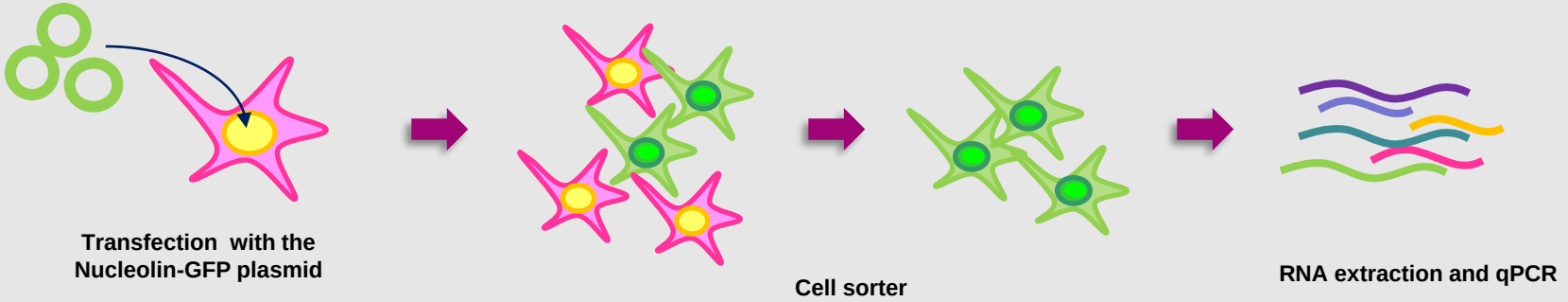
Gombert, MCB, 2003

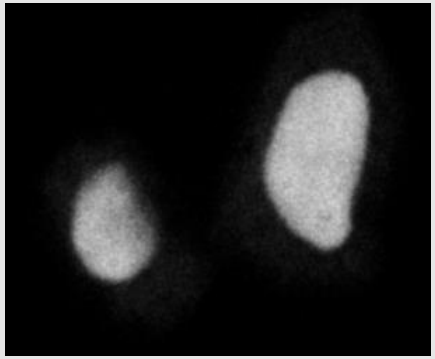
INTRANUCLEAR RELOCALIZATION AND GENE ACTIVATION IN CANCER



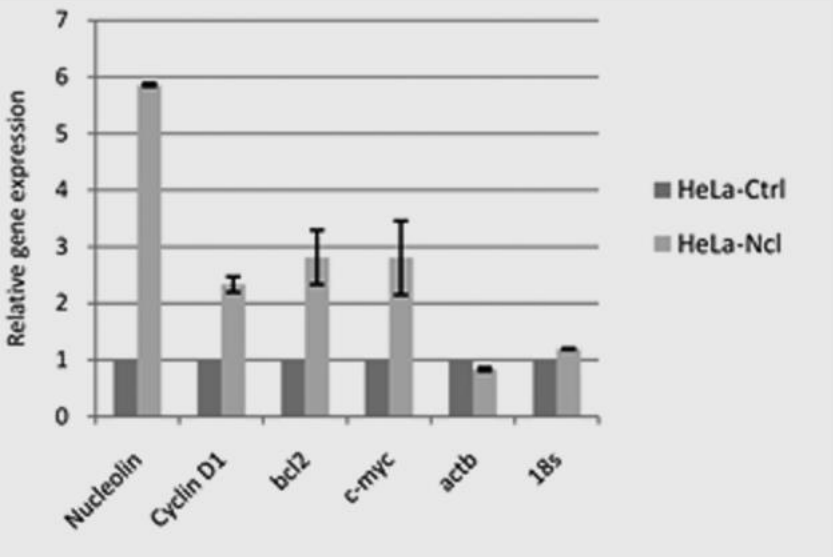
FACULTE DE MEDECINE PARIS-SUD
Institut de cancérologie GUSTAVE ROUSSY VILLEJUIF - www.igr.fr

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





Nucleolin-GFP



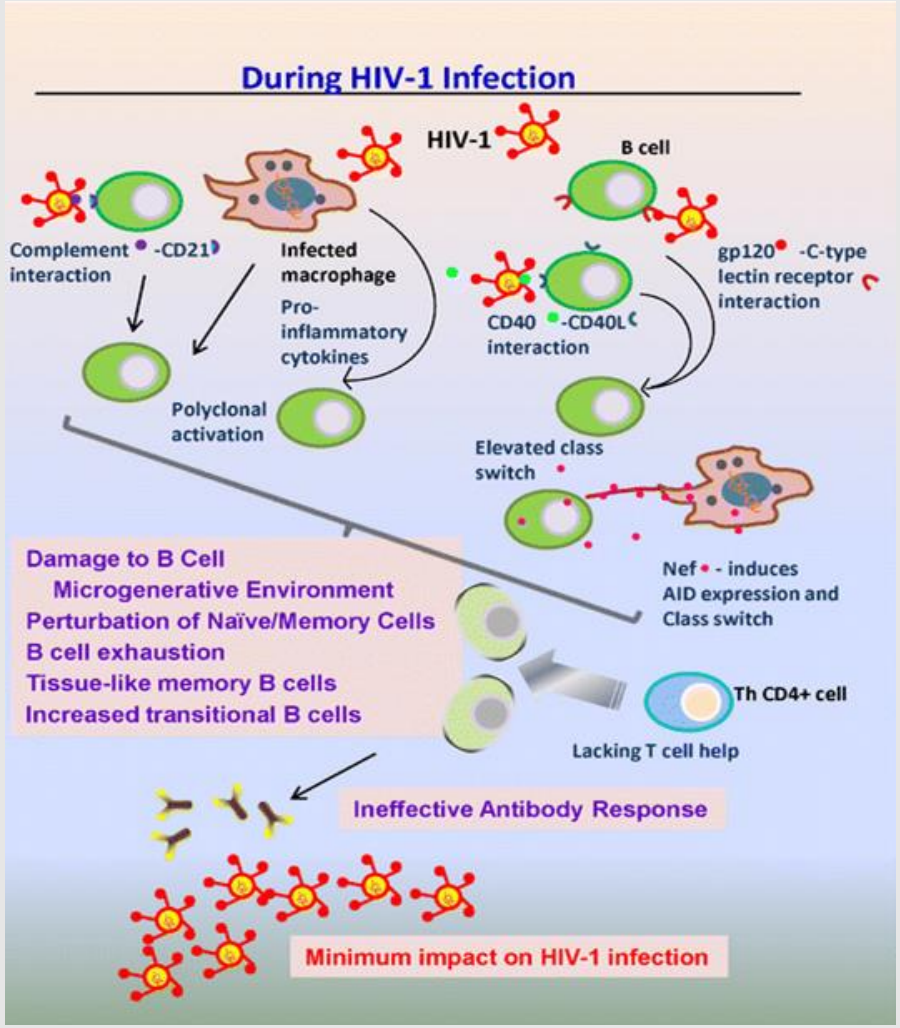
→Ectopic overexpression of nucleolin leads to its delocalization in the nucleus and overexpression of endogenous *CCND1, c-myc and bcl-2*

FACULTE DE MEDECINE PARIS-SUD

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

HIGH OCCURRENCE OF BURKITT LYMHOMA IN HIV PATIENTS: WHY?

