Mechanisms of non-monotonicity

Ana M. Soto

Mechanism

A mechanism is a type of explanation by means of a causal chain, and may involve multiple levels of organization.

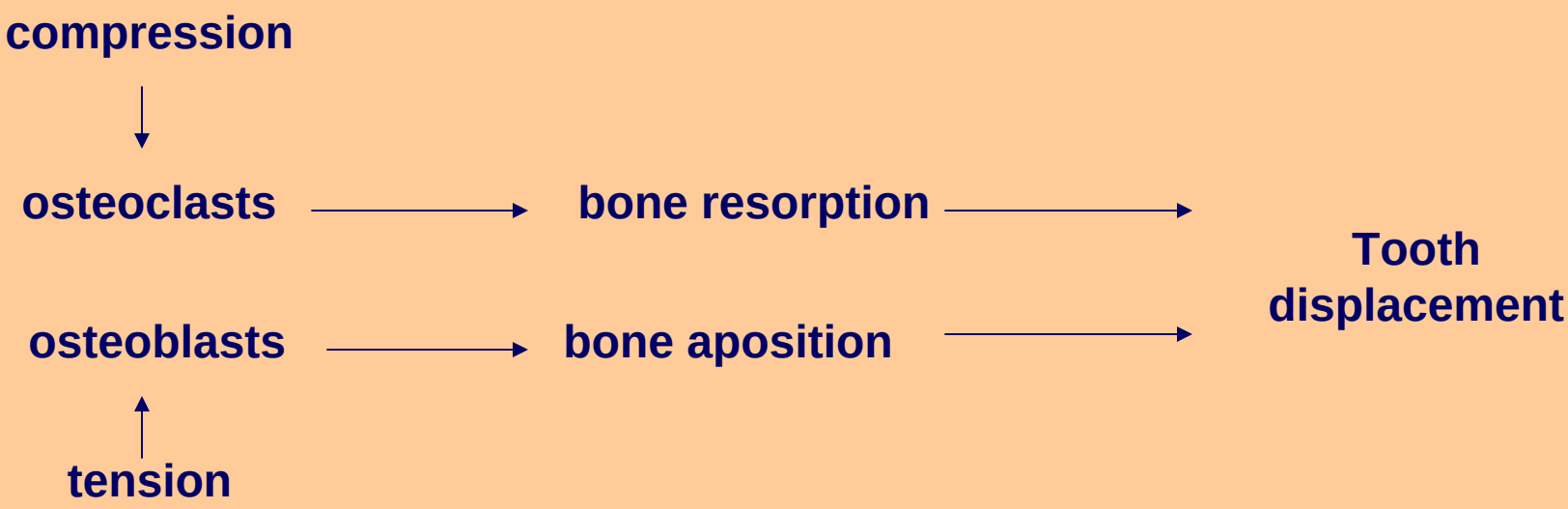
Mechanism

A mechanism **does not** necessarily involve molecules:

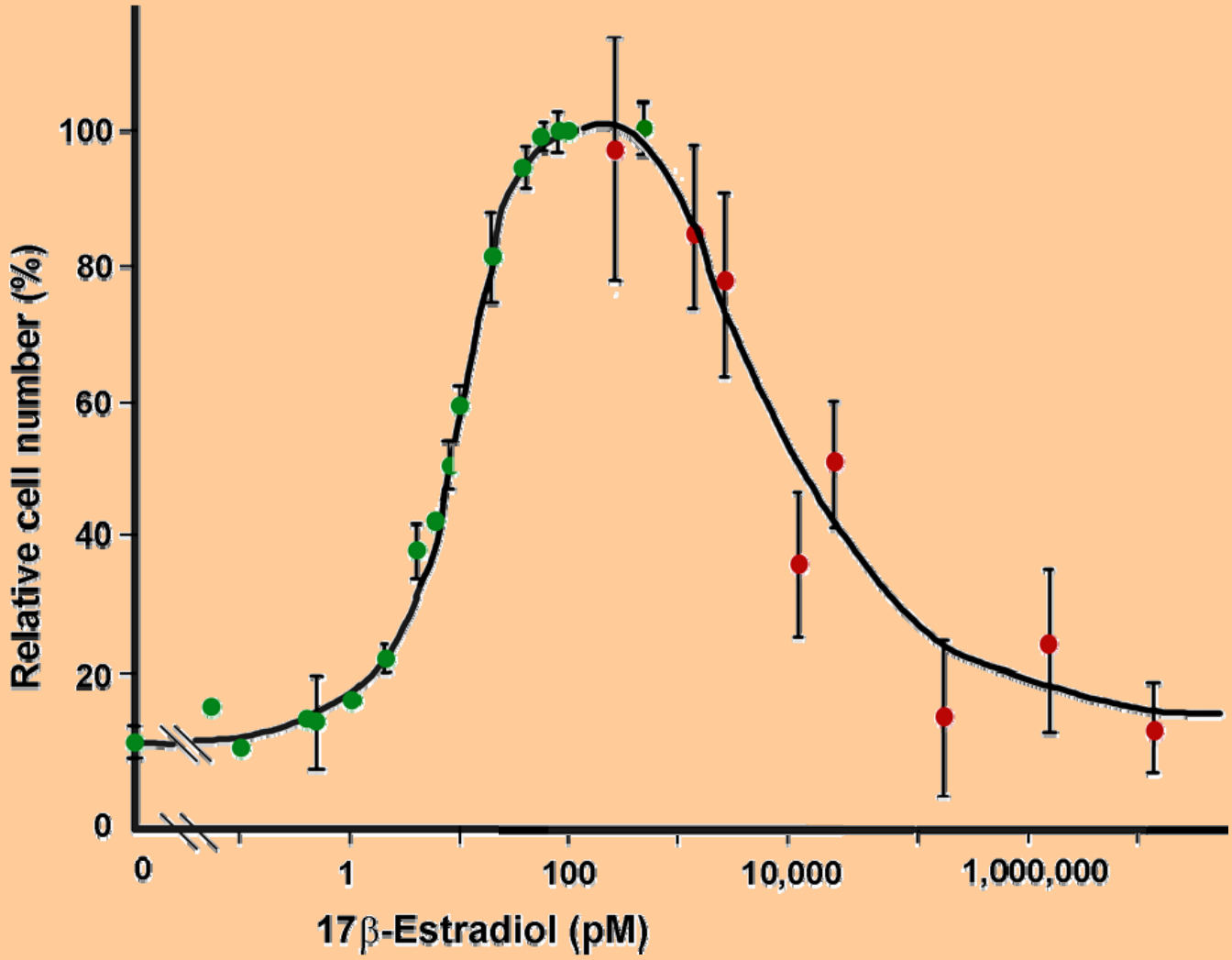
The works of an articulation (joint) can be best and totally explained in terms of biomechanics- every day, throughout the world, thousands of hips are replaced with man-made (metal and synthetic polymers) prostheses.

A mechanism is a type of explanation by means of a causal chain, and may involve multiple levels of organization.

EXAMPLE FROM ORTHODONTICS



A typical monotonic dose-response curve?



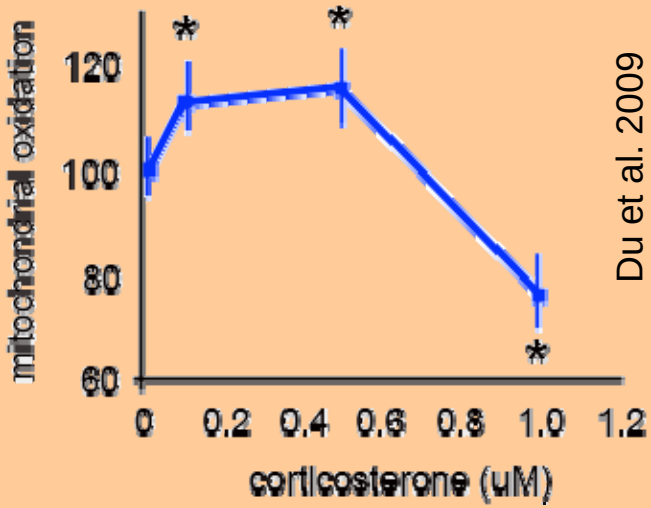
Modified from Coser et al. 2003

Modified from Amara et al. 1987

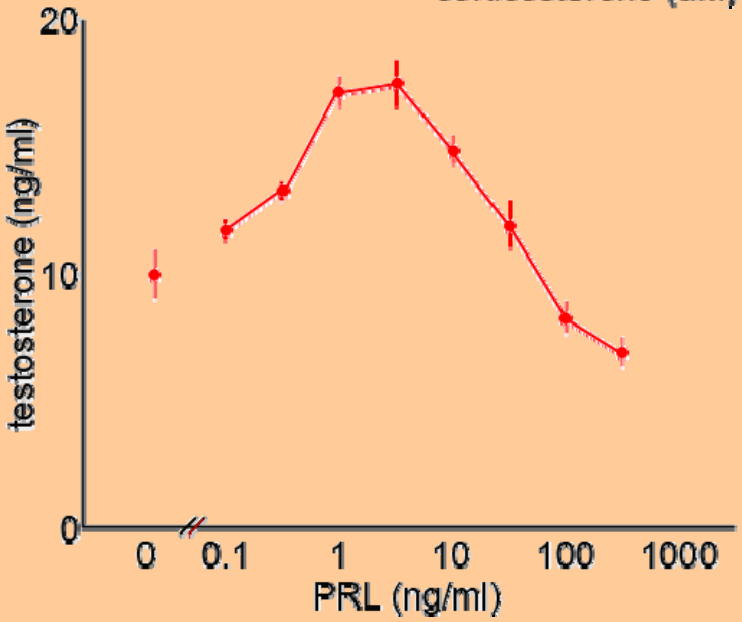
Non-monotonic dose-response curves (NMDRC) are remarkably common in endocrinology

