REGULATION OF ESTROGEN PRODUCTION IN UTERINE FIBROIDS

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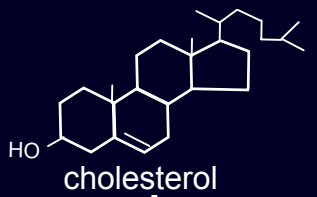


cholesterol

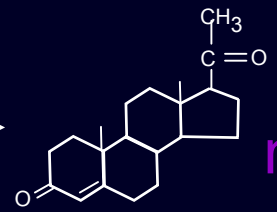
StAR

P450scc

MITOCHONDRION

pregnenolone

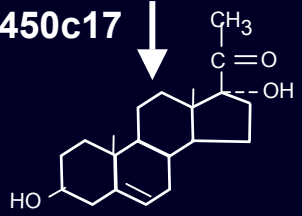
3β–HSD-II



PROGESTERONE

nmol/L

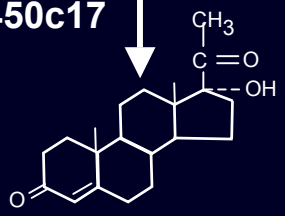
P450c17



17-hydroxypregnenolone

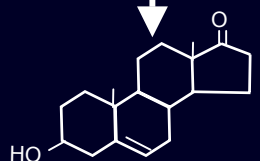
3β–HSD-II

P450c17



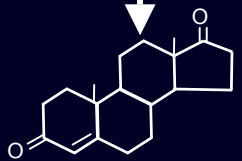
17-hydroxyprogesterone

P450c17



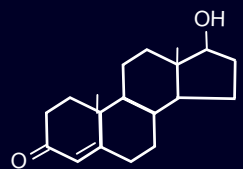
dehydroepiandrosterone

3β–HSD-II



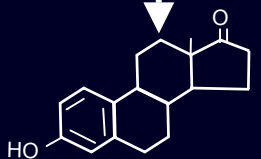
androstenedione

17β–HSD-1



testosterone

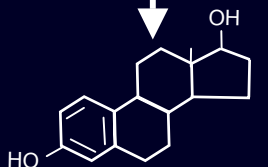
P450arom



estrone

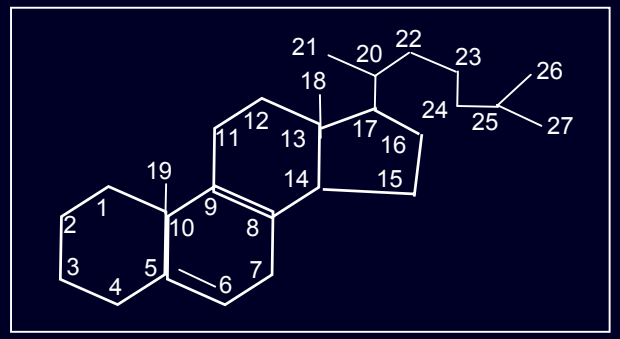
17β–HSD-1

P450arom



ESTRADIOL

pmol/L



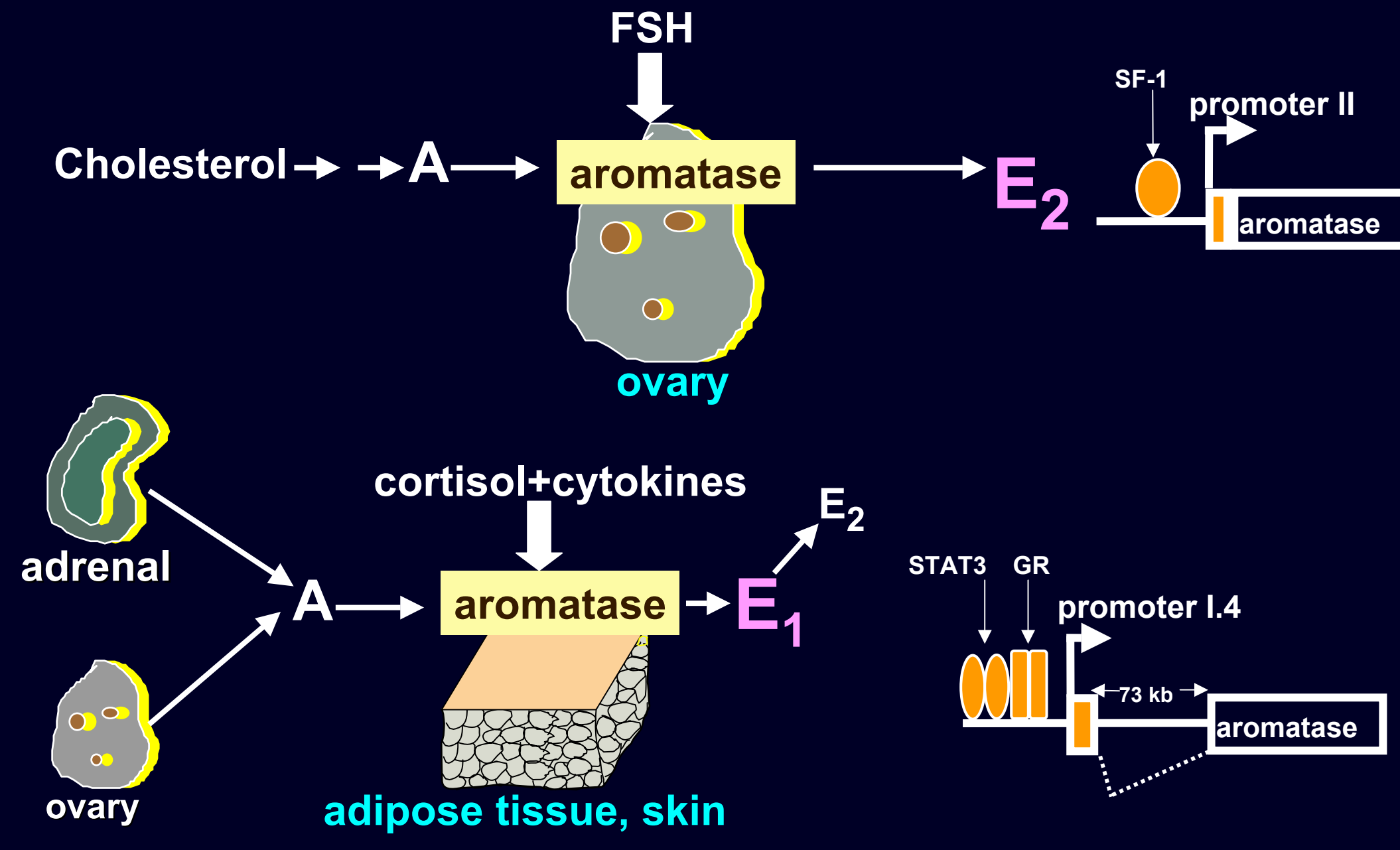
Recent Breast Cancer Trials: Tamoxifen vs Aromatase Inhibitors

		No of Participants	Response to Tx		Time to Prog	
			AromIn	TAM	AromIn	TAM
1st line therapy in advanced disease	2001 Letrozole vs TAM	907	49%*	38%	9.5 mo*	6 mo
	2000 Anastrozole vs TAM	353	59%*	46%	11.1 mo*	5.6 mo
	2001 Anastrozole vs TAM	668	56%	55%	8.2 mo	8.3 mo
	2000 Anastrozole vs TAM	121	34%*	27%	10.6 mo*	5.3 mo
	2000 Exemestane vs TAM	107	52%	41%	8.9 mo	5.2 mo
Neoadj tx	2001 Letrozole vs TAM	250	60%*	41%		

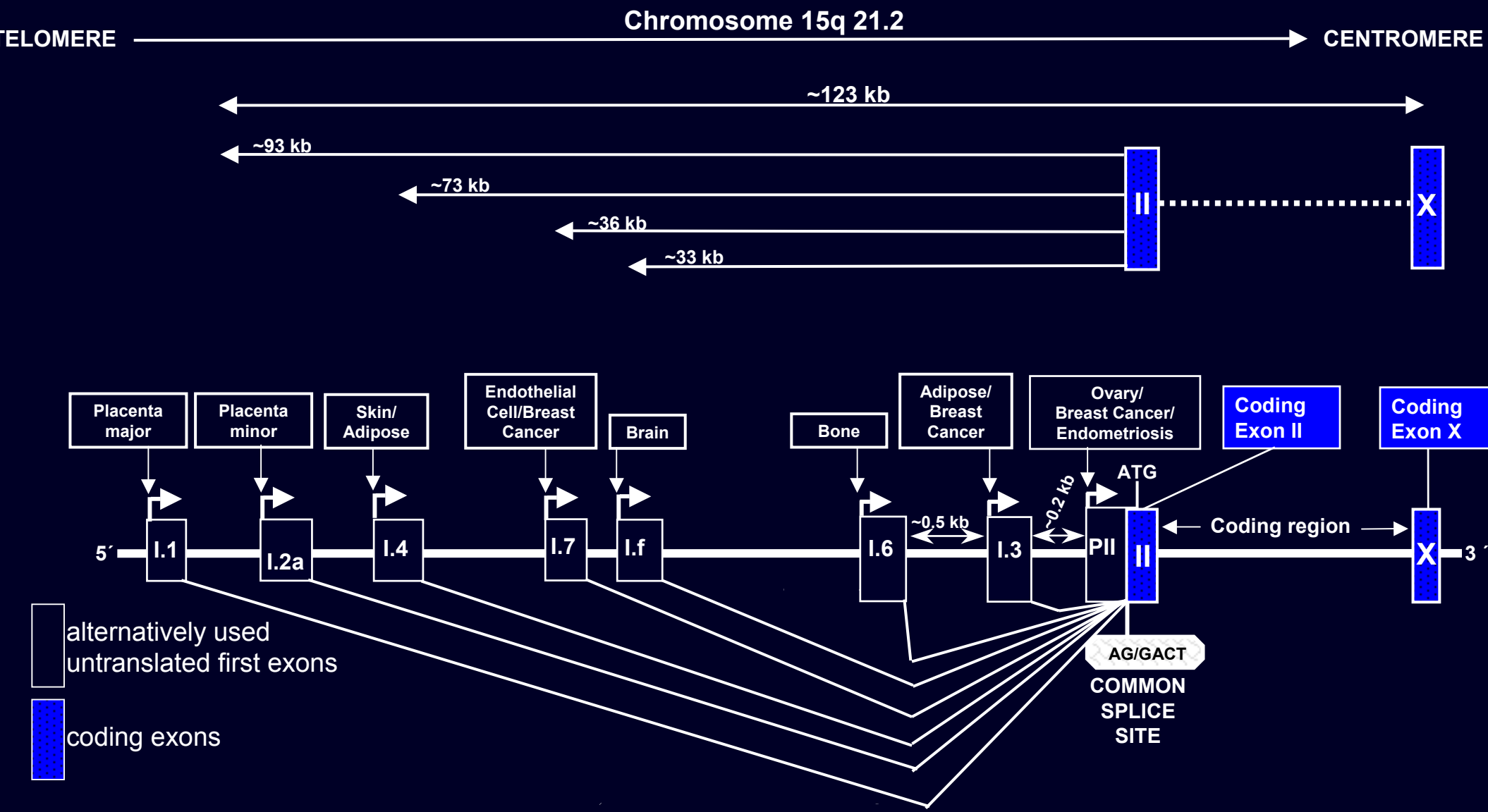
			No of Recurrences	
	ATAC		AromIn	TAM
Adj tx	2002 Anastrozole vs TAM	9366	223*	264

			No of Contralat Breast Ca	
			AromIn	TAM
Contralat ca prevention	2002 Anastrozole vs TAM	9366	14*	33

