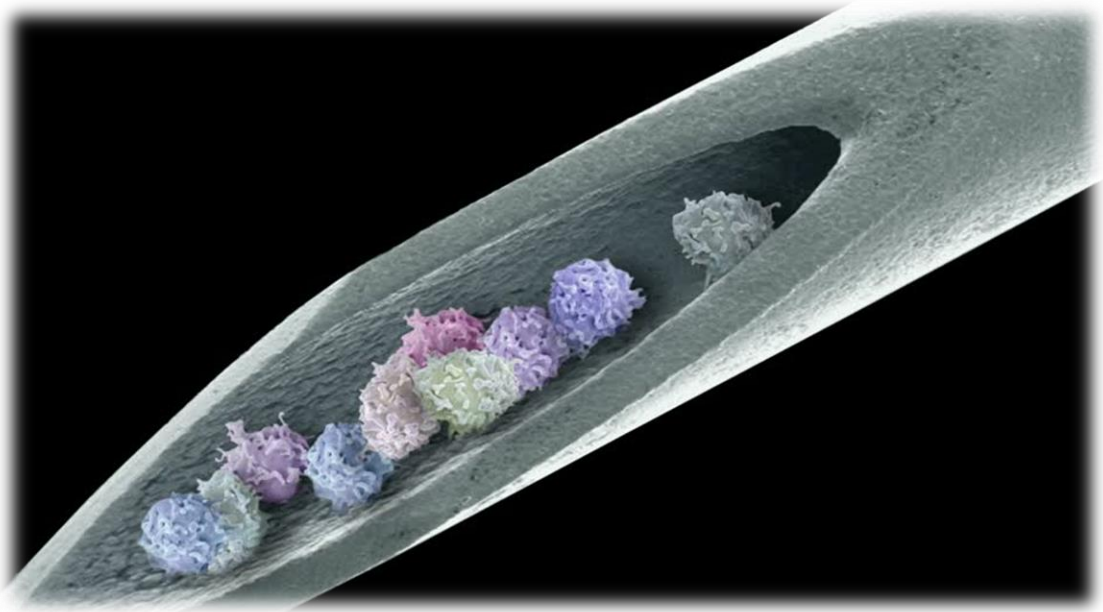

HEMATOPOIESIS AND IT'S REGULATION

Hematopoietic System and Disorders (MED202)

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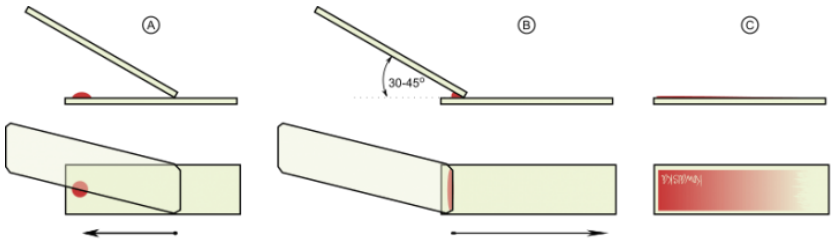
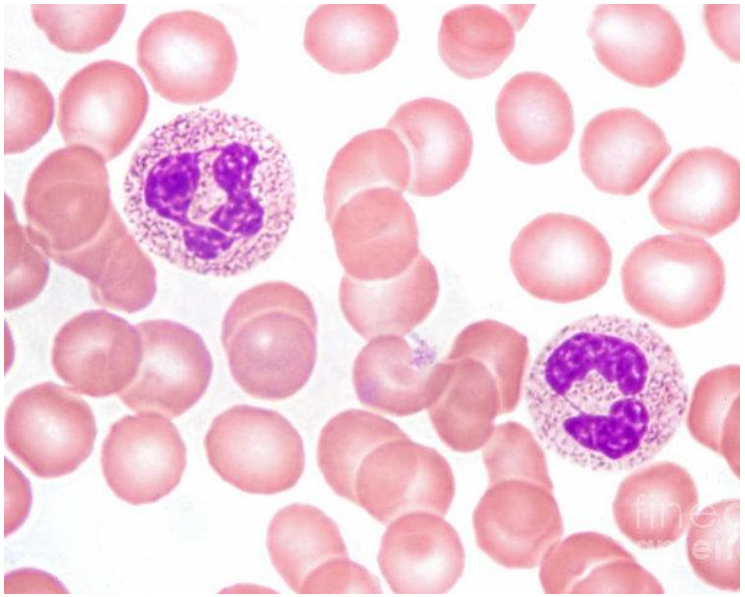
Lecture outline