Examples *in vitro*

Estradiol
DHT
Androstenedione
Corticosterone
Insulin
Progesterone
Prolactin
hCG
T3
Growth hormone



Du et al. 2009

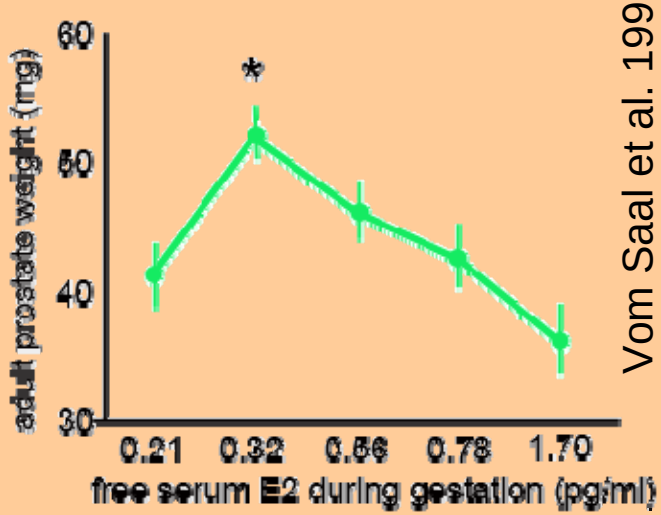


Welsh et al. 1986

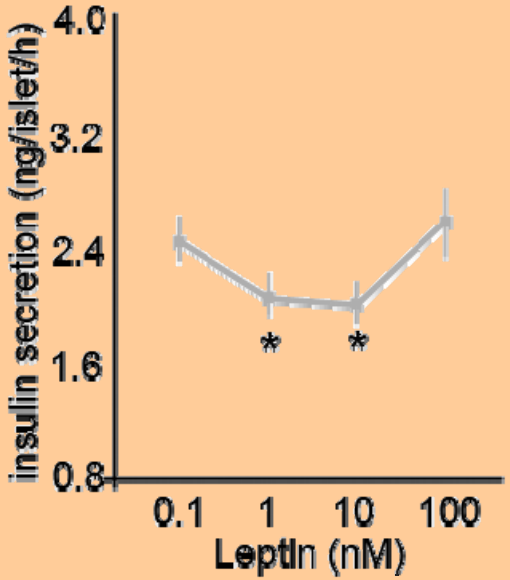
Non-monotonic dose-response curves (NMDRC) are remarkably common in endocrinology

Examples *in vivo* (animals)

Estradiol
Testosterone
Hydroxyandrosterone
Corticosterone
Glucocorticoid
Leptin
Oxytocin
Melatonin
Dopamine



Vom Saal et al. 1997

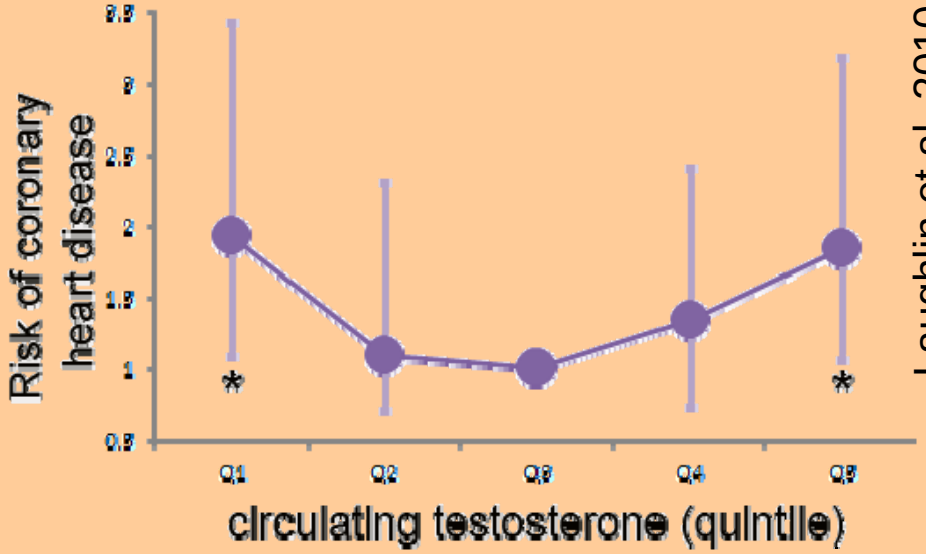


Pallett et al. 1997

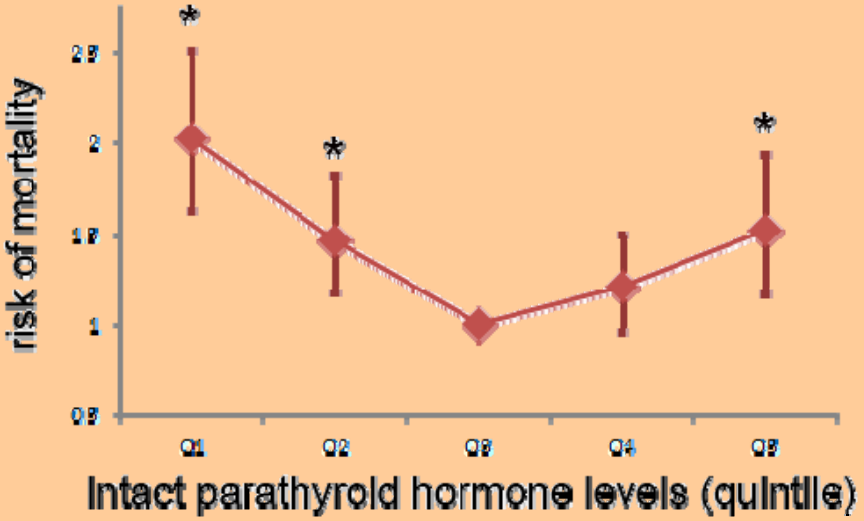
Non-monotonic dose-response curves (NMDRC) are remarkably common in endocrinology

