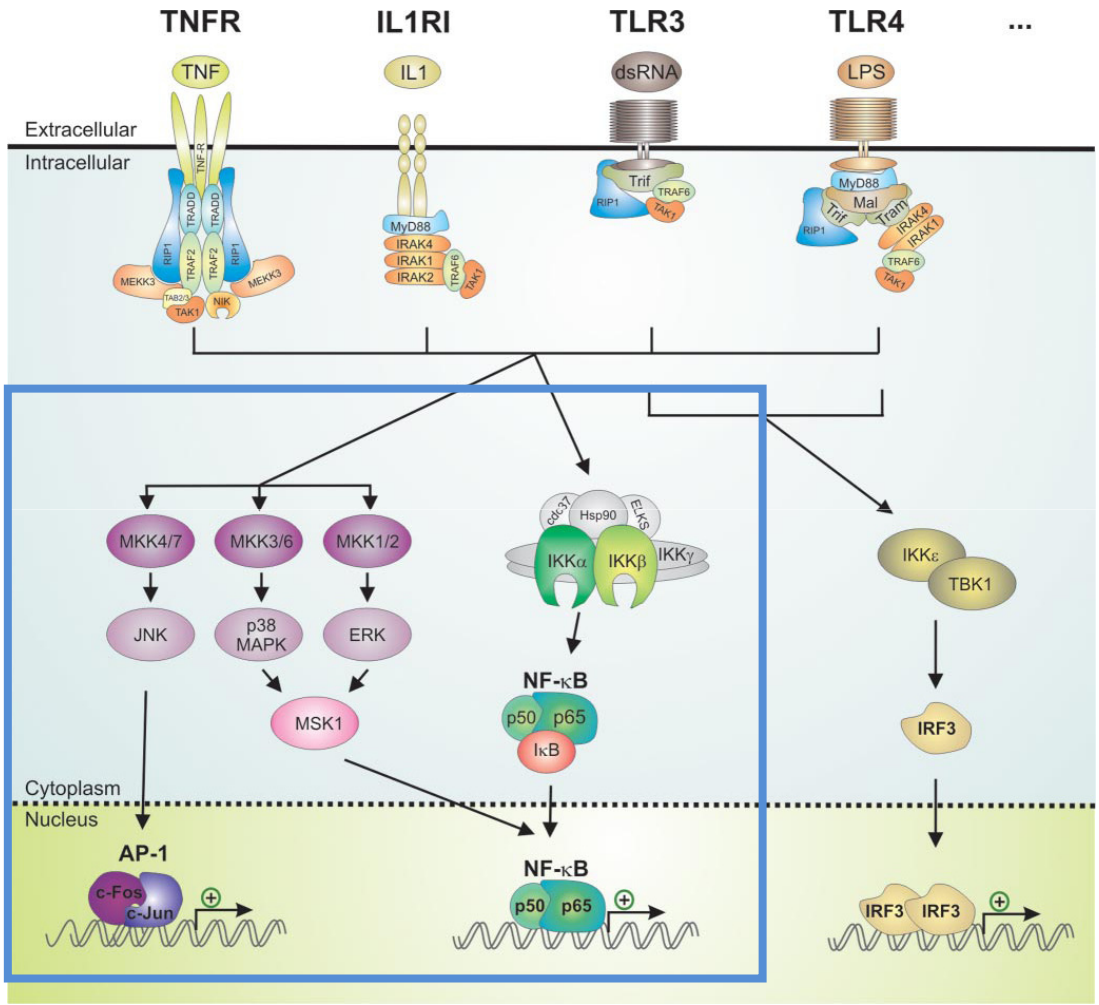
Selective modulation of the Glucocorticoid Receptor can distinguish between transrepression of NF-κB and AP-1

Ilse M. Beck

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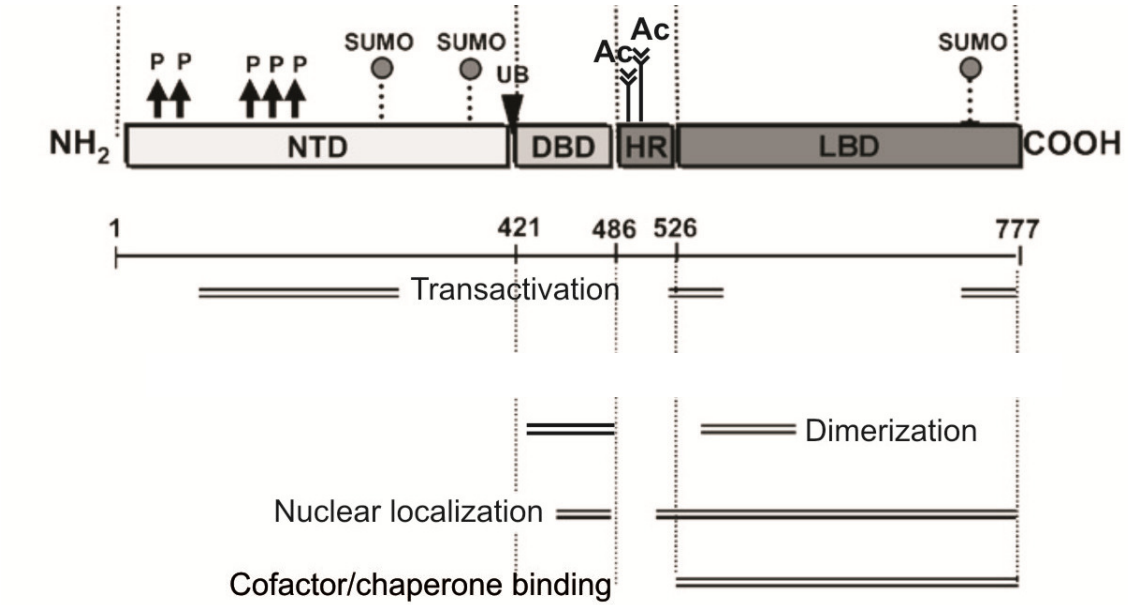
Inflammatory signalling pathways



Source:Beck et al., Endocrine Reviews, 2009

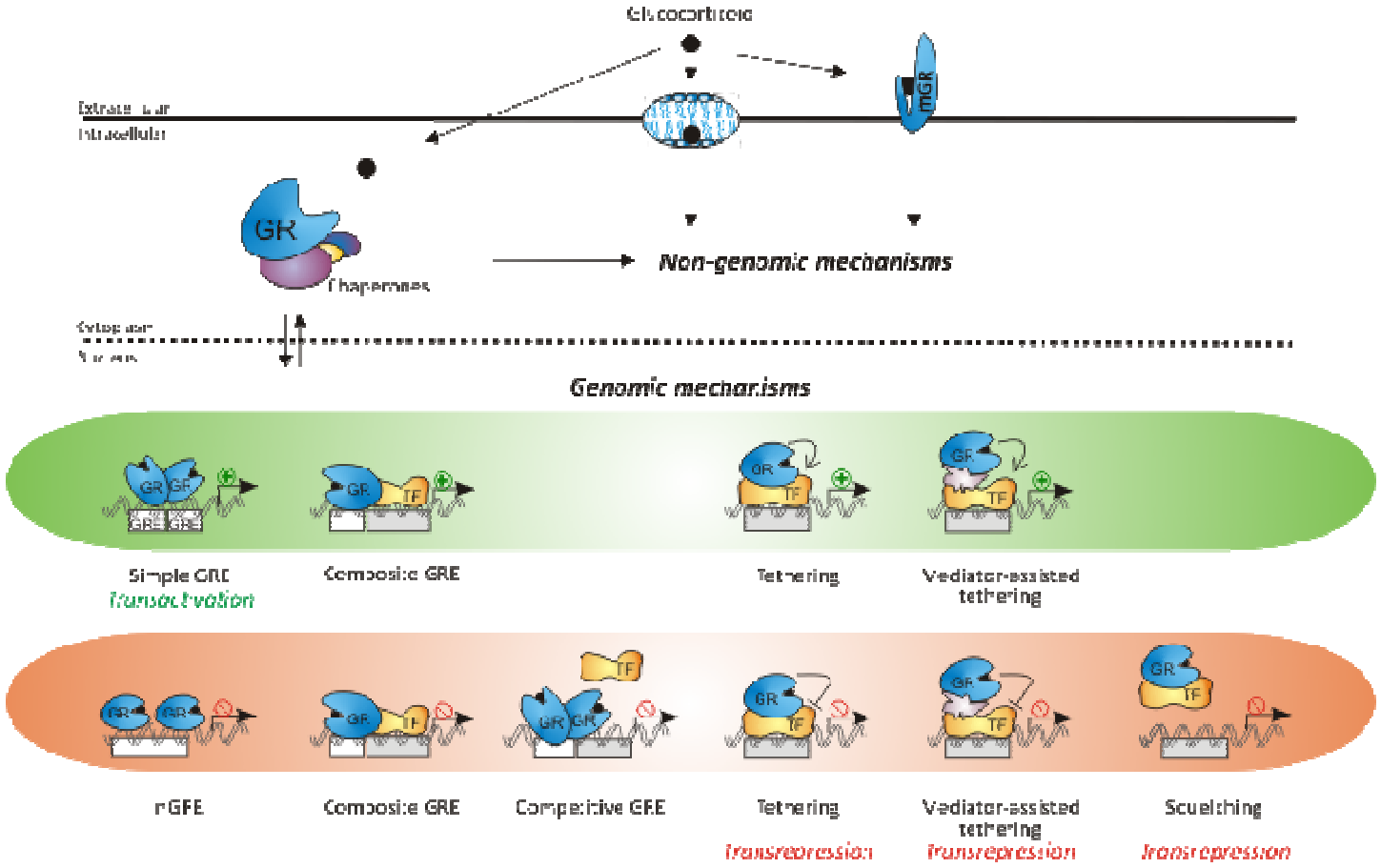
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The Glucocorticoid Receptor



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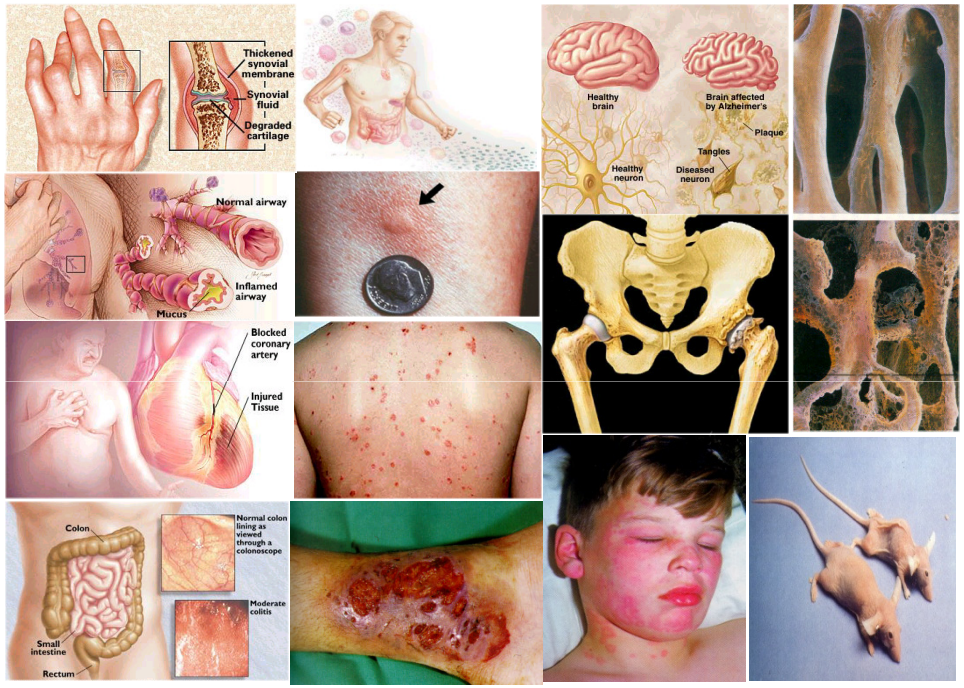
Glucocorticoid Receptor-mediated transcriptional mechanisms