- What is hematopoiesis?
- Hematopoietic organs and hematopoiesis during human development
- Generation of blood cells from HSCs
- Regulation of hematopoiesis by colony stimulating factors
- Erythropoiesis
  - Regulation of erythrocyte production
  - Erythropoietin (EPO)
  - **Hypoxia response**
- Leukopoiesis and its regulation
- Thrombopoiesis and its regulation





- If you spread a drop of anticoagulated blood thinly on a glass slide (peripheral blood smear), you can detect under the microscope the cellular elements of blood.
- Mature cell types are easily recognized: erythrocytes, granulocytes (neutrophils, eosinophils, and basophils), lymphocytes, monocytes, and platelets.

Hematopoiesis is the process of generation of all the cell types present in the blood.

- ✓ Hematopoiesis has been studied intensely for over a century using a variety of model systems.
- ✓ There is conservation of the overall hematopoietic process between vertebrates, although some differences do exist
- ✓ Comparative approach
- ✓ Understanding the mechanisms that regulate self-renewal and lineage specification of HSCs is key for developing treatments for many human diseases

For furher information: Understanding the regulation of vertebrate hematopoiesis and blood disorders – big lessons from a small fish. FEBS Letters (2016). https://doi.org/10.1002/1873-3468.12415

Hematopoiesis is the process of generation of all the cell types present in the blood.

- ✓ The rate of hematopoiesis depends on the body's needs.
- ✓ The body continually manufactures new blood cells to replace the old ones.
- ✓ About 1% of the body's blood cells must be replaced every day: >100 billion cells/day
- ✓ WBCs have the shortest life span, sometimes surviving just a few hours to a few days, while RBCs can last up to 120 days or so.

Hematopoiesis

- ✓ Begins with embryogenesis and continues throughout life.
- ✓ Because of the diversity of cell types generated, hematopoiesis serves multiple roles.
  - carriage of gases
  - immune responses
  - hemostasis

Hematopoietic organs during human development

- ✓ Hematopoietic tissue is derived from mesoderm
- ✓ Blood development in mammals may be divided into two developmental processes:
  1) The primitive hematopoiesis
  2) The definitive hematopoiesis

Primitive hematopoiesis

- Starts at 19th day of development, lasts in 8 weeks
- Involves an erythroid progenitor, gives rise to erythrocytes and macrophages during early embryonic development.
- Erythroid progenitor cells first appear in blood islands in the extra-embryonic **yolk sac**
- Early erythroid progenitors are not pluripotent and do not have renewal capability

Development 2013 140: 2463-2467; doi: 10.1242/dev.083147

Primitive hematopoiesis

- The primary purpose is to produce RBCs that can facilitate tissue oxygenation as the embryo undergoes rapid growth
- Does not require EPO

Development 2013 140: 2463-2467; doi: 10.1242/dev.083147

Definitive hematopoiesis

- Occurs later in development
- There is a transient wave of definitive hematopoiesis that occurs in the blood islands and produces progenitors called erythroid-myeloid progenitors (EMPs)
- Definitive hematopoiesis later involves HSCs, which are multipotent and can give rise to all blood lineages of the adult organism

Development 2013 140: 2463-2467; doi: 10.1242/dev.083147

Definitive hematopoiesis

- HSCs are born at aorta-gonad-mesonephros of the developing embryo
- They migrate to the fetal liver and then to the bone marrow, which is the location for HSCs in adults

Development 2013 140: 2463-2467; doi: 10.1242/dev.083147

Hematopoiesis during development: *Summary*

- ✓ In the early embryonic life, HSCs first appear in **yolk sac** and mesodermal tissue of the **aorta-gonad-mesonephros region**
- ✓ These stem cells then migrate and colonize in a series of early hematopoietic sites including **liver**, **thymus**, **spleen**, and **omentum**
- ✓ They eventually reside in **bone marrow** as their permanent home, where they give rise to sequential generations of blood cells throughout adult life.

