Molecular Hematopathology
Lymphomas

December 21, 2004

Translocations

Small or large fragment of a chromosome fuses with another chromosome

The fusion is viable

The fusion chromosome is faithfully replicated during subsequent cell generations and, if this gives the cell a growth advantage, a unique large monoclonal population is created

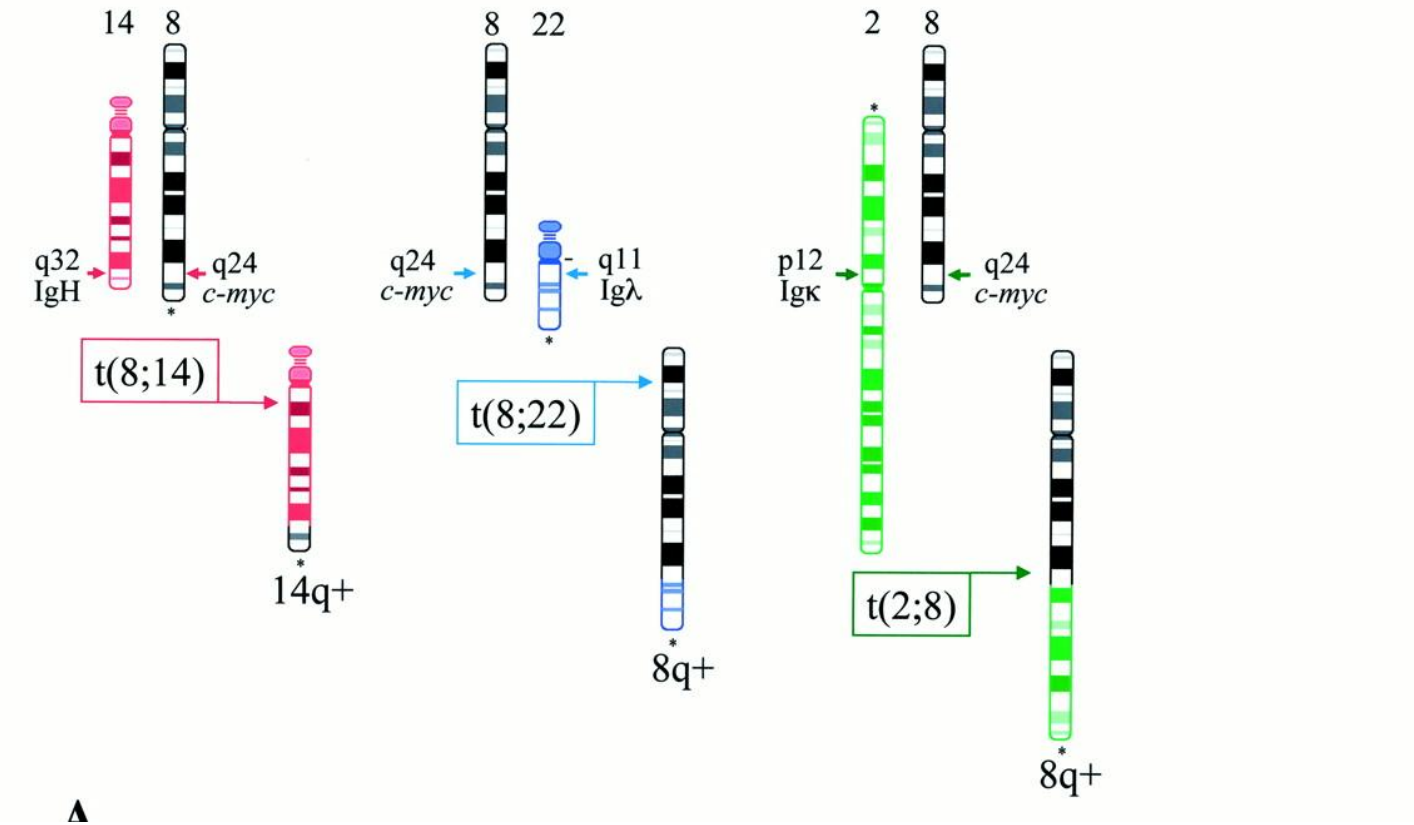
Translocations

There are 2 general types of translocations seen in non-Hodgkin's lymphomas:

1) Intact oncogene on one chromosome translocated beside a gene on another chromosome, usually on an antigen receptor gene, eg Burkitt's or follicular lymphoma

2) Two genes on separate chromosomes are disrupted, with portions of both genes fused into one "fusion gene" which encodes a novel protein, eg anaplastic large cell lymphoma

Detection of *c-myc*/Ig chromosomal translocations in Burkitt's



Hecht, J. L. et al. J Clin Oncol; 18:3707-3721 2000

Mechanism of Chromosomal Translocation

Mistakes made during B lymphocyte processing

1) Marrow - VDJ rearrangement in precursor B cells

2) Lymph node - V segment somatic hypermutation
- IgH class switching
- receptor editing

Mistakes made during T lymphocyte processing

1) Thymus - VDJ rearrangements in progenitor T cells
- no class switching
- only rare somatic hypermutation

B-cell Processing

In order to generate a unique surface Ig, precursor B cells must conjoin V, D, and J segments of genomic DNA on chromosome 14q for IgH and the V and J segments for κ and λ light chains on chromosomes 2p and 22q

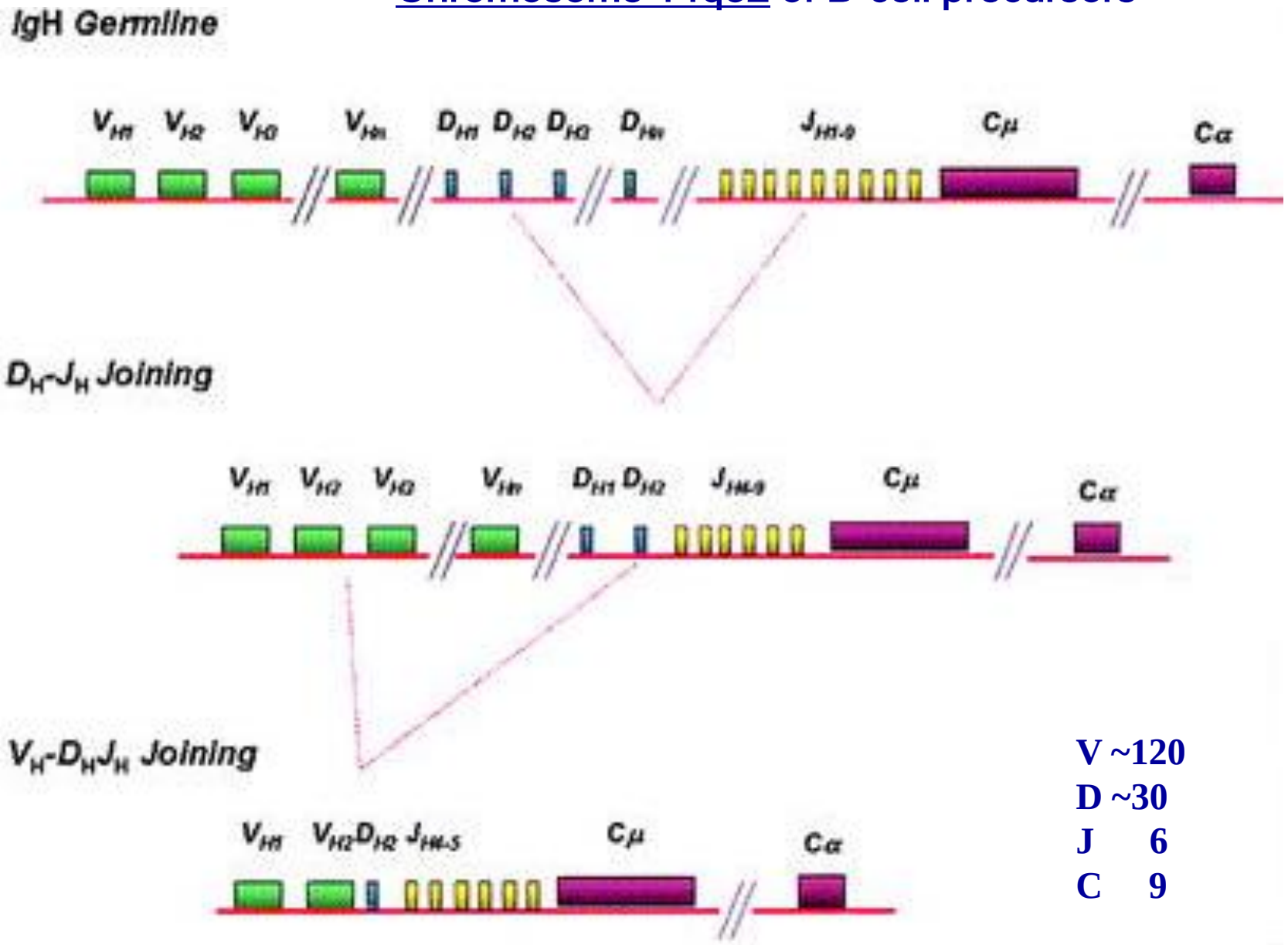
Genomic dsDNA is broken and rejoined by 2 endonucleases (*recombination-activating genes*, RAG 1 and RAG 2)

over long distances

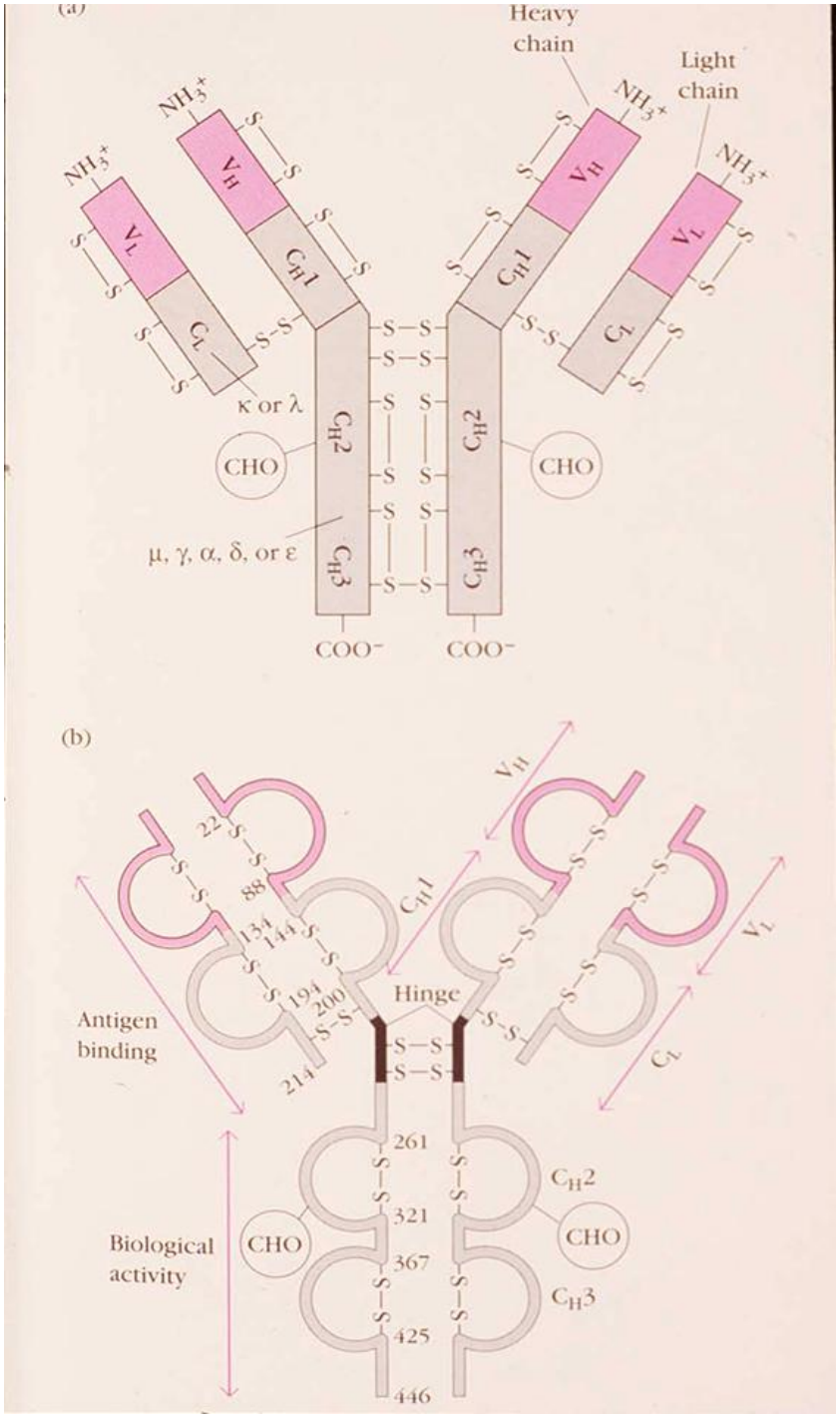
dsDNA breaks occur at recognition signal sequences (RSS) that are adjacent to V, D, J sequences

