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Tkáňové inženýrství: cesta k náhradám tkání a orgánů

Petr Vaňhara

Ústav histologie a embryologie| Lékařská fakulta | Masarykova univerzita
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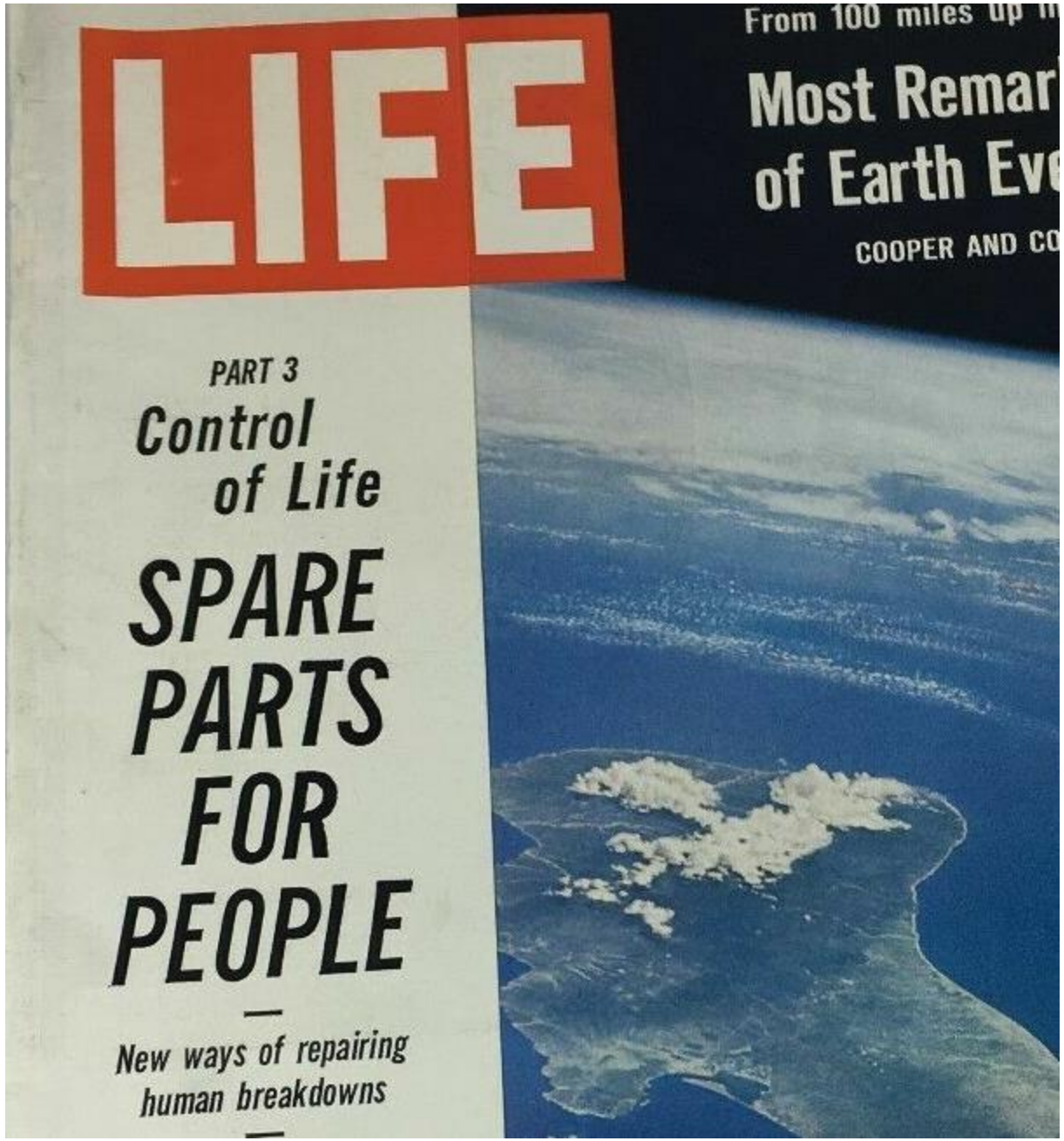
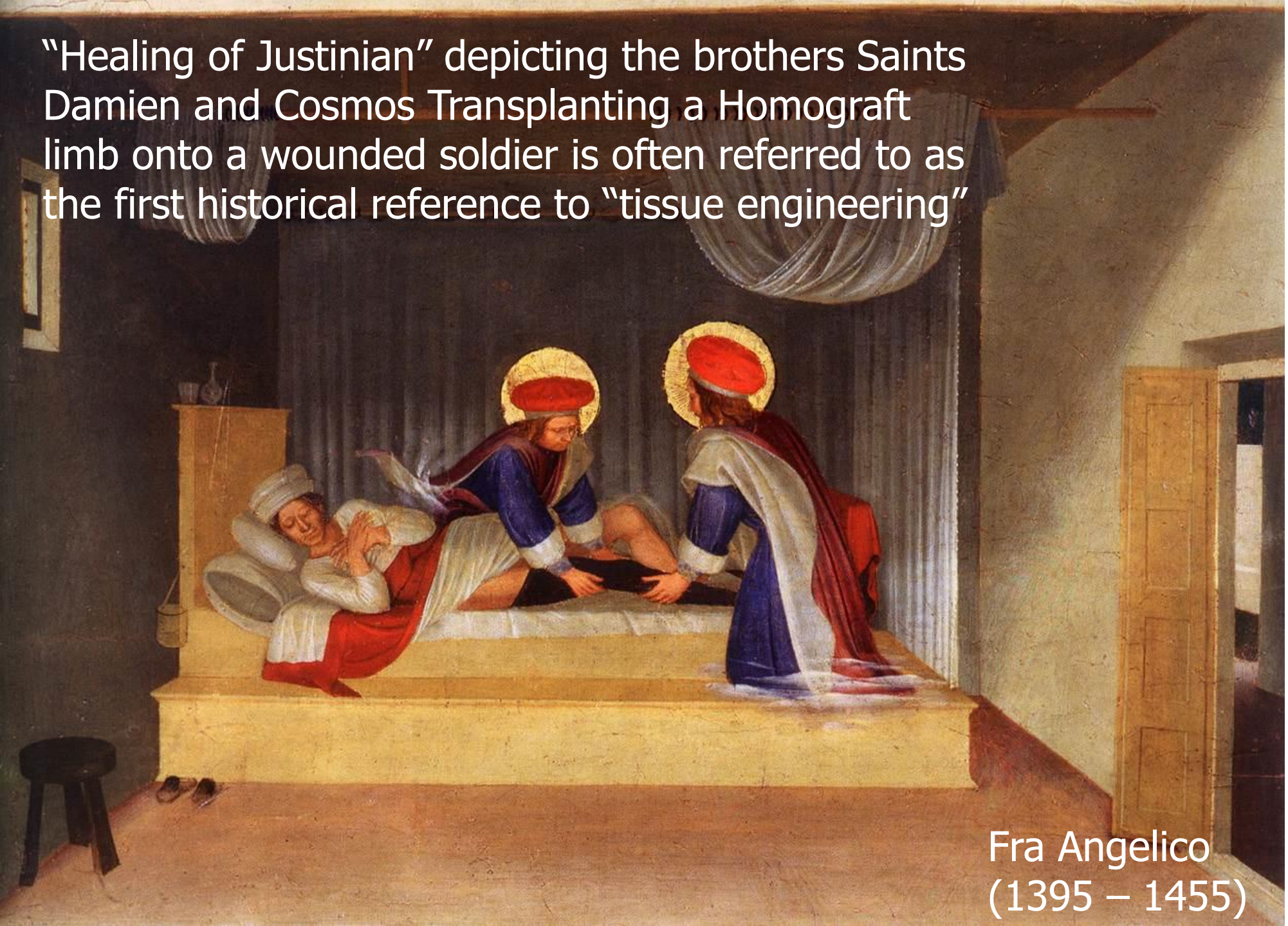
I. Základní principy vývoje tkání



9th June 2022

FRANKENSTEIN'S MONSTER Robots 'to wear living skin' to make human-like and aid relations

ROBOTS of the future could be coated in 'living skin' that makes them look and feel human.


"Healing of Justinian" depicting the brothers Saints Damien and Cosmos Transplanting a Homograft limb onto a wounded soldier is often referred to as the first historical reference to "tissue engineering"
Fra Angelico
(1395 – 1455)

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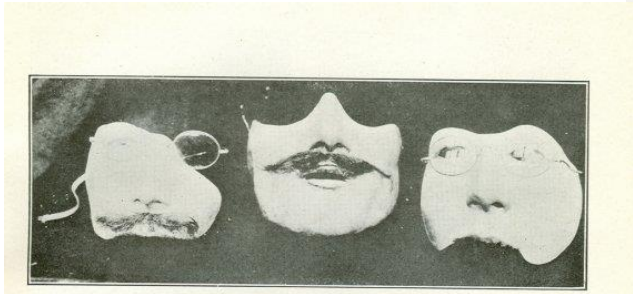
Pokusy o náhrady tkání a orgánů nejsou recentní záležitost



Masks made by Mrs Ladd, of the American Red Cross, Paris.

Driver F.

88–3

Credit: Wikipedia

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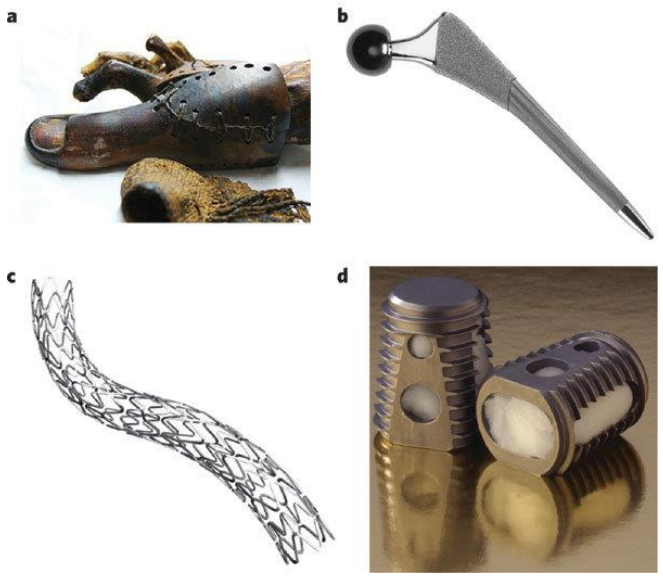
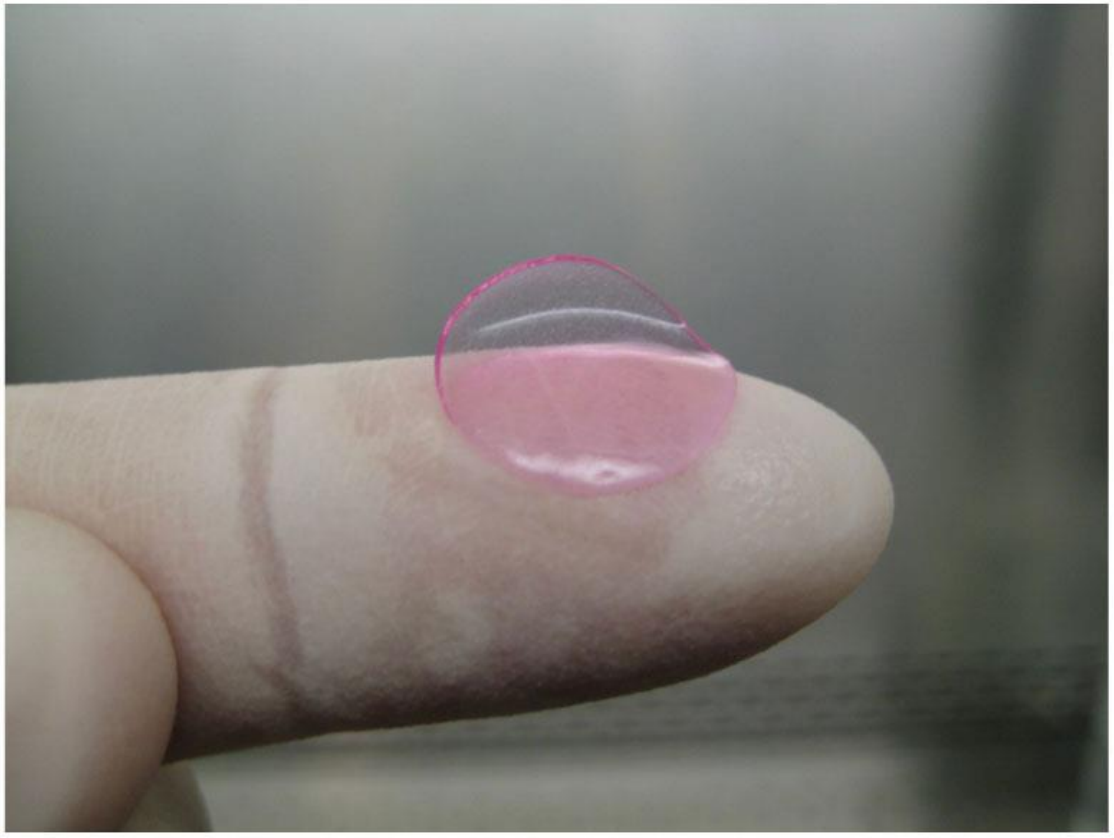
Biomateriály



Tkáňové inženýrství využívá **buňky**, **biomateriály** a **biochemické, chemické a fyzikální podněty,** a jejich kombinace, ke tvorbě tkání a tkáním podobným struktur.



