Molecular and Cell Biology of Tumors

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6. Genetic instability

Hallmarks of cancer

(1) Sustaining proliferative signaling
(2) Evading growth suppressors
(3) Resisting cell death
(4) Enabling replicative immortality
(5) Inducing angiogenesis
(6) Activating invasion and metastasis
(7) Genome instability and mutation
(8) Tumor-promoting inflammation
(9) Deregulating cellular energetics
(10) Avoiding immune destruction

Hanahan D. and Weinberg R.A., *Cell* 144 (2011) 646-674

Hallmarks of cancer

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Hanahan D. and Weinberg R.A., *Cell* 144 (2011) 646-674

Carl O. Nordling

- 1953: it was first speculated that neoplastic transformation does not occur in a single step
- An architect Carl O. Nordling studied deaths frekvencies for cancer patients of different age (from 25 to 74 years) and found the death rate increased proportionally with the sixth power of the age.
- he deduced that a cancer cell was the end-result of at least six successive mutations

Nordling C.O. A new theory on cancer–inducing mechanism. *Br.J.Cancer* 7: 93-112, **1953**.

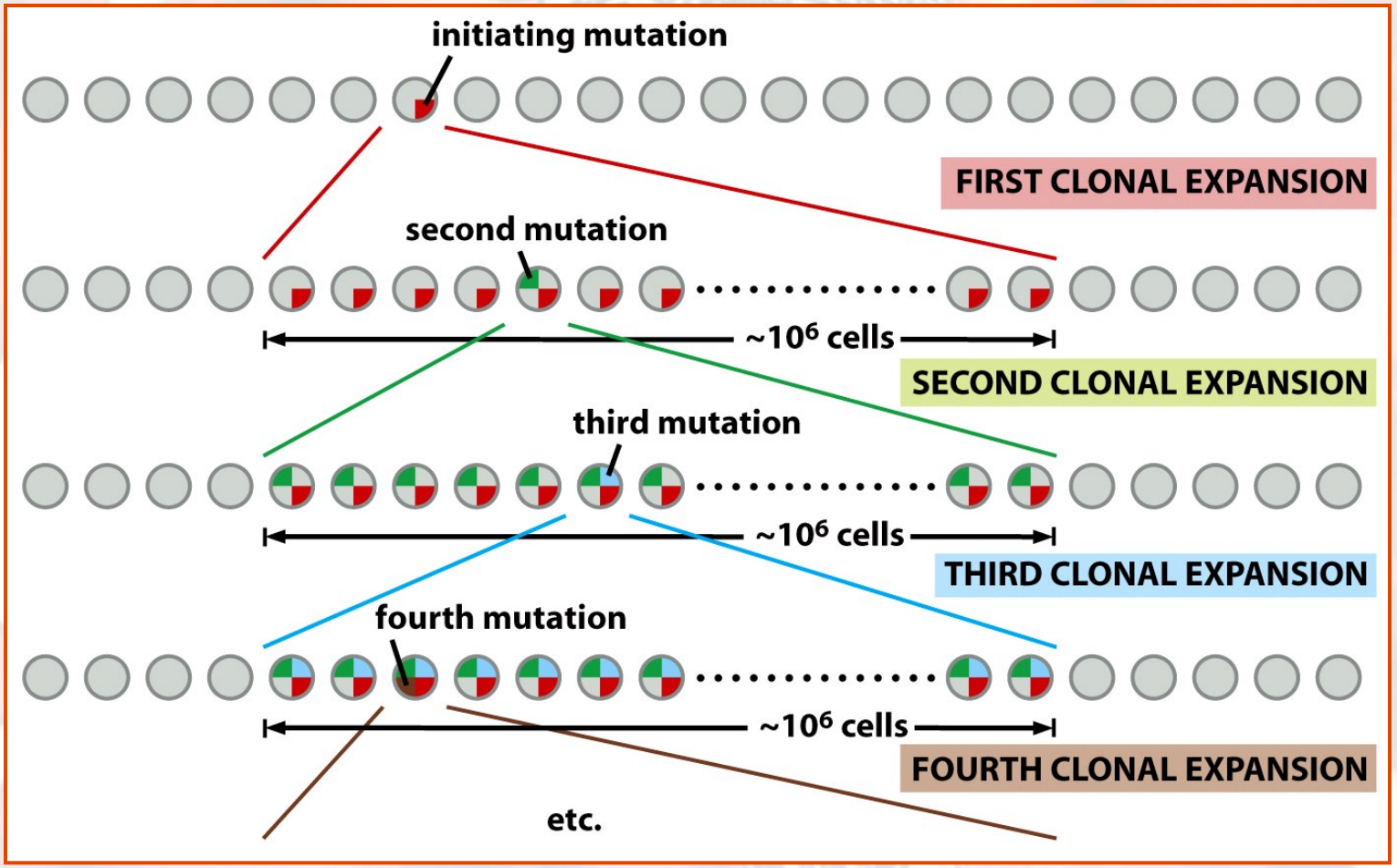
Genetic instability of tumors

- Tumors develop via gradual accumulation of genetic (and epigenetic) changes of specific genes driving cell division, cell death and other important cellular functions.

- Calculations based on the known mutation rate in somatic cells (10^{-6} per gene per cell generation / 10^{-9} per nucleotide per cell generation) showed that such accumution of mutations would not be possible during lifetime...

- What is the mechanism of this accumulation?

Multistep cancerogenesis accompanied by sequence of clonal expansions



Weinberg RA. The Biology of Cancer. *Garland Science* 2007

Genetic instability tumors

1. Accumulation of all necessary mutations is achieved by normal mutation rates in combination with waves of clonal expansion, that may be caused by positive selection of „precancerous“ cells.

2. Accumulation is enabled by genetic instability (i.e. „mutator hypothesis“). Instability means increased inherent rate of genetic change.

- Most tumors are genetically instable

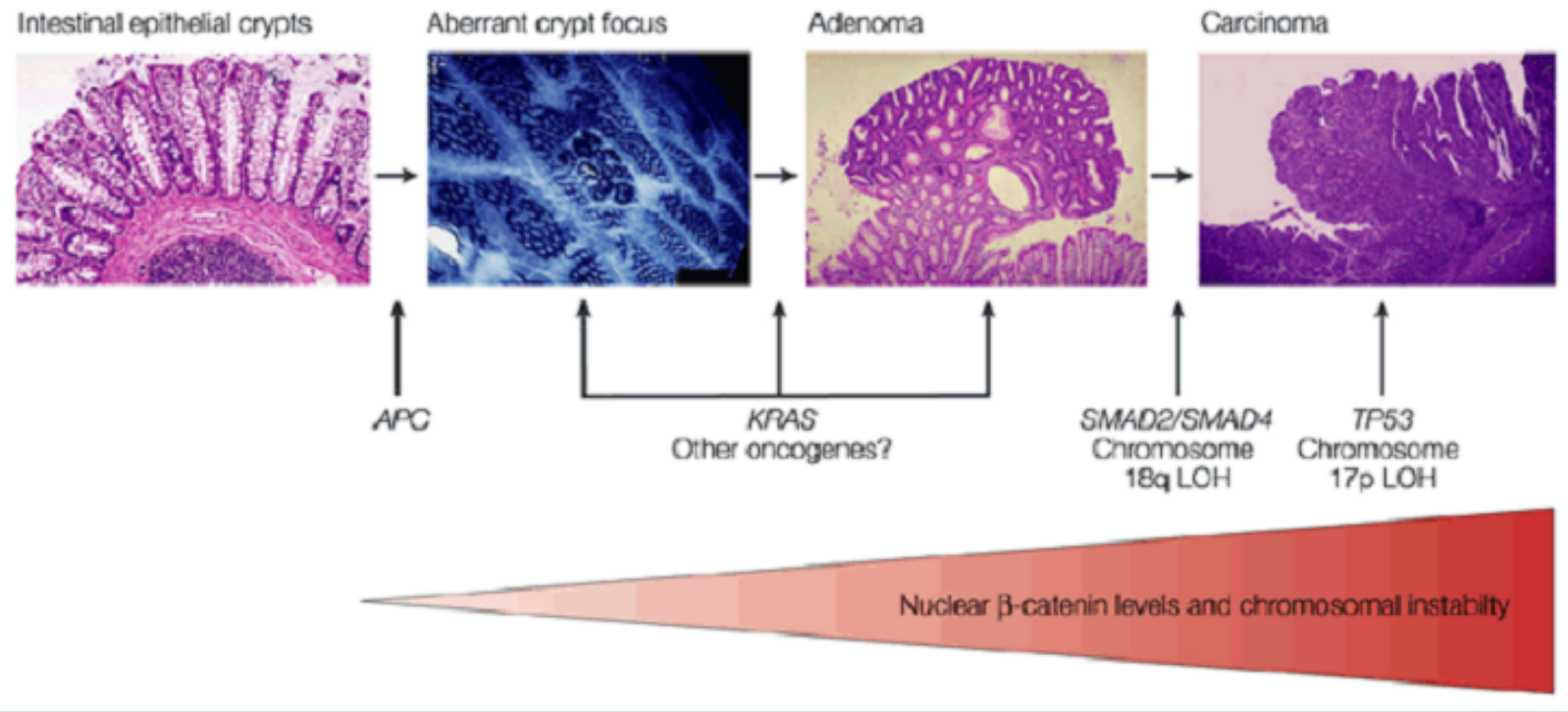
Level of genetic instability

- Absence of genetic instability would not ensure sufficient genetic variability to overcome additional selection barriers during multilevel cancerogenesis.
- Excessive instability would cause extensive DNA damage and subsequently induction of apoptosis.

- Similar findings achieved during research of bacterial **fitness** (reproduction capacity): there must be balance between positive and negative effects of genetic variability (secured by mutations) − sufficient variability to survive in changing and selective environment but not too high to threaten viability.