ESTROGEN PRODUCTION IN HUMAN TISSUES

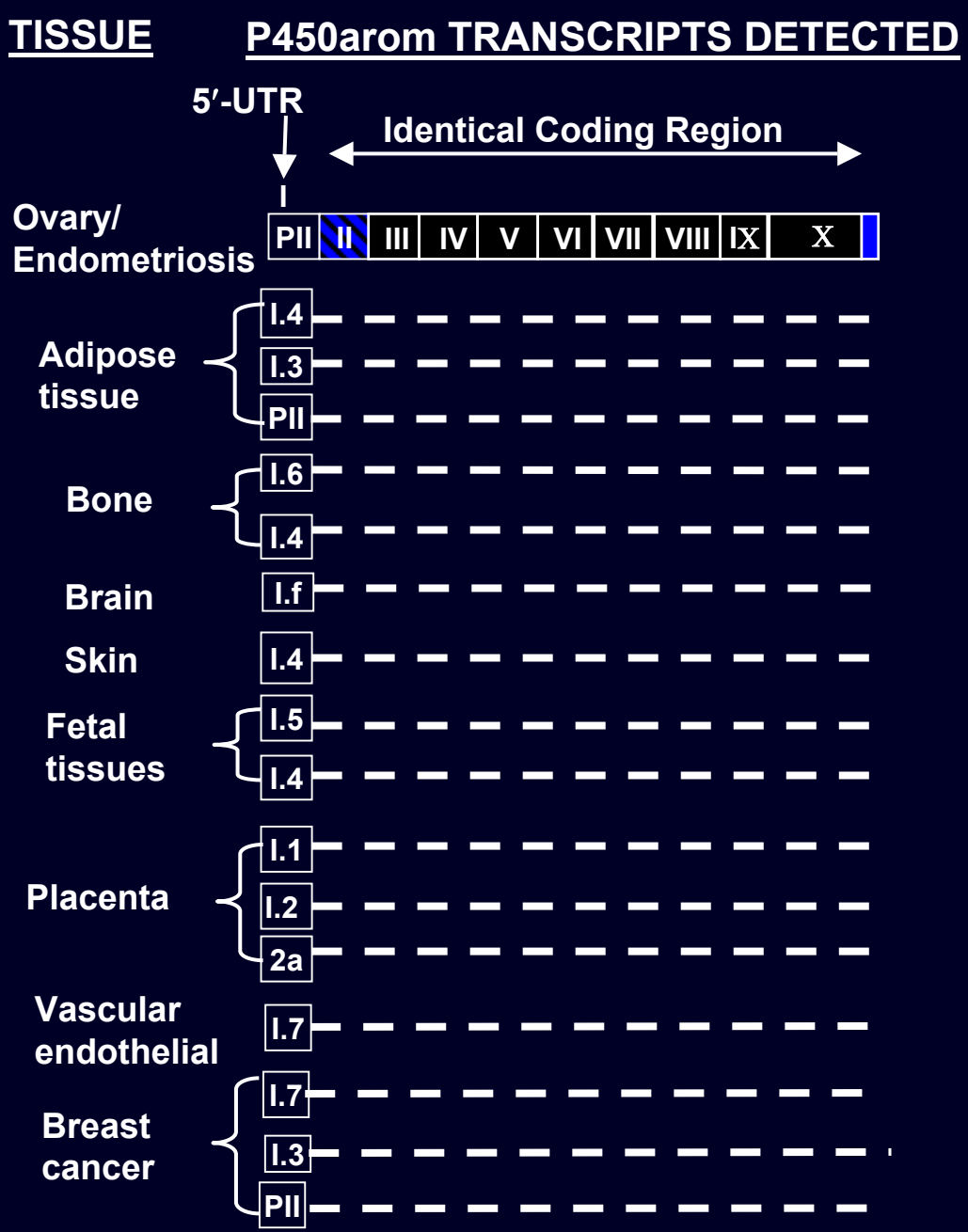


CYP19 (aromatase) GENE

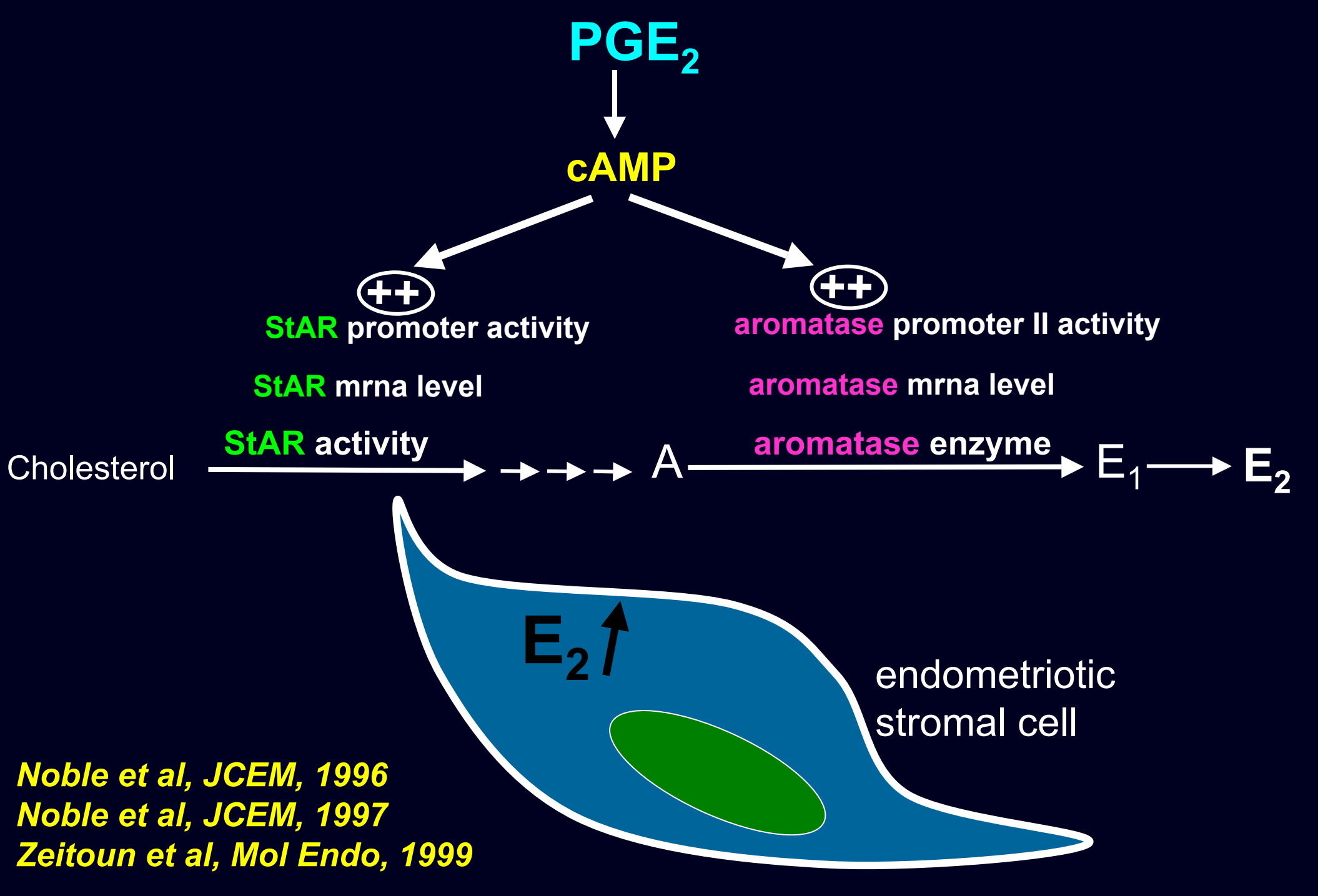


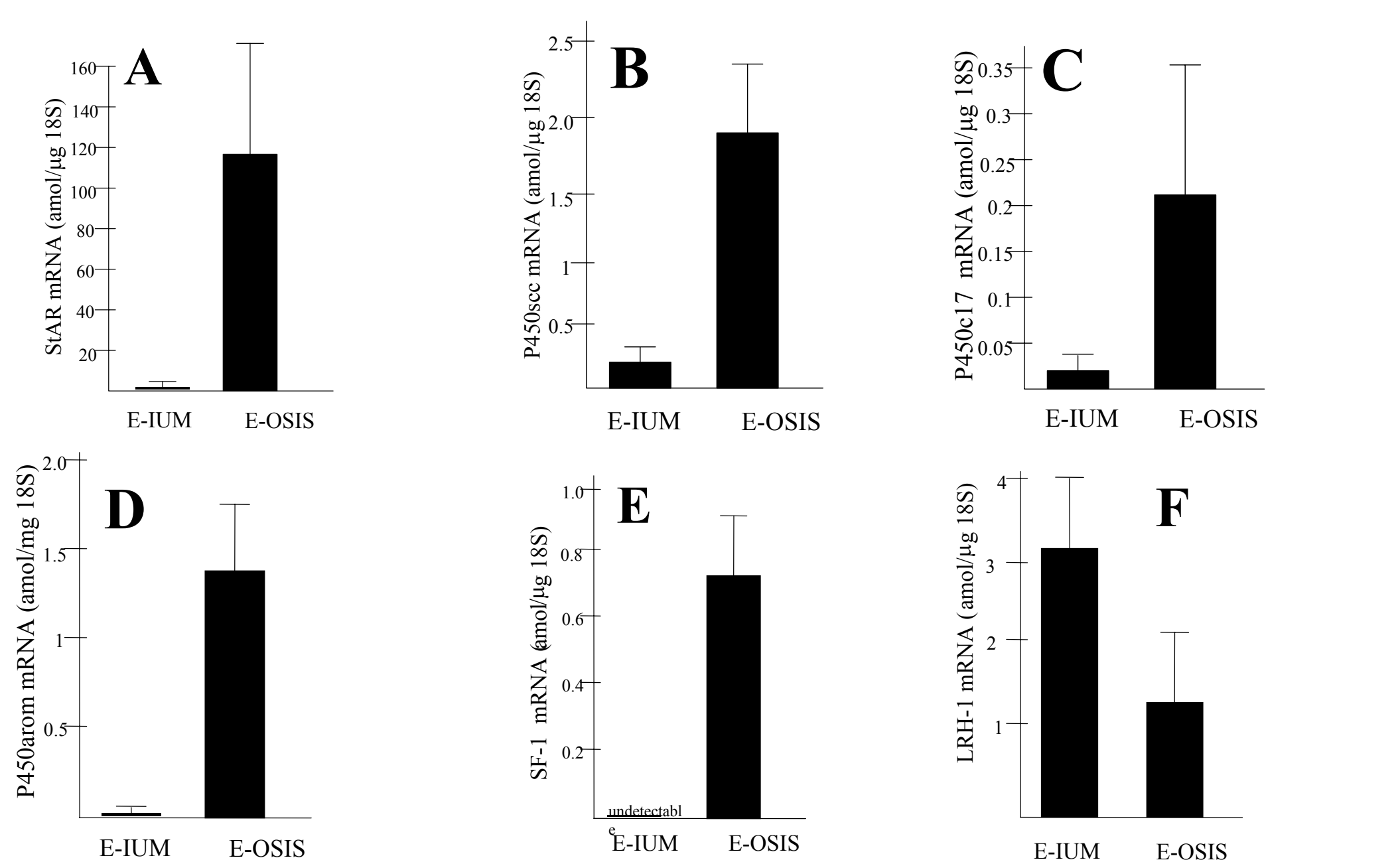
Sebastian et al, JCEM, 2001

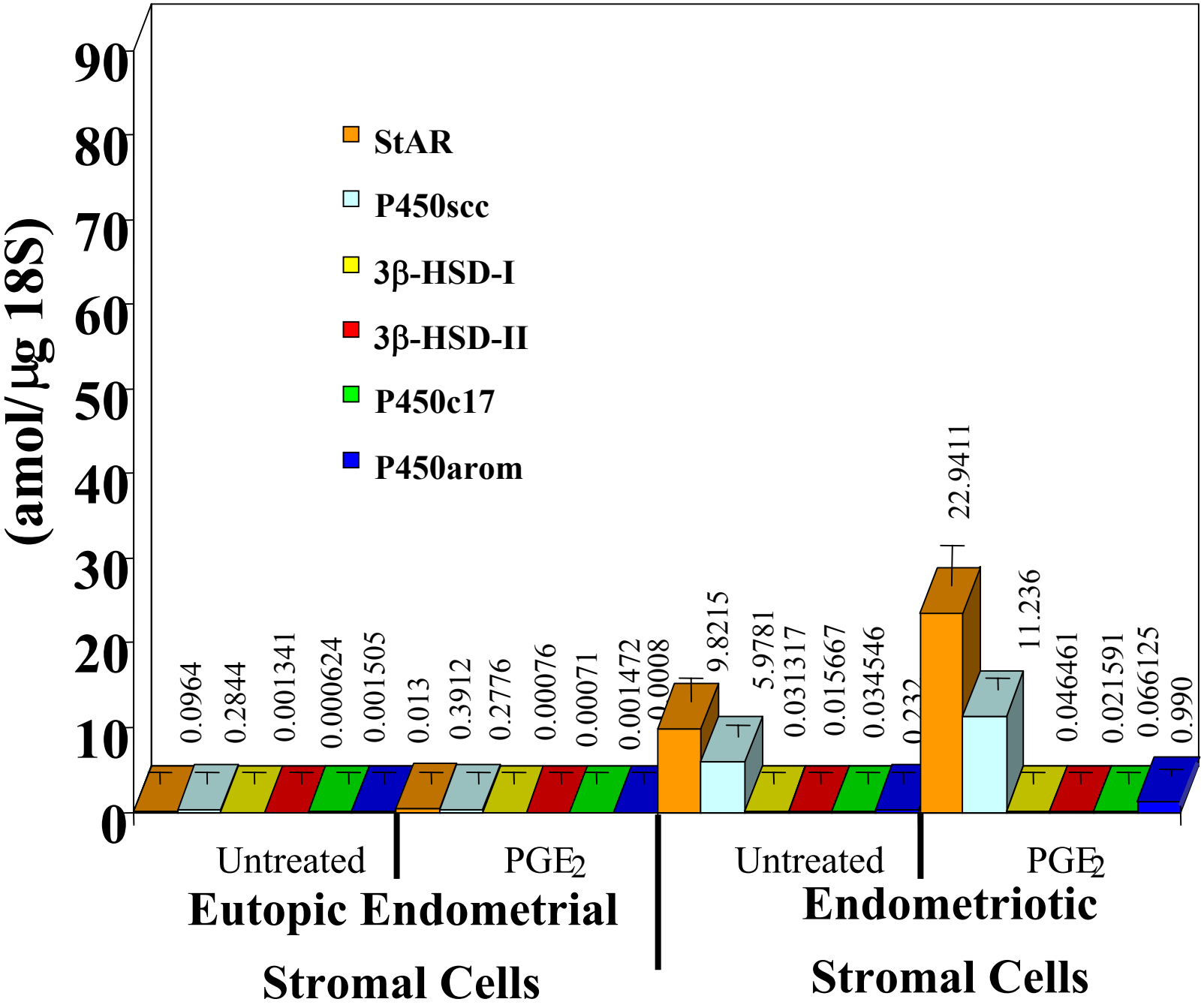
P450arom mRNA

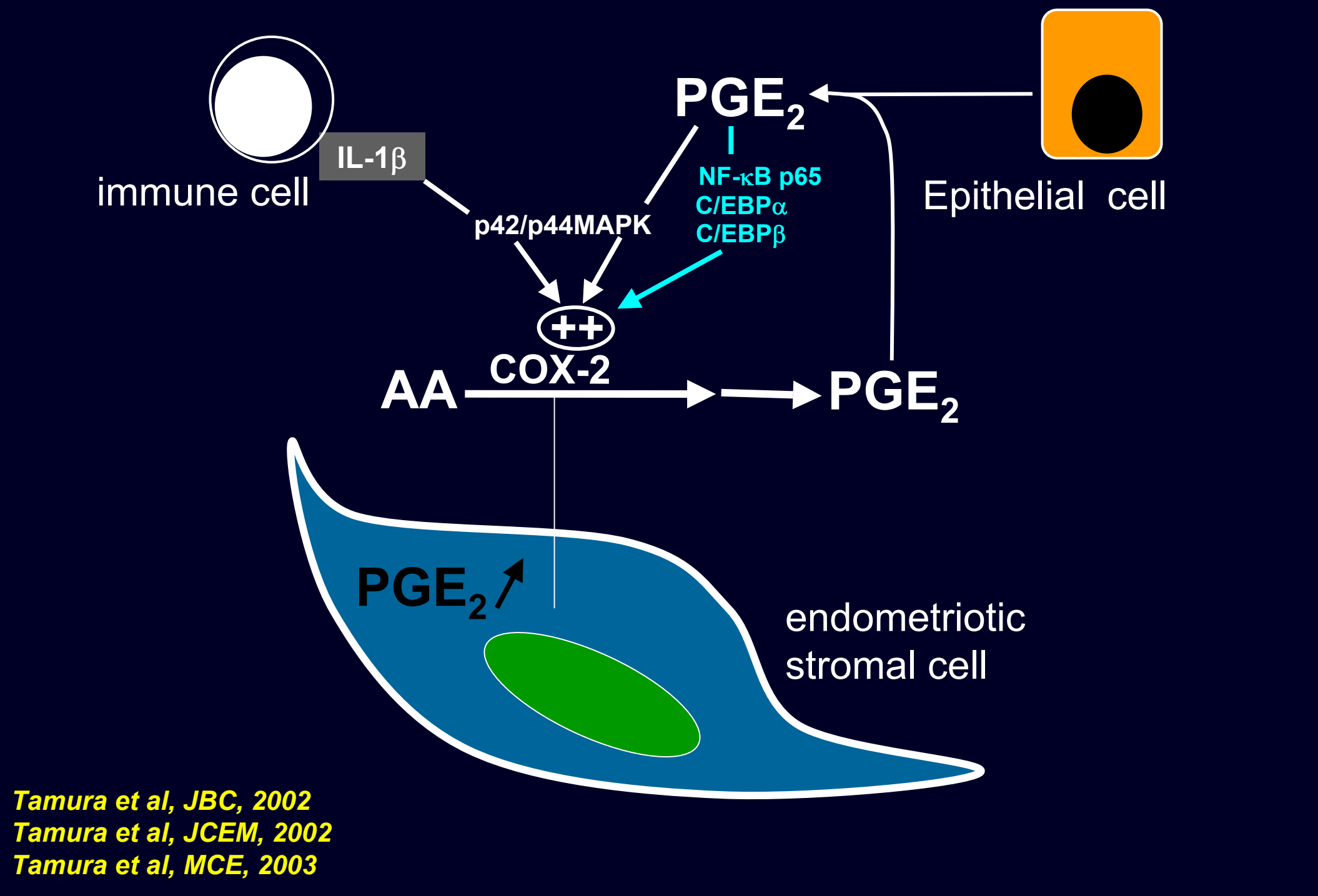


Sebastian et al, JCEM, 2001

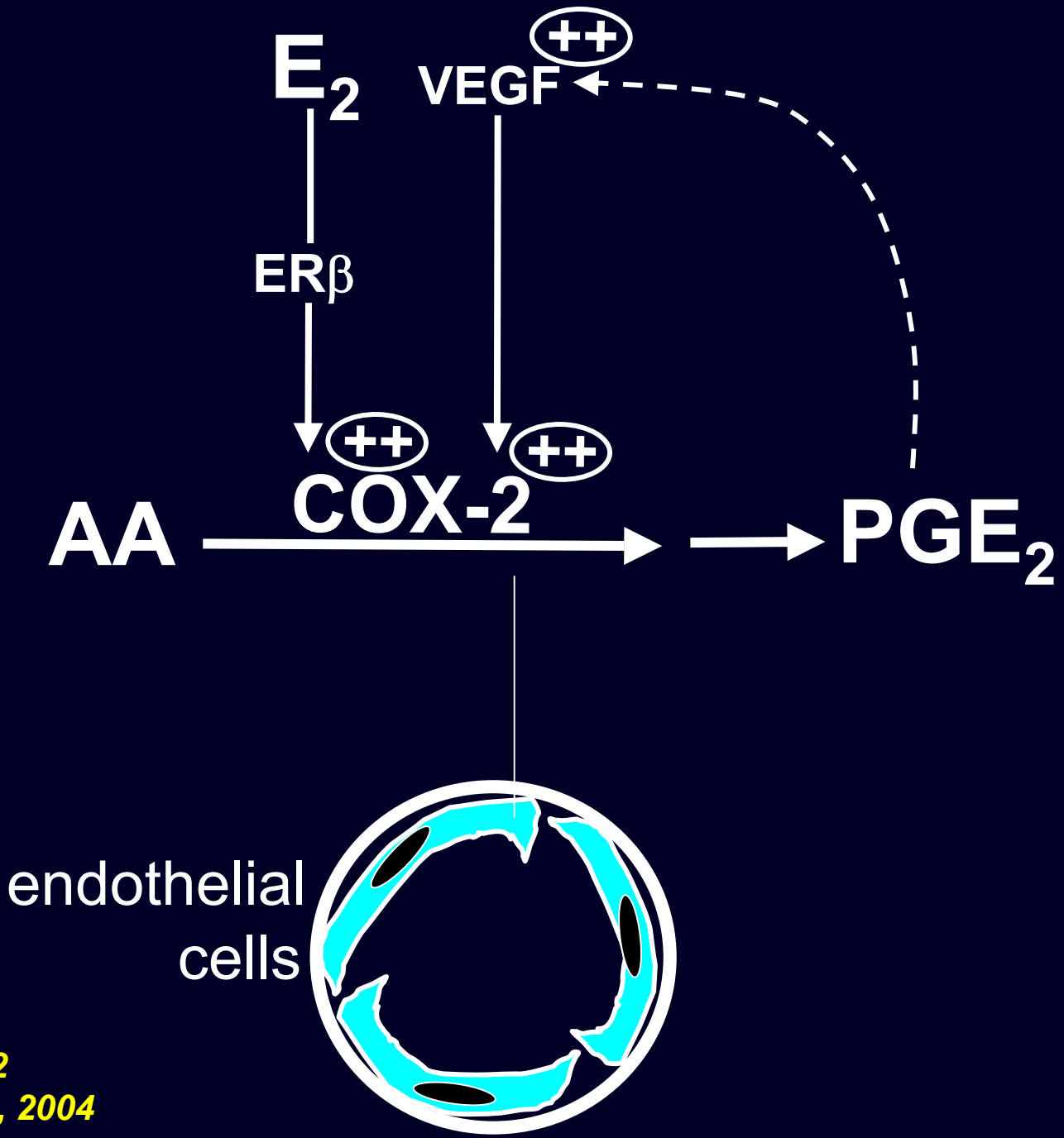

PGE2
cAMP
++
++
StAR promoter activity
StAR mrna level
StAR activity
aromatase promoter II activity
aromatase mrna level
aromatase enzyme
Cholesterol
A
E1
E2
E2
endometriotic
stromal cell
Noble et al, JCEM, 1996
Noble et al, JCEM, 1997
Zeitoun et al, Mol Endo, 1999


A
StAR mRNA (amol/µg 18S)
160
140
120
100
80
60
40
20
E-IUM
E-OSIS
B
