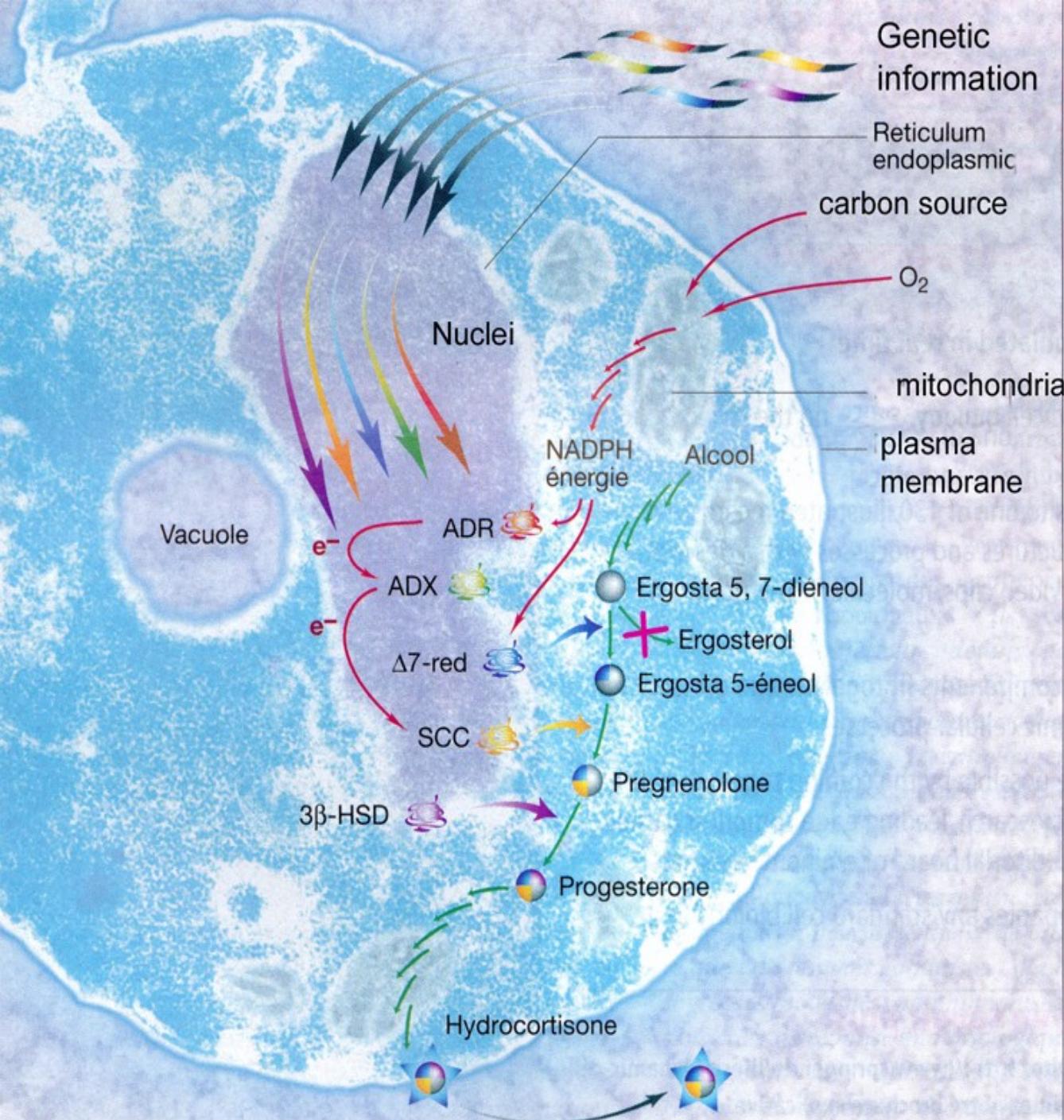


Reconstruction in yeast of human steroid metabolic pathway as a tool for drug discovery and biosynthesis

Denis POMPON

Laboratoire d'Ingénierie des
Protéines Membranaires
CGM-CNRS
Gif-sur-Yvette, France.

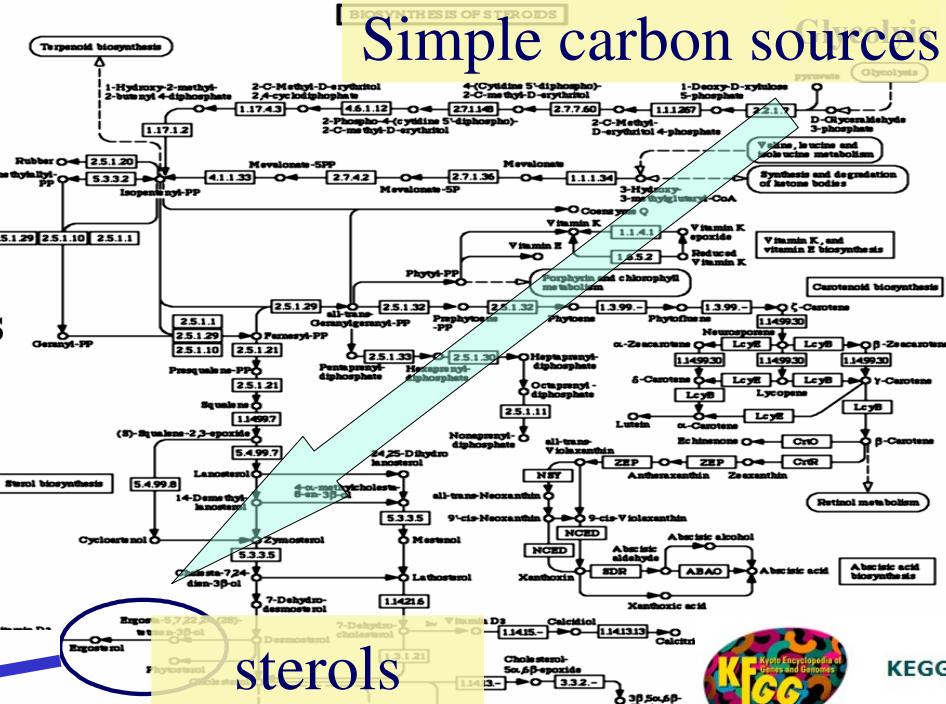


Simple carbon sources

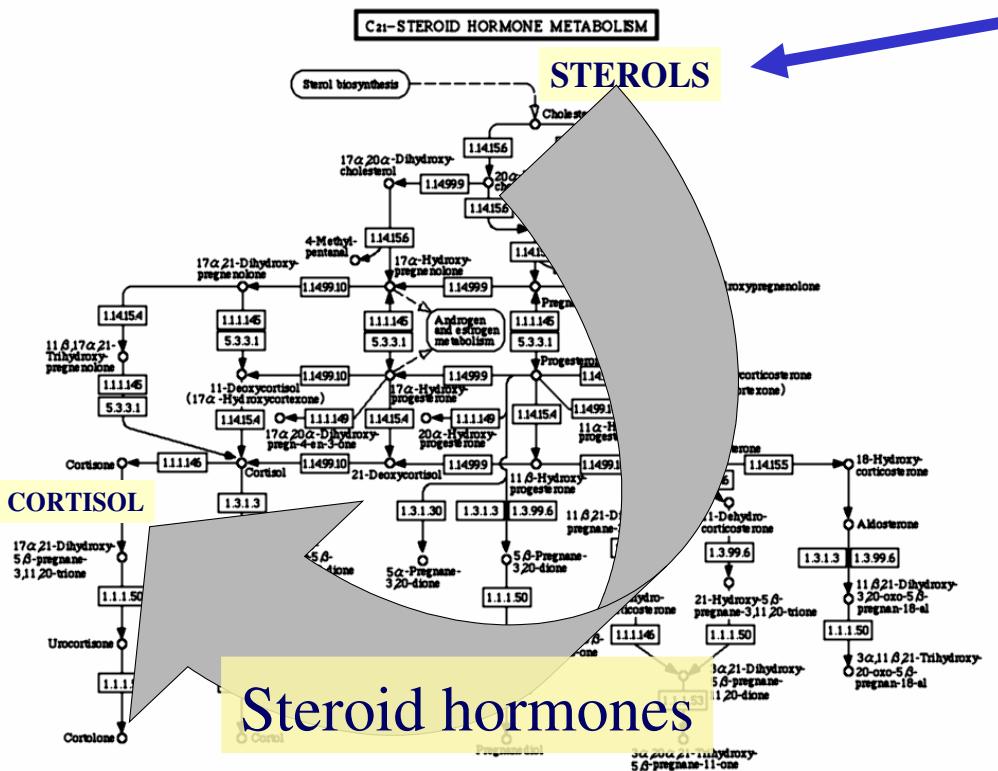
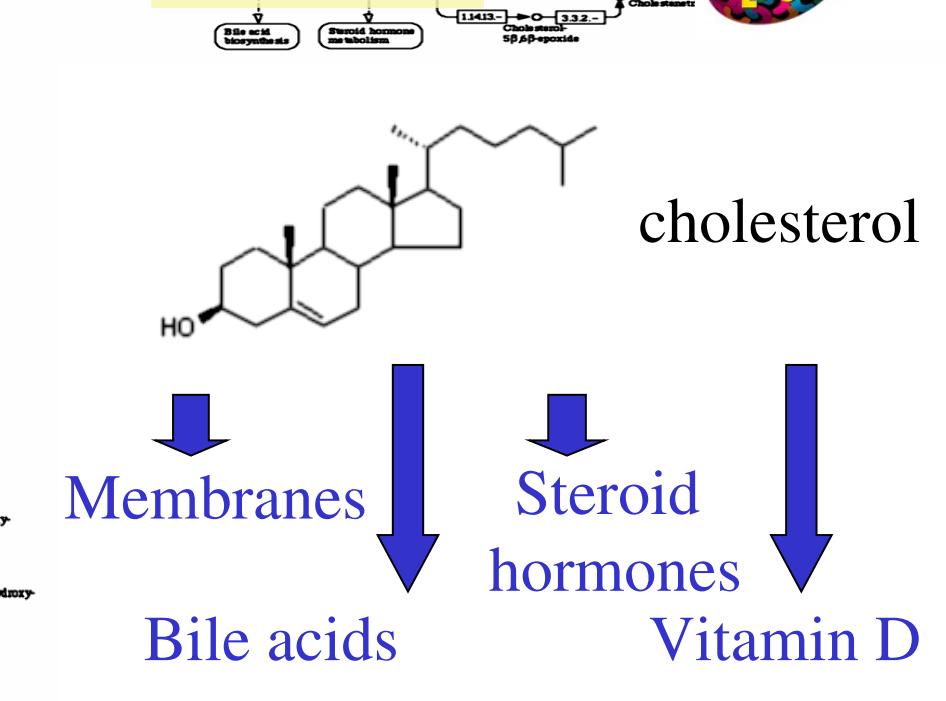
Sterol and steroid hormone biosynthesis pathway in animals

Sterol biosynthesis is a major target for drugs
(cholesterol lowering & antifungal drug)