- Principle „**just-right instability**“
- Extent of genetic instability increases during cancerogenesis

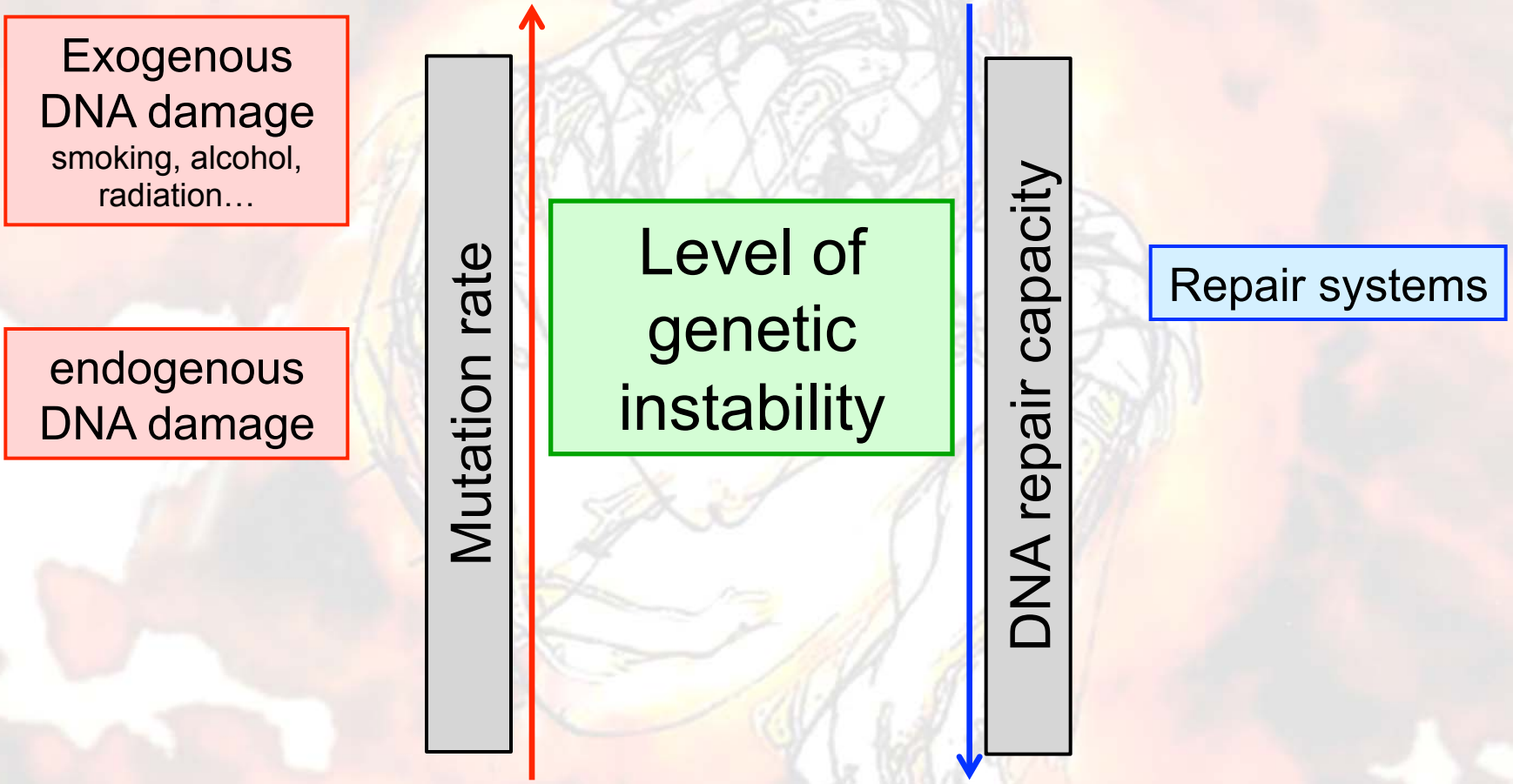
Genetic model of CRC cancerogenesis



Increasing tolerance to the increasing levels of ß-catenin and to chromosomal instability!

Fodde R et al, *Nat Rev Cancer* 1 (2001) 55-66

Level of genetic instability



Mutator hypothesis

mutator hypothesis

Accumulation of mutations is enabled by enhanced genetic instability, that results from (germinal or somatic) defect in DNA repair systems and cell cycle checkpoints

Significance of DNA repair and cell cycle checkpoints is confirmed by the fact, that congenital defects in these systems predispose to cancer development (hereditary cancer syndromes).

Types of genetic changes in tumors

1. Minor changes in DNA sequence

   missense mutations, small deletions and insertions (e.g. missense mutations of K-*ras* occur in 80 % of pancreatic tumors, missense mutations of *TP53* are present in almost 50% of all tumors..)

2. Changes in chromosome numbers

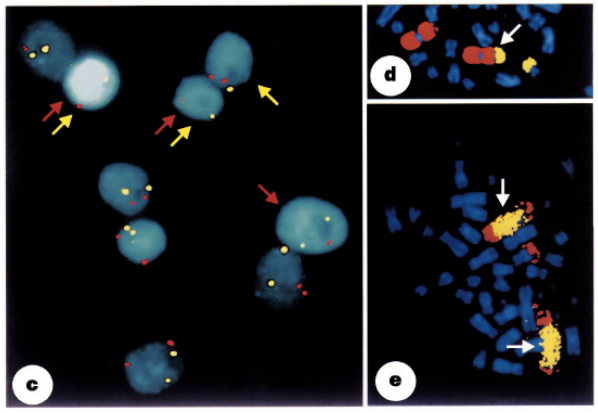
   loss or gain of whole chromosomes (e.g. Loss of chromosome 10 in glioblastomas associated with inactivation of tumor suppressor *PTEN*; gain of chromosome 7 in papillary renal cancer associated with duplication of oncogene c-Met)

3. Chromosomal translocations

   fusion of (parts of) different chromosomes or parts of the same chromosome that are normally not connected (may lead to fusions between different genes) (e.g. Philadelphia chromosome in leukemias)

4. Gene amplification

   amplification of N-*myc* in 30 % of neuroblastomas



- **Genetic instability has multiple levels**

Lengauer C et al, *Nature* 396 (1998) 643-649

1. DNA sequence instability

- This type of instability is rather rare in tumors, if present it has serious impact. Errors occur during DNA replication (error-prone vs error-free DNA polymerases − proof-reading) and because of imperfect DNA repair systems.
- Defects in DNA polymerases are not common in tumors,
  but 2 main systems of DNA damage repair may be disrupted:

1. Nucleotide-excision repair - **NER** − responsible for NER-associated instability - **NIN**
2. Mismatch repair - **MMR** − responsible for microsatellite instability (**MIN**)

DNA sequence instability

- **Nucleotide excision repair** - **NER** − associated with „NER-associated instability“ - **NIN**

Xeroderma pigmentosum

- **Mismatch repair** - **MMR** − associated with microsatellite instability (**MIN**)

Hereditary non-polyposis colorectal carcinoma - HNPCC = Lynch syndrome

Nucleotide excision repair – NER

Xeroderma pigmentosum

X

Mismatch repair - MMR

Lynch syndrome

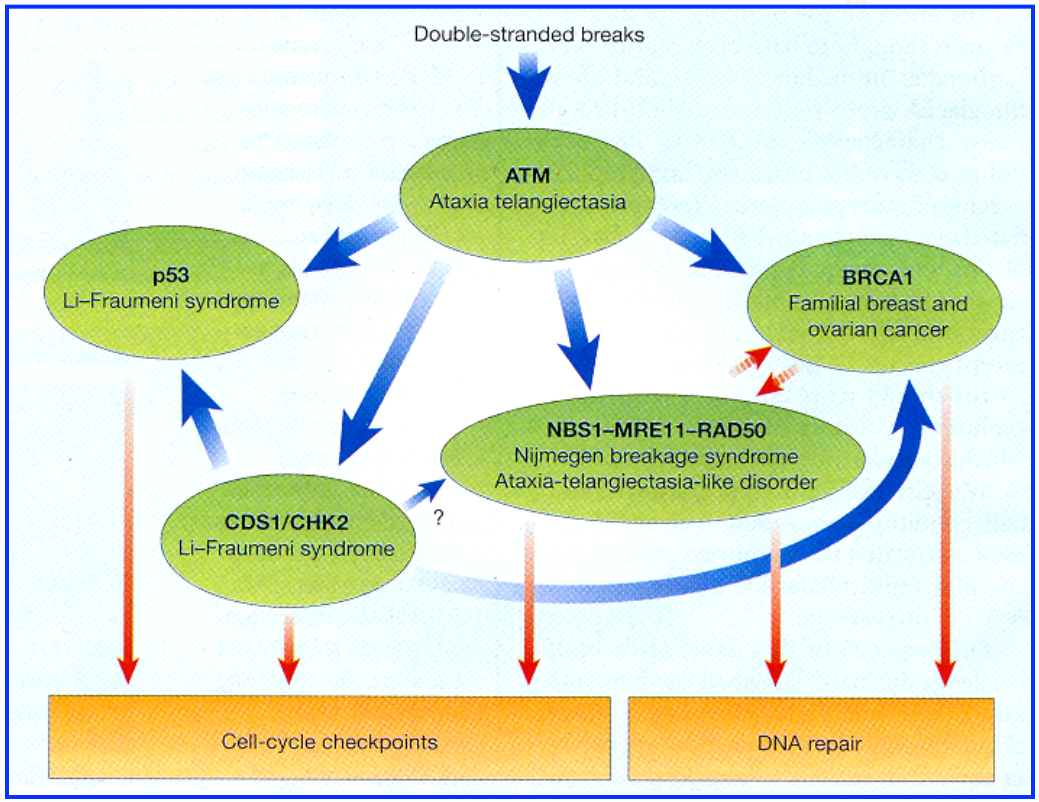
- Mutations of **MMR and NER genes** are **recessive** (at the cellular level!), i.e. one "functional" allele is sufficient to preserve normal repair, only after inactivation of second allele there is accumulation of mutations.
- Carriers of one germ-line mutation in MMR genes are predisposed for cancer − Lynch syndrome is **dominant**!

- × heterozygots in **NER** genes do not have increased risk of cancer!!

(it may be explained by the fact that the second mutation is not per se enough to increase the rate of mutations, there must be an exogenous mutagenic factor, e.g. UV!)