- ✓ gp-120 can interact with CD21 expressed on B cells (Moir.S and al 2000)
- ✓ HIV-1 causes B-cell hyperactivation (Schnittan and 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: Change in B cell receptors.



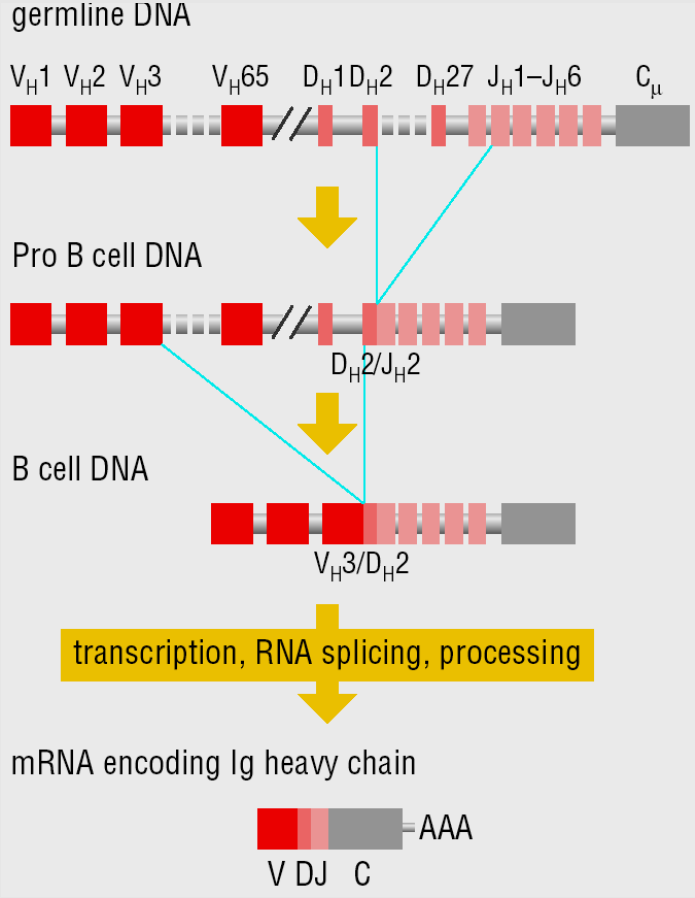
Shen ,2011

FACULTE DE MEDECINE PARIS-SUD

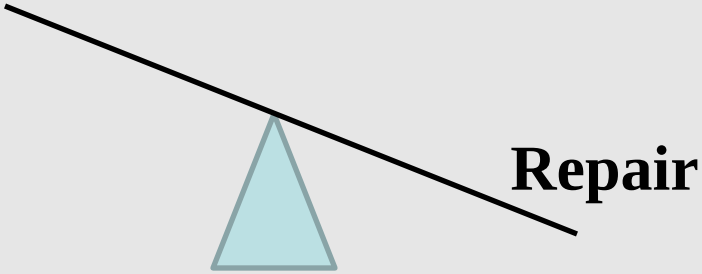
Institut de cancérologie GUSTAVE ROUSSY VILLEJUIF - www.igr.fr

SOMATIC CELL

B LYMPHOCYTE

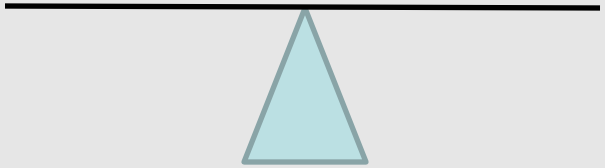


Recombination

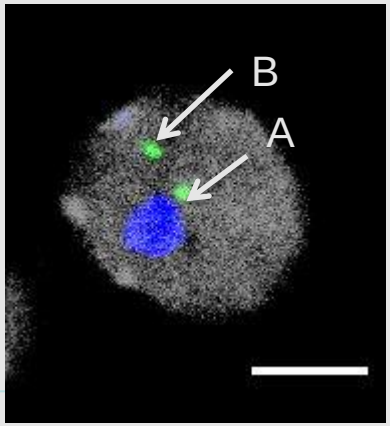


Repair

Recombination **Repair**



WHAT IS THE ROLE OF THE NUCLEOLUS IN NORMAL B-LYMPHOCYTES?



Gray-nucleus, green-IgH, blue-B23

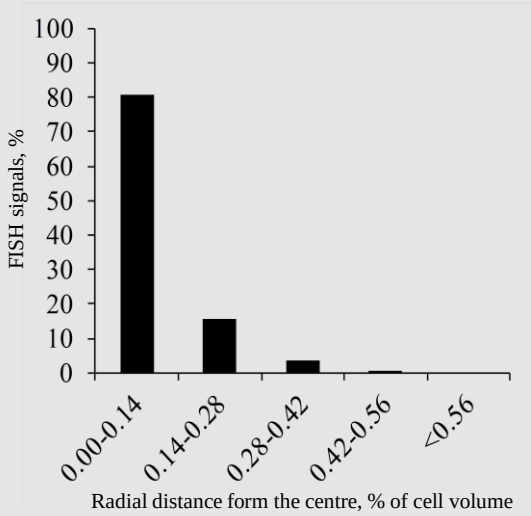
One IgH allele is always present in the perinucleolar region in normal B-lymphocytes

→ ***A role in regulation of transcription (repression/activation)?***

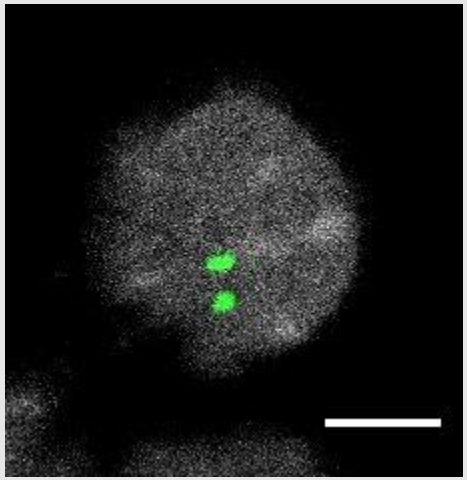
→ ***A role in allelic exclusion?***

→ ***A role in somatic hypermutation and class switch recombination?***

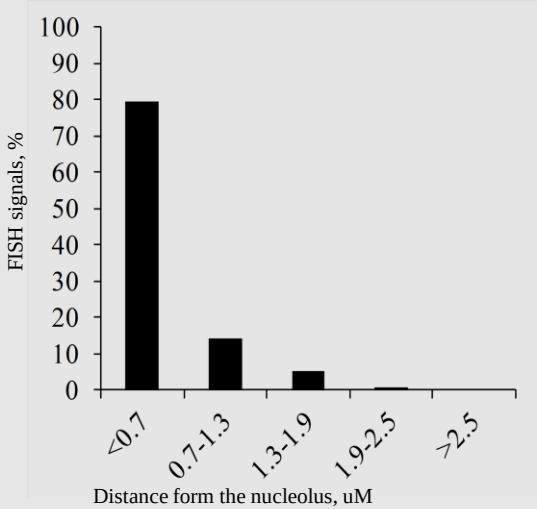
BOTH IgH ALLELES ARE LOCATED IN THE CENTER OF THE NUCLEUS; ONE ALLELE IS ALWAYS LOCATED CLOSE TO THE NUCLEOLUS

