

Gene pathways targeted during phthalate-induced testicular dysgenesis

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Presentation Outline

- **Phthalate profile study**
- **Time course**
- **Promoter analysis**
- **Species comparison**

Public Health Issue

- **Reported increases in the incidence of cryptorchidism, hypospadias, prostate and testicular cancer, and reduced fertility.**
- **Testicular dysgenesis syndrome (TDS)**
 - **Disruption of male reproductive tract development during fetal life by genetic or environmental factors.**

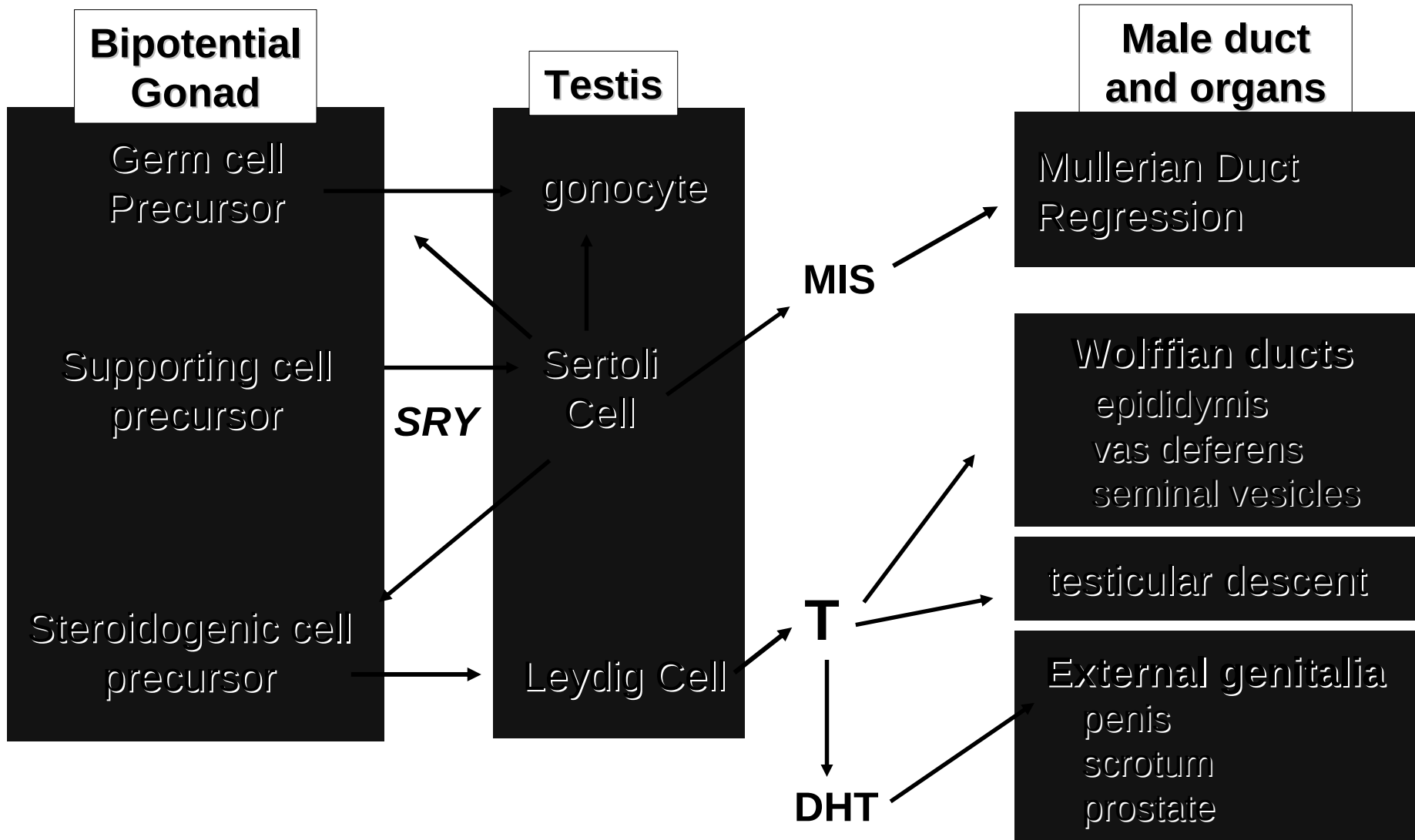
Laboratory Observations

- **Exposure of fetal male rats to antiandrogens leads to reproductive disorders that are similar to those of concern in the human male population.**
 - **Cryptorchidism, reproductive tract defects, reduced fertility, cancer**

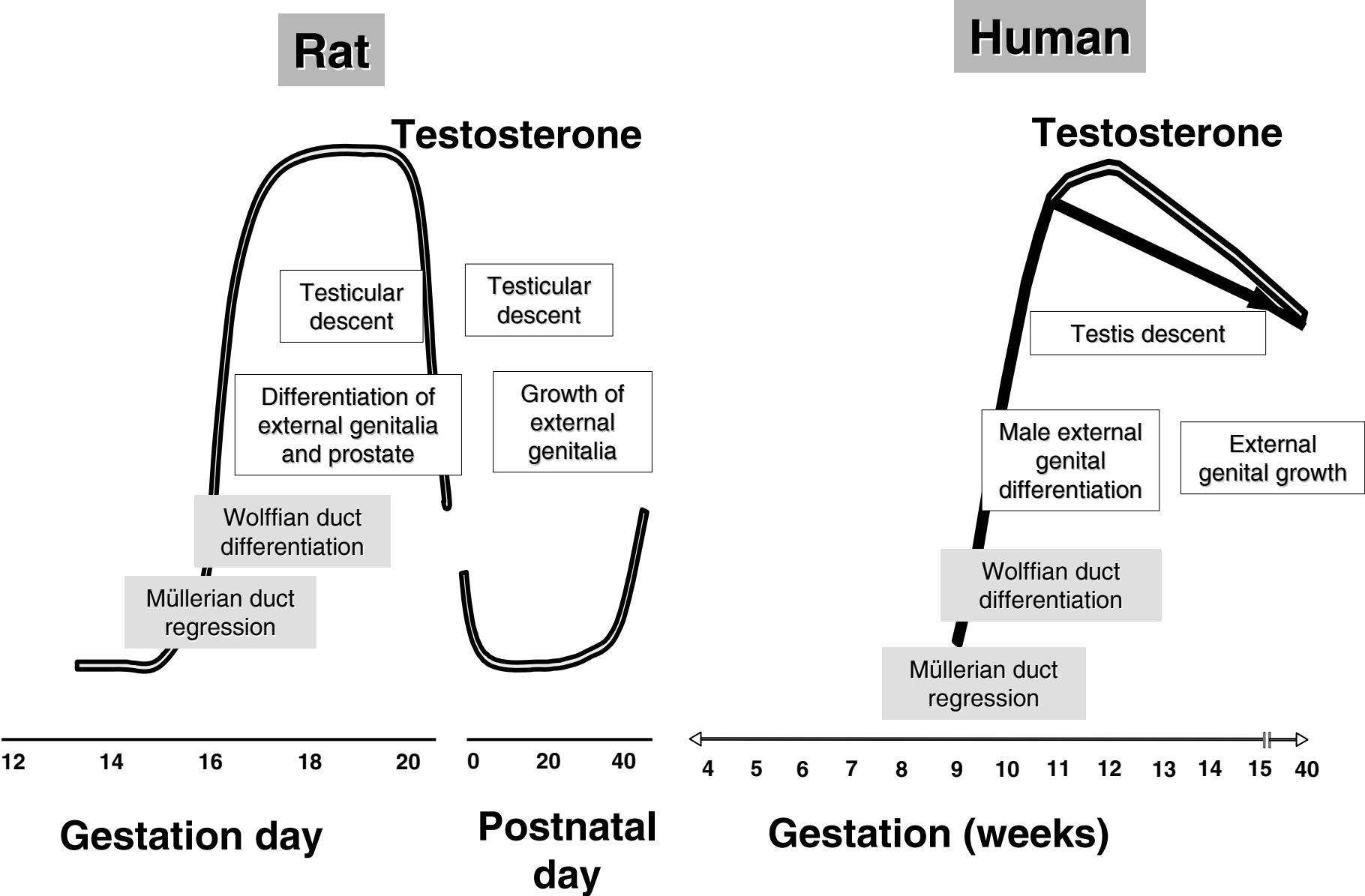
Environmental Antiandrogens

- **Insecticide metabolites (p,p'-DDE, HPTE)**
- **Fungicides (Vinclozolin, Procymidone)**
- **Herbicide (Linuron)**
- **Some Imidazole and Triazole antifungals**
- **Phthalate esters (DBP; DEHP)**
- **Organophosphate pesticides (Fenitrothion)**

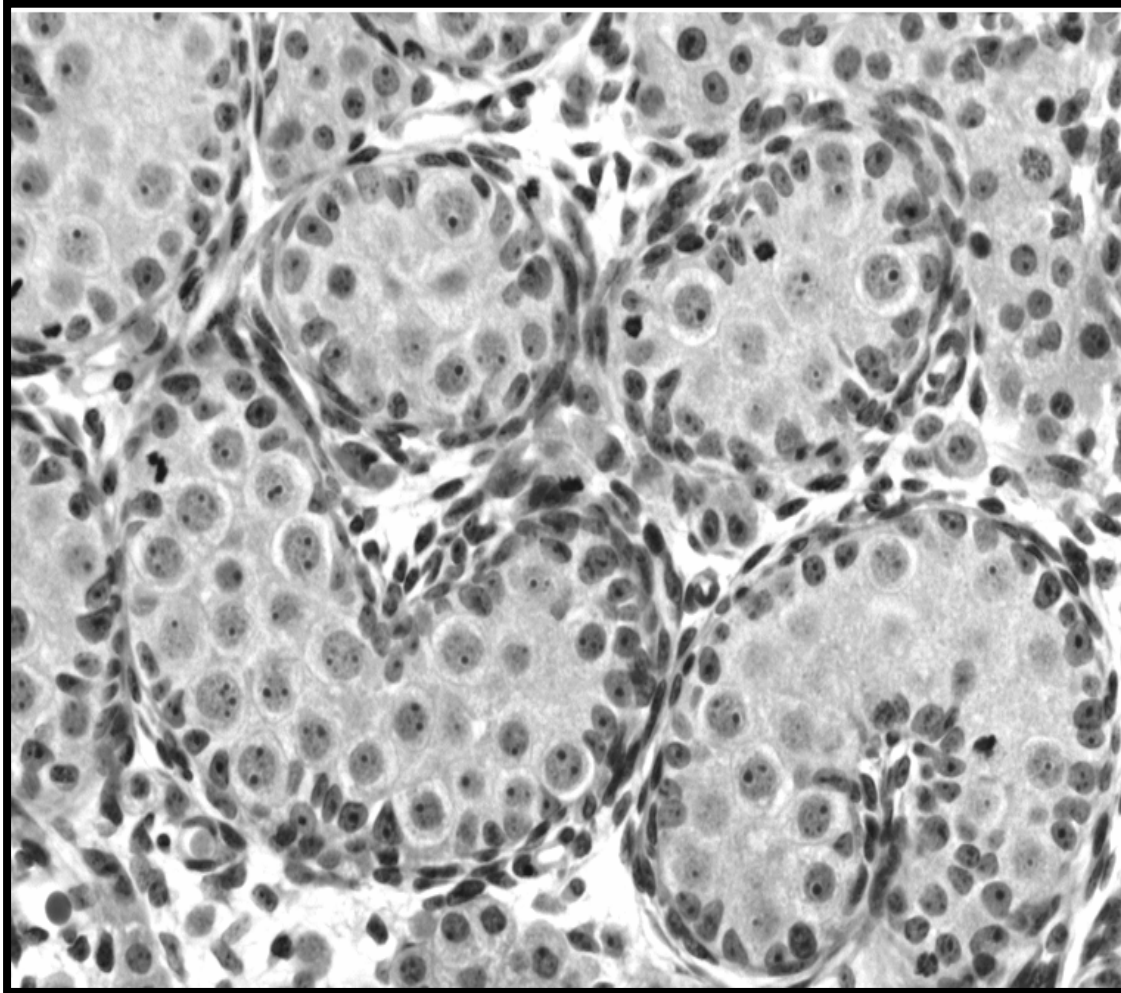
Development of the Male Reproductive Tract



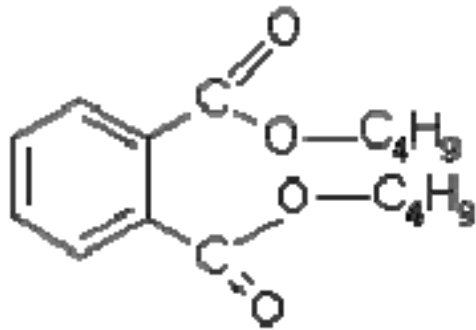
Development of the Male Reproductive Tract



Fetal Testis



Phthalates



- **Widely used plasticizers that impart flexibility and strength to a variety of consumer goods and plastics**
- **Found in food packaging, clothing, paints, toys and personal care products**
- **Anti-androgenic**
- **Does not bind to androgen receptor**

Phthalates Disrupt Male Rat Reproductive Development

- **Cryptorchidism**
- **Male reproductive tract malformations**
 - Epididymal malformations
 - hypospadias
- **Testicular dysgenesis**
 - Regions of Leydig cell hyperplasia
 - Multinucleated gonocytes
 - Abnormal seminiferous cord formation

Decrease in Anogenital Distance Among Male Infants with Prenatal Phthalate Exposure

**Swan SH, Main KM, Liu F, Stewart SL, Kruse RL, Calafat
AM, Mao CS, Redmon JB, Ternand CL, Sullivan S,
Teague JL, and the Study for Future Families Research
Team.**

Environ Health Perspect

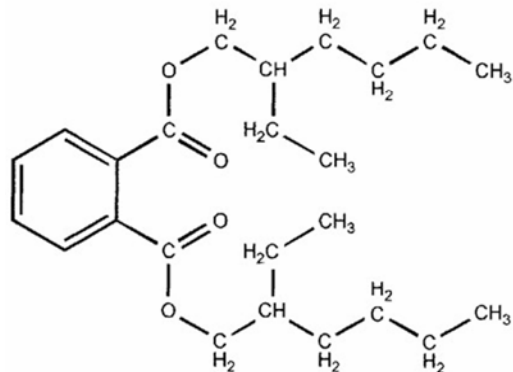
Phthalate Profile Study

Phthalate Ester	Reproductive effects
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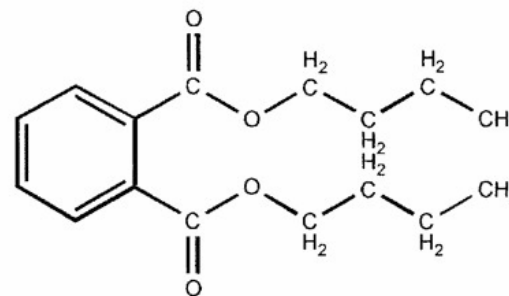
Dimethyl	-
Diethyl	-
Dioctyl- <i>tere</i>	-
Di- <i>n</i> - butyl	+
benzobutyl	+
Di- <i>n</i> - pentyl	+
Di-(2-ethylhexyl)	+