Hematopoiesis in adult

- Blood cells begin their lives in the **bone marrow** from a single type of cell: ***pluripotent hematopoietic stem cell (HSCs) ($CD34^+$, $CD38^-$, $HLA\text{-}DR^-$)***
- All the cells of the circulating blood are eventually derived from HSCs

Two separate and distinct pluripotent stem cells are simultaneously at work in bone marrow:

1. Hematopoietic
2. Stromal

✓These two systems not only co-exist but closely interact with each other.

✓Stromal cells are composed of a heterogeneous cell population including adipocytes, fibroblast-like cells, endothelial cells, and osteoblasts.

✓They produce a number of cytokines and a group of proteins that are involved in facilitating cell– cell interactions and presenting the cytokines and growth factors to the hematopoietic progenitor cells

Atlas of Hematopathology: Morphology, Immunophenotype, Cytogenetics, and Molecular Approaches 2013, Pages 1-24

Self renewal potential of HSCs

- As HSCs reproduce, a small portion of them remains exactly like the original pluripotential cells and is retained in the bone marrow to maintain a supply of these
- Their numbers diminish with age

Caocci and Nasa (2017) https://doi.org/10.4084/mjhid.2017.032

Most of the reproduced cells, however, differentiate to form the populations of progenitor cells which are **committed** to the main marrow cell lines: erythroid, granulocytic and monocytic, megakaryocytic, and lymphocytic

Migration and homing of HSCs into the bone marrow microenvironment.

Caocci and Nasa (2017) https://doi.org/10.4084/mjhid.2017.032

The earlier progenitor cells are multipotent, but as division and differentiation proceed, later progenitors are formed that are committed to three, two, or one cell line.

When grown in culture, different committed stem cells will produce colonies of specific types of blood cells

Colony-forming unit–erythrocyte (CFU-E): A committed stem cell that produces erythrocytes

Colony-forming unit–erythrocyte (CFU-GM): A committed stem cell that produces granulocytes and monocytes

Colony-forming unit–megakaryocytes (CFU-M): A committed stem cell that produces megakaryoctes and platelets

Soluble factors known as **cytokines** guide the development of each lineage.

- Growth and reproduction of the different stem cells are controlled by multiple cytokines called ***hematopoietic growth factors*** *or* **colony-stimulating factors**

- They are produced within the **bone marrow** microenvironment and by **extramedullary sources**, including T cells, macrophages, endothelial cells, and fibroblasts.

- Many of the cytokines have overlapping functions.

- Proteins that induce cell differentiation are called differentiation inducers

- Each of these differentiation inducers causes one type of committed stem cell to differentiate one or more steps toward a final adult blood cell
  - Thrombopoietin (TPO)
  - Erythropoietin (EPO)

GM-CSF (Granulocyte Macrophage-CSF)

- ✓ is a glycoprotein synthesized by T-lymphocytes, monocytes, fibroblasts, and endothelial cells
- ✓ stimulates proliferation and differentiation of a common myeloid progenitor
- ✓ promotes the production of neutrophils, eosinophils, and monocytes, and macrophages (i.e. WBCs).

G-CSF (Granulocyte-CSF)

- ✓ is a glycoprotein that affects primarily neutrophils
- ✓ is synthesized by monocytes, fibroblasts, and endothelial cells
- ✓ promotes the production of neutrophils, eosinophils, and monocytes-macrophages.

M-CSF (Macrophage-CSF)

- ✓ is synthesized by monocytes, fibroblasts, and endothelial cells
- ✓ Promotes monocyte and macrophage proliferation and cell survival

IL-3

- ✓ also known as multi-CSF
- ✓ has a broad effect on multiple lineages.
- ✓ the liver and the kidney constitutively produce this glycoprotein.

