IL-6 type cytokine signaling through the gp130-Jak-STAT pathway and its regulation

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UNI FREIBURG

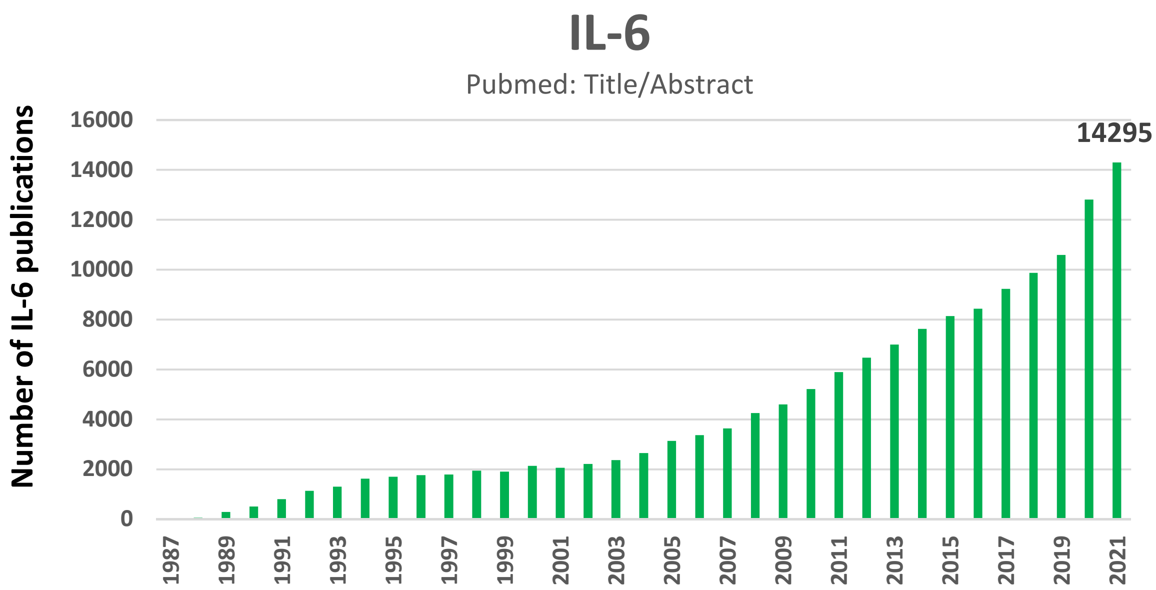
1969-1988 and 2008-present

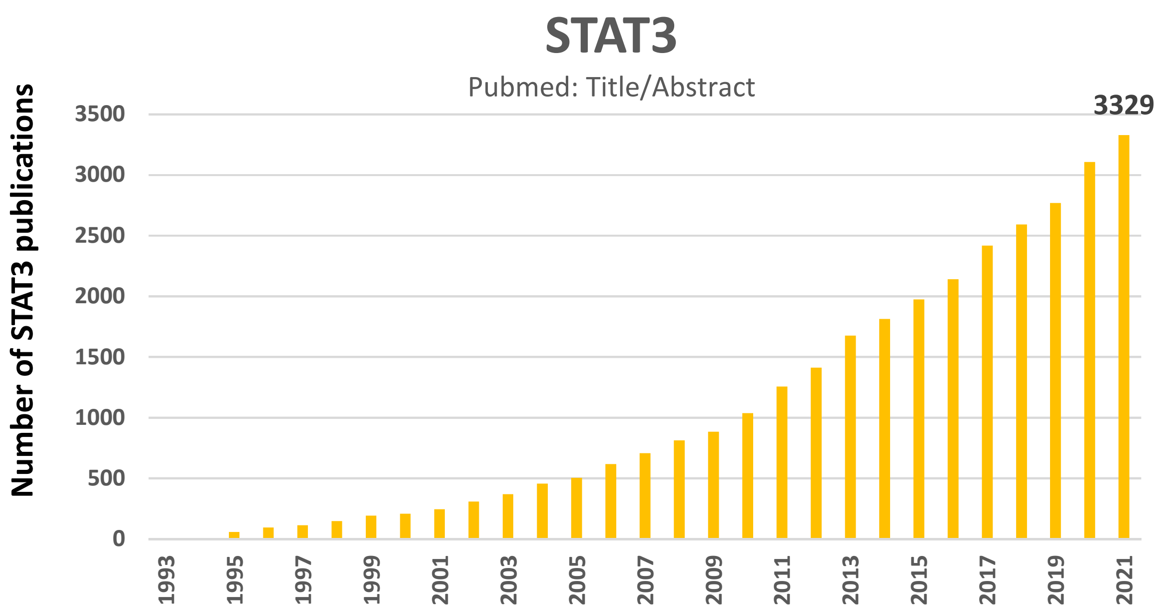
1988-2008

RHEINISCH-WESTFÄLISCHE TECHNISCHE HOCHSCHULE
RWTH AACHEN

SFB 542

IL-6 and STAT3 are still important molecules





Outline: Interleukin-6 signal transduction and its regulation

Part 1: **Molecular mechanisms of IL-6 signal transduction**

- **The acute phase response**
- **Identification of HSF as IL-6**
- **Structure and function of IL-6**
- **Acute phase protein synthesis induced by IL-6**
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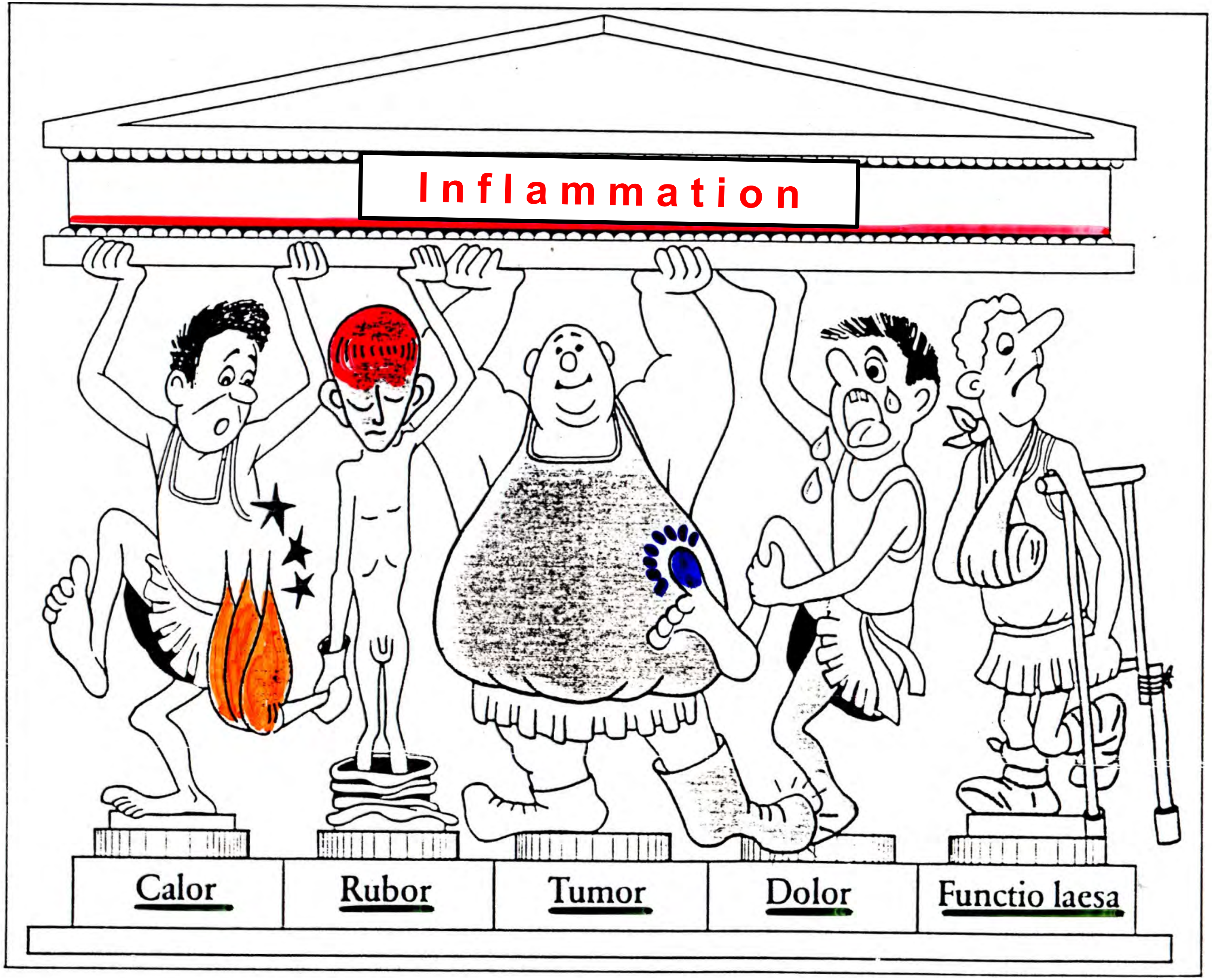
- **Formation of the IL-6-receptor complex**
- **Design of a highly potent IL-6 antagonist**
- **Molecular mechanisms of IL-6 signal transduction**
- **Nuclear translocation of STAT3-YFP**

Part 2: **Regulation of IL-6 signal transduction**

The acute phase response

Acute exsudative inflammatory reaction



Celsus (25 BC): The <u>four cardinal symptoms</u> of inflammation: fever-redness-swelling-pain

For the defense of diseases the evolution did not invent a thick skin, but the inflammatory reaction.

Inflammation brings healing and disease at the same time: 240 mio (3%) people suffer, 8 billion people profit from it.

Acute and chronic inflammatory and degenerative diseases are still therapeutical problems which cause high costs:

- Sepsis
- Rheumatoid arthritis (RA), Osteoarthritis (OA)
- Morbus Crohn, Ulcerative colitis

The acute phase response to injury and infection

The acute inflammation process

Noxa

Tissue injury
Trauma
Infection
Neoplasma

Local Reaction

Activation of
Blood cells:
Monocytes
Granulocytes
Lymphocytes
Thrombocytes

Tissue cells:
Macrophages
Mast cells
Lymphocytes
Endothelial cells
Fibroblasts

Release of
Inflammatory mediators:
Histamine
Serotonine
Leukotrienes
Prostaglandines
Oxygen radicals
Proteinases
Cytokines

- Growth factors
- Interleukins
- Interferons
- Chemokines

Systemic Reaction

Fever
Pain

Activation of
Immune system
Endocrine system
Hematopoiesis

Induction of
Acute phase proteins

- C-reactive protein
- Serum amyloid A
- LPS binding protein
- Fibrinogen
- Haptoglobin
- α_1-antichymotrypsin
- Interleukin-1-RA

Major human and rat acute phase proteins

Human

- C-reactive protein
- serum amyloid A
- LPS binding protein
- fibrinogen
- haptoglobin
- α_1-antichymotrypsin
- complement 3

LPS, Lipopolyssacharide

Rat

- α_2-macroglobulin
- LPS binding protein
- α_1-acid glycoprotein
- cysteine proteinase inhibitor
- serine proteinase inhibitor 2.3
- tissue inhibitor of metalloproteinases-1

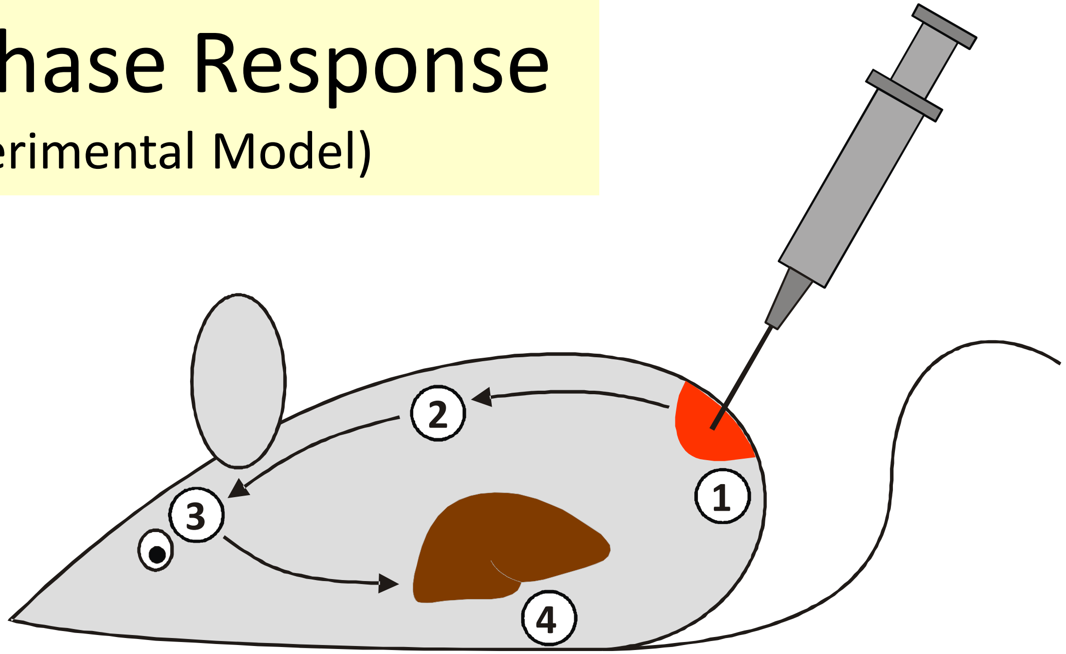
Negative acute phase proteins

- albumin
- transthyretin
- transferrin
- fetuin / α_2HS-glycoprotein
- apolipoprotein A-I (HDL)
- retinol binding protein

HDL, high density lipoprotein

Acute Phase Response

(Experimental Model)



(1) Local response

(2) Systemic distribution of inflammatory mediators

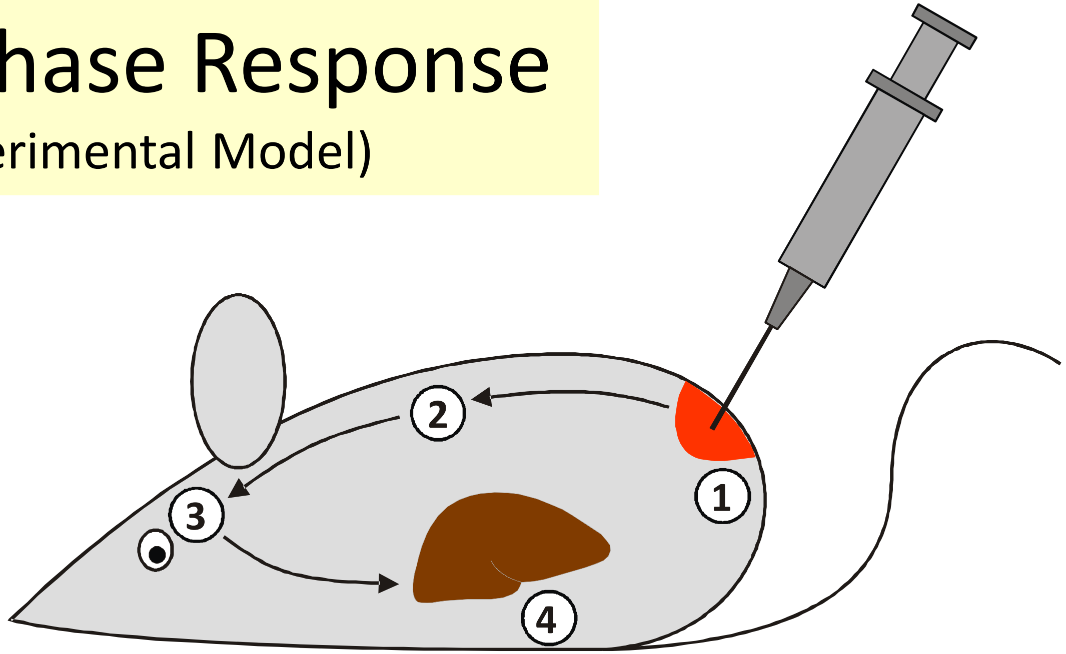
(3) Activation of CNS and endocrine hormone system

(4) Hepatic acute phase resonse

CNS, central nervous system

Modified from Heinz Baumann

Acute Phase Response
(Experimental Model)



(1) Local response turpentine injection

(2) Systemic distribution of inflammatory mediators IL-1, TNFα, IL-6, CKs

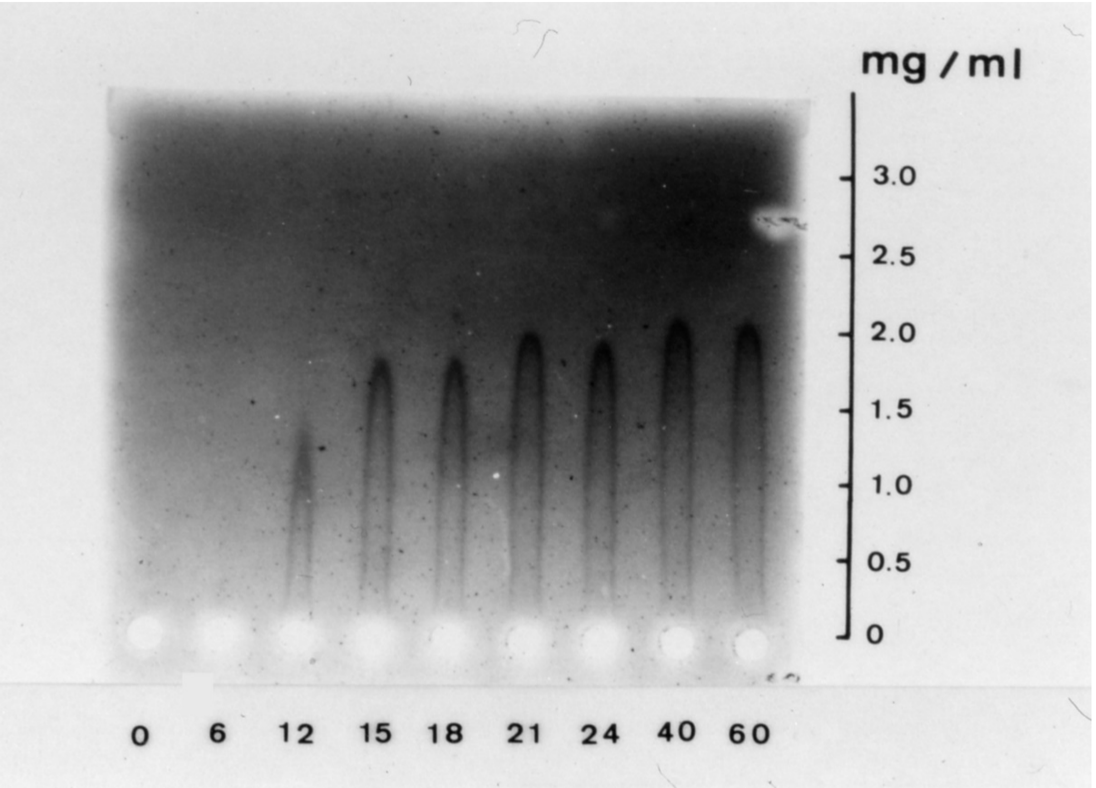
(3) Activation of CNS and endocrine hormone system ACTH, glucocorticoids

(4) Hepatic acute phase resonse acute phase proteins

CNS, central nervous system IL, Interleukine CK, Chemokines ACTH, Adrenocorticotropin

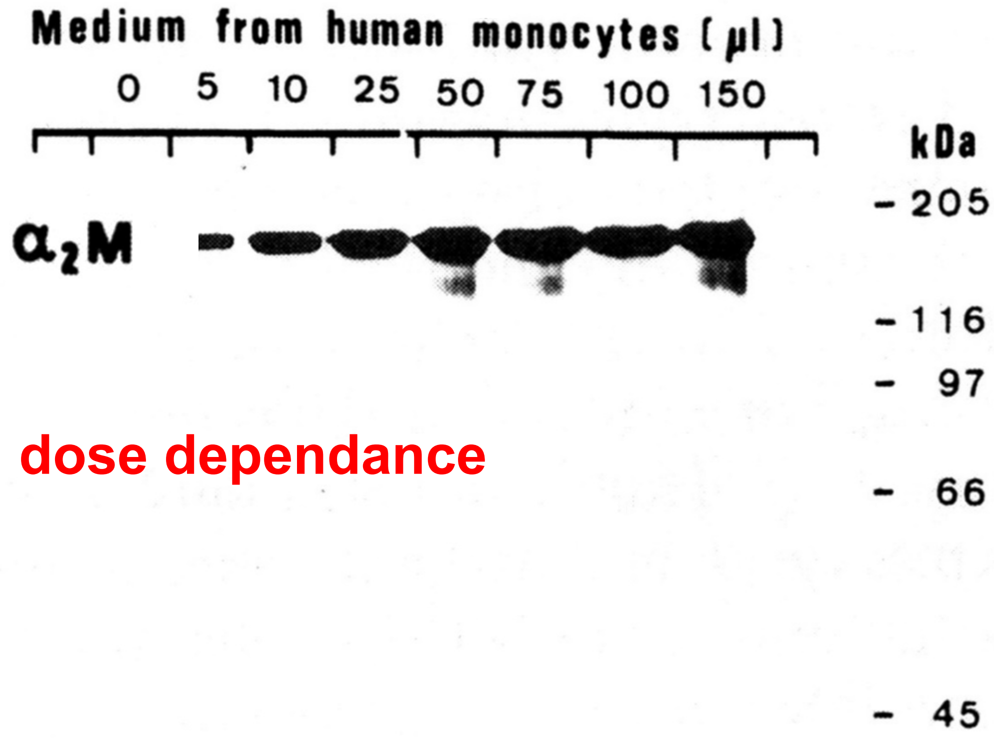
Rat model (*in vivo*)

serum α_2M



hours after turpentine

Rat hepatocytes



dose dependance

Conditioned medium from LPS-stimulated human monocytes added to rat hepatocytes → α2M – protein biosynthesis was measured

α_2M, α_2-macroglobulin

Northoff et al. (1987) Eur J Immunol 17, 707 - 711

THE JOURNAL OF BIOLOGICAL CHEMISTRY
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Vol. 260, No. 10, Issue of May 25, pp. 6200-6205, 1985
Printed in U.S.A.

Molecular Cloning of cDNA Sequences for Rat α_2-Macroglobulin and Measurement of Its Transcription during Experimental Inflammation*

97 citations

(07/2022)

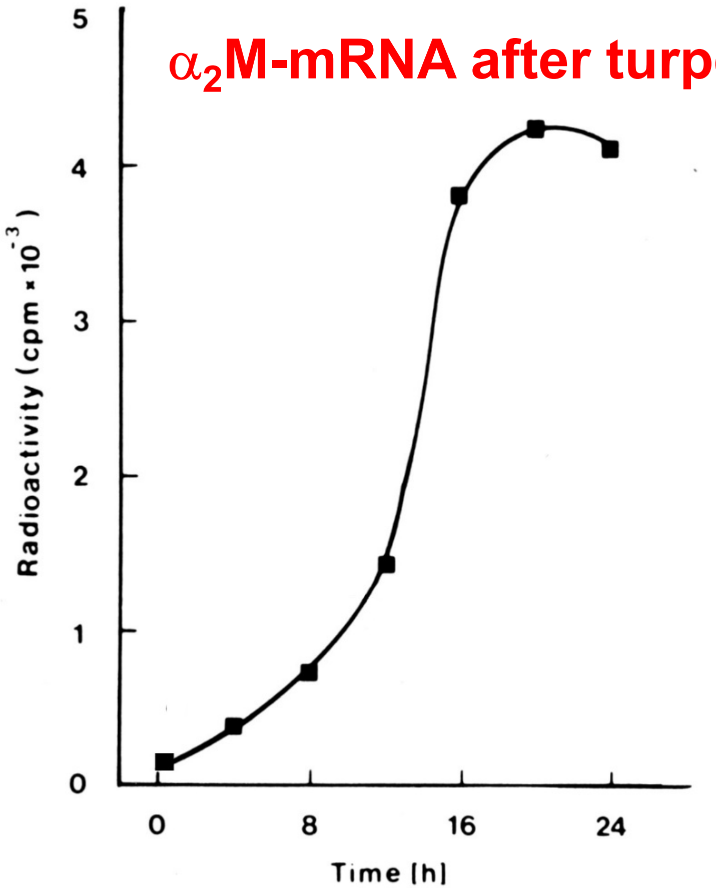
in vivo in rat liver

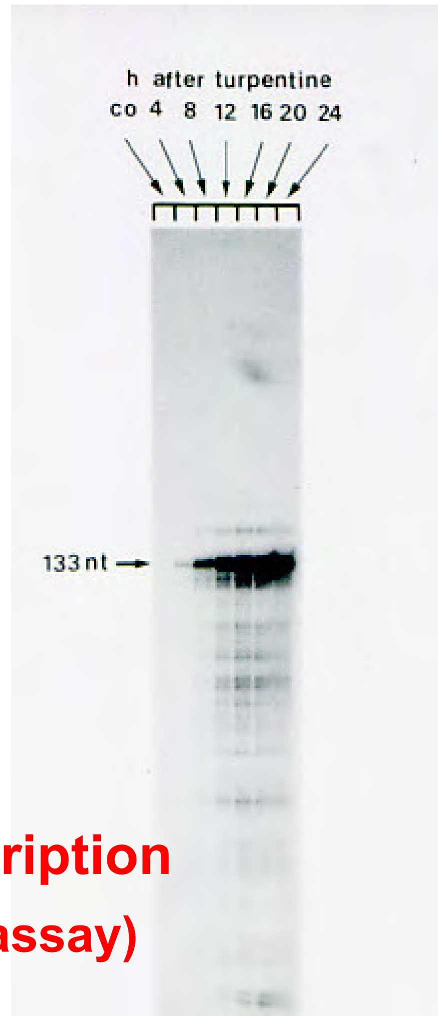
(Received for publication, December 4, 1984)

Wolfgang Northemann, Michael Heisig, Dieter Kunz, and Peter C. Heinrich

From the Biochemisches Institut, Universität Freiburg, Hermann-Herder-Str. 7, D-7800 Freiburg i. Br., Federal Republic of Germany

α_2M-mRNA after turpentine

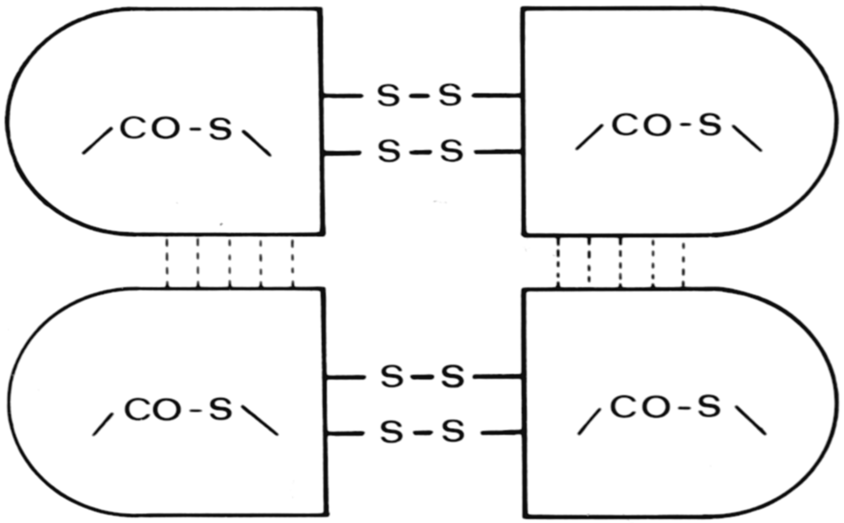


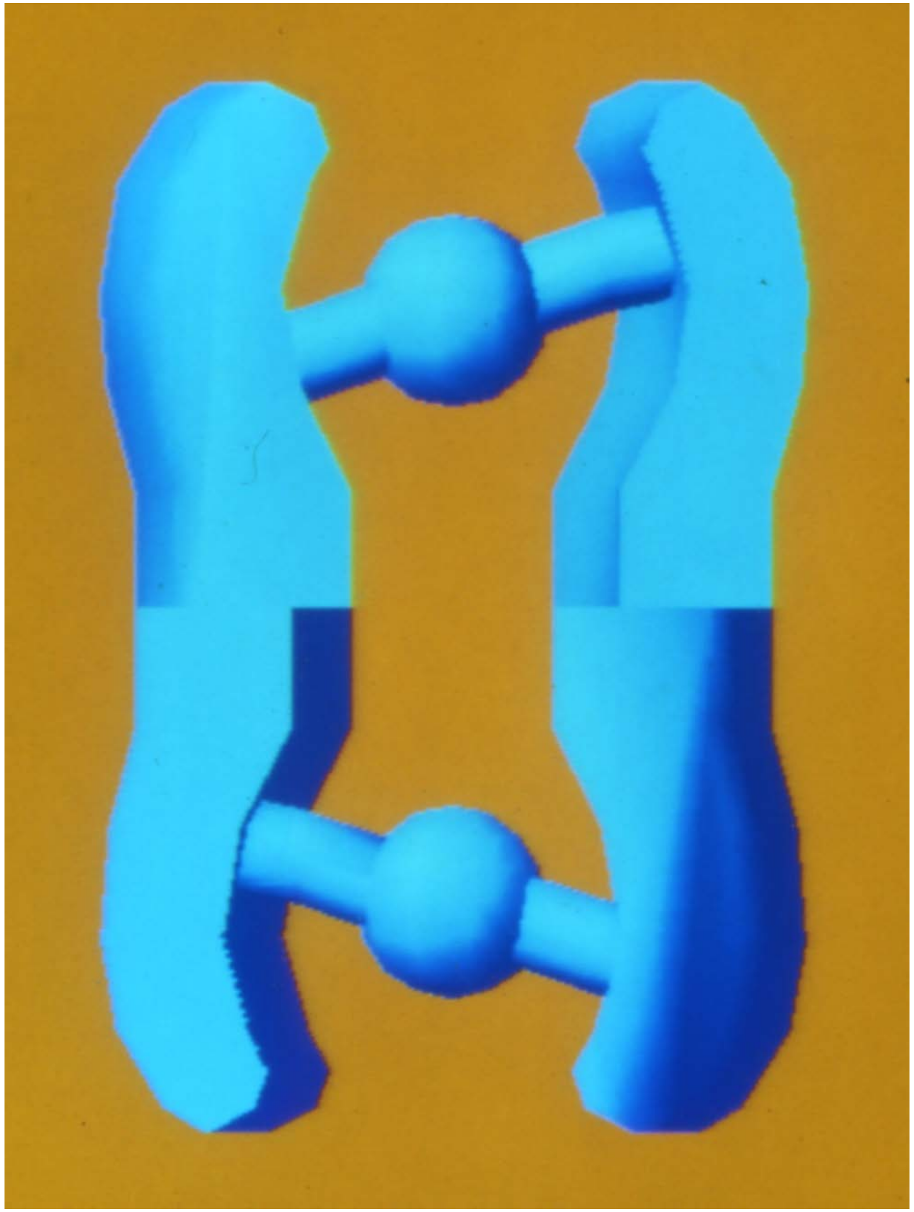


α_2M-gene transcription
(RNA protection assay)

Properties of α_2-Macroglobulin

- dimer of dimers
- molecular weight: 728 kDa
- 4 identical subunits of 182 kDa
- 1 thio-ester bond per subunit
- 4 interchain S-S bridges
- about 15% carbohydrate
- 4-8 moles Zn^{++} per mol
- serine -
SH -
metallo -
acid - } proteases are inhibited by cage formation





α_2M model in 1985

Properties of α_2-Macroglobulin

- dimer of dimers
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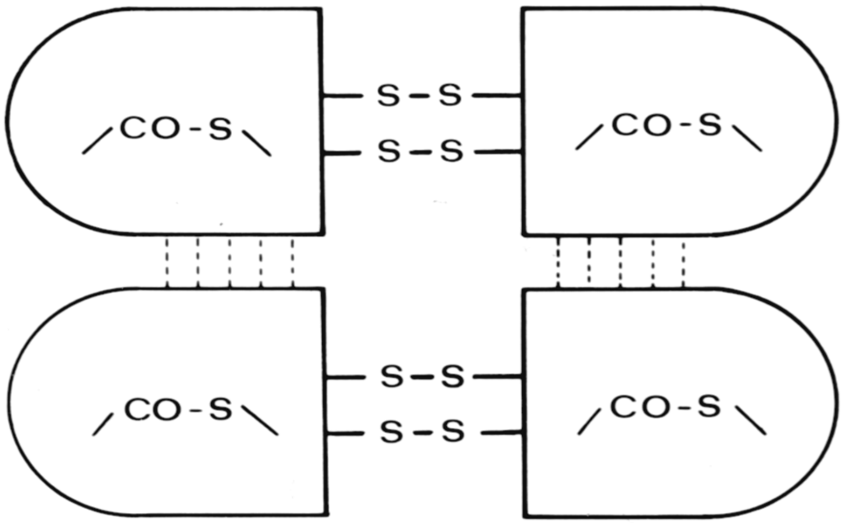

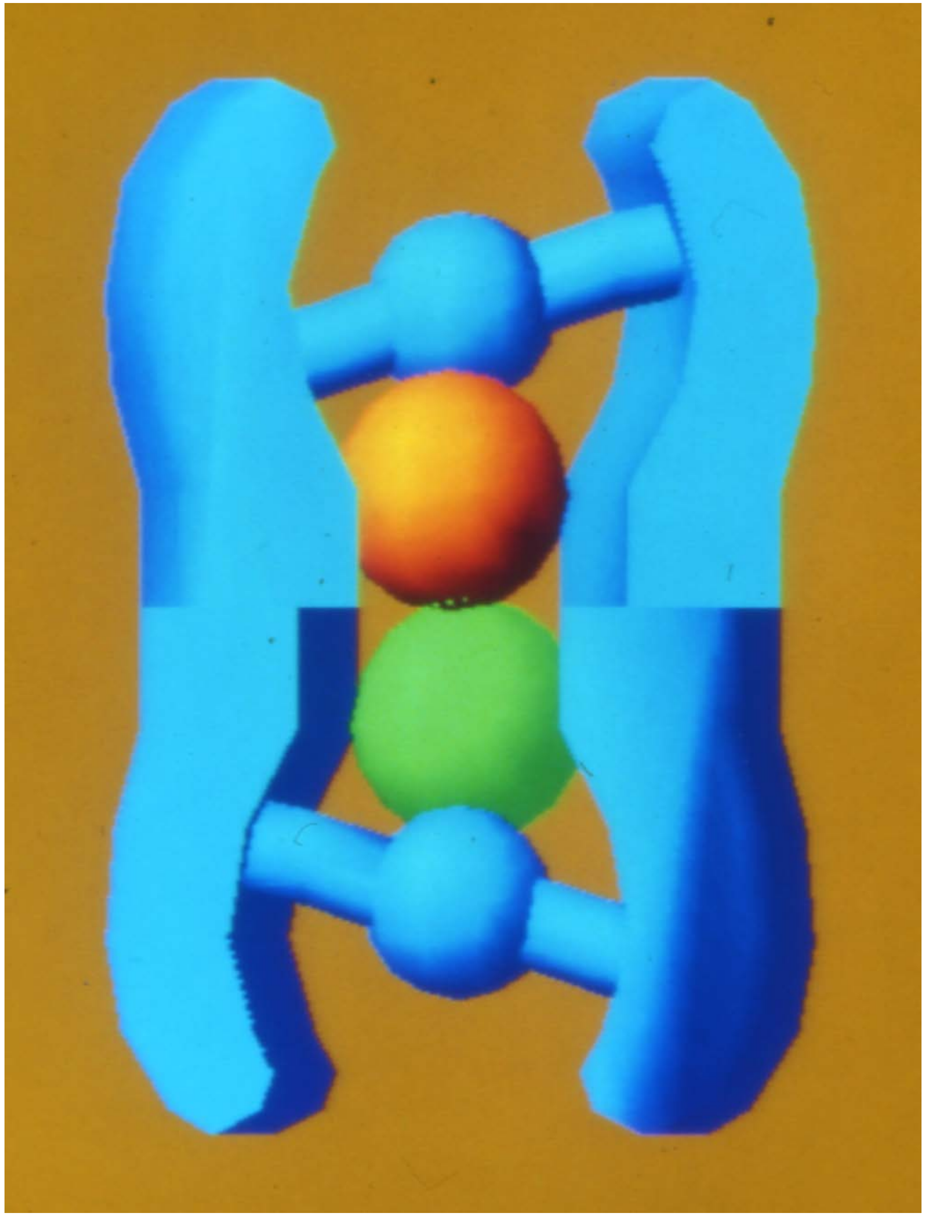

α_2M model in 1985

During our efforts with the purification of HSF from CM of LPS-stimulated monocytes

we excluded IL1-β, TNFα, TGFβ and IFNα,β,γ and found in rat hepatocyte primary cultures that BSF-2/IL-6 induced the expression of acute phase proteins at the level of mRNA and protein.

HSF,	hepatocyte stimulatory factor	IL,	interleukin	IFN,	interferon
CM,	conditioned medium	TNF,	tumor necrosis factor	BSF,	B-cell stimulatory factor
LPS,	lipopolysaccharide	TGF,	transforming growth factor		

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Part 2: **Regulation of IL-6 signal transduction**

Volume 221, number 1, 18–22 FEB 05045 August 1987

1987

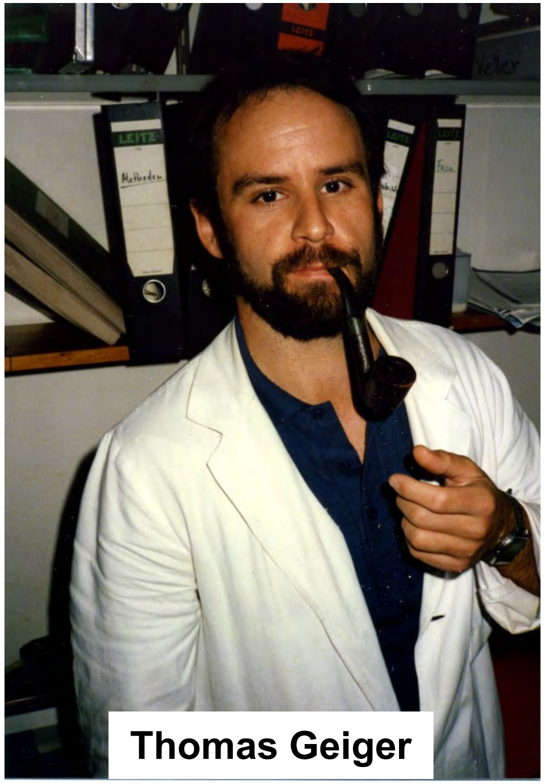
Recombinant human B cell stimulatory factor 2 (BSF-2/IFN-β2) regulates β-fibrinogen and albumin mRNA levels in Fao-9 cells

Tilo Andus, Thomas Geiger, Toshio Hirano*, Hinnak Northoff[+], Ursula Ganter[°], Joachim Bauer[°], Tadamitsu Kishimoto* and Peter C. Heinrich

*Biochemisches Institut, Universität Freiburg, Hermann-Herder-Str. 7, D-7800 Freibrug, FRG, *Institute for Molecular and Cellular Biology, Division of Immunology, Osaka University, 1-3, Yamada-oka, Suita, Osaka 565, Japan, [+]Hinnak Northoff, Blutspendezentrale des DRK, Oberer Eselsberg, D-7900 Ulm* and [°]*Medizinische Universitätsklinik, Hugstetter Str. 55, D-7800 Freiburg, FRG*

Received 23 June 1987; revised version received 9 July 1987

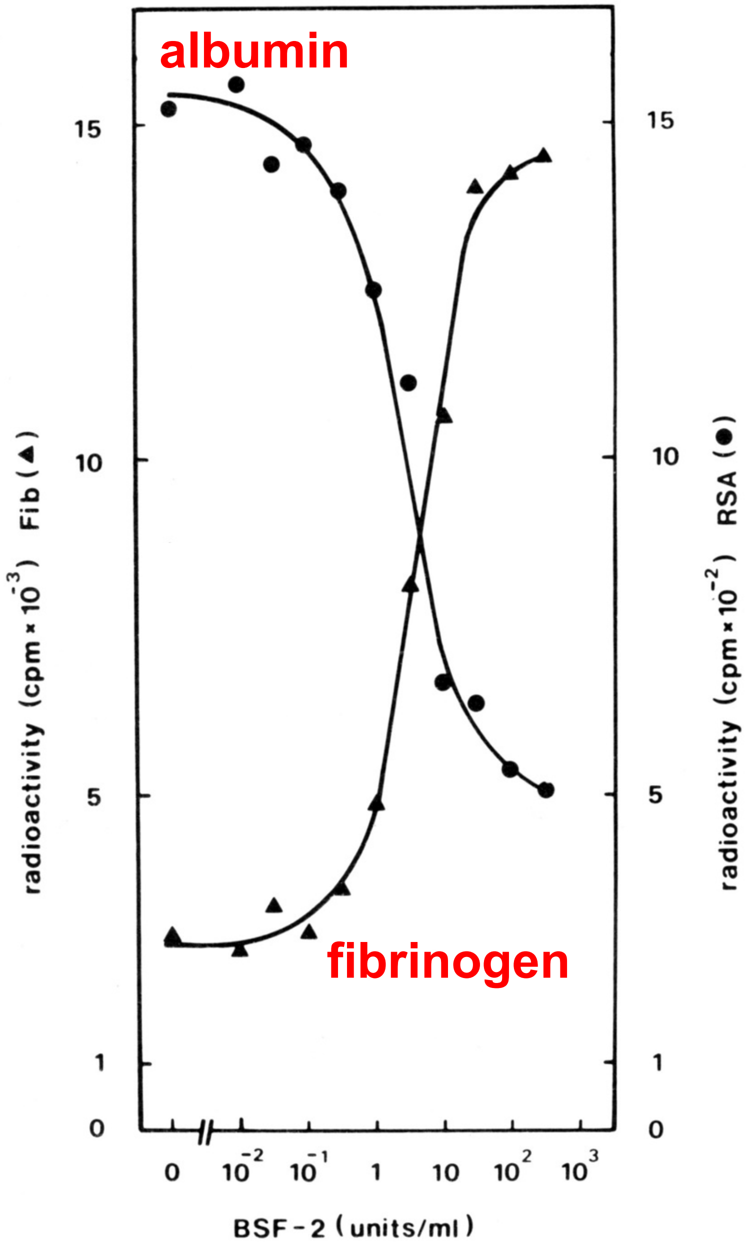
Tilo Andus



Thomas Geiger

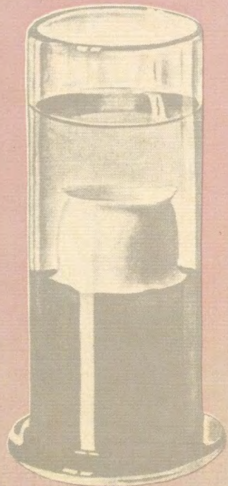
Fao-9 cells, hepatoma cells

mRNA - levels



Regulation of the Acute Phase and Immune Responses:

INTERLEUKIN-6



Editors
Pravinkumar B. Sehgal
Gerd Grieninger
Giovanna Tosato

Annals of the New York Academy of Sciences

Volume 557

New York, Dec 1988

ANNALS OF THE NEW YORK ACADEMY OF SCIENCES

Volume 557

REGULATION OF THE ACUTE PHASE AND IMMUNE RESPONSES: INTERLEUKIN-6

Edited by Pravinkumar B. Sehgal, Gerd Grieninger, and Giovanna Tosato

The New York Academy of Sciences
New York, New York
1989

New York, Dec 1988

HSF ≡ BSF-2 ≡ 26kD protein ≡ HPGF ≡ IFNβ2 ≡ IL- 6

Toshio Hirano, Tadamitsu Kishimoto

Walter Fiers

Leo Sachs

Michel Revel

ANNALS OF THE NEW YORK ACADEMY OF SCIENCES

Volume 557

REGULATION OF THE ACUTE PHASE AND IMMUNE RESPONSES: INTERLEUKIN-6

Edited by Pravinkumar B. Sehgal, Gerd Grieninger, and Giovanna Tosato

The New York Academy of Sciences
New York, New York
1989

INTERLEUKIN-6-SYNONYMS

Hepatocyte stimulating factor	**HSF**
B-cell stimulatory factor-2	**BSF-2**
Interferon β2	**IFNβ2**
26 kD protein	
Hybridoma-plasmacytoma growth factor	**HPGF**
Myeloid blood cell differentiation-inducing protein	**MGI-2A**

All these different biological activities turned out to be exerted by the same molecule/cytokine designated interleukin-6

Synonymes of „Interleukin-6“

26 kDa protein

BCDF (B-cell differentiation factors)

BCSF (B-cell stimulating factor)

BSF-2 (B-cell stimulating factor-2)

BSF-p2 (B-cell stimulating factor p2)

CDF (CAT development factor , choline acetyltransferase development factor)

CDF (cytolytic differentiation factor for T-lymphocytes)

CDF (cytolytic T-lymphocyte differentiation factor)

CDF (cytotoxic T-cell differentiation factor)

CPA (colony promoting activity)

CSF-309 (hematopoietic colony stimulating factor-309)

DIF (differentiation inducing factor)

Differentiation inducing factor for human monoblastic leukemia cells

FDGI (fibroblast-derived growth inhibitor)

Fibroblast derived differentiation inducing factor for human monoblastic leukemia cells

FSF (fibronectin stimulating factor)

HGF (hybridoma growth factor)

