

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

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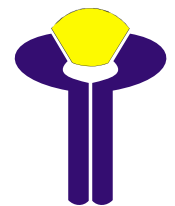
URL: <http://www.biochemie.uni-freiburg.de/ag/heinrich>



1969-1988 and 2008-present



1988-2008

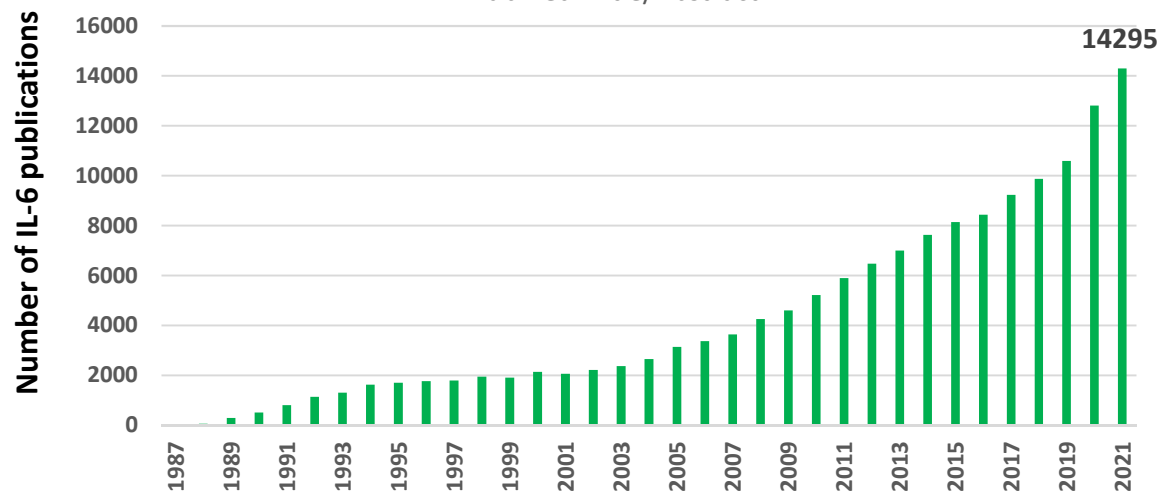


SFB 542

IL-6 and STAT3 are still important molecules

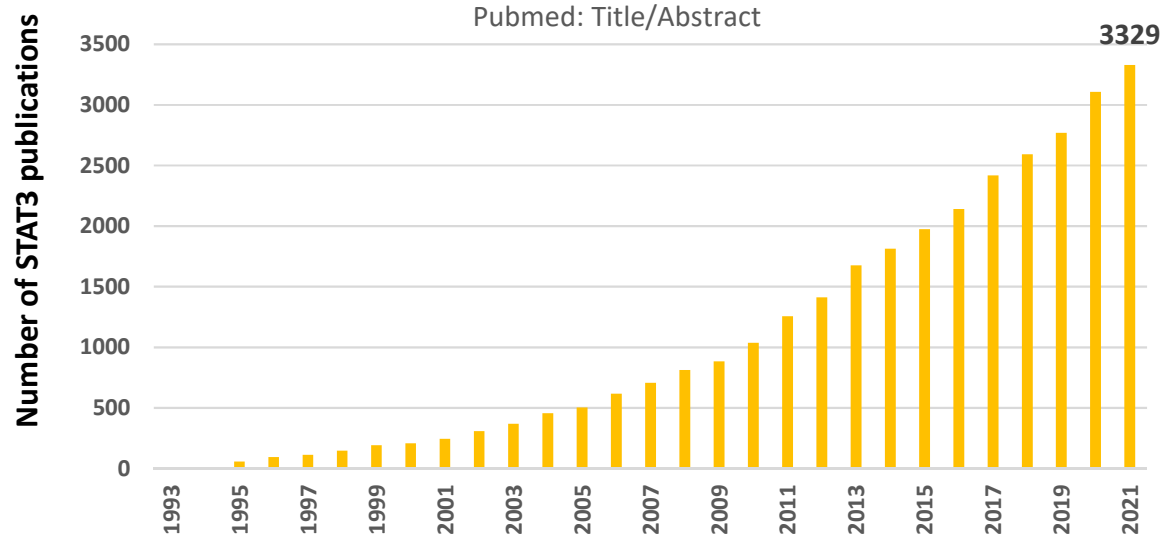
IL-6

Pubmed: Title/Abstract



STAT3

Pubmed: Title/Abstract



PART 1

Molecular Mechanism of IL-6 Signaling

Outline: Interleukin-6 signal transduction and its regulation

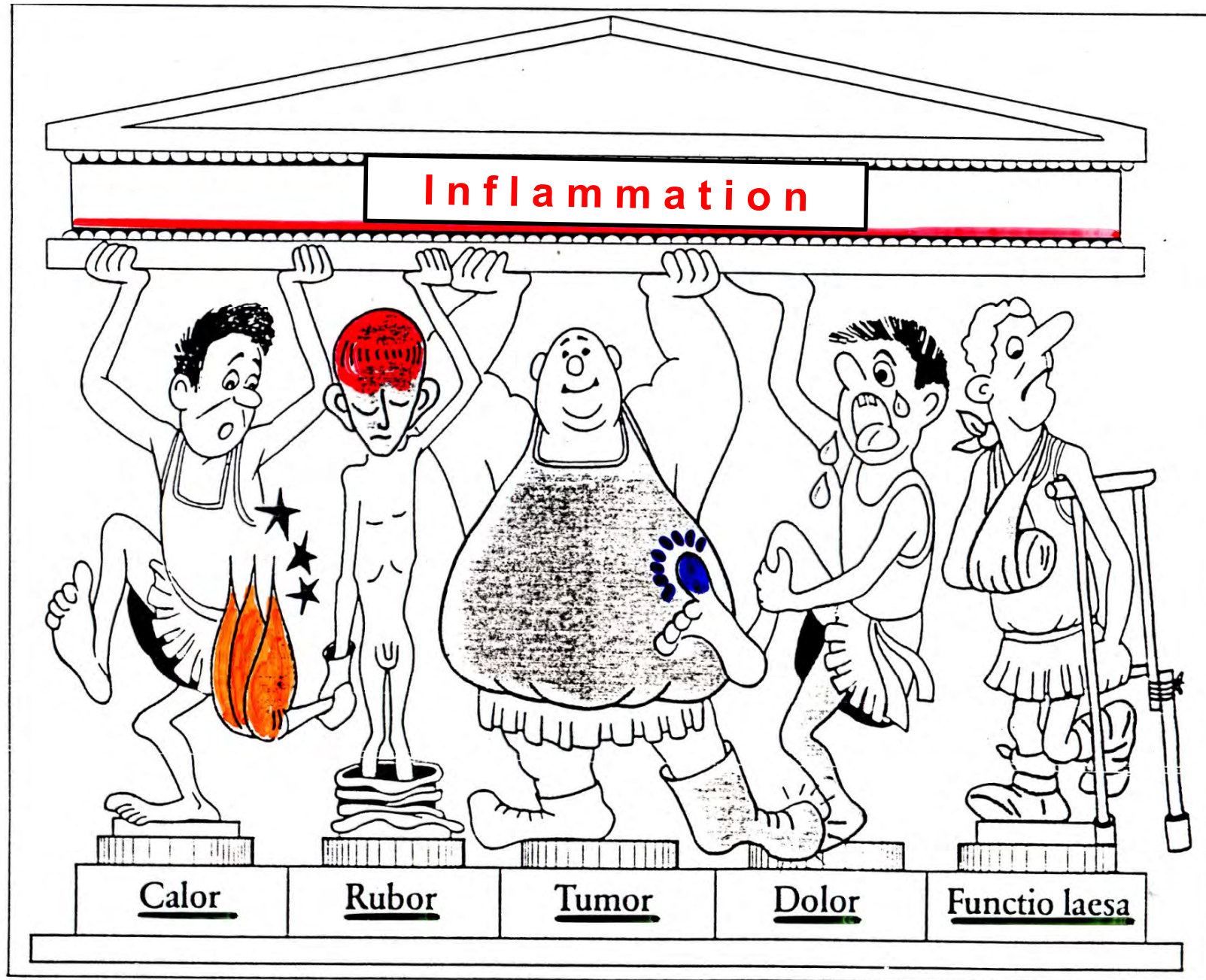
Part 1: Molecular mechanisms of IL-6 signal transduction

- **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

The acute phase response

Acute exsudative inflammatory reaction



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

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

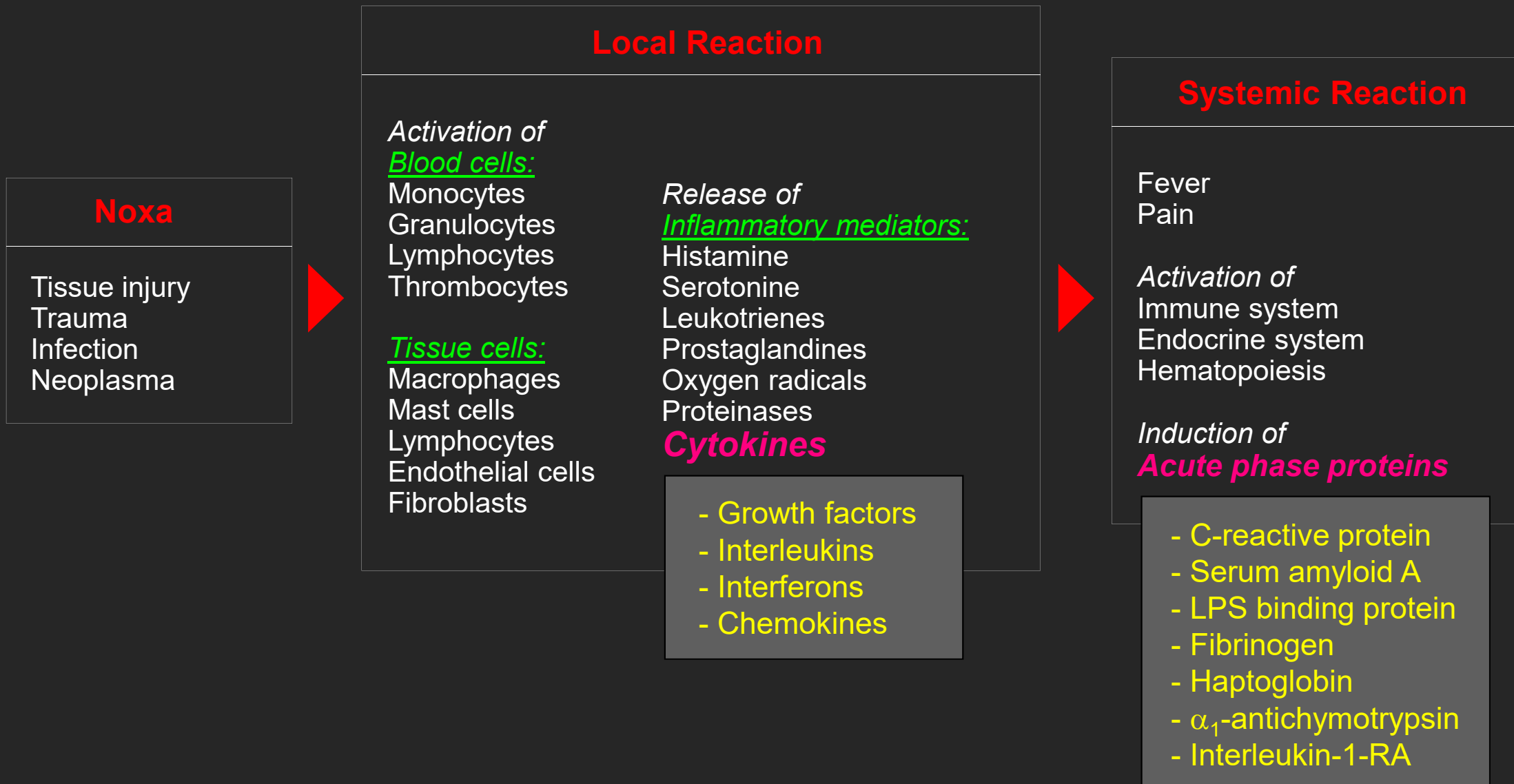
Inflammation brings healing and disease at the same time:
240 mio (3%) people suffer, 8 billion people profit from it.

