# Genetic instability and Cancer

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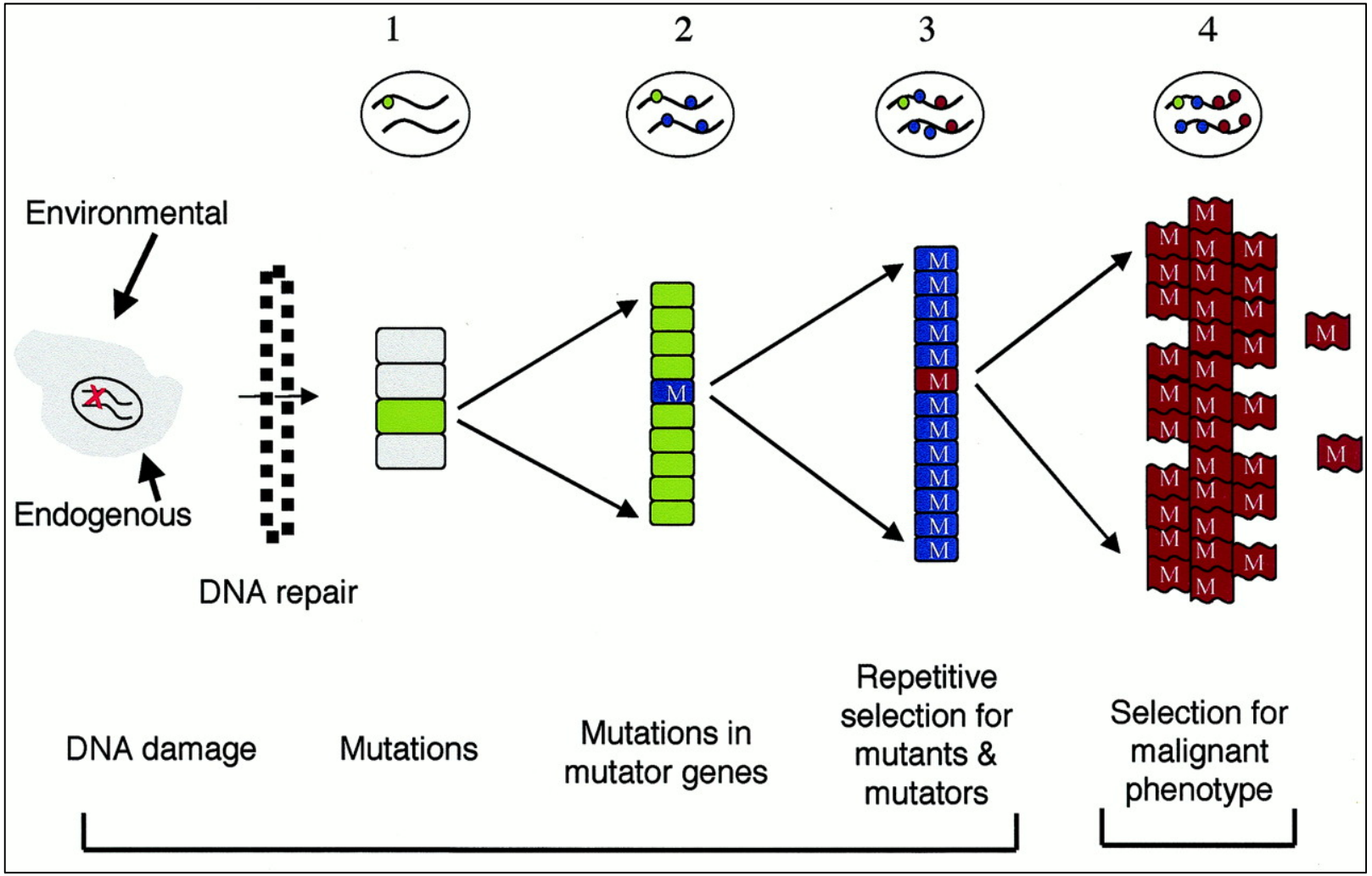
**DKFZ**
**Abteilung Tumorgenetik**

**The ultimate goal of cell division for somatic cells is accurately duplicate the genome and then accurately divide it into the two daughter cells**




**Mutation accumulation during tumor progression**

# Learning aims:

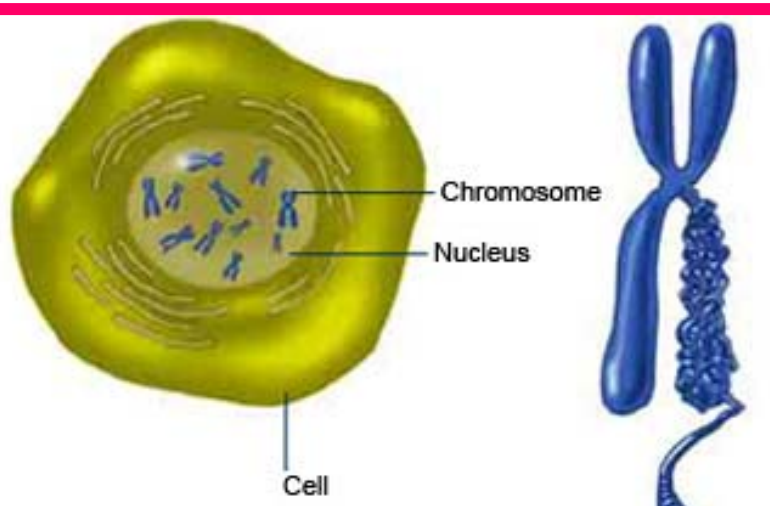
- **Knowing the types of genetic instability in cancer cells**
- **Knowing the major molecular mechanisms which are responsible for DNA damage repair**

# Genetic instability

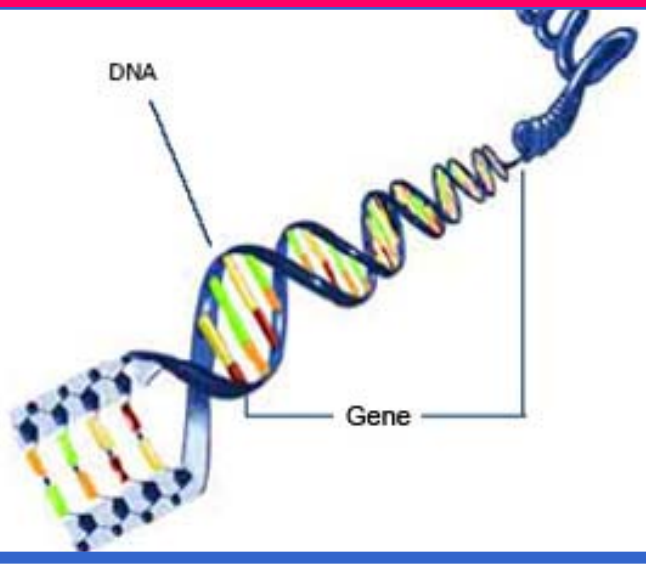
- Genetic instability is a hallmark of tumor development
- Carcinogenesis is the multistep process characterized by the accumulation of genetic alterations from preneoplastic lesions to advanced tumors
- Increased instability can result in accumulation of mutations in critical genes, such as oncogenes and tumor suppressor genes

# Genetic instability

**Genetic instability refers to a range of genetic alterations from point mutations to chromosome rearrangements**




**instability at the chromosomal level**




**instability at the nucleotide level**

# Genetic instability

## Types of genetic instability

### Instability at the nucleotide level

Nucleotide instability (NIN) → Defects of Base excision repair (BER)

Defects of Nucleotide excision repair (NER)

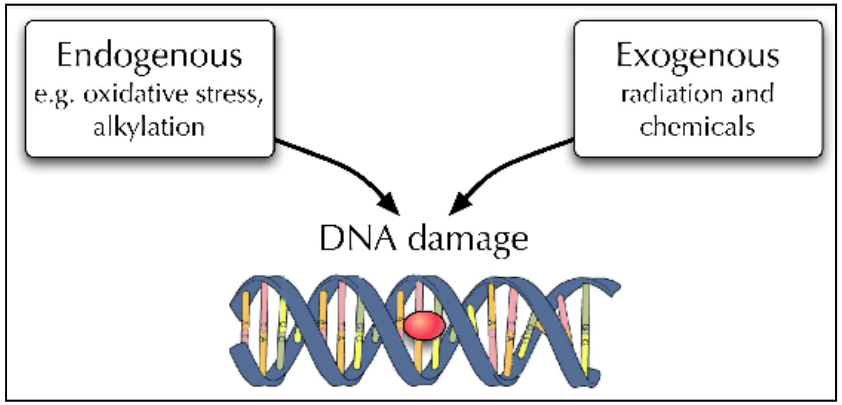
Microsatellite instability (MIN) → Defects of Mismatch repair (MMR)

### Instability at the chromosomal level

Chromosomal instability (CIN) → Defects of several DNA repair and mitotic control pathways

# Genetic instability
## Types of DNA damage

**All cells can sustain DNA damage from various sources that are classified as either endogenous or exogenous in origin**




**Endogenous damage includes:**

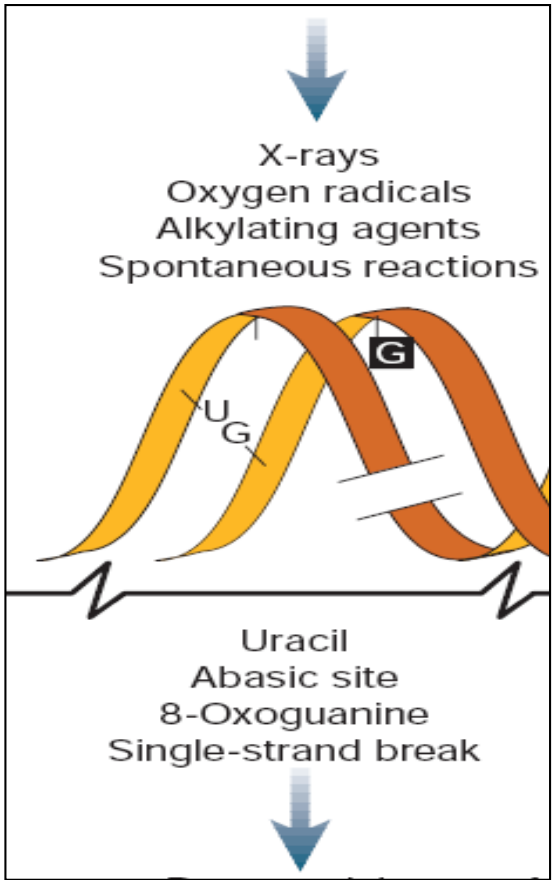
- incorporation of incorrect nucleotides during DNA replication
- alterations in the chemistry of A, G, C (deamination, generating xanthine, hypoxanthine or uracil)
- incorporation of uracil instead of thymine

**Environmental (exogenous) damage includes:**

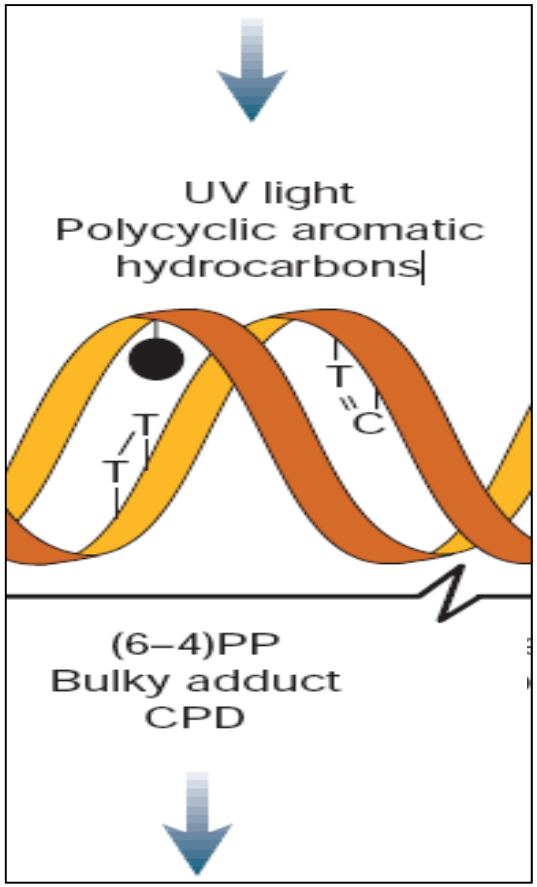
- ionizing radiation
- ultraviolet radiation
- naturally occurring and man-made chemicals that are reactive with DNA

# Nucleotide instability (NIN)

**Instability at the nucleotide level occurs due to faulty DNA repair pathways such as base excision repair and nucleotide excision repair**




**Base excision repair (BER)**

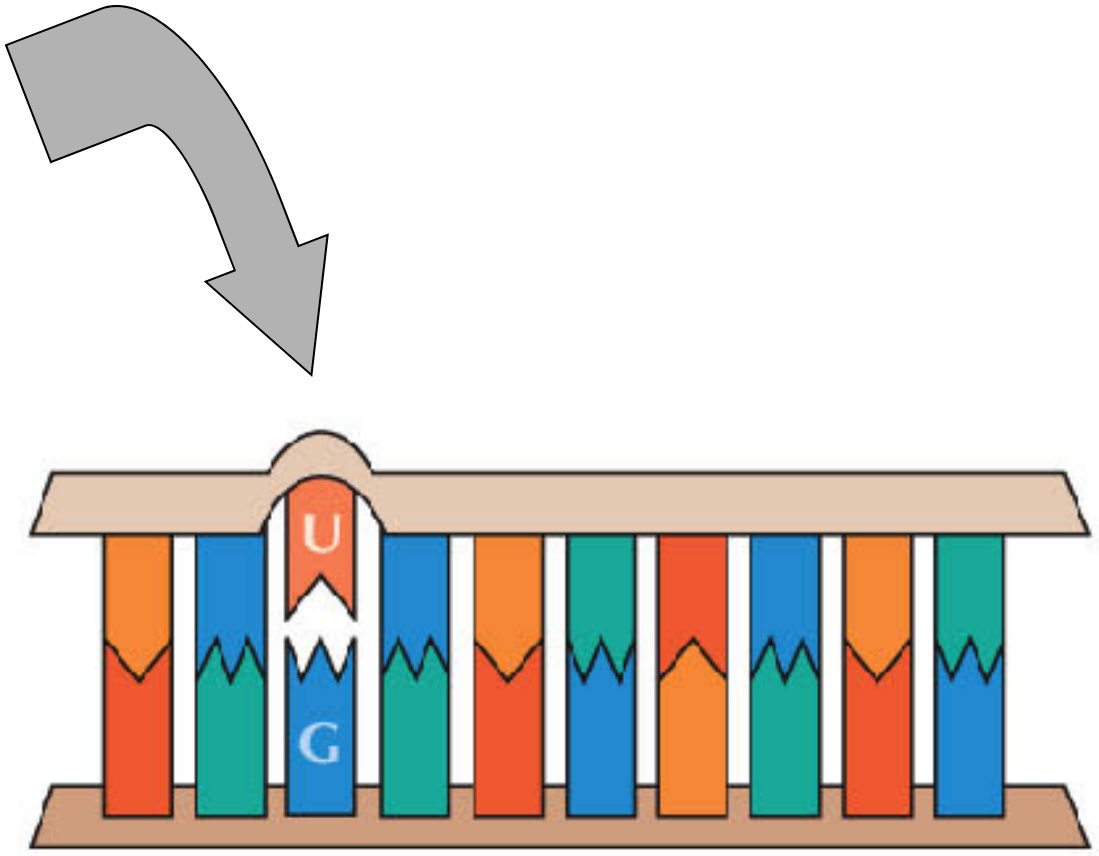



**Nucleotide excision repair (NER)**

**The various known sources of spontaneous base damage are estimated to alter about 25,000 bases per cell per day out of the $3 \times 10^9$ bases in the genome**

# Nucleotide instability (NIN)

**R**ecognition
**R**emoving
**R**esynthesis

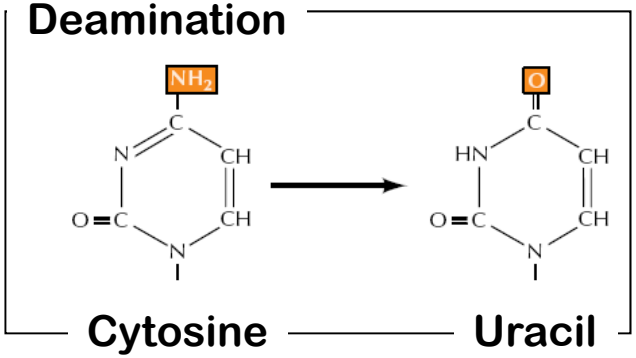


# Nucleotide instability (NIN)
## Base Excision Repair (BER)

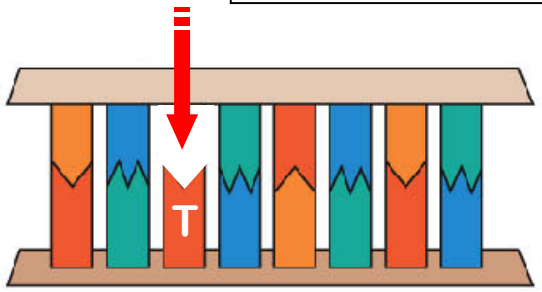
**Base excision repair (BER) is mainly responsible for repairing damage induced by endogenous metabolic processes such as methylation, deamination, reactive oxygen species (ROS) and hydrolysis.**

**Most frequent lesion is depurination. 5,000 purines are lost this way each day in a typical human cell.**

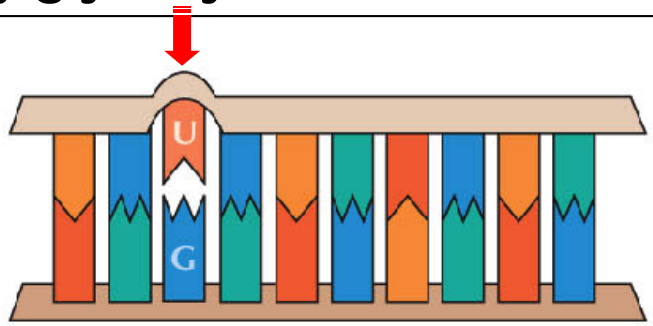
**Hydrolysis of the beta-N-glycosidic link between a purine base (Adenine or Guanine)**