A mini bioengineered human liver that can be implanted into mice. Source: Sangeeta Bhatia, MIT

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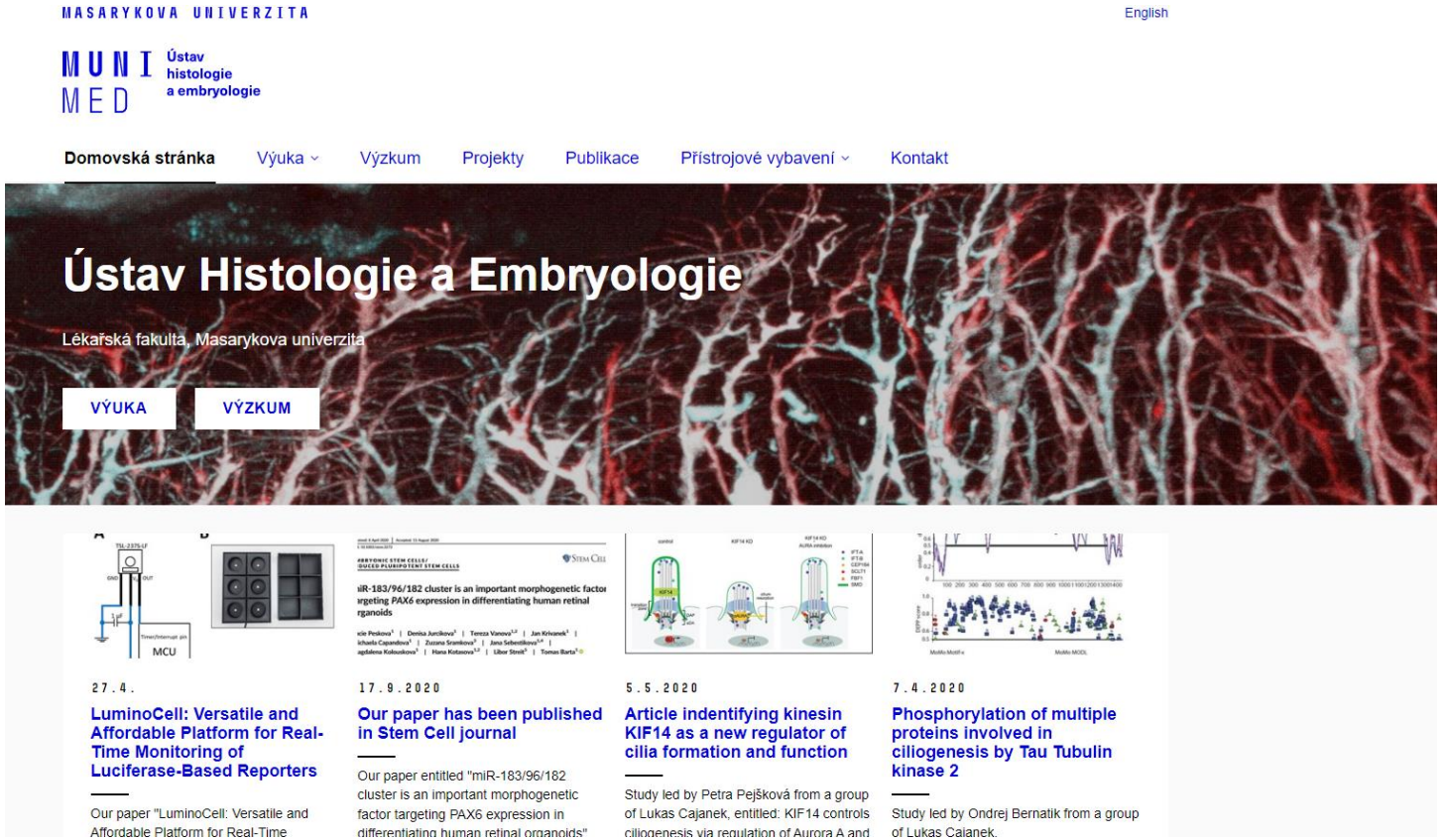
Princip náhrady tkání a orgánů není nový, současné medicíně se však nabízí nové postupy postavené na **pochopení struktury a funkce tkání.**

Doc. MVDr. Aleš Hampl, CSc.

www.histology.med.muni.cz



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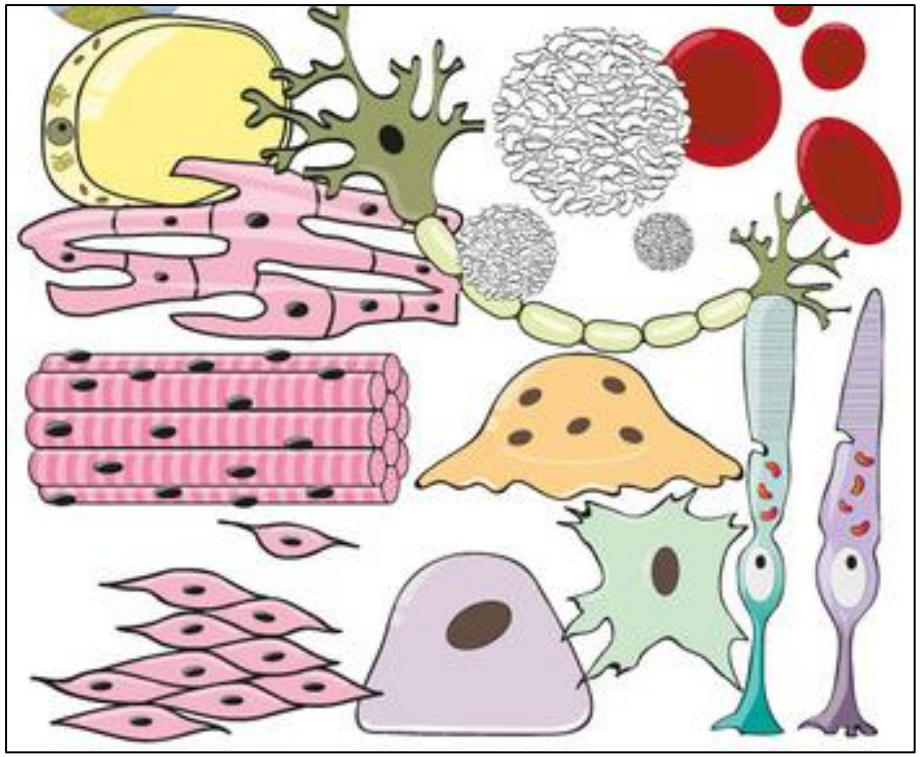
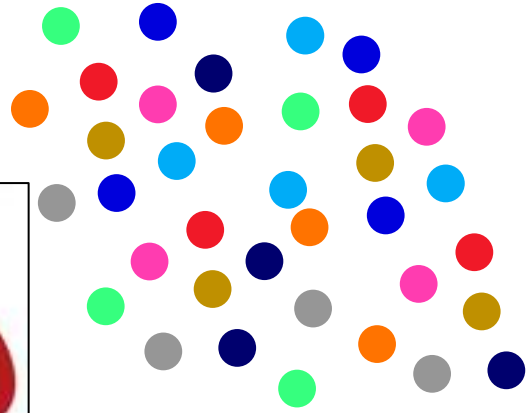
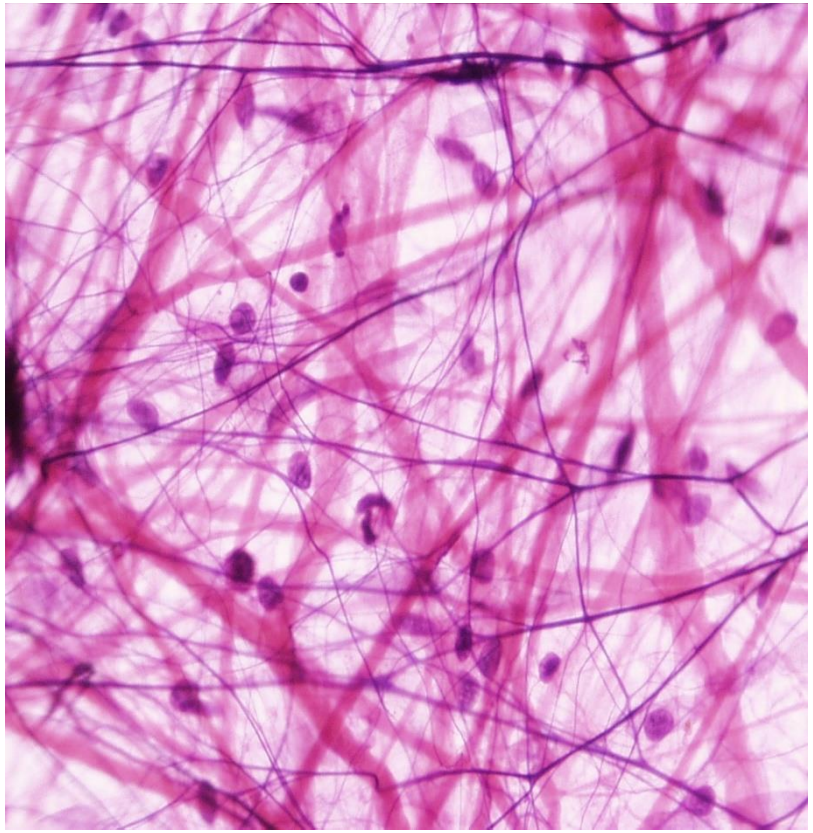
SOLUBILNÍ SIGNÁLNÍ MOLEKULY