Source: Ratman et al. 2013

The Glucocorticoid Receptor: clinical utility

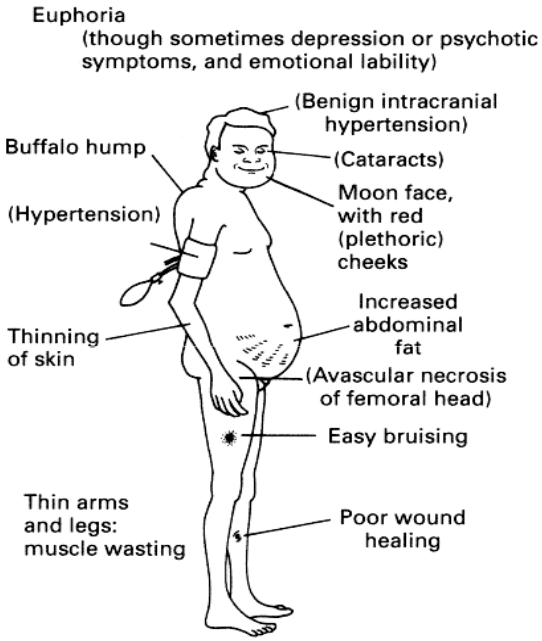
Indications



TNF-implicated/NF-κB-mediated disorders

-Rheumatoid arthritis
-Vascular inflammation
-Asthma
-Skin inflammation
-Inflammatory bowel diseases
-Neurodegenerative diseases

Adverse effects

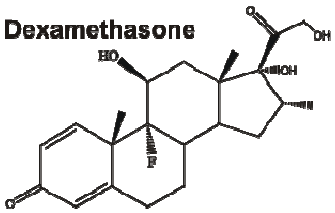


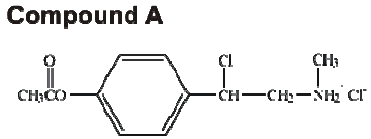
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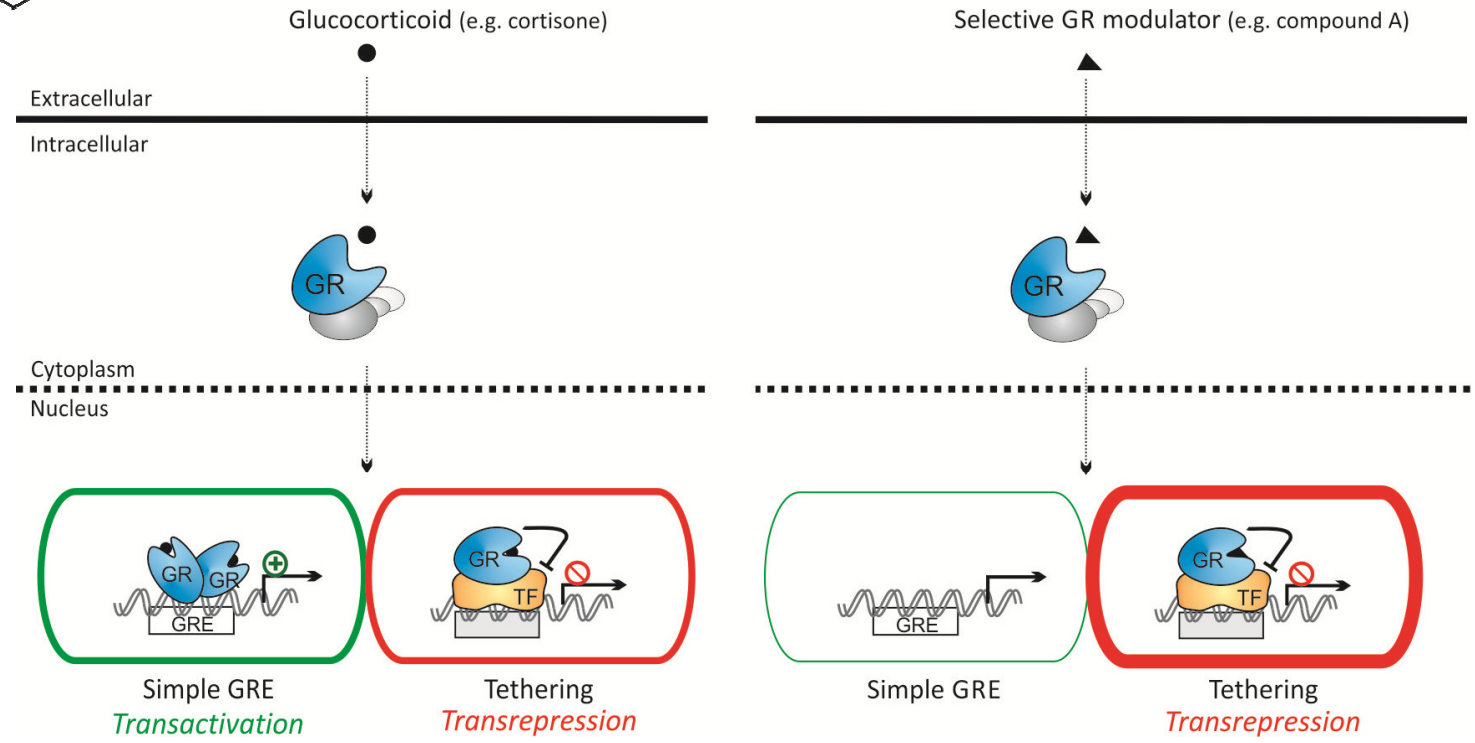
Osteoporosis
Tendency to hyperglycaemia
Negative nitrogen balance
Increased appetite
Increased susceptibility to infection
Obesity

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CpdA is a non-steroidal GR modulator that supports GR-mediated gene repression but no classic GR-mediated transactivation



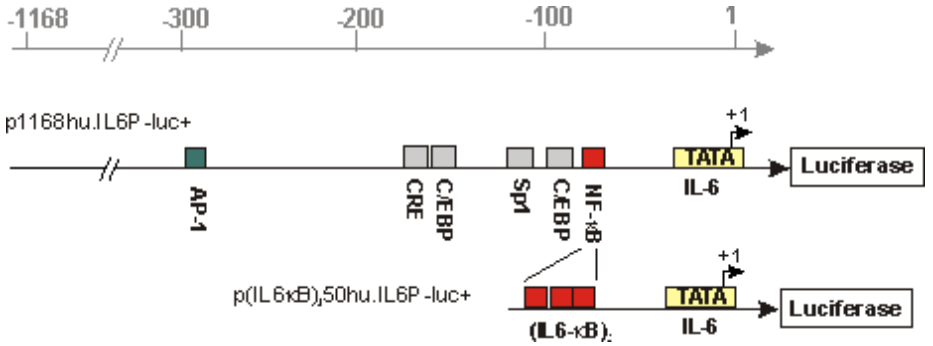


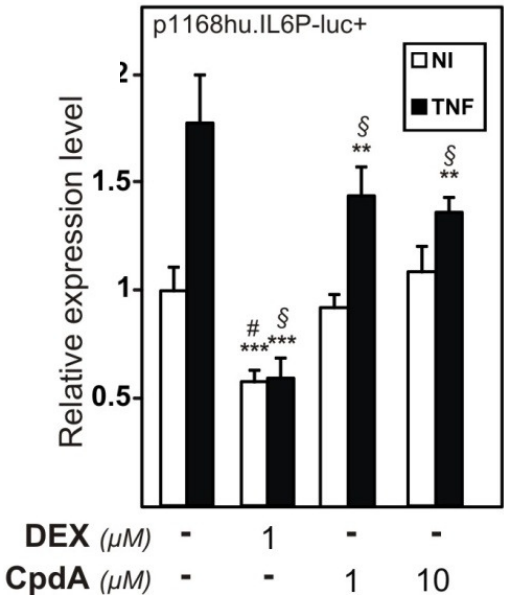


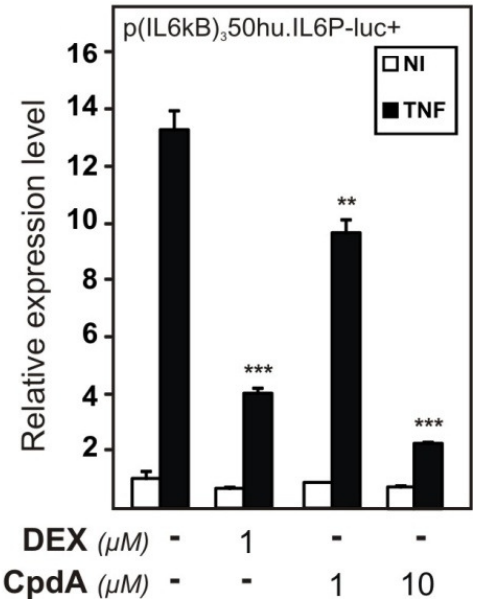
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Compound A favours selective GR transrepression of NF-κB in the human IL-6 promoter