Phthalate esters

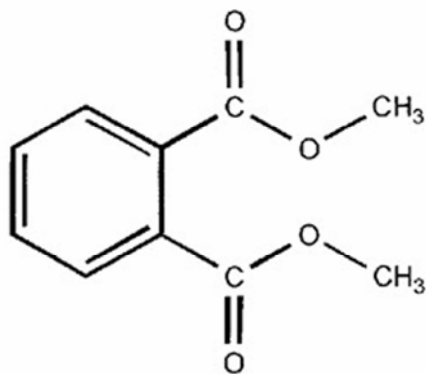
DEHP



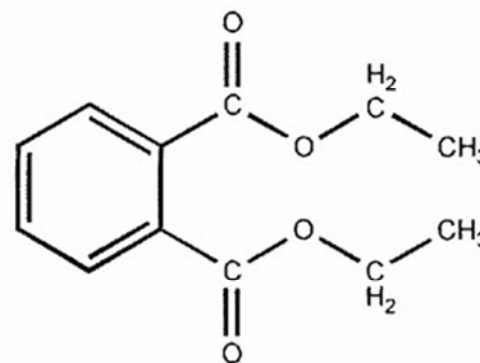
DBP



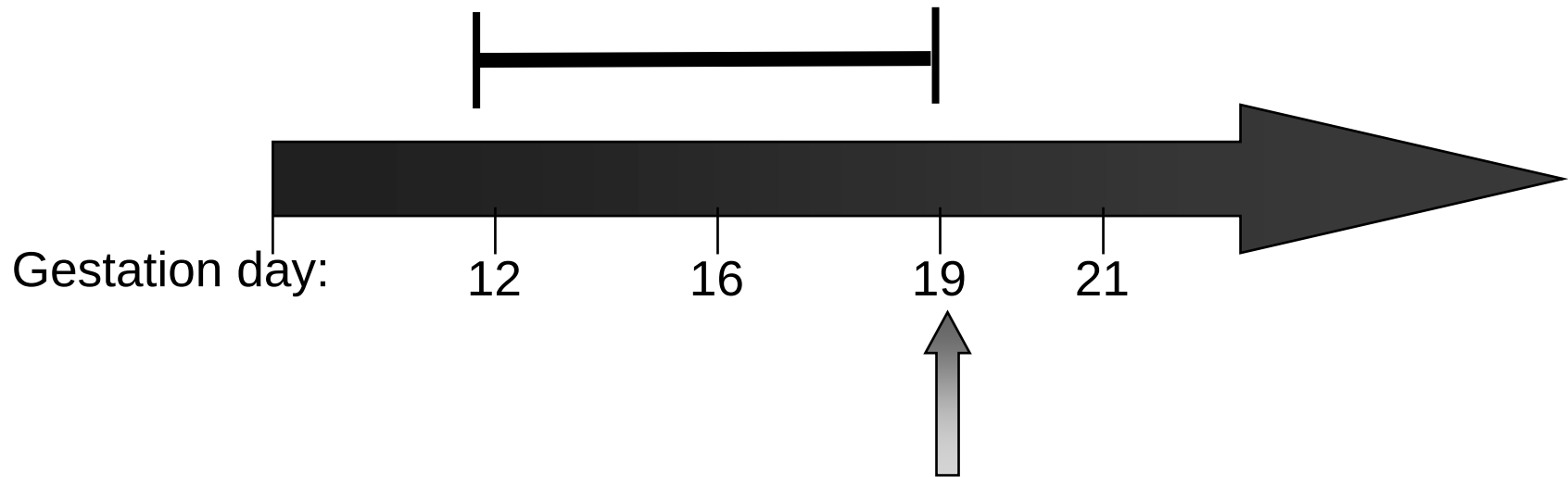
DMP



DEP



Study Design



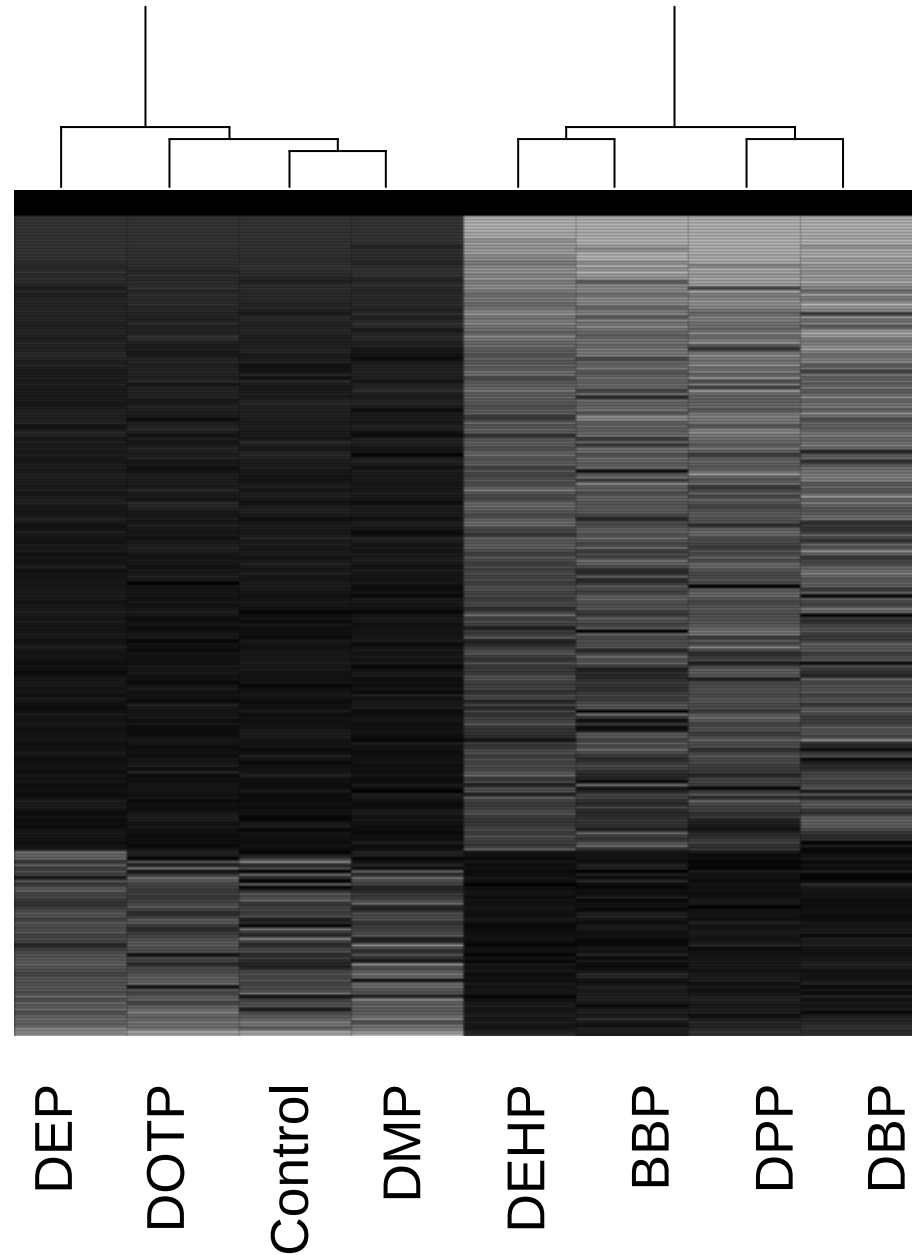
Isolate and snap freeze testis for RNA isolation

Affymetrix microarray results

- Investigated approximately 30,000 genes
- 391 genes significantly altered (1.3%)
 - 225 unknown transcribed sequences
- DMP, DEP, DOTP
 - Not significantly different from control or each other.
- DBP, BBP, DPP, DEHP
 - All significantly different from control and DMP, DEP, DOTP
 - But not each other

391 unique genes

- Universal mean determined for each gene
- Red = increased relative to mean
- Green = reduced

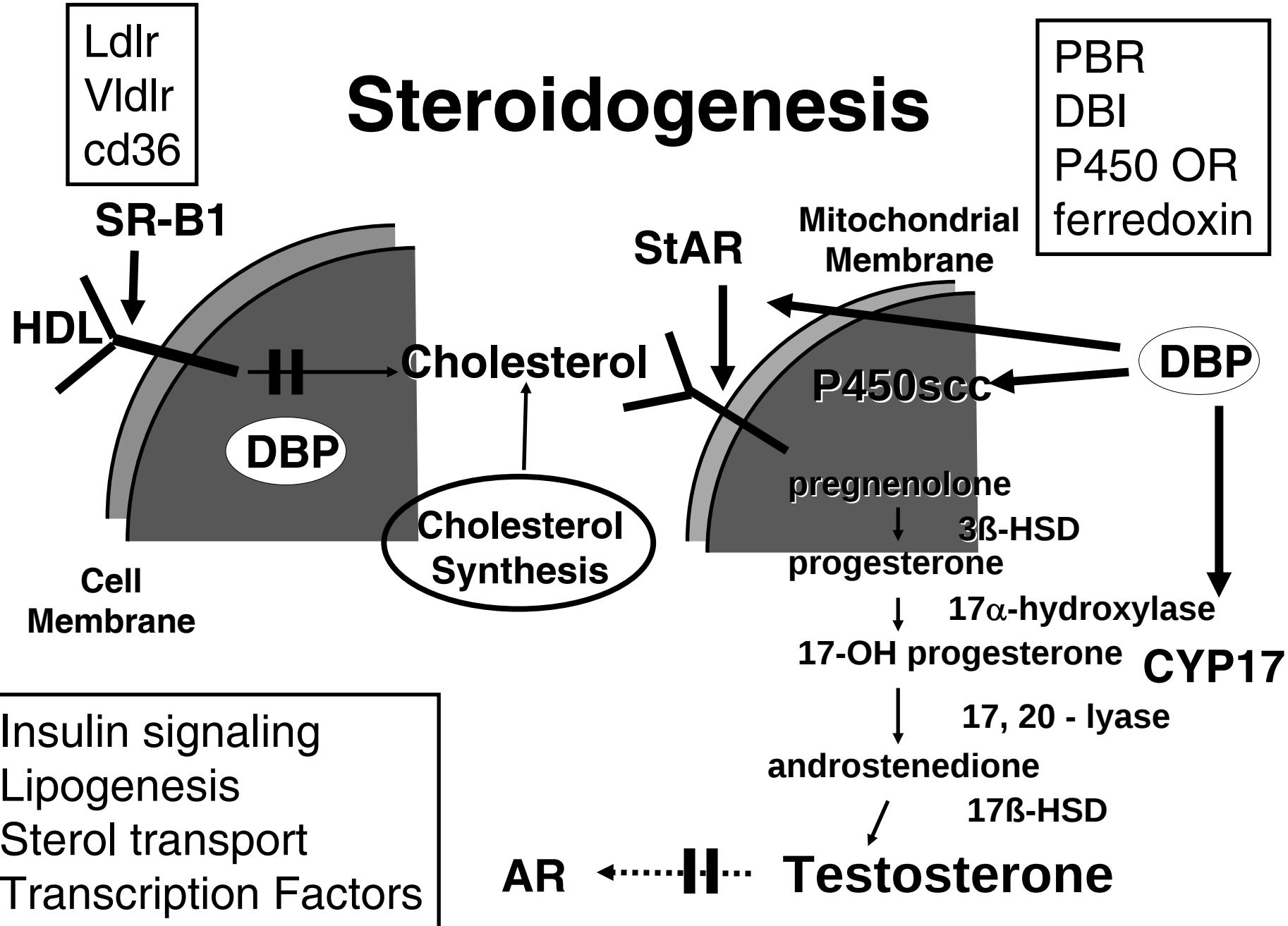


Gene Ontology

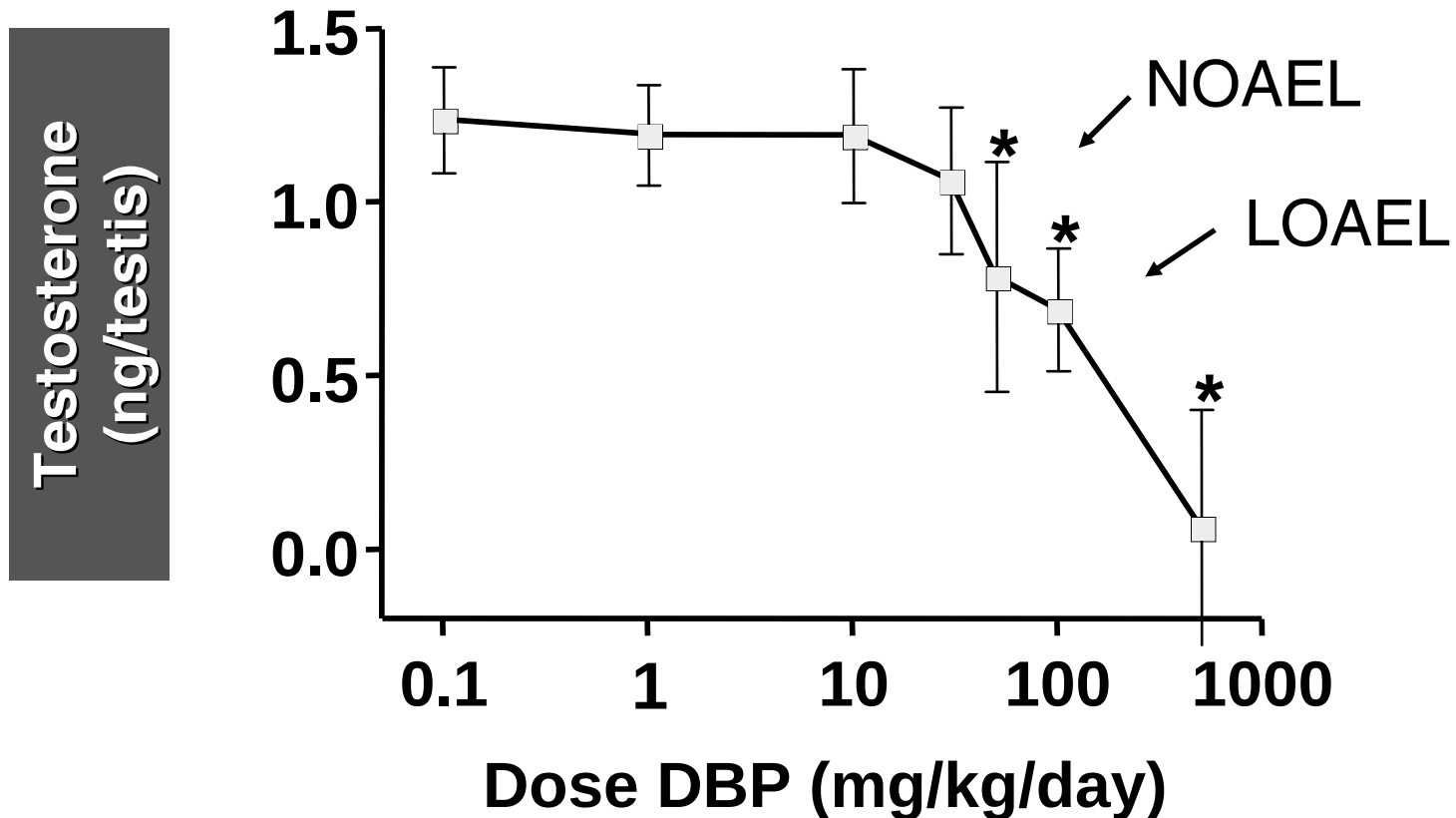
167 genes

- Lipid, cholesterol, sterol homeostasis - - - - 31 (19 %) ↓
- Steroidogenesis - - - - - 12 (7 %) ↓
- Lipid, cholesterol, sterol transport - - - - - 10 (6 %) ↓
- Transcription Factors - - - - - 9 (5 %)
- Signal Transduction - - - - - 22 (13 %)
- Oxidative Stress - - - - - 11 (7 %) ↓
- Cytoskeleton - - - - - 13 (8 %)
- Unclassified - - - - - 59 (35 %)