HGI (Hepatocyte growth inhibitory factor)

HPGF (hybridoma/plasmacytoma growth factor)

HSF (hepatocyte stimulating factor)

HSF-1 (hepatocyte stimulating factor-1)

IFN-beta-2 (Interferon-beta-2)

ILHP1 (Interleukin-hemopoietin-1)

ILHP1 (interleukin-hybridoma/plasmacytoma-1)

L-GI factor (murine lung-derived growth inhibitory factor)

L-HGF (L929-derived hybridoma growth factor

MGI-2A (Macrophage-granulocyte inducer-2A)

Mk potentiator

Myeloma GF (myeloma growth factor)

Natural killer cell activity-augmenting factor

NKAF (natural killer cell activating factor)

PCT-GF (plasmacytoma growth factor)

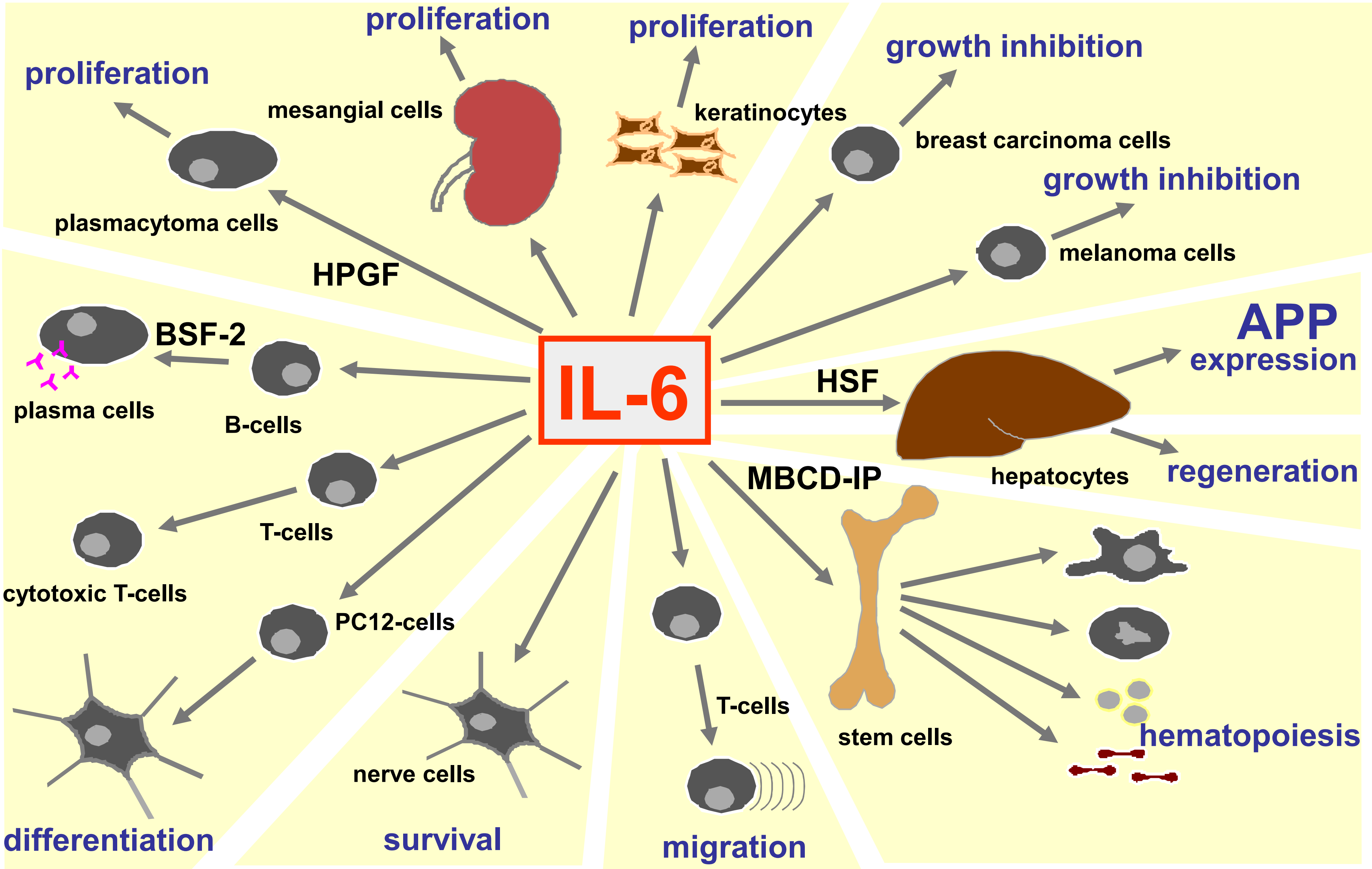
TAF (T-cell activating factor)

Thymocyte growth factor

TSF (thymocyte stimulating factor)

WI-26-VA4 factor

All these different biological activities turned out to be exerted by the same molecule/cytokine, designated interleukin-6



MBCD-IP, myeloid blood cell diff.-inducing protein ; BSF-2, B-cell stimulatory factor-2; HPGF, hybridoma plasmacytoma growth factor

IL-6 producing cells

- T-cells
- monocytes/ macrophages
- neutrophils
- synovial fibroblasts
- osteoblasts (not osteoclasts)
- endothelial cells
- adipocytes
- skeletal muscle cells

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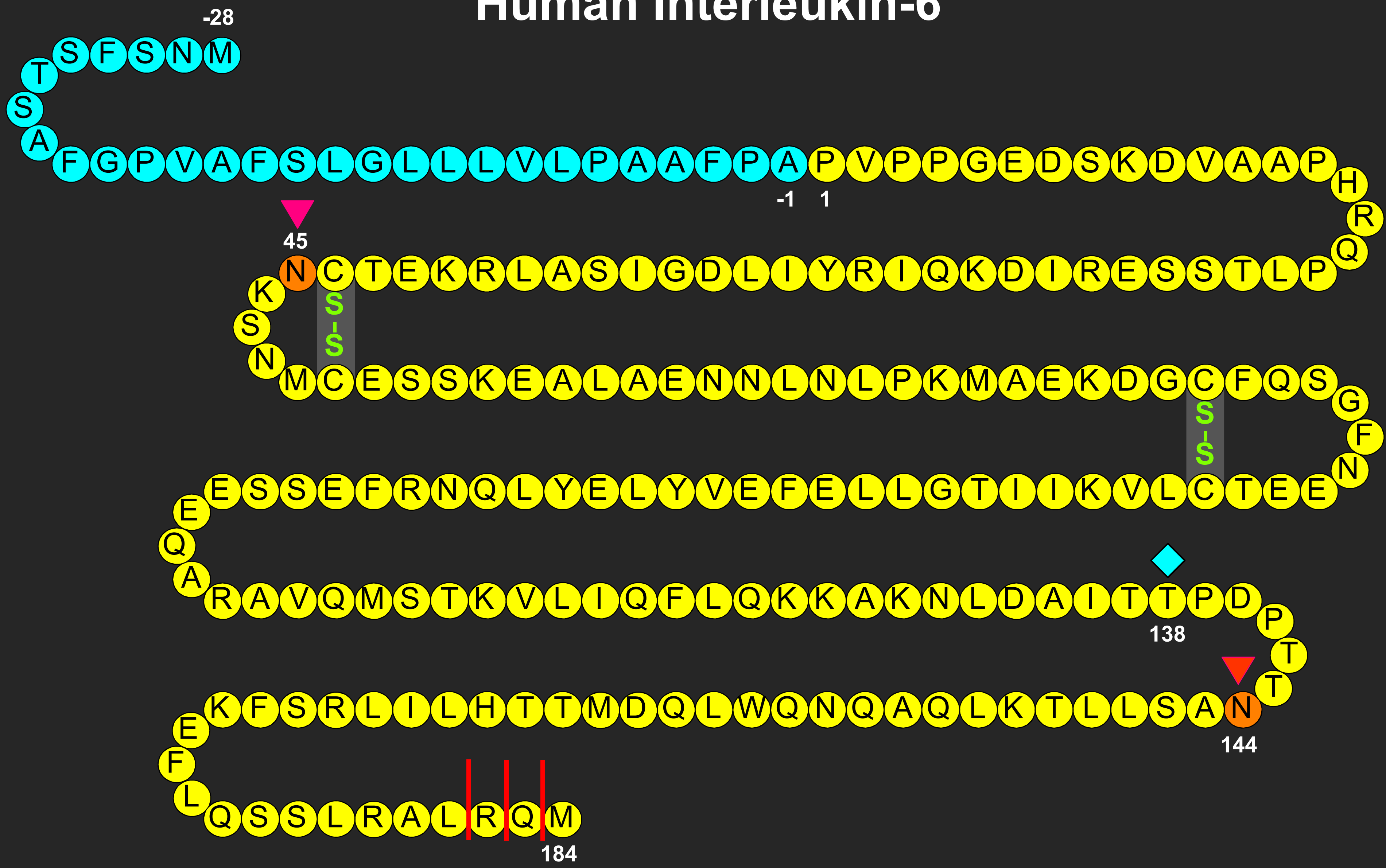
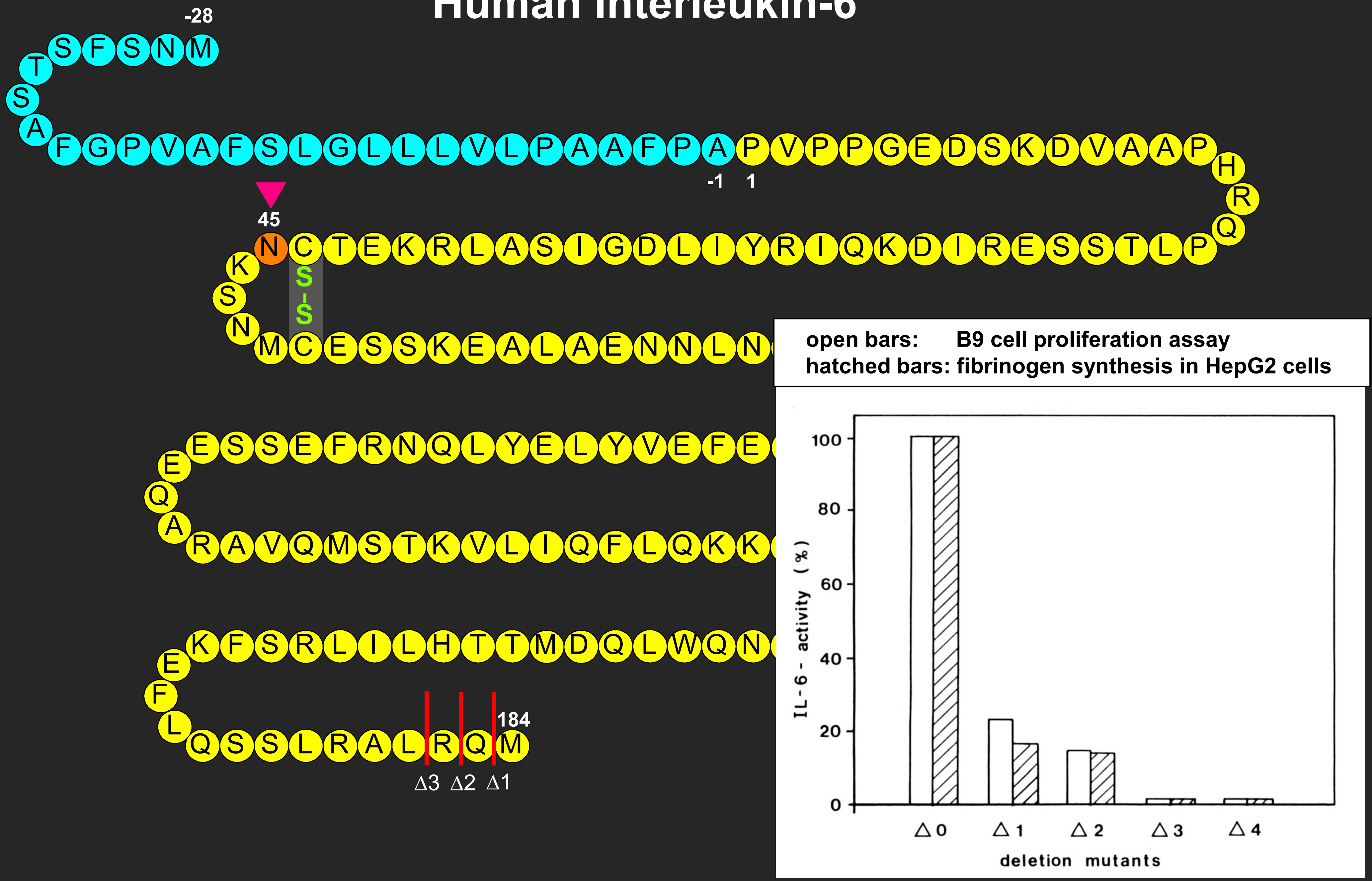
Part 2: **Regulation of IL-6 signal transduction**

Structure / function studies on IL-6

Human Interleukin-6



Human Interleukin-6

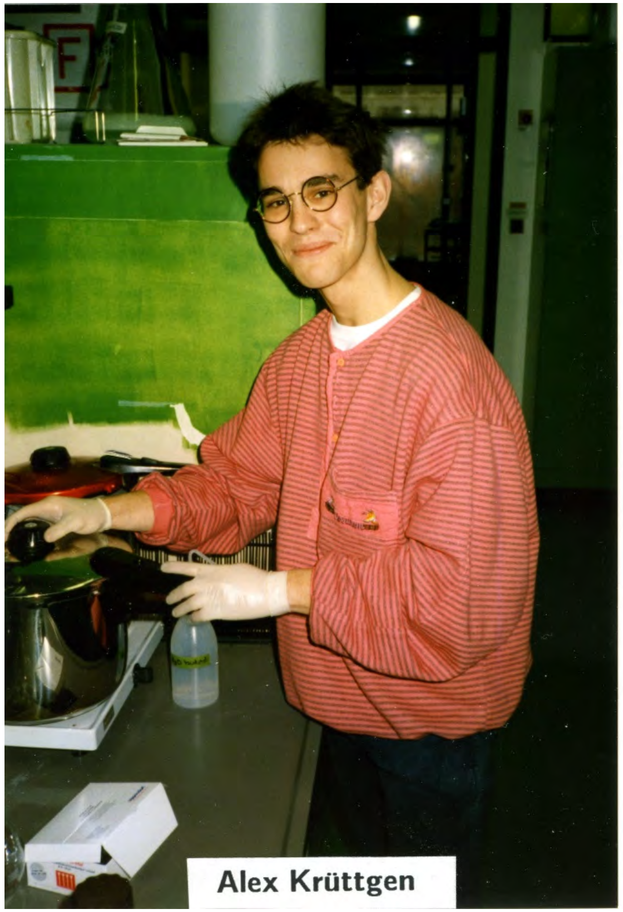


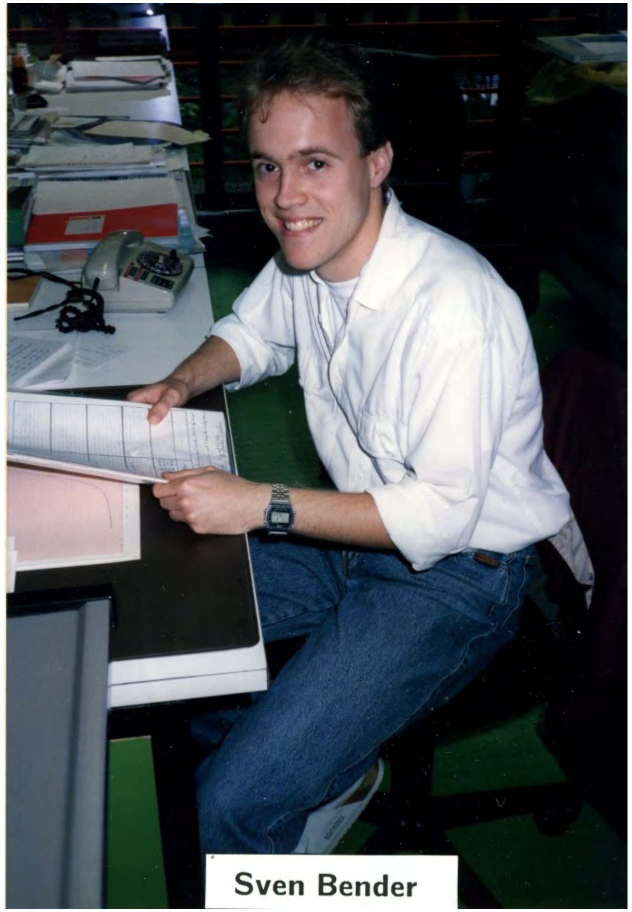
Volume 273, number 1,2, 95-98 FEBS 09028 October 1990

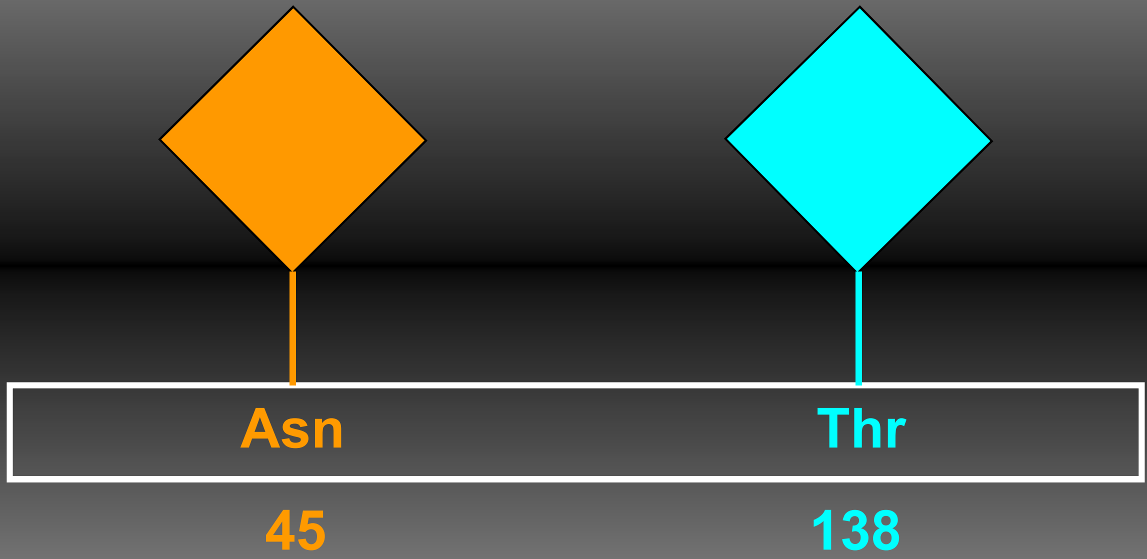
The three carboxy-terminal amino acids of human interleukin-6 are essential for its biological activity

Alex Krüttgen, Stefan Rose-John, Gabi Dufhues, Sven Bender, Claudia Lütticken, Peter Freyer and Peter C. Heinrich

Department of Biochemistry, RWTH Aachen, Klinikum, Pauwelsstrasse 30, 5100 Aachen, FRG



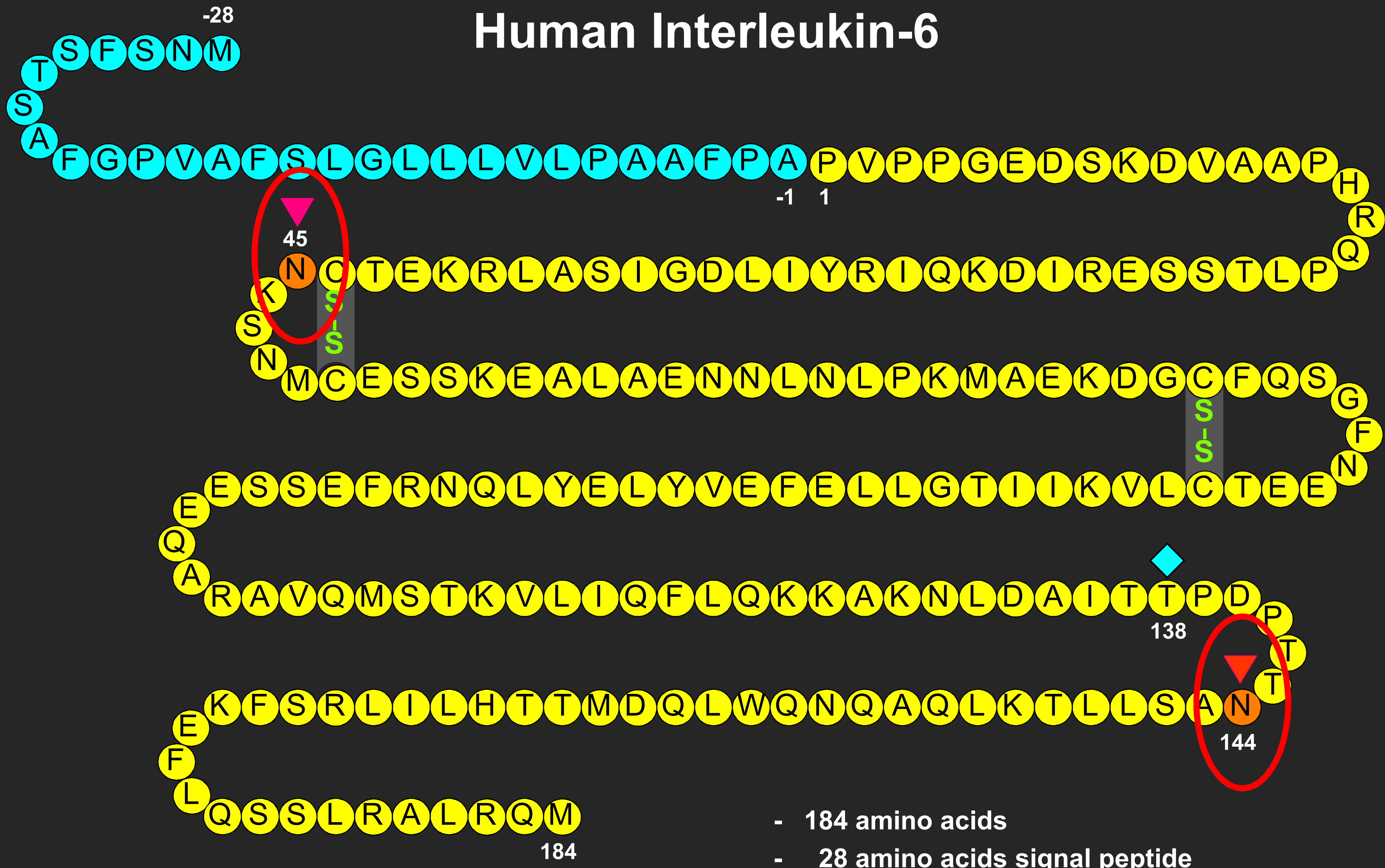





Gabi Dufhues

Heinrich, P.C., Dufhues, G., Flohe, S., Horn, F., Krause, E., Krüttgen, A., Legres, L., Lenz, D., Lütticken, C., Schooltink, H., Stoyan, T., Conradt, H.S., Rose-John, S. (1991)
Interleukin-6, its hepatic receptor and the acute phase response of the liver
42nd Mosbacher Colloquium on "Molecular Aspects of Inflammation"

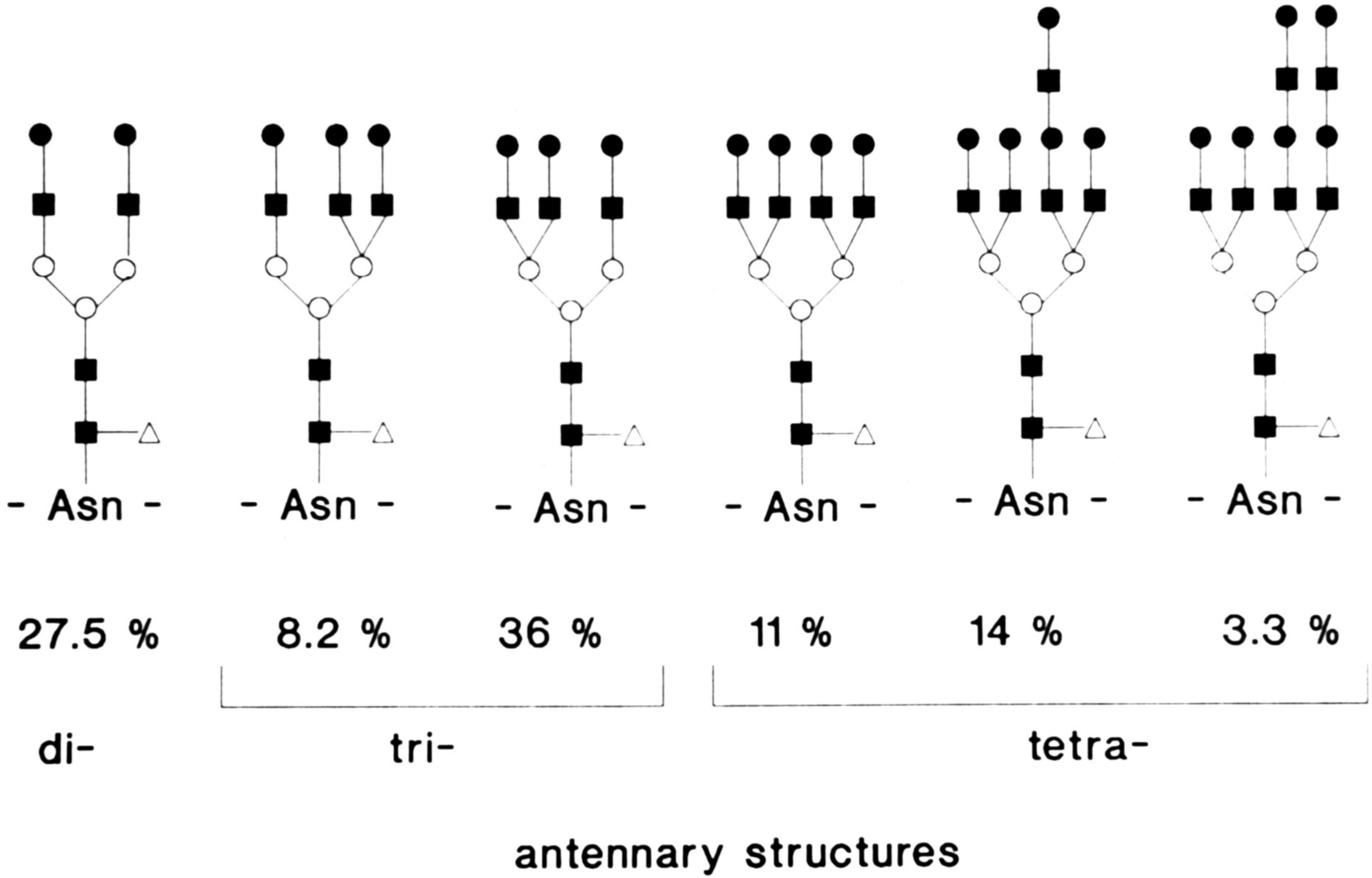
Human Interleukin-6



- 184 amino acids
- 28 amino acids signal peptide
- 2 potential N-glycosylation sites
- several O-glycosylation sites
- many different cells synthesize and secret IL-6
- pleiotropic actions

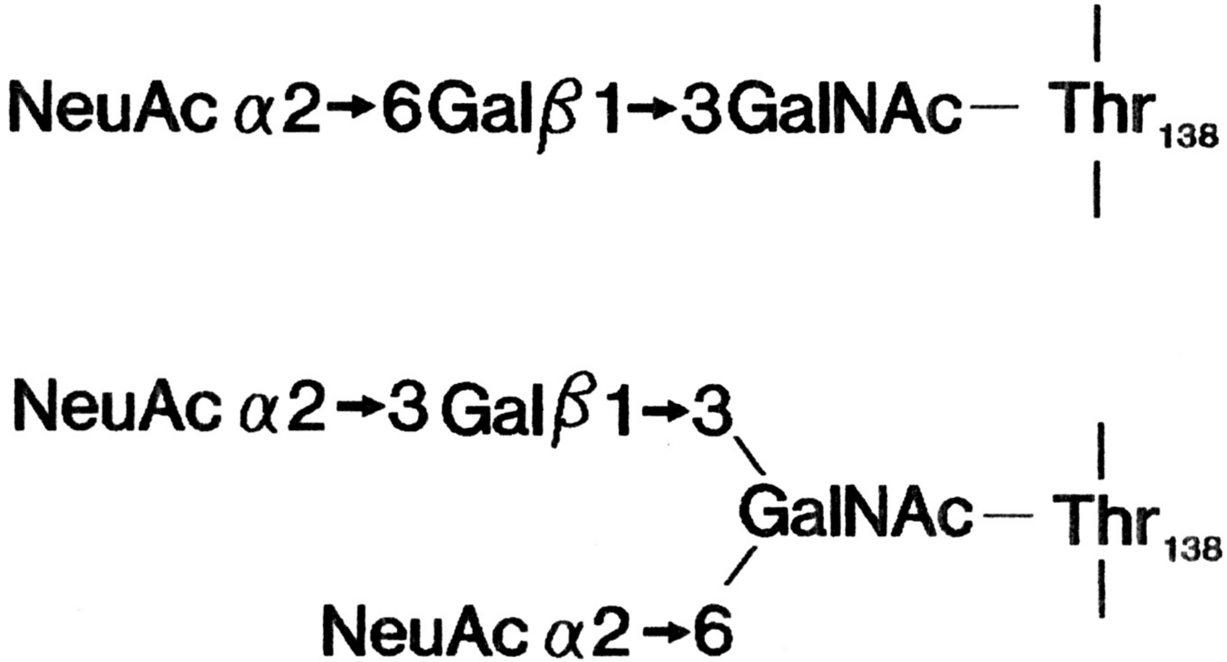
Dufhues et al. (1991) 42. Mosbach Colloquium pp129-145 Springer Verlag

Antennary structures of N-linked carbohydrate side chains



Harald Conradt, Gabi Dufhues 1991

Structures of O-linked carbohydrate side chains



Harald Conradt, Gabi Dufhues 1991

Comparison of glycosylated and unglycosylated recombinant IL-6

	Biol. Activity		Half-life
	B9 [%]	HepG2 [%]	[min]
glycosylated IL-6	100	100	5
desialylated IL-6	98	63	n.d.
unglycosylated IL-6	33	26	3

Xaver Schiel

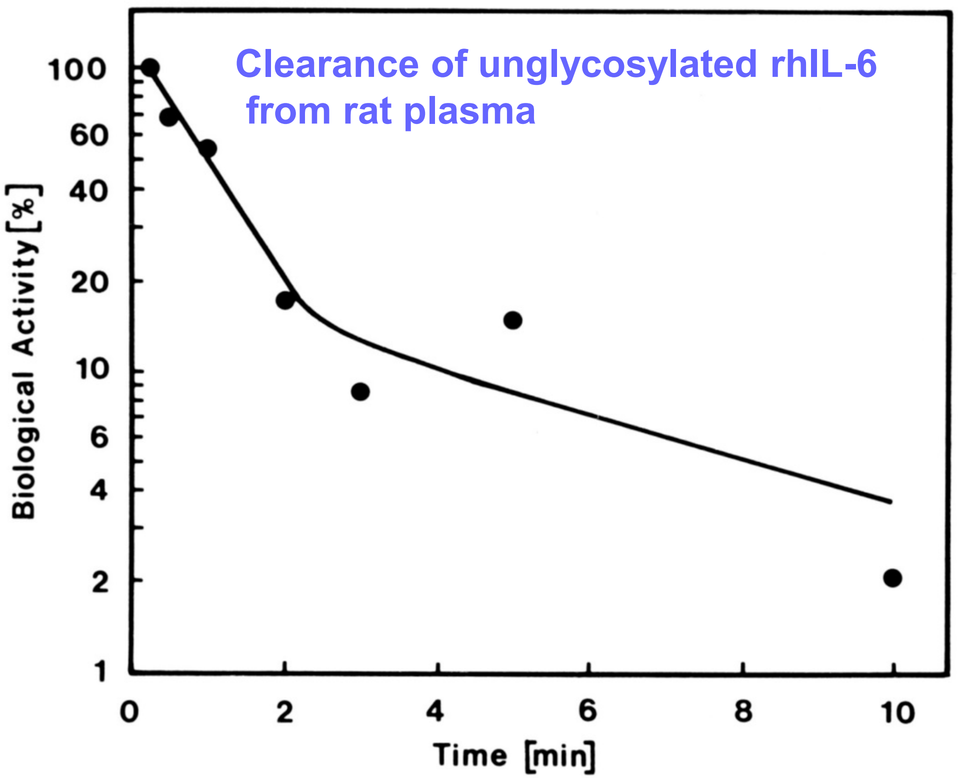
Eur. J. Biochem. *177*, 357 – 361 (1988)

Plasma clearance, organ distribution and target cells of interleukin-6/hepatocyte-stimulating factor in the rat

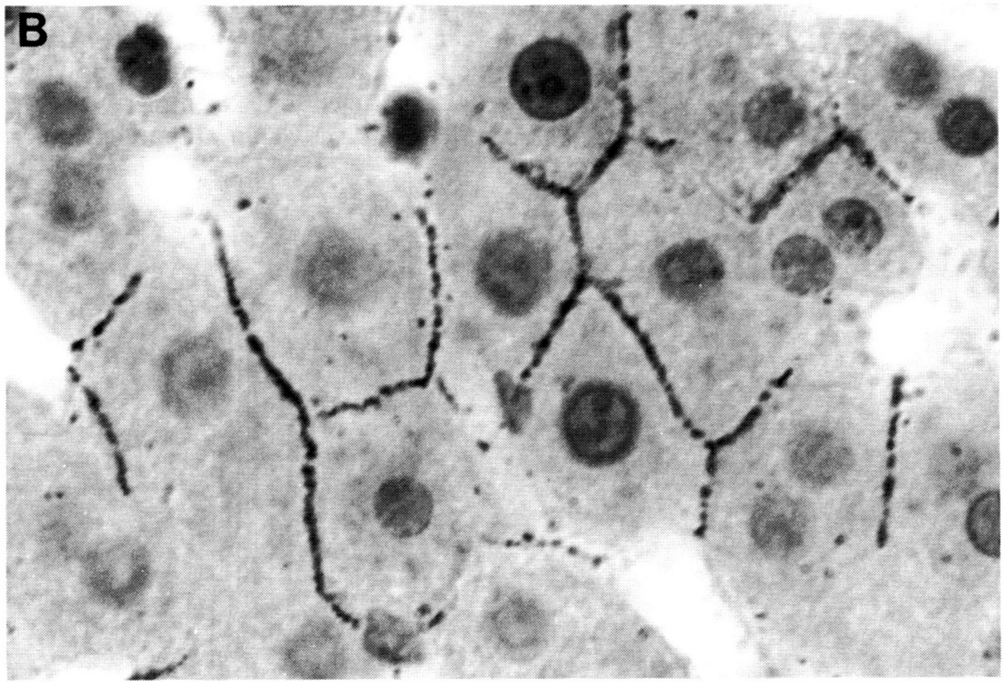
José V. CASTELL[1], Thomas GEIGER[2], Volker GROSS[2], Tilo ANDUS[2], Eicke WALTER[2], Toshio HIRANO[3], Tadamitsu KISHIMOTO[3] and Peter C. HEINRICH[1]

[1] Biochemisches Institut and [2] Medizinische Klinik II der Universität Freiburg
[3] Institute for Molecular and Cellular Biology, Osaka University

(Received March 18/June 24, 1988) – EJB 88 0319

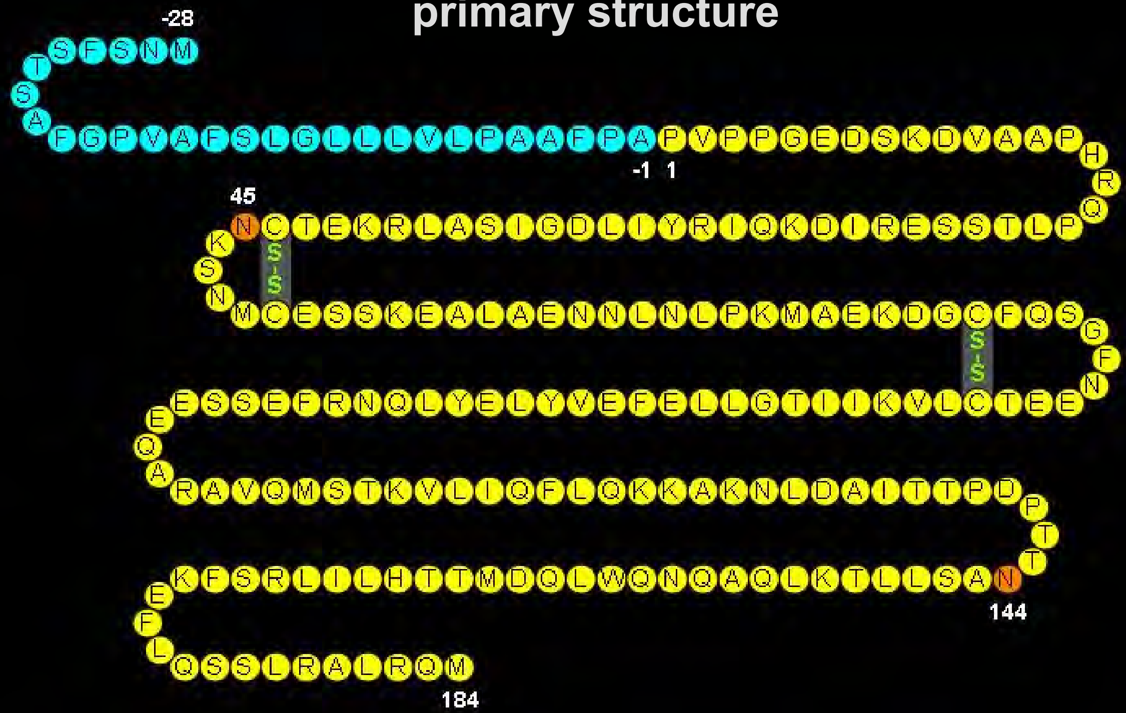


Distribution of ^{125}I- rhIL-6 in rat liver



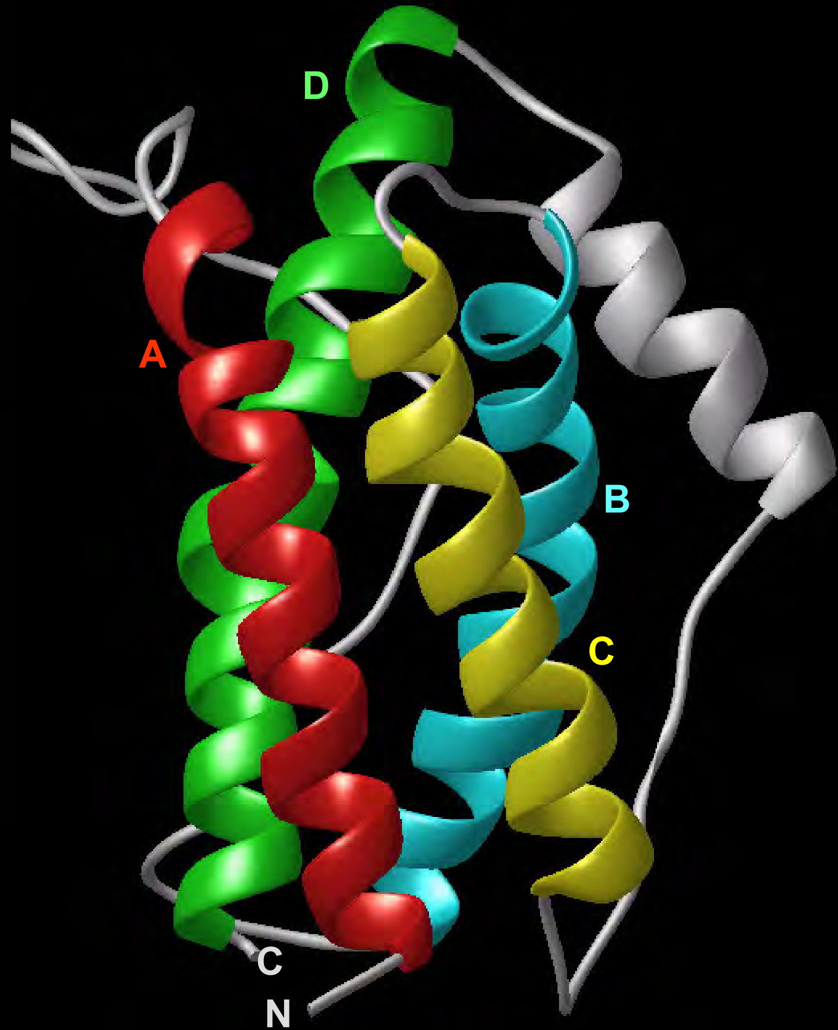
Human Interleukin-6

primary structure



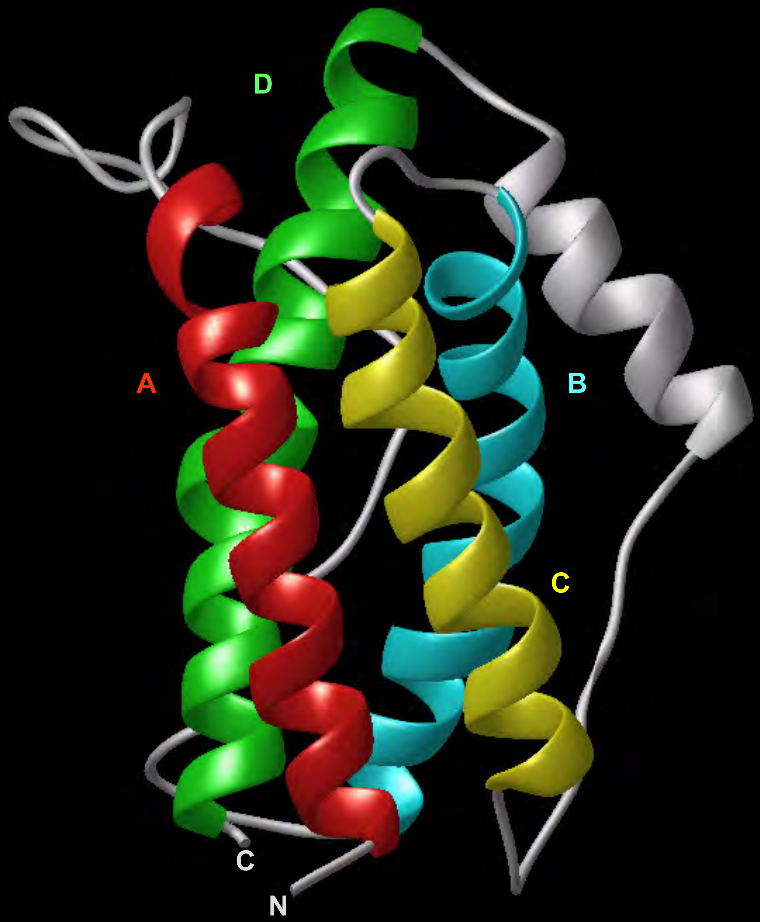
3D – structure

a bundle of 4 antiparallel helices



- single gene on chromosome 7p21
- 5 exons
- 1.3 kb poly(A) RNA
- 184 amino acids
- 28 amino acids signal peptide
- 2 N-glycosylation sites
- several O-glycosylation sites
- many different cells synthesize and secret IL-6
- pleiotropic actions

Straight helix subfamily



Interleukin-6 * (1995)

New members of the IL-6-family were discovered:

LIF — Leukemia inhibitory factor
(Baumann et al. 1989)

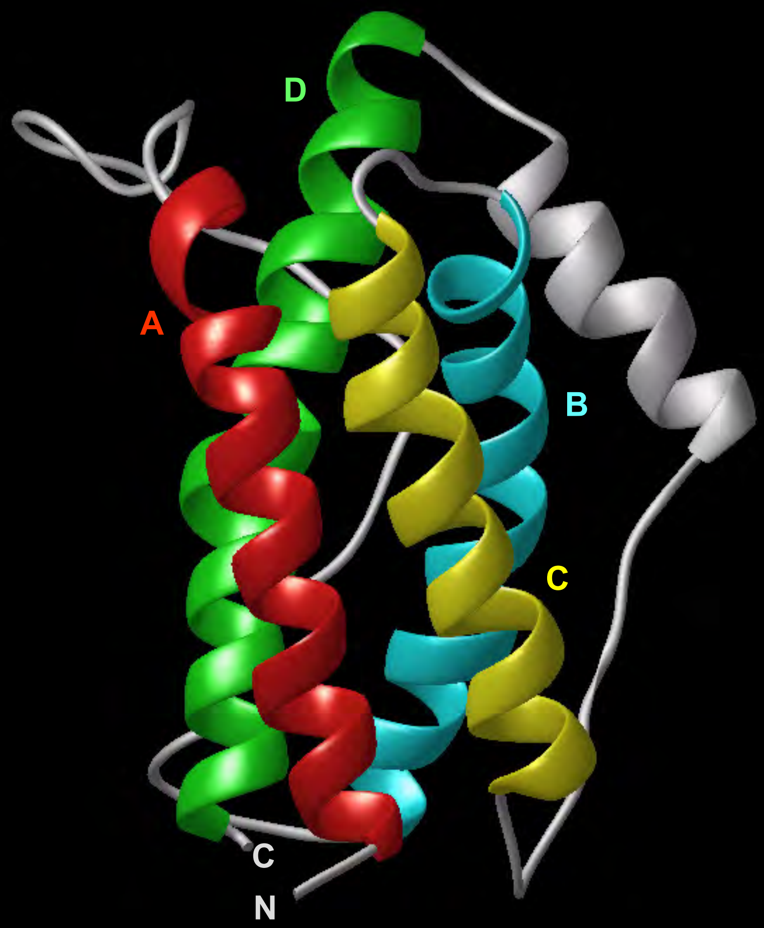
IL-11 — Interleukin 11
(Baumann et al. 1991)

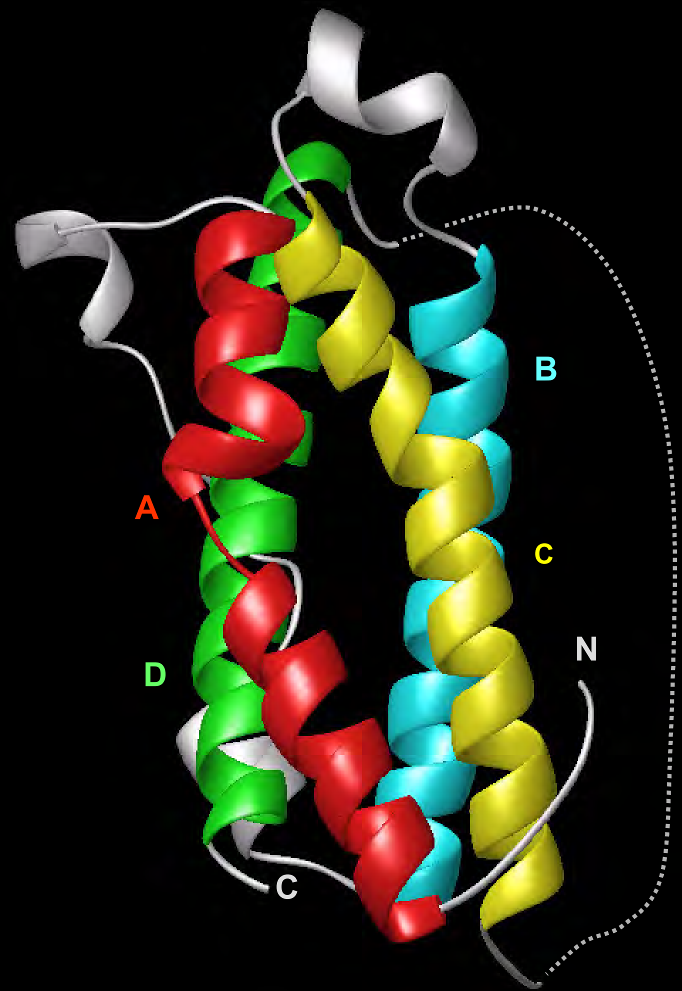
CNTF — Ciliary neurotrophic factor
(Bazan 1991)

OSM — Oncostatin M
(Richards et al. 1992)

CT — Cardiotrophin

* 3D structures solved





IL-6	**(1997)**	Somers et al. EMBO J	(X-ray)
		Xu et al. J Mol Biol	(NMR)
IL-11	**(2020)**	Metcalfe et al. J. Biol. Chem.	(X-ray)
Viral IL-6	**(2001)**	Garcia lab	(X-ray)

LIF	**(1994)**	Robinson et. al. Cell	(X-ray)
CNTF	**(1996)**	Mcdonald et al.	(X-ray)
OSM	**(2000)**	Deller et al.	(X-ray)
CT*			

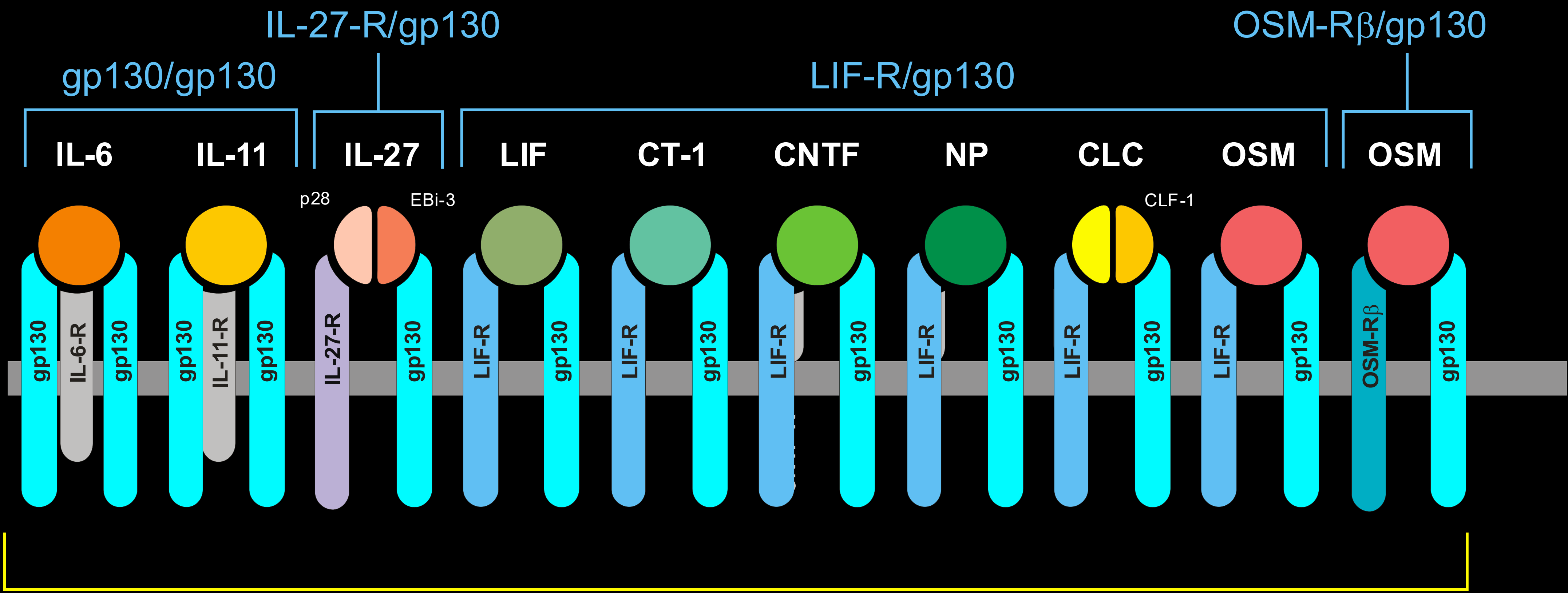
*** 3D structure not solved**

Why IL-6 type cytokine family?

