EB$_{\&}$T – Ecophysiology, Biochemistry and Toxicology
www.ecotox.be

Universiteit Antwerpen

7th Annual ecopa Workshop: “REACH for help: science back up?”
25-26 November 2006, Brussels

Development of a custom-made microarray as an alternative tool for screening of endocrine disruptors

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University of Antwerp
Belgium

REACH

- Introduction
- Objectives
- Results
- Conclusions
- Future work

Registration

Toxicity testing:

- Biodegradation
- Aquatic toxicity
- Irritation/corrosivity
- Reproduction/development

No specific endocrine endpoint

→ endocrine related !

Extra tests → validated!

- OECD Endocrine Disruption Testing and Assessment (EDTA)
  - TG407: Repeated dose (28 days) rat test → adapted for ED

Authorisation

Substances of very high concern:

- CMR, cat 1 and 2
- PBT, vPvB
- Equivalent endocrine disruptors

Expert judgement

↓

Focused testing

Endocrine disruption

- Introduction
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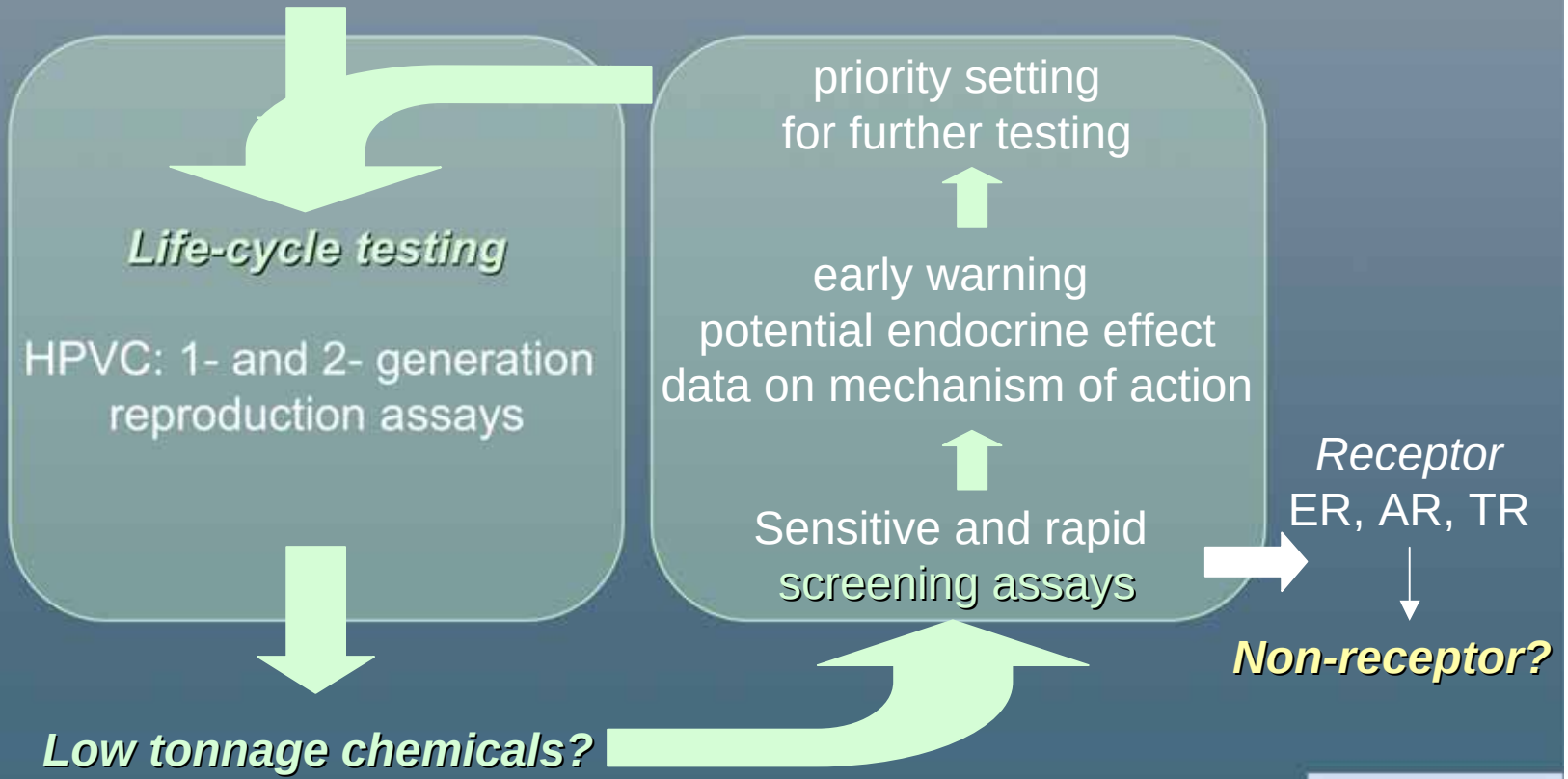
- Complex endocrine system
- Subtle effects at low concentrations → long term implications
- Action at the developmental stage → not expressed until adulthood



H295R

- Introduction
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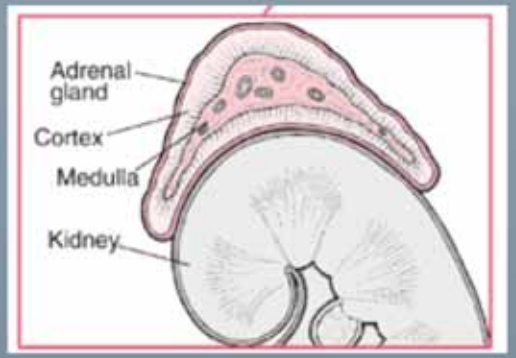
- Adrenocortical carcinoma cell line
- Complete pathway of steroidogenesis
  - → production of steroid hormones
  - → multiple endpoints for toxicity testing
- High basal P450 aromatase activity (Heneweer *et al.*, 2004)
- Expression of ER (α and β) and AR (Montanaro *et al.*, 2005)



What has been done?

