



Cell cycle, oncogenes & tumor markers

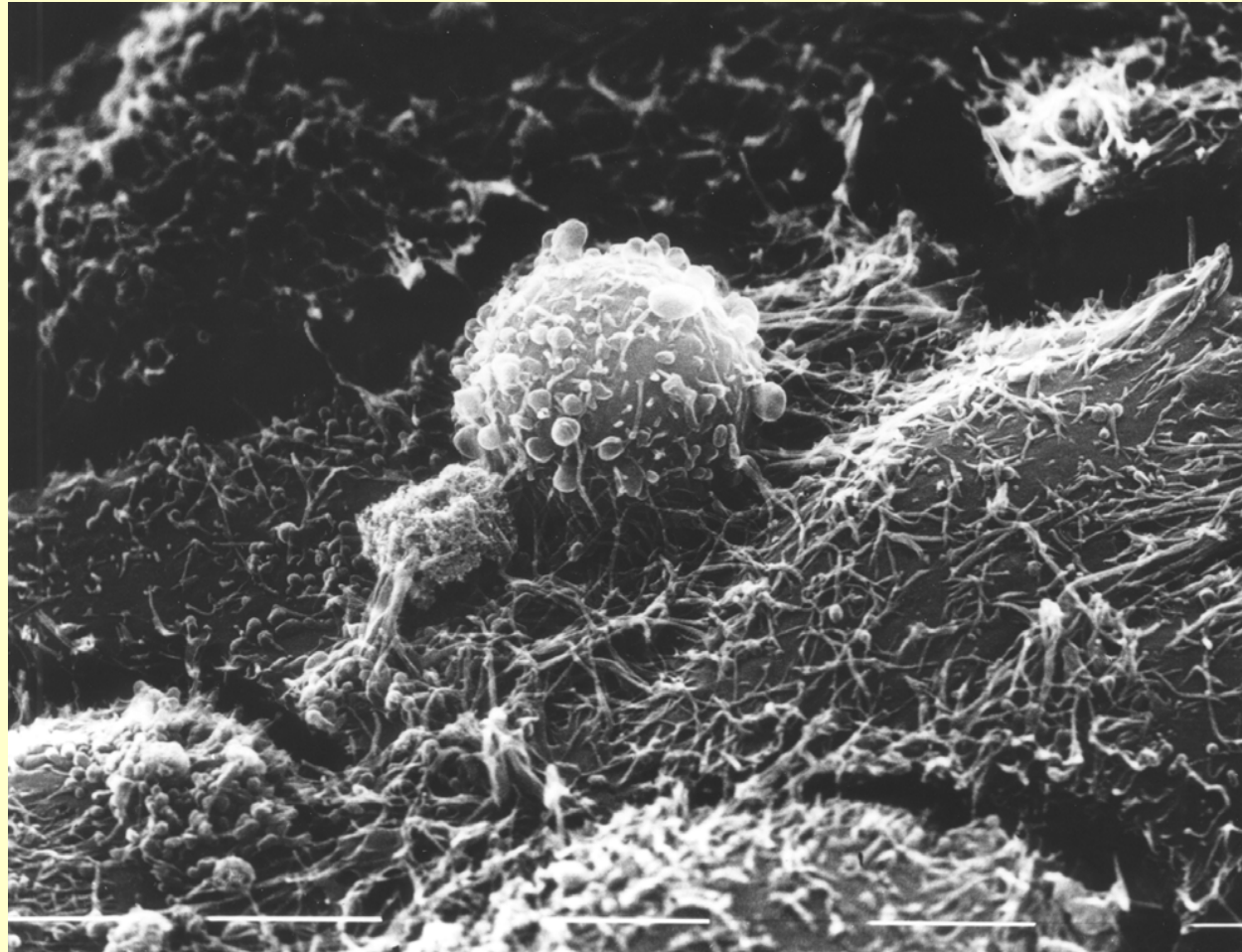
Potential diagnostic applications via TASNANO?

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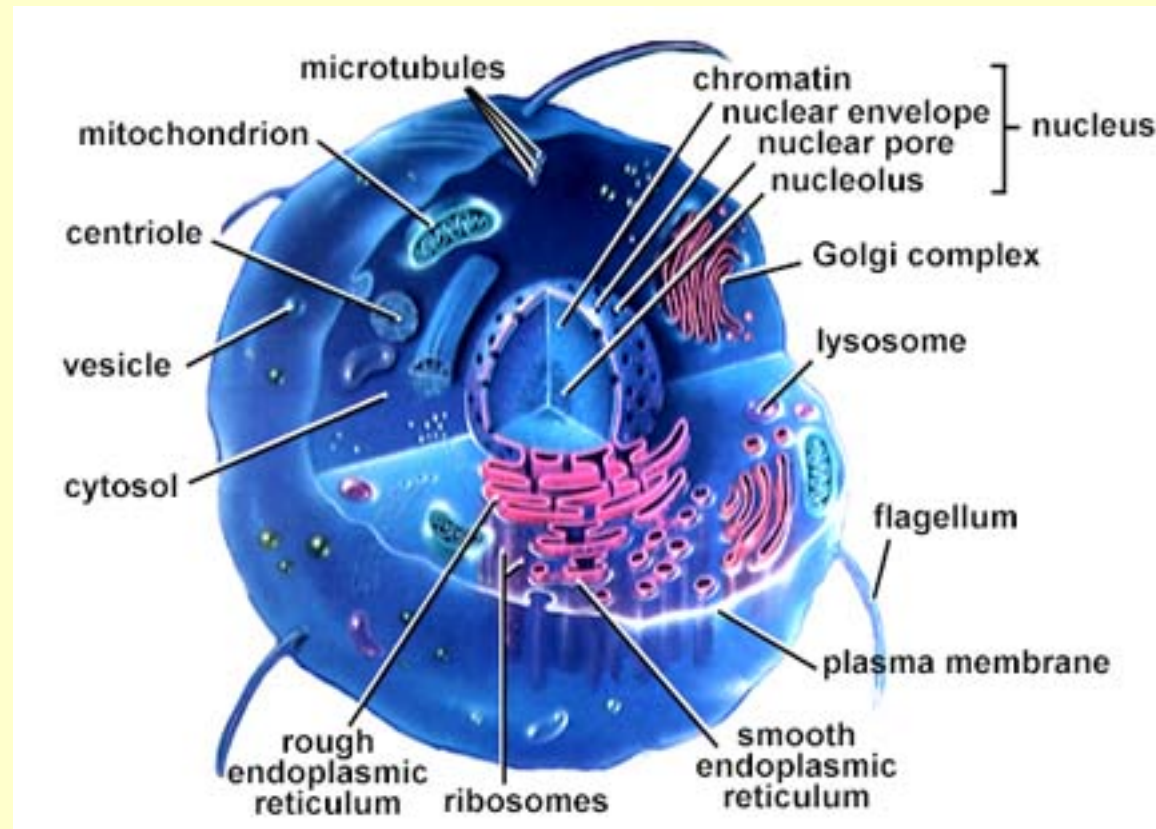
Mammary tumor cells (MaTu) *in vitro*



