University of BRISTOL

Functional analysis of the pig CYP2E1 promoter and identification of the transcription factors required for CYP2E1 expression

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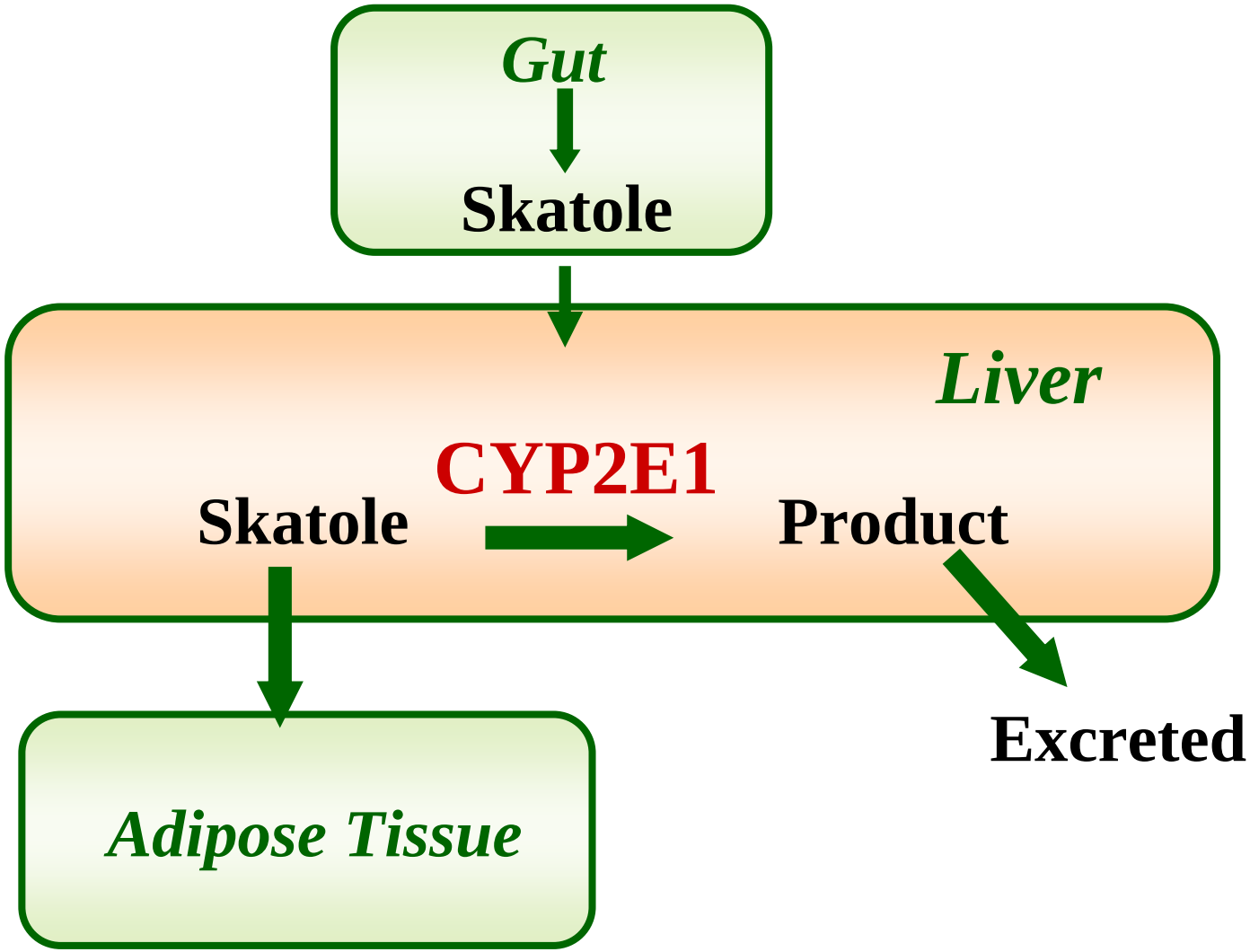
Boar Taint

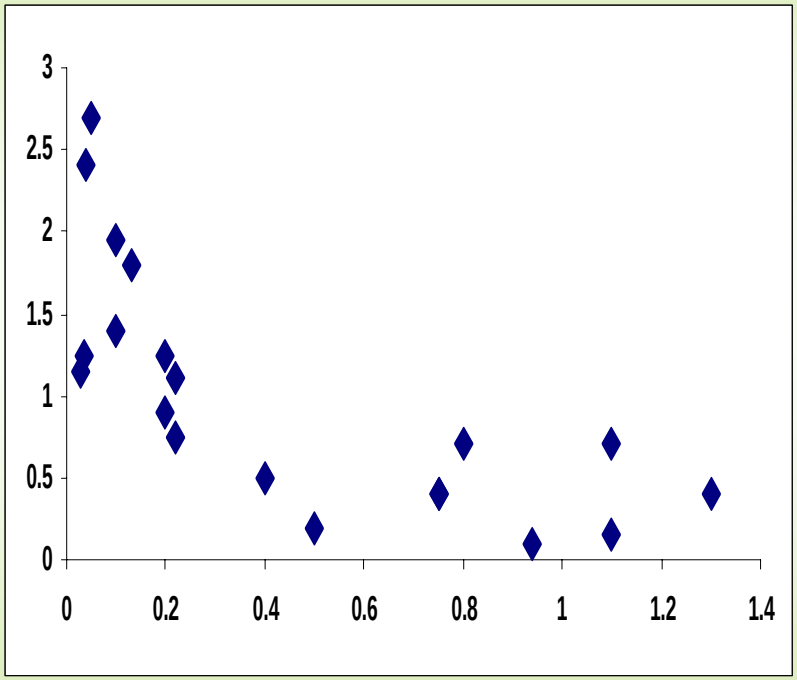
- **Is an offensive odour in the meat of 5-10% of uncastrated male pigs**
- **Is due to excessive accumulation of skatole and androstenone in adipose tissue**

Control of Skatole Accumulation