H295R: hormone profiling

↓

Predictive in vitro test

Aim: reduce/replace animal testing

↓

~~Minced testis steroidogenic assay~~

What do we do?

Toxicogenomics

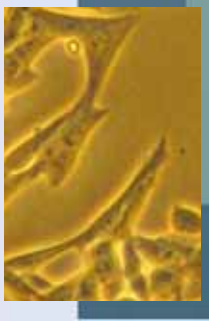
↓

Mechanistic in vitro test

Aim: add information

↓

Reduce animals by a more sustained approach



Objectives

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Exploration of H295R cell line

Toxicological endpoints?

Potential in evaluating endocrine disruption ?

↓

Development of a 'custom' H295R-specific array

↓

Evaluation

Chemical-specific response profiles?

Discriminative power?

↓

Performance of H295R in a toxicogenomics approach

Mechanistic information?

Classification of chemicals?

Experimental set-up

- Introduction
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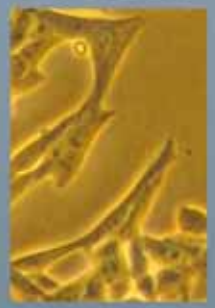
H295R



Model chemicals

8Br-cAMP
Ketoconazole
Atrazine
Genistein
Lindane

RNA extraction
cDNA labelling

control
exposed

Hybridization

Agilent Whole Human Genome Array
44K, 60mer oligo's
Microarray Facility (MAF, VIB)

Scanning

Results

- Introduction
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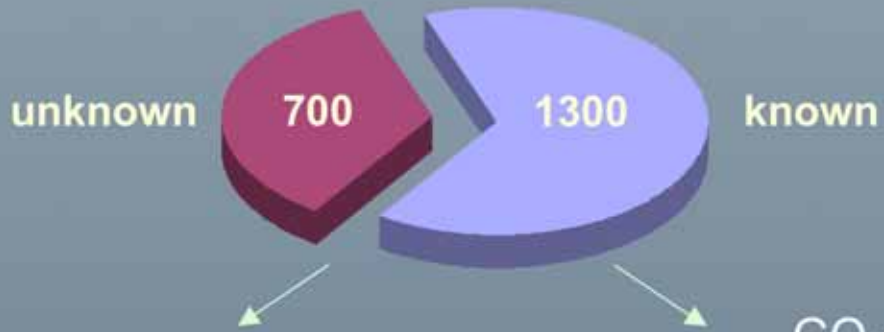

	Up	*Down*
8Br-cAMP	292	508
Lindane	474	598
Atrazine	105	252
Ketoconazole	104	262
Genistein	77	168
	1075	***1788***

GO-annotaties
(EASE, GenMAPP)

Steroid synthesis/metabolism
Cholesterol synthesis/metabolism
Hormone activity
Receptor binding/activity
Response to external stimulus
Cell growth/proliferation
Signal transduction
Sperm motility
Growth factor activity