DEX=strong synthetic glucocorticoid & GR agonist



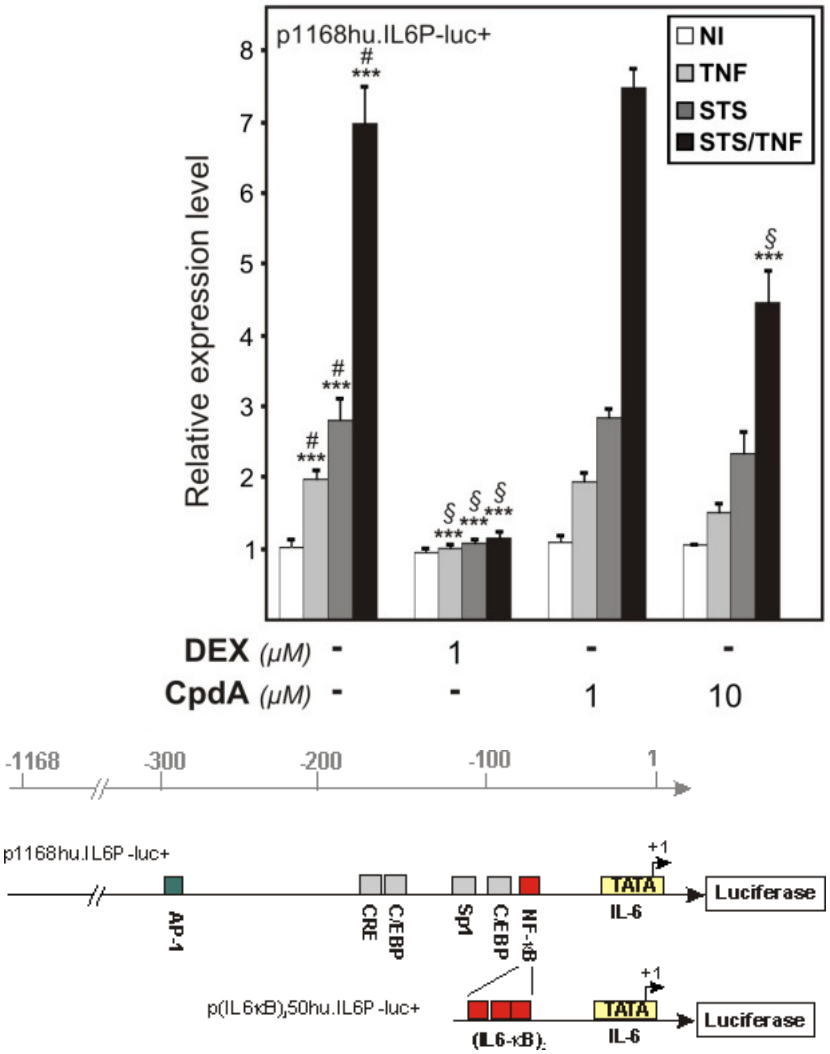


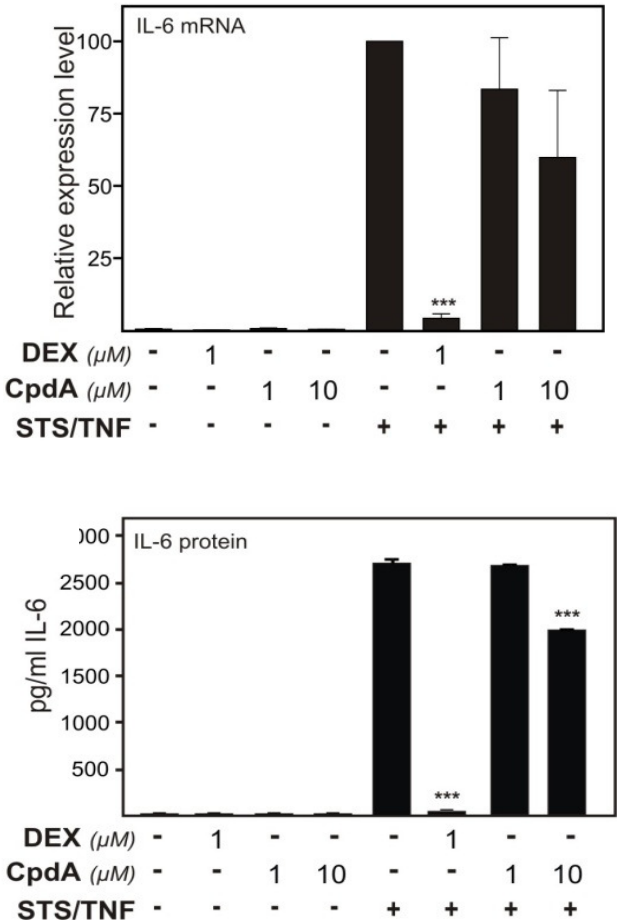


L929sA, murine fibrosarcoma cells

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A strong AP-1-activating signal driving the IL-6 promoter does not concur with an efficient transrepression by **CpdA**-activated GR





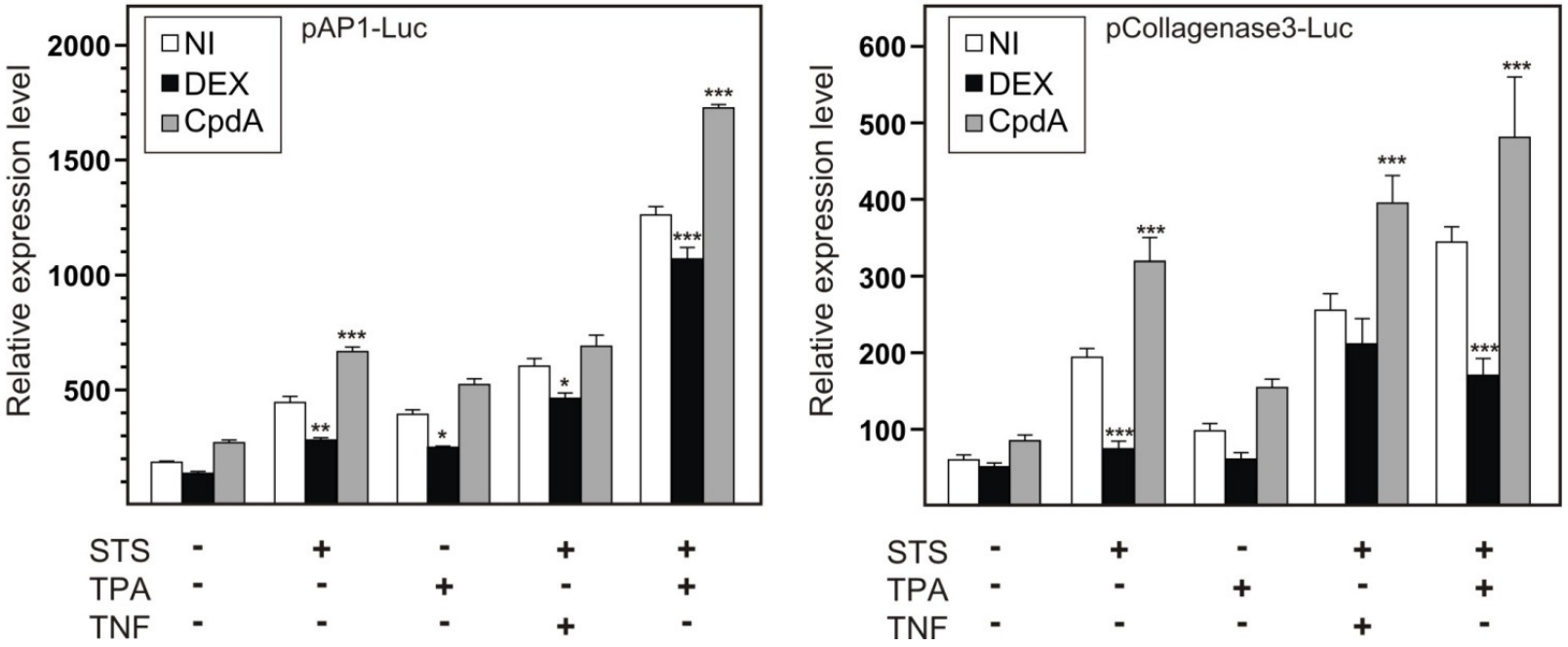
L929sA, murine fibrosarcoma cells

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In contrast to DEX,
Compound A does not transrepress AP-1-driven promoter activity

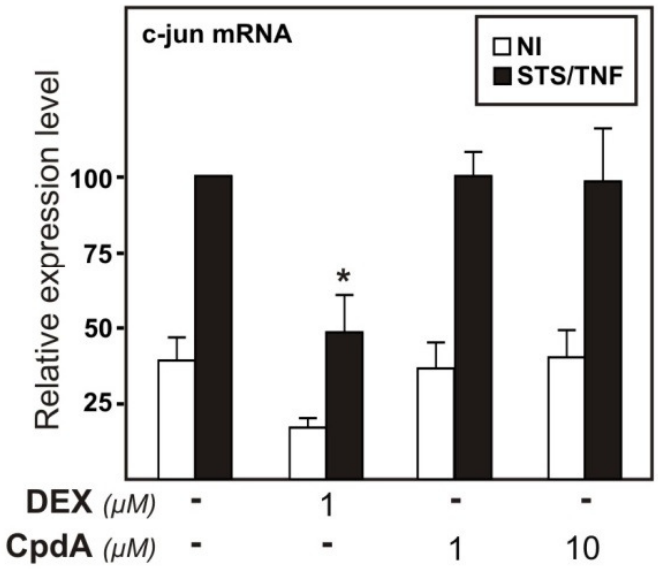


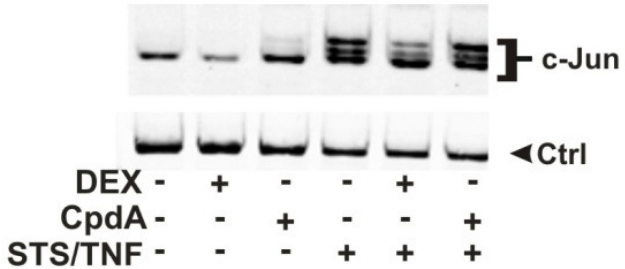
The divergent transrepression characteristics of DEX and CpdA are largely maintained across different AP-1 reporter gene models, regardless of the AP-1-activating stimuli.

L929sA, murine fibrosarcoma cells

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In contrast to DEX, **Compound A** sustains AP-1-induced gene and protein expression

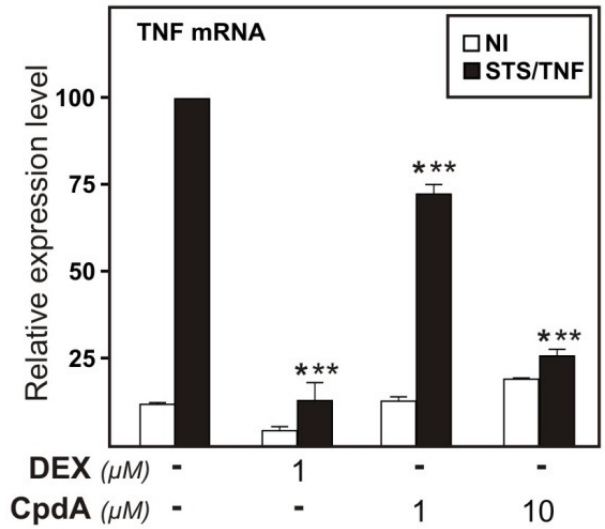


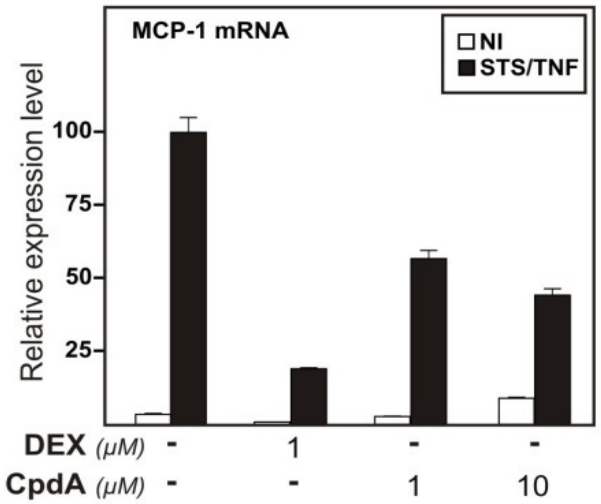


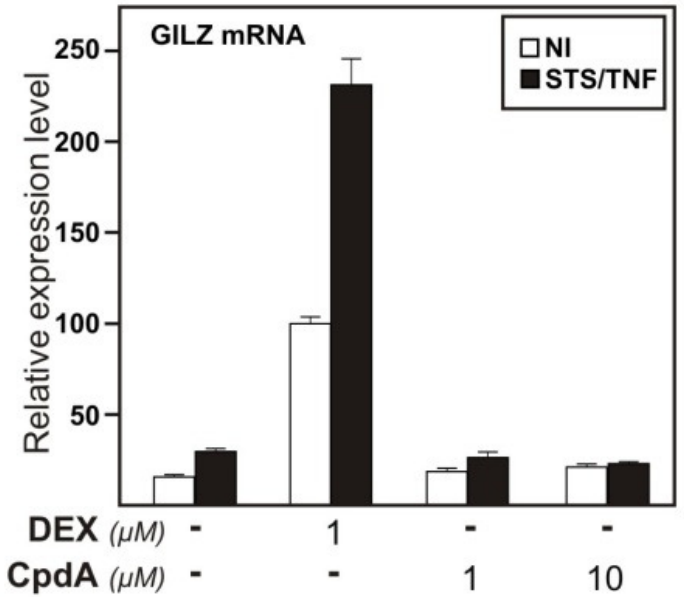
L929sA, murine fibrosarcoma cells

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Confirming **Compound A** 's functionality and dissociated character