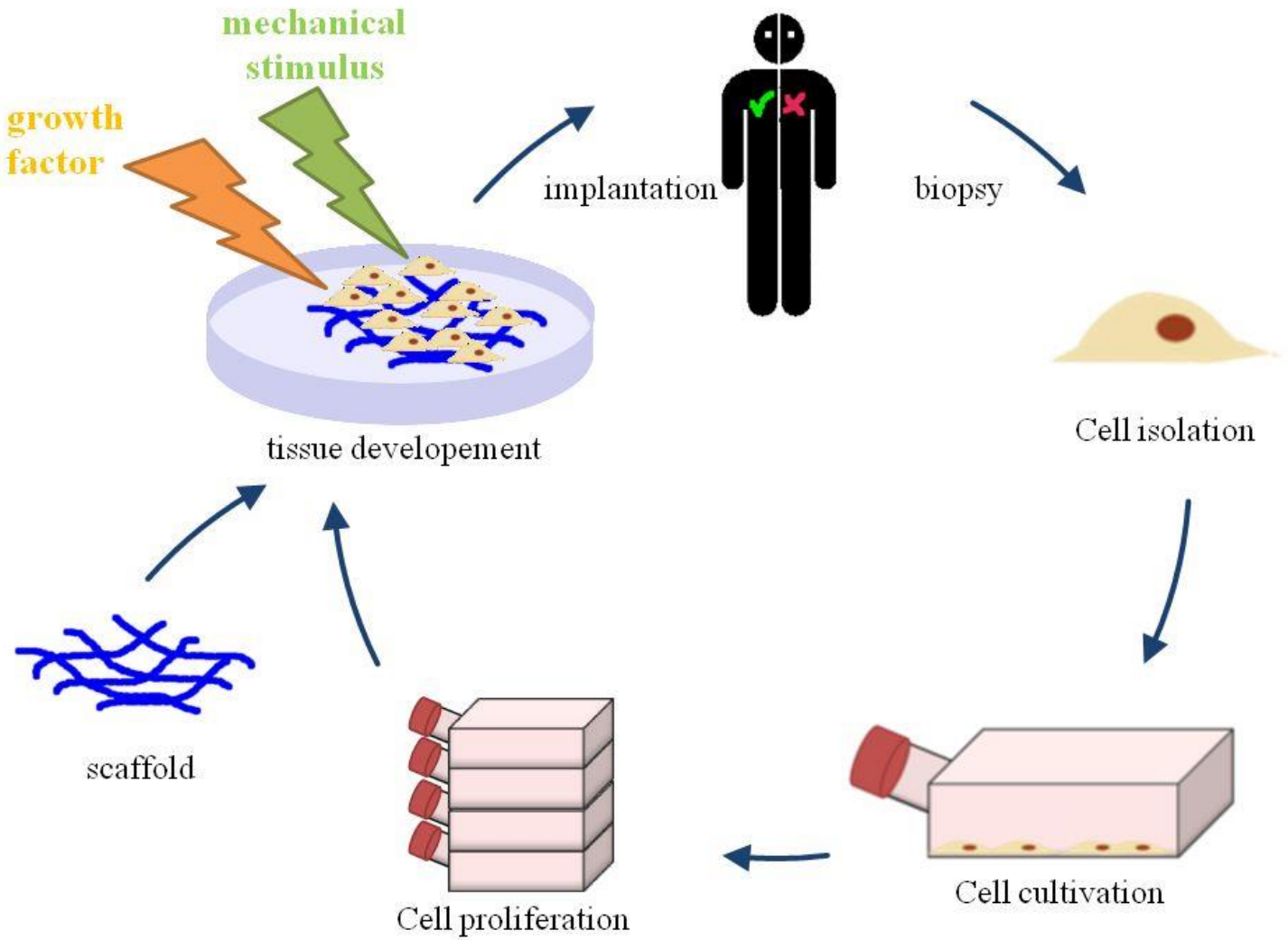
BUŇKY



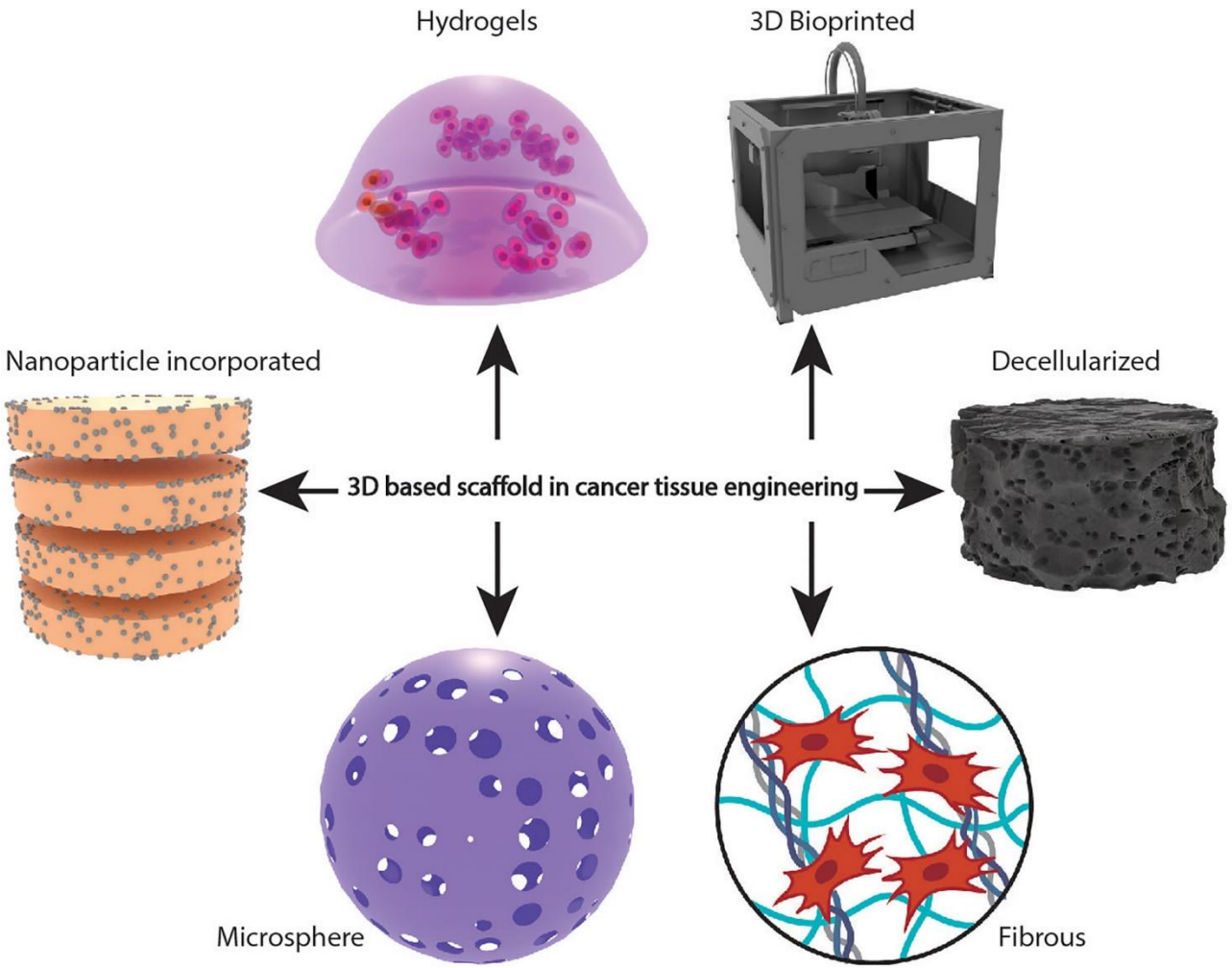


EXTRACELULÁRNÍ MATRIX

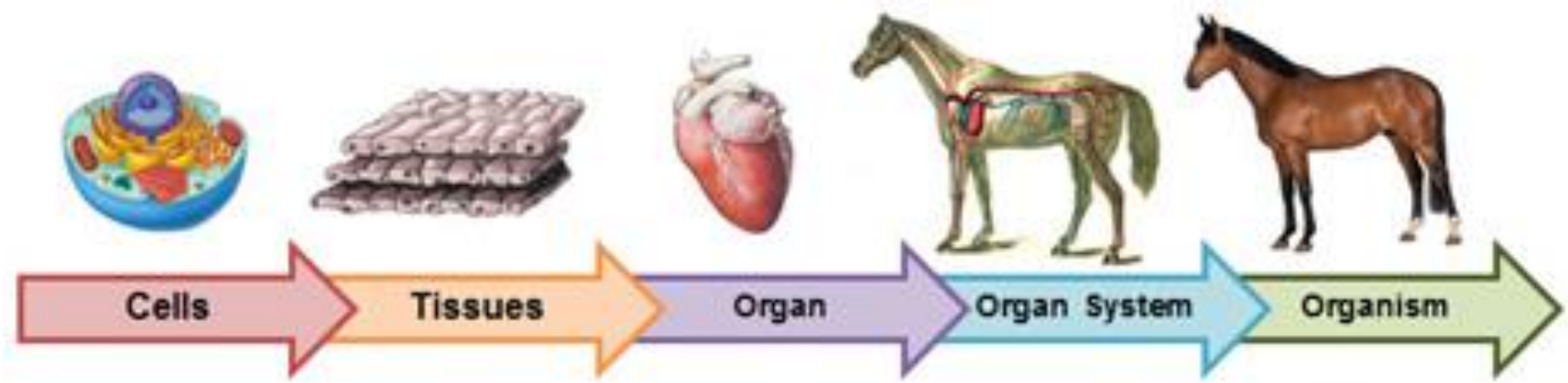




https://doi.org/10.3389/fonc.2021.733652



https://doi.org/10.3389/fonc.2021.733652



Buňky

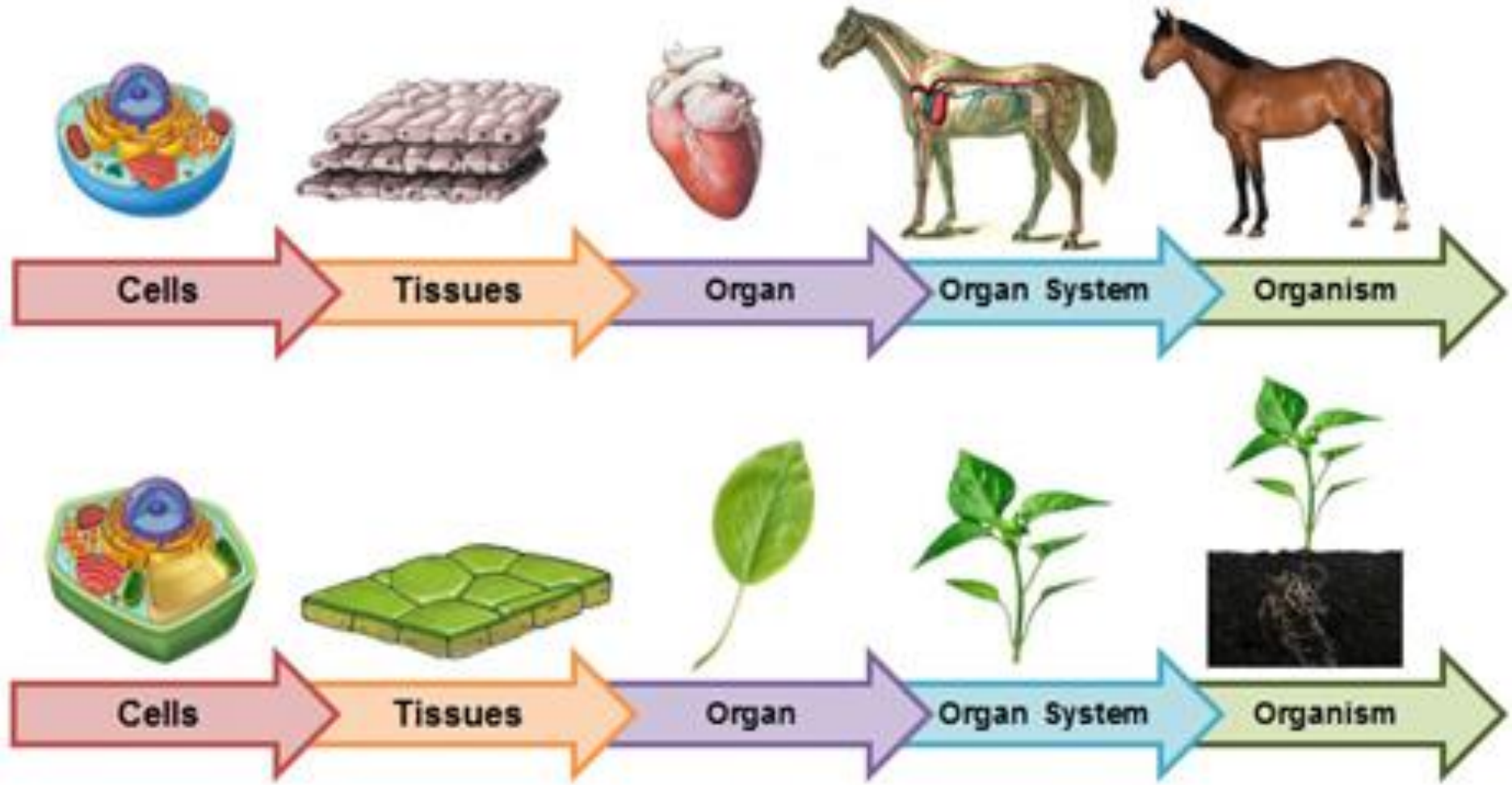
- 6×10^{13} buněk v lidském těle
- více než 200 různých typů

Tkáně

- Funkční seskupení morfologicky podobných buněk

Orgány

- Funkční kombinace různých tkání


Cells
Tissues
Organ
Organ System
Organism
Cells
Tissues
Organ
Organ System
Organism

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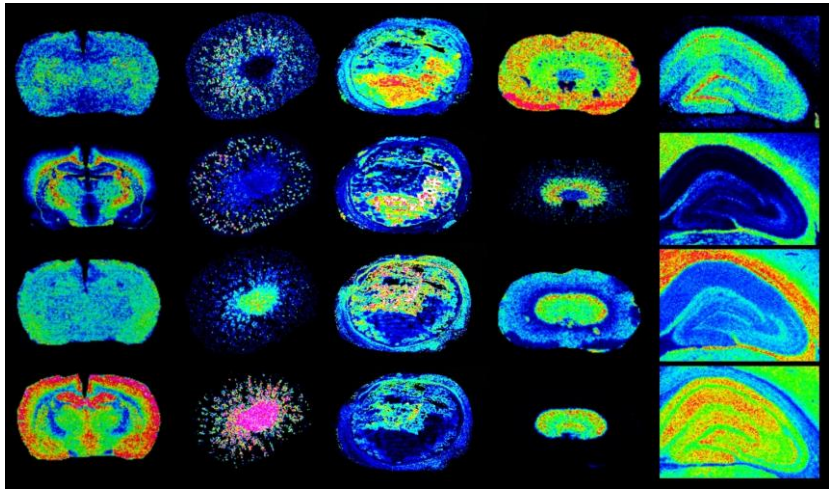
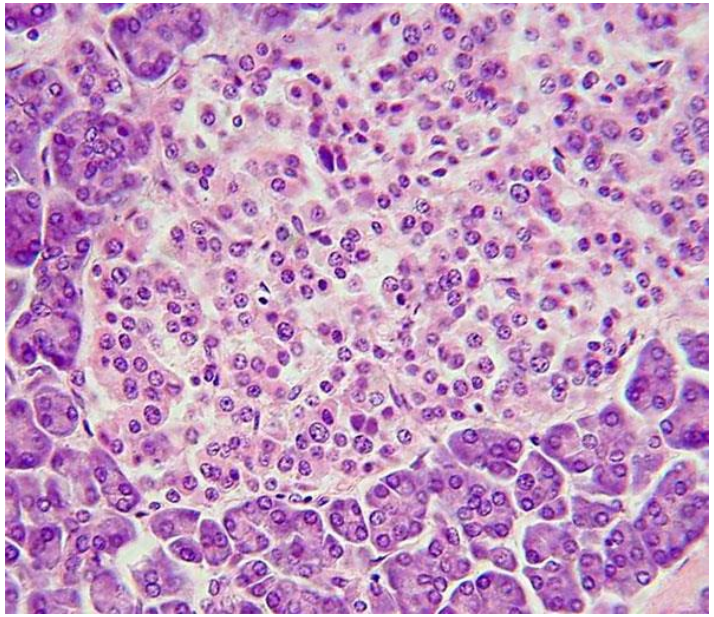
TKÁNĚ

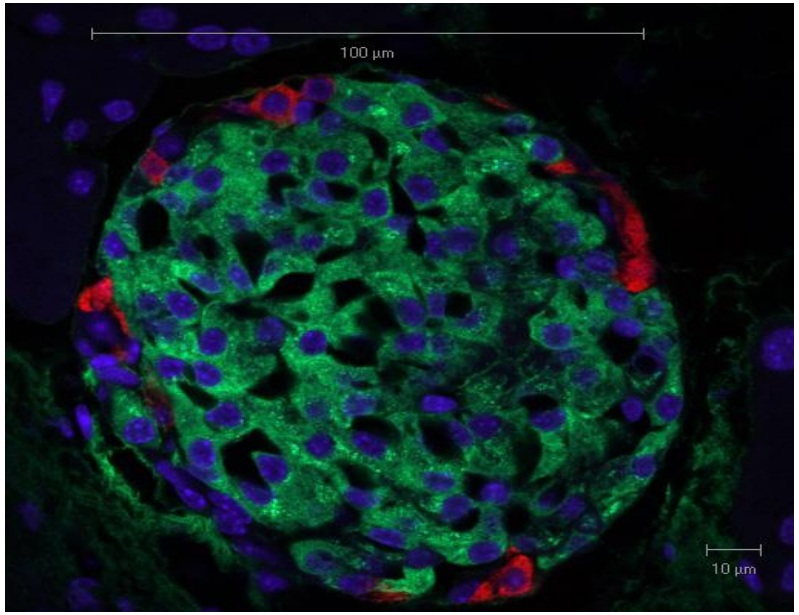
Funkční, **trojrozměrné**, organizované seskupení morfologicky podobných buněk a jejich produktů a derivátů

klasická histologická definice tkání je založena na mikroskopické vizualizaci



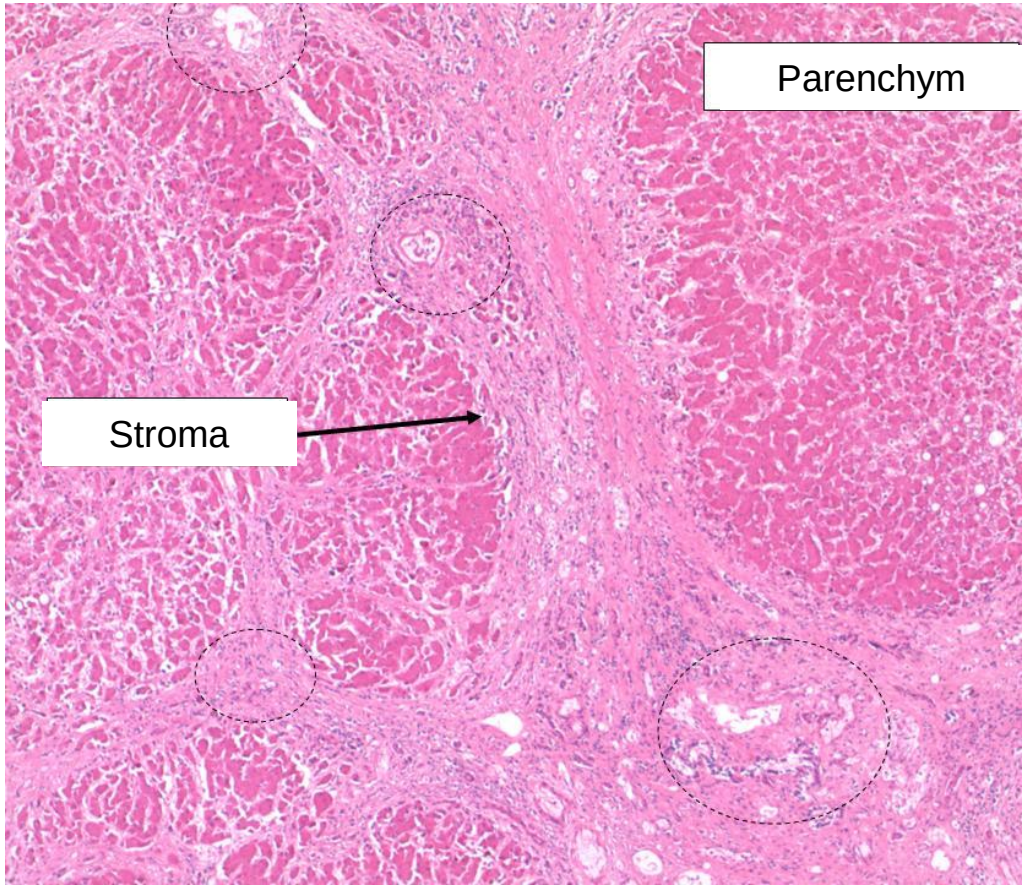
Credit: Wikipedia, ImaBiotech





Parenchym: vlastní funkční tkáň konkrétního orgánu (jaterní, plicní, pankreatický, ledvinný parenchym)