Examples from epidemiology

Testosterone (free)
TSH
Leptin
Insulin
Cortisol
Parathyroid hormone



Laughlin et al. 2010

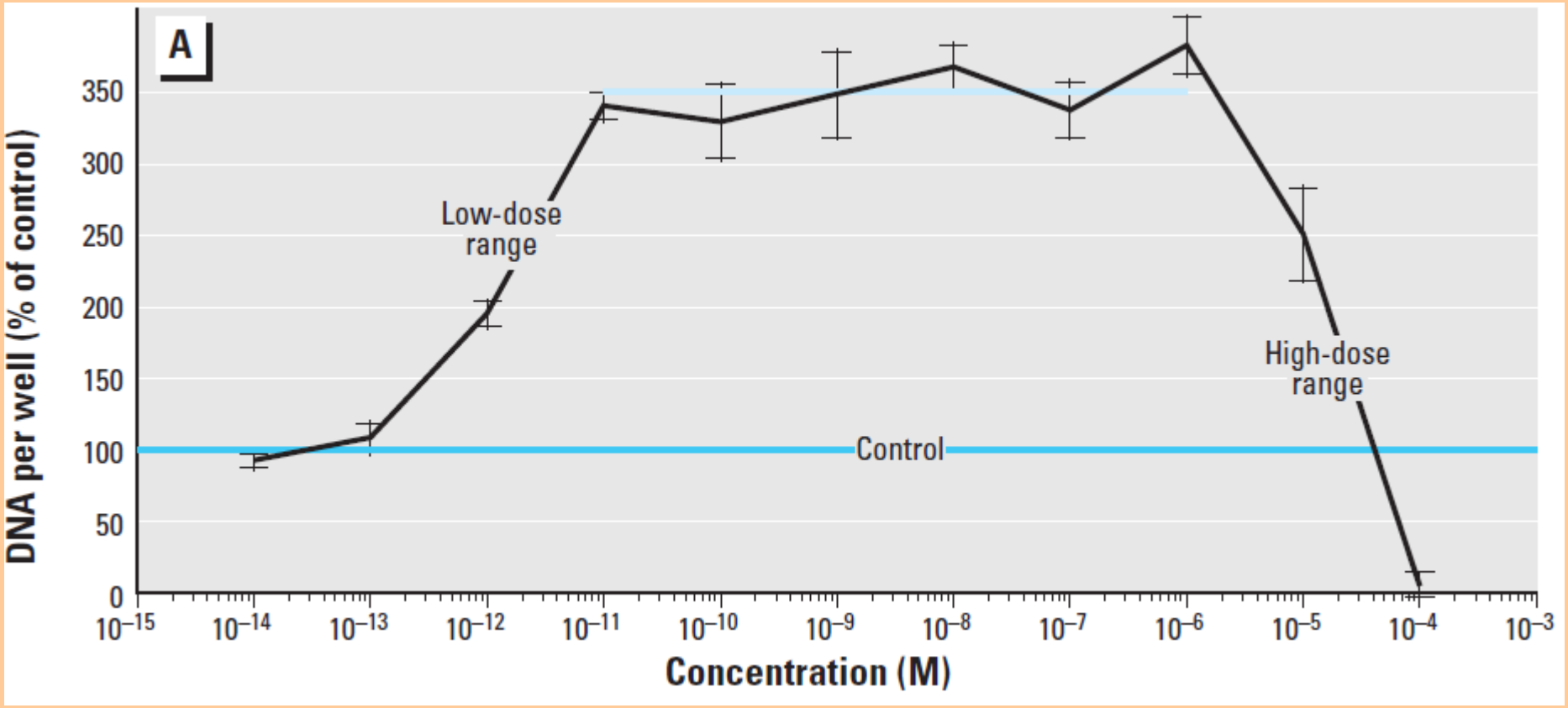


Floege et al. 2011

NMDRC: Mechanisms

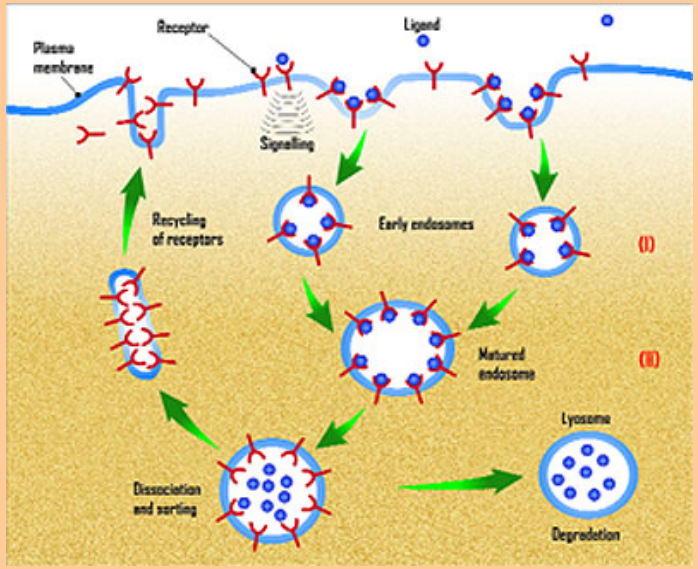
1. Cytotoxicity
2. Receptor down-regulation & desensitization
3. Cell- and tissue-specific receptors and cofactors
4. Receptor selectivity
5. Receptor competition
6. Endocrine negative feedback loops
7. Tissue interactions

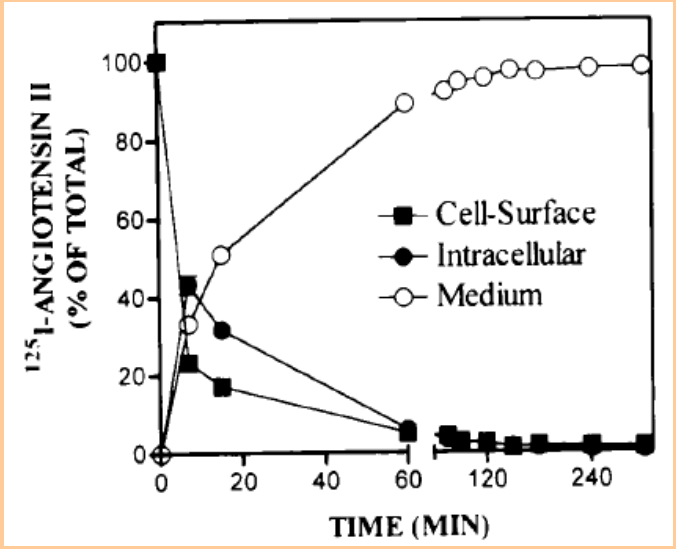
1. Cytotoxicity

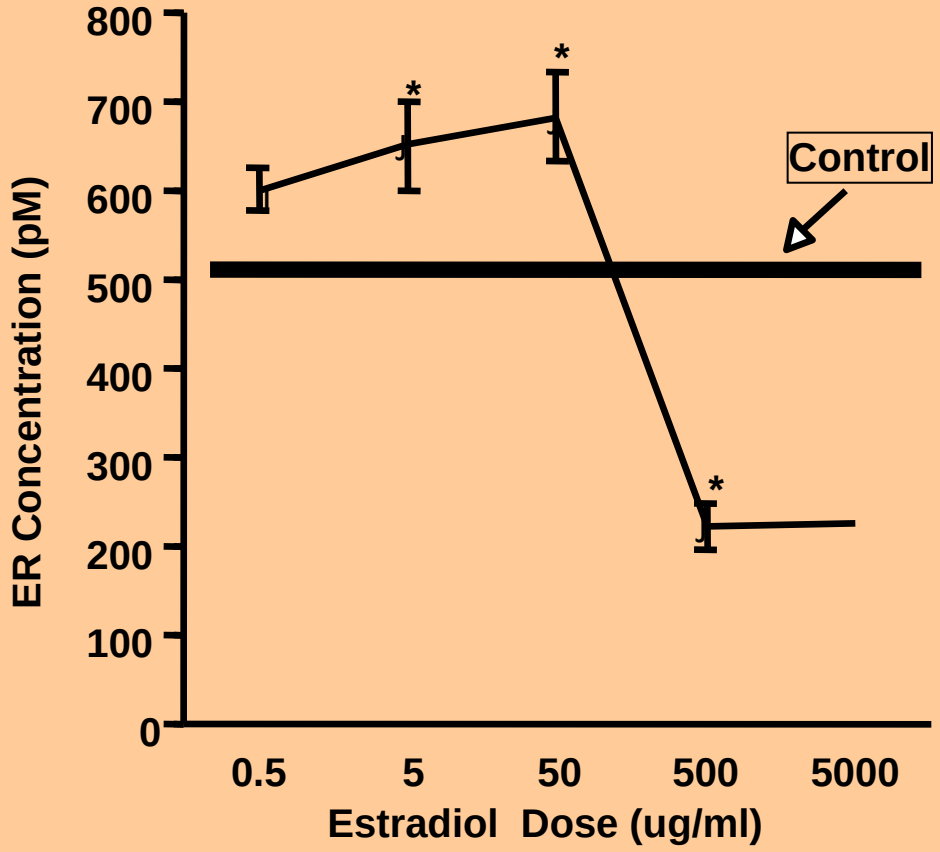


Welshons et al. 2003 [Ref 38]