Steroidogenesis



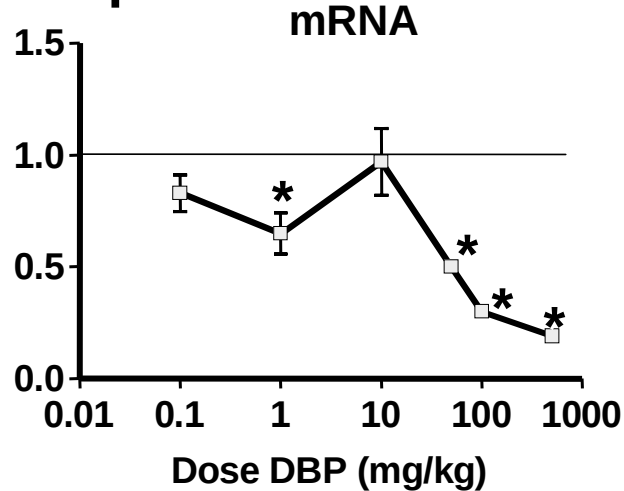
Substantial Reductions in Testosterone Occur Before Developmental Endpoints



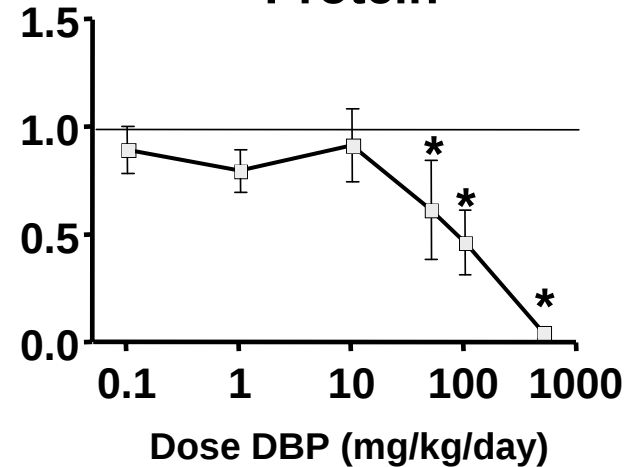
Cholesterol transport

Scavenger Receptor

Relative
Expression

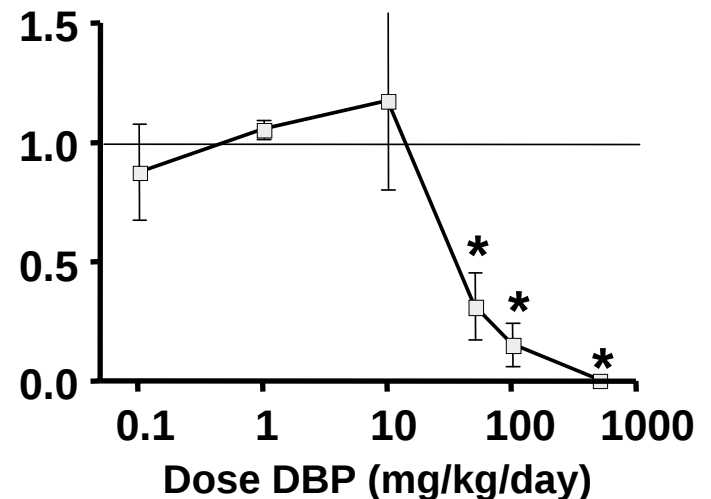
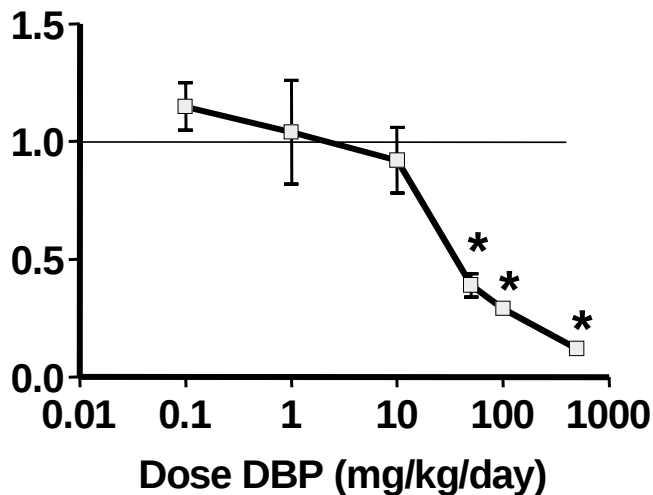