P450scc mRNA (amol/µg 18S)
2.5
2.0
1.5
1
0.5
E-IUM
E-OSIS
C
P450c17 mRNA (amol/µg 18S)
0.35
0.3
0.25
0.2
0.15
0.1
0.05
E-IUM
E-OSIS
D
P450arom mRNA (amol/mg 18S)
2.0
1.5
1
0.5
E-IUM
E-OSIS
E
SF-1 mRNA (amol/µg 18S)
1.0
0.8
0.6
0.4
0.2
undetectable
E-IUM
E-OSIS
F
LRH-1 mRNA (amol/µg 18S)
4
3
2
1
E-IUM
E-OSIS

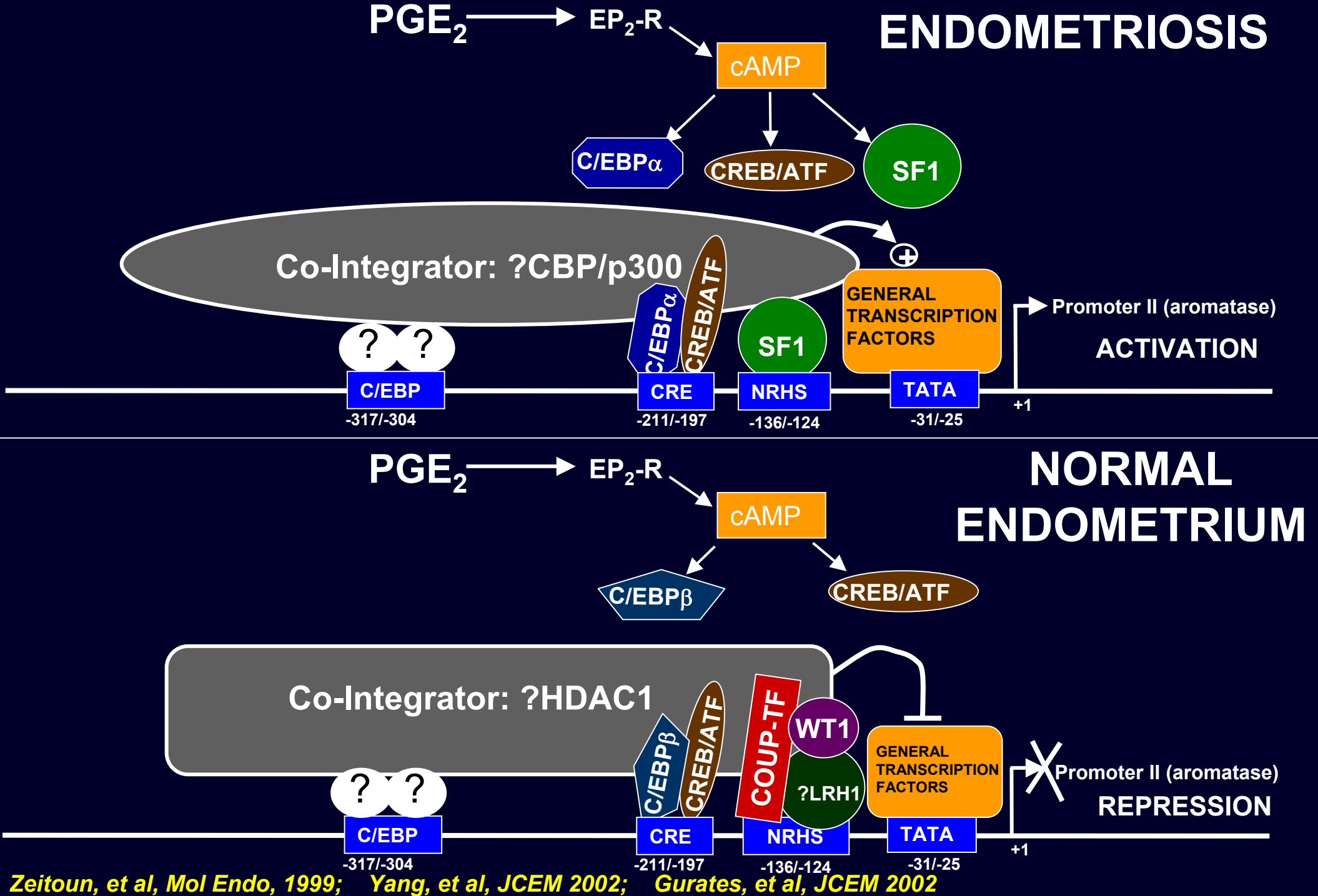

(amol/μg 18S)
90
80
70
60
50
40
30
20
10
0
StAR
P450scc
3β-HSD-I
3β-HSD-II
P450c17
P450arom
0.0964
0.2844
0.001341
0.000624
0.001505
0.013
0.3912
0.2776
0.00076
0.00071
0.001472
0.0008
9.8215
5.9781
0.031317
0.015667
0.034546
0.232
22.9411
11.236
0.046461
0.021591
0.066125
0.990
Untreated
PGE2
Untreated
PGE2
Eutopic Endometrial Stromal Cells
Endometriotic Stromal Cells