DNA sequence instability

- Repair of DNA double strand breaks by **homologous recombination**



DNA sequence instability

- Repair of DNA double strand breaks by **homologous recombination**

Ataxia Telangiectasia

Nijmegen breakage syndrome

AT-like disorder

Hereditary breast and ovarian cancer syndrome (*BRCA1*, *BRCA2*)

DNA sequence instability

Bloom syndrome

Werner syndrome

Rothmund-Thomson syndrome

Fanconi anemia

DNA sequence instability and …

Li-Fraumeni syndrome (*TP53*)

- p53 is associated with **all** types of DNA repair: NER, MMR, HR a NHEJ
- Model of p53 as a cellular rheostat: guarantees appropriate cellular response:

1. Minor damage: p53 activates repair systems
2. More extensive damage: stabilization of p53 leads to the DNA repair and cell cyle arrest
3. Unrepairable DNA damage: p53 induces apoptosis or senescence

Sengupta S., Harris C.C. p53: Traffic cop at the crossroads of DNA repair and recombination. *Nat. Rev. Cancer* 6: 44-55, **2005**

2. Chromosomal instability - CIN

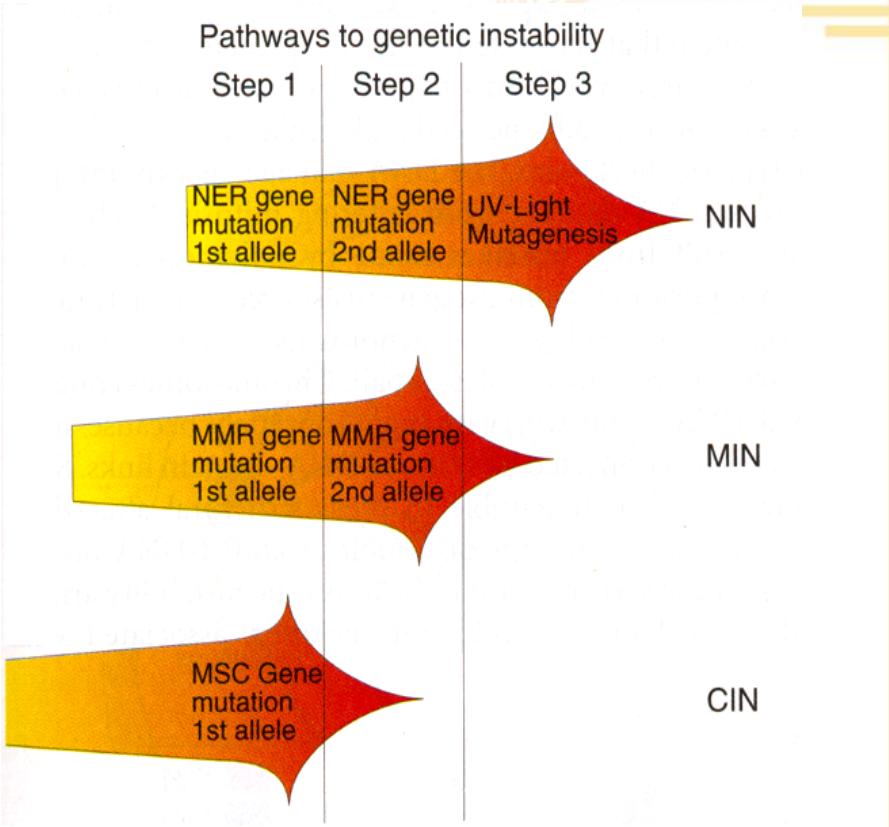
- In comparison with NIN and MIN are chromosomal changes much **more common** in tumors − about **85 %** of human CRCs are aneuploid.
- Common is loss of chromosome due to the LOH

  ⇒ Not always is the incorrect caryotype result of CIN!
- In CRCs and endometrial cancer there is an inverse correlation between MIN and CIN: tumors with defects in MMR are diploid and exhibit normal rate of chromosomal rearrangements, while tumors without defects in MMR are often aneuploid and have high frequency of chromosomal abnormalities. ⇒ At least in these two cancer types are MIN and CIN equal in respect of induction of genetic instability
- Both types of instability occur rather early during cancerogenesis and promote accumulation of genetic changes during later stages

Relation between MIN and CIN

Fusion of cells with CIN and MIN generates cells with CIN:

- Defects of **MIN** are complemented by MMR system of „CIN cells“
- **CIN** phenotype is dominant: it indicates that to „achieve“ CIN only one mution may be sufficient



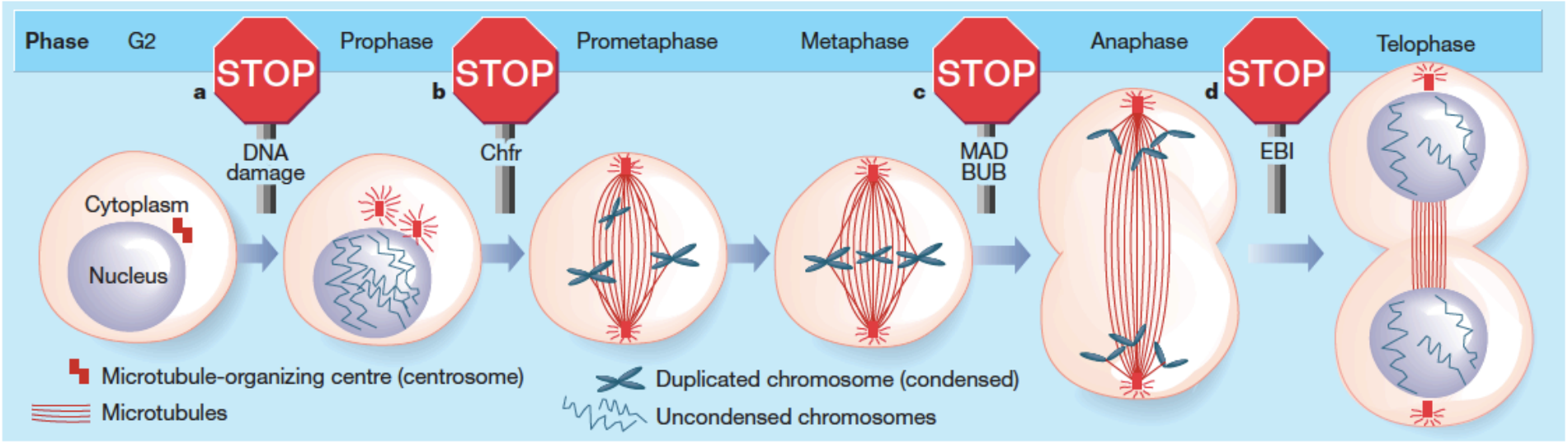
Lengauer C et al, *Nature* 396 (1998) 643-649

Molecular mechanism of CIN

- In yeasts CIN may be caused by mutation of one of 100 genes: genes involved in chromatin condensation, sister chromatid cohesion, kinetochore assembly, centrosome and microtubule structure, etc

- During cell cycle there are several **checkpoints** that serve as surveillance mechanisms to monitor its proper progression: growth to the appropriate cell size, the replication and integrity of the chromosomes, and their accurate segregation at mitosis.

Checkpoints during mitosis



A. Delays entry into mitosis until any damaged DNA is repaired

B. Chromosomes are not condesened if microtubules are disturbed

C. Prevents chromosome separation until the chromosomes are attached correctly to the spindle

D. Separation of cells is delayed if spindle is incorrectly orientated

Cortez D and Elledge SJ, *Nature* 406 (2000) 354-355

Restriction checkpoint vs. Other checkpoint

Restriction checkpoint (G1 checkpoint, Start, Major checkpoint):

- proliferation
- quiscence, resting state
- differentiation
- senescence
- cell death

Restriction checpoint vs.other checkpoints

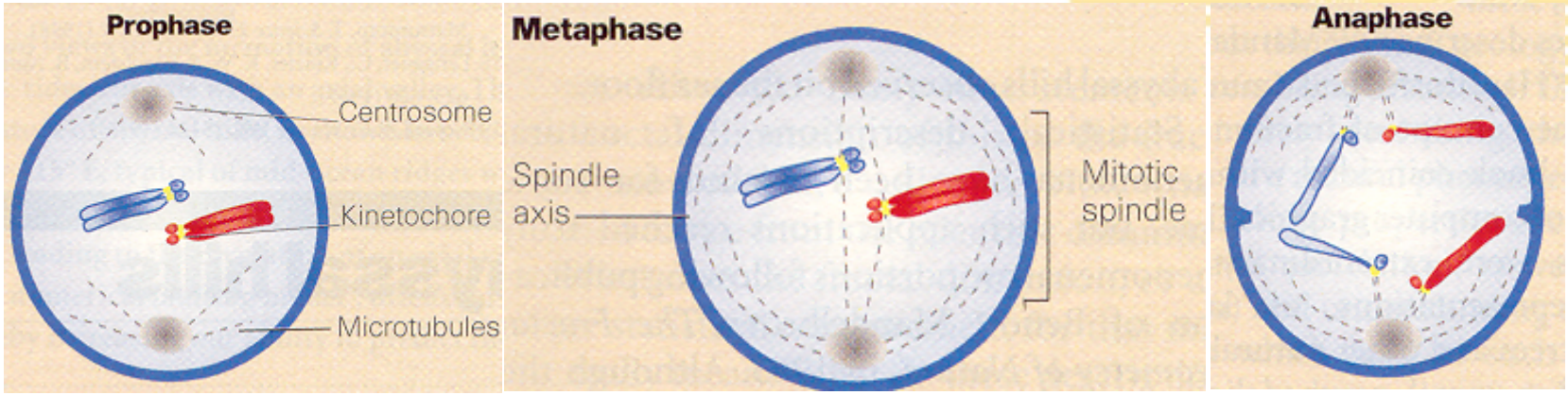
- In restriction checkpoint cell cycle may be stopped
- In other checkpoints cell cycle is delayed

Spindle checkpoint

- *metaphase checkpoint* or *spindle checkpoint* or *spindle assembly checkpoint* or *mitotic checkpoint* ensures **correct segregation of chromosomes**
- it prevents anaphase movement of sister chromatids to the cell poles, unless:
  - correct assembly of the mitotic spindle;
  - attachment of all chromosomes to the mitotic spindle in a bipolar manner;
  - congression of all chromosomes at the metaphase plate.

- Chromosomes are attached via **kinetochores**: protein structures that assemble on the centromeric DNA
- Kinetochores that are not linked to the microtubules of spindle form signaling complex transducing „***wait anaphase signal*“**, that halts separation of chromatids until all kinetochores are attached.