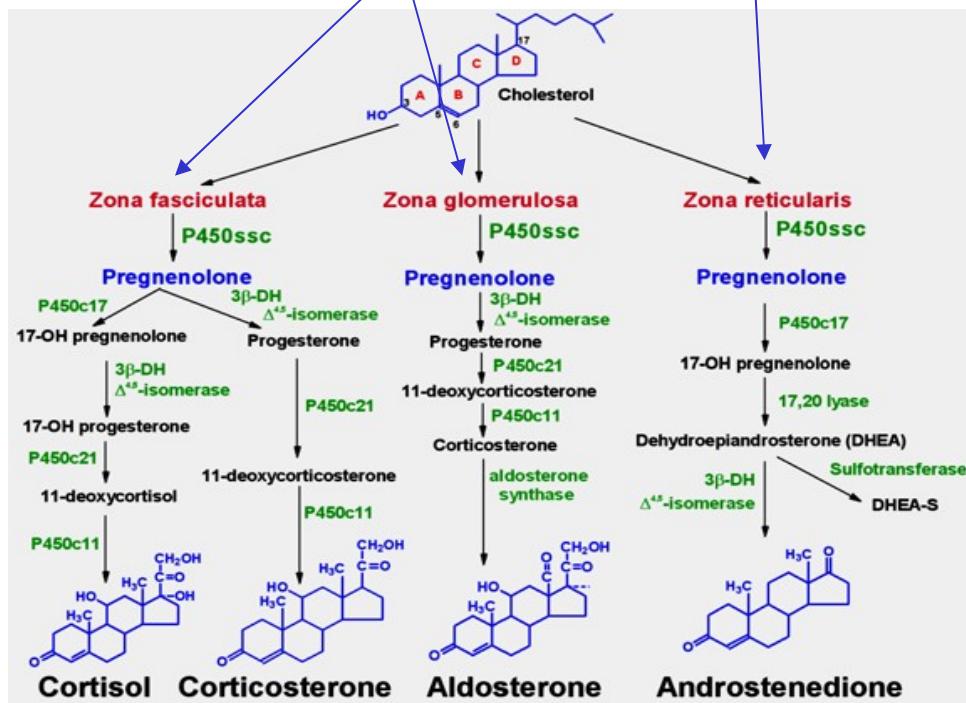
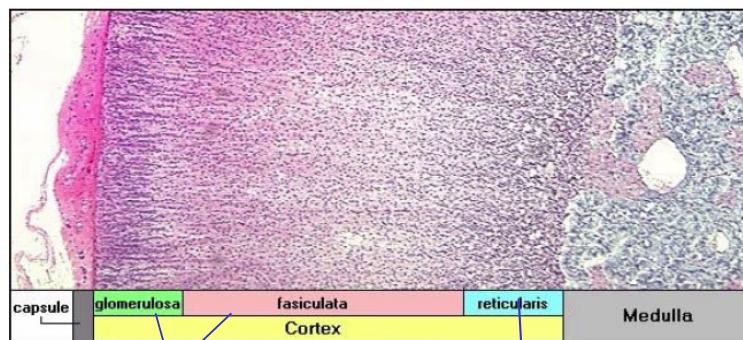
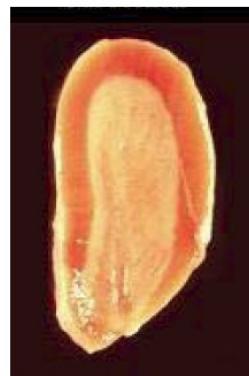
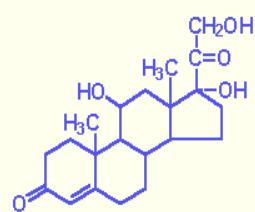
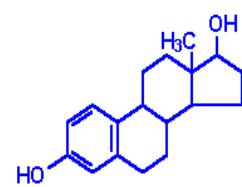
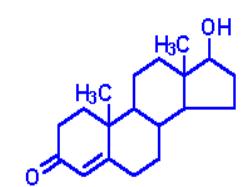
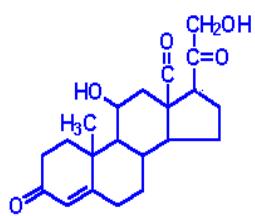
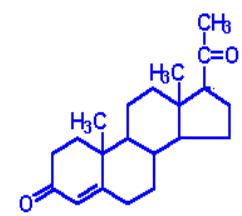
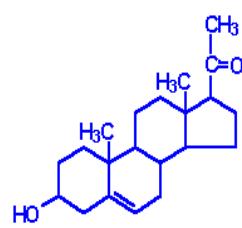
Defect in sterol synthesis or in transport is found in several genetic diseases

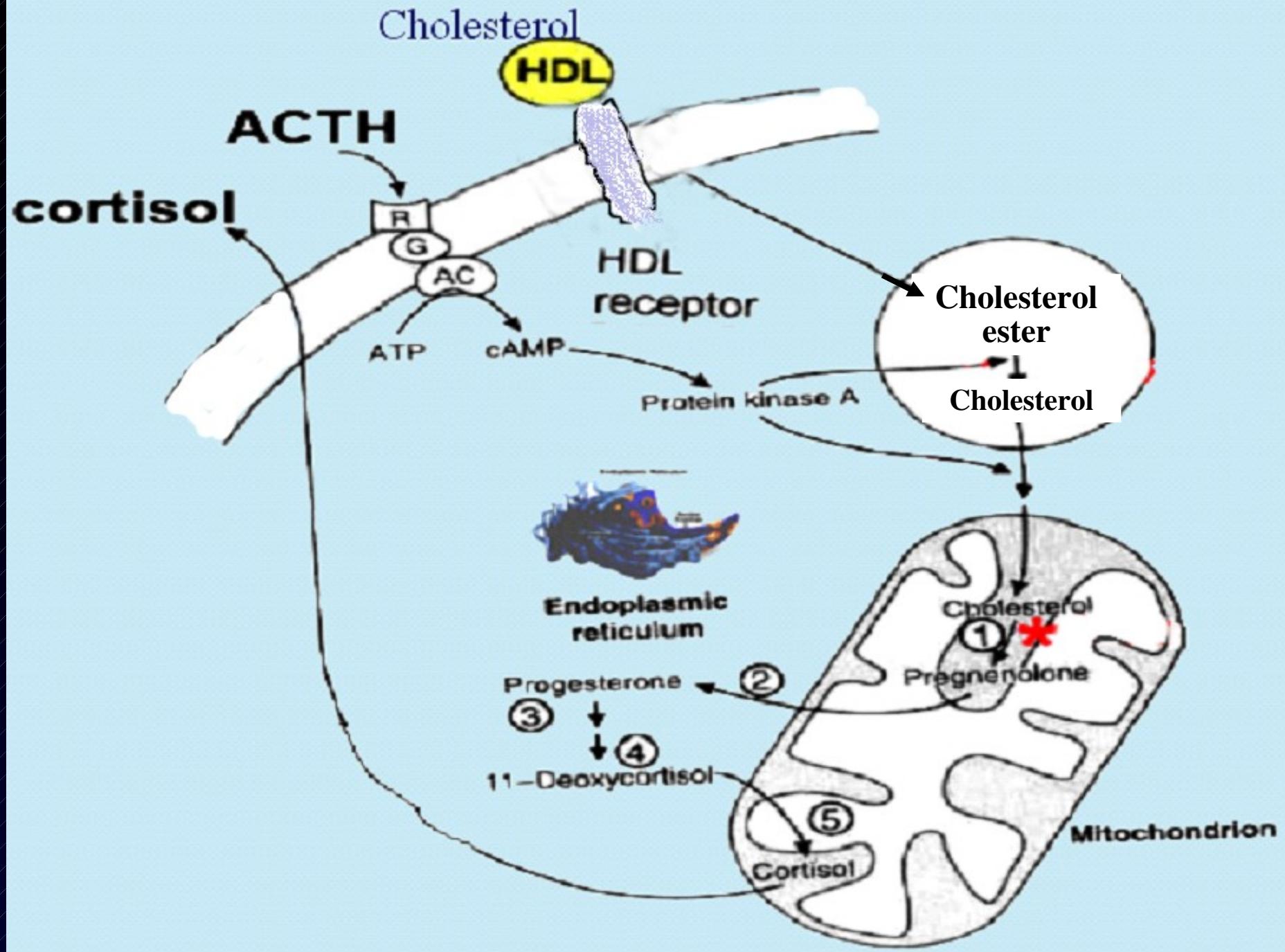


KEGG

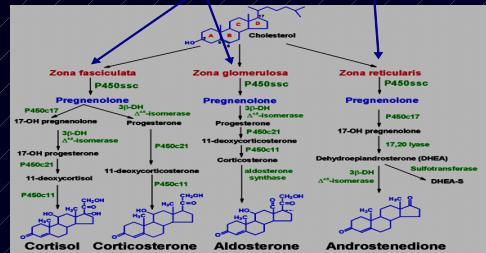
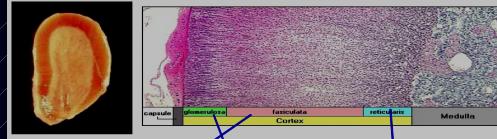


Adrenal gland

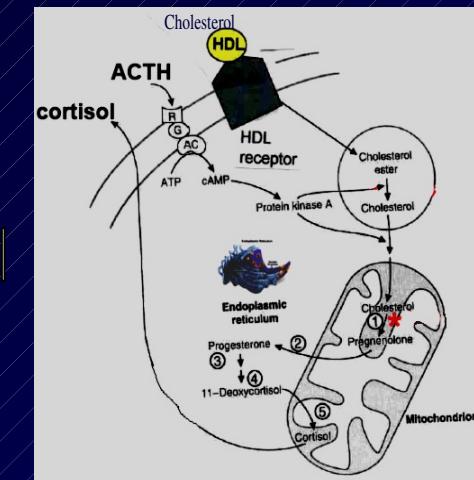
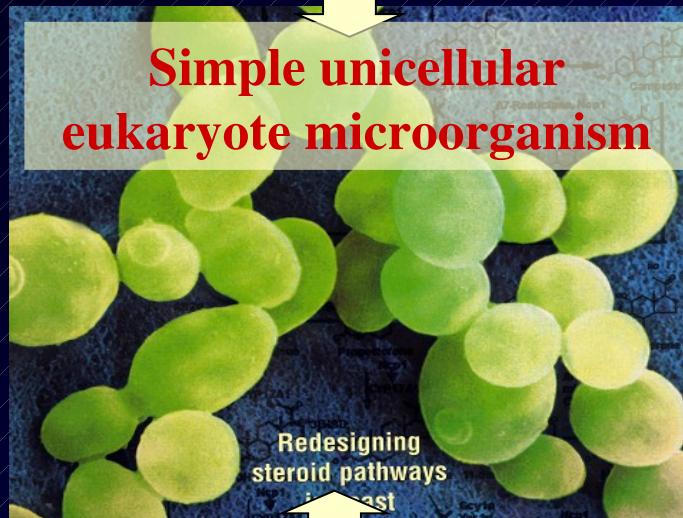
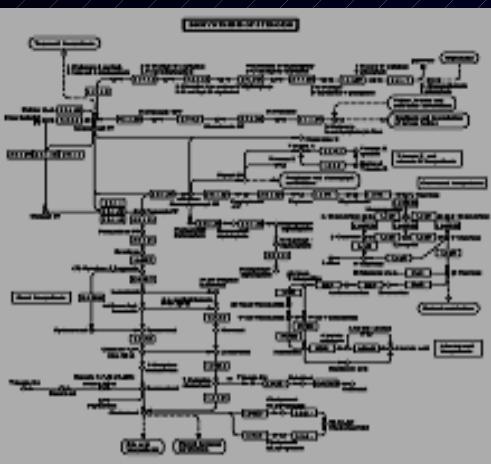




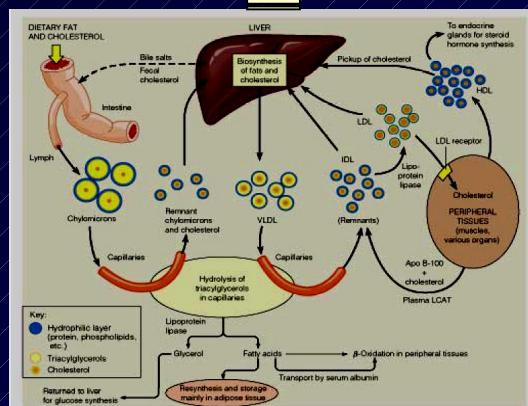
High organized
tissue dependent
spatial organization



Multiple
subcellular
biosynthesis
location



Highly branched
multi-step
biosynthetic pathway
with complex
regulation



Multi- organ
transport &
metabolism

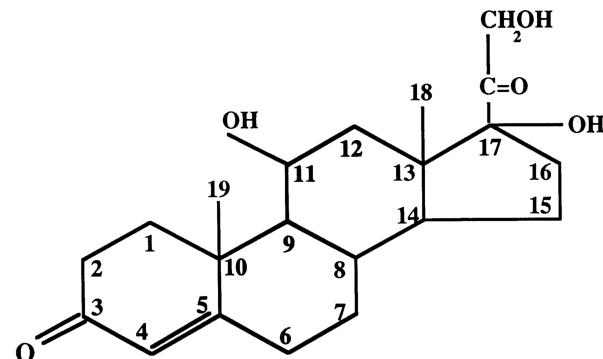
Make Yeast



Human

TOOLS

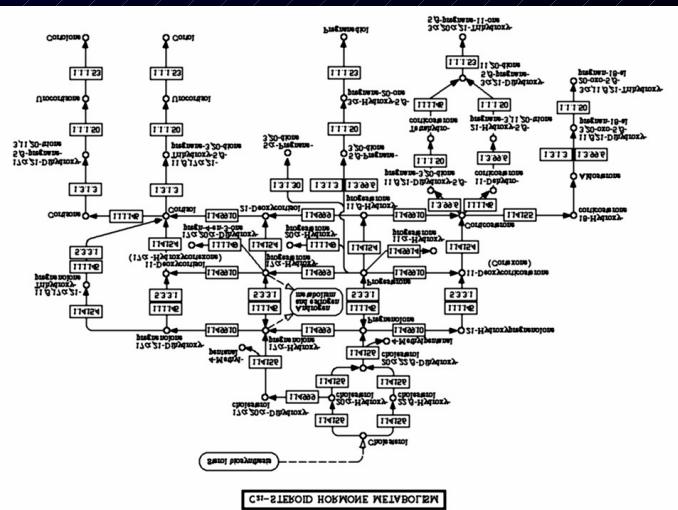
Redesigning
animal steroid hormones
biosynthesis for yeast



Cortisol

MODELS

Humanization
of yeast sterol
biosynthesis



MOLGENIUM MONOSOON GIBSON-16

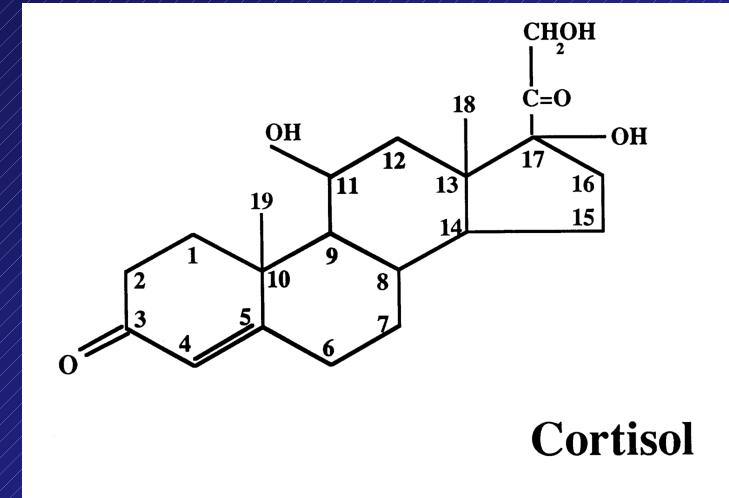
Redesigning animal steroid hormones biosynthesis for yeast

Self-sufficient biosynthesis
from simple carbon source

Mimetic but not necessarily a
copy of natural process

Target a single end-product
instead of the natural
hormones spectra

Optimized for productivity
and not to be a model



Biosynthesis involved large number
of membrane bound enzymes
needing eukaryote environment.