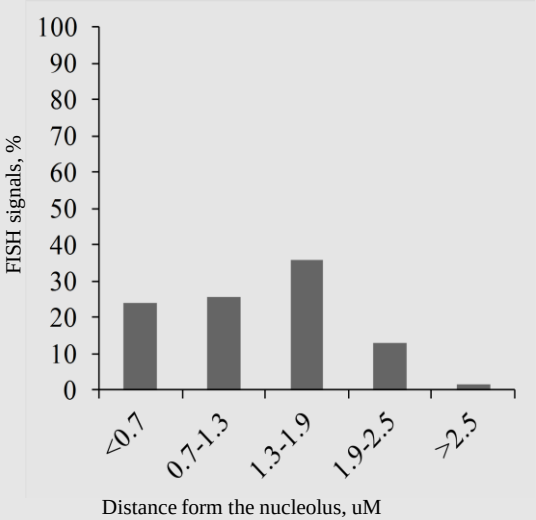


n=120

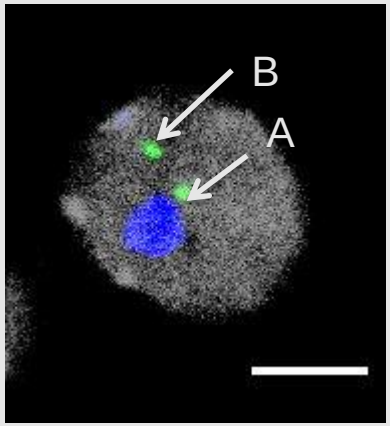


Gray-nucleus, green-IgH, blue-B23





n=120



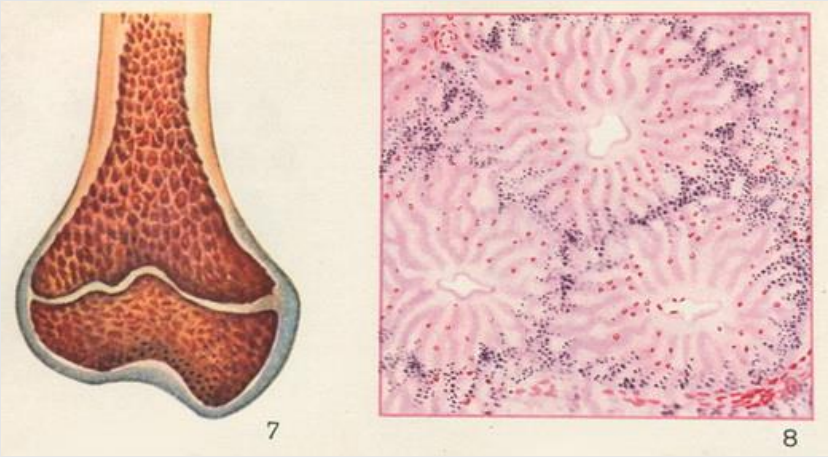
n=120

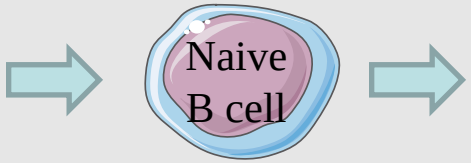
FACULTE DE MEDECINE PARIS-SUD

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

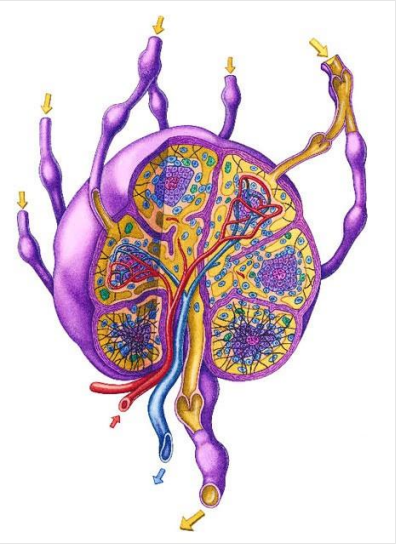
B-CELL DIFFERENTIATION

Bone marrow



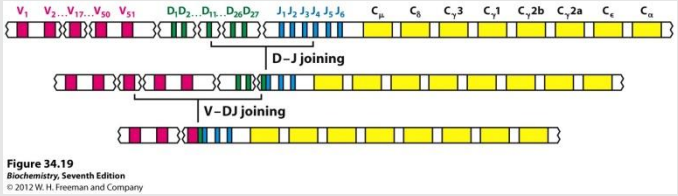


Lymph node

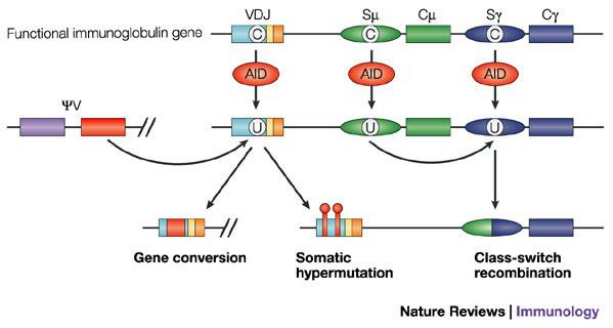


B-cell maturation

V-D-J Recombination



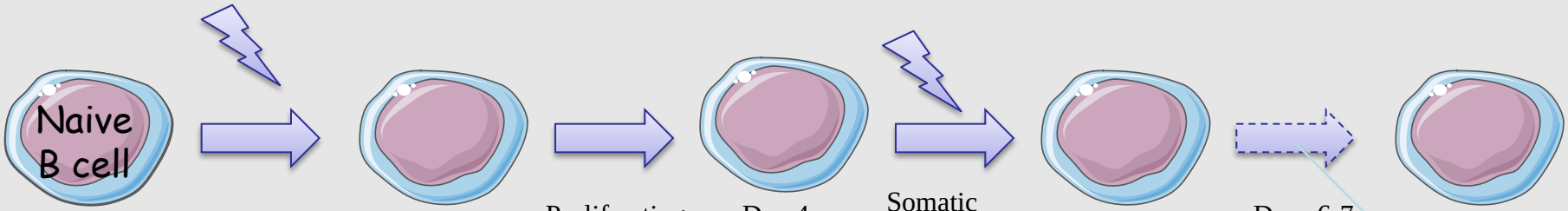
Class switch recombination and
Somatic hypermutation



AN *IN VITRO* SYSTEM OF HUMAN B-LYMPHOCYTE DIFFERENTIATION

<u>Stim1</u>
IL-2, CD40L, CpG and Anti Fab'2

<u>Stim2</u>
IL2, IL4 and IL10



Day 0

Human naive B-cells from blood.