Protein

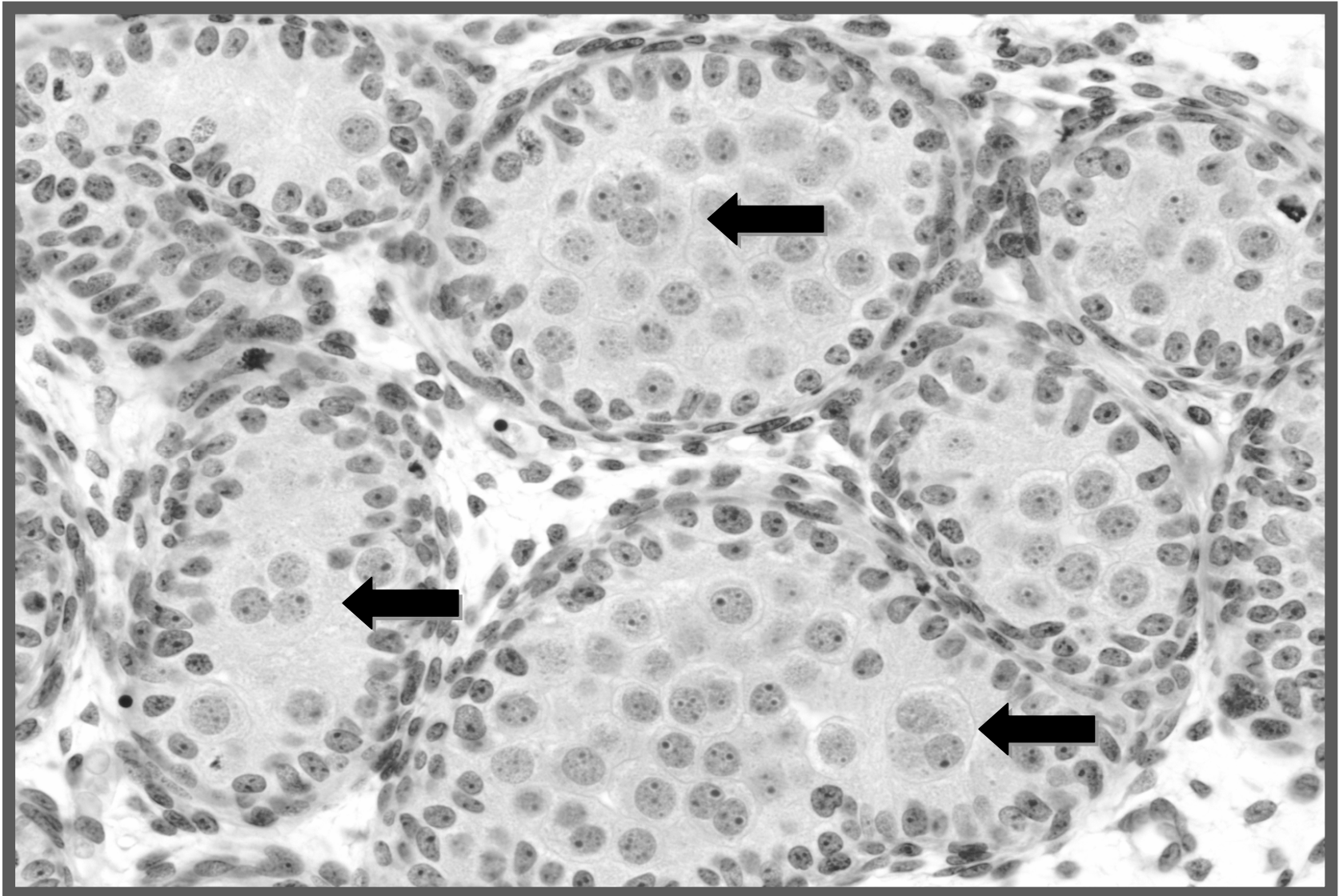


StAR

Relative
Expression

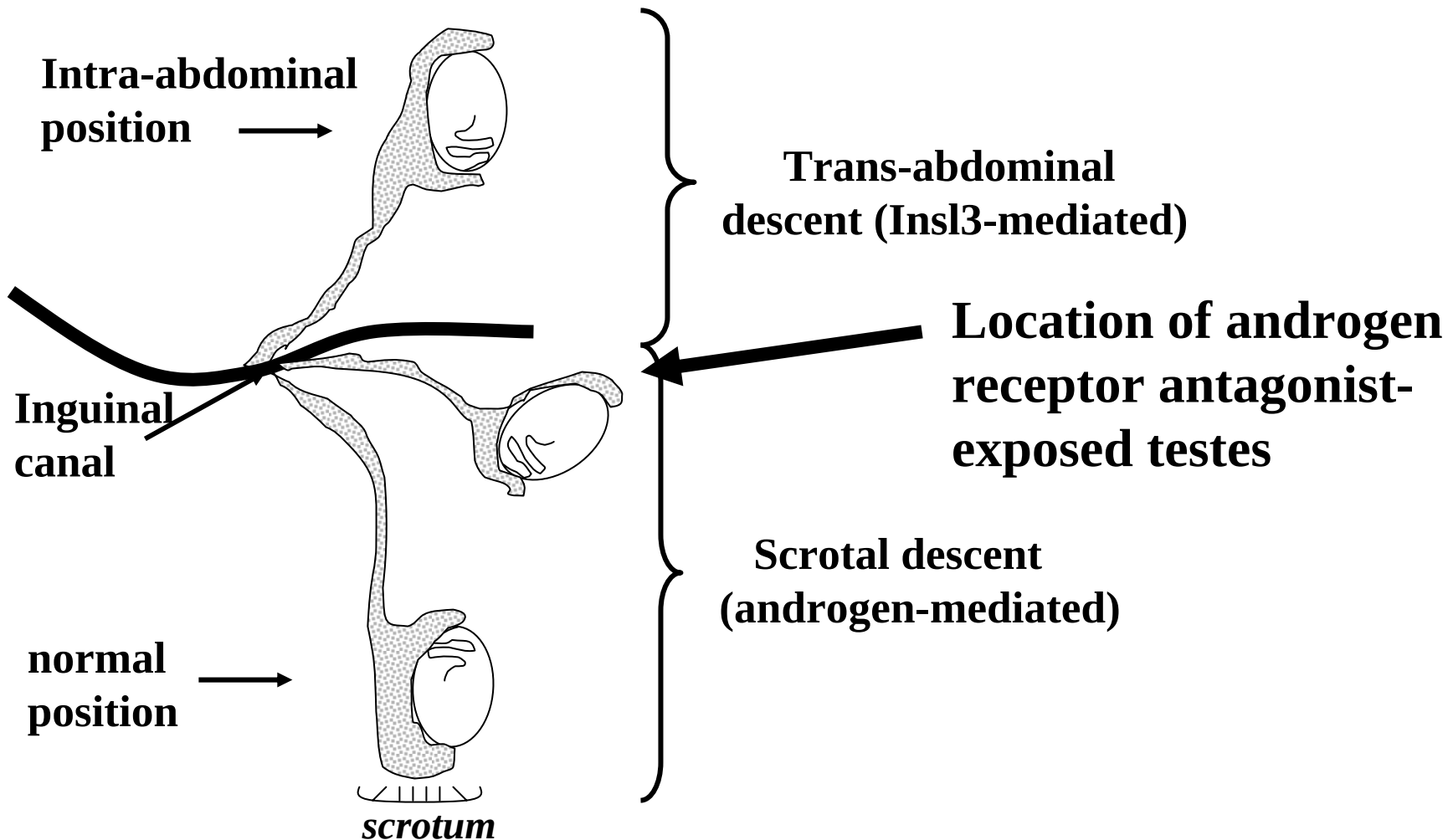


Multinucleated Gonocytes

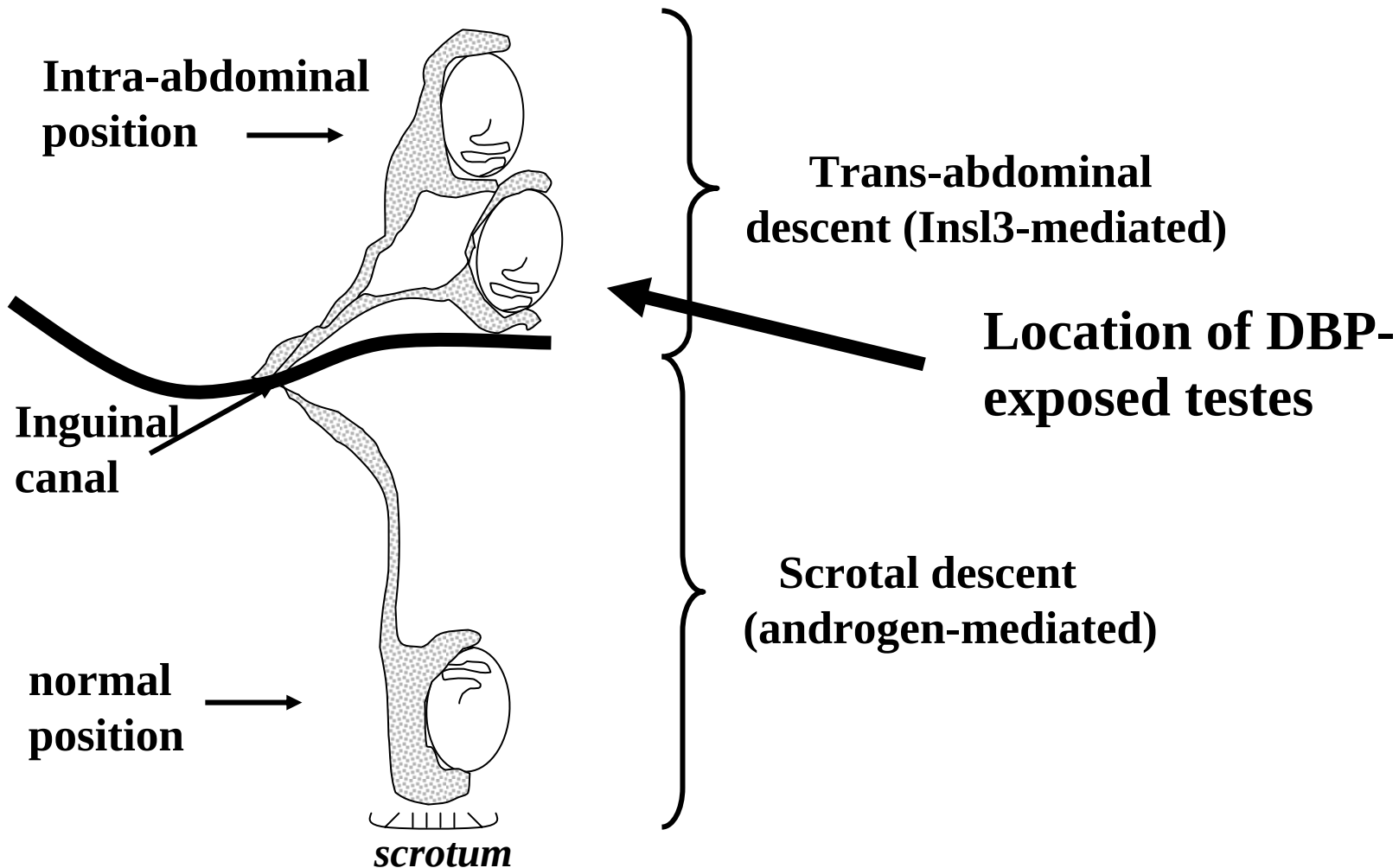


DBP-induced cryptorchidism

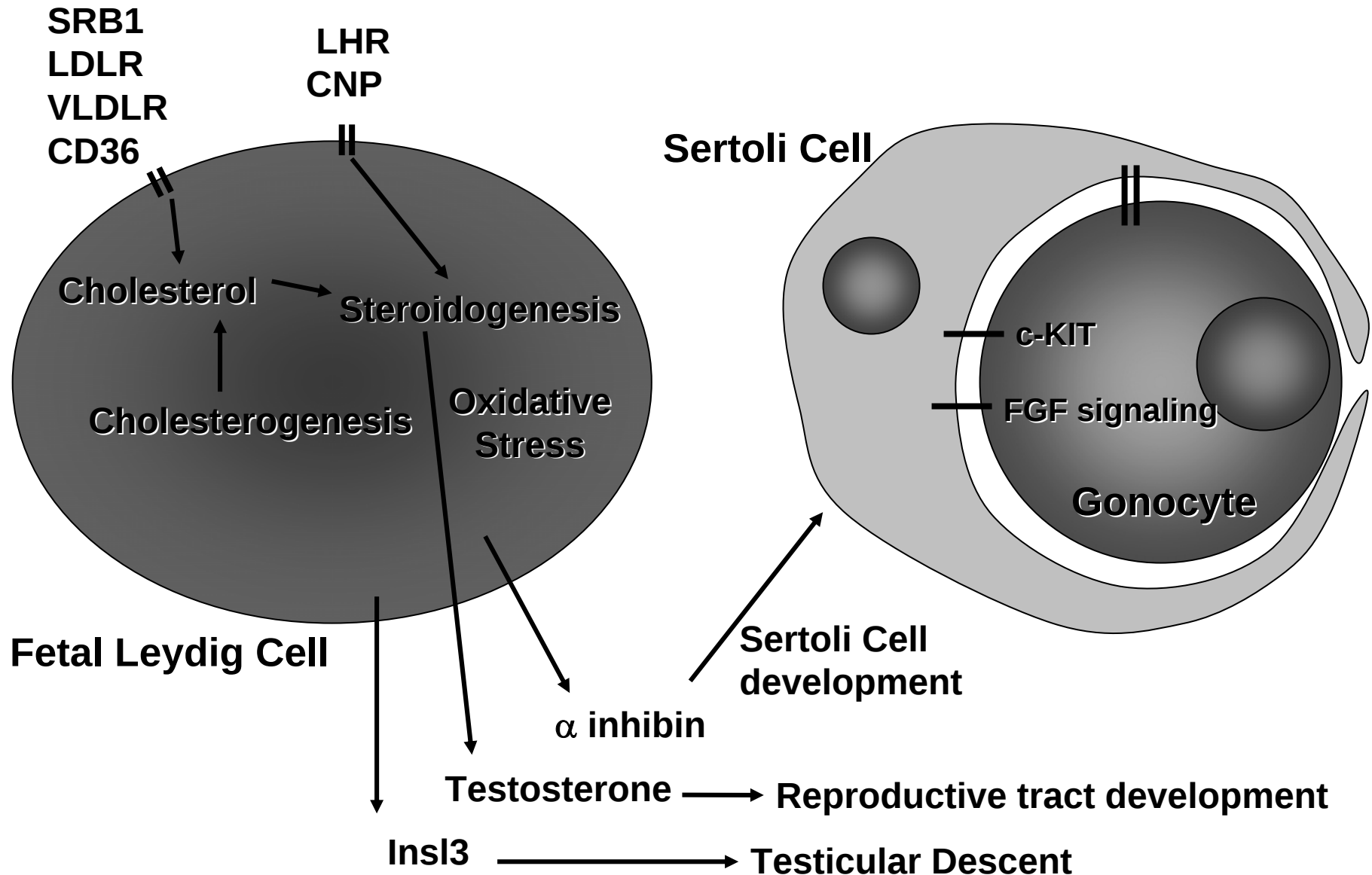
Role of insl3 in Testicular Descent



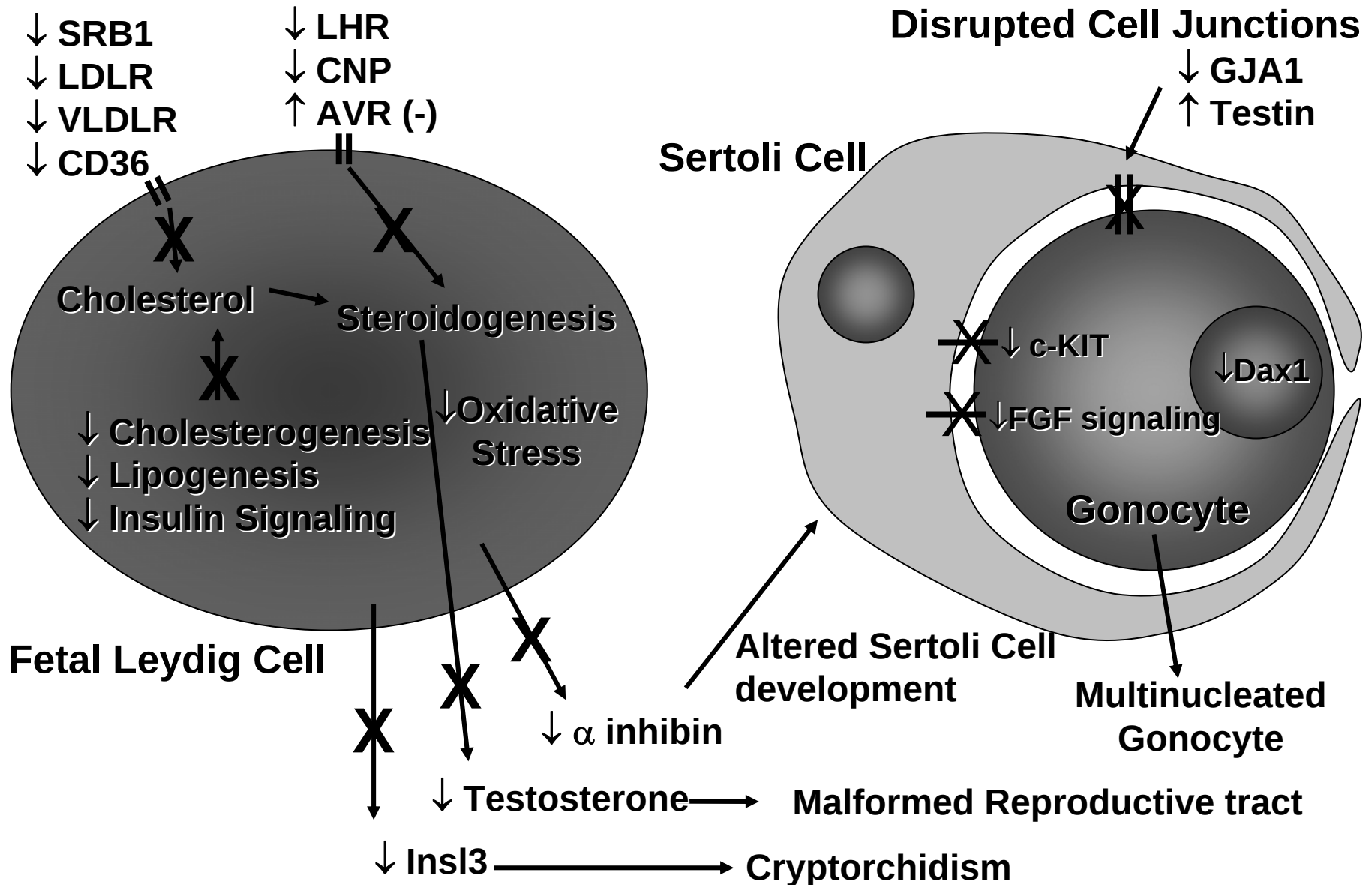
Role of insl3 in Testicular Descent



Phthalate-Induced Testicular Dysgenesis



Phthalate-Induced Testicular Dysgenesis

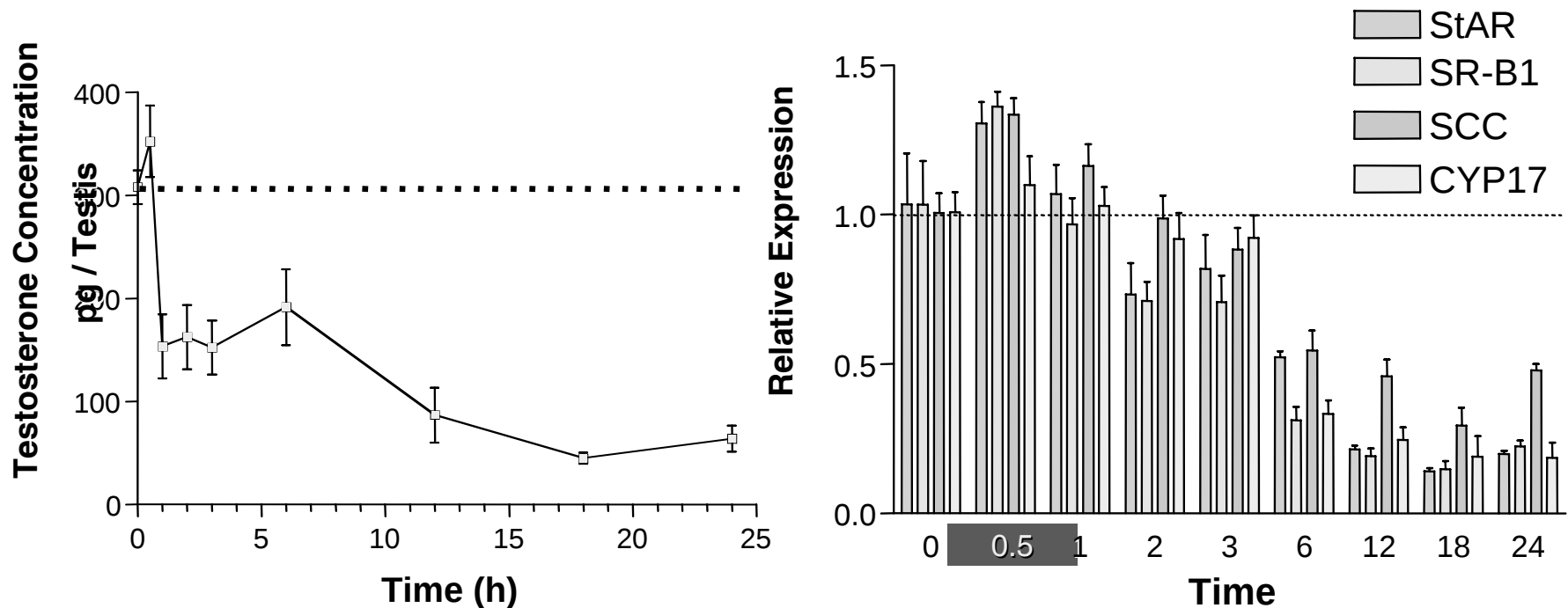


Early gene changes in the fetal testis after DBP exposure

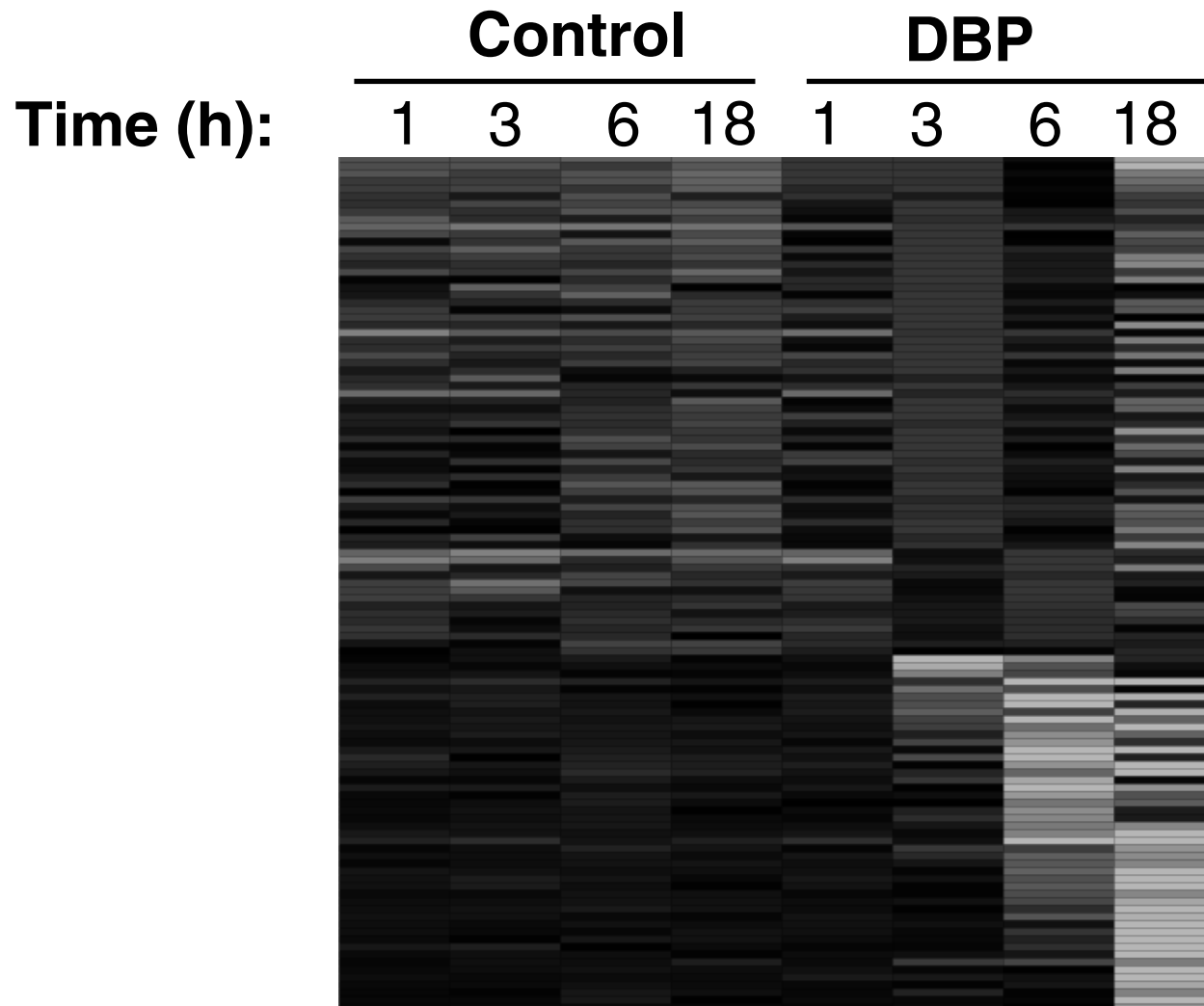
Time Course

- 500 mg/kg/day
- 0, 0.5, 1, 3, 6, 12, 18, & 24 h
- Between gd 18 and 19
- Testosterone RIA
- RT-PCR
- Microarrays at 1, 3, 6, 18 h