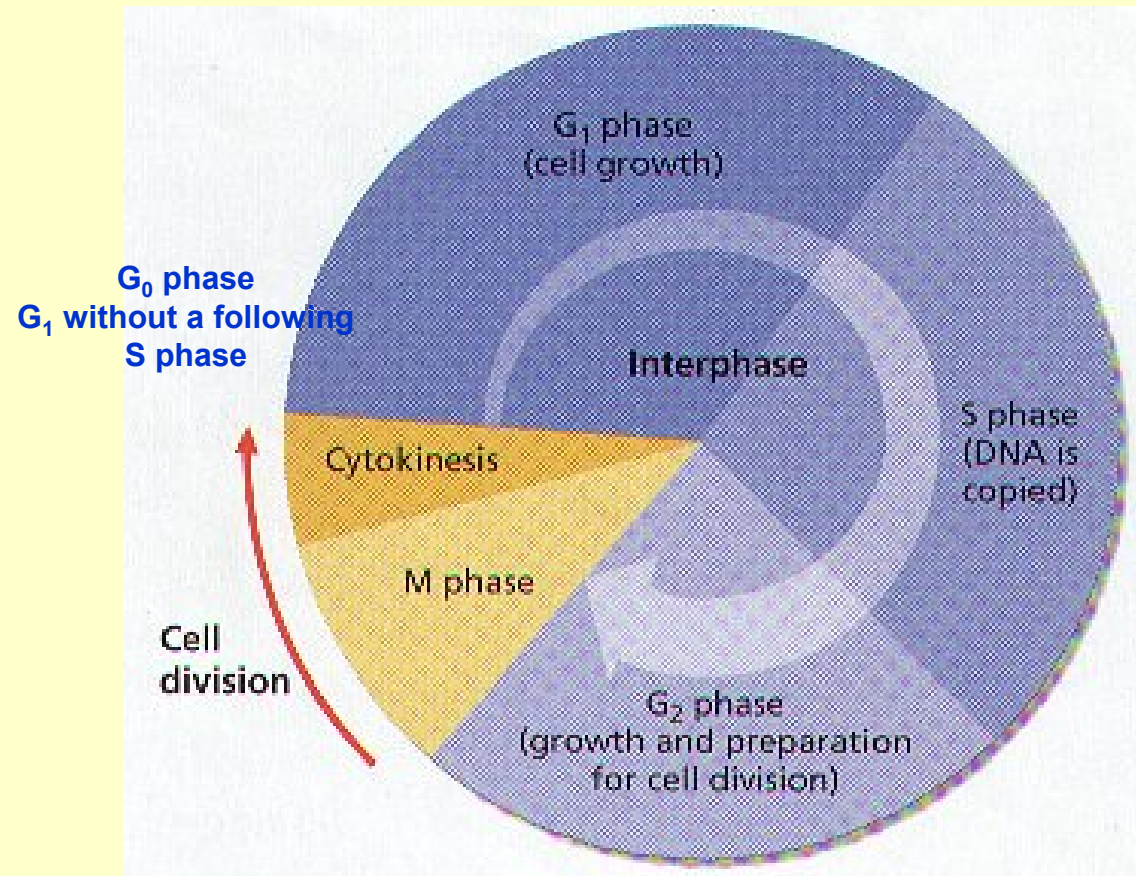
Marker: 5 μ m

H. Breter, unpublished

Model of a eucaryotic cell

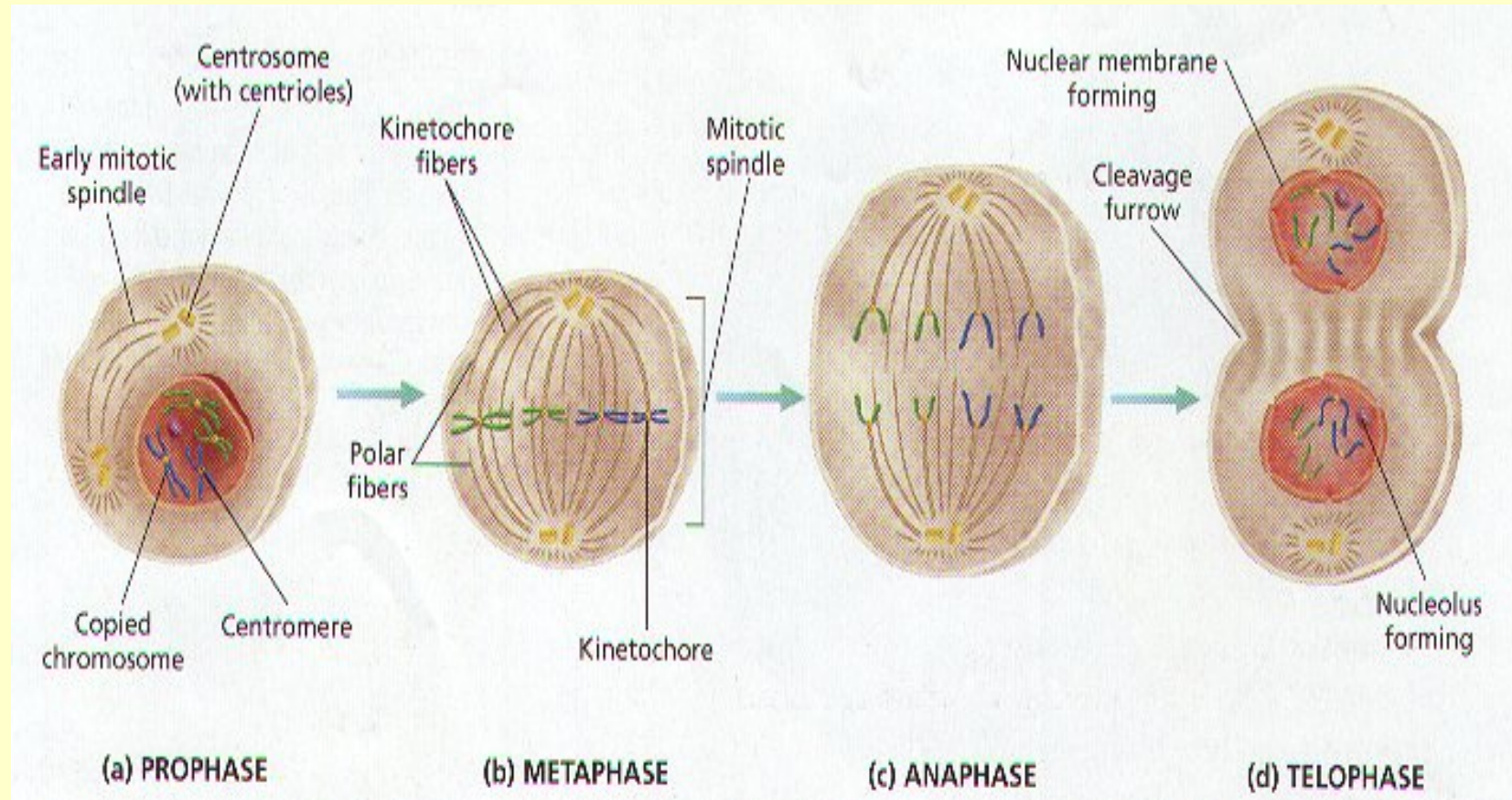


Model of the cell cycle



**proliferation
vs.
differentiation**

Mitosis



Control of eucaryotic cell proliferation

- **Equilibrated balance between GFs and GIs**
 - GF - growth (proliferation) stimulating factor**
 - GI - growth (proliferation) inhibiting factor (e.g. MDGI, TGF β ...)**

- **Apoptosis (programmed cell death)**

For every cell there is a time to live and a time to die.

There are two ways in which cells die:

- they are killed by injurious agents
- they are induced to commit suicide

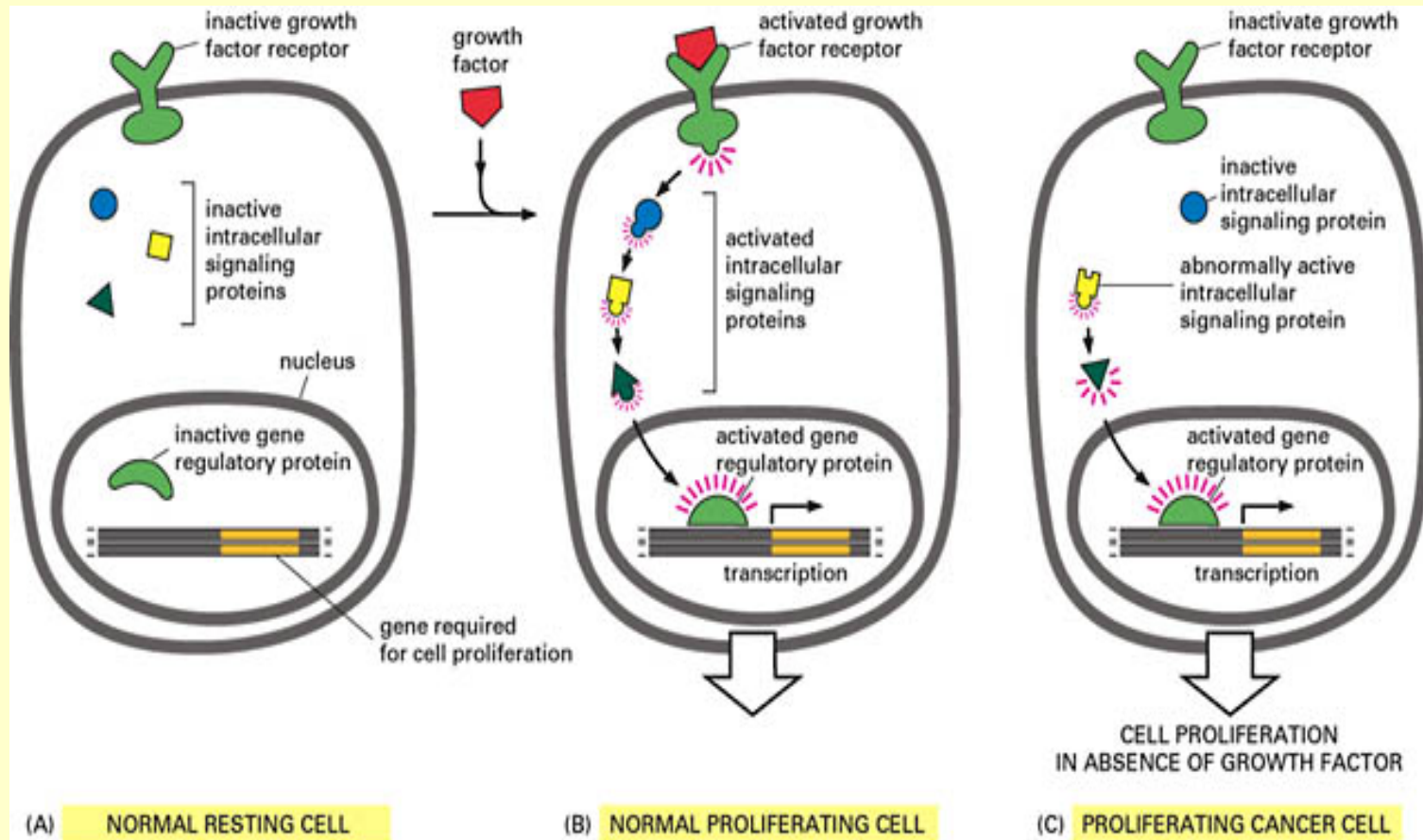
depends on

... the balance between the withdrawal of positive signals (needed for continued survival) and the receipt of negative signals

Apoptosis and cancer

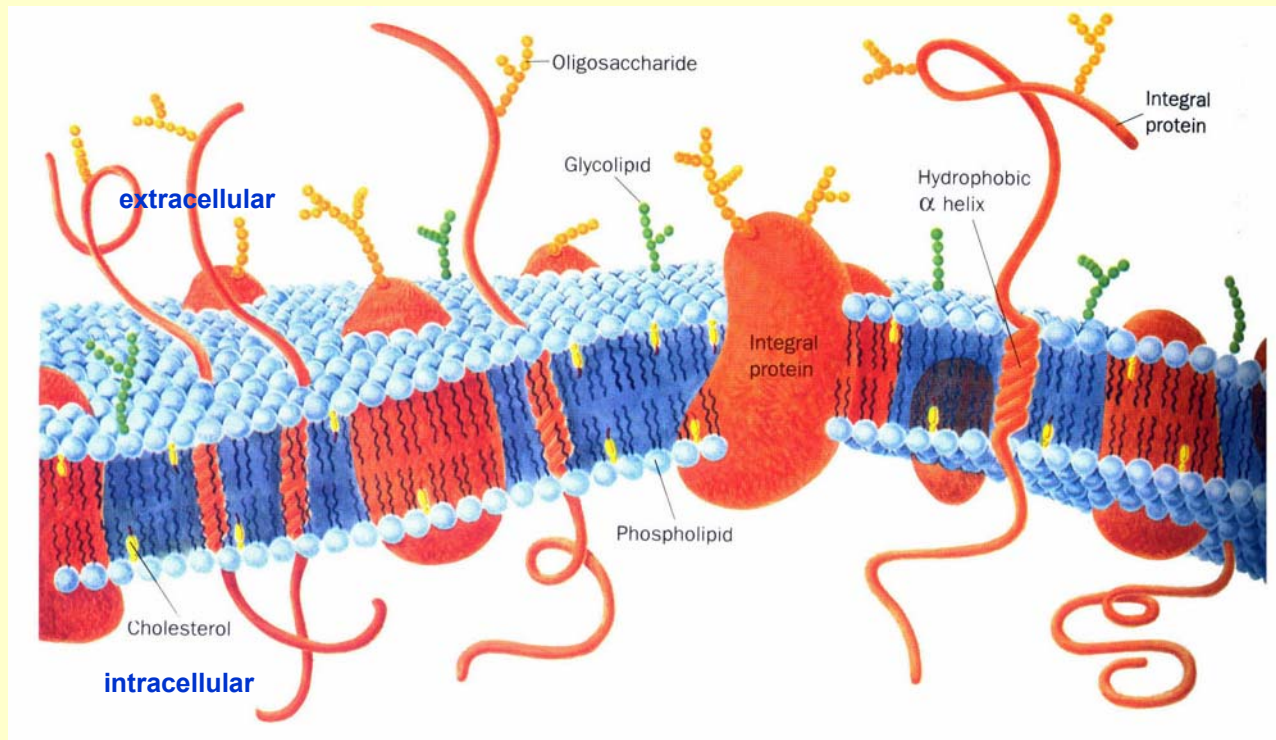
some viruses (EBV, HPV) associated with cancers use tricks to prevent apoptosis of the cells which they have transformed

Eucaryotic cell proliferation



© 1998 by Alberts, Bray, Johnson, Lewis, Roff, Roberts, Walter

Model of the plasma membrane of animal cells (fluid mosaic model)



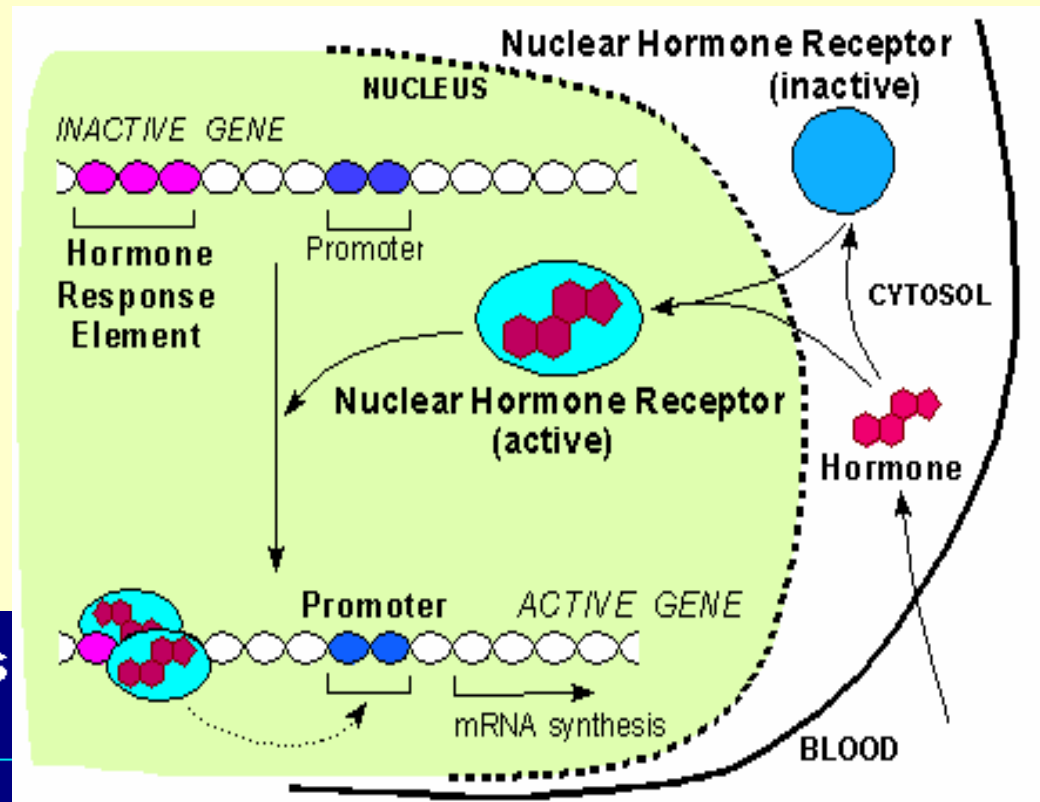
**integral & peripheral
proteins**

**glycolipids
glycoproteins**

carbohydrates

according to LJ Kleinsmith & VM Kish (Harper Collins 1995: Principles Cell Molecular Biology)