Day 1

Activated B-cells prior proliferation

Proliferating B-cells.

Day 4

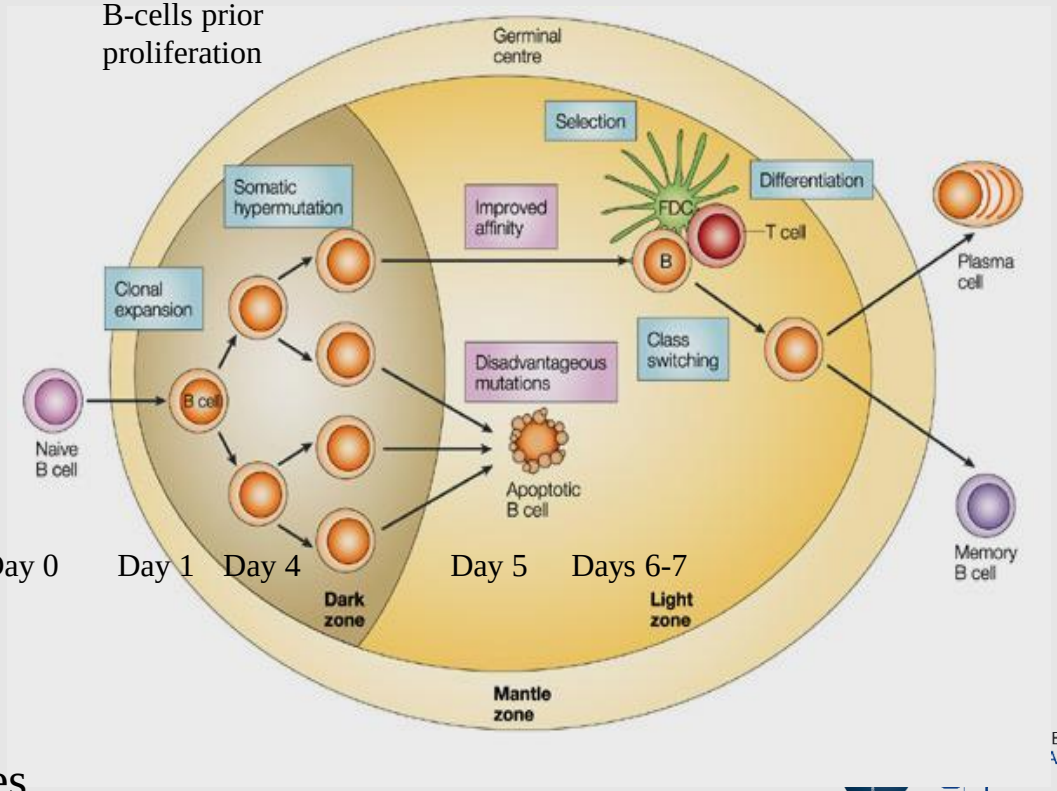
Somatic Hypermutation

Day 5

Days 6-7

90% of cells undergo apoptosis. Remaining live cells undergo CSR.