No cholesterol in yeast

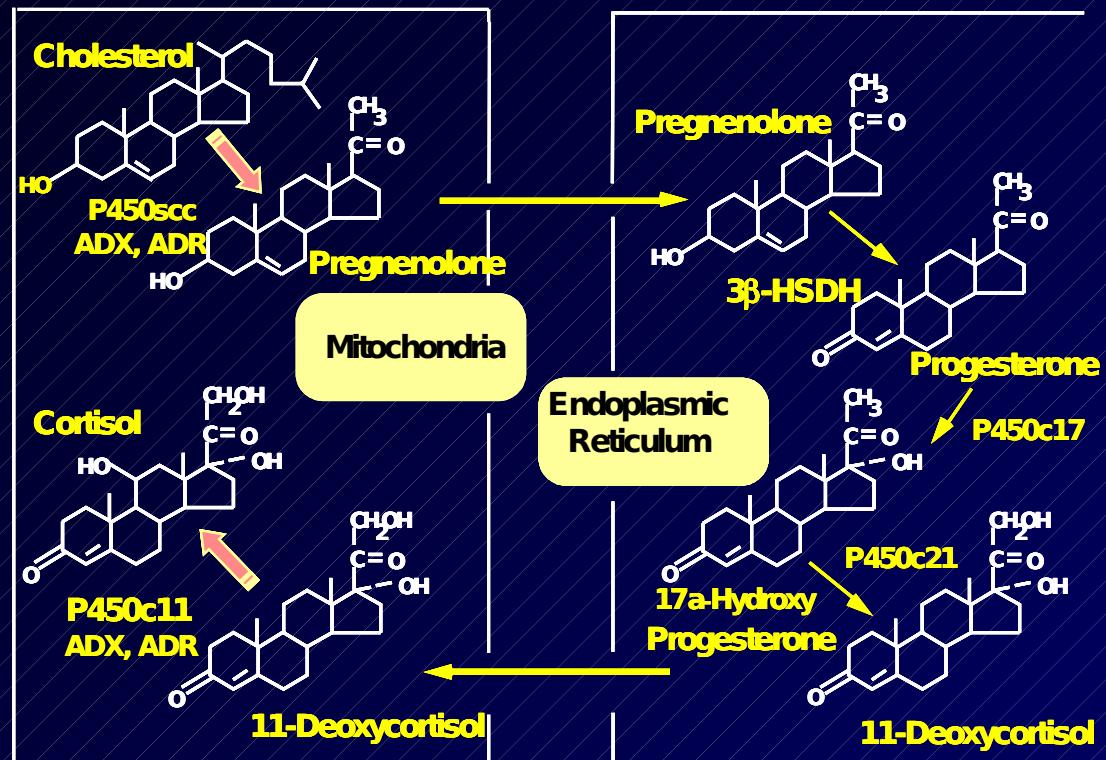
Required enzymes absent

Required electron transport chains absent

Dealing with
subcellular transport of
heterologous enzymes

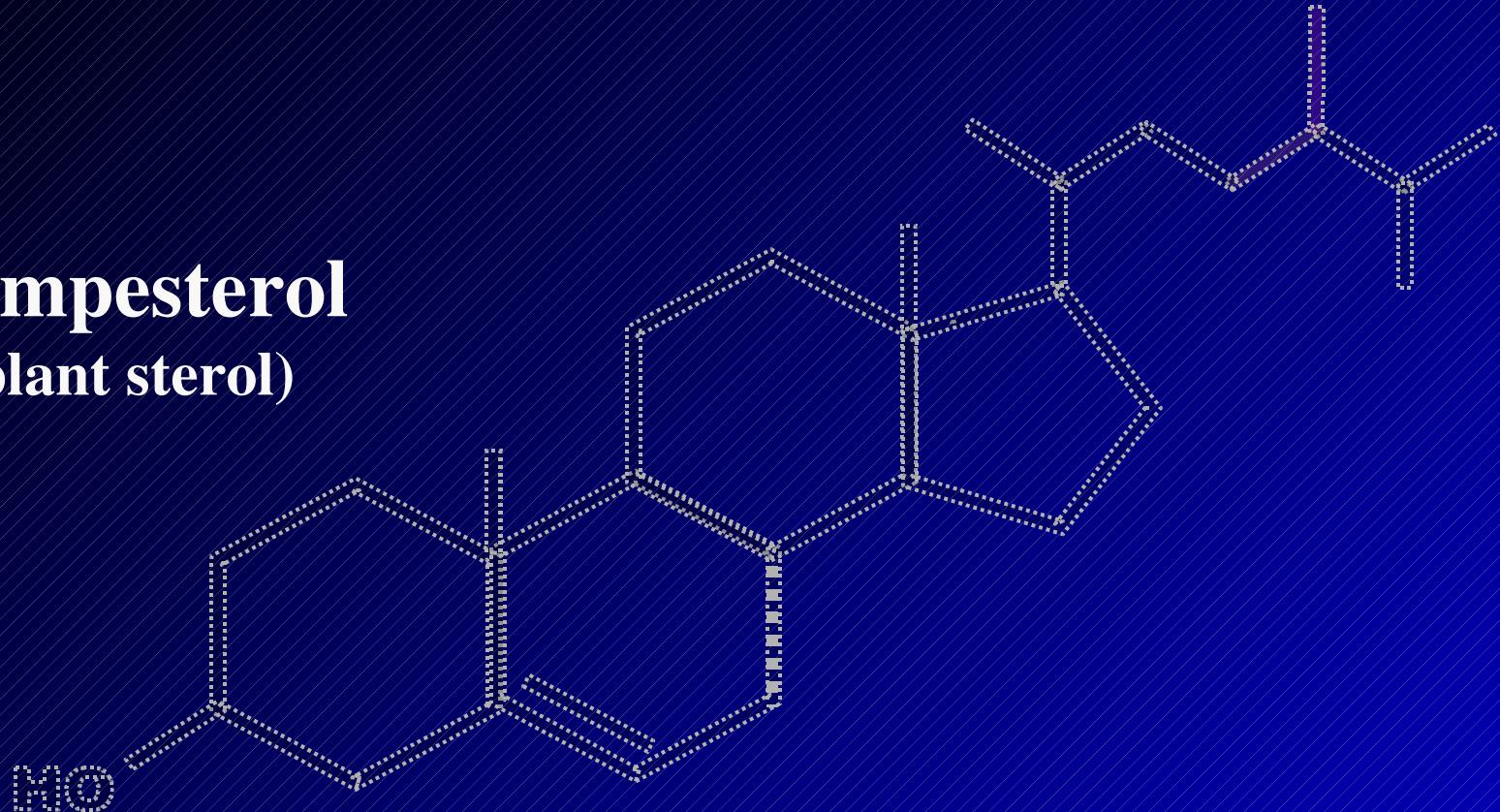
Dealing with
subcellular traffic of
lipophilic intermediates

Cortisol biosynthesis in animals

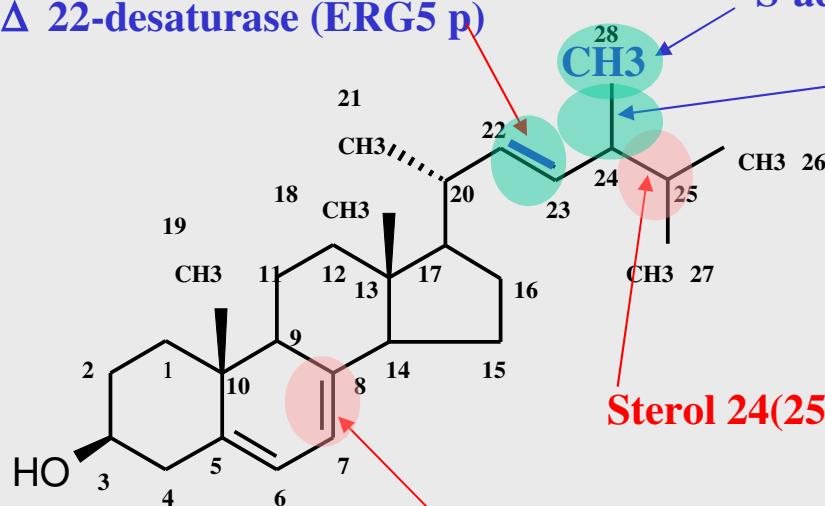


Substitute for cholesterol with a yeast metabolism derived sterol

Campesterol (plant sterol)



Δ 22-desaturase (ERG5 p)



S-adenosyl sterol methylene transferase (ERG6 p)

Sterol 24(28) reductase

Sterol 24(25) reductase

Sterol Δ 7 reductase

Ergosterol (yeast)

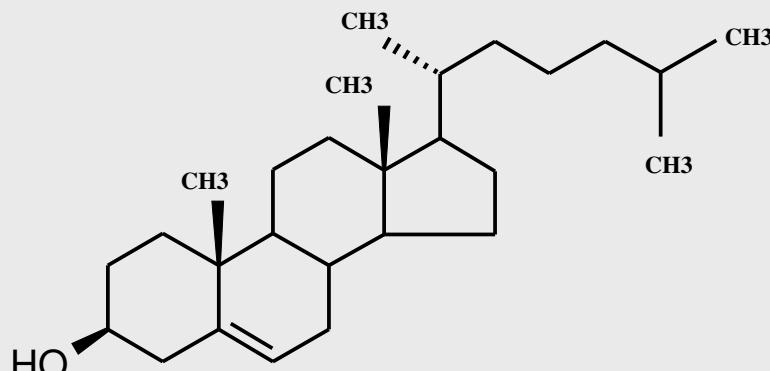
Gene critical for differences



Only present in yeast

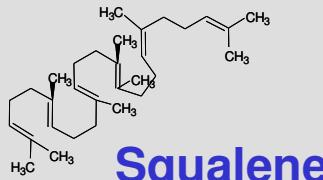
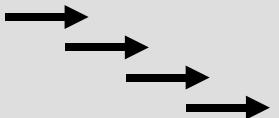


Only present in animals



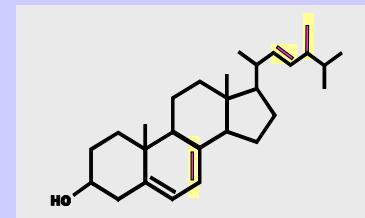
Cholesterol (animals)

Simple carbon sources



Squalene

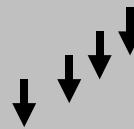
ERGOSTEROL



Common to
yeast and animals

STEROLS

Lanosterol

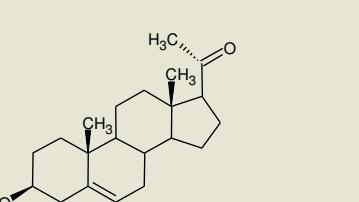
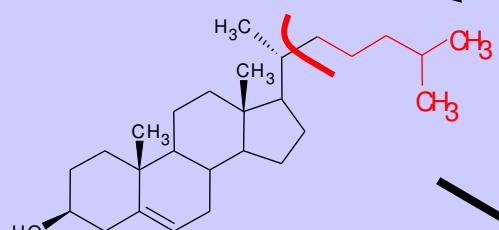