Spindle checkpoint



a. Prophase: mitotic spindle is being formed from microtubule organising centre around cetrosomes at the cell poles

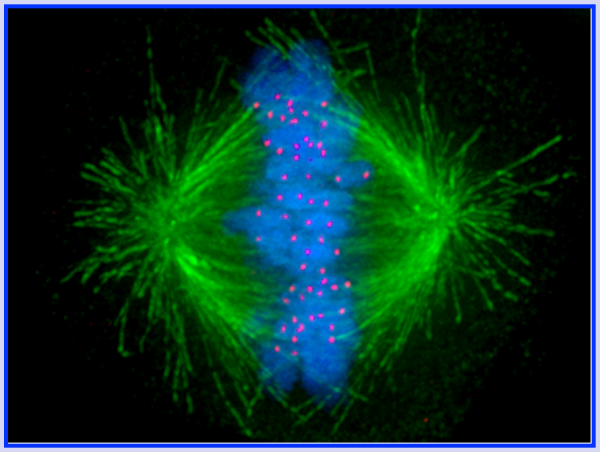
b. Metaphase: mitotic spindle is finished and chromosomes are attached to the spindle microtubules at kinetochores

c. Anaphase: chromatids are separated and pulled towards cell poles, kinetochores first

Orr-Weaver TL and Weinberg RA, *Nature* 392 (1998) 223-224

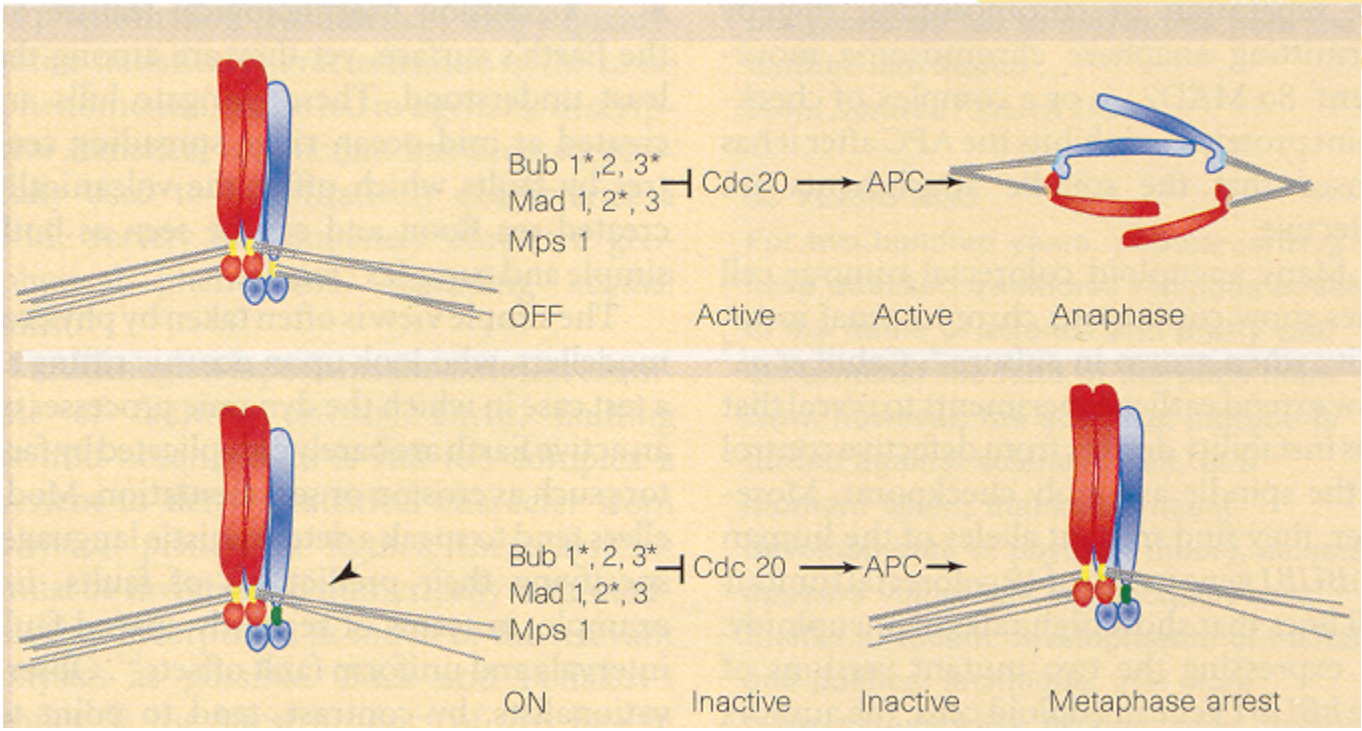
Kinetochores

- **Kinetochores** are large multiprotein complexes assembled on centromeres of chromosomes during mitosis or meiosis. They allow bipolar attachement to the microtubules of mitotic spindle and help with the movement to the opposite cell poles during anaphase.



kinetochores red, microtubules green, chromosomes blue

Spindle checkpoint



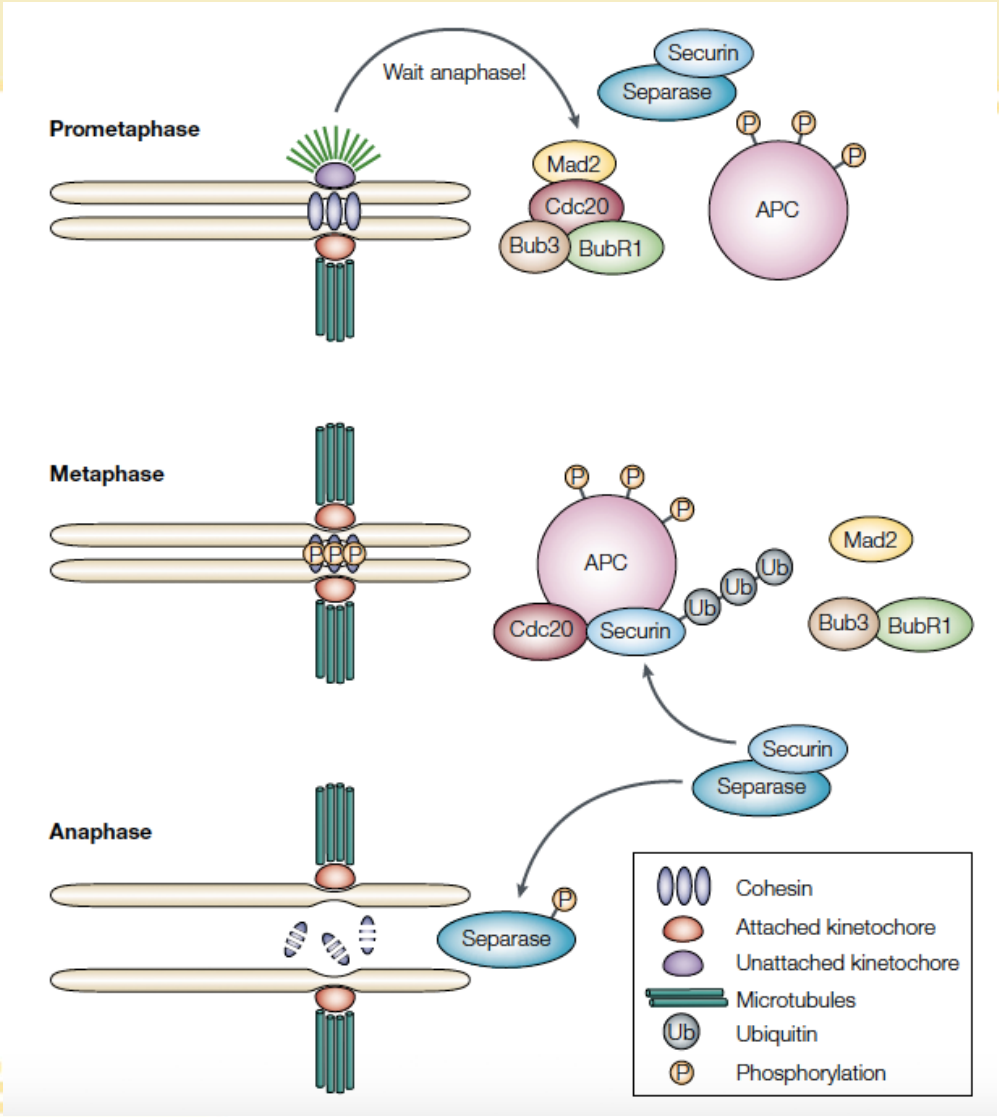
Proteins **Bub** and **Mad** monitor the correct attachment of chromatids to spindle. They prevent activation of **APC** (**APC/C, Anaphase-Promoting Complex**) if not all kinetochores are anchored to spindle.

Orr-Weaver TL and Weinberg RA, *Nature* 392 (1998) 223-224

Spindle checkpoint

- Key event in the spindle checkpoint is inhibion of ubiquitin ligase complex **APC/C** (*anaphase-promoting complex/cyclosome*): multiprotein complex that is active during the transition to anaphase.
- When all kinetochores are attached to the spindle, protein **Cdc20** is released from inhibitory complex with checkpoint proteins **BubR1**, **Bub3** (*budding uninhibited by benomyl*) and **Mad2** (*mitotic arrest deficient*)
- **Cdc20** binds and activates APC/C for interaction with cyclin B and securin
- Activated **APC/C** ubiquitinylates **securin** that functions as inhibitor of separase
- **Separase** then cleaves **cohesin** and sister chromatids are no longer connected − segregation

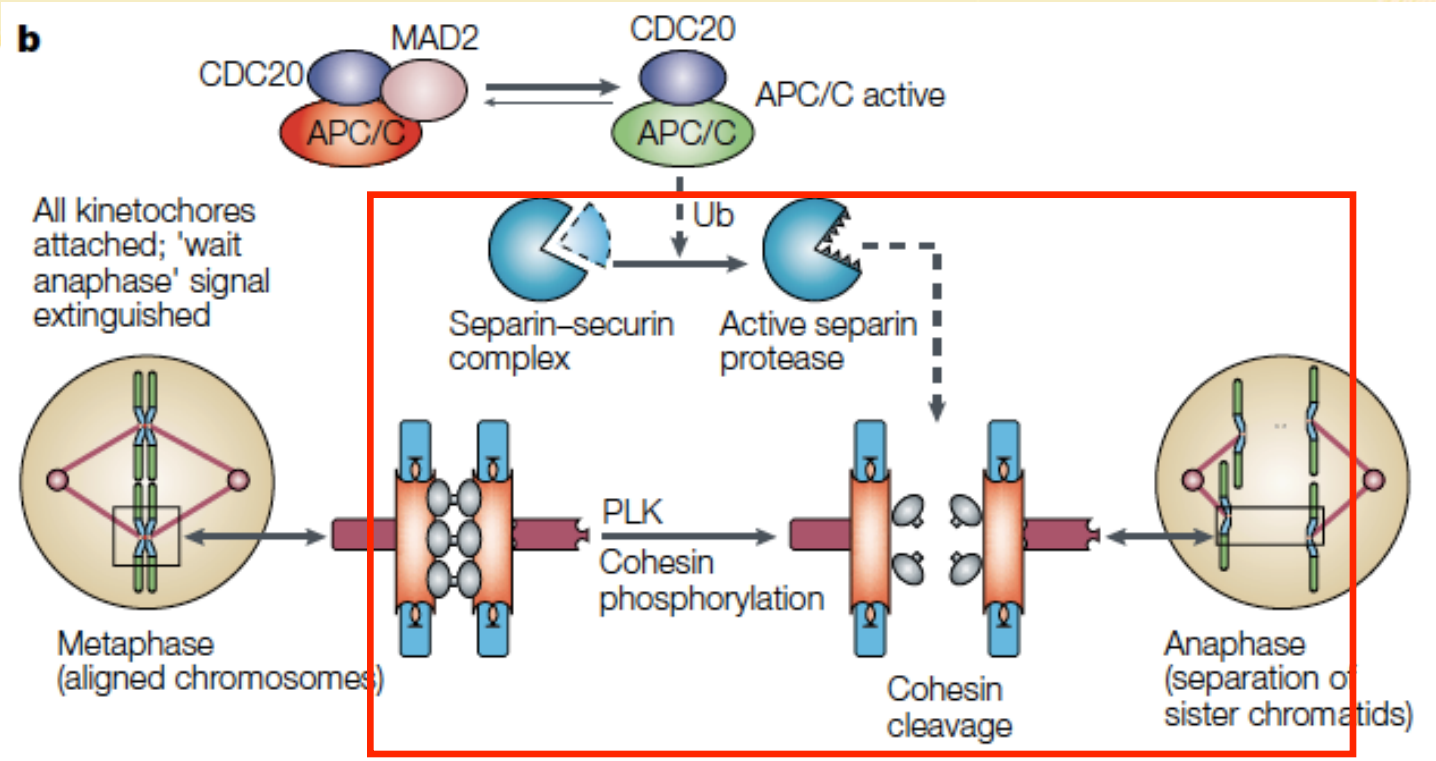
Spindle checkpoint



After attachment of the last kinetochore, the Cdc20-APC/C becomes activated thus triggering degradation of securin, that results in release and activation of separase and proteolytic cleavage of its substrate cohesin.