(3' to V, 5' and 3' to D, and 5' to J)

Chromosome 14q32 of B-cell precursors



V ~120
D ~30
J 6
C 9



Immunoglobulin Protein Structure

Recombination

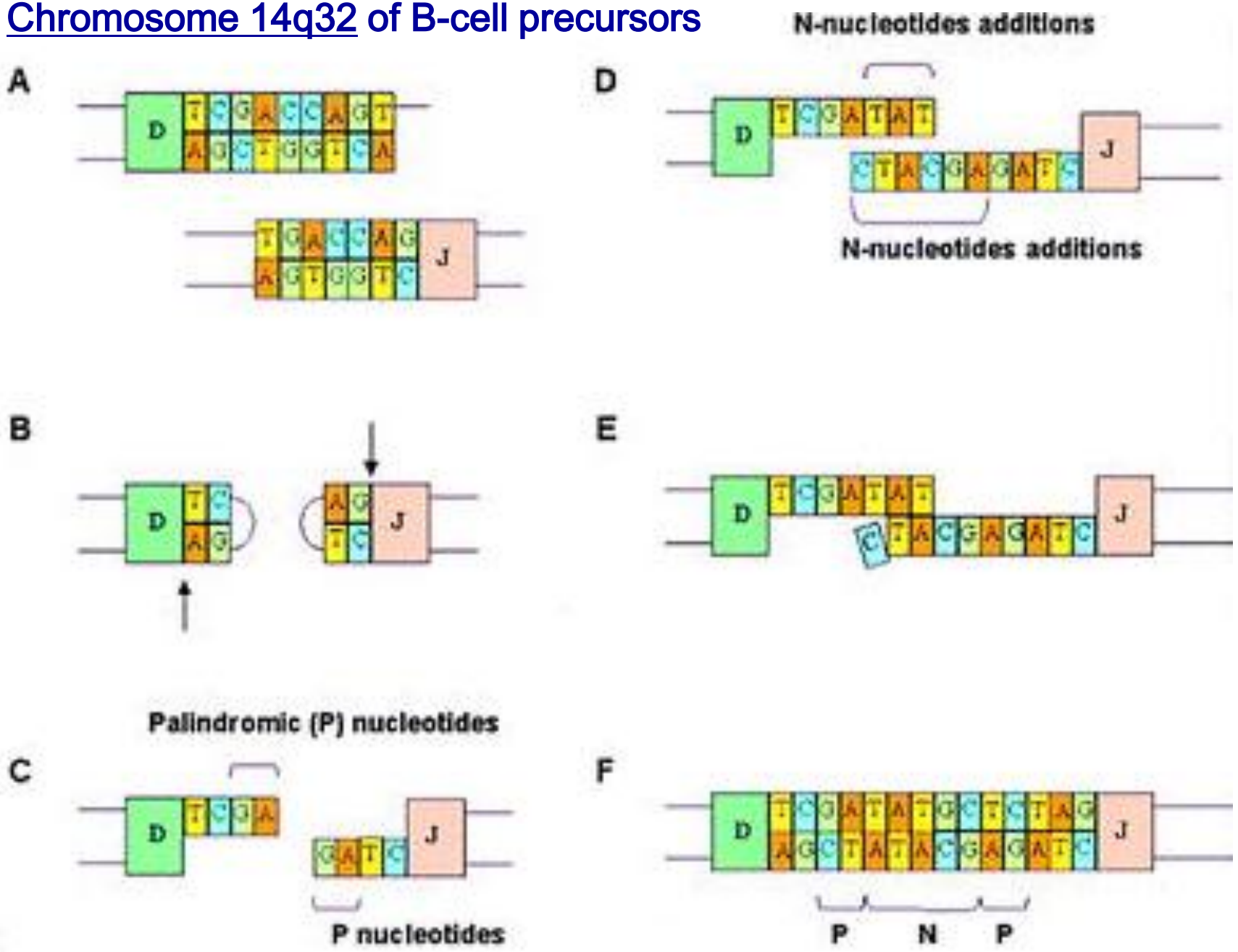
RSS consists of a 12-13bp spacer region flanked by a conserved 7mer on one end and a conserved 9mer on the other end

Recombination requires one RSS of each type

Recombination also requires:

- DNA ligase IV
- DNA dependent protein kinase
- Ku (Ku70/ Ku80 heterodimer), that assoc with DNADPK
- XRCC4 (X-ray repair complementary defective in Chinese hamster 4), a DNA repair protein

Chromosome 14q32 of B-cell precursors



VDJ Recombination Steps

RAG breaks the dsDNA and then forms an internal hairpin loop at each end

Hairpin opens up as ssDNA with a 3'overhang

Nucleotides are then added (by TdT) or removed (DNA exonuclease) from the 3' overhang to create diversity

3' overhangs from the two strands then join as the best fit possible to form dsDNA, with some of the nucleotides cut out; AKA "coding end processing"

Gaps filled with DNA polymerase and DNA ligase

VDJ Recombination Steps

During normal everyday VDJ recombination in these precursor B or T cells, these dsDNA breaks occur

Rarely the dsDNA breaks fuse with the wrong gene → chromosomal translocation

If this translocation puts an Ig or TCR gene next to a proto-oncogene, the cells may acquire a growth advantage and become clonal, eg DLBCL, MCL, BL, precursor T- cell lymphoblastic lymphoma

VDJ Recombination Steps

At least 2 mechanisms can lead to a translocation:

1) Oncogene has a functional RSS sequence that is recognized by RAG, eg in:

precursor T cell lymphoblastic lymphoma
t(10;14)(q24;q11)

2) Oncogene gets a dsDNA break by other mechanism of cleavage (not by RAG), eg in:

mantle cell lymphoma
t(11;14)(q13:q32)

Translocations

Why do they occur in proto-oncogene regions?

Unknown, but theories:

(1) Some oncogenes may have adjacent crossover hotspot instigator sequences that promote recombination, eg: chi sequences around

bcl-2 of **t(14;18)** follicular lymphoma

bcl-1 of **t(11;14)** mantle cell lymphoma

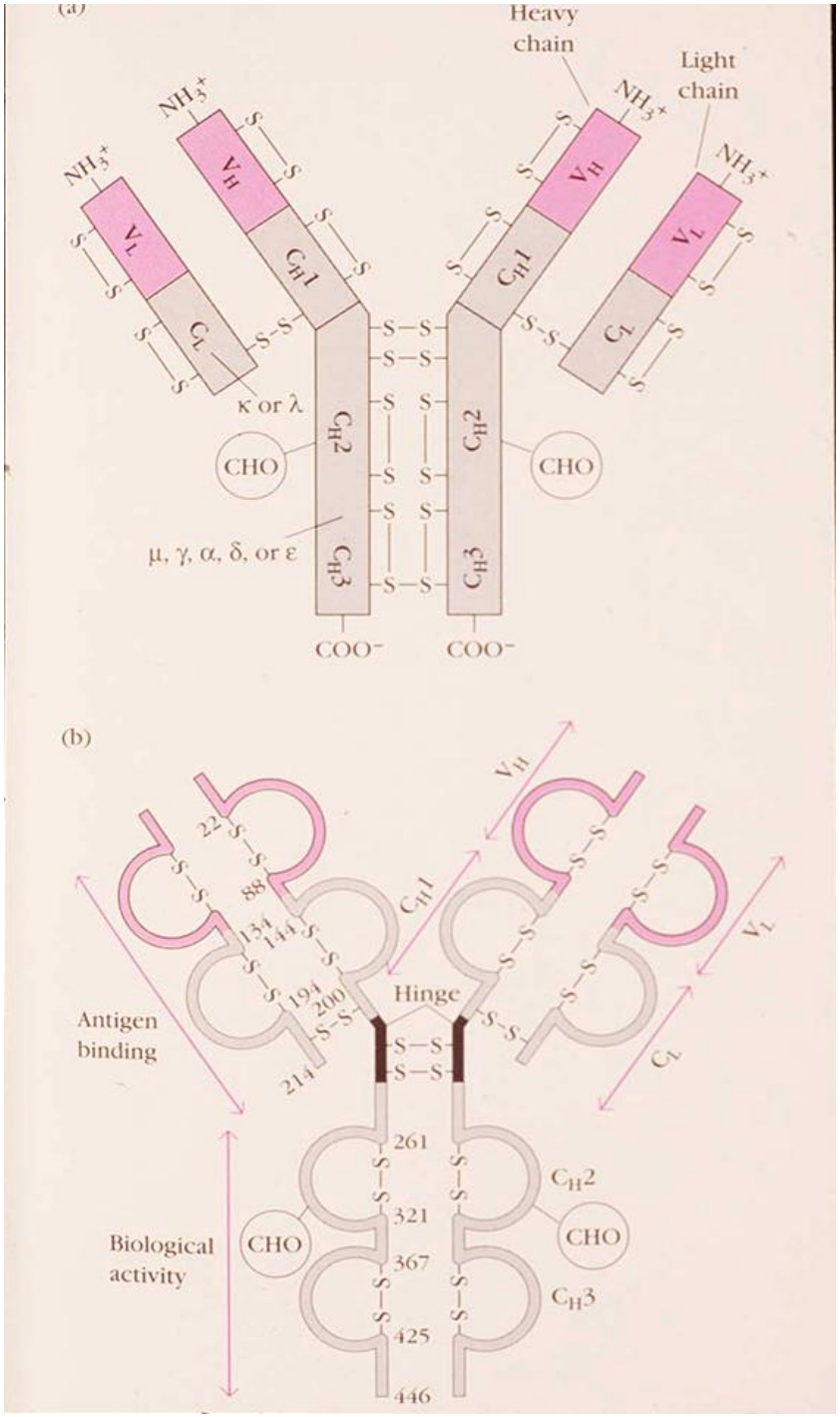
(2) RAG enzyme transpositional activity

dsDNA Breaks in More Mature B cells

Germinal center cells normally undergo somatic hypermutation

- single nucleotides or short oligos inserted/deleted from V segment by enzyme, after Ag exposure (creates diversity)
- requires dsDNA break

- possibly *c-myc* translocation to IgH gene in endem Burkitt's
- possibly *bcl-6* translocation to IgH gene in DLBCL
- possibly *bcl-2* translocation to IgH in follicular lymphoma