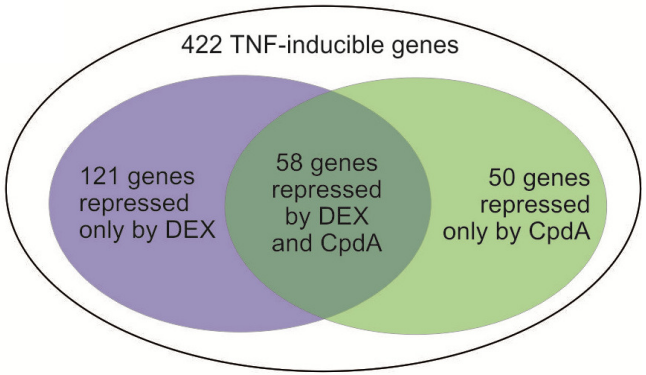
L929sA, murine fibrosarcoma cells

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Discrimination in promoter motifs between DEX-and CpdA-targeted gene promoters

A549
Transcriptome Agilent array analysis

Inductions:
Solv, DEX, CpdA,
TNF, DEX/TNF and CpdA/TNF



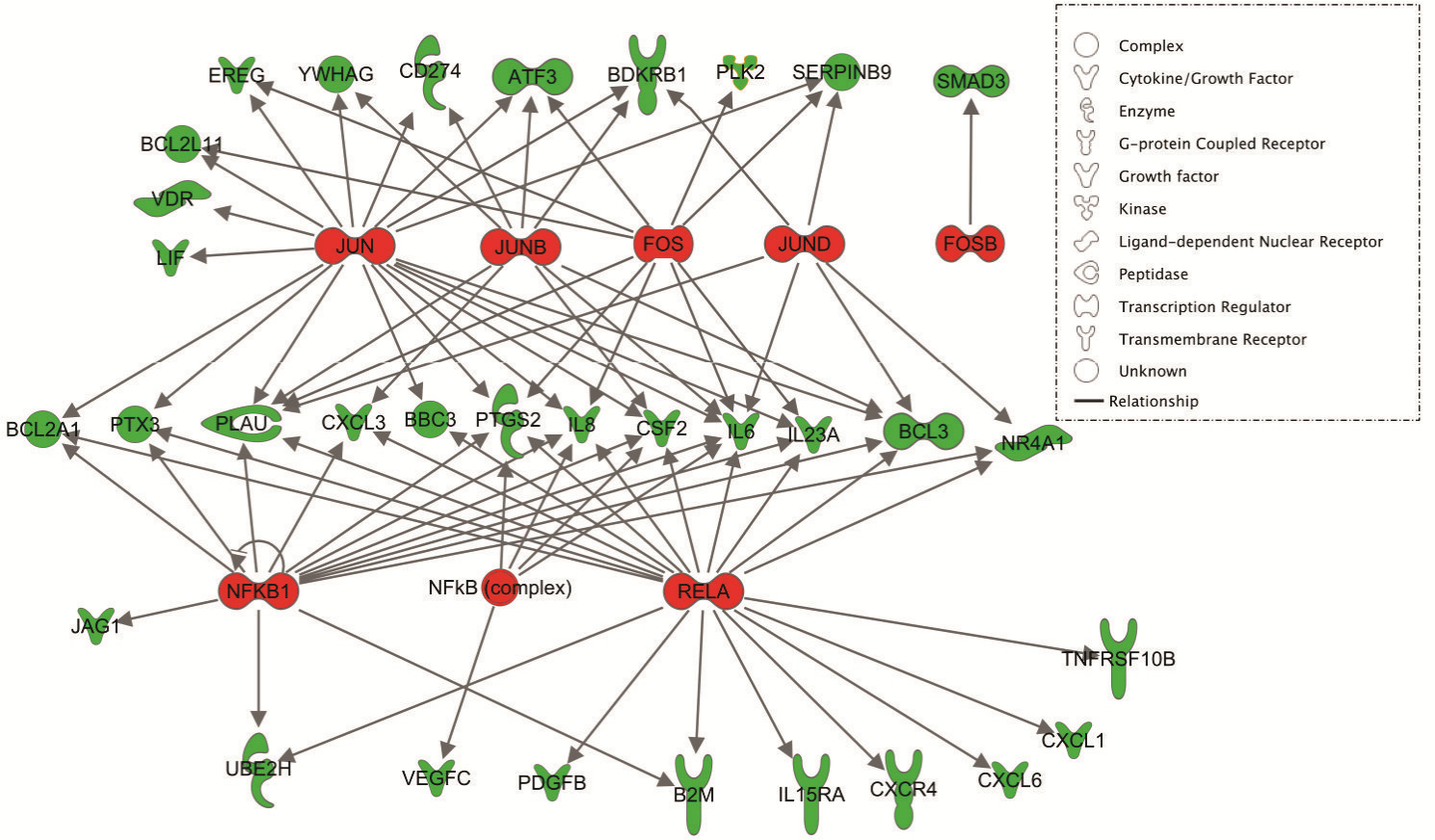
Pscan analysis

A549, human lung adenocarcinoma cells

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Discrimination in promoter motifs between DEX-and CpdA-targeted gene promoters

Genes **responding to DEX**, but not or to a lesser extent to CpdA



Transcriptome Agilent array analysis

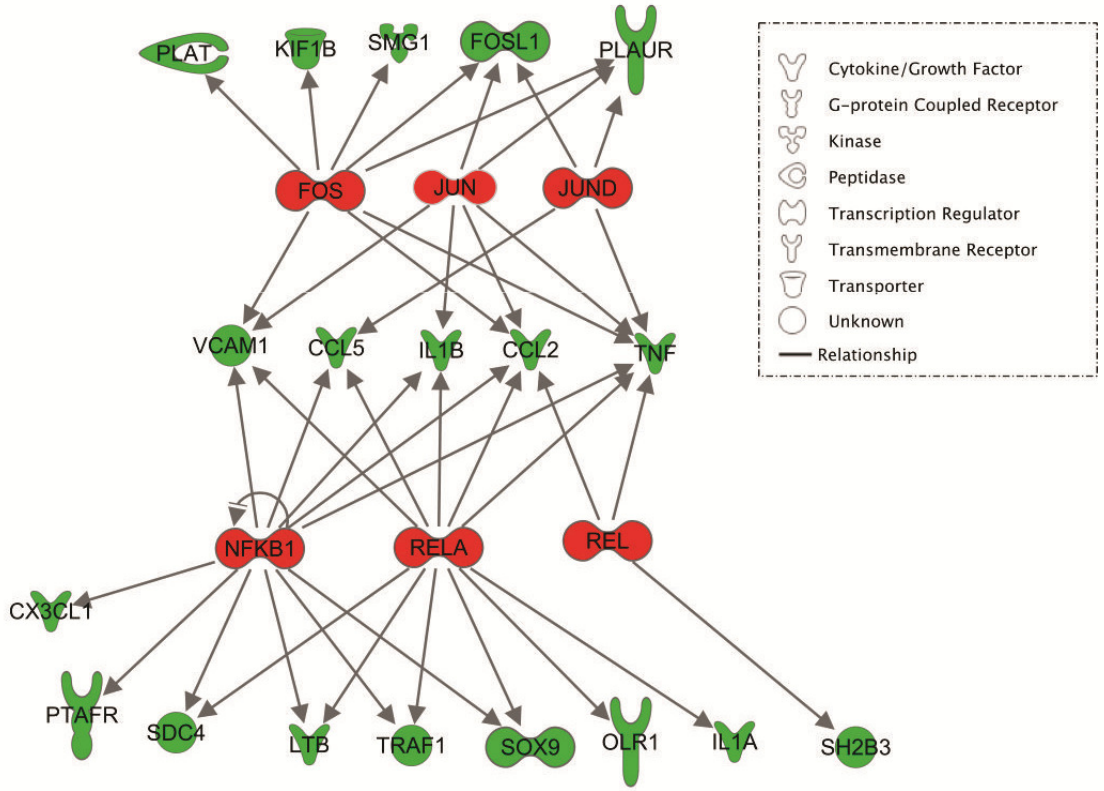
IPA : Ingenuity pathway analysis

A549, human lung adenocarcinoma cells

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Discrimination in promoter motifs between DEX-and CpdA-targeted gene promoters

Gene expression **repressed by both DEX and CpdA**



Transcriptome Agilent array analysis

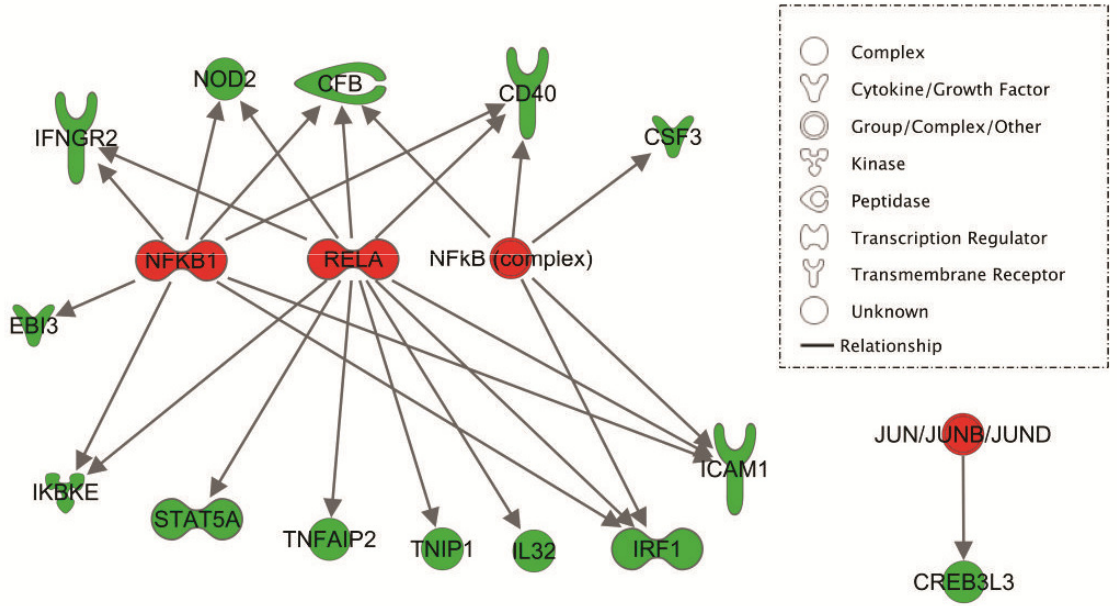
IPA : Ingenuity pathway analysis

A549, human lung adenocarcinoma cells

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Discrimination in promoter motifs between DEX-and CpdA-targeted gene promoters