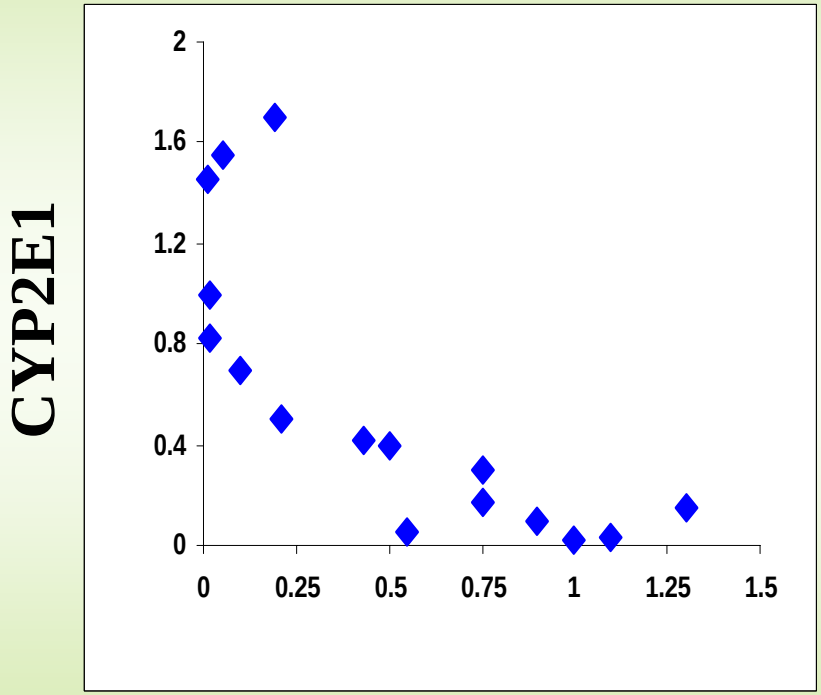


CYP2E1 Expression

Protein



mRNA



Objectives

- **Functional analyses of pig CYP2E1 promoter**
- **Identification of transcription factors required for pig CYP2E1 expression**
- **Investigation of effect of androstenone on pig CYP2E1 promoter activity**

5’-Flanking Region of the Pig CYP2E1

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-850                                                    CCCCA GAAACAACCT