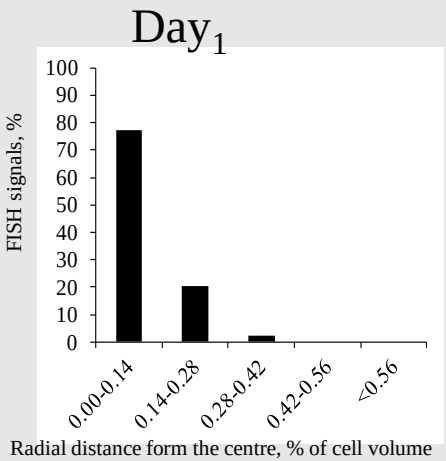
Plasmocytes

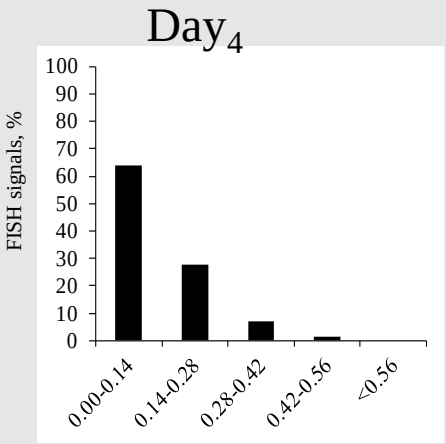


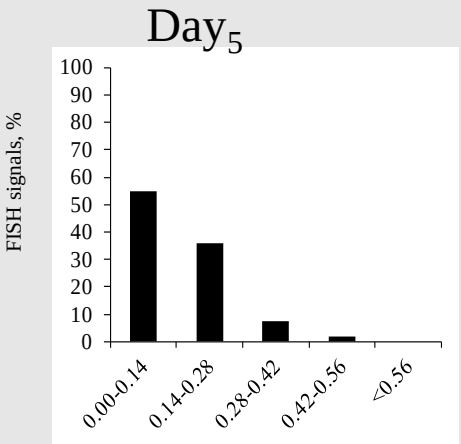
Day 0 Day 1 Day 4 Day 5 Days 6-7

Thierry Fest, Rennes

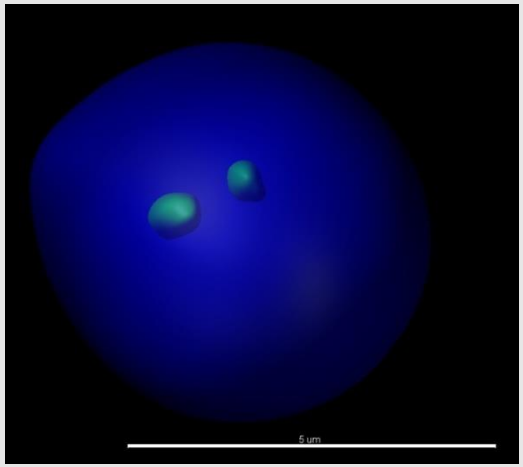
IgH RADIAL POSITIONING DURING B-LYMPHOCYTE DIFFERENTIATION

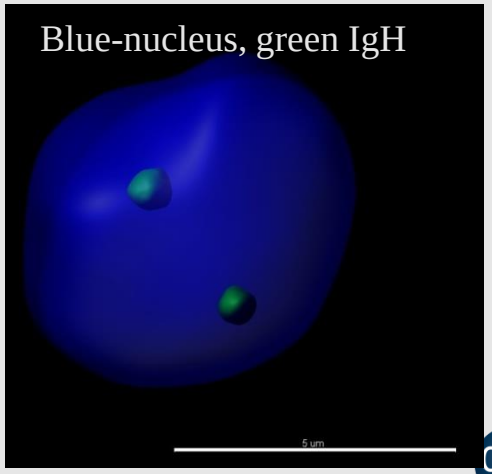


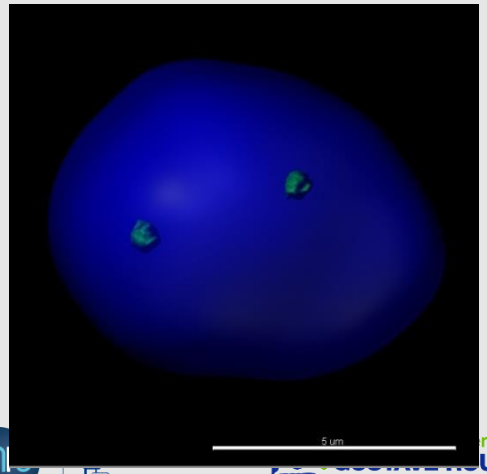




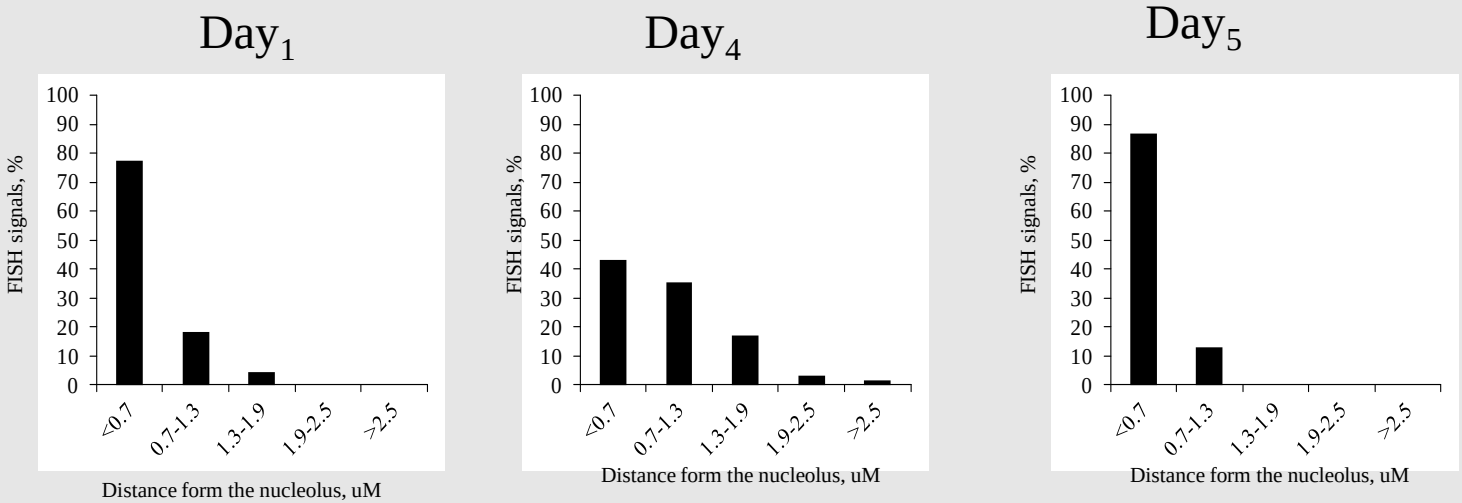
n=120

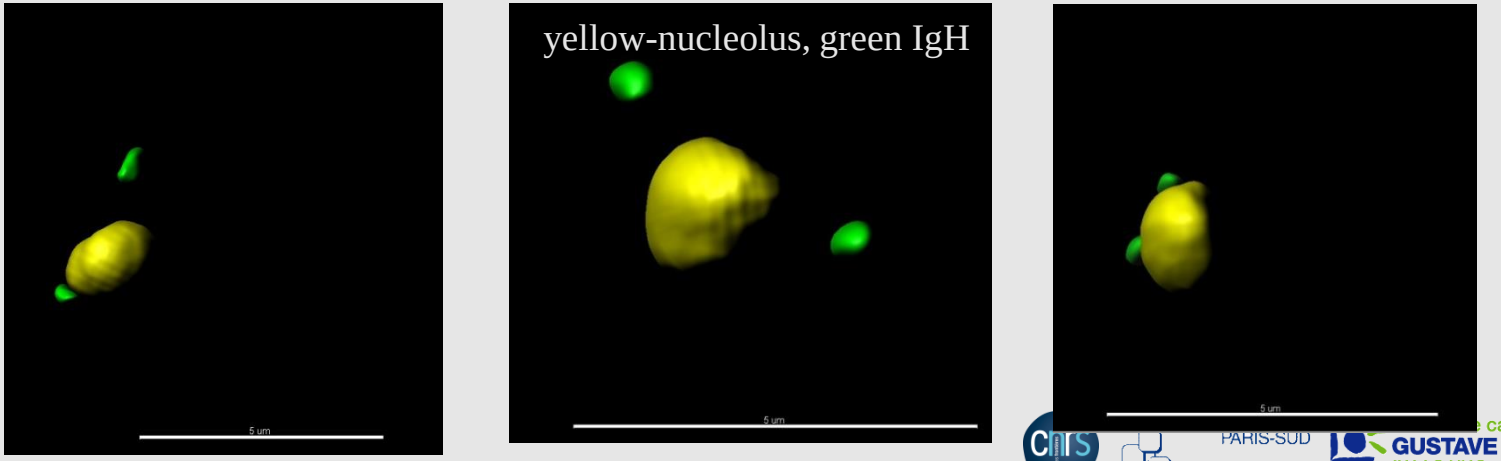




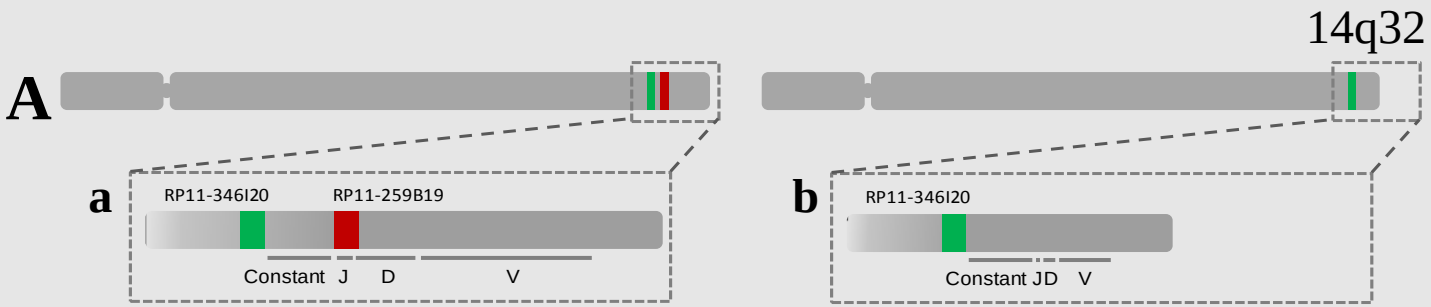


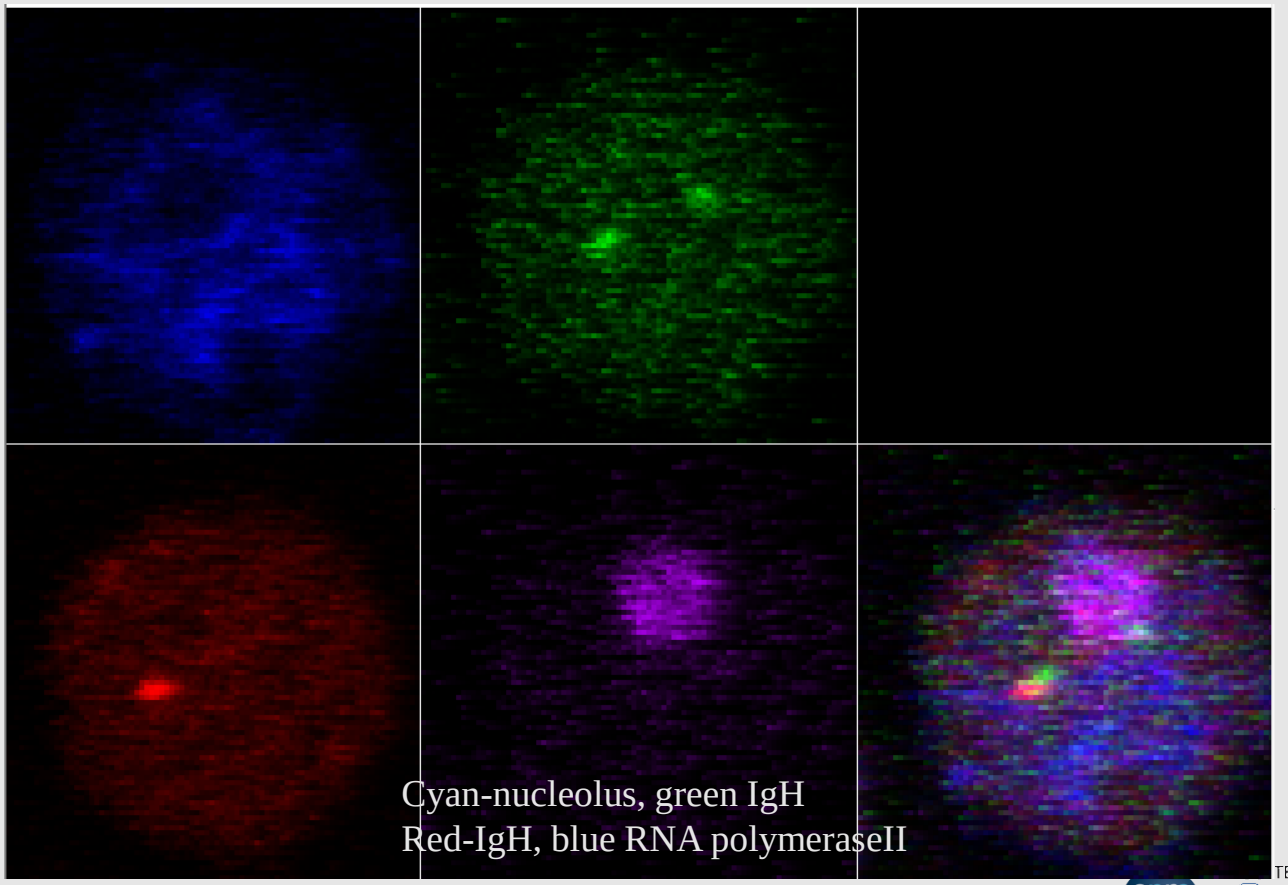
IgH PROXIMITY TO THE NUCLEOLUS CHANGES B-LYMPHOCYTE DIFFERENTIATION

