Molecular Disease Mechanisms

Lecture 3: Receptor-mediated carcinogenesis and epigenetics

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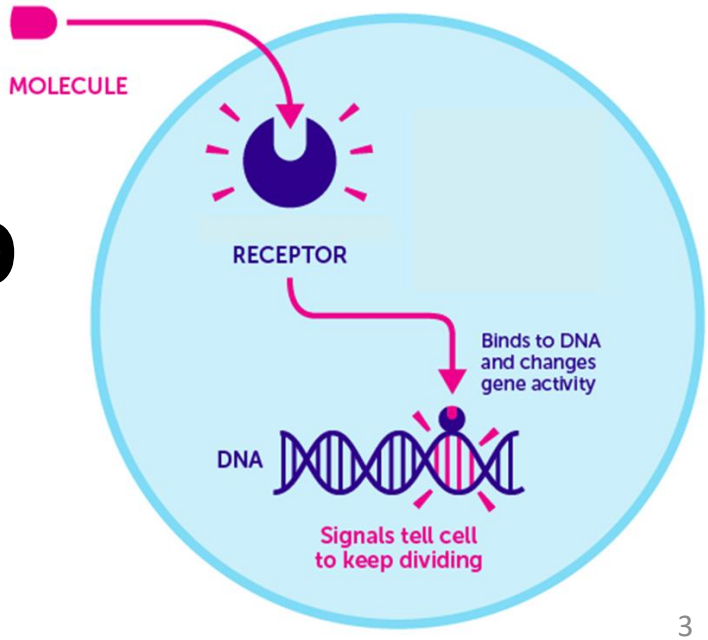
Topics for lecture 3

1. Receptor-mediated carcinogenesis
   -Aryl Hydrocarbon Receptor
2. Hormone-mediated carcinogenesis
   -Breast cancer risk factors
   -Endocrine disrupting compounds
3. Epigenetic mechanism of carcinogenesis
   -Epigenetic modifications
   -The cancer epigenome

Lecture 3, Part 1

RECEPTOR-MEDIATED CARCINOGENESIS



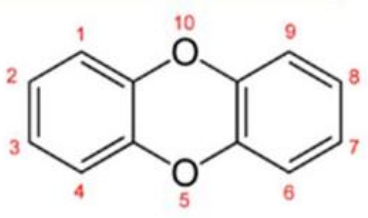
Aryl hydrocarbon receptor

- AHR protein encoded by the AHR gene
- **Ligand-activated** transcription factor (the ligand-receptor complex migrates from the cytoplasm to the nucleus)
- Activates the expression of multiple phase I and II xenobiotic chemical metabolizing enzyme genes (e.g. CYP1A1 gene)
- In the cytosol AHR is inactive, binds to ligand and transports to nucleus where it is active
- Ligands are generally planar aromatic hydrocarbon compounds
  - Dioxin
  - Plant flavonoids
  - Polyphenolic compounds
  - PAHs
  - Endogenous ligands of AhR include tryptophan-derived metabolites

Dioxins bind to AhR

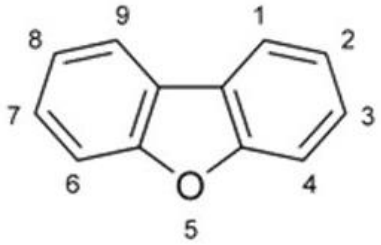
- Dioxins: a family of compounds with diverse chlorine substitutions at the benzene rings in PCDDs and PCDFs (210 possible types)

- PCDD:



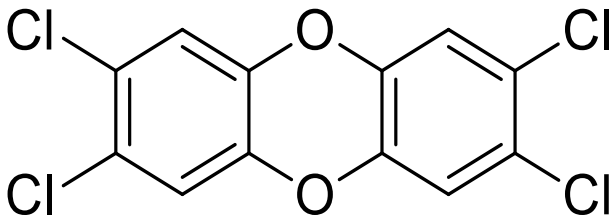
polychlorinated dibenzo-p-dioxin (75)

- PCDF:



polychlorinated dibenzofurans (135)

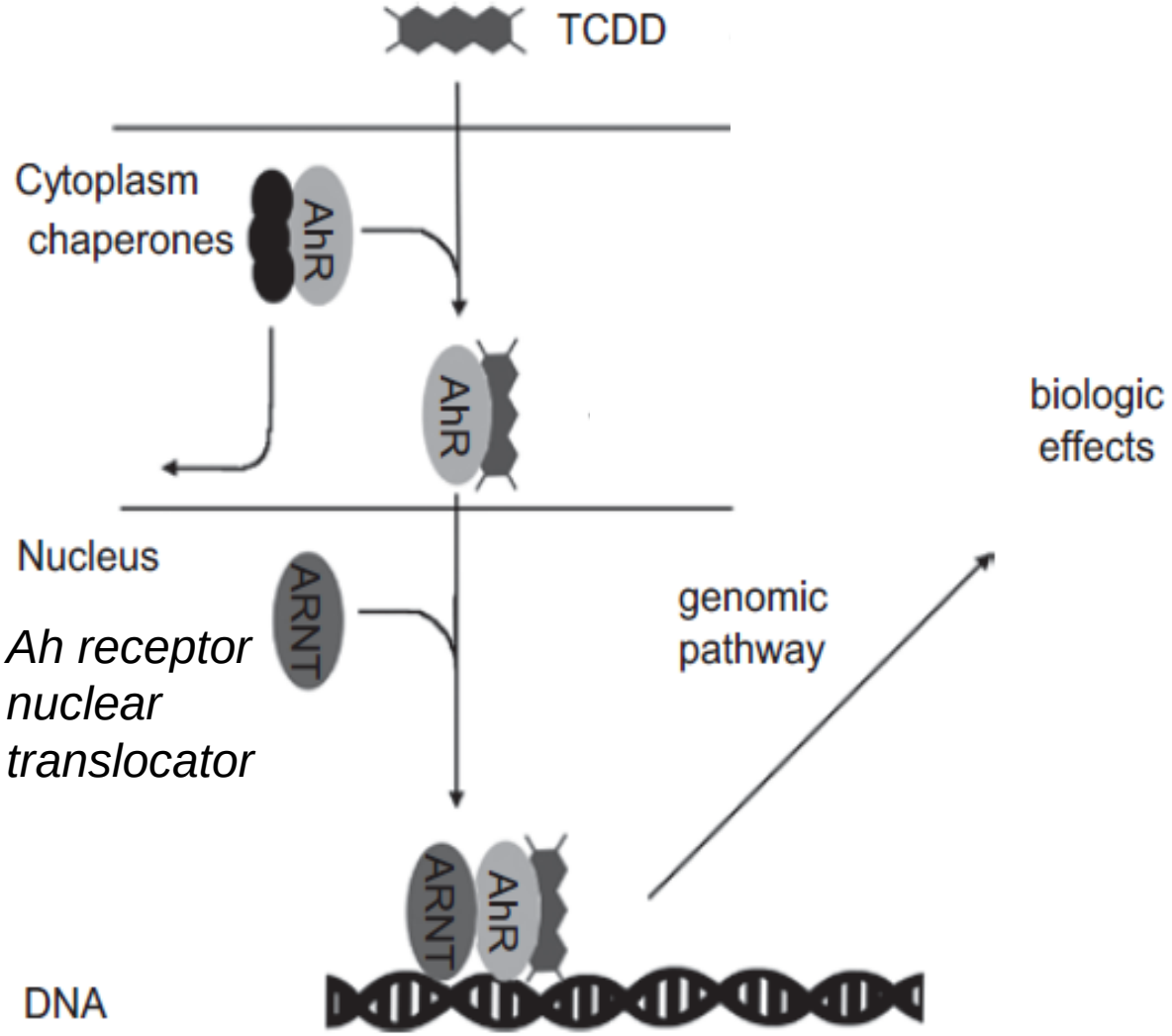
TCDD – proto-typical example



2,3,7,8 tetrachlorodibenzo-*p*-dioxine (TCDD)