Immunoglobulin Protein Structure

dsDNA Breaks in More Mature B cells

Germinal center B cells switch IgM → IgG or IgA or IgE after antigen exposure

This recombination requires a dsDNA break in the germ ctr

DNA between Cμ and Cγ or Cα or Cε is cut out

- switch (S) regions around C genes contain microsatellites

Translocations into S regions of IgH described in:

Sporadic Burkitts'	*c-myc*	**t(8;14)(q24;q32)**
B-CLL	*bcl-3*	**t(14;19)(q32;q13)**
DLBCL	*bcl-6*	**t(3;14)(q27;q32)**
PC myeloma	*mum/irf4*	**t(6;14)(p25;q32)**

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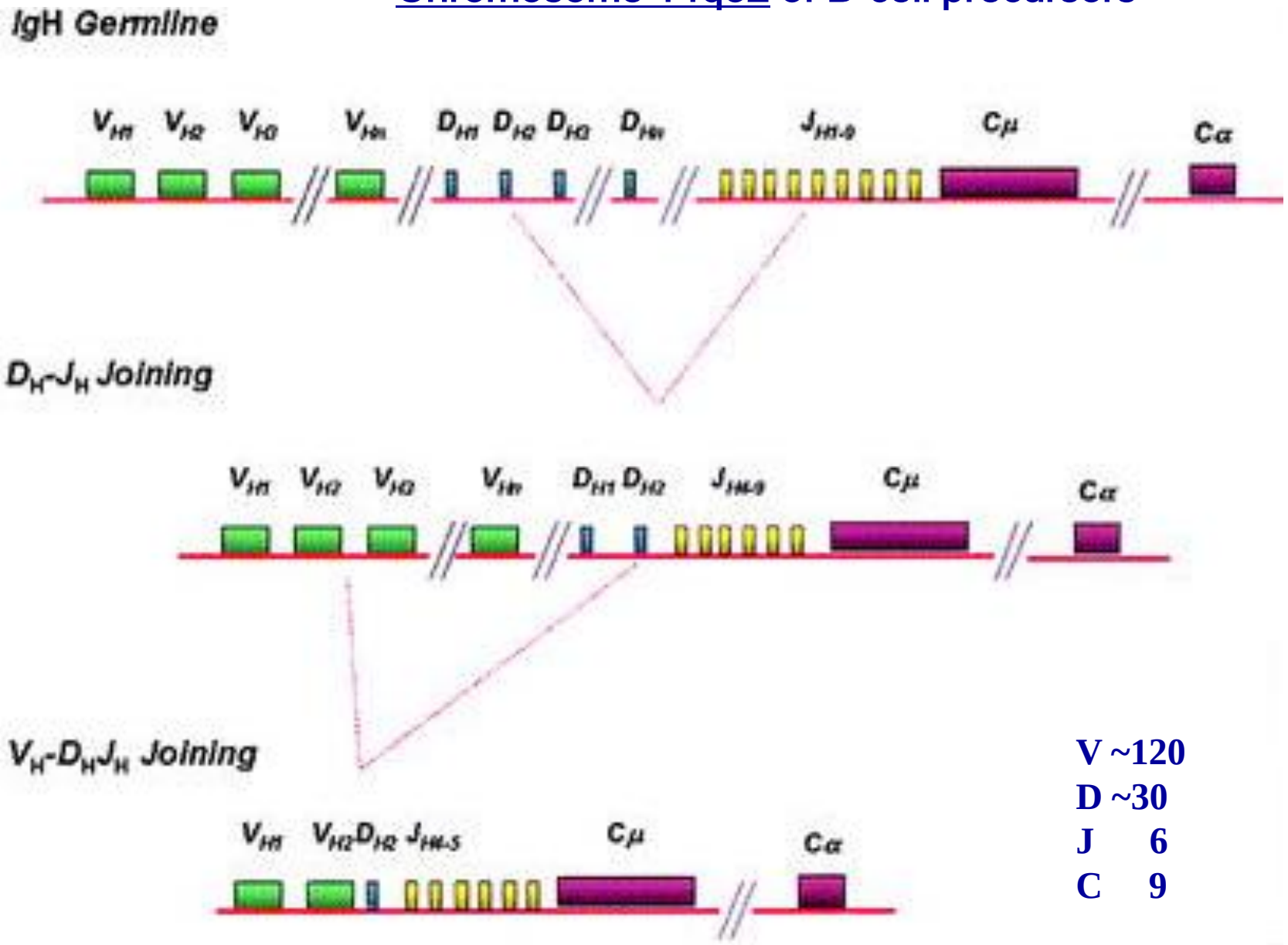

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PC myeloma	*mum/irf4*	**t(6;14)(p25;q13)**

Other dsDNA Breaks in B cells

Receptor editing

dsDNA breaks also must occur when a B cell switches from κ to λ light chain or vice versa

Why Do Other dsDNA Breaks Occur at Specific Sites?

Unknown

Breakpoints usually occur within the introns; in some genes the breakpoints appear to cluster in certain regions of gene

Theories, none proven:

- Homologous recombination between Alu elements
- Cleavage at purine/pyrimidine repeat sequences
- Topoisomerase II subunit errors during DNA replication
- Non-homologous DNA repair during replication

Must create fusion gene with growth advantage

How Do We Determine if Monoclonal Population with Translocation?

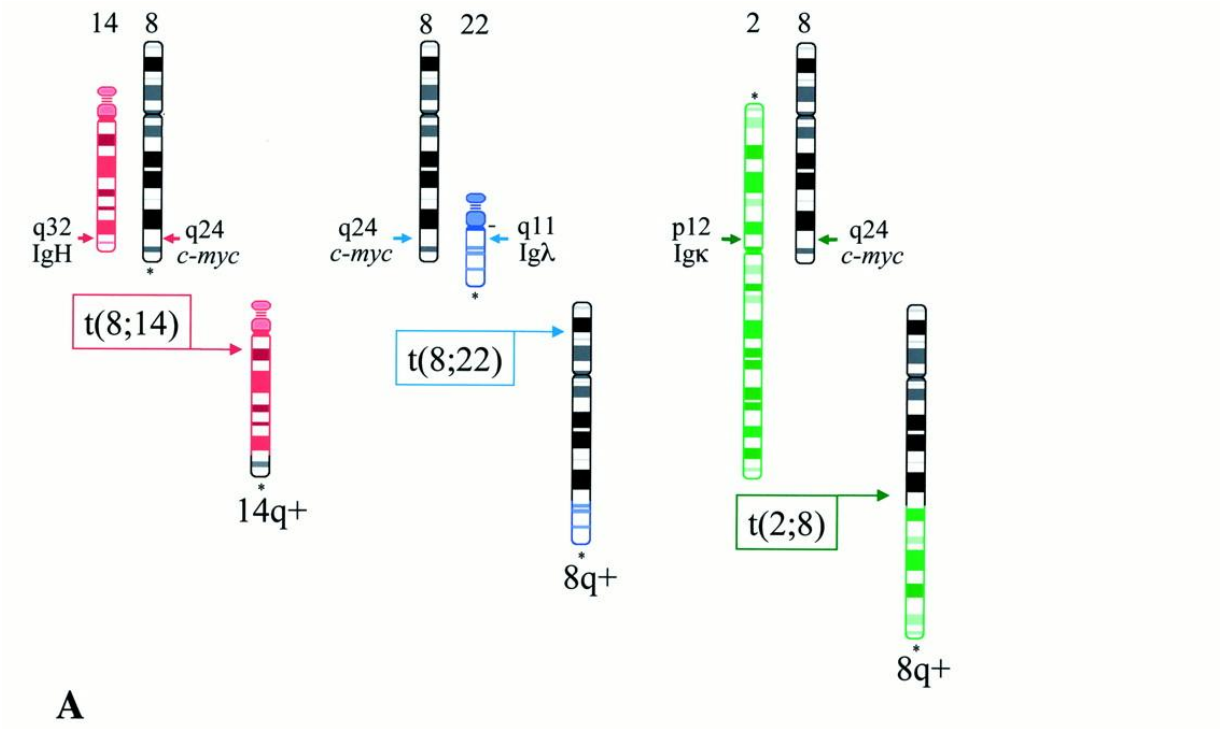
Cytogenetics

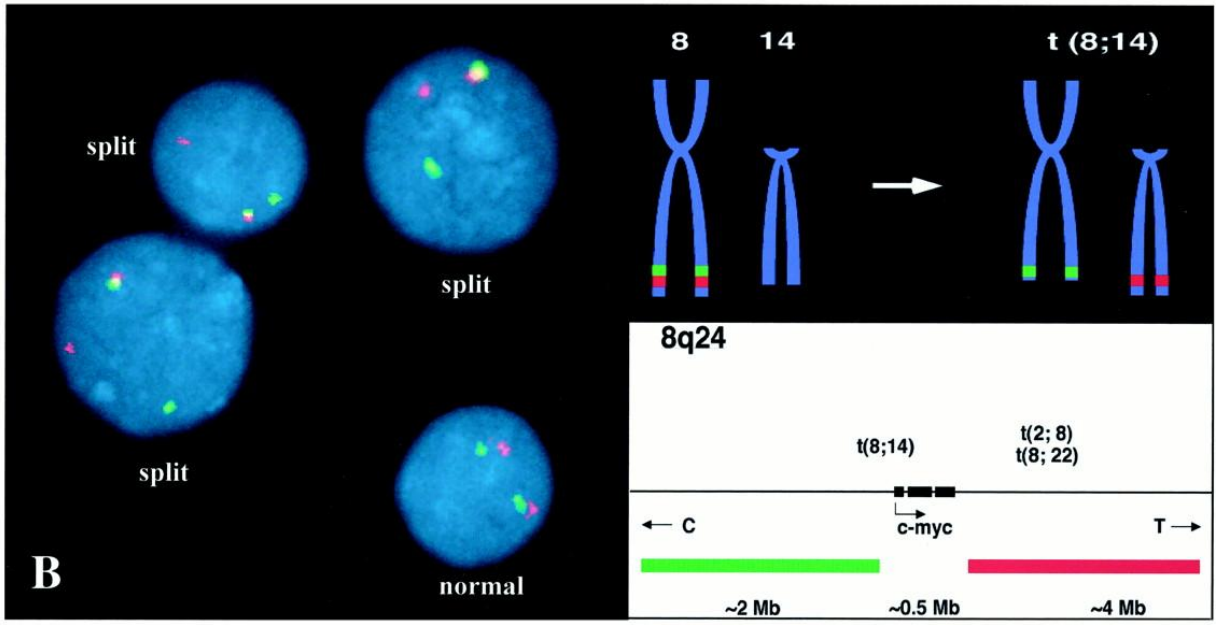
FISH - fluorescence in situ hybridization

RFLP-SB – restriction fragment length polymorphism- Southern blot

PCR or RT-PCR – need primers and probes

- PCR with primers straddling CDR3; run the product on:
- polyacrylamide gel - EtBr stained - look for single strong band
- ABI 310 capillary gel electrophoresis - fluorescent marker - look for large peak