Steroid hormone activation of gene transcription



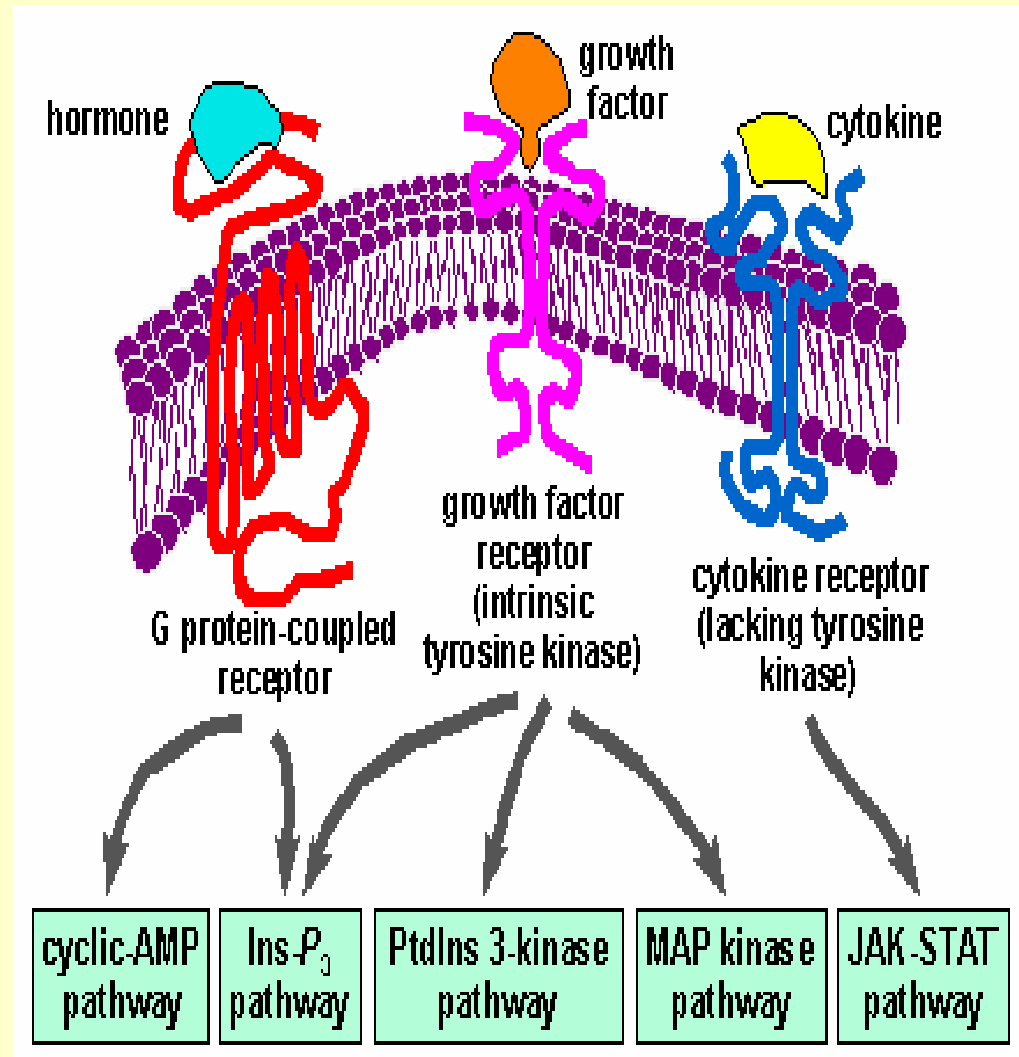
Hormone Response Elements

| Element | DNA sequence |
|---|---|
| GRE glucocorticoid | 5'-AGAACA nnnTGTTCT -3' |
| Specific amino acids in DNA binding domain include Val, Ser, and Gly. | |
| ERE estrogen | 5'-AGGTCAnnnTGACCT-3' |
| TRE thyroid hormone | 5'-AGGTCATGACCT-3' |

**Signal transduction
via
cell surface receptors**

signal transduction cascades

**additional mechanisms
but not shown
receptor-mediated endocytosis
(clathrin-coated pits)**



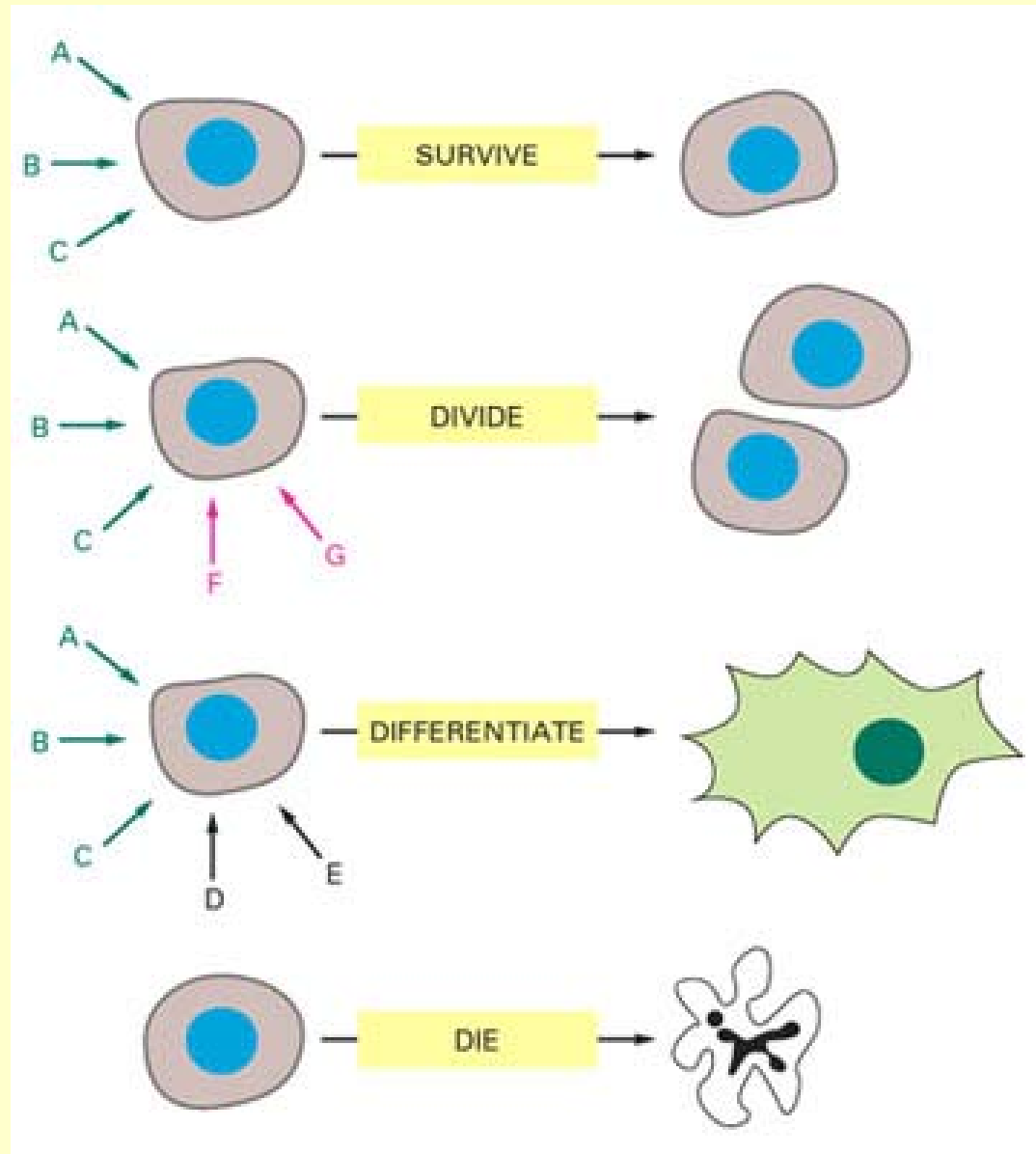


e.g. unknown constituents
of FCS

e.g. EGF, NGF, PDGF

e.g. TGF β (system)

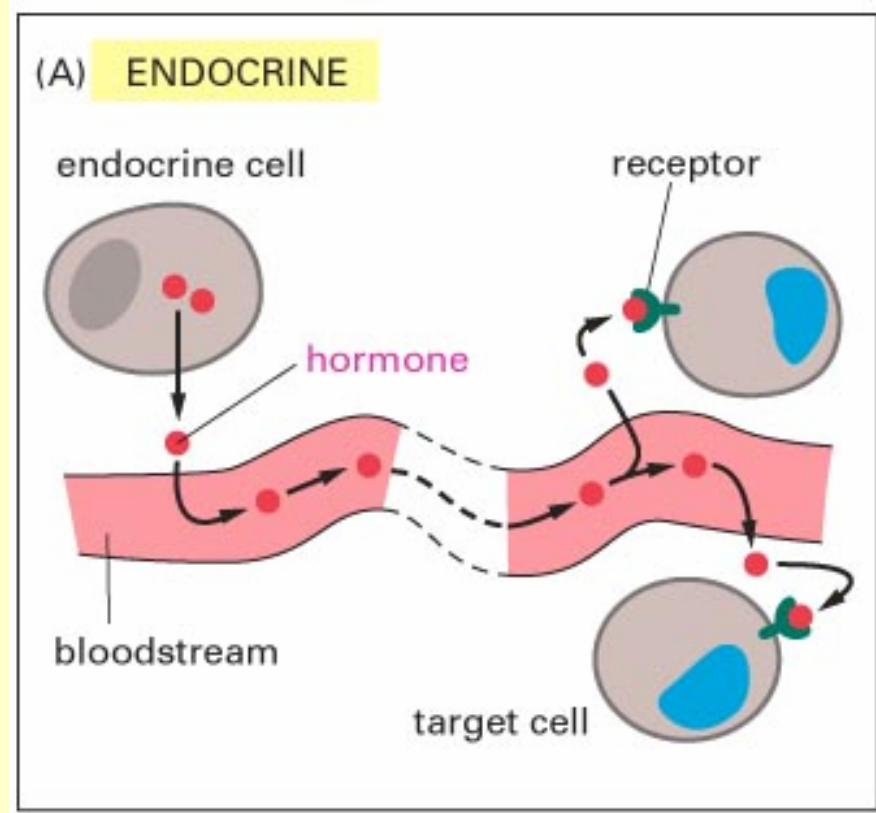
e.g. TNF α , TNF β



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Endocrine regulation of cell growth