Cholesterol uptake

- Introduction
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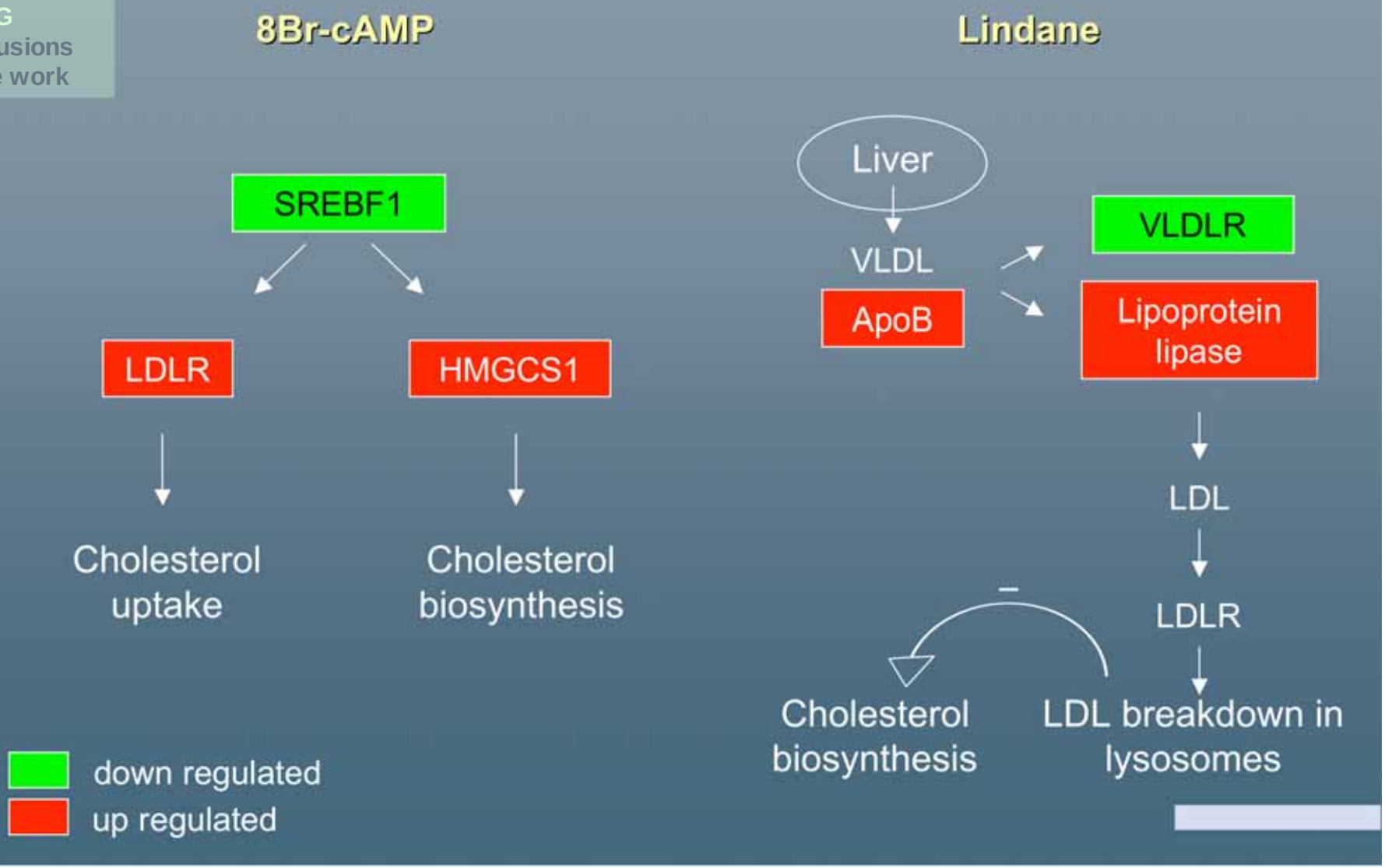

Cholesterol biosynthesis

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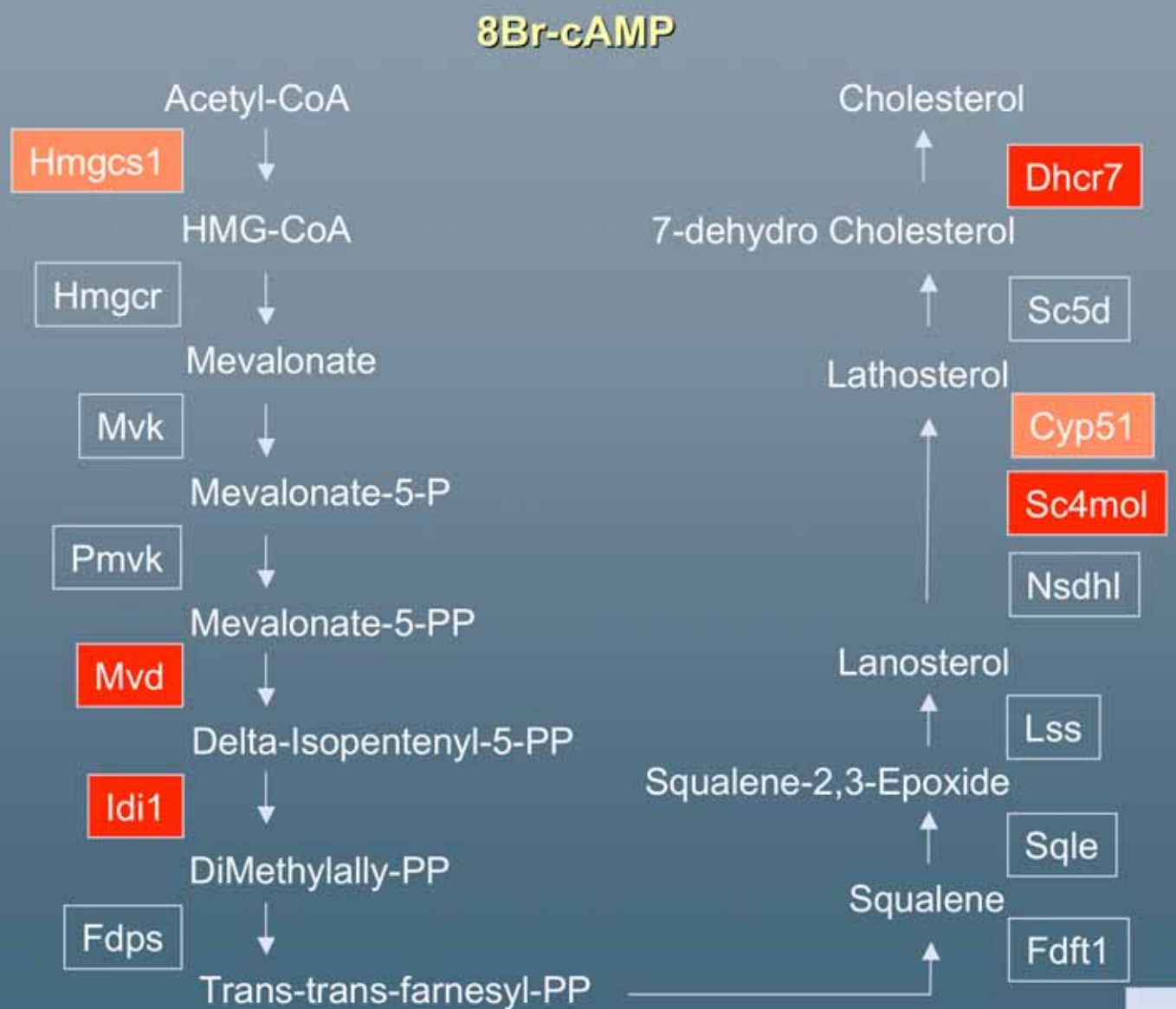