-800 AACAGAAAGG TGAATGTAAA TAGTTTGGAG CTCTTATTTT AAATGAGAAT
-750 GTCCACACAC ATTAGCACAG ATTTAAACAA ACACAGTTAA AATACTAATT
-700 TTTTTTTTAG AATTTGACAA AATGACTCTA AACAGTAATT ATCCCTTAAT
-650 TTTCTACAGT AAAAATATAC CCTTTTTTGG TAGTAATCAG AGATGAACTT
-600 TTTTGAAATT TGTCAACTCT TTTCCTTTCT CTTTTCCTCC CCCACTGAAT
-550 TTGCCAGTTG ATTTCCCAAA GTGGAGTGAA ATTCAGATAC TGAATTTCCC
-500 TTCTCTGGCC CATGAGGCTG GCTGCTGATG ACTCAGTACC ACTGGGGTTG
-450 CTCAGACAGA CCTGCTCGGA GGCTGAGAGT TGCACCAGGA GATGGAGCAA
-400 GACGGTCGGC ACATCATTGA TGTCGCCTTA CATAAATCCT ACCCCAAACA
-350 AACCCATGTA AATATGACCT TCTTGTCCAA CCAAGGTAAA GGAGAGGACA
-300 GTTCCCCACC CTATGTTCTG ACCTCTGGGT TGGTGGAGCT AAACTGGATG
-250 ACATGTTTTA CTGACATTGG TGCAGGTGTC AGCAGCCAGT GTTGGCAGAG
-200 CCCAGGCTAG AGGAAGTGAG TGTCTGGATG GAGTTCTAAG GGGTAACCGC
                HNF-4                                   HNF-1
-150 CTCAGGGATC AGCCTTTGAA CTGATAGCCA ACAGCAGCTA ATAATAAACC
-100 TATATCTTGG GCTGGAGGAA AAGGAAGGTG GCATTGGTTG GCTGGTCACC