IL-5

- ✓ CSF-E
- ✓ a homodimeric glycoprotein, sustains the terminal differentiation of eosinophilic precursors.

January 2012Growth factors (Chur, Switzerland) 30(2):63-75 DOI: 10.3109/08977194.2011.649919

Cytokine	Primary effect
GM-CSF[1]	Granulocyte and macrophage colony formation, functional enhancement of mature forms
G-CSF[2]	Granulocyte colony formation, functional enhancement of granulocytes
M-CSF (CSF-1)[3]	Macrophage colony formation, functional enhancement of monocytes and macrophages
Erythropoietin (EPO)	Erythropoiesis, possible enhancement of megakaryocyte proliferation
Thrombopoietin (TPO)	Megakaryocyte proliferation, platelet production
Steel factor (c-kit ligand)	Stem cell and mast cell proliferations
Interleukin (IL)-1	Promoter of hematopoiesis, inducer of other factors, B- and T-cell regulators, endogenous pyogen
IL-2	T-cell growth factor, may inhibit G/M colony formation and erythropoiesis
IL-3 (multi-CSF)	G/M colony formation, syngeneic effects on EPO, eosinophil, mast cell, and megakaryocyte colony formation
IL-4	B-cell proliferation, IgE production
IL-5	Eosinophil growth and B-cell differentiation
IL-6	B-cell differentiation, synergestic effects on IL-1
IL-7	Development of B- and T-cell precursors
IL-8	Granulocyte chemotactic factor
IL-9	Growth of mast cells and T-cells
IL-10	Inhibitor of inflammatory and immune responses
IL-11	Synergestic effects on growth of stem cells and megakaryocytes
IL-12	Promoter of Th1 and suppressor of Th2 functions

Cytokine	Primary effect
IL-13	B-cell proliferation, IgE production
IL-15	Activates T-cells, neutrophils and macrophages
IL-16	Chemotactic factor for helper T-cells
IL-17	Promotes T-cell proliferation, pro-inflammatory activities
IL-18	Activates T-cells, neutrophils, and fibroblasts
IL-19	Member of IL-10 family, transcriptional activator of IL-10
IL-20	Member of IL-10 family with epidermal function
IL-21	Improves proliferation of T- and B-cells, and enhances NK cytotoxic activities
IL-22	Member of IL-10 family; induces inflammatory responses
IL-23	Activates autoimmune responses
IL-24	Member of IL-10 family, tumor suppressor molecule
IL-25	Capable of amplifying allergic inflammation
IL-26	Member of IL-10 family; plays a role in mucosal and cutaneous immunity
TGF-β[4]	Suppresses BFU-E, CFU-S, and HPP-CFC
Interferons	Suppress BFU-E, CFU-GEMM, and CFU-GM
TNF[5]-α and -β	Suppress BFU-E, CFU-GEMM, and CFU-GM
PGE[6]-1 and -2	Suppress GFU-GM, GFU-G, and GFU-M
Lactoferrin	Suppresses release of IL-1

Postnatal Hematopoiesis

- Prenatal

  Mesoblastic hematopoiesis: First 3 months; yolk sac

  Hepatic hematopoiesis : 3-6 months; Liver, spleen and lymphoid organs

  Myeloid hematopoiesis : Last 3 months; Liver and red bone marrow

- Postnatal

By age 6 : Marrow caviteis in all bones

By age 20: Red marrow in the cavities of the long bones, and marrow in flat bones

After the age of 20: Only in the marrow of flat bones (e.g., vertebra, iliaca). The marrow of the long bones is lubricated and becomes yellow marrow

Genesis of Red Blood Cells: **Erythropoiesis**

- Erythroprotein (EPO) supports erythropoiesis or red blood cell development
- EPO is not absolutely required for early commitment of progenitor cells to the erythroid lineage; it is essential for the differentiation of burst-forming unit–erythroid cells (BFU-Es) to colony-forming unit–erythroid cells (CFU-Es) or **proerythroblasts** (also known as pronormoblasts), which still lack hemoglobin.
- The further maturation of cells downstream of proerythroblasts does not require EPO.