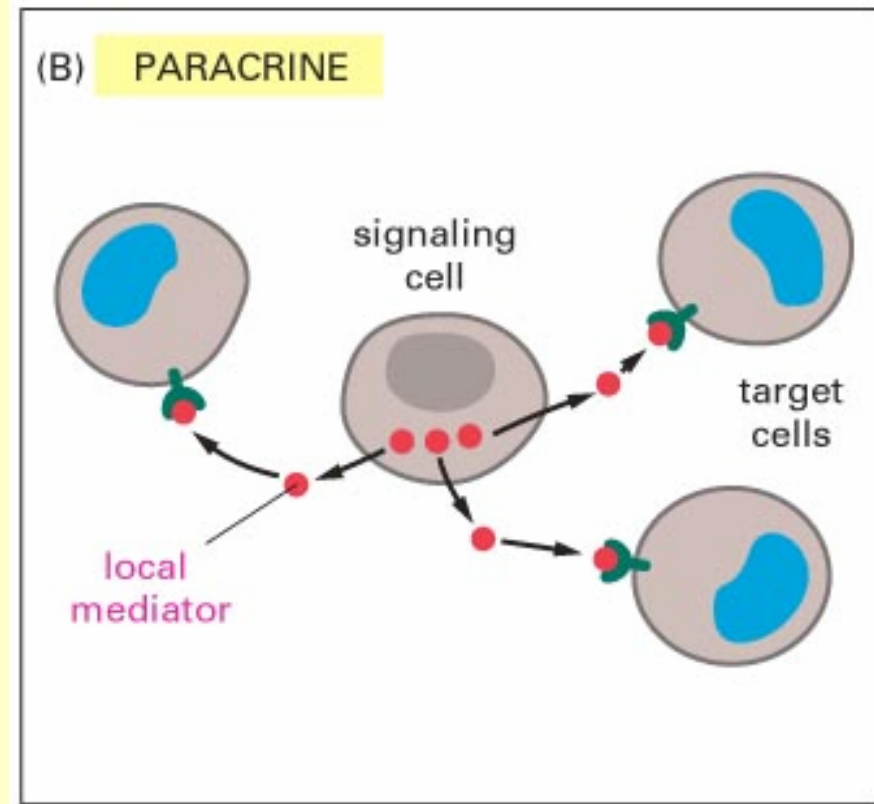
insulin
glucagon
hcG
prolactin



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Paracrine regulation of cell growth or differentiation

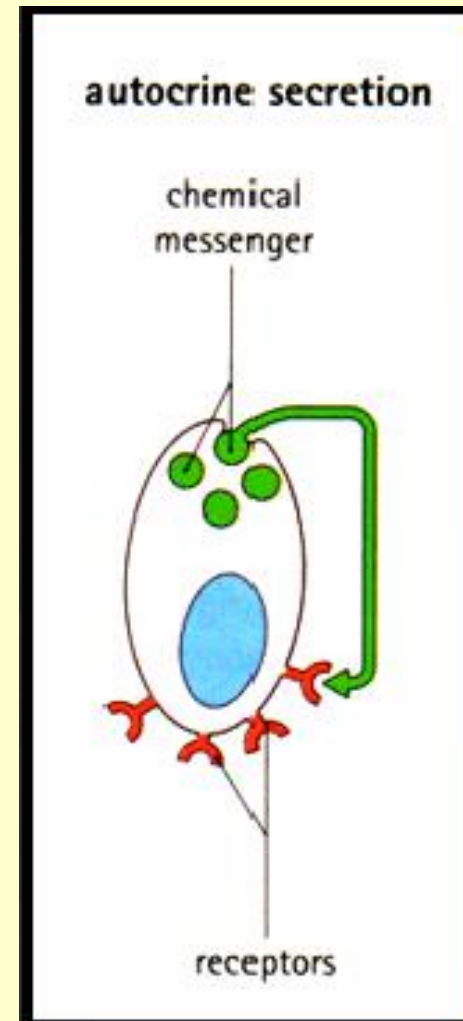
e.g. FGF7 in breast
FGF family (differentiation)



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Autocrine regulation of cell growth

e.g. interleukin 8 for ovarian cancer
gastrin for colorectal cancer
heparin-binding EGF-like growth factor for urothelial cells



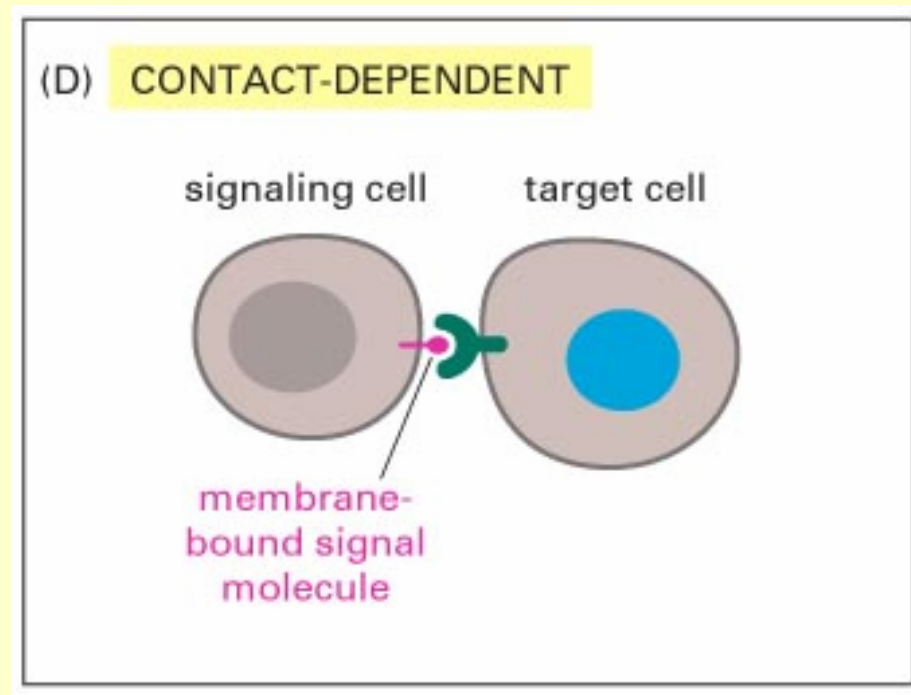
Stevens & Lowe 2nd, p. 251

Contact-dependent regulation of cell growth

**contact inhibition
(not in tumor cells)**

**e.g. for corneal endothelial cells
(after loss of contact TGF β 2)**

e.g. FAK (fokal adhesion kinase)