2. Receptor down-regulation



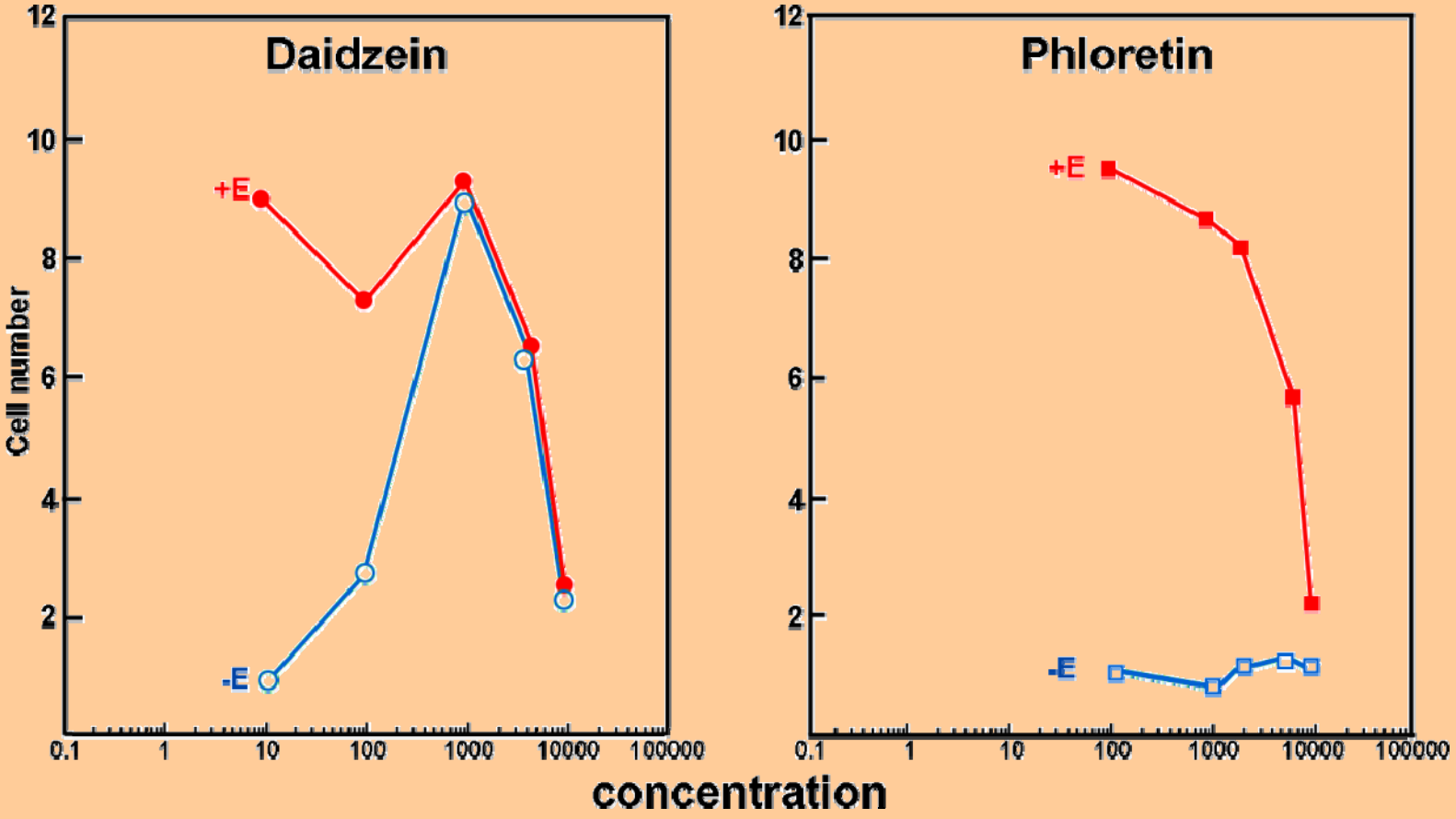




Medlock et al. 1991 [FDA scientists at NCTR]

Modrall et al. 2001 [Ref 529]

3. Cell- and tissue-specific receptors and cofactors: phytoestrogens and the proliferation of MCF7 cells



+E2= 100 pM

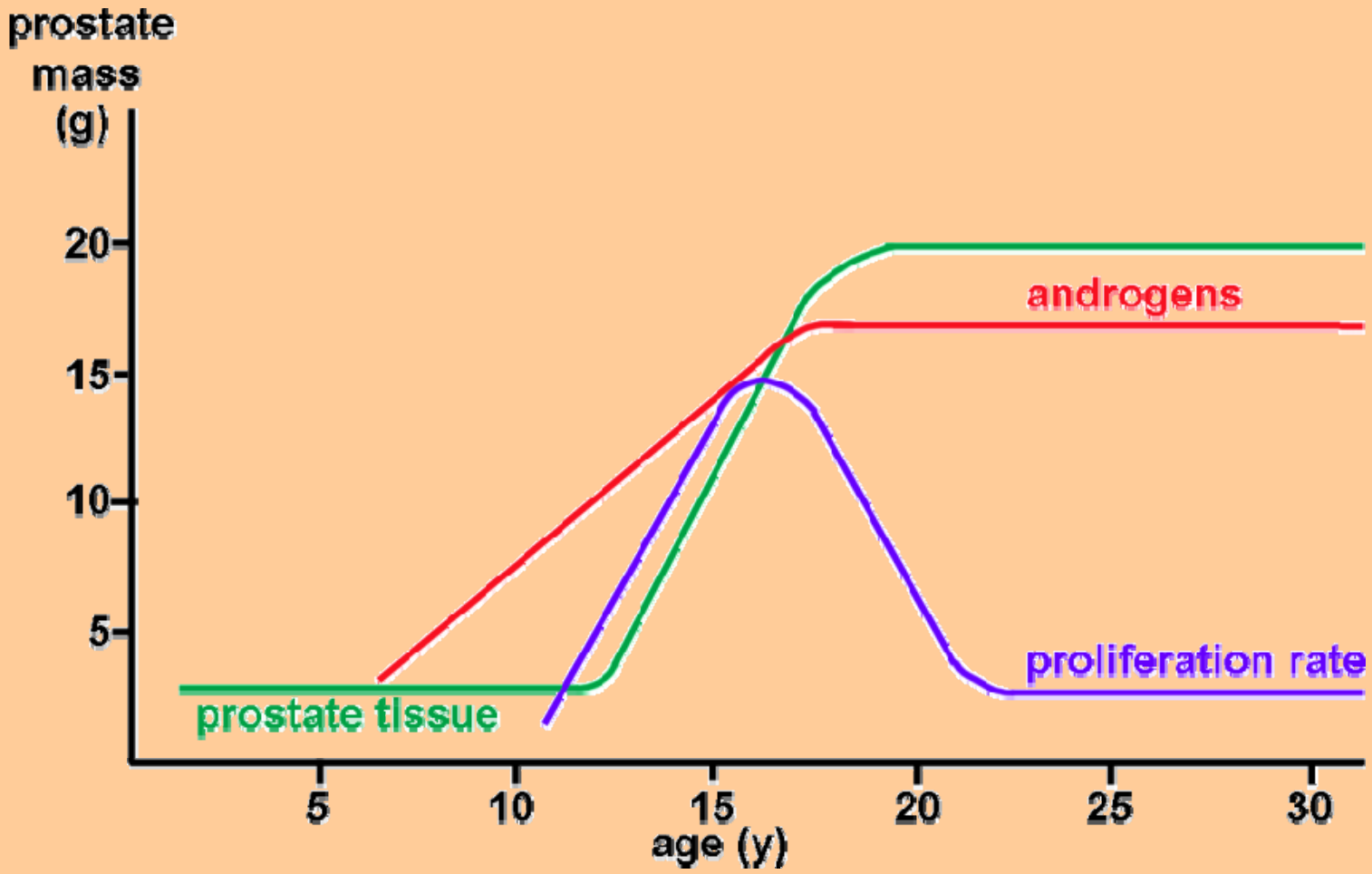
low doses=increased cell proliferation (ER-mediated)

High doses=inhibition of cell proliferation (non-ER mediated)

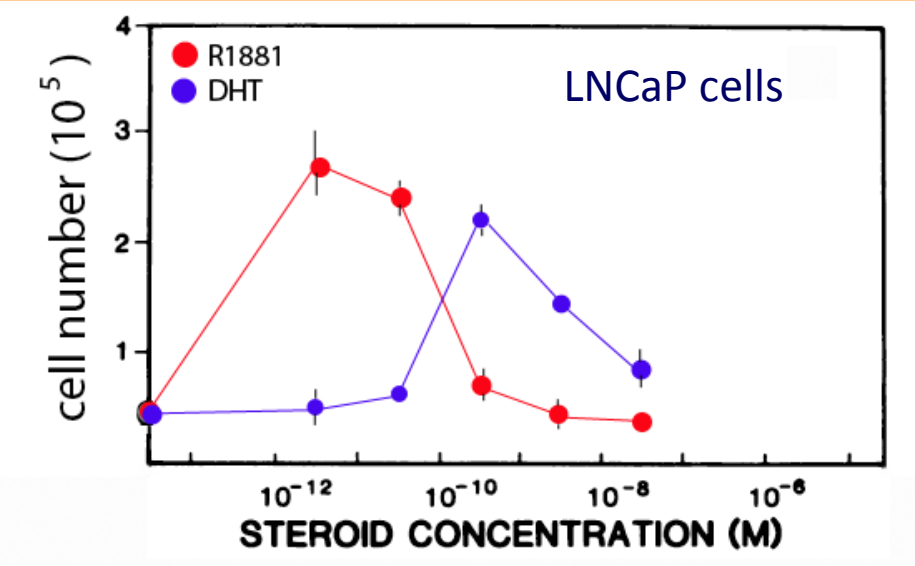
The ER-independent effect is inhibition of cell proliferation

Treatment	% G1	% S	% G2M
Control (5% CD-FBS)	89.9	5.2	4.9
100 pmol/L E2	58.0	30.1	11.9
10 µmol/L daidzein	88.6	6.5	4.9
10 µmol/L daidzein + 100 pmol/L E2	89.9	4.8	5.3
10 µmol/L genistein	91.0	5.4	3.5
10 µmol/L genistein + 100 pmol/L E2	90.4	5.7	3.9