Genes **responding to CpdA**, but not or to a lesser extent to DEX



Transcriptome Agilent array analysis

IPA : Ingenuity pathway analysis

A549, human lung adenocarcinoma cells

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Discrimination in promoter motifs between DEX-and CpdA-targeted gene promoters

Presence of NF-κB family member motifs in promoters of FOS/JUN target genes

Symbol	Score	Position	Sequence	Strand
SMG1	0.91	-239	GGGGATCTCCA	-
KIF1B	0.88	-432	GGGGGTCACCC	+
PLAT	0.86	-435	GGGGCACCTCC	+
FOSL1	0.84	-154	GGGGCTCCACC	+
PLAUR	0.81	-432	GGGGTTTCACC	+
CREB3L3	0.82	-201	GGGGTACCTCC	-

Transcriptome Agilent array analysis

Pscan analysis

A549, human lung adenocarcinoma cells

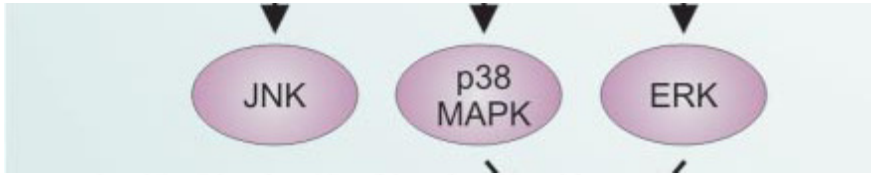


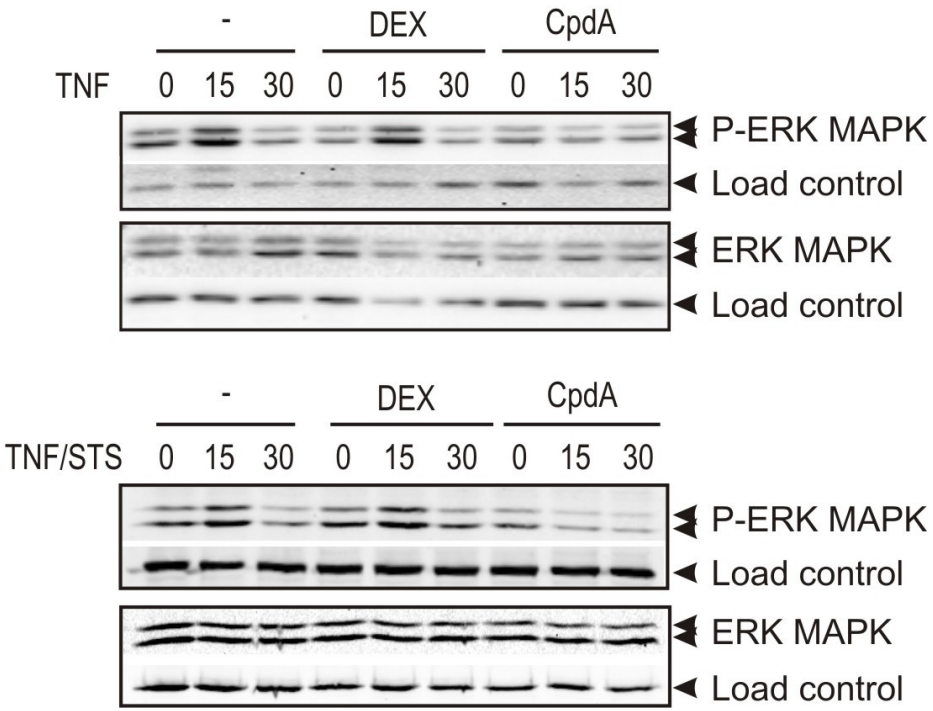






Compound A blocks ERK activation in L929sA



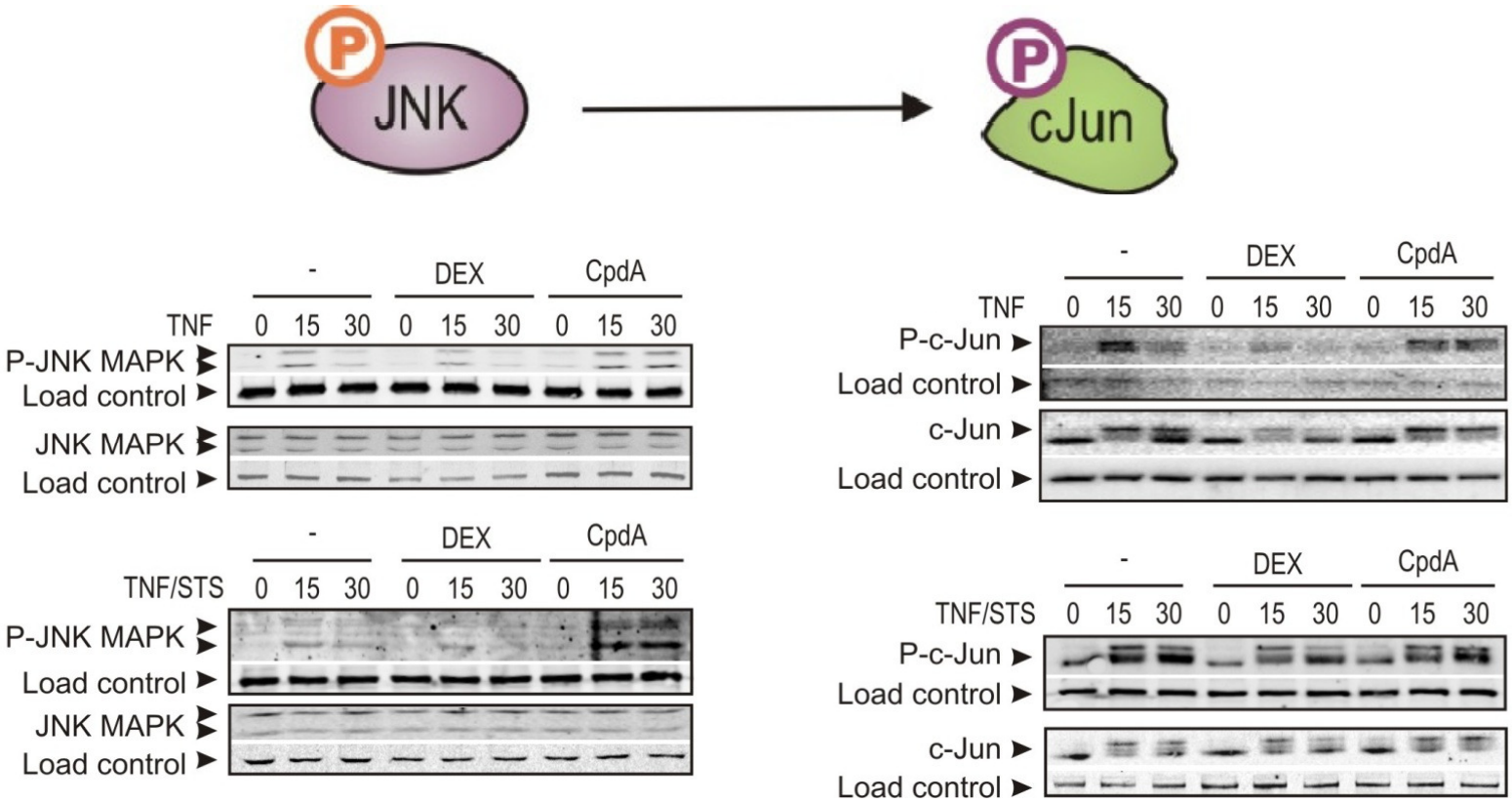


Note: activation of p38 is neither affected by DEX nor by CpdA in L929sA

L929sA, murine fibrosarcoma cells

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Yet, **Compound A** sustains JNK activation in L929sA



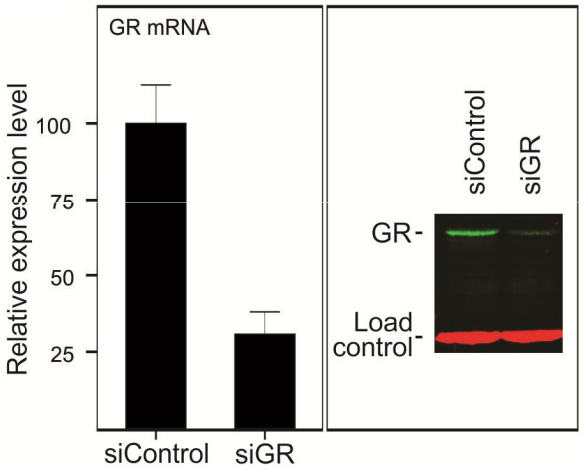
(Caelles et al., 1997:
DEX blocks JNK activation)

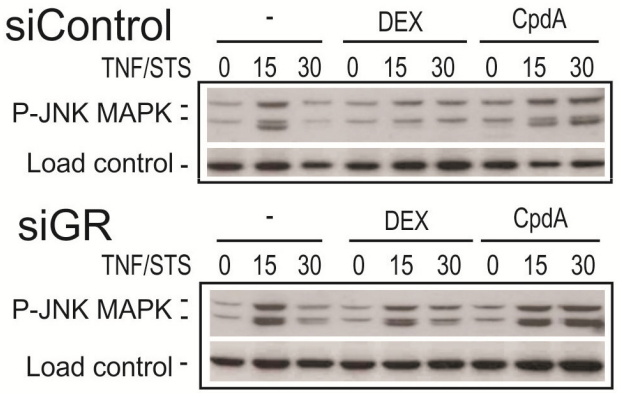
L929sA, murine fibrosarcoma cells

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GR plays a role in DEX-mediated P-JNK modulation

But GR knockdown could not impact the CpdA-mediated sustained P-JNK





L929sA, murine fibrosarcoma cells

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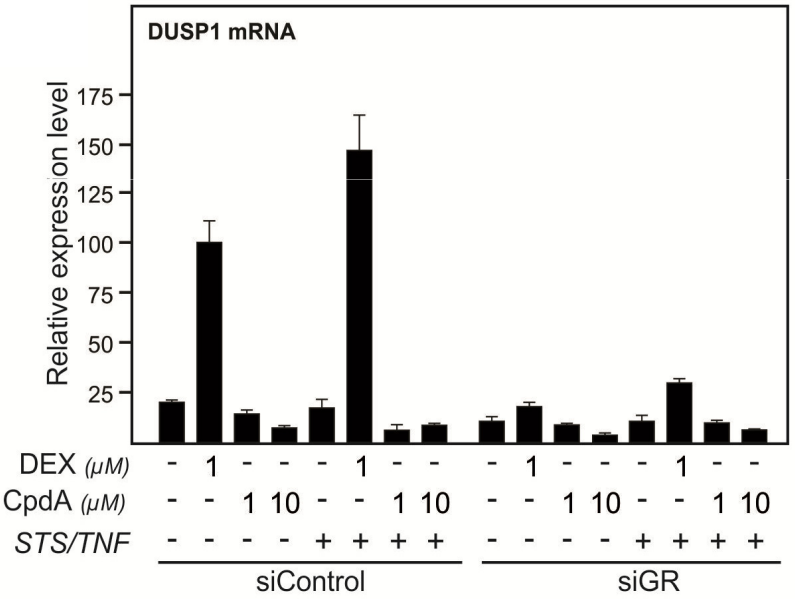
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GR plays a role in DEX-mediated P-JNK modulation