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



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

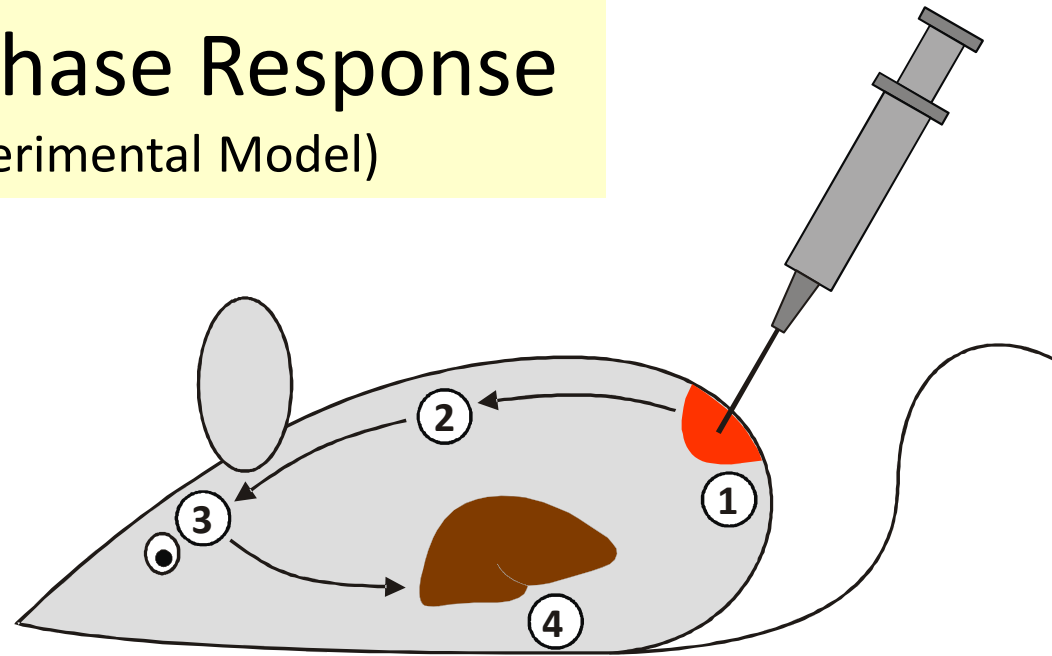
- α_2 -macroglobulin
- LPS binding protein
- α_1 -acid glycoprotein
- cysteine proteinase inhibitor
- serine proteinase inhibitor 2.3
- tissue inhibitor of metalloproteinases-1

Negative acute phase proteins

- albumin
- transthyretin
- transferrin
- fetuin / α_2 HS-glycoprotein
- apolipoprotein A-I (HDL)
- retinol binding protein

Acute Phase Response

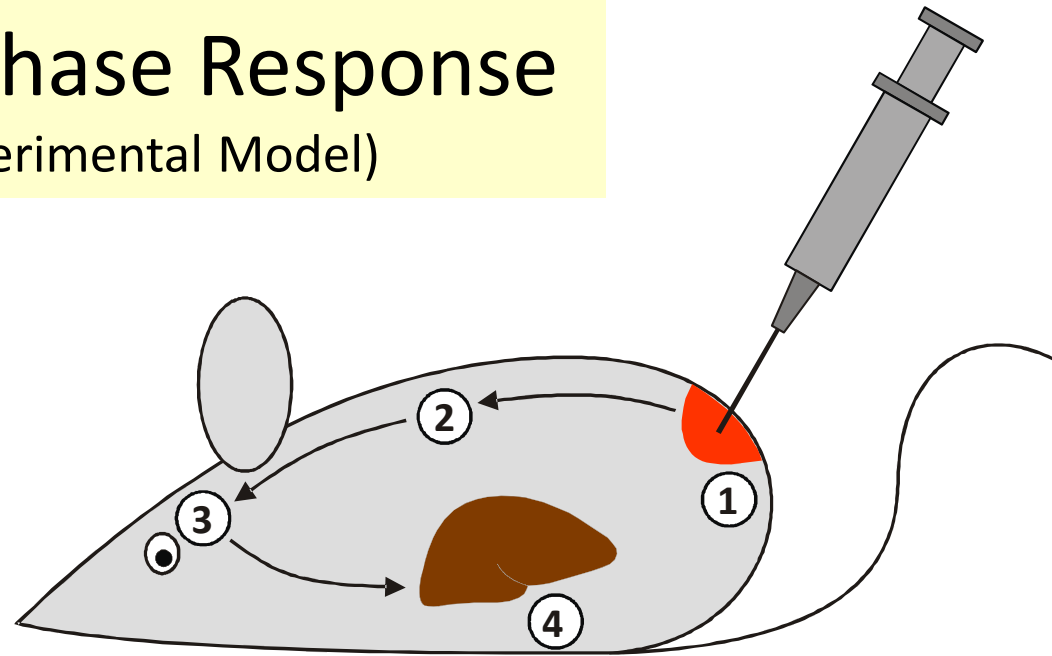
(Experimental Model)



- (1) Local response**
- (2) Systemic distribution of inflammatory mediators**
- (3) Activation of CNS and endocrine hormone system**
- (4) Hepatic acute phase response**

Acute Phase Response

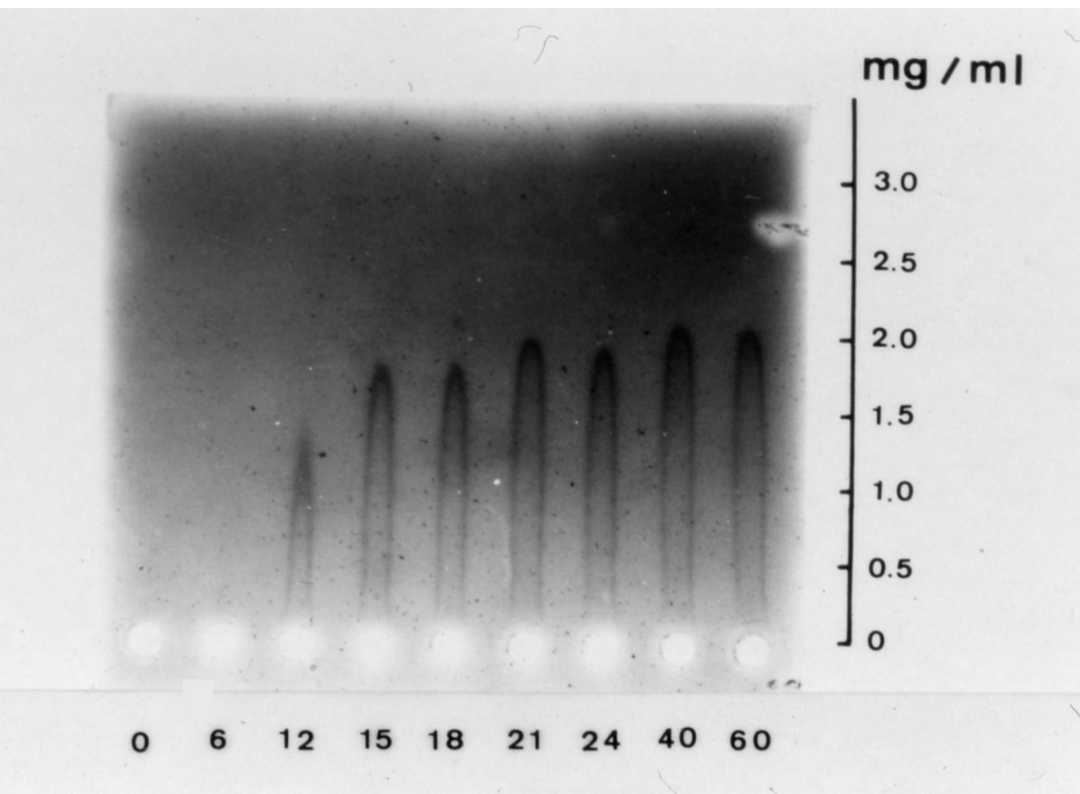
(Experimental Model)



- (1) Local response **turpentine injection**
- (2) Systemic distribution of inflammatory mediators **IL-1, TNF α , IL-6, CKs**
- (3) Activation of CNS and endocrine hormone system **ACTH, glucocorticoids**
- (4) Hepatic acute phase response **acute phase proteins**

Rat model (*in vivo*)

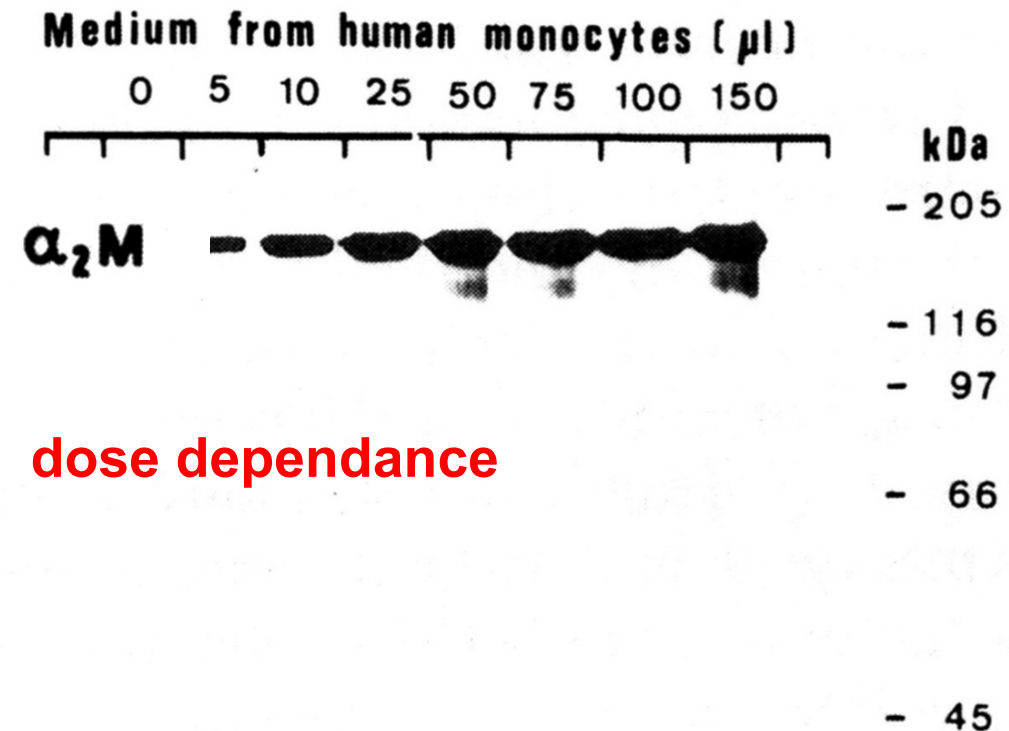
serum α_2 M



hours after turpentine

α_2 M, α_2 -macroglobulin

Rat hepatocytes



dose dependance

Conditioned medium from LPS-stimulated human monocytes added to rat hepatocytes
→ α_2 M – protein biosynthesis was measured

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

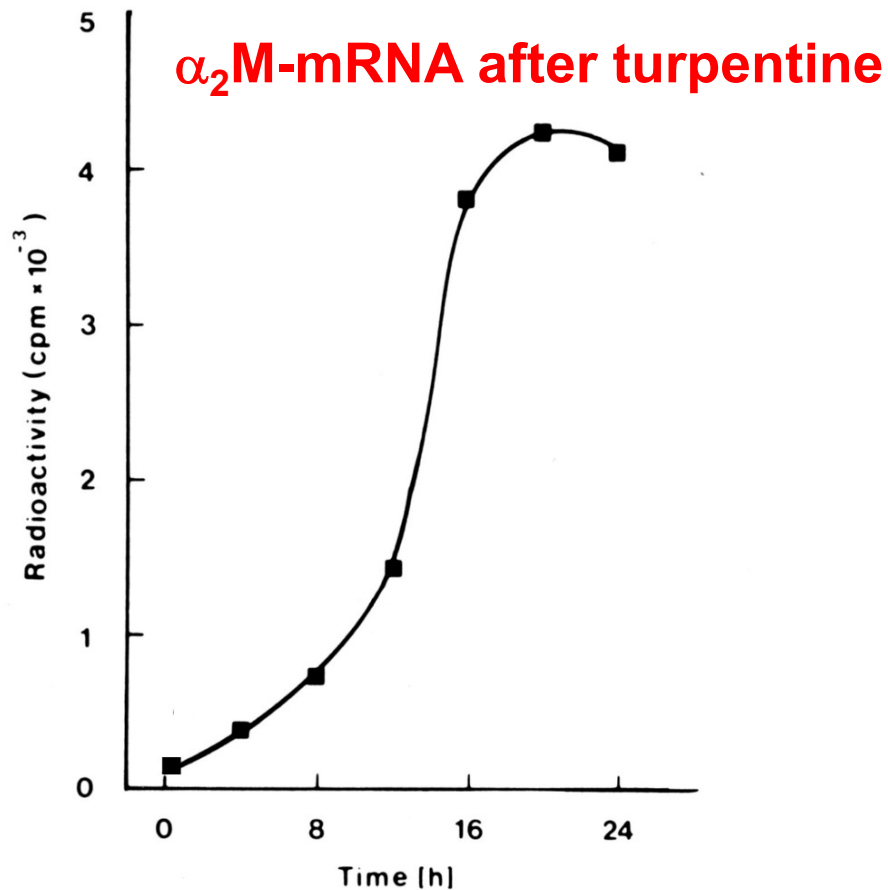
97 citations
(07/2022)