FISH of *c-myc*/Ig translocations in Burkitt's

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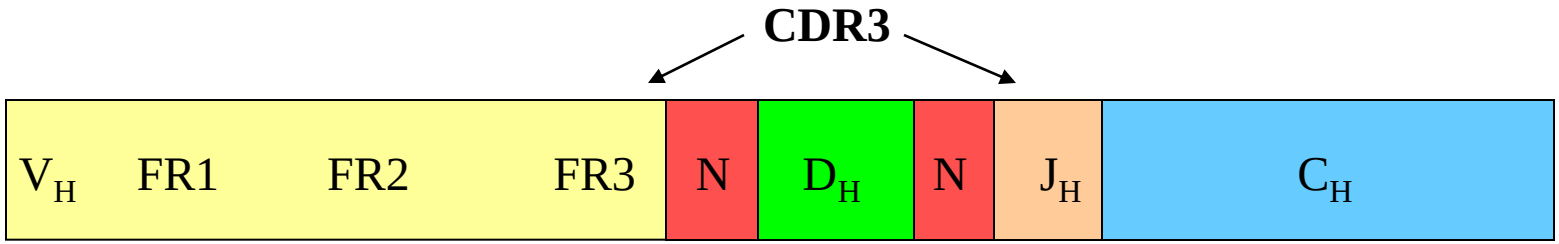
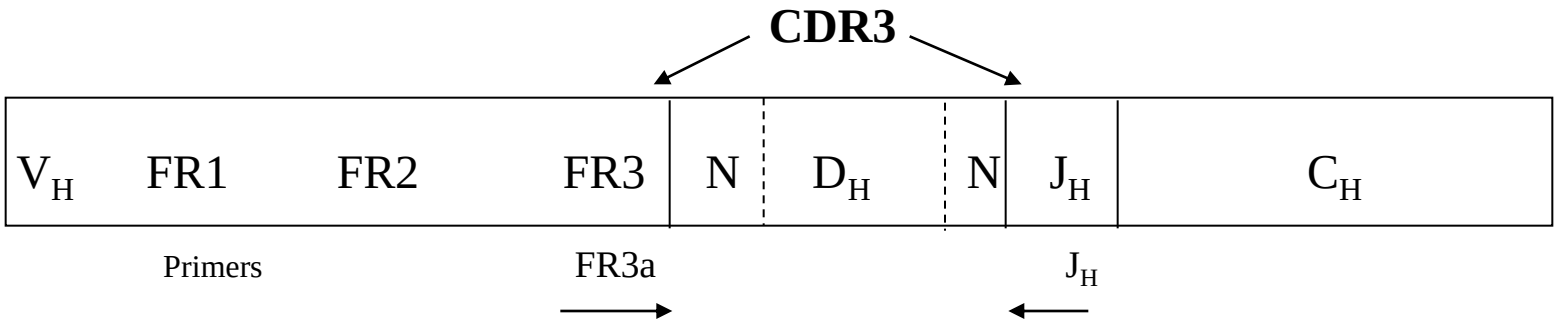
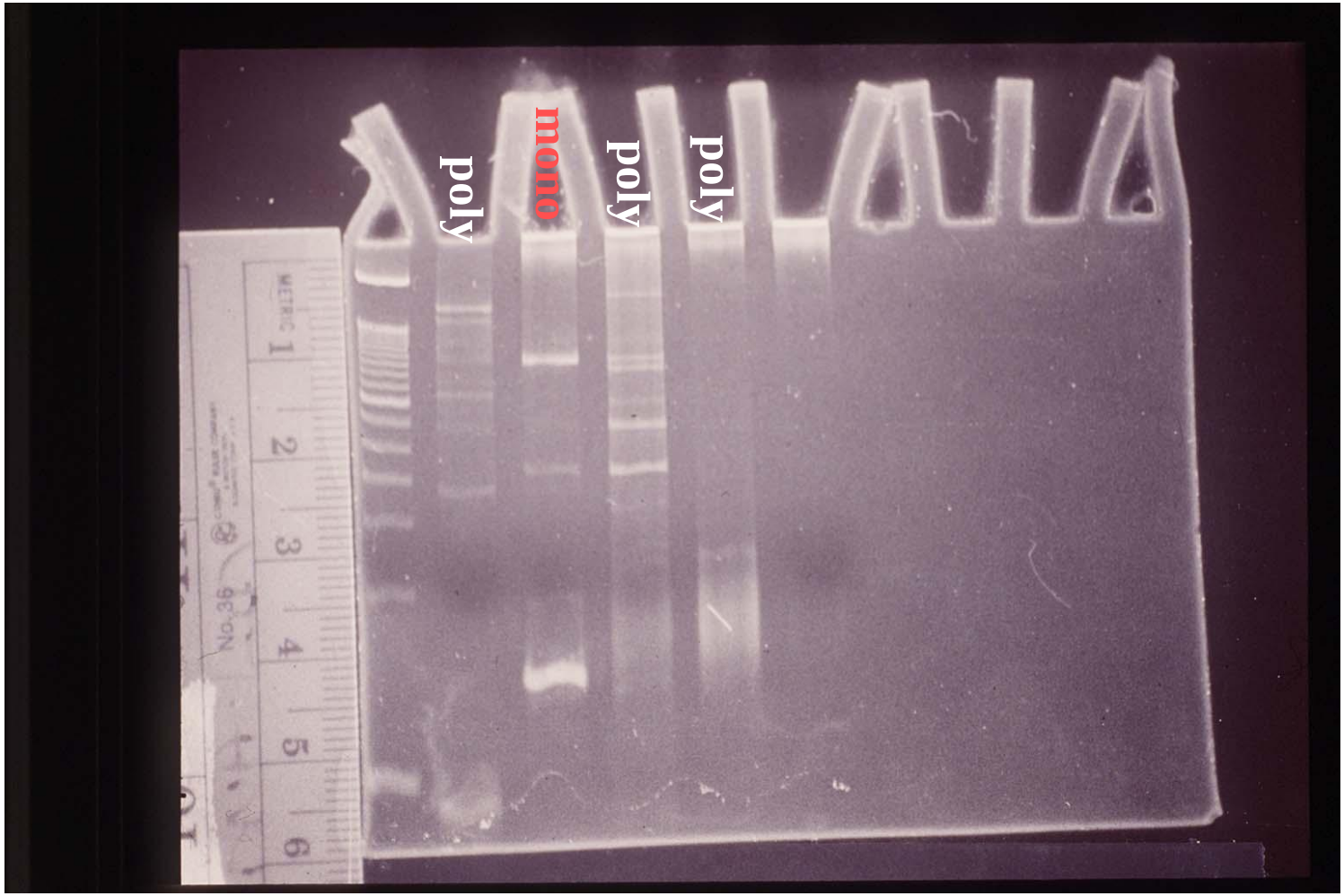
PCR of IgH Gene ± Translocation



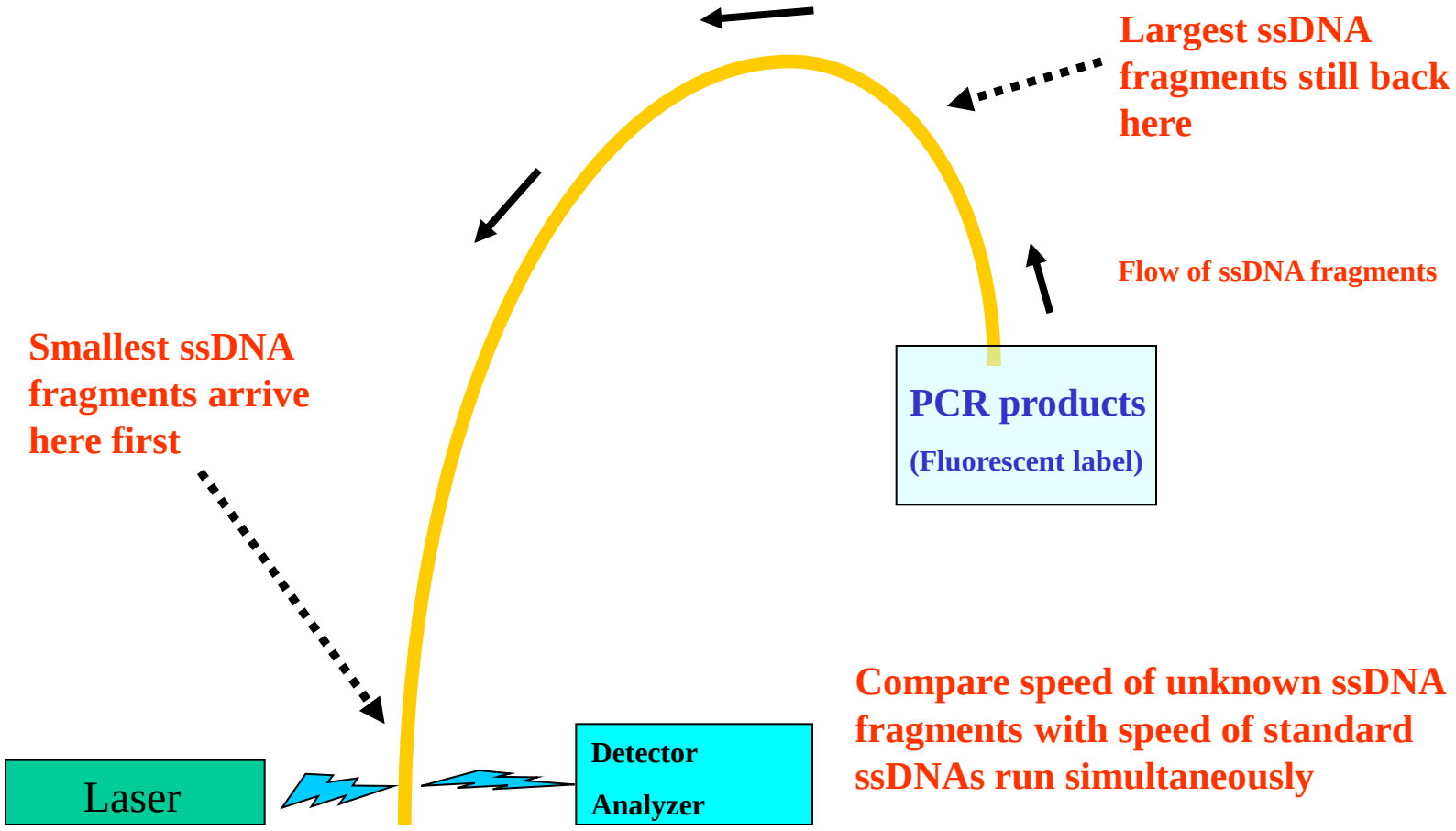
- BUT, point of cleavage is flexible
- AND, a few nucleotides in the coding regions may get trimmed during fusion
- ALSO, a few nucleotides (≤ 15 N) may be added during recombination between