Stroma: okolní podpůrná, intersticiální tkáň



Příklad: jaterní tkáň

Parenchym:
Funkční komponenta

- Hepatocyty
- Sinusoidy a přidružené struktury

Stroma:
Podpůrná komponenta

- Vazivo a s ním spojené struktury
- Cévy
- Nervy
- Žlučovody

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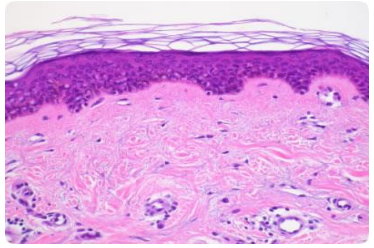
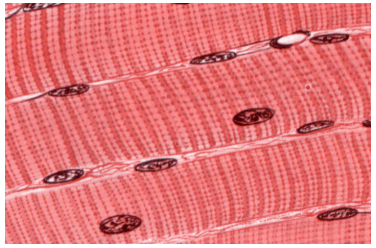
SOUČASNÁ KLASIFIKACE TKÁNÍ

Epitelová



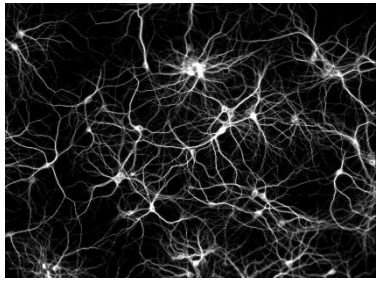
Kontinuální, avaskulární vrstvy buněk s různou funkcí, orientovaných do volného prostoru, se specifickými mezibuněčnými spoji a minimem mezibuněčného prostoru a ECM
Deriváty všech tří zárodečných listů

Svalová



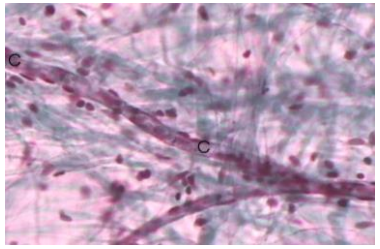
Obsahují myofibrily → schopnost kontrakce
Derivát mezodermu - KS, myokard, mezenchymu - HS
Výjimečně ektoderm (např. m. sphincter a m. dilatator pupillae)

Nervová



Neurony a neuroglie
Příjem a přenos elektrického vzruchu
Derivát ektodermu, výjimečně mezenchymu (mikroglie)

Pojivová



Dominantní přítomnost extracelulární matrix
Vazivo, chrupavka, kost, tuková tkáň
Derivát zejména mezenchymu

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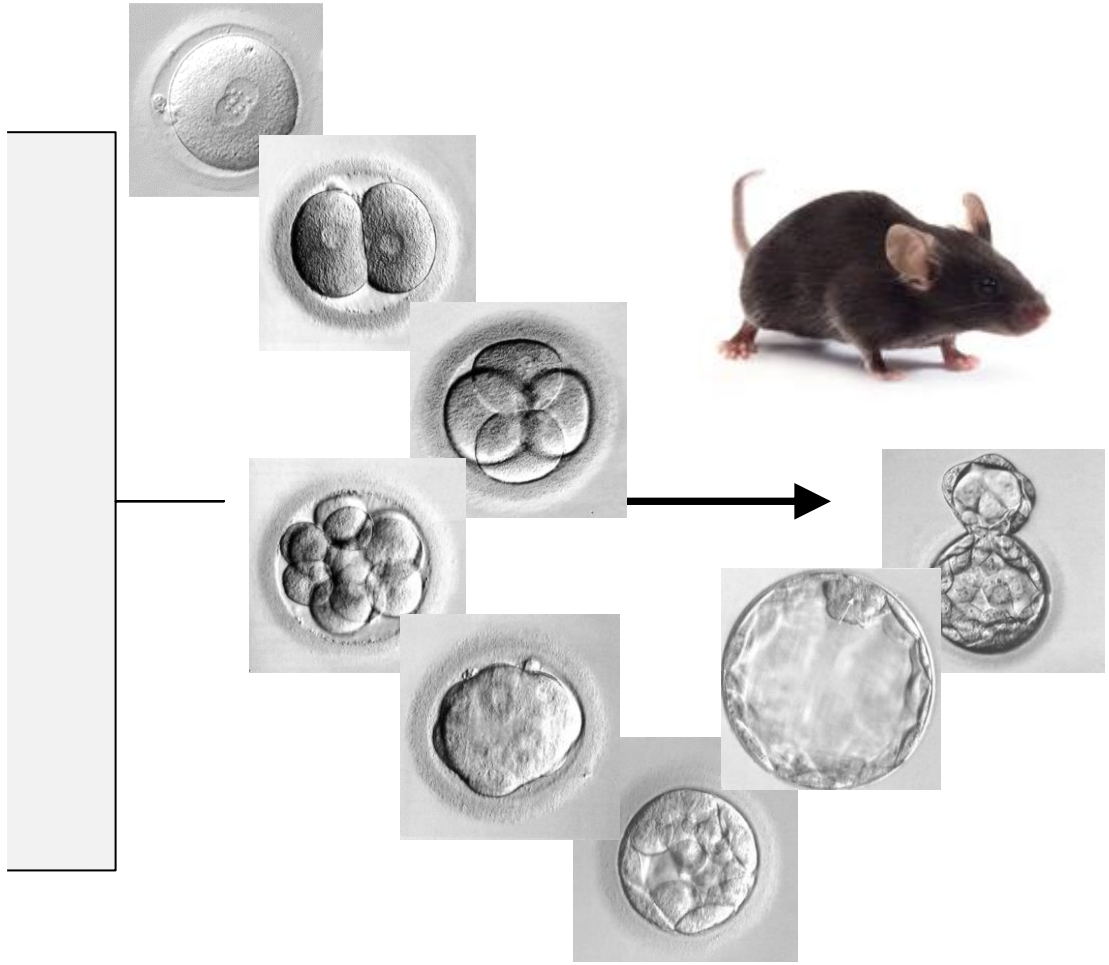
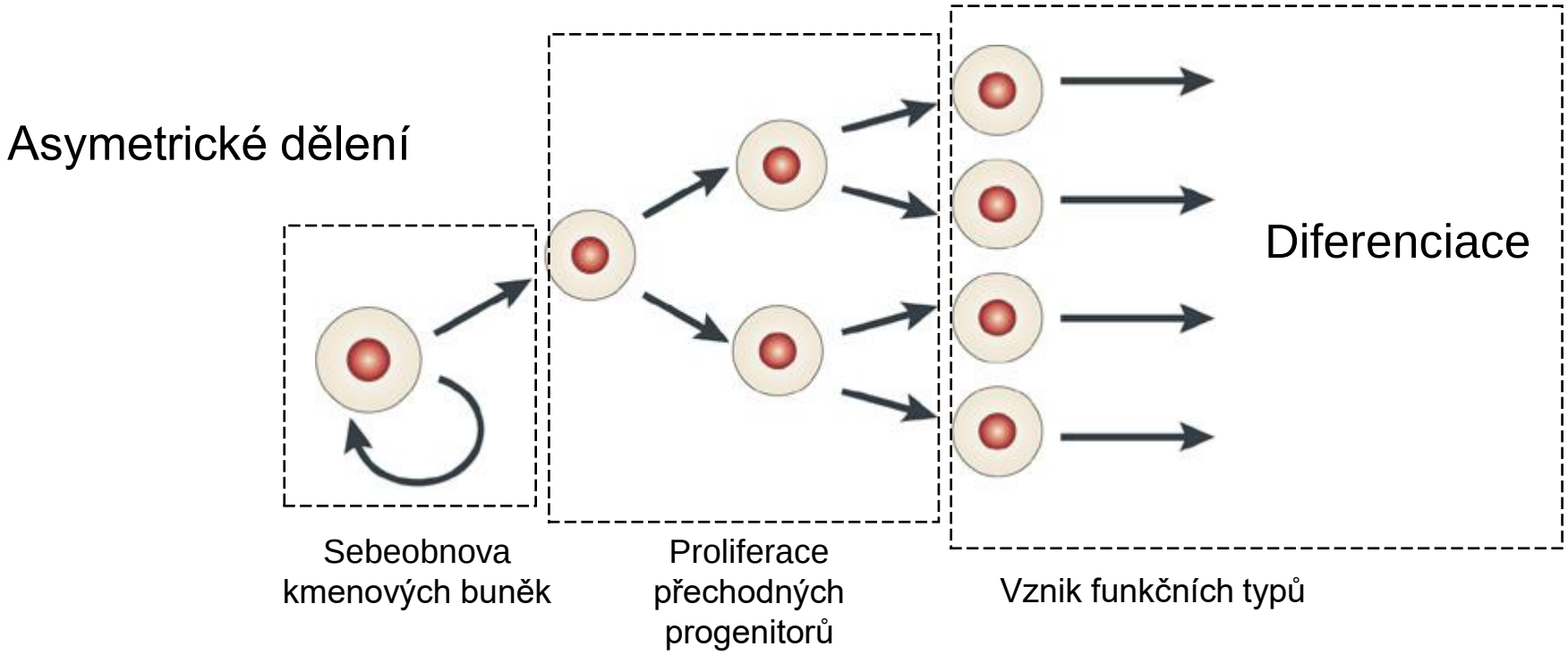
PRINCIPY HISTOGENEZE

- Proliferace
- Diferenciace
- Migrace
- Apoptóza
- Definice tkáňových vzorů



FUNKČNÍ BUŇKY TKÁNÍ DIFERENCUJÍ Z KMENOVÝCH BUNĚK



Knoblich JA. Asymmetric cell division during animal development. 2001. Nat Rev Mol Cell Biol

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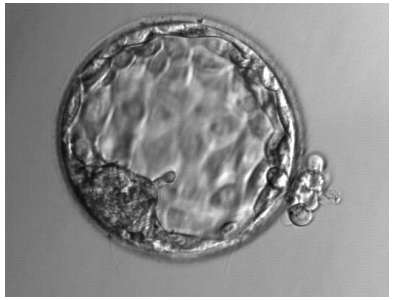
KMENOVÉ BUŇKY

Totipotence

- Všechny buňky těla včetně extraembryonálních tkání
- Zygota, blastomery a raná stádia embryogeneze

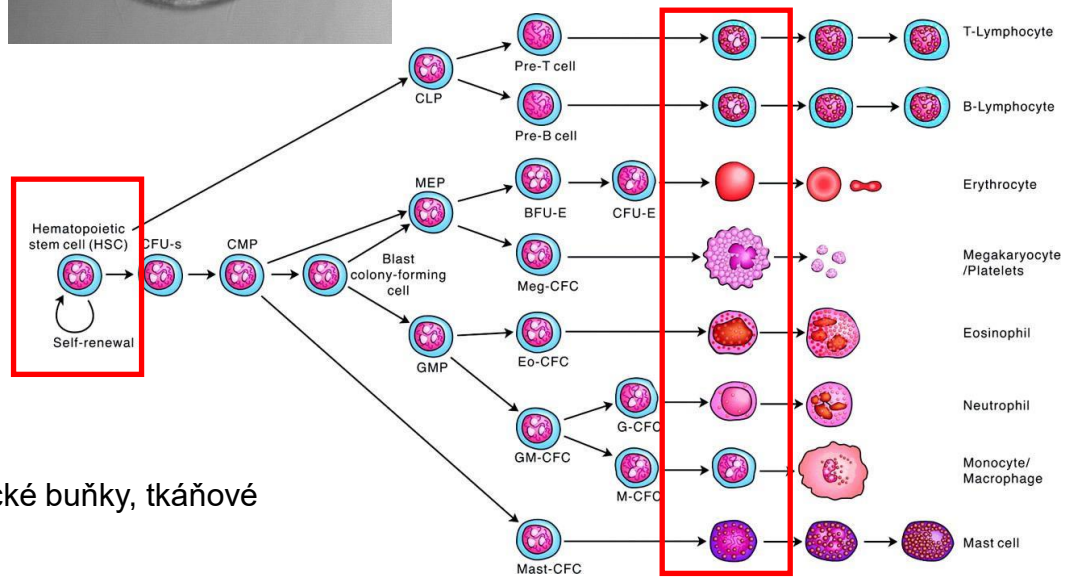


Pluripotence

- Všechny buňky těla s výjimkou trofoblastu
- Blastocysta – *Inner cell mass* - ICM (embryoblast)

Multipotence

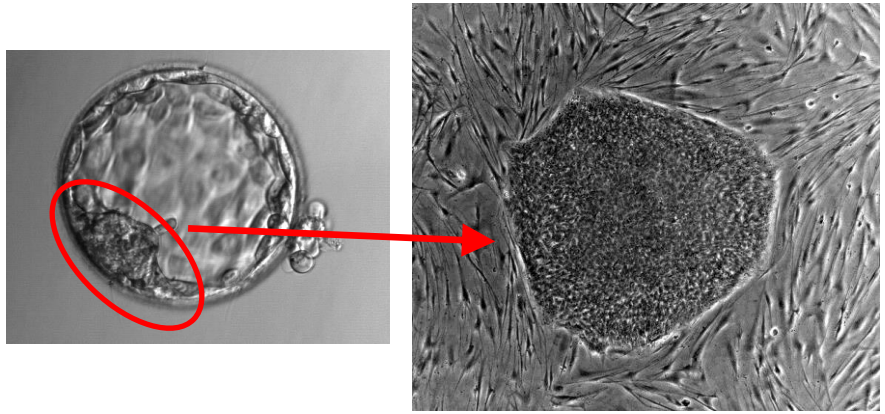
- Různé buněčné typy v rámci tkáně
- Mesenchymální SC, hematopoietické SC



Oligo- a unipotence

- Jeden nebo několik buněčných typů – hematopoietické buňky, tkáňové prekurzory (obnova epitelů apod.)