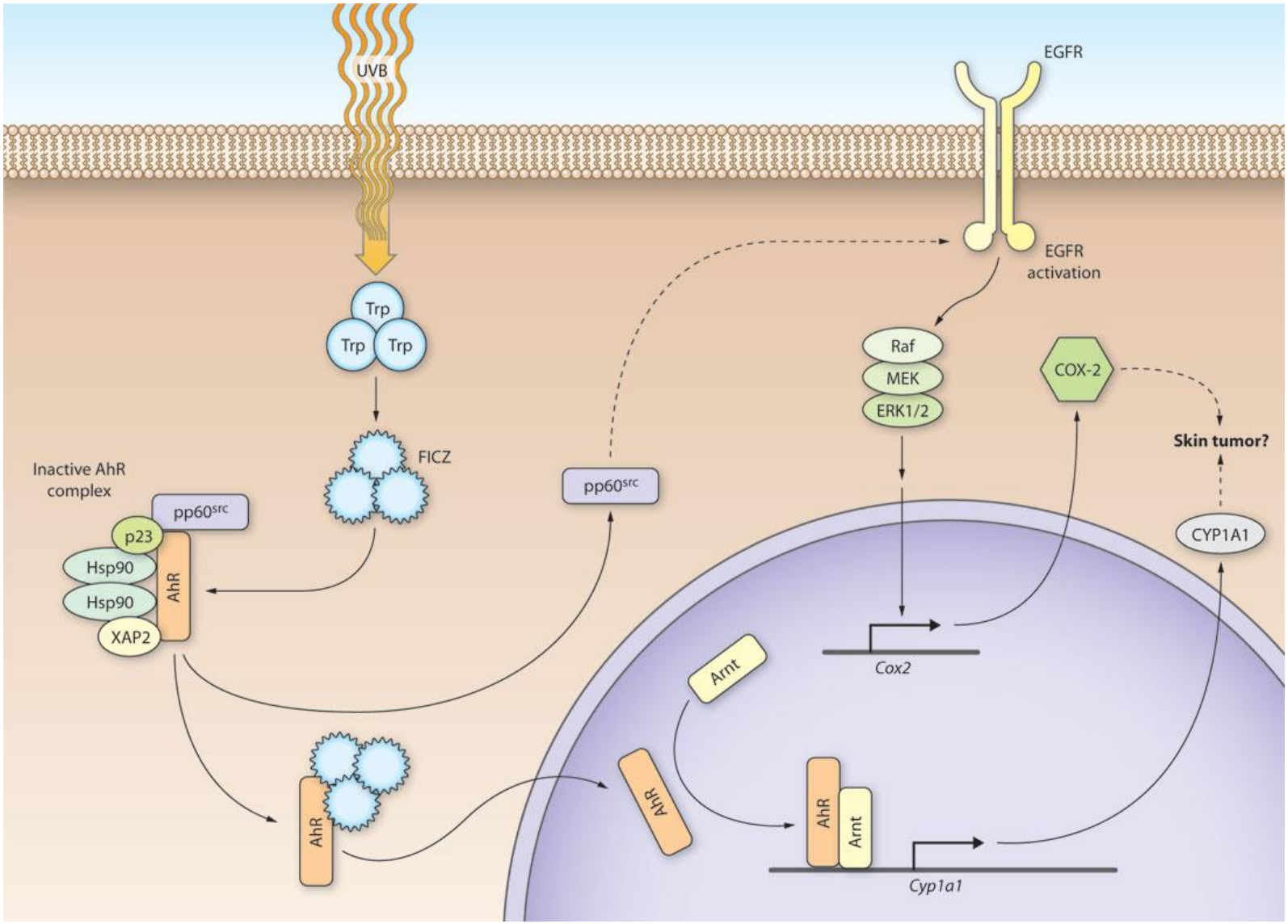
- Industry byproduct, have been used as pesticides since before WWII
- Can be detected anywhere in the environment; most human exposure is from food (accumulate in fat)
- Very high lipophilicity and stability
  - (half life TCDD ~ 7 years)
- Classified as known human carcinogen
- Classification based on mechanistic considerations focusing on the Ah receptor --> receptor-mediated mechanism of carcinogenesis

Aryl hydrocarbon signaling



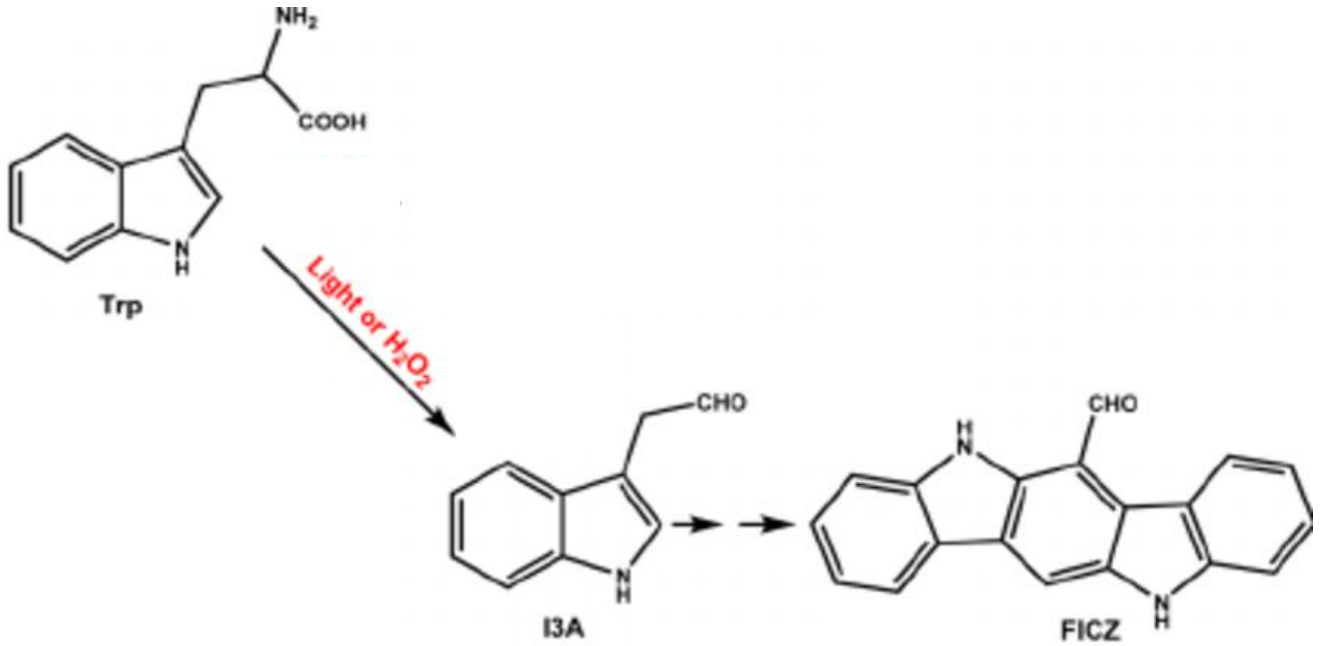
Chopra and Schrenk, Crit Rev Toxicol, 2011, 41, 292

AHR activation by UV exposure



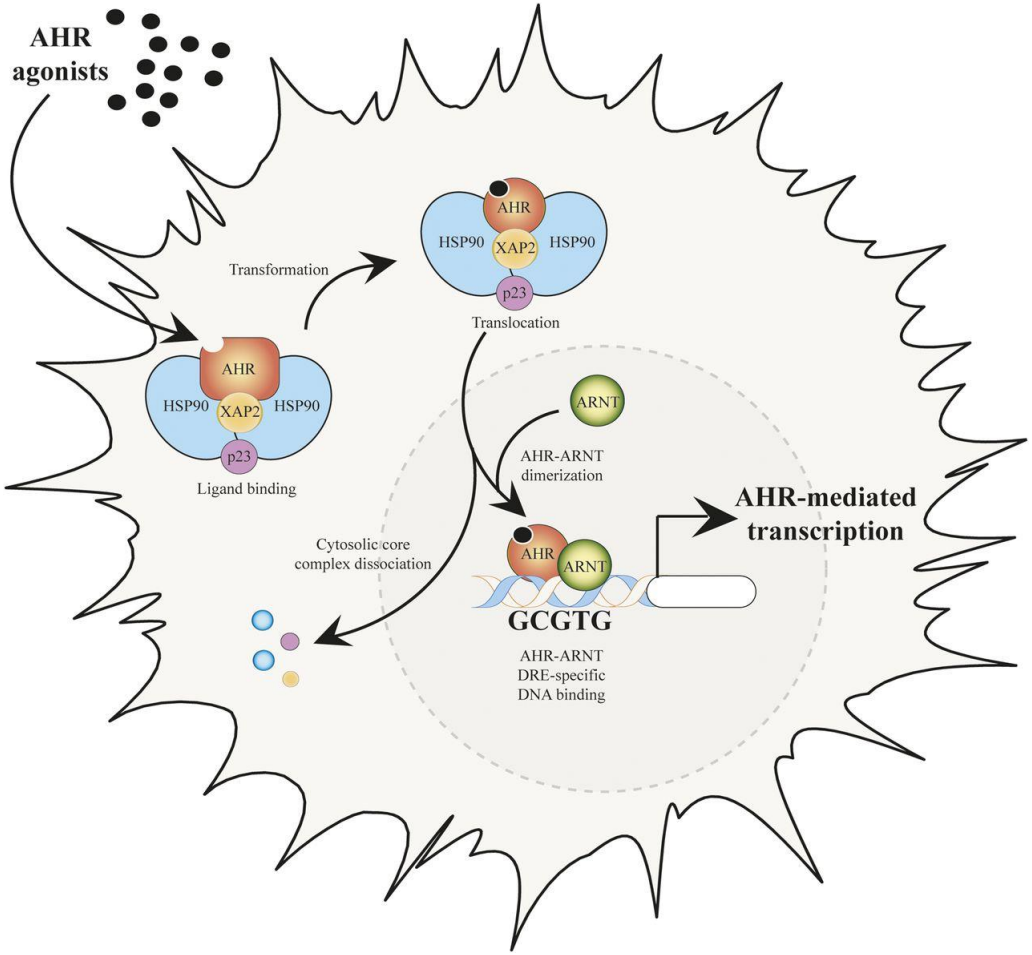
The Aryl Hydrocarbon Receptor: An Illuminating Effector of the UVB Response