**Recognized by a repair nuclease**



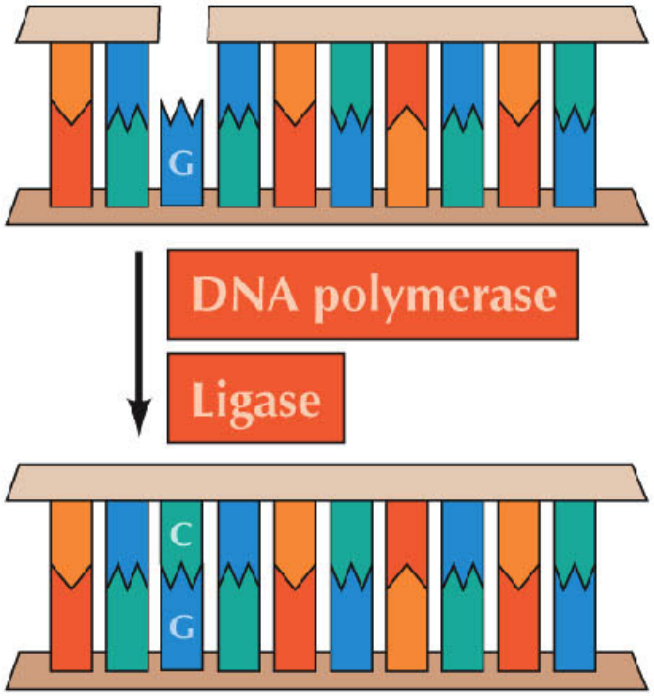

**Recognized and Removed by glycosylases**




**11 DNA glycosylases have been identified in mammals**

# Nucleotide instability (NIN)
## Base Excision Repair (BER)

**Multiple proteins contribute to BER pathway and enable it to correct non-bulky damaged nucleotides.**




**Resynthesis**

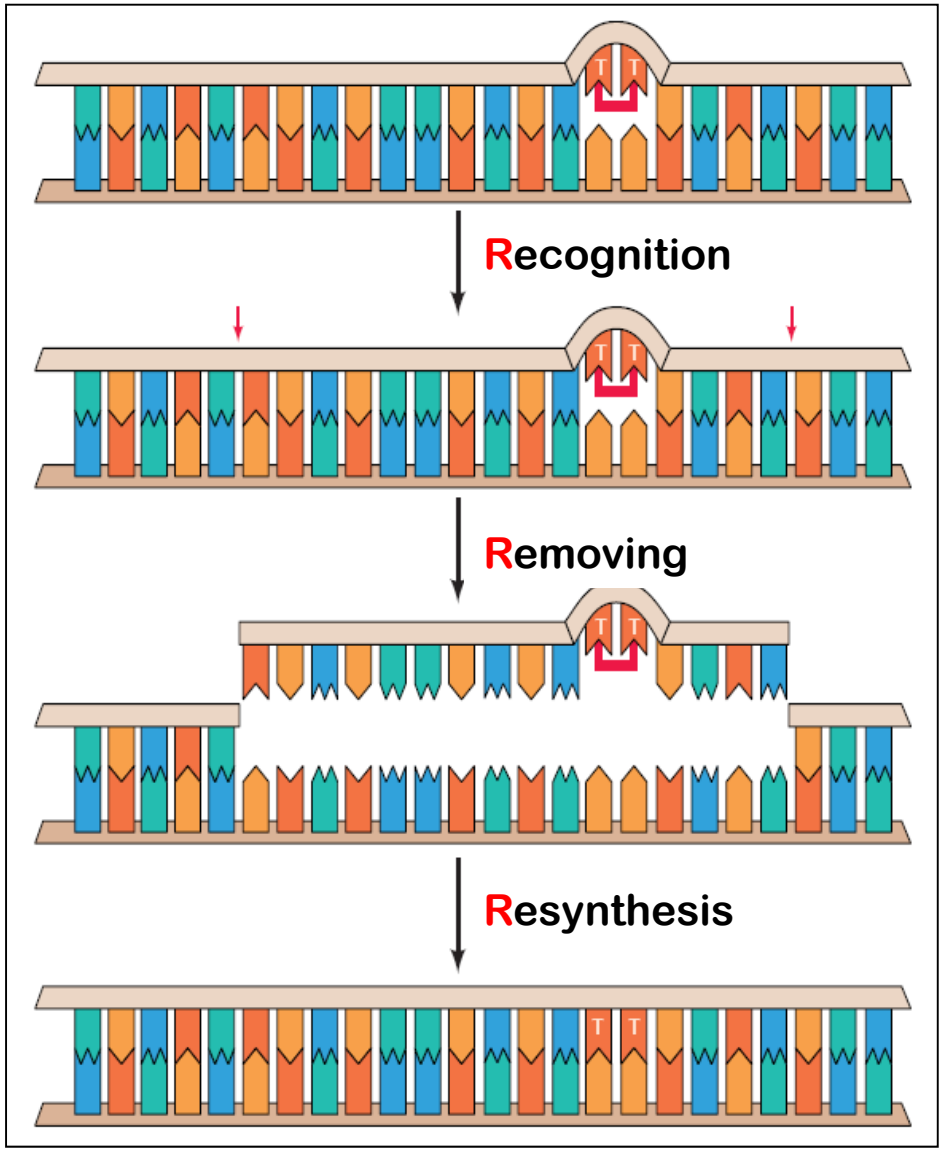
**Biallelic inactivation of MYH (glycosylase) can lead to an autosomal recessive form of inherited colorectal cancer known as MYH-associate polyposis (MAP) .**

# Nucleotide instability (NIN)
## Nucleotide Excision Repair (NER)

**NER has a broader specificity in recognition and repair of lesions that are caused by exogenous damage (UV light, chemicals) and gives rise the DNA cross links/bulky adducts.**

**Recognition**
**Removing**
**Resynthesis**

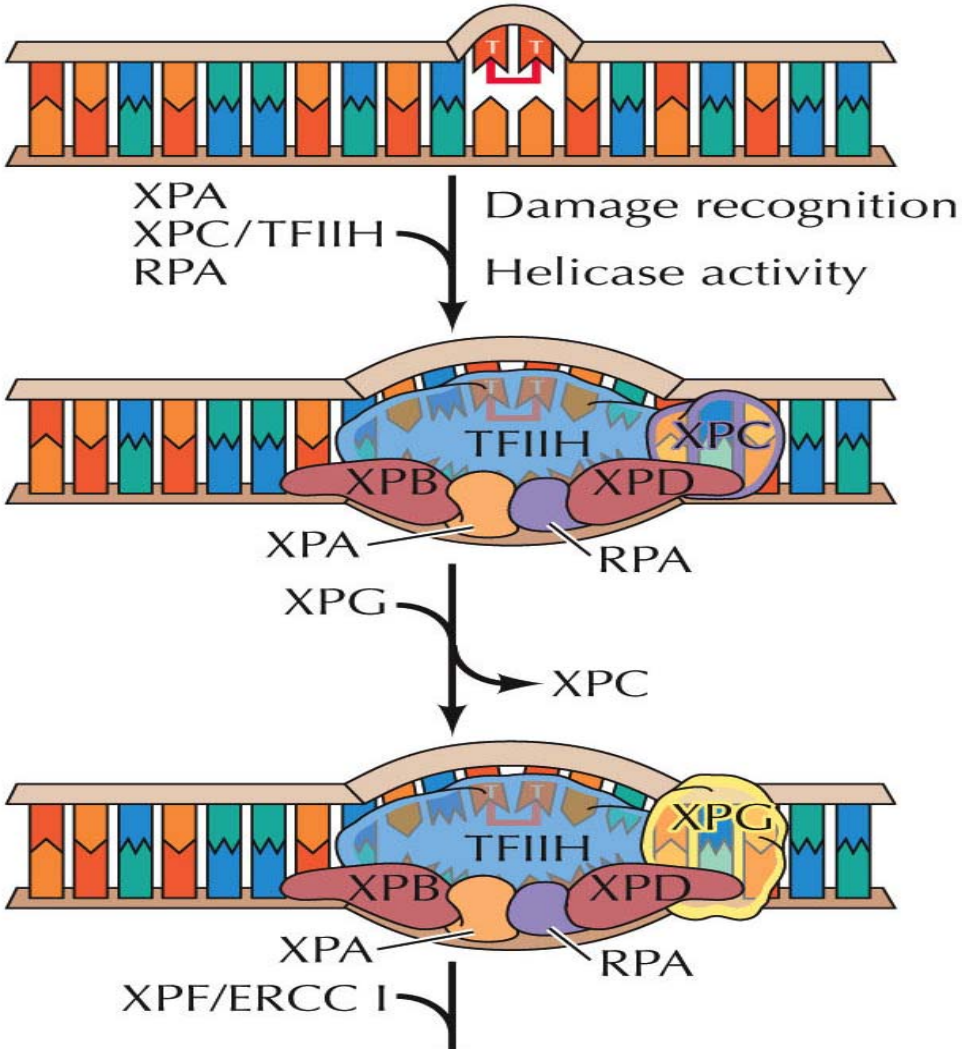
# Nucleotide instability (NIN)
## Nucleotide Excision Repair (NER)




*THE CELL, Fourth Edition,*

# Nucleotide instability (NIN)
## Nucleotide Excision Repair (NER)

**The NER pathway is a multi-step process and as many as 30 proteins assemble at the damaged site in a stepwise fashion.**




THE CELL, Fourth Edition,

**7 nucleotide excision repair genes: XPA-XPB-XPC-XPD-XPE-XPF-XPG**

# Nucleotide instability (NIN)
## Xeroderma Pigmentosum

- Individuals born with defects in the NER pathway develop a syndrome known as Xeroderma Pigmentosum (XP). Inherited defects in any one of the 7 nucleotide excision repair XPA-XPB-XPC-XPD-XPE-XPF-XPG genes have been implicated in this disease

- XP patients have a very high susceptibility to developing cancer in areas of skin exposed to the sun. The median age at which skin tumours arise in these patients is 8 years, compared with a average of 60 years observed in the normal population

# Microsatellite instability (MIN)

**Microsatellite instability (MIN or MSI) is associated with the occurrence of unrepaired deletions/extentions in microsatellite repeat sequences resulting in variable lengths of these repeats.**

# Microsatellite instability (MIN)

## Human genome composition

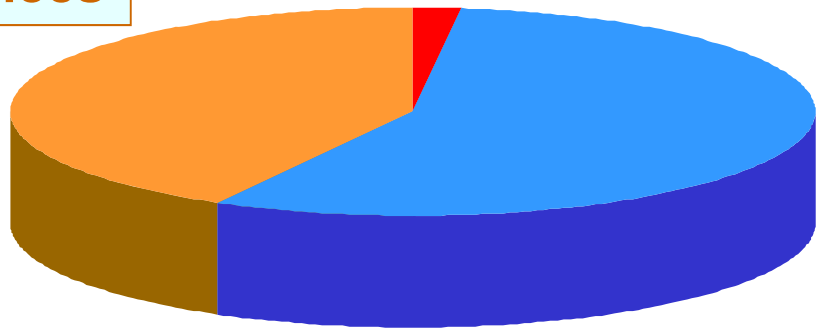
~3.2 x $10^9$ (3.2 billion) nucleotide pairs

23 pairs of chromosomes (each chromosome is a single DNA molecule)

~30.000 genes

Less than 2% of the genome codes for proteins.

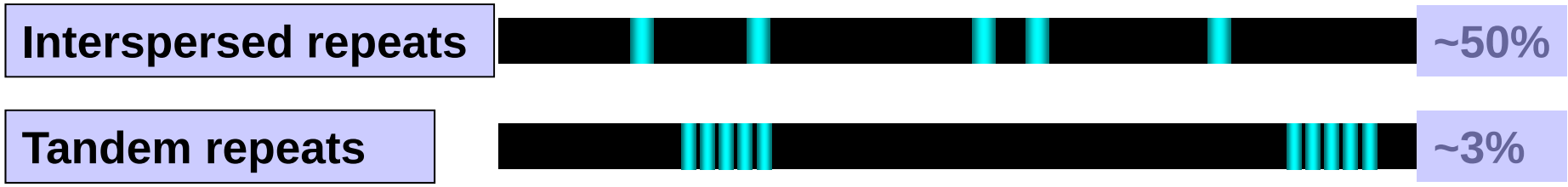
Other sequences



Repeated sequences that do not code for proteins make up >50% of the human genome.

# Microsatellite instability (MIN)

## Repeats in human genome

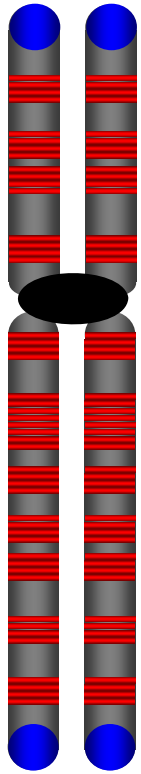



### Satellites

long series of tandem repeats, hundreds of kb in length

### Minisatellites

Minisatellites form clusters up to 20 kb in length, with repeat units up to 25 bp;



### Microsatellites

Microsatellite clusters are shorter, usually < 150 bp, and the repeat unit is usually 1-4 bp.

# Microsatellite instability (MIN)

## Microsatellites

Short tandem repeats (STRs)  Simple sequence repeats (SSR)

- are sequences made up of a single sequence motif (1-4 bp) which is repeated many times side-by-side
- can be repeated 10 to 100 times
- In the human genome poly(A)/poly(T) stretches are the most common repeat types

5´- A A A A A A A A A A A A -3´
3´- T T T T T T T T T T T T´-5´

- CA nucleotide repeats are very frequent in human genome (every few thousand base pairs)

5´- CA CA CA CA CA CA CA CA -3´
3´- GT GT GT GT GT GT GT GT -5´

# Microsatellite instability (MIN)

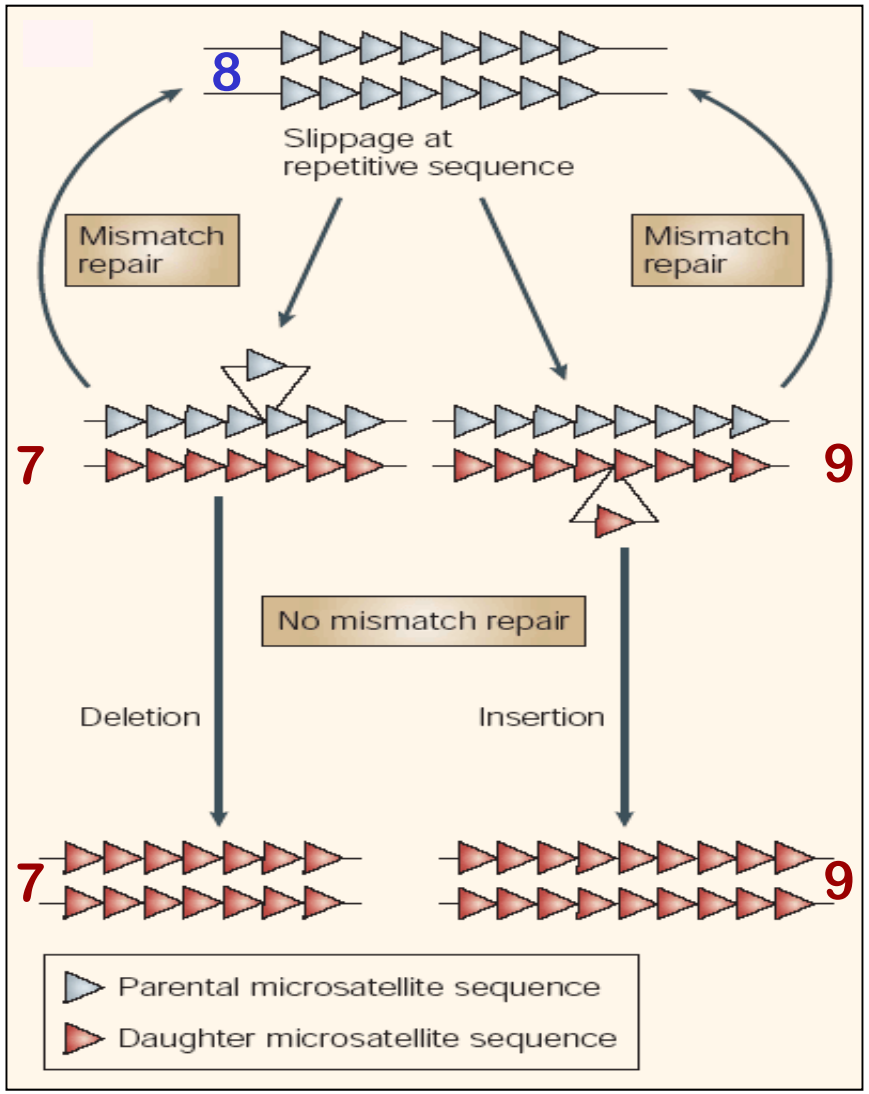
## Microsatellite variability

- No two humans alive today have exactly the same combination of microsatellite length variants: if enough microsatellites are examined then a unique genetic profile can be established for every person. The only exceptions are genetically identical twins.

# Microsatellite instability (MIN)

## Why microsatellites are unstable?

**The mechanism responsible for microsatellite variability is replication slippage (polymerase slippage)**

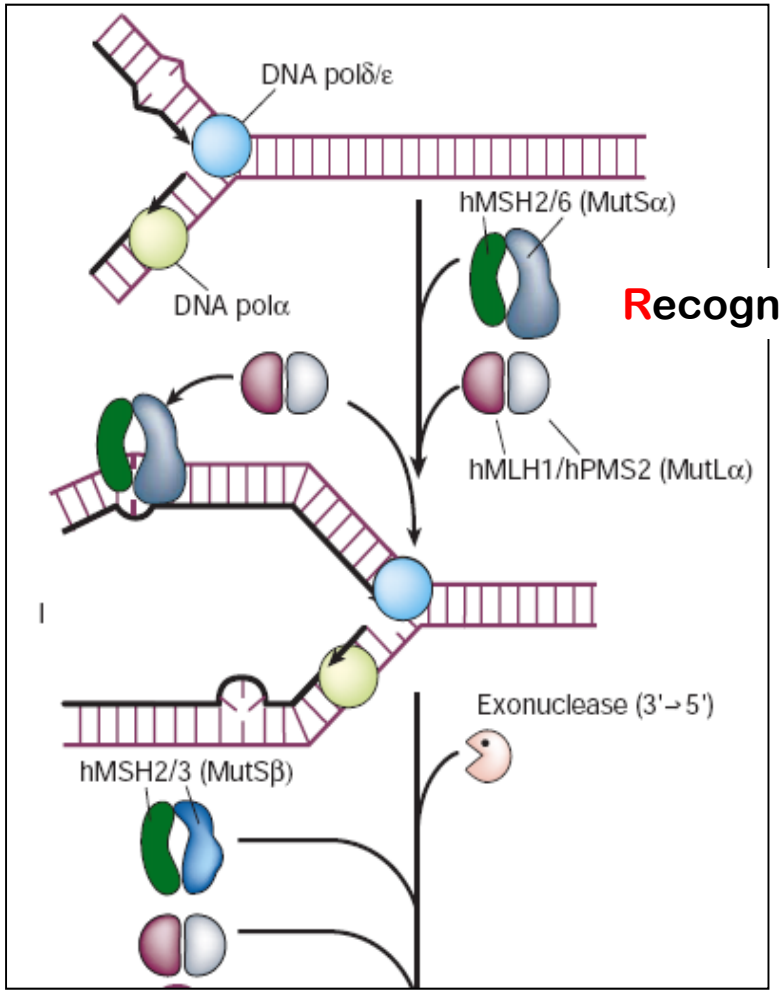



**Template strand and its copy shift their relative positions so that part of the template is either copied twice or missed out.**

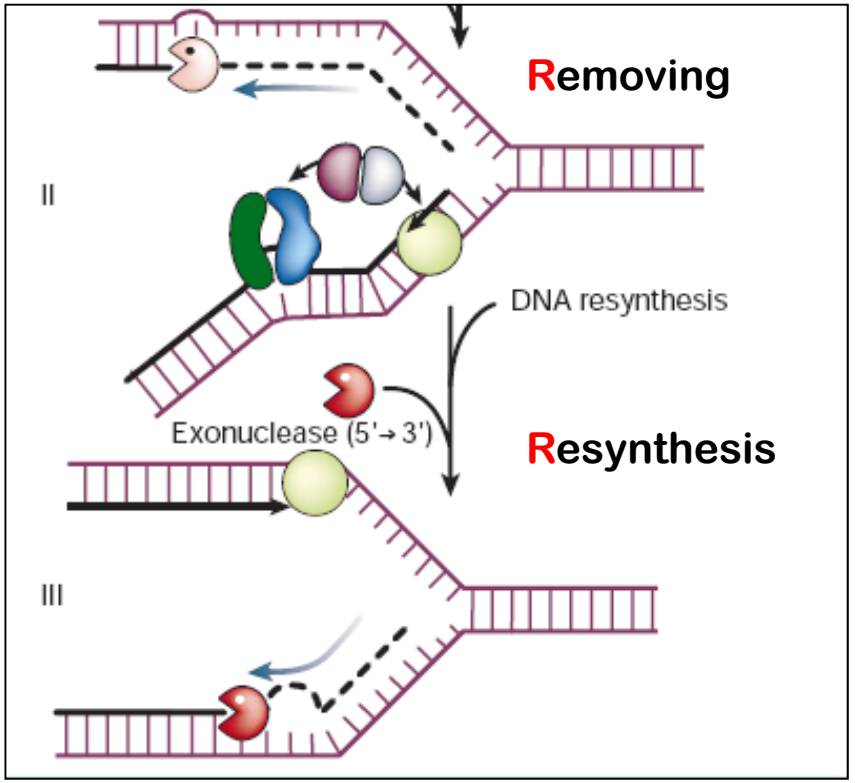
# Microsatellite instability (MIN)
## Mismatch repair (MMR)

- Mismatch repair (MMR) has a central role in maintaining genomic stability by repairing DNA replication errors.
- It is a post-replicative mechanism capable of eliminating base-base mismatches and insertion/deletion loops that arise during DNA synthesis.