in vivo in rat liver

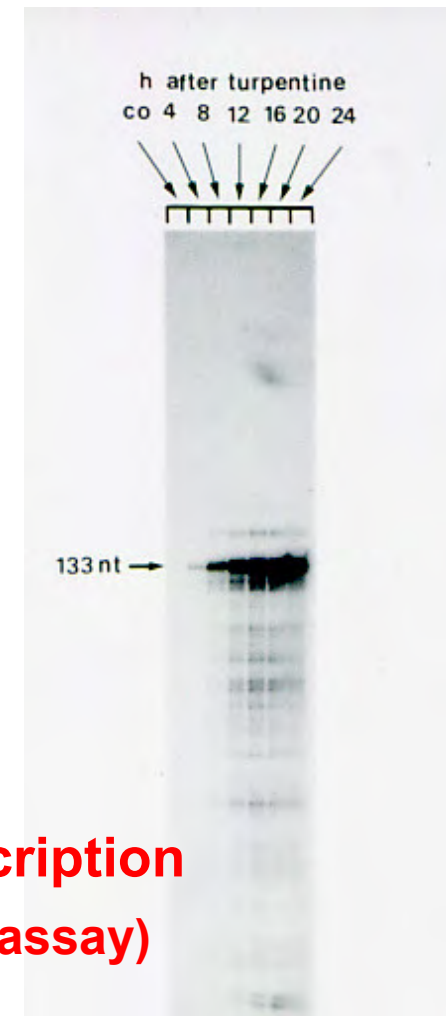
(Received for publication, December 4, 1984)

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

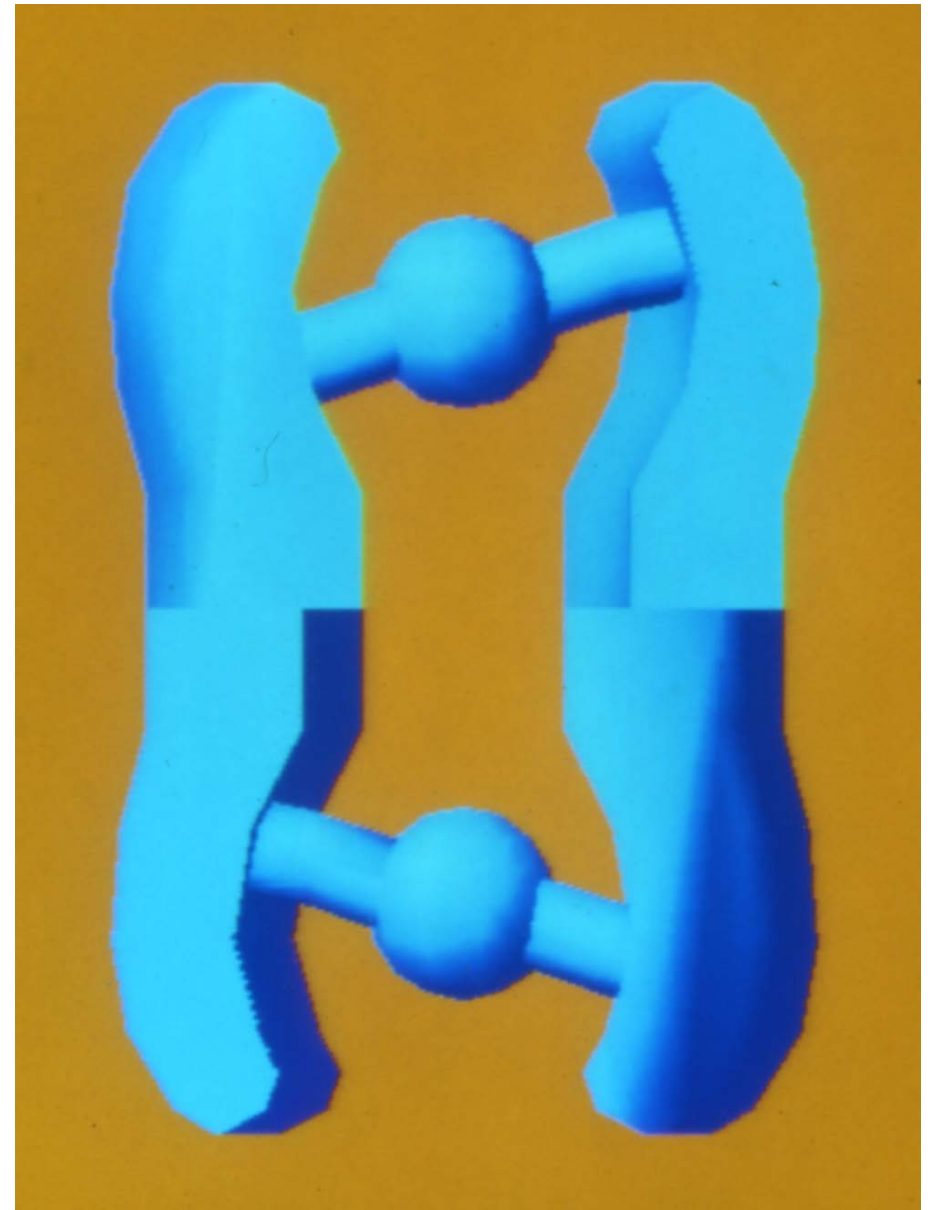
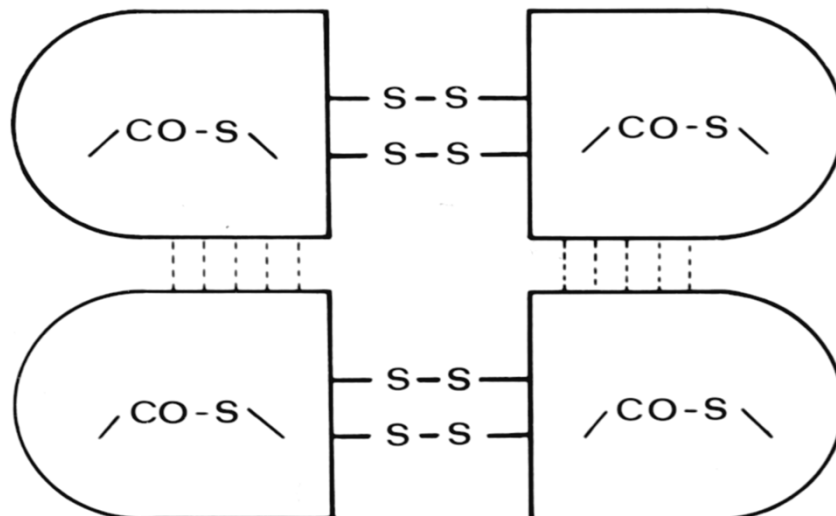


α_2 M-gene transcription
(RNA protection assay)



Properties of α_2 -Macroglobulin

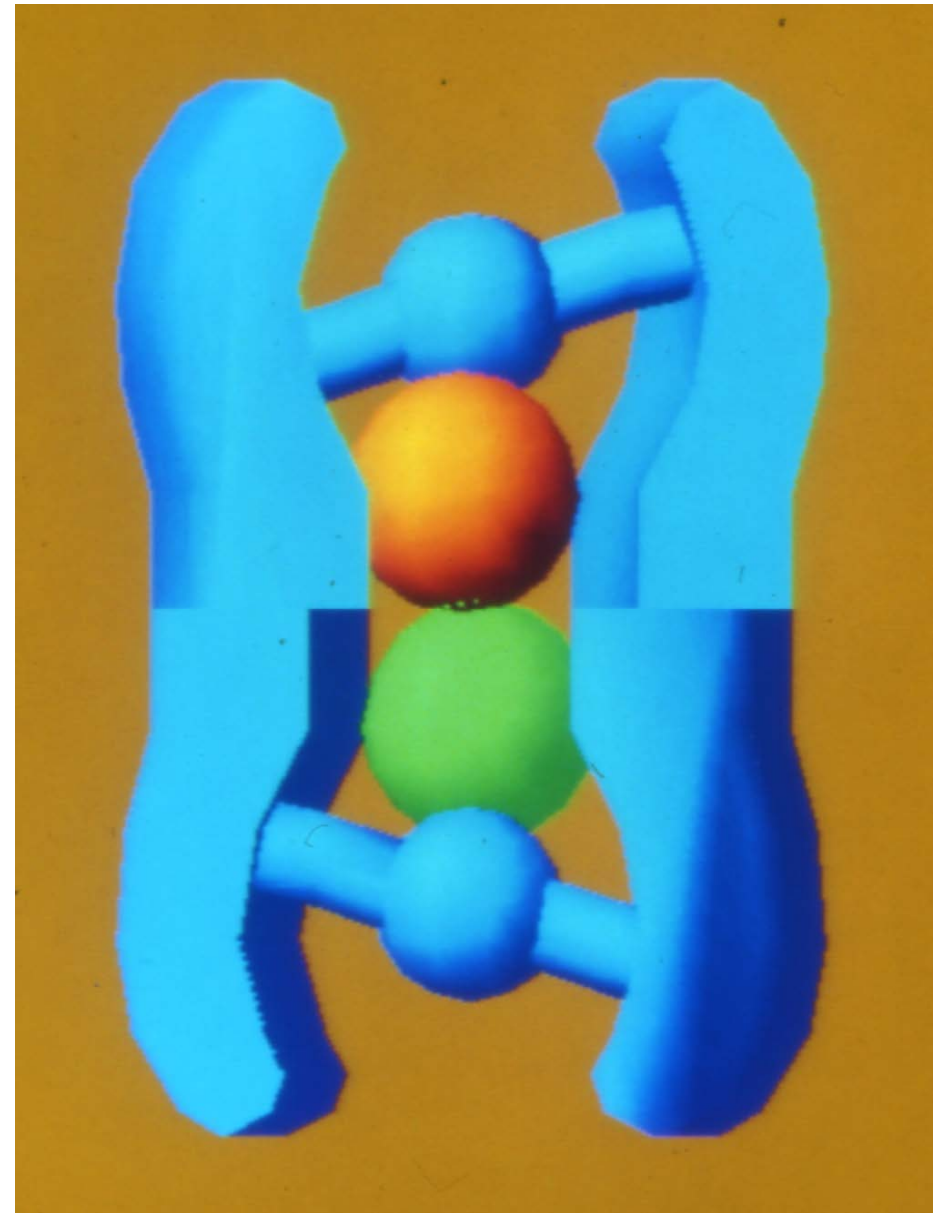
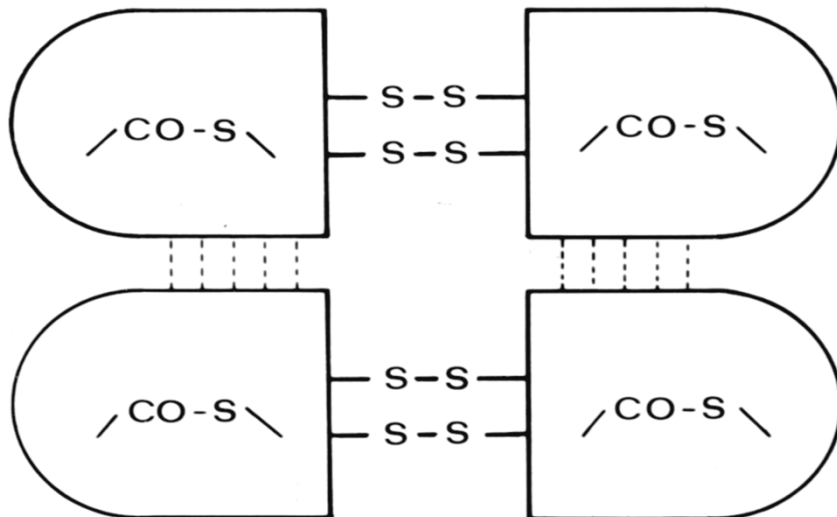
- dimer of dimers
 - molecular mass: 728 kDa
 - 4 identical subunits of 182 kDa
 - 1 thio-ester bond per subunit
 - 4 interchain S-S bridges
 - about 15% carbohydrate
 - 4-8 moles Zn^{++} per mol
 - serine -
SH -
metallo -
acid -
- proteases are inhibited
by cage formation



α_2 M model in 1985

Properties of α_2 -Macroglobulin

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by cage formation



α_2 M model in 1985

During our efforts with the purification of HSF from CM of LPS-stimulated monocytes we excluded IL1- β , TNF α , TGF β and IFN α,β,γ and found in rat hepatocyte primary cultures that BSF-2/IL-6 induced the expression of acute phase proteins at the levels of mRNA and protein.