Tryptophan metabolite FICZ activates AHR



Evidence for New Light-Independent Pathways for Generation of the Endogenous Aryl Hydrocarbon Receptor Agonist FICZ

AHR-mediated transcription



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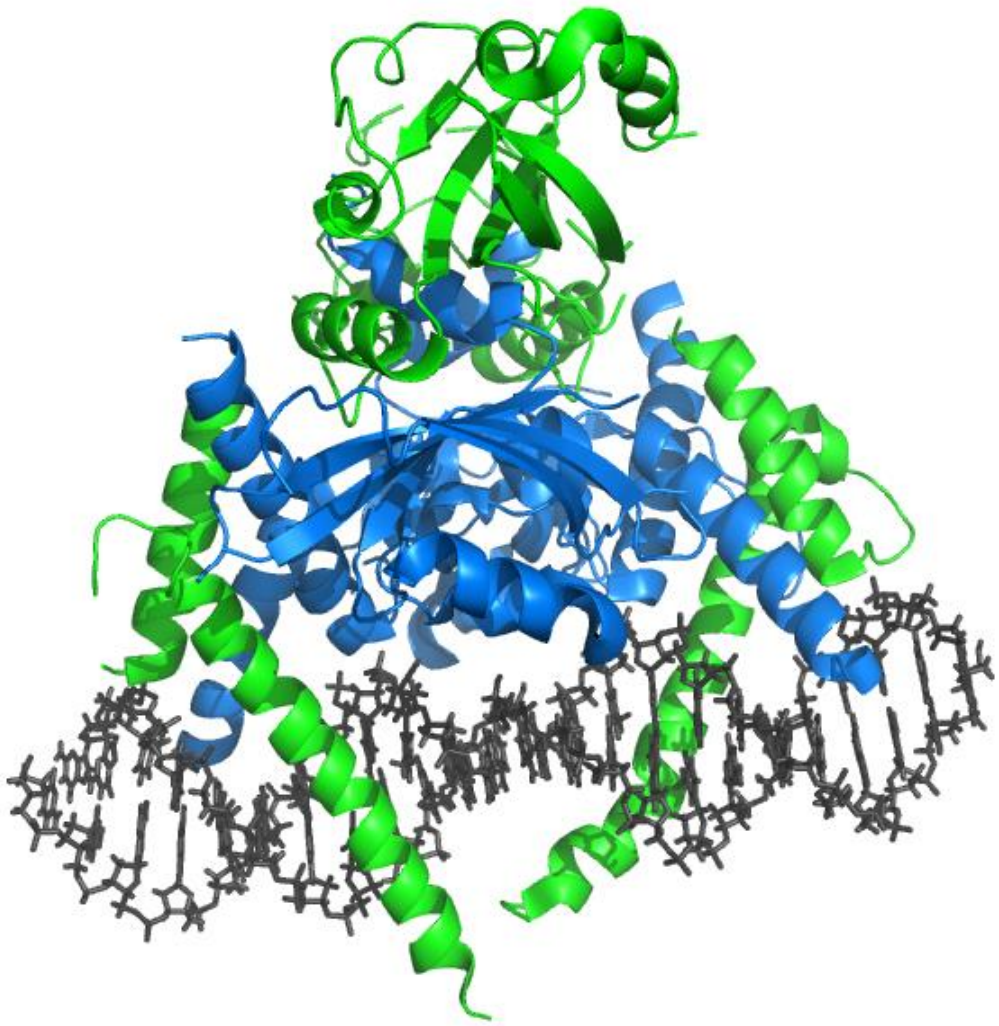
Aryl hydrocarbon receptor



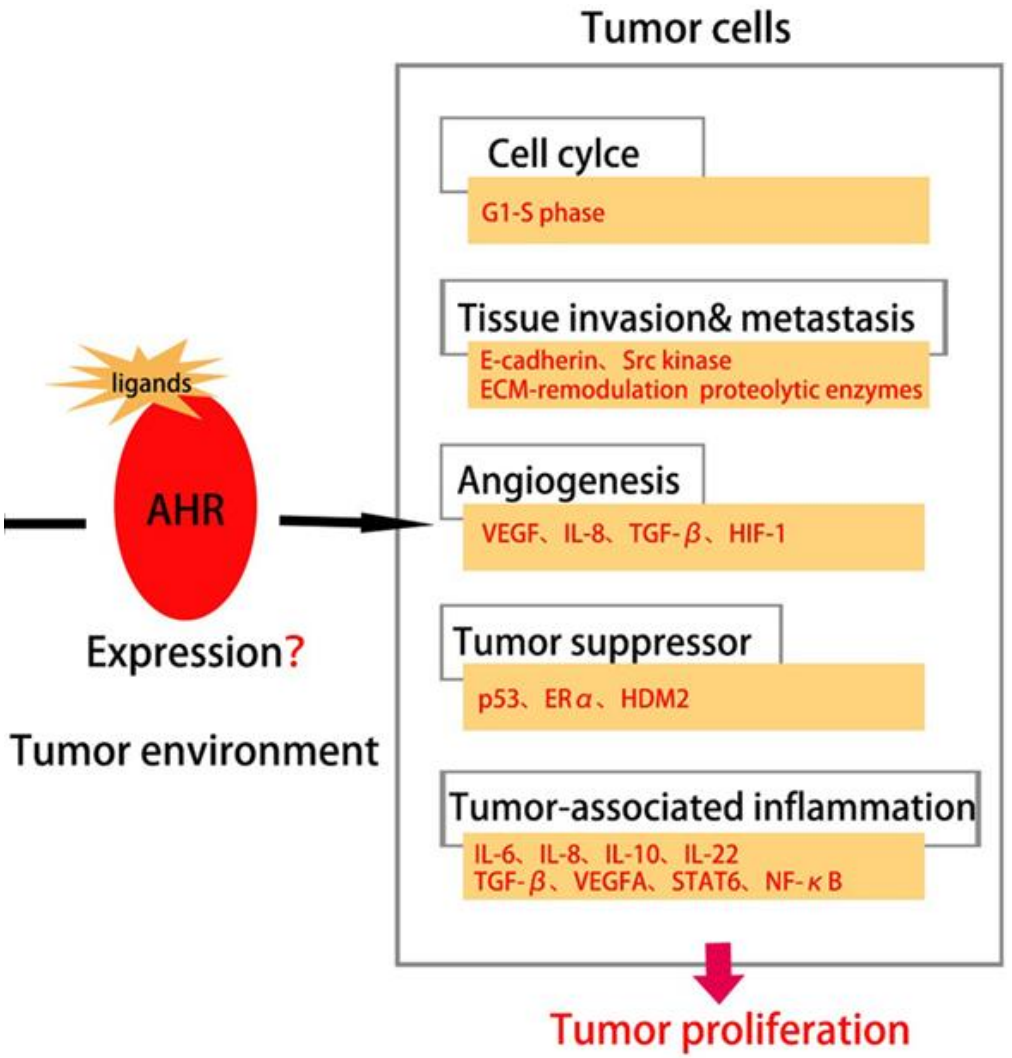
AhR

ARNT

PDB code: 5NJ8

AhR promotes tumorigenesis

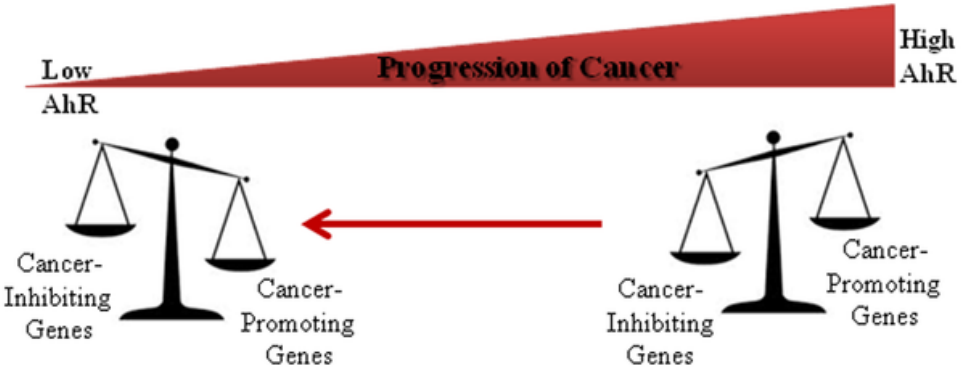
Overexpression and constitutive activation of the AhR have been observed in various tumor types