# Microsatellite instability (MIN)
## Mismatch repair (MMR)



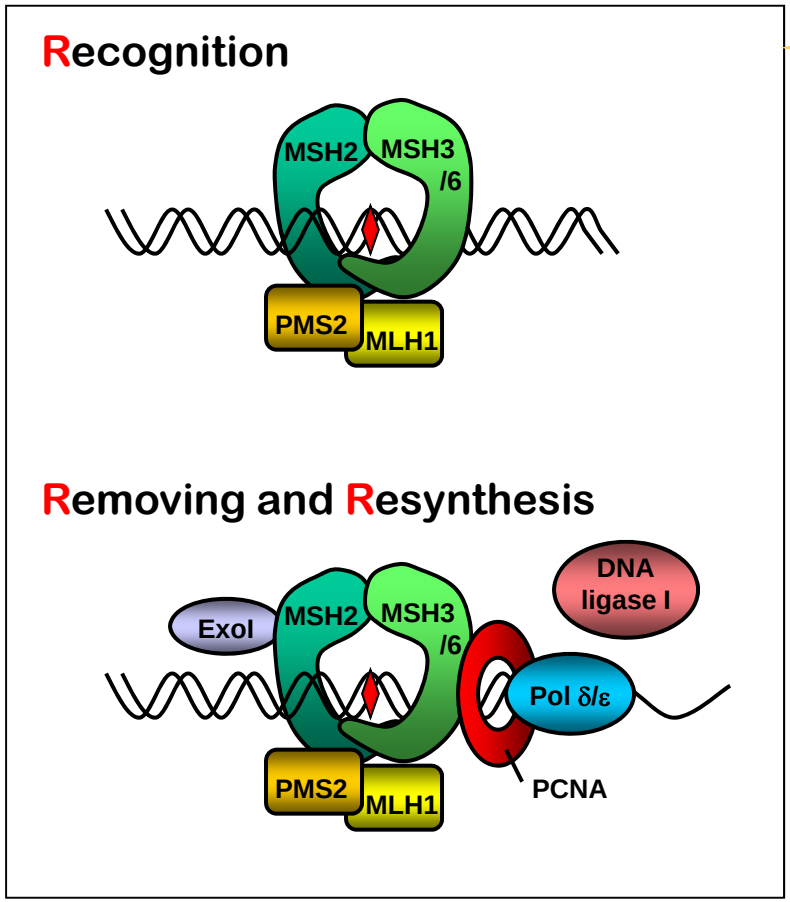

**Recognition**




**Removing**

**Resynthesis**

# Microsatellite instability (MIN)
## Mismatch repair proteins



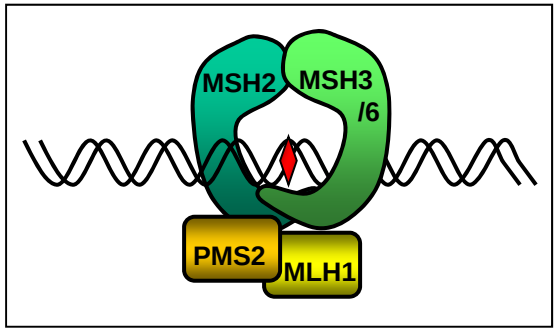

- **MSH2-MSH3 complex**, which preferentially recognizes insertion/deletion loops
- **MLH1 and PMS2** form a heterodimer that is then able to bind to the MSH2 heterodimers.

# Microsatellite instability (MIN)
## Hereditary non-polyposis colorectal cancer (HNPCC)

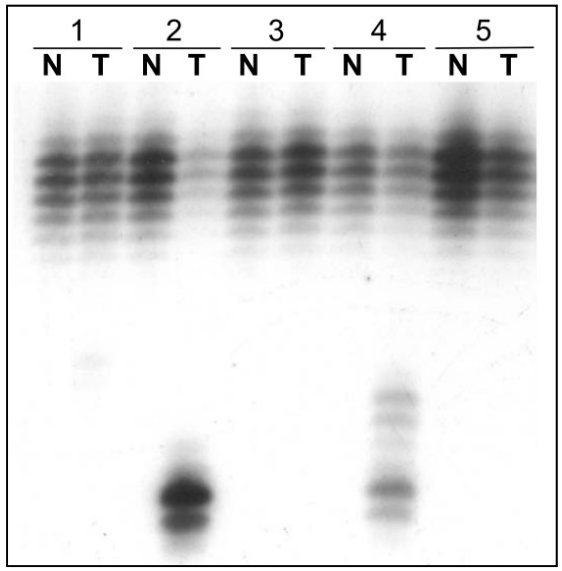
- Germline mutations in the MMR genes are associated with the inherited cancer syndrome, hereditary non-polyposis colorectal cancer (HNPCC). Instability of microsatellite repeats is seen in tumours of as many as 85% of patients with HNPCC, making it a hallmark feature of this syndrome HNPCC, which accounts for about 2% of all CRC cases.

MIN is a key factor in several cancers including colorectal, endometrial, ovarian and gastric cancers

# Microsatellite instability (MIN)
## Hereditary non-polyposis colorectal cancer (HNPCC)




HNPCC is frequently associated with defects in the genes encoding MSH2 (about 35% of cases) and MLH1 (about 60% of cases)

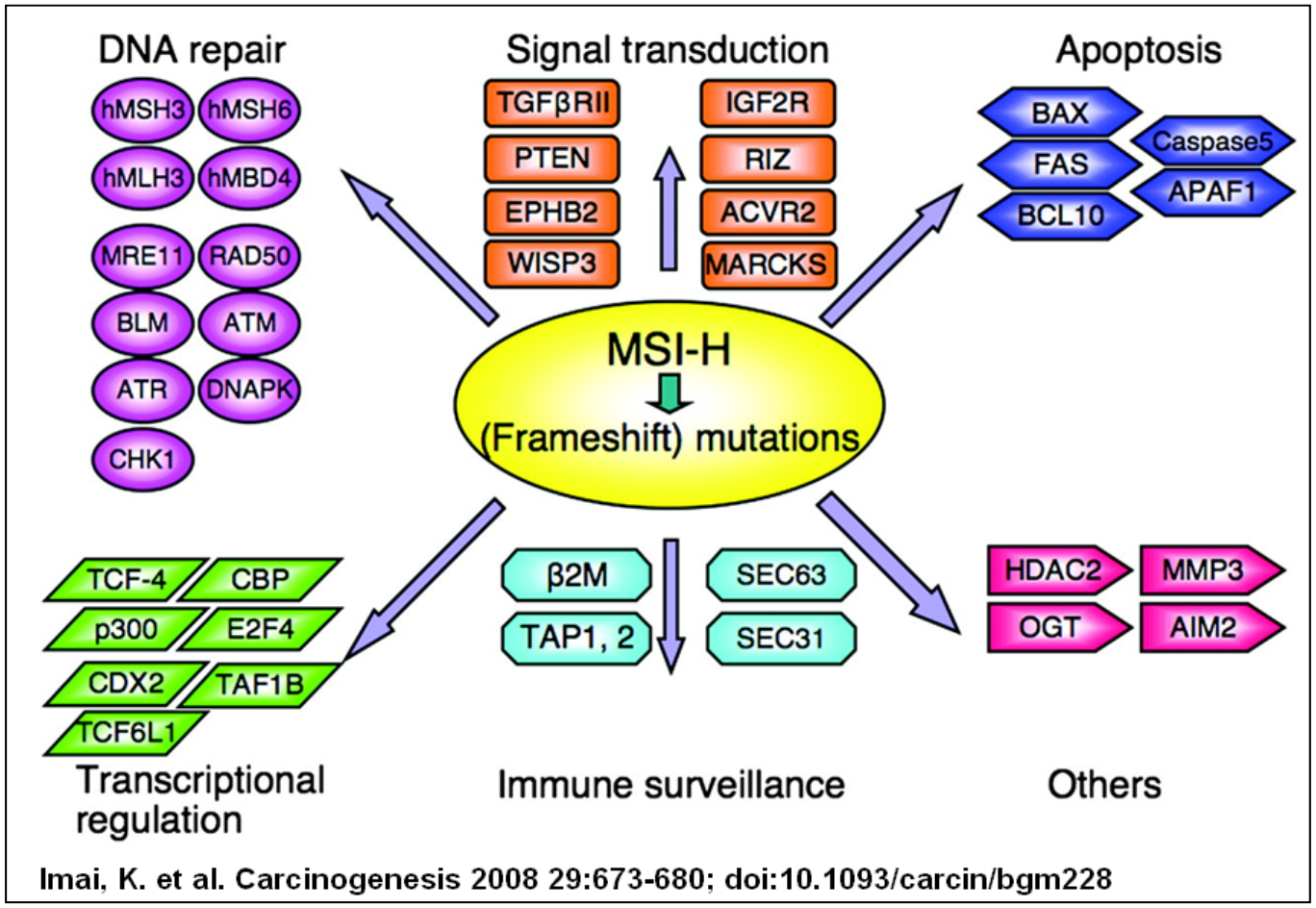



The instability appeared as a substantial or a minor change in repeat length

Tumors can carry more than 100,000 such mutations.

# Microsatellite instability (MIN)
## Hereditary non-polyposis colorectal cancer (HNPCC)

**Instability at coding microsatellites in target genes causes frameshift mutations and functional inactivation of affected proteins, thereby providing a selective growth advantage to MMR-deficient cells**




Imai, K. et al. Carcinogenesis 2008 29:673-680; doi:10.1093/carcin/bgm228

# Genetic instability

## Instability at the nucleotide level

**Nucleotide instability (NIN)** → **Defects of Base excision repair (BER)**

**Defects of Nucleotide excision repair (NER)**
Xeroderma Pigmentosum

**Microsatellite instability (MIN)** → **Defects of Mismatch repair (MMR)**
Hereditary non-polyposis colorectal cancer (HNPCC)

# Chromosomal instability (CIN)

## What is chromosomal instability?

- All malignant tumour types have been shown to contain chromosomal aberrations, either a gain or a loss of chromosomes or chromosomal pieces
- In most cases, a subset of these abnormalities are shared by all cells of a tumour, indicating that a step-wise accumulation of cytogenetic changes have occurred during tumour growth
- Chromosomal instability is a state of continuous formation of novel chromosomal aberrations during mitosis

# Chromosomal instability (CIN)

## Two types of chromosomal instability

Abnormal mitotic mechanisms may result in numerical or structural aberrations in the daughter cells

- Numerical chromosomal instability involves changes in the chromosome number that lead to chromosome gain or loss
- Structural chromosomal instability involves changes in the genetic linkage of two DNA fragments

# Chromosomal instability (CIN)

## Numerical instability

- Proper chromosome segregation is required to maintain the appropriate number of chromosomes from one cell generation to the next and to prevent

  Aneuploidy ⟶ the condition in which a cell has gained or lost one or several chromosomes during cell division or

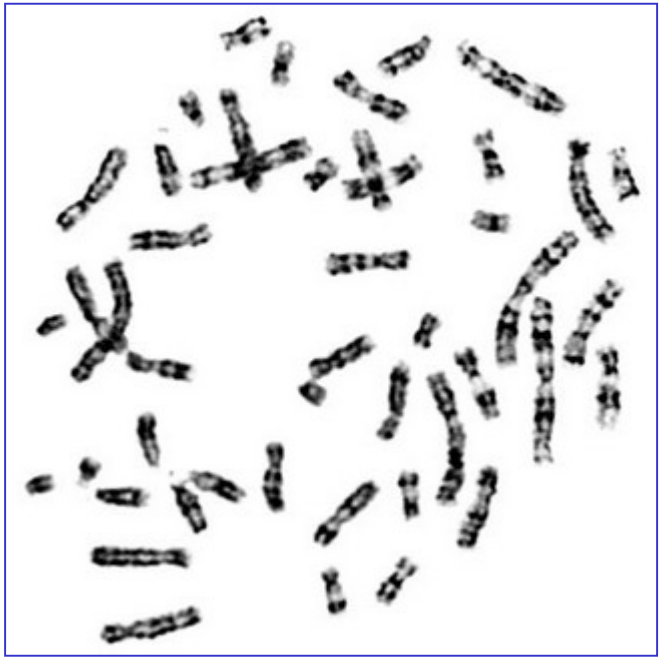
  Polyploidy ⟶ the condition in which a cell has gained one or more haploid sets of chromosomes

- Aneuploidy/polyploidy is a hallmark associated with birth defects and cancer, and is observed at relatively high frequencies in human somatic cells

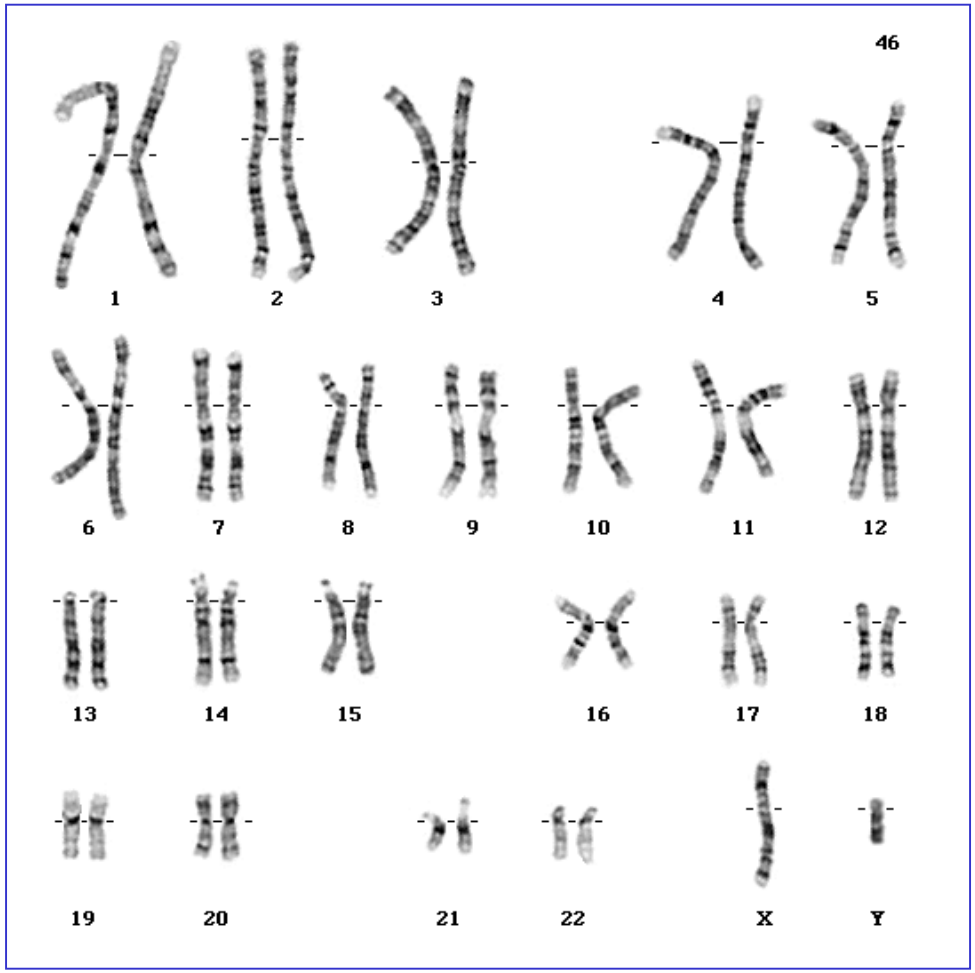
# Chromosomal instability (CIN)

## Numerical instability

Metaphase spread



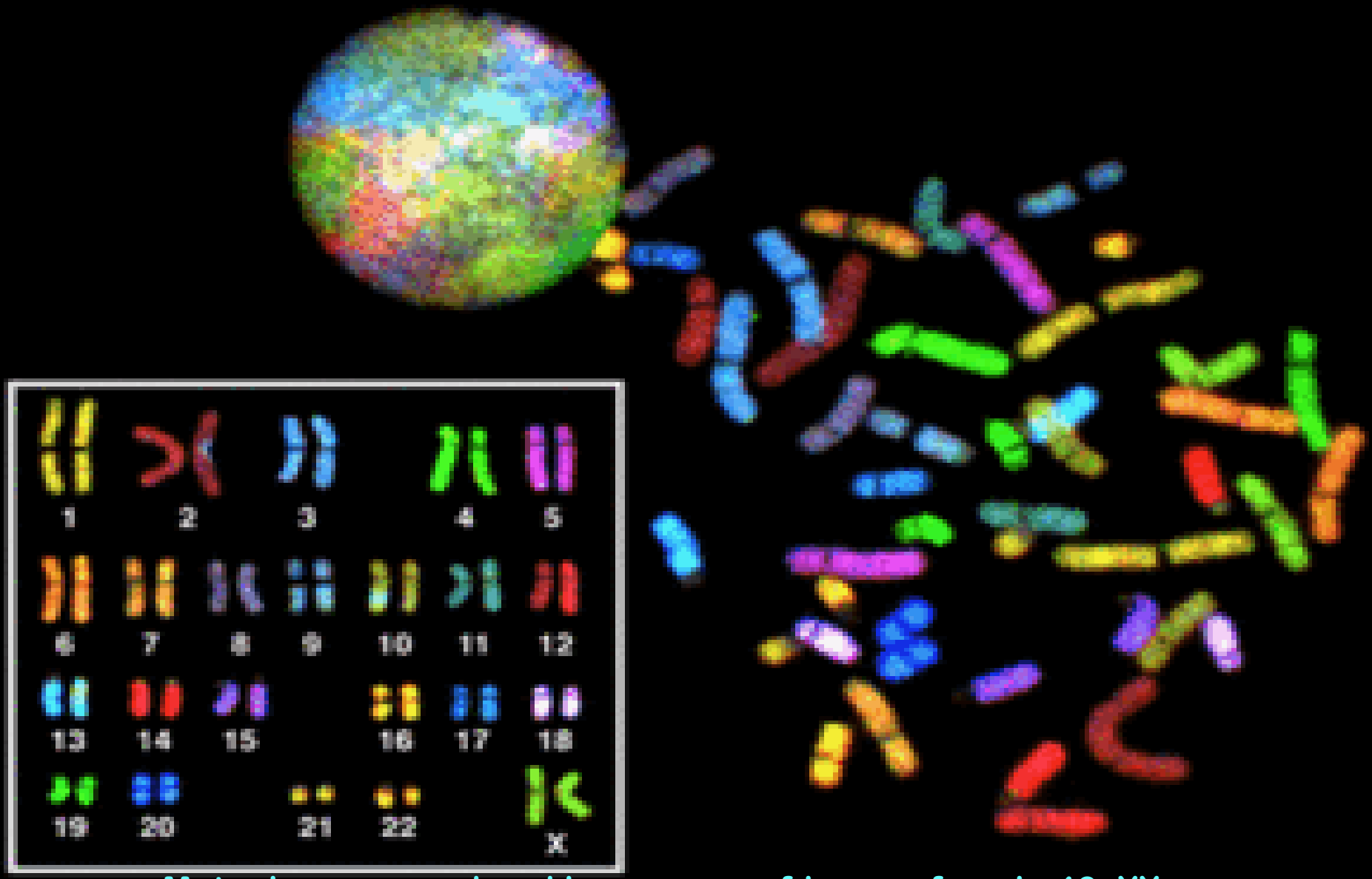
Karyogram of human male 46, XY




The rate of spontaneous aneuploidy in human somatic cells is estimated of between 0.1% and 0.8%