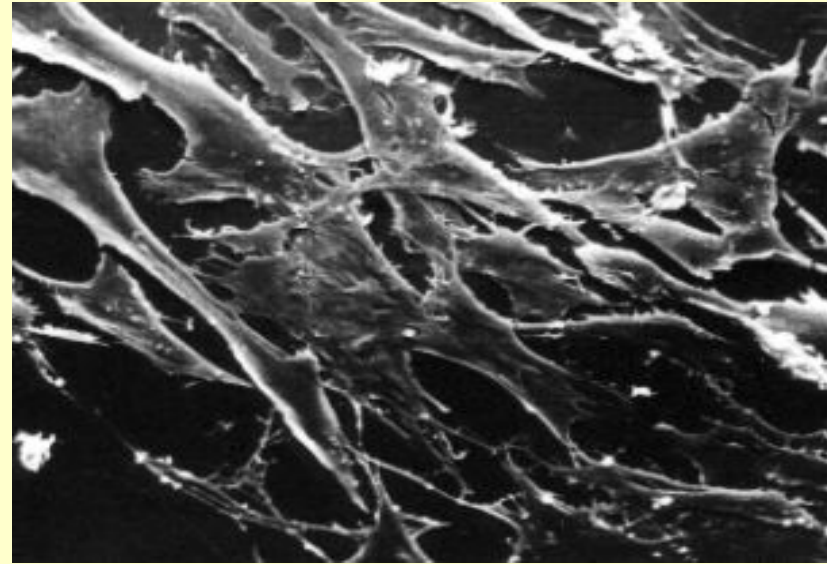


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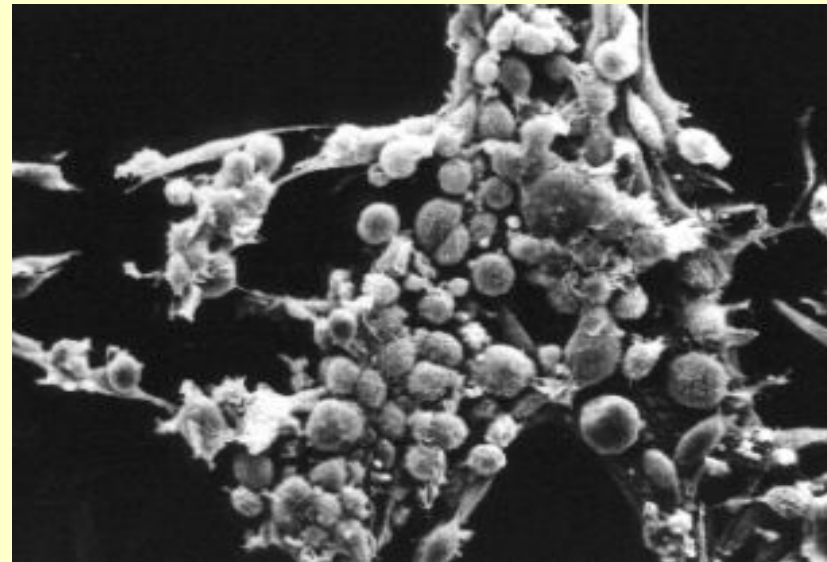
Contact inhibition