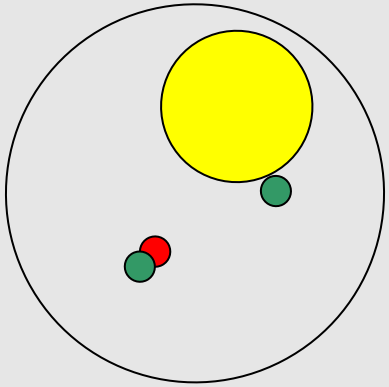




DETECTING THE PRODUCTIVE ALLELE



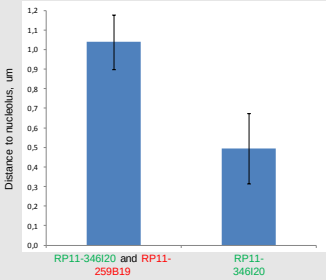


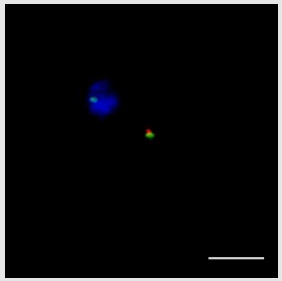


PRODUCTIVE IgH ALLELE ASSOCIATES WITH THE NUCLEOLUS ON DAY5

Day 0

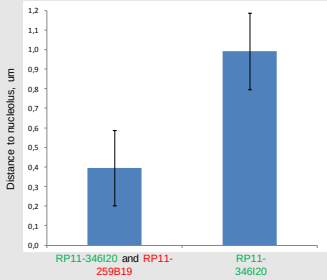
p= 0,05; n=78

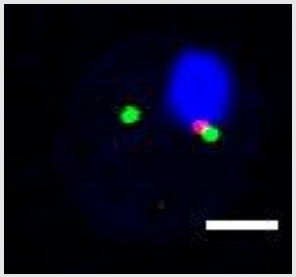




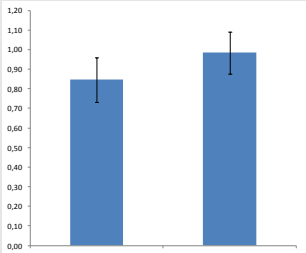
Day 1

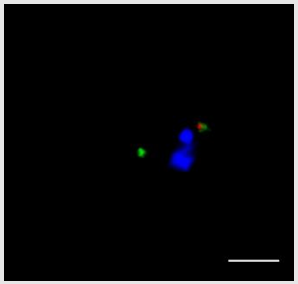
p= 0,02; n= 76





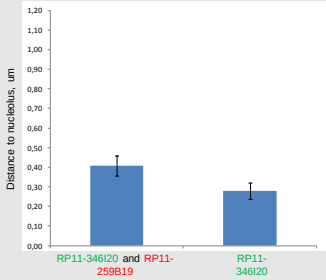
Day 4

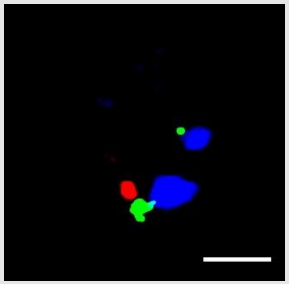




Day 5

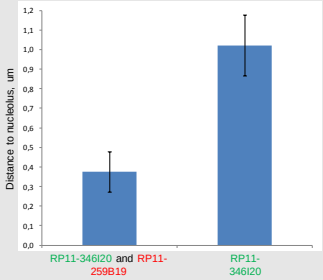