                          TATA box
-50  CTCCTTCTCA AGGATGCATT ATAAAAGGCT GCCTCTCCAC AGGAGCATCT
-0   CCACACATTG AAAGATCCCC TGAAGGAGCC ATG
```

5’-Flanking Region of the Pig CYP2E1

CYP2E1 Promoter Analyses

CYP2E1 Promoter fragments (PCR)

⇩

Ligation into the promoter site of pGL3-basic vector (containing luciferase cDNA)

⇩

CYP2E1 promoter-controlled luciferase reporter constructs

CYP2E1 Promoter Analyses

CYP 2E1 promoter constructs

⇩

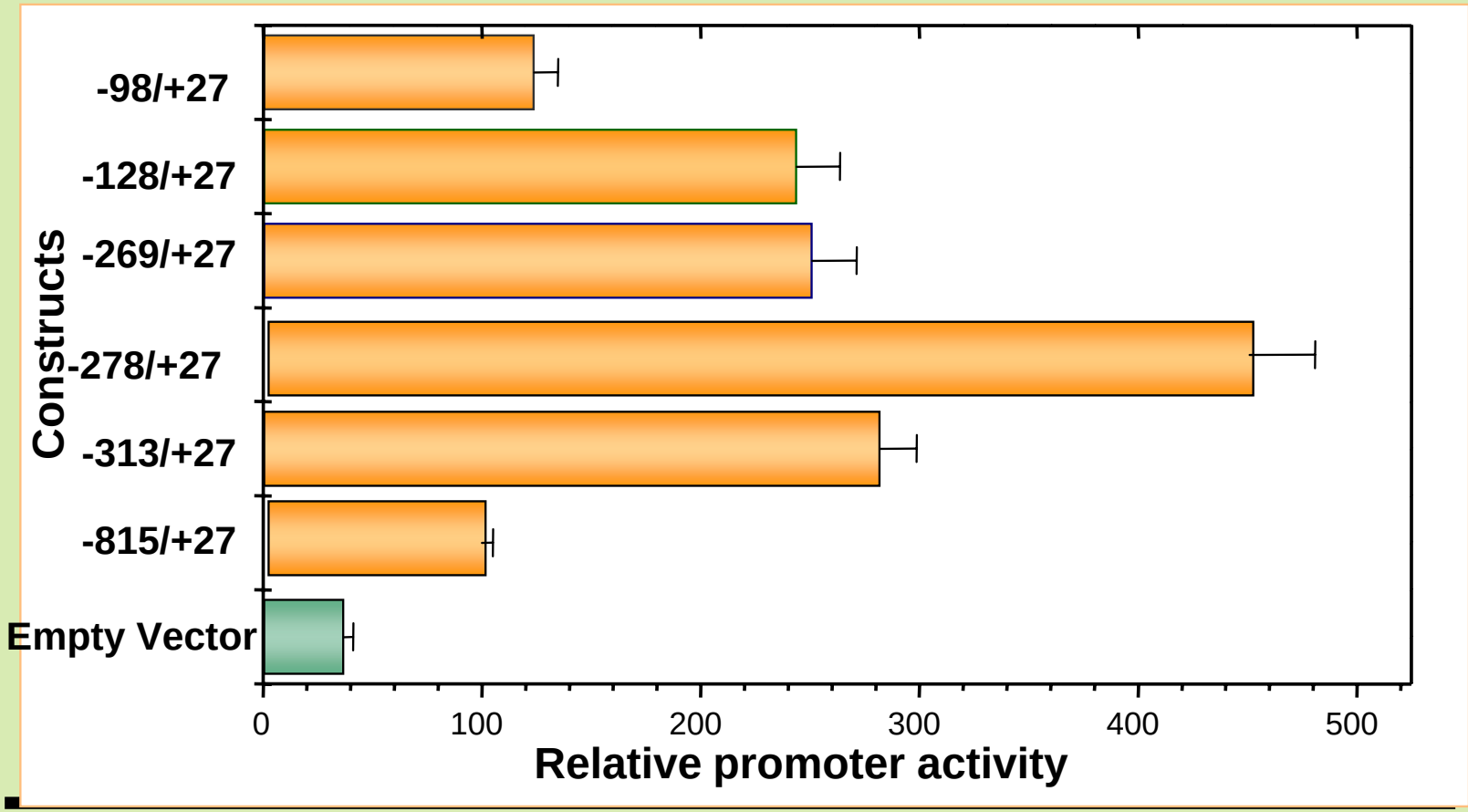