Zymosterol



Yeast specific

Cholesterol



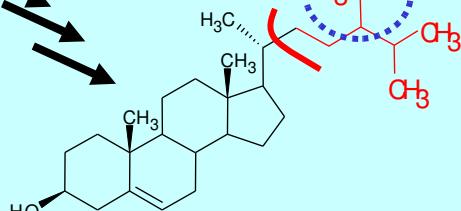
Pregnenolone

Steroid
biosynthesis

Hydrocortisone

Animal specific

Campesterol



Engineered yeast

Zymosterol

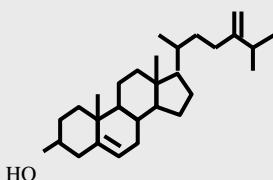
SAM-transferase

Δ 8-7 isomerase

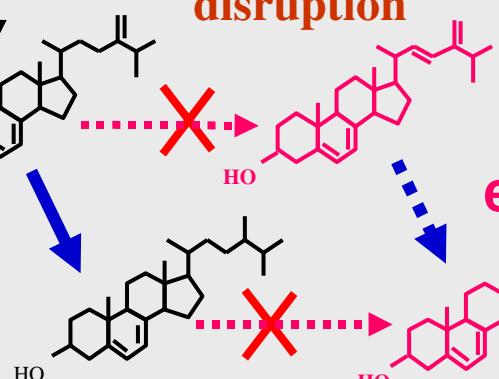
Δ 5-desaturase, cyt. b5

NADPH

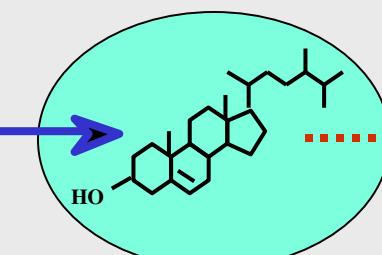
A. thaliana
 Δ 7- reductase



Δ 22-desaturase
disruption

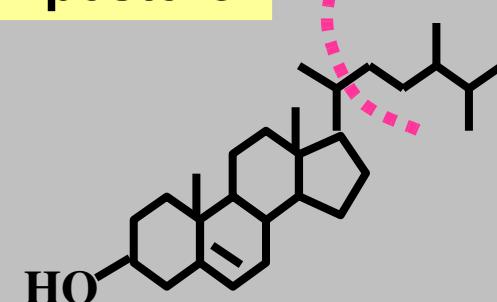


ergosterol



Ergosta 5-ene ol
(campesterol)

Campesterol



NADP⁺

ADR

HO

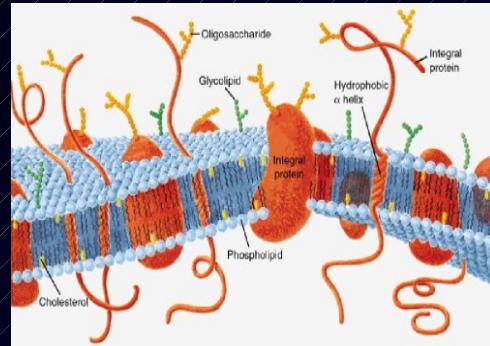
P450 SCC



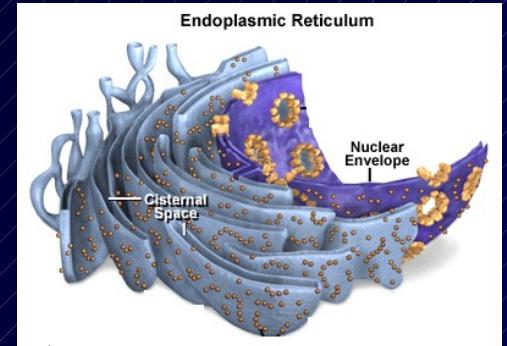
Pregnenolone

Dealing with intracellular targeting and compartmentalization

Plasma membrane



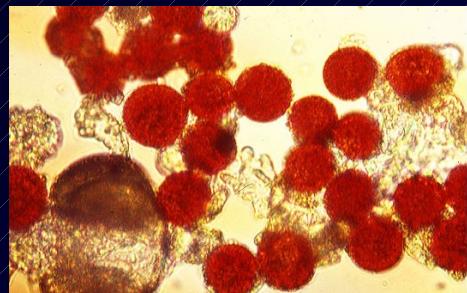
Endoplasmic reticulum



Exogenous sterols

sterols

storage



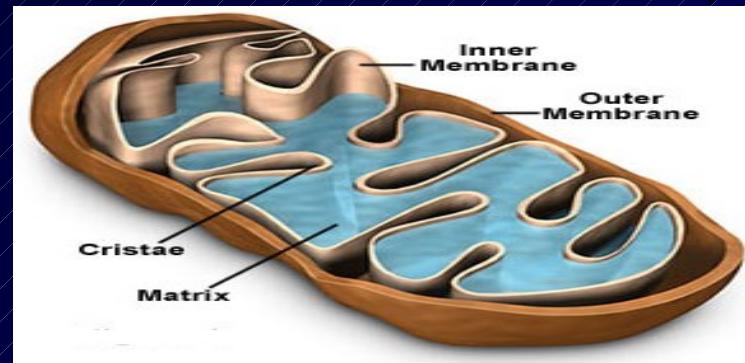
Lipid droplet

sterols

neosynthesis

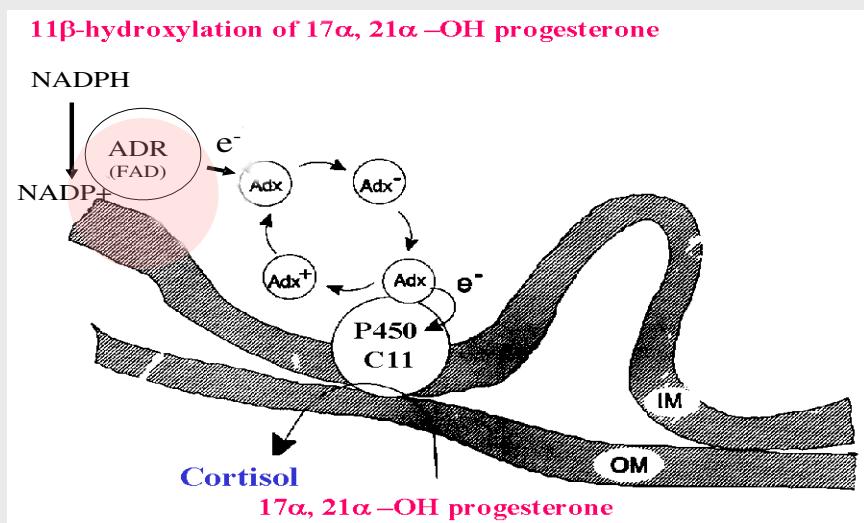
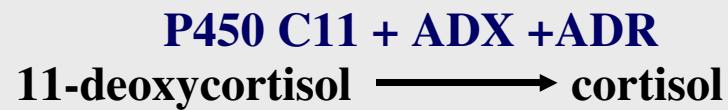
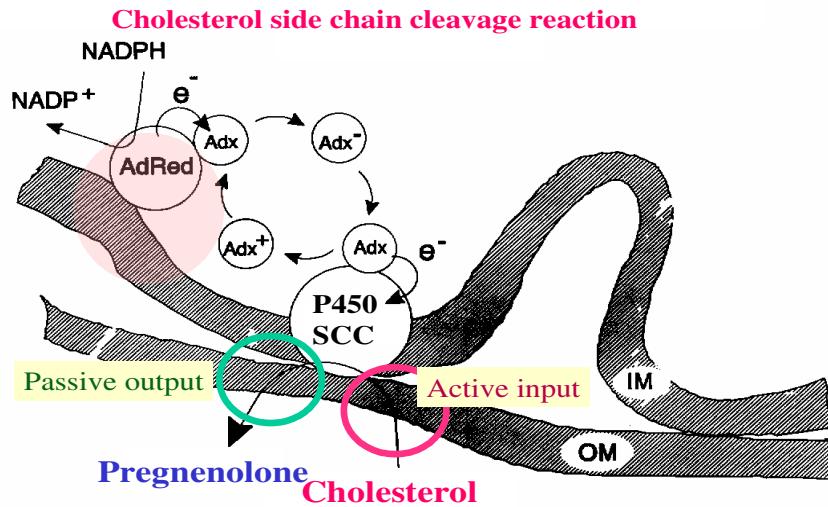
progesterone

11-deoxy cortisol



Mitochondria

Two similar reactions occur on internal mitochondrial membrane in animal and involve the same electron transfer chain

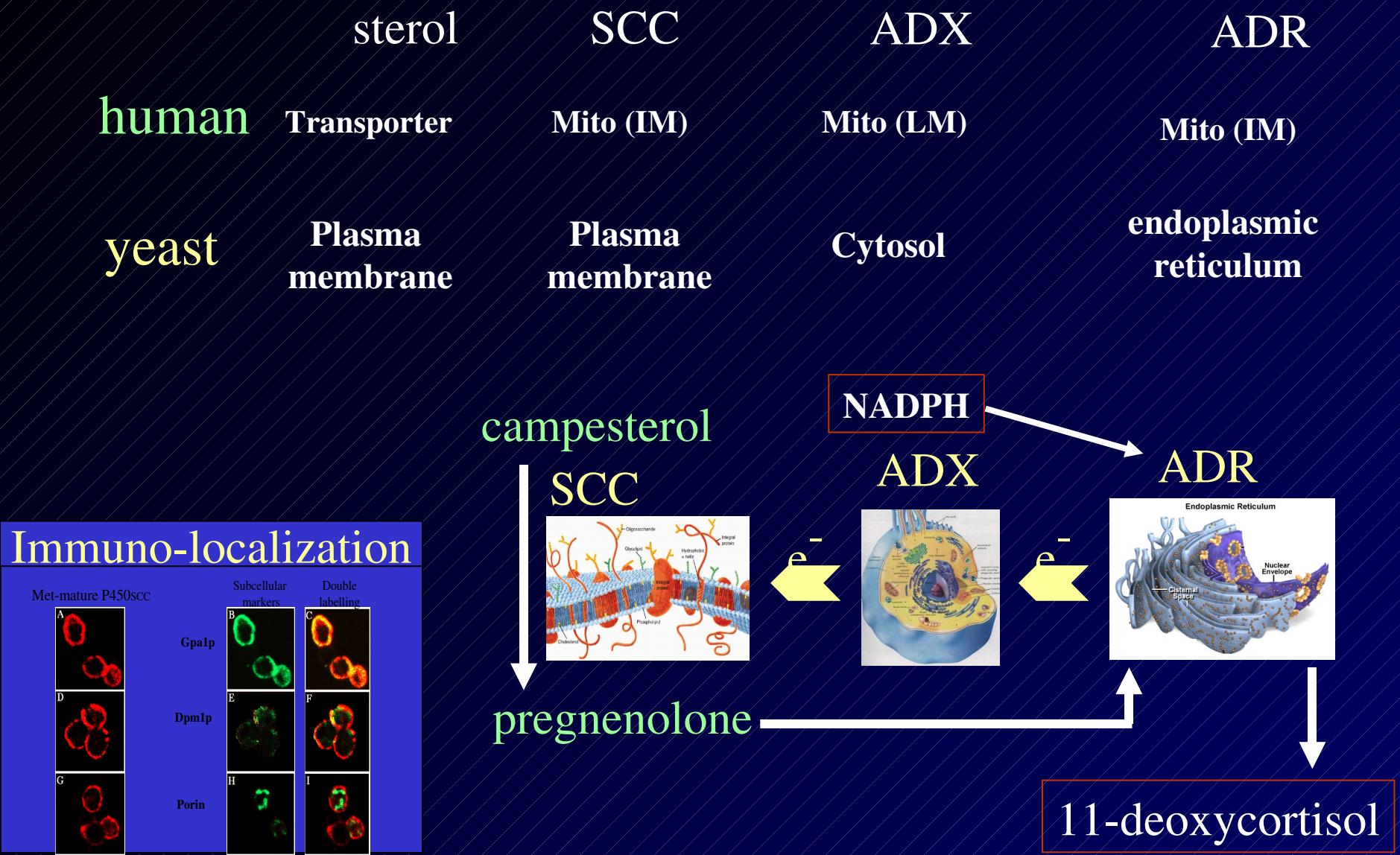


Cholesterol, in contrast to oxysteroids, is insoluble and needs complex and function critical transport machinery to enter mitochondria in animals.