# Chromosomal instability (CIN)

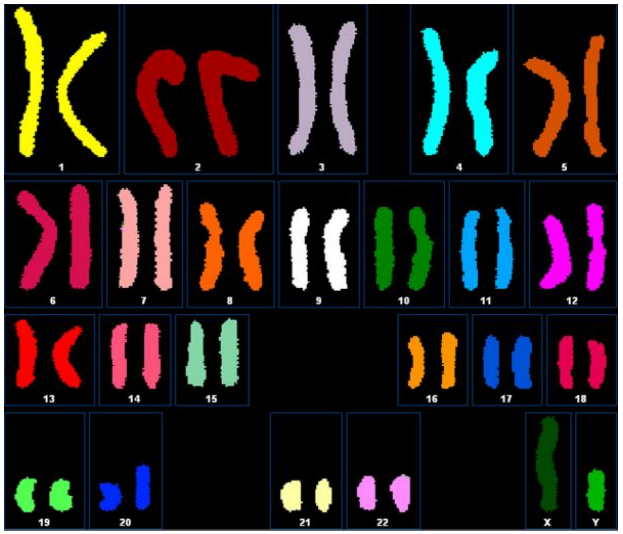
## Numerical instability




Metaphase spread and karyogram of human female 46, XX

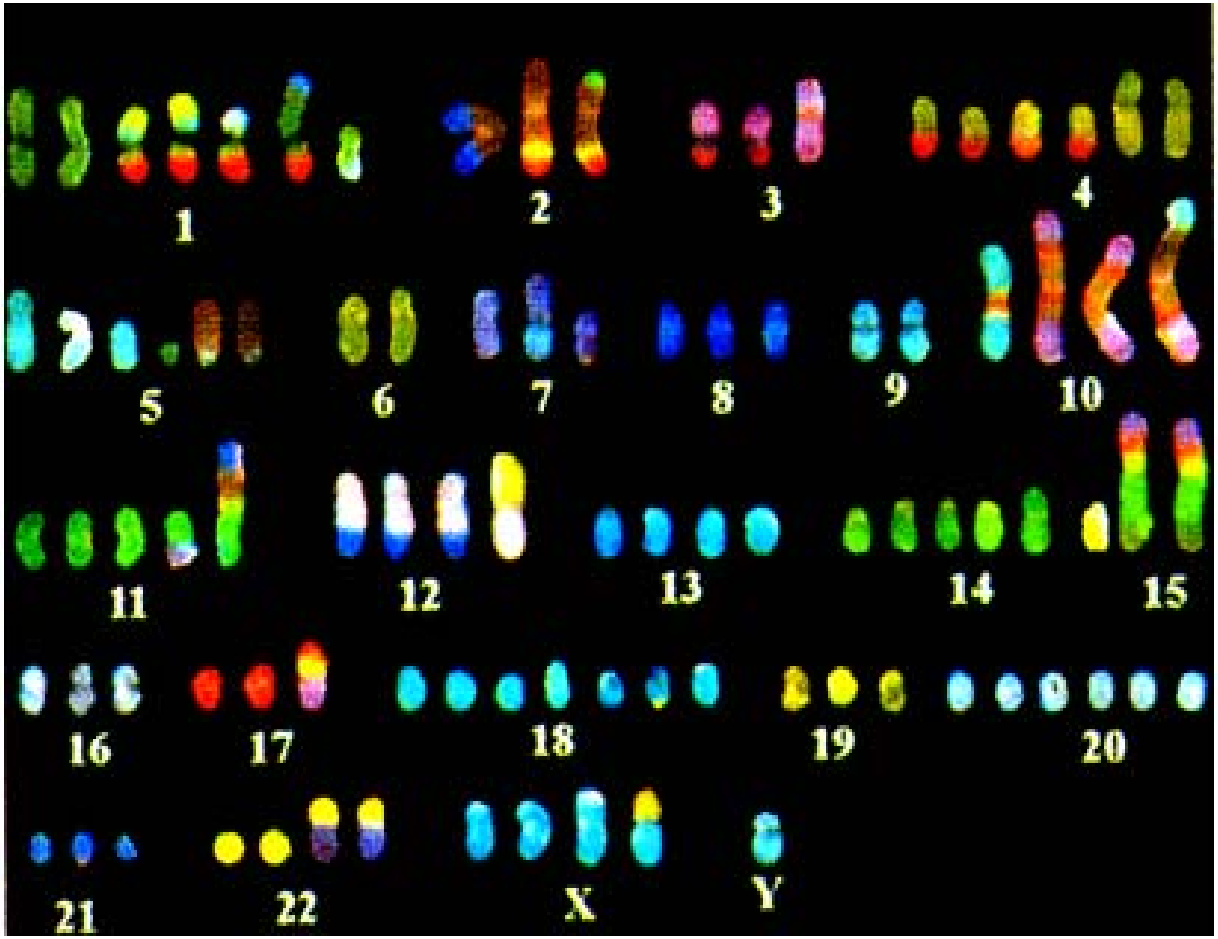
# Chromosomal instability (CIN)

## Numerical instability



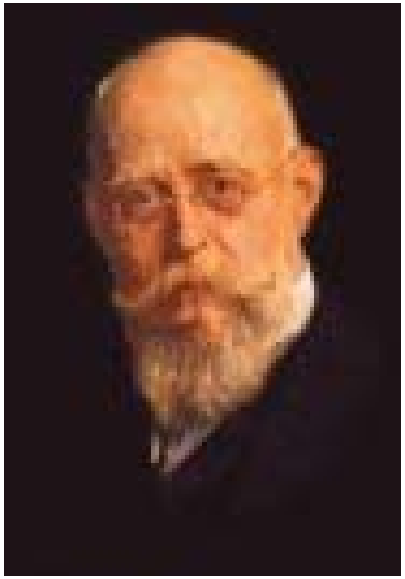
normal cell
2n=46

tumor cell
2n=94



# Chromosomal instability (CIN)

David Paul von Hansemann



von Hansemann, D. Ueber Asymmetrische Zelltheilung in epithel Krebsen und deren biologische Bedeutung. Virchow's Arch. Path. Anat. 119, 299 (1890)

described in detail the aberrant mitotic figures of different carcinoma samples (multipolar mitoses, asymmetric anaphase figures)

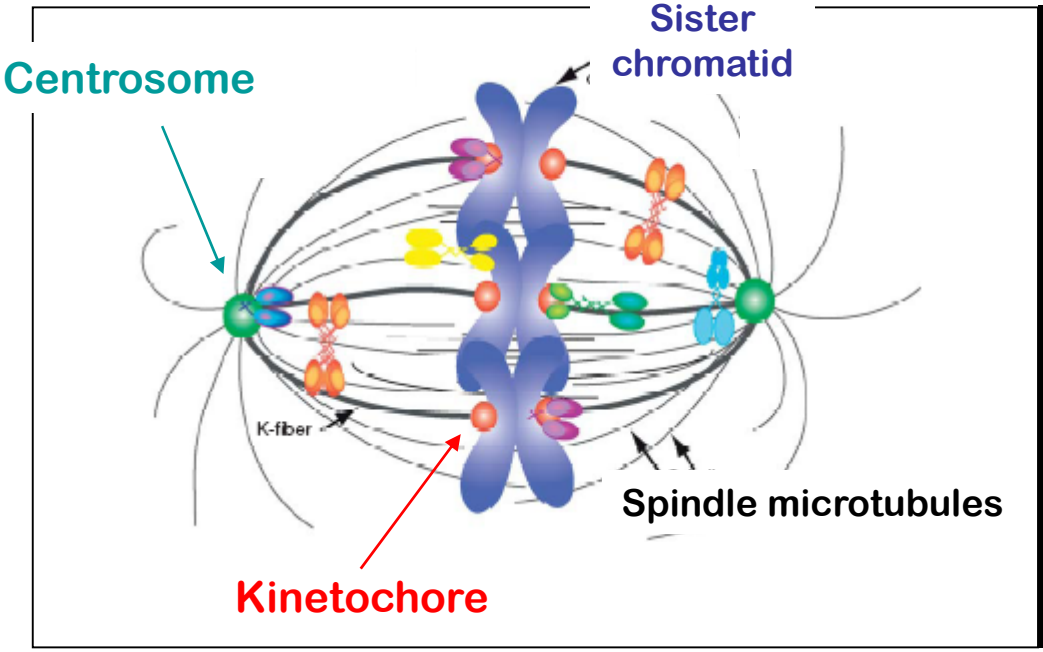
Theodor Boveri

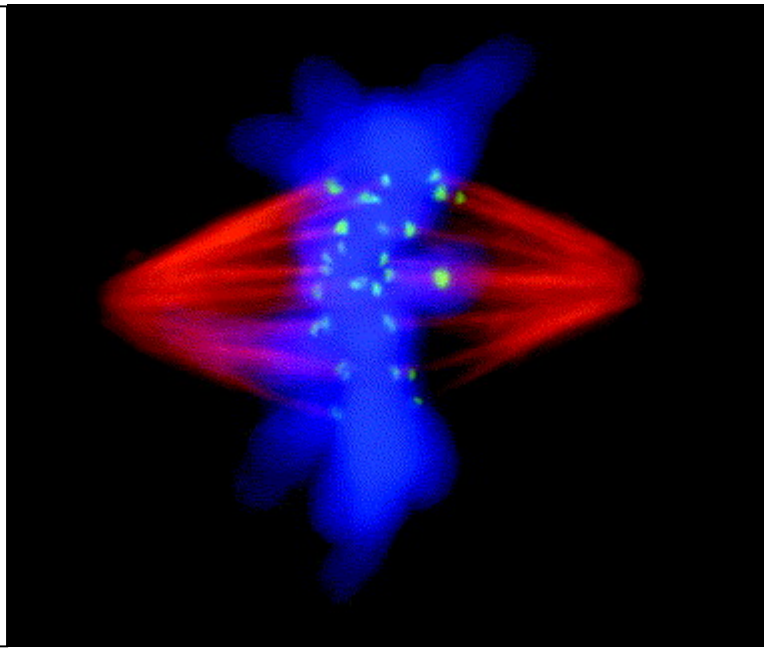
Boveri, T. Zur Frage der Entstehung Maligner Tumoren (Gustav Fisher, Jena, 1914)

postulated the association between aberrant mitoses and malignant tumours

# Chromosomal instability (CIN)
## Mitotic spindle

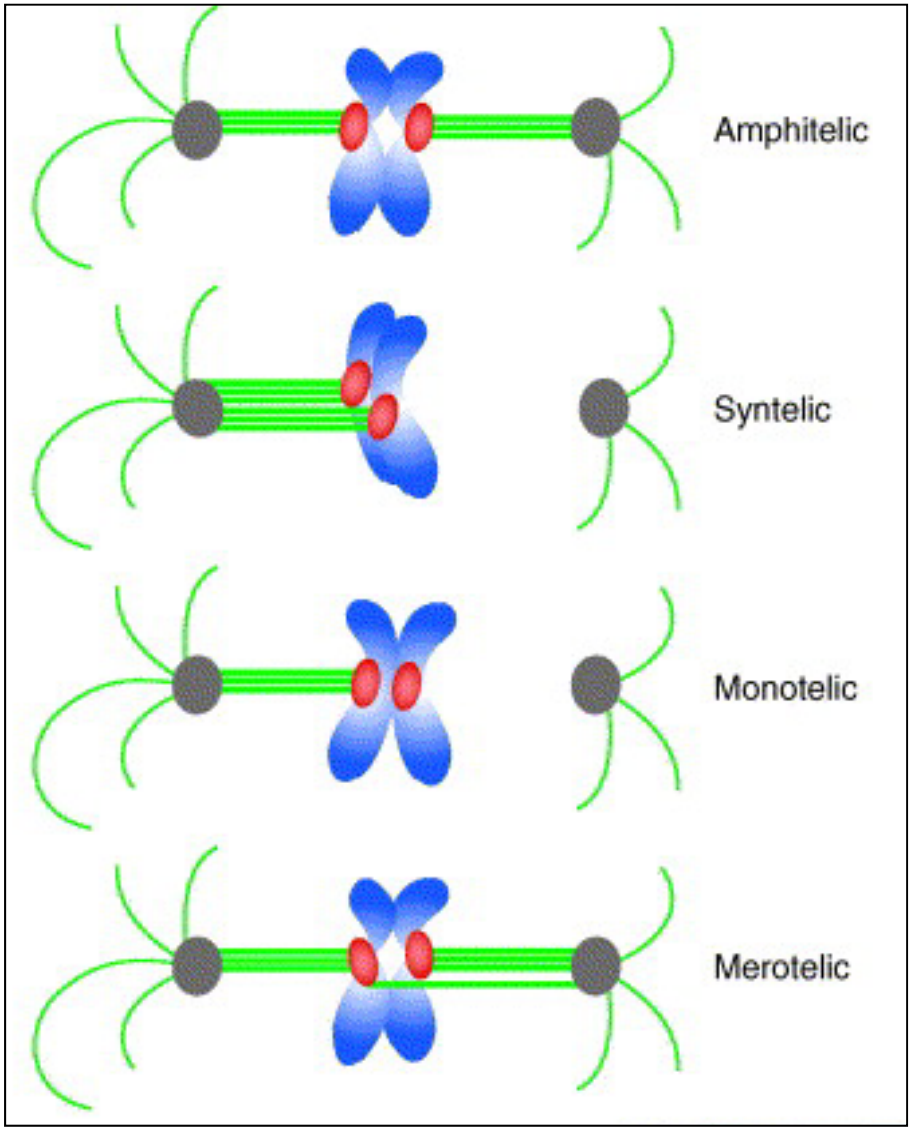
### The key components of the mitotic spindle



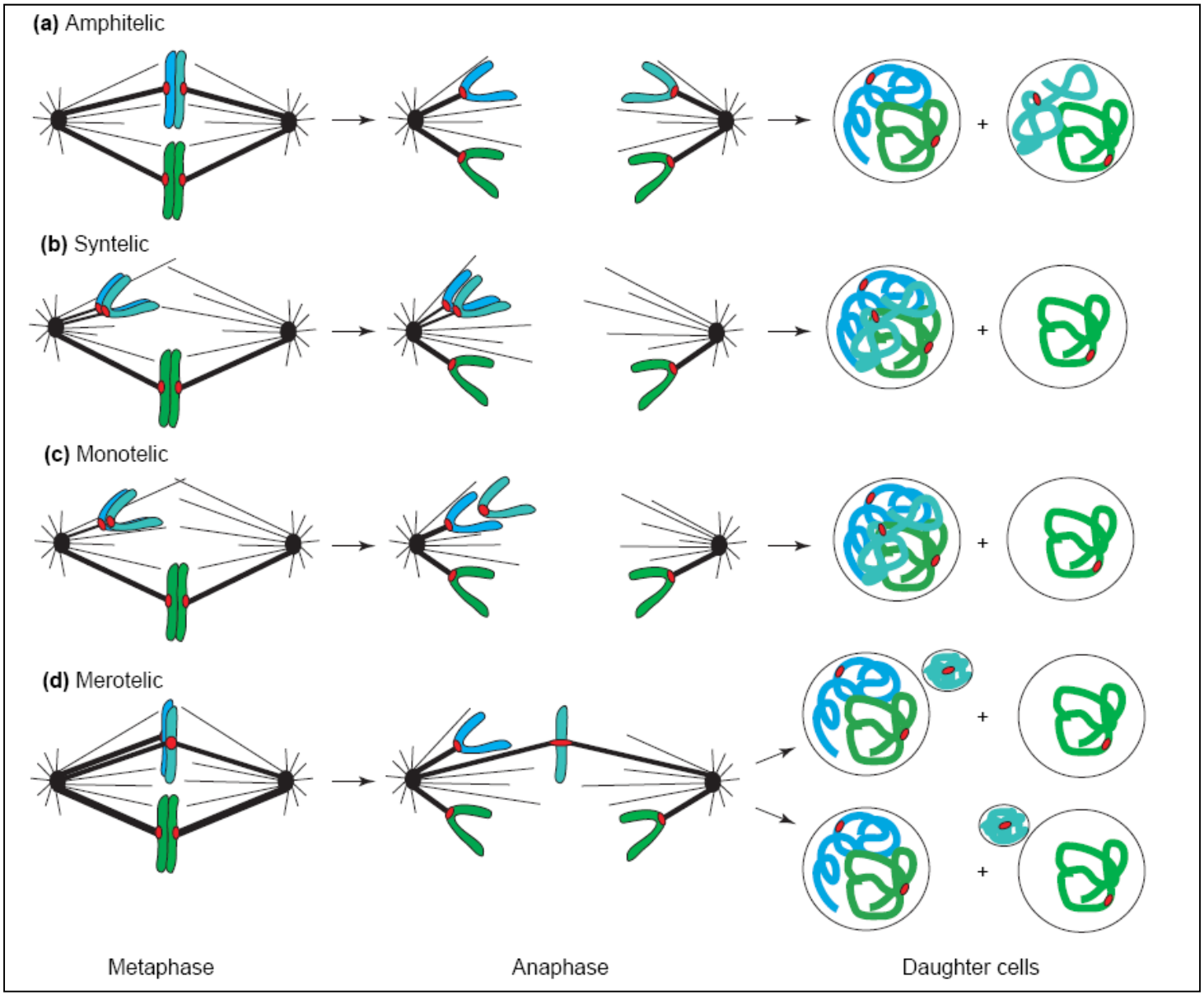
# Chromosomal instability (CIN)