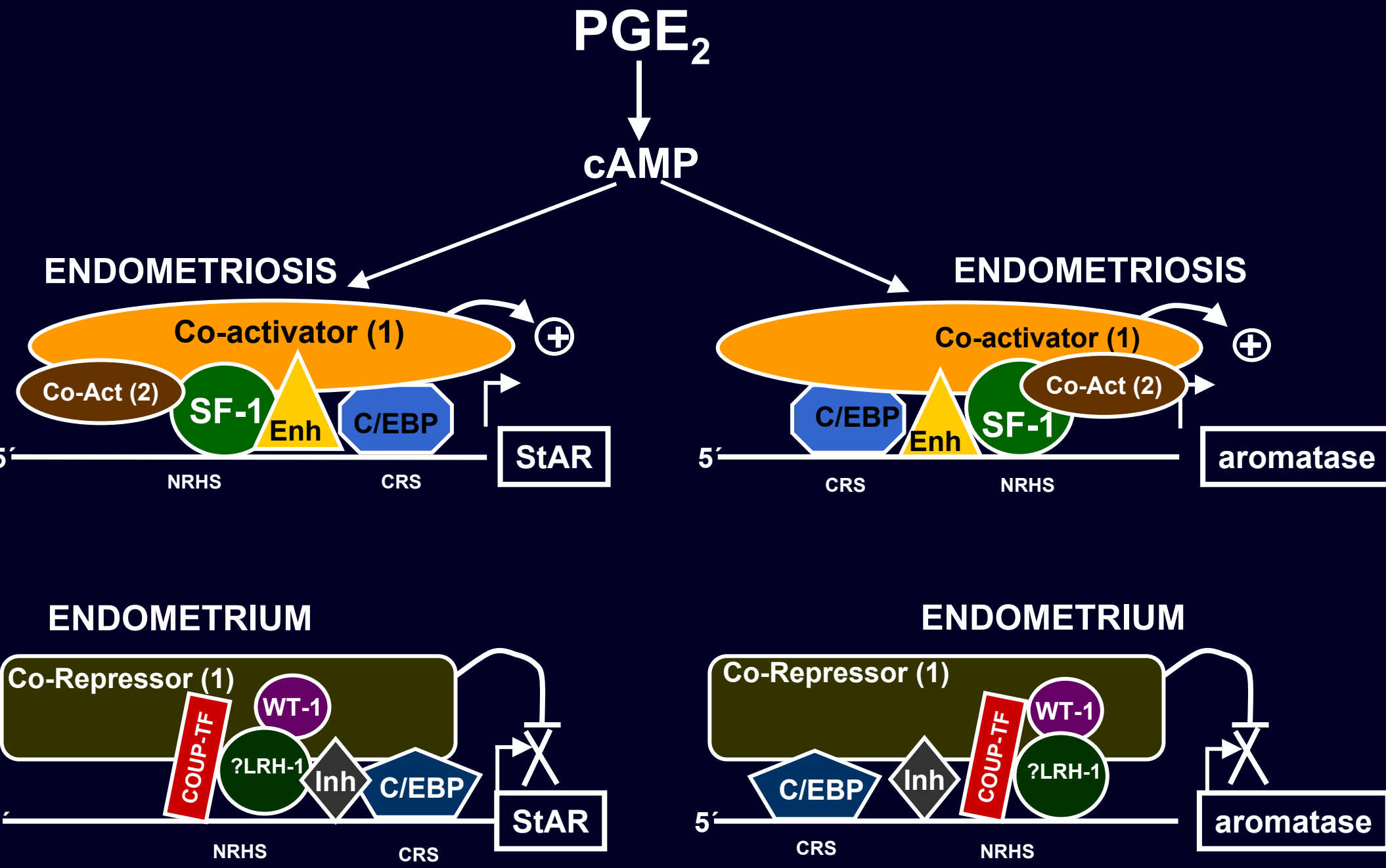
Tamura et al, JBC, 2002
Tamura et al, JCEM, 2002
Tamura et al, MCE, 2003

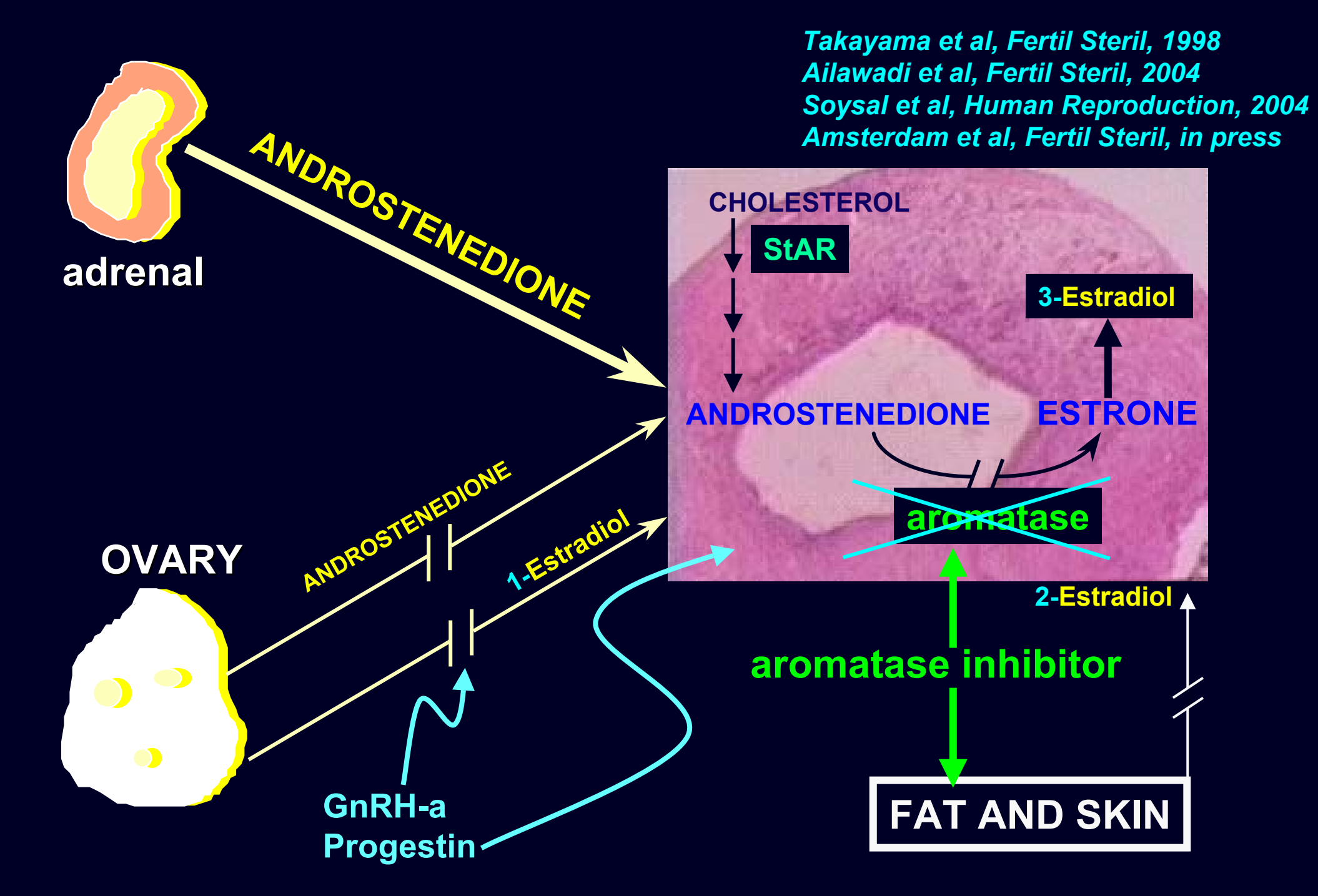


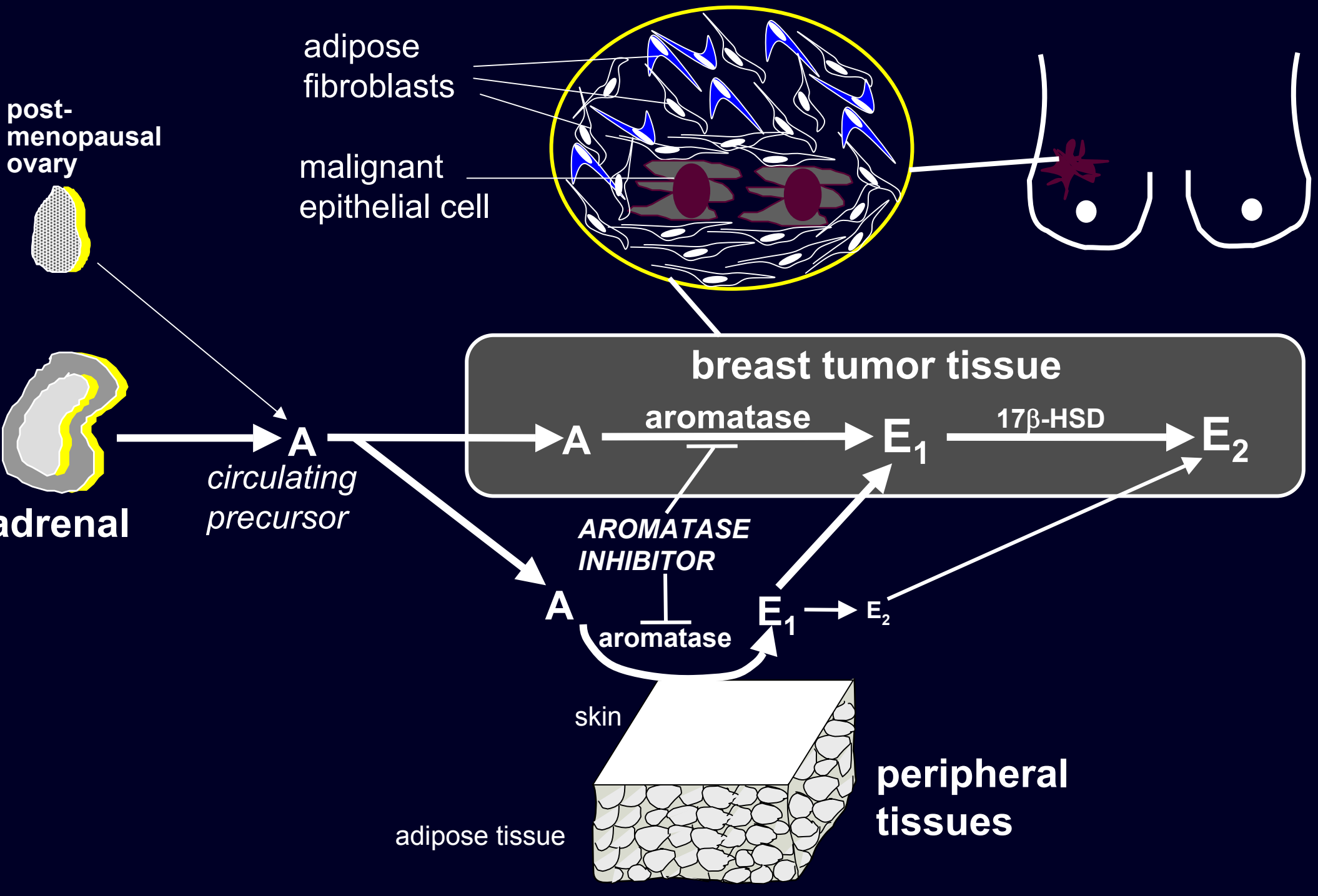
Tamura et al, JCEM, 2002
Tamura et al, Fertil Steril, 2004