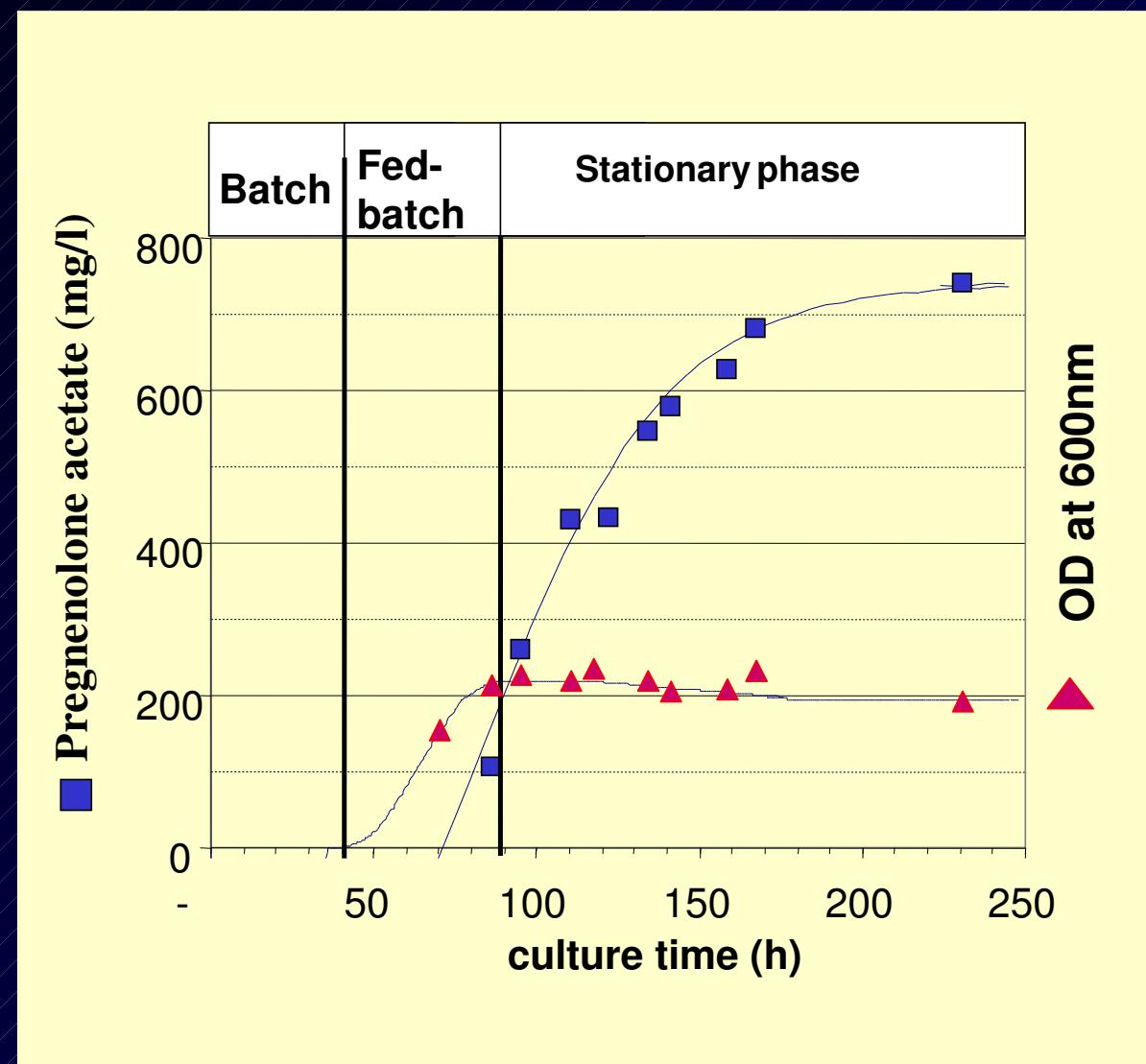
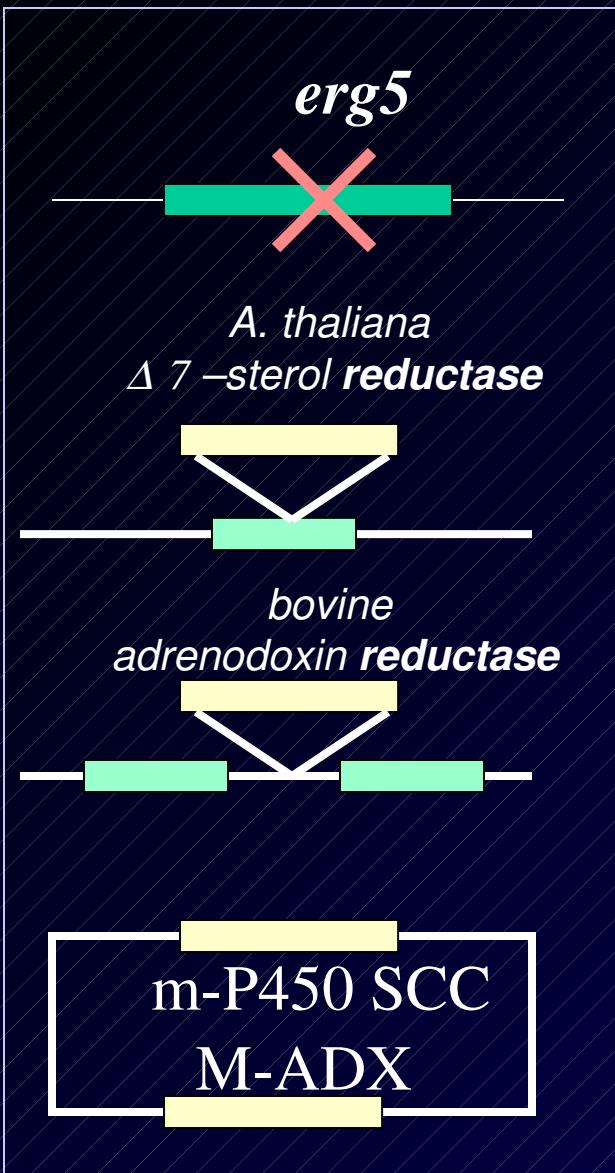
- Intra-mitochondrial transport of cholesterol (campesterol) cannot be rebuilt in yeast
- Signal sequence engineering allowed mitochondrial import in yeast of the 4 required components of the animal system but AdRed enzyme does not fold properly in yeast mitochondria

Yeast reconstruction of the campesterol side chain cleavage

Electron transport chain



Pregnenolone acetate production by the strain CA10/pCD63



Yeast reconstruction of the 11β -hydroxylase activity

Electron transport chain

P450 C11

ADX

ADR

human

Mito (I.M.)

Mito (matrix)

Mito (I.M.)

yeast

COX fusion
Mito (I.M.)

COX fusion
Mito (matrix)

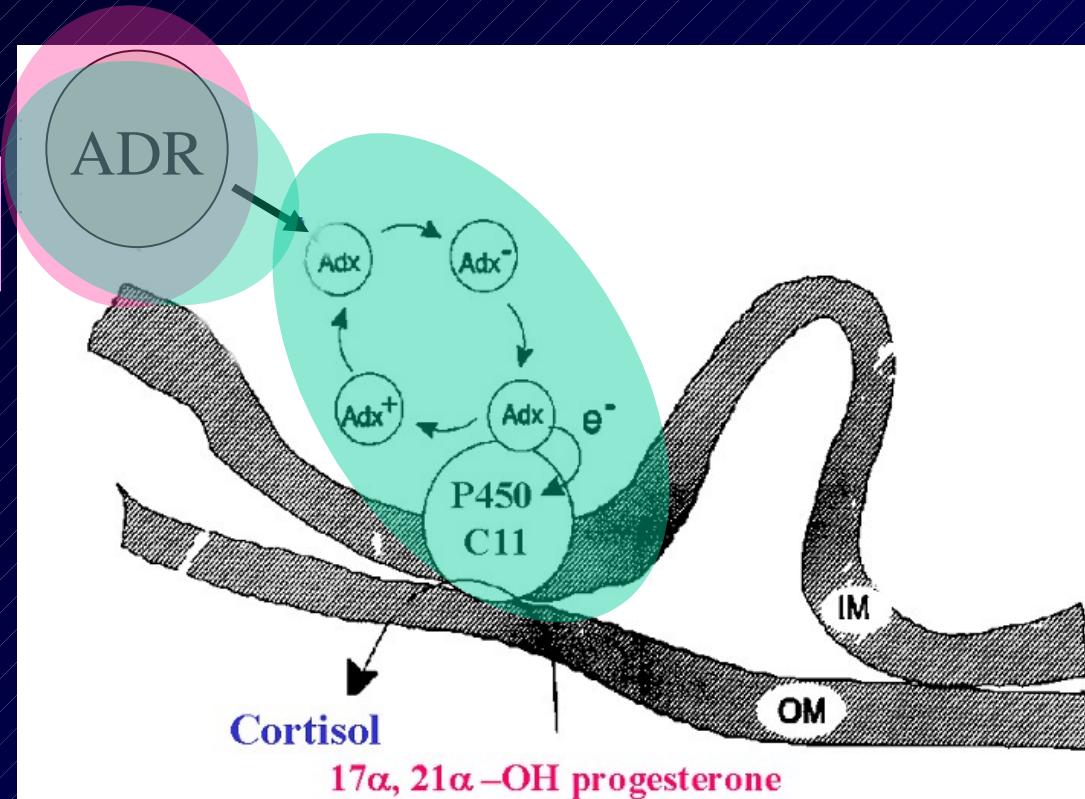
Endogenous ARH1p
Mito (I.M.)

P450 C11 does not fold outside of mitochondrial context

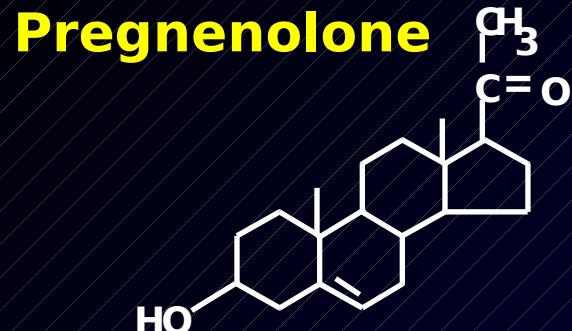
ADR does not fold inside of the mitochondrial context

Yeast ARH1p has an unrelated essential function in yeast in iron transport but shows a significant sequence similarity with human ADR.

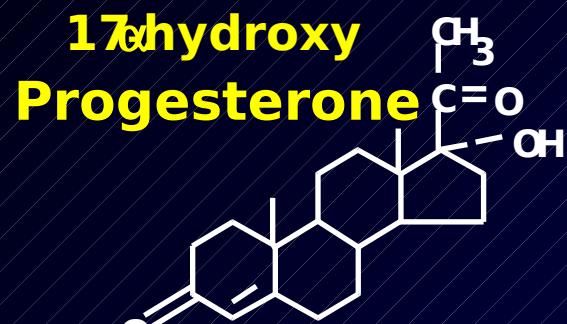
Experiment demonstrated that ARH1p can nicely substitute for human ADR in the mammalian electron transfer chain.