## Types of kinetochore-microtubule attachments



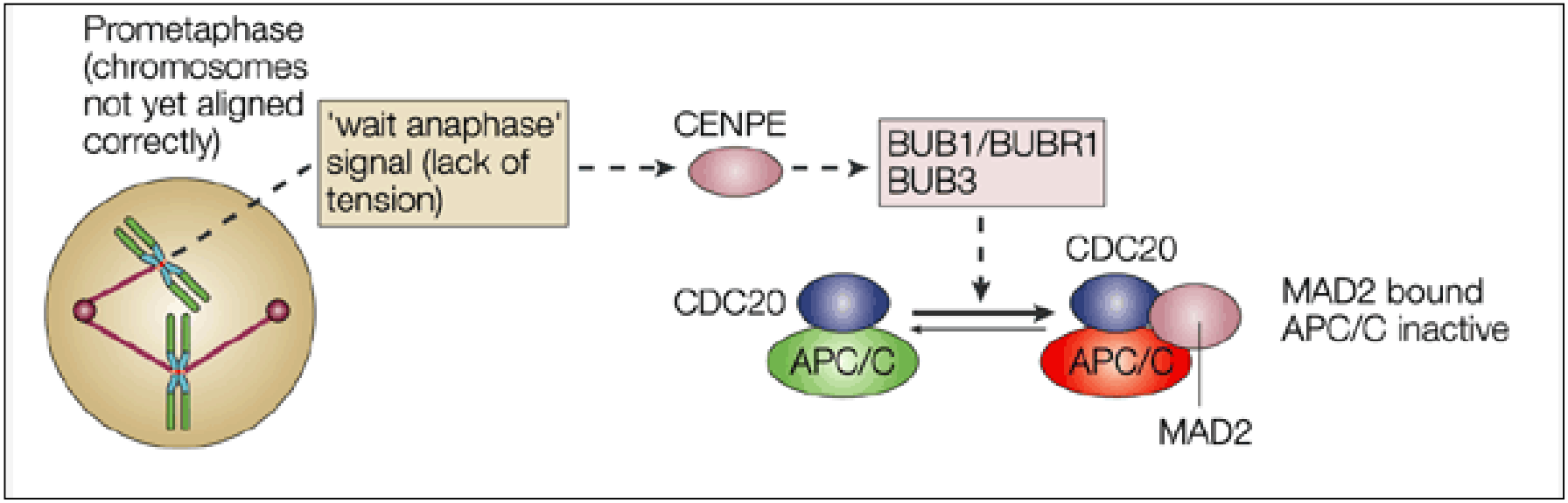

**Vertebrate cell kinetochores have multiple microtubule-attachment sites (~20)**

# Chromosome segregation produced by different kinetochore-microtubule attachments



# Chromosomal instability (CIN)
## Numerical instability

### The mitotic spindle checkpoint regulates the metaphase-to-anaphase transition

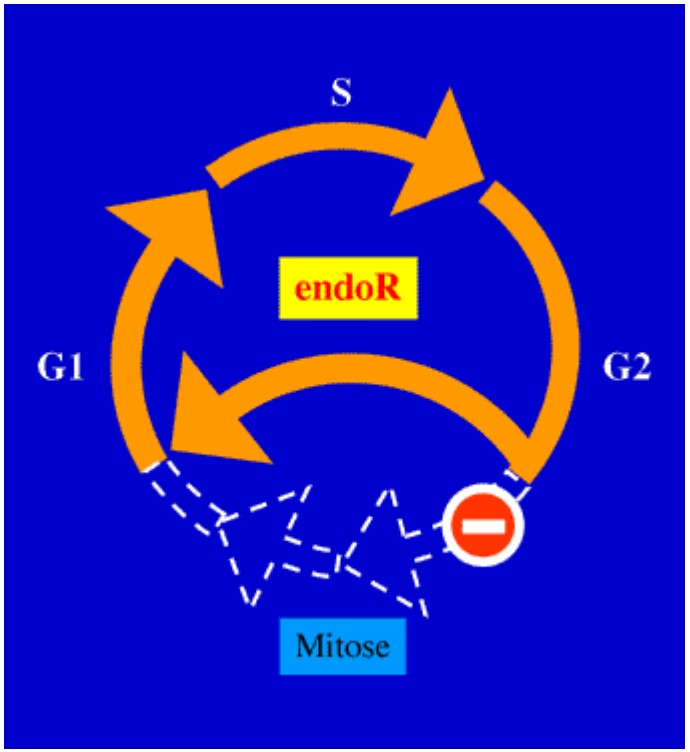


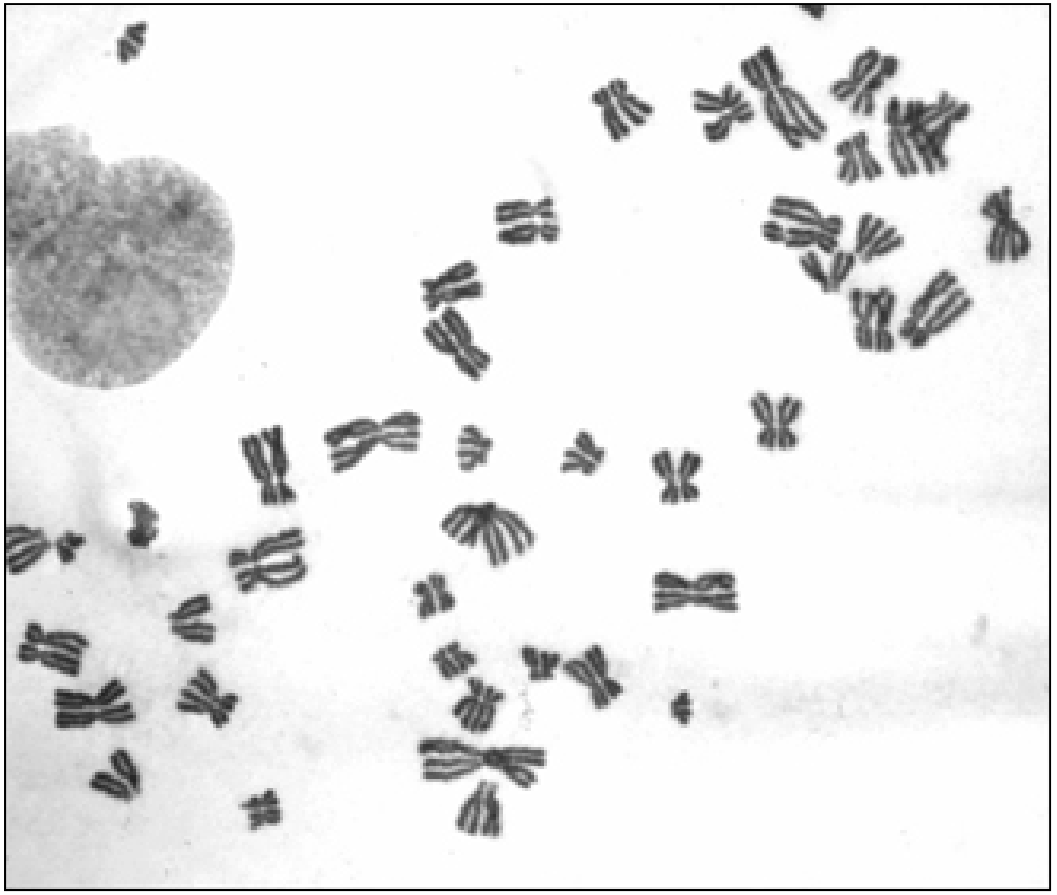

Unattached chromosomes generate a signal that delays progress to anaphase until all sister chromatids are attached to the spindle apparatus. This signal is transduced by a relay of spindle-checkpoint proteins that include CENPE and the MAD/BUB proteins. This ultimately results in inhibition of the anaphase-promoting complex/cyclosome (APC/C).

# Chromosomal instability (CIN)

## Numerical instability

**DNA endoreduplication** → **polyploidy**

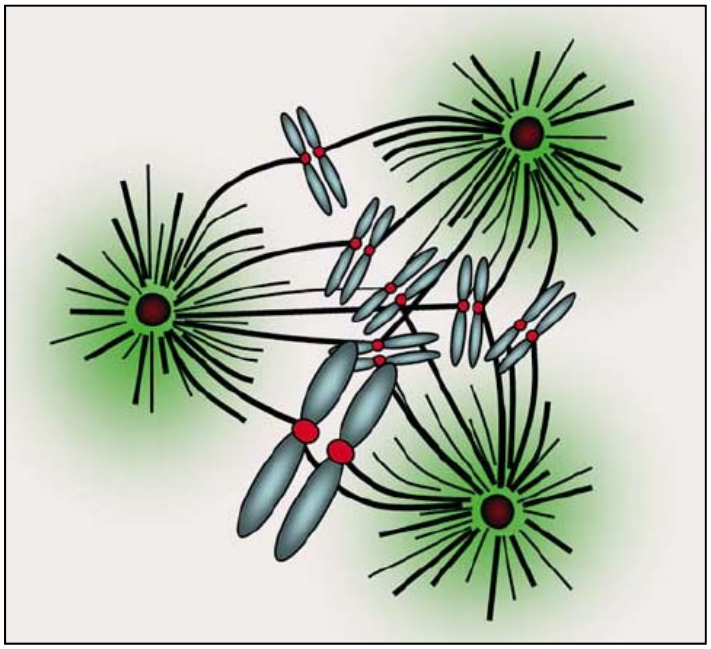


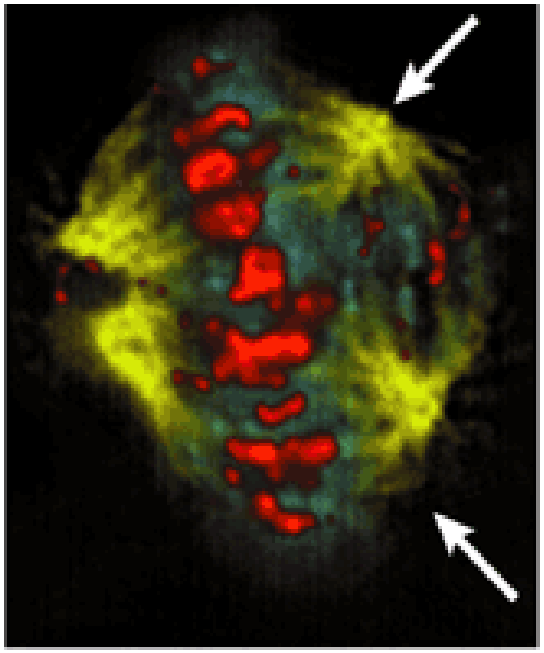



**Defects in chromosome segregation and cytokinetic proteins**

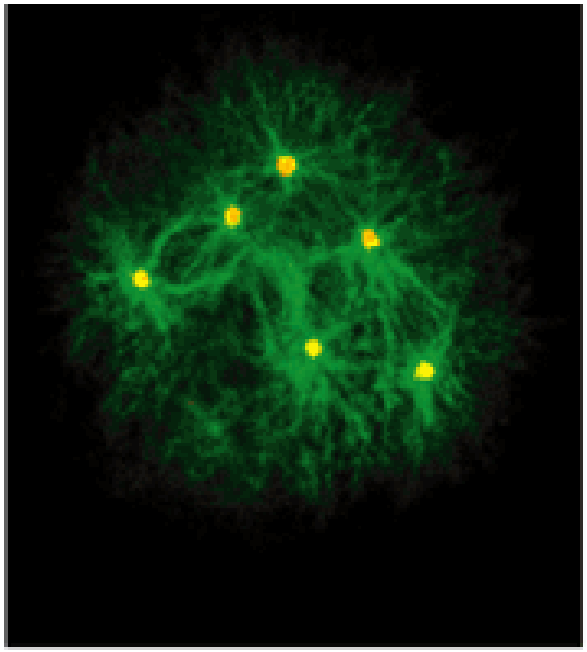
# Chromosomal instability (CIN)
## Numerical instability

**Centrosomes** act as poles of the mitotic spindle apparatus to regulate its assembly and function. Defects in the number, structure, and function of centrosomes are associated with many human tumors.





tetrapolar mitosis



centrosome amplification

# Chromosomal instability (CIN)
## Numerical instability

**Numerical aberrations can be caused by:**

- kinetochore–microtubule misattachments through mitosis is a major cause of aneuploidy.
- the replication of DNA without the subsequent completion of mitosis and cell division (DNA endoreduplication)
- multipolar divisions associated with abnormal number or structure of centrosomes

# Chromosomal instability (CIN)

## Numerical instability

- More than 100 genes can cause chromosomal instability (CIN). These include genes that are involved in spindle assembly and dynamics, cell-cycle regulation and mitotic checkpoint control. Cancer cells have numerous defects in their genetic stability mechanisms.

# Chromosomal instability (CIN)

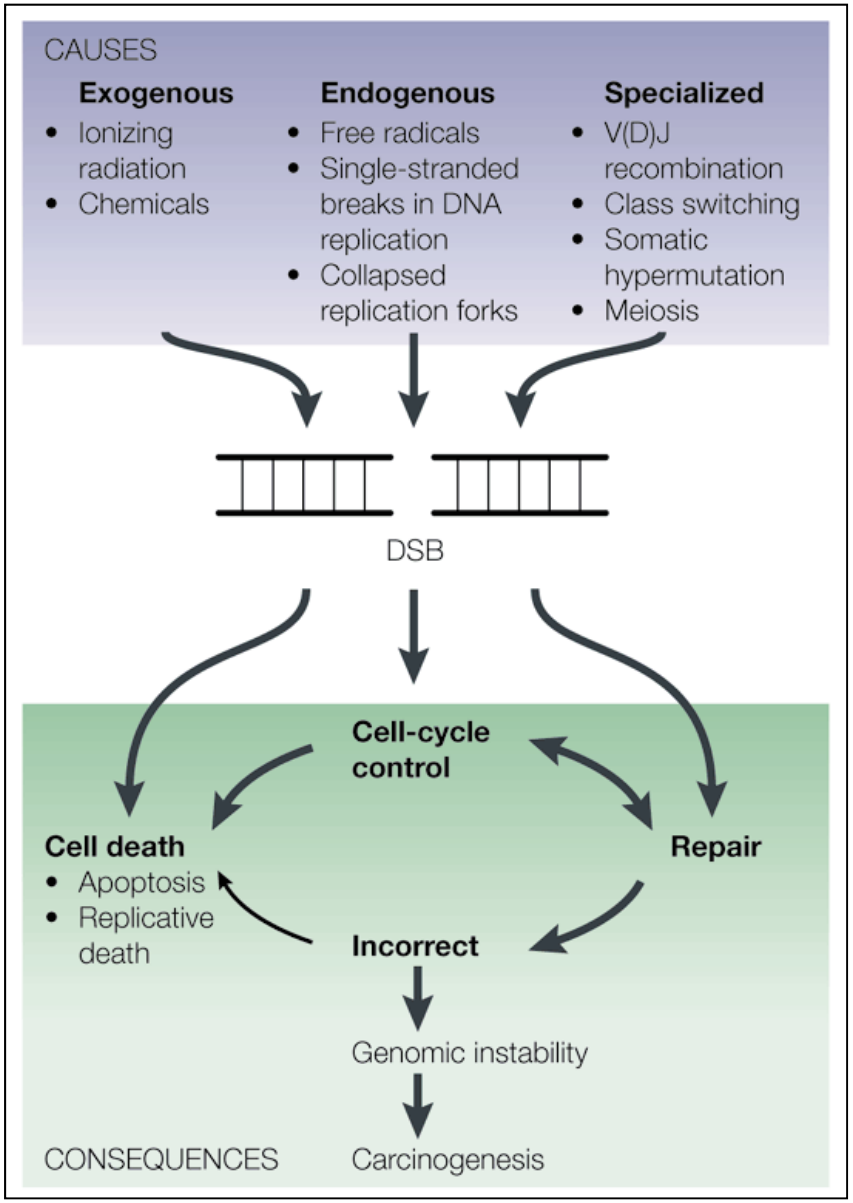
## Structural instability

- Structural chromosomal instability refers to events that involve changes in the genetic linkage of two DNA fragments.

- Structural chromosomal instability is generated by double-strand DNA breaks (DBS).

# Chromosomal instability (CIN)

## Structural instability

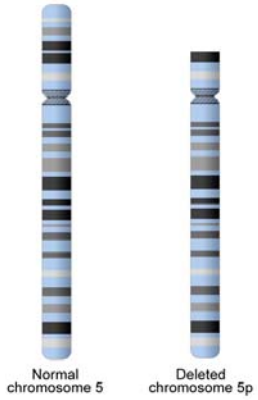



**Inaccurate repair can lead to mutations and/or chromosomal aberrations (genome instability) that can contribute to carcinogenesis**