1. All members of the IL-6 type cytokine family are characterized by a 3D structure which consists of a bundle of 4 anti-parallel helices, although the primary sequences are different.

2. The receptors of the IL-6 type cytokines contain two gp130 or one gp130 and one LIFRβ molecule.

Family of IL-6 type cytokines



Receptors sharing the signal transducer gp130

LIF, Leukemia inhibitory factor CT, Cardiotrophin CNTF, Ciliary neurotrophic factor OSM, Oncostatin M

Ciba Foundation Symposium 167

1992

POLYFUNCTIONAL CYTOKINES: IL-6 AND LIF

Outline: Interleukin-6 signal transduction and its regulation

Part 1: **Molecular mechanisms of IL-6 signal transduction**

- **The acute phase response**
- **Identification of HSF as IL-6**
- **Structure and function of IL-6**
- **Acute phase protein synthesis induced by IL-6**
- **Molecular mechanism of IL-6 induced APP expression**
- **Formation of the IL-6-receptor complex**
- **Design of a highly potent IL-6 antagonist**
- **Molecular mechanisms of IL-6 signal transduction**
- **Nuclear translocation of STAT3-YFP**

Part 2: **Regulation of IL-6 signal transduction**

Acute phase protein synthesis induced by IL-6

Eur. J. Biochem. *173*, 287 – 293 (1988)
 1988

Regulation of synthesis and secretion of major rat acute-phase proteins by recombinant human interleukin-6 (BSF-2/IL-6) in hepatocyte primary cultures

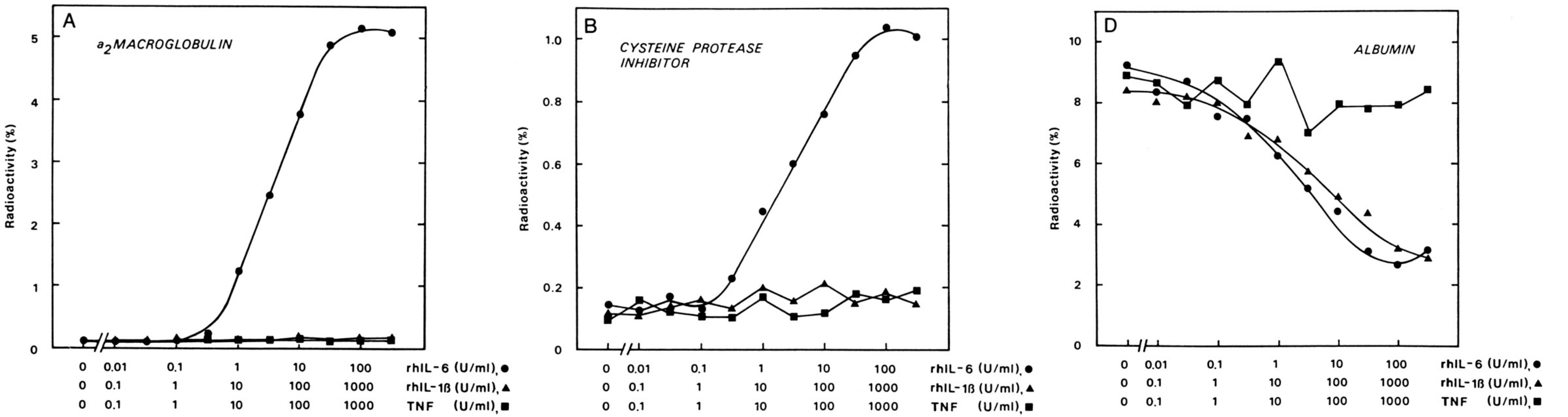
Tilo ANDUS[1], Thomas GEIGER[1], Toshio HIRANO[2], Tadamitsu KISHIMOTO[2], Thuy-Anh TRAN-THI[1], Karl DECKER[1] and Peter C. HEINRICH[1]

[1] Biochemisches Institut, Universität Freiburg
[2] Institute for Molecular and Cellular Biology, Division of Immunology, Osaka University

178 citations
(07/2022)

(Received November 2, 1987/January 15, 1988) — EJB 87 1216

α_2M-protein CPI - protein albumin - protein



THE JOURNAL OF BIOLOGICAL CHEMISTRY

Vol. 260, No. 10, Issue of May 25, pp. 6200–6205, 1985
Printed in U.S.A.

Molecular Cloning of cDNA Sequences for Rat α_2-Macroglobulin and Measurement of Its Transcription during Experimental Inflammation*

(Received for publication, December 4, 1984)

Wolfgang Northemann, Michael Heisig, Dieter Kunz, and Peter C. Heinrich
From the Biochemisches Institut, Universität Freiburg, Hermann-Herder-Str. 7, D-7800 Freiburg i. Br., Federal Republic of Germany

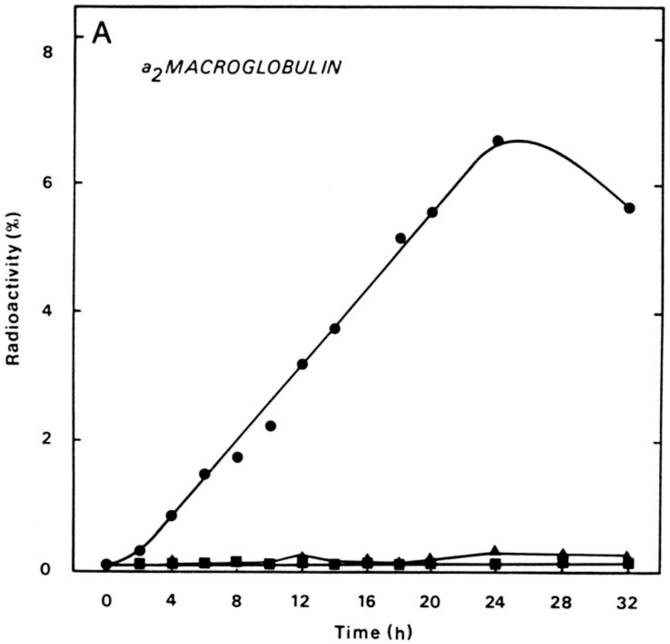
Tilo Andus,
Thomas Geiger,
Toshio Hirano○**,**
Tadamitsu Kishimoto○ **and**
Peter C. Heinrich+

Biochemical Institute, University of Freiburg+ **and**
Institute for Molecular and Cellular Biology, Osaka University○**,**
Division of Immunology, Osaka

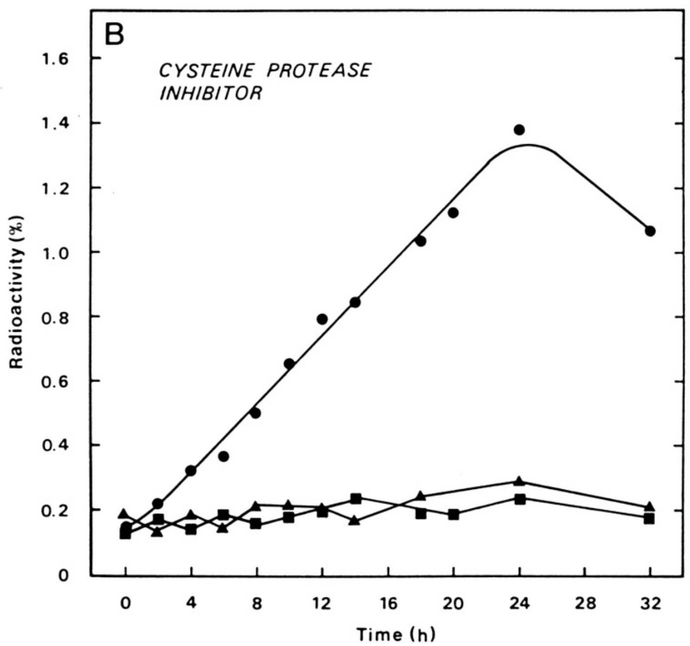
Action of recombinant human interleukin 6, interleukin 1β and tumor necrosis factor α on the mRNA induction of acute-phase proteins*

in rat hepatocytes

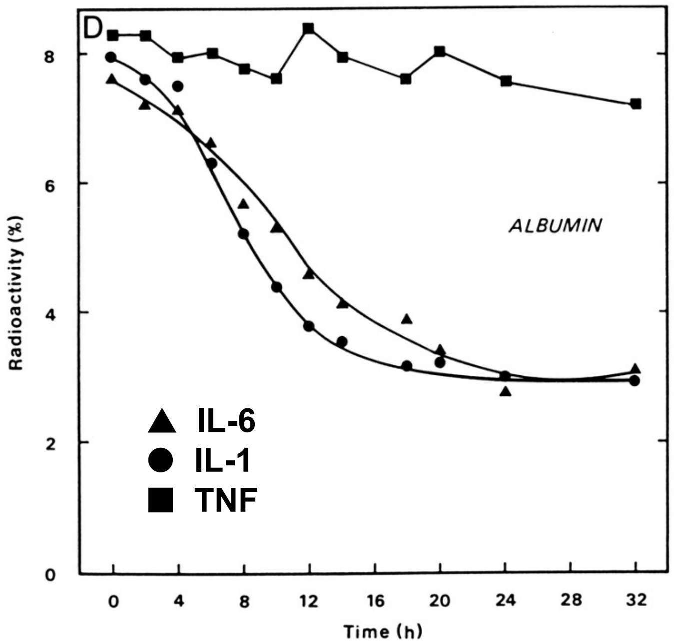
α_2M - mRNA



CPI - mRNA



albumin - mRNA



time dependence after rhIL-6

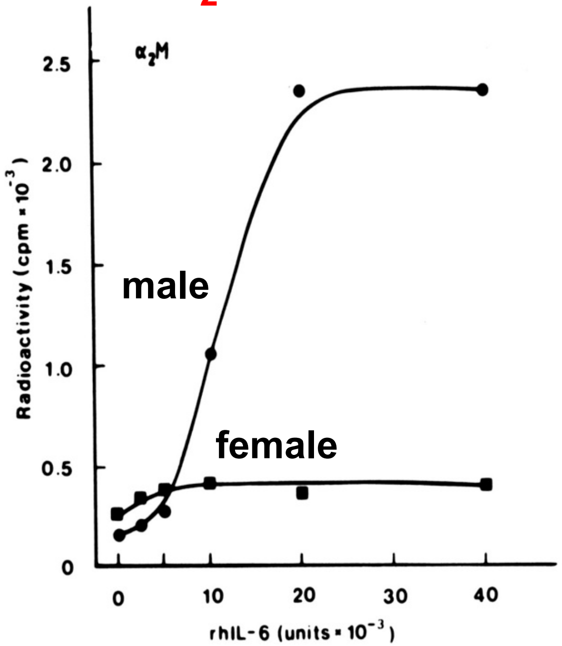
Thomas Geiger,
Tilo Andus,
Jan Klapproth,
Toshio Hirano[+],
Tadamitsu Kishimoto[+] and
Peter C. Heinrich

Biochemisches Institut, Universität Freiburg, Freiburg, and Institute for Molecular and Cellular Biology, Osaka University[+], Division of Immunology, Osaka

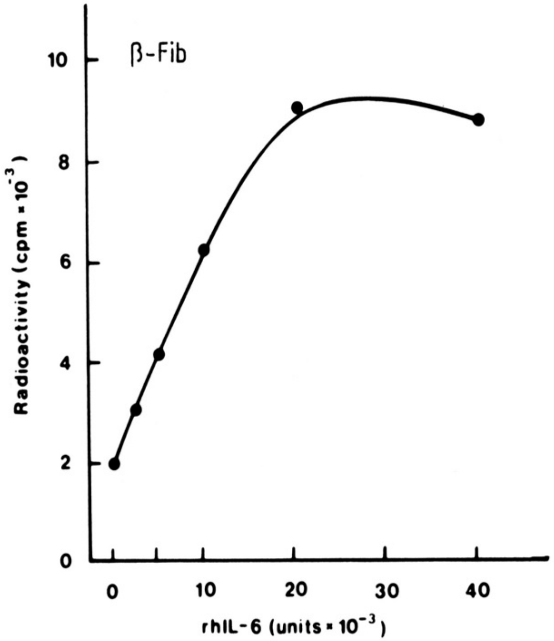
Induction of rat acute-phase proteins by interleukin 6 *in vivo**

(rat)

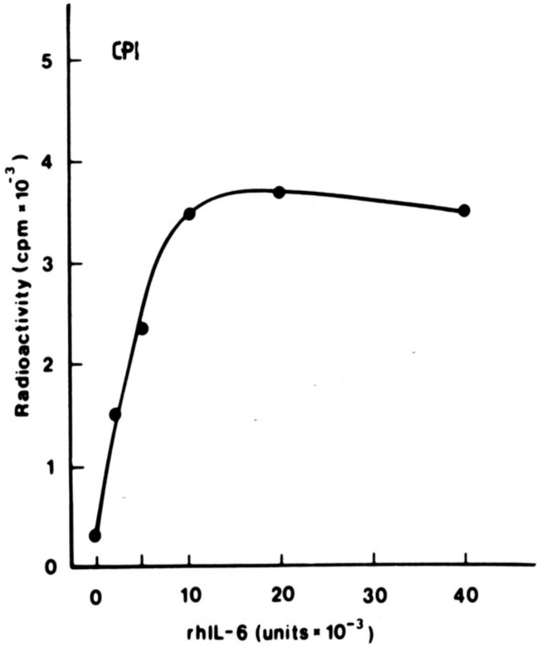
α_2M-mRNA



Fibrinogen-mRNA



CPI-mRNA



rhIL-6 dose dependence

Volume 242, number 2, 237–239 FEB 06595 January 1989

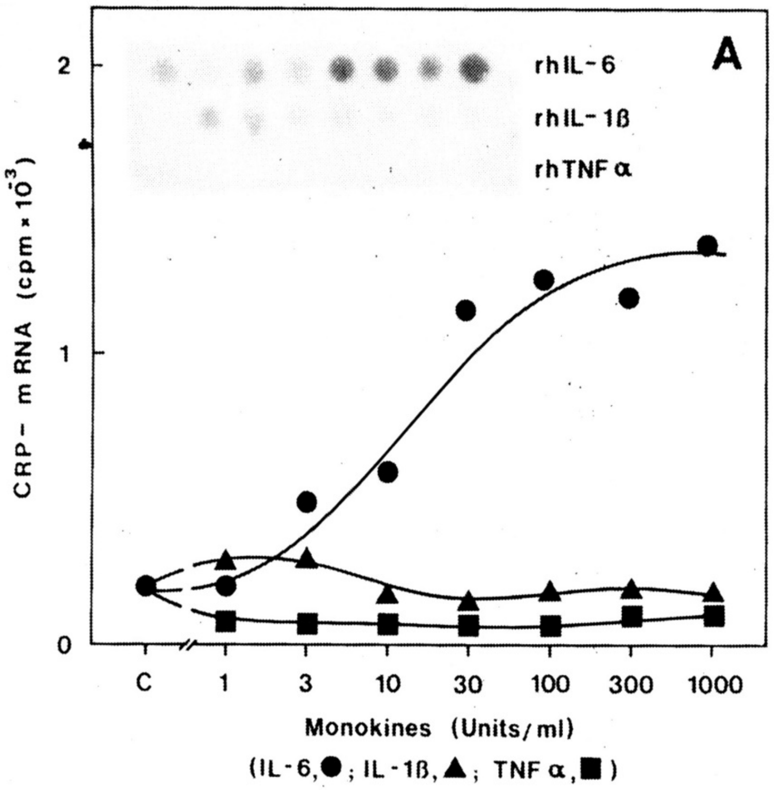
Interleukin-6 is the major regulator of acute phase protein synthesis in adult human hepatocytes

José V. Castell, Maria J. Gómez-Lechón[+], Martina David, Tilo Andus[°], Thomas Geiger[°], Ramón Trullenque*, Ricardo Fabra* and Peter C. Heinrich

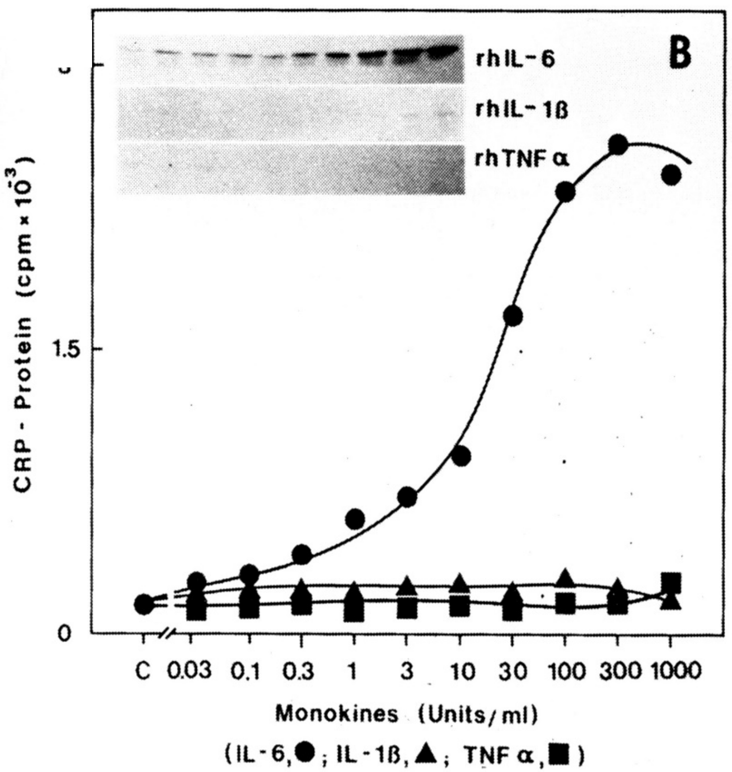
Biochemisches Institut, Universität Freiburg, Hermann-Herder-Str. 7, D-7800 Freiburg, FRG, [+]Centro de Investigación, Hospital La Fe, INSALUD, Avda. de Campanar, 21, E-46009 Valencia, Spain, [°]Medizinische Klinik der Universität Freiburg, Hugstetter Str. 55, D-7800 Freiburg, FRG and **Servicio de Cirugia, Hospital General de Valencia, Avda. del Cid s/n, E-46018 Valencia, Spain*

Received 12 October 1988

CRP- mRNA



CRP- protein

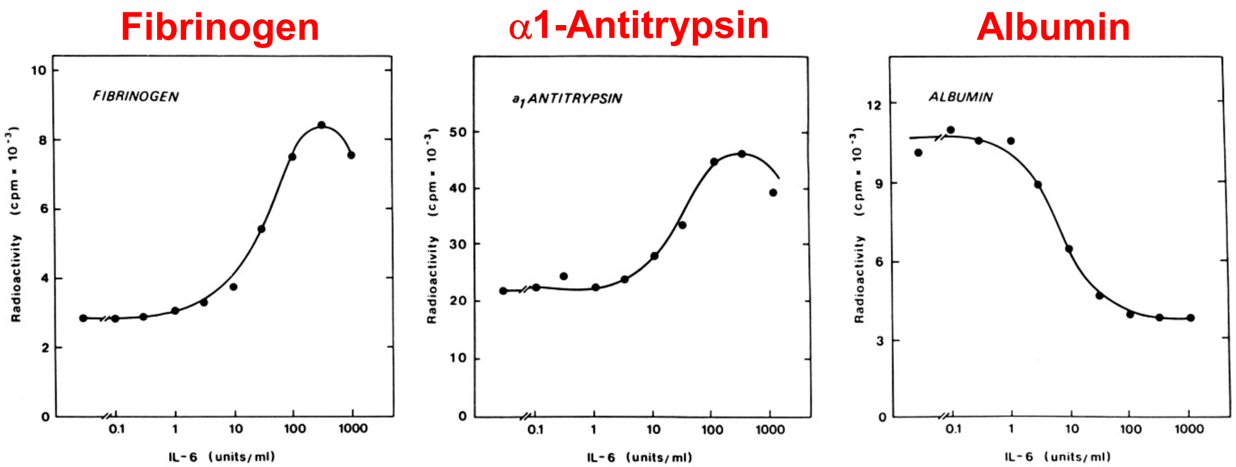


Reprinted from HEPATOLOGY, St. Louis
Vol. 12, No. 5, pp. 1179-1186, November, 1990, (Printed in the U.S.A.)

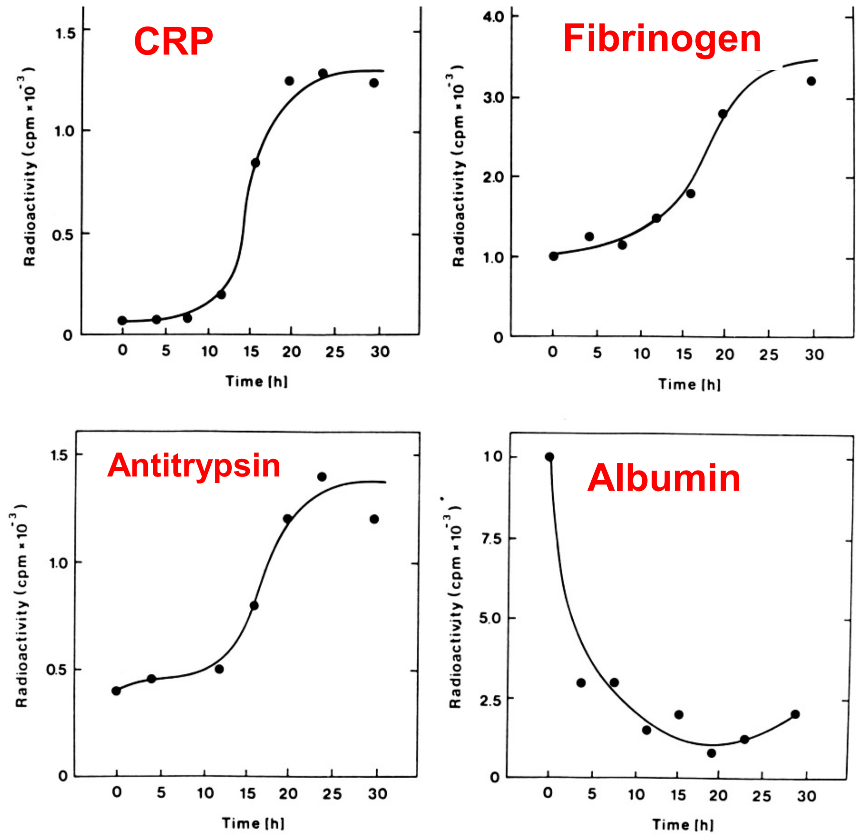
Acute-phase Response of Human Hepatocytes: Regulation of Acute-phase Protein Synthesis By Interleukin-6

JOSÉ V. CASTELL,[1] MARIA JOSÉ GÓMEZ-LECHÓN,[2] MARTINA DAVID,[1] RICARDO FABRA,[3] RAMÓN TRULLENQUE[3] AND PETER C. HEINRICH[1]

Human hepatocytes



IL-6 dose dependence



time dependence after IL-6

IL-6 type cytokine family: overlapping biological effects

Cytokine-mediated effect	IL-6	IL-11	LIF	OSM	CNTF	CT-1
Maintenance of ES cell pluripotency	-/+	-	+	+	+	+
Macrophage differentiation in M1 cells	+	-	+	+	-/+	+
Growth promotion of myeloma cells	+	+	+	+	+	nd
Promotion of thrombopoiesis	+	+	+	+	nd	nd
Induction of hepatic acute phase proteins	+	+	+	+	+	+
Induction of ACTH secretion *in vivo*	+	+	+	+	+	+
Induction of ACTH secretion *in vitro*	-/+	+	+	+	nd	nd
Neural differentiation	+	+	+	+	+	+
Induction of bone loss/osteclast formation	+	+	+	+	nd	nd
Induction of cardiac hypertrophy *in vitro*	-/+	+	+	+	-/+	+

Auernhammer and Melmed 2000

Outline: Interleukin-6 signal transduction and its regulation

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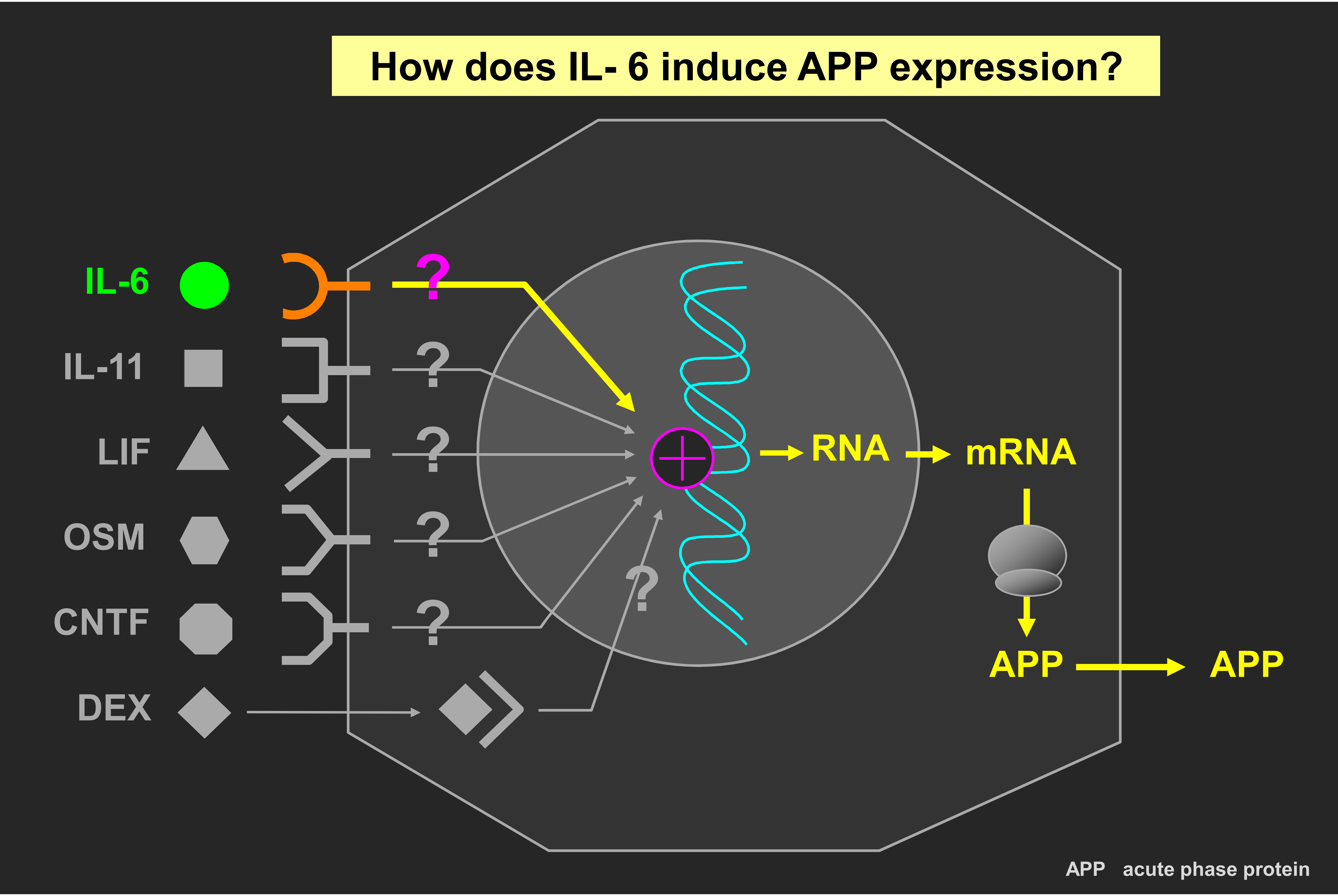
Part 2: **Regulation of IL-6 signal transduction**

Molecular mechanism of IL-6 induced APP expression in liver cells

APP, acute phase protein


How does IL- 6 induce APP expression?
IL-6
IL-11
LIF
OSM
CNTF
DEX
?
?
?
?
?
?
RNA
mRNA
APP
APP
APP acute phase protein

Reprinted from Biochemistry, 1988, *27*, 9194.

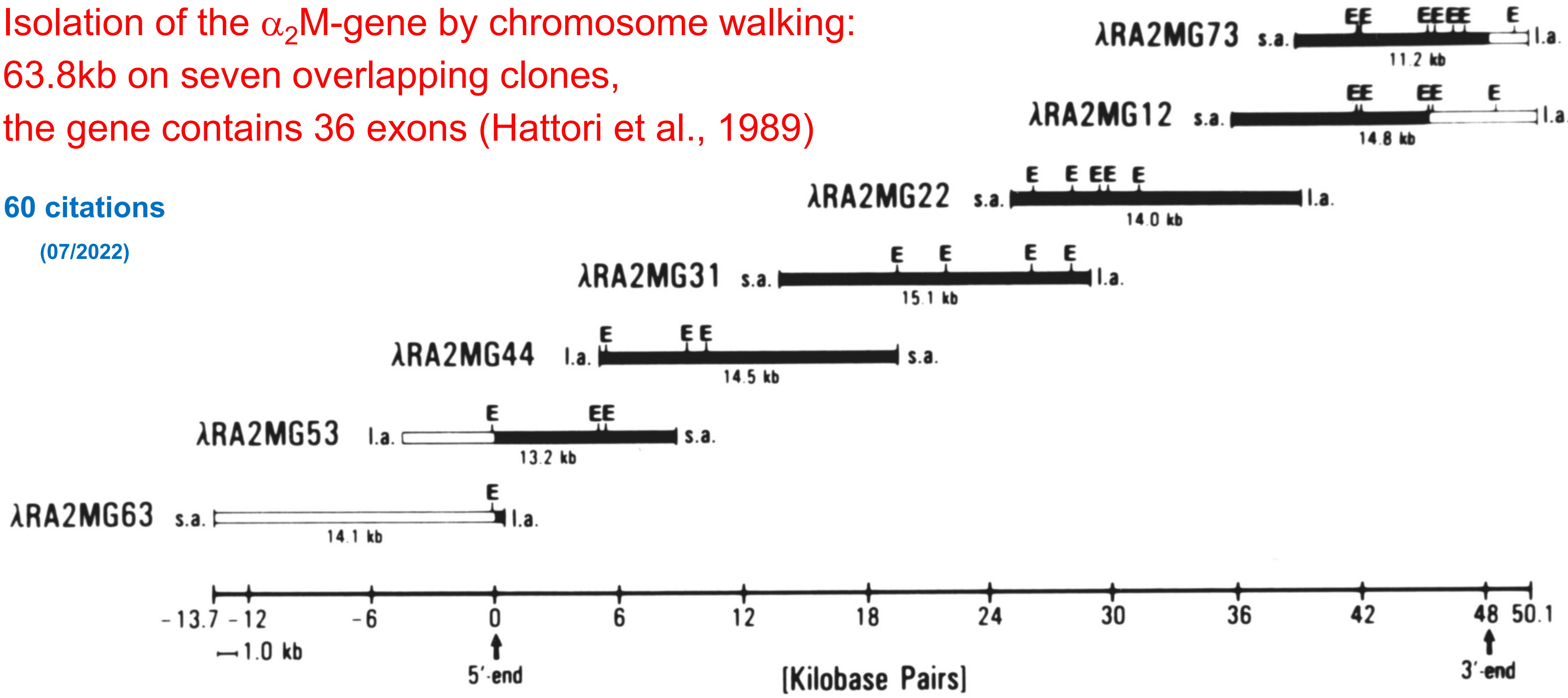
Structure and Acute-Phase Regulation of the Rat α_2-Macroglobulin Gene†

Wolfgang Northemann,‡ Brian R. Shiels,‡,§ Todd A. Braciak,‡ Richard W. Hanson,‖ Peter C. Heinrich,⊥ and Georg H. Fey*,‡

Department of Immunology, Research Institute of Scripps Clinic, La Jolla, California 92037, Department of Biochemistry, School of Medicine, Case Western Reserve University, Cleveland, Ohio 44106, and Institute of Biochemistry, University of Freiburg, Freiburg, West Germany

Received April 1, 1988; Revised Manuscript Received July 28, 1988

Isolation of the α_2M-gene by chromosome walking:
63.8kb on seven overlapping clones,
the gene contains 36 exons (Hattori et al., 1989)



Volume 17 Number 3 1989 Nucleic Acids Research

Identification of the promoter sequences involved in the interleukin-6 dependent expression of the rat α_2-macroglobulin gene

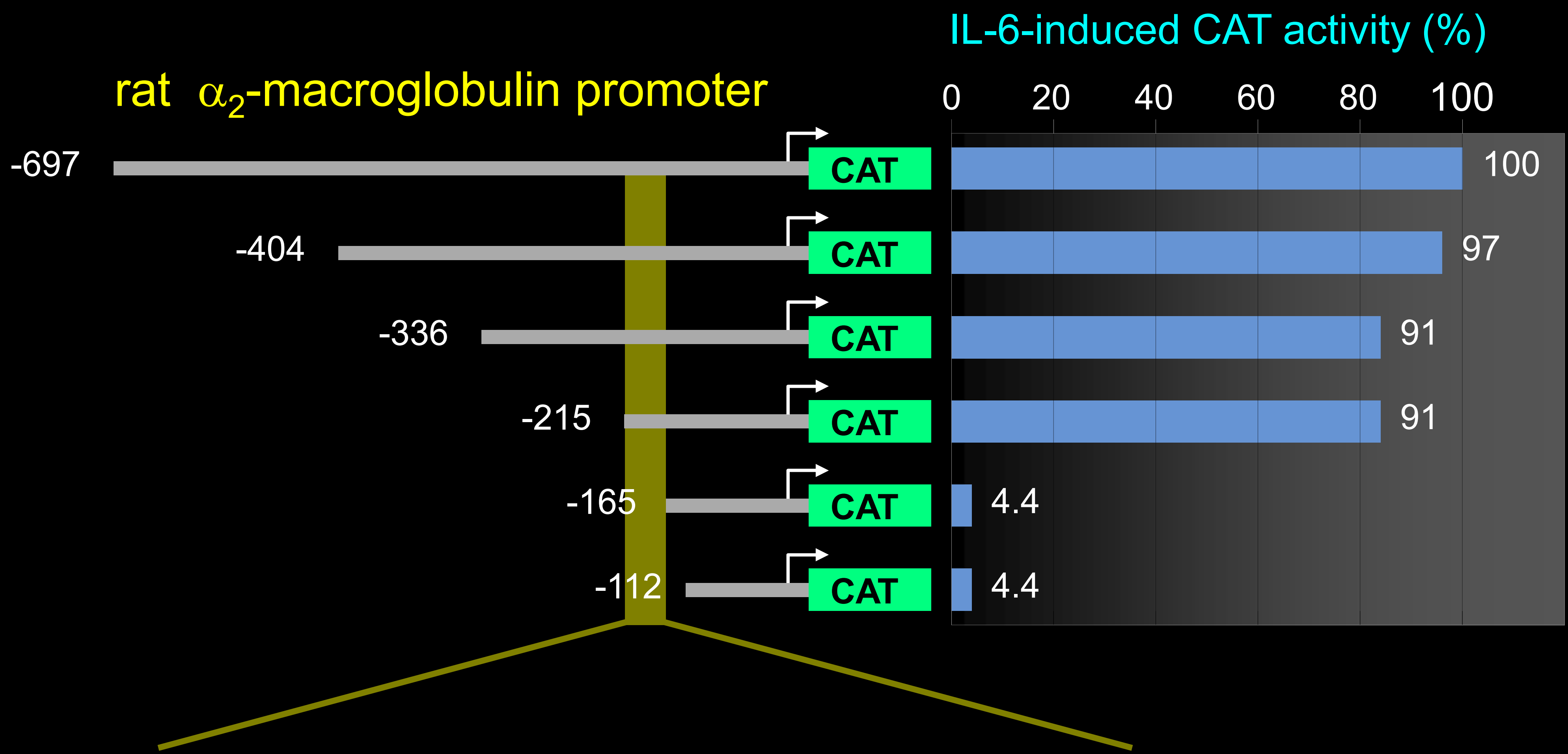
Dieter Kunz, René Zimmermann[1], Michael Heisig and Peter C.Heinrich[1]*

Biochemisches Institut, Universität Freiburg, D-7800 Freiburg and [1]Institut für Biochemie, Neuklinikum Aachen, Pauwelsstrasse, D-5100 Aachen, FRG

Received September 28, 1988; Revised December 20, 1988; Accepted January 13, 1989 Acc. no.+

Search for an acute phase response element (APRE) in the α_2-macroglobulin-promoter

Analysis of the α_2-macroglobulin promoter



GTAACTGGAAAGTCCTTAATCCTTCTGGGAAT

Acute-Phase Response Element (APRE)

transfected HepG2 cells

1988 start in Aachen

Friedemann Horn

Promoter sequences of APP genes

T	T	C	T	G	G	G	A	A	rat α_2M "core"
T	A	A	C	T	G	G	A	A	rat α_2M "core-like"
T	T	A	C	G	G	G	A	A	human α_2M
G	T	A	C	G	G	T	A	A	human α_2M
T	T	C	T	G	G	G	A	A	rat α_1-acid glycoprotein ("C")
T	T	A	C	T	G	G	A	A	human haptoglobin ("B")
G	T	A	C	T	G	G	A	A	human γ-fibrinogen, IL-6RE
T	T	C	T	G	G	T	A	A	human α_1-ACT, IL-6RE
T	T	C / A	C	N	G	G / T	A	A	**palindromic consensus**