The microsomal part of the cortisol biosynthesis



3 β -HSDH



P450c17

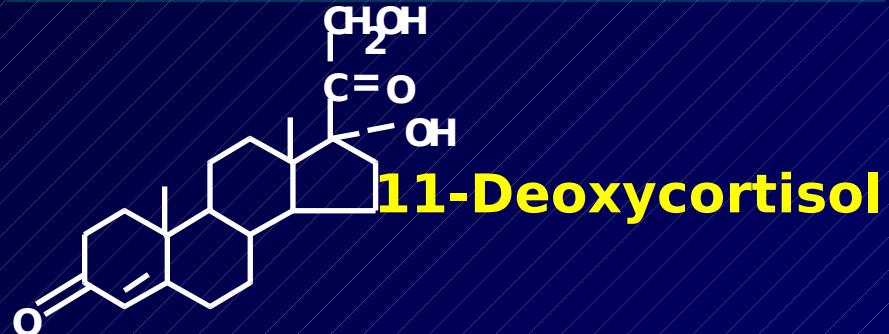
electrons

CPR

NADPH

NADP⁺

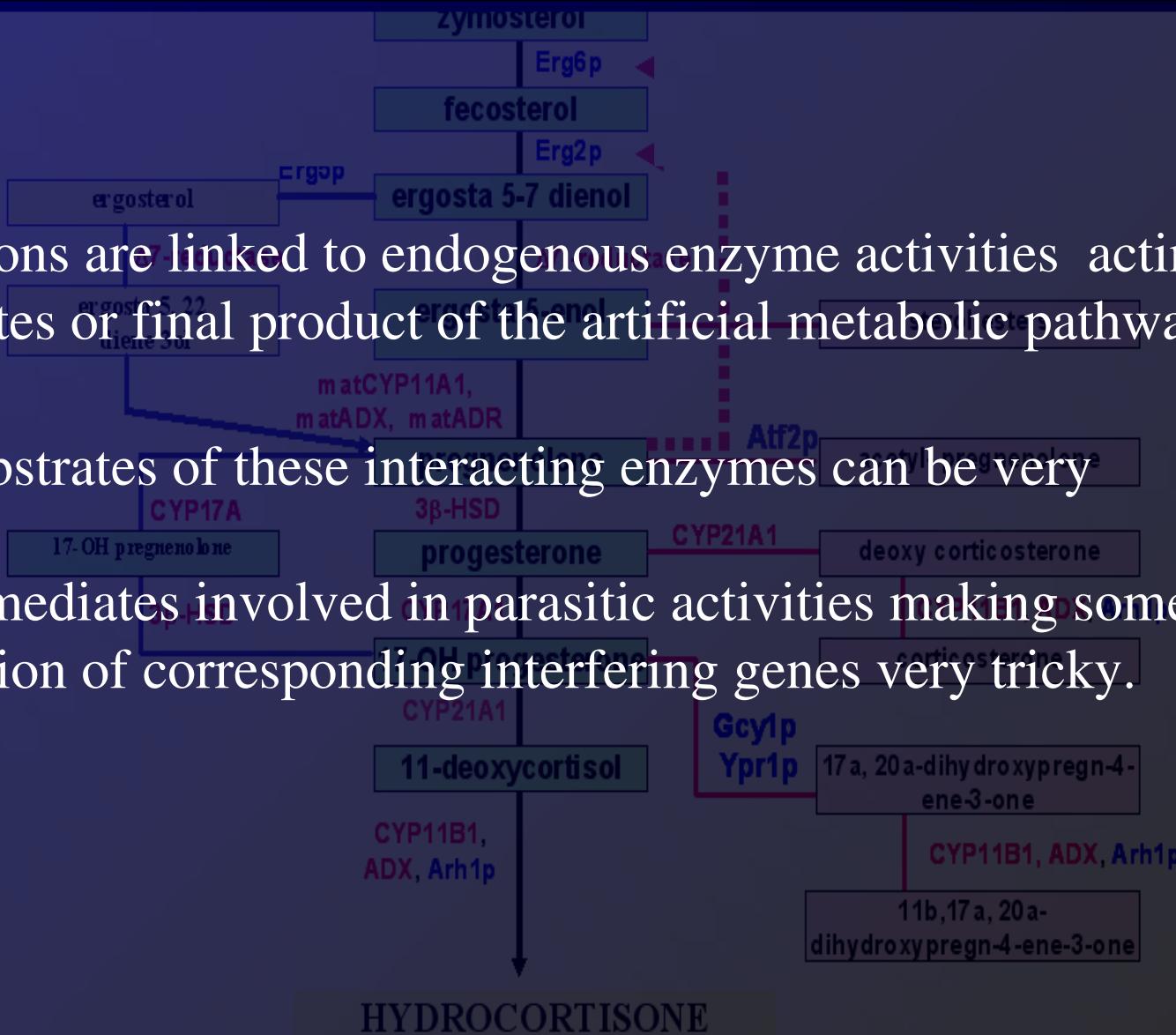
P450c21



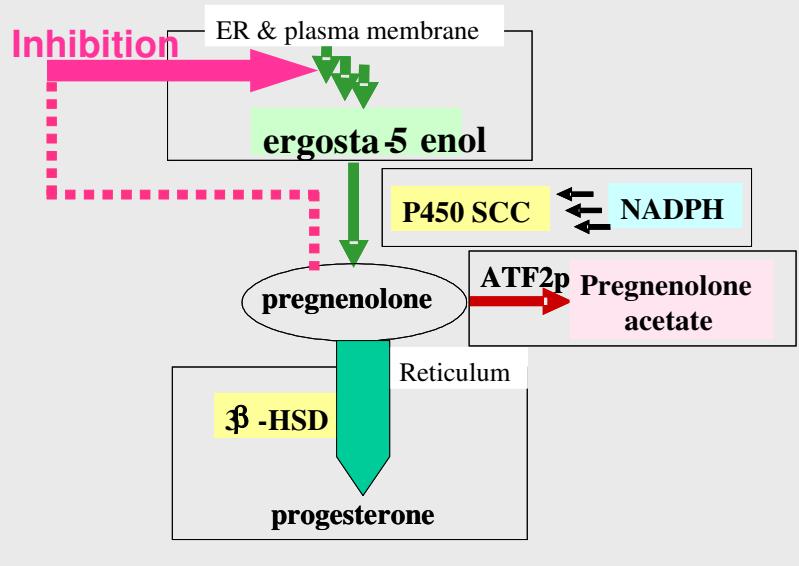
Side reactions and end-point control.

Side reactions are linked to endogenous enzyme activities acting on intermediates or final product of the artificial metabolic pathways

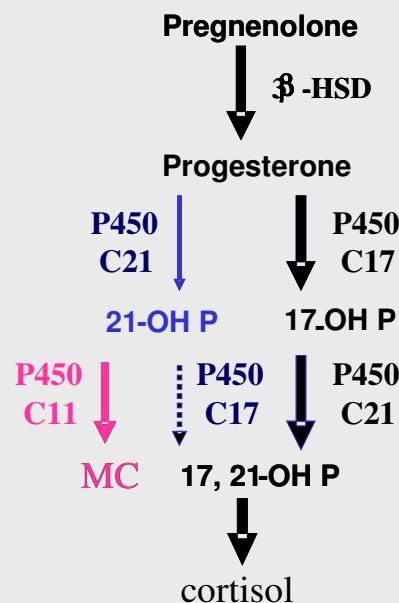
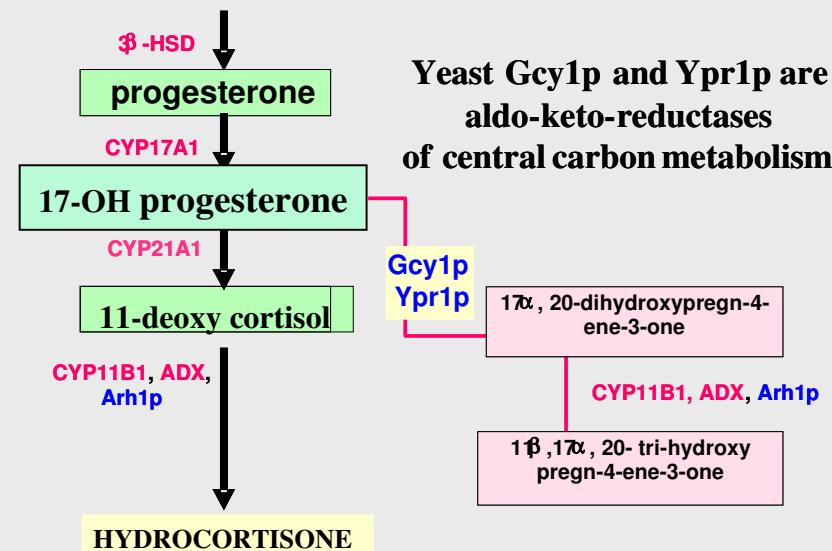
Natural substrates of these interacting enzymes can be very different from intermediates involved in parasitic activities making sometime identification of corresponding interfering genes very tricky.



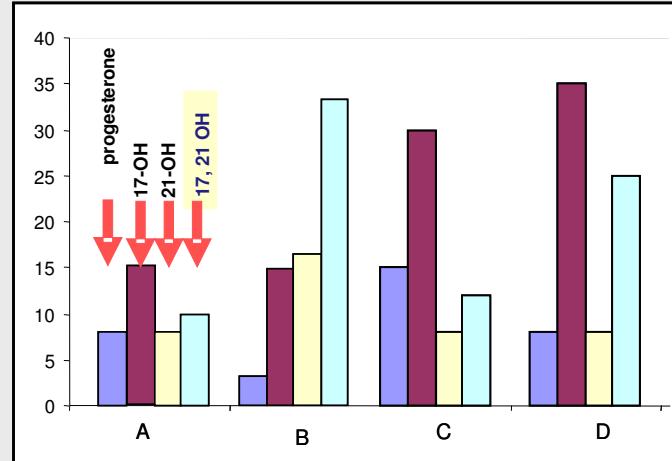
Unexpected retro-inhibition of sterol synthesis by pregnenolone upon disruption of parasitic Atf2p acetylase activity.



Parasitic aldo-ketoreductase activity from enzymes of central carbon cycle acting on steroid



Gene dosage effects

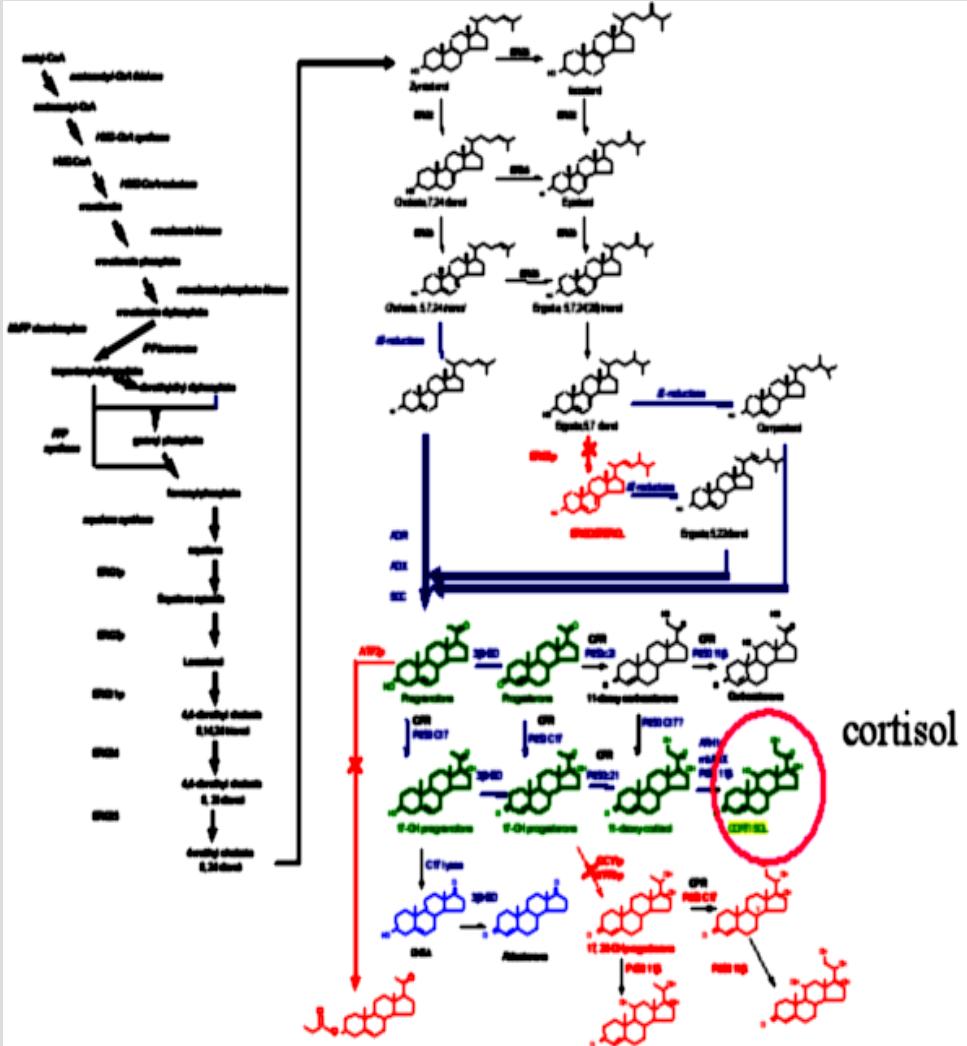


Gene dosage effect on a branched pathway dramatically affect the output of the biosynthesis

In the absence of regulation tight tuning is requested to accumulate correct end-product

Global pathway engineering of yeast for cortisol production

native parts  engineered parts



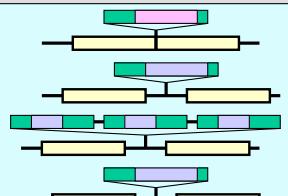
Genomic integrations of transgenes

A. thaliana $\Delta 7$ -sterol reductase

Bovine P450 C17

Bovine P450 C21

Yeast & bovine COX VIp-ADX



Plasmids

Mature P450 SCC

Mature ADX

Bovine

3 β -HSD

&

Mature ADR

human

COX VIp-P450 C11



Overexpression by promoter exchanges

Yeast

ARH1



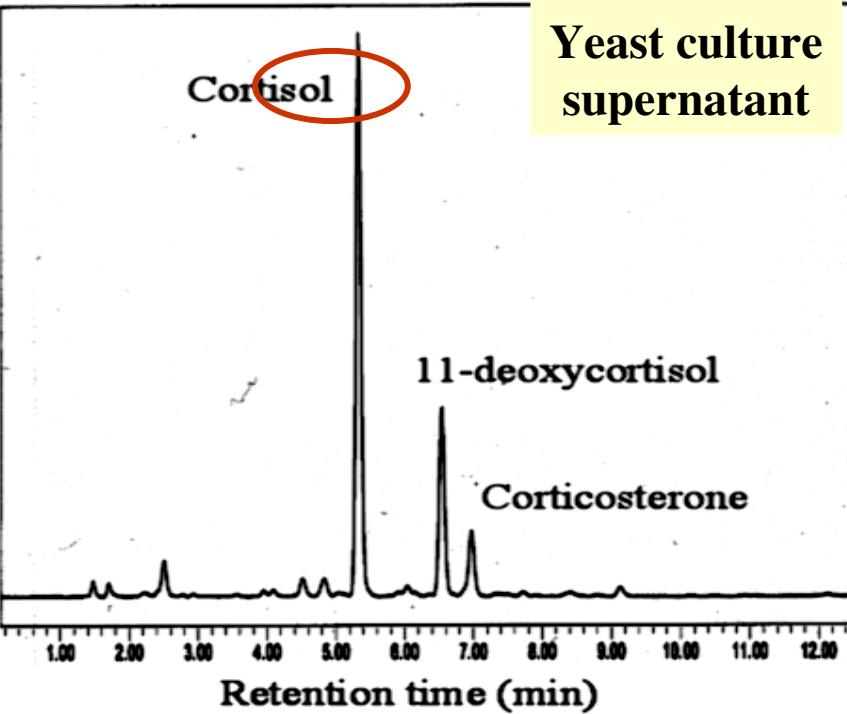
Gene inactivation

Yeast $\Delta 22$ -desaturase

Yeast Aldo-ketoreductase (gcy1)