# Chromosomal instability (CIN)

## Types of chromosomal rearrangements in tumor cells

### Deletions



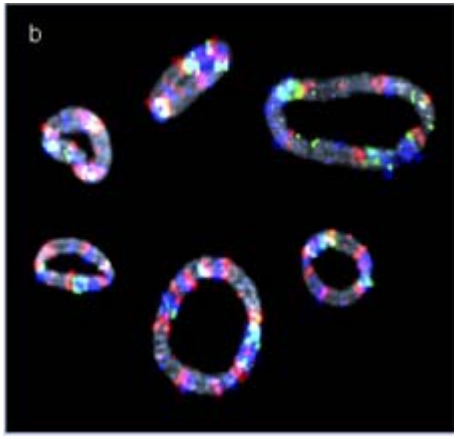

### Translocations

### Insertions

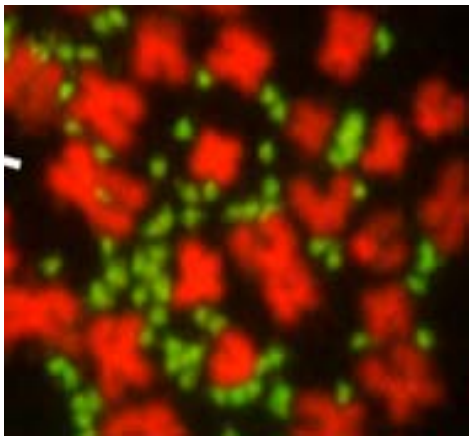
### Inversions

### Duplications

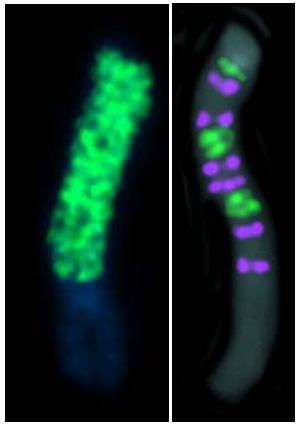
### Ring chromosomes




### Double minutes - DM

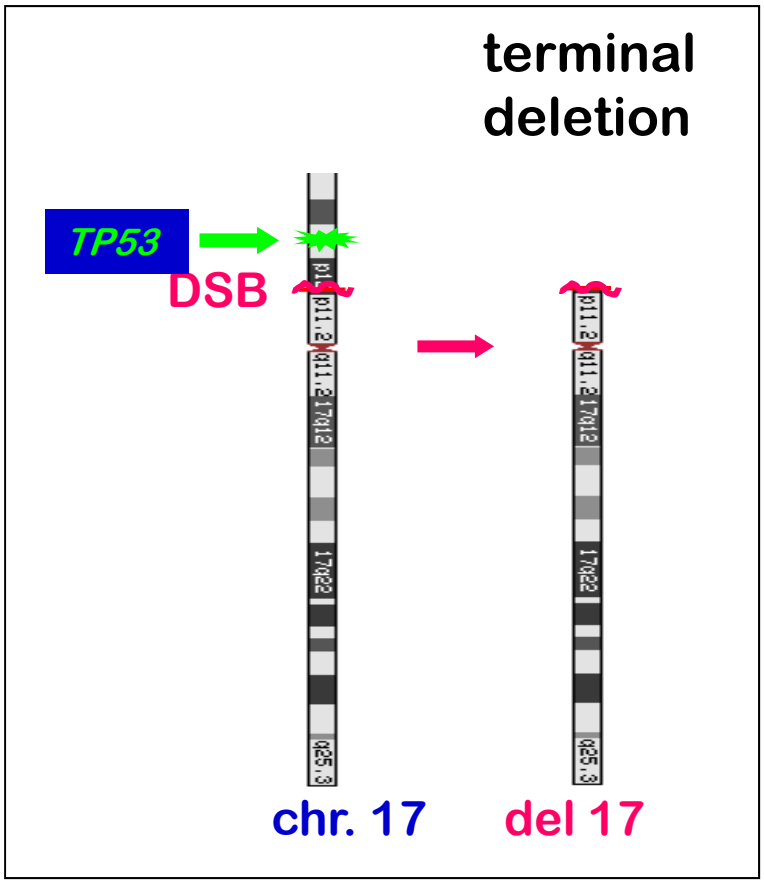


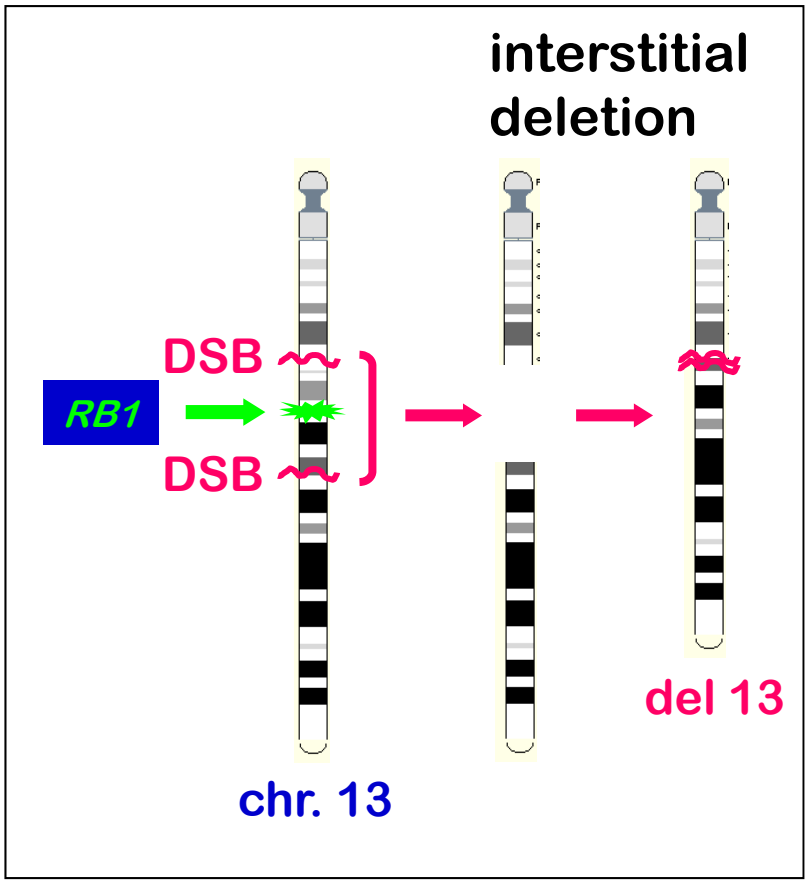
### Homogeniously stained regions - HSR



# Chromosomal instability (CIN)

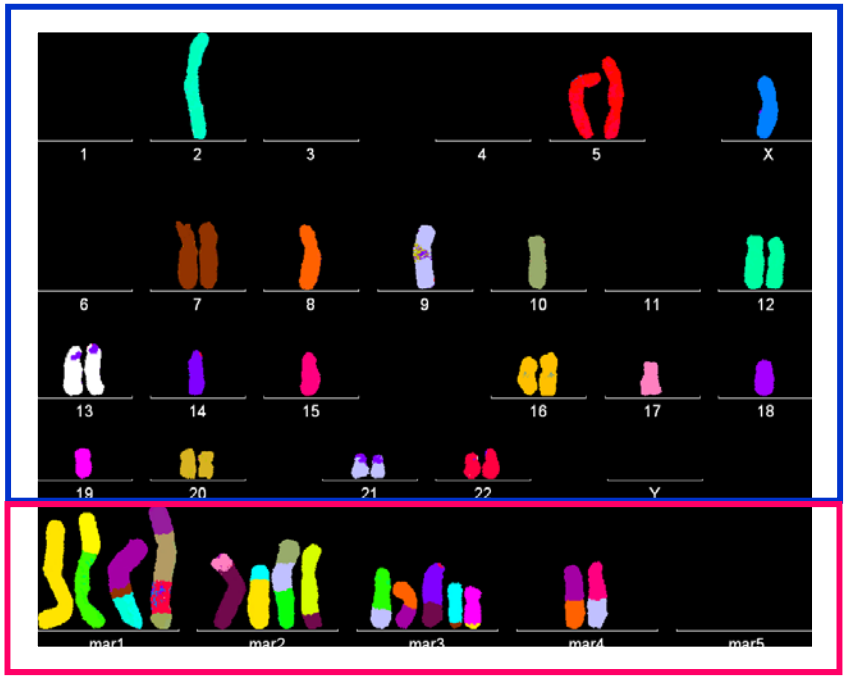
## Deletions of tumor suppressor genes



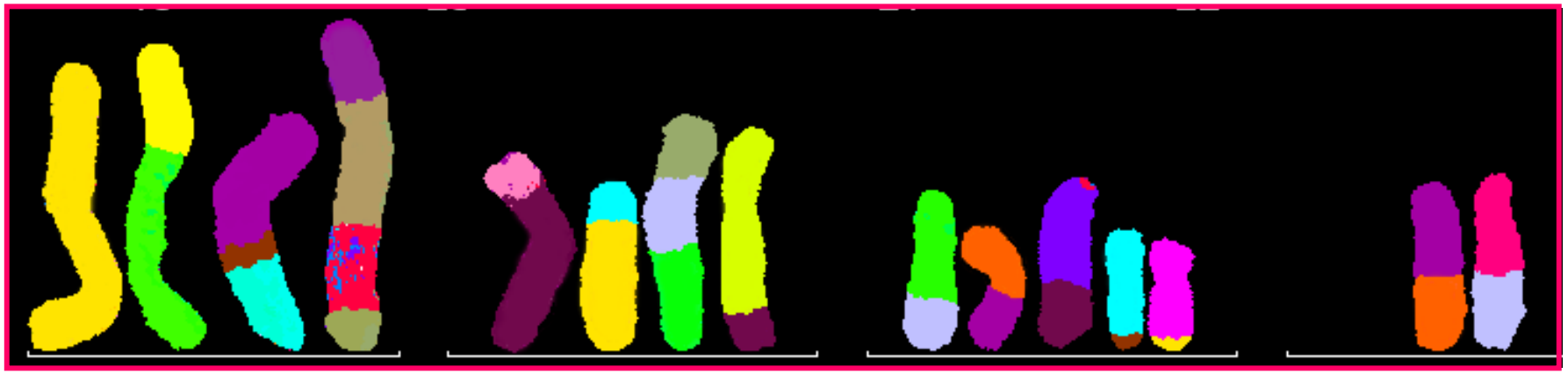

Allelic deletions of tumor suppressor genes have been observed frequently in a variety of human tumors. These losses contribute to the development of human cancer. Two of the most frequently deleted chromosomal loci contain the tumor suppressor genes TP53 and retinoblastoma (RB1).

# Chromosomal instability (CIN)
## Translocations




Normal chromosomes

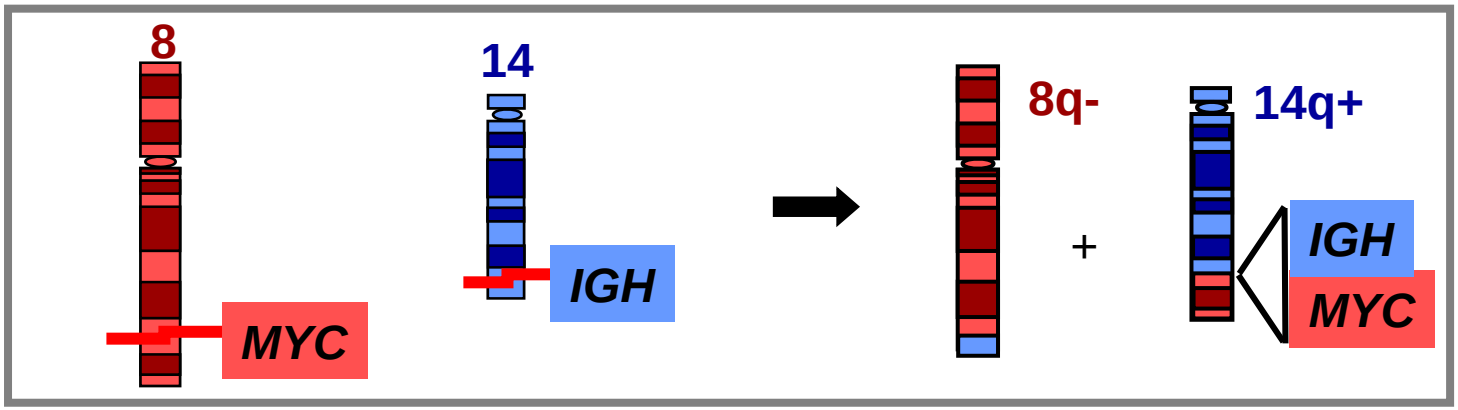
Marker chromosomes

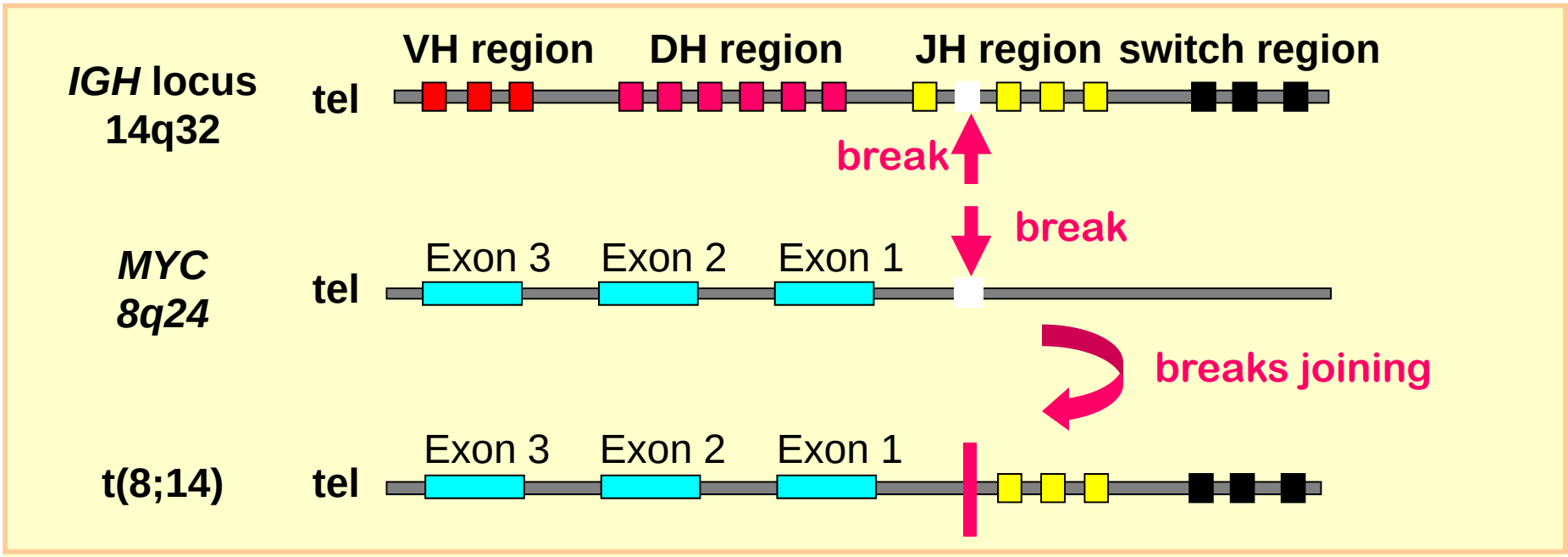


# Chromosomal instability (CIN)

## Translocation leads to the overexpression of oncogene

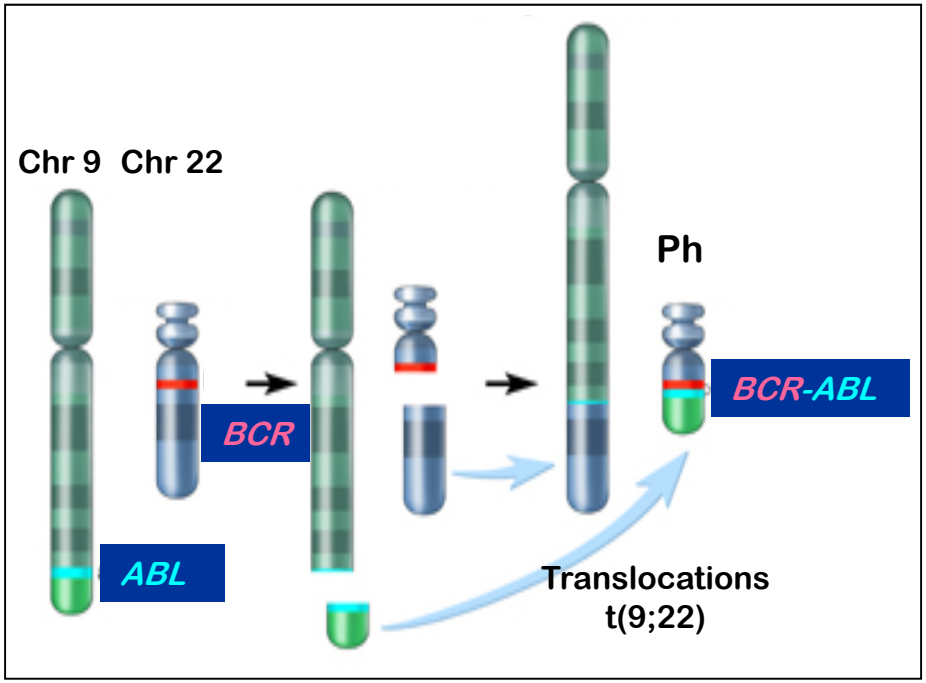
**Up-regulation of *MYC* expression in Burkitt lymphoma**

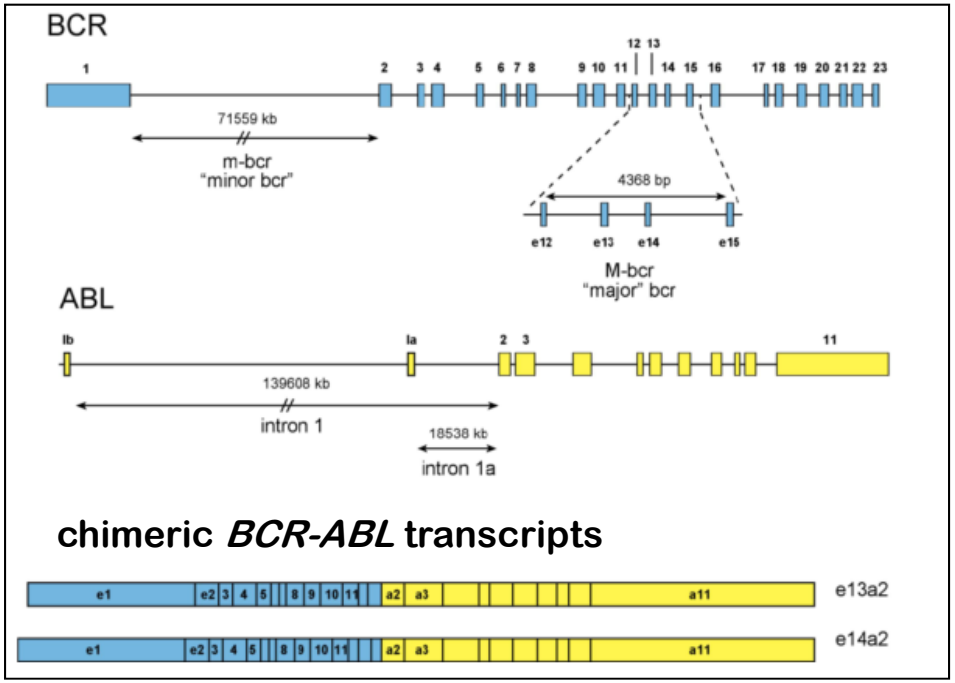


# Chromosomal instability (CIN)
## Translocations produce new functional genes

**Chronic myeloid leukemia (CML) is characterised by the occurrence of the Philadelphia (Ph) chromosome in about 95% of CML patients**