Cholesterol biosynthesis

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Steroid biosynthesis

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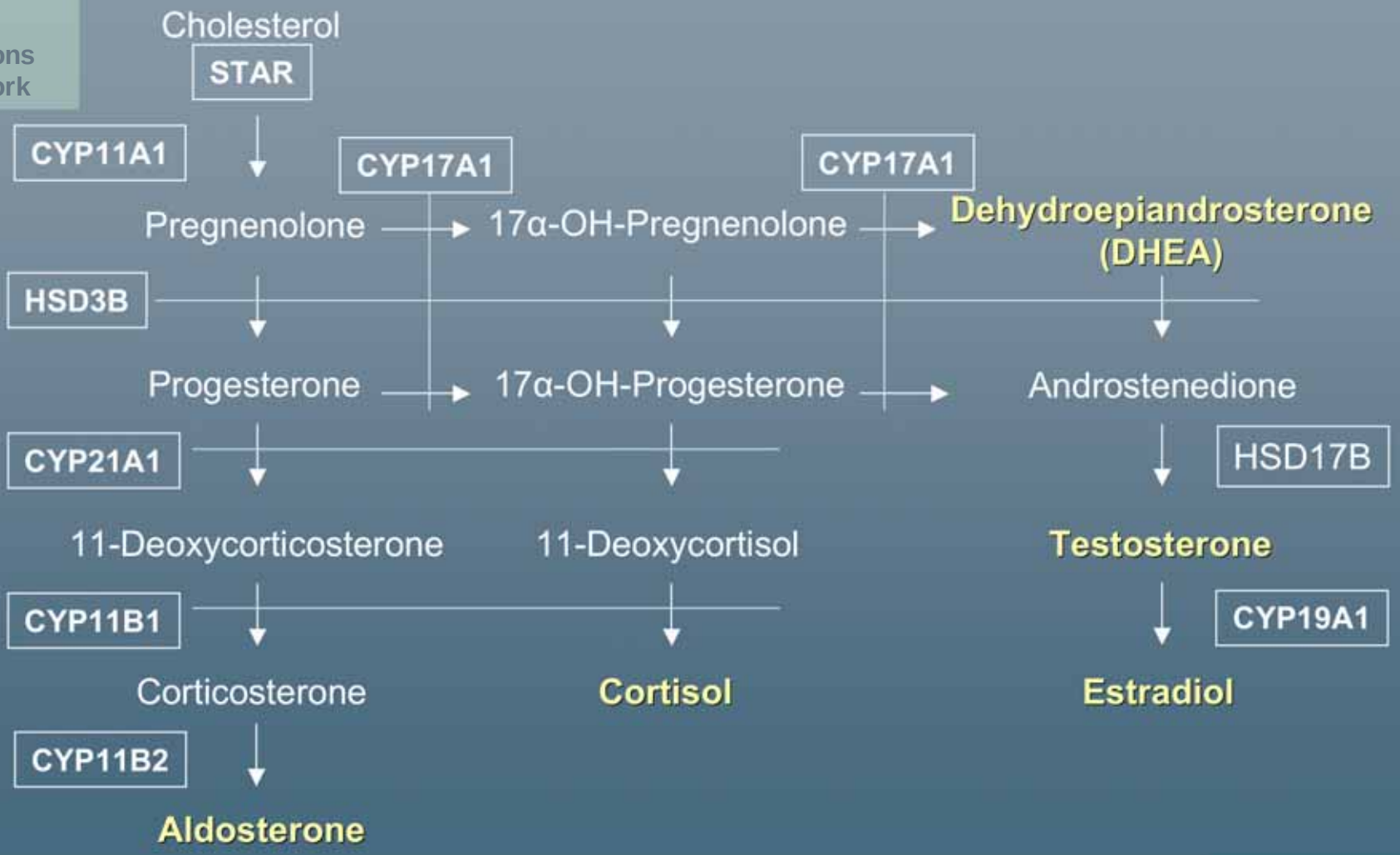