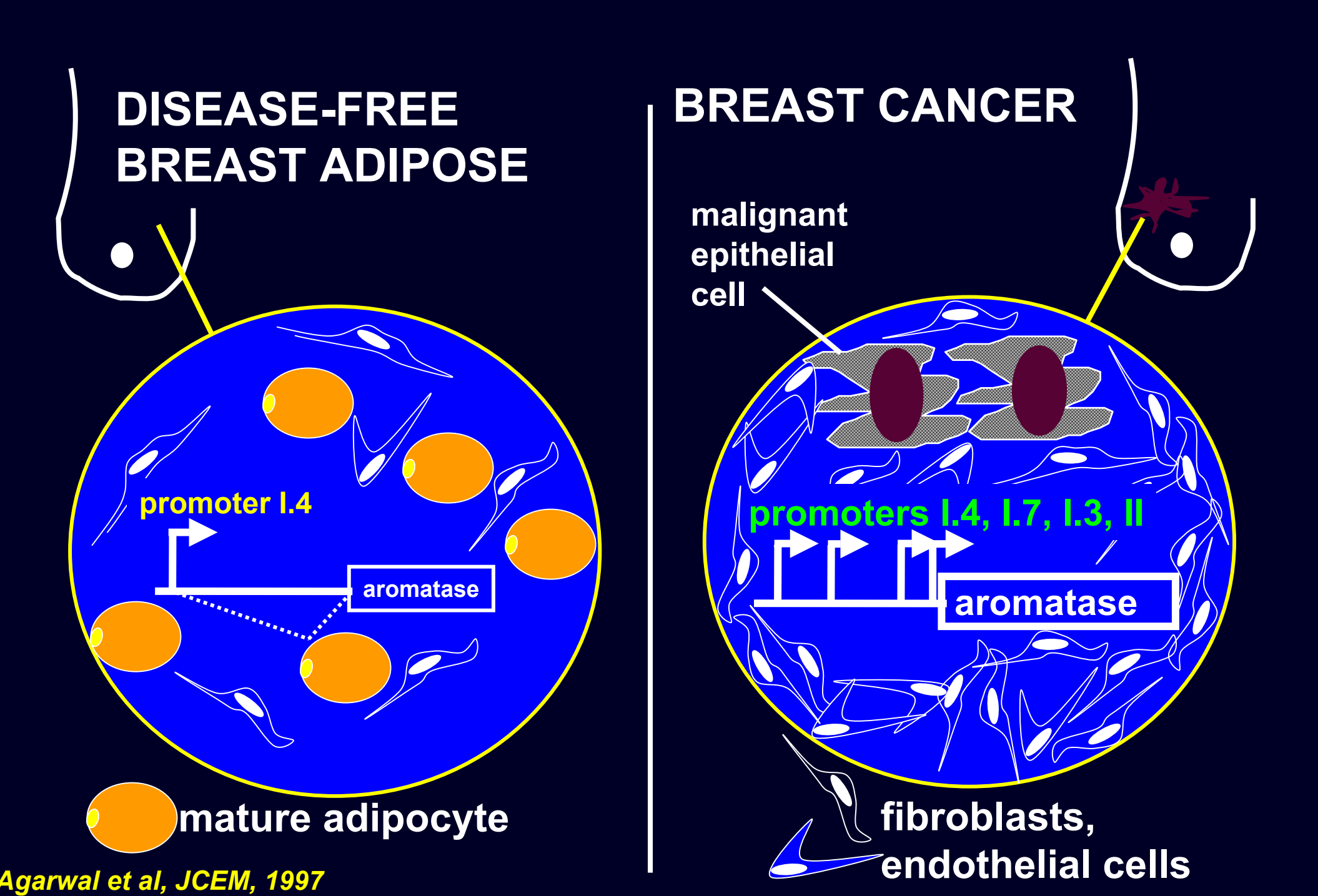


Zeitoun, et al, Mol Endo, 1999; Yang, et al, JCEM 2002; Gurates, et al, JCEM 2002

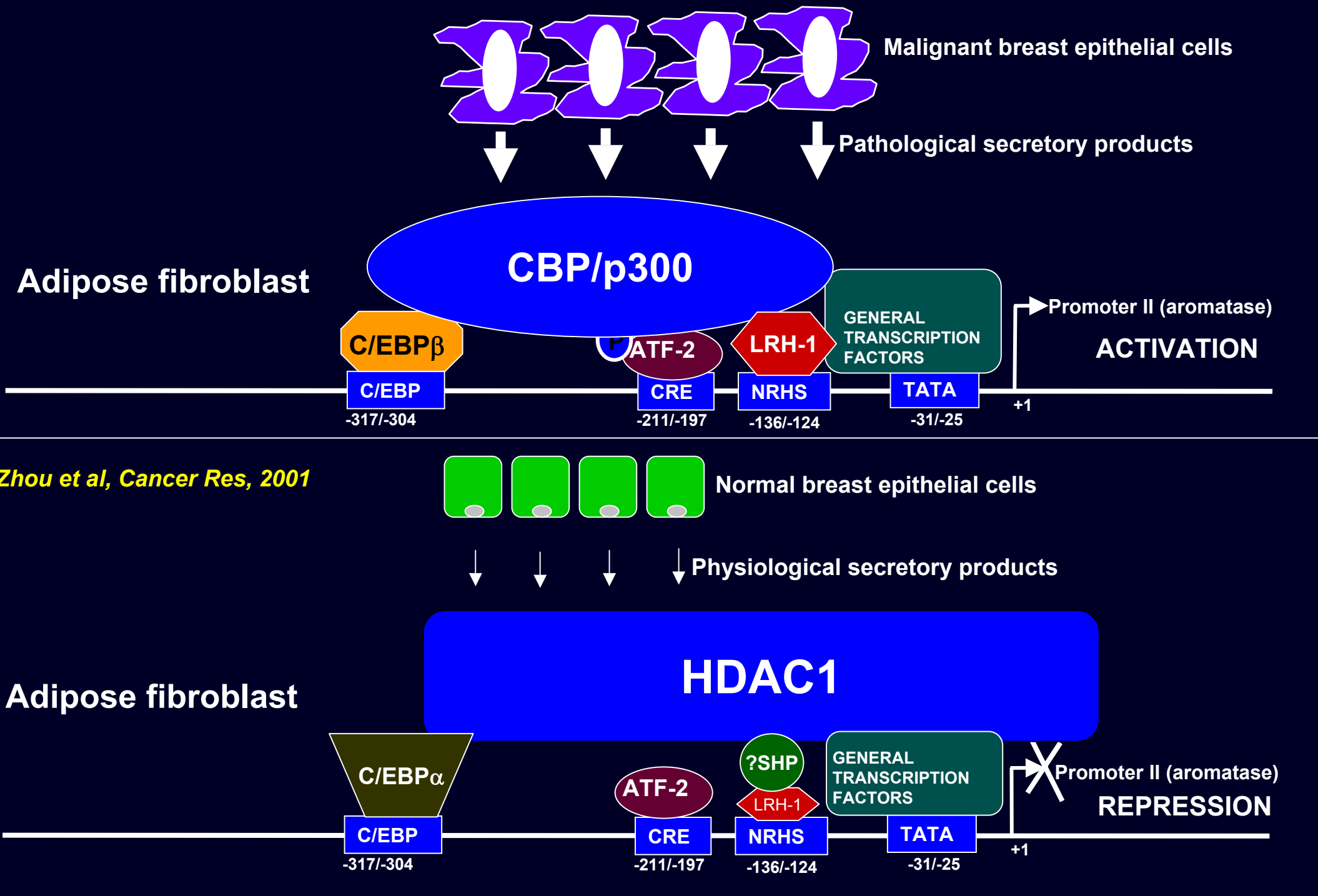

PGE2
cAMP
ENDOMETRIOSIS
Co-activator (1)
Co-Act (2)
SF-1
Enh
C/EBP
5´
NRHS
CRS
StAR
ENDOMETRIOSIS
Co-activator (1)
C/EBP
Enh
SF-1
Co-Act (2)
5´
CRS
NRHS
aromatase
ENDOMETRIUM
Co-Repressor (1)
WT-1
COUP-TF
?LRH-1
Inh
C/EBP
5´
NRHS
CRS
StAR
ENDOMETRIUM
Co-Repressor (1)
WT-1
COUP-TF
?LRH-1
C/EBP
Inh
5´
CRS
NRHS
aromatase

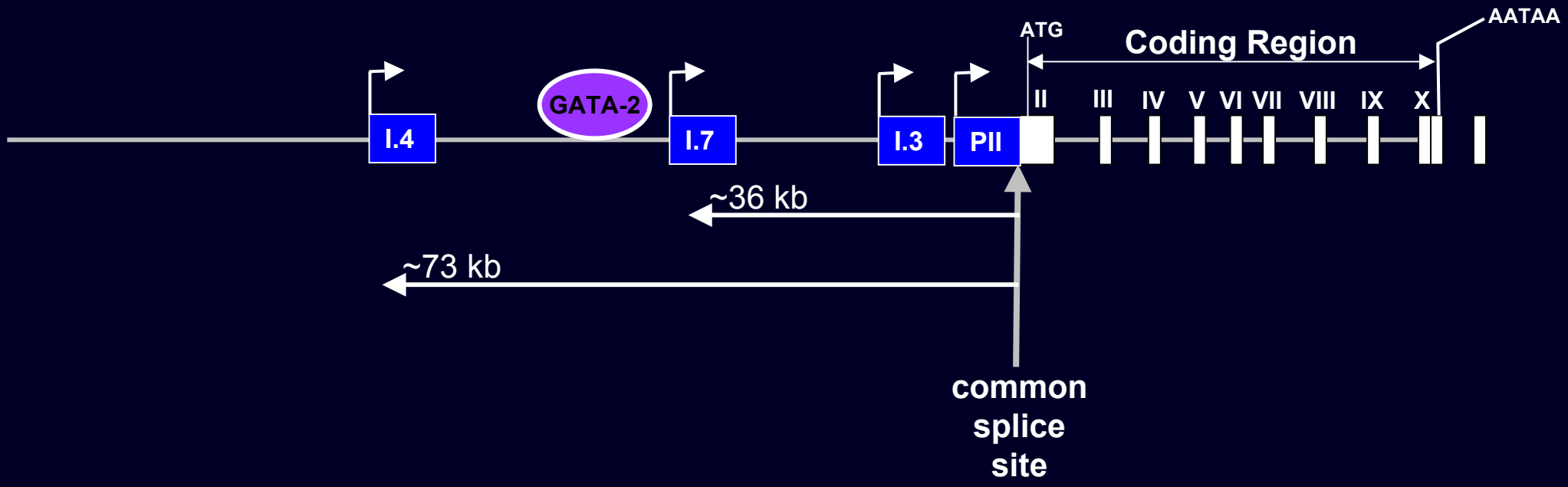

Takayama et al, Fertil Steril, 1998
Ailawadi et al, Fertil Steril, 2004
Soysal et al, Human Reproduction, 2004
Amsterdam et al, Fertil Steril, in press
adrenal
ANDROSTENEDIONE
CHOLESTEROL
StAR
3-Estradiol
ANDROSTENEDIONE
ESTRONE
aromatase
2-Estradiol
OVARY
ANDROSTENEDIONE
1-Estradiol
aromatase inhibitor
GnRH-a
Progestin
FAT AND SKIN


adipose
fibroblasts
malignant
epithelial cell
post-
menopausal
ovary
adrenal
A
circulating
precursor
breast tumor tissue
A
aromatase
E1
17β-HSD
E2
AROMATASE
INHIBITOR
A
aromatase
E1
E2
skin
adipose tissue
peripheral
tissues



Agarwal et al, JCEM, 1997


Malignant breast epithelial cells
Pathological secretory products
Adipose fibroblast
CBP/p300
C/EBPβ
ATF-2
LRH-1
GENERAL TRANSCRIPTION FACTORS
Promoter II (aromatase)
ACTIVATION
C/EBP
CRE
NRHS
TATA
-317/-304
-211/-197
-136/-124
-31/-25
+1
Zhou et al, Cancer Res, 2001
Normal breast epithelial cells
Physiological secretory products
Adipose fibroblast
HDAC1
C/EBPα
?SHP
LRH-1
ATF-2
GENERAL TRANSCRIPTION FACTORS
Promoter II (aromatase)
REPRESSION
C/EBP
CRE
NRHS
TATA
-317/-304
-211/-197
-136/-124
-31/-25
+1



DISTRIBUTION OF AROMATASE PROMOTERS IN BREAST CANCER SAMPLES (n=5):

Number of 5´-RACE Clones sequenced	I.7	PII	I.3	I.4	Total
Breast Cancer 1	4	3	4	3	14
Normal Breast				10	10

5´-RACE/ Colony hybridization	Percentage of I.7-specific clones
Breast Cancer 2	39%
Breast Cancer 3	42%
Breast Cancer 4	42%
Breast Cancer 5	54%

Sebastian et al, Mol Endo, 2002

TOTAL AROMATASE mRNA LEVELS:

NORMAL BREAST →

5´-UTR:

I.4	arom coding

aromatase mRNA species in normal breast **+**

BREAST CANCER →

5´-UTR:

PII	arom coding
I.3	arom coding
I.7	arom coding
I.4	arom coding

aromatase mRNA species in breast cancer **++++**

LEIOMYOMAS AND ESTROGEN-PROGESTERONE

- Leiomyoma growth is dependent on ovarian steroids.
- Conditions associated with increased estrogen and progesterone (e.g., pregnancy and HRT) result in growth of leiomyomata.
- On the other hand, conditions in which ovarian steroid concentrations are diminished (menopause and GnRHa therapy) result in a decrease in size of the leiomyomata.

LEIOMYOMAS AND ESTROGEN-PROGESTERONE

- The ovary represents a very important source of estrogen and progesterone necessary for leiomyoma growth.

- Leiomyoma tissues are themselves a source of estrogen.

- The tissue concentrations of estrogens are elevated in leiomyoma nodules compared with levels in the surrounding myometrium.