cultured 3T3-cells

normal 3T3-cells

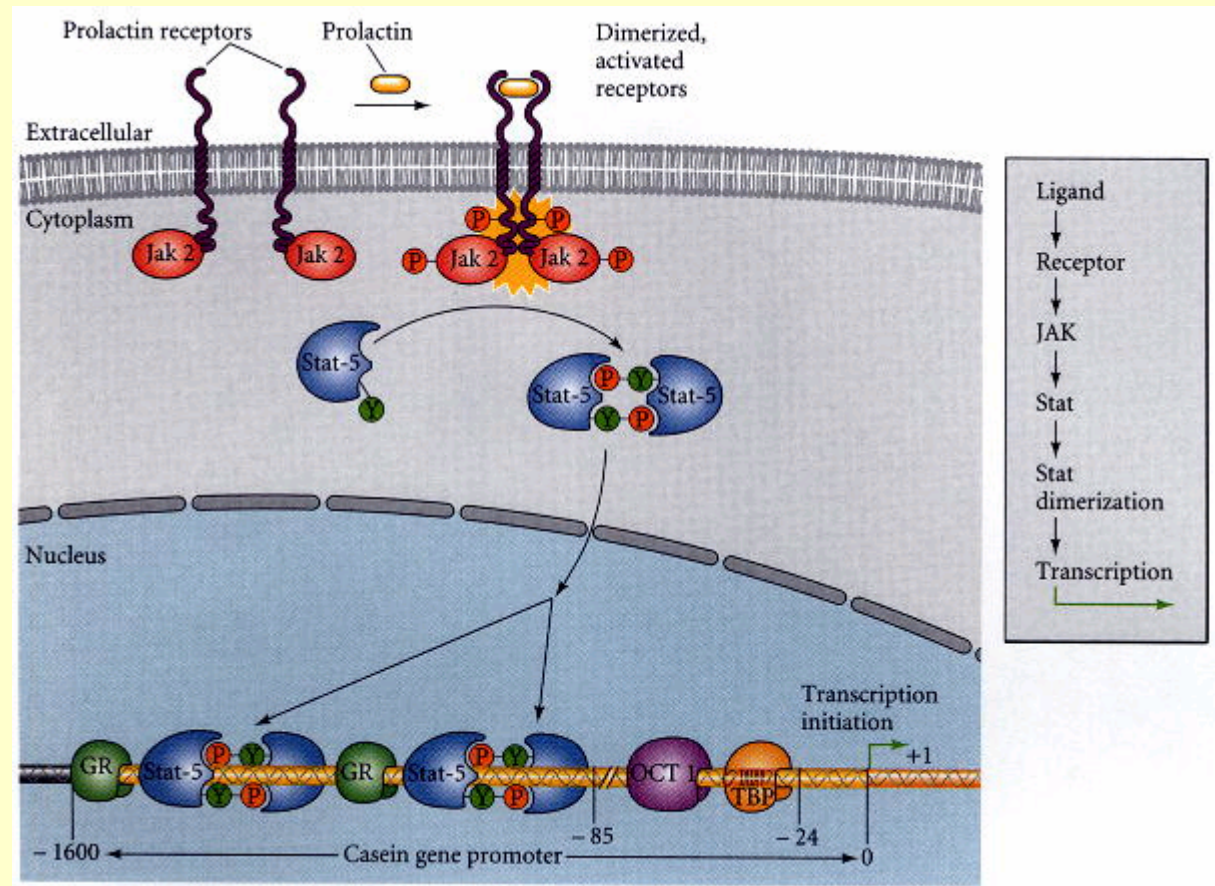


3T3-cells transformed *in vitro*



G. Steven Martin

Prolactin-induced activation of the synthesis of casein

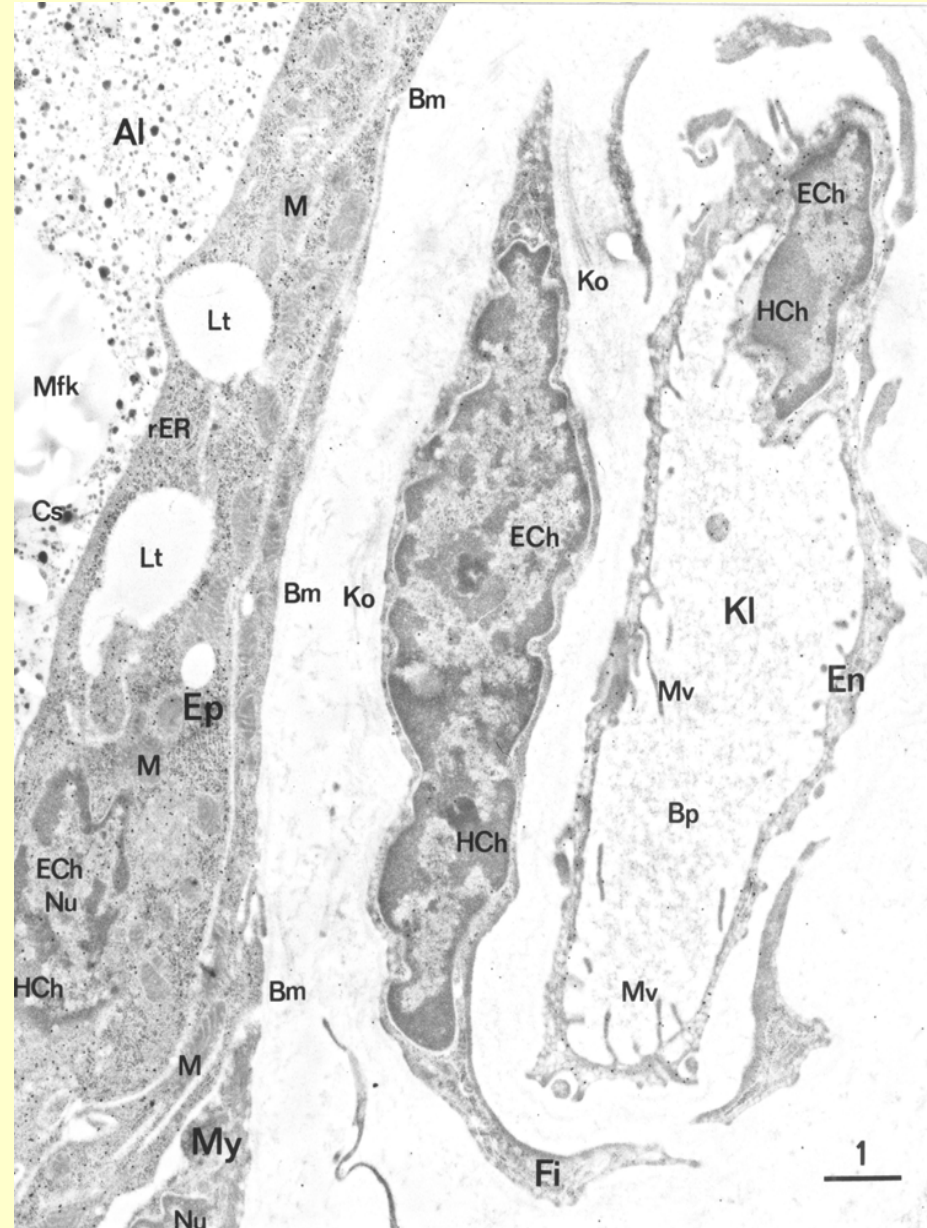


**Lactating mammary
gland**

**anti-MDGI antibodies
Au15-pA**

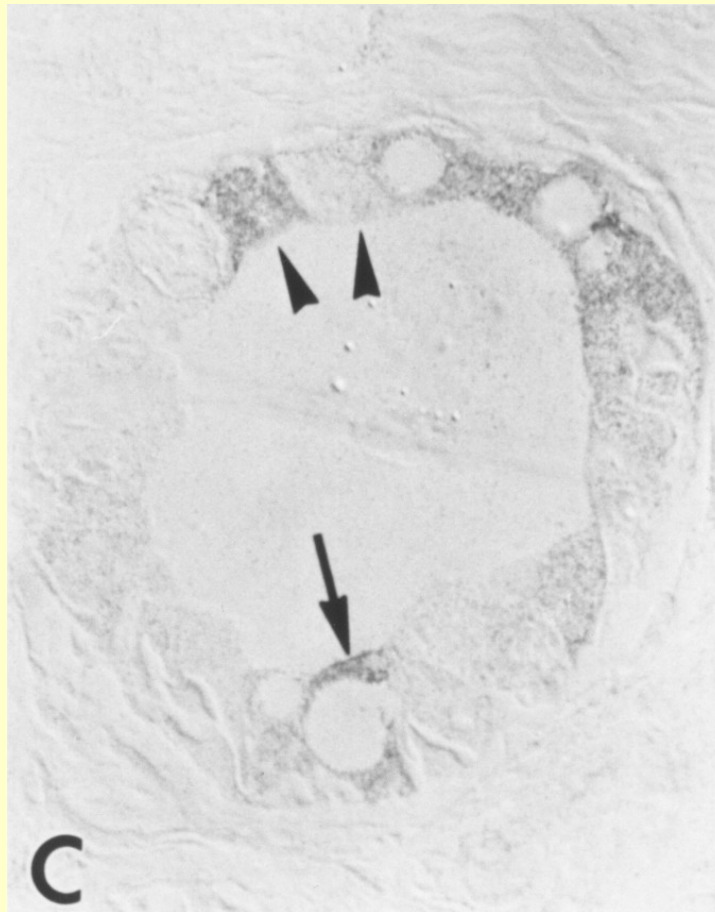
Cs – casein micelles

marker: 1 μ m



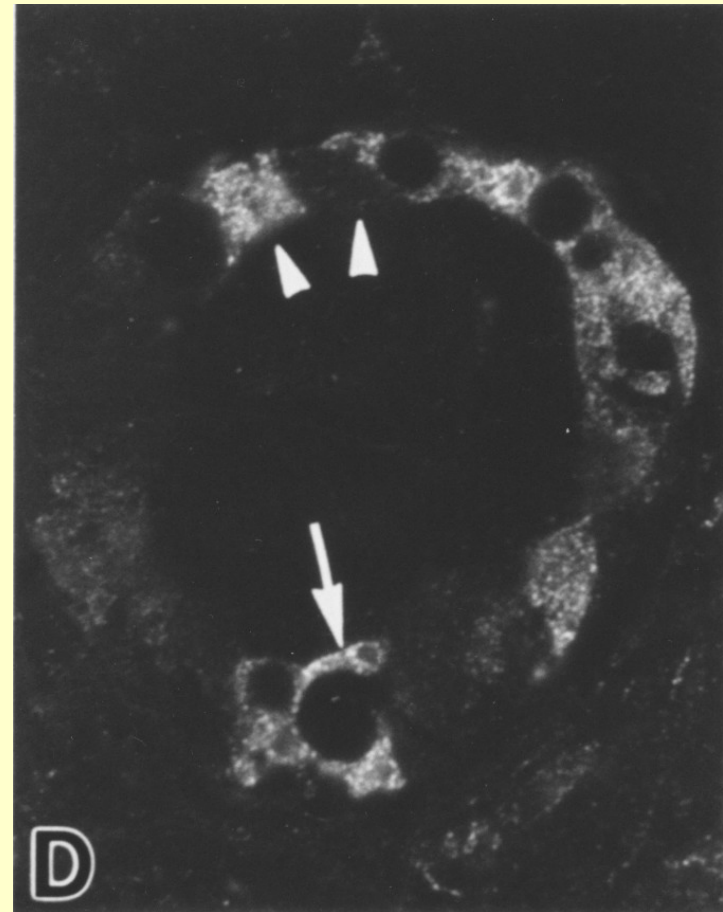
Breter & Erdmann (Cell Tissue Res [277](#):457-464)

Proliferation inhibition in the mammary gland



Erdmann & Breter (Cell Tissue Res [272](#):383-389)

DIC



Epipolarization

Proto-oncogenes, oncogenes, tumor suppressors

Oncogene

- a modified gene that increases the malignancy of a tumor cell
- oncogenes, usually involved in early stages of cancer, increase the chance that a normal cell develops into a tumor cell

SIS (PGFB)

ERB2

jun, fos

SRC/RAF

Ras

PDGF

receptor for EGF

AP-1 (heterodimer; transcription factor)

fusion protein (kinase)

membrane-associated G-protein

Proto-oncogene

- a normal gene that can become an oncogene, either after mutation or increased expression
- often involved in signal transduction and execution of mitogenic signals, usually through its gene product
- upon activation, it (or its product) becomes a tumor inducing agent, an oncogene

Tumor suppressor gene (most prominent is p53; BRCA1 & BRCA2)

- a gene that reduces the probability that a cell in a multicellular organism will become a tumor cell
- a mutation or deletion of such a gene increases the probability of the formation of a tumor
- tumor suppressors have a dampening or repressive effect on the regulation of the cell cycle or promote apoptosis

Tumor markers

substances, usually proteins, that are produced by the body in response to cancer growth or by the cancer tissue itself

e.g. CEA carcinoembryonic antigen
 PSA prostate specific antigen

some tumor markers are specific for one type of cancer
other are seen in several cancer types

many of these well-known markers are seen in non-cancerous conditions as well

GOAL: to be able to **SCREEN** for and **DIAGNOSE** cancer **EARLY**
when it is in the most treatable conditions
before it had a chance to grow and spread

Importance of tumor marker detection for

- Screening
- Diagnosis
- Stage
- Prognosis
- Guide treatment
- Monitor treatment
- Determine recurrence

ELISA (EIA) for detection of proteins (enzyme linked immunosorbent assay)

ELISA is the most widely used immunodiagnostic technique in many different clinical applications, including diagnosis of HIV, Hepatitis and others as well as hormones, tumor markers or the screening of blood banks etc.

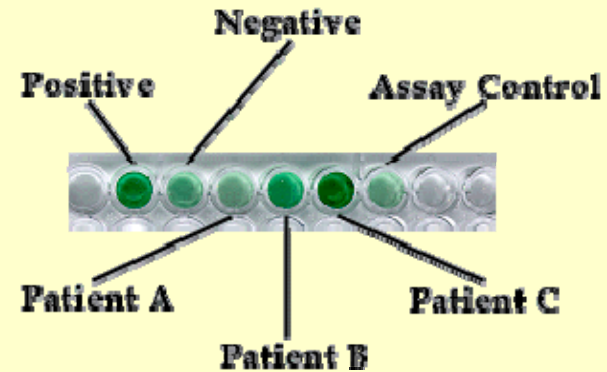
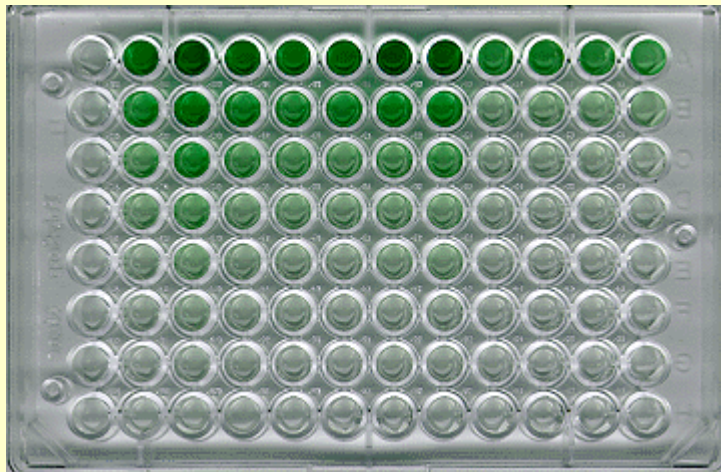
The most commonly used format is the microplate ELISA commonly known as „micro-ELISA“.