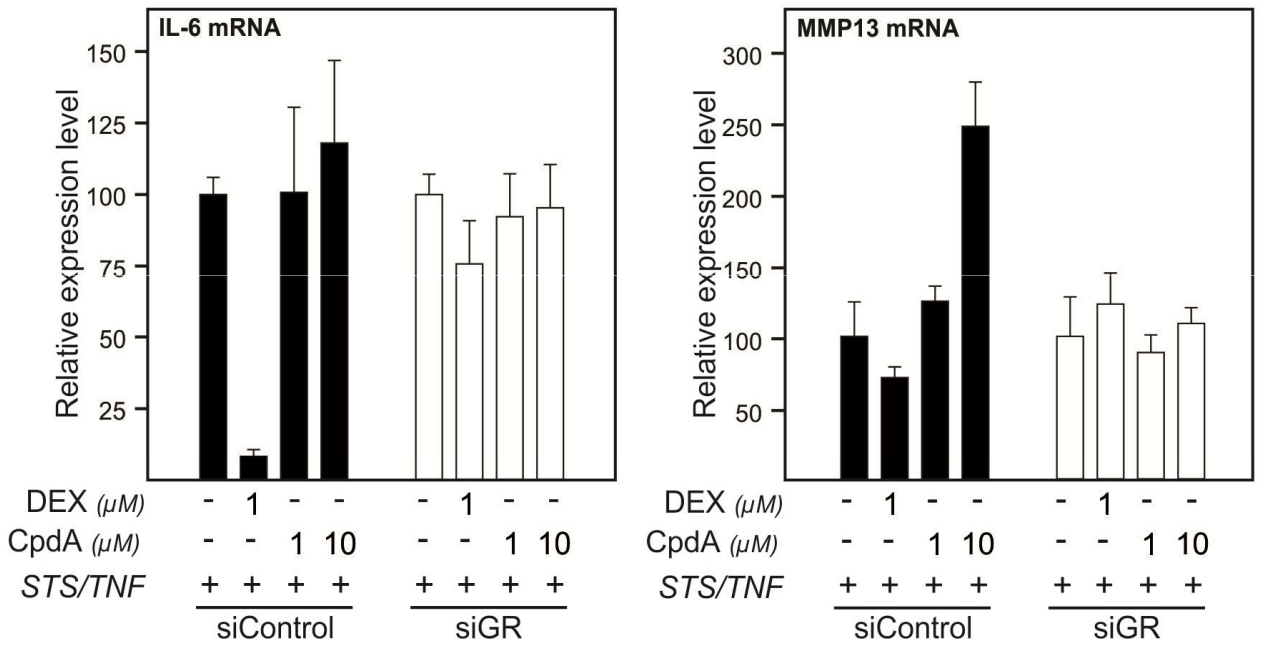
But GR knockdown could not impact the CpdA-mediated sustained P-JNK

→ Absence of DUSP1 /MKP1 regulation



L929sA, murine fibrosarcoma cells

GR is essential to mediate the gene expression modulation effect of DEX and Compound A



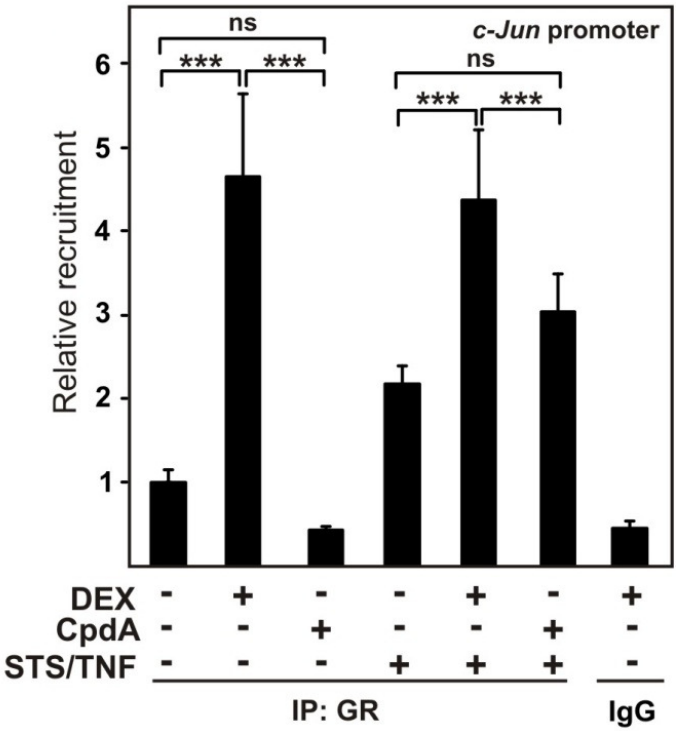
L929sA, murine fibrosarcoma cells
Similar results in A549 cells for IL-6, IL-8 and c-Jun

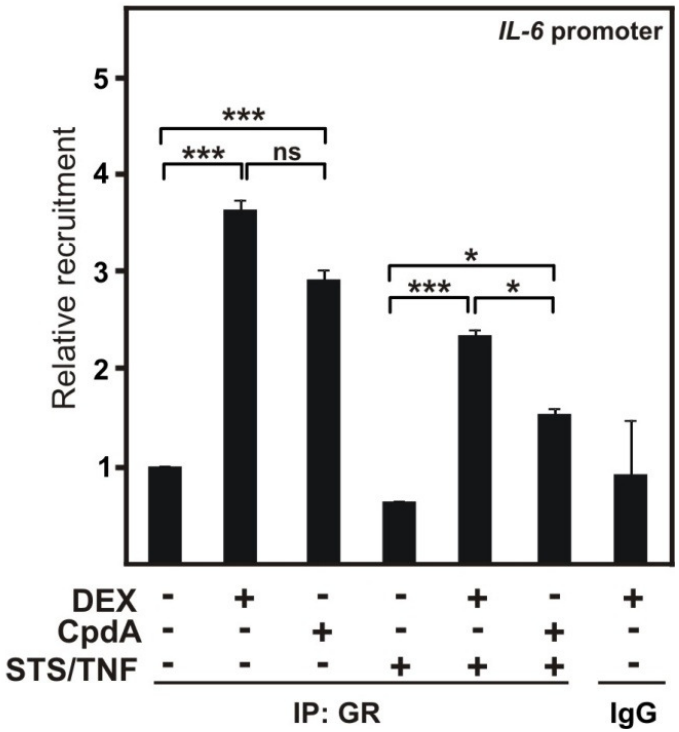
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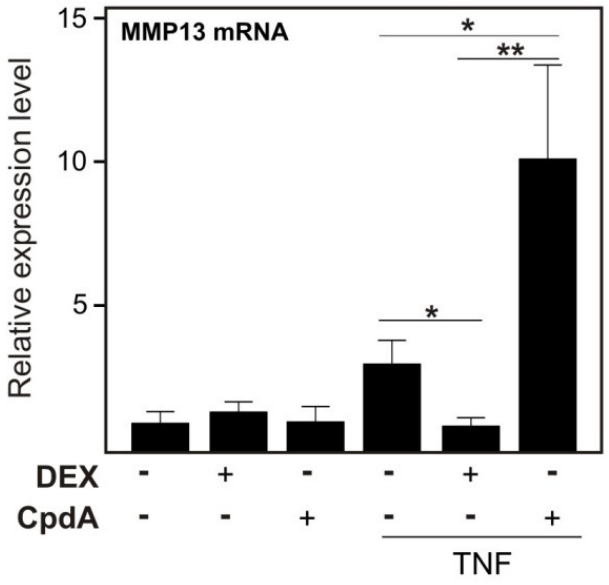
Compound A, in contrast to DEX, does not support GR recruitment onto the AP-1-dependent *c-jun* gene promoter

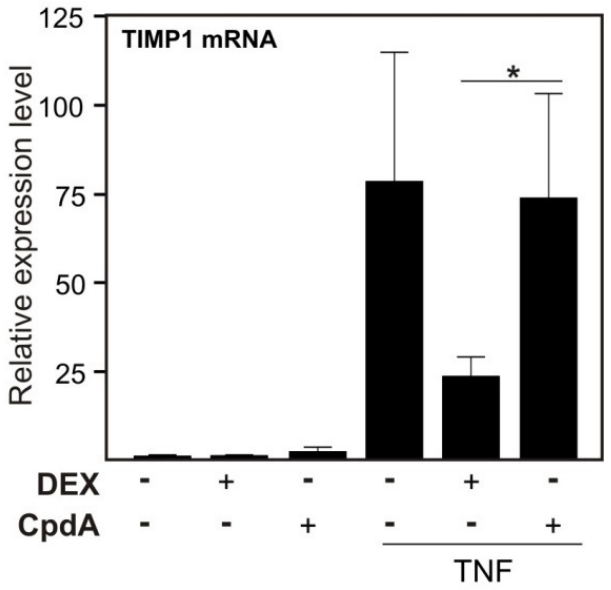




L929sA, murine fibrosarcoma cells
ChIP analysis

TNF lethality model: Compound A and DEX differentially modulate AP-1-regulated gene expression