http://www.embryology.ch/anglais/evorimplantation/furchung01.html

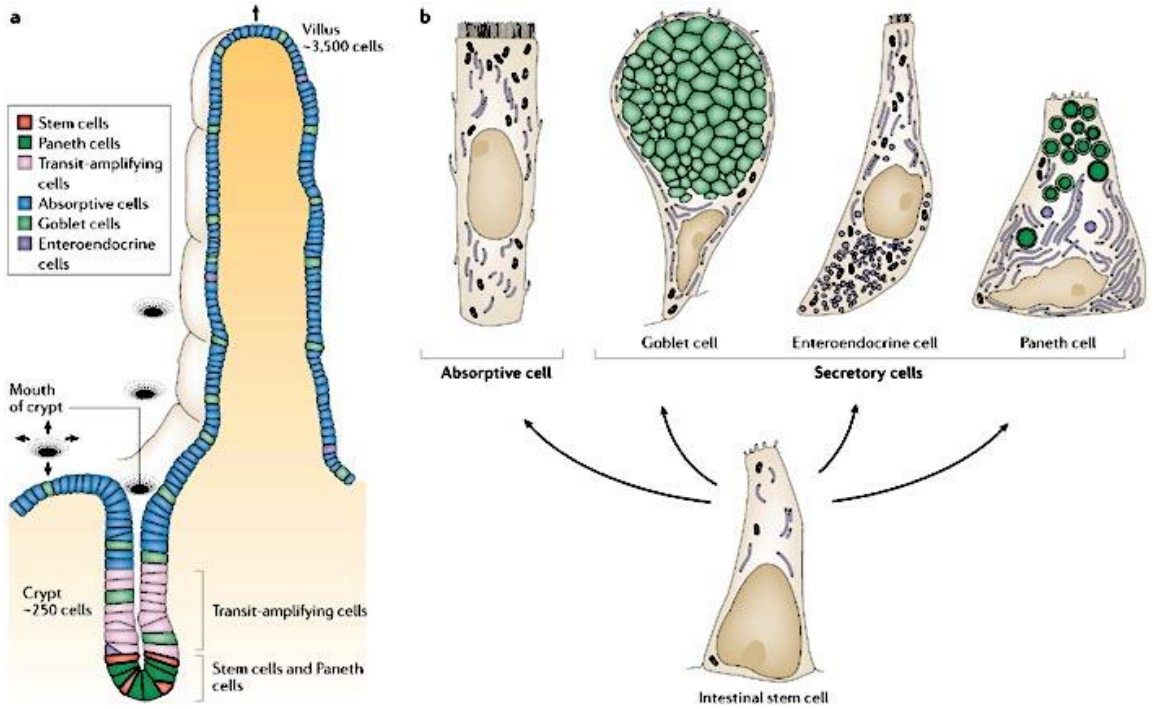


Embryonální kmenové buňky (ESCs)

- odvozeny z embryoblastu (ICM) blastocysty
- pluripotentní
- model rané embryogeneze a histogeneze, význam pro regenerativní medicínu
- kultivační artefakt, *in vivo* se nevyskytují

Tkáňové (adultní) kmenové buňky

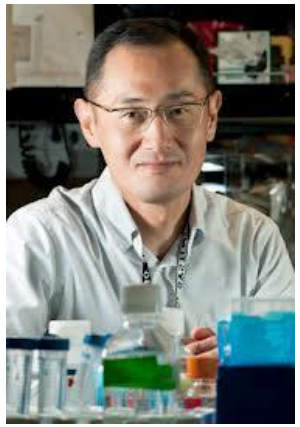
- regenerace a obnova tkání
- GIT, CNS, mezenchym
- regenerativní medicína, nádorová biologie

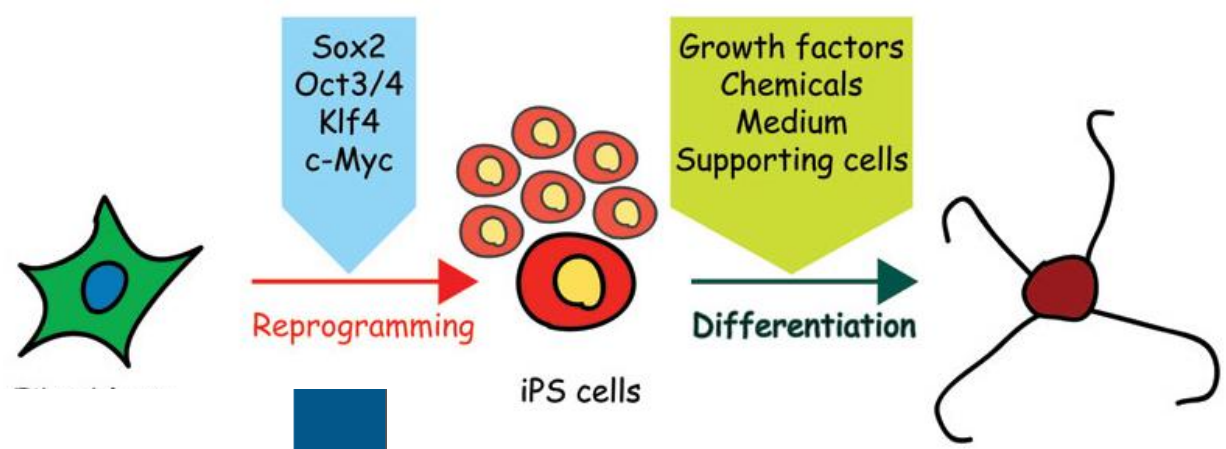


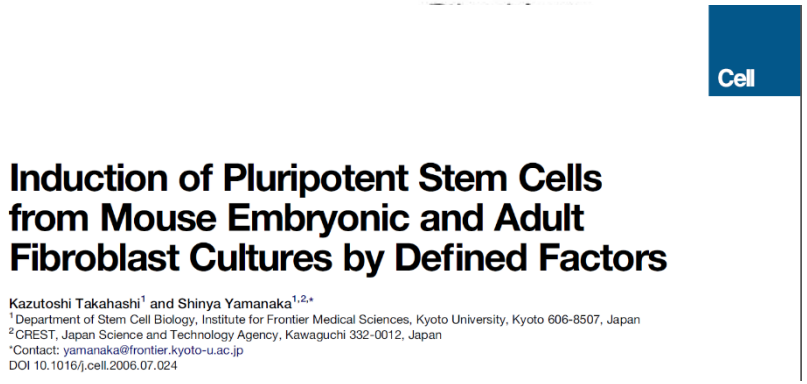
Indukované pluripotentní kmenové buňky (IPSc)

- dospělá diferencovaná buňka (fibroblast) je převedena do pluripotentního stavu
- diferenciace do žádaného buněčného typu
- regenerativní medicína, buněčná a genová terapie

Nobel prize 2012






Cell

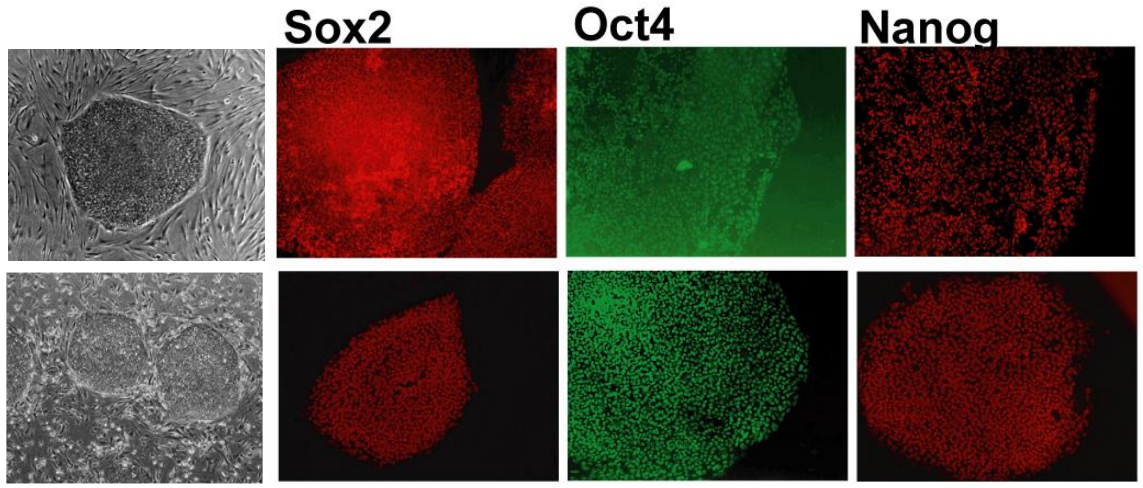
Induction of Pluripotent Stem Cells from Mouse Embryonic and Adult Fibroblast Cultures by Defined Factors

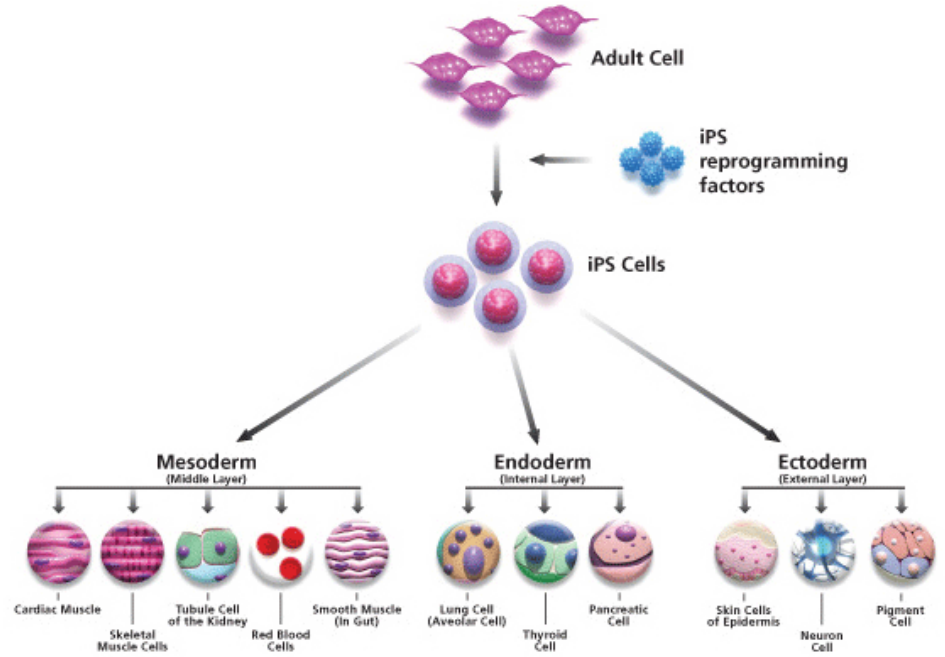
Kazutoshi Takahashi[1] and Shinya Yamanaka[1,2,*]
[1]Department of Stem Cell Biology, Institute for Frontier Medical Sciences, Kyoto University, Kyoto 606-8507, Japan
[2]CREST, Japan Science and Technology Agency, Kawaguchi 332-0012, Japan
*Contact: yamanaka@frontier.kyoto-u.ac.jp
DOI 10.1016/j.cell.2006.07.024

Reprogramace

hESCs

hiPSCs





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Nádorové kmenové buňky

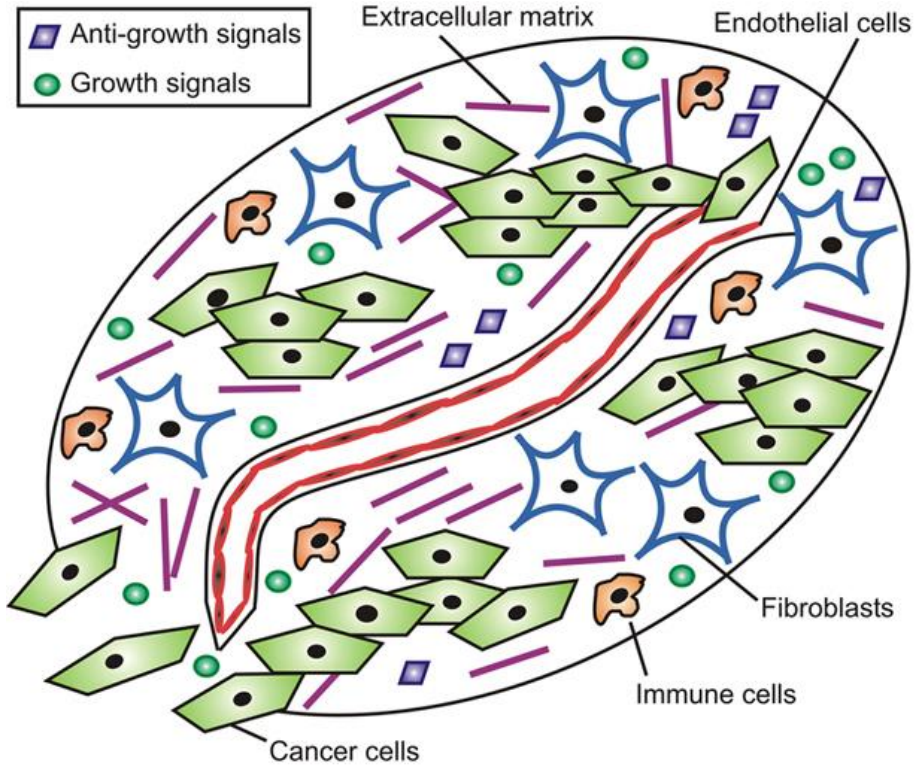
- solidní tumor je vždy heterogenní

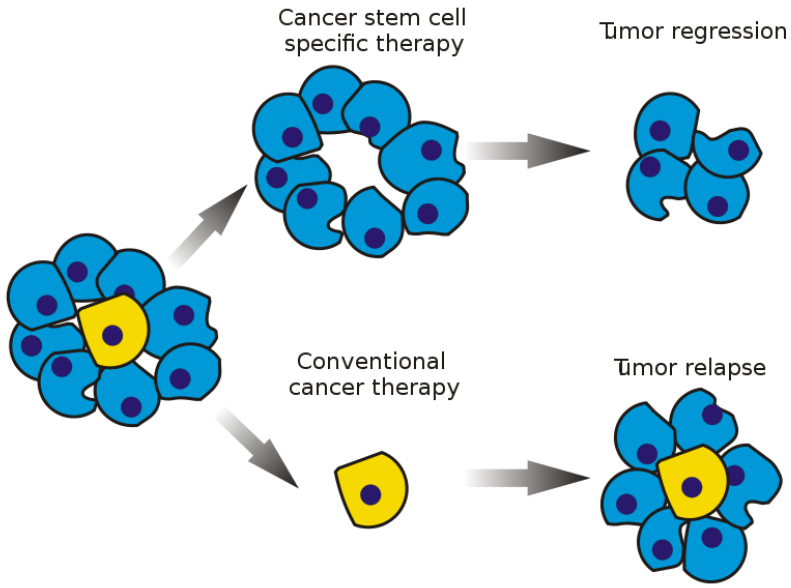
- malá populace buněk s charakterem CSC může znovu iniciovat růst tumoru a být příčinou selhání terapie

Tkáňová kmenová buňka

Sebeobnova
Quiescence
Multipotence
Nízká četnost (<1%)
Dlouhá životnost
Rezistence k terapii
Tumorigenicita
Vysoká proliferativní kapacita