HSF, hepatocyte stimulatory factor

CM, conditioned medium

LPS, lipopolysaccharide

IL, interleukin

TNF, tumor necrosis factor

TGF, transforming growth factor

IFN, interferon

BSF, B-cell stimulatory factor

Outline: Interleukin-6 signal transduction and its regulation

Part 1: Molecular mechanisms of IL-6 signal transduction

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- Nuclear translocation of STAT3-YFP

Part 2: Regulation of IL-6 signal transduction

1987

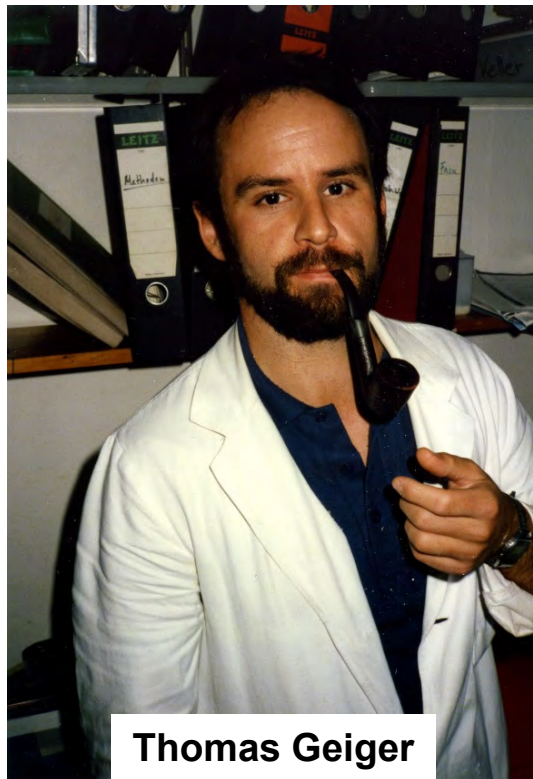
Recombinant human B cell stimulatory factor 2 (BSF-2/IFN- β 2) regulates β -fibrinogen and albumin mRNA levels in Fao-9 cells

332 citations (07/2022)

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

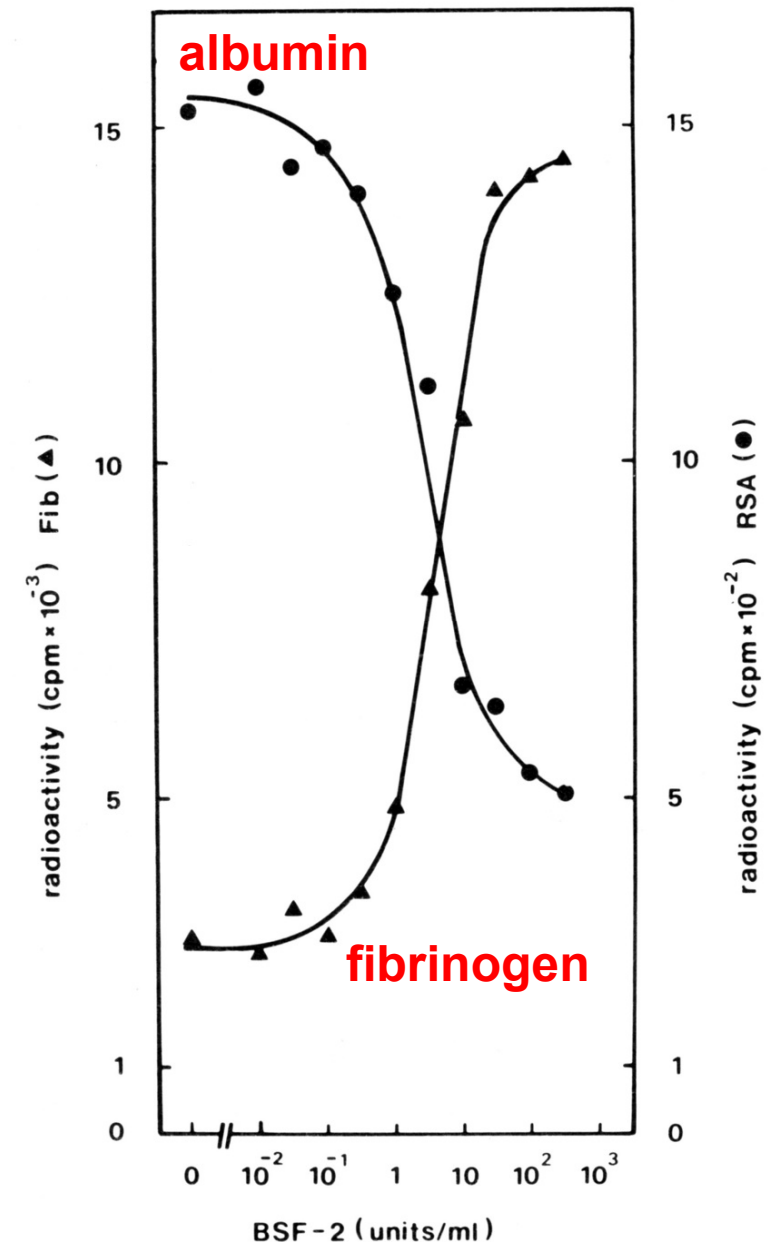
Biochemisches Institut, Universität Freiburg, Hermann-Herder-Str. 7, D-7800 Freiburg, FRG, *Institute for Molecular and Cellular Biology, Division of Immunology, Osaka University, 1-3, Yamada-oka, Suita, Osaka 565, Japan, ⁺Hinnak Northhoff, Blutspendezentrale des DRK, Oberer Eselsberg, D-7900 Ulm and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

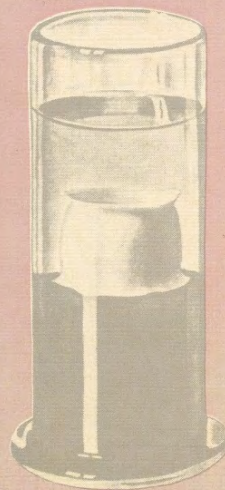
Editors

Pravinkumar B. Sehgal

Gerd Grieninger

Giovanna Tosato

Annals of the
New York
Academy of
Sciences



New York, Dec 1988

ANNALS OF THE NEW YORK ACADEMY OF SCIENCES

Volume 557

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

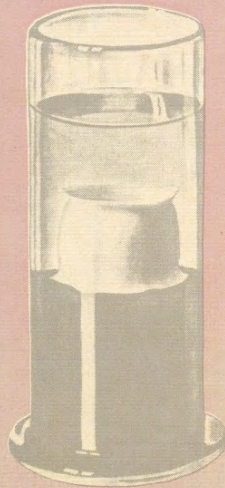
*Edited by Pravinkumar B. Sehgal, Gerd Grieninger,
and Giovanna Tosato*



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

Regulation
of
the
Acute
Phase
and
Immune
Responses:

INTERLEUKIN-6



HSF $\equiv$ BSF-2 $\equiv$ 26kD protein $\equiv$ HPGF $\equiv$ IFN β 2 $\equiv$ IL-6

Toshio Hirano,
Tadamitsu Kishimoto

Walter Fiers

Annals of the
New York
Academy of
Sciences

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*

Leo Sachs

Michel Revel



*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 β 2	IFN β 2
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)

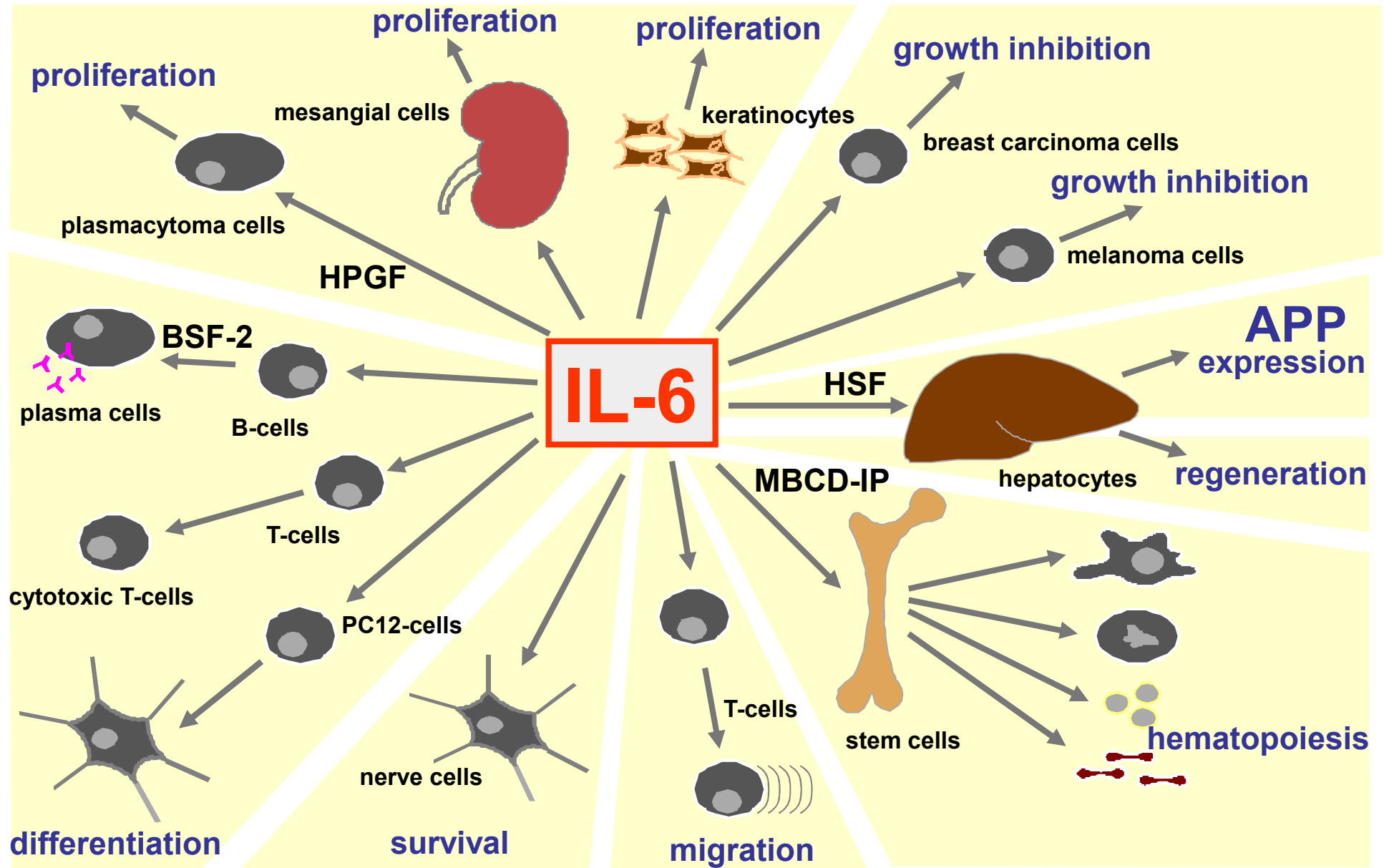
TAF (T-cell activating factor)