HISTORY - AROMATASE

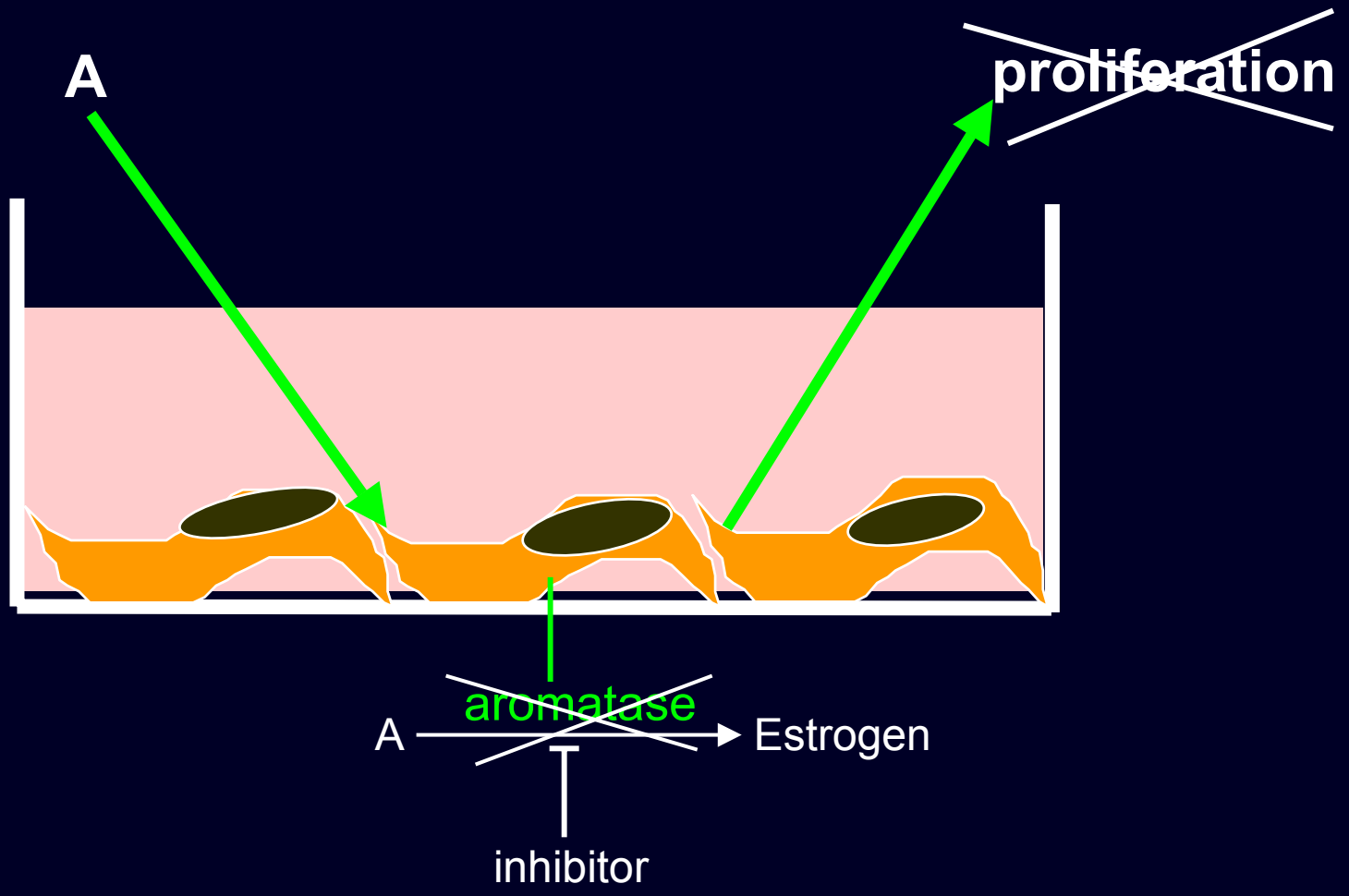
- Leiomyoma tissue possesses high aromatase enzyme activity, whereas normal myometrium displays no activity (Folkerd et al and Yamamato et al, 1984).

- We demonstrated that LSMCs in culture are capable of aromatizing androstenedione to estrogen (Bulun et al, 1994).

- Levels of aromatase mRNA are 1.5-25 times higher in leiomyomata than in surrounding myometrium (Bulun et al, 1994).

- Immunoreactive aromatase is strongly expressed in smooth muscle cells of leiomyoma and in smooth muscle cells of the tunica intima of arterioles, but not in other cell components such as blood cells infiltrating leiomyoma tissues (Shozu et al, 2000).

Primary Leiomyoma Smooth Muscle Cells

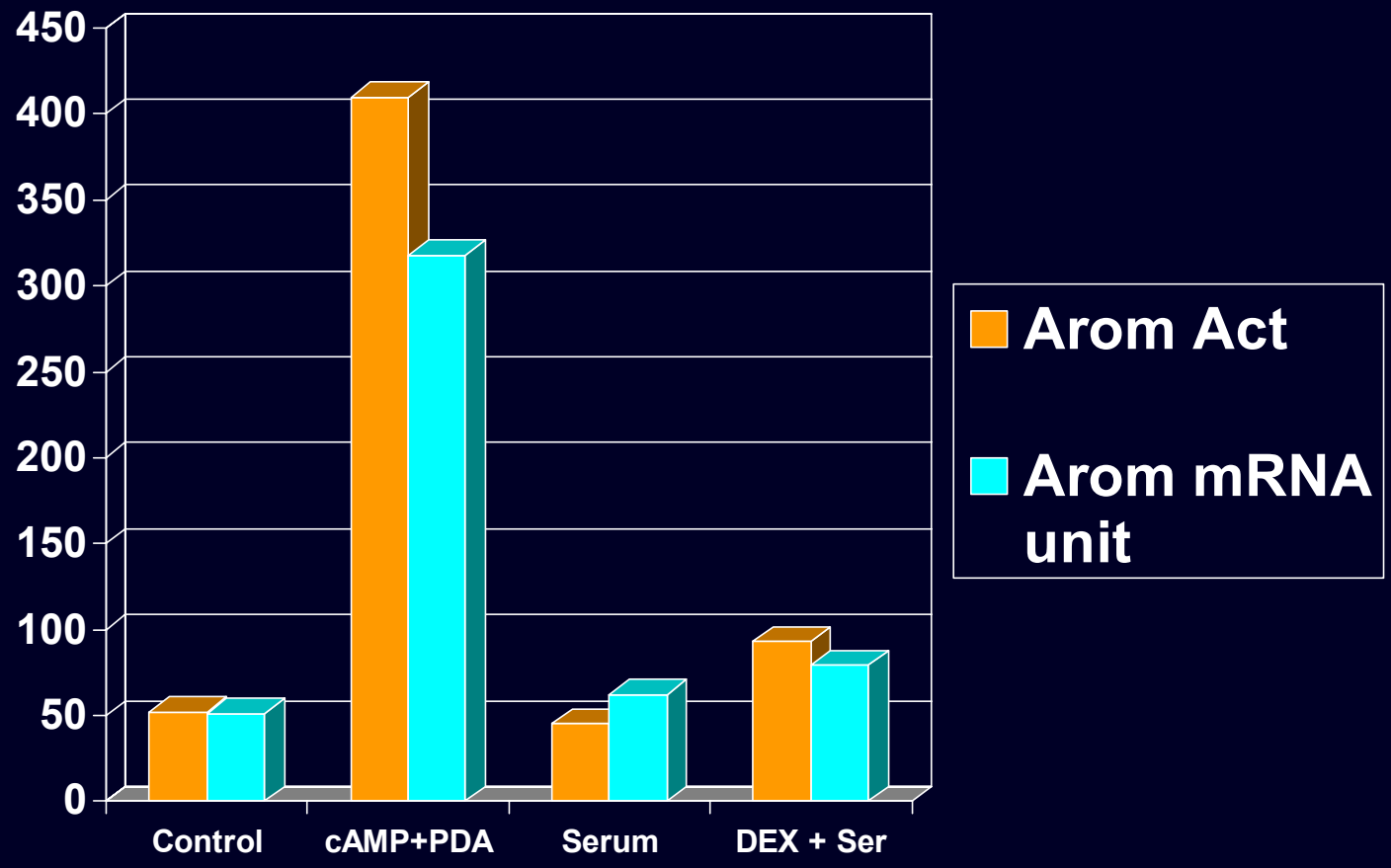


Shozu et al, JCEM, 2000

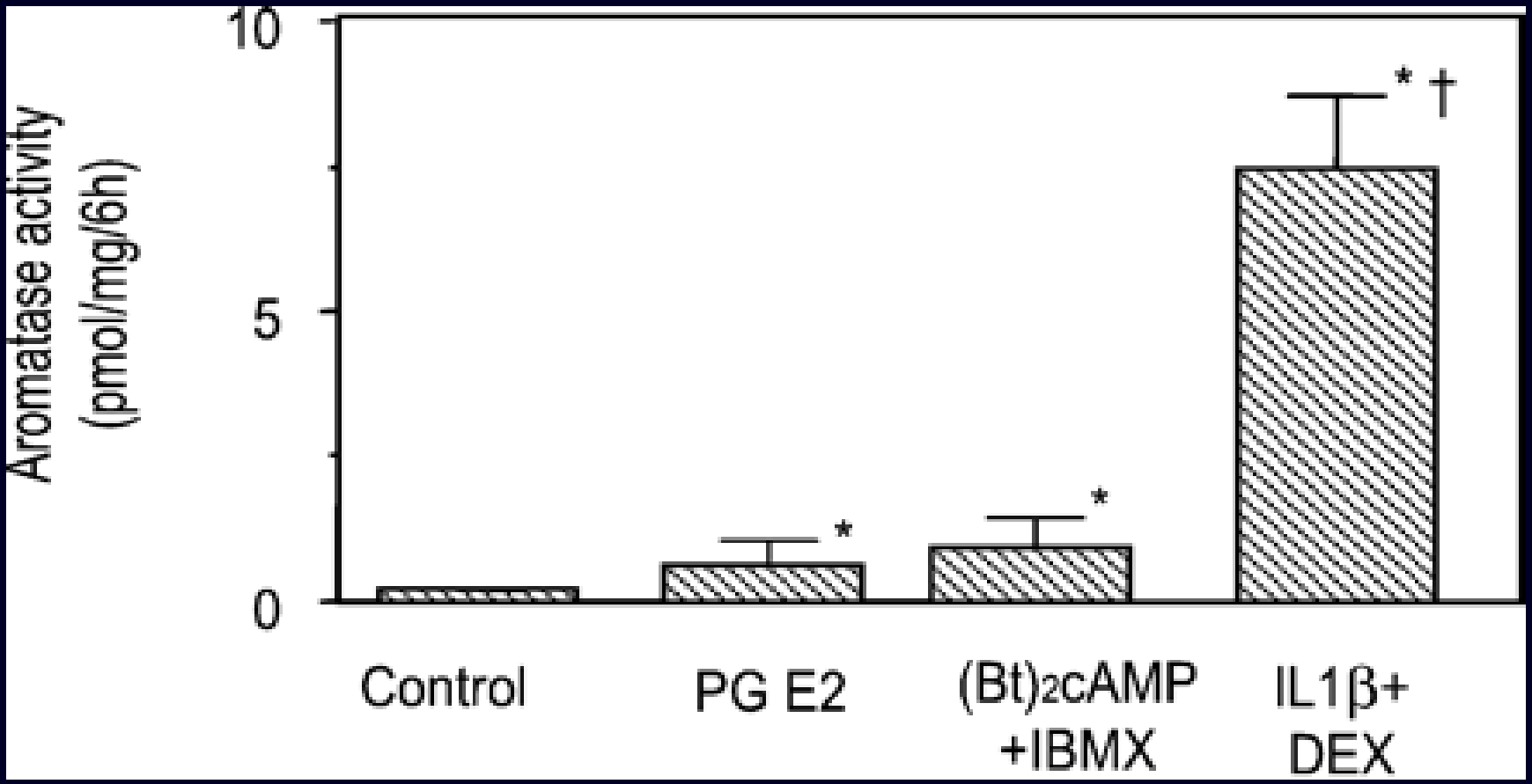
Uterine leiomyoma in a perimenopausal Japanese woman shrank significantly (>50%) after treatment with the aromatase inhibitor fadrozole.