MOLECULAR AND CELLULAR BIOLOGY, Jan. 1993, p. 276–288
0270-7306/93/010276-13$02.00/0

Vol. 13, No. 1

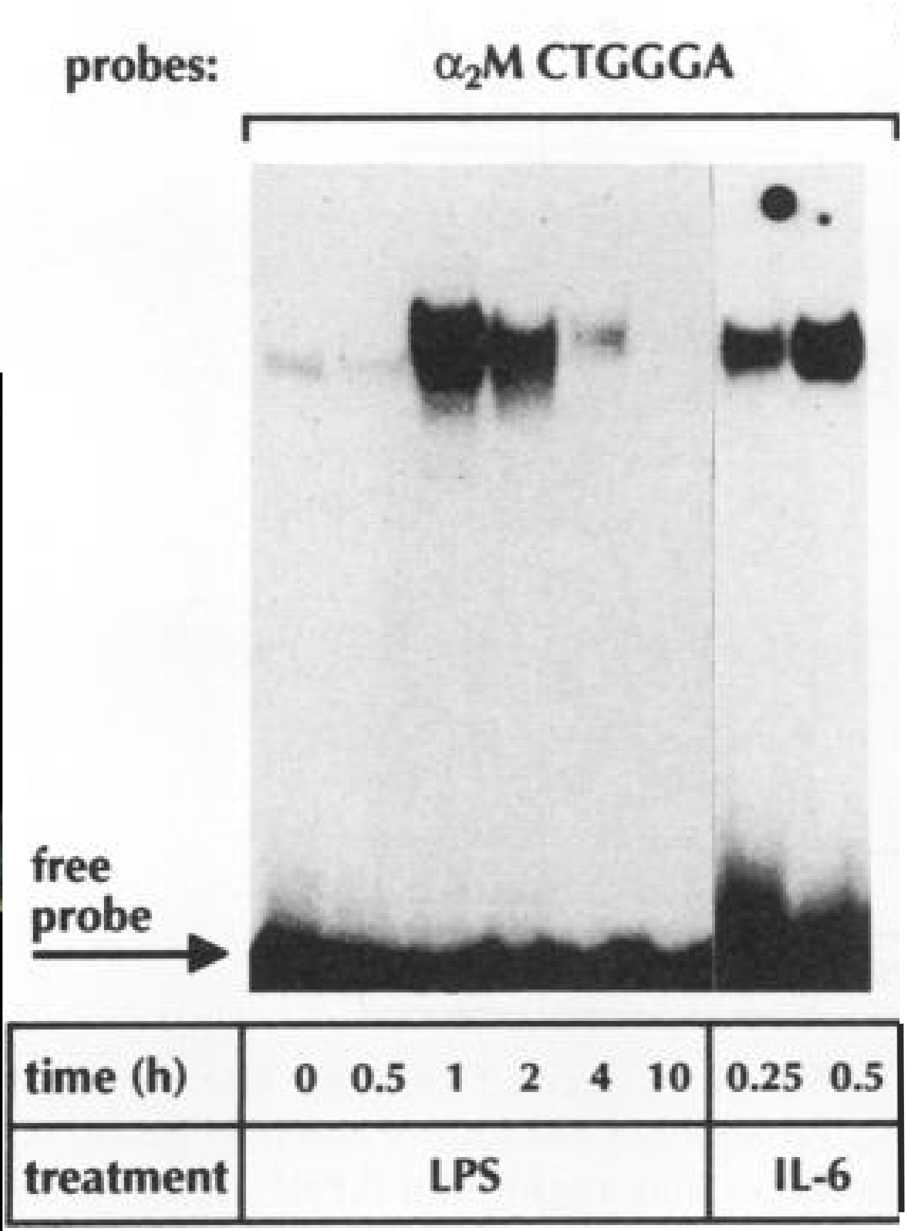
Acute-Phase Response Factor, a Nuclear Factor Binding to Acute-Phase Response Elements, Is Rapidly Activated by Interleukin-6 at the Posttranslational Level

URSULA M. WEGENKA, JAN BUSCHMANN, CLAUDIA LÜTTICKEN, PETER C. HEINRICH,* AND FRIEDEMANN HORN

Institute for Biochemistry, RWTH Aachen, Pauwelsstrasse 30, D-5100 Aachen, Germany

1993

Received 15 June 1992/Returned for modification 17 September 1992/Accepted 16 October 1992



injection of LPS or IL-6 into rats

Molecular and Cellular Biology, May 1994, p. 3186–3196
0270-7306/94/$04.00+0

Vol. 14, No. 5

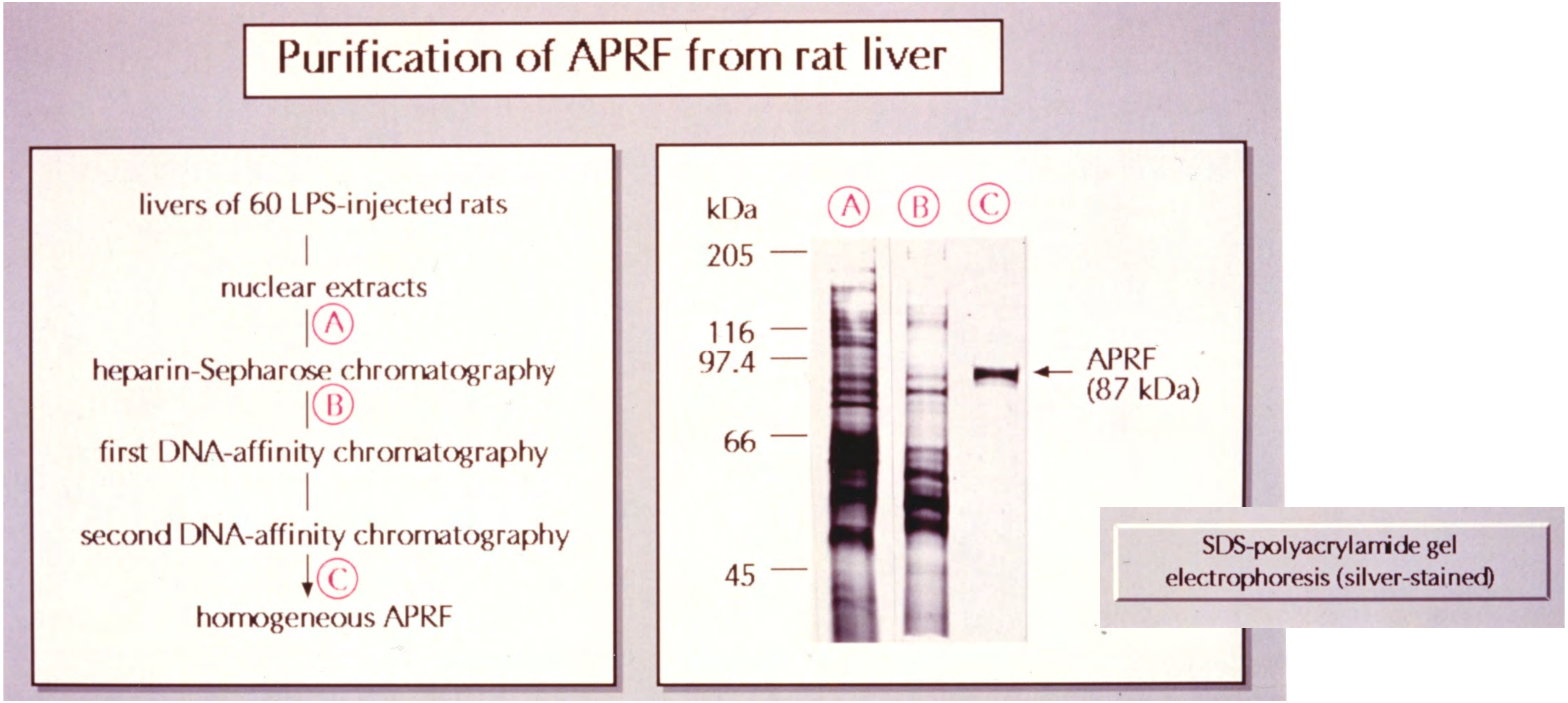
1994

The Interleukin-6-Activated Acute-Phase Response Factor Is Antigenically and Functionally Related to Members of the Signal Transducer and Activator of Transcription (STAT) Family

URSULA M. WEGENKA,[1] CLAUDIA LÜTTICKEN,[1] JAN BUSCHMANN,[1] JUPING YUAN,[1]† FRIEDRICH LOTTSPEICH,[2] WERNER MÜLLER-ESTERL,[3] CHRIS SCHINDLER,[4] ELKE ROEB,[1] PETER C. HEINRICH,[1]* and FRIEDEMANN HORN[1]*

251 citations

(07/2022)



Reprint Series
7 January 1994, Volume 263, pp. 89–92

SCIENCE

1994

Association of Transcription Factor APRF and Protein Kinase Jak1 with the Interleukin-6 Signal Transducer gp 130

Claudia Lütticken, Ursula M. Wegenka, Juping Yuan, Jan Buschmann, Chris Schindler, Andrew Ziemiecki, Ailsa G. Harpur, Andrew F. Wilks, Kiyoshi Yasukawa, Tetsuya Taga, Tadamitsu Kishimoto, Giovanna Barbieri, Sandra Pellegrini, Michael Sendtner, Peter C. Heinrich, and Friedemann Horn*


Claudia Lütticken


Ursula Wegenka


Juping Yuan


Friedemann Horn

RED-HOT RESEARCH PAPERS OF 1994

Title	# of Citations
1. A. Kamb *et al.*, "A cell cycle regulator potentially involved in genesis of many tumor types," *Science*, 15 April 1994.	71
2. N. Stahl *et al.*, "Association and activation of Jak-Tyk kinases by CNTF-LIF-OSM–IL-6 β receptor components," *Science*, 7 January 1994.	64
3. C. Lütticken *et al.*, "Association of transcription factor APRF and protein kinase Jak1 with the interleukin-6 signal transducer gp130," *Science*, 7 January 1994.	52
4. N. Papadopoulos *et al.*, "Mutation of a *mutL* homolog in hereditary colon cancer," *Science*, 18 March 1994.	46

Reprint Series
7 January 1994, Volume 263, pp. 89–92

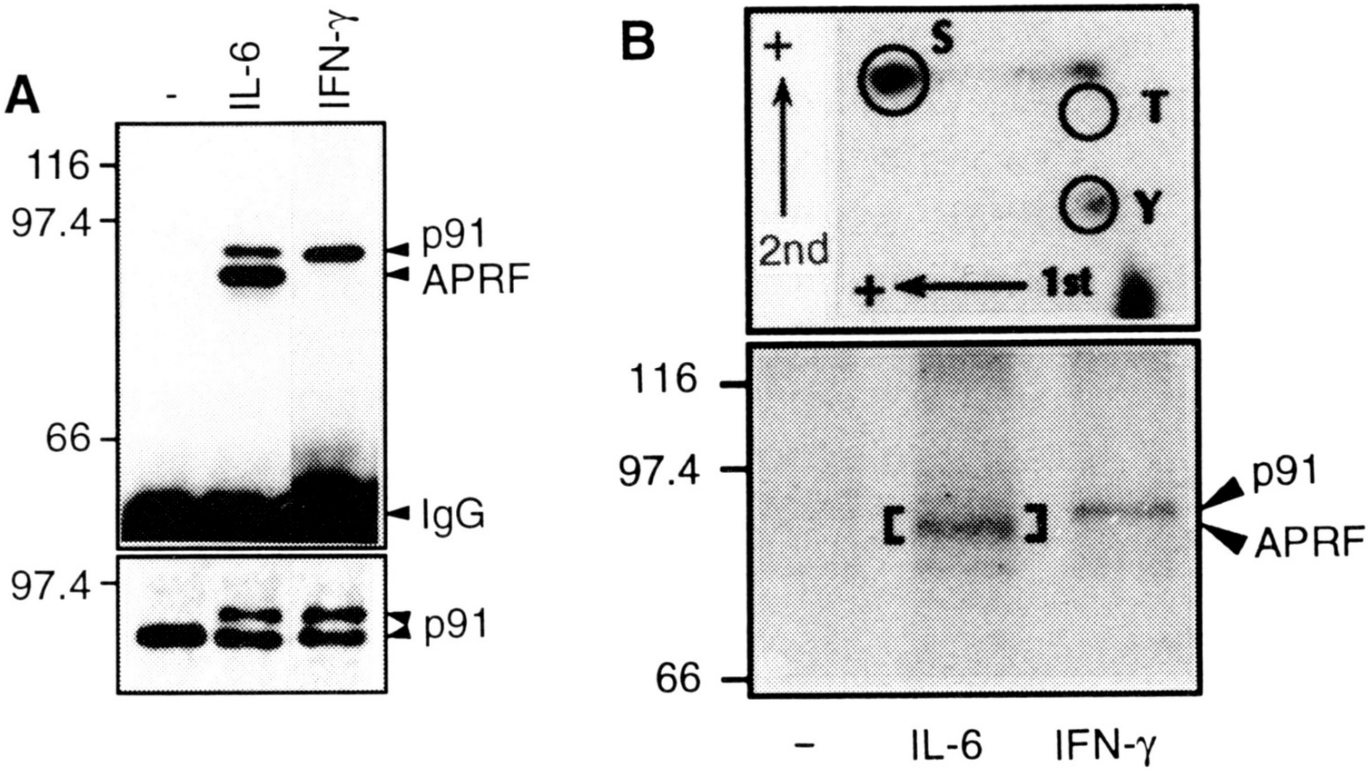
SCIENCE

Association of Transcription Factor APRF and Protein Kinase Jak1 with the Interleukin-6 Signal Transducer gp 130

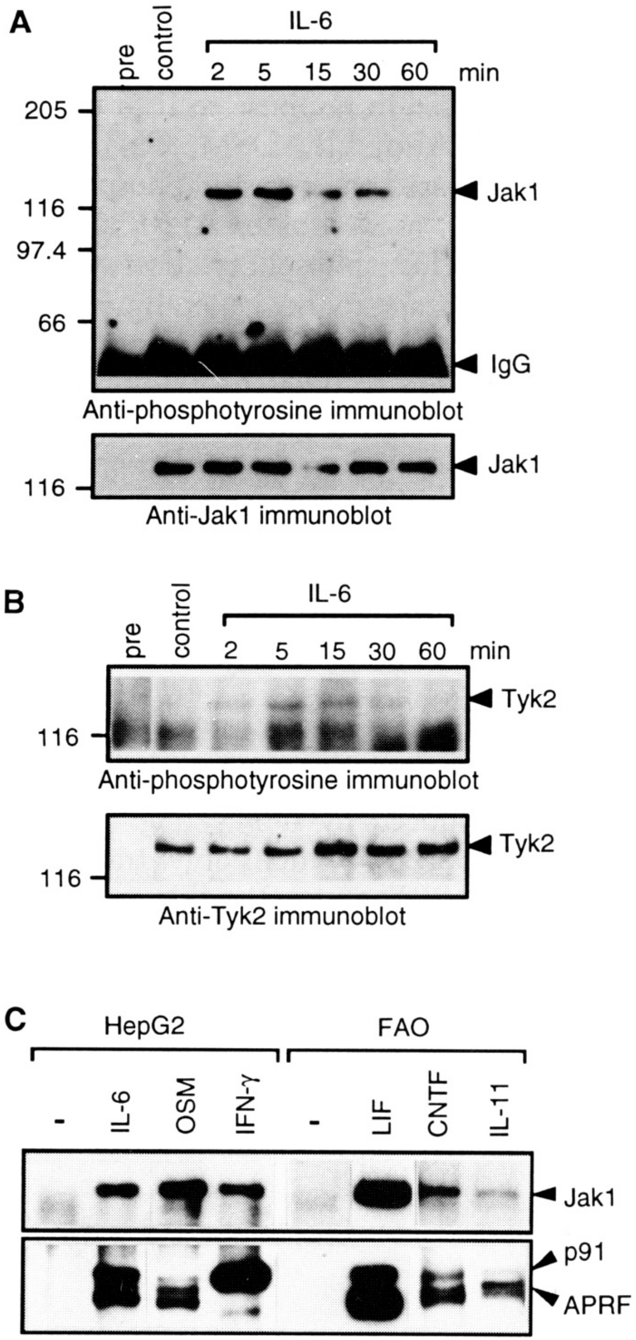
Claudia Lütticken, Ursula M. Wegenka, Juping Yuan, Jan Buschmann, Chris Schindler, Andrew Ziemiecki, Ailsa G. Harpur, Andrew F. Wilks, Kiyoshi Yasukawa, Tetsuya Taga, Tadamitsu Kishimoto, Giovanna Barbieri, Sandra Pellegrini, Michael Sendtner, Peter C. Heinrich, and Friedemann Horn*

Y-P of APRF and ISGF-3α

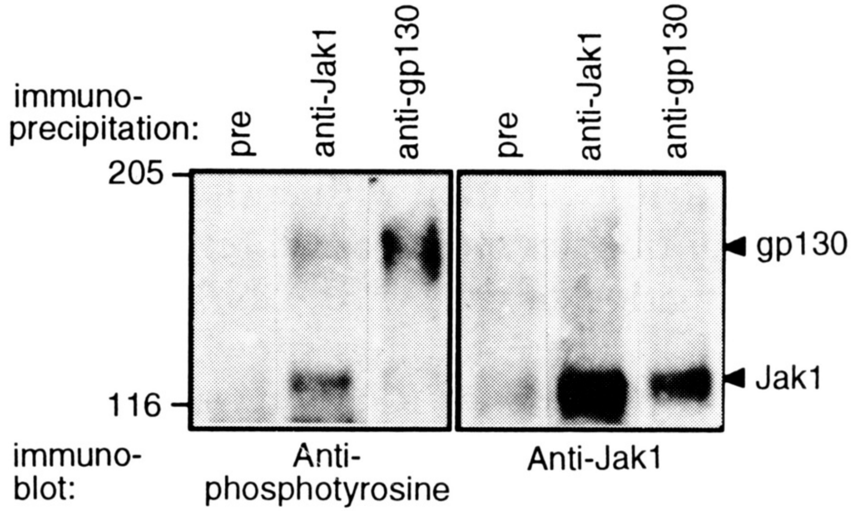
P-aa-analysis of APRF



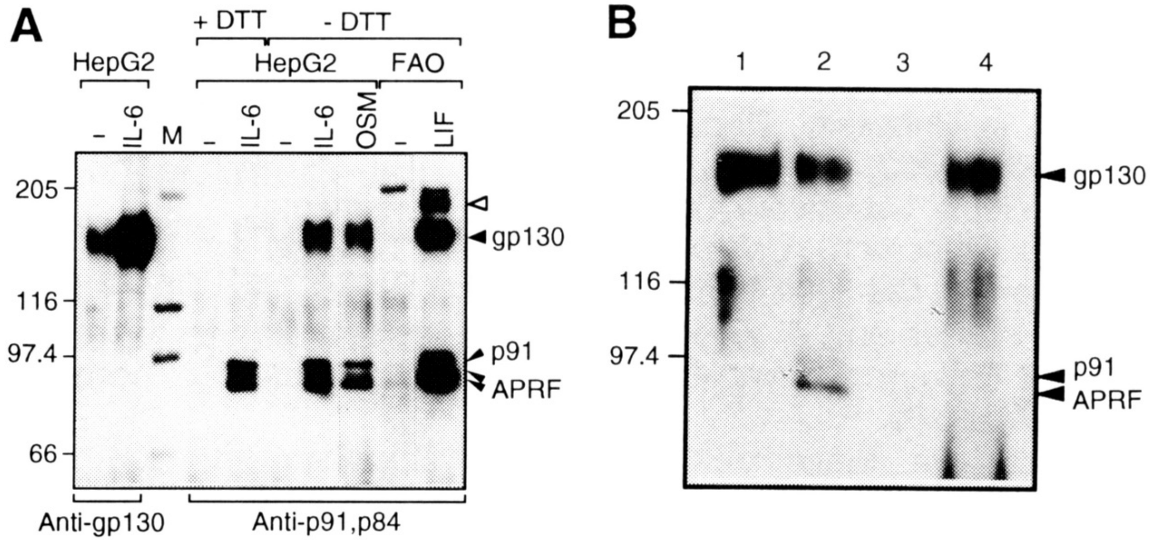
Y-P of Jak1 and Tyk2 after IL-6



Association of Jak1 with gp130



Coprecipitation of gp130 with APRF after IL-6



Summary of Wegenka et al. (1993)

- APRF is Y- and S-phosphorylated after IL-6 stimulation of HepG2 cells
- APRF coprecipitates with gp130 after IL-6
- JAK1 and Tyk2 are Y-phosphorylated after IL-6
- JAK1 associates with gp130 after IL-6
- The observations made for IL-6 are also valid for IL-11, LIF, OSM and CNTF

> conclusion: IL-6 signals via the JAK / STAT pathway

The JAK / STAT pathway was first described by Jim Darnell for the the interferon-gamma signal transduction

IL-6 signals via the JAK / STAT pathway

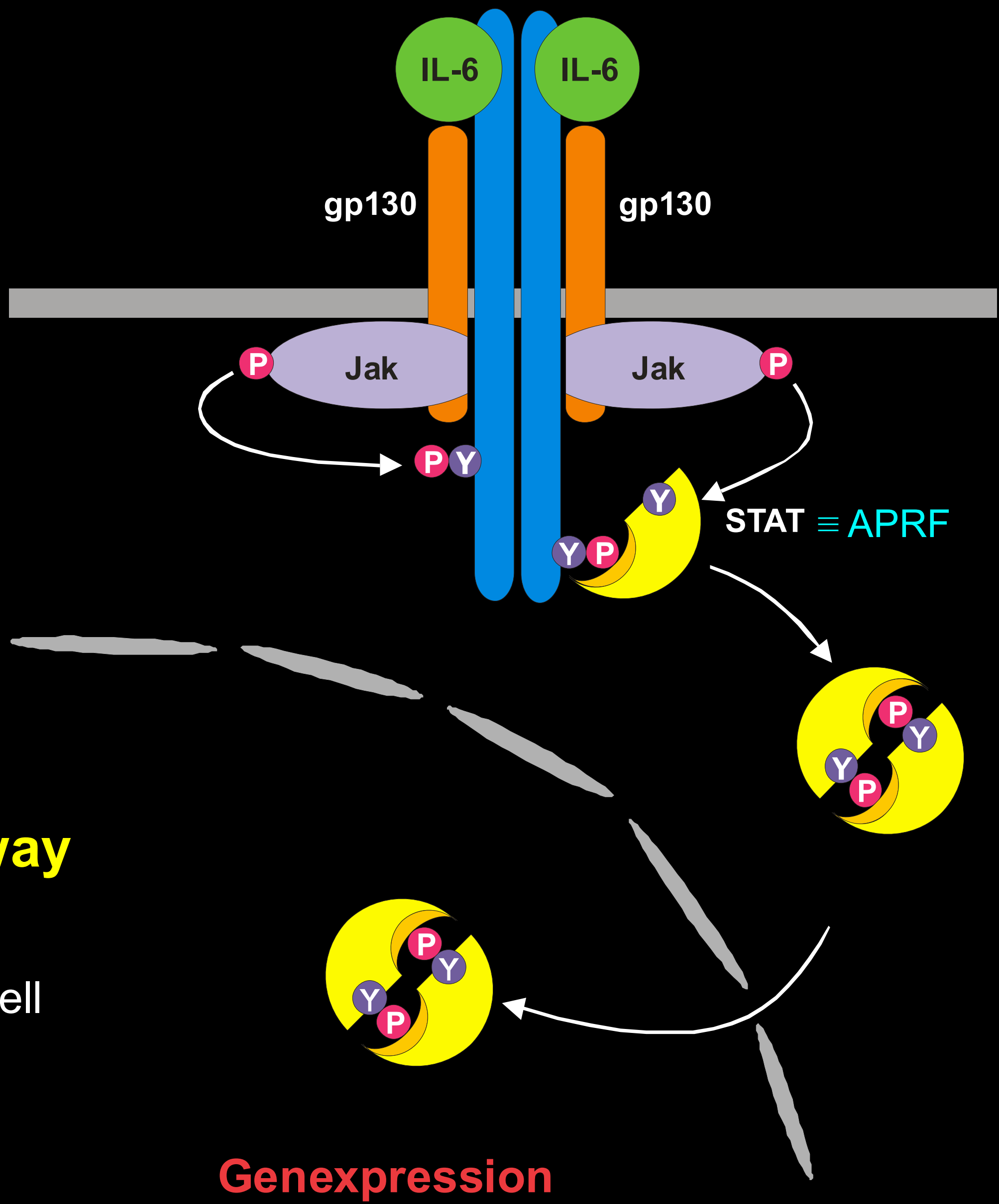
After IL-6 stimulation of HepG2 cells:

- APRF is Tyr- and Ser- phosphorylated
- APRF coprecipitates with gp130
- JAK1 and Tyk2 are Tyr-phosphorylated
- JAK1 associates with gp130

The observations made for IL-6 are also valid for IL-11, LIF, OSM and CNTF

IL-6 signals via the JAK / STAT pathway

The JAK / STAT pathway was first described by Jim Darnell for the the interferon-γ signal transduction, which uses STAT1 and STAT2 as transcription factors



In the middle of APRF – cDNA-cloning we were scooped by Kishimoto‘s group. According to our purification procedure published in 1993 and with the information on an amino acid sequence of a small APRF-oligopeptide (which we had sent to Friedrich Lottspeich, Munich for sequencing) - which we had given to our „colleagues“ in Osaka - Akira et al. purified APRF from 3000 !!! mice after LPS injection and cloned the cDNA of APRF (Cell, 1994)

APRF turned out to be a new STAT- factor: STAT-3

Although we lost the APRF-cloning race,
we succeeded in the elucidation of the molecular mechanism of IL-6 signaling.

Before the discussion on the IL-6 signaling pathway in detail the IL-6 receptor complex will be introduced.

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- **Molecular mechanisms of IL-6 signal transduction**
- **Nuclear translocation of STAT3-YFP**

Part 2: **Regulation of IL-6 signal transduction**

Formation of the IL-6-receptor complex

The **IL-6 receptor** consists of 2 subunits:

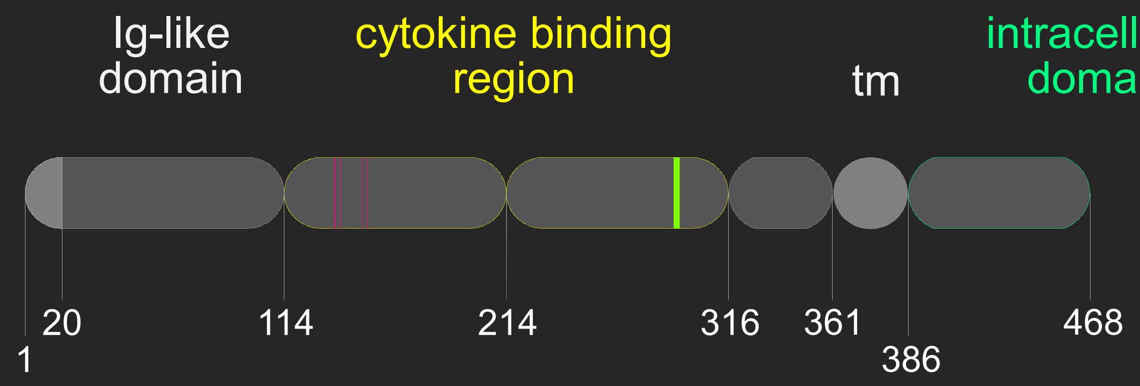
- an α-receptor (**gp80**)
  which binds the ligand IL-6 with low affinity and a

- β-receptor, more frequently designated as
  signal transducer glycoprotein130 (**gp130**)
  which has hardly any affinity for IL-6

However, both subunits together bind IL-6 with high affinity.

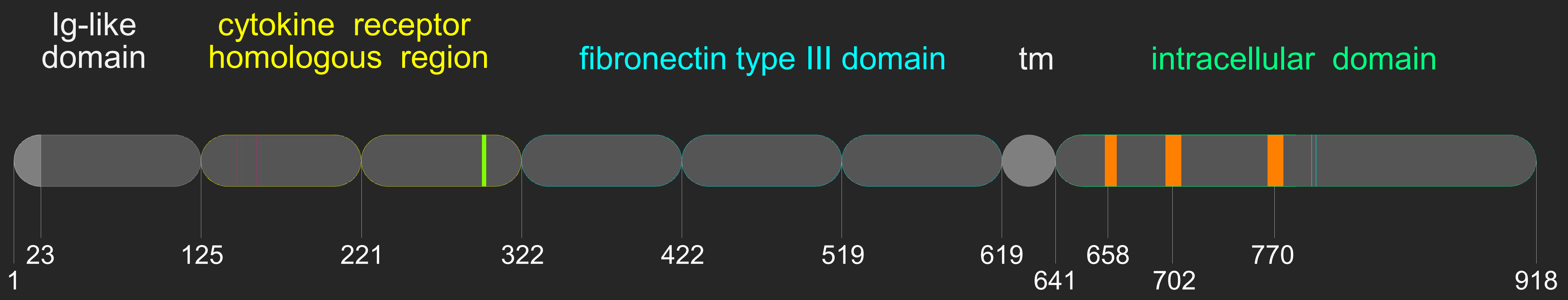
The IL-6R subunits gp80 and gp130

IL-6Rα / gp80



Yamasaki et al. (1988) Sci 241, 825

IL-6Rβ / gp130



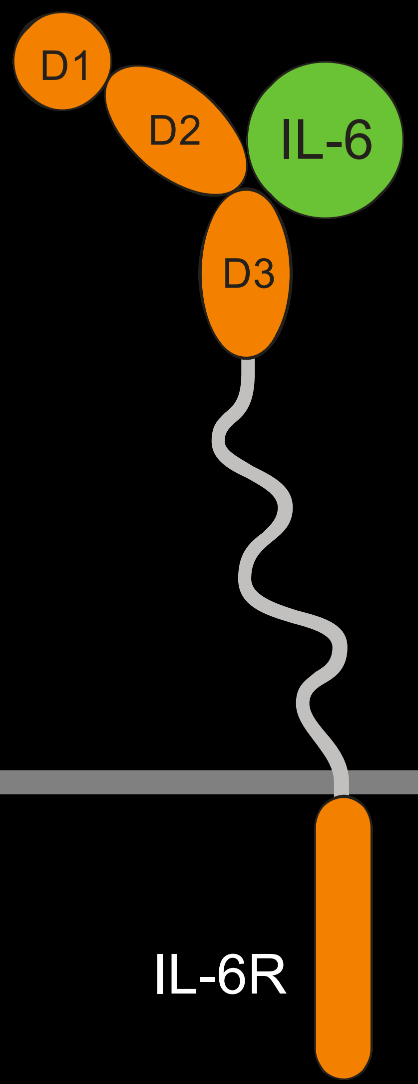
Hibi et al. (1990) Cell 63, 1149

tm, transmembrane region

Identification of the interaction sites of IL-6, IL-6Rα and gp130 and their assembly to a signaling-competent complex

The following slide summarizes the results from 13 publications of our lab during 12 years.

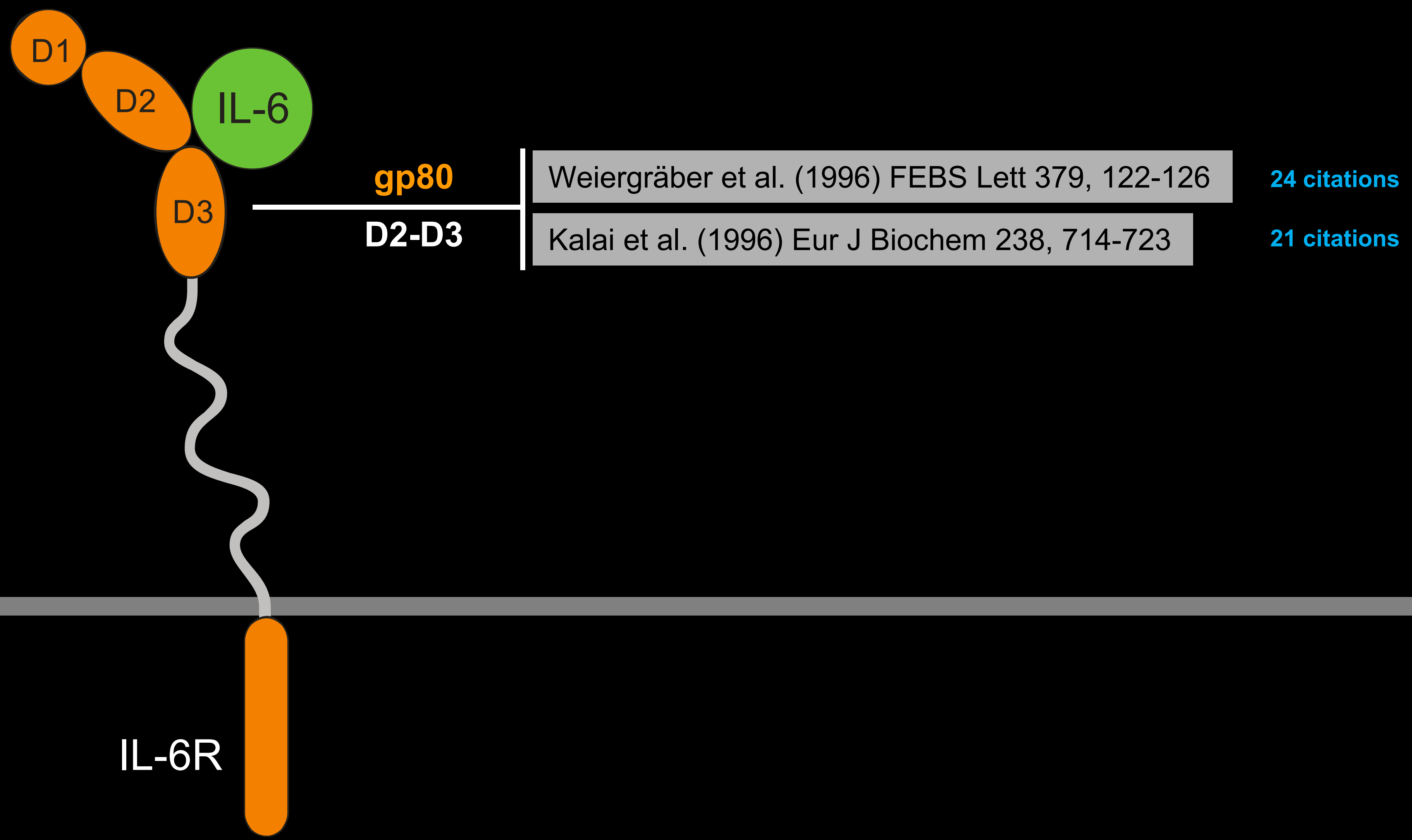
Structural assembly of the functional human IL-6 hexamer signaling complex



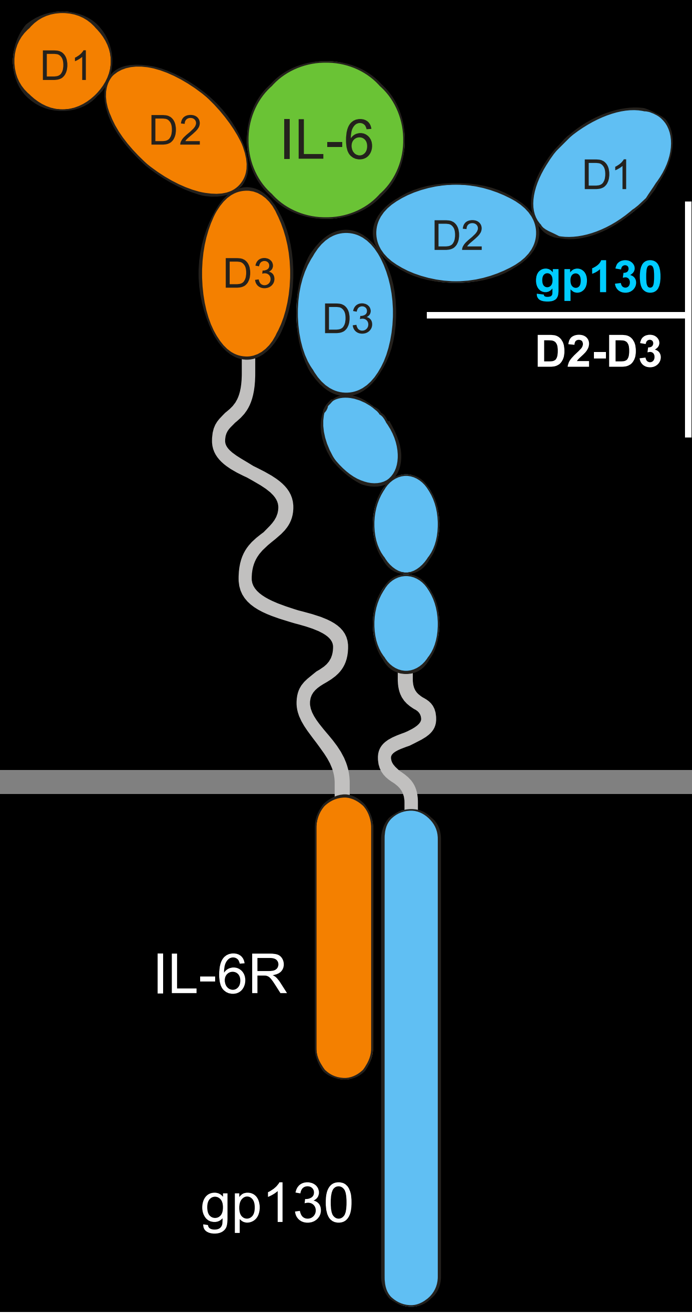
IL-6

- Krüttgen et al. (1990) FEBS Lett 262, 323-326 — 75 citations
- Krüttgen et al. (1990) FEBS Lett 273, 95-98 — 68 citations
- Lütticken et al. (1991) FEBS Lett 282, 265-267 — 47 citations
- Van Dam et al. (1993) J Biol Chem 268, 15285-15290 — 101 citations

Structural assembly of the functional human IL-6 hexamer signaling complex



Structural assembly of the functional human IL-6 hexamer signaling complex

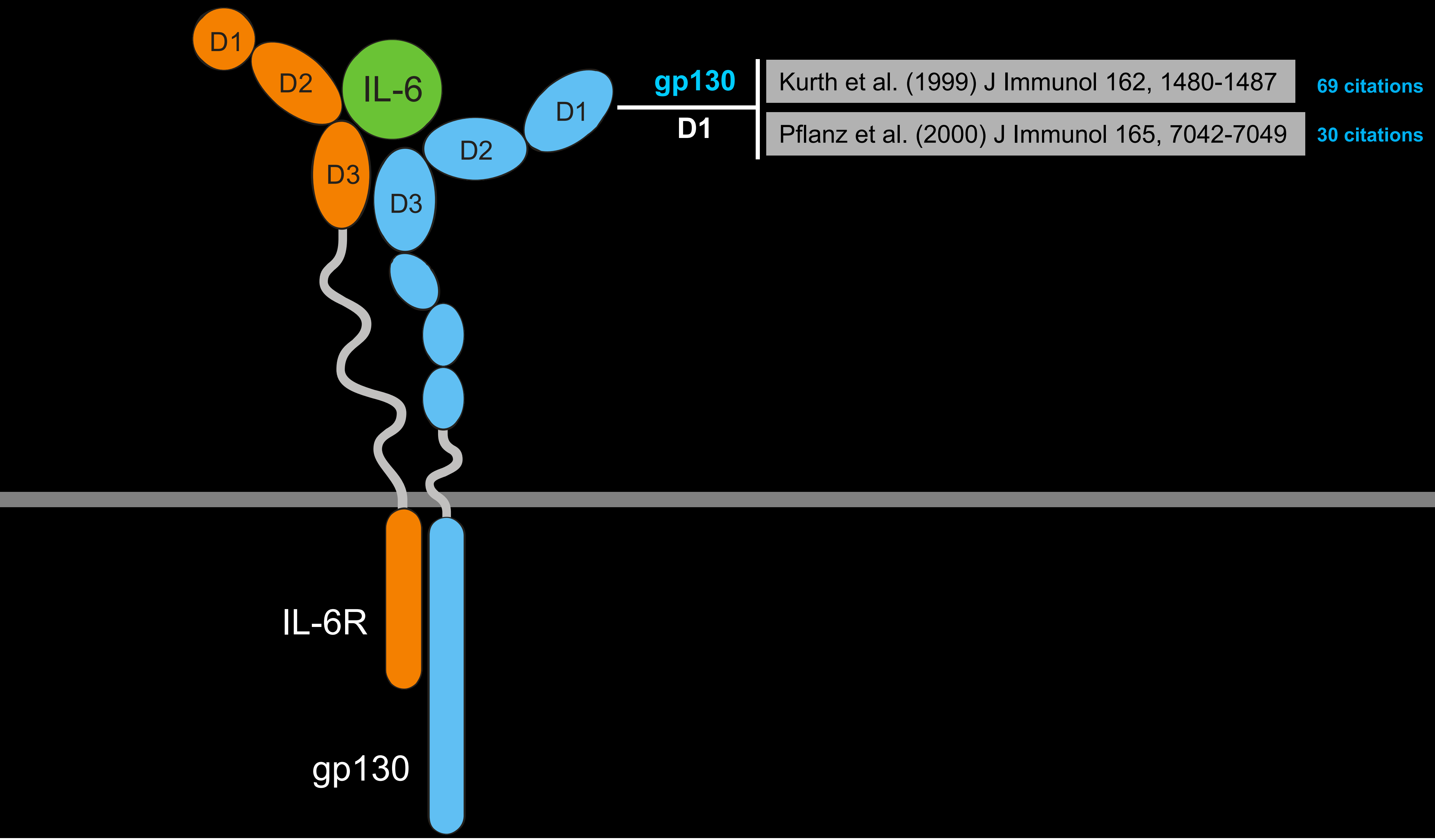


Horsten et al. (1997) J Biol Chem 272, 23748-23757 — 21 citations

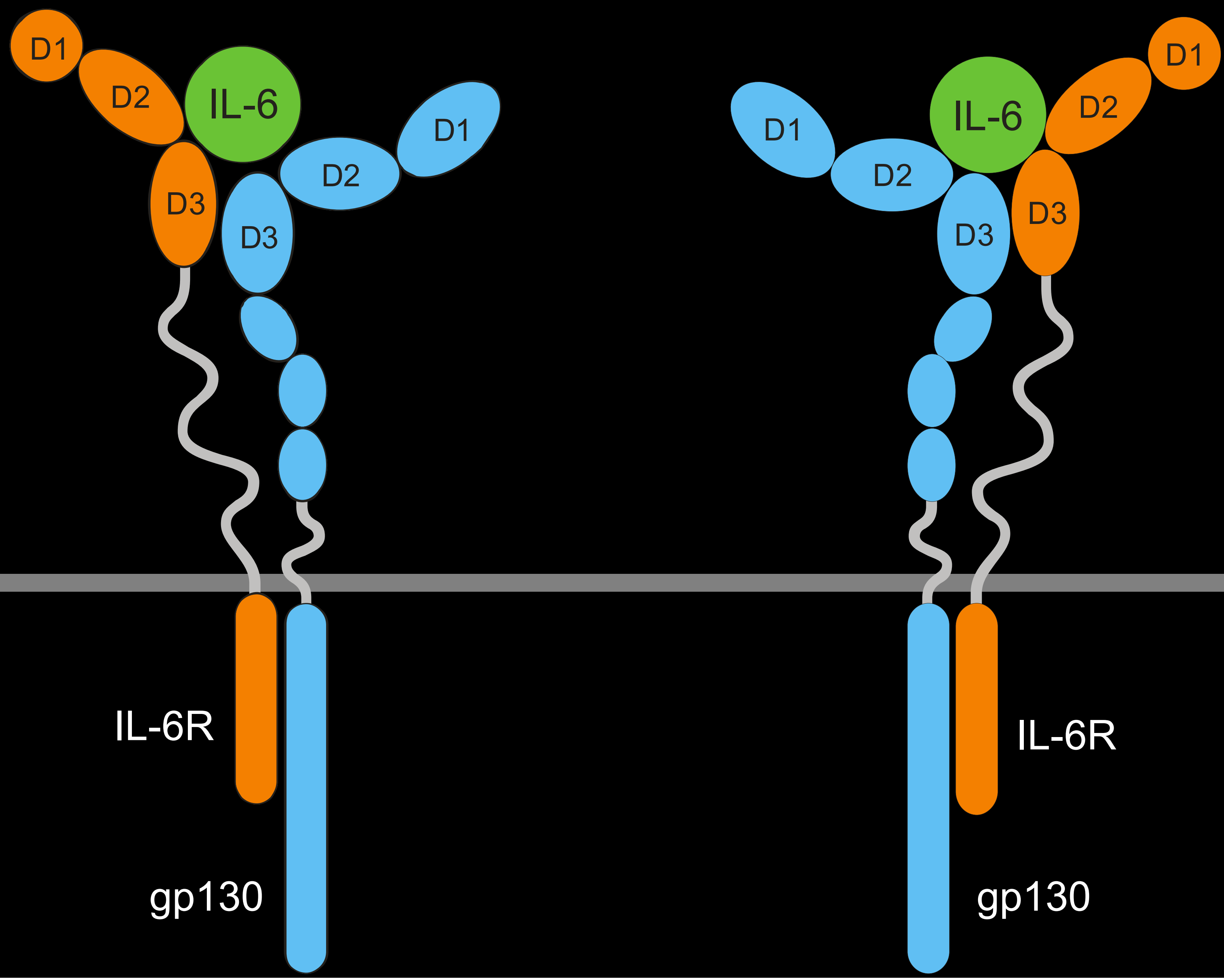
Müller-Newen et al. (1997) Eur J Biochem 247, 425-431 — 8 citations