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**

Outline: Interleukin-6 signal transduction and its regulation

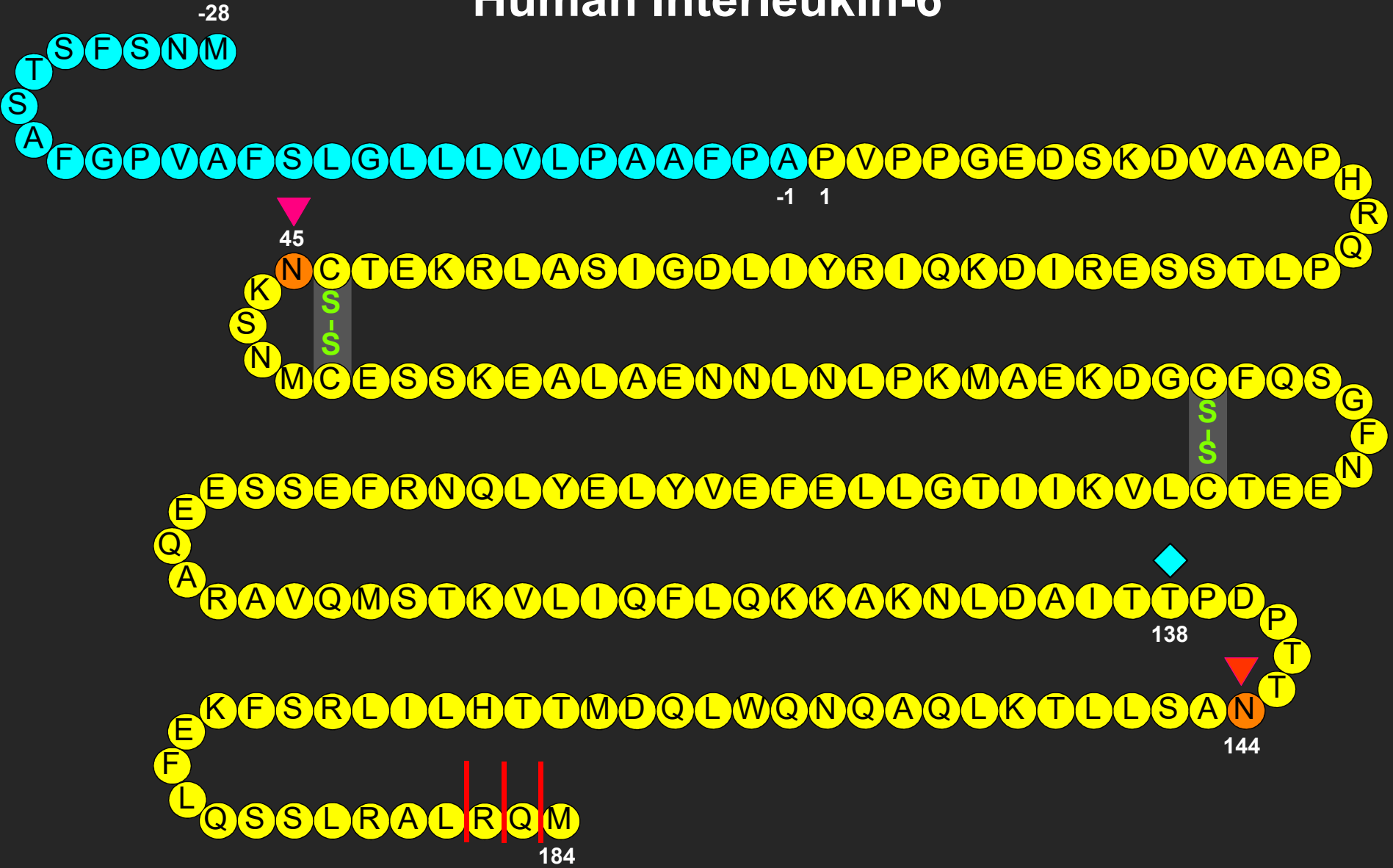
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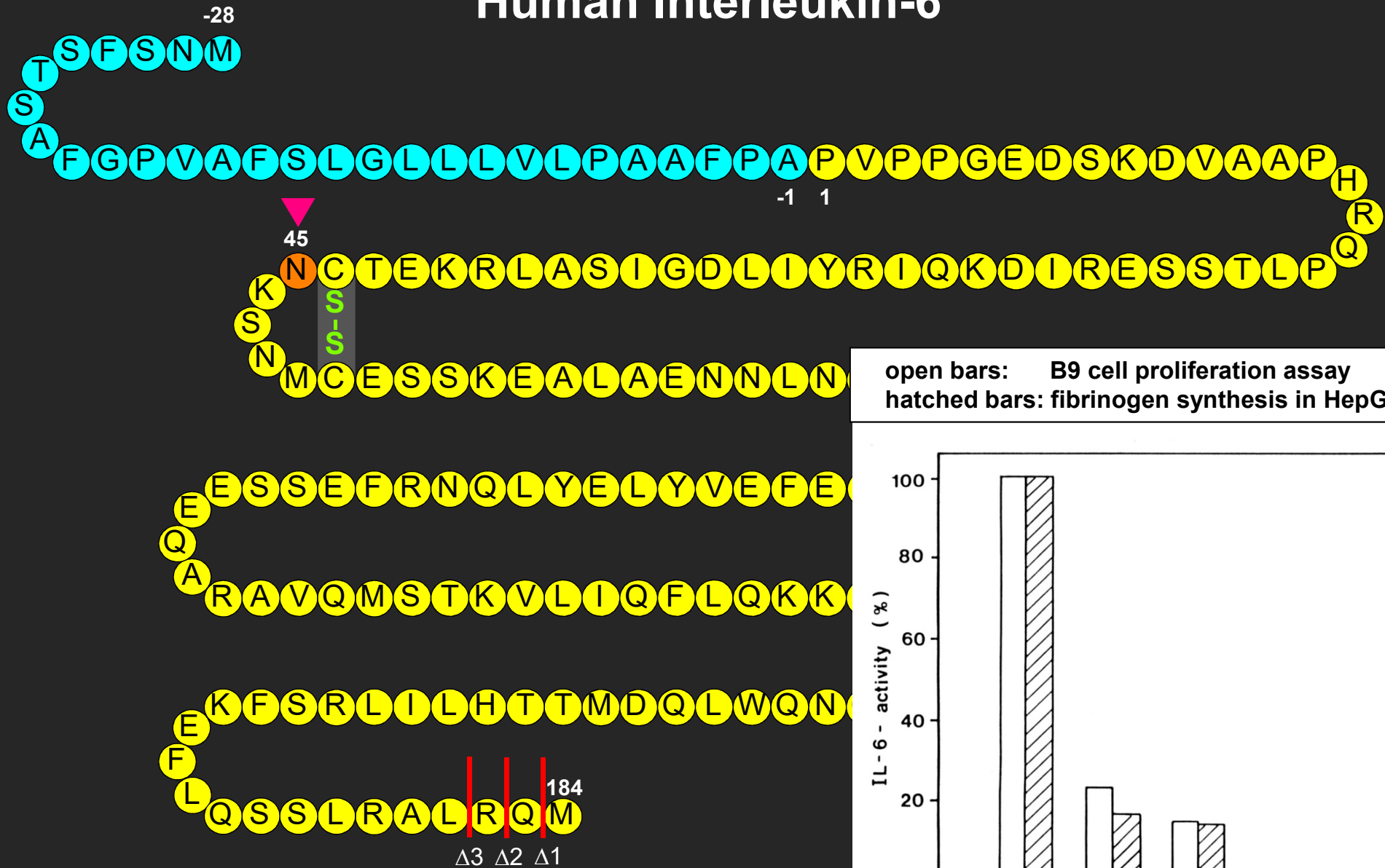
Part 2: Regulation of IL-6 signal transduction

Structure / function studies on IL-6

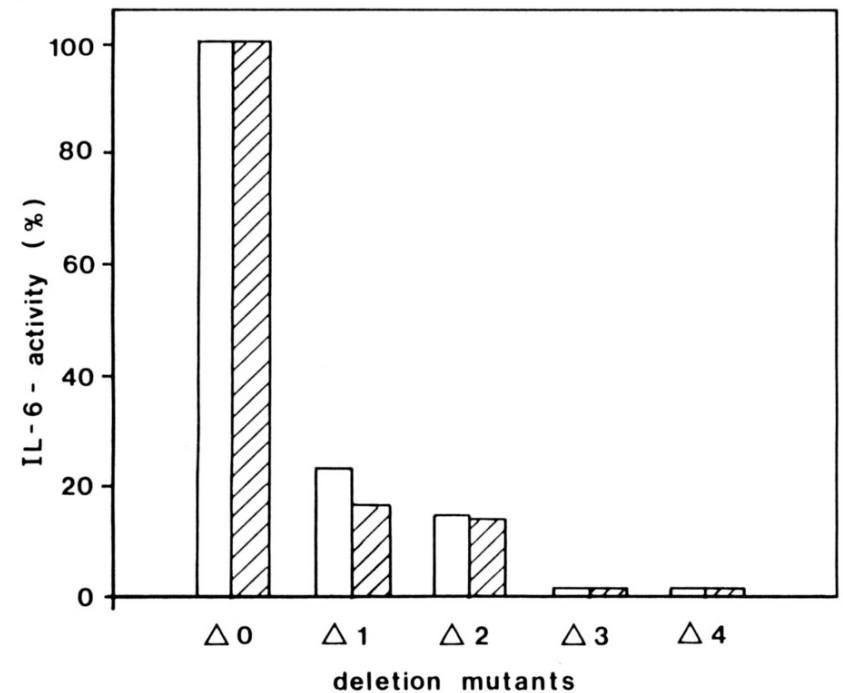
Human Interleukin-6



Human Interleukin-6



open bars: B9 cell proliferation assay
hatched bars: fibrinogen synthesis in HepG2 cells



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

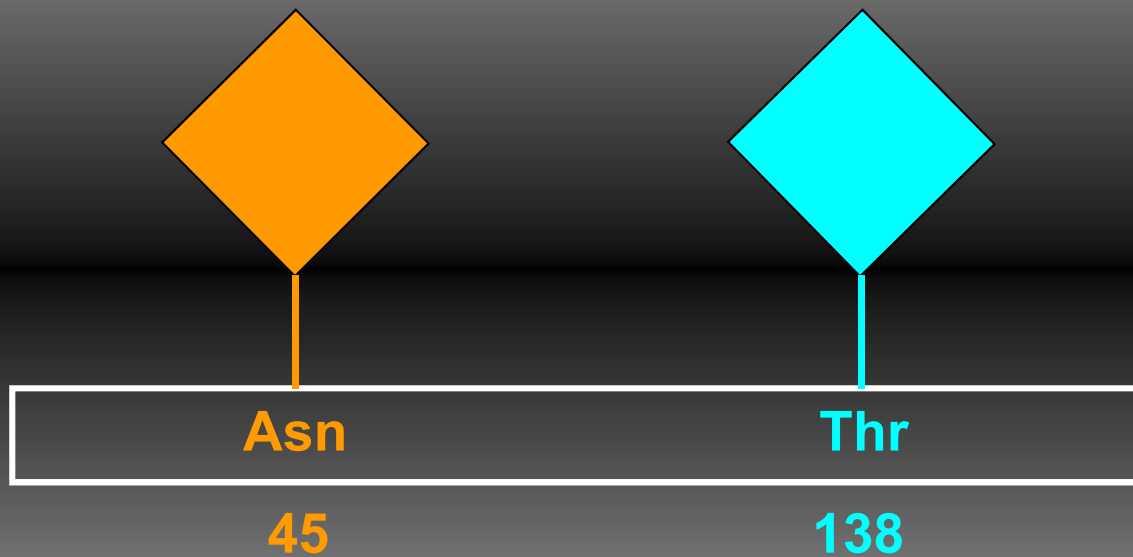
68 citations

(07/2022)