Hepatoma cells

(HepG2)

⇩

Promoter activity

CYP2E1 Promoter Analysis



Regulatory Elements in CYP2E1 Promoter

Region between -128 and -98

HNF-1 binding sequence

Region between -300 and -260

COUP-TF1 binding sequence

Gel Mobility Shift Assay

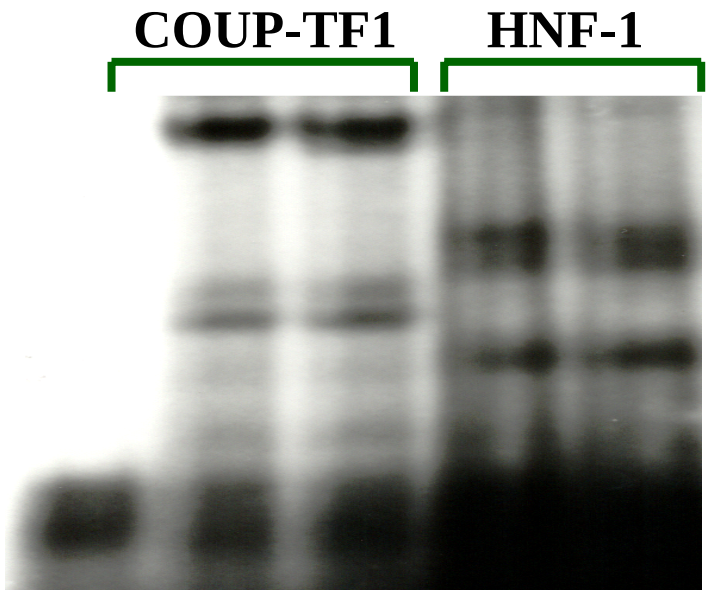
Synthetic Oligonucleotides

+

Liver Nuclear Extract

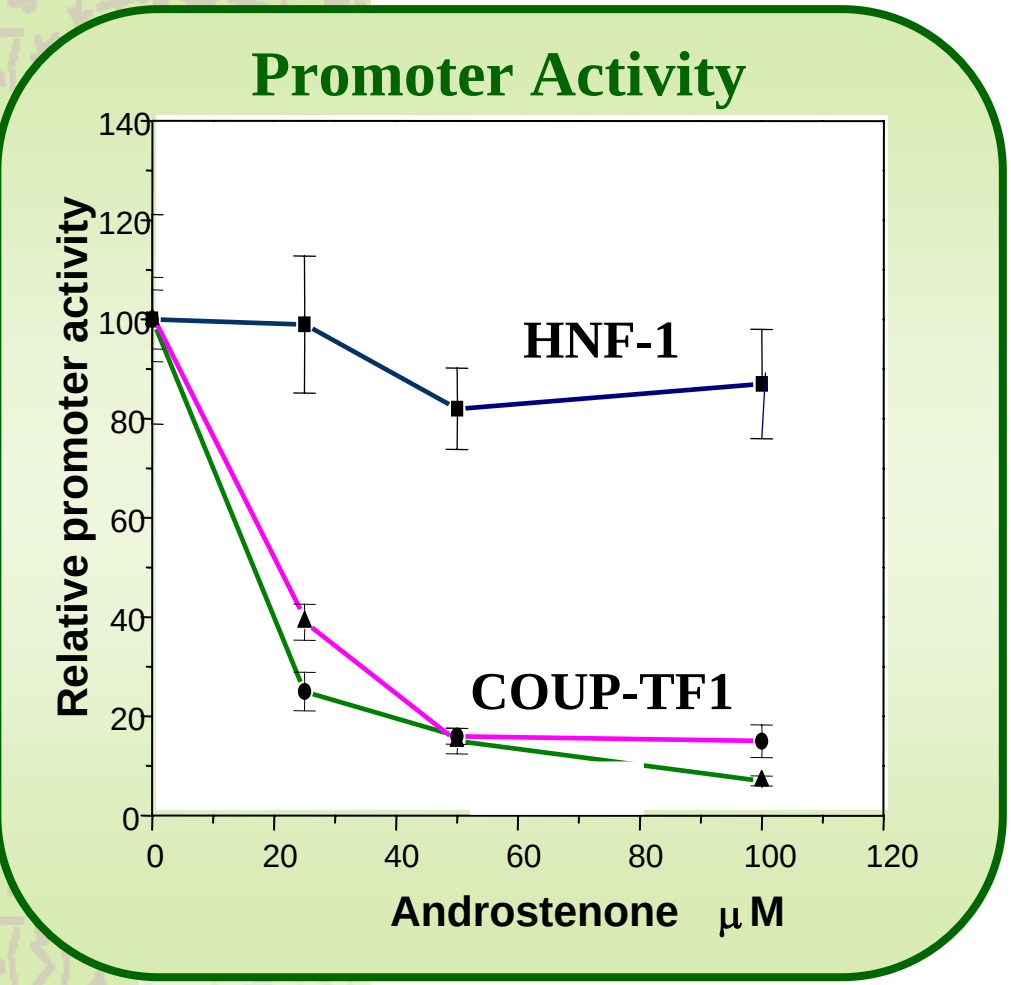
Polyacrylamide Gel

Gel Mobility Shift Assay



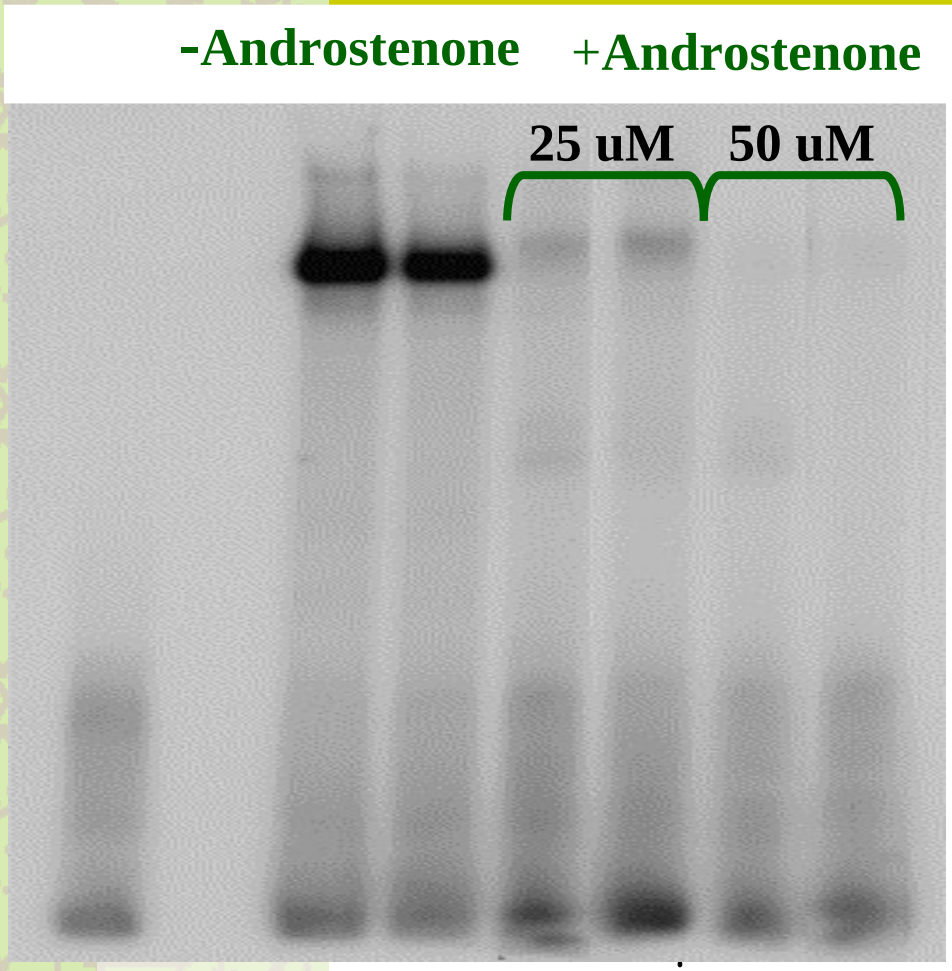