Musacchio A and Hardwick KG, *Nat Rev Mol Cell Biol*. 2002;3(10):731-41.

Spindle checkpoint



Separase, also known as separin, is a cysteine protease responsible for triggering anaphase by hydrolysing cohesin, which is the protein responsible for binding sister chromatids

Jallepalli PV and Lengauer C, *Nat Rev Cancer* 1 (2001) 109-117

Spindle checkpoint

- **Securin** prevents separation of sister chromatids by binding **separin**/**separase** − cystein protease that catalyzes cleavage of multiprotein complex **cohesin**. Cohesin bridges form immediately after DNA replication in S phase, bind sister chromatids and endure (at centromeric region) till anaphase. Securin functions as inhibitor of separase.

- Activation of APC/C at the onset of anaphase triggers degradation of securin and release of separase − degradation of cohesin −chromatid segregation

Spindle checkpoint

- **Securin** have another function:

Securin mutations cause chromosome nondisjunction! No separation of chromatids, if securin is inactive!

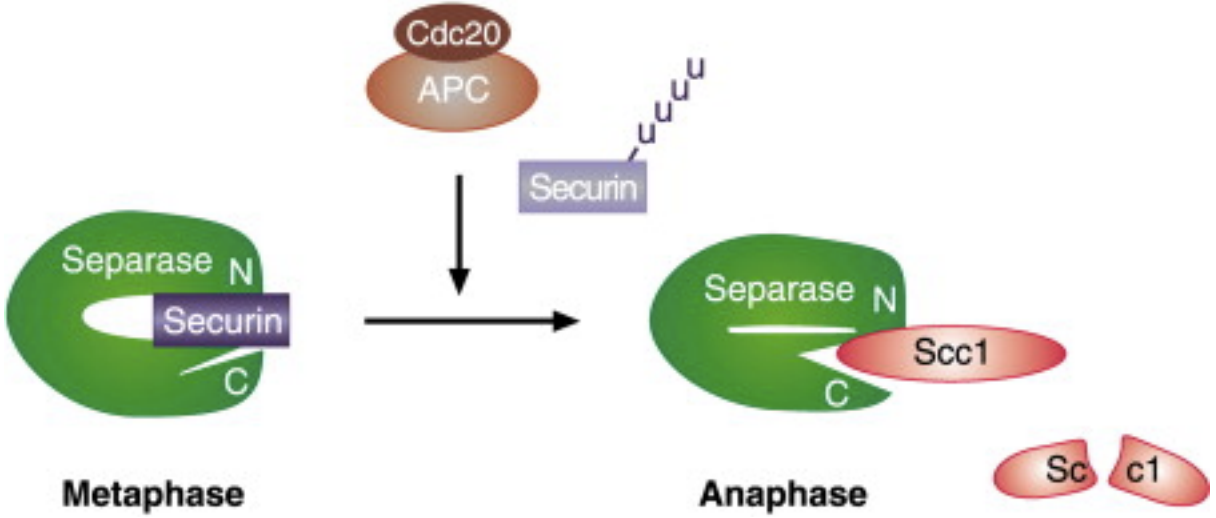
Securin is required for correct localization and/or activation of separase ⇒ dual impact of interaction securin:separase – securin is necessary **for full activation („priming“) of separase** and also acts as **separase inhibitor** = double protection of correct chromatin segregation!!

Model of interaction separase – securin

Securin functions as a chaperone for separase − ensures that separase adopts its proper fold required for proteolytic activity (and promotes its nuclear accumulation)

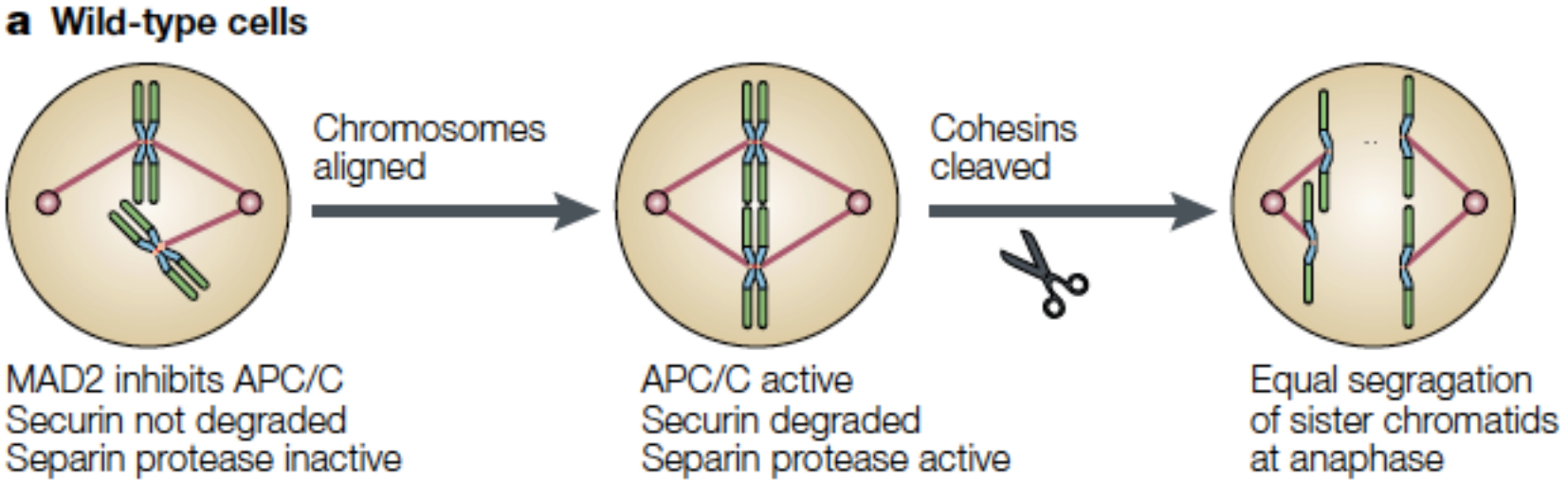
But:

Inhibits proteolytic activity by disrupting the interaction between its N- and C-terminus and preventing interaction with its substrate (**Scc1** is a subunit of cohesin).



Hornig N et al, *Curr Biol* (2002)

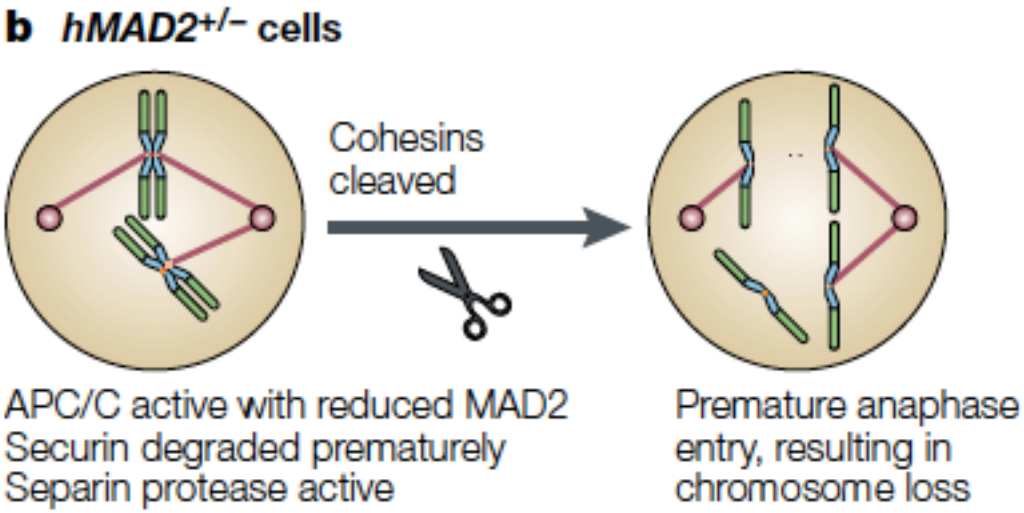
Spindle checkpoint



In normal cell is segreġation of sister chromatids achieved by activation of APC/C − securin − separase axis.

Jallepalli PV and Lengauer C, *Nat Rev Cancer* 1 (2001) 109-117

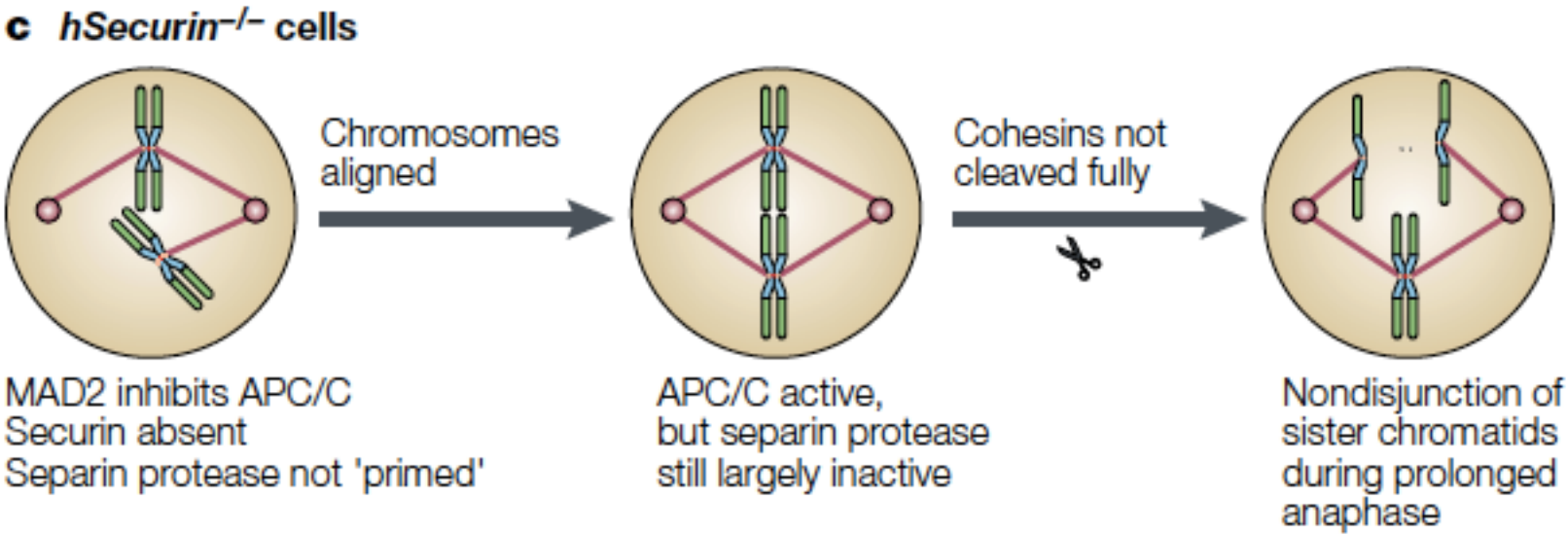
Spindle checkpoint



In cell with only 1 functional allelle of Mad2 (heterozygot!!) there is not sufficient inhibition of APC/C, thus securin is prematurely degraded and chromatids separated without proper attachment to spindle, leading to chromosome losses.