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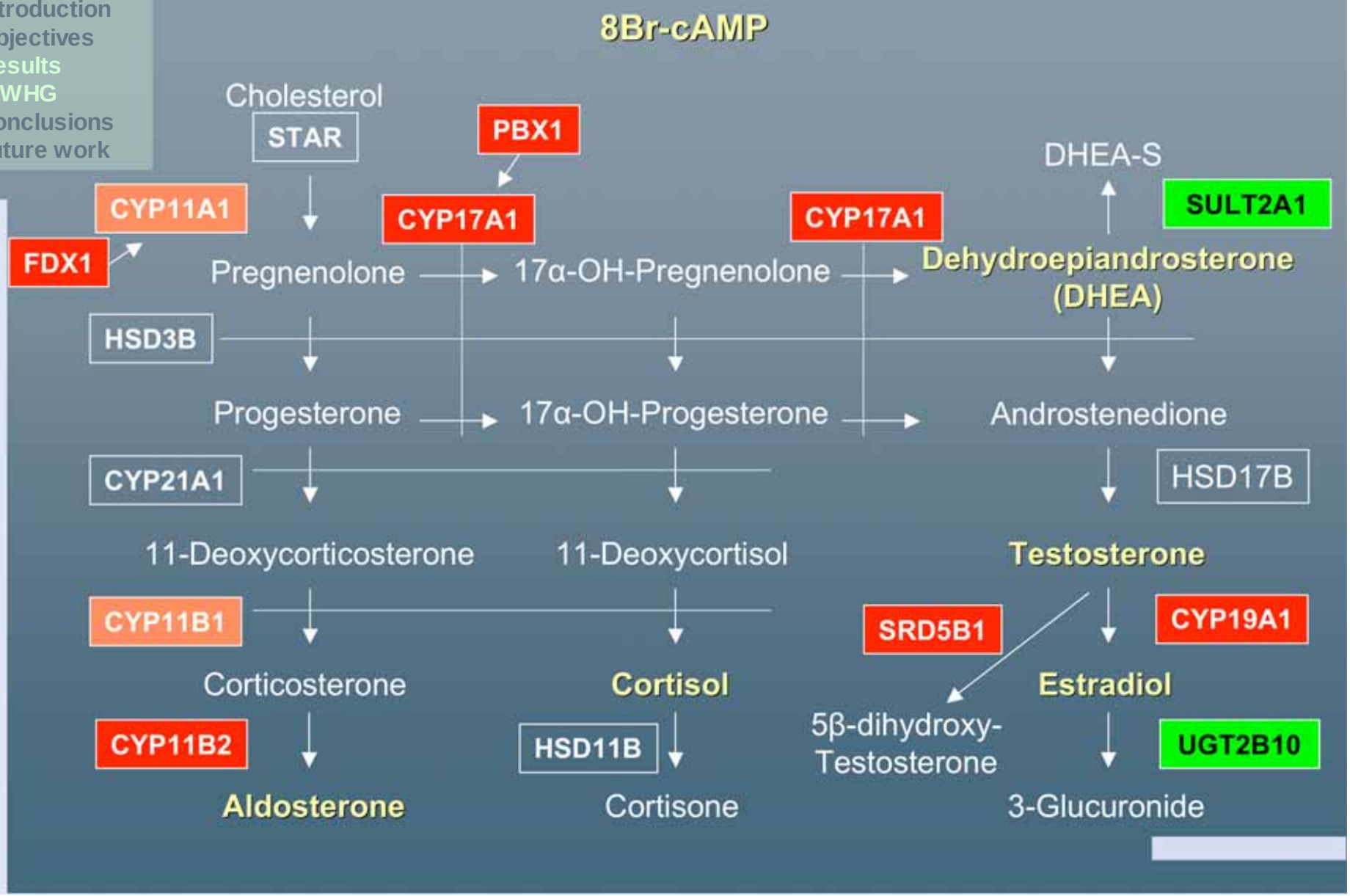

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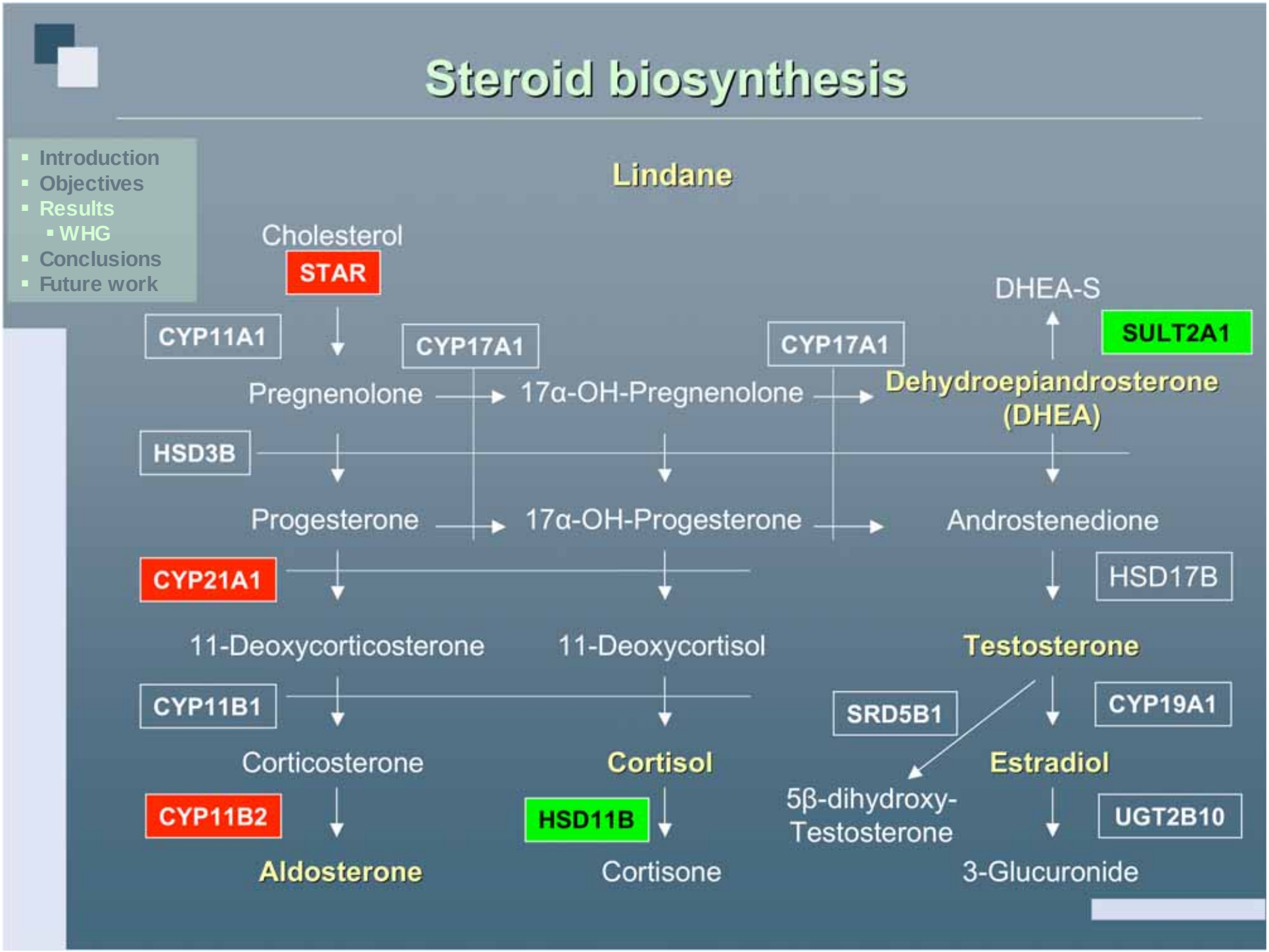