Genesis of Red Blood Cells: **Erythropoiesis**

- Once the **proerythroblast** has been formed, it divides multiple times, eventually forming many mature RBCs.
- The first generation cells are called ***basophil erythroblasts*** because they stain with basic dyes
- The cell at this time has accumulated very little hemoglobin.
- In the succeeding generations, the cells become filled with hemoglobin to a concentration of about 34 %
- The nucleus condenses to a small size, and its final reminant is absorbed or extruded from the cell.
- At the same time, the endoplasmic reticulum is also reabsorbed. The cell at this stage is called a ***reticulocyte***

Genesis of Red Blood Cells: **Erythropoiesis**

- During this reticulocyte stage, the cells pass from the bone marrow into the blood capillaries by ***diapedesis*** (squeezing through the pores of the capillary membrane).
- The remaining basophilic material in the reticulocyte normally disappears within 1 to 2 days, and the cell is then a ***mature erythrocyte.***

Erythropoietin Regulates Red Blood Cell Production

The total mass of RBCs in the circulatory system is regulated within narrow limits

1) an adequate number of RBCs should always be available to provide sufficient transport of oxygen from the lungs to the tissues
2) the cells should not become so numerous that they impede blood flow.

Tissue Oxygenation Is the Most Essential Regulator of RBC Production

Conditions that decrease the quantity of oxygen transported to the tissues ordinarily increase the rate of RBC production

Erythropoietin Stimulates Red Blood Cell Production, and Its Formation Increases in Response to Hypoxia

- The principal stimulus for RBC production in low oxygen states is a circulating hormone called ***erythropoietin***
- glycoprotein MW= 34,000 Da
- EPO is formed mainly in the kidneys (90%), and the remainder is formed mainly in the liver.
- Renal tissue hypoxia leads to increased tissue levels of ***hypoxia-inducible factor–1*** **(HIF1)**
- **HIF1 is a transcription factor that binds to** *hypoxia response element* containing genes including EPO.

Ill. Niklas Elmehed. © Nobel Media.

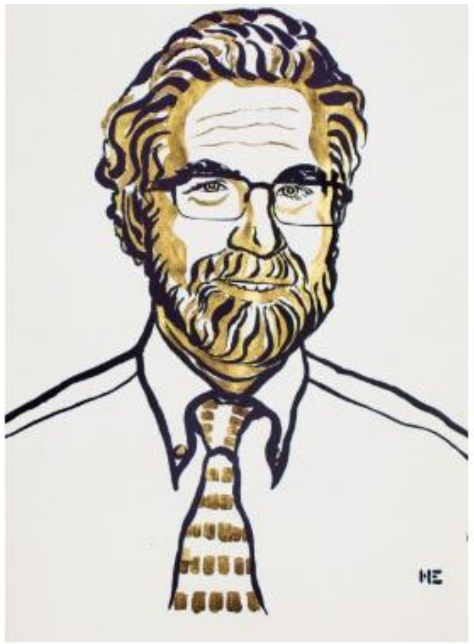
William G. Kaelin Jr

Prize share: 1/3

Ill. Niklas Elmehed. © Nobel Media.

Sir Peter J. Ratcliffe

Prize share: 1/3



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Gregg L. Semenza

Prize share: 1/3

The Nobel Prize in Physiology or Medicine 2019 was awarded jointly to William G. Kaelin Jr, Sir Peter J. Ratcliffe and Gregg L. Semenza "for their discoveries of how cells sense and adapt to oxygen availability."

- Hypoxia in other parts of the body, but not in the kidneys, stimulates kidney **erythropoietin** secretion, which suggests that there might be some non-renal sensor that sends an additional signal to the kidneys to produce this hormone.

- In particular, both norepinephrine and epinephrine and several of the prostaglandins stimulate EPO production.

Q: What happens when both kidneys are removed from a person or when the kidneys are destroyed by renal disease?

A: The person invariably becomes very **anemic.** Because the 10 % of the normal EPO formed in other tissues (mainly in the liver) is sufficient to cause only one third to one half the RBC formation needed by the body.