PAGE Gel of PCR Products after amplification of FR3 in the IgH Gene



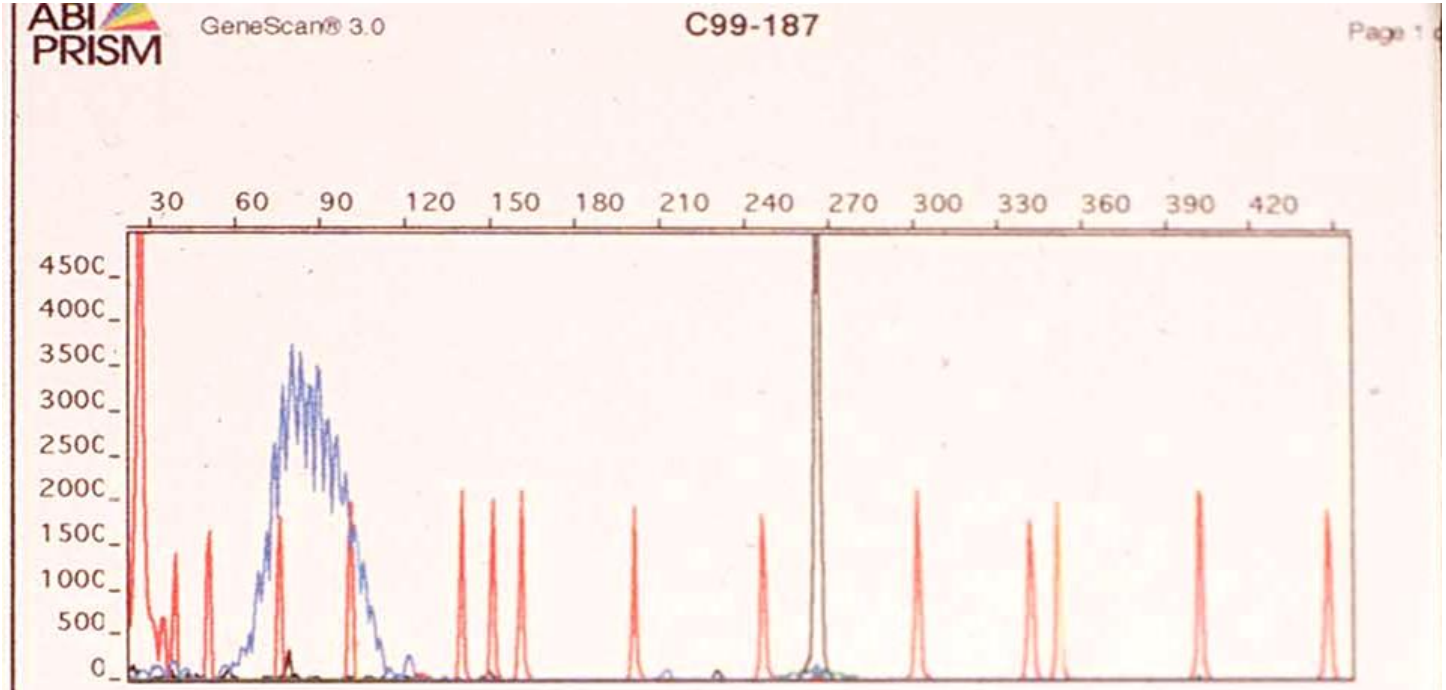
Capillary Gel Electrophoresis



Compare speed of unknown ssDNA fragments with speed of standard ssDNAs run simultaneously

SMALLER= FASTER

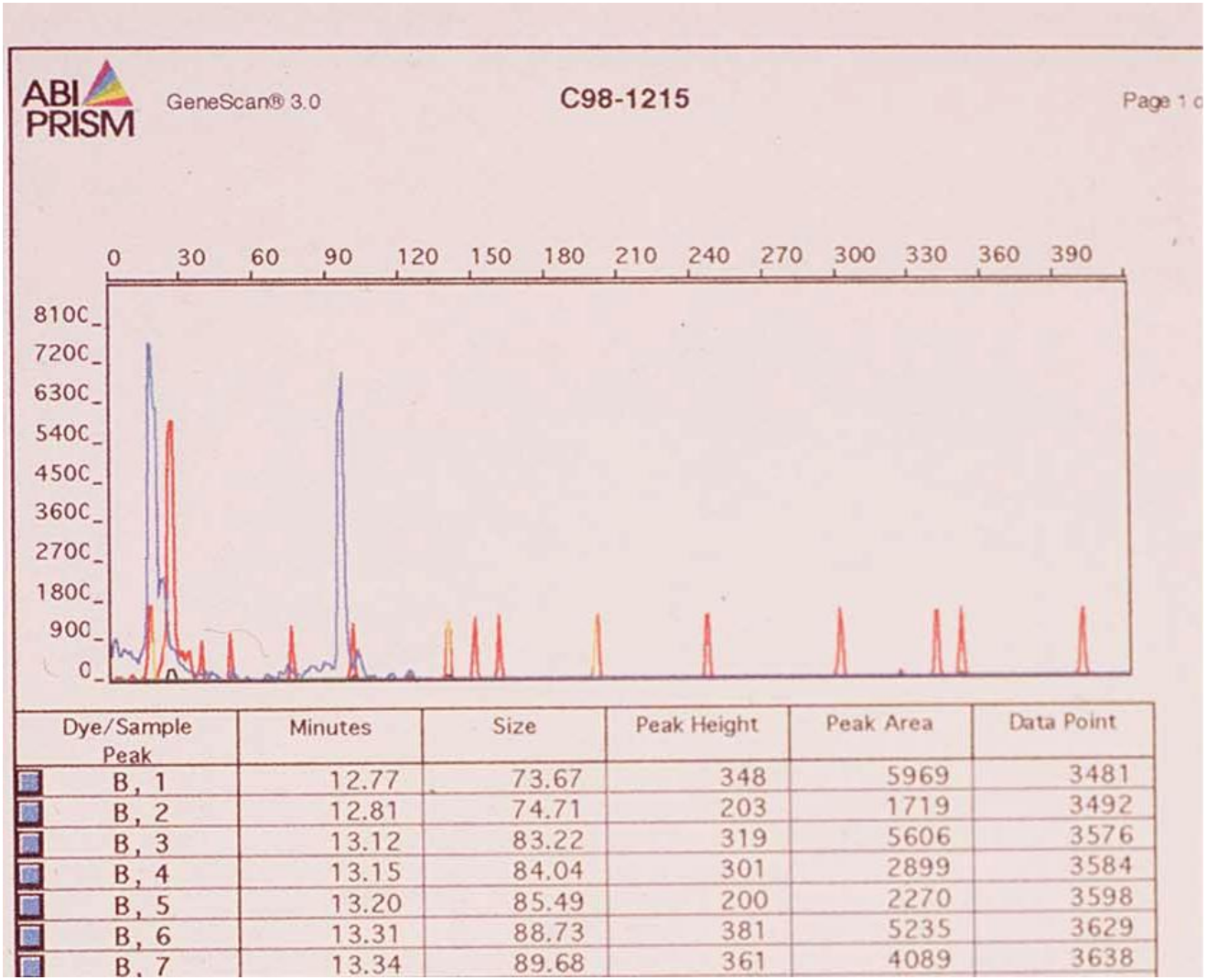
Capillary gel electrophoresis of PCR products after amplification of FR3 in the IgH gene



Dye/Sample Peak	Minutes	Size	Peak Height	Peak Area	Data Point
B, 1	11.93	61.65	372	8215	3252
B, 2	12.04	64.47	514	9288	3283
B, 3	12.15	67.26	1211	23288	3313
B, 4	12.27	70.30	1,680	32528	3345
B, 5	12.38	73.12	2670	54243	3374
B, 6	12.49	76.25	3325	66427	3405
B, 7	12.60	79.31	3751	81276	3434
B, 8	12.71	82.51	3675	78408	3464

Polyclonal

Capillary gel electrophoresis of PCR products after amplification of FR3 in the IgH gene



Dye/Sample Peak	Minutes	Size	Peak Height	Peak Area	Data Point
B, 1	12.77	73.67	348	5969	3481
B, 2	12.81	74.71	203	1719	3492
B, 3	13.12	83.22	319	5606	3576
B, 4	13.15	84.04	301	2899	3584
B, 5	13.20	85.49	200	2270	3598
B, 6	13.31	88.73	381	5235	3629
B, 7	13.34	89.68	361	4089	3638

Monoclonal

Follicular Lymphoma

t(14;18)(q32;q21) in 90% of cases

Translocates the *bcl-2* gene on 18 next to enhancer and promotor elements of the IgH gene on chr 14

Results in the over-expression of the Bcl-2 protein, which has anti-apoptotic function

Necessary but not sufficient for tumor formation

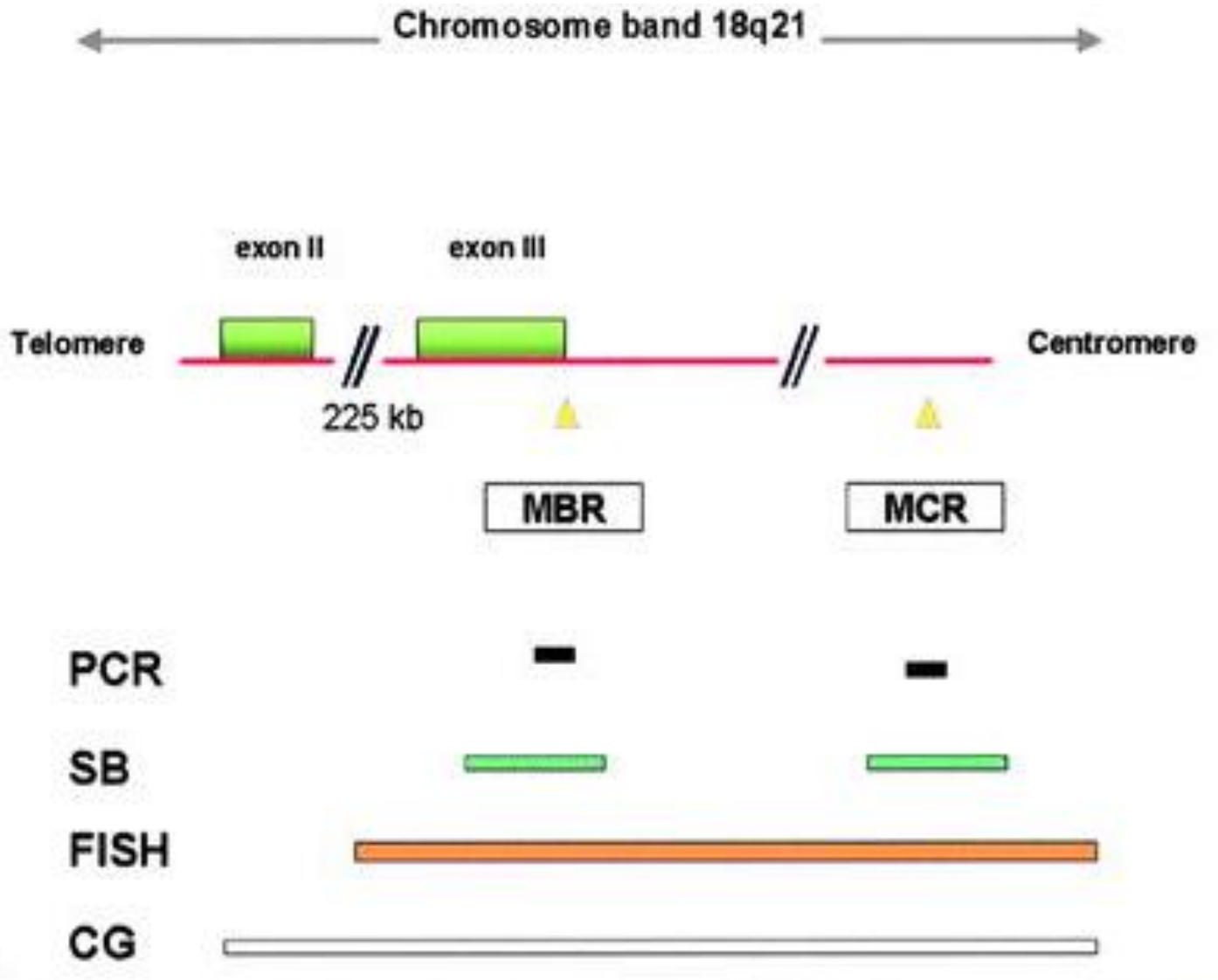
Major breakpoint cluster region (MBR) – 60% of cases

Minor breakpoint cluster region (MCR) – 15% of cases

Intermediate breakpoint cluster region – small % of cases

Detect by cytogenetics, FISH, PCR, quant PCR

Follicular Lymphoma



CLL and SLL

Translocations in only 5% of cases

Most have 13q14 deletion, trisomy 12, or 11q deletion

Translocations:

-t(2;18)(p12;q21) Igκ gene translocated onto *bcl-2* gene

-**t(18;22)(q21;q11)** Igλ gene translocated onto *bcl-2* gene

-t(14;19)(q32;q13) *bcl-3* gene translocated onto IgH gene

-result in Bcl-2 or Bcl-3 overexpression

The t(14;19) seen in young age, poorer Px

Detection by cytogenetics, RFLP-Southern blot, PCR

Mantle Cell

t(11;14)(q13;q32) in nearly all cases

Translocates the cell cycle regulator gene known as *cyclin D1/ccnd-1/bcl-1/prad1* of chr 11 onto the enhancer of the IgH gene of chr 14 near the J region

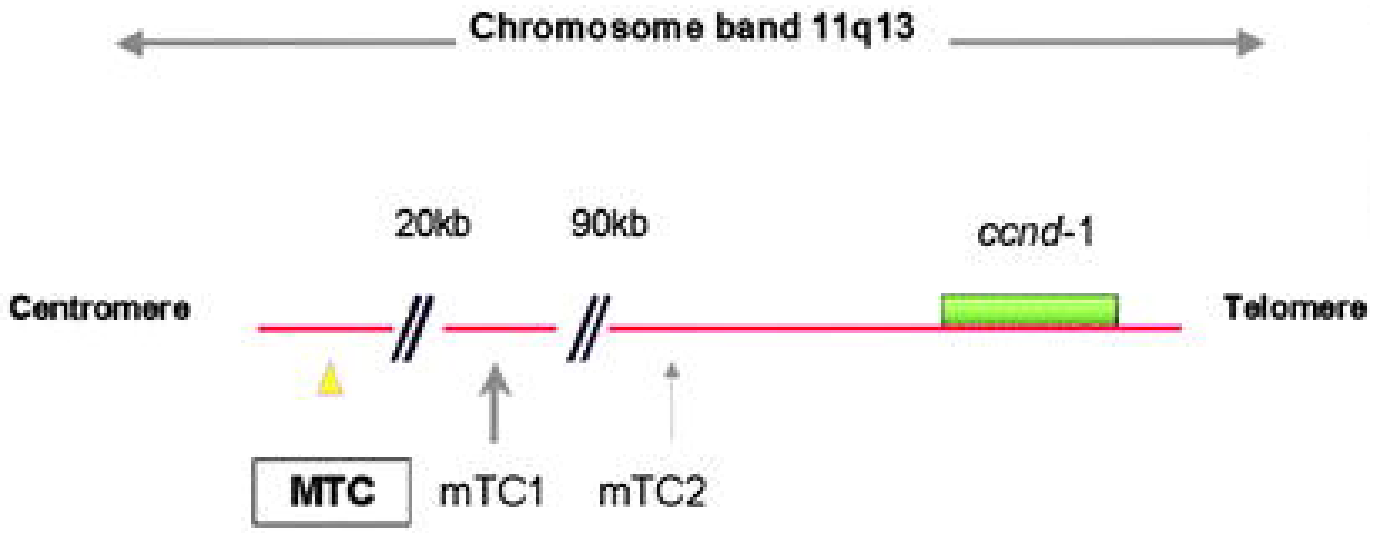
Results in cyclin D1 overexpression

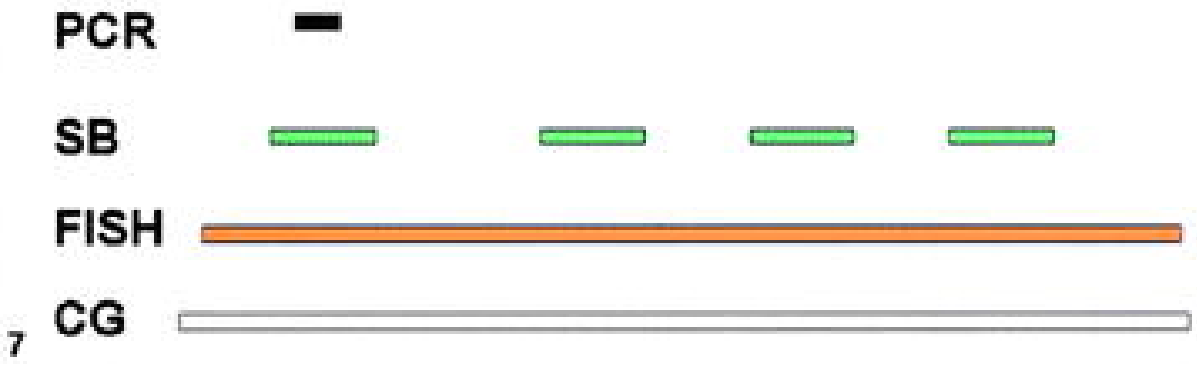
Major translocation cluster region in 11q13 - 50% of cases

Detection by cytogenetics, FISH, RFLP-SB, PCR, and quantitative RT-PCR

Immunohistochemistry – not great sensitivity

Mantle Cell Lymphoma





MALT Lymphoma

Multiple translocations described

t(11;18)(q21;q21) 35% of MALTs (most common one)

api2 gene on chr 11 translocates to *malt1* gene on chr18

api= inhibitor of apoptosis

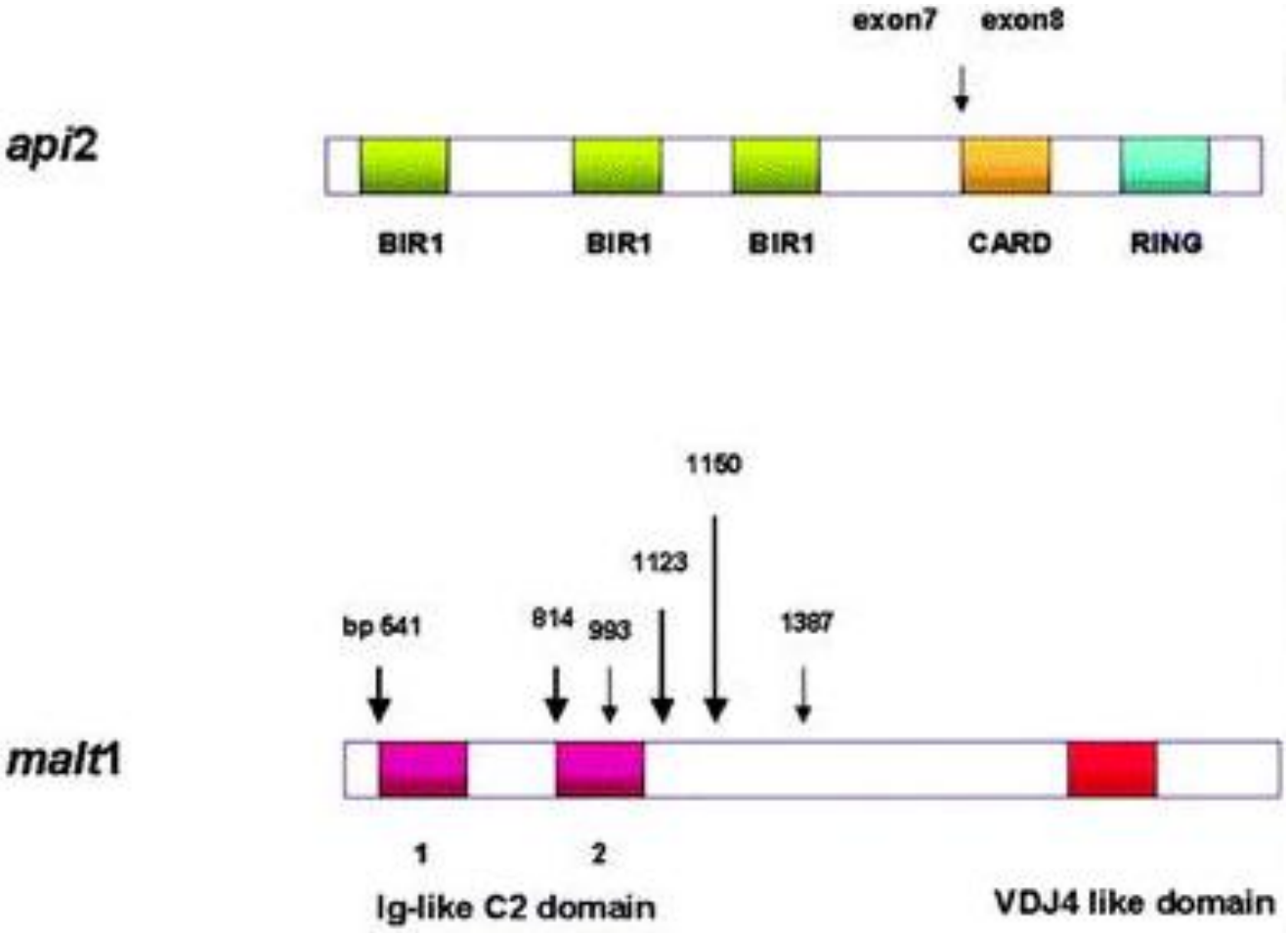
BIR= baculovirus inhibitor of apoptosis repeat CARD= caspase recruitment domain RING= really interesting new gene

malt1 functions in signaling pathway; structure is similar to Ig receptor

malt1 has 4 main breakpoint sites in t(11;18)(q21;q21)

malt1-*api2* fusion leads to increased apoptosis inhibition, and NF-*k*B probably involved

Malt Lymphoma



MALT Lymphoma

t(11;18)(q21;q21) 35% of MALTs (most common one)

malt1 function is unknown; probably involved in BCR signaling

malt1 has 4 main breakpoint sites in t(11;18)(q21;q21)

The *malt1-api2* translocation results in fusion protein, which functions to increase apoptosis inhibition and probably activate transcription factor NF-*k*B

Prognosis:

- t(11;18) = usually not *H pylori* driven, no response to Abx
  = rare for high grade BCL to arise in these MALTs
- non t(11;18) = usually H pylori driven, do respond to Abx
  = transform to high grade BCL more often