Nádorová kmenová buňka





Angiogenesis

Inflammation

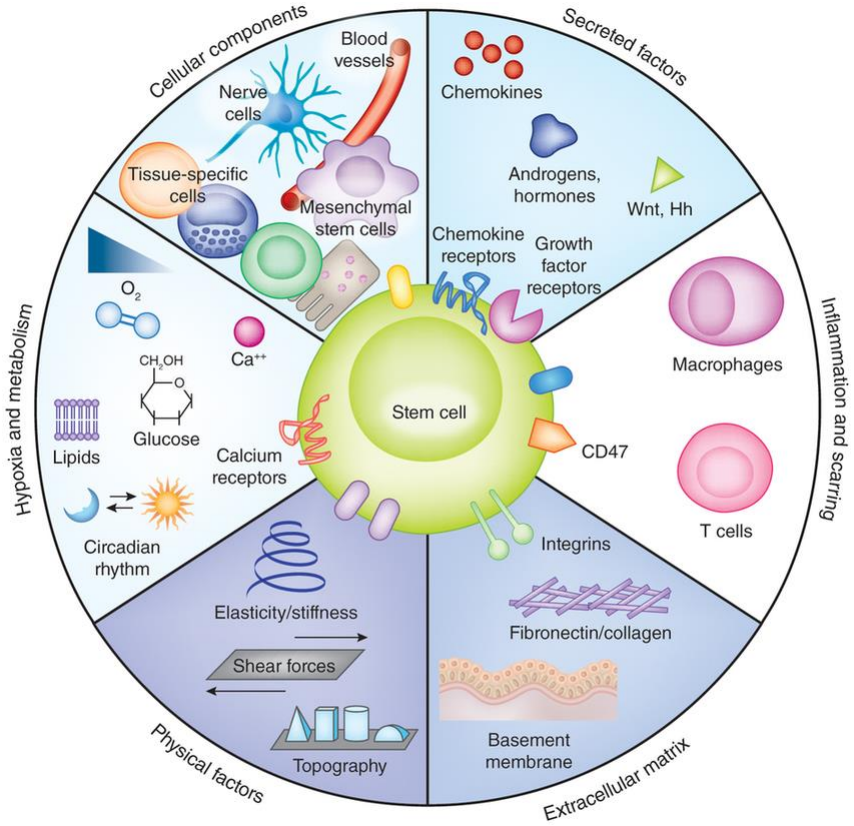
Invasion and metastasis

Self-sufficiency in growth signals

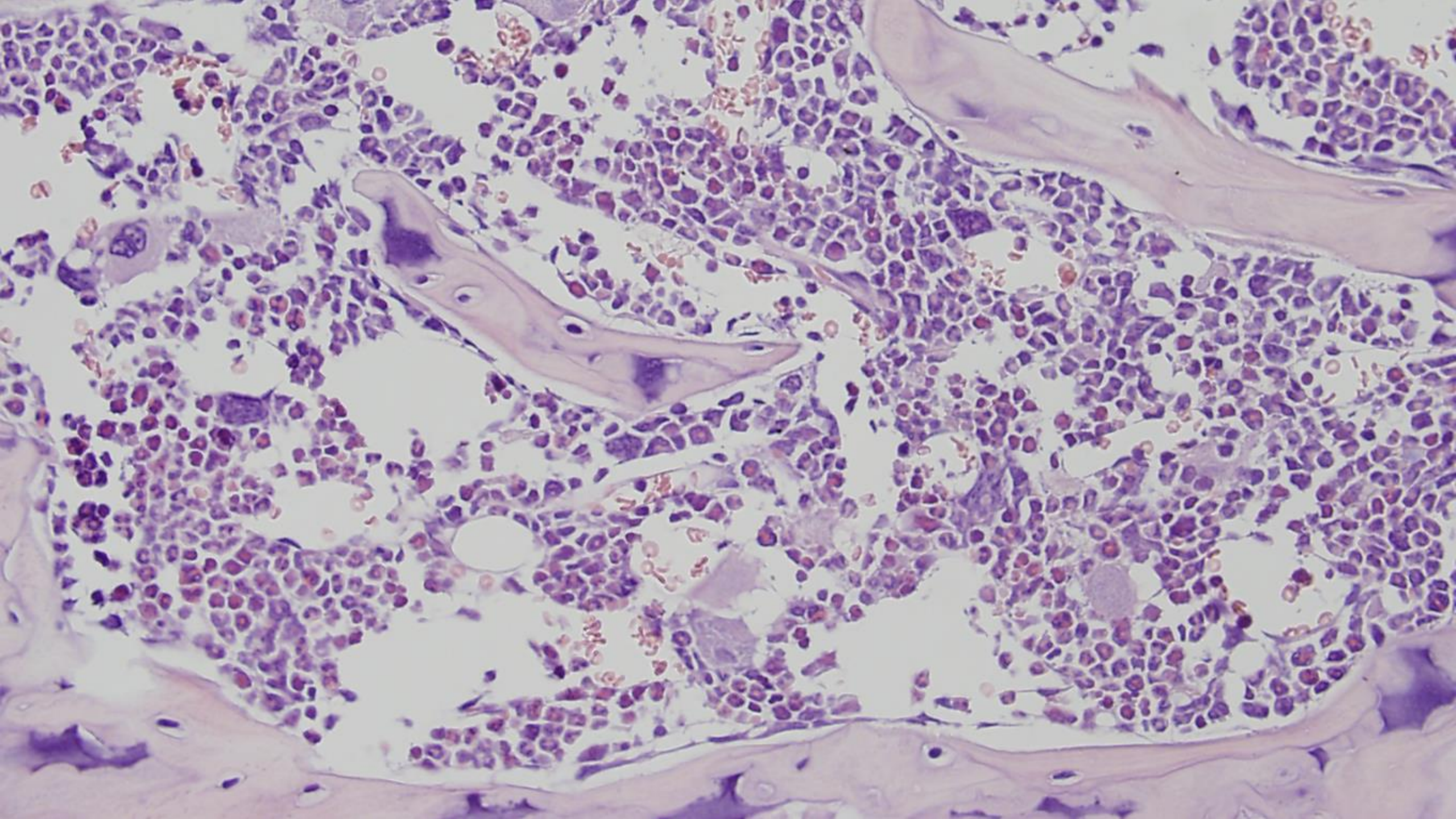
Insensitivity to anti-growth signals

Do vlastní mikroskopické stavby tkání se tedy promítá velké množství **biologických** a **fyzikálně-chemických** parametrů

Stem cell niche

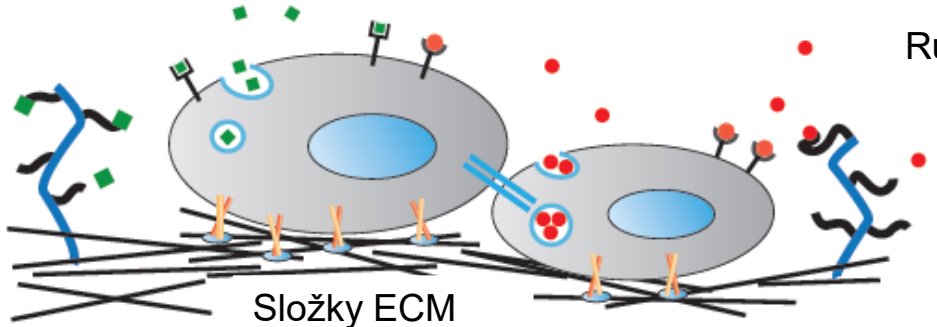


- Procesy embryonálního vývoje
- Mezibuněčné interakce
- Prostorové uspořádání (dimenzionalita)
- Gradienty morfogenů
- Epigenetický profil
- Dynamika genové exprese
- Parciální tlaky plynů
- Složení ECM
- Mechanická stimulace
- Perfuze a intersticiální toky
- Lokální imunitní odpověď
- Metabolity
- …

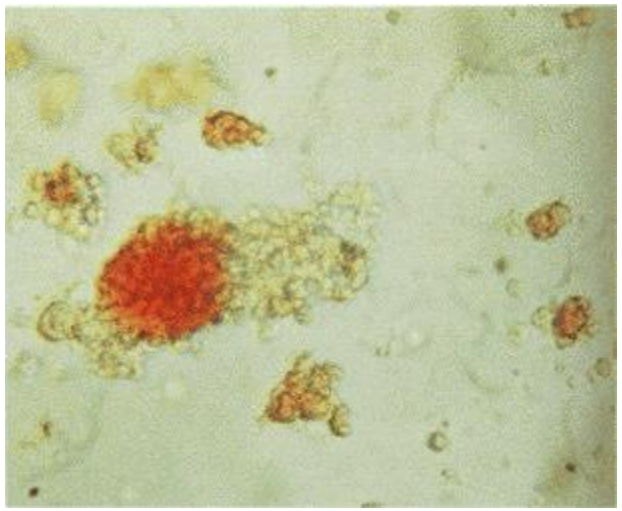


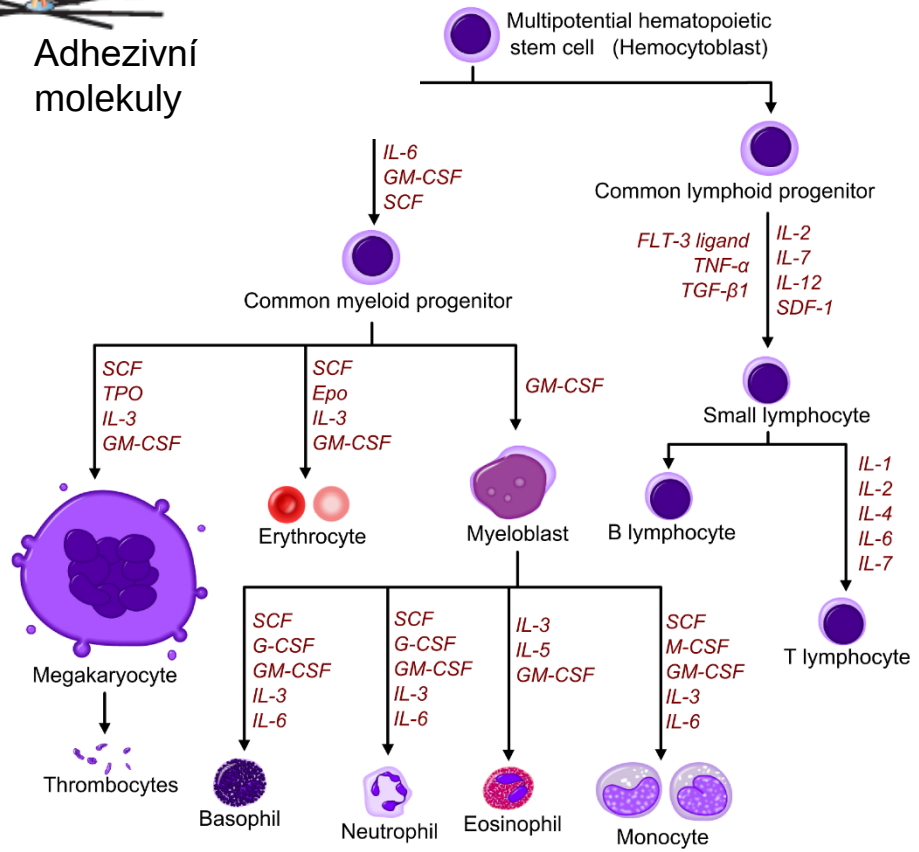
Příklad: krvetvorba v kostní dřeni

Mezibuněčné interakce



Růstové faktory





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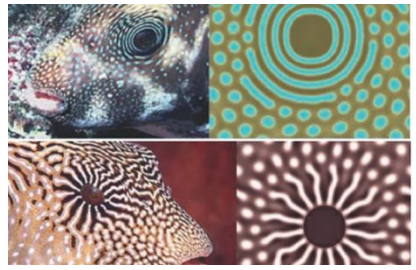
Reakčně-difúzní systém

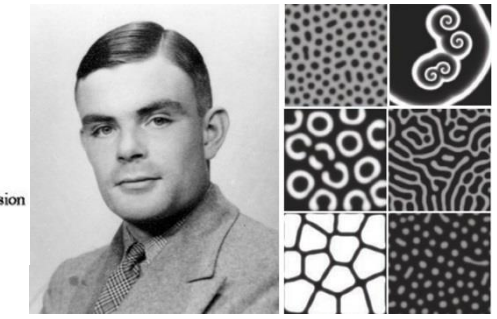


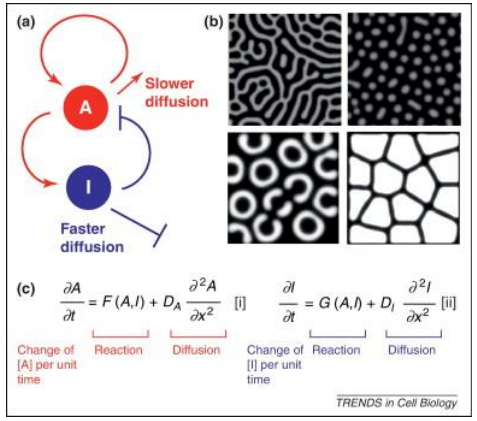
$$\frac{\partial u}{\partial t} = F(u,v) - d_u v + D_u \Delta u$$

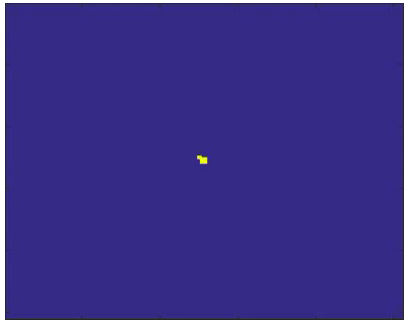
$$\frac{\partial v}{\partial t} = G(u,v) - d_v v + D_v \Delta v$$

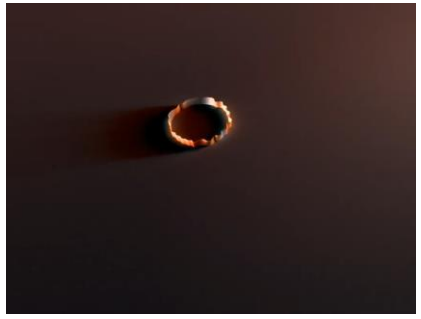
Rate of concentration change | Production | Degradation | Diffusion

Reaction









THE CHEMICAL BASIS OF MORPHOGENESIS

By A. M. TURING, F.R.S. *University of Manchester*

(*Received* 9 *November* 1951—*Revised* 15 *March* 1952)

It is suggested that a system of chemical substances, called morphogens, reacting together and diffusing through a tissue, is adequate to account for the main phenomena of morphogenesis. Such a system, although it may originally be quite homogeneous, may later develop a pattern or structure due to an instability of the homogeneous equilibrium, which is triggered off by random disturbances. Such reaction-diffusion systems are considered in some detail in the case of an isolated ring of cells, a mathematically convenient, though biologically unusual system. The investigation is chiefly concerned with the onset of instability. It is found that there are six essentially different forms which this may take. In the most interesting form stationary waves appear on the ring. It is suggested that this might account, for instance, for the tentacle patterns on *Hydra* and for whorled leaves. A system of reactions and diffusion on a sphere is also considered. Such a system appears to account for gastrulation. Another reaction system in two dimensions gives rise to patterns reminiscent of dappling. It is also suggested that stationary waves in two dimensions could account for the phenomena of phyllotaxis.