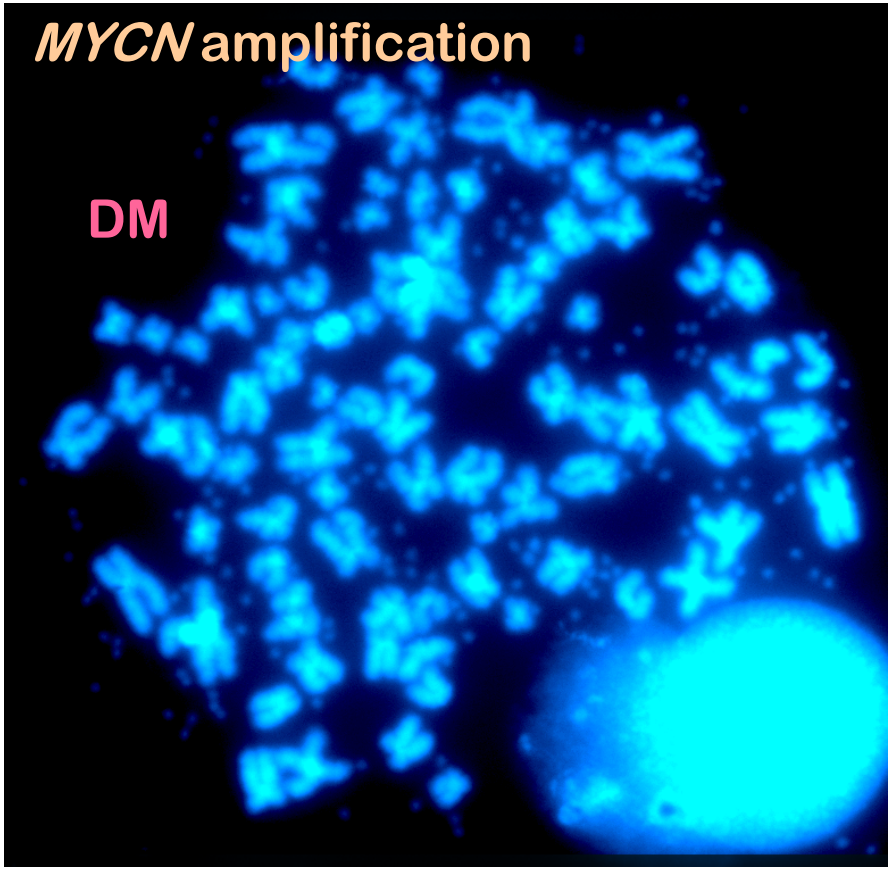


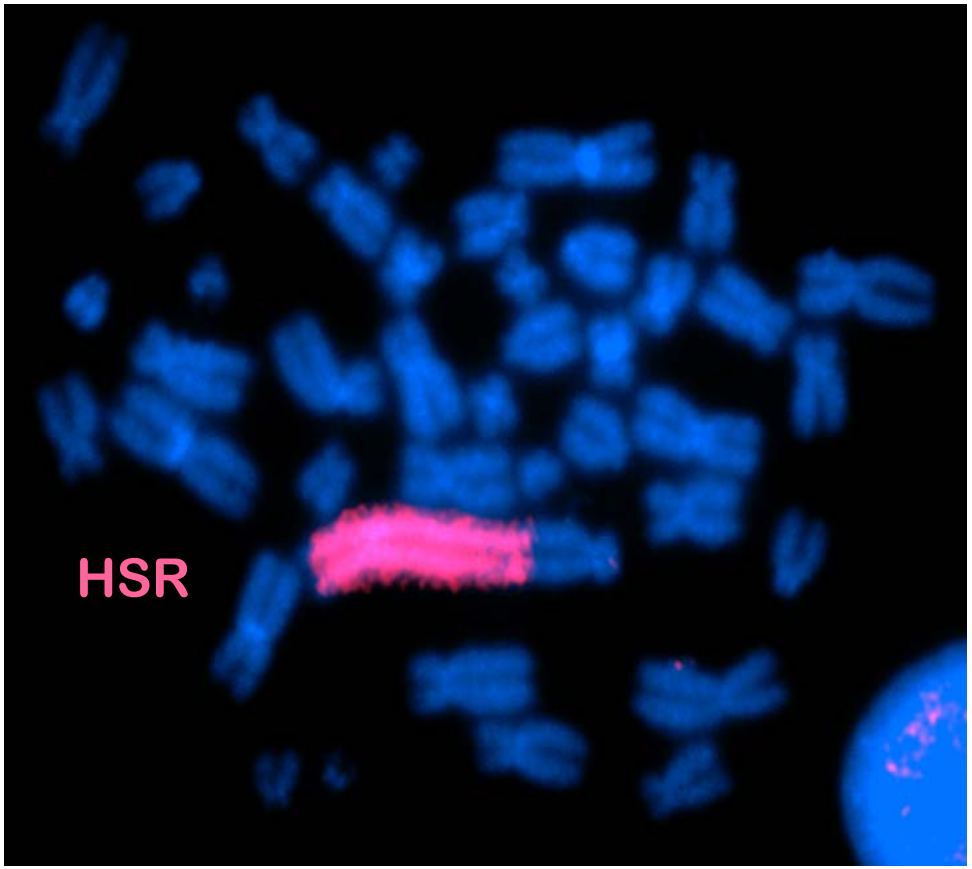



# Chromosomal instability (CIN)

## Oncogene amplification in tumor cells

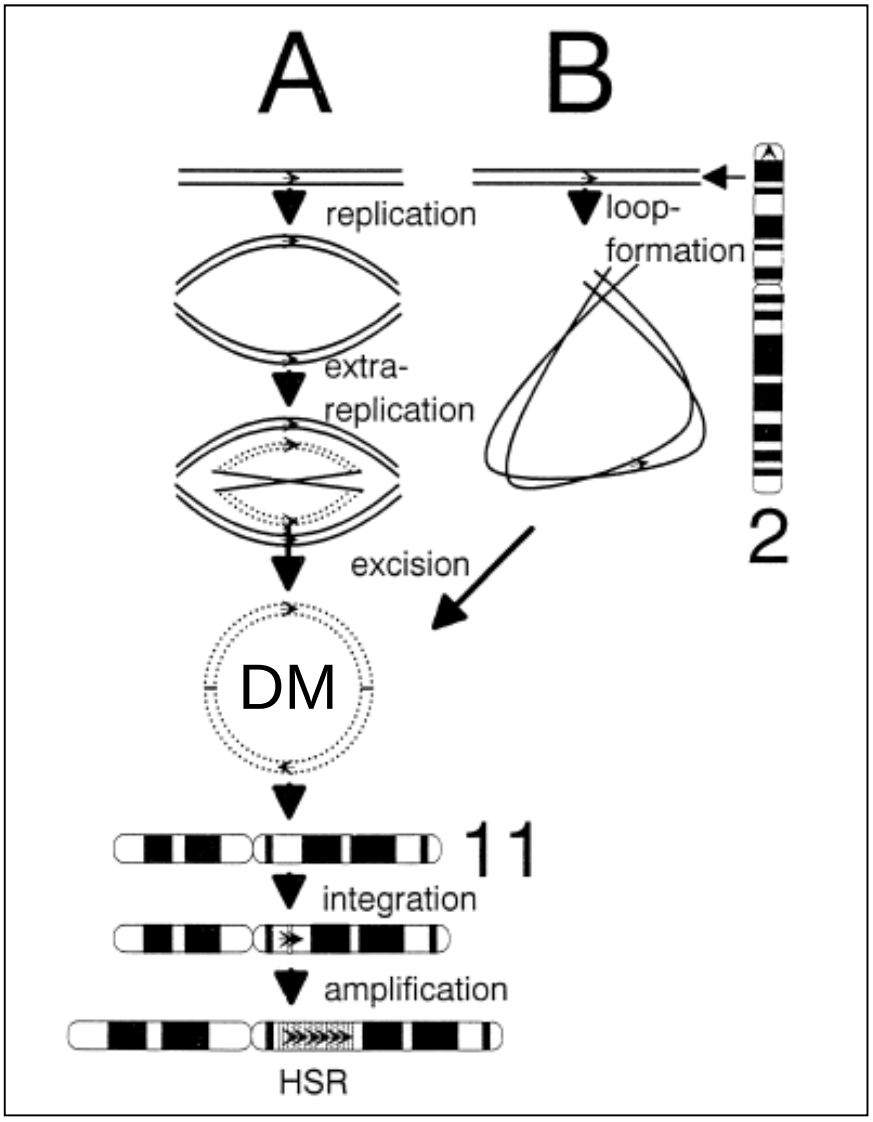
**Gene amplification leads to increased gene expression through alteration of gene copy number.**



# Chromosomal instability (CIN)

## Model for oncogene amplification in tumor cells




**A replication-exision model proposes the local extrareplication of DNA with following exision from the replication structure.**

# Chromosomal instability (CIN)

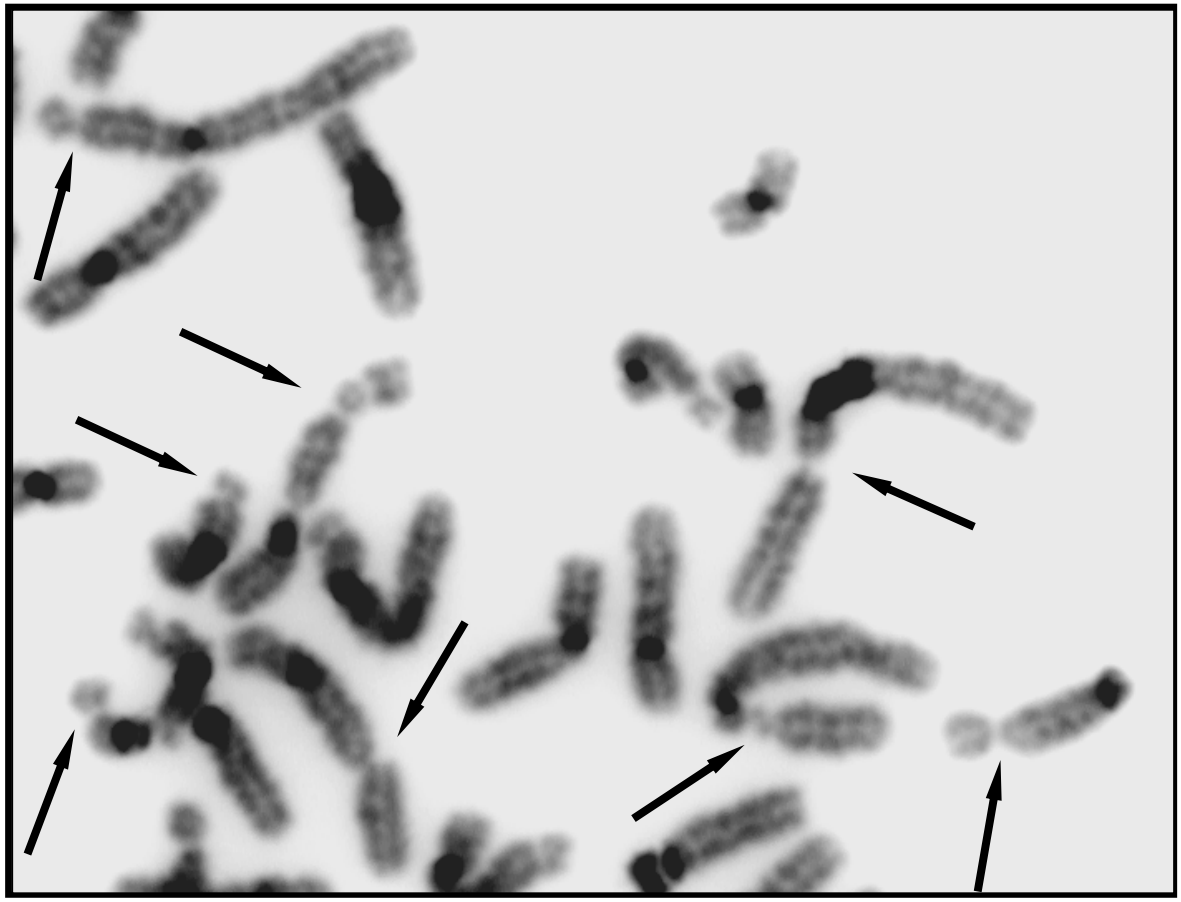
**The cause and mechanism of the majority of SCR in cancer cells is unknown**

| | |
|---|---|
| Specific translocations in lymphoma and leukemia cells | → False recognized sequences by *RAG1/RAG2* recombinase (VDJ recombination) |
| Highly transcribed genes | → Defects of transcription-coupled repair (TCR) |
| Fragile sites | → Defects of DNA replication process |

# Chromosomal instability (CIN)

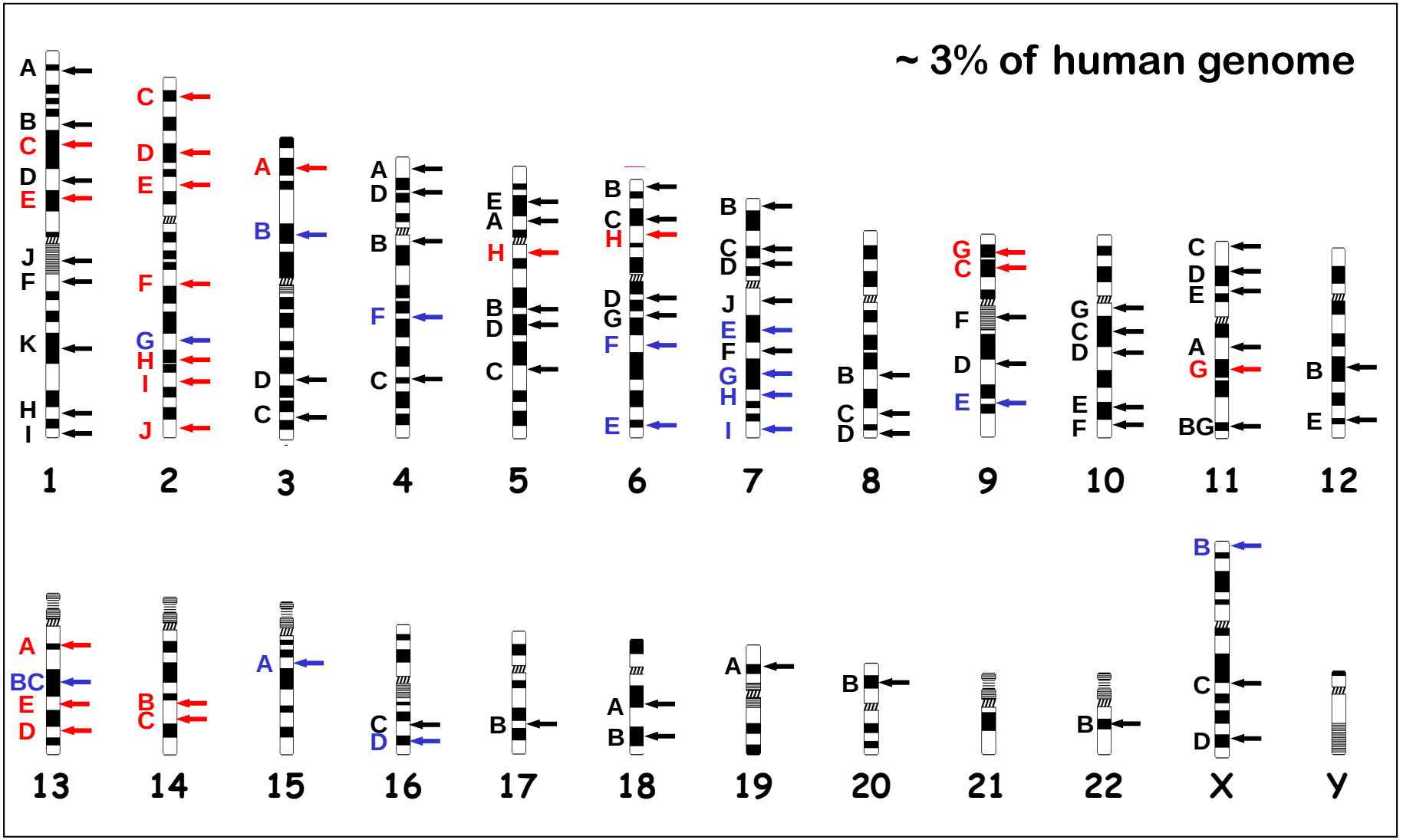
## Common fragile sites

Fragile sites are specific loci that preferentially exhibit gaps and breaks on metaphase chromosomes under conditions of replication stress



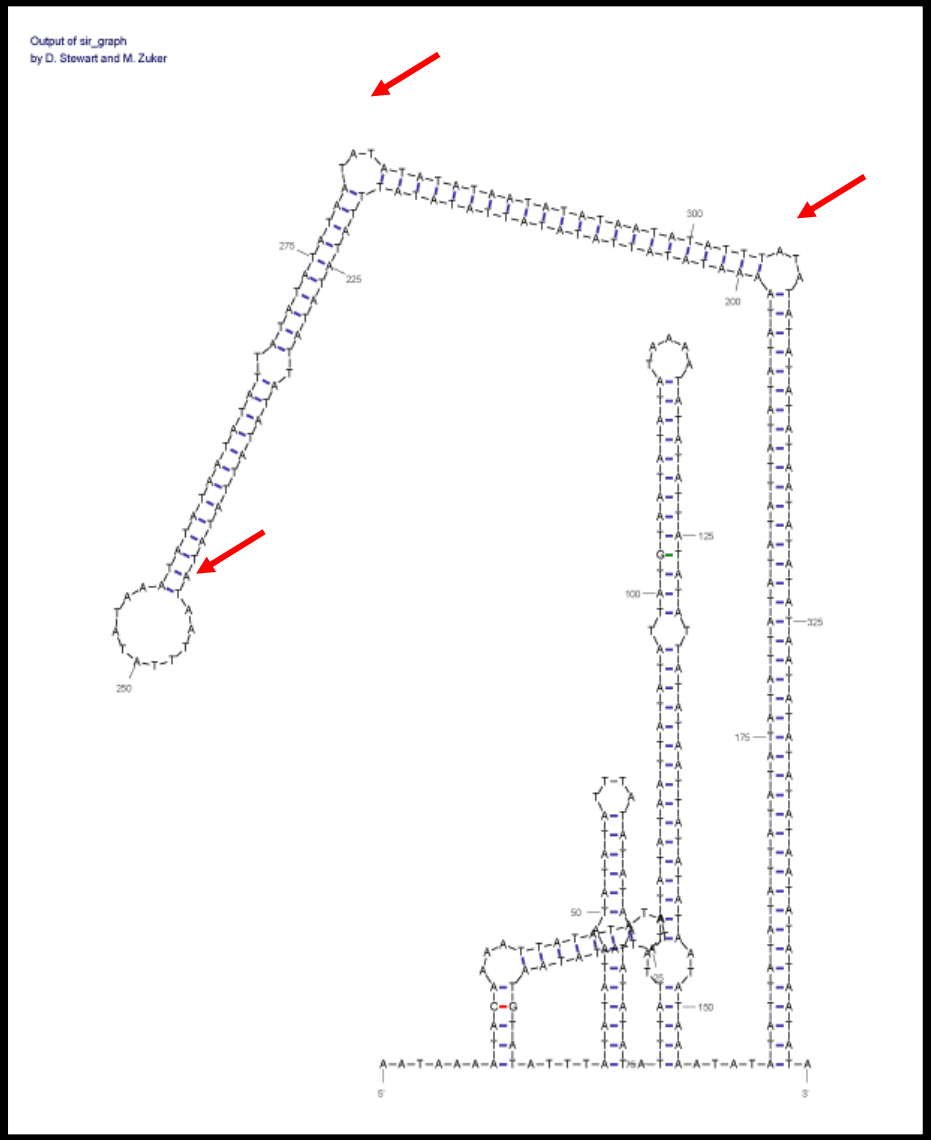
# Chromosomal instability (CIN)

## 89 common fragile sites in human genome



# Chromosomal instability (CIN)

## Common fragile sites form secondary DNA structures




Why non-B DNA structures could cause fragility?

- Strong secondary structures lead to stalling of replication forks
- Single-stranded DNA is vulnerable to endonucleases

# Chromosomal instability (CIN)
## Common fragile sites genes *FHIT* and *WWOX*

***FRA3B* – *FHIT*** (fragile histidine triad)

**genomic sequence 1.5 Mb;  transcript 1.1kb**

**-heterozygous and homozygous deletions in adenocarcinomas, gastric cancers, head and neck squamous cell carcinomas, lung cancers and B-cell lymphomas**

**-rare translocations in hepatocellular, esophageal and breast carcinomas**

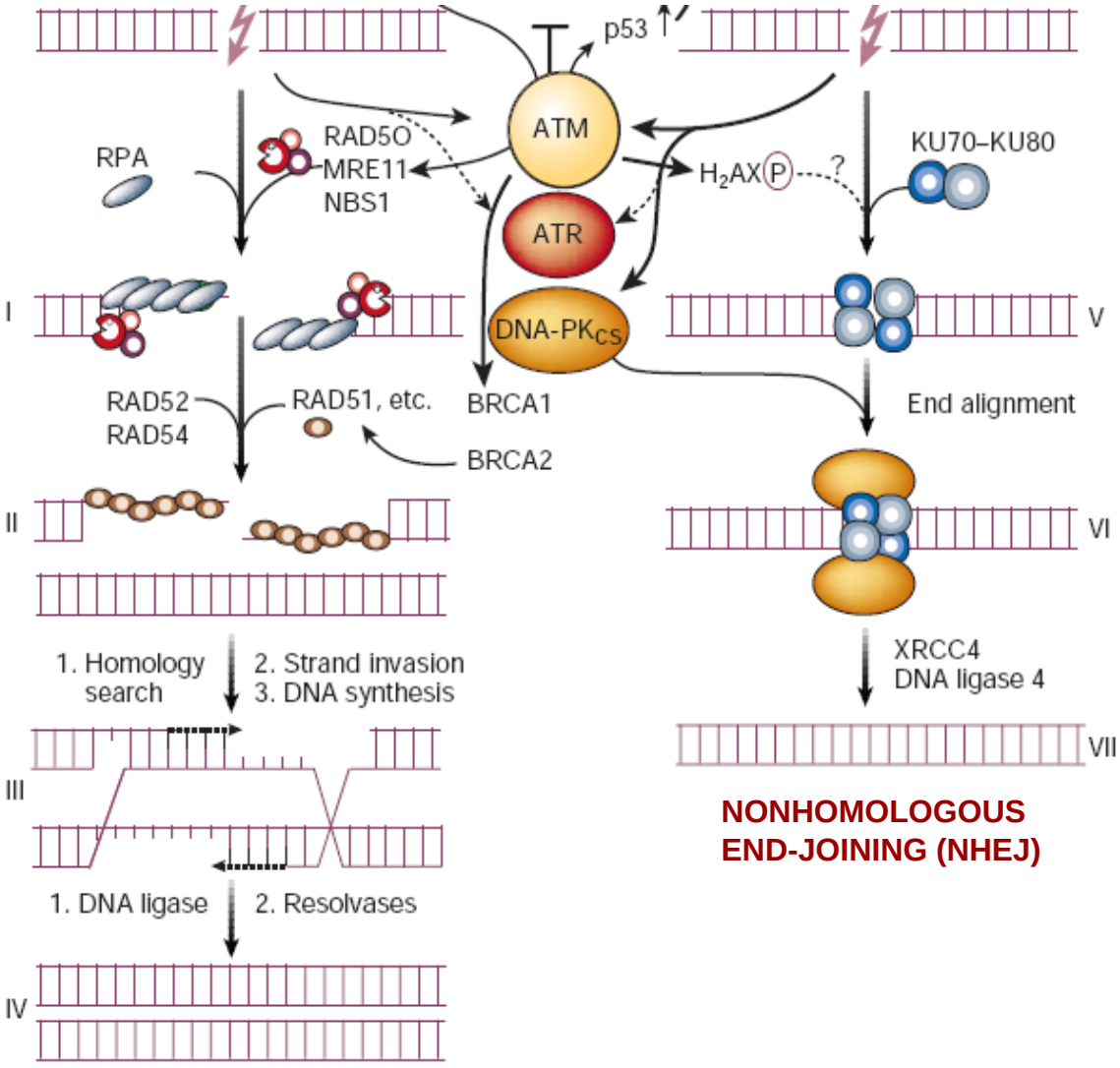
***FRA16D - WWOX*** (WW domain-containing oxidoreductase)