Kernebeck et al. (1999) Protein Science 8, 5-12 — 27 citations

Structural assembly of the functional human IL-6 hexamer signaling complex



Structural assembly of the functional human IL-6 hexamer signaling complex



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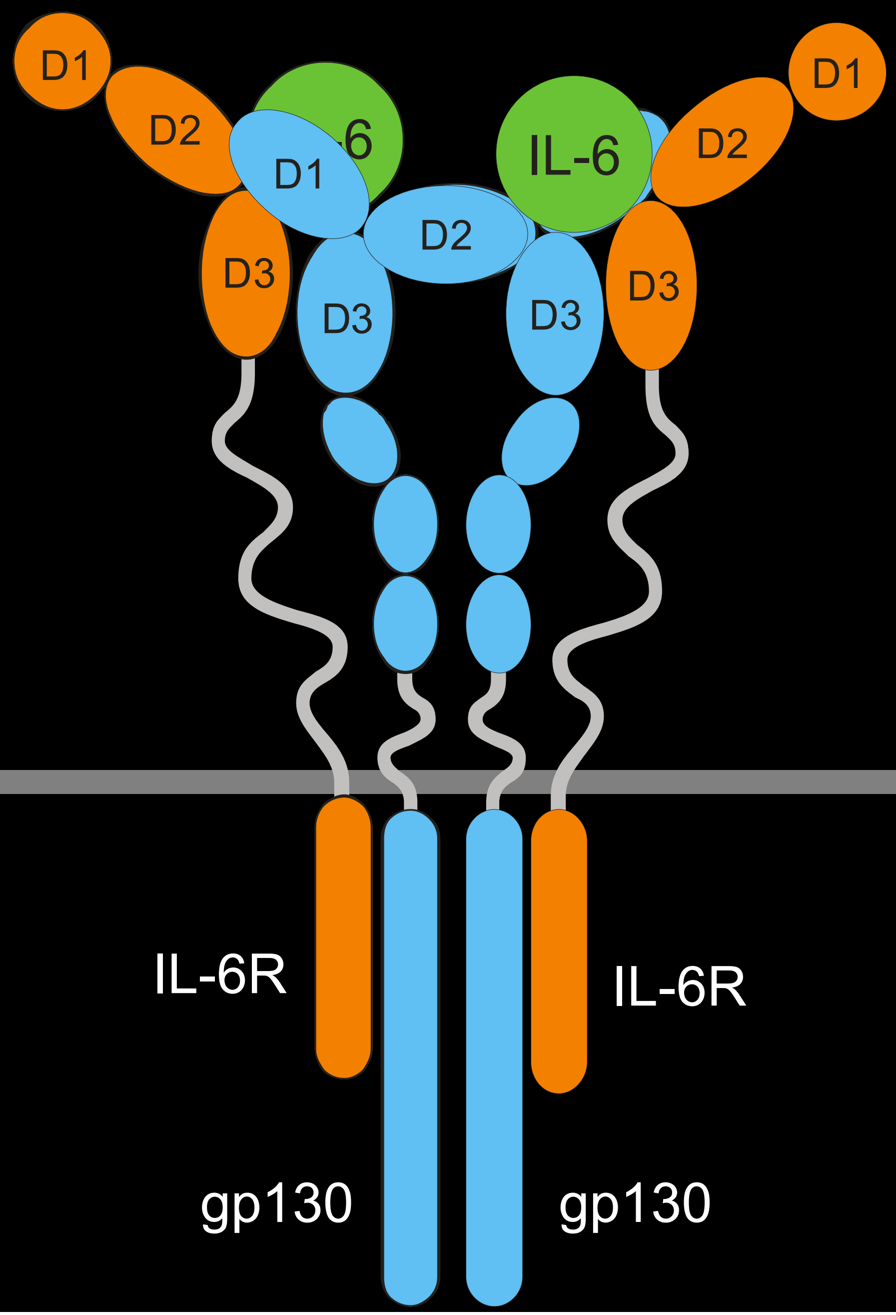
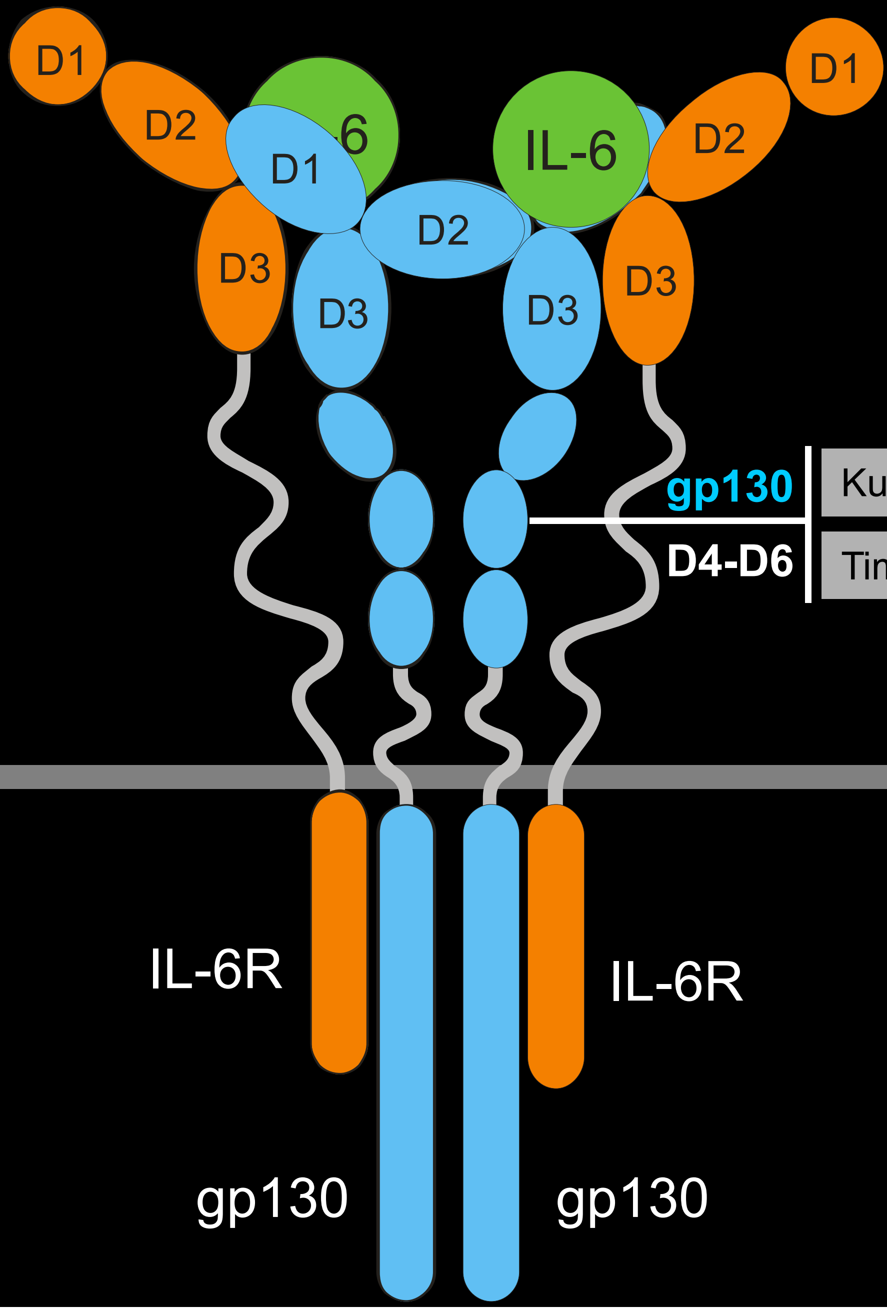


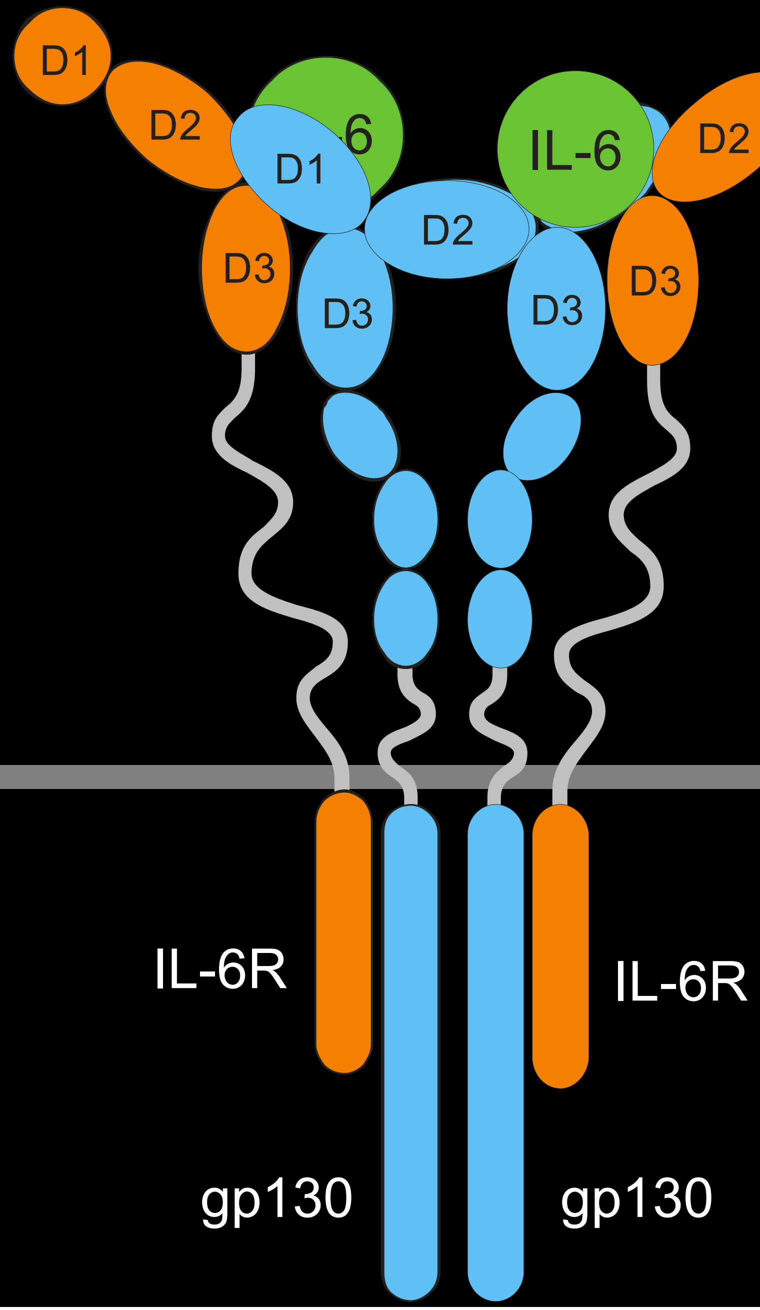
Structural assembly of the functional human IL-6 hexamer signaling complex

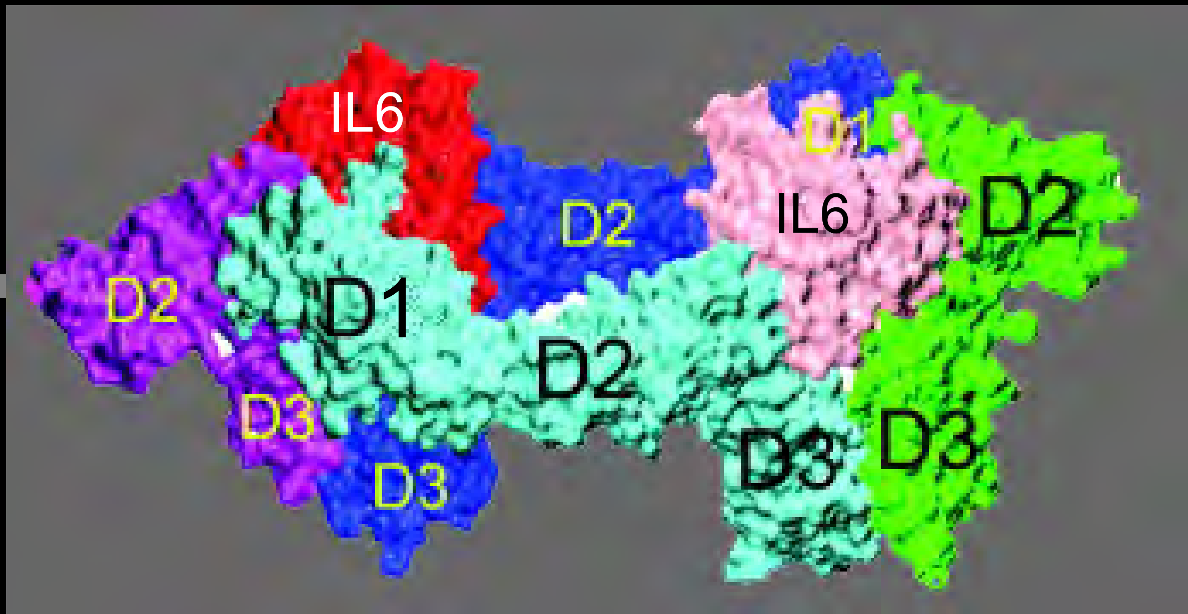


Kurth et al. (2000) J Immunol 164, 273-282

Timmermann et al. (2002) Eur J Biochem 269, 2716-2726

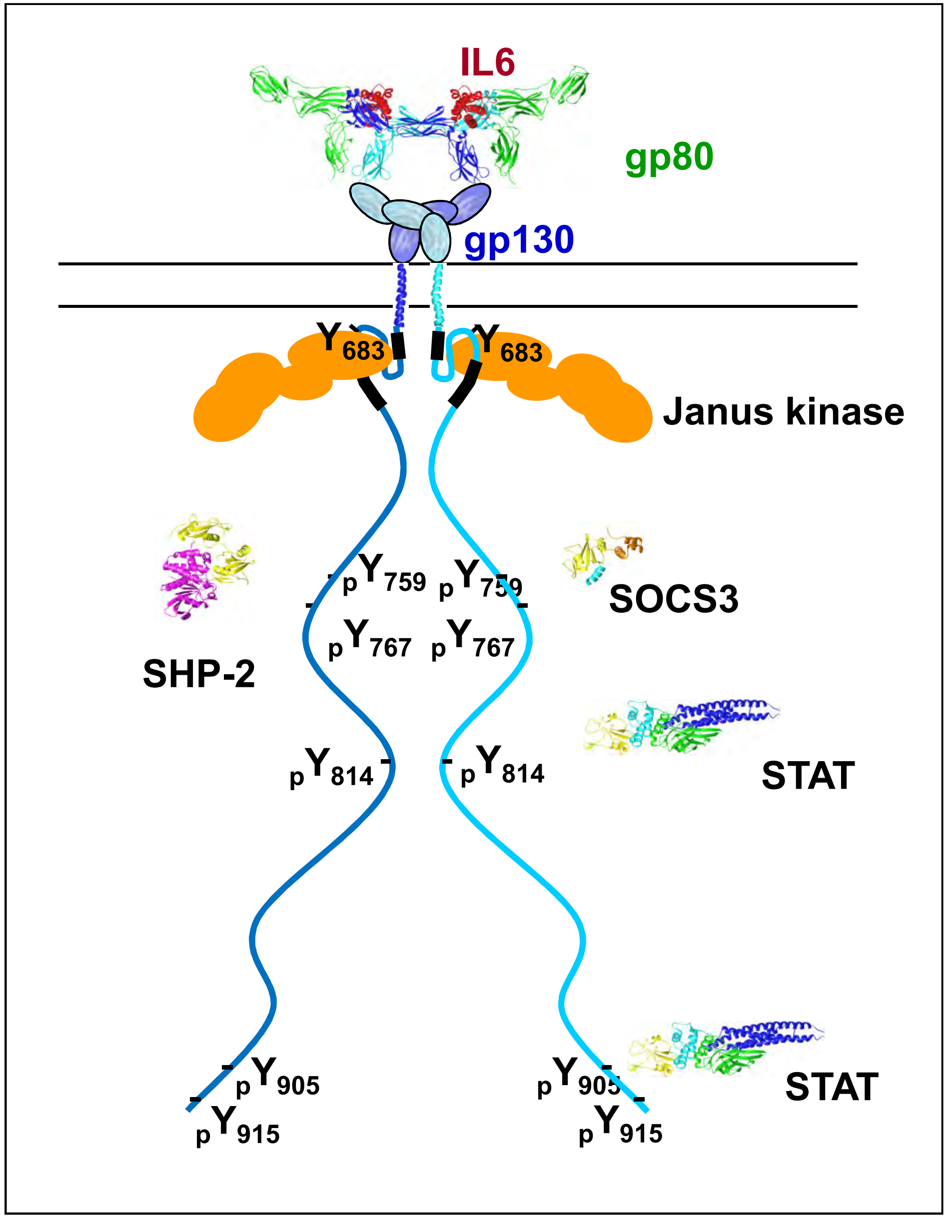
Structural assembly of the functional human IL-6 hexamer signaling complex

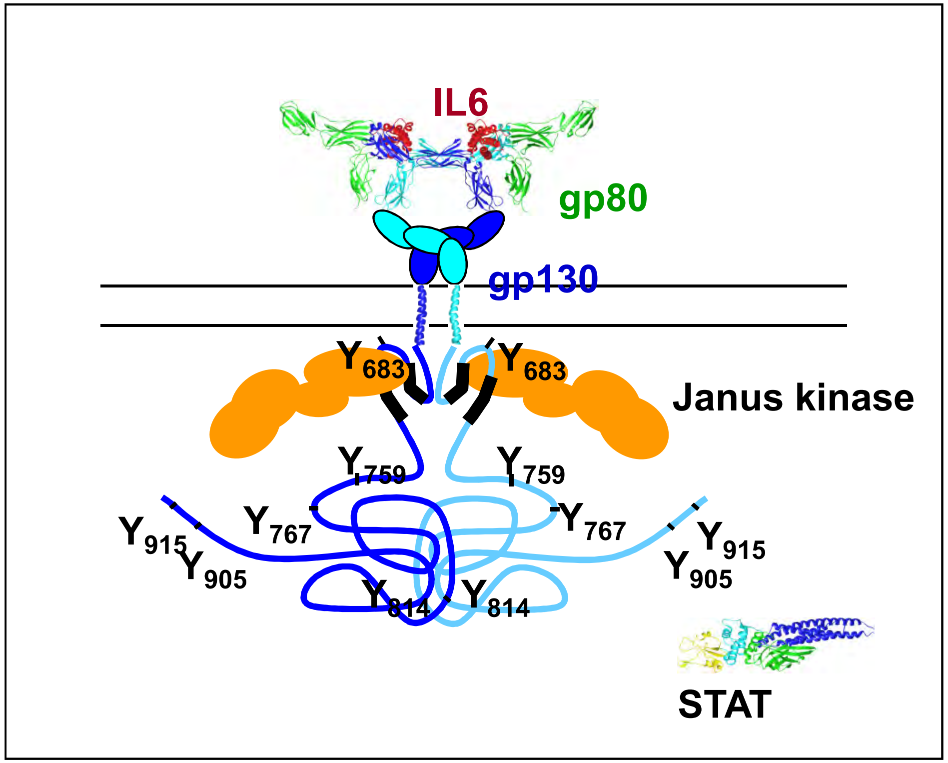




Boulanger et al. (2003) Science 300, 2101 - 2104

There is presently no information on the structure and conformational states of the cytoplasmic regions of cytokine receptors





Are structural properties important for activation and modulation of cytokine signaling ?

Heinrich PC (2003) Biochem J. 2294 citations (07/2022)

FRET study with CFP- and YFP-tagged gp130

A FRET signal was detected between YFP- and CFP- tagged gp130 at the plasma membrane of unstimulated cells which does not increase upon IL-6 stimulation

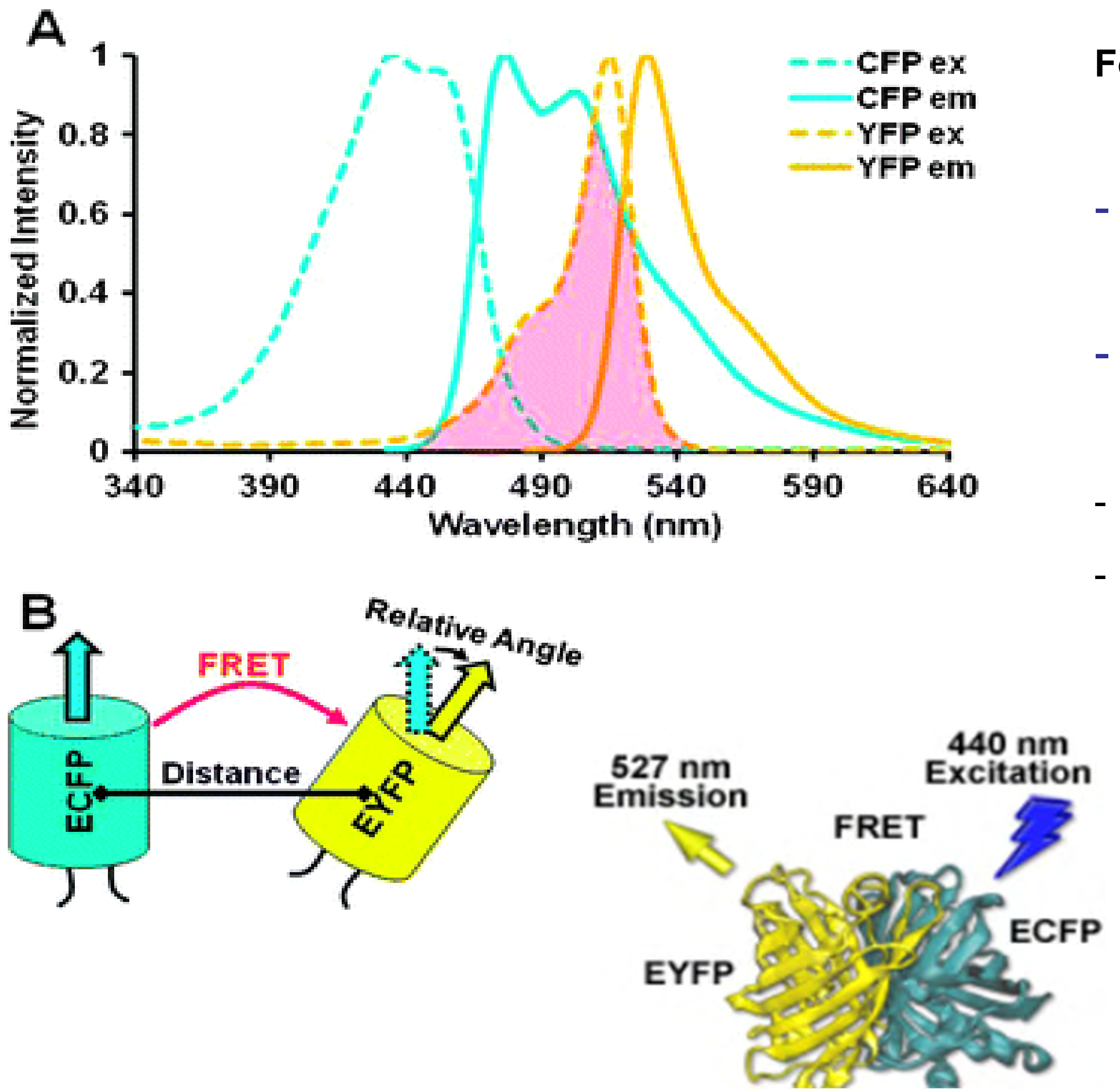
>>> We concluded that

gp130 is present as a preformed dimer on the cell membrane

Giese et al. J Cell Science (2005) 118, 5129-5149 **62 citations** (07/2022)

FRET, fluorescence resonance energy transfer

protein-protein interaction → FRET



Förster/Fluorescence Resonance Energy Transfer

- Energy transfer between fluorophores based on dipole-dipole coupling <u>not</u> on photons or radiation
- Emission spectrum of the donor must overlap with the excitation spectrum of the acceptor
- Relative orientation of the fluorophores matters
- Extremely distance-sensitive:

$$E = \frac{1}{1 + (r/R_0)^6}$$

E: FRET efficiency
r: distance
R_0: Förster distance where E=50%

Dimerization of the cytokine receptors gp130 and LIFR analysed in single cells 2005

Bernd Giese*, Christoph Roderburg*, Michael Sommerauer, Saskia B. Wortmann, Silke Metz, Peter C. Heinrich and Gerhard Müller-Newen‡

Institut für Biochemie, Universitätsklinikum RWTH Aachen, Pauwelsstraße 30, 52074 Aachen, Germany

*These authors contributed equally to this work

‡Author for correspondence (e-mail: mueller-newen@rwth-aachen.de)

Accepted 09 August 2005

Journal of Cell Science 118, 5129-5140 Published by The Company of Biologists 2005

doi:10.1242/jcs.02628

62 citations (07/2022)

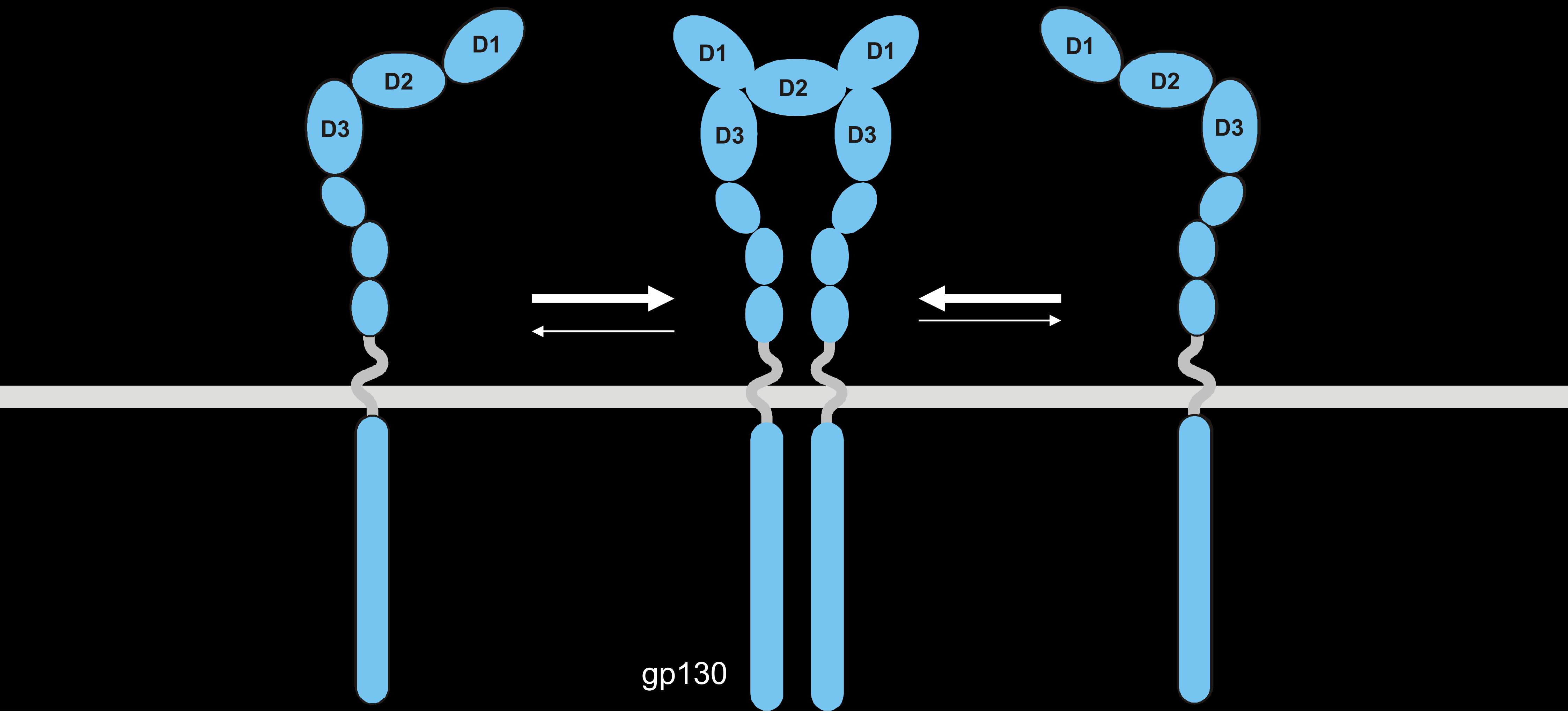
Journal of Cell Science

Summary

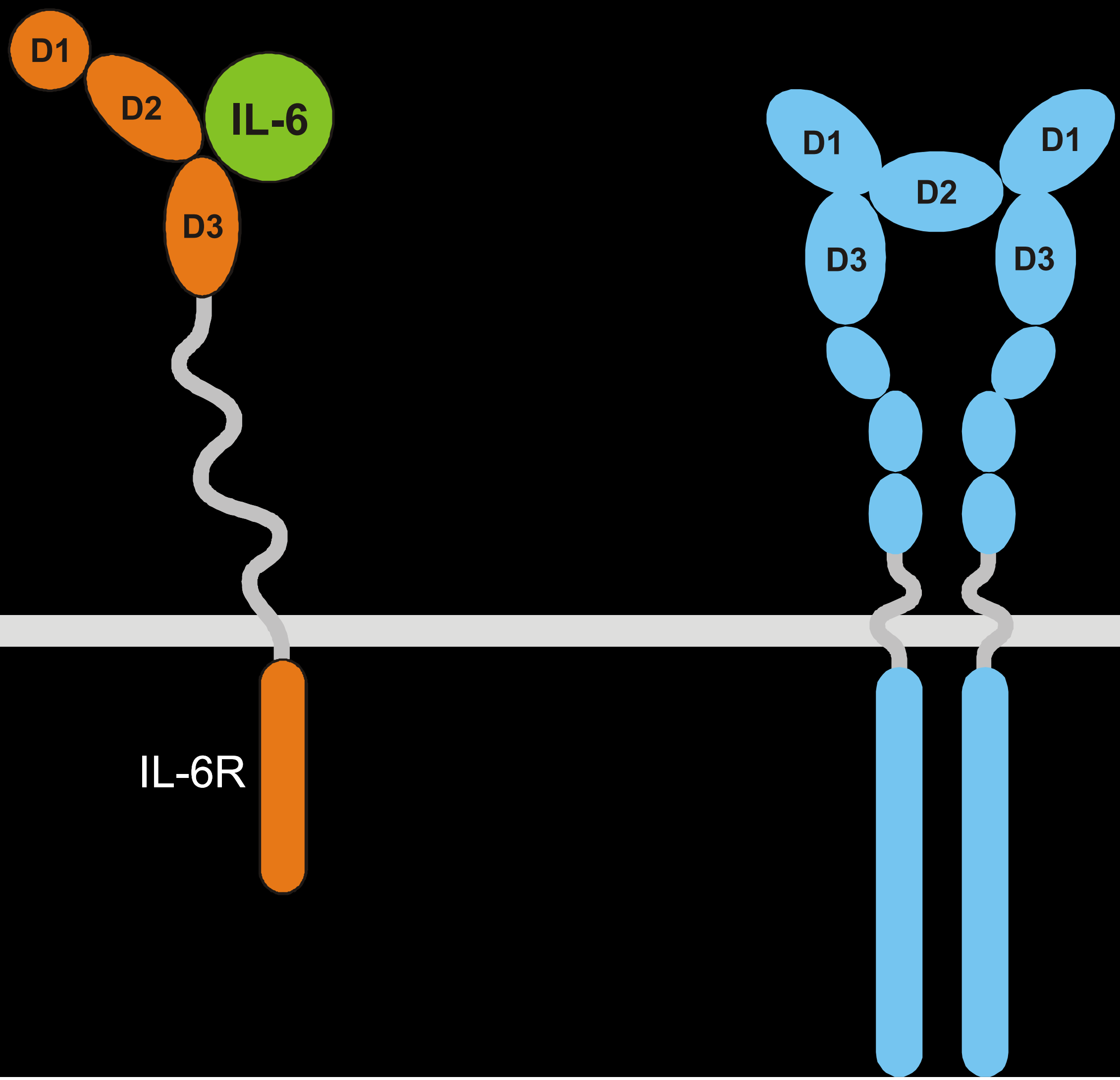
The cytokine receptor gp130 is the shared signalling subunit of the IL-6-type cytokines. Interleukin-6 (IL-6) signals through gp130 homodimers whereas leukaemia inhibitory factor (LIF) exerts its action through a heterodimer of gp130 and the LIF receptor (LIFR). Related haematopoietic receptors such as the erythropoietin receptor have been described as preformed dimers in the plasma membrane. Here we investigated gp130 homodimerization and heterodimerization with the LIFR by fluorescence resonance energy transfer (FRET) and bimolecular fluorescence complementation (BiFC). We detected a FRET signal between YFP- and CFP-tagged gp130 at the plasma membrane of unstimulated cells that does not increase upon IL-6 stimulation. However, FRET between YFP-tagged gp130 and CFP-tagged LIFR considerably increased upon LIF stimulation. Using a BiFC approach that detects stable interactions we show that fluorescence complementation of gp130 constructs tagged with matching 'halves' of fluorescent proteins increases upon IL-6 stimulation. Taken together, these findings suggest that transient gp130 homodimers on the plasma membrane are stabilized by IL-6 whereas heterodimerization of gp130 with the LIFR is mainly triggered by the ligand. This view is supported by the observation that the simultaneous action of two IL-6 binding domains on two gp130 molecules is required to efficiently recruit a fluorescent IL-6 (YFP-IL-6) to the plasma membrane.

Key words: Cytokine receptor, Gp130, LIFR, Dimerization, FRET, BiFC

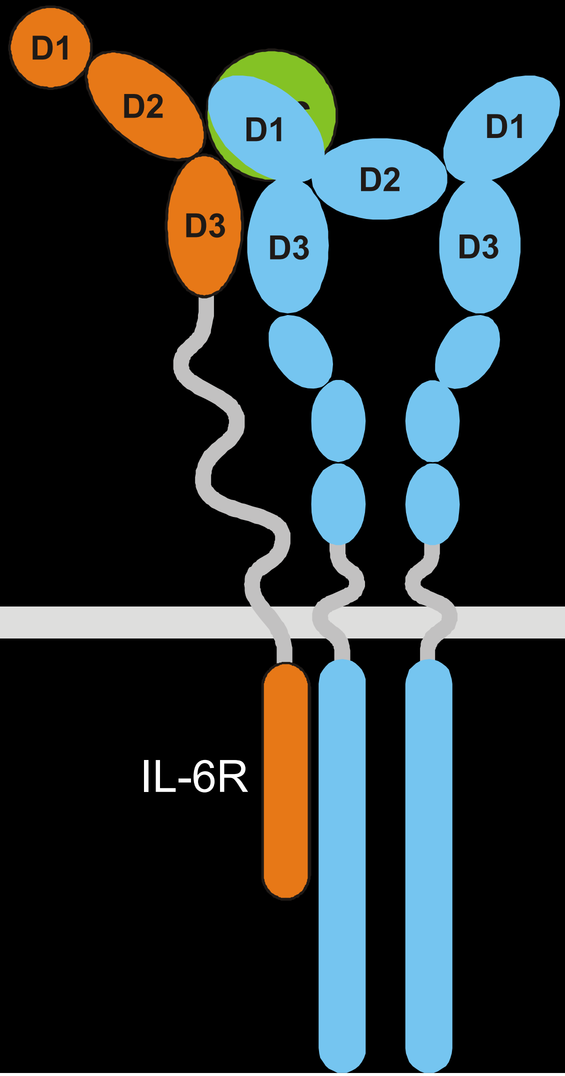
gp130 is present as a preformed dimer



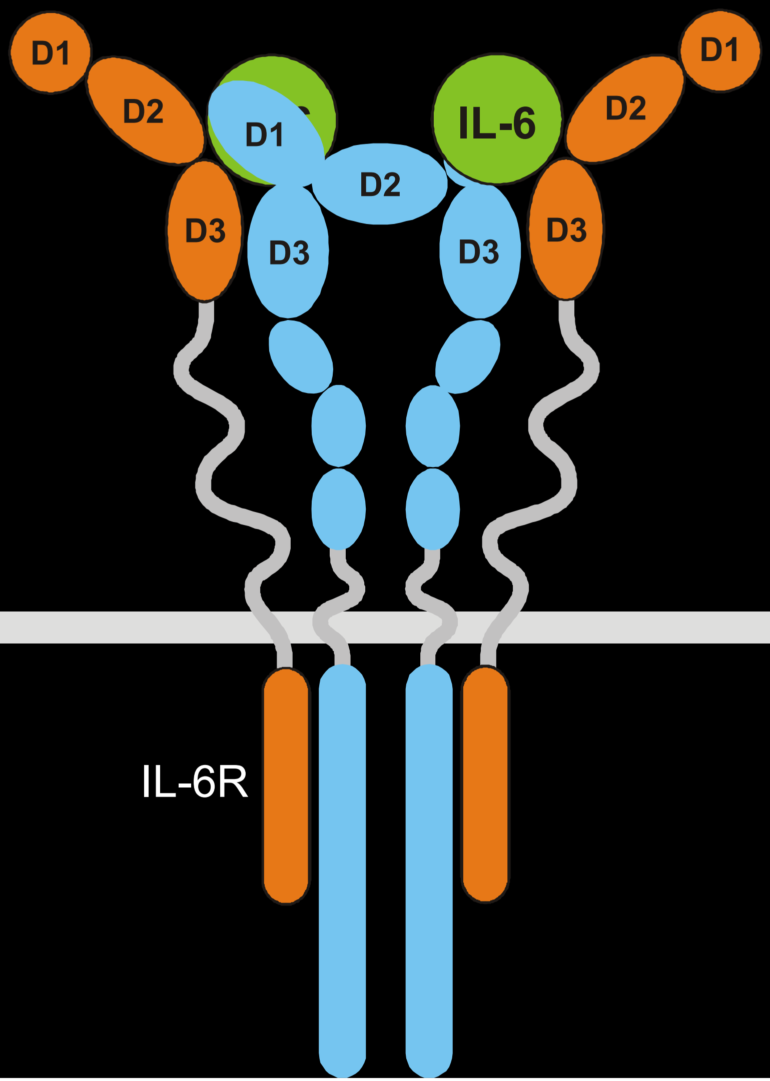
gp130 is present as a preformed dimer



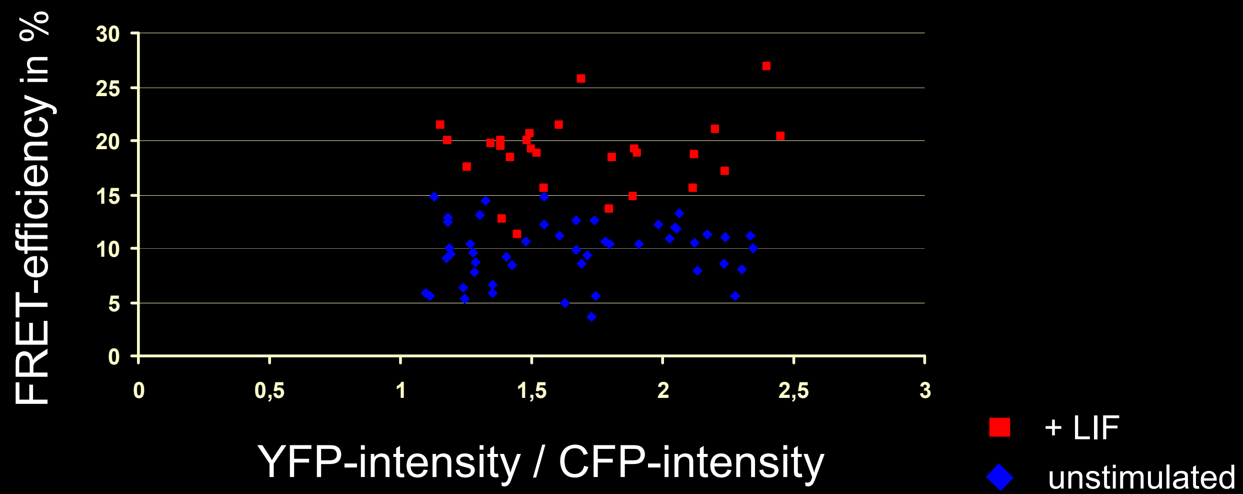
gp130 is present as a preformed dimer

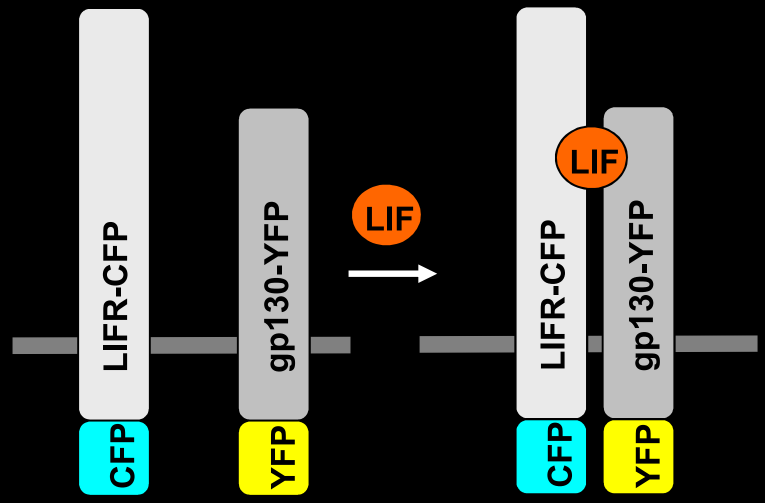


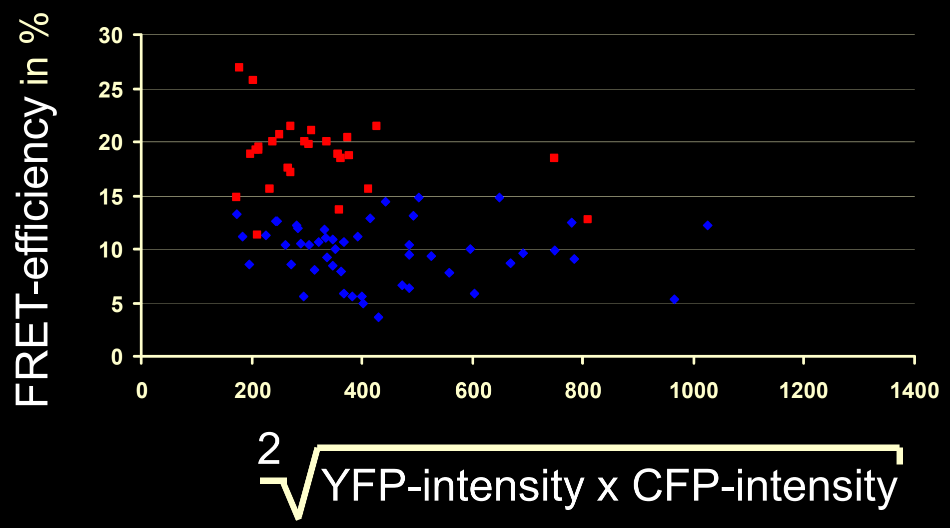
gp130 is present as a preformed dimer

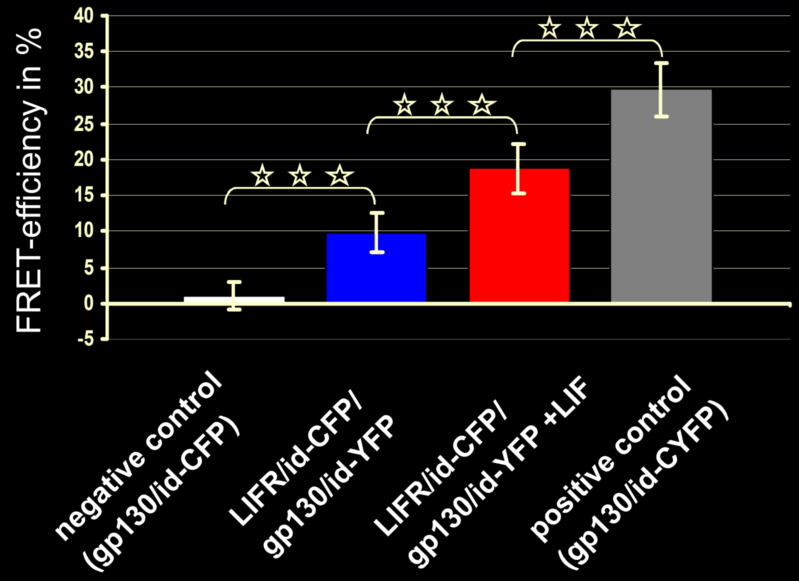


Detection of ligand-induced LIFR/gp130 heterodimerization by FRET









Outline: Interleukin-6 signal transduction and its regulation

Part 1: **Molecular mechanisms of IL-6 signal transduction**

- **The acute phase response**
- **Identification of HSF as IL-6**
- **Structure and function of IL-6**
- **Acute phase protein synthesis induced by IL-6**
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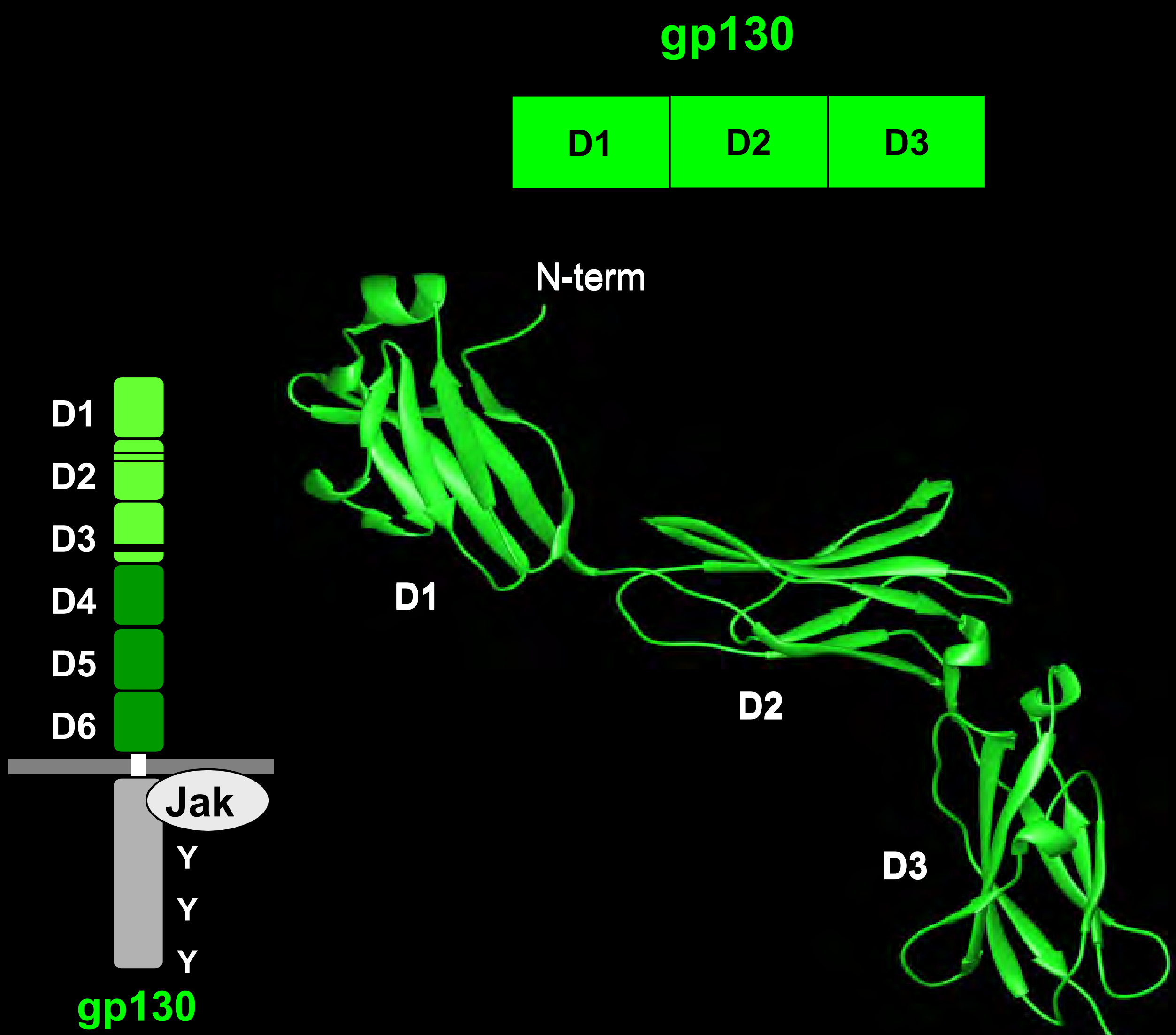
- **Design of a highly potent IL-6 antagonist**
- **Molecular mechanisms of IL-6 signal transduction**
- **Nuclear translocation of STAT3-YFP**