The purpose of this paper is to discuss a possible mechanism by which the genes of a zygote may determine the anatomical structure of the resulting organism. The theory does not make any new hypotheses; it merely suggests that certain well-known physical laws are sufficient to account for many of the facts. The full understanding of the paper requires a good knowledge of mathematics, some biology, and some elementary chemistry. Since readers cannot be expected to be experts in all of these subjects, a number of elementary facts are explained, which can be found in text-books, but whose omission would make the paper difficult reading.

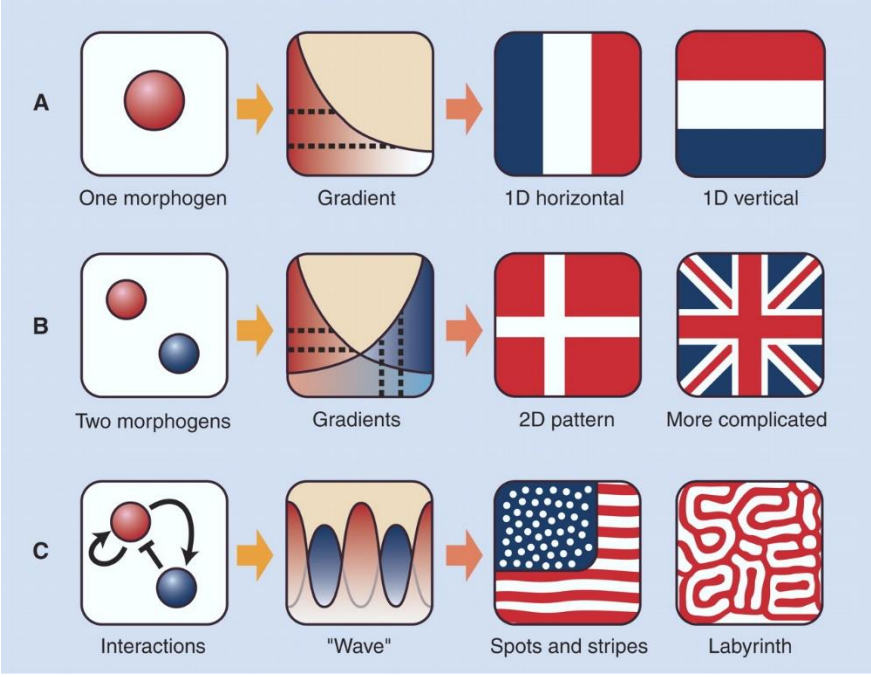
1. A model of the embryo. Morphogens

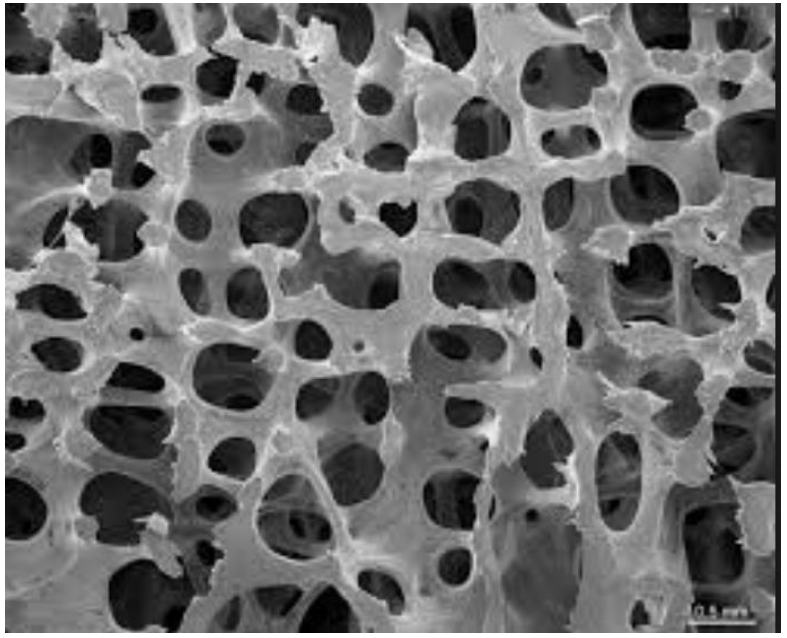
In this section a mathematical model of the growing embryo will be described. This model will be a simplification and an idealization, and consequently a falsification. It is to be hoped that the features retained for discussion are those of greatest importance in the present state of knowledge.

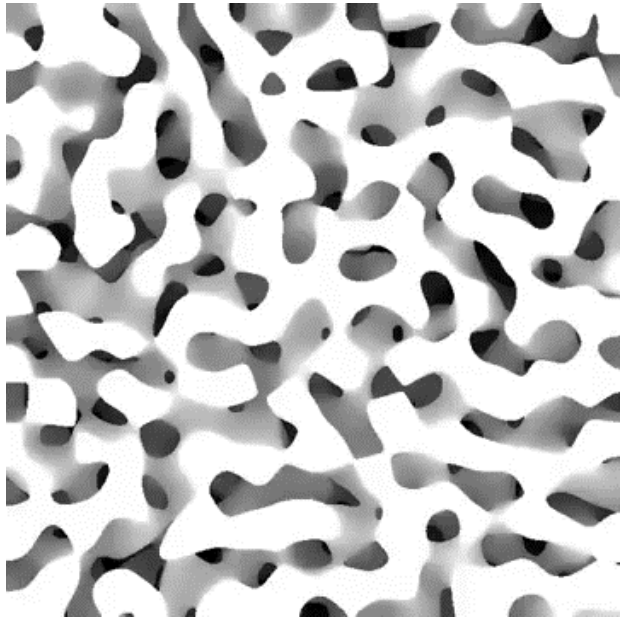
The model takes two slightly different forms. In one of them the cell theory is recognized but the cells are idealized into geometrical points. In the other the matter of the organism is imagined as continuously distributed. The cells are not, however, completely ignored, for various physical and physico-chemical characteristics of the matter as a whole are assumed to have values appropriate to the cellular matter.

MUNI MED

Ústav histologie a embryologie







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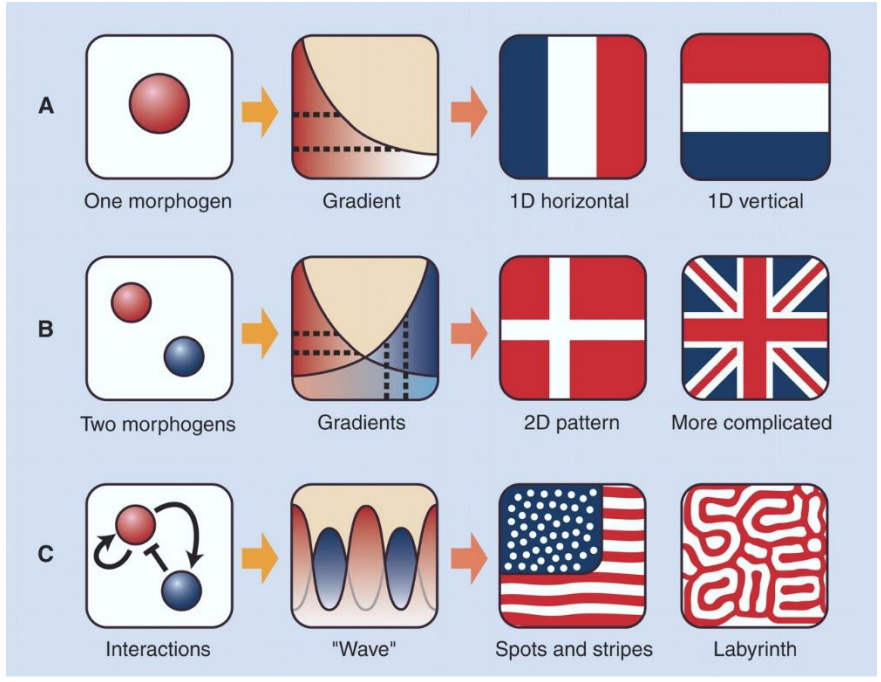
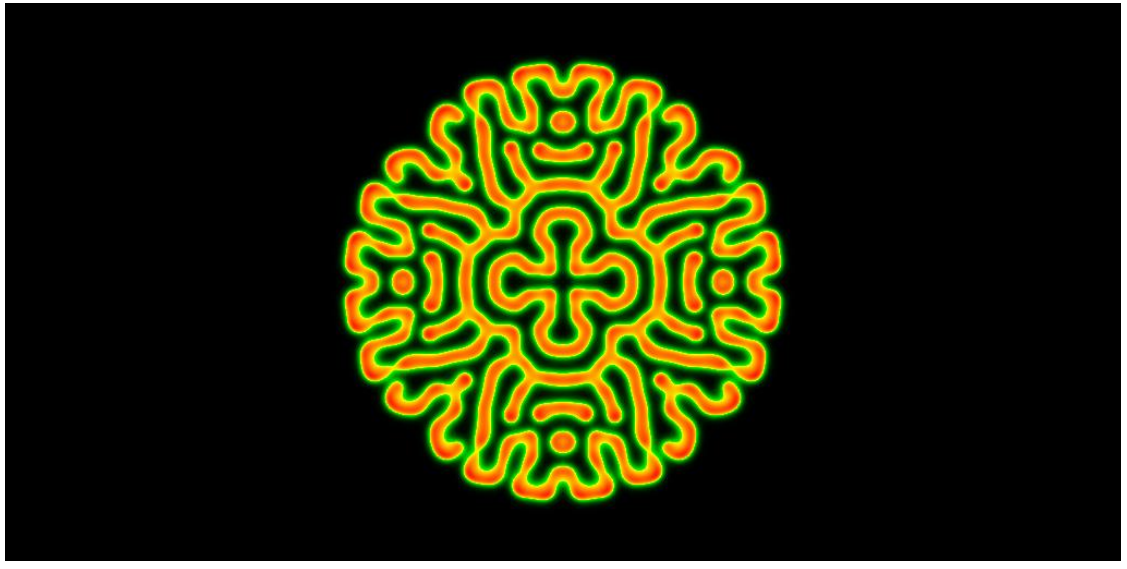
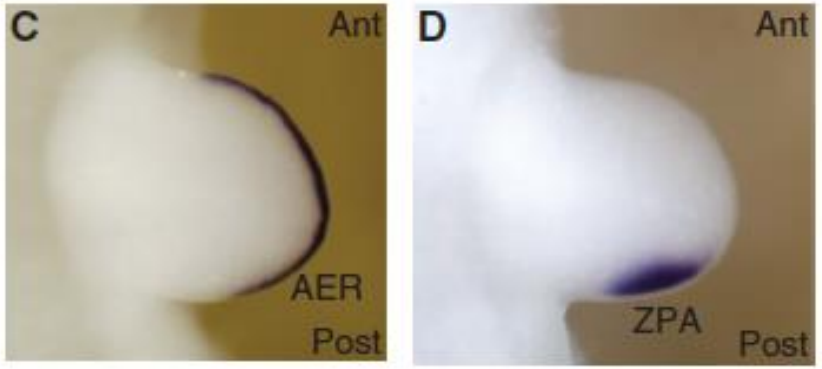