Jallepalli PV and Lengauer C, *Nat Rev Cancer* 1 (2001) 109-117

Spindle checkpoint



Cell completely deficient in securin cannot „prime“ separase for full activation thus cohesin bridges are incompletely cleaved and sister chromatids are not separated (mitotic nondisjuction) that leads to aneuploidy.

Jallepalli PV and Lengauer C, *Nat Rev Cancer* 1 (2001) 109-117

Spindle checkpoint alterations in cancer

- Downregulation of ***hMad2*** found in some breast cancers. **Haploinsufficiency** probably plays role *Mad2* mutation phenotype
- Some CRCs have somatic mutations etiher in ***hBub1*** or ***hBubR1***. Mutations of *hBub1* are **dominant**.
- **hMad1** is targeted for degradation by protein Tax, product of T-lymphotropic virus type 1: this results in spindle checkpoint defects in adult T cell leukemias.
- Gene encoding **securin** was first described as *PTTG* (*pituitary tumour-transforming gene*) and it is highly expressed in some tumors (overproduction of securin probably cause missegregation of chromatids).

Spindle checkpoint alterations in cancer

- Gene encoding components of mitotic checkpoint are rarely mutated in cancer, but frequently up/downregulated (↑ and ↓)
- Level of **Mad2** must be tightly controlled, both ↑ and ↓ enhanced cancer development. In mice higher Mad2 levels result in wider range of tumor types and more agressive tumors. High levels of Mad2 cause not only changes in chromosome numbers (aneuploidy) but also chromosomal rearrangements (breaks, end-to-end fusions)
- Molecular mechanism (high Mad2 levels): (1) insufficient destruction of cyclin B and securin − reduced separese activity −separation of chromatids without cohesin desintegration ⇒ breaks (2) failure of cytokinesis - tetraploidy

Cancer Cell
Previews

Rb Loss Causes Cancer by Driving Mitosis Mad

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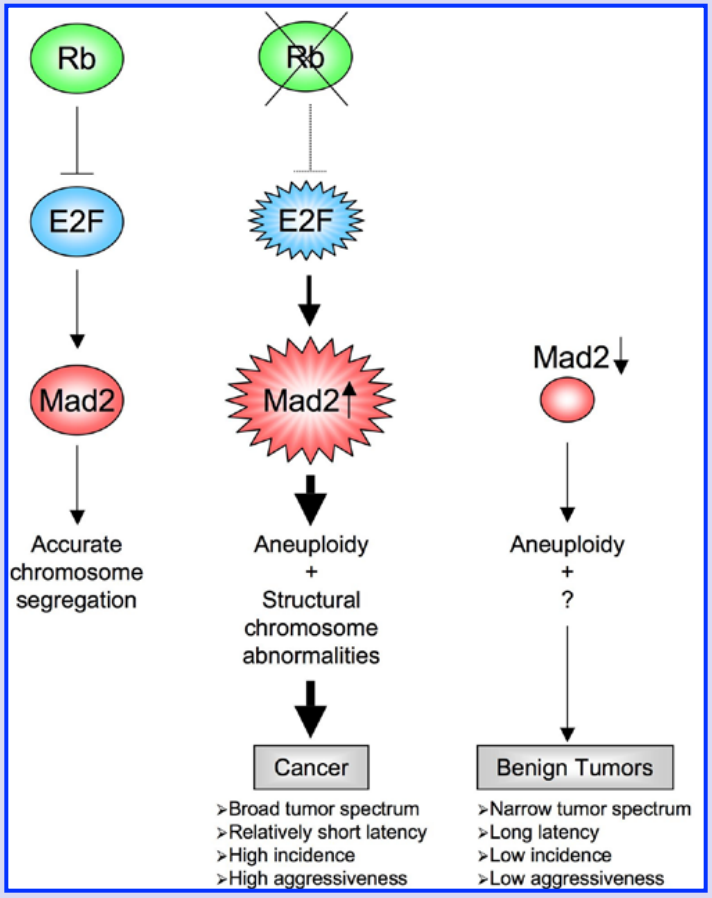
DOI 10.1016/j.ccr.2006.12.006



- Gene ***mad2*** is transactivated by **E2F1** and thus highly overexpressed in tumors with inactivated RB.

Van Deursen JM, Rb Loss causes cancer by driving mitosis Mad, Cancer Cell 11, pp. 1-2, 2007

APC and CIN

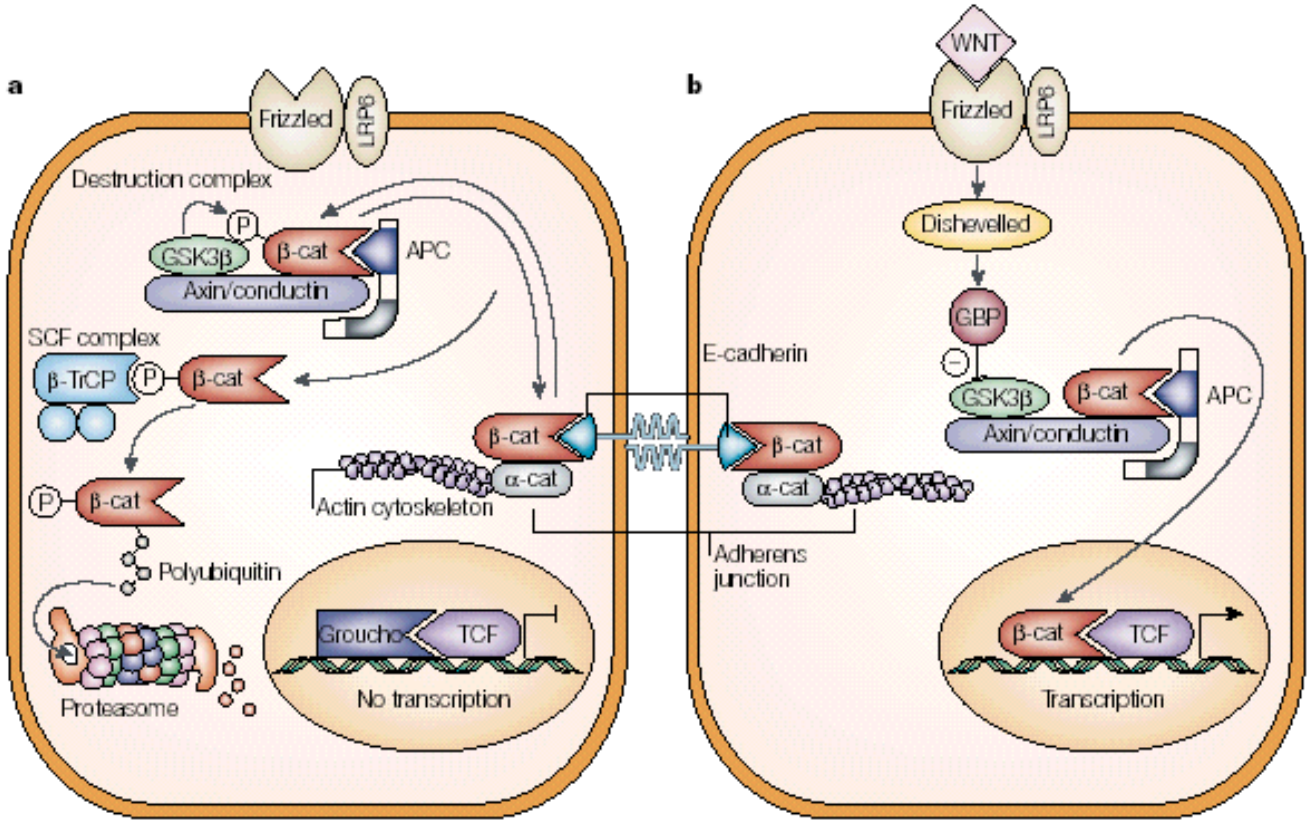
- Germ-line mutations of *APC* (adenomatous polyposis coli) cause FAP (*familial adenomatous polyposis*), somatic mutations are the most common (early) genetic changes in colorectal cancer.
- APC has 2 functions:

  1. regulation of WNT signaling via binding to **β-catenin** – target genes of this pathway are Myc and cyclin D1 (⇒ increased proliferation)