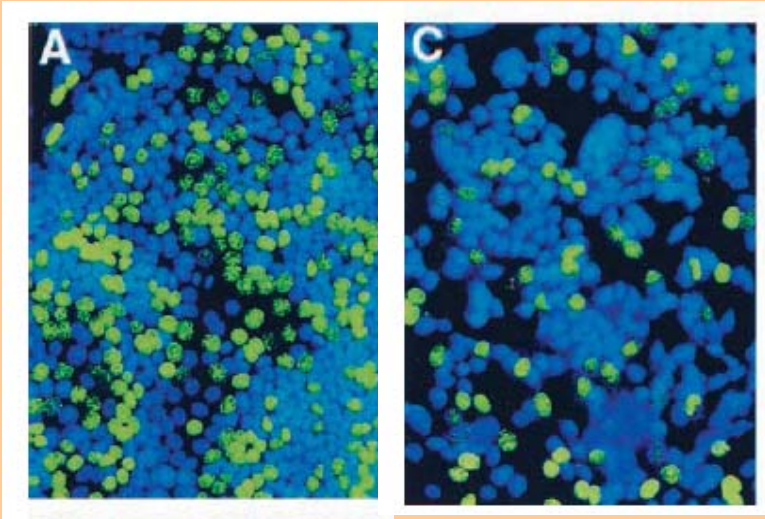
Same receptor, different androgen-receptor occupancy



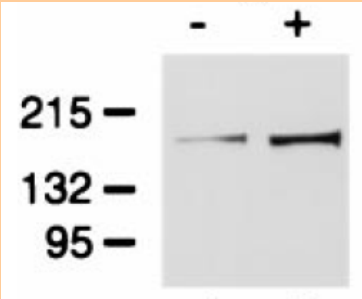
Same receptor, different androgen-receptor occupancy (cell culture)



Sonnenschein et al. 1989 [Ref 499]

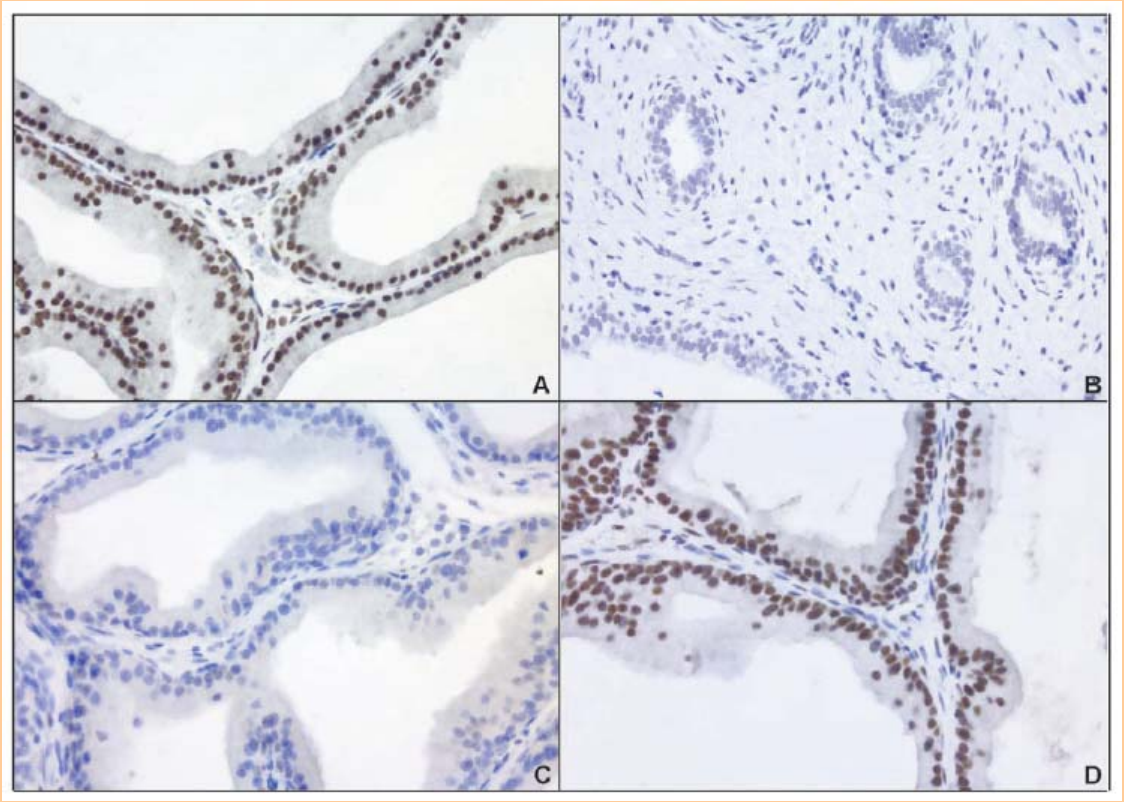


Geck et al 2000 [Ref 56]



At high doses androgens induce a transcription factor, **aprin**, that inhibits cell proliferation producing a Go arrest

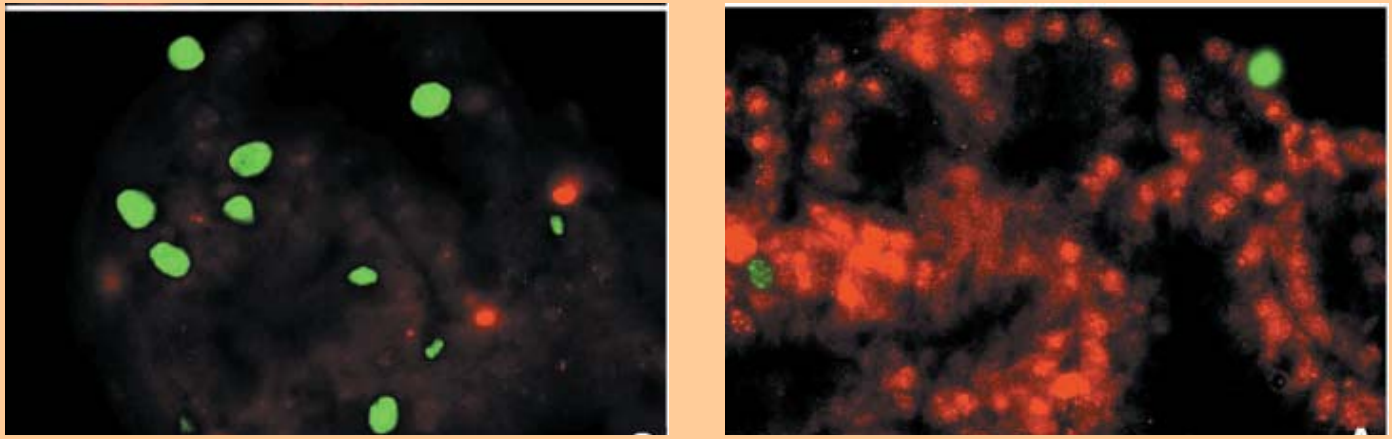
Dissociation of the proliferative and quiescence-promoting effect of androgen in the rat prostate



Aprin expression in the rat prostate. A, Intact adult rat; B, chronically castrated rat; C, maximal cell proliferation induced by 3 d androgen replacement in a castrated rat; D, proliferative shutoff after 7 d of androgen treatment in a castrated rat.

Maffini et al, Endocrinology 2002, 143:2708–2714

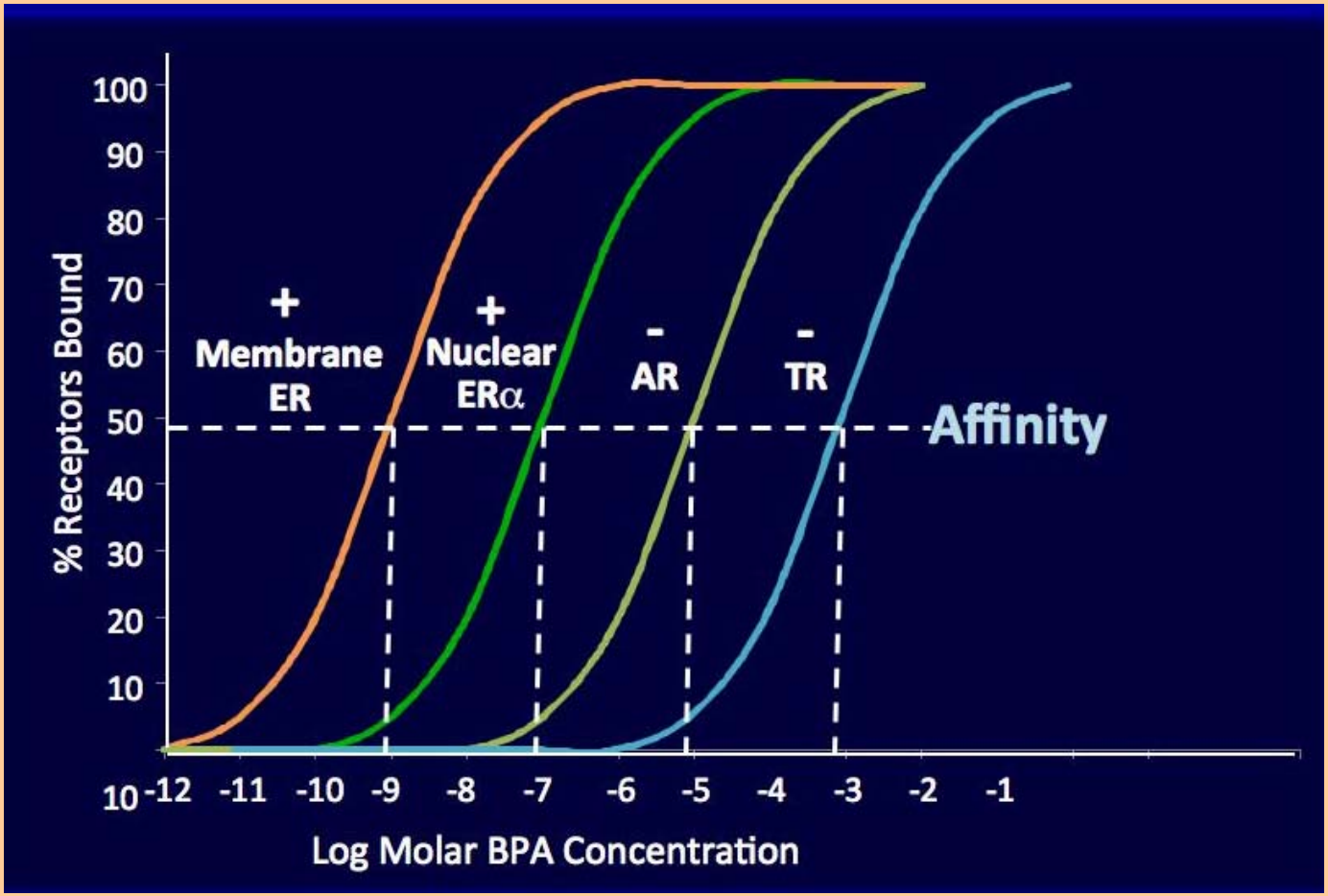
Dissociation of the proliferative and quiescence-promoting effect of androgen in the rat prostate