MALT Lymphoma

t(14;18)(q32;q21) 20% of MALTs

malt1 gene on chr 18 translocated onto IgH gene on chr 14

Results in *malt1* overexpression

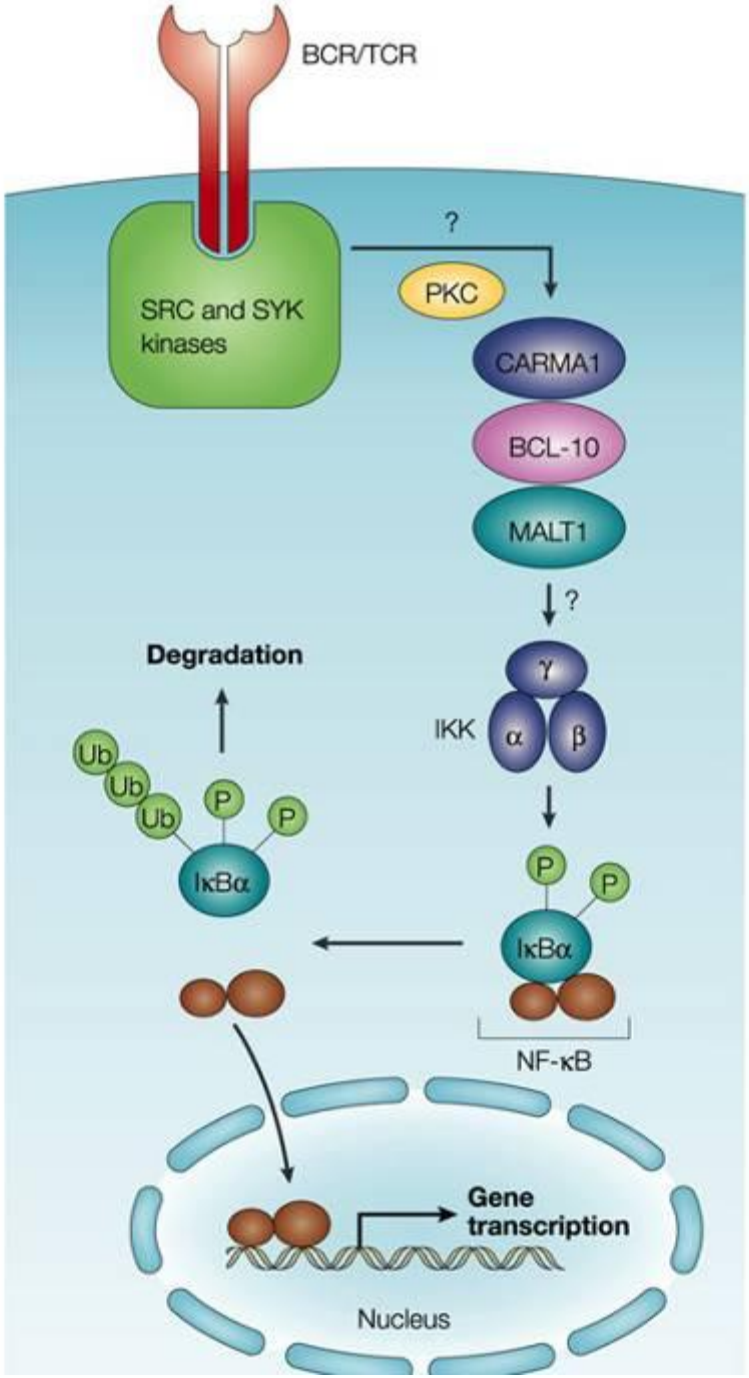
t(1;14)(p22;q32) <5%, new

bcl-10 on chr 1 translocated onto IgH on chr 14

Results in *bcl-10* overexpression due to IgH enhancer

Normal Bcl-10 protein is a signaling protein, activates NF-*k*B, which promotes cell activation/replication

bcl-10-IgH fusion disrupts Bcl-10 protein function, may cause Bcl-10 deregulation→NF-*k*B activation, leading to cell proliferation?



Malt1 and **Bcl-10** are thought to be part of signaling pathway for **NF-*k*B**

MALT Lymphoma

t(1;2)(q22;p12) rare, new

Translocation of *bcl-10* gene on chr 1 onto Igκ gene on chr 2

Detection of all four translocations mainly by cytogenetics

- IHC to detect Bcl-10:
  - normal B cells = Bcl-10 in cytoplasm
  - MALT lymphoma = Bcl-10 in nucleus in t(1;14) and t(11;18) translocations

Diffuse Large B-cell Lymphoma

Over 25 translocations reported, 40% of which involve *bcl-6*

30% have the **t(14;18)(q32;q21)** seen in FL, prob transformed FL

bcl-6 translocations seen with IgH, Igκ, Igλ, hsp90, eif4A11, et al

bcl-6 translocations and mutations also seen in MALT, FL, BL, CLL

In fact, bcl-6 alterations are the most common genetic defect in BCLs

Normal bcl-6 is transcription repressor that regulates germinal center B-cell formation and development, antibody-affinity maturation, T_H cell responses, plasma cell maturation

Diffuse Large B-cell Lymphoma

In DLBCL, *bcl-6* gets translocated onto the promoter regions of the other genes, but the *bcl-6* coding region remains intact

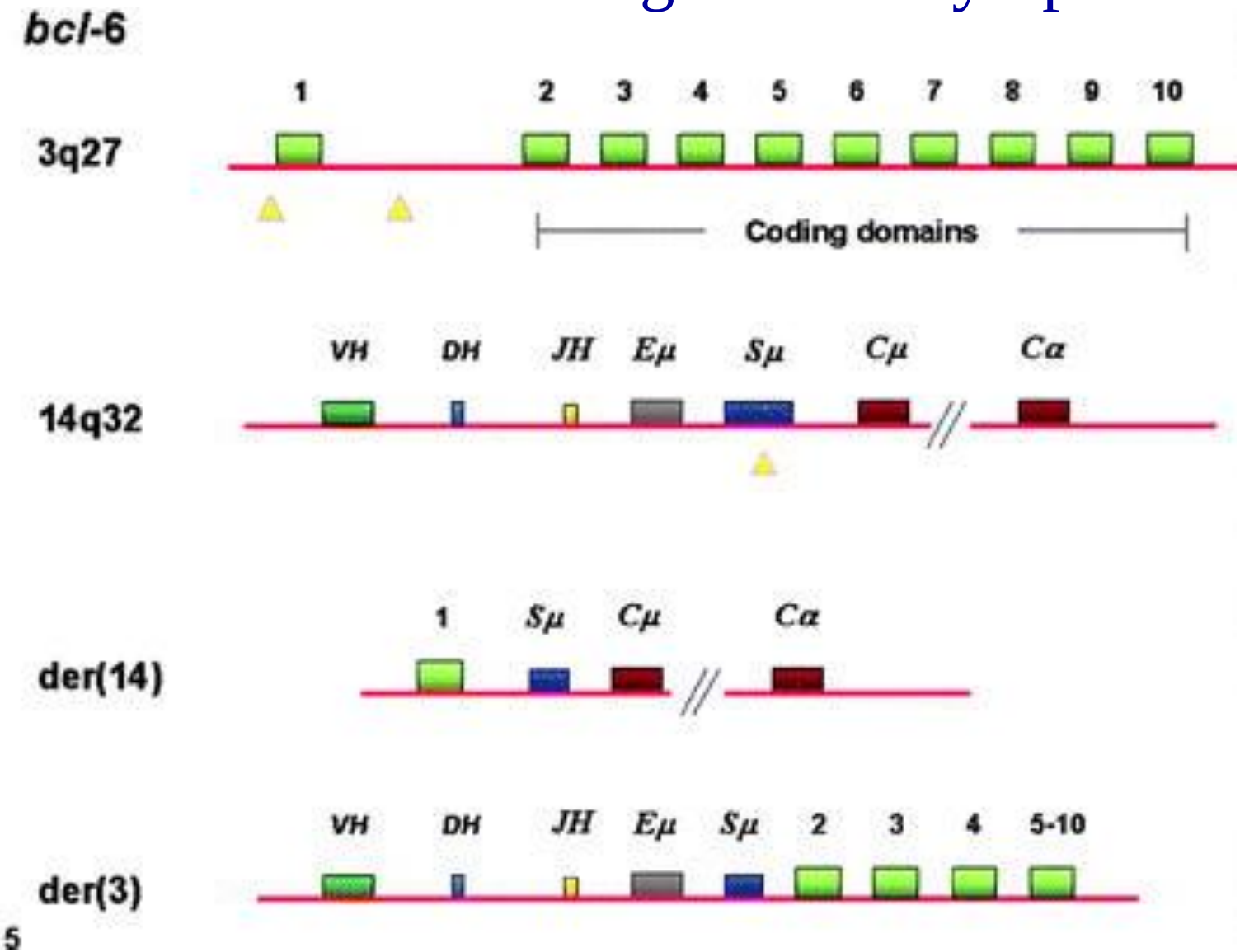
Felt that *bcl-6* translocation onto other gene's promoter causes disregulation of *bcl-6* and this leads to lymphoma

About 20% of DLBCL are Ig gene - *bcl-6* gene translocations:

- Ig gene translocations have better prognosis
- Non-Ig translocations have worse prognosis

Detection – RFLP-SB, FISH; not PCR (need too many primers)

Diffuse Large B-cell Lymphoma



Translocations in DLBCL

Translocation	Involved Genes
t(14;18)(q32;q21)	*Ig*H and *bcl*-2
t(3;4)(q27;p13)	*bcl*-6 and *rhoh/ttf*
t(3;6)(p27;p21.3)	*bcl*-6 and *H4* histone
t(3;6)(p27;p21.2)	*bcl*-6 and *pim*-1
t(3;7)(p27;p12)	*bcl*-6 and *ikaros*
t(3;11)(p27;q23.1)	*bcl*-6 and *bob/obf*1
t(3;3)(p27;q29)	*bcl*-6 and *tfrr*
t(3;13)(q27;q14)	*bcl*-6 and 1-*plastin*
t(3;16)(p27;p11)	*bcl*-6 and *Il*-21*r*
t(3;12)(p27;12q23-24.1)	*bcl*-6 and *α-nac*
t(3;18)(p27;p11.2)	*bcl*-6 and *eif*4AII
t(3;14)(p27;q32)	*bcl*-6 and *hsp*89*α*
t(3;6)(p27;p12)	*bcl*-6 and *hsp*90*β*
t(3;16)(p27;p13)	*bcl*-6 and *cIIta*
t(3;14)(p27;q32)	*bcl*-6 and *Ig*H
t(3;2)(p27;p12)	*bcl*-6 and *Igκ*
t(3;22)(p27;q11)	*bcl*-6 and *Igλ*
t(14;15)(q32;q11-13)	*Ig*H and *bcl*-8
t(1;22)(q22;q11)	*fcgr*IIb and *Igλ*
t(1;14)(q21;q32)	*muc*-1 and *Ig*H
t(10;14)(q24;q32)	*nf-κb*2 and *Ig*H
t(1;13)(p32;q14)	—
t(1;7)(q21;q22)	—
der(6)t(6;8)(q11;q11)	—
t(5;16)(?;q11-q12)	—
t(19;22)(q13;q11-q13)	—

Burkitt's Lymphoma

t(8;14)(q24;q32) 85% of all cases most common type

c-myc gene on chr 8 translocates onto IgH gene on chr 14

Breakpoints on 14q and 8q vary, endemic vs sporadic BL

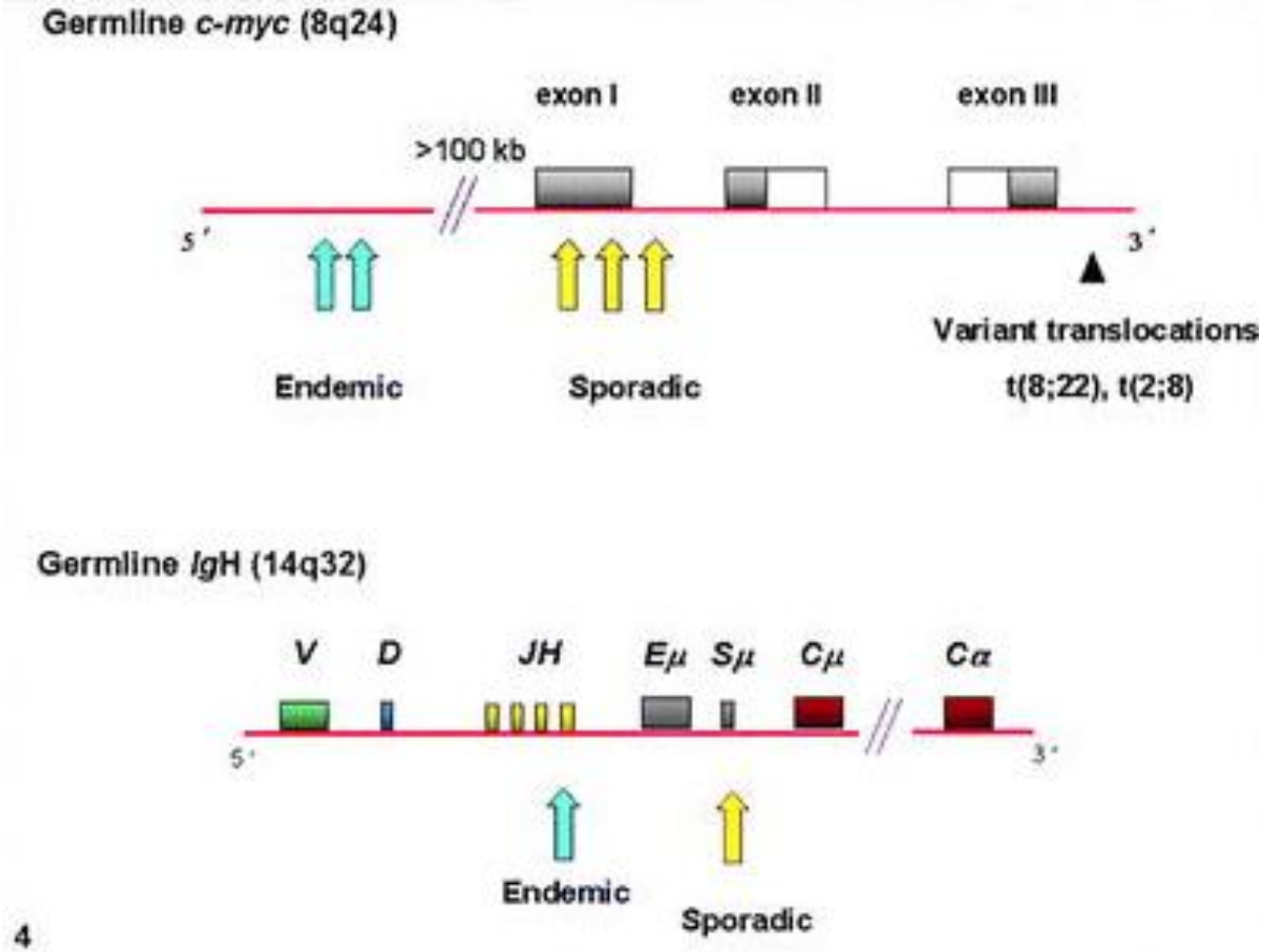
sporadic = 5'end or in *c-myc* $\rightarrow$ C or S region of IgH gene