The Aryl Hydrocarbon Receptor and Tumor Immunity. 2018

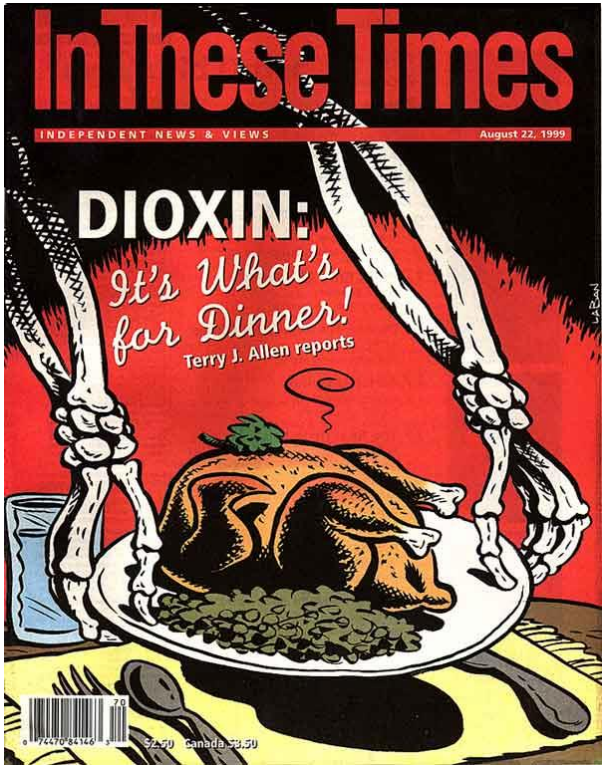
Why can stimulating AHR be bad?

- Sustained hyperactivation situation:
  - Long human half life (dioxin ~10 y)
  - high binding affinity
- Induces expression of enzymes that bioactivate other carcinogens
- Induces growth factors
- Promotes inflammation signals
- Overexpression of AHR and its target growth factors characterized in cancer cells



Dioxin contamination scandal: Belgium 1999

- farms in Belgium ordered to destroy livestock given feed contaminated with dioxin
- bans on Belgian agricultural exports of eggs, chickens, pork and beef
- contamination thought to have come from tanks used to hold animal fats for producing animal feeds
- tanks previously used to hold industrial oil containing dioxins
- tanks not sufficiently cleaned so animal fats became contaminated with the dioxin-bearing oil residues
- tainted animal feeds were supplied to hundreds of Belgian farms, and were also exported to France, Holland and Germany
- The food crisis precipitated a massive political crisis inside Belgium



WORLD

Food Scare Is Leaving Tables Bare in Belgium

By THE ASSOCIATED PRESS JUNE 6, 1999

What's for dinner? No local steaks for the main course. No chicken. Nothing with eggs in it. No Belgian waffles for dessert. Forget pastries and ice cream.

The entire nation is wondering what to eat these days after those foods and more were pulled from supermarket shelves or considered too suspect to eat because of cancer-causing dioxin feared to have spread through the Belgian food chain due to contaminated animal feed.

"No chicken, no pork, no eggs, no beef!" screamed a banner headline in the daily La Derniere Heure this week.

"And that jar of mayonnaise?" Health Minister Luc van den Bossche said. "Better not touch it."

The Government met Friday night to decide whether to withdraw all beef and pork products from the markets, after doing so with poultry and eggs earlier this week -- leaving shoppers staring at empty shelves instead of stocking up on weekend goodies.

https://www.nytimes.com/1999/06/06/world/food-scare-is-leaving-tables-bare-in-belgium.html

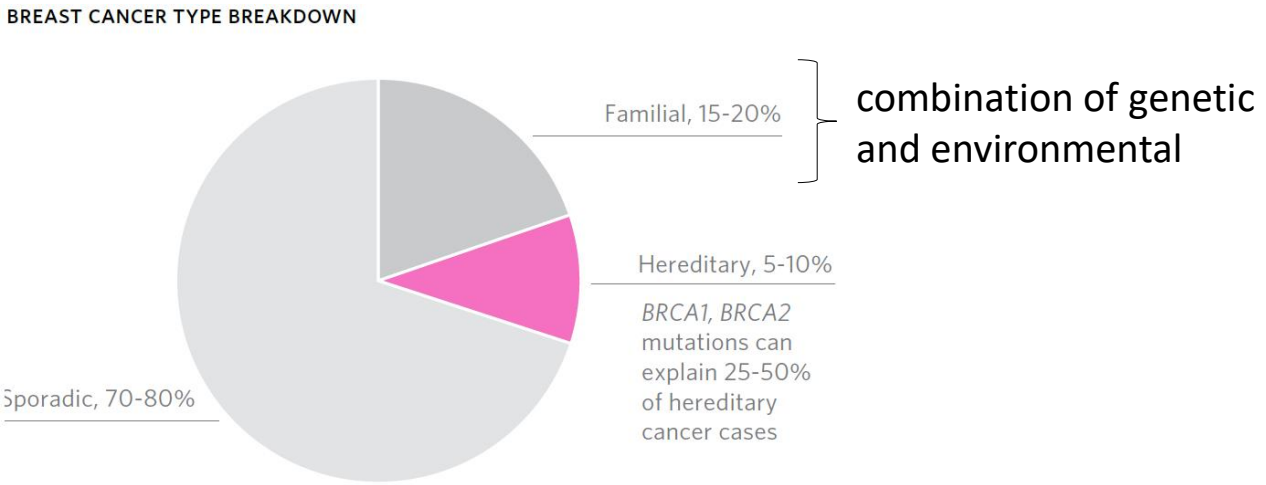
Lecture 3, Part 2

HORMONE-MEDIATED CARCINOGENESIS

Breast
Prostate

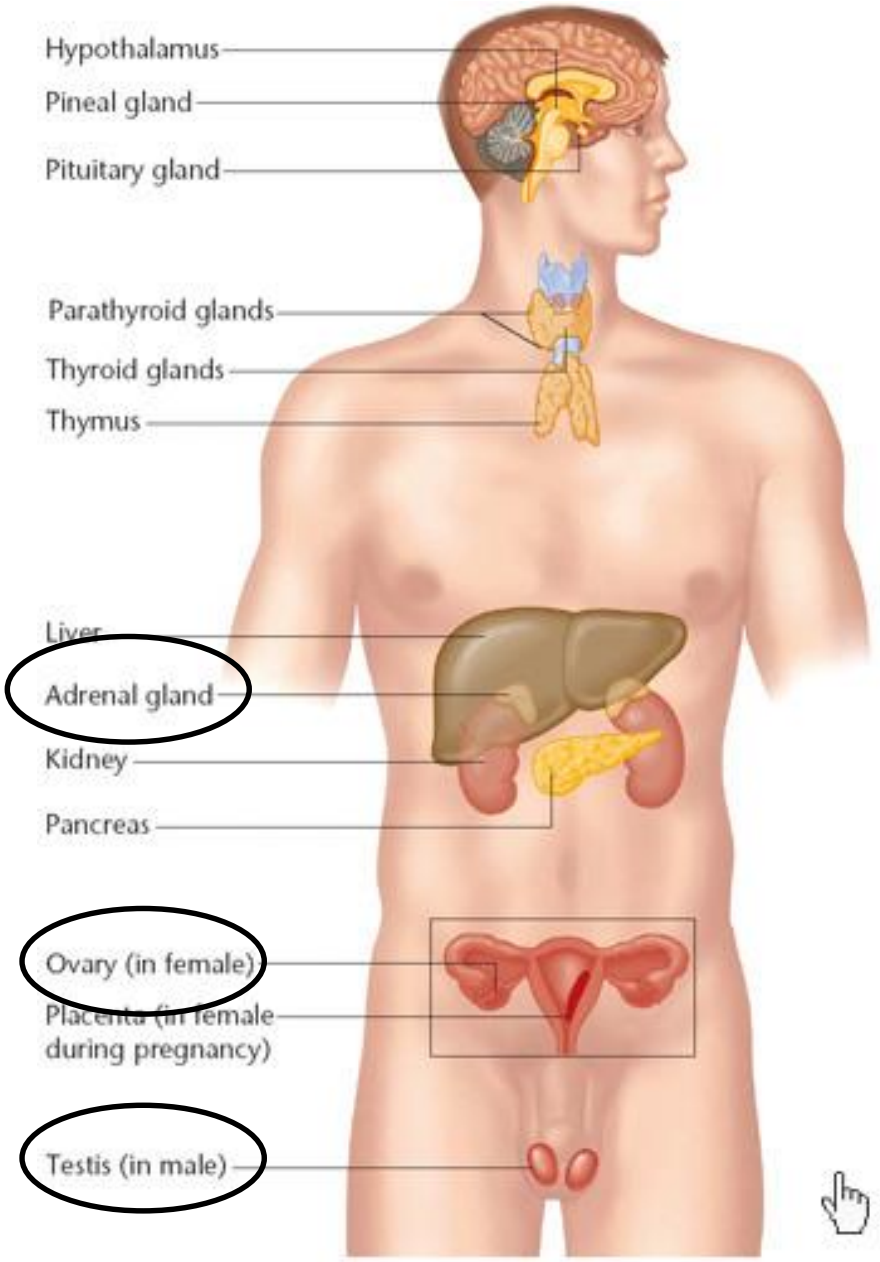
Hormone dependent cancers

Breast Cancer: Risk Factors



http://www.breastlink.com/breast-cancer-101/risk-factors/

- prolonged exposure to high levels of estrogens
- in obese women, adipose tissue a major source of estrogens that increase breast cancer risk
- women who receive hormone replacement therapy (HRT) are more likely to develop breast cancer than those who have never used HRT
- higher levels of blood estrogen in post-menopausal women are associated with higher Breast Cancer Incidence
- Increased rate of Breast Cancer in women taking estrogens