Steroid/xenobiotic metabolism

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P450 mono-oxygenases

- CYP3A4
- CYP3A5 — Induced by glucocorticoids
- CYP3A7 — Hydroxylates testosterone, DHEA-3-sulfate → estriol

SULT1C1

ABC-transporters

- ABCA1 — Cholesterol efflux
- ABCC4 — Multi-drug resistance, cellular detoxification
- ABCD2 — Peroxisomal import of FA

UDP glycosyltransferases

- UGT2B4 — Estrogen metabolism
- UGT2B17 — Steroid metabolism

Results

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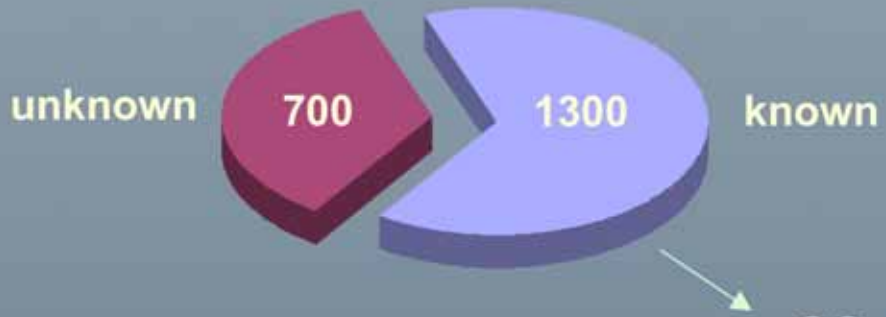

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Endocrine regulation

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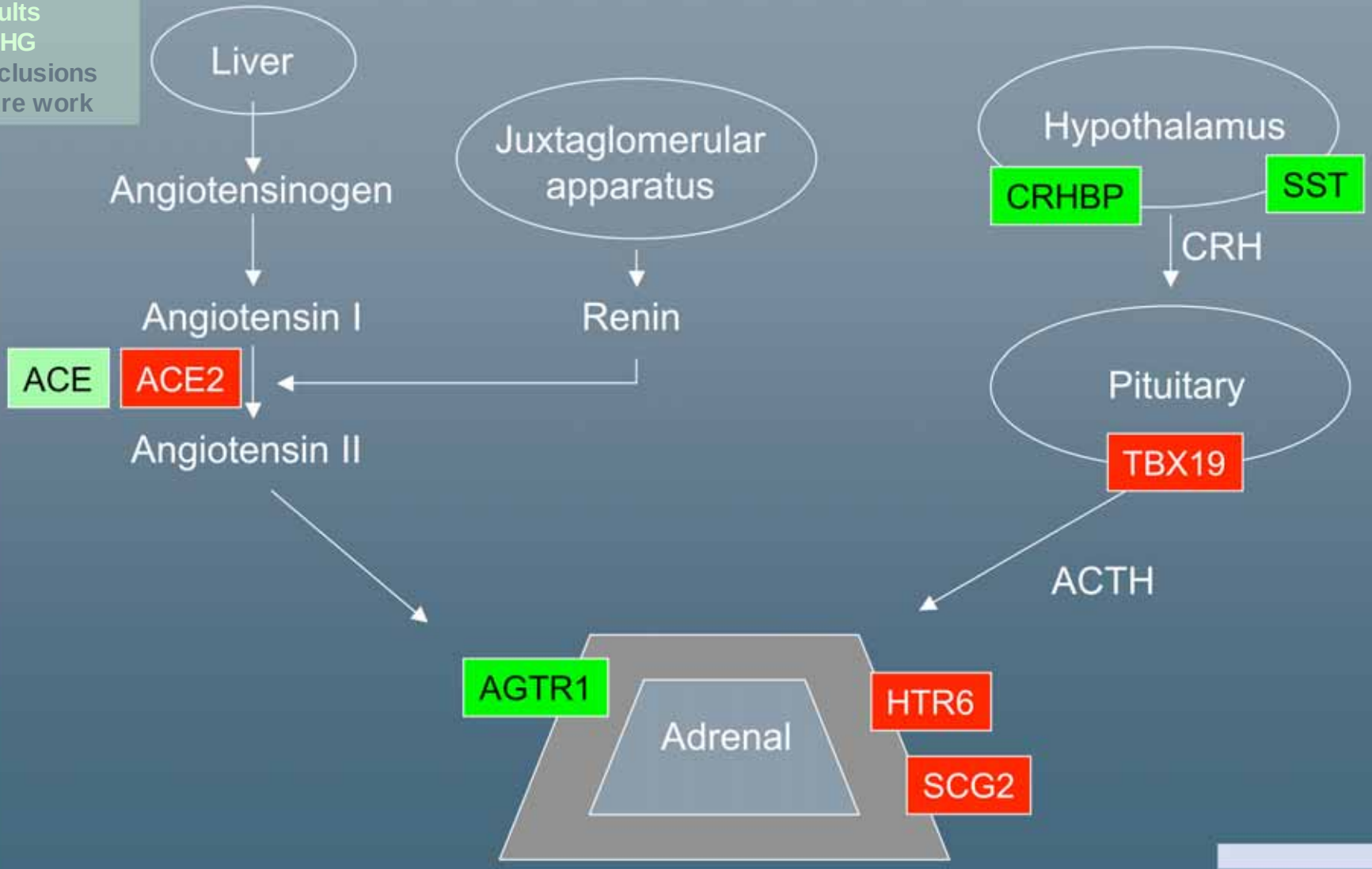