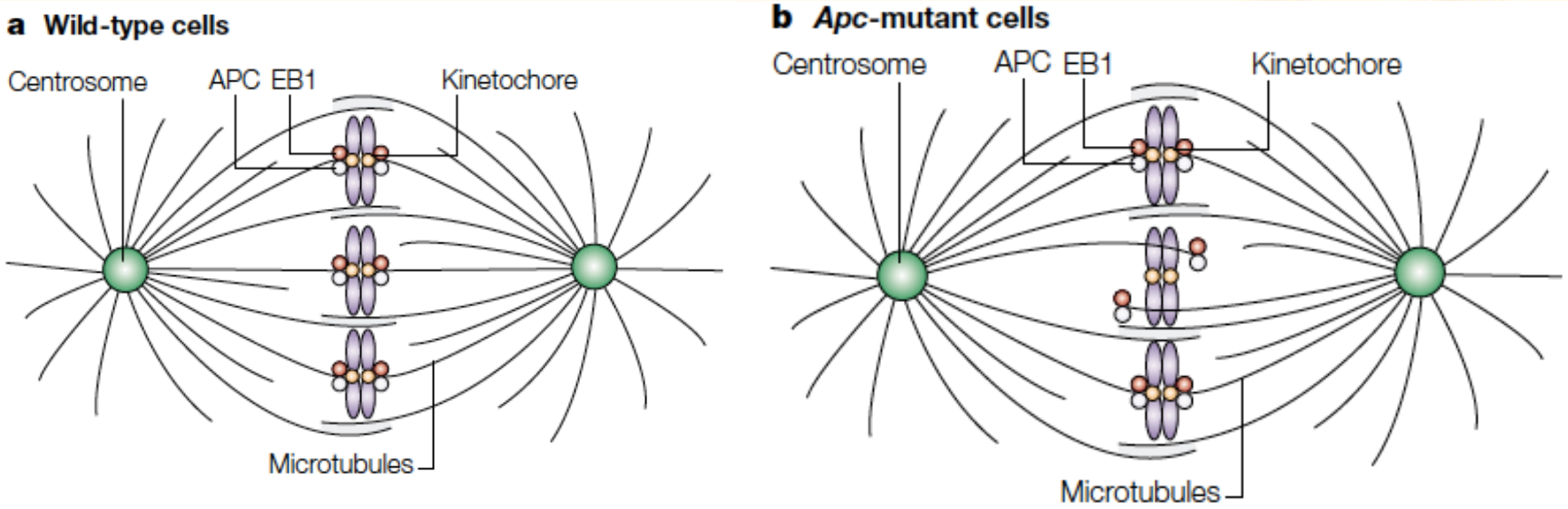
  2. APC is localized to the kinetochores of metaphasic chromosomes via binding of its C-teminus to the **EB1 protein** − it mediates binding of kinetochore to the microtubules; mutant APC proteins (C-terminally truncated forms) cannot bind to EB1 − the attachement of kinetochore to spindle is disrupted

Dual function of APC in cell

A. Regulation of levels of free β-catenin



B. APC function in mitotic spindle



A APC accumulates at the kinetochore, where it facilitates the binding of spindle microtubules to the kinetochore by interacting with the microtubule-associated protein EB1.

B In cells that express a truncated form of APC, the interaction between kinetochores and spindle microtubules is disrupted, leading to CIN.

Fodde R et al, *Nat Rev Cancer* 1 (2001) 55-66

APC and CIN

- Mutation of *APC* gives cell dual „benefit“: increased proliferation and genetic instability.

- CRCs with mutated β-catenin and without mutated APC...

do not progress that fast as APC mutated CRCs − they have enhanced cell division rate (thus advantage in respect to clonal expasion) (*gatekeeper function*), but slower progression to the more aggresive stages due to lack of genetic variability/instability (*caretaker*).

Multiple centrosomes

- Presence of multiple centrosomes (more than 2) in one cell leads to the defects in mitotic spindle organisation (multipolar) − missegregation of chromosomes

  ⇒ results in increased genetic instability CIN

Contribution of E6 and E7 proteins to transformation by papillomaviruses

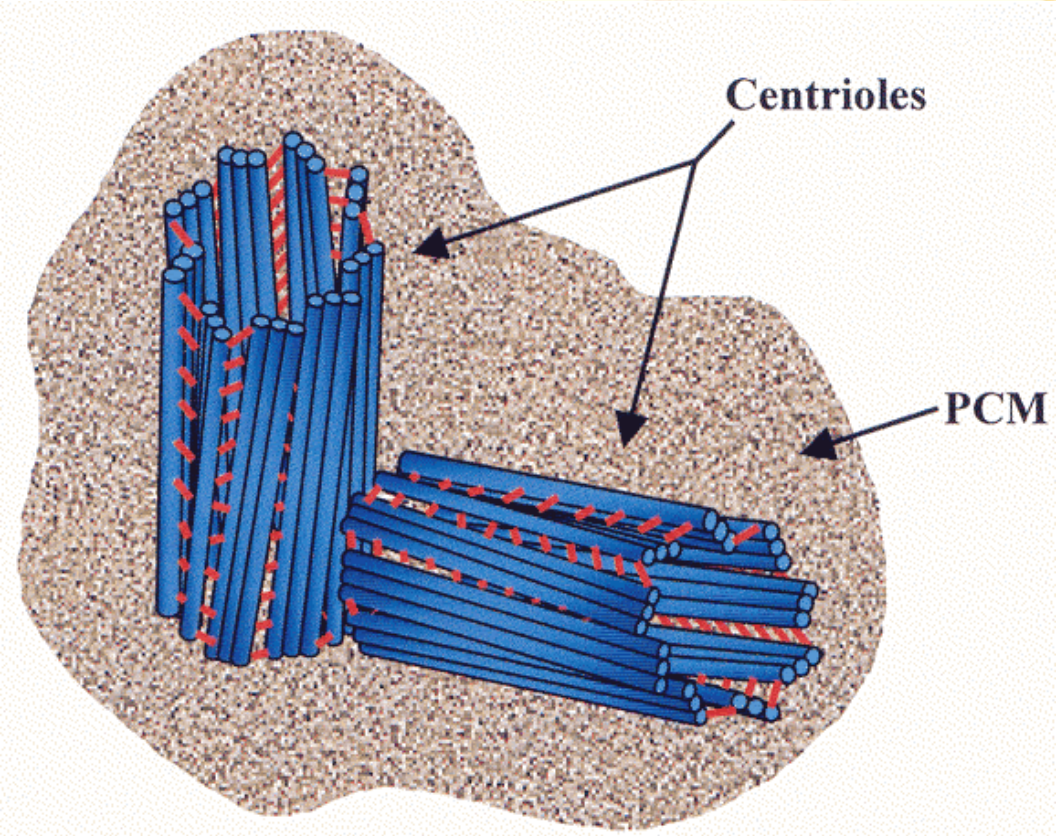
E6 - inactivates **p53** (disrupted: blok G_1, apoptosis, genetic stability)
- interacts with **p300/CBP** (homeostasis pertrubation)
- activates expression of **hTERT** (telomerase activation)
- inactivates **p16^{ink}** (distrubed cell cycle control)
- interacts with **Bak** (inhibition of apoptosis)
- interacts with **E6BP/ERC-55** (inhibits diferentiation)
- induces degradation of **hDlg** (and with other proteins with PDZ motif) (change of morphology, induction of motility)

E7 − binds protein **RB**
- inactivation of **p21^{Cip}** and **p27^{Kip}** (disconnected proliferation and differentiation)
- prevent inhibitory effect of **TGF-β** on cell growth
- cause formation of multiple centrosomes

Centrosome

- Small organels consisting of two **centriols** (9 triplets of microtubules) and surrounding dense protein matrix = **pericentriolar material** (PCM)

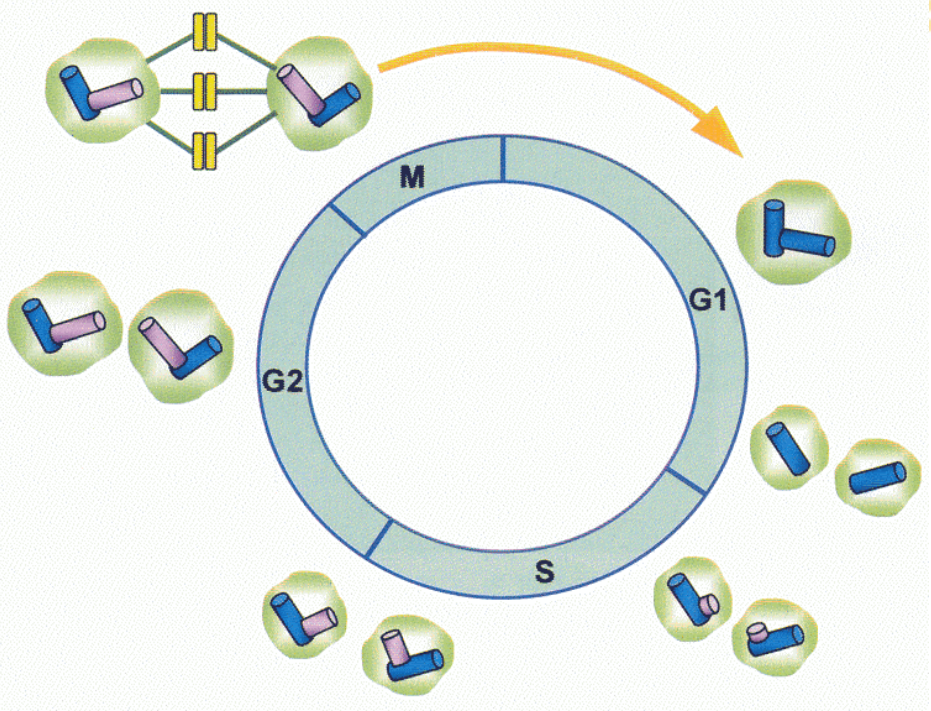
- Functions as microtubules organization centre, determine polarity and orientation of microtubules during interphase regulates mitotic spindle assembly



Fukasawa K, *Oncogene* 21 (2002) 6140-6145

Centrosome duplication

- After mitosis each daughter cell has one centrosome, this is duplicated in late G1 (after restriction checkpoint) and early S phase
- Daugther centrioles form in the vicinity of pre-existing (mother) centrioles and grow during S an G2 phases



Fukasawa K, *Oncogene* 21 (2002) 6140-6145

Mechanisms leading to the multiple centrosomes

1. Deregulation of cell cycle checkpoint for duplication of centrosomes
   - Check of initiation od centorosome duplication
   - Suppression of centrosome re-duplication