Left: maximal cell proliferation induced by 3 d androgen replacement in a castrated rat; Right, proliferative shutoff after 7 d of androgen treatment in a castrated rat. Green: BrdU, red: Aprin

Maffini et al, Endocrinology 2002, 143:2708–2714

4. Receptor selectivity: BPA

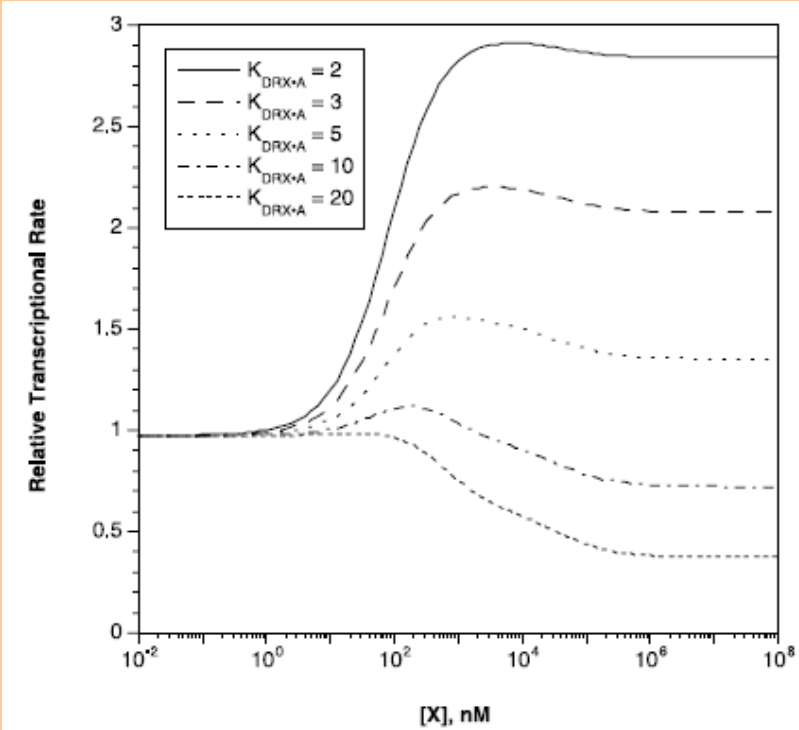


Fred Vom Saal

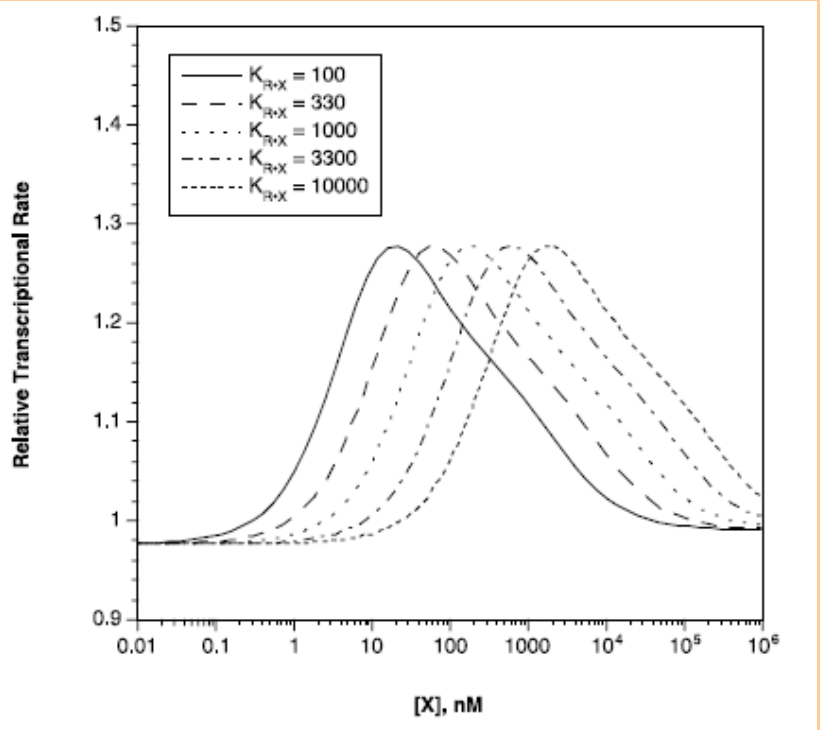
4. Receptor selectivity: BPA

- Very low dose: membrane ERs (Nadal, Watson)
- Intermediate dose: nuclear ERs (Shioda)
- High dose TR (ref 249)
- High dose: AR (ref 521)

5. competition with endogenous hormones for receptors



Changing affinity of receptor for a co-activator

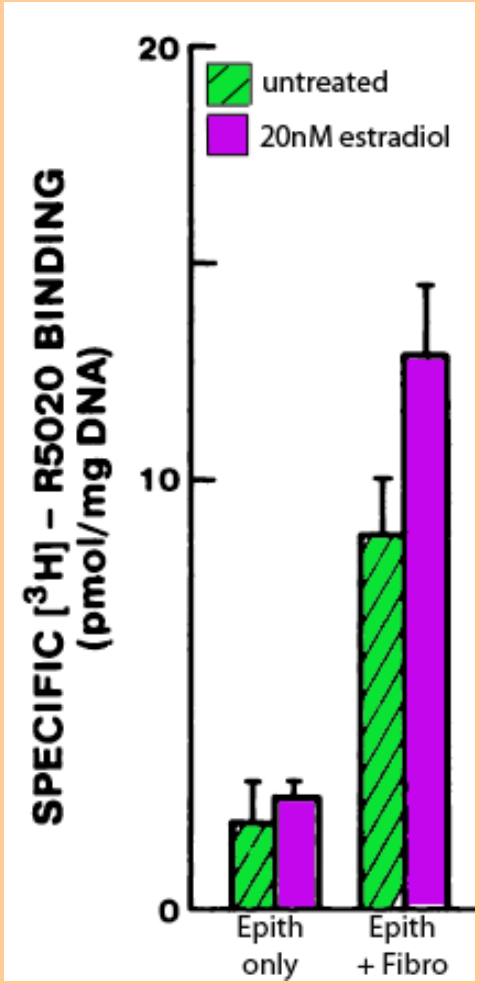


Changing affinity of receptor for a xenobiotic

Kohn & Melnick 2002 [Ref 42]