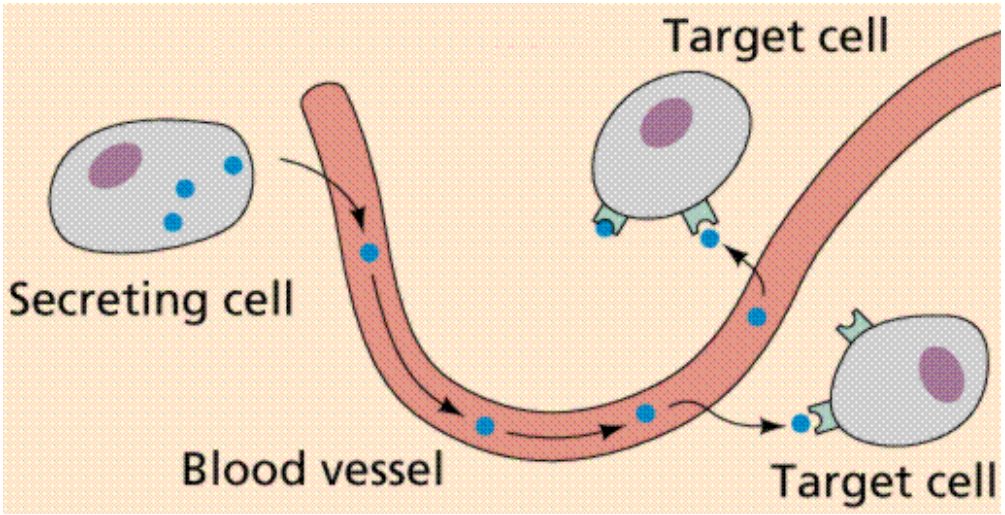


Secretion of hormones

- chemical messengers
- secreted into the blood by specialized cells in endocrine glands
- generally act on remote organ sites and alter rates of processes in target cells
- act at very low concentrations
  - nano to picomolar range (10^{9} to 10^{12})
- control growth, development, metabolism, reproduction, and regulation of homeostasis

http://e.hormone.tulane.edu/learning/endocrine-system.html

Hormones act by binding receptors on or in target cells

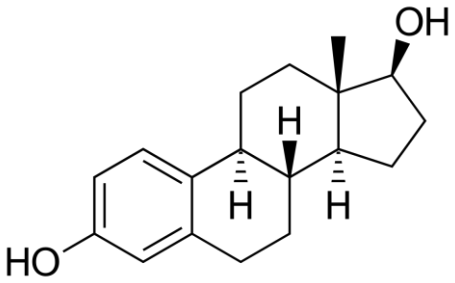


- Controlling the rates of enzymatic reactions
- Controlling the movement of ions or molecules across membranes
- Controlling gene expression and protein synthesis

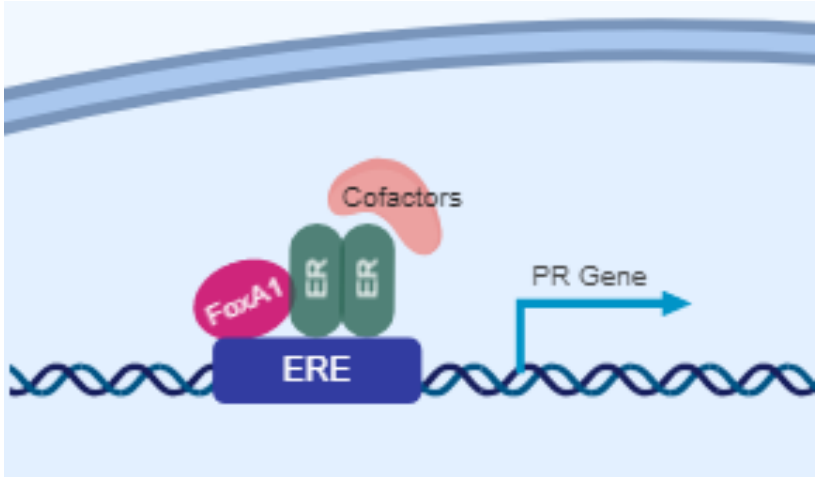
http://tsbiomed.blogspot.ch/2012/12/notes-in-endocrinologyhormones.html

Estrogen binds to the ER Transcription Factor

- Estrogen receptor (ER) is a transcription factor that regulates gene expression
  - (classic ER target is the PR gene)
- Progesterone receptor (PR) is a biomarker for ER function

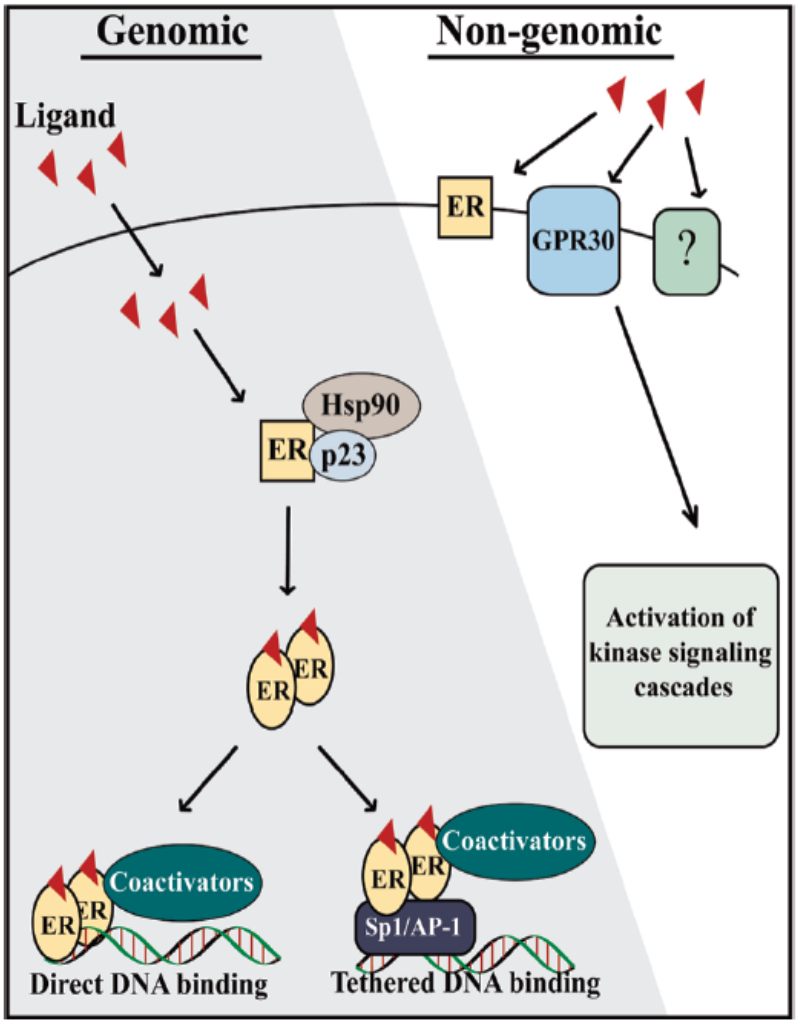


Estradiol (E2)