Yeast Aldo-ketoreductase (ypr1)

Yeast O-acetyl transferase (atf2)



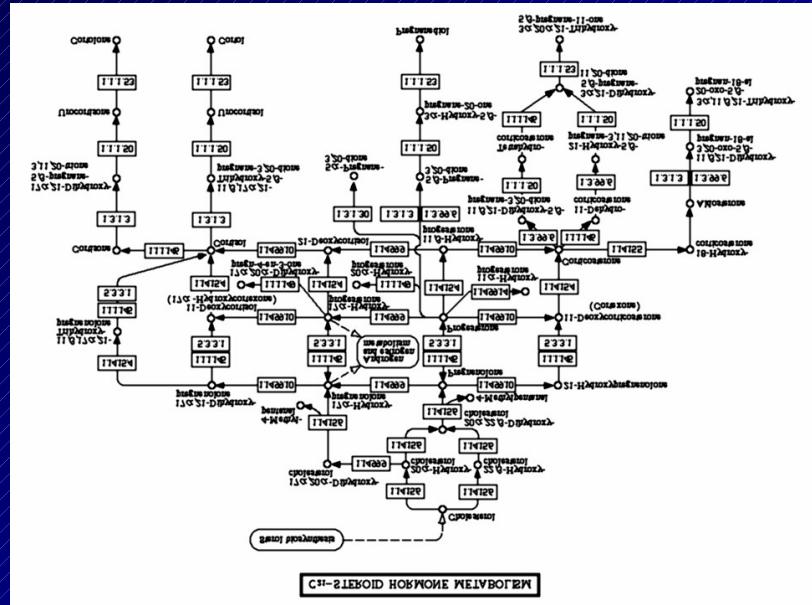
Humanization of yeast sterol biosynthesis

Mimic as closely as possible
human behavior.

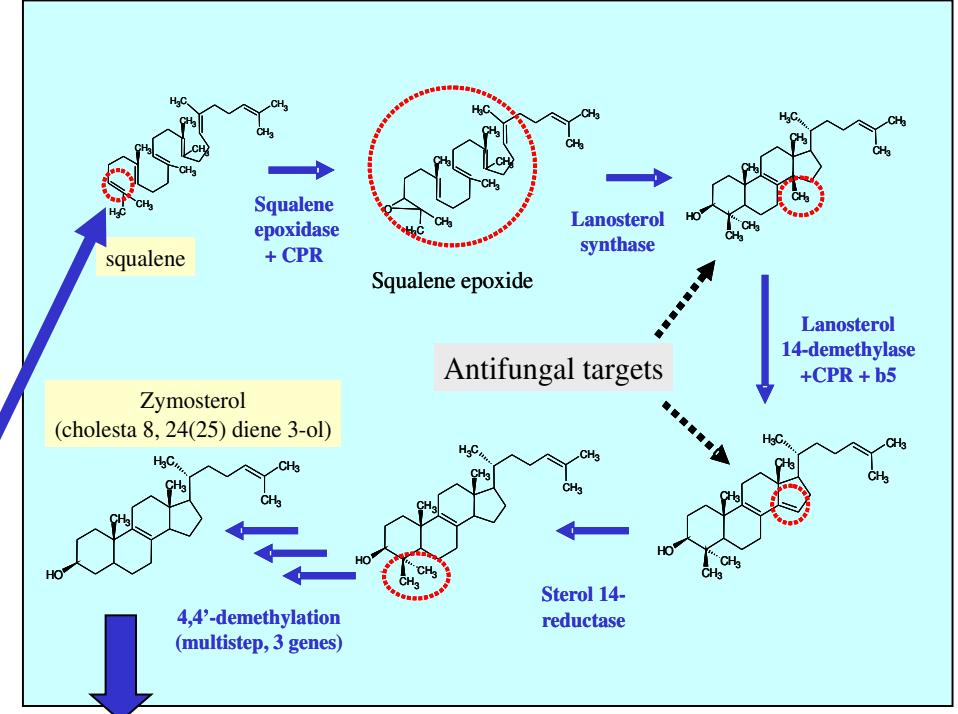
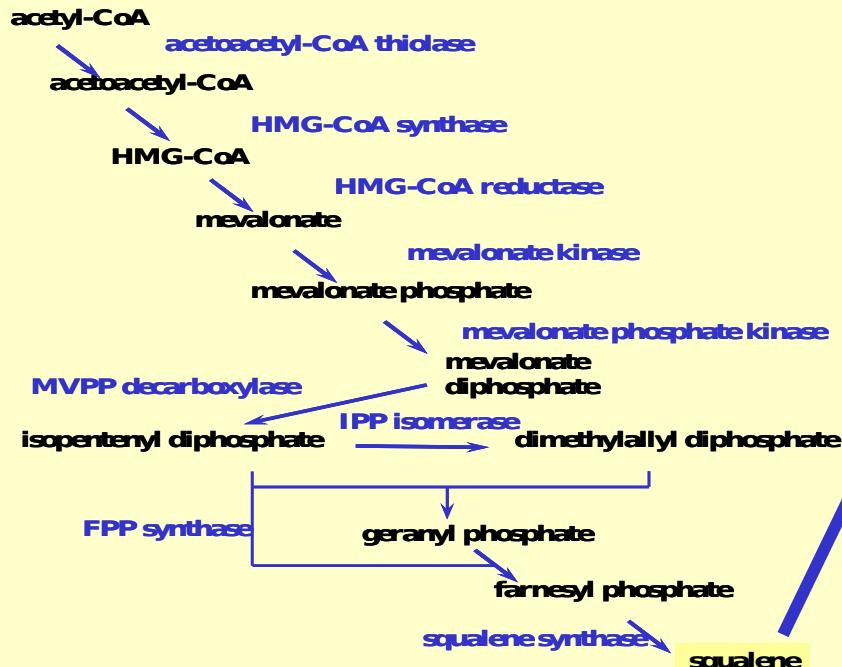
“In vivo” metabolome model
aware of compartmentalization

Basic and applied tool for
drug design.

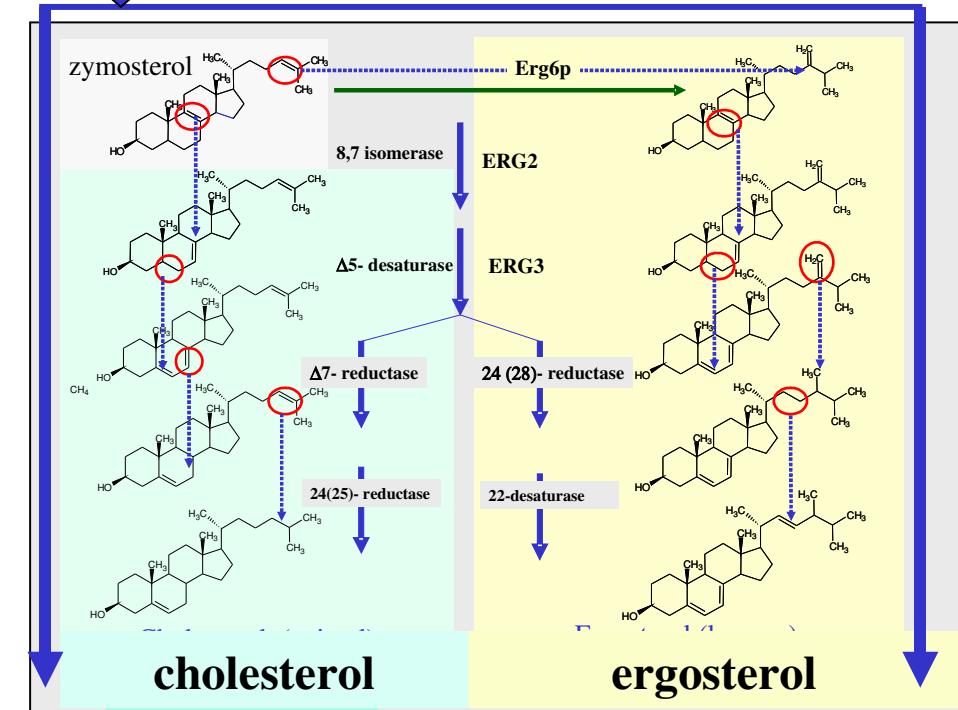
Approach the complexity real
complexity. No optimization
for specific end-product



Conservative approach taking
advantage of a model
eukaryote organism

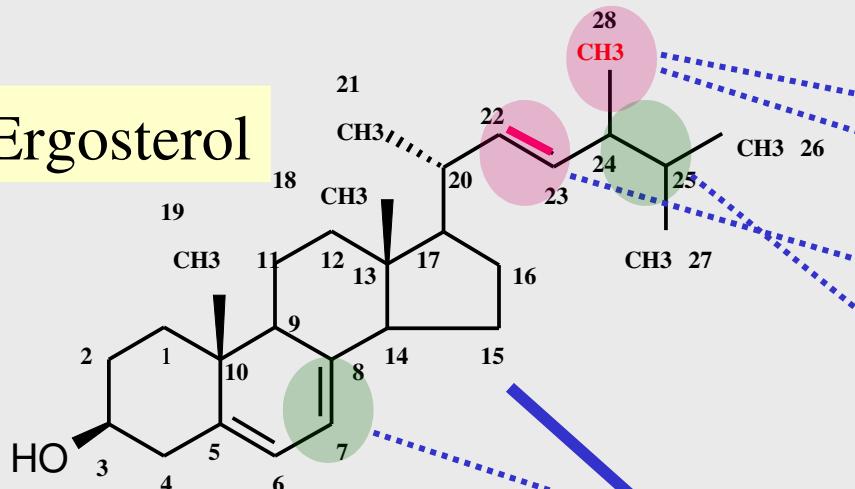


Sterol biosynthesis in animal and yeast



Disruptions of yeast genes

Ergosterol



SA M-sterol transferase

24(28)-sterol reductase

Δ 22-sterol desaturase

Additions of human gene

Δ 24(25)-sterol reductase

Δ 7-sterol reductase

Humanisation of yeast genes

HMG CoA reductase

Statin drugs

Lanosterol demethylase

Antifungal drugs

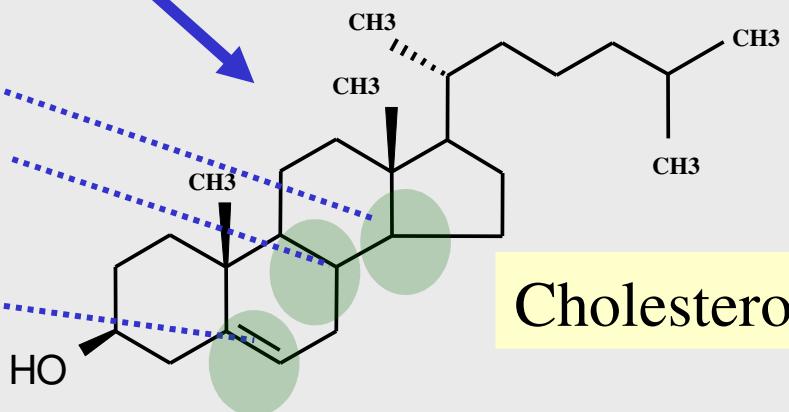
Sterol 8,7 isomerase

Sigma receptor analog

Sterol 5-desaturase

Cytochrome b5

P450-reductase



Cholesterol

Redox environment

Human	Yeast	Action
HMG-CoA reductase	HMG-CoA reductase	substitution
		
Lanosterol demethylase	Lanosterol demethylase	substitution
		
absent	SAM-sterol transferase	deletion
	Δ 7-8 sterol isomerase	substitution
	Δ 5- sterol desaturase	substitution
	absent	introduction
absent	Δ 22- sterol desaturase	substitution
	absent	introduction
absent	Δ 24(28)-sterol reductase	deletion