Shozu et al, Fertil Steril 2003

REGULATION OF AROMATASE ACTIVITY AND EXPRESSION

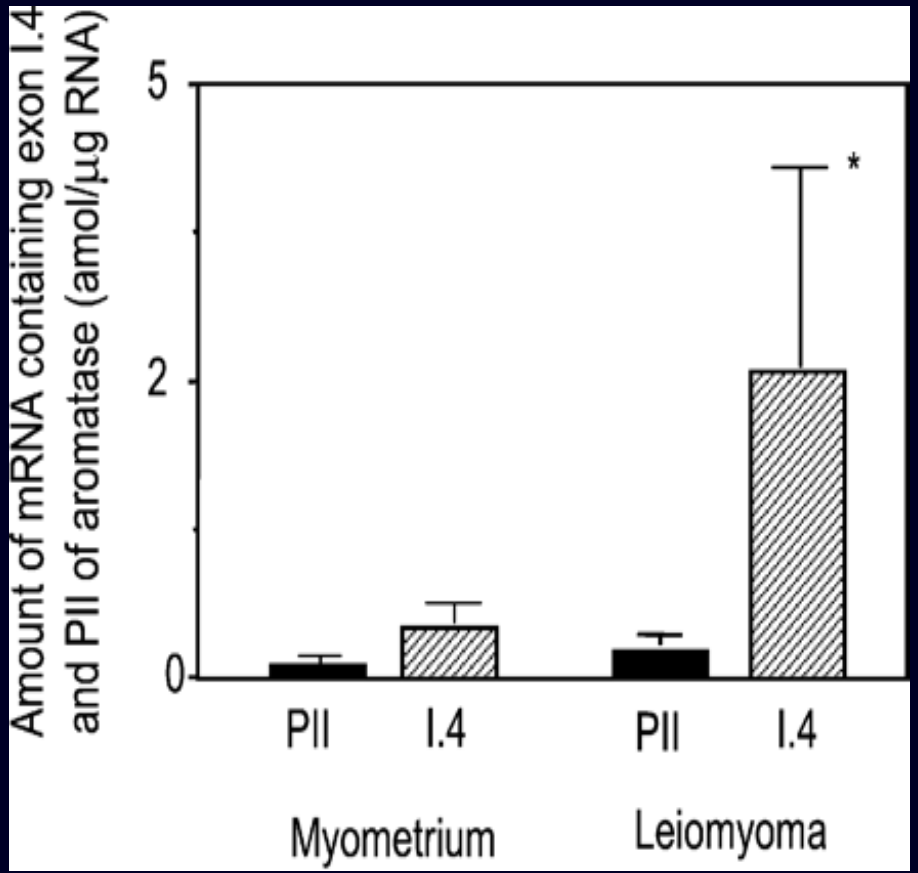


Bulun et al, JCEM 1994



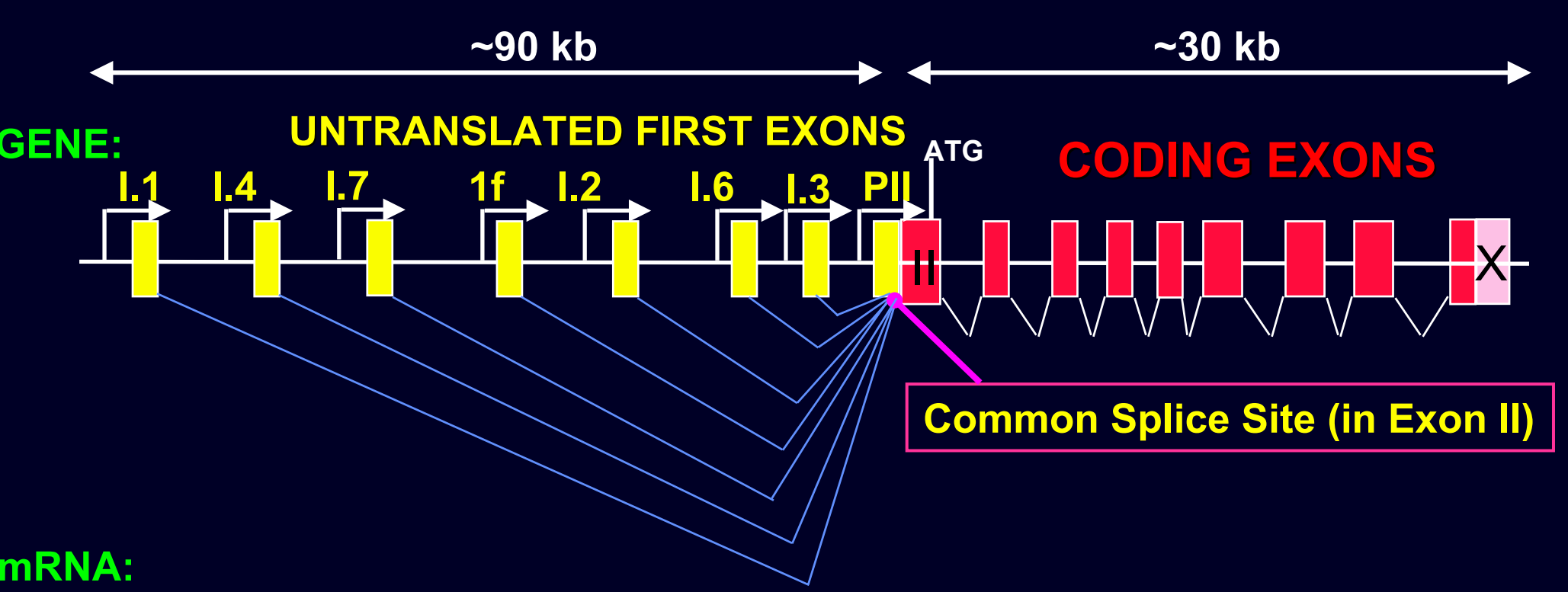
Shozu et al, JCEM 2002

Identification of Promoter Use Specific for Leiomyoma Tissue from Japanese Women



- Real-Time PCR: 16 of 20 nodules contained only I.4-specific mRNA.
- The remaining 4 leiomyoma nodules were positive for both PII and I.4-specific mRNA.
- 5´-RACE: 5 out of 6 leiomyomata from Japanese patients contained I.4, whereas only 2/6 contained PII-specific mRNA.

Shozu et al, JCEM 2002



mRNA:

Placenta:

I.1	Arom Coding Region

Adipose Tissue:

I.4	Arom Coding Region

Ovary/endometriosis:

PII	Arom Coding Region

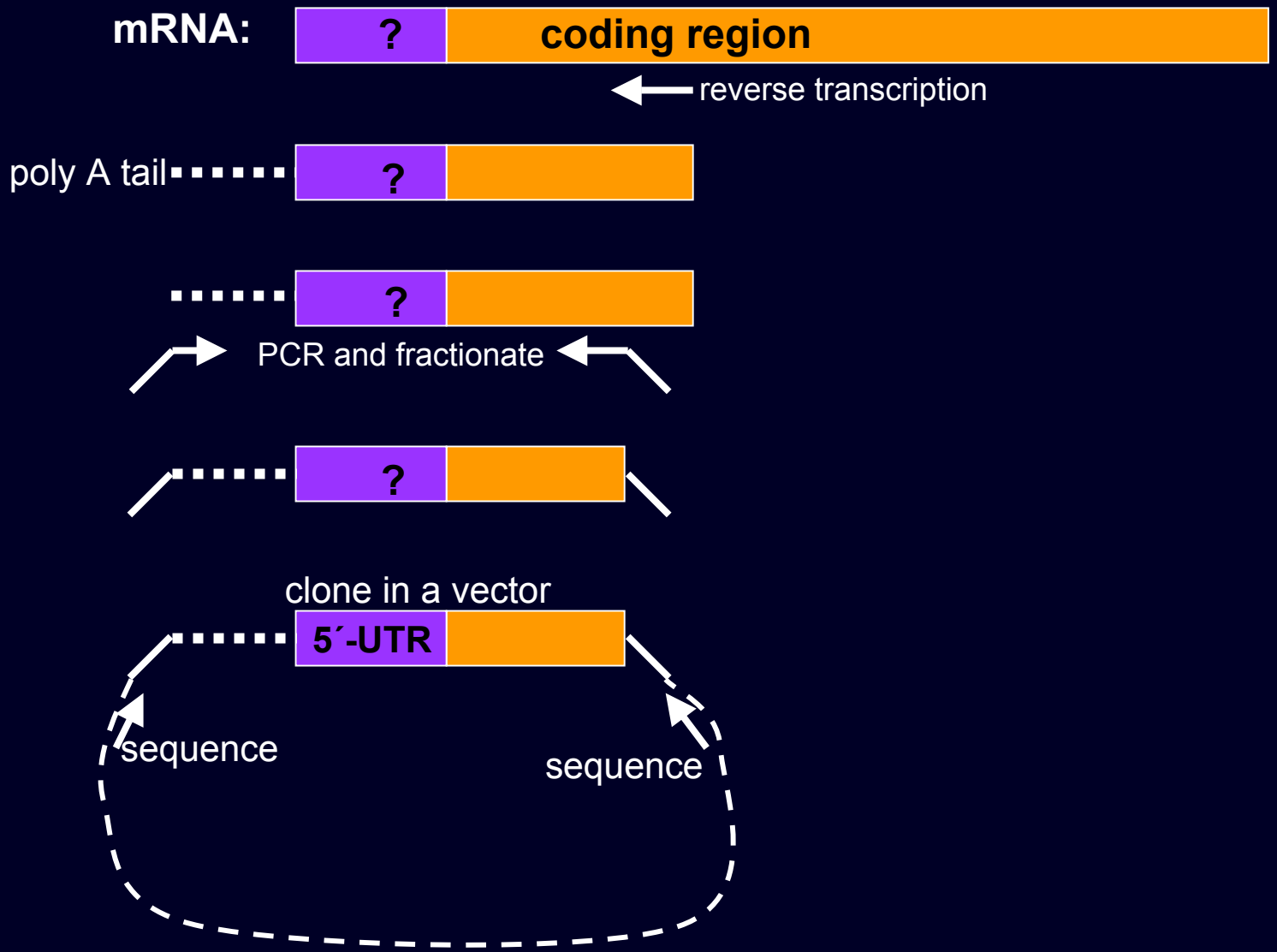
Brain:

I.f	Arom Coding Region

Breast cancer:

I.3	Arom Coding Region
PII	Arom Coding Region
I.7	Arom Coding Region

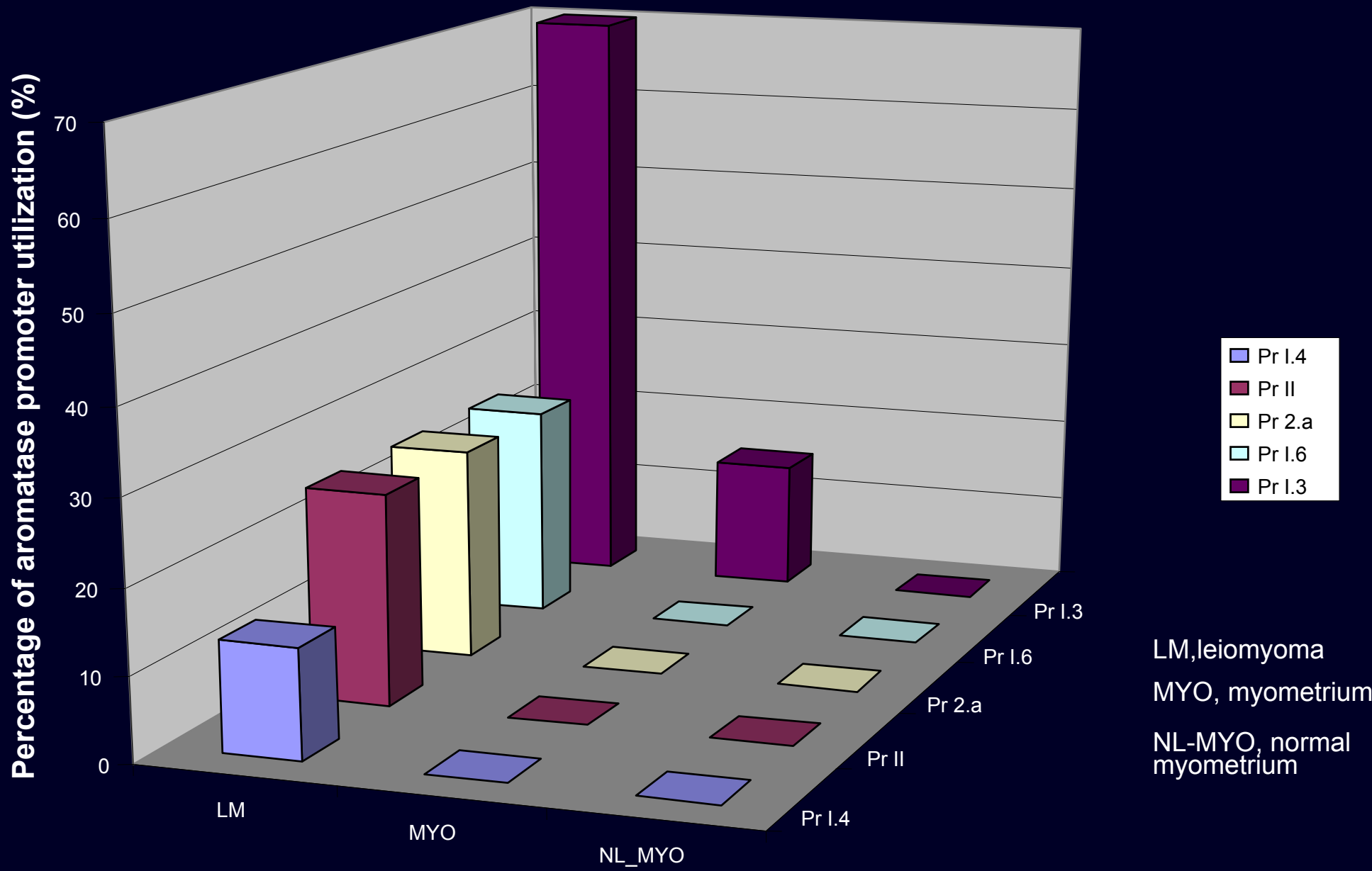
5´-RACE


mRNA:
?
coding region
reverse transcription
poly A tail
?
?
PCR and fractionate
?
clone in a vector
5´-UTR
sequence
sequence