**genomic sequence 1.3 Mb;  transcript 1.1kb**

**-heterozygous and homozygous deletions in breast, prostate, lung, stomach, ovary, colon and pancreatic carcinomas**

**-translocations in 25% of all multiple myelomas**

# Chromosomal instability (CIN)
## Double Strand Break (DSB) Repair




**DSB repair genes associated with cancer:**

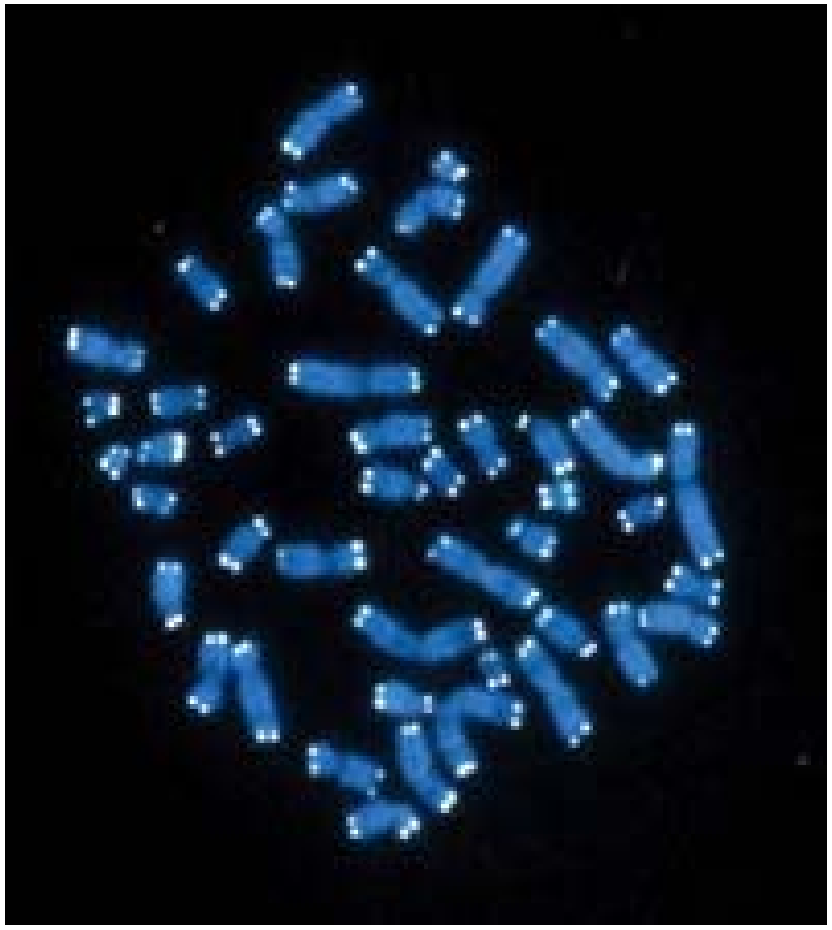
*ATM*
*ATR*
*MRE11*
*NBS1*
*BRCA1*
*BRCA2*
ReqQ-like helicases
DNA Ligase IV

**Structural chromosomal aberrations**

# Chromosomal instability (CIN)

## Telomere dysfunction

All eukaryotic chromosomes are capped by telomeres, structures composed of DNA and associated proteins comprising the ends of each linear chromosome



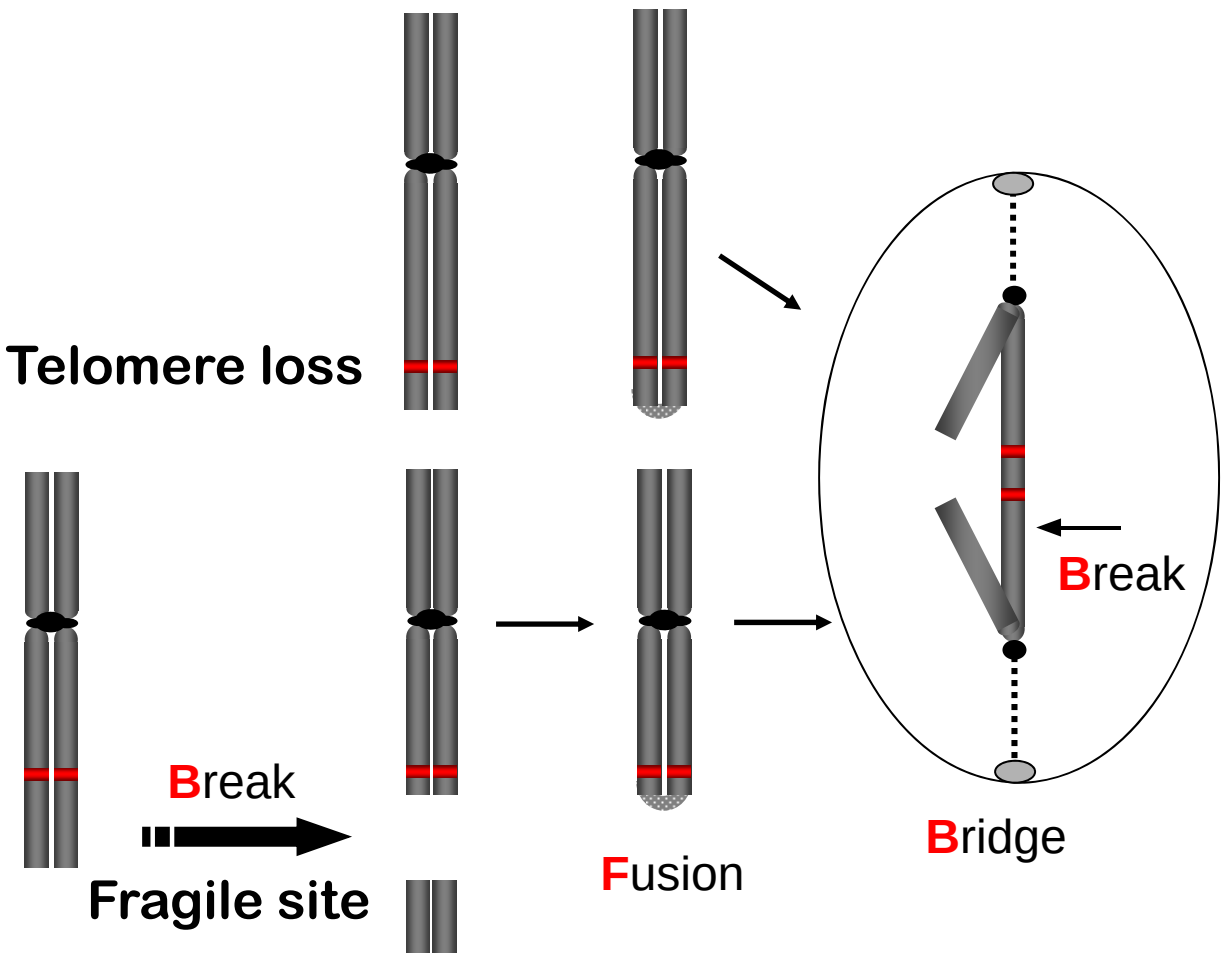
Ends of linear chromosomes composed of a (TTAGGG) repeat

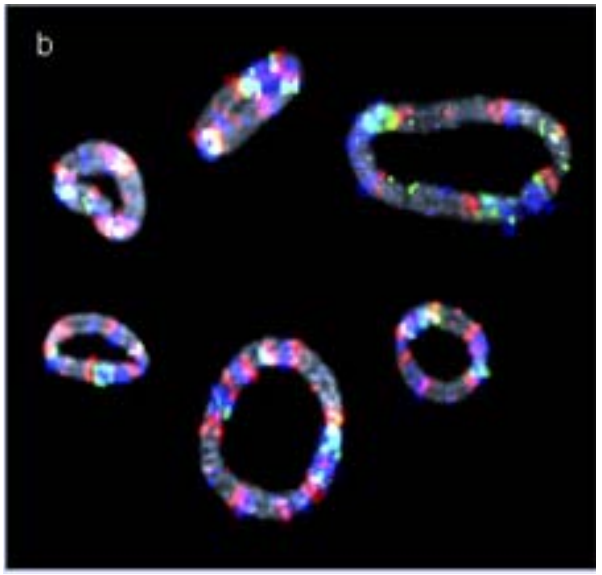
Hexamer unit present in as many as 2,000 copies (up to 15 kb of DNA)

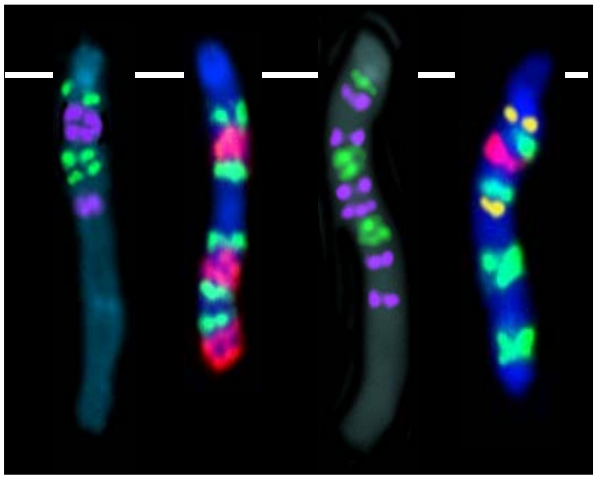
50-150 bp of terminal DNA lost with each passage through the cell cycle

Telomeres protect chromosome ends from fusion, and loss of telomeres can lead to genomic instability and tumour formation

# Chromosomal instability (CIN)
## Breakage-Fusion-Bridge Cycles



# Chromosomal instability (CIN)

## Structural instability

- An elevated frequency of structural chromosome aberrations could be directly caused by an abnormally high incidence of DNA double-strand breaks
- Increases in HR-mediated events or end-joining between non-homologous DNA fragments can result in gross chromosomal rearrangements (GCRs) such as translocations, inversions, deletions or amplifications.
- Despite the broad spectrum of proteins and breakpoints that are associated with rearrangements, the common feature is their association with replication stress. Replication failures seem to be the primary cause of cancer (fragile sites!?)

# Chromosomal instability (CIN)

## Numerical instability

Changes in chromosome number

## Defects of mitotic control pathways

chromosome segregation

centrosome duplication

cell cycle checkpoint

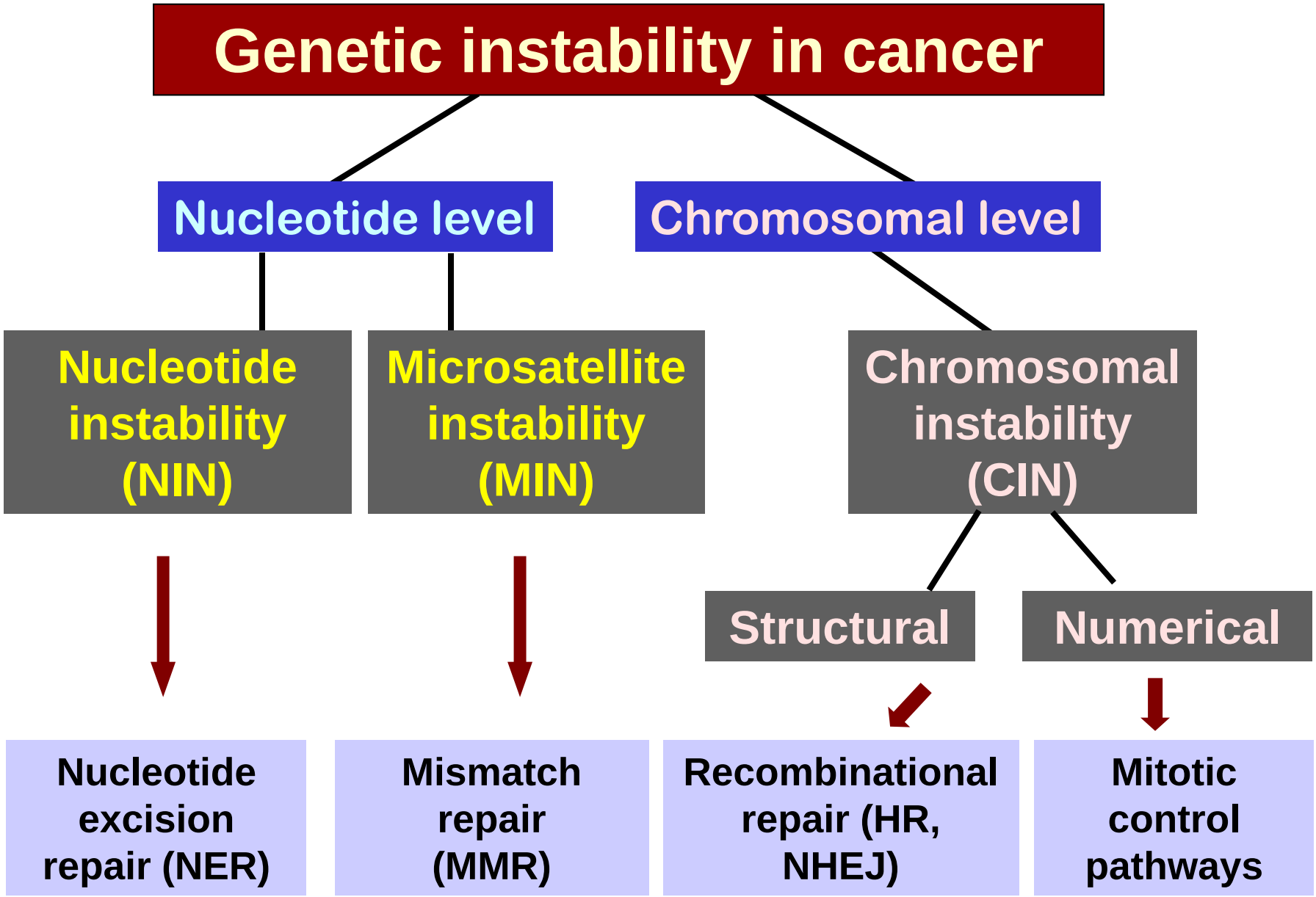
## Structural instability

Deletions
Translocations
Amplifications
Telomere dysfunction

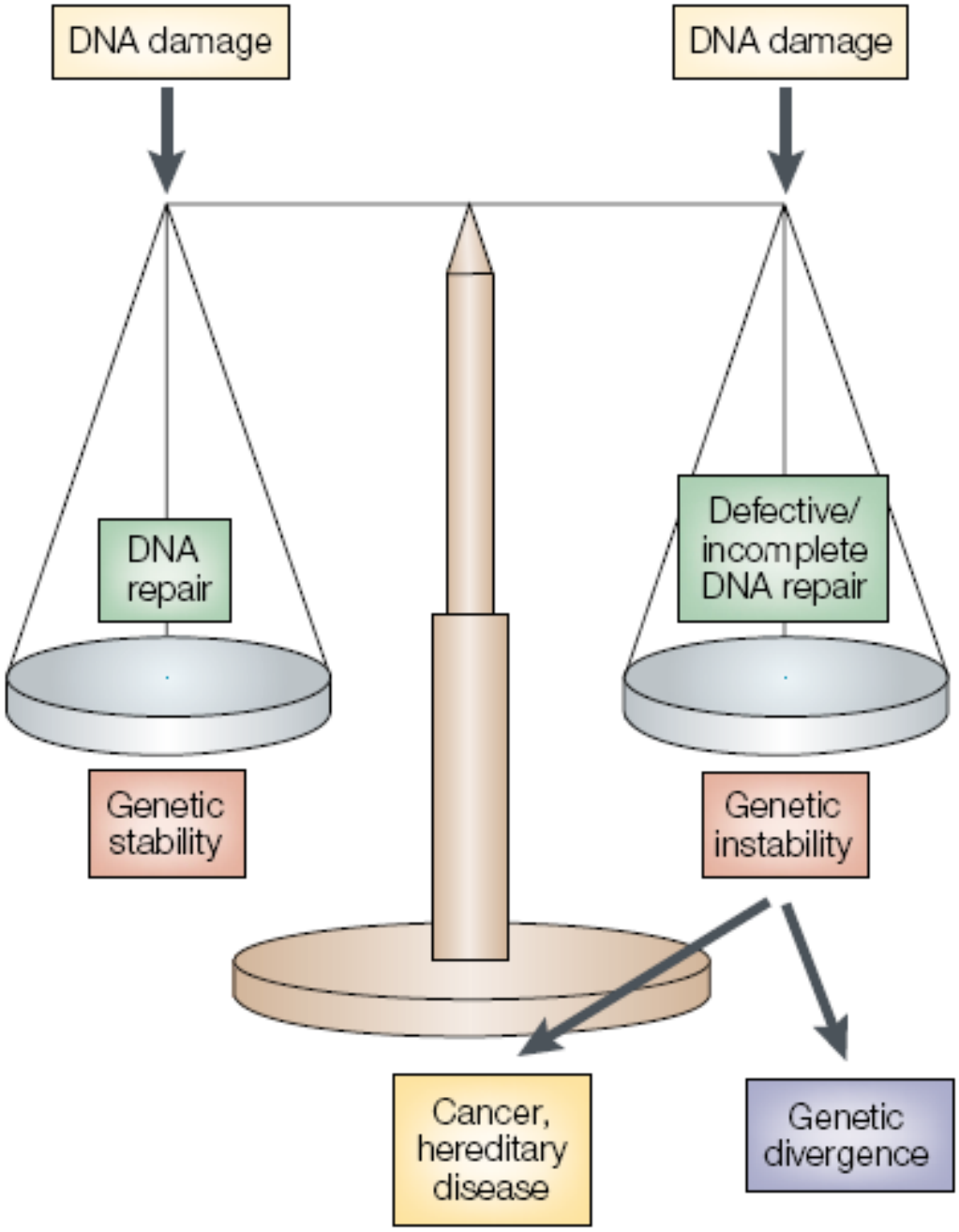
## Defects of repair DNA double-strand breaks

homologous recombination (HR)

non-homologous end joining (NHEJ)




controlled by hundreds of genes


DNA damage
DNA damage
DNA repair
Defective/ incomplete DNA repair
Genetic stability
Genetic instability
Cancer, hereditary disease
Genetic divergence