Part 2: **Regulation of IL-6 signal transduction**

Design of a highly potent IL-6 antagonist

Human IL-6 Receptor Fusion Protein (hIL-6RFP)



Ancey et al. (2003) J Biol Chem 278, 16 968 -16 972

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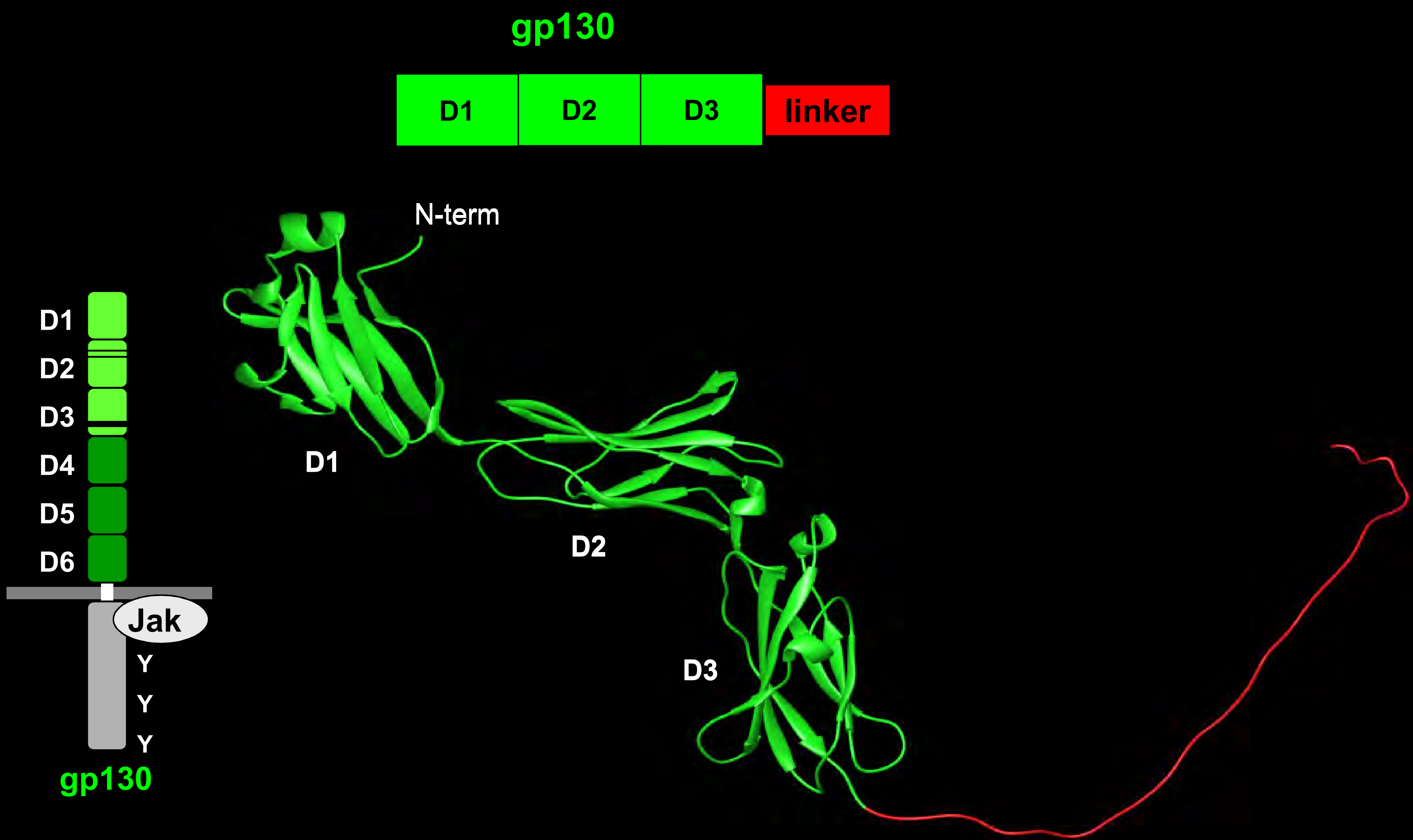

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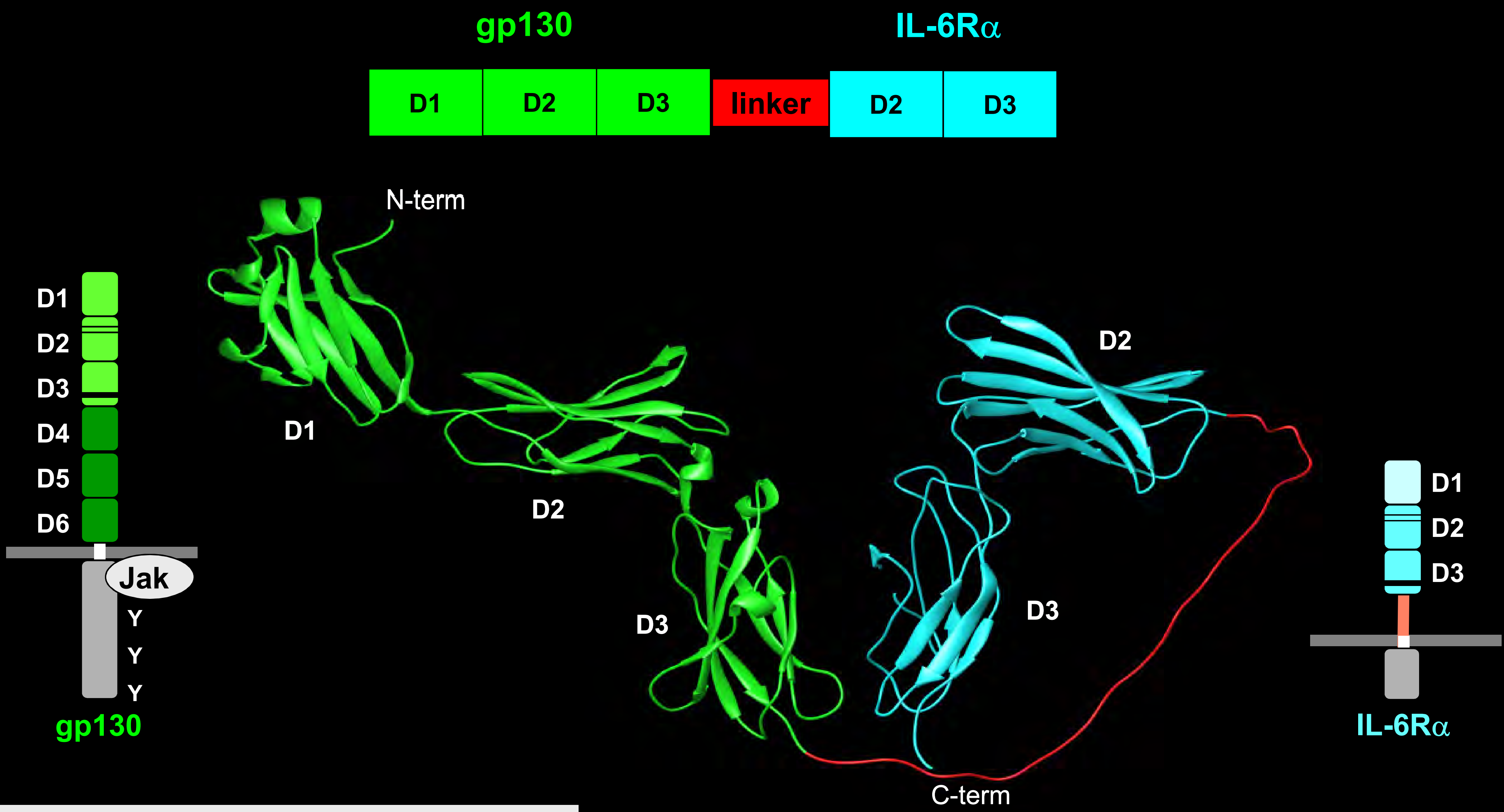

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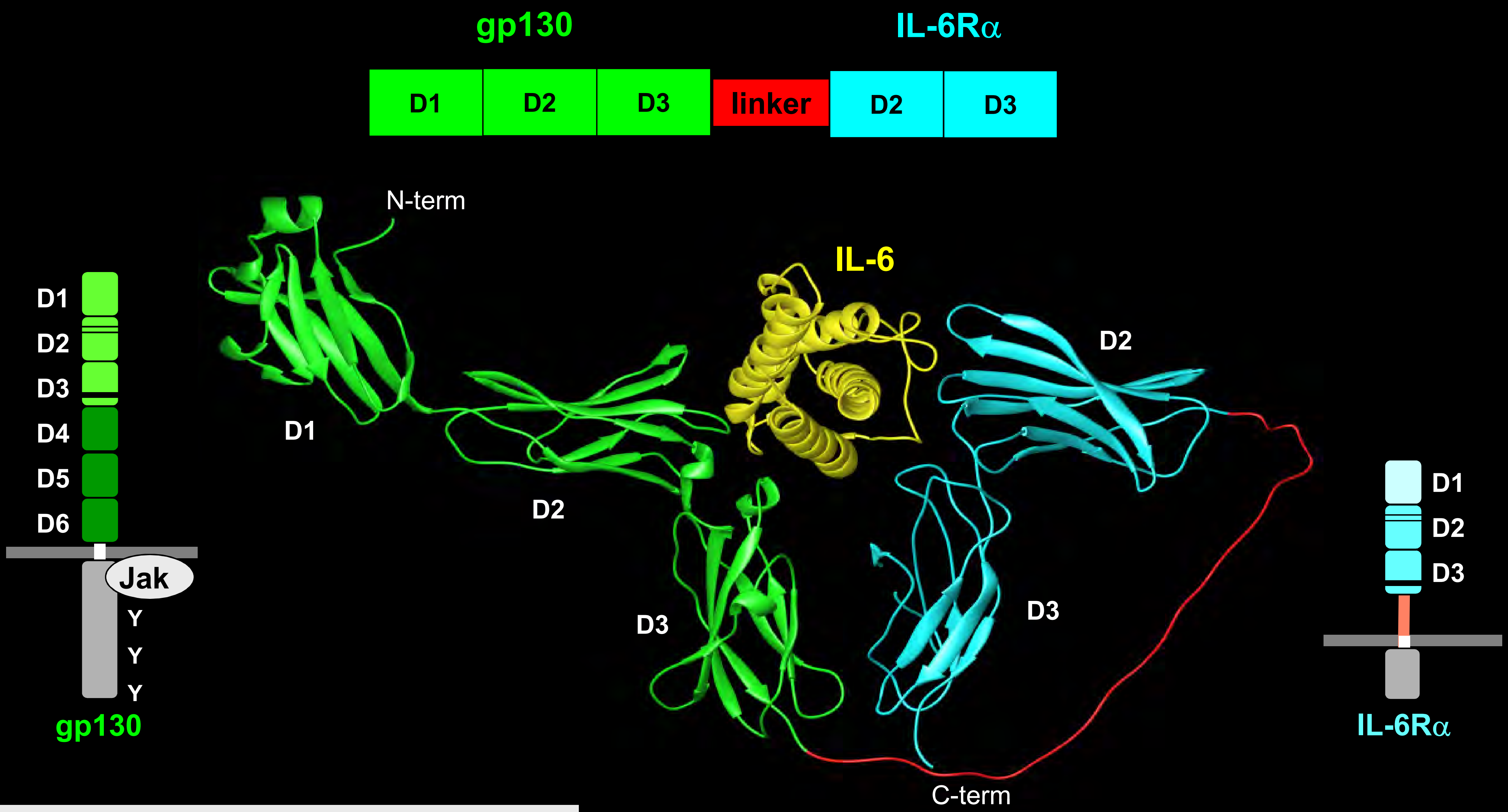
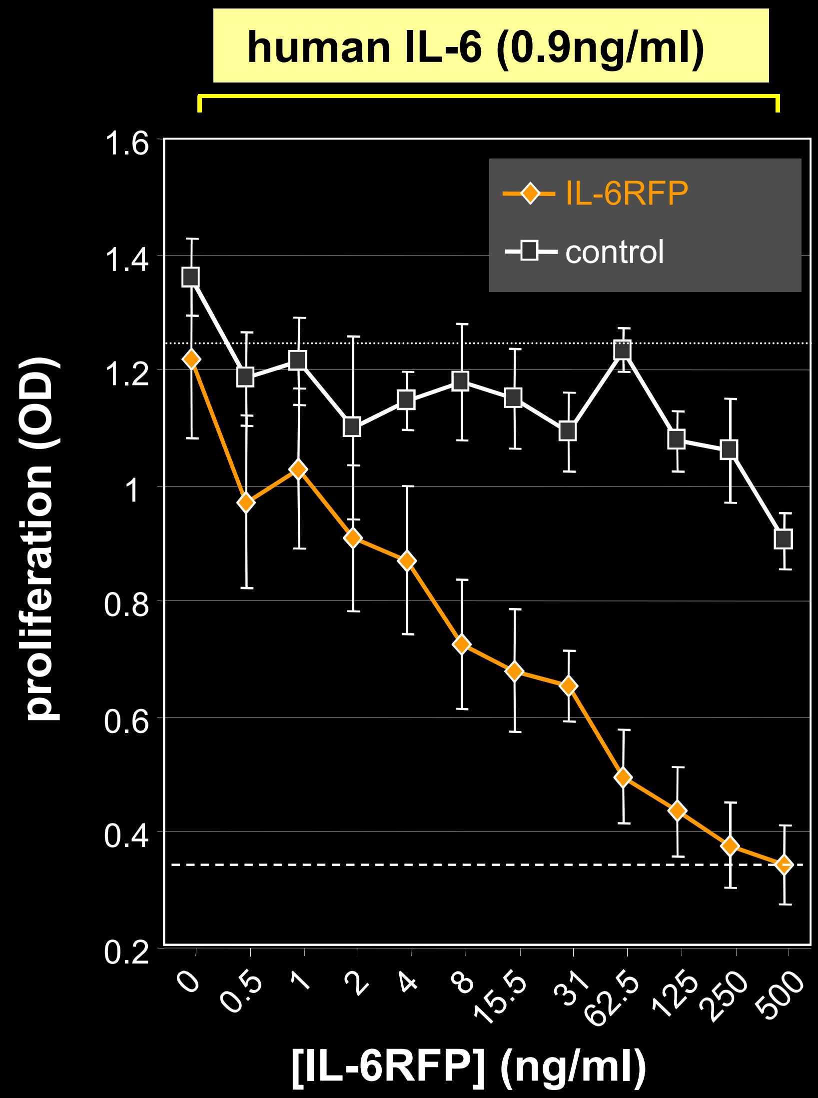


Ancey et al. (2003) J Biol Chem 278, 16 968 -16 972

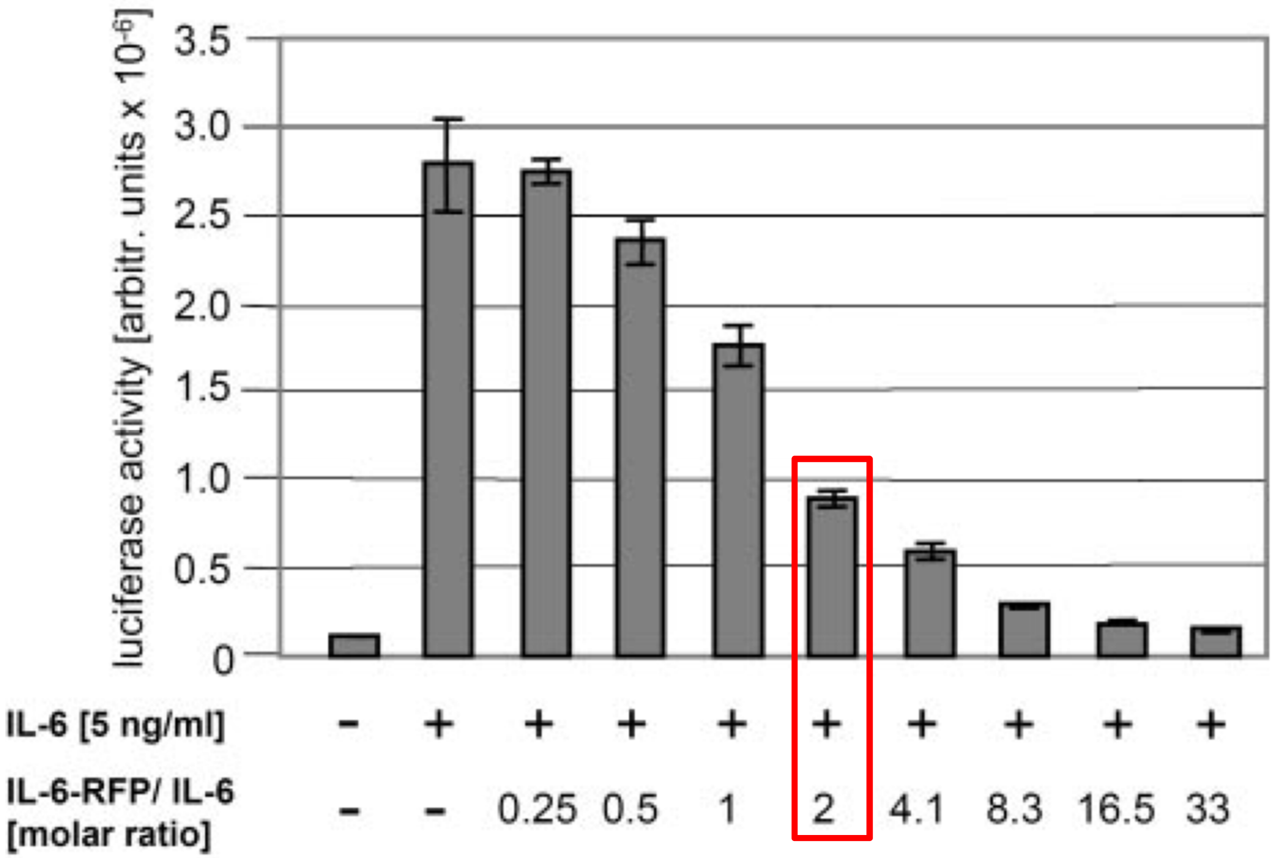
The human IL-6RFP inhibits cell proliferation mediated by human IL-6

Ba/F3-gp130-IL-6R



Ancey et al. (2003) J Biol Chem 278, 16 968 -16 972

IL-6 receptor fusion protein inhibits acute phase protein gene induction in HepG2 cells



Metz et al. (2007) J Biol Chem 282, 1238-1248

The Journal of Biological Chemistry

Vol. 278, No. 19, Issue of May 9, pp. 16968–16972, 2003
Printed in U.S.A.

A Fusion Protein of the gp130 and Interleukin-6Rα Ligand-binding Domains Acts as a Potent Interleukin-6 Inhibitor*

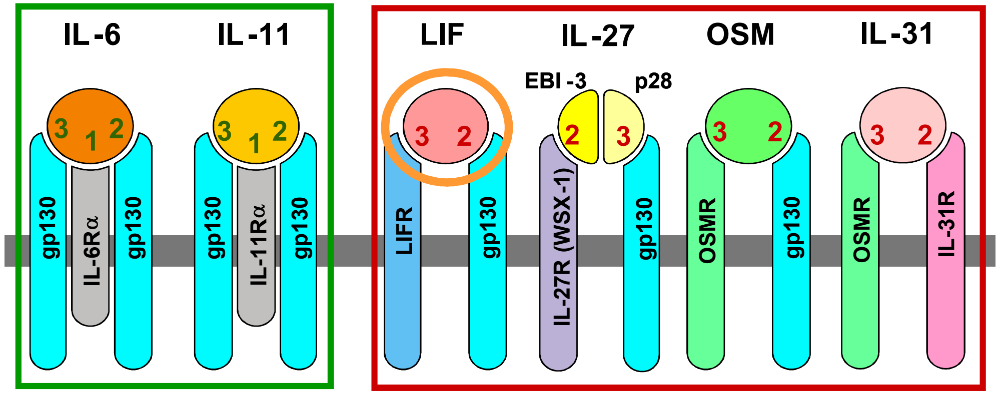
Received for publication, February 19, 2003, and in revised form, March 17, 2003
Published, JBC Papers in Press, March 19, 2003, DOI 10.1074/jbc.C300081200

Cécile Ancey, Andrea Küster, Serge Haan, Andreas Herrmann, Peter C. Heinrich‡, and Gerhard Müller-Newen‡

From the Institut für Biochemie, Universitätsklinikum Rheinisch-Westfälische Technische Hochschule Aachen, Pauwelsstrasse 30, 52057 Aachen, Germany

This inhibitor strategy was also applicable to other cytokines that signal *via* heteromeric receptor complexes, e.g. LIF-R and OSM-R

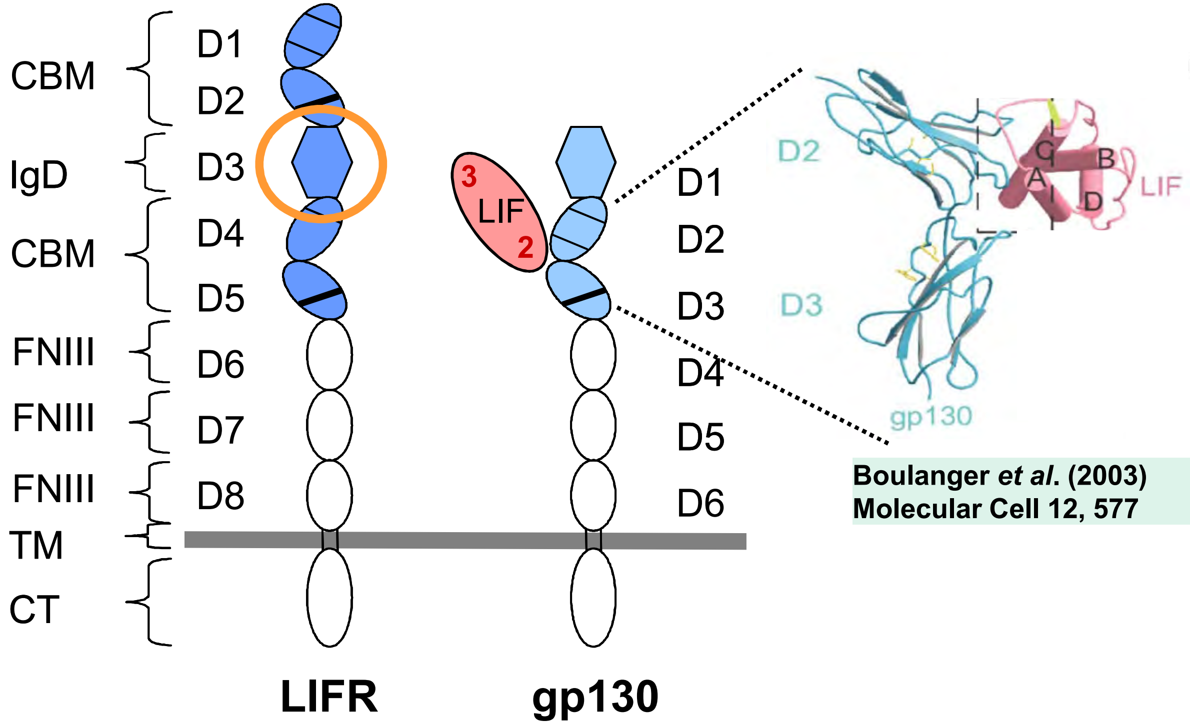
mLIF-RFP: a prototypic site II/III inhibitor



Cytokines with **3** receptor binding sites (sites I, II und III)

Cytokines with **2** receptor binding sites (sites II und III)

Owczarek et al. (1997)
J. Biol. Chem. 272, 23976



Boulanger *et al.* (2003)
Molecular Cell 12, 577

CBM, cytokine-binding module

Ig, immunoglobulin

FNIII, fibronectin type III

Metz S. et al. (2008) J. Biol. Chem. 283, 5985-5995

Outline: Interleukin-6 signal transduction and its regulation

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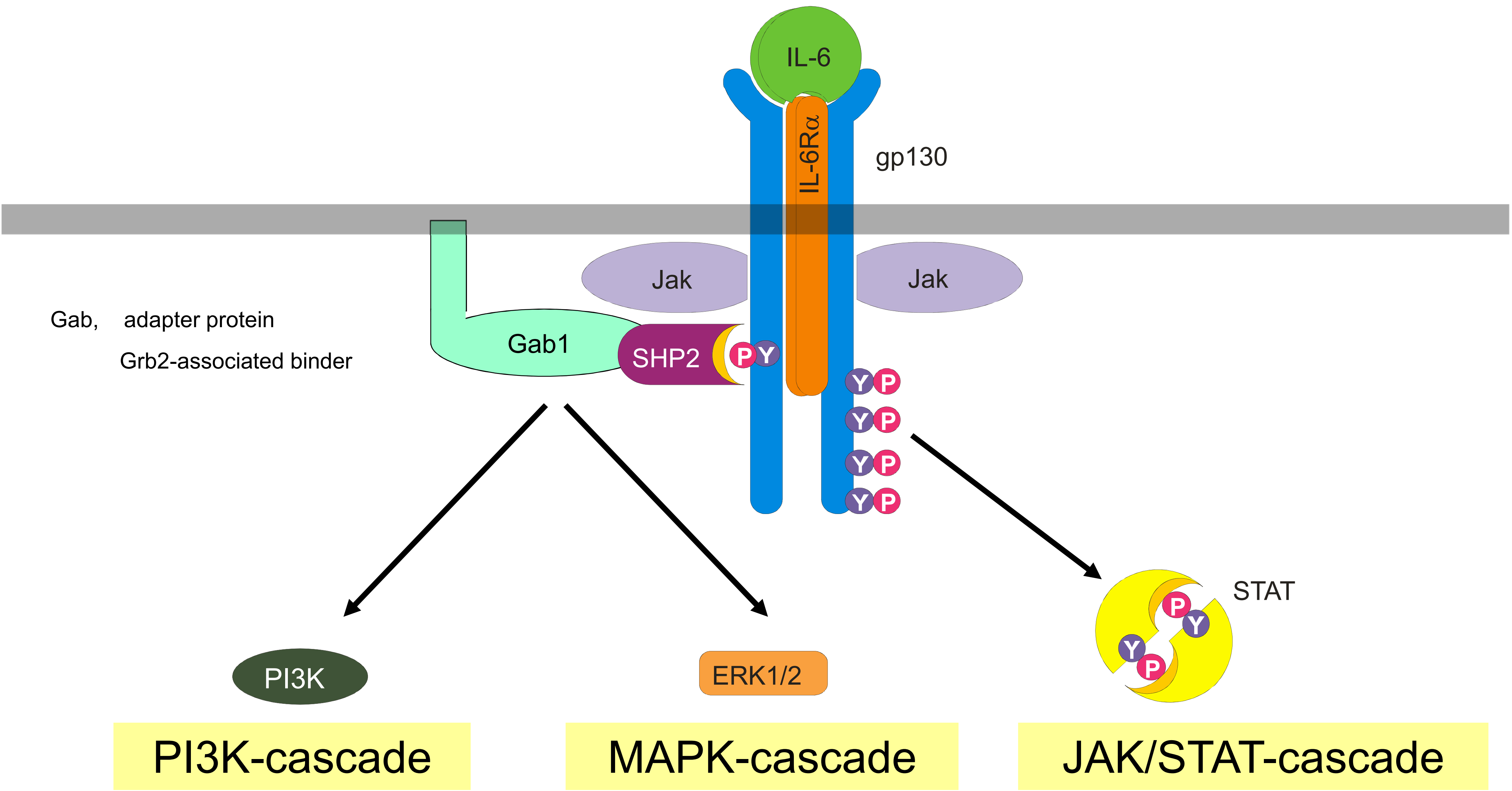
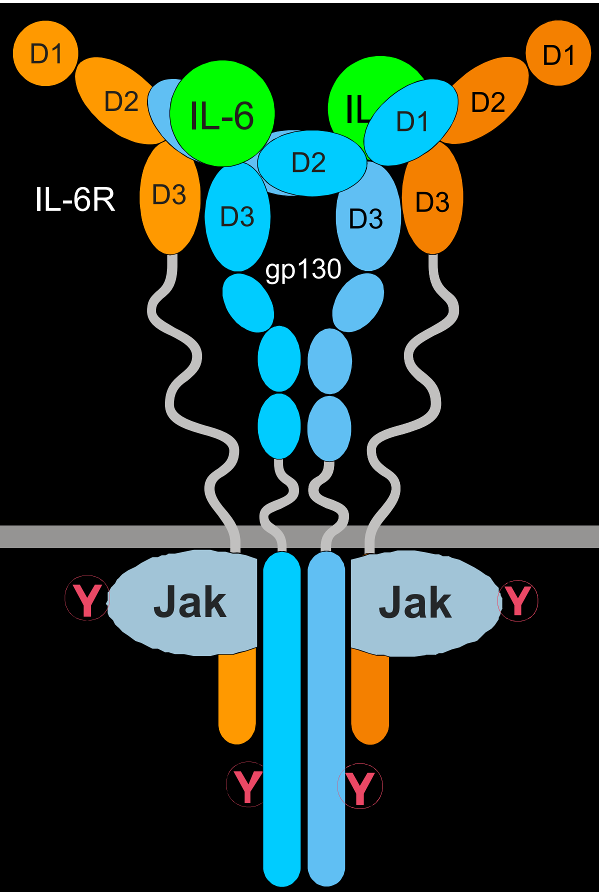
- **Nuclear translocation of STAT3-YFP**

Part 2: **Regulation of IL-6 signal transduction**

Molecular mechanism of IL-6 signal transduction

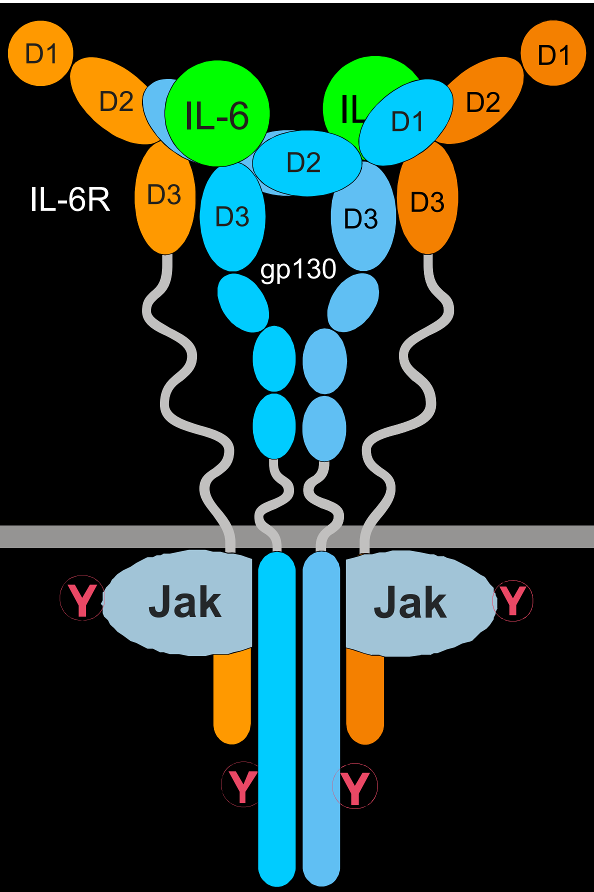
IL-6-initiated signaling pathways





Lütticken et al. (1994) Science 263, 89-92 740 citations (07/2022)

Wegenka et al. (1993) Mol Cell Biol 13, 276-288 517 citations (07/2022)



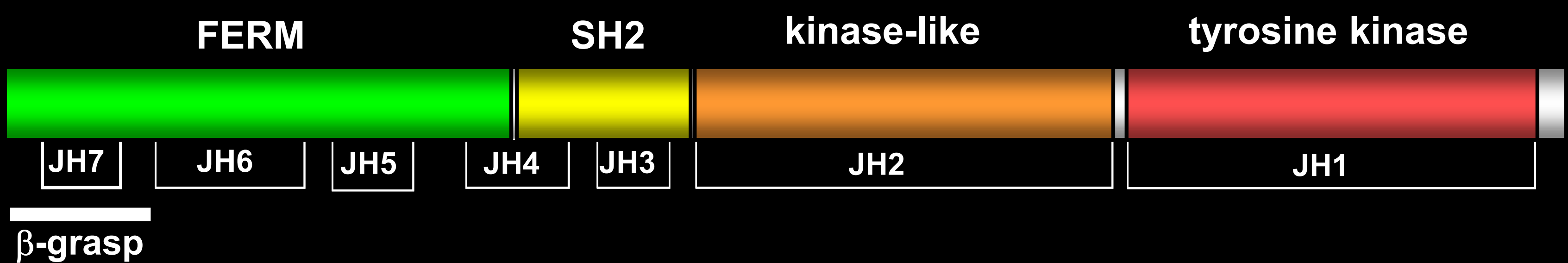
Lütticken et al. (1994) Science 263, 89-92 — 740 citations (07/2022)

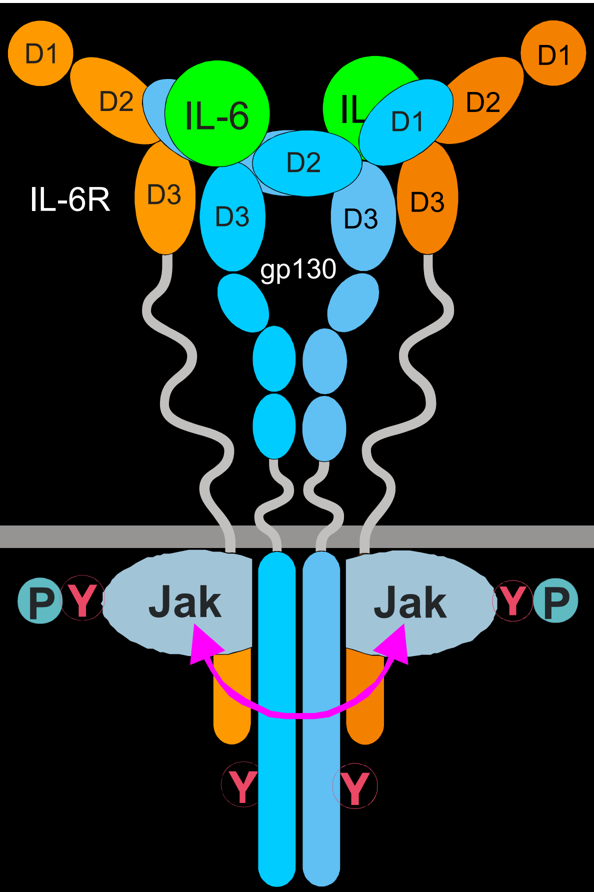
Wegenka et al. (1993) Mol Cell Biol 13, 276-288 — 517 citations (07/2022)

Janus

Roman god of “doors and gates“

Jak = Janus kinase





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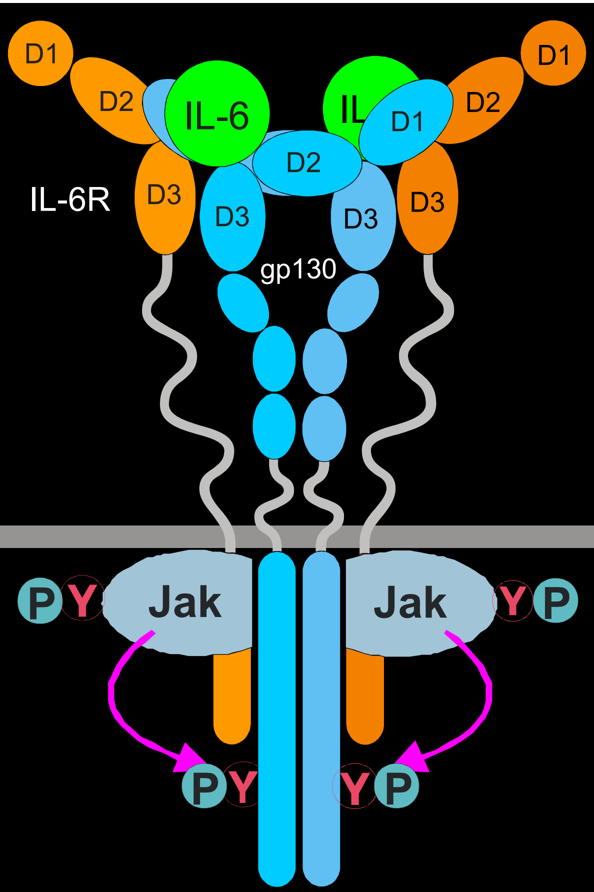

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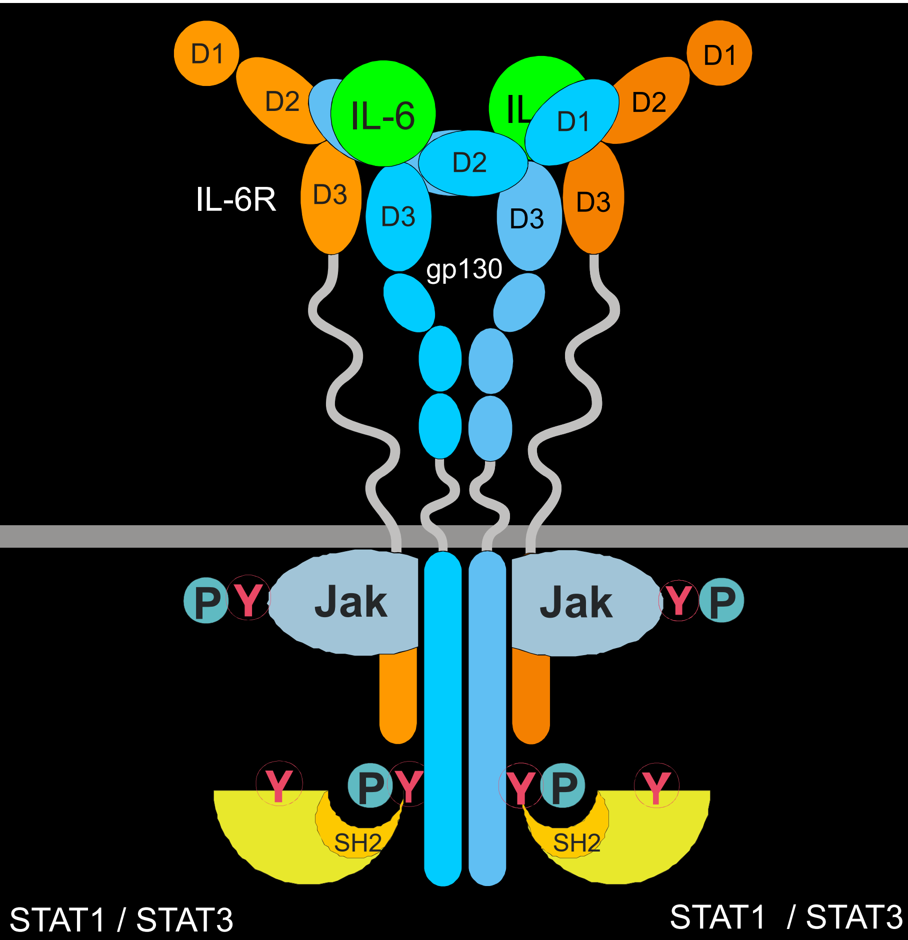
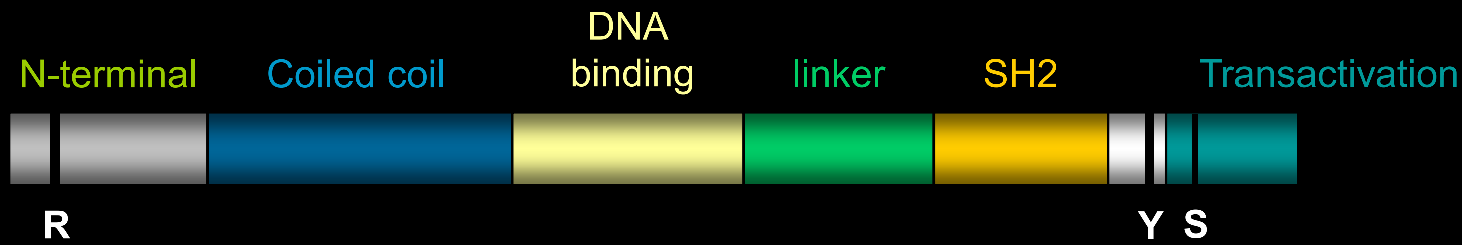
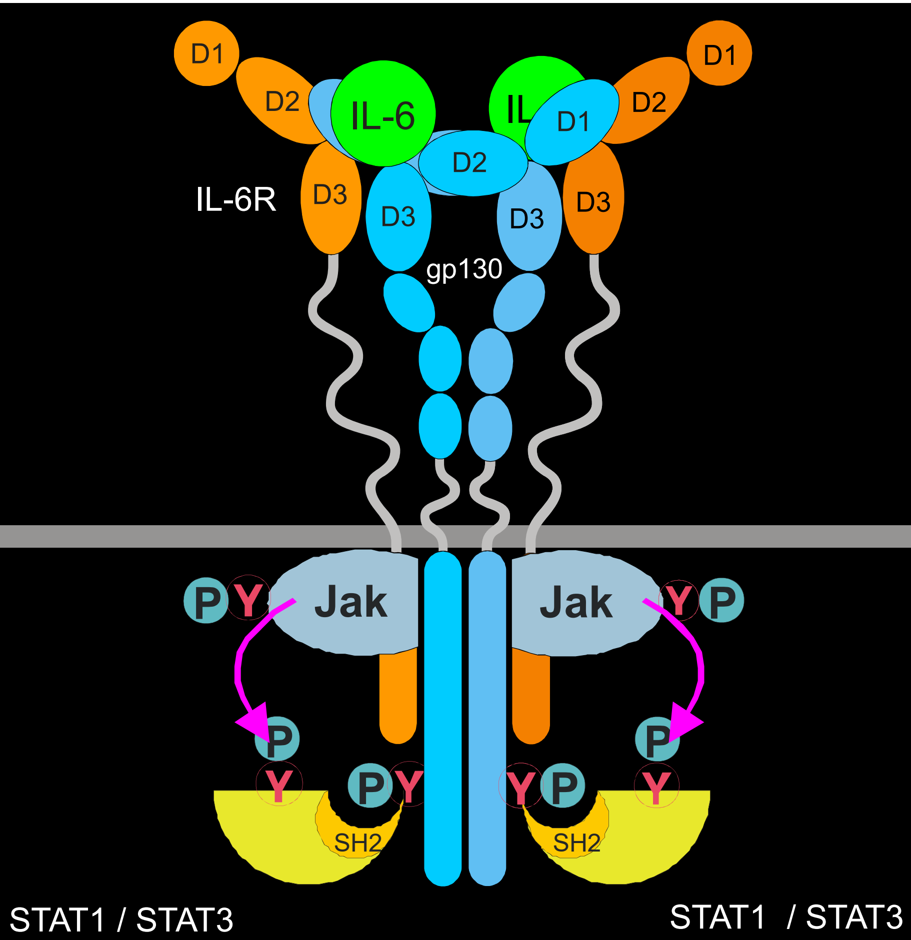


Lütticken et al. (1994) Science 263, 89-92 691 citations (2018)

Wegenka et al. (1993) Mol Cell Biol 13, 276-288 487 citations (2018)