Endocrine regulation

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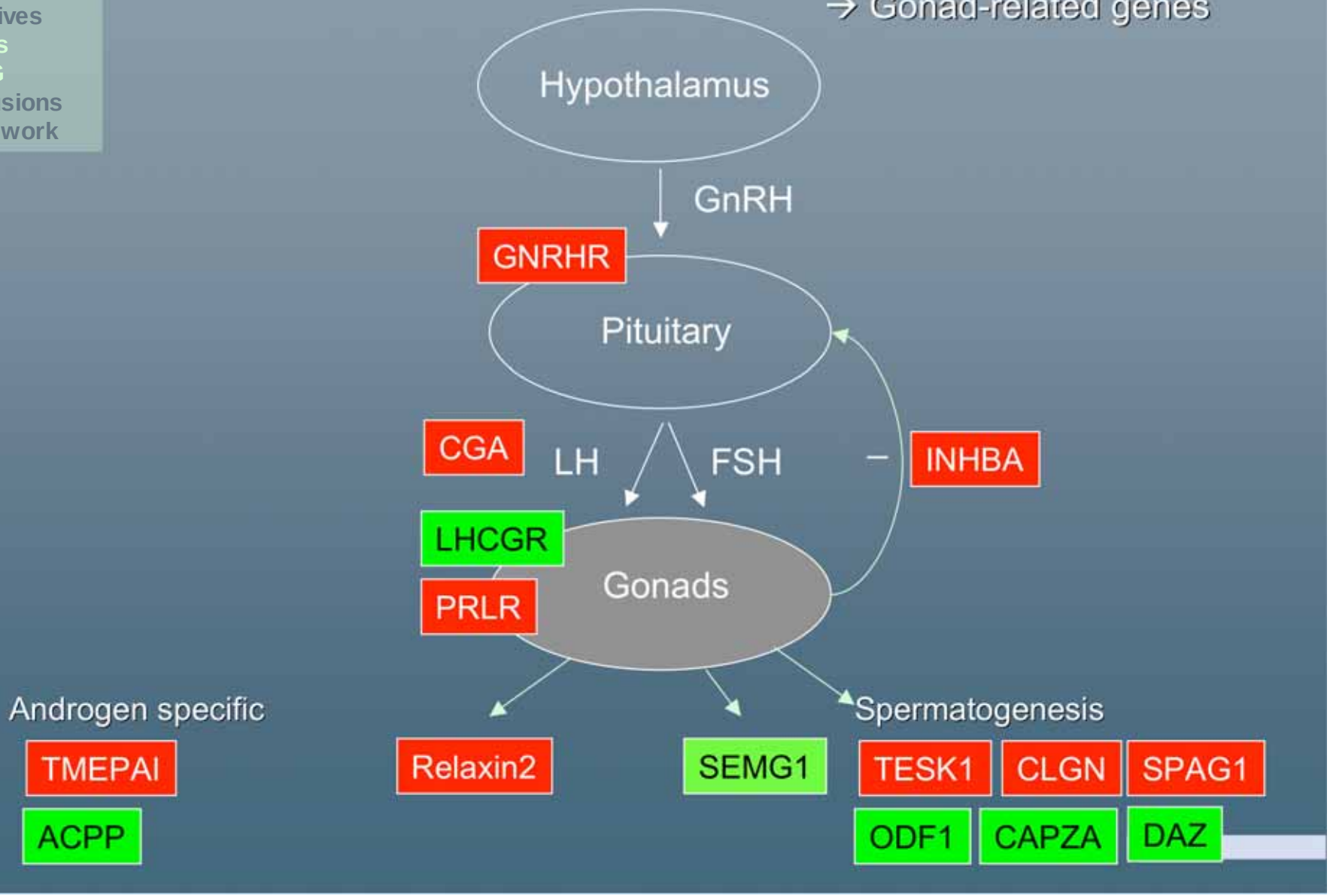