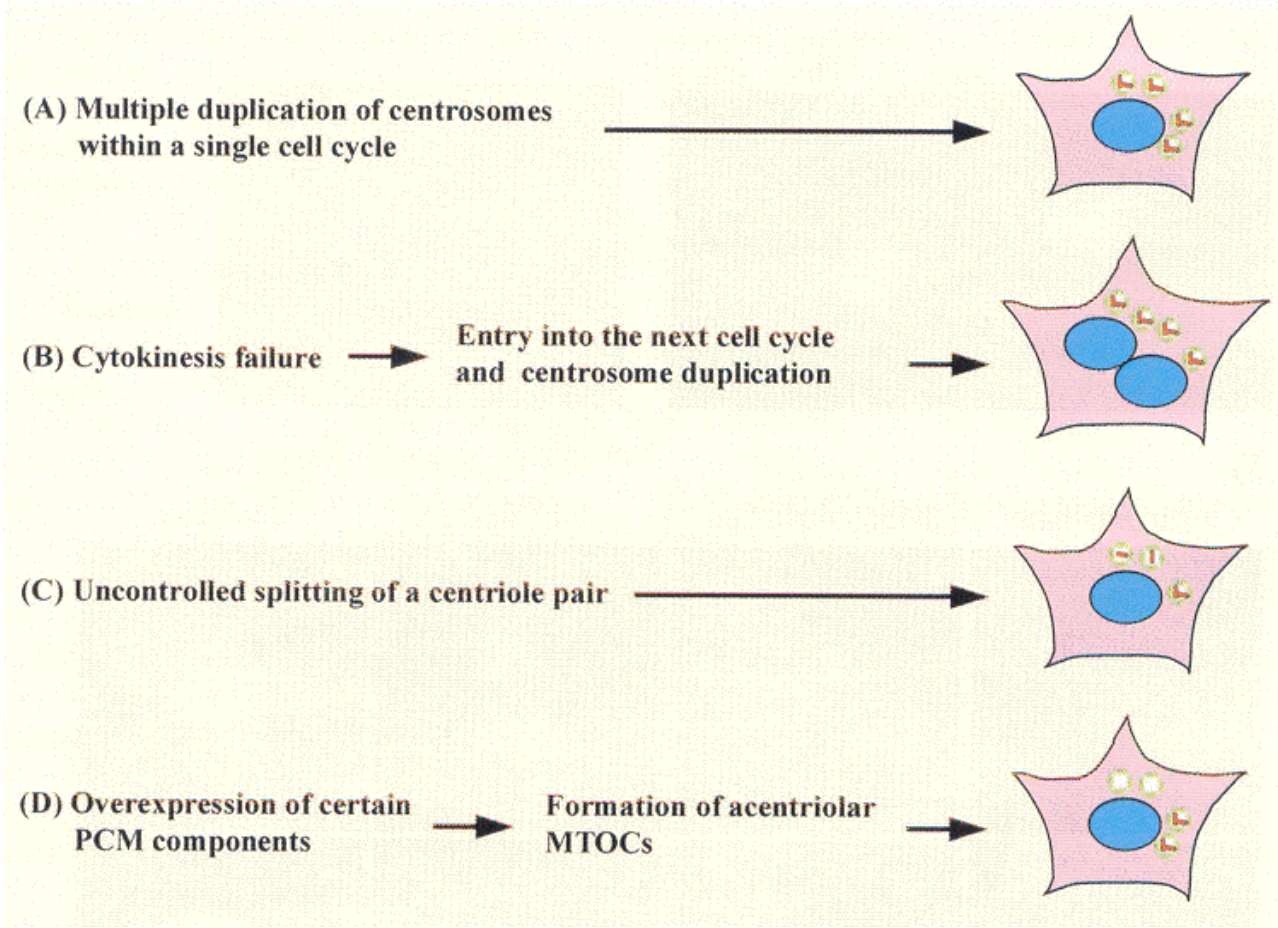
2. Failure of cytokinesis

3. Uncontrolled splitting of centriol pair

4. Overexpression of certain PCM component and formation of acentriolar centrosome

➢ Multiple centrosomes as a cause of aneuploidy were observed in breast, lung, prostate, colorectal, brain tumors,

Mechanisms leading to the multiple centrosomes



Fukasawa K, *Oncogene* 21 (2002) 6140-6145

Regulation of centrosome duplication

- For regulation of centrosome duplication during cell cycle is critical function of **Cdk2/cyclin E**.
- Substrate of Cdk2/cyclin E is **nucleophosmin**, this protein is associated with unduplicated centrosomes and dissociates from them upon phosphorylation by Cdk2/cyclin E.
- In some breast, prostate and head and neck cancer mutation of ***TP53*** correlates with presence of multiplicated centrosomes (possibly via target **p21^{Waf-1}** - inhibitor of Cdk2/cyclin E).

- Human homolog (BTAK/STK-15) of *Droshophila* gene **aurora2/ STK-15** regulates structure of centrosomes and segregation of chromosomes and is **overexpressed (amplified)** in some cancers.

- Some cancers **overexpress** kinase **PLK1** (**Polo-like kinase**) that regulates centrosome maturation.

3. Chromosomal translocation

A. Simple type

- Specific rearrangement of chromosome segment in specific type of tumor. Common in leukemias and lymphomas, sometimes in some sarcomas. Typical for cancer type, used as diagnostic tool.
- Specific translocations may by caused by ionizing radiation, e.g. some thyroid cancer in children after Chernobyl accident have specific translcation of chromosome 10 resulting in fusion gene consisting of *RET* oncogene.
- This specific translocations are not associated with genetic instability, are likely the results of errors during VDJ recombination.

3. Chromosomal translocation
B. complex type

- Common in solid tumors. Translocations include more that 2 chromosomes, are random, different in individual tumors of one histological type. Large parts of chromosomes are deleted, often also "*marker chromosomes*" that contains complex rearrangements of several chromosomes.
- They result in losses and gains of chromosomes similarly as during CIN, in addition new genes may be produced.
- Molecular mechanism is not completely known, presumably cells enter mitosis without previous repair of ds breaks. Candidate molecular players: ***ATM***, ***ATR***, ***BRCA1***, ***BRCA2***, ***TP53***.

4. Gene amplifications

- Occur in some cancer types in later phases and sometimes underlies the acquisition of (chemotherapy-) resistance
- Most common genes undergoing amplification are **N-*myc***, ***erbB*** (***HER2***) and ***ras***, less frequently ***abl***, ***myb***, ***MET***, ***GLI1***,
- Generally amplifications are features of late tumors, associated with aggressive types and poor prognosis

Gene amplifications

- Gene amplifications are more common in cells with inactivated **p53**. In cells with functional p53 is the presence of amplicon sensed as DNA damage and may induce apoptosis. Mutation of p53 may thus allow survival of cells with amplifications and promote their accumulation during subsequent cell divisions. This is a specific type of „amplification instability“ different from CIN.

Level of genetic instability

Exogenous DNA damage
smoking, alcohol, radiation…

endogenous DNA damage

Mutation rate

Level of genetic instability

DNA repair capacity

Repair systems

Level of genetic instability

Exogenous DNA damage
smoking, alcohol, radiation…

endogenous DNA damage

Proliferation rate: DNA replication stress

Mutation rate

Level of genetic instability

DNA repair capacity

Repair systems

Accumulation of somatic mutations during cancerogenesis

1. Mutator hypothesis

Accumulation of mutations is enabled by enhanced genetic instability, that results from (germinal or somatic) defect in DNA repair systems and cell cycle checkpoints

Significance of DNA repair and cell cycle checkpoints is confirmed by the fact, that congenital defects in these systems predispose to cancer development (hereditary cancer syndromes).

2. Model of DNA replication stress induced by oncogenes

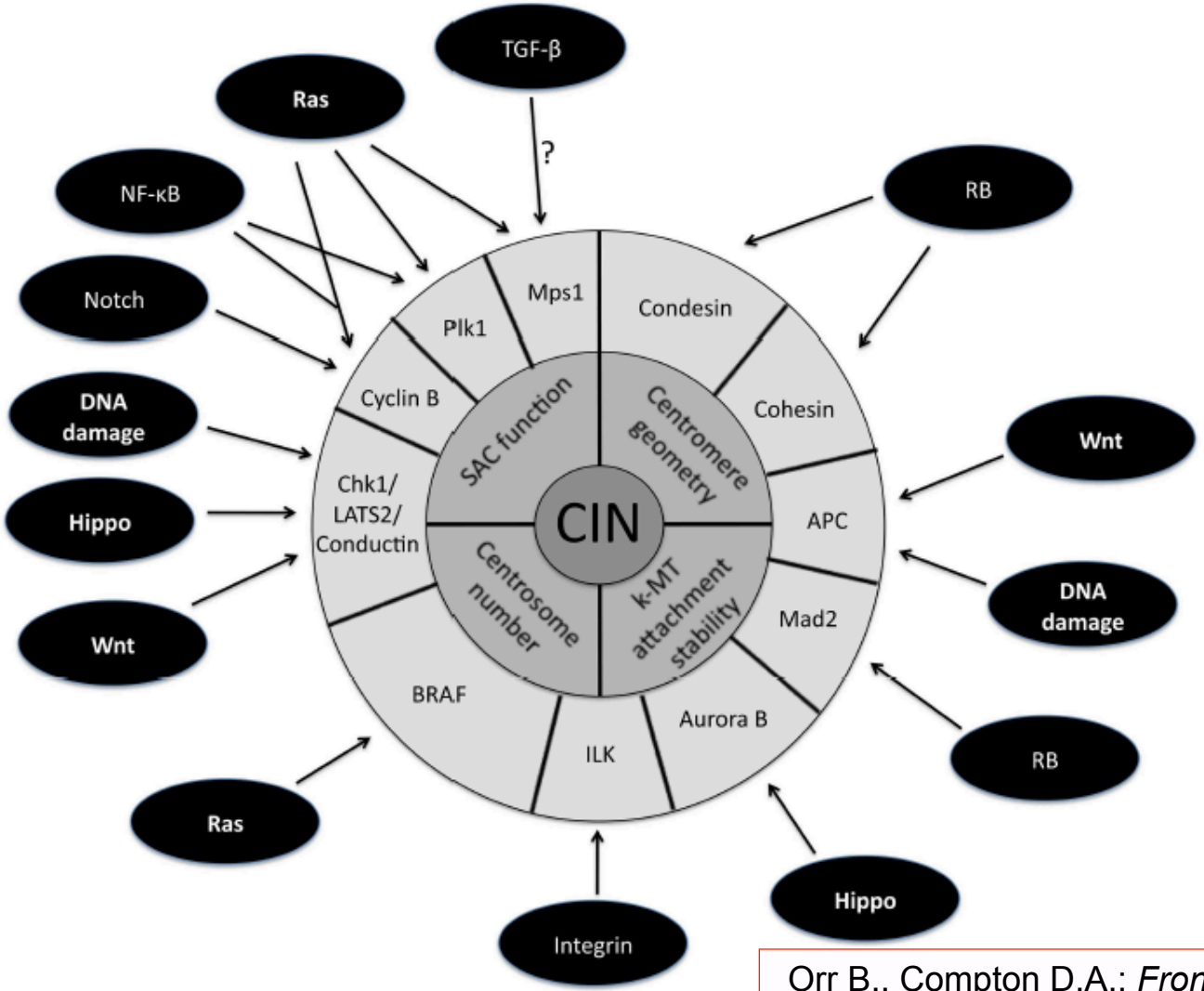
oncogenic pathways (cancer-driver mutations) have dual impact:

1. Stimulate growth/ cell division
2. Induce genetic instability, mostly CIN, by reducing mitotic fidelity

Orr B., Compton D.A.: *Frontiers in Oncology* 3 (2013) 164

Negrini S et al.: *Nat Rev Mol Cell Biol* 11 (2010) 220-228

Activated oncogenes (deregulated cell division) induce DNA replication stress



Orr B., Compton D.A.: *Frontiers in Oncology* 3 (2013) 164

Thank you for attention!