STAT = **S**ignal **T**ransducer and **A**ctivator of **T**ranscription





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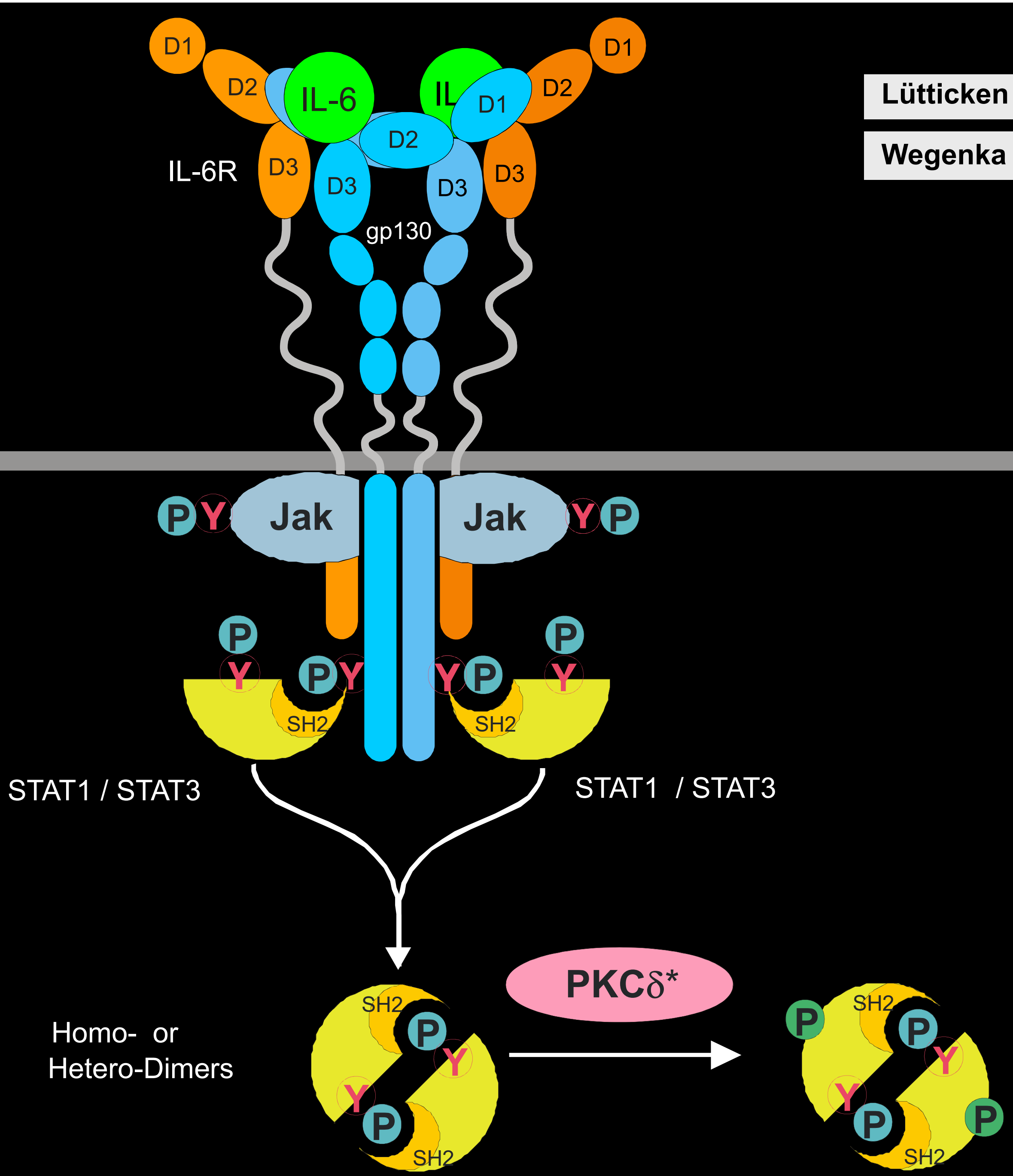

*Ca-CM-kinase II and MAP-kinases may also be involved

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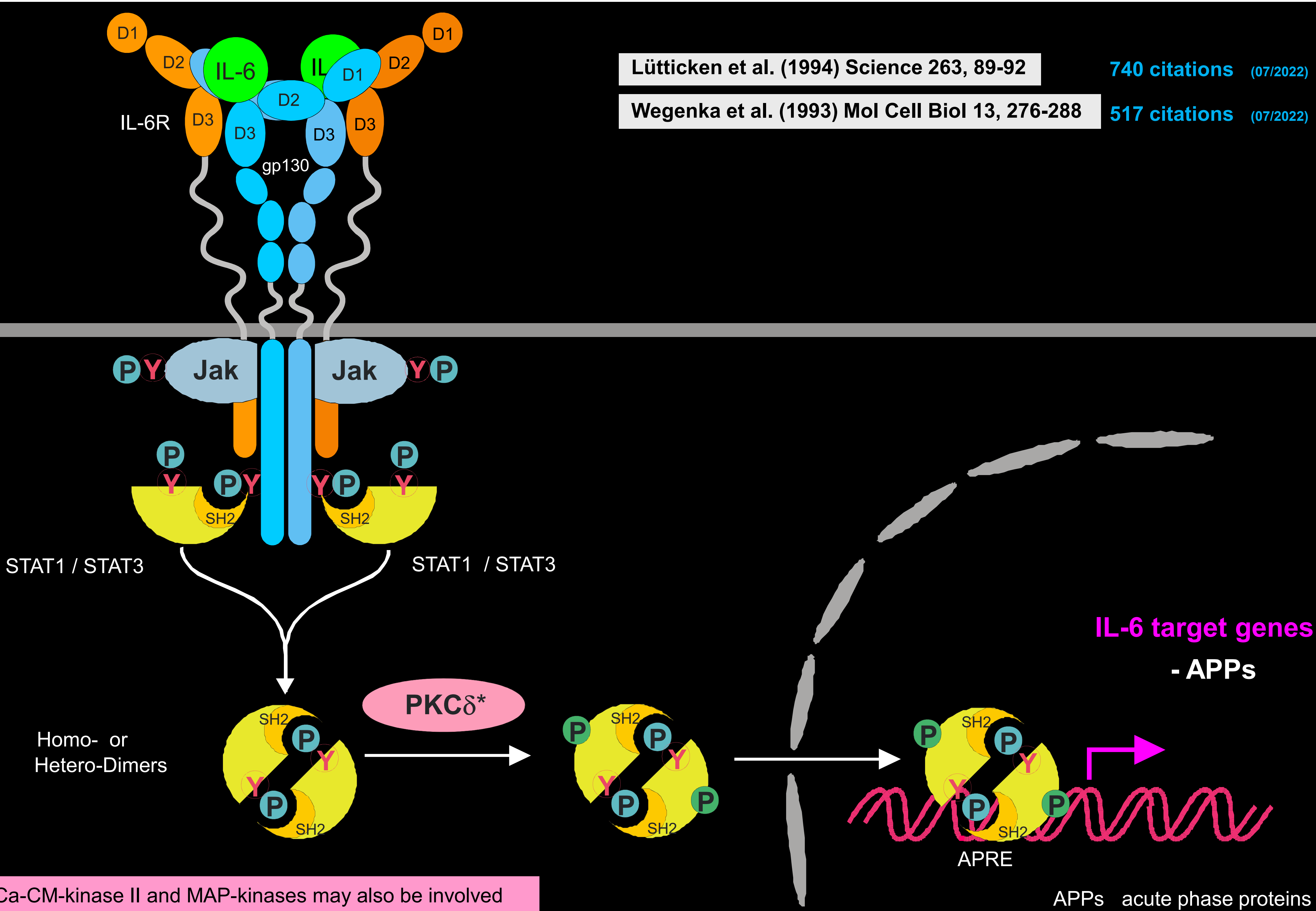

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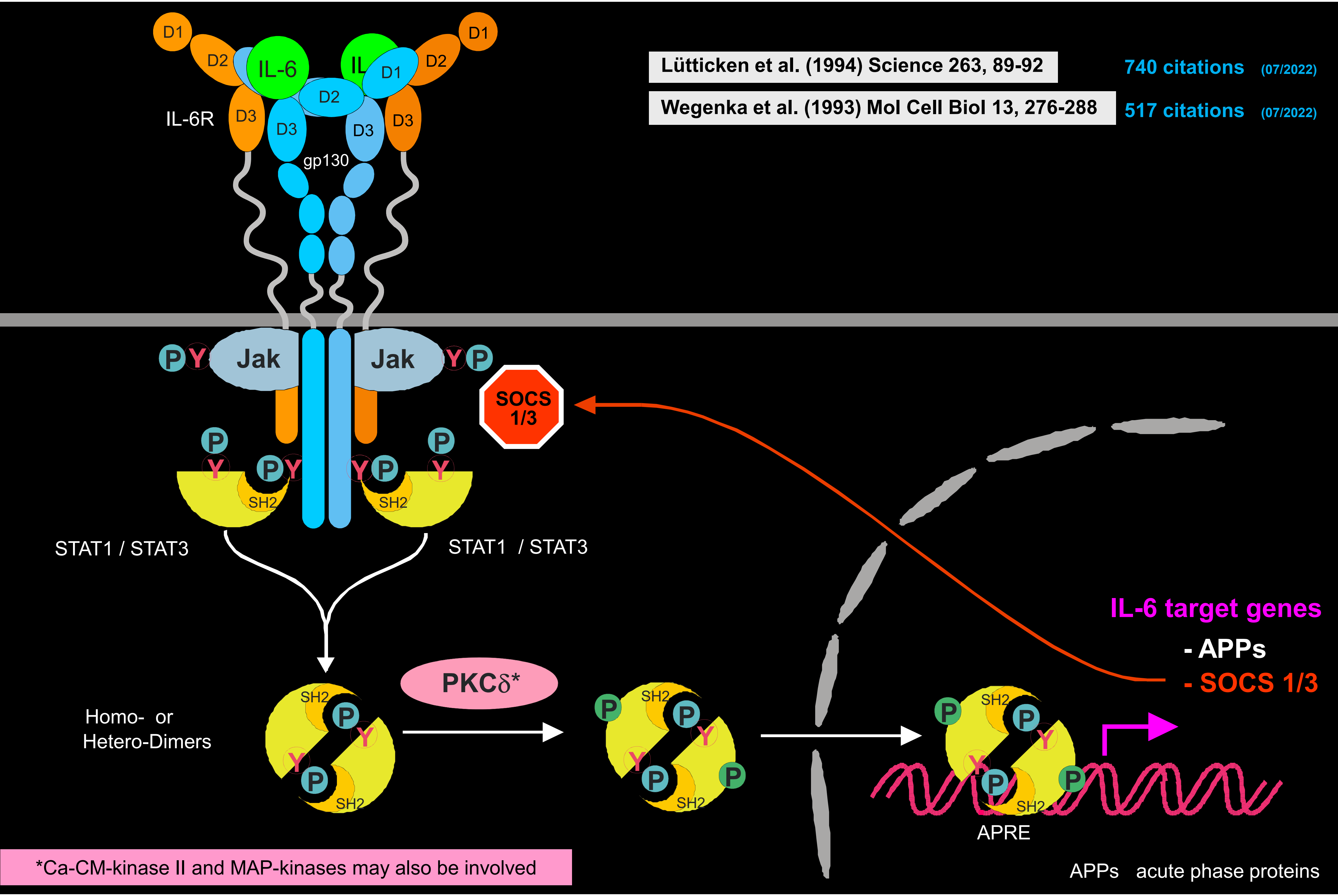

Lütticken et al. (1994) Science 263, 89-92
740 citations (07/2022)
Wegenka et al. (1993) Mol Cell Biol 13, 276-288
517 citations (07/2022)
D1
D2
IL-6
IL
D1
D2
D1
D2
IL-6R
D3
D3
D3
D3
gp130
Jak
Jak
P
Y
SOCS 1/3
SH2
SH2
STAT1 / STAT3
STAT1 / STAT3
Homo- or
Hetero-Dimers
PKCδ*
APRE
IL-6 target genes
- APPs
- SOCS 1/3
*Ca-CM-kinase II and MAP-kinases may also be involved
APPs acute phase proteins

Which Janus kinase phosphorylates gp130 ?

There are 4 JAK tyrosine kinases in mammalian cells

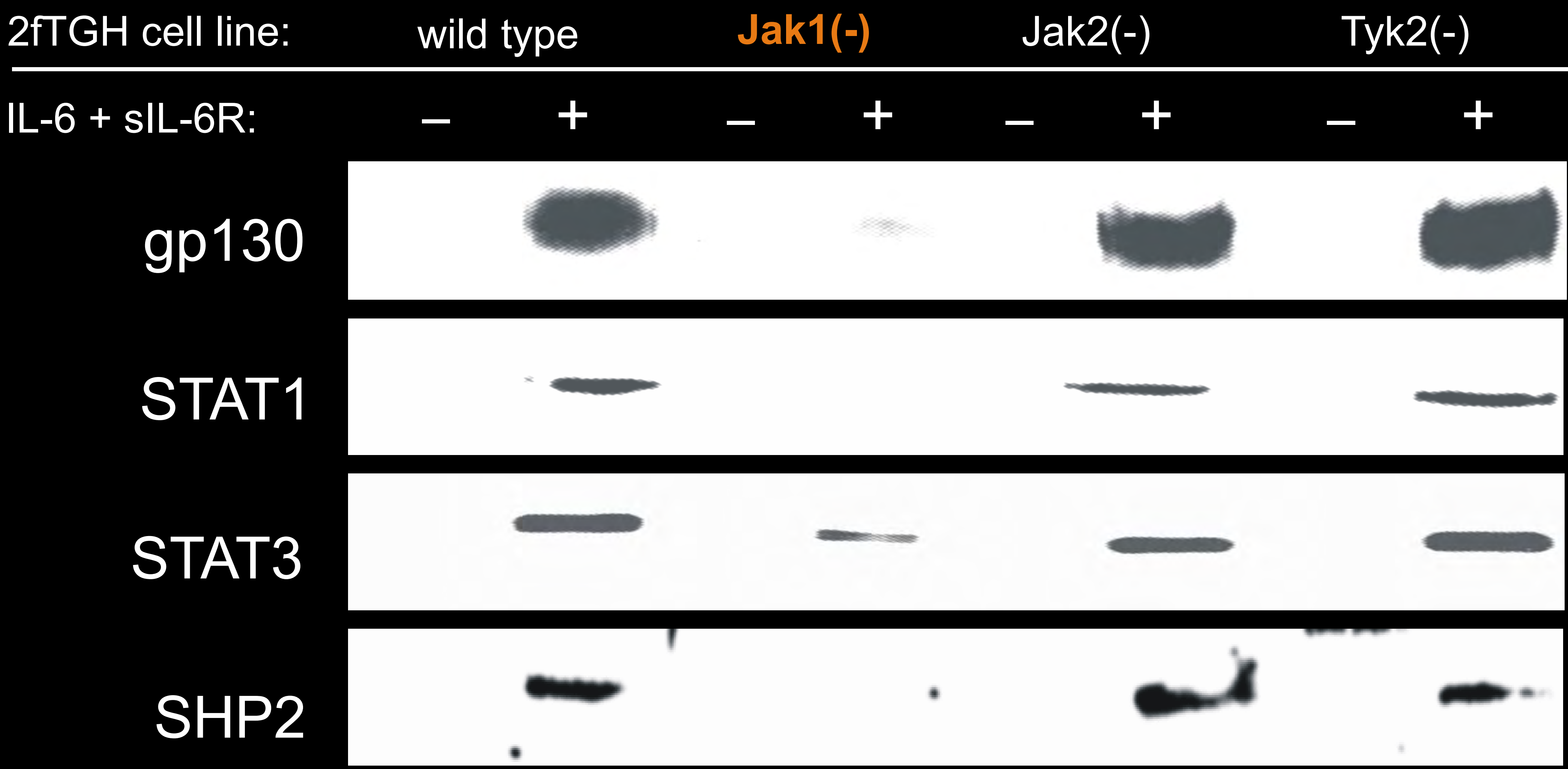
JAK 1

JAK 2

JAK 3 **only present in immune cells**

TYK 2

Central role of Jak1 for IL-6 signaling



anti-phosphotyrosine immunoblots

Guschin et al. (1995) EMBO J 14, 1421 - 1429

human fibrosarcoma cells

Jak1 k.o. mice

- perinatal lethality
- decrease in birth rate
- smaller thymus
- impaired response to cytokine signaling via gp130, γc and interferon receptors

Rodig et al. (1998) Cell

Jak2 k.o. mice

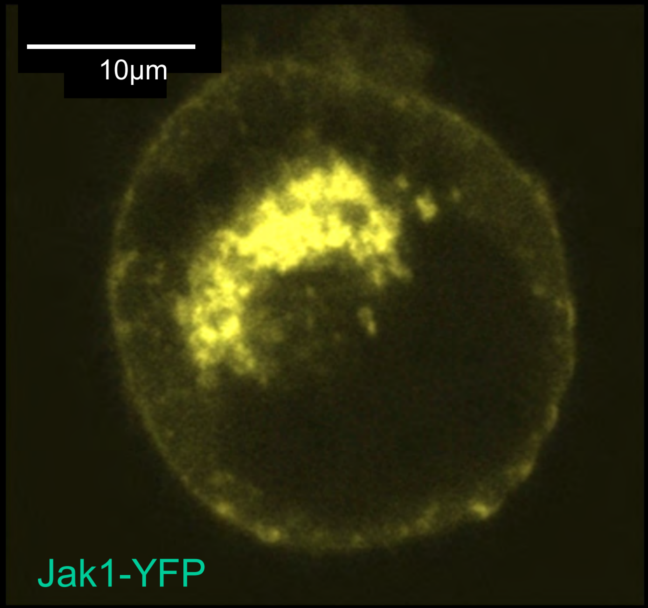
- embryonic lethality
- absence of definitive erythropoiesis
- impaired response to Epo, Tpo, IL-3, GM-CSF and IFNγ

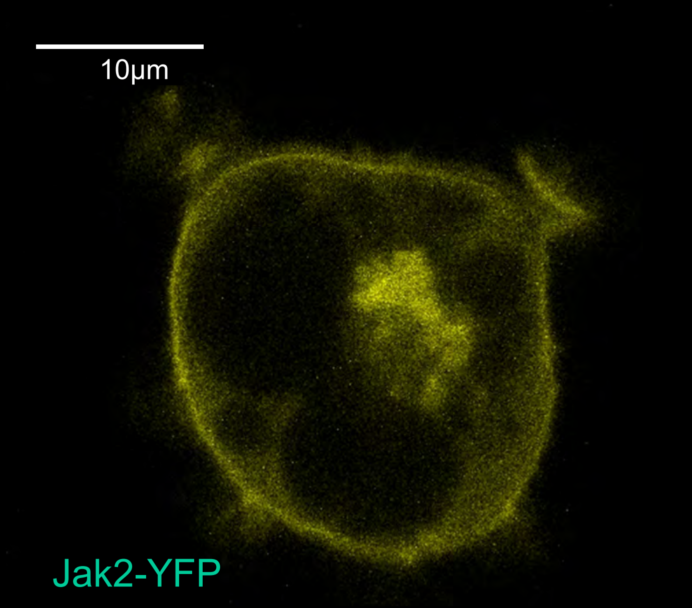
Parganas et al., Neubauer et al. (1998) Cell

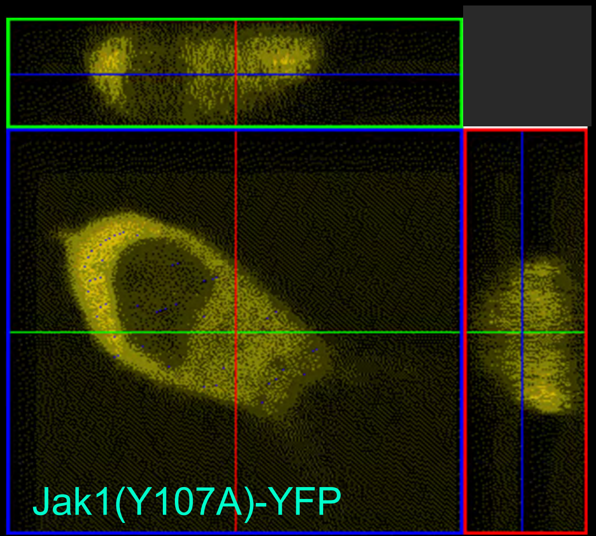
Interestingly, the heterodimeric LIFR binds
at least 3 of the 4 JAK family members,
JAK1, JAK2, and TYK2.
In the absence of JAK1, but not JAK2 or TYK2
LIF signaling is abrogated,
exactly like in the case of IL-6 receptor gp130.
This indicates that JAK1 has a dominant role.

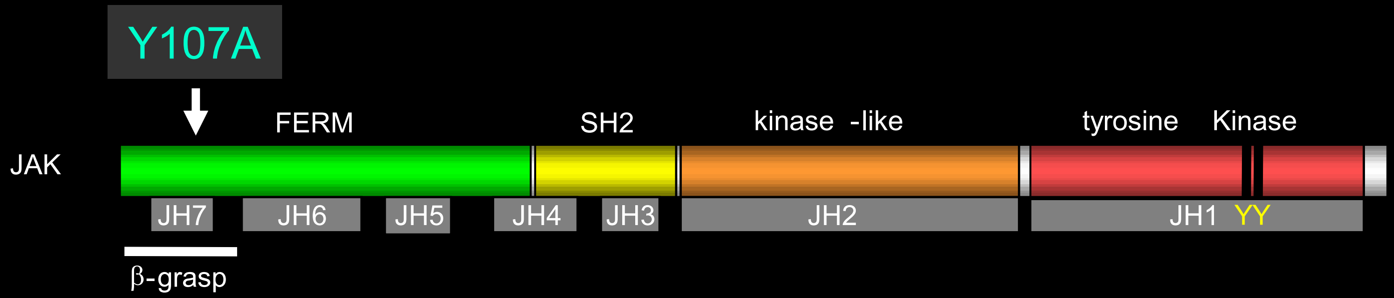
gp130 - Jak interaction

Jak1 and Jak2 are membrane-associated kinases





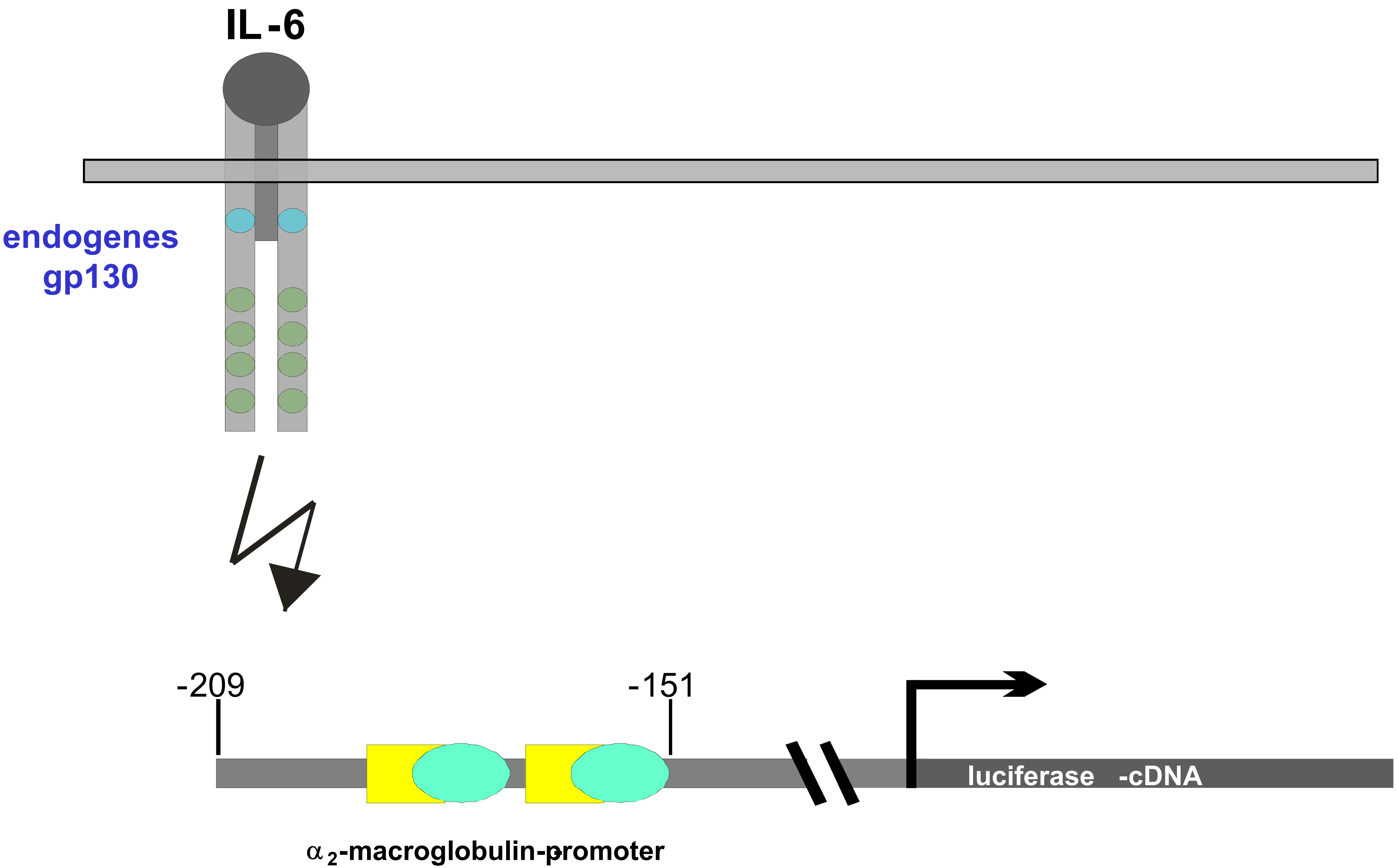




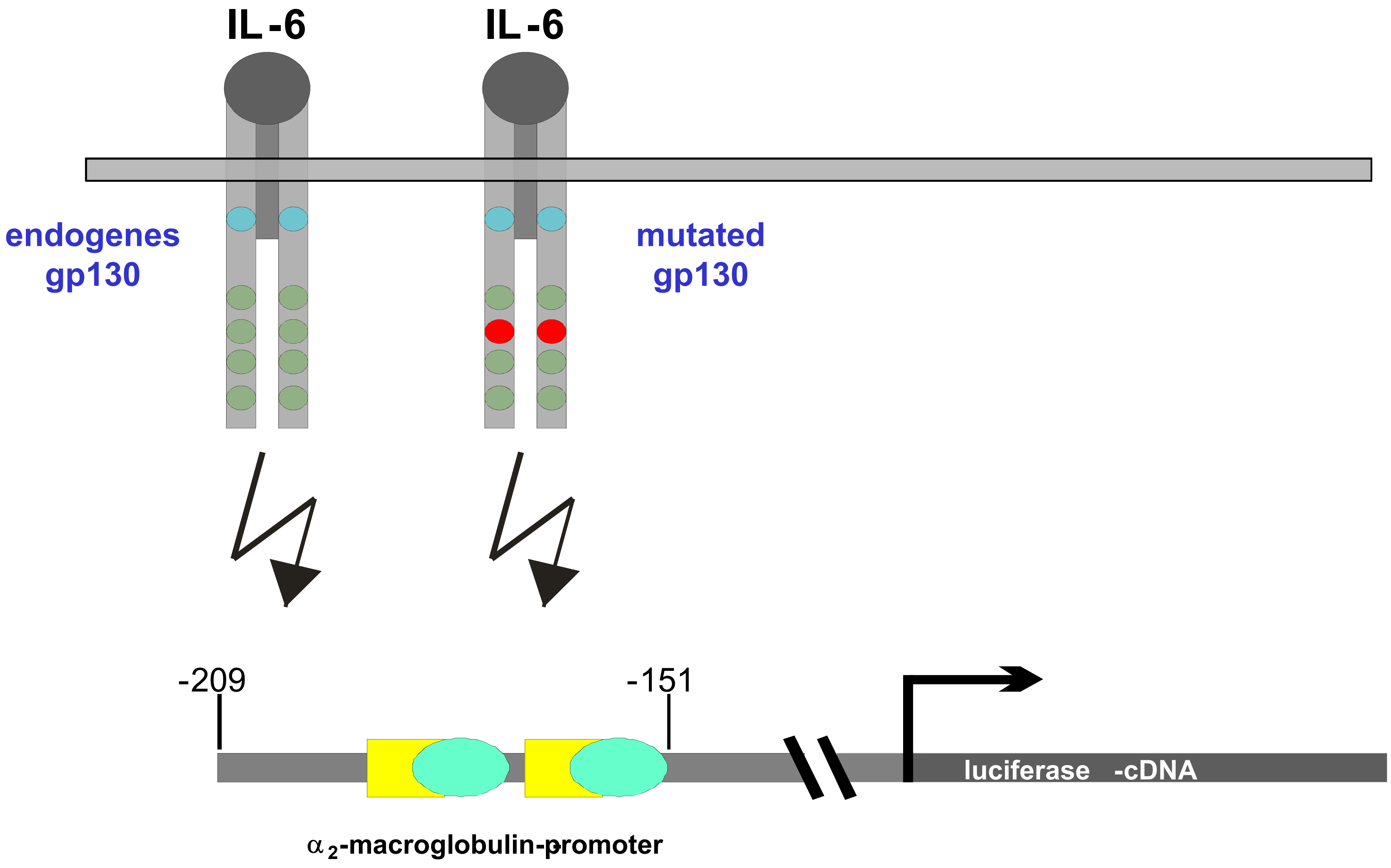
Laser scanning microscopy in living COS7 cells

Giese et al. (2003) J Biol Chem 278, 39205

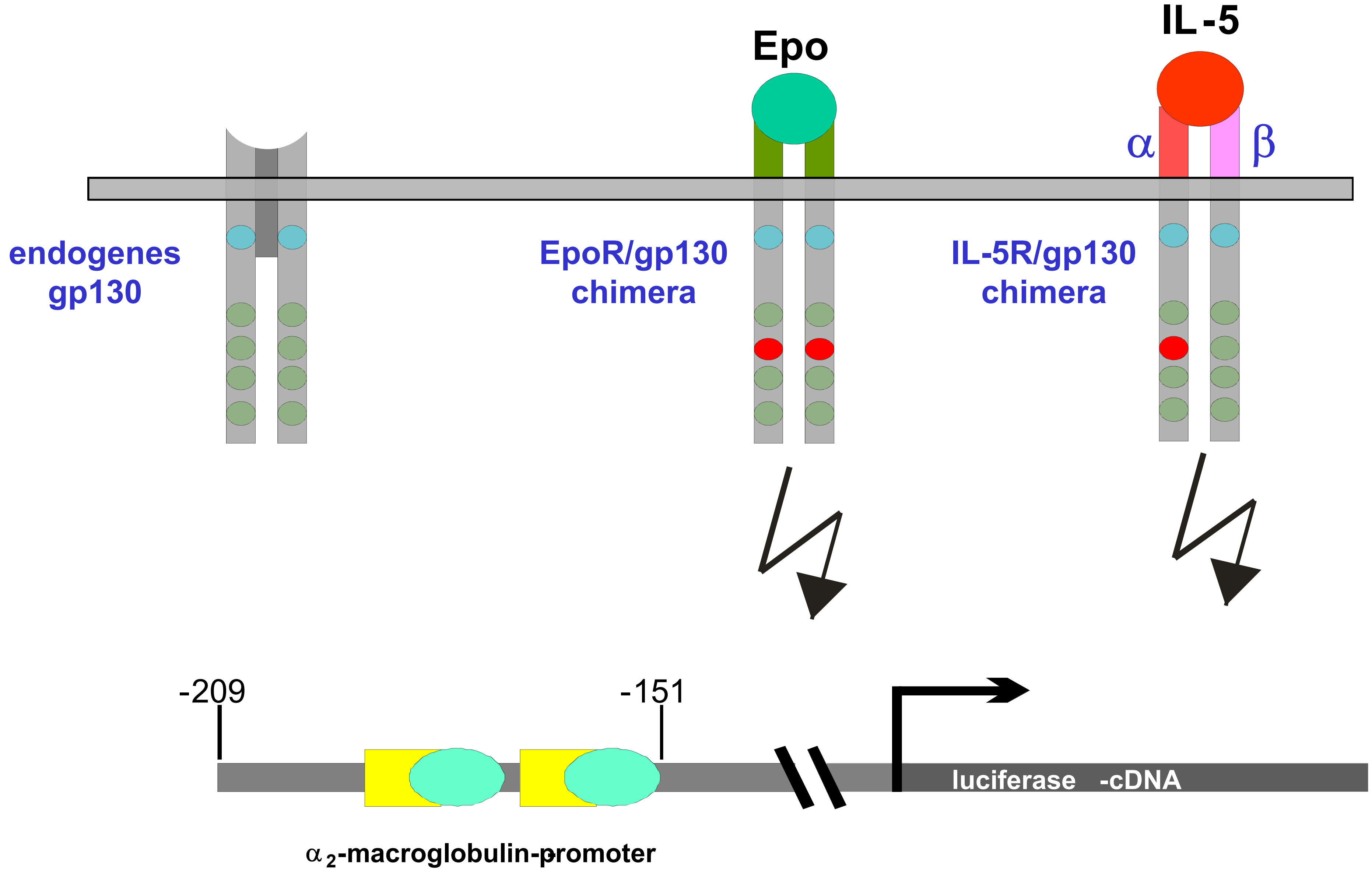
Using chimeric receptor systems

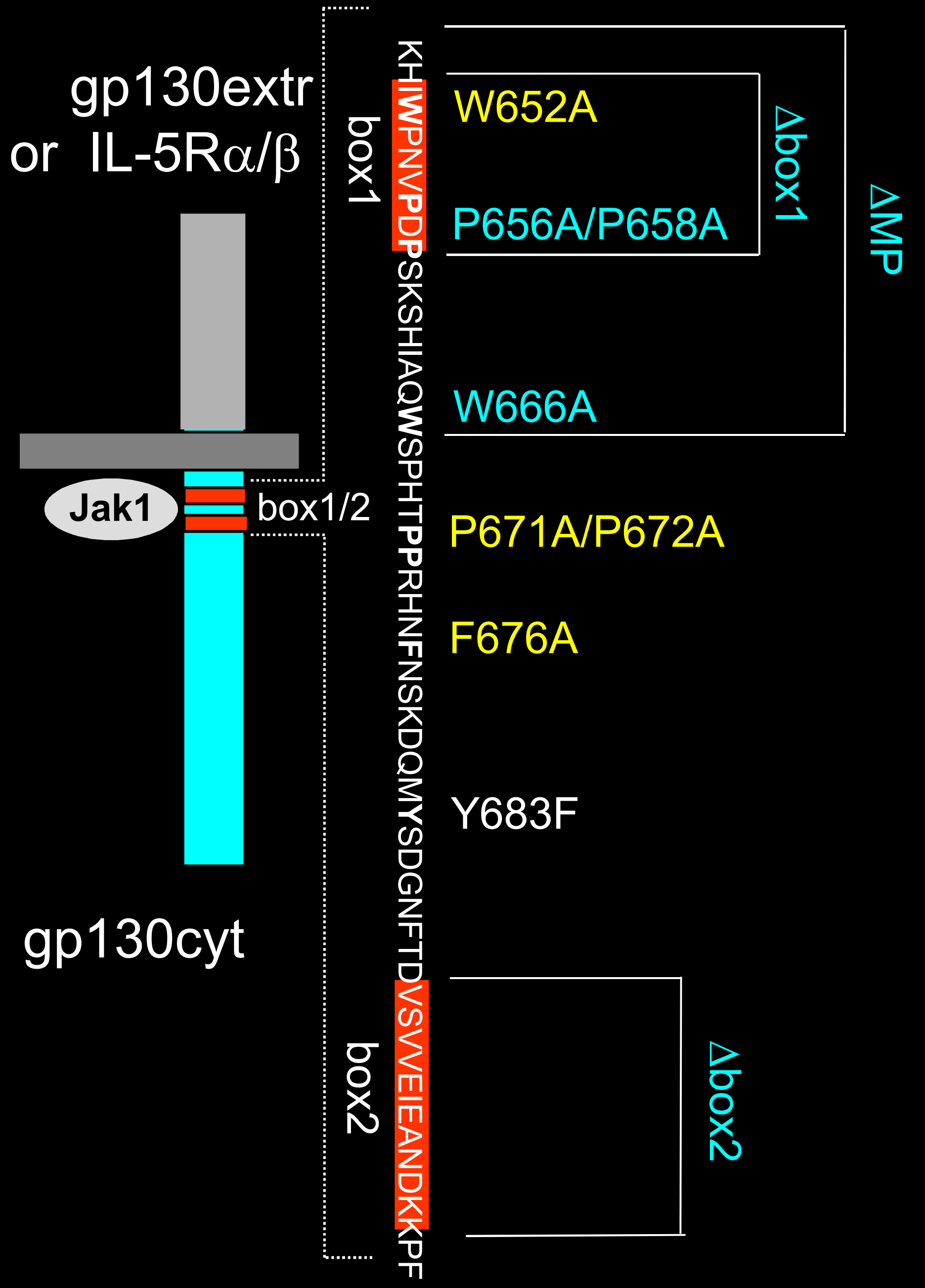


Using chimeric receptor systems



Using chimeric receptor systems





Mutational analysis of the membrane-proximal region of gp130

Δbox1
P656A/P658A
ΔMP
W666A
Δbox2
} no Jak association

W652A
P671A/P672A
F676A
} normal Jak association, signal transduction impaired

Y683F no effect

chimaeric IL-5Rα/β - gp130 expressed in COS-7 cells

C. Haan et al. (2002) Biochem. J. 361, 105 - 111

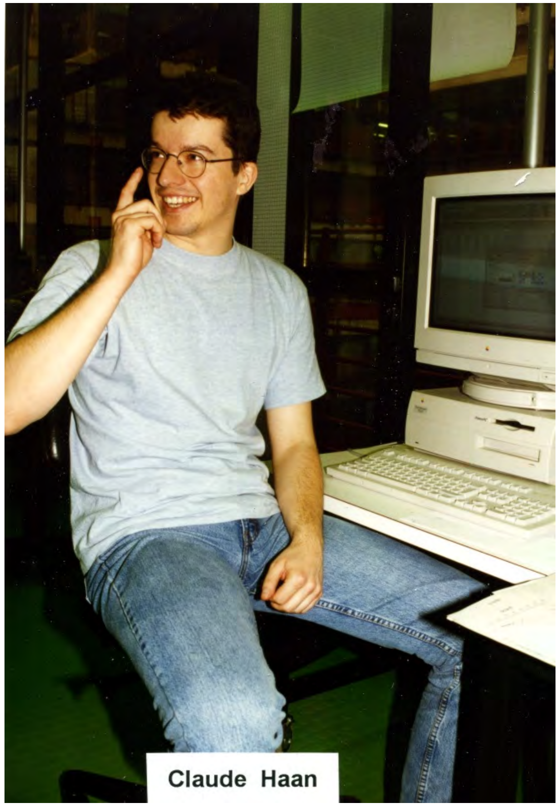
THE JOURNAL OF BIOLOGICAL CHEMISTRY

Vol. 276, No. 40, Issue of October 5, pp. 37451–37458, 2001
Printed in U.S.A.