Nuclear extract protein binds to oligonucleotides corresponding to the binding sites for HNF-1 and COUP-TF1

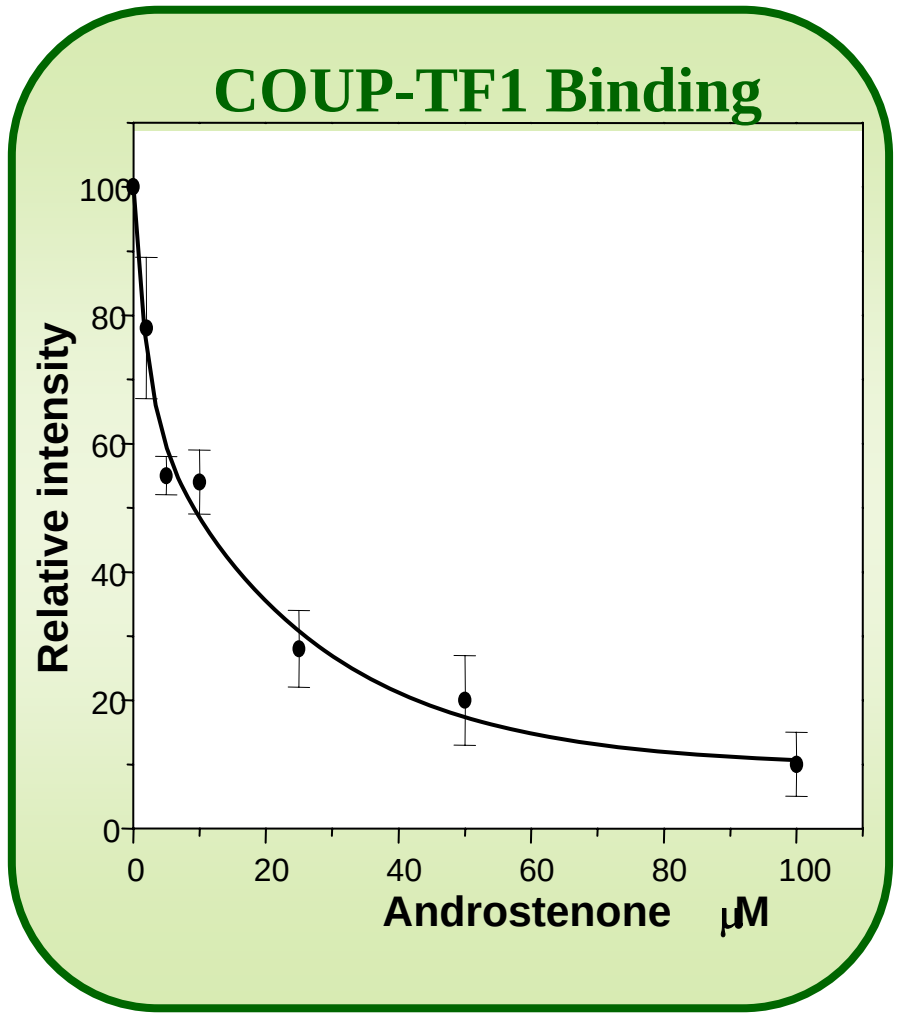
Effect of Androstenone on CYP2E1 Promoter Activity



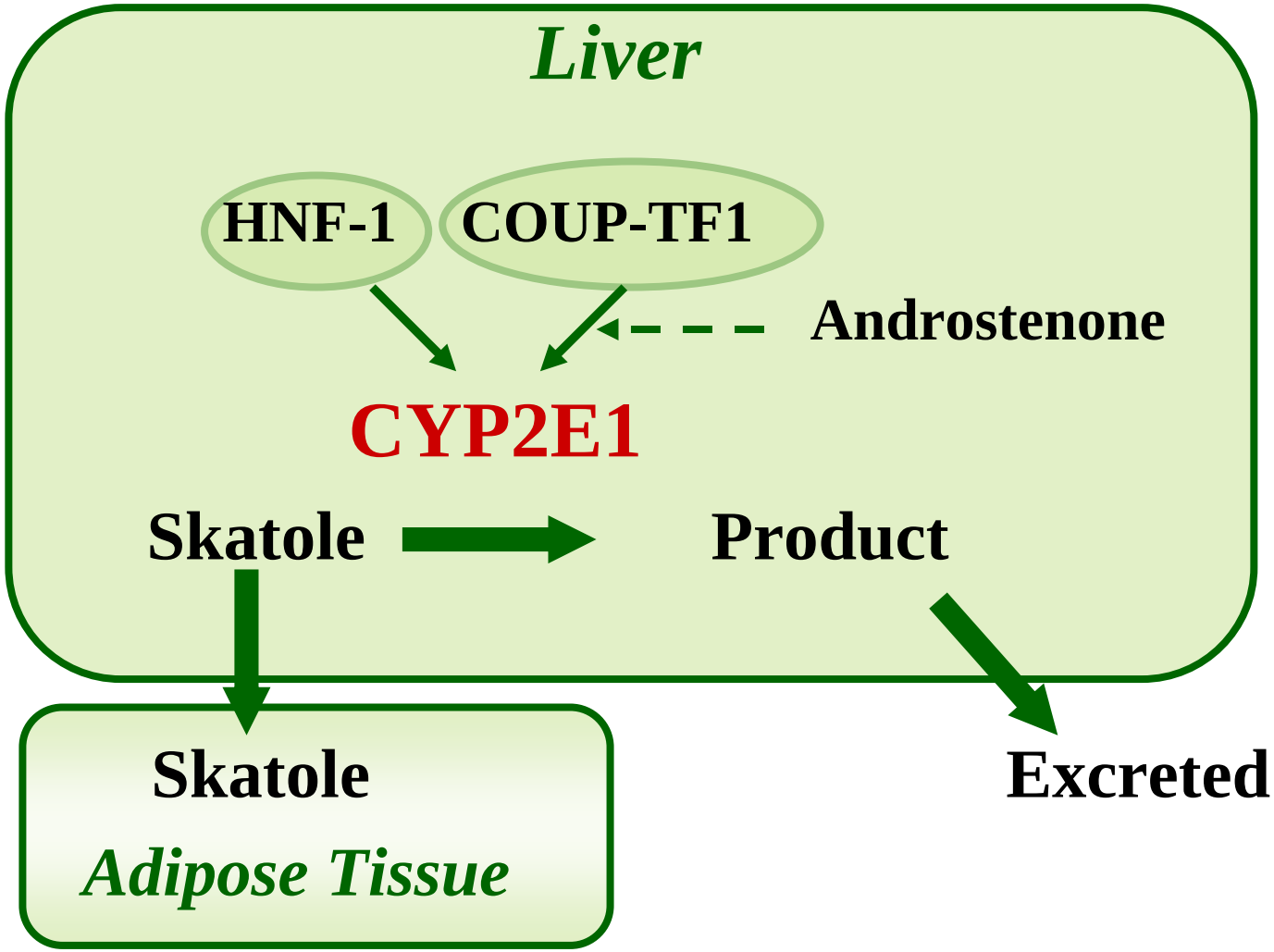
Androstenone inhibits the promoter activity of the constructs with a binding site for COUP-TF1

Effect of Androstenone on COUP-TF1 Binding





Control of Skatole Accumulation



Acknowledgments

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