endemic = far 5' end of *c-myc* $\rightarrow$ J region of IgH gene

t(2;8)(p12;q24) *c-myc* gene translocated onto Igκ gene

t(8;22)(q24;q11) *c-myc* gene translocated onto Igλ gene

Burkitt's Lymphoma



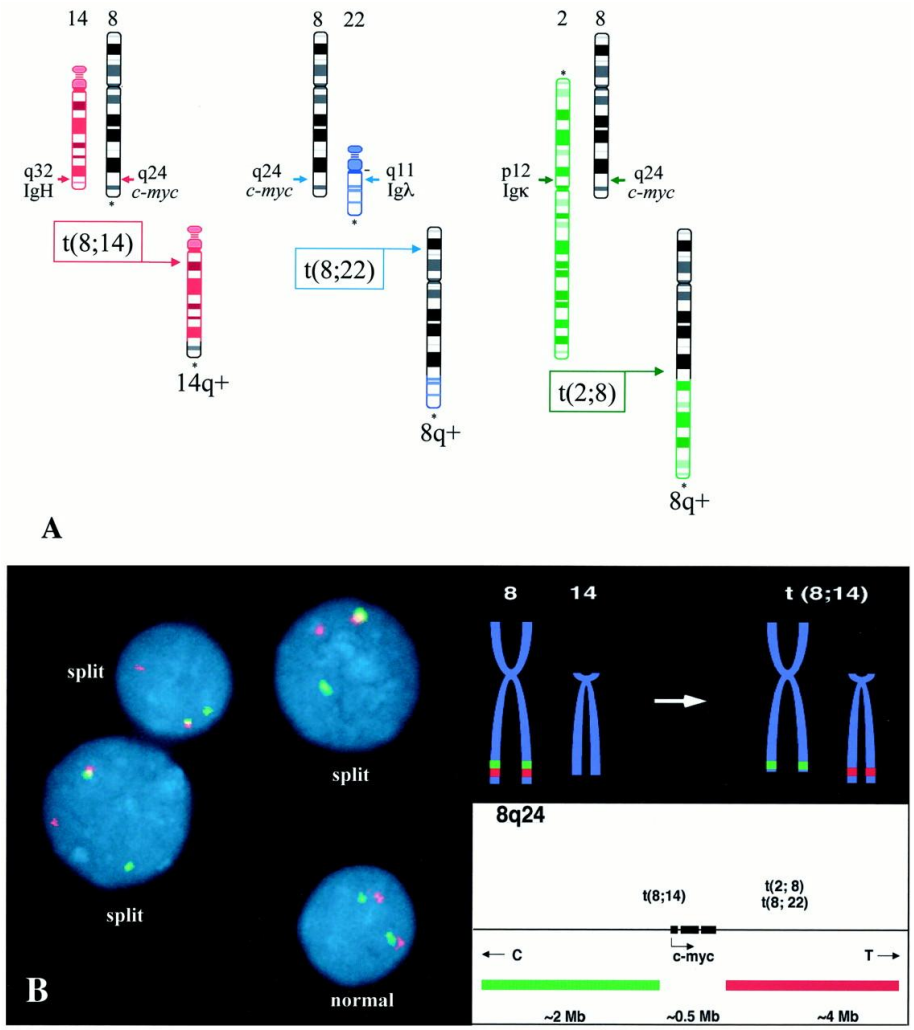
Burkitt's Lymphoma

Pathogenesis- in all 3 the *c-myc* gene is overexpressed due to translocation next to Ig gene enhancers

Normal *c-myc* regulates cell proliferation, differentiation, apoptosis, cell cycle progression

Detection – cytogenetics, FISH, RFLP-SB on some cases, but not PCR (too many primers needed)

Detection of *c-myc*/Ig chromosomal translocations in Burkitt's



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Anaplastic Large Cell Lymphoma

t(2;5)(p23;q35) most common, in 50-70% of cases

Nucleophosmin (*npm*) gene of chr 5 translocated onto anaplastic lymphoma kinase (*alk*) gene of chr 2

Normal NPM = rRNA processing; nuclear transport protein

Normal ALK = membane protein with intracytoplasmic tyrosine kinase; only expressed in CNS and PNS

ALK expression in hematopoeitic cells is abnormal

Anaplastic Large Cell Lymphoma

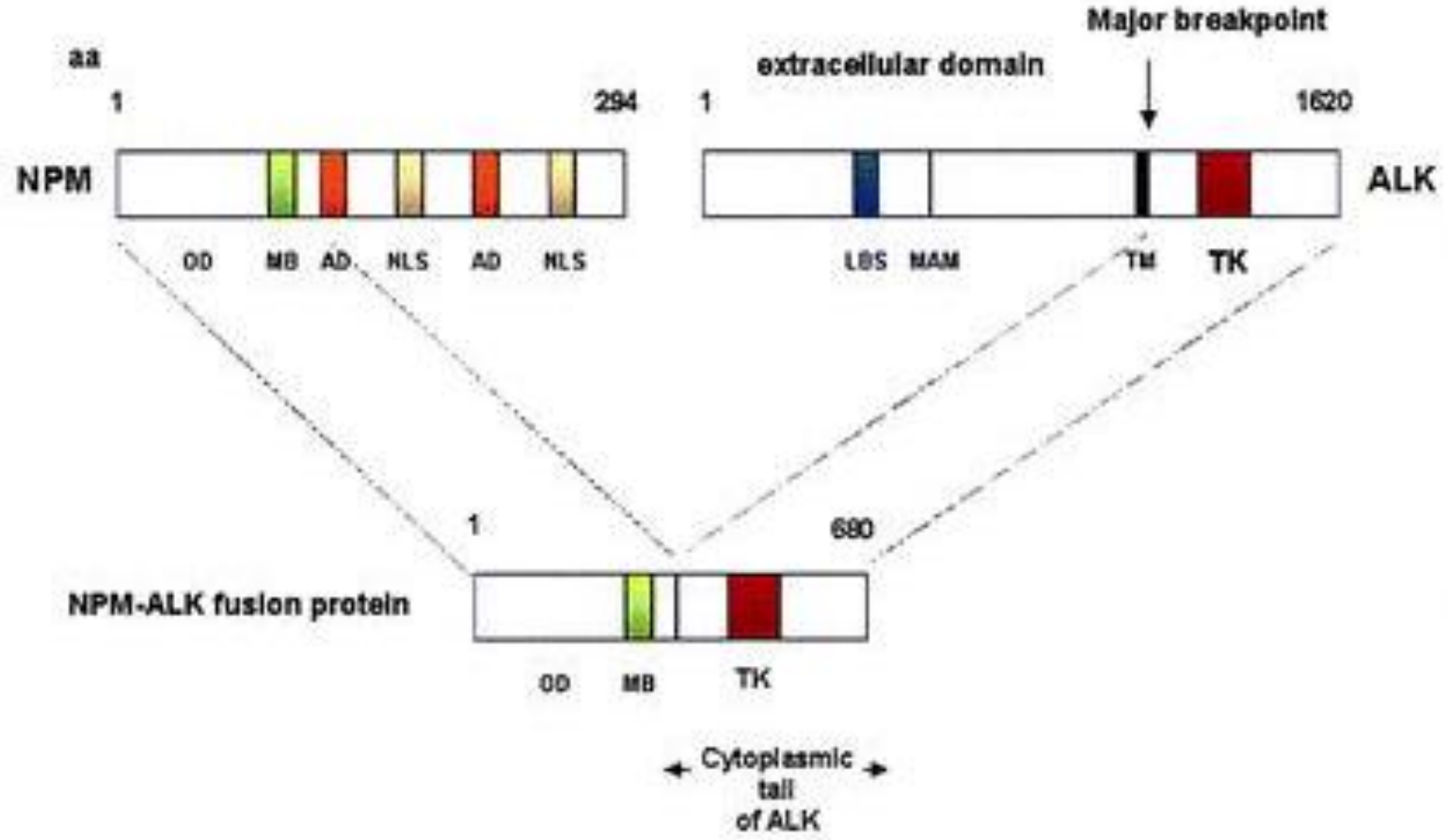
Fusion of *npm* and *alk* genes leads to encoded NPM-ALK fusion protein expression; *npm* promoter drives NPM-ALK transcription

NPM-ALK fusion protein is thus constantly expressed and ALK's tyrosine kinase activity turned on all the time

ALK tyrosine kinase stimulates downstream mitogen signaling and constant proliferation signals to the B-cell

Detection – cytogenetics, FISH, IHC (usually both cytoplasmic and nuclear stain), but not PCR

Anaplastic Large Cell Lymphoma



Plasma Cell Myeloma

Several translocations reported and most involve IgH on 14q32

t(11;14)(q13;q32) 30% of cases

ccnd-1 (cyclin D1) gene on chr 11 transloc onto IgH on chr 14

ccnd-1 breakpoints not the same as those in mantle cell

IgH gene breakpoints are in the S (switch) region

t(4;14)(p16;q32) 25% of cases

fgfr3 on chr 4 translocated onto IgH of chr 14

fgfr3 (fribroblast growth factor receptor 3) gene is disregulated

Plasma Cell Myeloma

t(6;14)(p25;q32) 20% of cases

mum/irf4 gene of chr 6 translocated onto IgH gene

mum/irf4 (multiple myeloma gene 1/interferon regulatory factor 4) dysregulated due to IgH enhancer

t(14;16)(q32;q23) 10% of cases

c-maf gene on chr 16 translocated onto IgH gene

c-Maf (musculoaponeurotic fibrosarcoma) protein is a transcription factor that helps regulate expression of cyclin D1 and IL-4

Plasma Cell Myeloma

These translocations seen in MGUS and PC myeloma

They are likely initiating translocations, and further mutations needed for full blown myeloma

Myelomas also frequently have non-translocation chromosomal defects

Detection – FISH usually; cytogenetics; PCR works some of the time