Generation of White Blood Cells: **Leukopoiesis**

- WBCs are formed in bone marrow
- Myeloid cells (Granulocytes)
  - have a life span of 12 hrs-3days
    - Neutrophils
    - Eosinophils
    - Basophils
    - Monocytes
- Lymphoid cells (Agranulocytes)
  - have a life span of 100-300 days

Normal indices for WBC

	Differential	Absolute counts
Lymphocytes	20-44%	1.2-3.4 x $10^3/\mu l$
Monocytes	2-9%	0.11-0.59 x $10^3/\mu l$
Neutrophils	50-70%	1.4-6.5 x $10^3/\mu l$
Bands	2-6%	0-0.7 x $10^3/\mu l$
Eosinophils	0-4%	0-0.5 x $10^3/\mu l$
Basophils	0-2%	0-0.2 x $10^3/\mu l$

Absolute count = %/100 x WBC

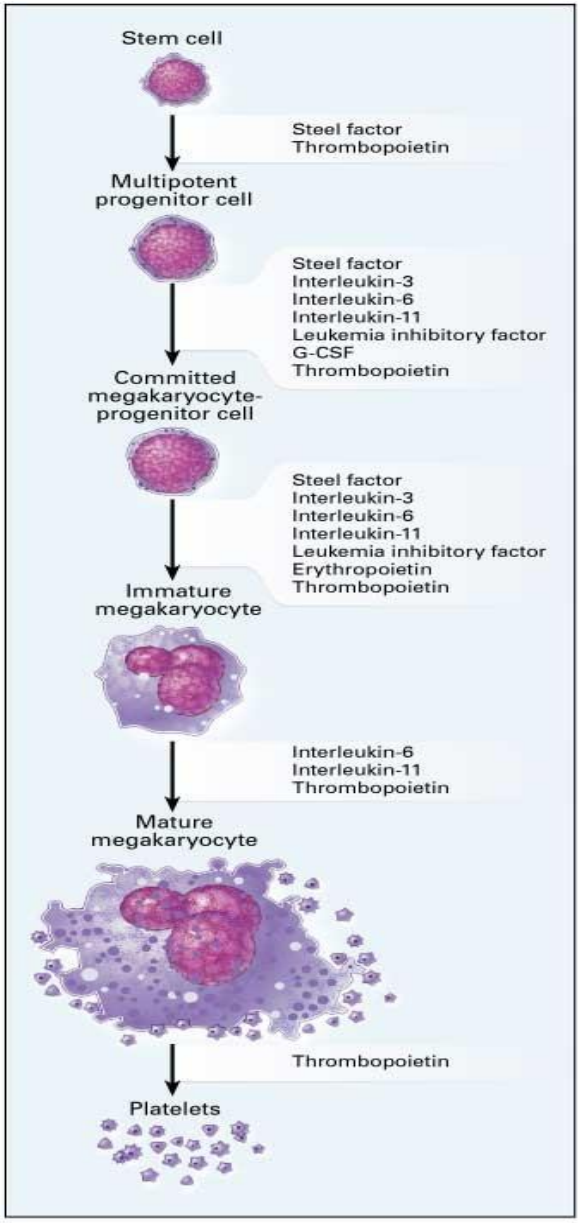
Generation of White Blood Cells: **Leukopoiesis**

- Leukopoiesis is regulated by production of interleukins and colony-stimulating factors (CSF)

Generation of Platelets: **Trombopoiesis**

- Megakaryocytes → 1000-3000 platelet
- Megakaryocytes are made in the bone marrow
- Lineage: Myeloid
- Count: 0.3-3% of bone marrow cells
- Maturation: endomitosis (no cytokinesis)
- While leaving and trying to squeeze they become platelets