- **first published in 1971**
 - **by Engvall & Perimann (Sweden)**
 - **by van Weemen & Schuurs (Netherlands)**
- **first commercial one in 1976**
 - **to detect Hepatitis B surface antigen**

ELISA

microplate

8 x 12 matrix of 96 wells
(each 1 cm high, 0.7 cm diameter)
up to 70 μ l per well



next steps in case of positive results

- re-testing
- Western-blot

ELISA (EIA) principle

e.g. detection of anti-HIV antibodies



antigens (e.g. proteins of HIV)



primary antibodies (patient serum)

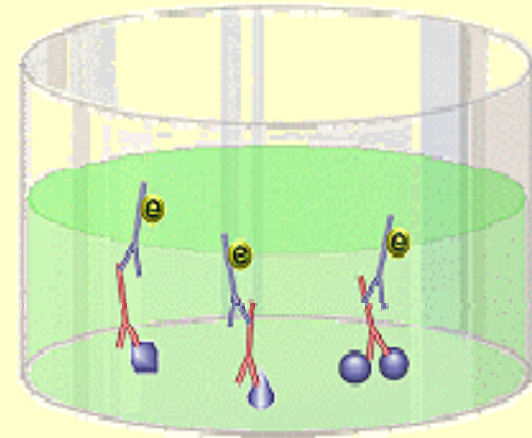


enzyme-labeled secondary antibodies

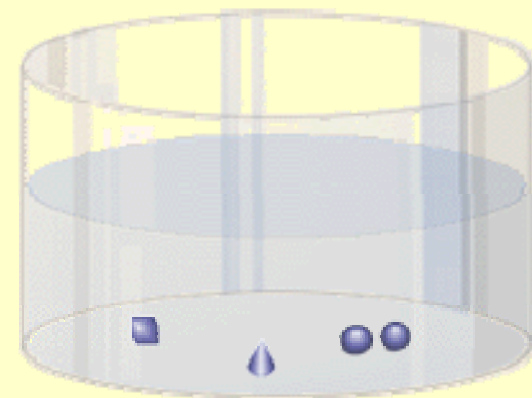


chromogen/substrate

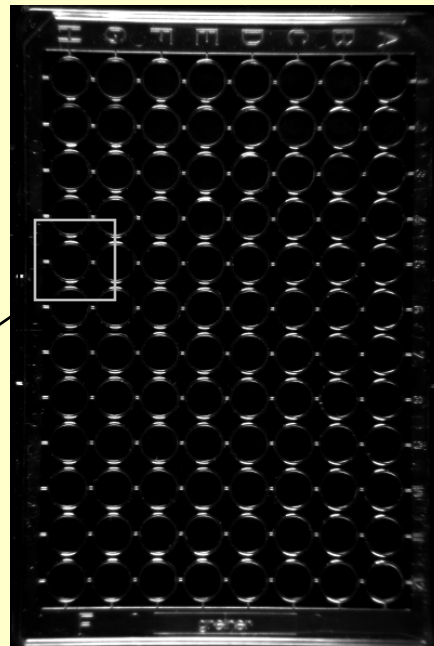
positive ELISA



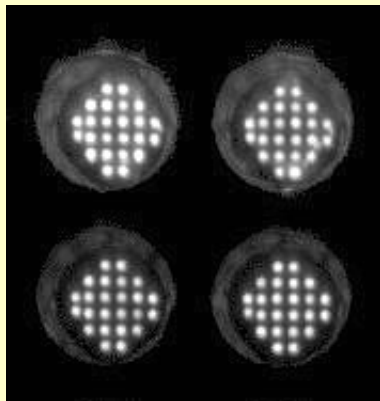
negative ELISA



Antibody Array Features by Spendlove Research Foundation



96 well plate



four enlarged wells

- chemiluminescent ELISA
- one spot equals one investigation
- up to 24 assays/well
- quantitative
- customizable analysis sw (Windows)

| Cancer | <u>A</u> <u>P</u> | <u>C</u> <u>E</u> <u>A</u> | <u>C</u> <u>A</u> <u>15-</u> <u>3</u> | <u>C</u> <u>A</u> <u>19-</u> <u>9</u> | <u>C</u> <u>A</u> <u>125</u> | <u>P</u> <u>S</u> <u>A</u> | <u>P</u> <u>S</u> <u>A</u> <u>F</u> | <u>P</u> <u>S</u> <u>A</u> <u>C</u> | <u>P</u> <u>A</u> <u>P</u> | <u>h</u> <u>T</u> <u>G</u> | <u>H</u> <u>C</u> <u>G</u> <u>b</u> | <u>F</u> <u>e</u> <u>r</u> | <u>N</u> <u>S</u> <u>E</u> | <u>I</u> <u>L</u> <u>-</u> <u>2</u> | <u>I</u> <u>L</u> <u>-</u> <u>6</u> | <u>B</u> <u>2</u> <u>M</u> | <u>A</u> <u>2</u> <u>M</u> | TOTAL |
|--------------------|----------------------|----------------------------------|--|--|------------------------------------|----------------------------------|--|--|----------------------------------|----------------------------------|--|----------------------------------|----------------------------------|--|--|----------------------------------|----------------------------------|-------|
| Lung | | | | | | | | | | | | | | | | | | 7 |
| Pancreas | | | | | | | | | | | | | | | | | | 5 |
| Kidney | | | | | | | | | | | | | | | | | | 6 |
| Breast | | | | | | | | | | | | | | | | | | 4 |
| Ovarian | | | | | | | | | | | | | | | | | | 7 |
| Cervical | | | | | | | | | | | | | | | | | | 2 |
| Uterine | | | | | | | | | | | | | | | | | | 4 |
| Testicular | | | | | | | | | | | | | | | | | | 4 |
| Prostate | | | | | | | | | | | | | | | | | | 5 |
| Liver | | | | | | | | | | | | | | | | | | 5 |
| Gastro | | | | | | | | | | | | | | | | | | 3 |
| Colorectal | | | | | | | | | | | | | | | | | | 5 |
| Bile Duct | | | | | | | | | | | | | | | | | | 1 |
| Thyroid | | | | | | | | | | | | | | | | | | 2 |
| Wilms Tumor | | | | | | | | | | | | | | | | | | 2 |
| Bladder | | | | | | | | | | | | | | | | | | 1 |
| Brain | | | | | | | | | | | | | | | | | | 1 |
| Larynx | | | | | | | | | | | | | | | | | | 1 |
| Bur kitts Lymphoma | | | | | | | | | | | | | | | | | | 1 |
| Neuroblastoma | | | | | | | | | | | | | | | | | | 2 |
| Leukemia | | | | | | | | | | | | | | | | | | 2 |
| Multiple Myeloma | | | | | | | | | | | | | | | | | | 3 |
| Lymphoma | | | | | | | | | | | | | | | | | | 1 |
| TOTAL / Marker | 5 | 8 | 5 | 7 | 9 | 1 | 2 | 1 | 2 | 2 | 10 | 6 | 4 | 2 | 4 | 5 | 1 | 74 |

Spendlove Research FOUNDATION

CEA - carcino embryonic antigen
 Ferr - Ferritin
 PSA - free prostatic specific antigen
 + prostate specific antigen
 + alpha(1)antichymotrypsin complex
 PSAF - free prostatic specific antigen
 A2M - alpha 2 macroglobulin
 B2M - beta 2 microglobulin
 ...



Immuno-PCR

chimera biotec GmbH

Biomedicinecentre Dortmund
Emil-Figge-Str. 76a
D-44227 Dortmund
www.cimera-biotec.com

LDN

Labor Diagnostika Nord GmbH & Co KG
Am Eichenhain 1
D-48531 Nordhorn
www.ldn.de

M. Adler et al: Detection of **femtogram amounts** of biogenic amines using sel-assembled DANN-protein nanostructures

with specific antibody-DNA conjugates ...

up to 1000-fold improvement of the detection limits accessible in conventional microplate format