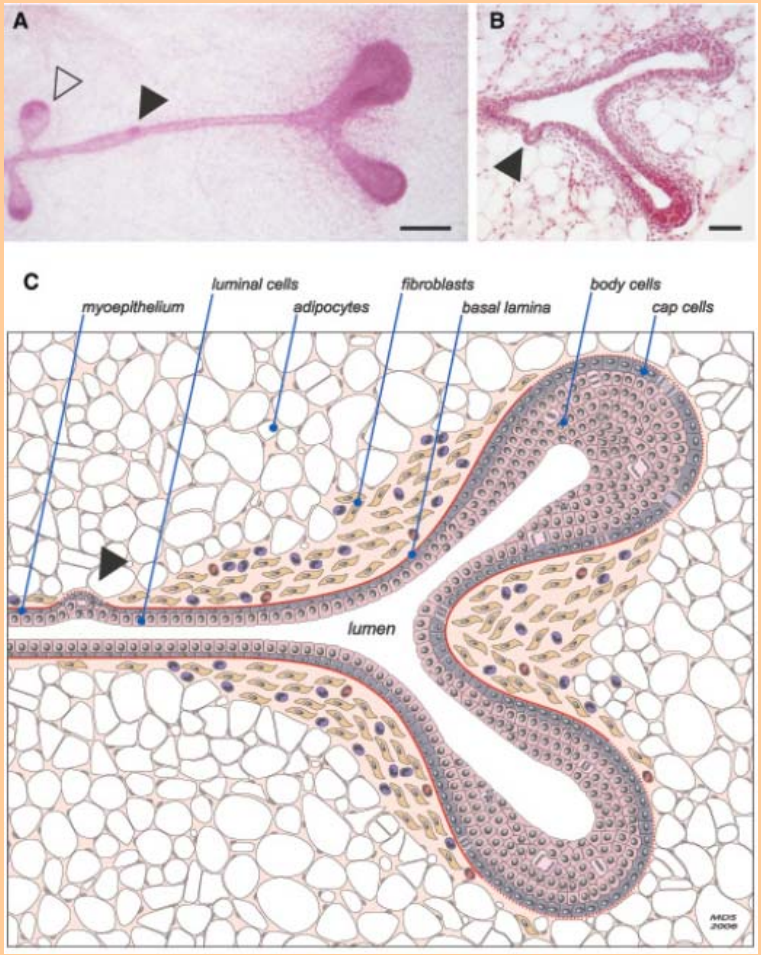
In the presence of natural hormone, at low concentrations, some unoccupied receptors bind to the EDC. At high concentrations, the EDC outcompetes the natural hormone.

6. Cell-cell interaction

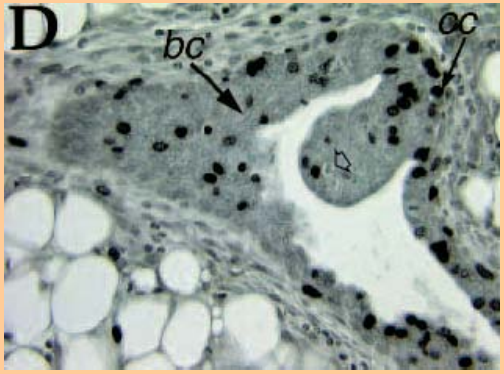


Haslam 1986 [Ref 519]

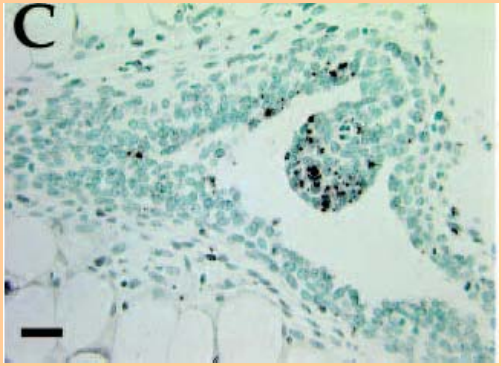
Branching morphogenesis in the mammary gland



Proliferation



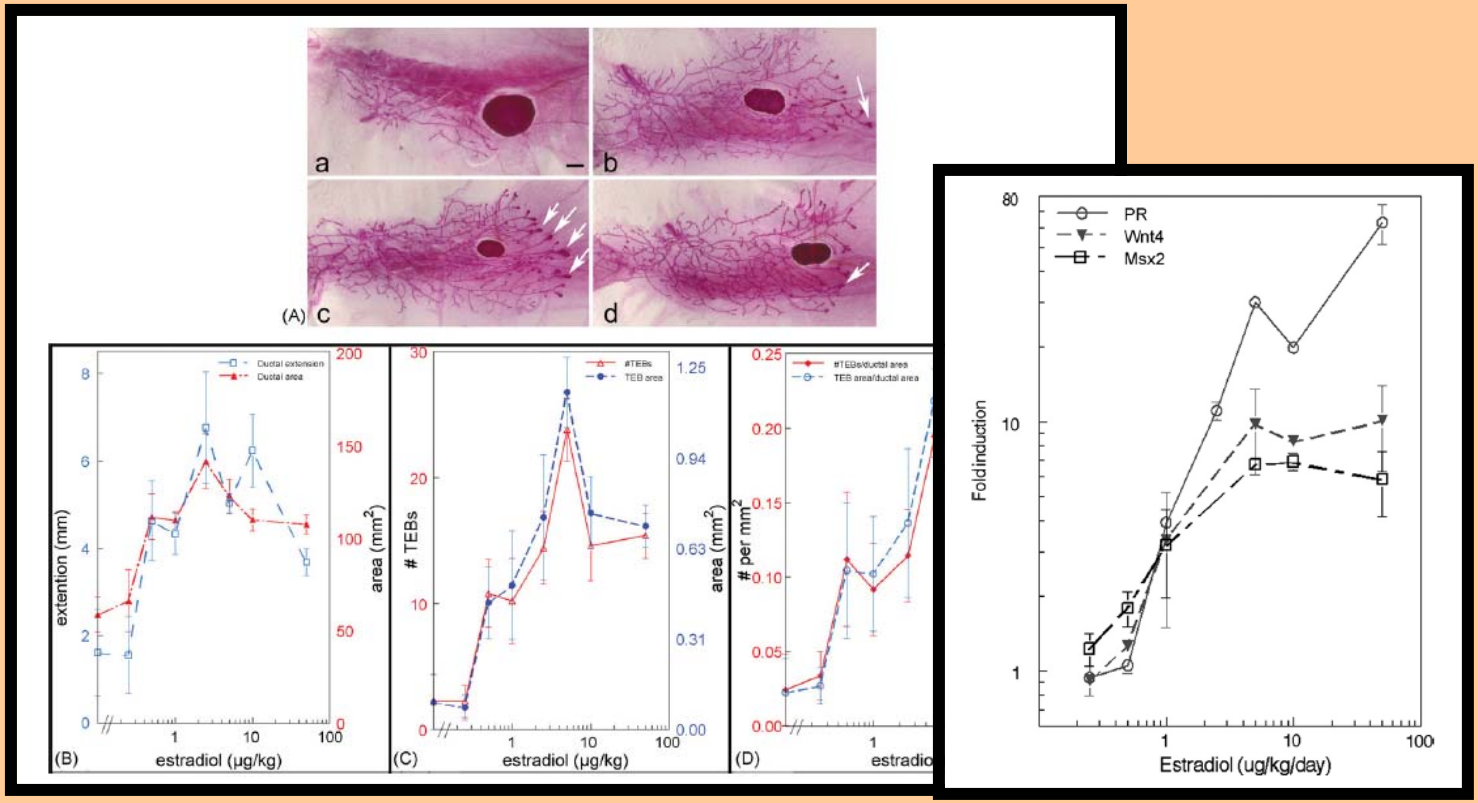
Cell Death



Sternlicht et al. Differentiation. 2006, 74:365-81.

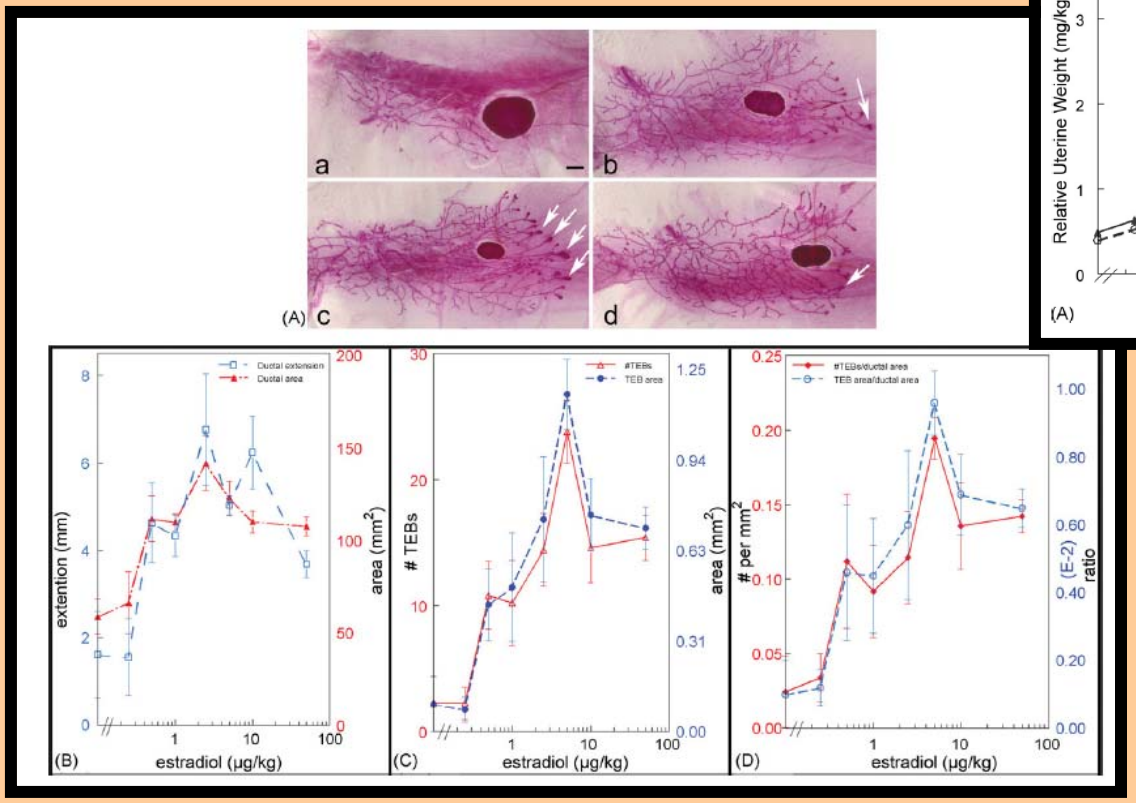
Humphreys RC et al, Development, 1996;122:4013-22