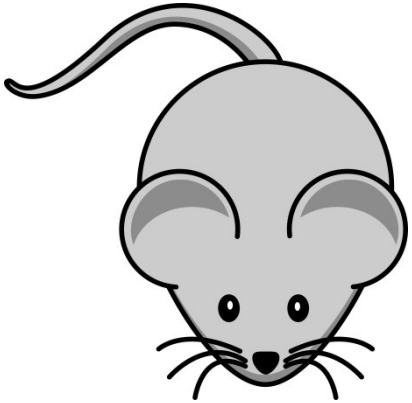




C57BL6/J mice, liver mRNA

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TNF lethality model



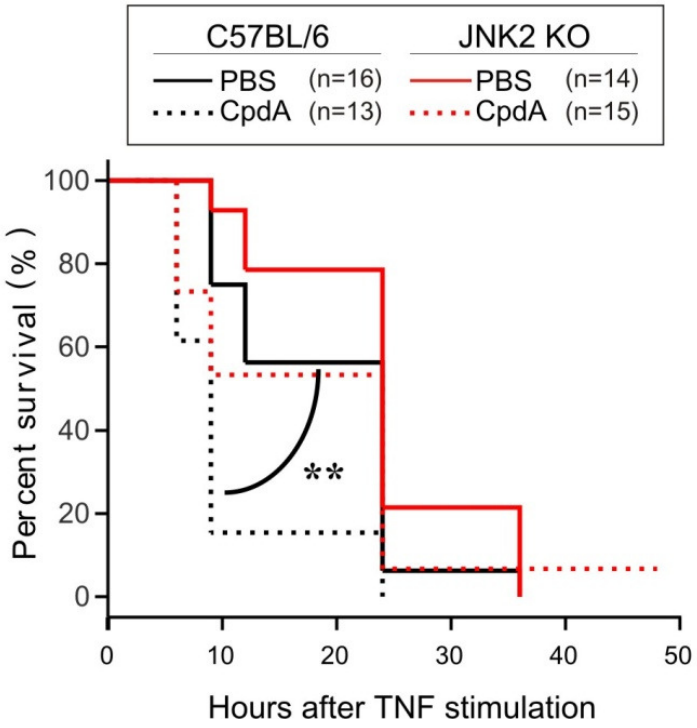
C57BL6/J mice

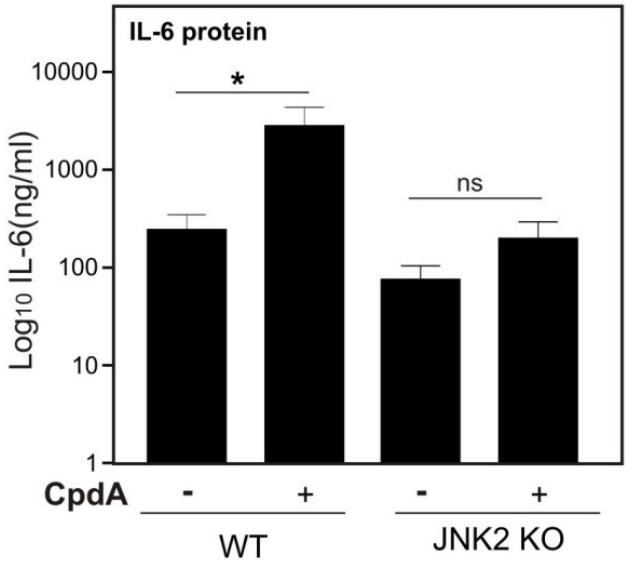
- GR dim/dim mice → increased sensitivity towards TNF-induced lethality
- DUSP1/MKP1-/- mice
  - → TNF-mediated lethality increased
  - → P-JNK2 enhanced
- JNK2 -/- mice : significant protection against TNF-induced lethality
- Control of JNK2 activity via a **GR dimerization-dependent** mechanism (*DUSP1*/MKP-1) protects against systemic TNF-induced lethality

(Vandevyver et al., 2012, JCI).

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JNK2 is involved in the **Compound A** -mediated sensitization to TNF toxicity



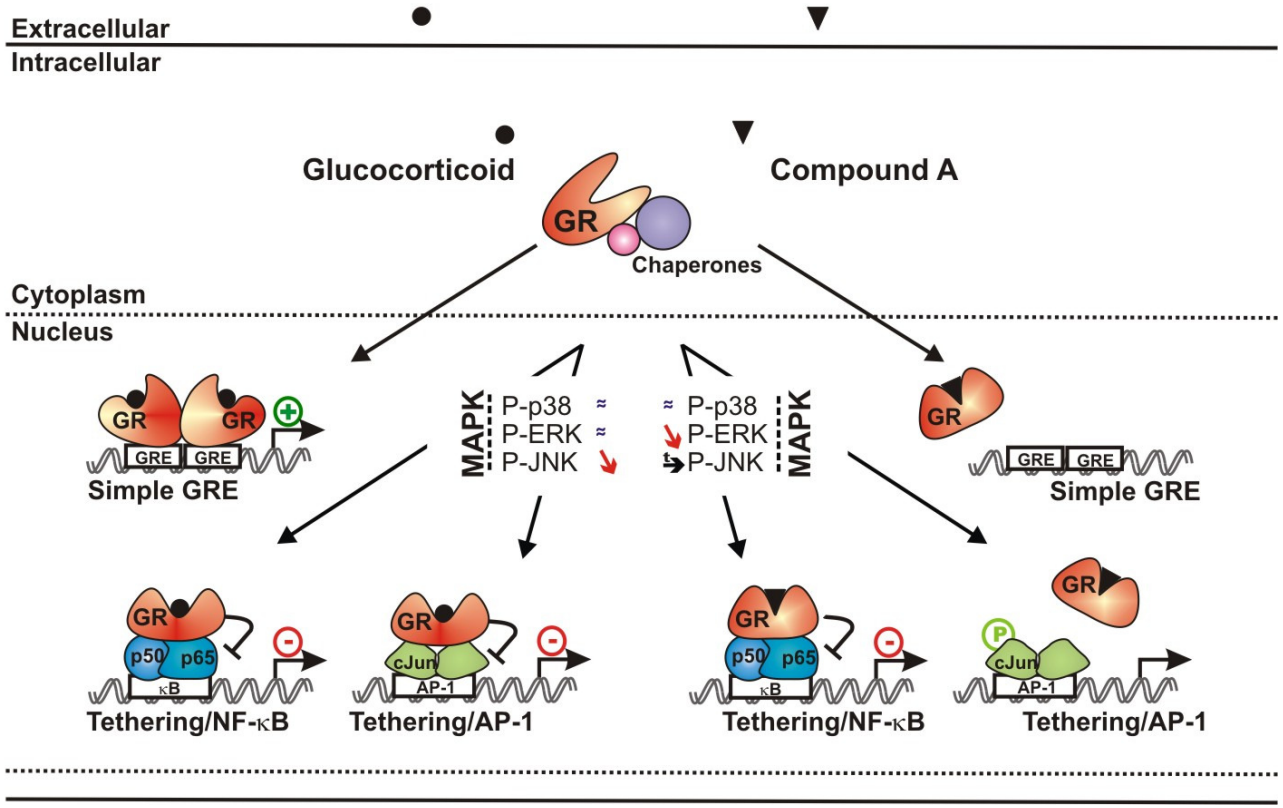


IL-6 serum protein levels as indicator for murine TNF sensitivity

C57BL6/J mice

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Concluding model



Our data support the hypothesis that a ligand-induced differential conformation of GR may expose different surfaces to yield a different transcription factor cross-talk profile

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Acknowledgements







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IL-6 gene activation by STS and TNF can be discriminated

STS=
a microbial alkaloid
and
protein kinase
inhibitor

STS skews the IL-6
promoter activity
towards a
AP-1-dependent gene
activation

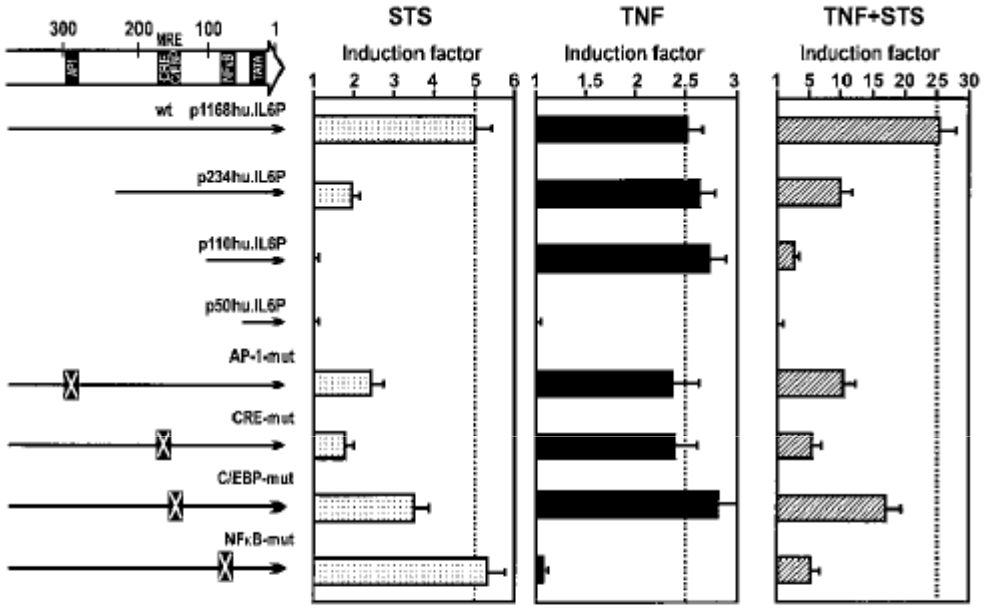


FIG. 3. **Localization of TNF- and STS-responsive elements in the IL-6 promoter.** Various IL-6 promoter-derived recombinant reporter gene constructs were used in induction experiments (*black boxes*, transcription factor-binding sites; *crossed boxes*, mutations of the transcription factor-binding sites yielding the point-mutated versions of p1168hu.IL6P-luc+). Stable cell pools of the promoter reporter gene constructs were left untreated or were induced with 2500 IU/ml TNF for 6 h, with 60 nM STS for 8 h, or added 2 h prior to TNF in a combined treatment.