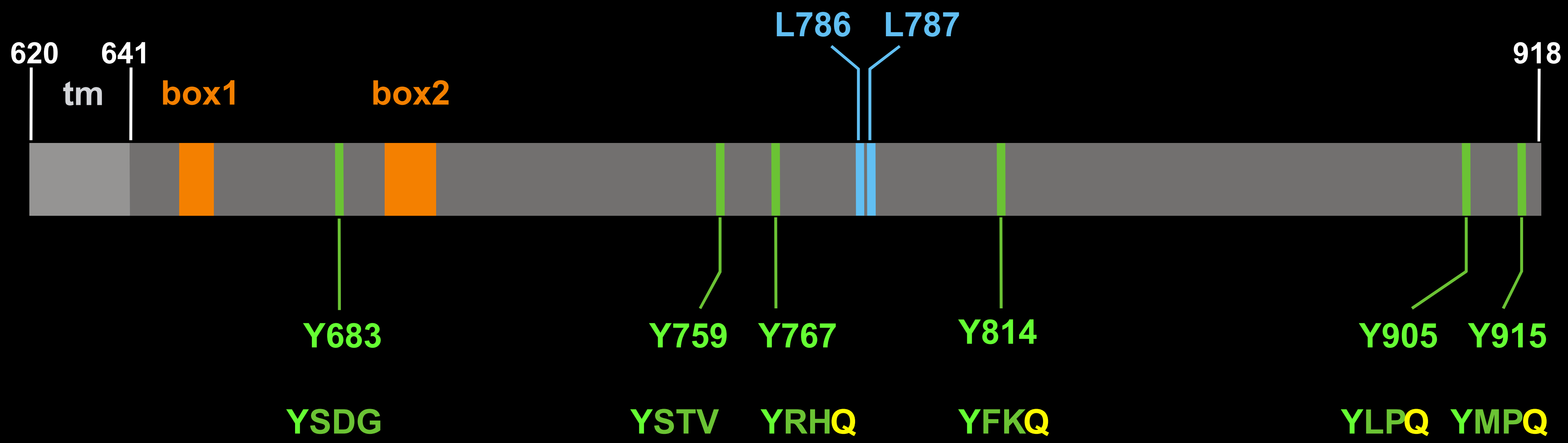
Mapping of a Region within the N Terminus of Jak1 Involved in Cytokine Receptor Interaction*

Received for publication, July 2, 2001
Published, JBC Papers in Press, July 23, 2001, DOI 10.1074/jbc.M106135200

Claude Haan‡§, Hayaatun Is'harc§¶, Heike M. Hermanns‡, Hildegard Schmitz-Van de Leur‡, Ian M. Kerr¶, Peter C. Heinrich‡, Joachim Grötzinger‡, and Iris Behrmann‡||



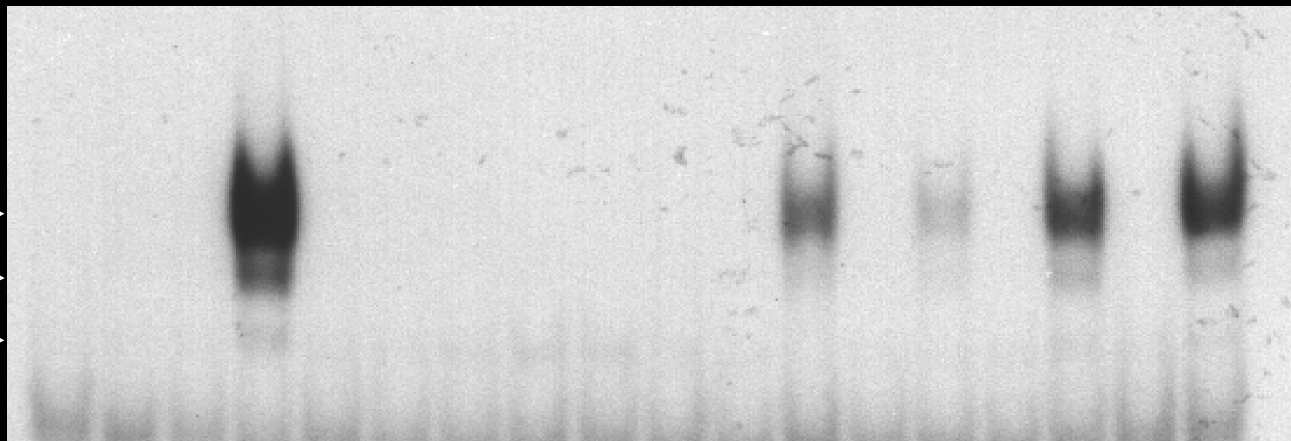
Which tyrosine in the cytoplasmic tail of gp130 activates which STAT factor ?



intracellular domain of gp130

Activation of STAT3 via individual tyrosine motifs in gp130

receptor	none									
		Y	F	Y	F	F	F	F	F	683
		Y	F	F	Y	F	F	F	F	759
		Y	F	F	F	Y	F	F	F	767
		Y	F	F	F	F	Y	F	F	814
		Y	F	F	F	F	F	Y	F	905
		Y	F	F	F	F	F	F	Y	915
IL-6/sIL-6R	- +	- +	- +	- +	- +	- +	- +	- +	- +	

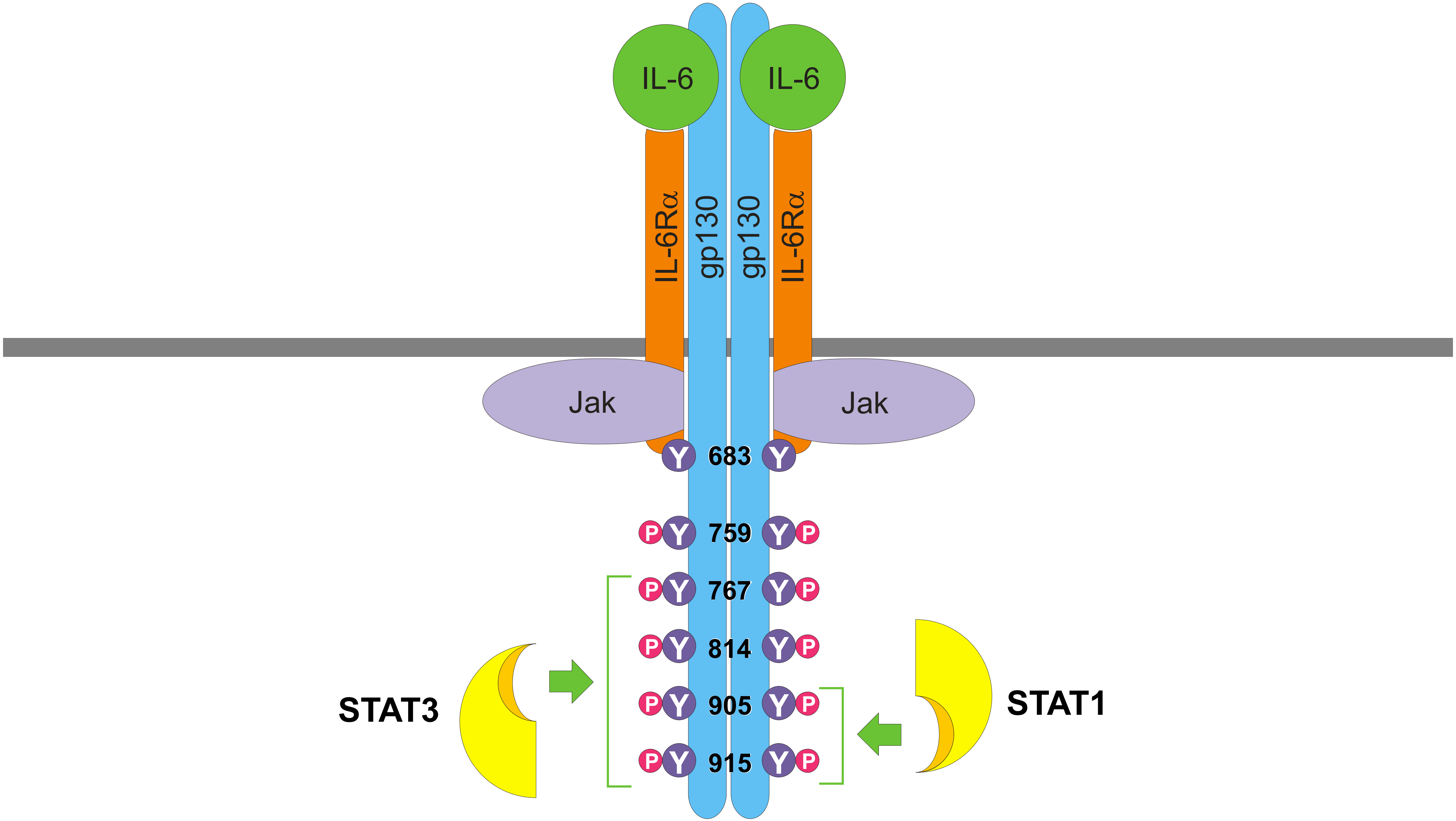


EMSA: m67 SIE

add-back mutants in stably transfected BaF3 cells + IL-6/solR

Schmitz et al. (2000) J Immunol 164, 848 - 854 **70 citations** (07/2022)

STAT3 and STAT1 recruitment sites in gp130



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Differential Activation of Acute Phase Response Factor/STAT3 and STAT1 via the Cytoplasmic Domain of the Interleukin 6 Signal Transducer gp130

I. DEFINITION OF A NOVEL PHOSPHOTYROSINE MOTIF MEDIATING STAT1 ACTIVATION*

225 citations
(07/2022)

(Received for publication, October 25, 1995, and in revised form, February 20, 1996)

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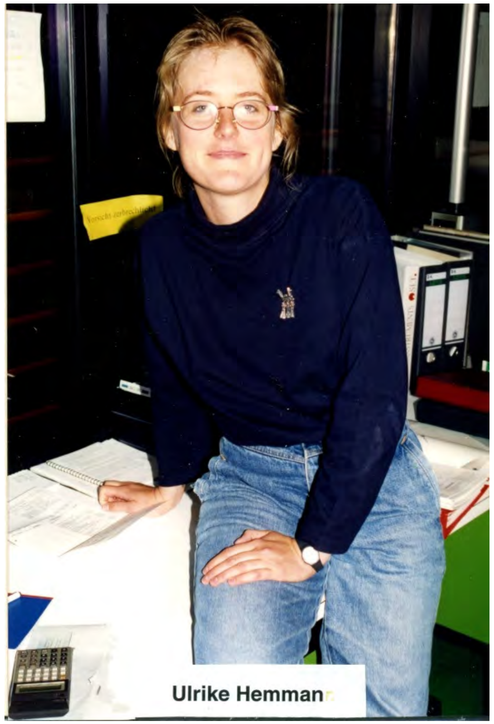
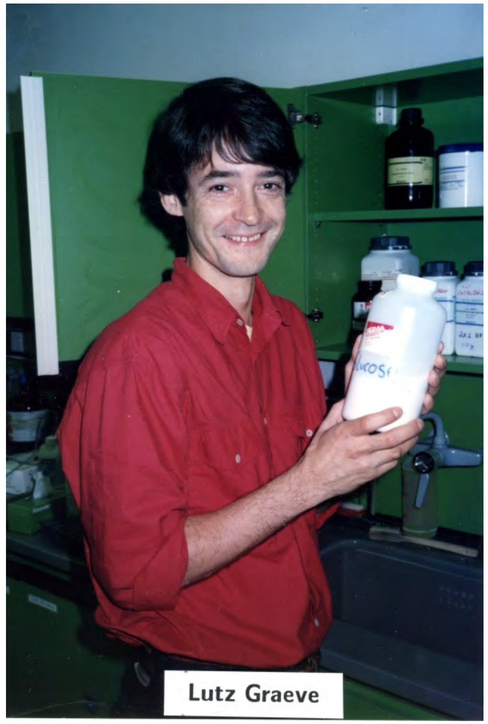

Ulrike Hemman

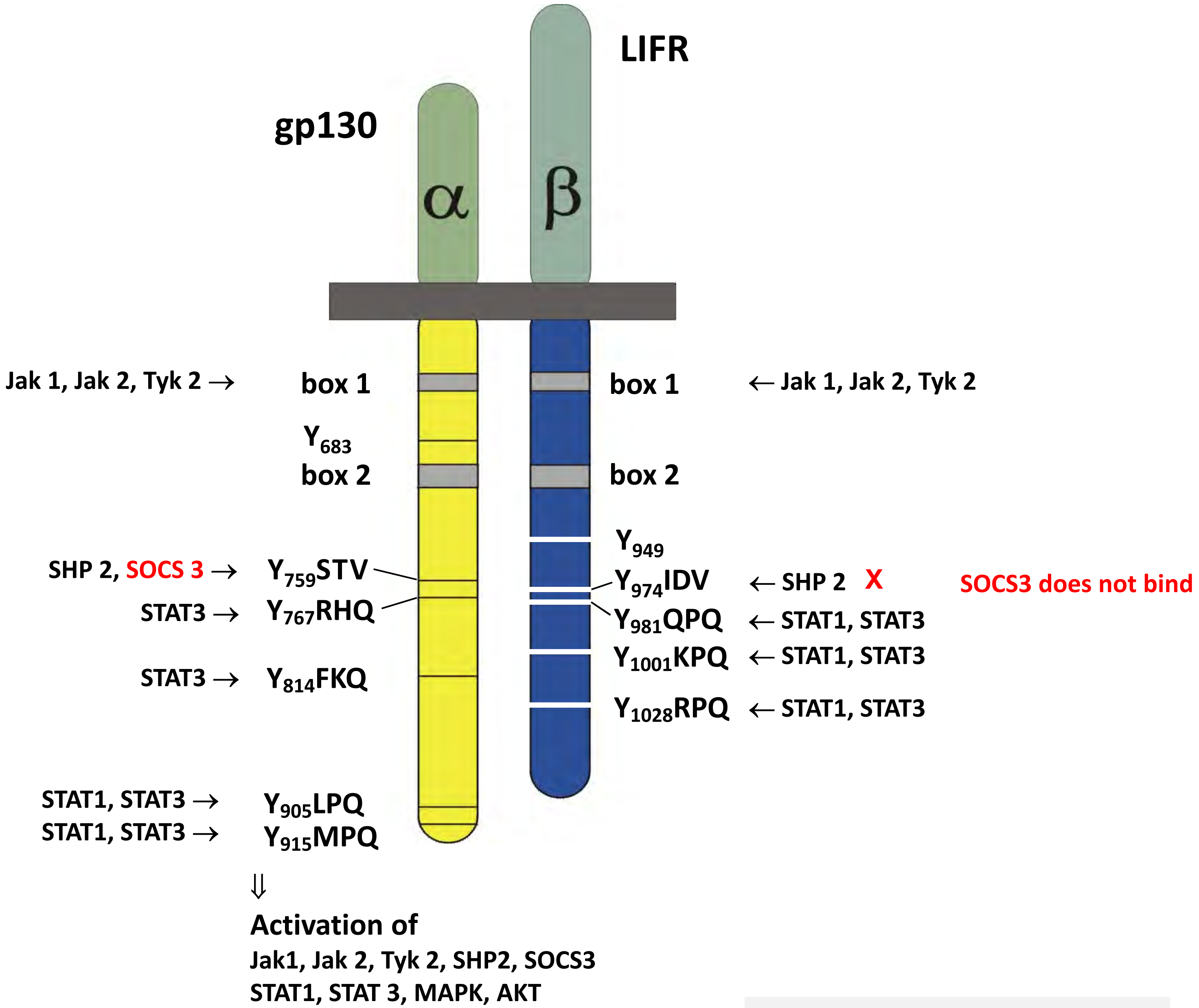

Lutz Graeve

The Cytoplasmic Tyrosine Motifs in Full-Length Glycoprotein 130 Have Different Roles in IL-6 Signal Transduction[1]

Jochen Schmitz, Heike Dahmen, Carsten Grimm, Cornelia Gendo, Gerhard Müller-Newen, Peter C. Heinrich,[2] and Fred Schaper

The Journal of Immunology, 2000, 164: 848-854

The LIF receptor complex



Clahsen et al. Cell Signal 2005 16 citations (07/2022)

Outline: Interleukin-6 signal transduction and its regulation

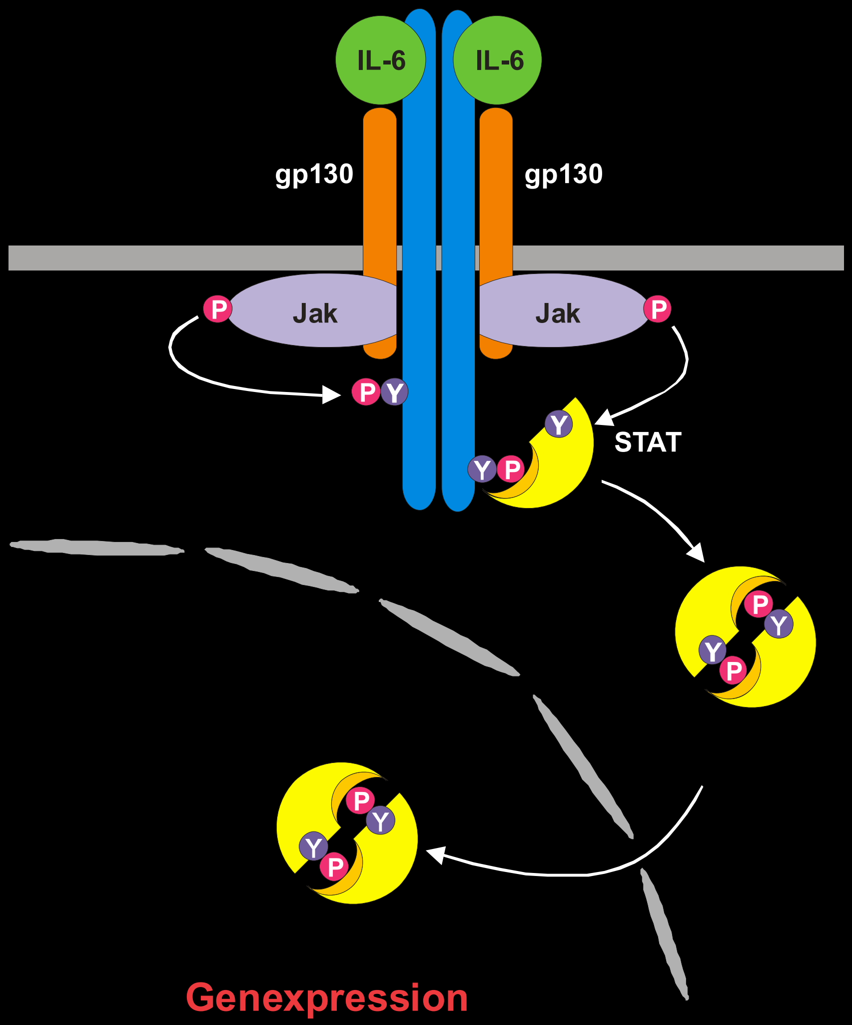
Part 1: **Molecular mechanisms of IL-6 signal transduction**

- **The acute phase response**
- **Identification of HSF as IL-6**
- **Structure and function of IL-6**
- **Acute phase protein synthesis induced by IL-6**
- **Molecular mechanism of IL-6 induced APP expression**
- **Formation of the IL-6-receptor complex**
- **Design of a highly potent IL-6 antagonist**
- **Molecular mechanisms of IL-6 signal transduction**
- **Nuclear translocation of STAT3-YFP**

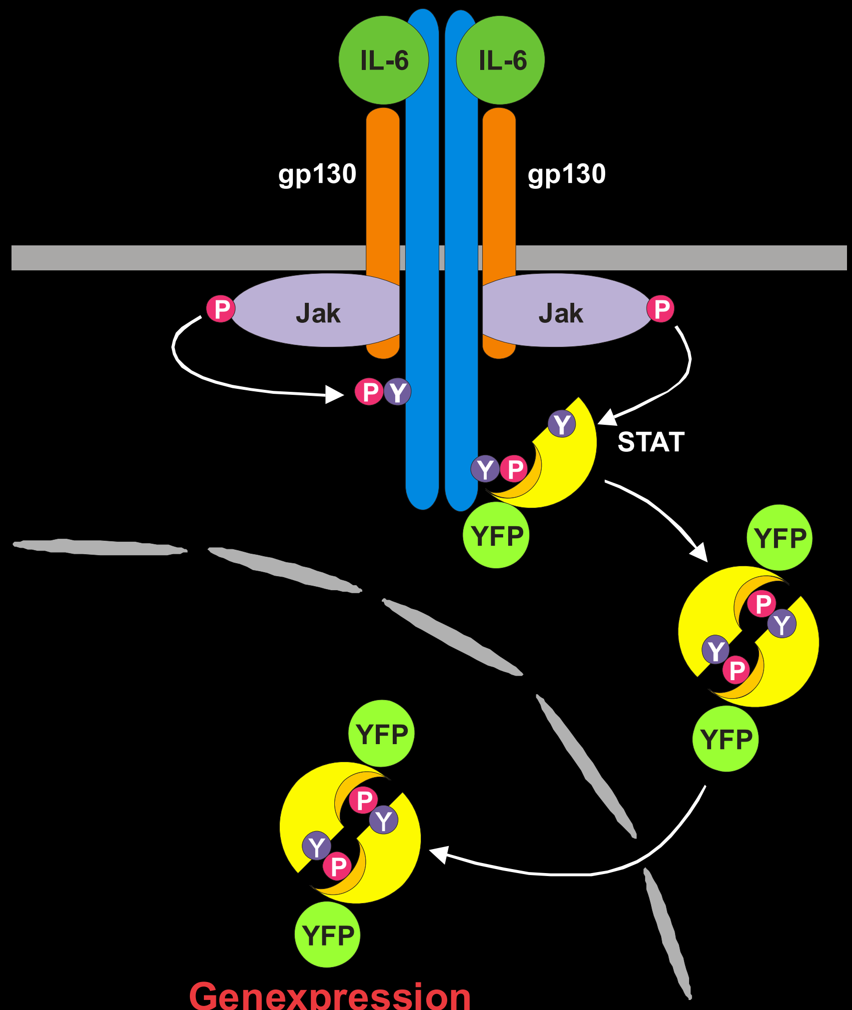
Part 2: **Regulation of IL-6 signal transduction**

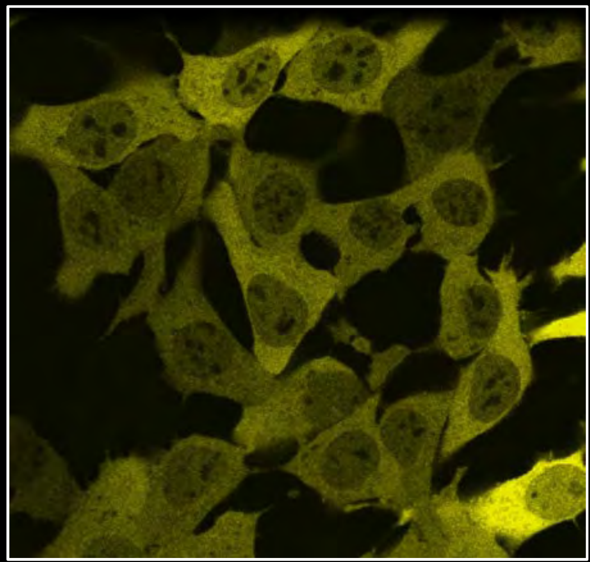
Nuclear translocation of STAT3 -YFP

Nuclear translocation of STAT 3

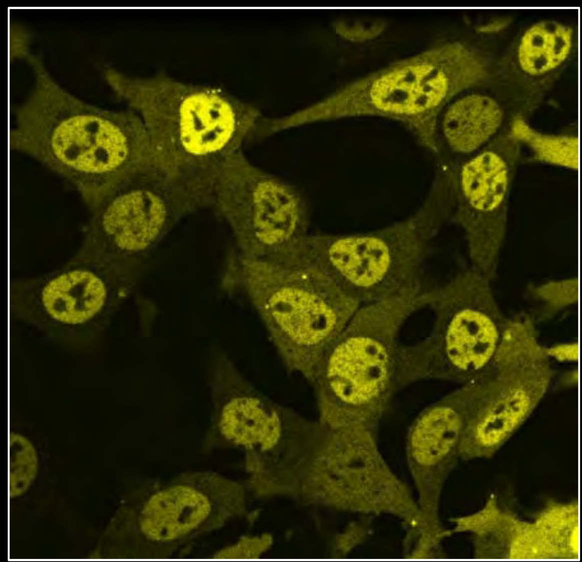


Nuclear translocation of STAT3-YFP





0 min

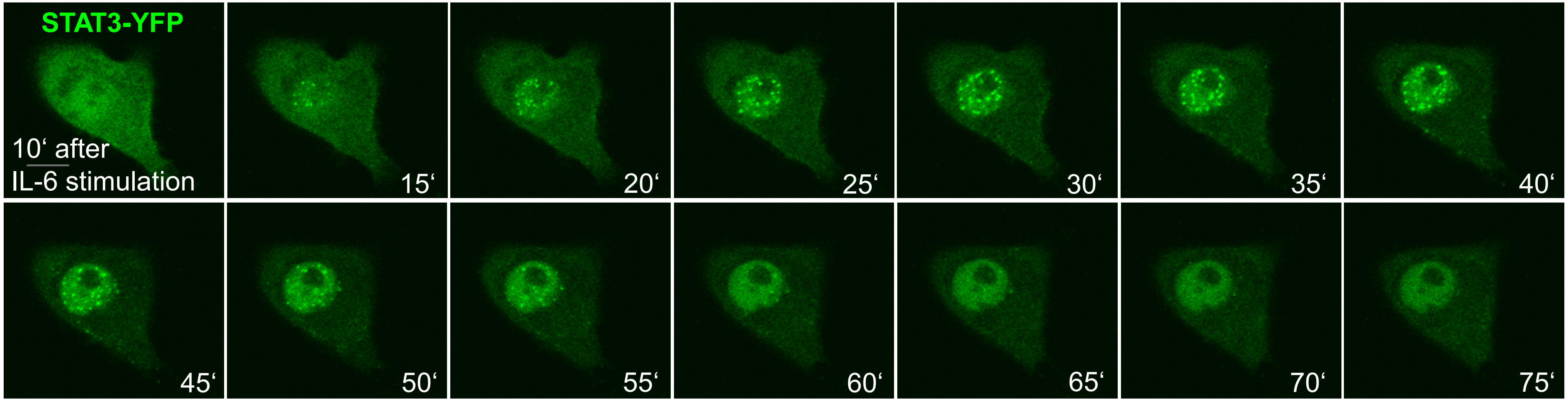


60 min

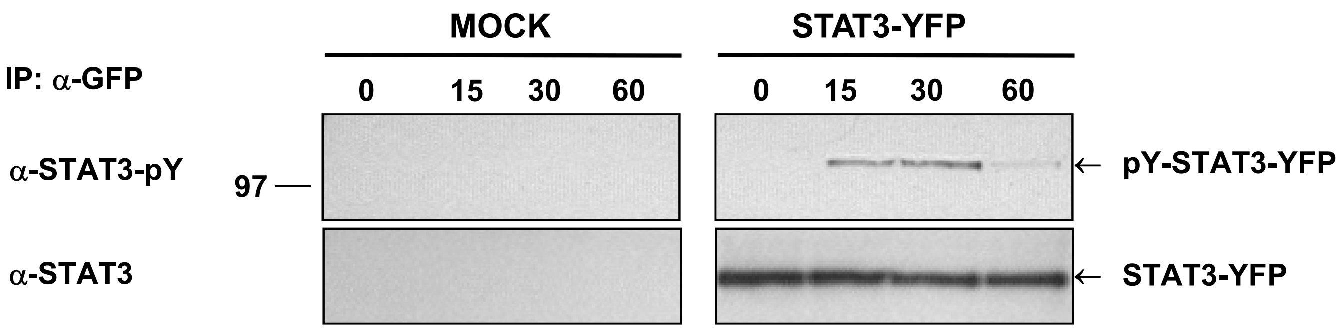
Lap time 60 mins HepG2 cells

Activated STAT3 in the nucleus transiently accumulates in dot-like structures

- **live-cell imaging: nuclear translocation of STAT3-YFP**



- **nuclear body formation correlates with tyrosine phosphorylation of STAT3**

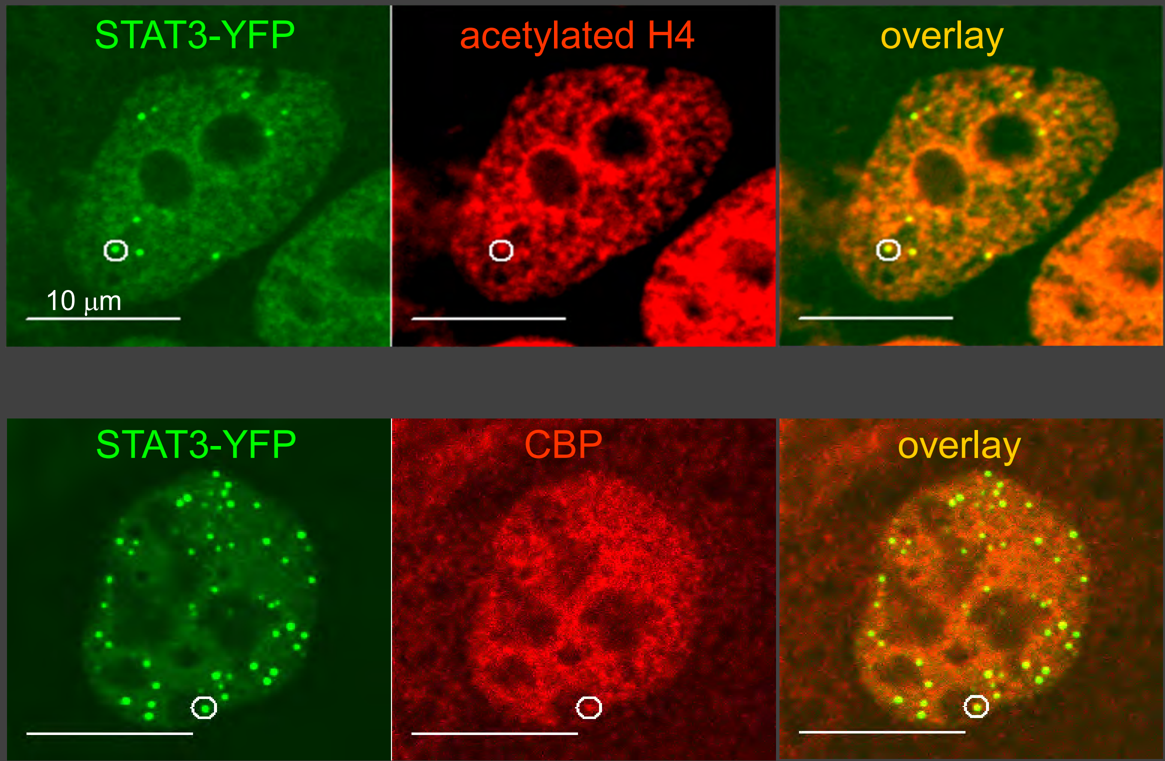


Herrmann et al. (2004) J Cell Sci 117, 339-349 51 citations (07/2022)

HepG2 cells

STAT3 nuclear bodies are associated with transcriptionally active chromatin

- immunofluorescence:



HepG2

CBP, CREB binding protein marker for transcriptional active chromatin

no co-localization with PML-bodies

Herrmann et al. (2004) J Cell Sci 117, 339-349

STAT3 is enriched in nuclear bodies

Andreas Herrmann[1], Ulrike Sommer[1], Albert L. Pranada[1], Bernd Giese[1], Andrea Küster[1], Serge Haan[1], W. Becker[2], Peter C. Heinrich[1] and Gerhard Müller-Newen[1,*]

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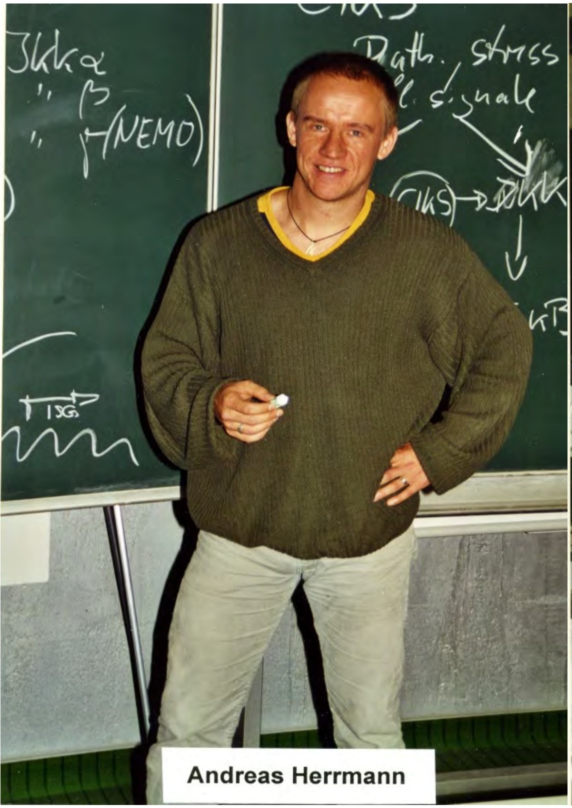
doi:10.1242/jcs.00833



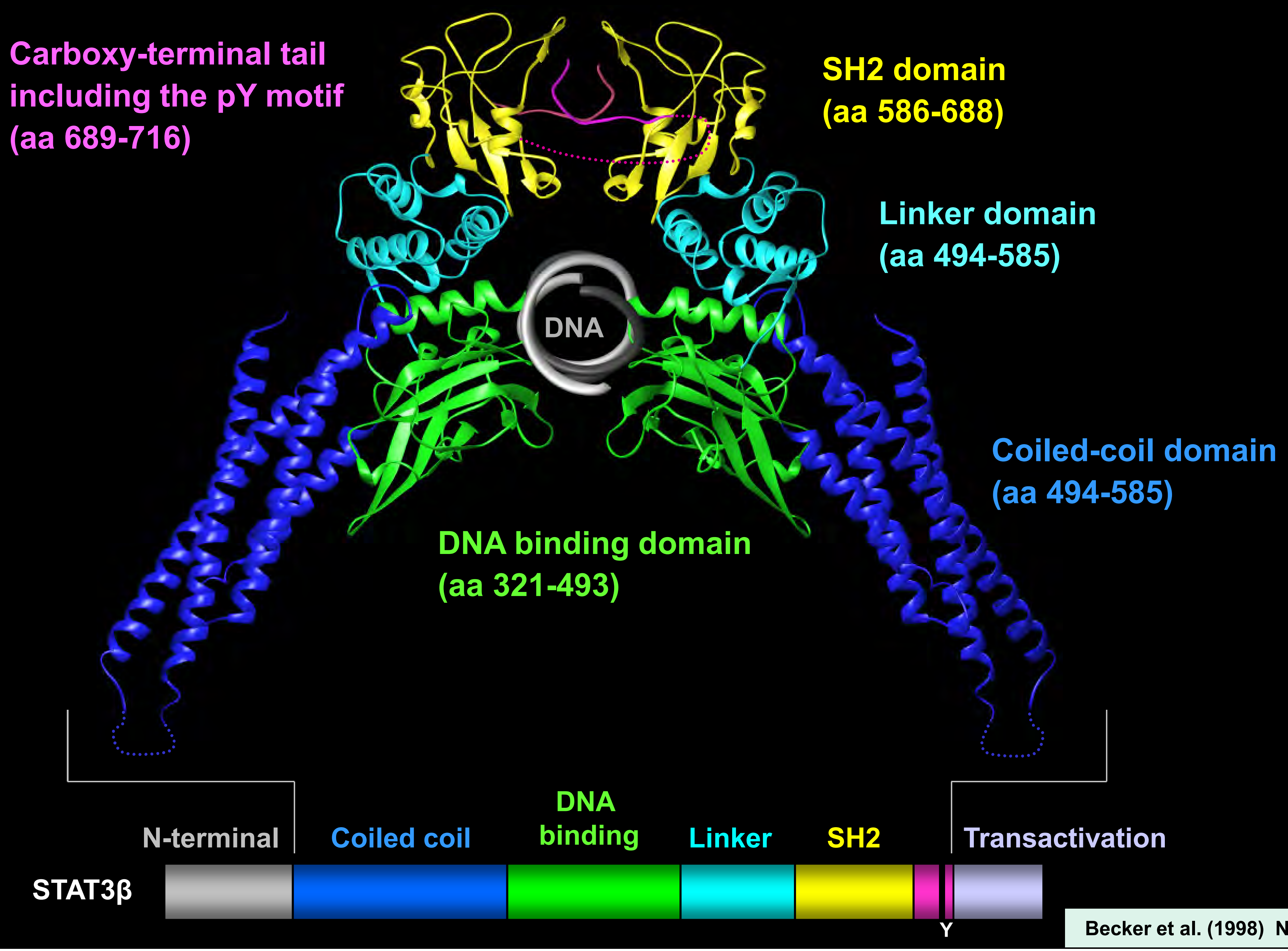
Andreas Herrmann

Ulrike Sommer

Albert Pranada

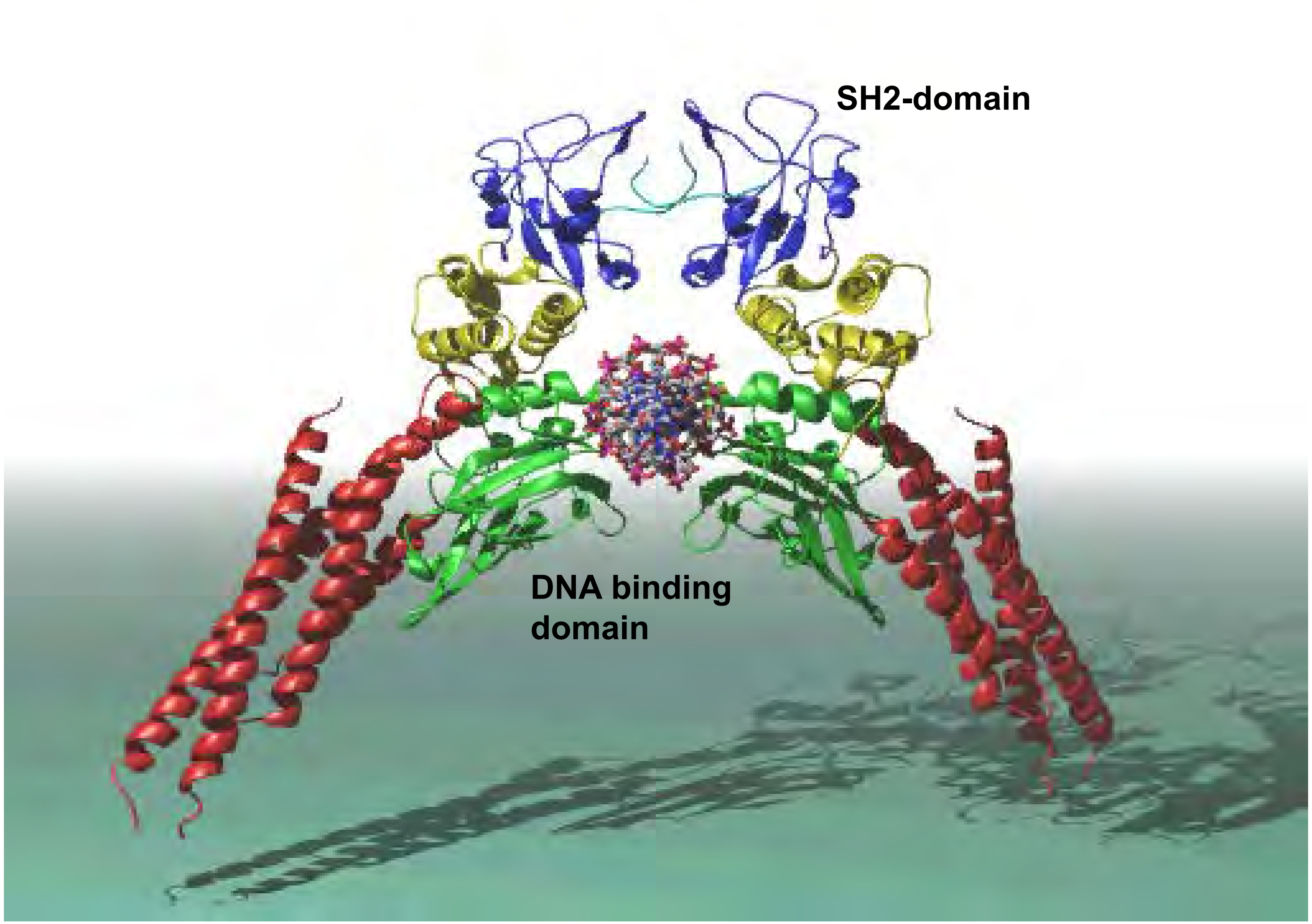
Bernd Giese

STAT3β homodimer bound to DNA



Becker et al. (1998) Nature 394, 145-151

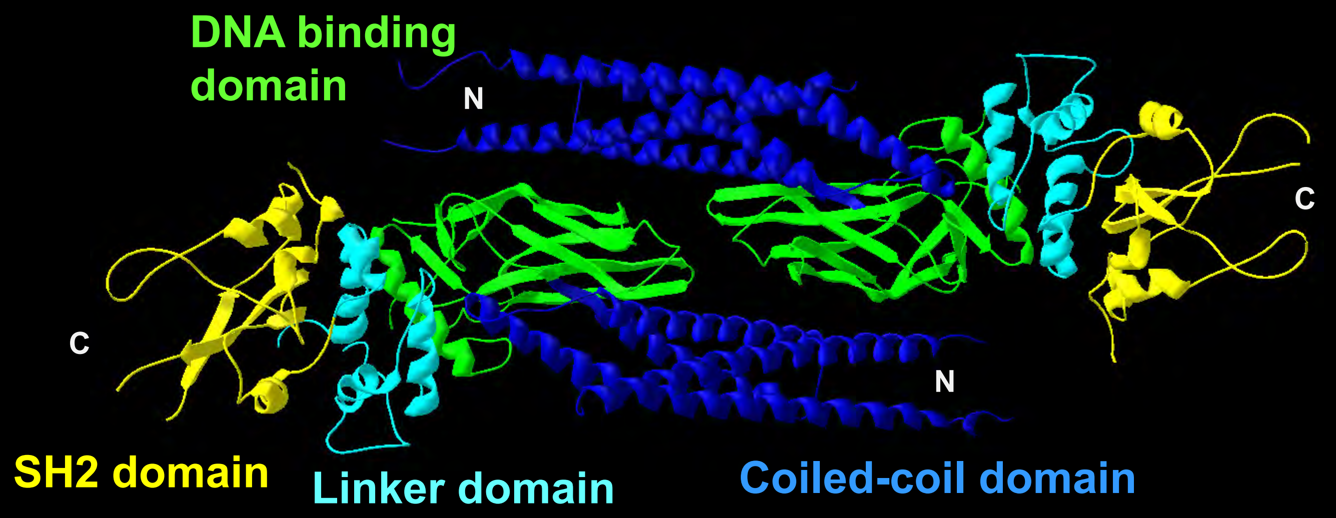
STAT3-β homodimer bound to DNA



Becker et al. (1998) Nature 394, 145-151

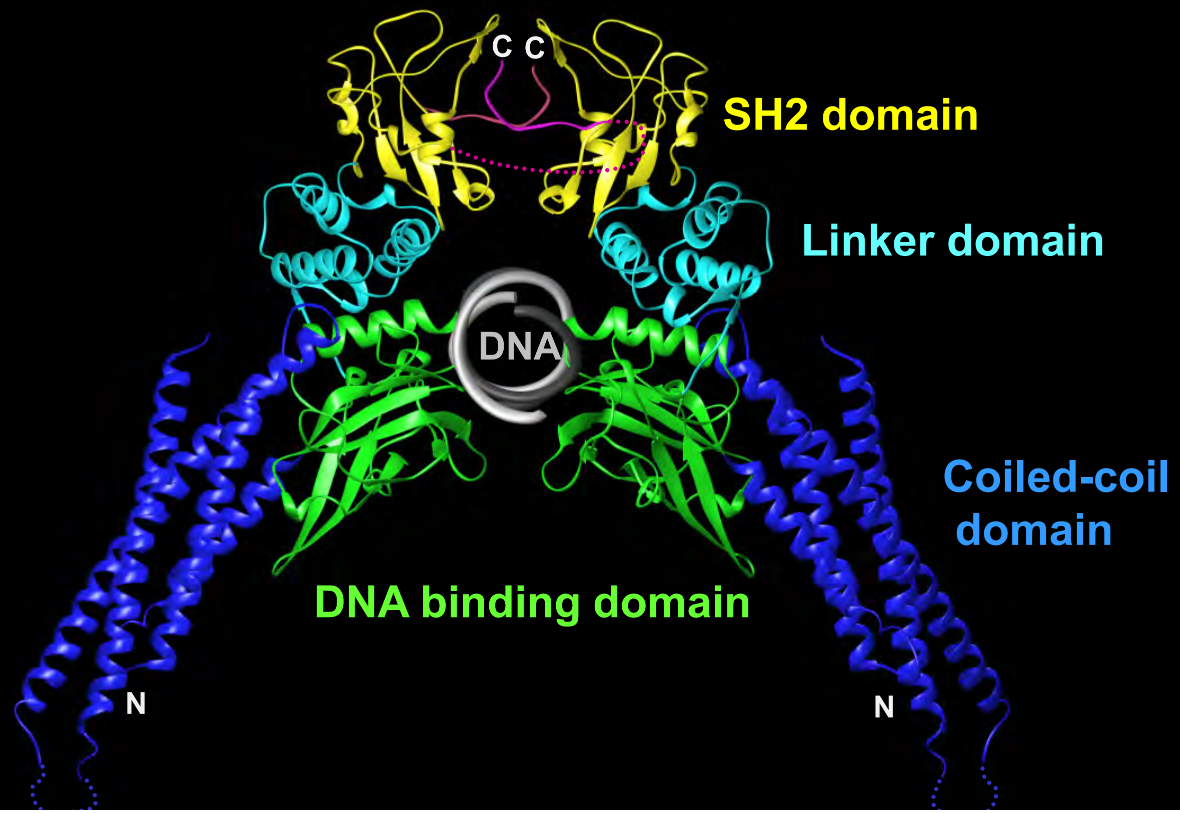
STAT factors undergo extensive conformational changes upon activation

non-phosphorylated STAT-dimer
(anti-parallel orientation)



STAT1: *Mao et al. 2005, Mol Cell 17, 761*
STAT5A: *Becker et al. 2005, J Biol Chem 280, 40782*

phosphorylated STAT-dimer
bound to DNA
(parallel orientation)

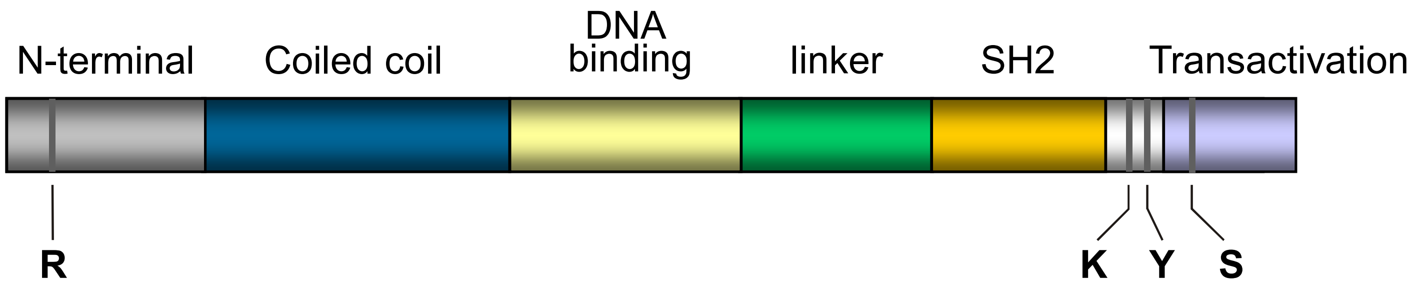


STAT1: *Chen et al. 1998, Cell 93, 827*
STAT3: *Becker et al. 1998, Nature 394, 145*

STAT family (Signal Transducer and Activator of Transcription)

Seven members: STAT1, 2, 3, 4, 5A, 5B, 6 plus splice- and proteolysis variants

Domain structure of STAT3



Post-translational modifications of STAT3

- Tyrosine(Y)-705 phosphorylation > dimerization
- Serine(S)-727 phosphorylation > transactivation
- Lysine(K)-685 acetylation > dimerization
- Arginine(R)-31 methylation > release of PIAS > not confirmed
- proteolytic processing > not confirmed
- proteasomal degradation > not confirmed
- tetramerization of the N-terminus

Constitutive activation of STAT3 in many human cancer cells

Tumour type	Activated STAT
Blood tumours	
Multiple myeloma	STAT1, STAT3
Leukaemias:	
HTLV-I-dependent	STAT3, STAT5
Erythroleukaemia	STAT1, STAT5
Acute myelogenous leukaemia (AML)	STAT1, STAT3, STAT5
Chronic myelogenous leukaemia (CML)	STAT5
Large granular lymphocyte leukaemia (LGL)	STAT3
Lymphomas:	
EBV-related/Burkitt's	STAT3
Mycosis fungoides	STAT3
Cutaneous T-cell lymphoma	STAT3
Non-Hodgkins lymphoma (NHL)	STAT3
Anaplastic large-cell lymphoma (ALCL)	STAT3
Solid tumours	
Breast cancer	STAT1, STAT3, STAT5
Head and neck cancer	STAT1, STAT3, STAT5
Melanoma	STAT3
Ovarian cancer	STAT3
Lung cancer	STAT3
Pancreatic cancer	STAT3
Prostate cancer	STAT3

Yu & Jove (2004) ***The STATs of cancer - New molecular targets come of age*** **Nature Reviews Cancer 4, 97-105**

2001, Blood

STAT3 is constitutively activated in Hodgkin cell lines

Dieter Kube, Udo Holtick, Martina Vockerodt, Tahamtan Ahmadi, Birgit Haier, Iris Behrmann, Peter C. Heinrich, Volker Diehl, and Hans Tesch

Hodgkin disease (HD) represents a malignant lymphoma in which the putative malignant Hodgkin and Reed-Sternberg cells are rare and surrounded by abundant reactive nonmalignant cells. It has been suggested that cytokines such as interleukin-6 (IL-6) are involved in the pathogenesis of the disease. The expression of the IL-6 receptor (IL-6R) complex and its link to the activation of signal transducers and activators of transcription (STAT) molecules in HD cell lines was investigated. Gel retardation and Western blot analyses revealed a high level of constitutively activated STAT3 in 5 of 7 HD cell lines, which could not be detected in Burkitt lymphoma cell lines. Different levels of IL-6R protein were measured in various HD cell lines: L428 and Dev cells were characterized by very low levels of gp80 and gp130, on KMH2 cells only gp130 but no gp80 was detected, whereas L540, L591, HDLM2, and L1236 were positive for both gp80 and gp130, suggesting a possible autocrine stimulation of STAT3. However, a further increase in STAT3 activation on IL-6 or IL-6/soluble IL-6R stimulation was not observed. Neutralizing monoclonal antibodies against IL-6, gp80, gp130, or both receptor subunits did not affect the proliferation or the constitutive activation of STAT molecules in HD cell lines. However, the tyrosine kinase inhibitor AG490 blocked the constitutive activation of STAT3 and inhibited spontaneous growth of HD tumor cells. The evidence suggests abnormal STAT signaling and growth regulation in Hodgkin cell lines. (Blood. 2001;98:762-770)

In tumour cells constitutive STAT3 activation can be accomplished through different signaling mechanisms

- autocrine/paracrine expression of ligands
- overexpression of receptors
- constitutive activation of receptor tyrosine kinases
- constitutive activation of tyrosine kinases
- disturbed shut-down mechanisms
- mutations in Janus kinases and STAT3

Alternative mechanisms of constitutive STAT3 activation have been described.