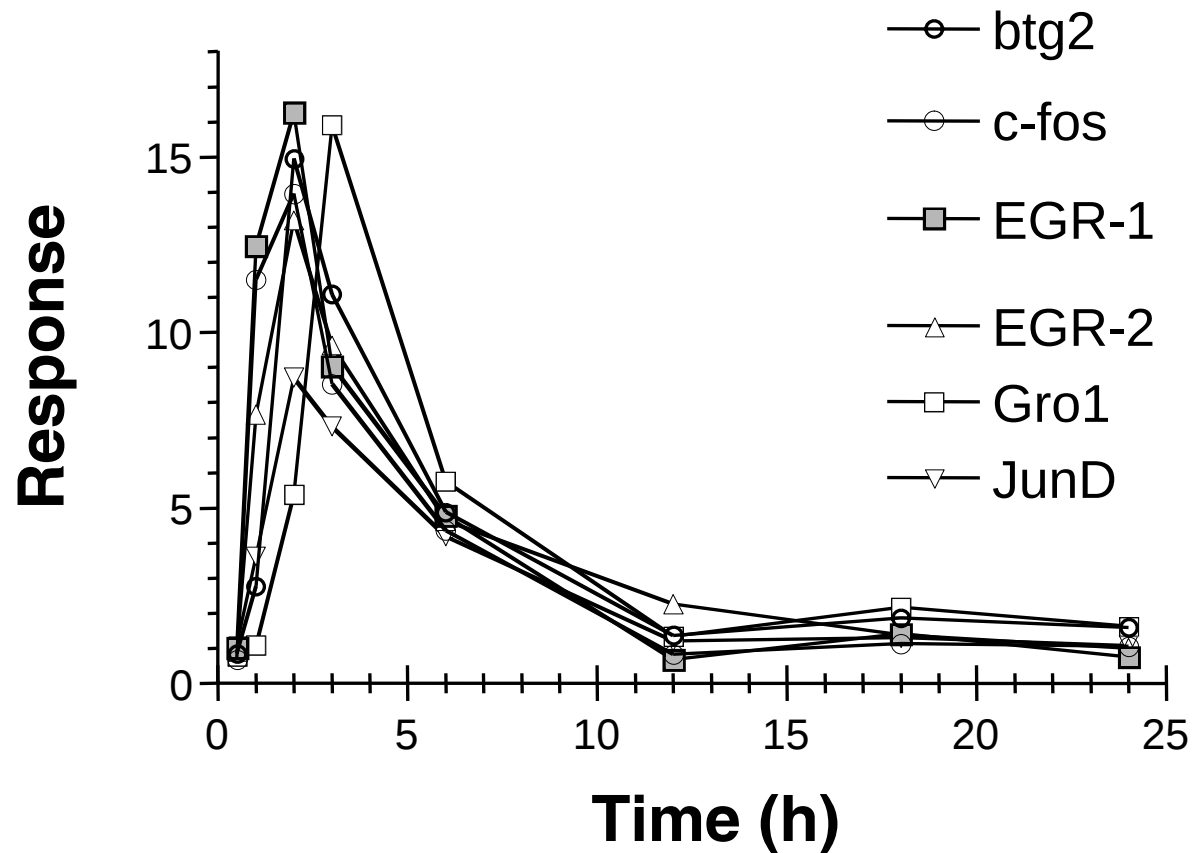
DBP causes a biphasic reduction in fetal testicular testosterone and gene expression



Time course heat map



DBP induces immediate early gene expression



Promoter Analysis

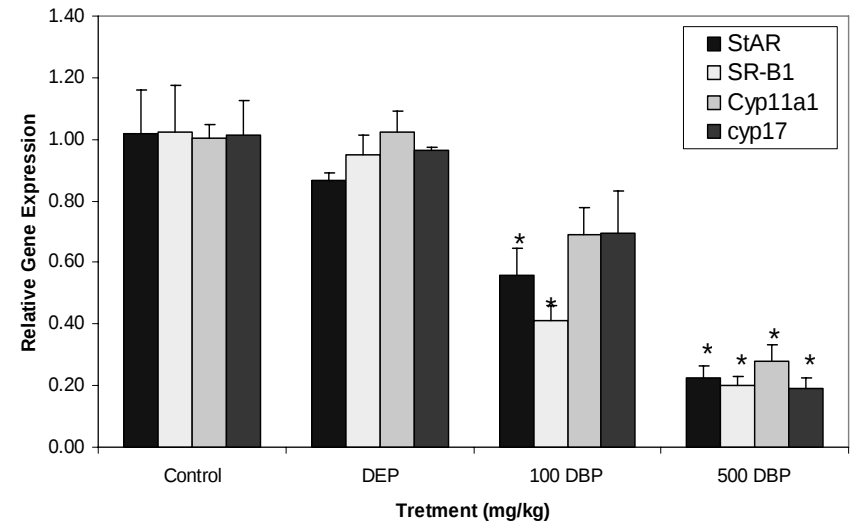
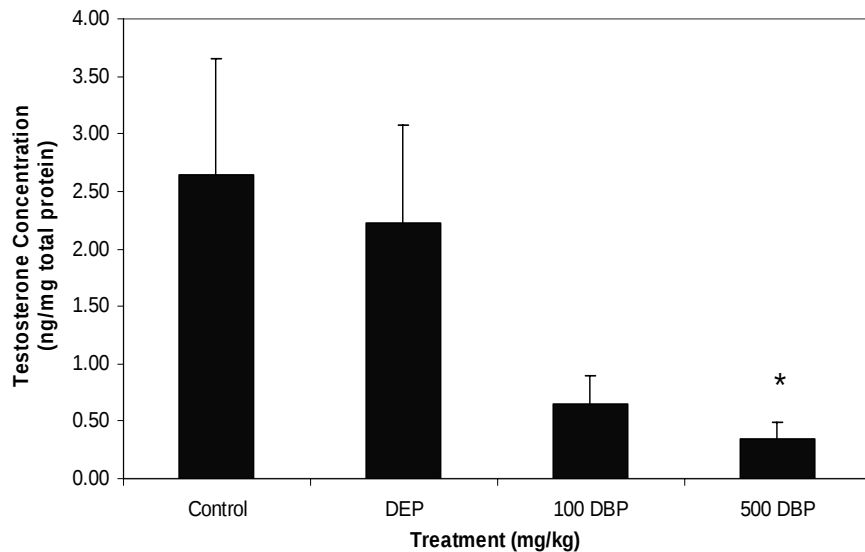
Dnase footprinting