Results

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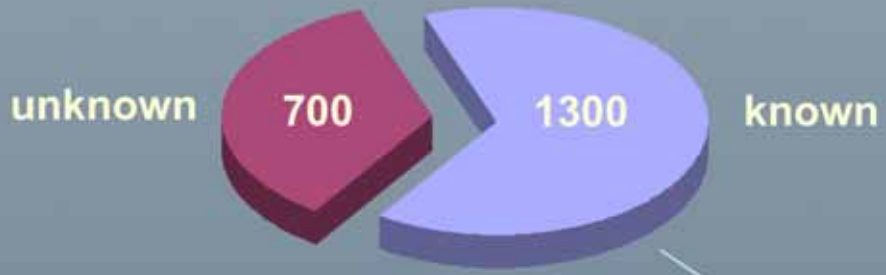

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Nuclear receptors

- **ERβ** (ESR2)
- **AHR** - **AHRR**epressor
- **PPARα – PPARγ**
- **NR0B1** (adrenal hypoplasia protein) RAR mediated
- **NR4A1 – NR4A2 – NR4A3**

  Steroid-thyroid hormone retinoid receptor superfamily
- **RARRES2** retinoic acid receptor responder

Design H295R array

- Introduction
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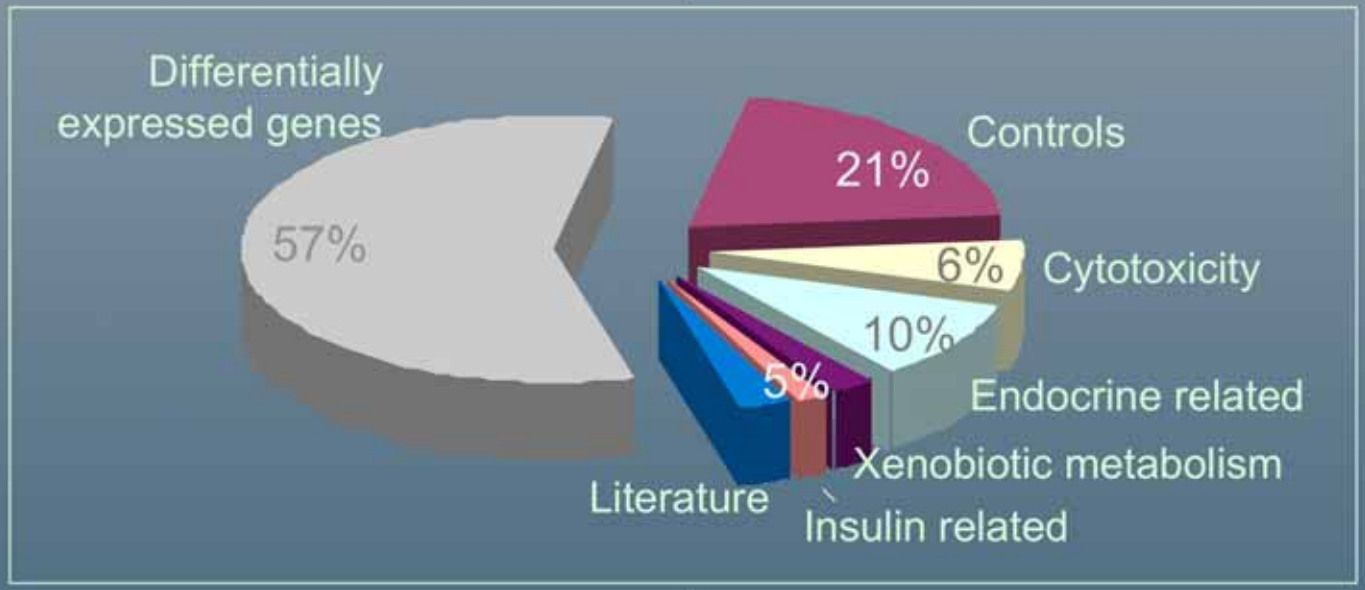
44K Whole Human Genome Array

Down scaling

1.9K H295R-specific array



Agilent 8-pack 1.9K array

8 arrays on 1 slide

Conclusions

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- **Diversity of toxicological endpoints present**
  - Cholesterol/lipid metabolism
  - Endocrine related
  - Nuclear receptors
- **Clear discrimination between 8Br-cAMP and lindane**

Promising towards using this H295R cell line in toxicogenomics approach

Future work

- Introduction
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Exploration of H295R cell line

Toxicological endpoints?

Potential in evaluating endocrine disruption ?

↓

Development of a 'custom' H295R-specific array

↓

≠ exposure conditions

5 concentrations

5 periods

(1h, 4h, 10h, 24h, 48h)

≠ cell conditions

passage #

media

serum batch

Chemical testing

≠ physicochemical properties

≠ MOA

Evaluation

Chemical-specific response profiles?

Discriminative power?

↓

Performance of H295R in a toxicogenomics approach

Future work

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Combination with differential proteome analysis (2D-DiGE) and hormone profiling

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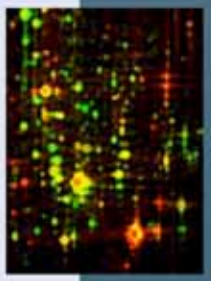
More comprehensive view on role of the gene profiles in endocrine function

↓

Identification of potential biomarkers



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