https://pmneila.github.io/jsexp/grayscott/

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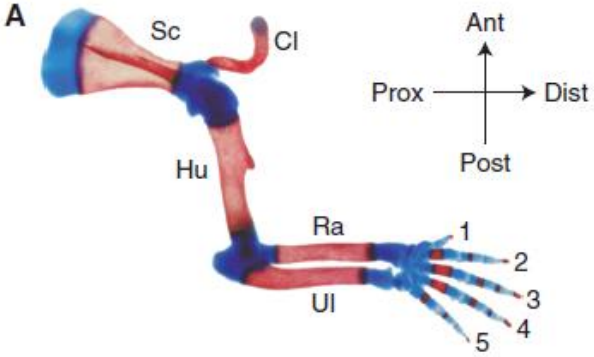
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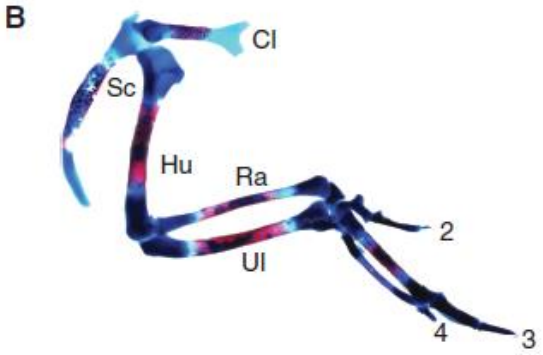
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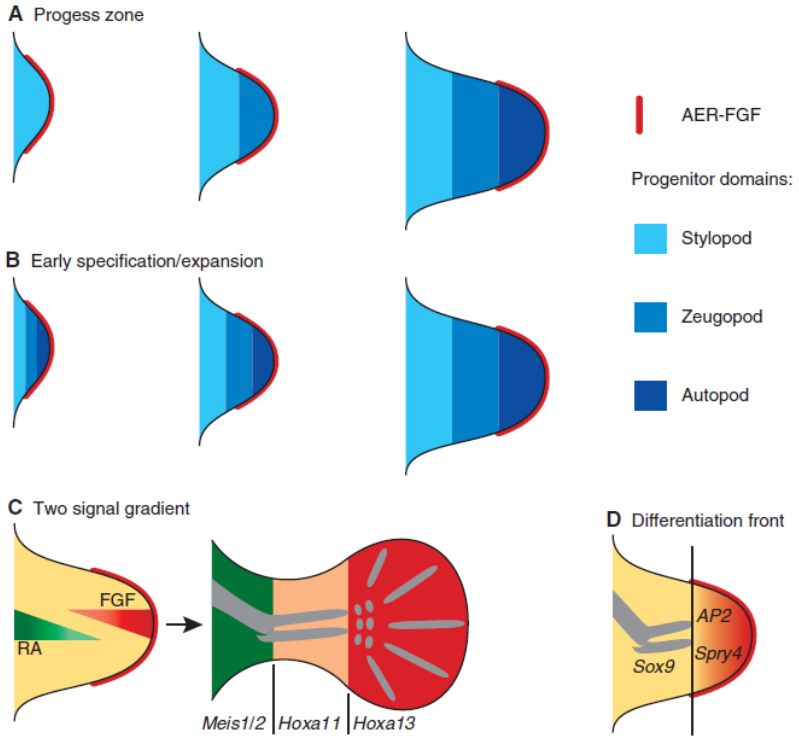
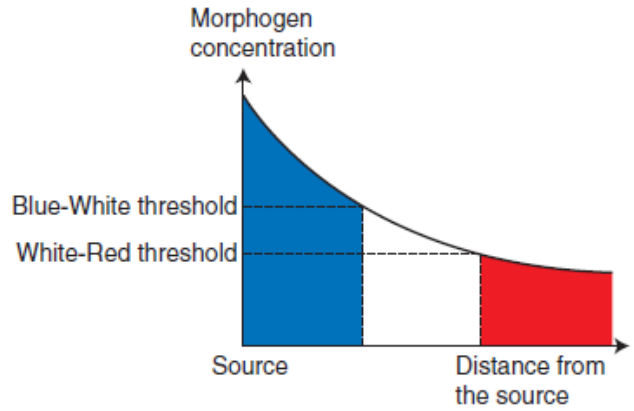
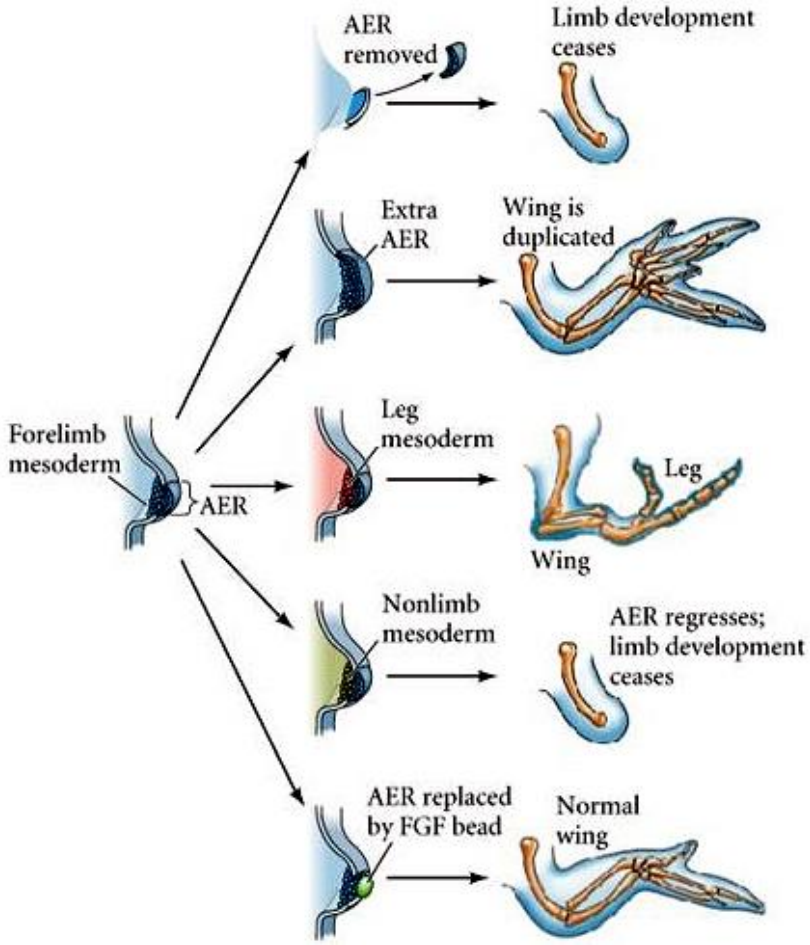
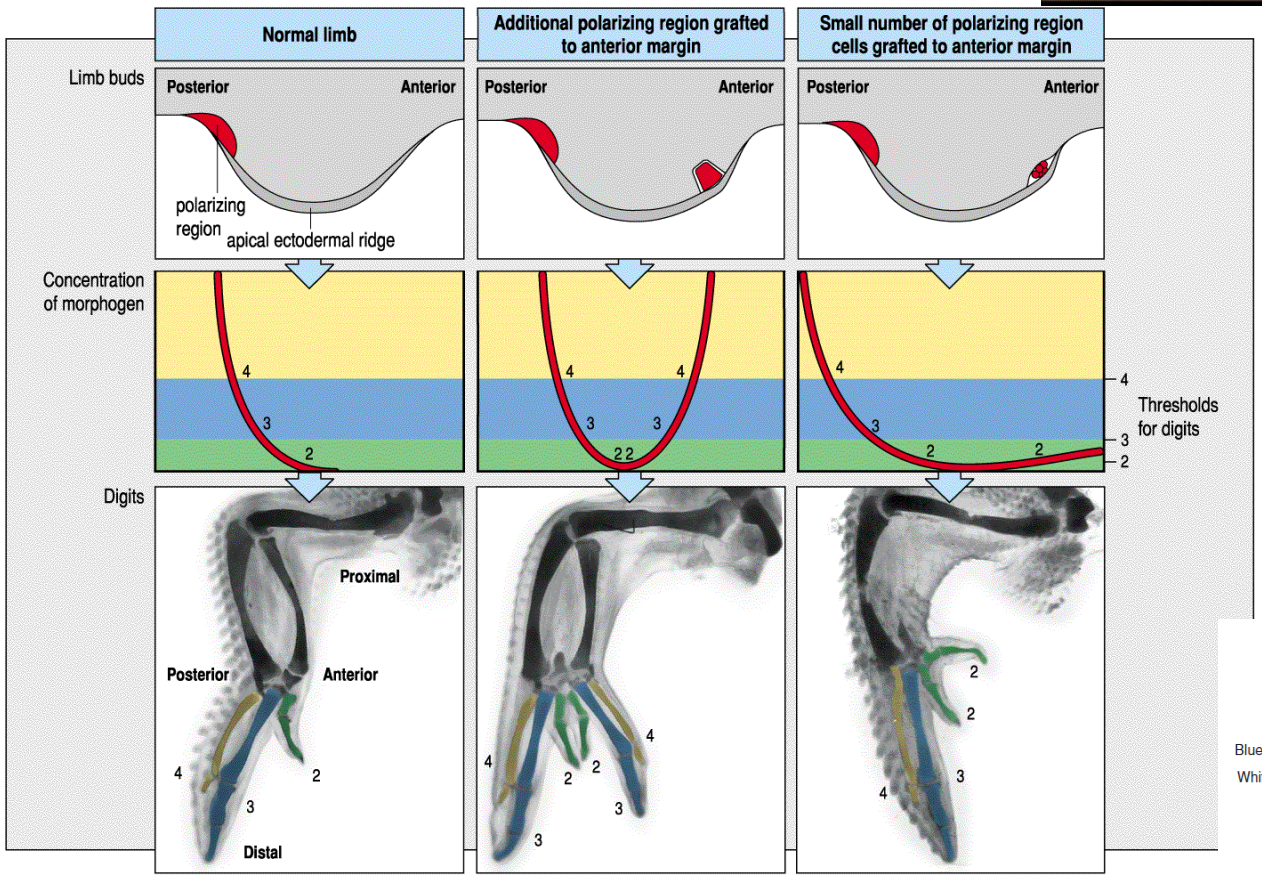
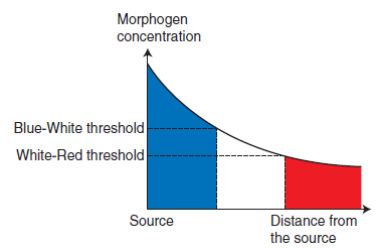
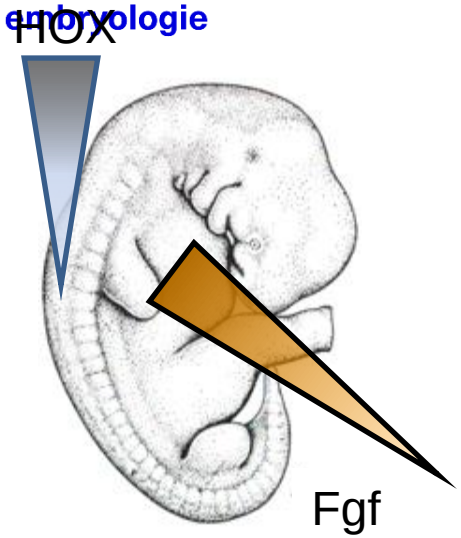

A Progess zone
B Early specification/expansion
AER-FGF
Progenitor domains:
Stylopod
Zeugopod
Autopod
C Two signal gradient
FGF
RA
Meis1/2
Hoxa11
Hoxa13
D Differentiation front
AP2
Sox9
Spry4

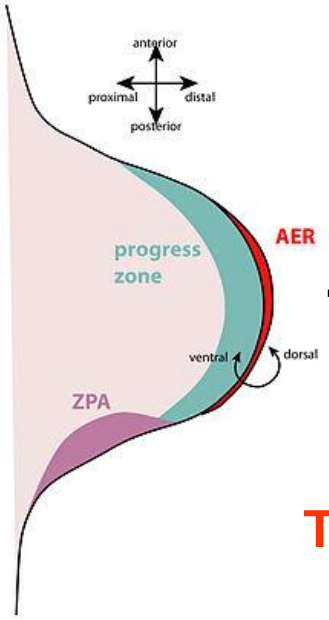

Morphogen concentration
Blue-White threshold
White-Red threshold
Source
Distance from the source


AER removed
Limb development ceases
Extra AER
Wing is duplicated
Forelimb mesoderm
AER
Leg mesoderm
Leg
Wing
Nonlimb mesoderm
AER regresses; limb development ceases
AER replaced by FGF bead
Normal wing


Normal limb
Additional polarizing region grafted to anterior margin
Small number of polarizing region cells grafted to anterior margin
Limb buds
Posterior
Anterior
polarizing region
apical ectodermal ridge
Concentration of morphogen
Thresholds for digits
Digits
Proximal
Distal


Morphogen concentration
Blue-White threshold
White-Red threshold
Source
Distance from the source

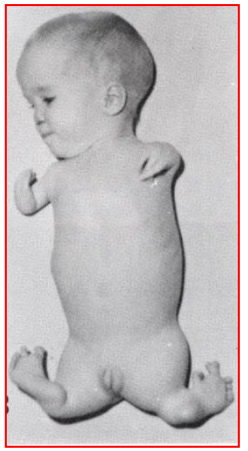




Proliferace

Vaskularizace

Thalidomid

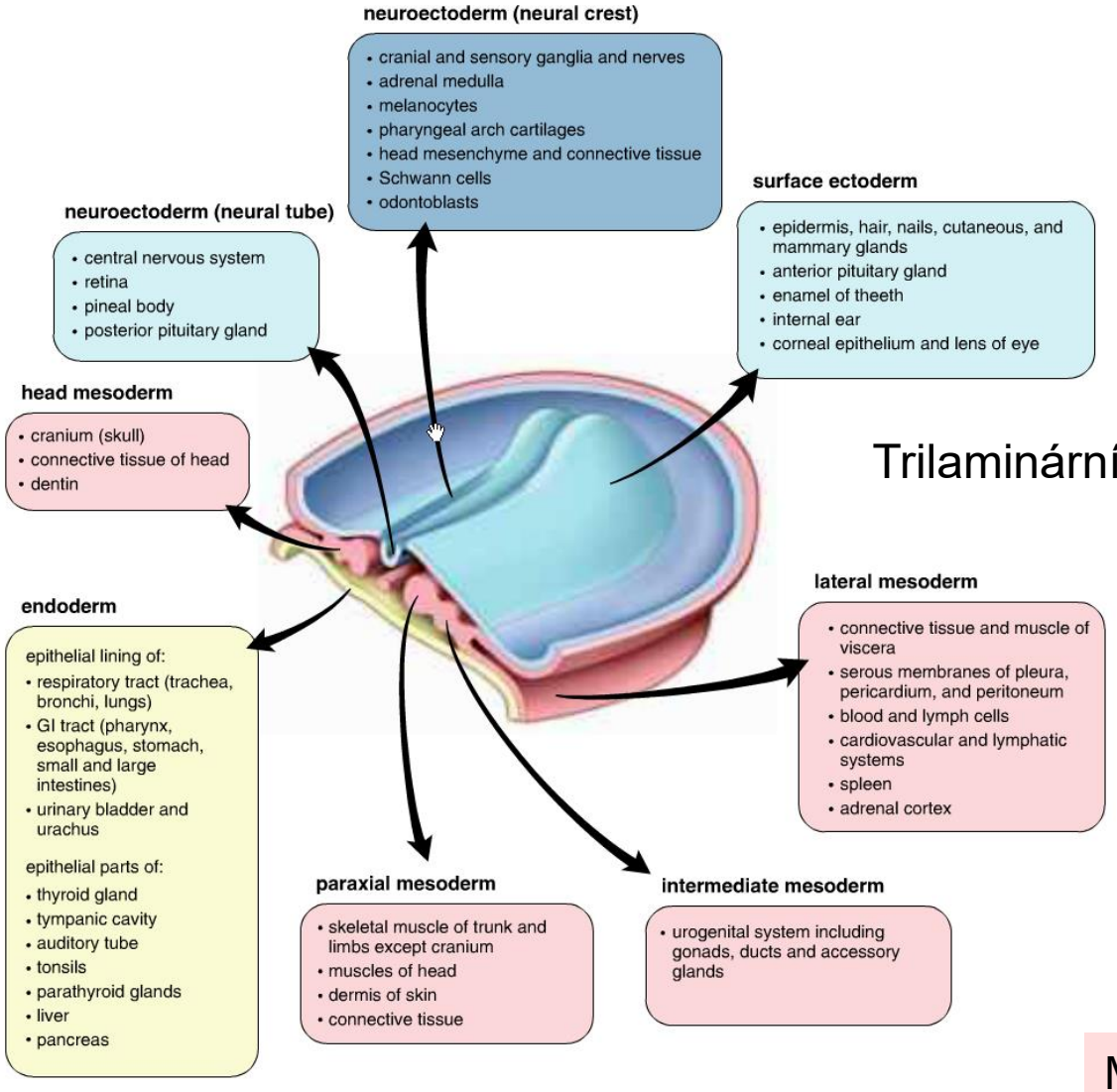


Thalidomidová embryopatie

- fokomelie
- amelie
- anocie/mikrocie
- anoftalmie/mikroftalmie
- poškození ledvin, srdce, GIT, genitálu

Frances Oldham Kelsey, FDA USA

Ektoderm



Trilaminární zárodečný disk

(3. týden)

Entoderm

Mesoderm