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



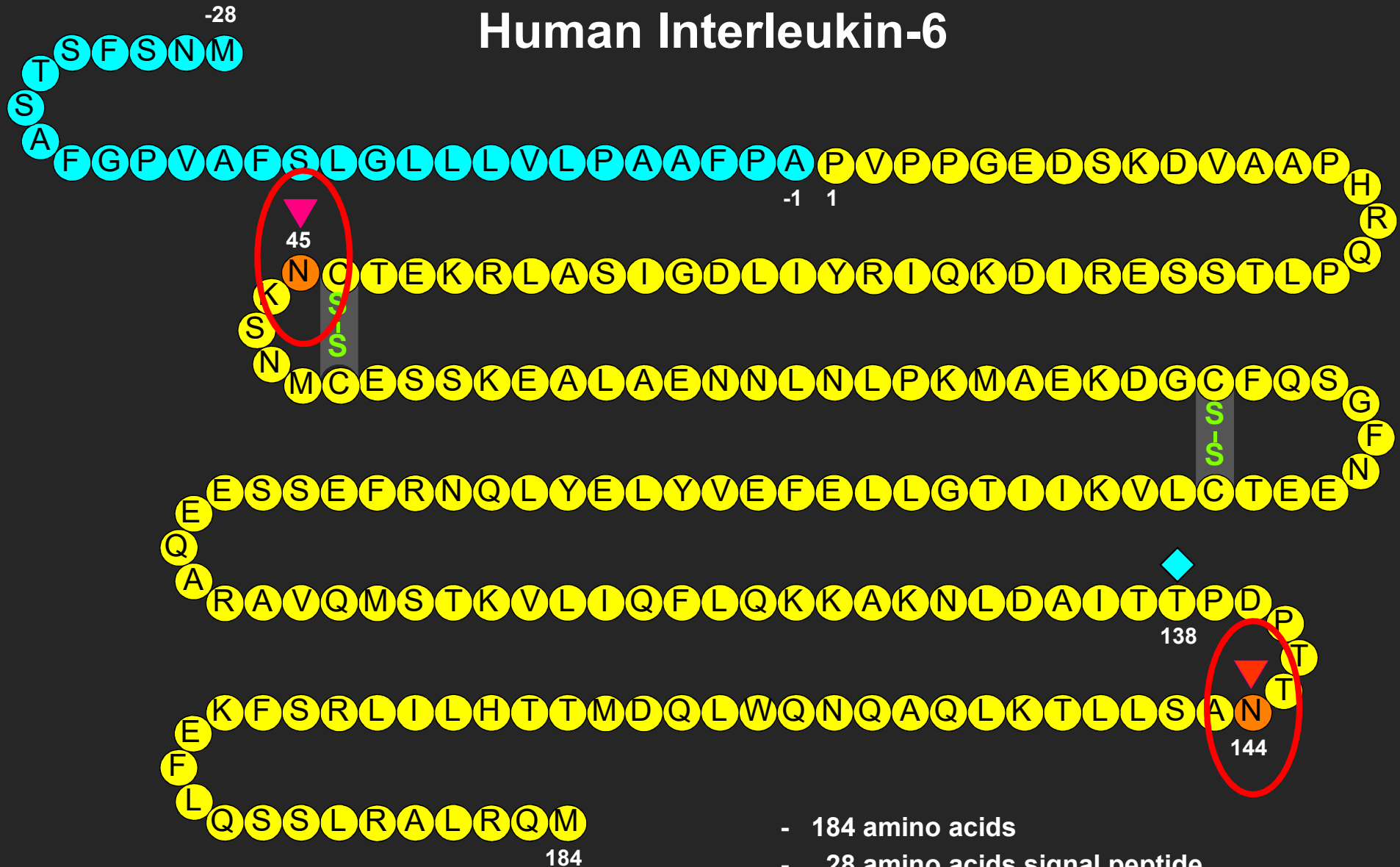


Heinrich, P.C., Dufhues, G., Flohe, S., Horn, F., Krause, E., Krüttgen, A., Legres, L., Lenz, D., Lütticken, C., Schooltink, H., Stoyan, T., Conradt, H.S., Rose-John, S. (1991) *Interleukin-6, its hepatic receptor and the acute phase response of the liver*
42nd Mosbacher Colloquium on "Molecular Aspects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



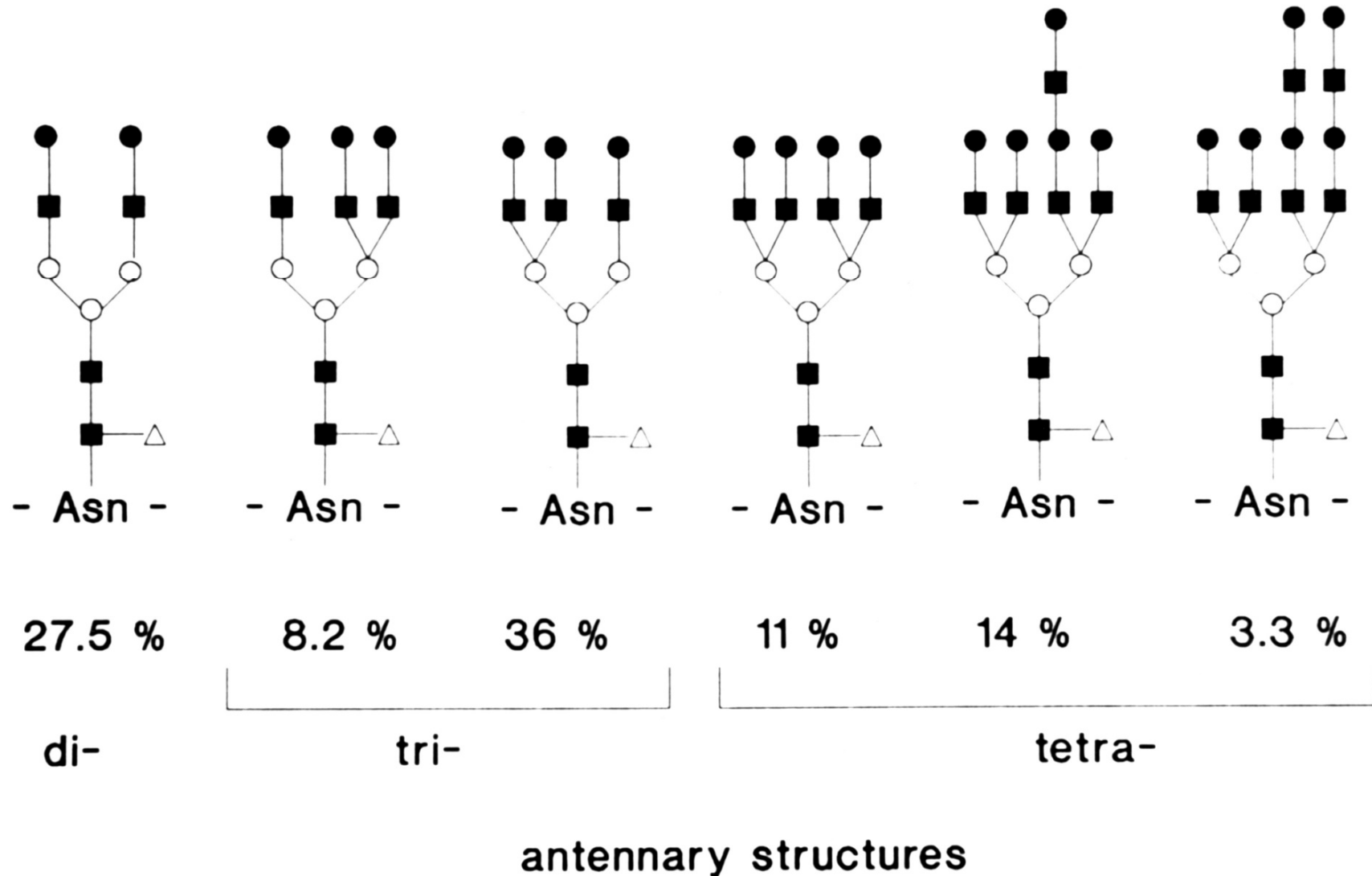
Gabi Dufhues

Human Interleukin-6

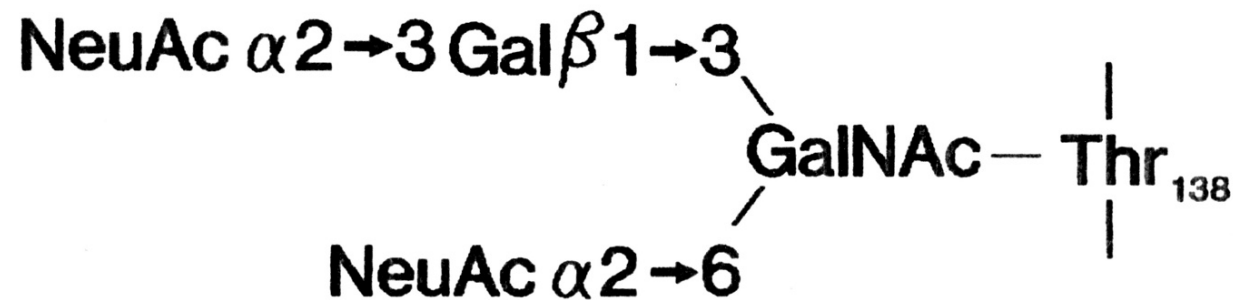
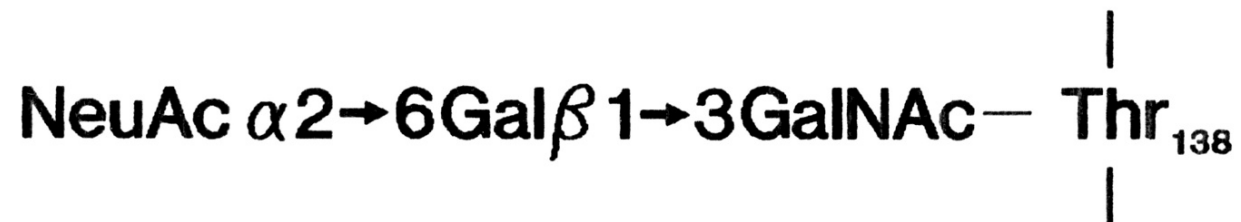


- 184 amino acids
- 28 amino acids signal peptide
- 2 potential N-glycosylation sites
- several O-glycosylation sites
- many different cells synthesize and secrete IL-6
- pleiotropic actions

Antennary structures of N-linked carbohydrate side chains



Structures of O-linked carbohydrate side chains



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

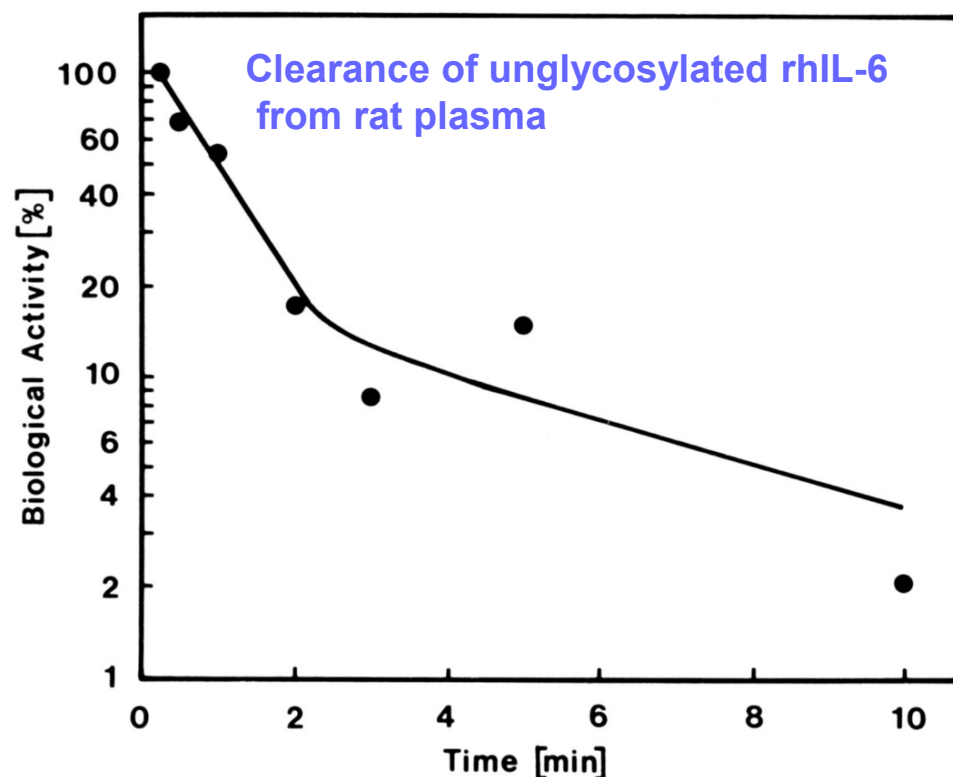
Plasma clearance, organ distribution and target cells of interleukin-6/hepatocyte-stimulating factor in the rat

José V. CASTELL¹, Thomas GEIGER², Volker GROSS², Tilo ANDUS², Eicke WALTER², Toshio HIRANO³, Tadamitsu KISHIMOTO³ and Peter C. HEINRICH¹

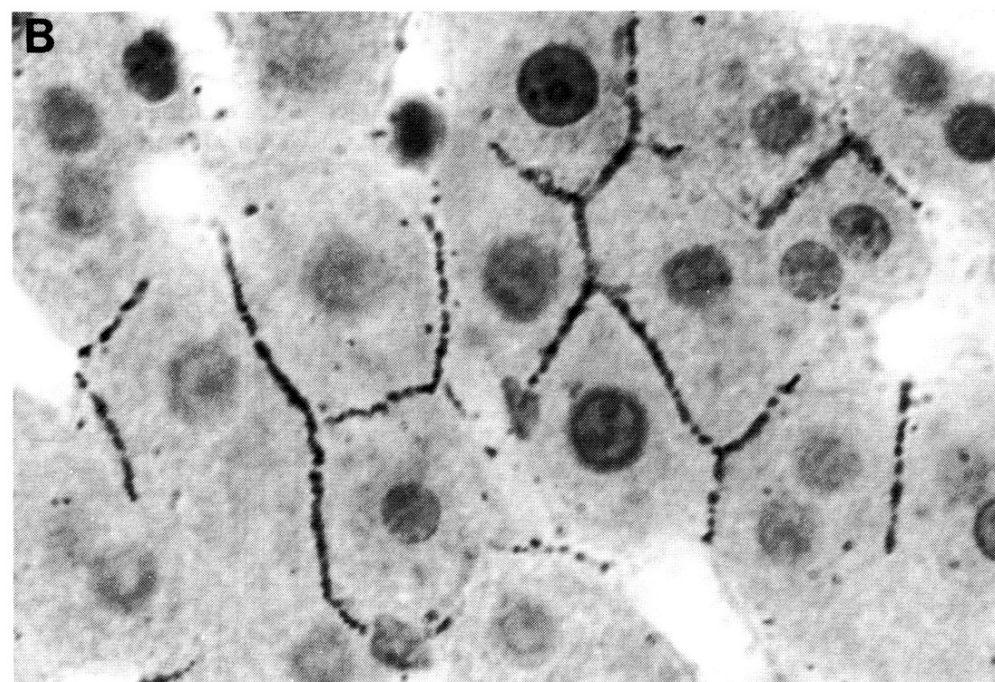
¹ Biochemisches Institut and ² Medizinische Klinik II der Universität Freiburg