Source:Vanden Berghe et al., 1999, JBC

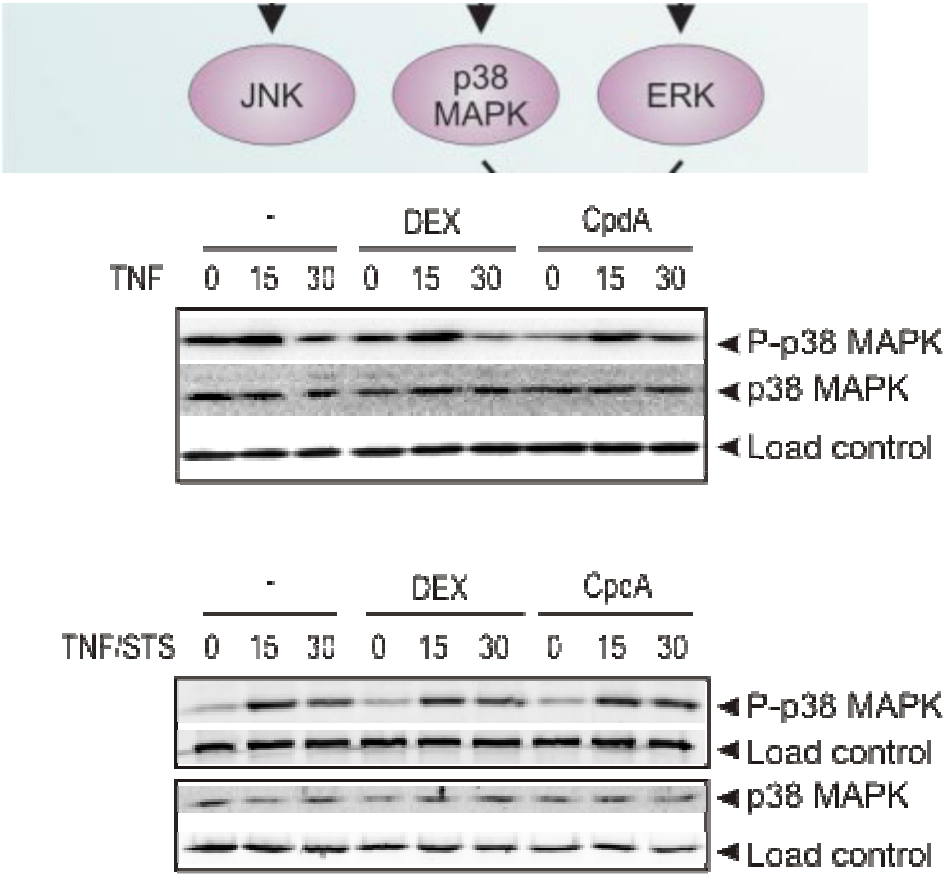
L929sA, murine fibrosarcoma cells

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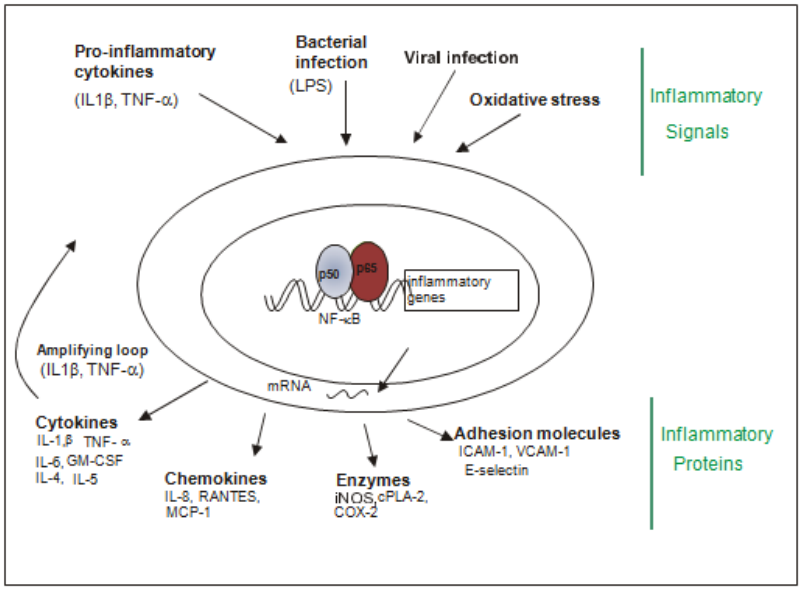
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CpdA nor DEX affect p38MAPK activation in L929sA



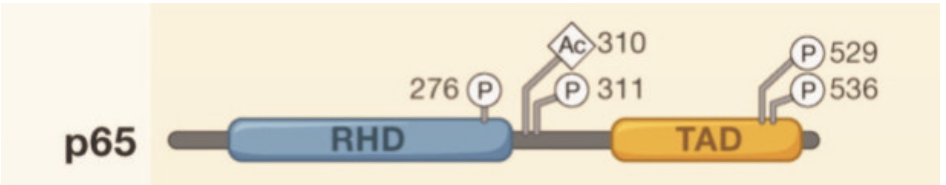
L929sA, murine fibrosarcoma cells

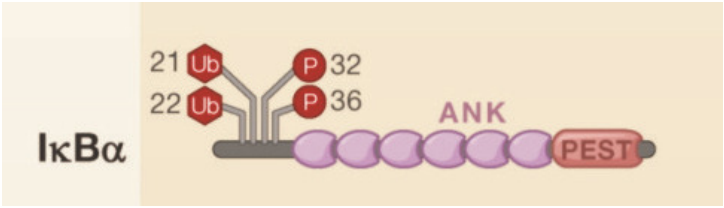
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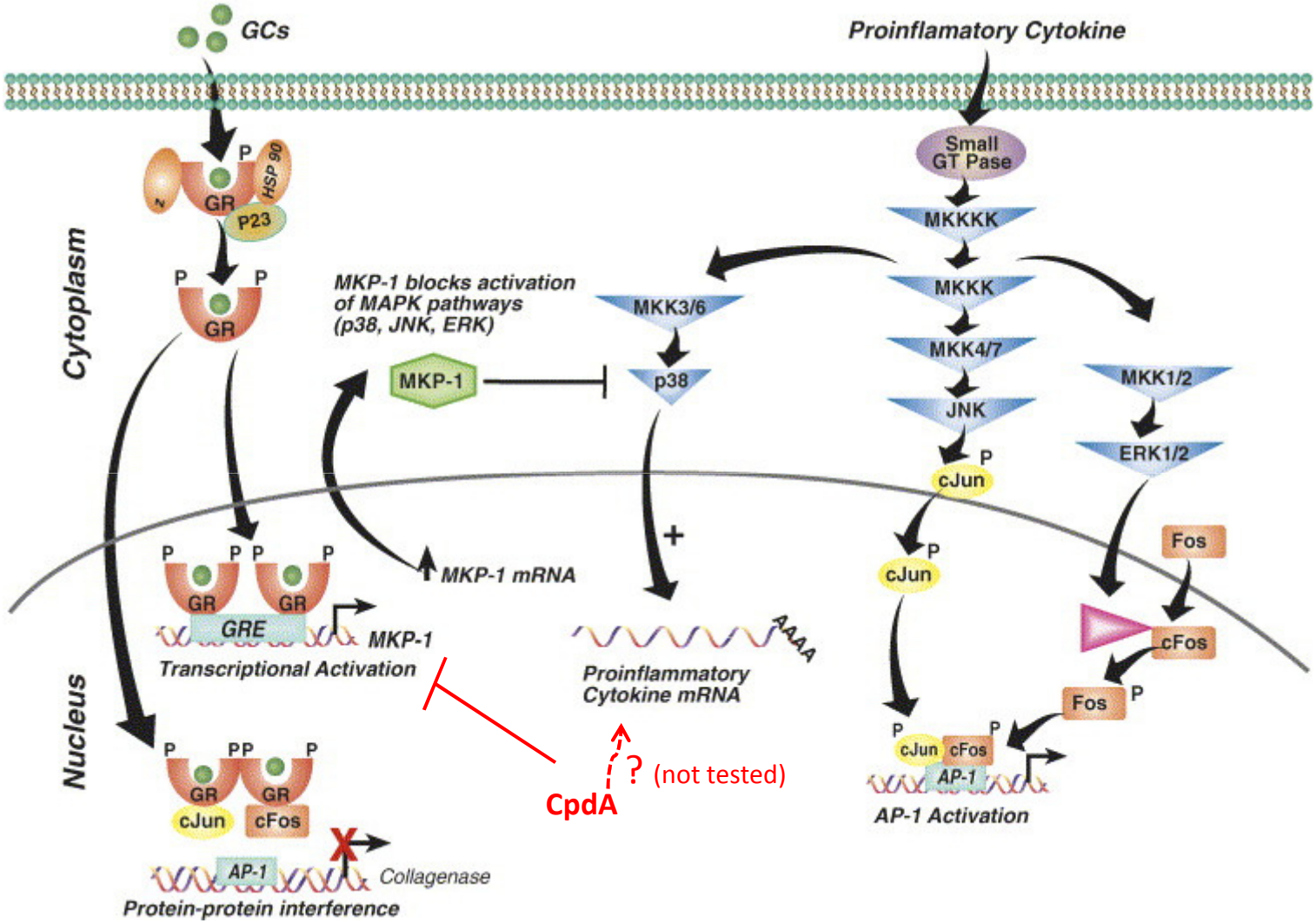
NF-κB is an important mediator of inflammation-associated diseases

- NF-κB transcription factor family → Rel-homology domain
  - **NF-κB p65** (RelA)
  - RelB
  - c-Rel
  - NF-κB p100/p52 (NF-κB2)
  - NF-κB p105/p50 (NF-κB1)
- NF-κB p65 – p50 heterodimer
- NF-κB p65-p50 – IκBα association
  - Masks mutual NLS domains → cytoplasmic localization

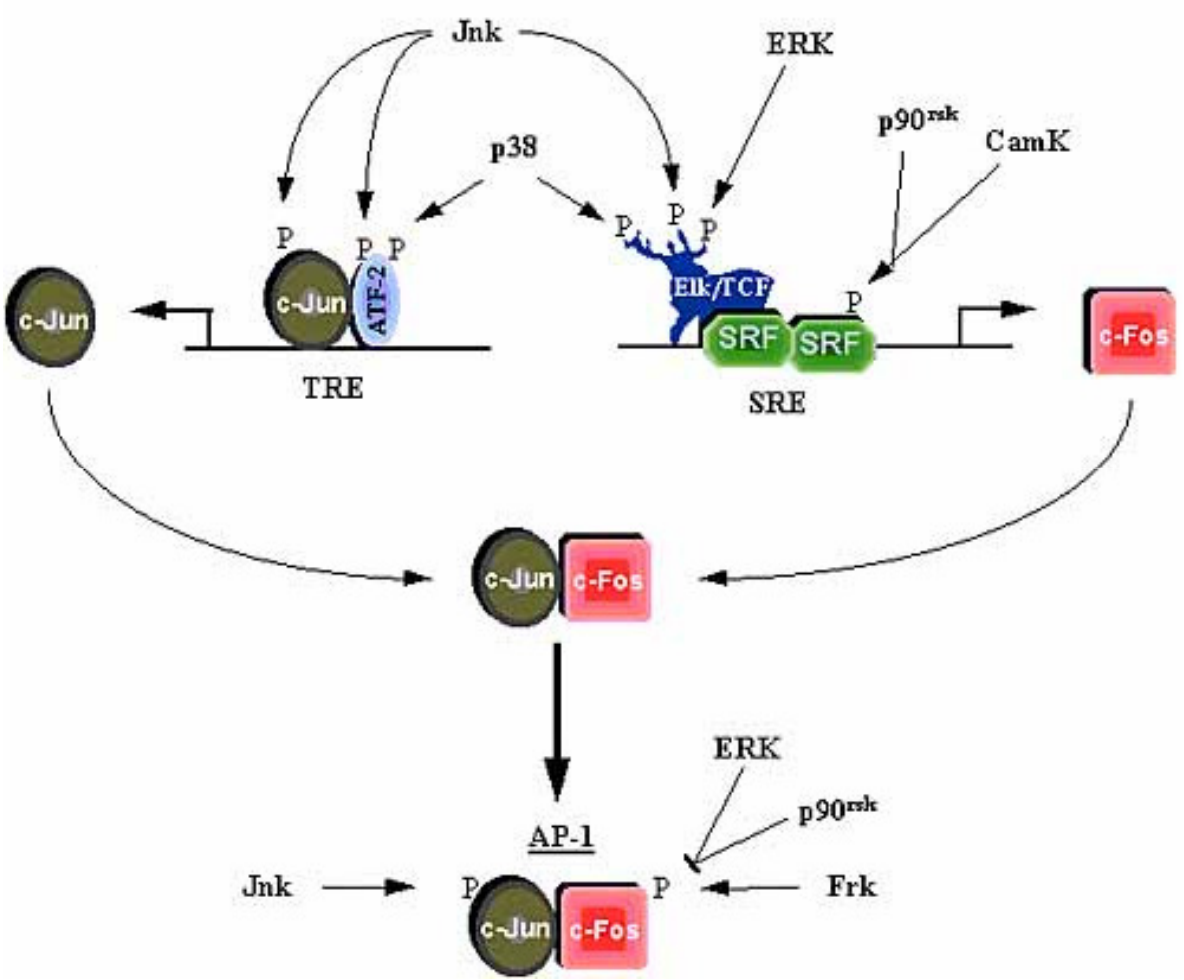




GR and MAPK and AP-1



Source: Smoak and Cidlowski, 2004



Source:Hipskind and Bilbe, Frontiers in Bioscience 1998

Supplementary information: stimulus-independent efficient repression of NF-κB by CpdA