Mechanism of endocrine modulation by estrogen agonist

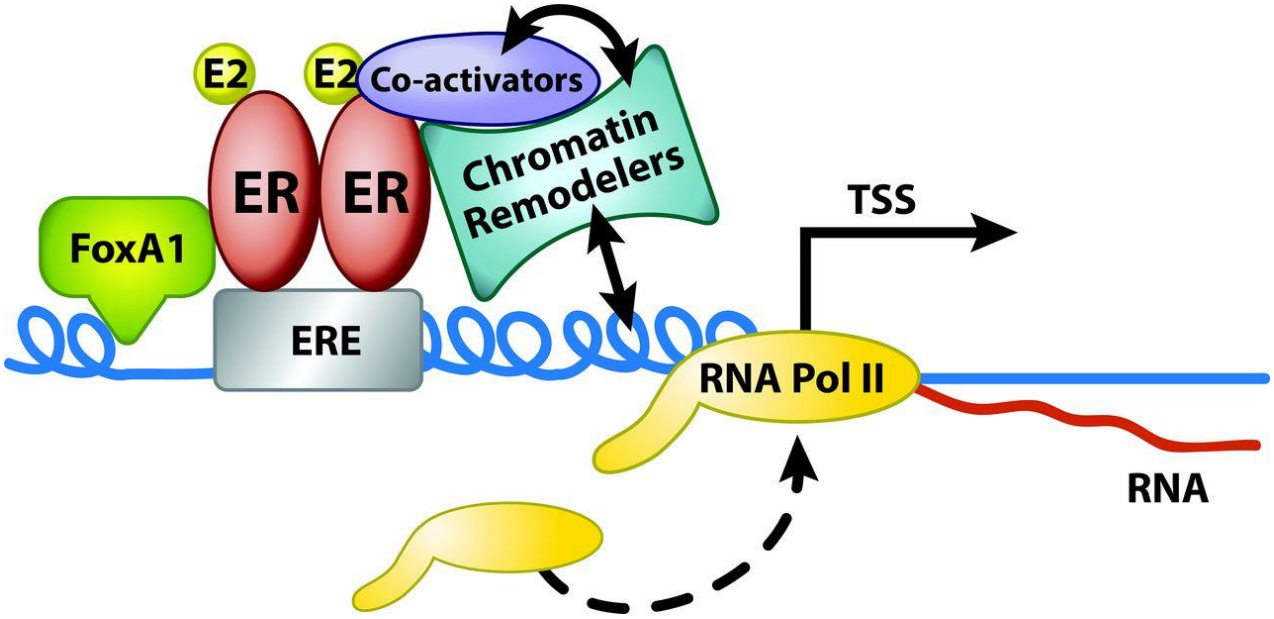
Intracellular
Estrogen receptors

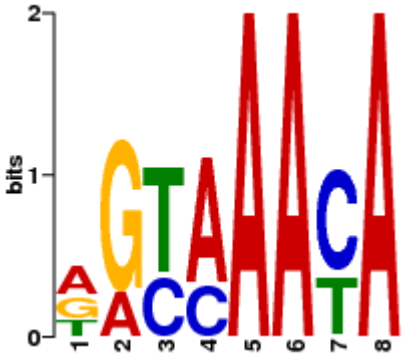


Membrane estrogen
Receptors (mER)

Shanle and Xu, Chem ResToxicol, 2011, 24, 6

Mapping ER binding sites





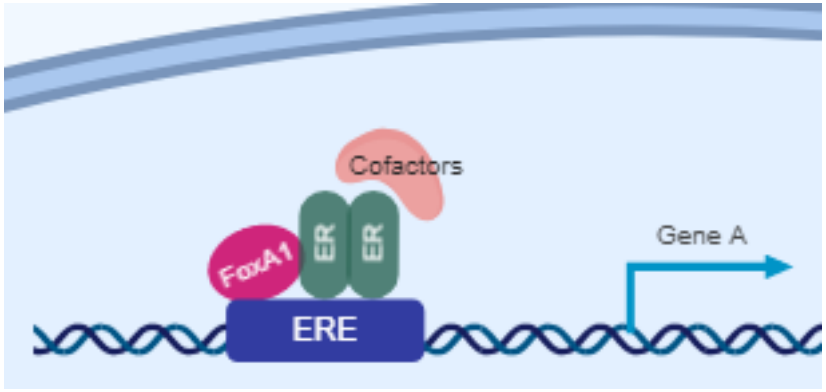
Forkhead motifs

FoxA1 – tethers ER to chromatinized DNA
-ER will not bind to chromatin (DNA + histones) without FoxA1
-ER will bind to DNA in a test tube

What's new in estrogen receptor action in the female reproductive tract. J Mol Endocrin. 2016

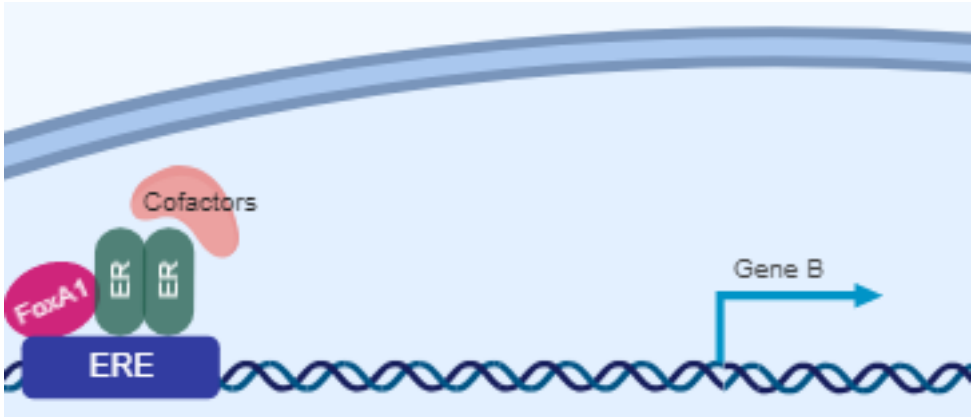
ER binds in different genomic regions depending on the cancer

ER +, PR + (better outcomes)



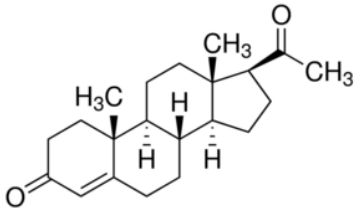
The movement is not random: It moves from one Fox1 site to another

ER +, PR – (poor outcomes)



This was surprising as progesterone is usually linked to pro-proliferation

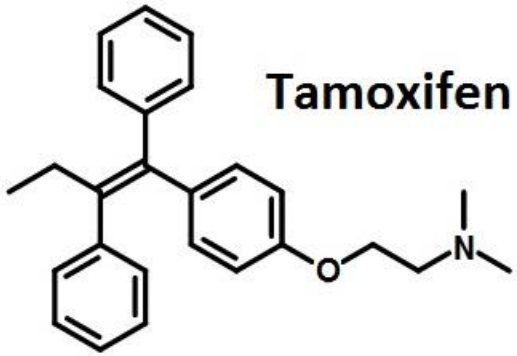
ER and progesterone



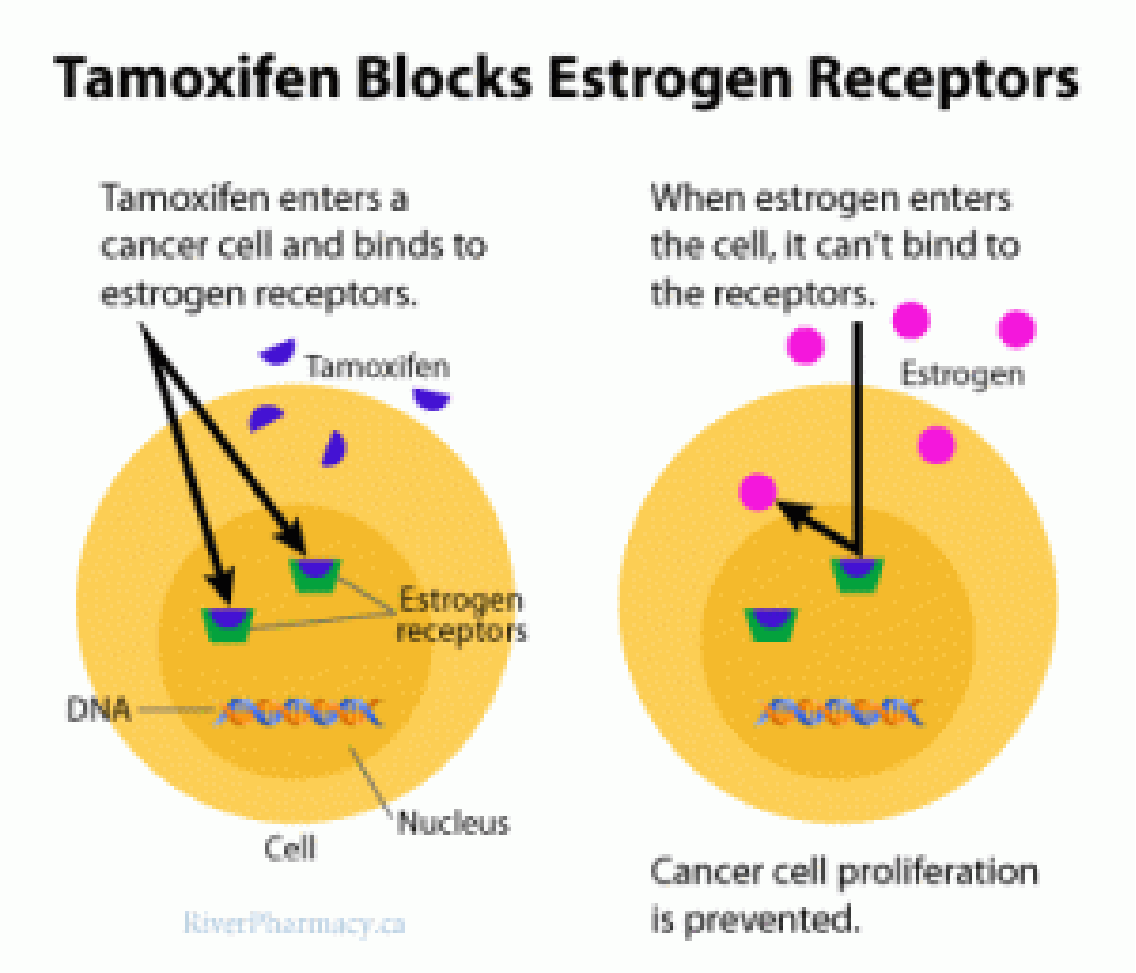
- Progesterone is generally pro-proliferative
- However, in cancer, progesterone is often anti-proliferative
  - (a re-wiring in cancer cells)
- Treating cancer cells with progesterone changes where ER binds -> Leading to better clinical outcomes
  - Progesterone brings PR and ER together for binding as TFs.