³ Institute for Molecular and Cellular Biology, Osaka University

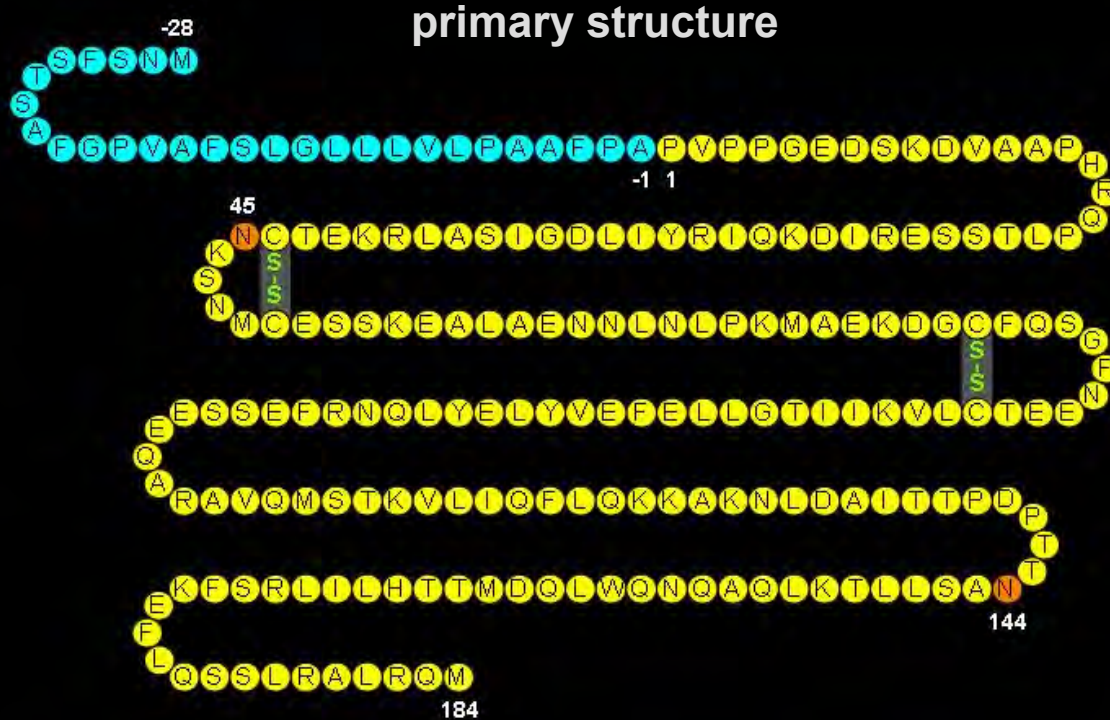
(Received March 18/June 24, 1988) — EJB 88 0319



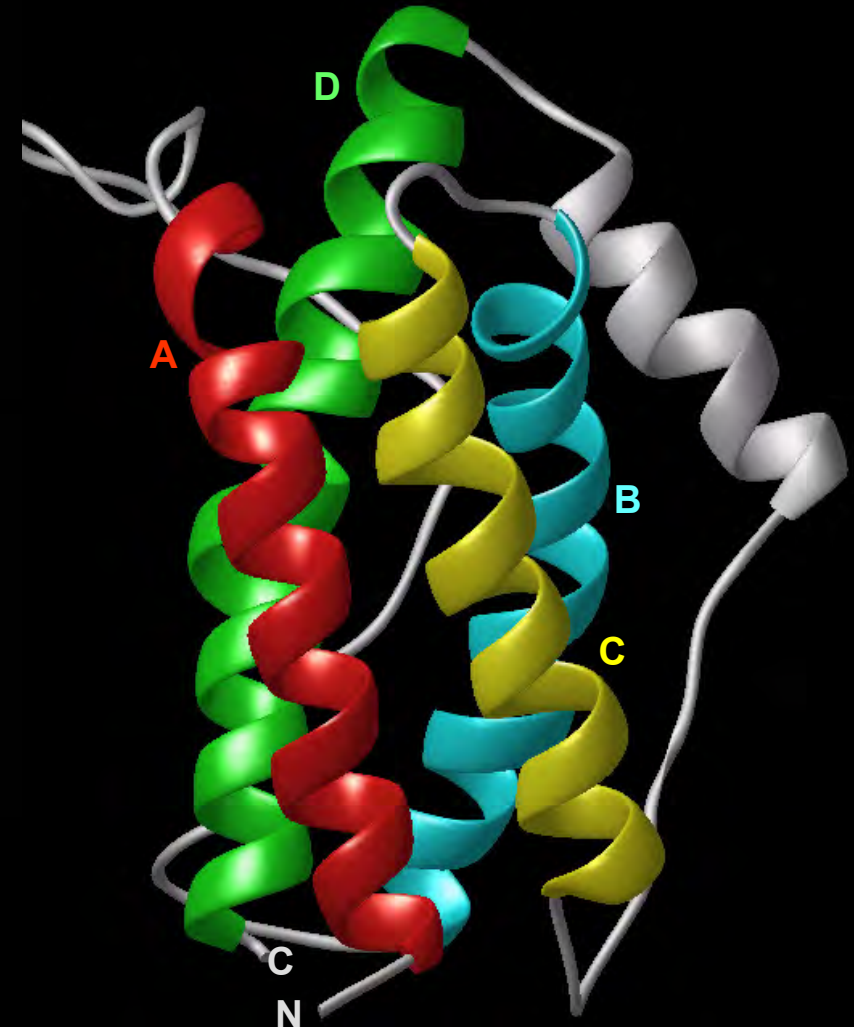
Distribution of ¹²⁵I- rhIL-6 in rat liver



Human Interleukin-6

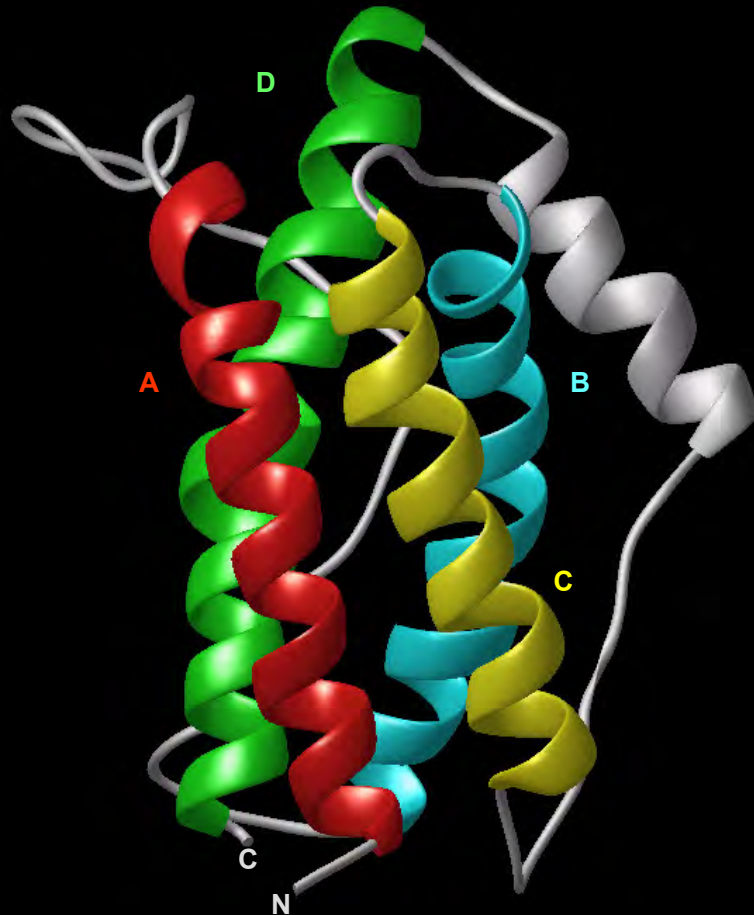


3D – structure
 a bundle of 4 antiparallel helices



- 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 secrete 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)

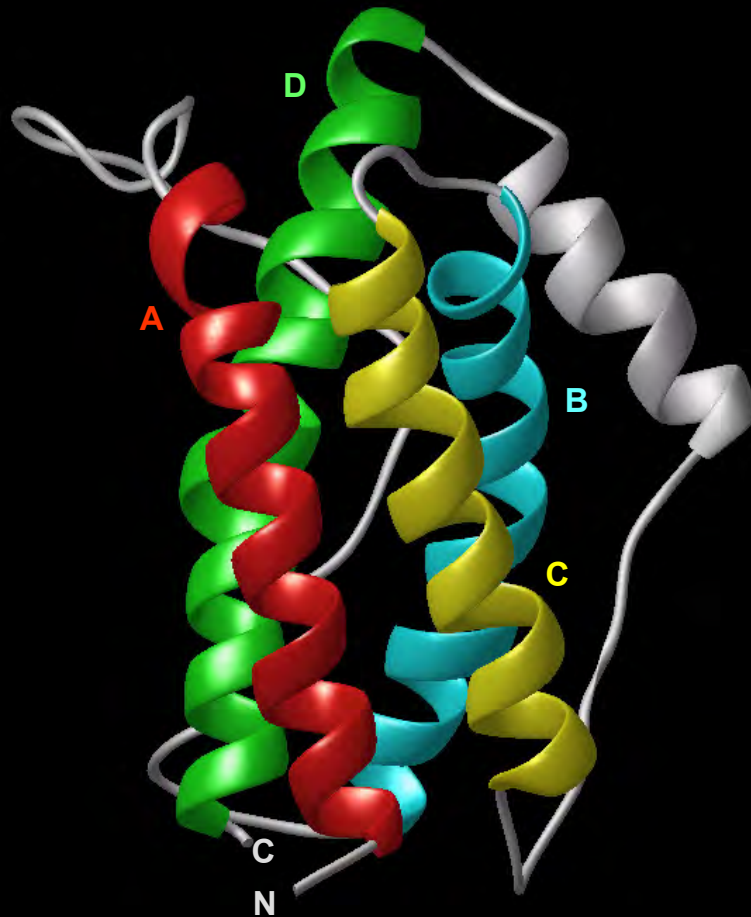
IL-11 **Interleukin 11**
(Baumann et al. 1991)

CNTF **Ciliary neurotrophic factor**
(Bazan 1991)

OSM **Oncostatin M**
(Richards et al. 1992)