Chromatin Immunoprecipitation

EMSA

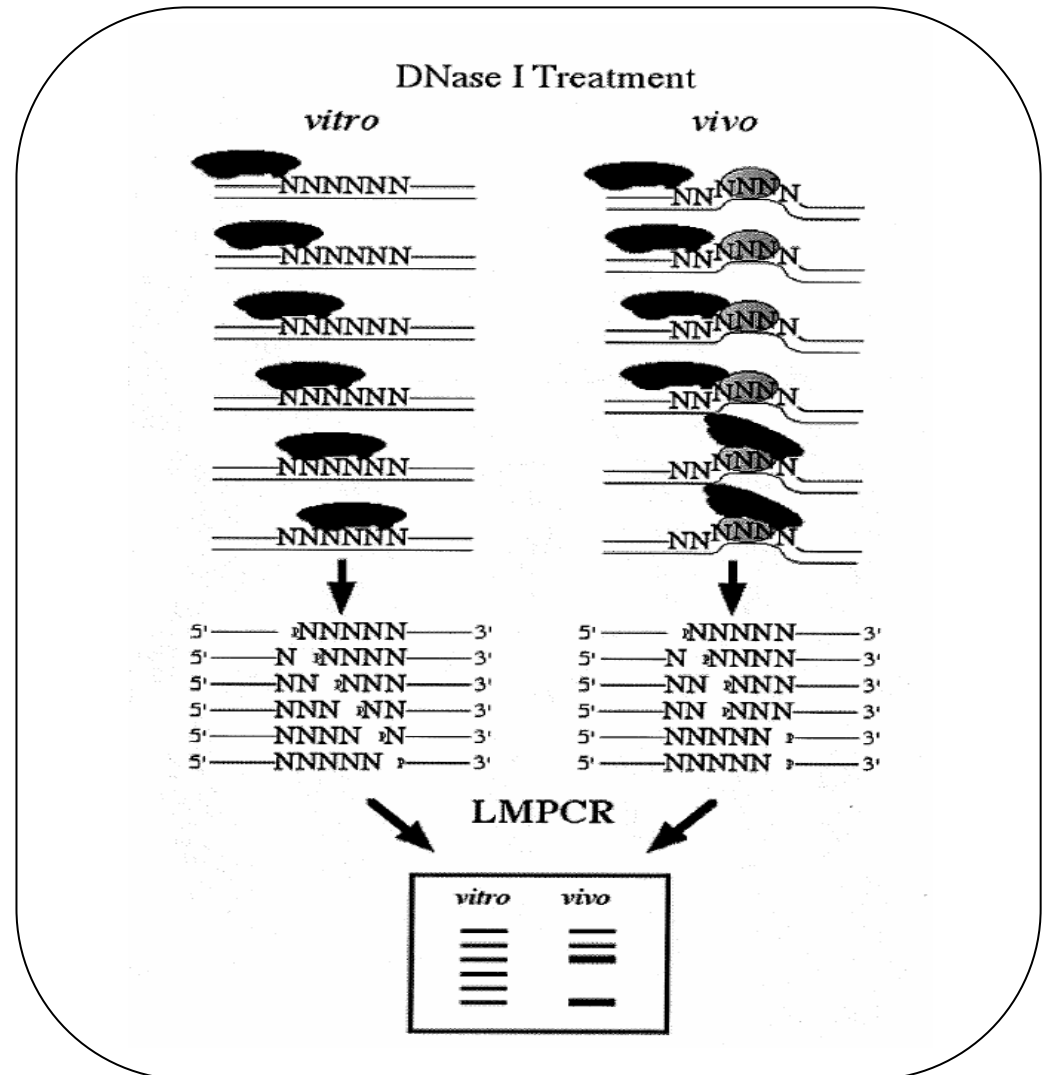
Western analysis

DBP inhibition of Testosterone biosynthesis pathway

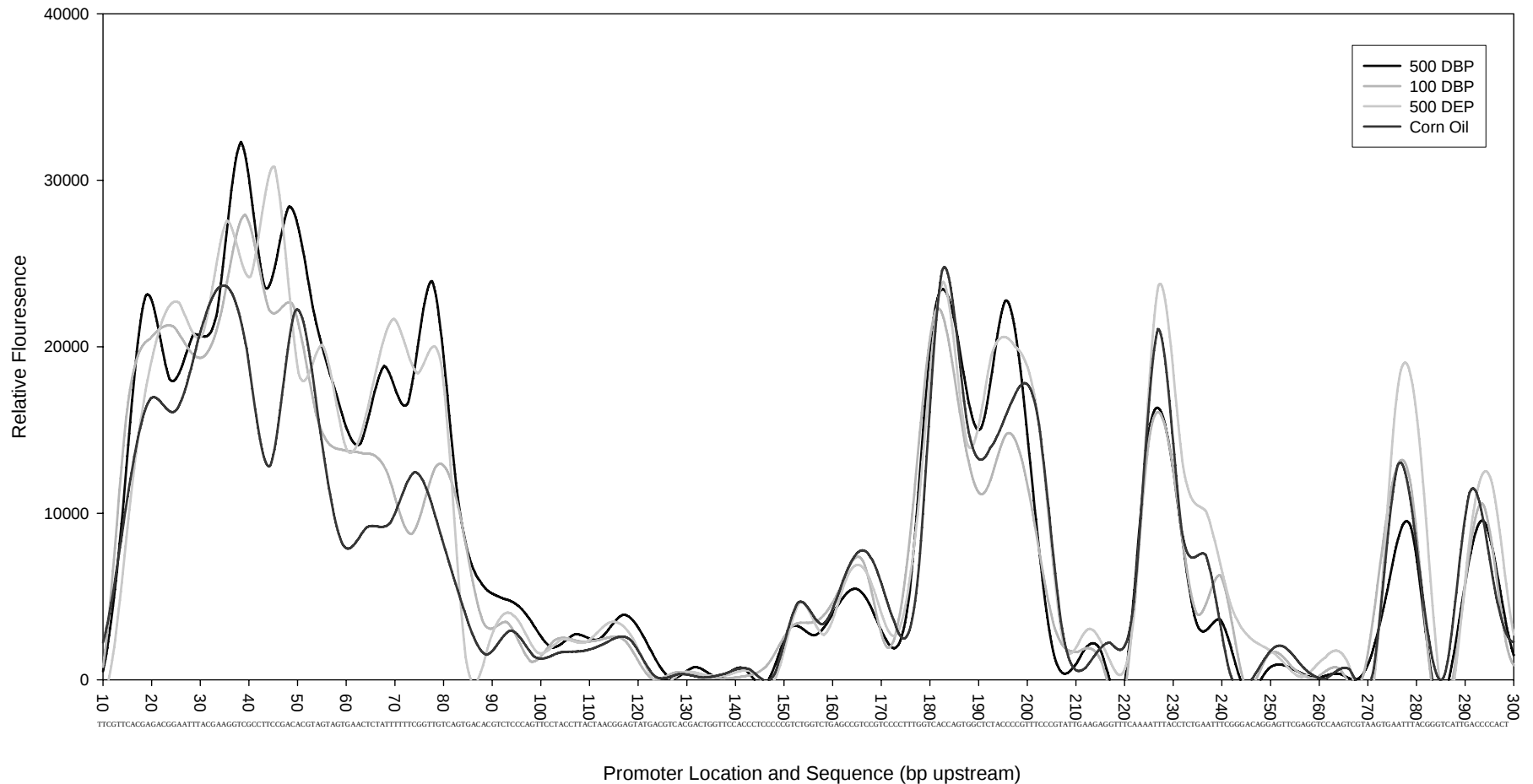


Automated DNase I Footprinting

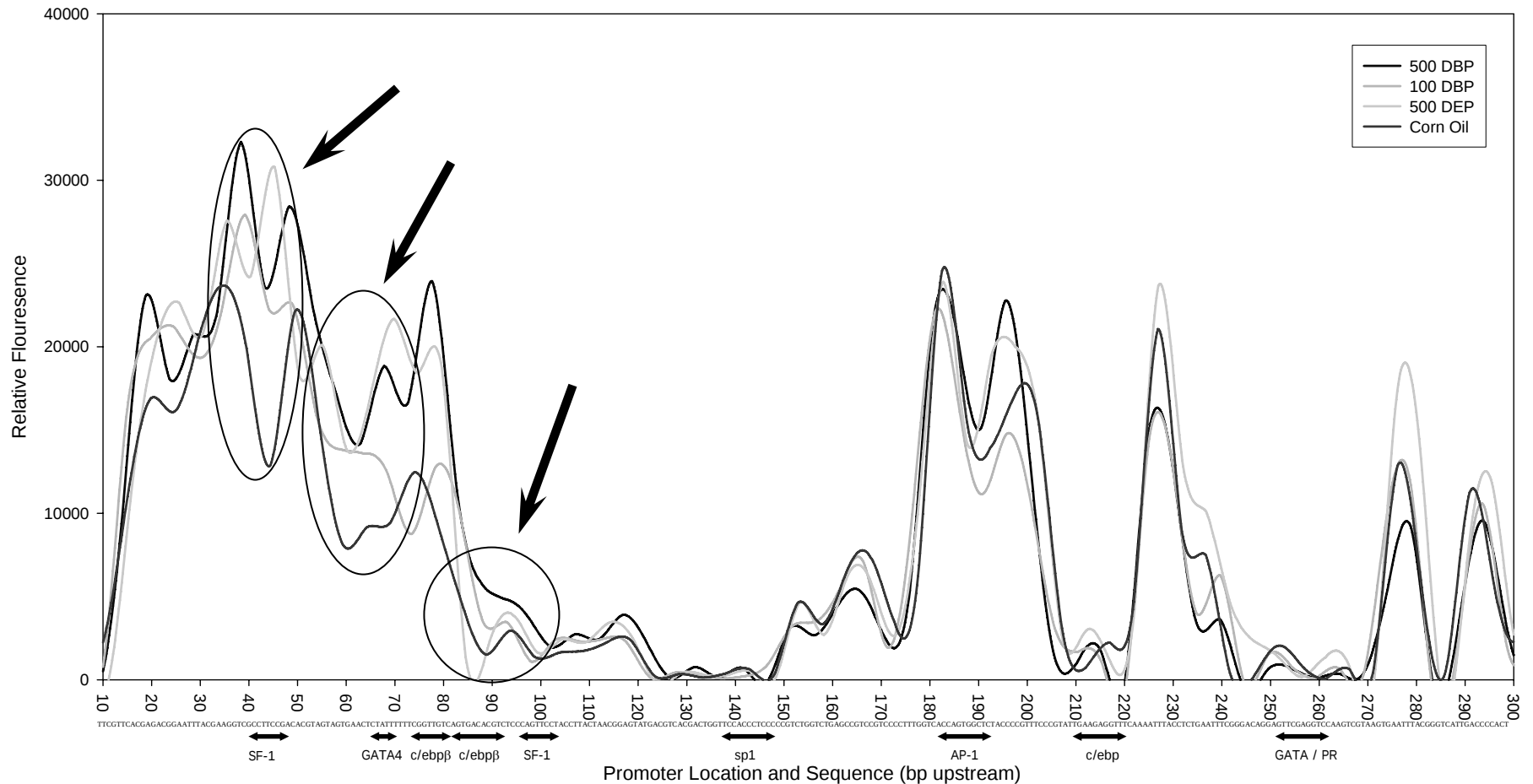
- DNA subjected to DNase digestion
- DNA regions bound by protein are protected from digestion
- DNA fragments are amplified with Ligation Mediated-PCR using promoter specific primers



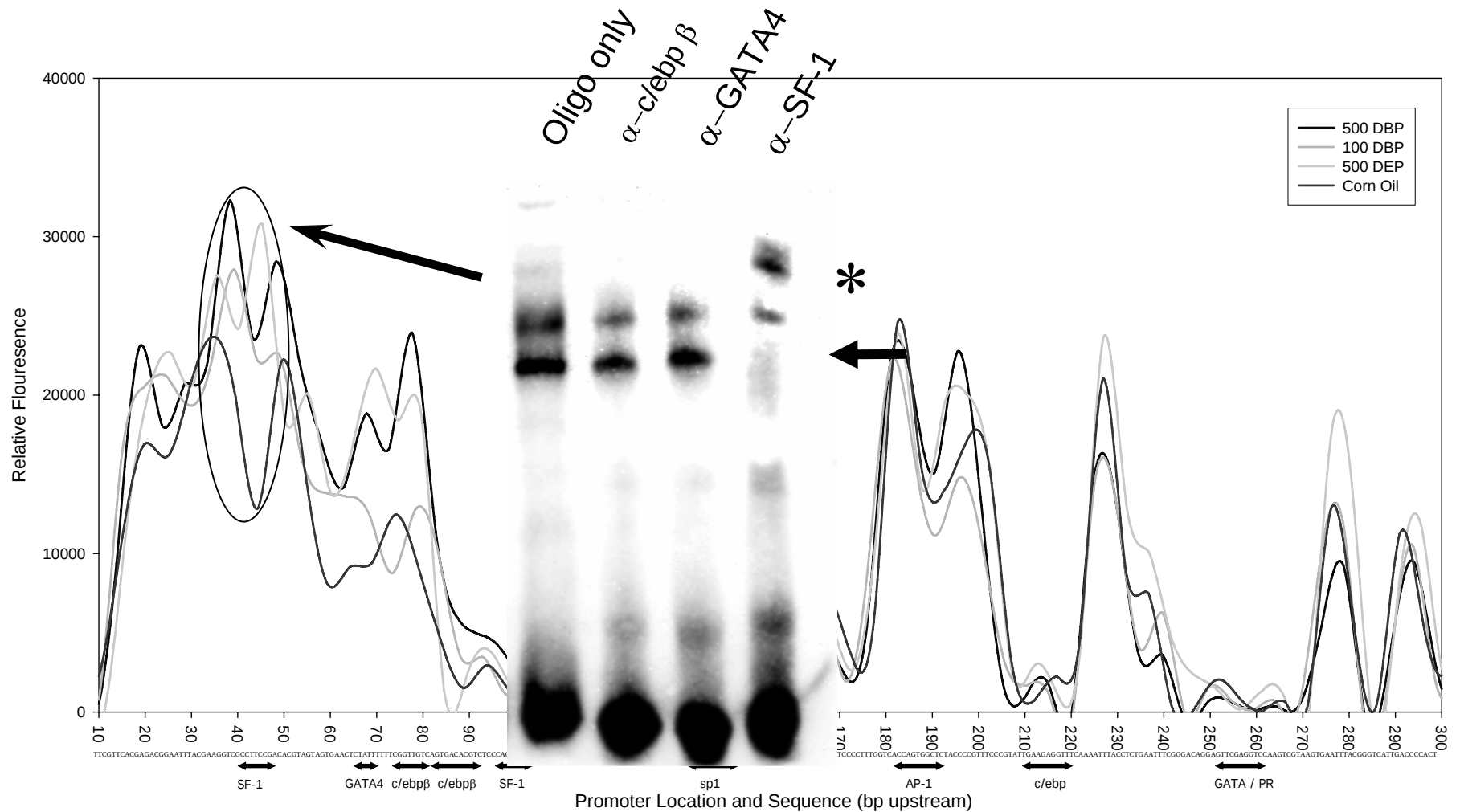
Changes in StAR promoter architecture using in vivo DNase footprinting



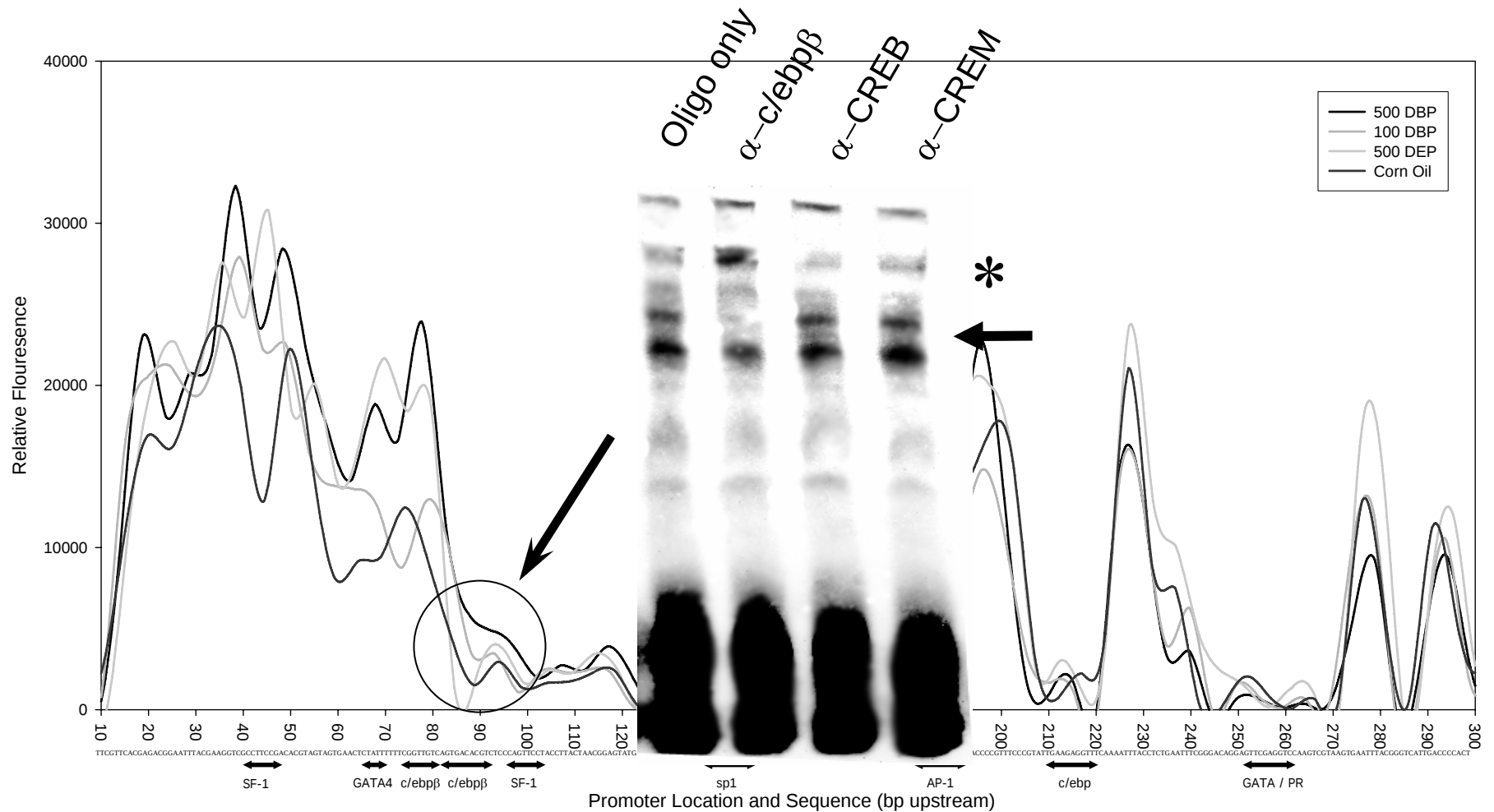
Changes in StAR promoter architecture using in vivo DNase footprinting



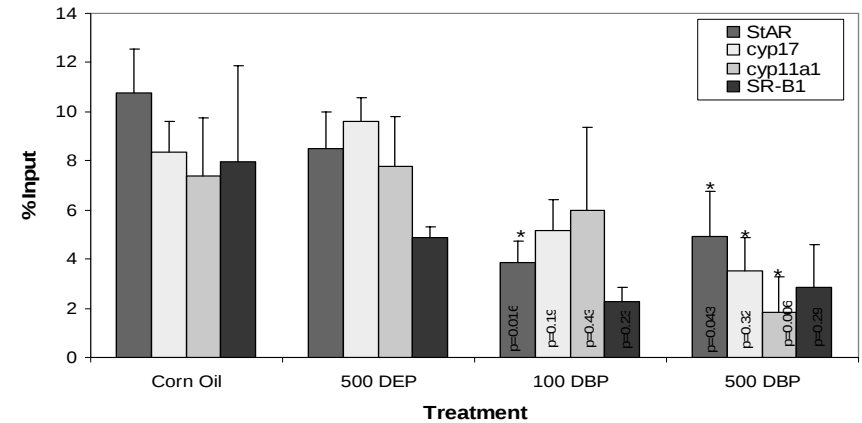
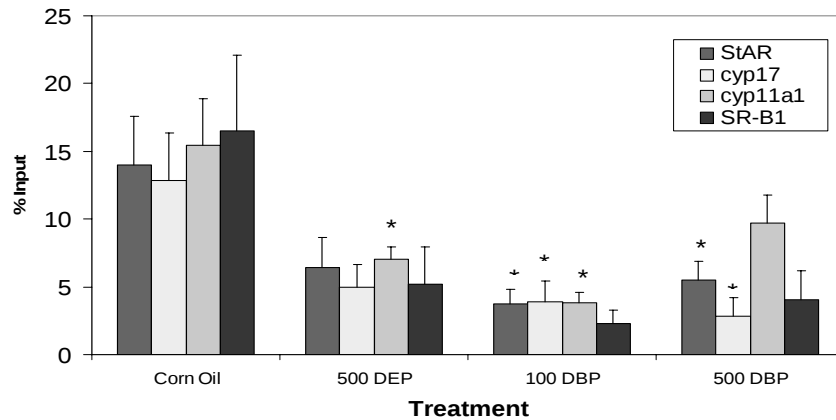
EMSA



EMSA

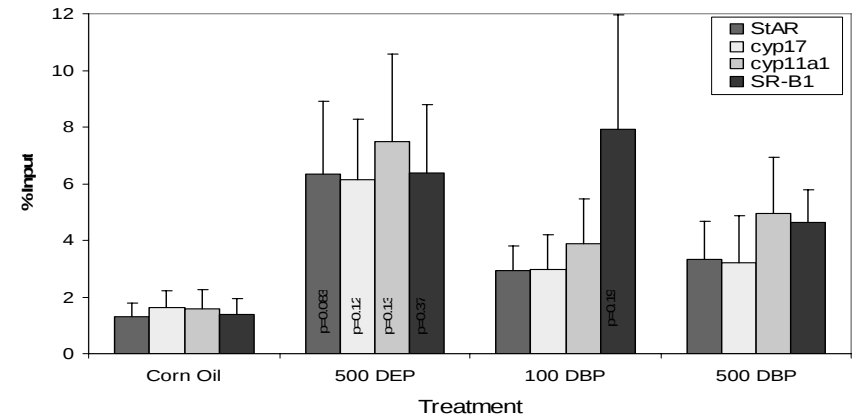
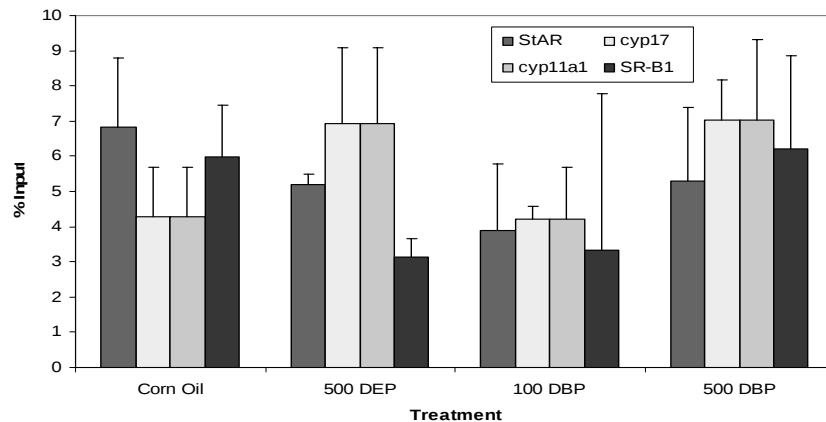