Tamoxifen Therapy

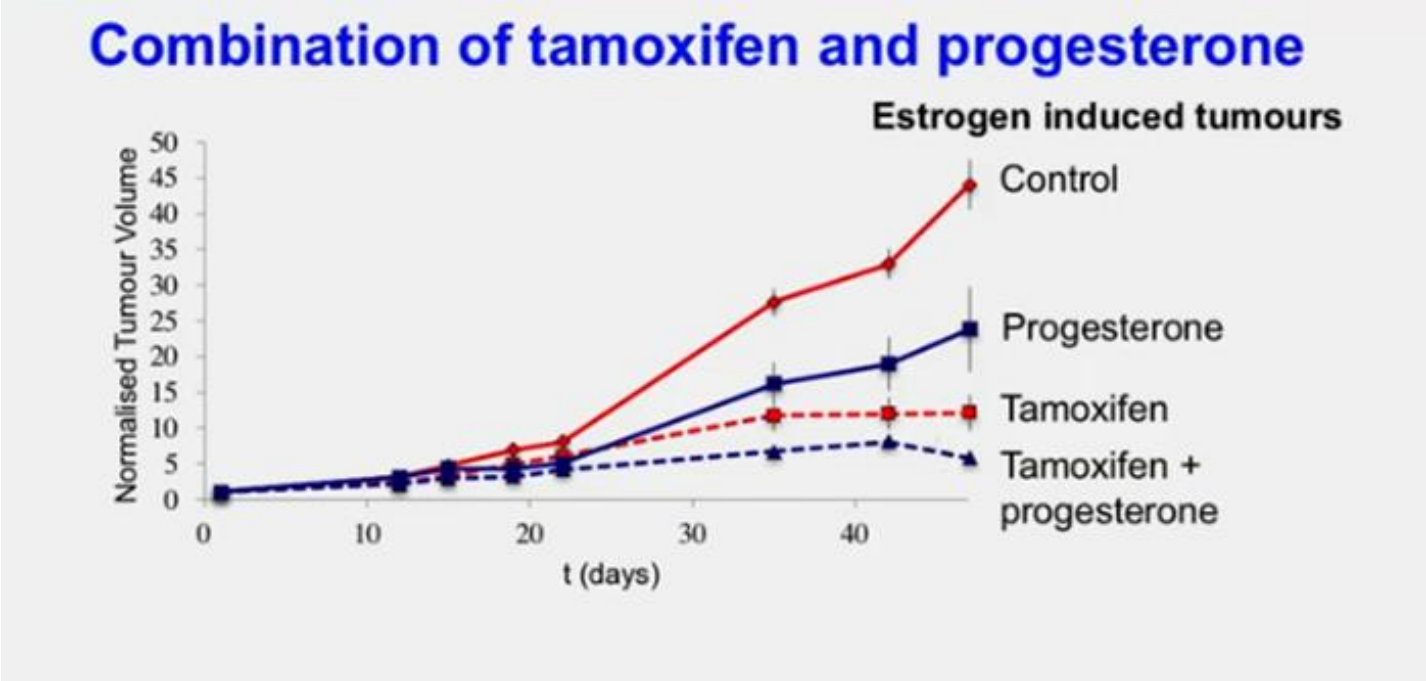
- used to prevent breast cancer in women and treat breast cancer in women and men
- Competitive inhibitor of estrogen that blocks the action of estrogens in several tissues, most notably the breast
- Therapeutic compound acting as a ligand for estrogen receptors (ER), but with a distinctly different spectrum of activities from the natural hormone, 17-β-estradiol
- Tamoxifen exhibits an anti-estrogen activity on mammary tissues in postmenopausal women