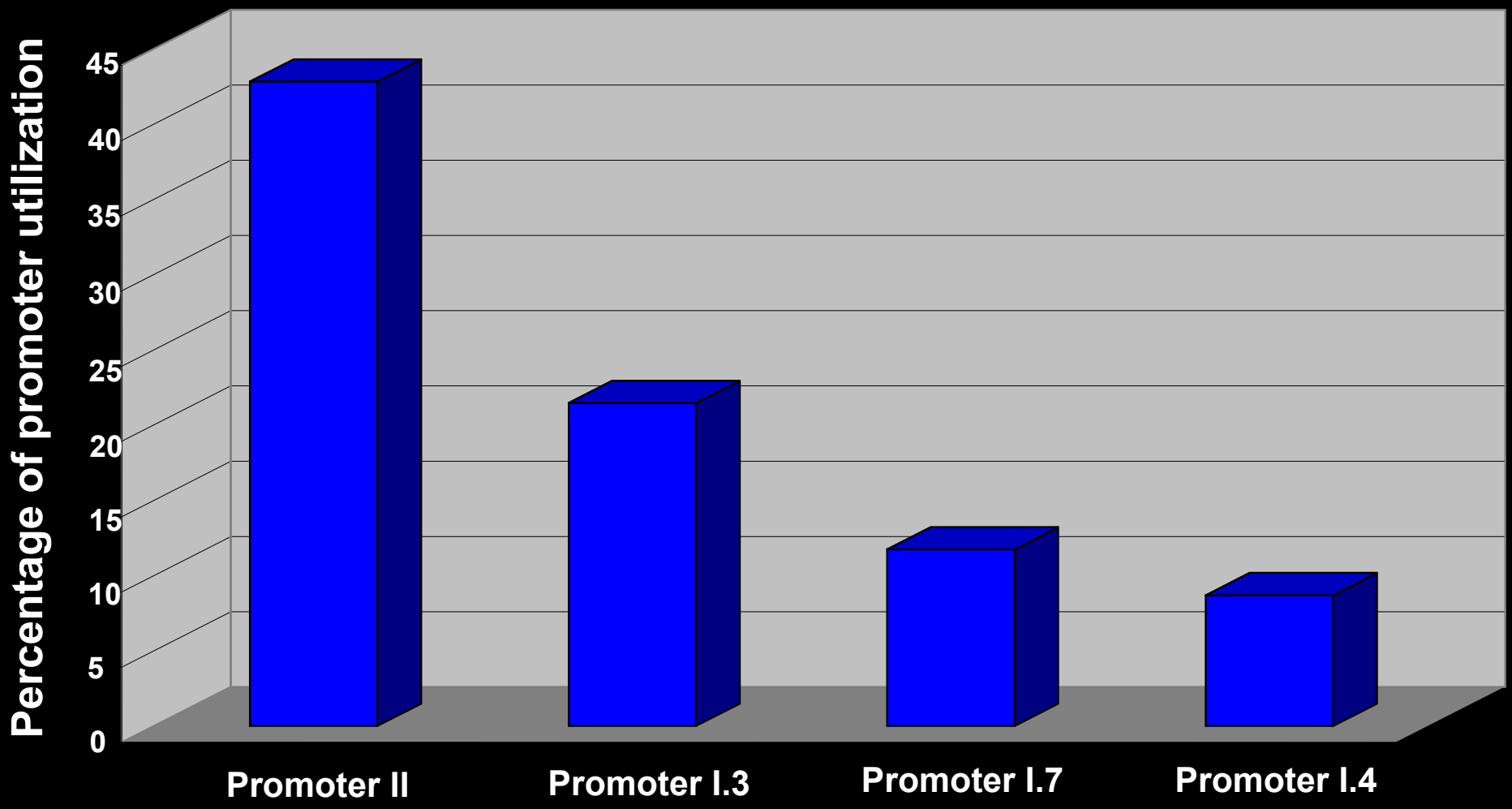
PROMOTER-SPECIFIC AROMATASE mRNA SPECIES BY 5´-RACE

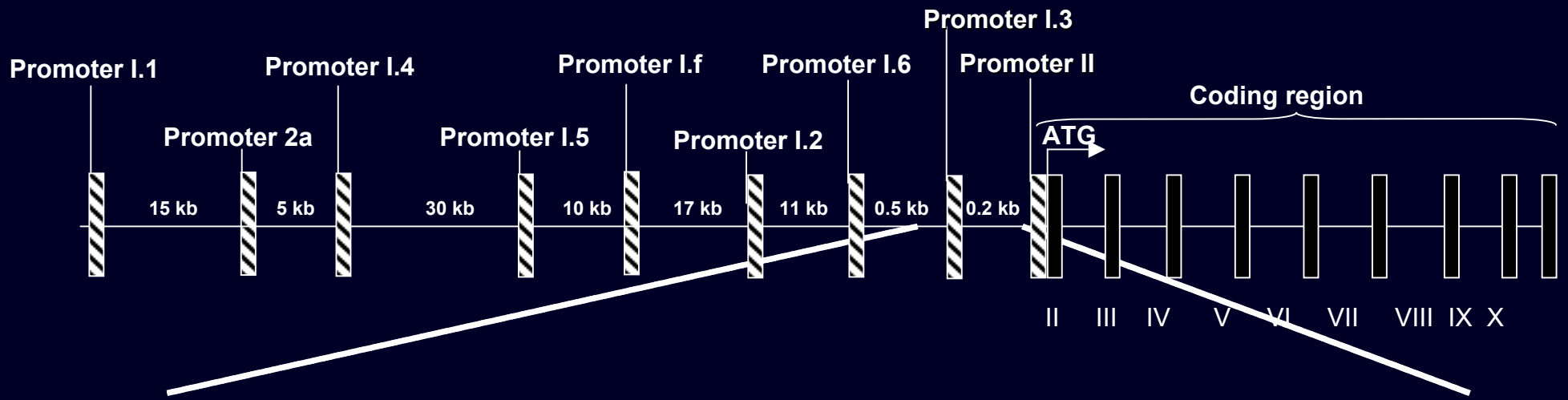
Patient	Tissue	Race	Age	Mens cycle	Pr I.3	Pr II	Pr I.6	Pr 2.a	Pr I.4
1	LM LM	AA AA	39 39	Follicular Follicular	(+)	(+)		(+)	
2	LM MYO	White White	42 42	Follicular Follicular	(+) (+)				
3	LM	Hispanic	65	Postmen	(+)				
4	LM	AA	41	Luteal	(+)			(+)	
5	LM	White	42	Luteal					(+)
6	LM	Hispanic	42	Luteal	(+)				
7	LM	AA	40	Luteal		(+)	(+)		
8	LM	White	54	Postmen	(+)		(+)		
9	LM	AA	45	Follicular	(+)				
10	LM	Hispanic	54	Postmen			(+)	(+)	
11	LM	White	38	Follicular					(+)
12	LM	AA	37	Follicular	(+)				
13	LM	AA	39	Follicular	(+)	(+)	(+)		
14	LM	AA	36	Luteal	(+)				
15	LM	AA	45	Follicular	(+)	(+)			
16	LM	AA	42	Luteal				(+)	
17	LM	White	42	Follicular	(+)				

PROMOTER-SPECIFIC AROMATASE mRNA SPECIES IN HUMAN UTERINE LEIOMYOMATA AND MYOMETRIAL TISSUES BY 5´-RACE (N=17)



REAL TIME PCR (N=28)





-576 gtccctaggg atactgttta atctggccca tggtacaaga gattttagat cttcattgaa

-516 gtcactagag atggcctgag tgagcacttt gaattcatta gacaactgat ggaaggctct

-456 gagaagacct caacgatgcc caagaaatgt gttcttactg tagaaactta ctattttgat

-396 caaaaaagtc attttggtca aaaaggggag ttgggagatt gccttttgt tttgaaattg

-336 atttggcttc aagggaaga**a gattgcctaa aca**aaacctg ctga**tgaagt ca**caaaatga

C/EBP (-317/-304)

CRE (-292/-285)

-276 ctccacctct ggaatgagct ttatttct**t ataa**tttggc aagaaatttg gctttcaatt

PI.3 TATA box

-216 gggaa**tgcac gtcactc**tac c**cactcaagg gca**ag**atgat aagg**ttctat cagaccaagc

CRE (-211/-199)

CRE(-195/-184)

GATA (-181/-173)

-156 gtctaaagga acctgagact **ctaccaaggt cag**aaatgct gcaattcaag ccaaaagatc

NRHS (-136/-124)

-96 tttcttgggc ttccttgttt tgacttgtaa ccataaatta gtcttgccta aatgtctgat

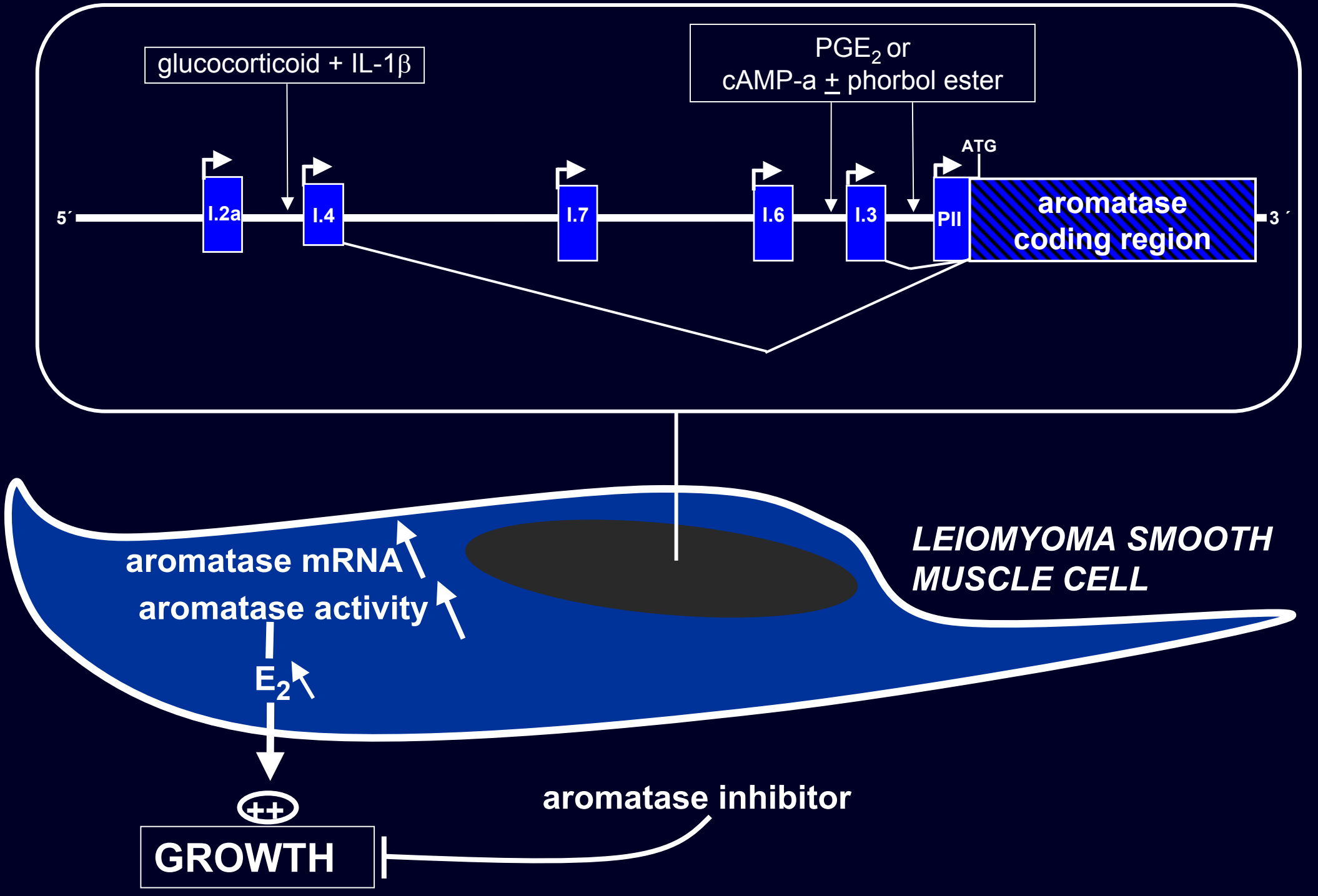
-36 caca**ttataa aa**cagtaagt gaatctgtac tgtacagcac

PII TATA box

AROMATASE EXPRESSION AND TRANSCRIPTION FACTORS

	Endometrium	Endometriosis
Aromatase	-	++++
SF-1	-	++++
LRH-1	++	++

	Myometrium	Leiomyoma
Aromatase	-	++++
SF-1	+	+
LRH-1	+	+


glucocorticoid + IL-1β
PGE2 or
cAMP-a ± phorbol ester
ATG
5´
I.2a
I.4
I.7
I.6
I.3
PII
aromatase
coding region
3´
aromatase mRNA
aromatase activity
E2
LEIOMYOMA SMOOTH
MUSCLE CELL
++
GROWTH
aromatase inhibitor

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