Chromatin Immunoprecipitation quantifies changes in specific TF binding



SF1

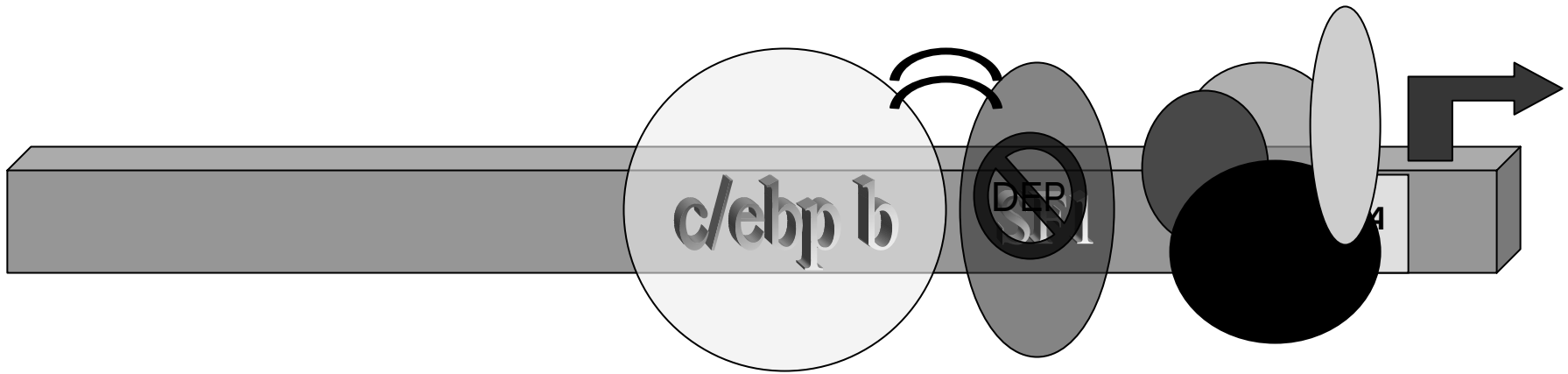
c/ebp β



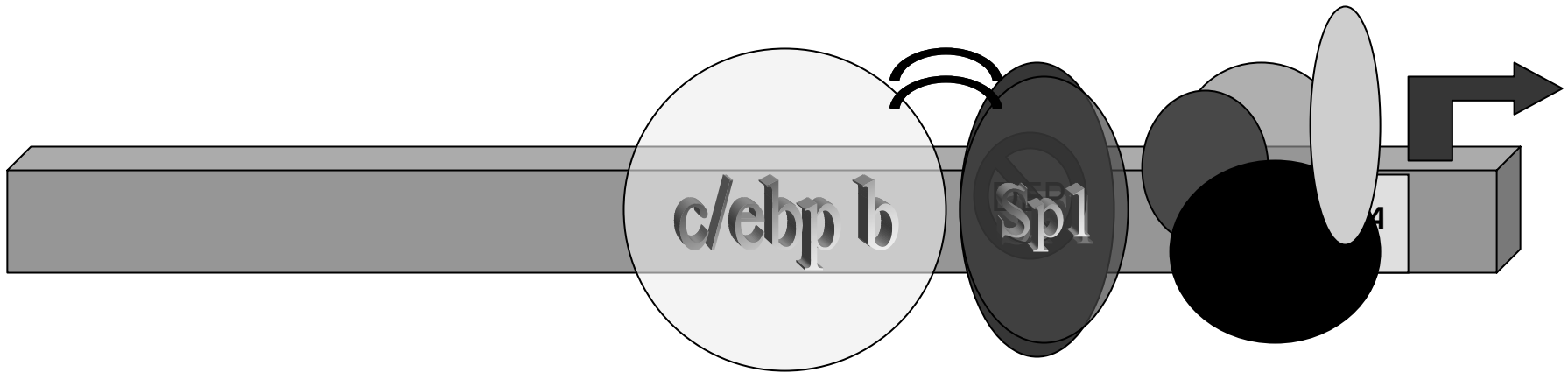
GATA4

sp1

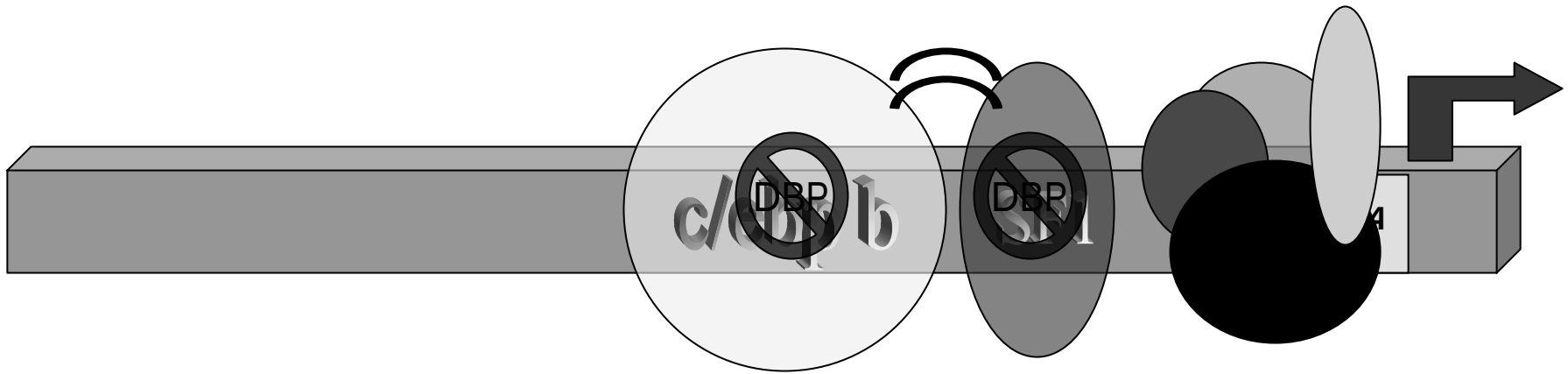
Proposed Mechanism



Proposed Mechanism



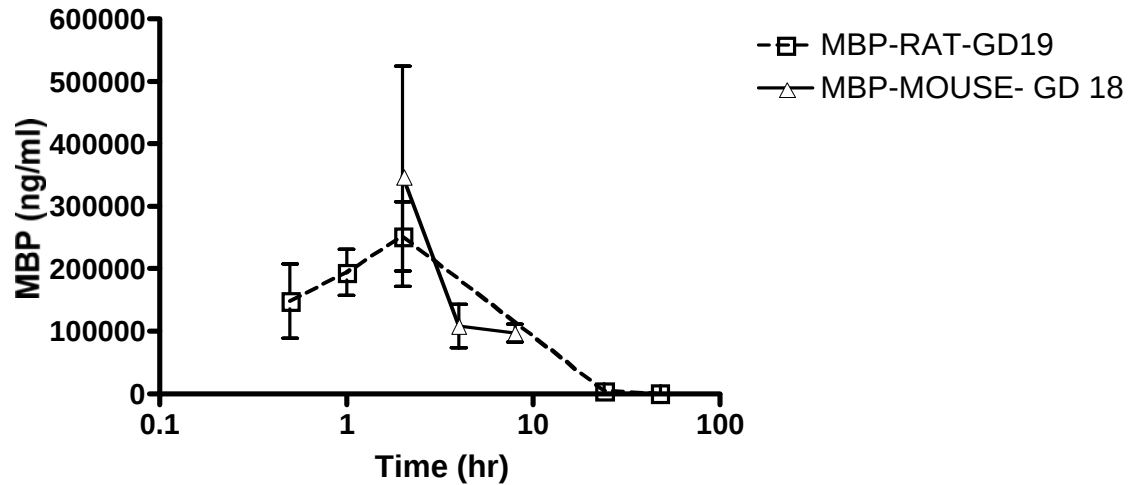
Proposed Mechanism



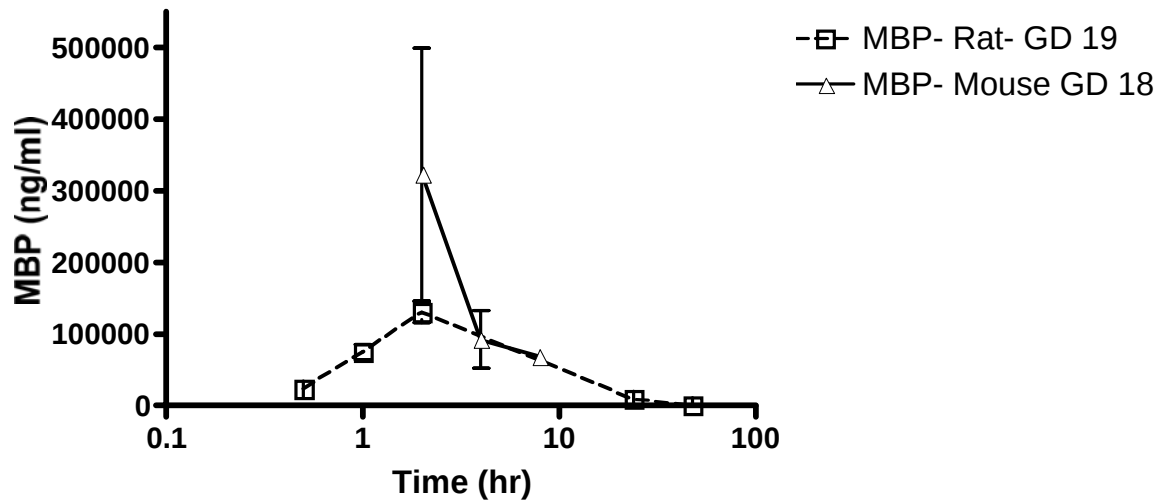
Effect of DBP on Mouse Fetal Testis

MBP Pharmacokinetics

Maternal Plasma- Single 500 mg/kg



Fetal Plasma- Single 500 mg/kg

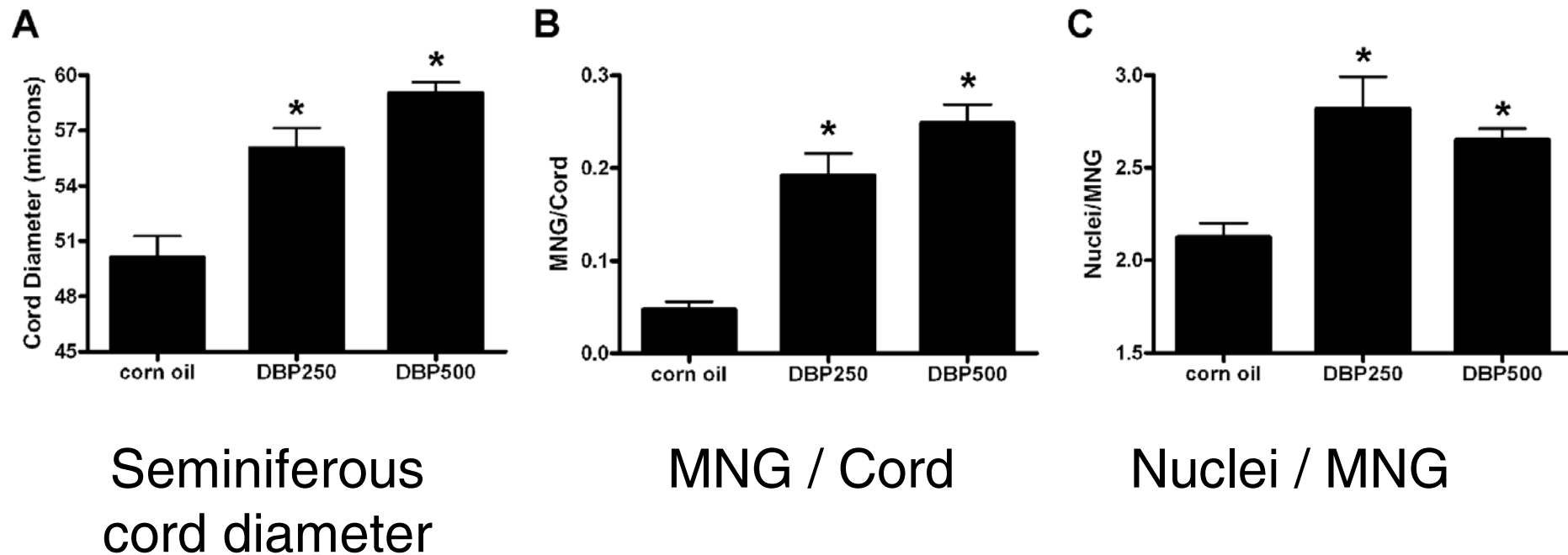


Gene Expression Following DBP Exposure

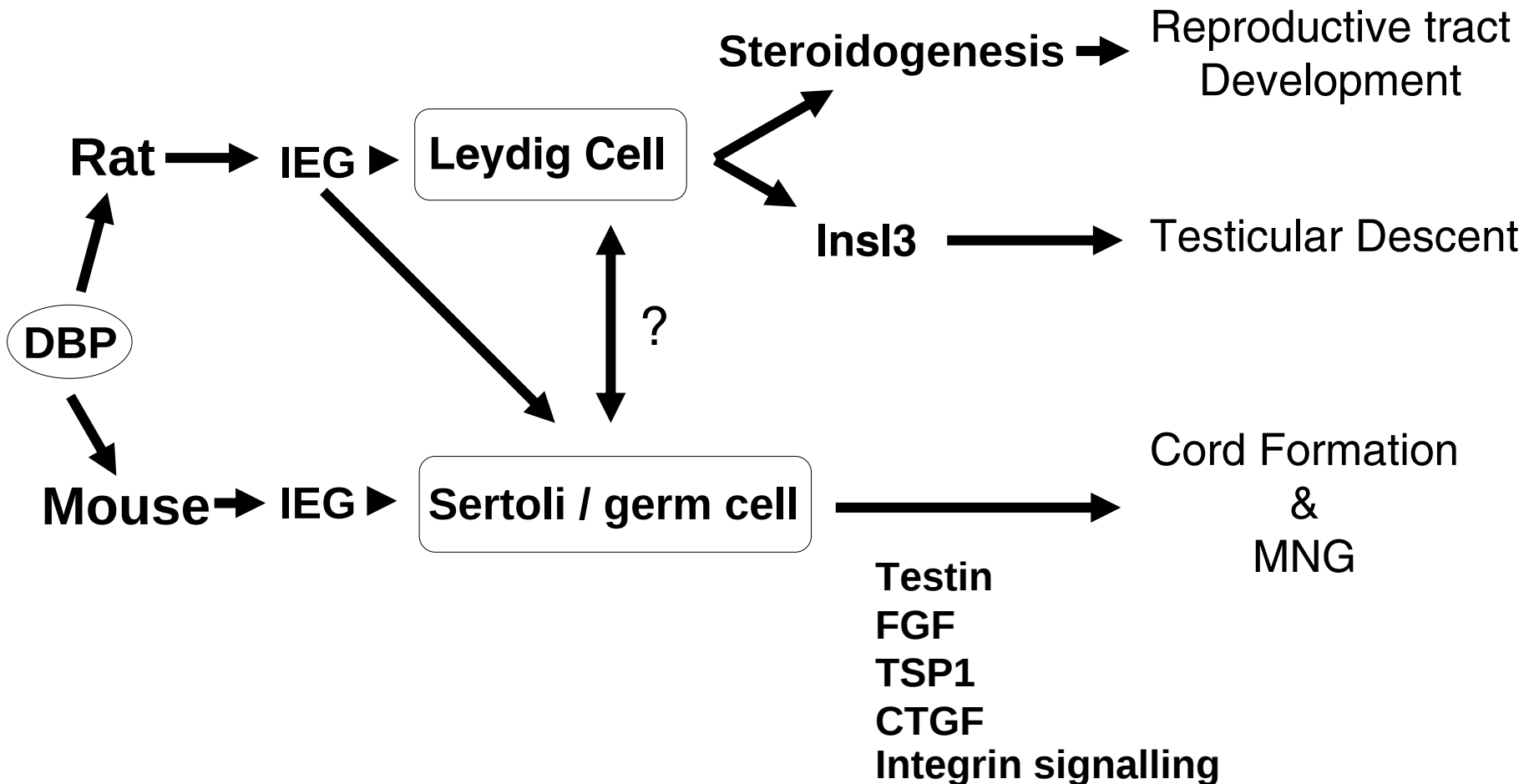
Lipid, Sterol, and Cholesterol homeostasis