Factors that stimulate thrombopoiesis

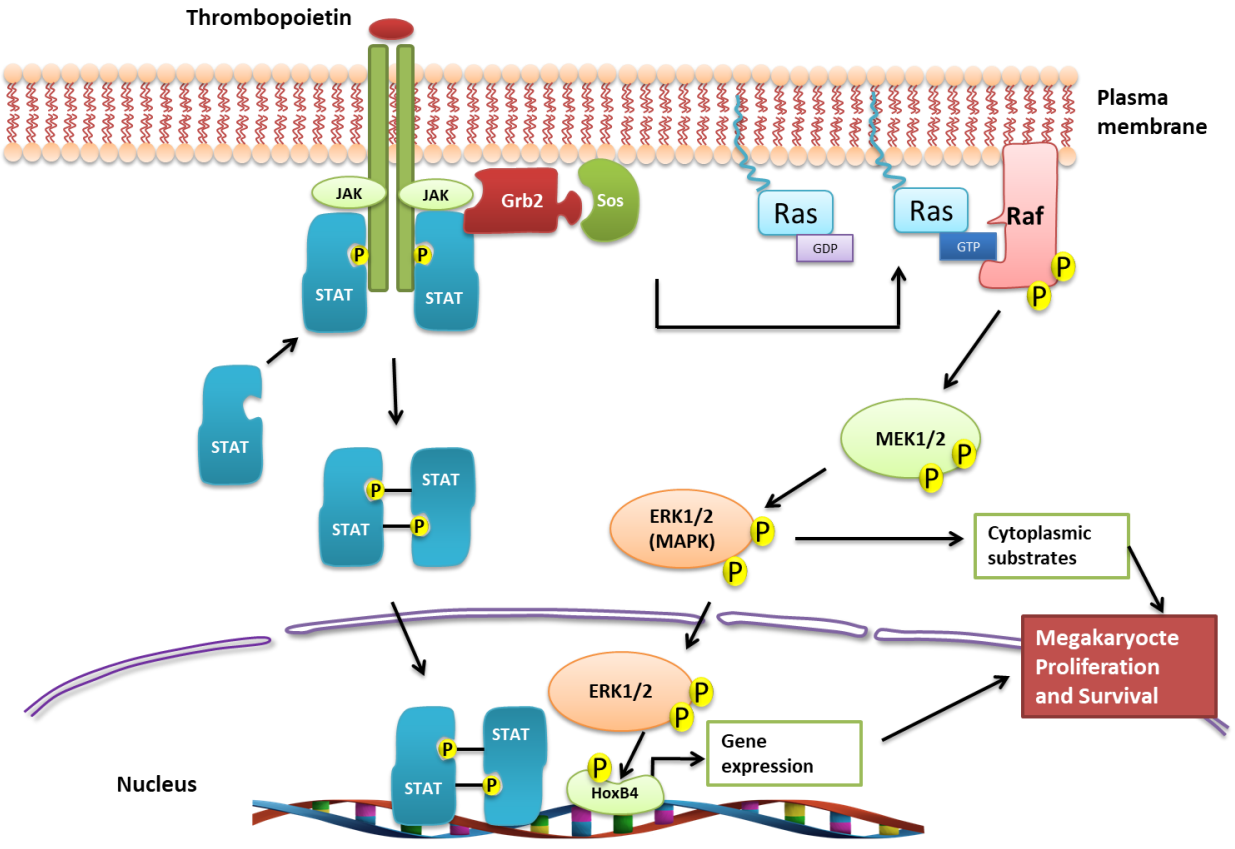


- Thrombopoietin (TPO)
- GM-CSF, IL-3, IL-6, IL-11
- Chemokines

TPO

- Produced in the liver, kidney, and bone marrow
- After binding to the surface of platelets and megakaryocytes through the c-mpl receptor, TPO is internalized and destroyed, thereby reducing further platelet and megakaryocyte exposure to the hormone

How does TPO do its job?



- Stimulation of the TPO receptor results in activation of a number of signaling pathways via Janus kinase type 2 (JAK2) and tyrosine kinase 2 (TYK2).
- Mitogen-activated protein kinase activation subsequently leads to changes in gene expression, promoting progression of stem cells along the megakaryocytic pathway, megakaryocyte maturation, and subsequent release of normally functioning platelets into the peripheral circulation