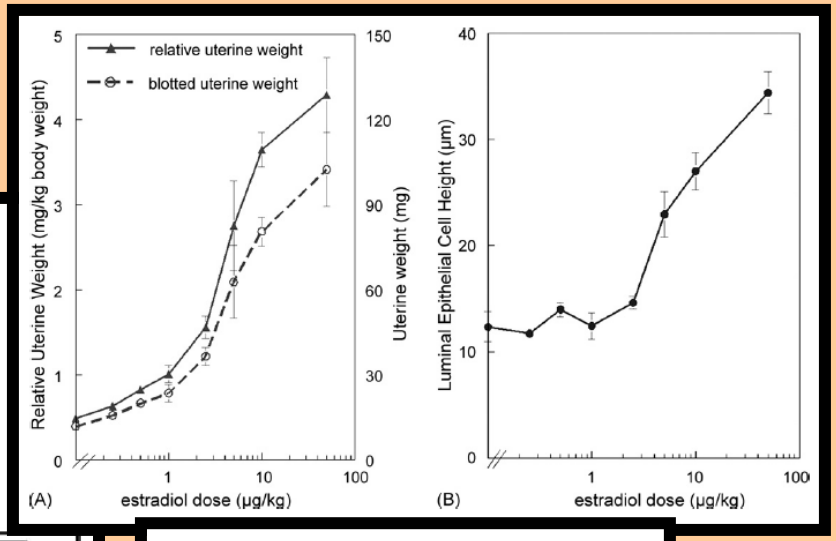
NMDRCs due to tissue interactions

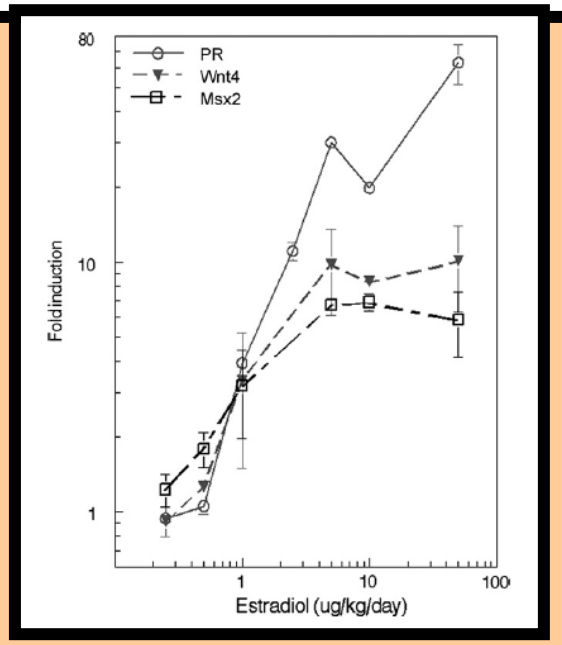


Vandenberg et al. 2006 [Ref 541]

NMDRCs are specific to doses examined, tissues & endpoints

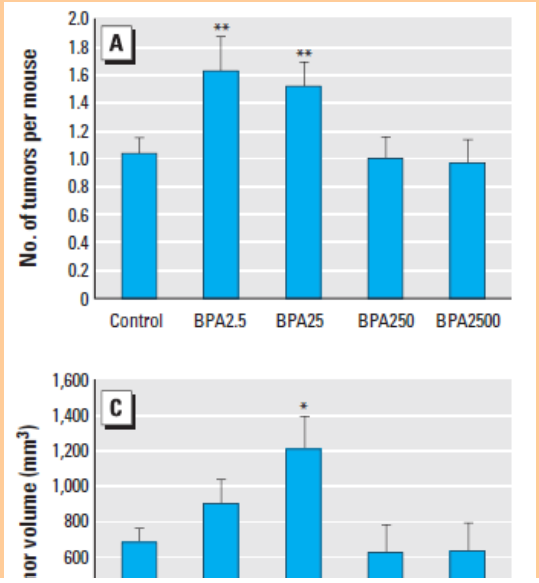


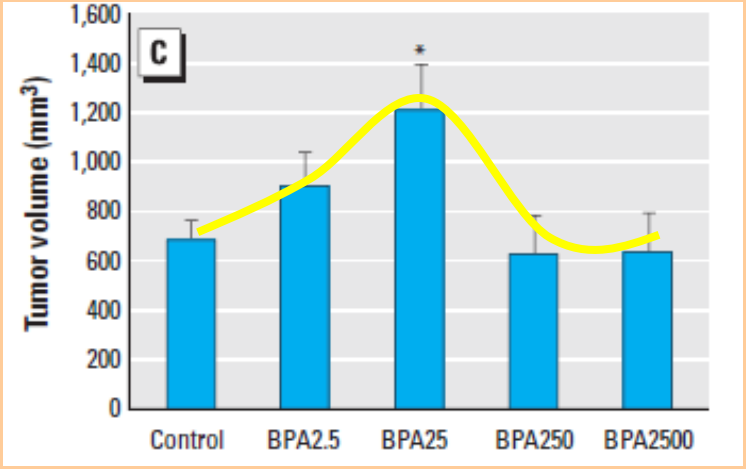


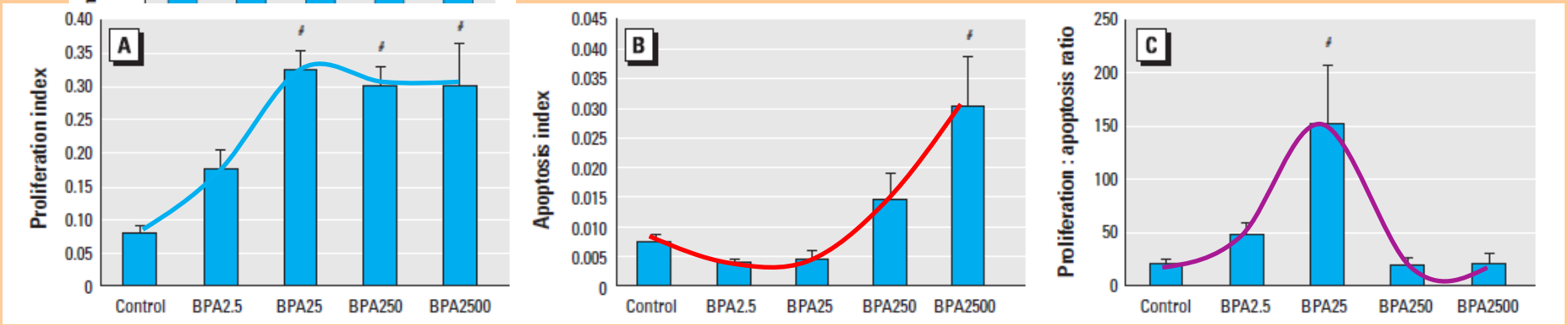


Vandenberg et al. 2006 [Ref 541]

The same mechanisms manifest in normalcy and in cancer







Jenkins et al. 2011 [Ref 293]

Mechanisms *In Vivo*

- Many of the same mechanisms that operate *in vitro* are observed *in vivo*.
- Tissues are more complicated: receptor expression changes at different developmental periods; multiple cell types are present and in contact with varying levels of receptor expression; tissue compartments interact and influence each other.

Do we need to know the underlying mechanisms in order to accept the existece of NMDRC?

Example: Since time immemorial, humans castrated animals to make them useful to them. Our ancestors **did know** that castrated animals would not reproduce. They **did not know** why. Mechanistic explanations were generated thousands of years after this practice became common.

Conclusion: No, we do not need to know mechanisms in order to accept the existence of a phenomenon!

Conclusions

- NMDRC occur at all levels of biological organization
- Both natural hormones and endocrine disruptors produce NMDRC
- The mechanisms underlying NMDRC are well understood
- The existence of a phenomenon is not defined by the mechanism producing it