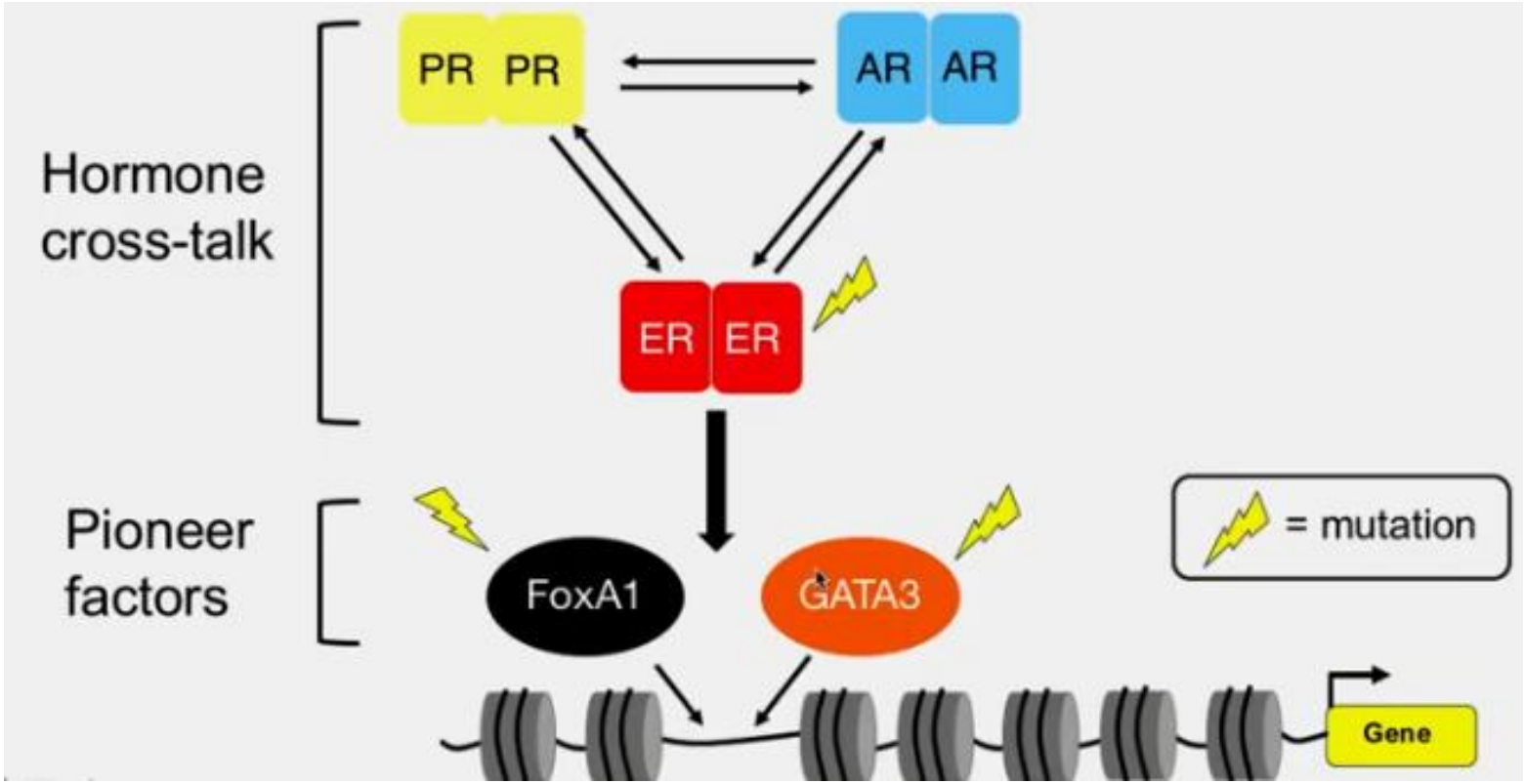
Mechanism of action of Tamoxifen



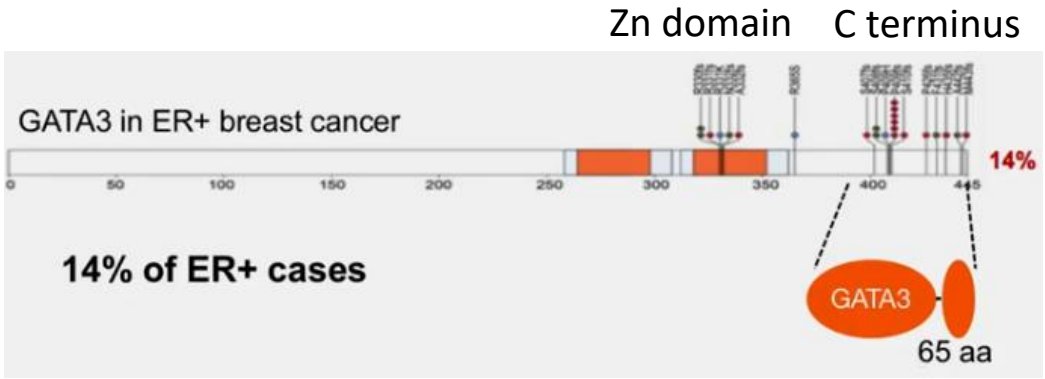
Tamoxifen and Progesterone



Pioneer factor GATA3 in ER binding

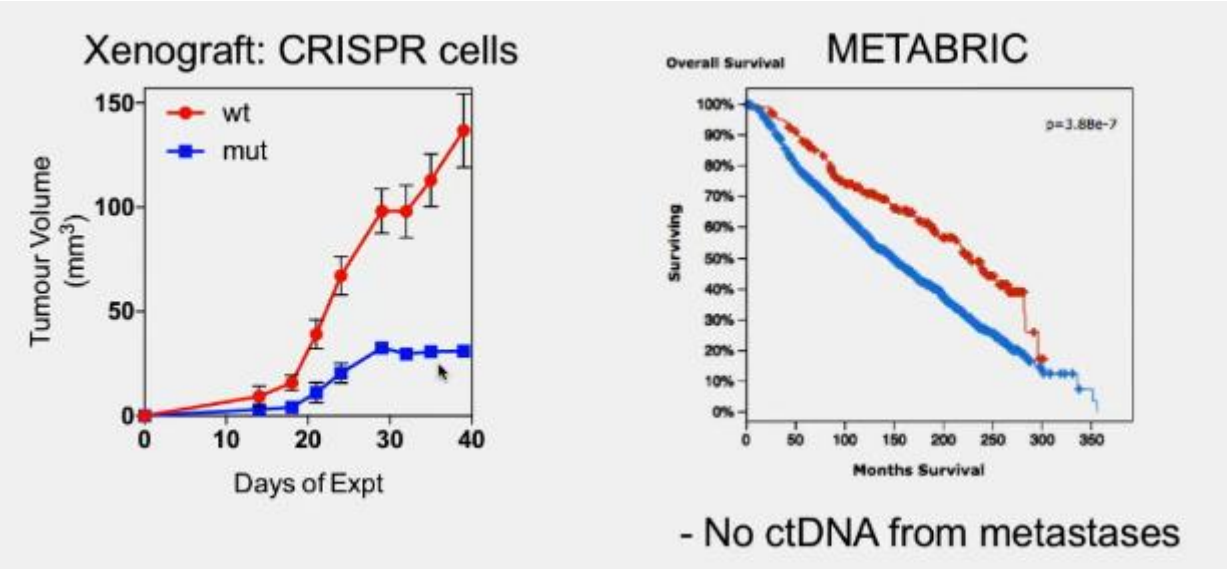


Mutations in GATA3

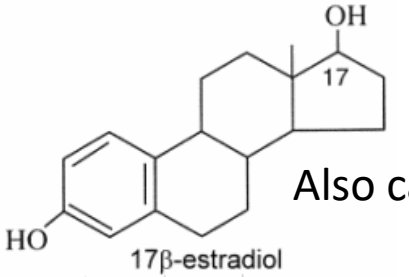


C terminal mutation:
+1 insertion = a frame shit
-changes stop codon
adding 65 aa to C-terminus
-This mutant protein is stable

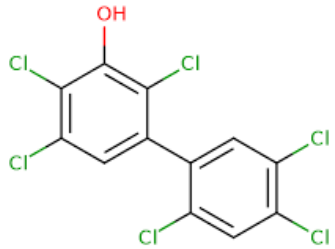
This mutant GATA3 is protective in cancer!



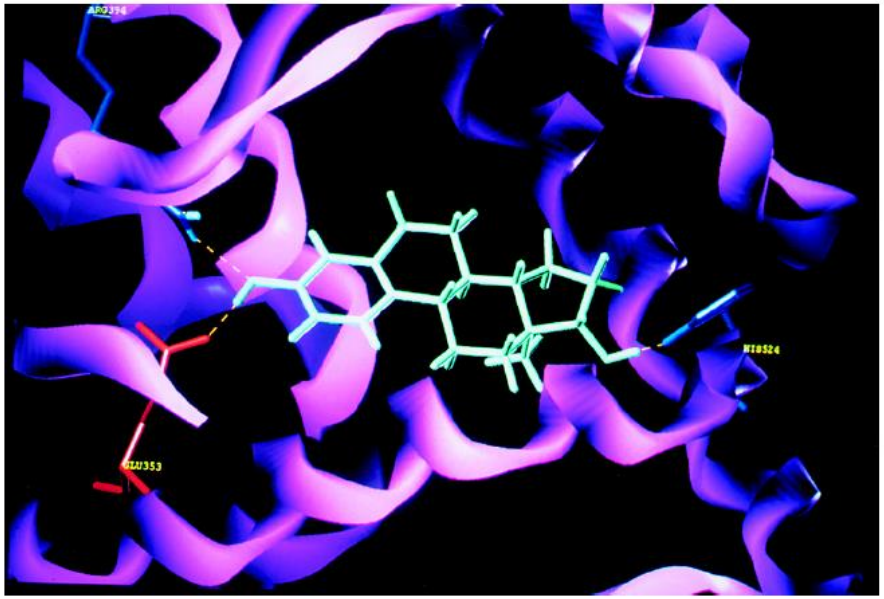
ER also binds to xenobiotics

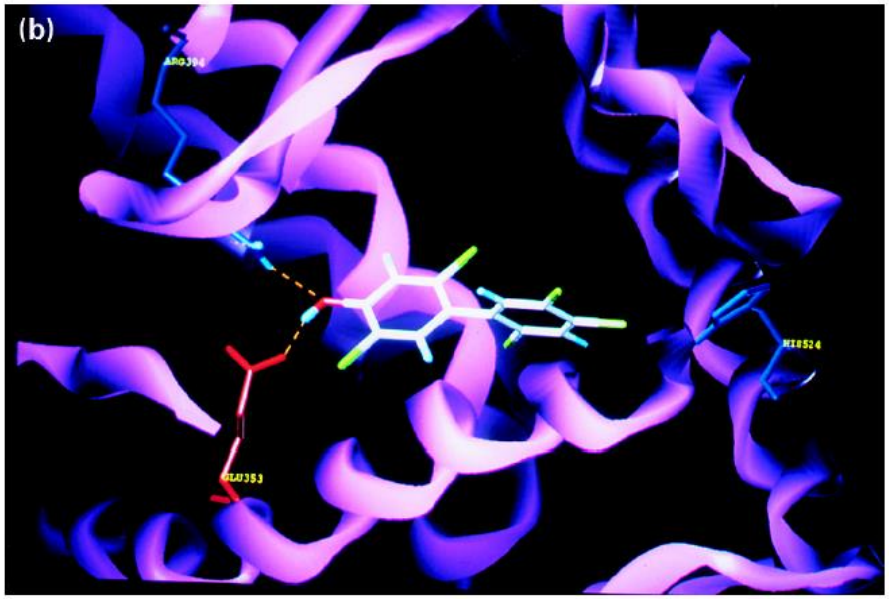


Also called E2



Polychlorinated Biphenyl (PCB)





PCBs - once widely used as dielectric and coolant fluids in electrical apparatus
-synthetic hormone mimics, like PCB, are similar binders like the natural ligand
-high fat solubility and stability can cause bioaccumulation in adipose tissue

Direct influence of endocrine disrupting compounds and ER signaling

- Industrial phenolics
  - bisphenol A
  - Alkyl phenols
- Phytoestrogens (dietary estrogens)
  - genistein
- Organochlorine pesticides
  - methoxychlor
  - DDT
- Pharmaceutical agents
  - tamoxifen
  - diethylstilbestrol (DES)

- ✓ Compete with endogenous estrogen for ER binding sites
- ✓ Shared E2 structural features determined by ER binding pocket
- ✓ Similar ligand binding pockets, but subtle differences in amino acids lining the pocket contribute to ligand selectivity