p=0,44; n= 47

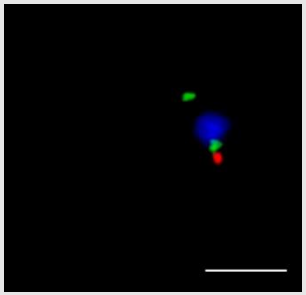




Plasmocytes

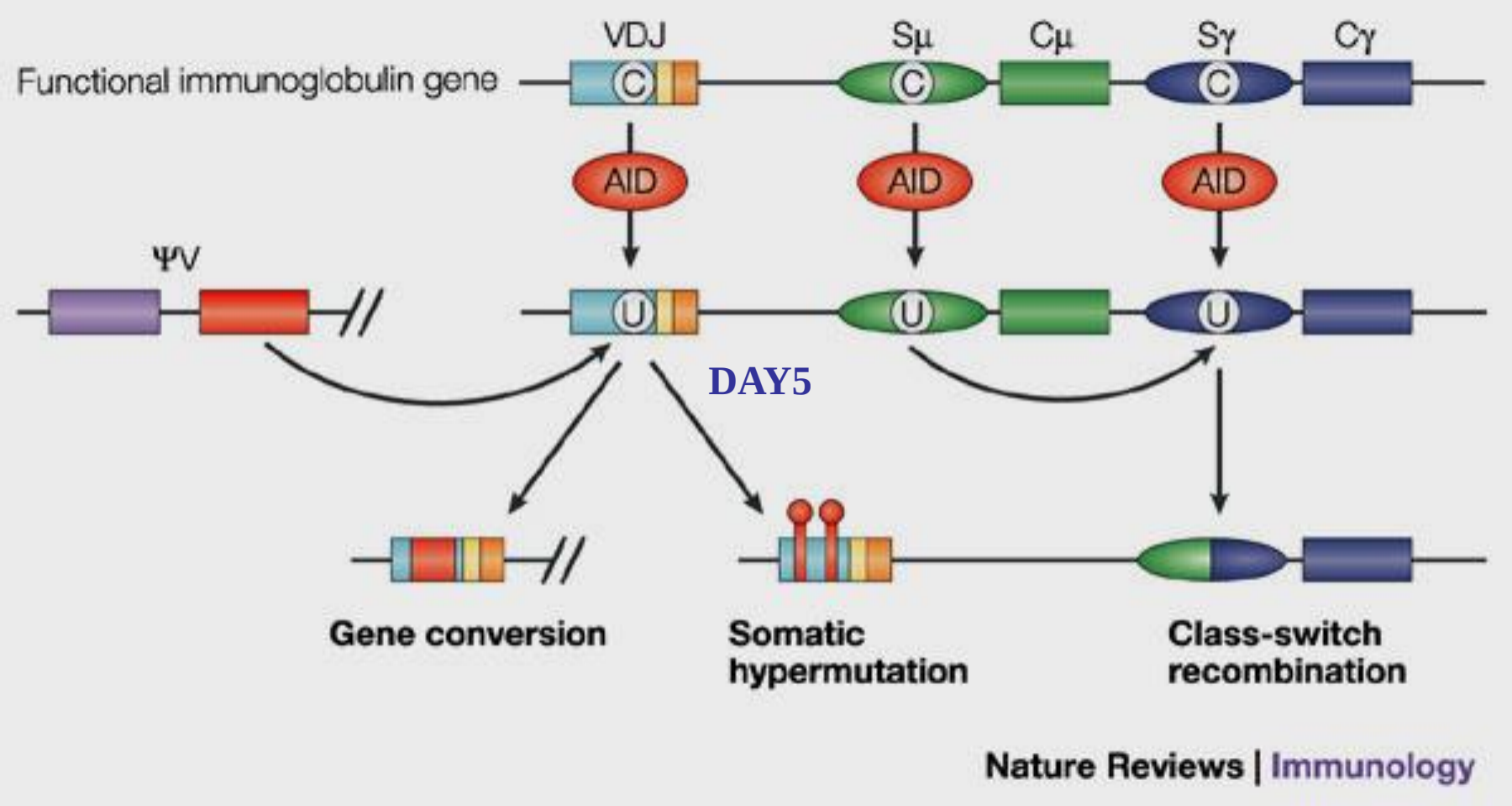
p= 0,12; n=6



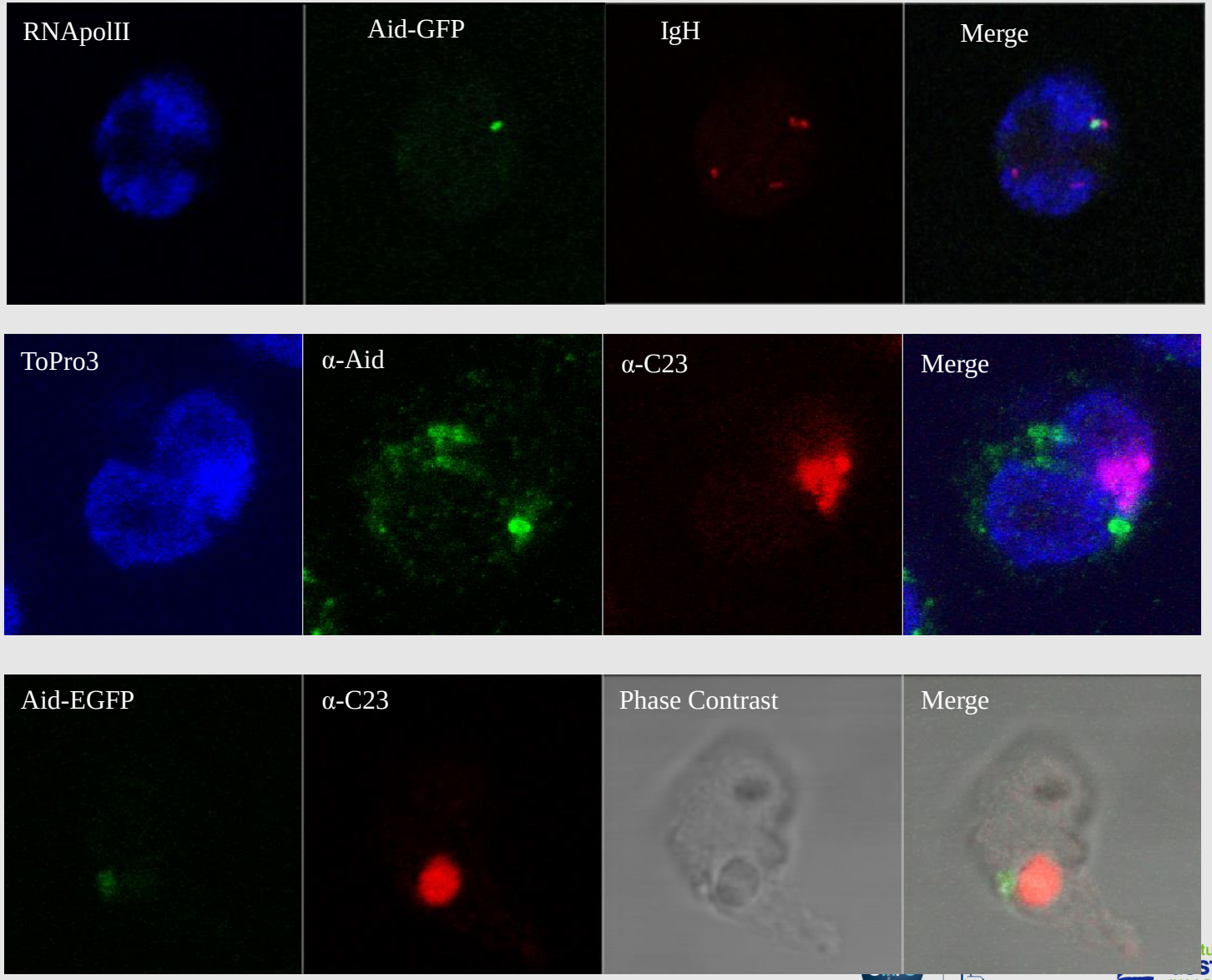


blue-nucleolus, green IgH, red - IgH

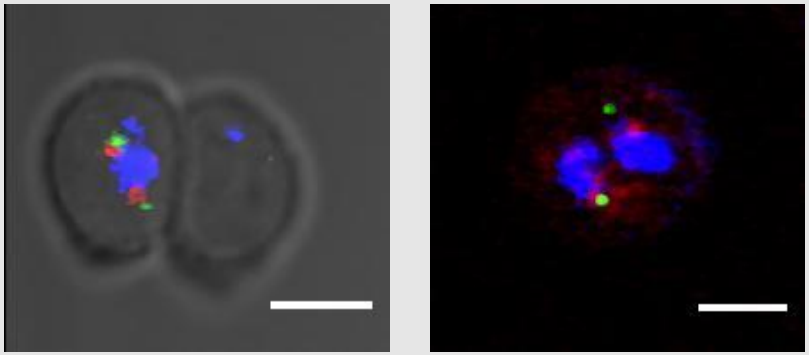
DAY5: SOMATIC HYPERMUTATION AND CLASS SWITCH RECOMBINATION



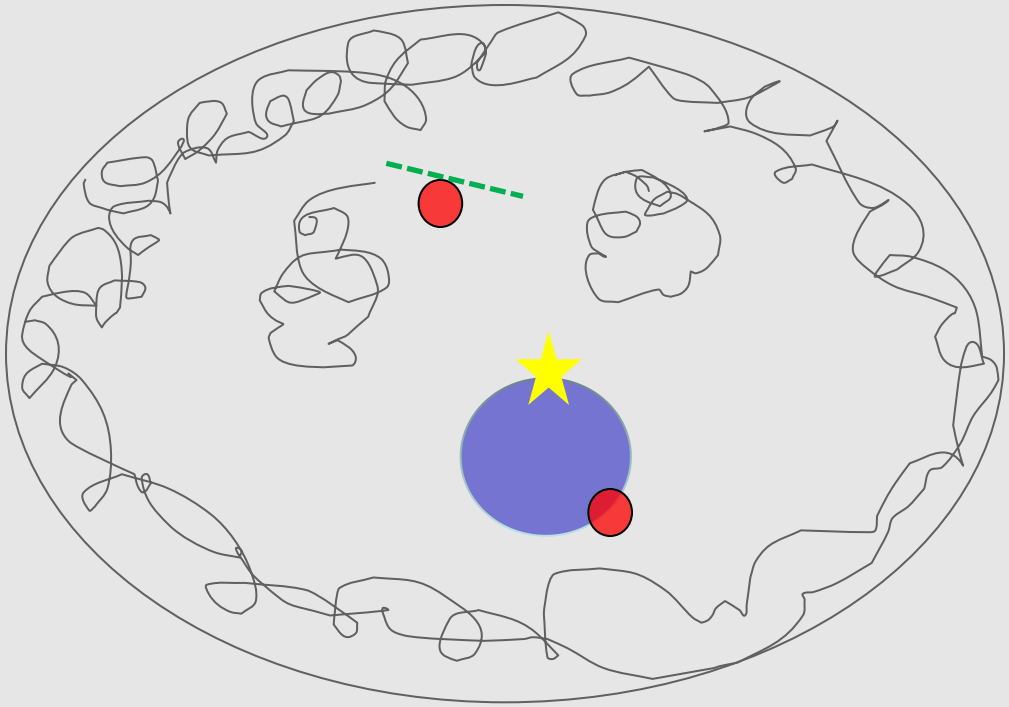
TRANSCRIPTIONALLY-ACTIVE IgH ALLELE AND AID ARE LOCATED CLOSE TO THE NUCLEOLUS



THE γH2AX FOCI SPECIFICALLY COLOCALIZE WITH IgH AND THE NUCLEOLUS IN DAY$_5$ B-LYMPHOCYTES



NUCLEOLUS (Anti-B23) γH2AX IgH



Equipe "Chromatine, Développement et Cancer "

(UMR-8126, IGR, Villejuif):

- **Marc Lipinski,** DR CNRS
- **Yegor Vassetzky,** DR CNRS
- **Chrystèle Bilhou-Nabera**, MCU-PH
- **Andrei Pichugin**, postdoctorant
- **Tatiana Tsfasman**, postdoctorante
- **Yara Bou Saada,** doctorante UPS
- **Diana Markozashvili,** doctorante (cotutelle Russie)
- **Ilya Sklyar,** doctorant (cotutelle Russie)
- **Rawan El-Amine**, doctorante (cotutelle Liban)
- **Carla Dib**, M2 UPS
- **Vincent Ribrag,** IGR, Villejuif
- **Sergey Razin,** IBG, Moscow
- **Olga Iarovaya,** IBG, Moscow
- **M.-N. Prioleau,** IJM, Paris
- **S. Bury-Moné,** ENS Cachan