Gene	Mouse	Rat	Gene Title
Dhcr7	↑	↓	7-dehydrocholesterol reductase
Aacs	↑	↓	acetoacetyl-CoA synthetase
Clps	↓	NS	colipase, pancreatic - lipid catabolism
Cyp51	↑	↓	cytochrome P450, family 51
Hsd17b7	↑	↓	hydroxysteroid (17-beta) dehydrogenase 7
Idi1	↑	↓	isopentenyl-diphosphate delta isomerase
Lss	↑	↓	lanosterol synthase
Stard4	↑	↓	StAR-related lipid transfer domain containing 4
Scd1	↑	↓	stearoyl-Coenzyme A desaturase 1
Scd3	↓	---	stearoyl-coenzyme A desaturase 3
Sc5d	↑	↓	sterol-C5-desaturase

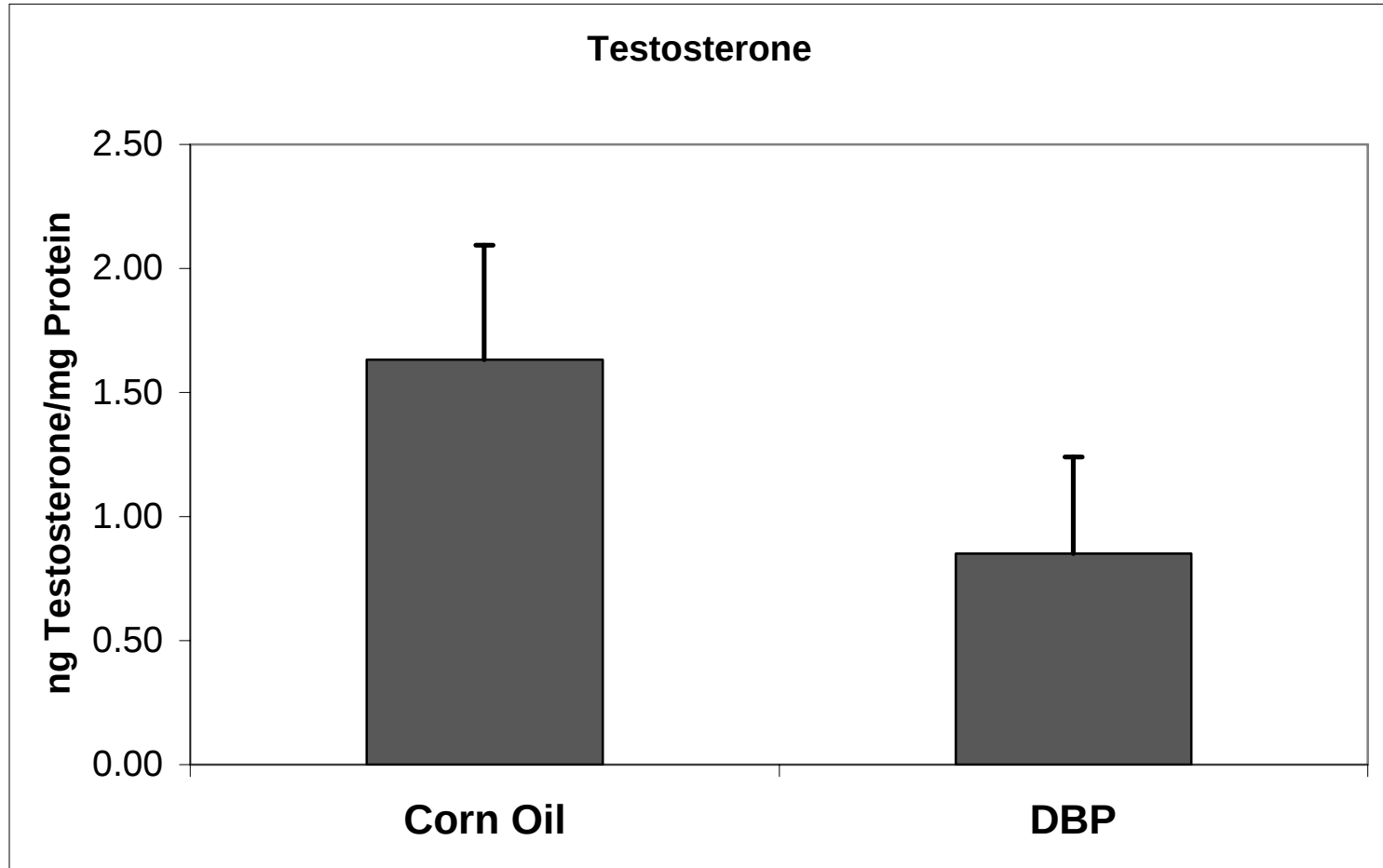
Multinucleated gonocytes in mouse fetal testis following DBP exposure



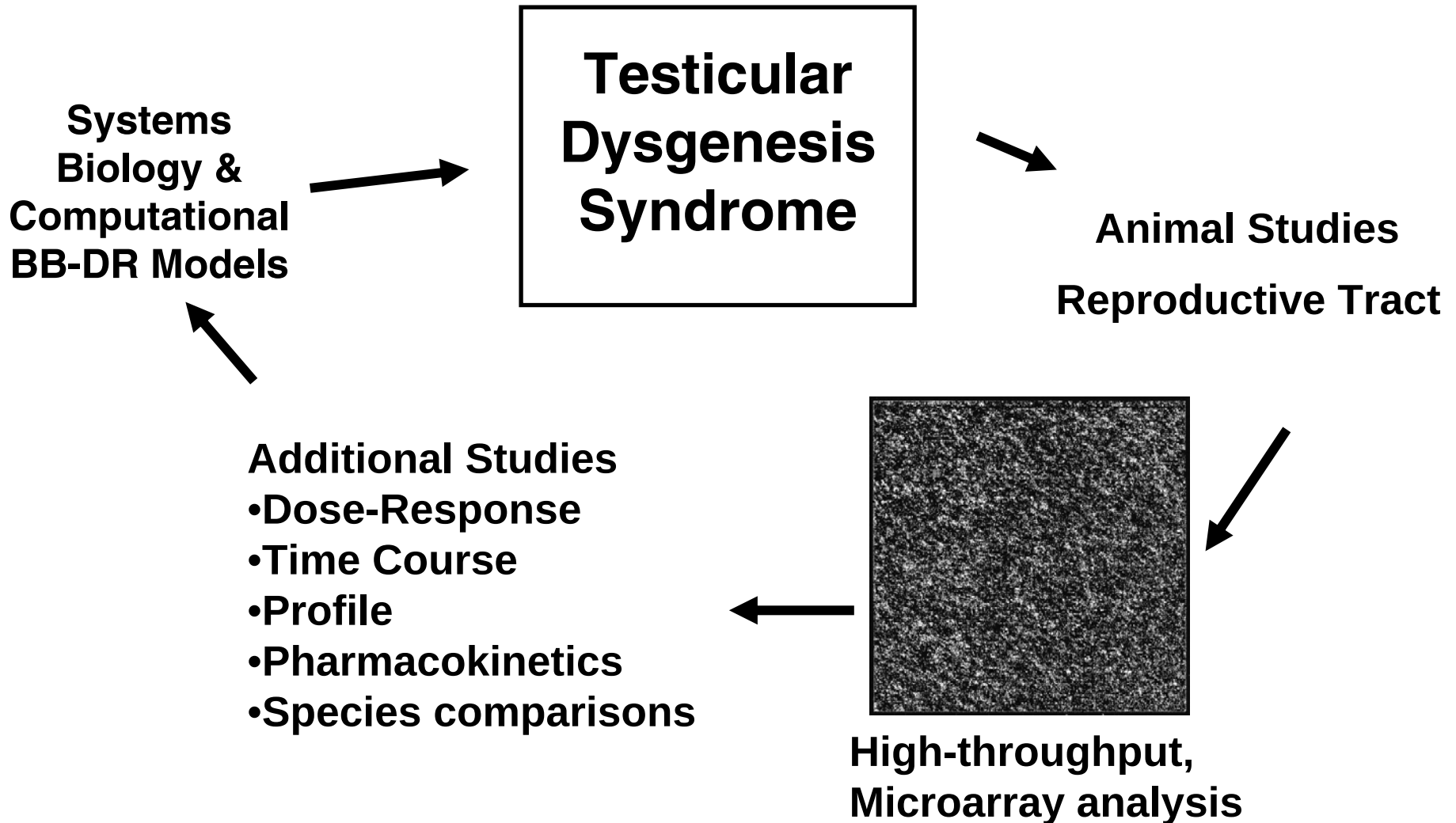
DBP targets common and divergent pathways in the Mouse and Rat



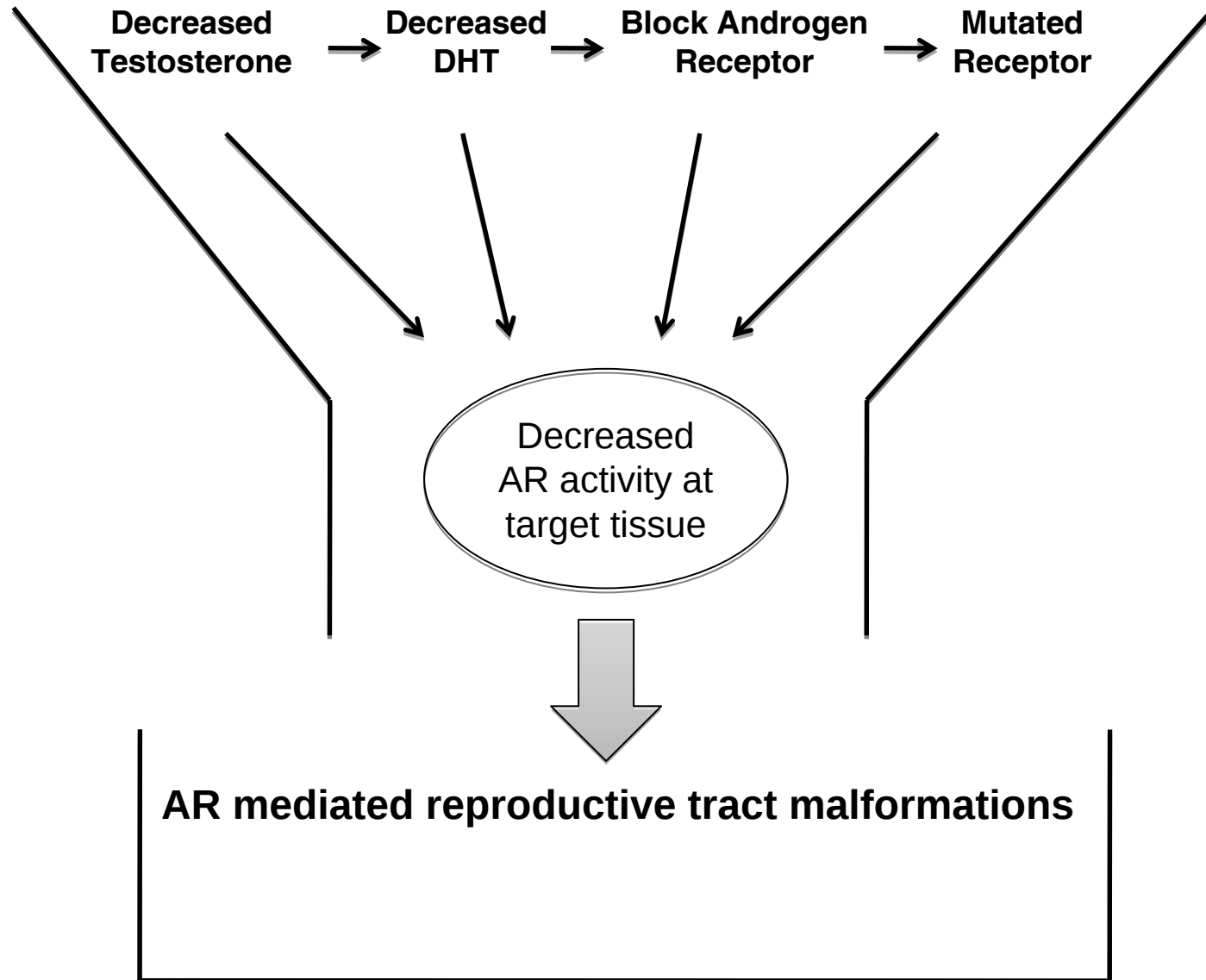
Testis transplant model



Application of Genomics to Reproductive Health Risks



Fetal Androgen Insufficiency



Acknowledgments

- Kamin Johnson
- Adam Kuhl
- Elena Kleyменова - GSK
- Jack Liu - GSK
- Susan Ross
- Kim Lehmann
- Janan Hensley
- Delong Liu
- Kim Boekelheide - Brown
- Susan Borghoff - ILS
- Paul Foster - NIEHS
- Chris Thompson - Sanofi-Aventis
- Valerie Shultz - Sanofi-Aventis
- Stan Young - NISS
- Russ Wolfinger - SAS
- ACC-LRI
- NIH R01 ES011803-01A1
- NIH R21 ES011754-01
- EPA STAR R830766