Cholesterol

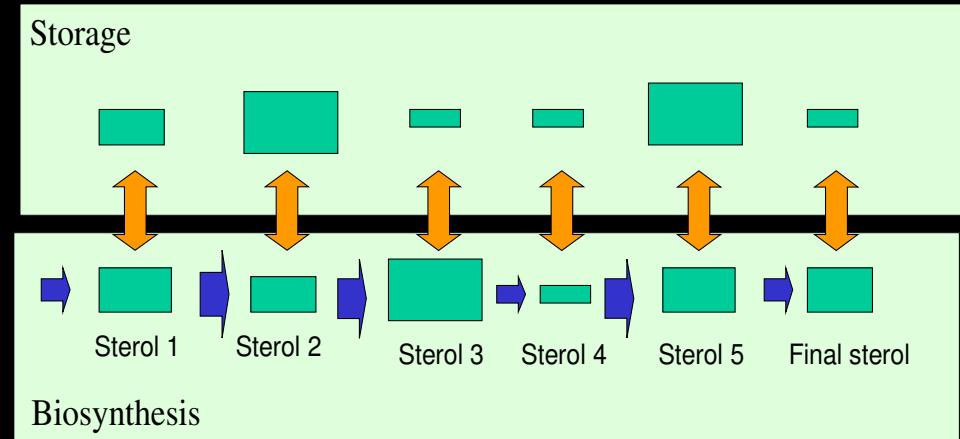
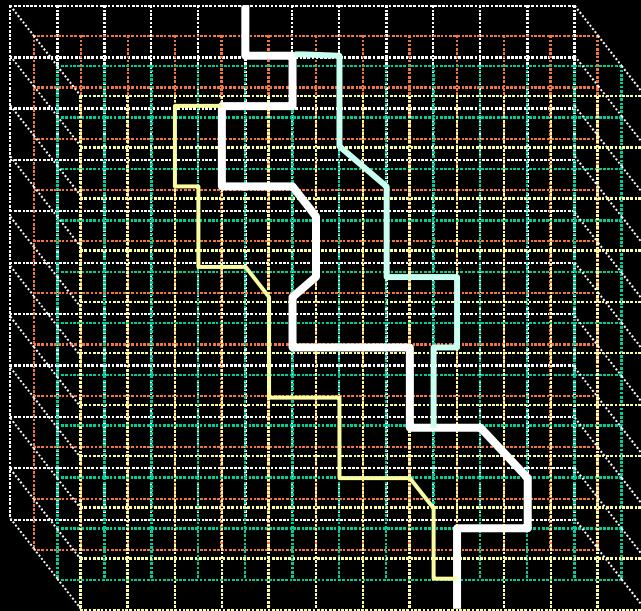
Ergosterol

Cholesterol

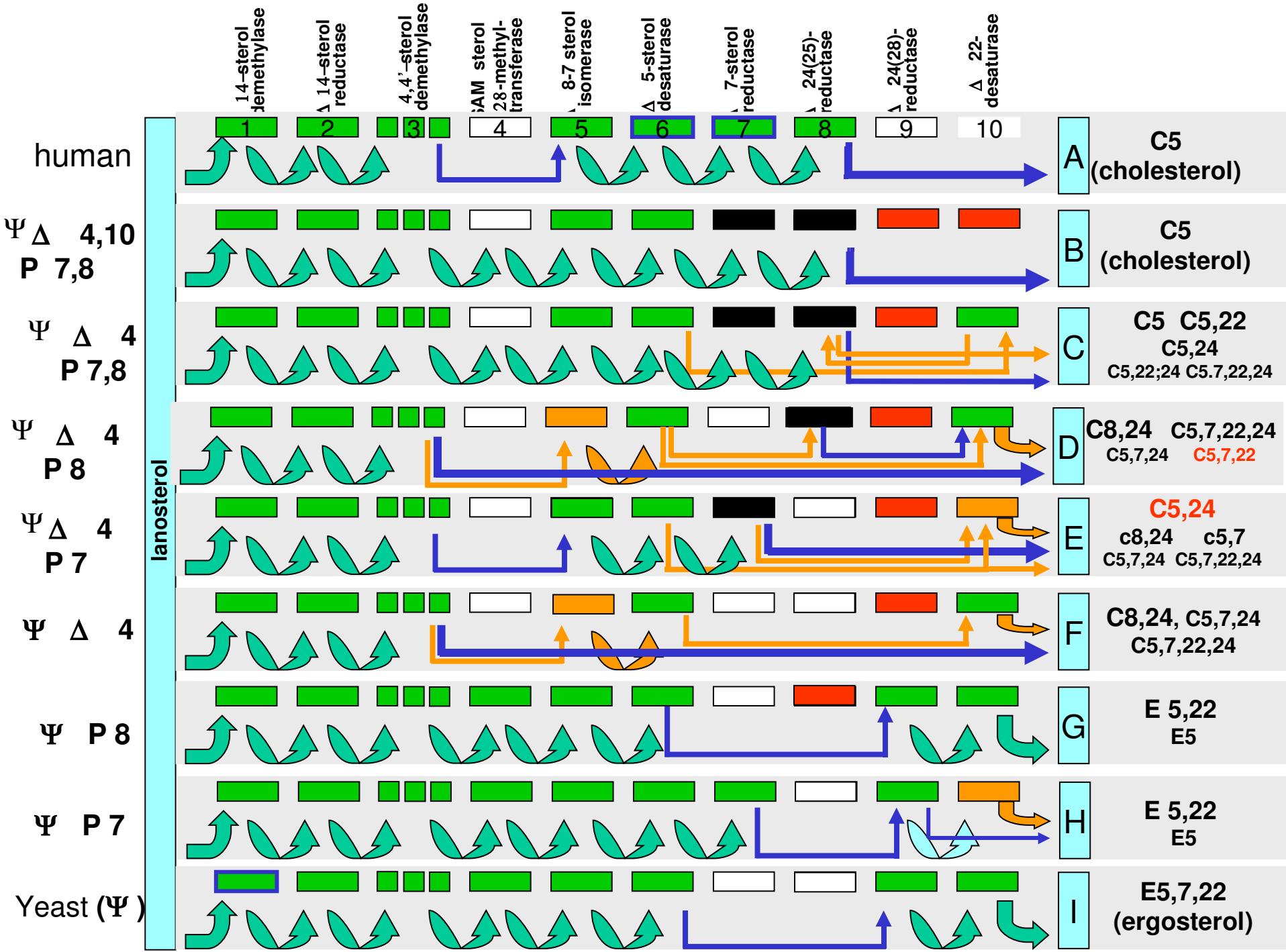
Sterol biosynthesis is a multi dimensional network

Enzymes have preferential but wide substrate specificities allowing multiple alternate paths

Storage-retrieval-transport mechanisms are tightly coupled to biosynthesis

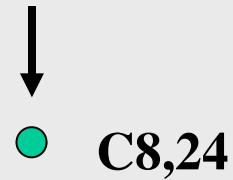


Pathological state or interaction with xenobiotics can reprogram sterol metabolic network leading to physiological dysfunctions



Non-physiological pathway accumulate sterol intermediates

Human

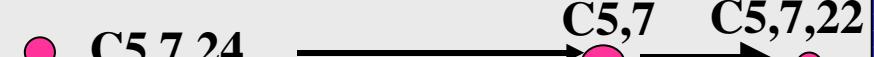


WT yeast



yeast

Expression of $\Delta 7$ - and $\Delta 24$ -reductase
Deletion of SAM-sterol transferase

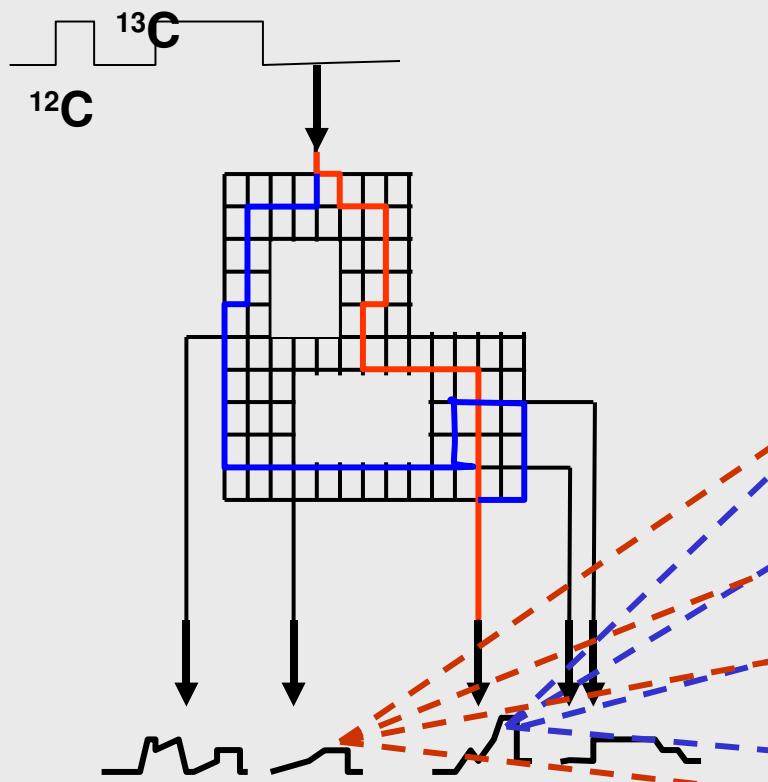


cholesterol

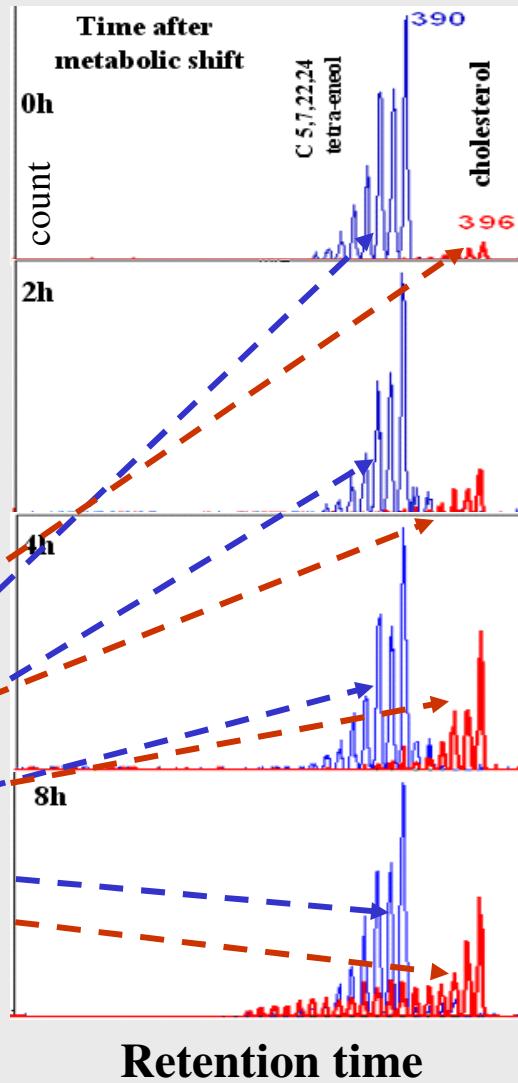
ergosterol

Analysis of pathologies or drug perturbations induced reprogramming of sterol metabolic network fluxes

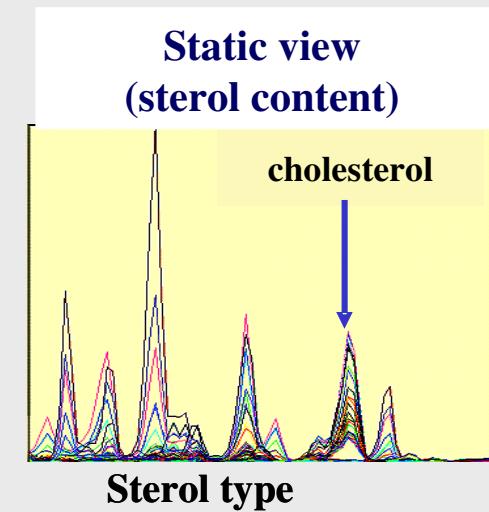
Isotope shift propagation analysis



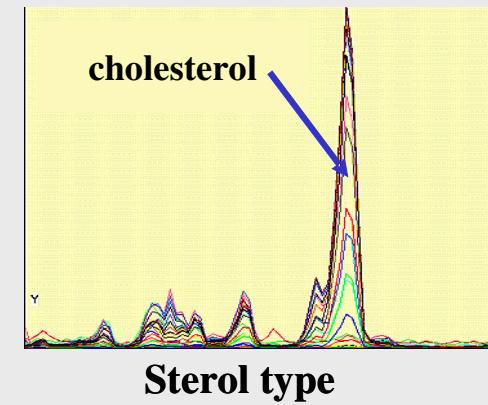
Time series of output



Static view (sterol content)



Dynamic view (synthesis rate)



Some concluding remarks

Metabolic network reconstruction in yeast constitutes a powerful tool both for drug development and production.

Nature selected biosynthetic strategies are not unique and efficient

artificial alternate self-sufficient routes can be build for biotechnological purposes.

Natural organ, cellular and subcellular compartmentalization is not a request for steroid hormone synthesis

Critical yeast functions controlling membrane integrity can be massively engineered keeping a viable host physiology.

Enzyme functions frequently exhibit surprising plasticity which can be both source of side reactions and advantage when reconstructing heterologous metabolic pathways.

Thank to

II

Steroid biosynthesis in yeast

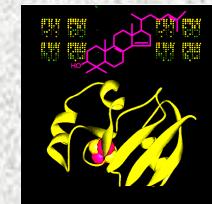


Humanized yeast

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