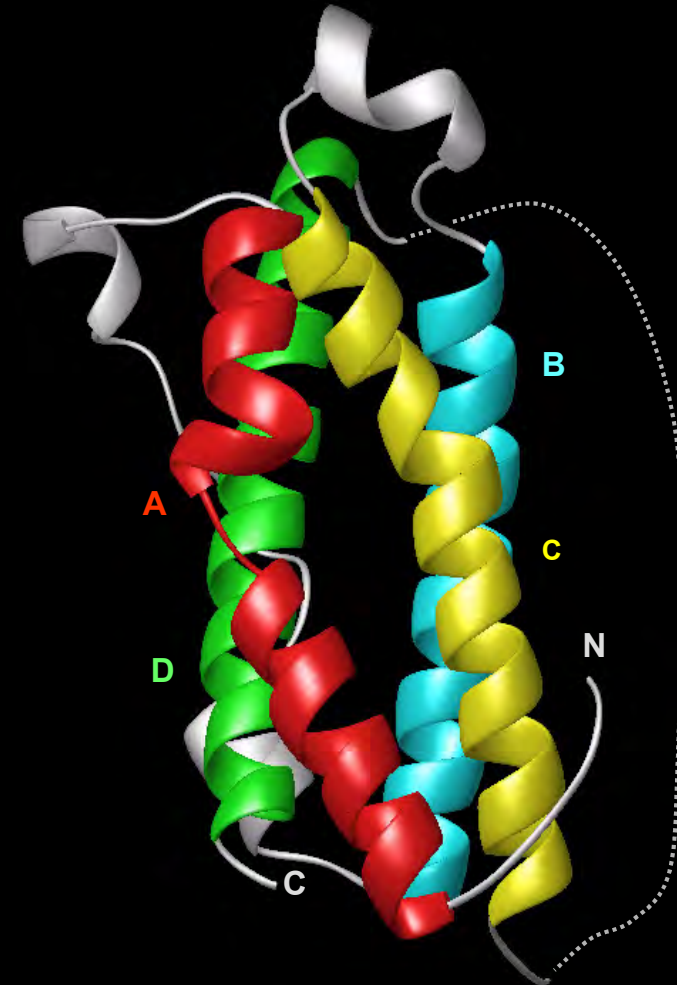
CT **Cardiotrophin**

Straight helix subfamily



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

Kinked helix subfamily



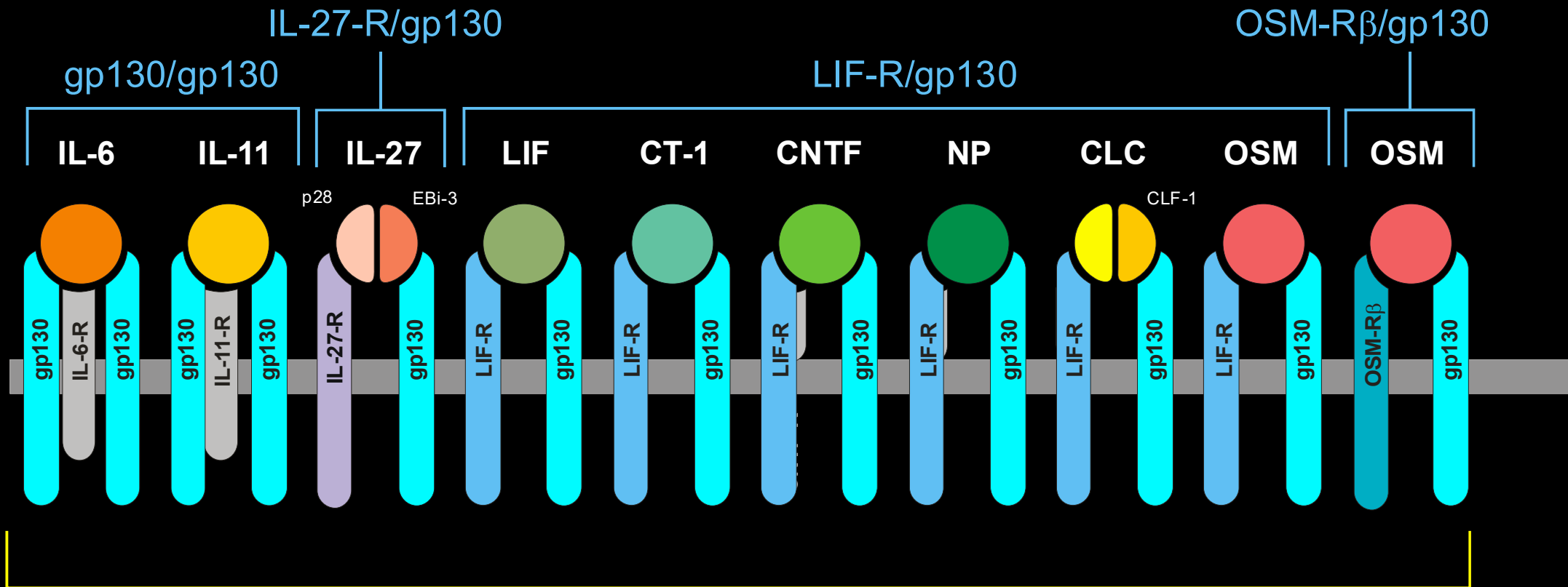
LIF	(1994)	Robinson et. al. Cell (X-ray)
CNTF	(1996)	Mcdonald et al. (X-ray)
OSM	(2000)	Deller et al. (X-ray)
CT*		

* 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 LIFR β 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

1992 LONDON



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

Acute phase protein synthesis
induced by IL-6

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 ANDUS¹, Thomas GEIGER¹, Toshio HIRANO², Tadamitsu KISHIMOTO², Thuy-Anh TRAN-THI¹, Karl DECKER¹
and Peter C. HEINRICH¹

¹ Biochemisches Institut, Universität Freiburg

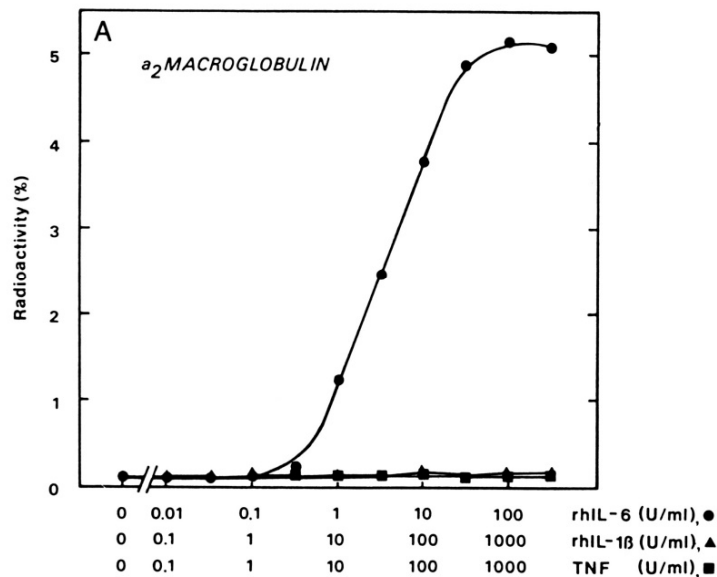
² Institute for Molecular and Cellular Biology, Division of Immunology, Osaka University

178 citations

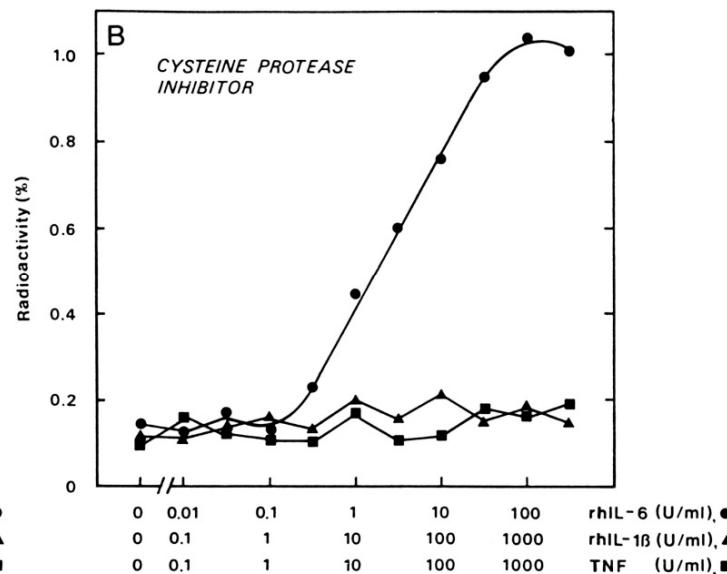
(07/2022)

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

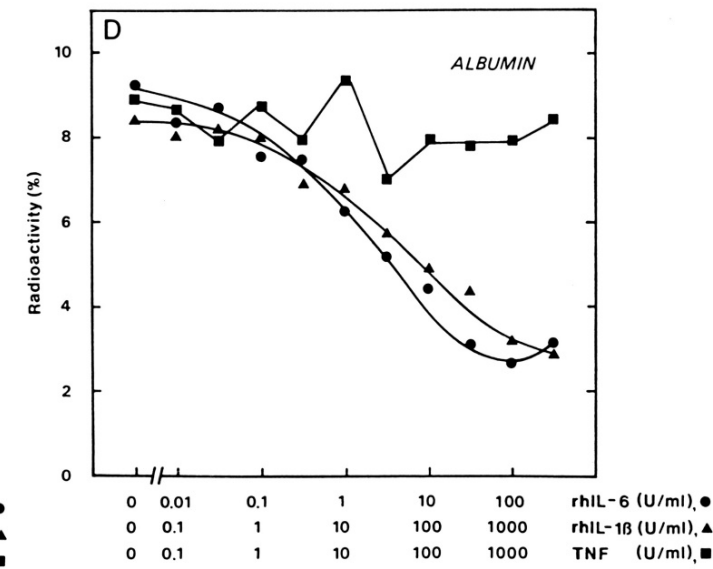
α_2 M-protein



CPI - protein



albumin - protein



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

(Received for publication, December 4, 1984)

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(07/2022)



Michael Heisig



Wolfgang Northemann



Dieter Kunz

Tilo Andus,
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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

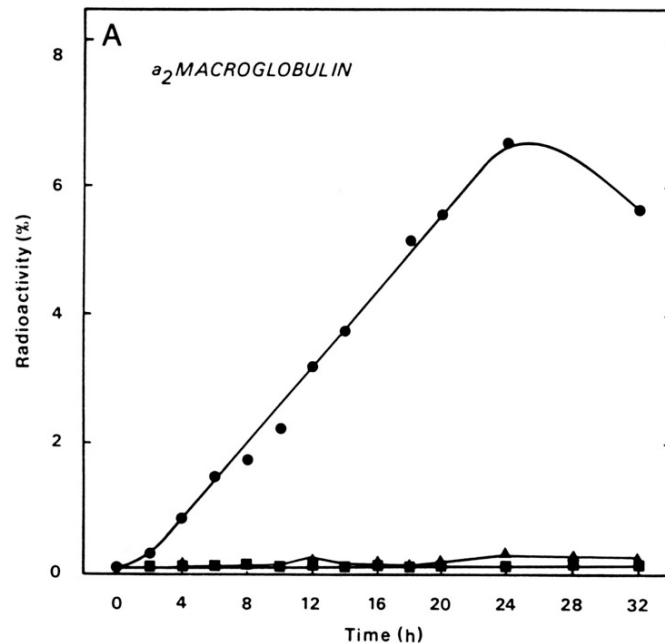
Action of recombinant human interleukin 6, interleukin 1 β and tumor necrosis factor α on the mRNA induction of acute-phase proteins*

261 citations

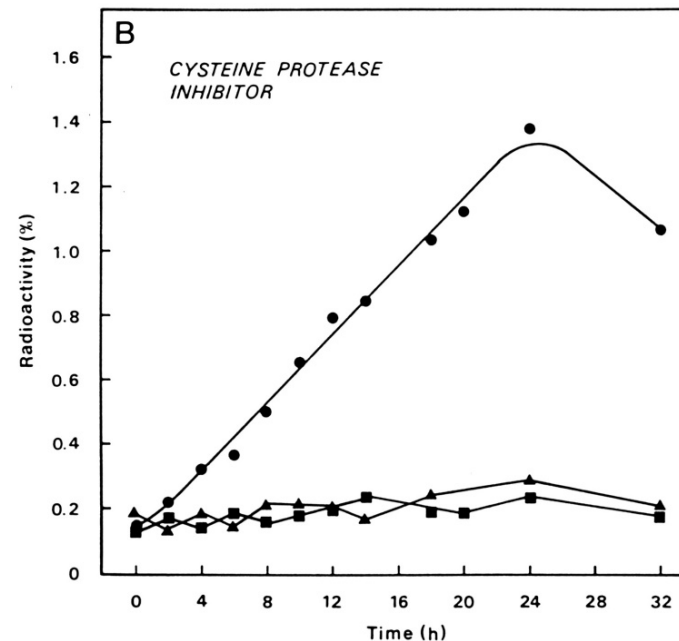
(07/2022)

in rat hepatocytes

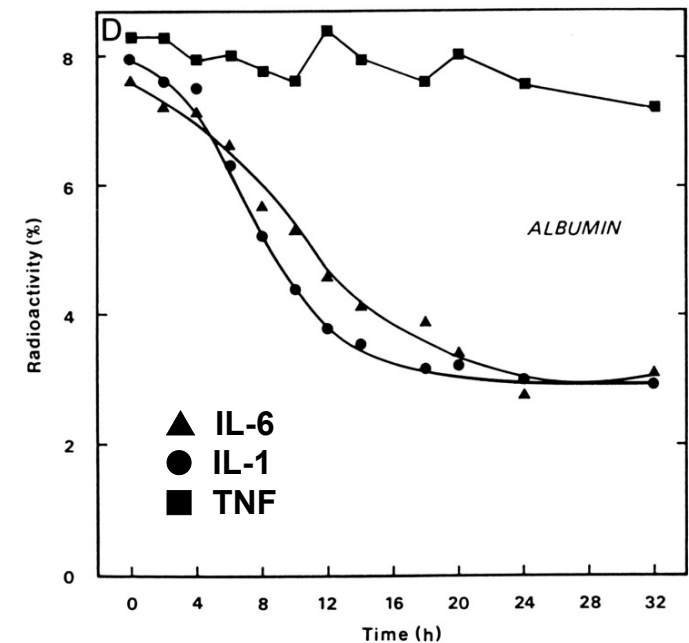
α_2 M - mRNA



CPI - mRNA



albumin - mRNA



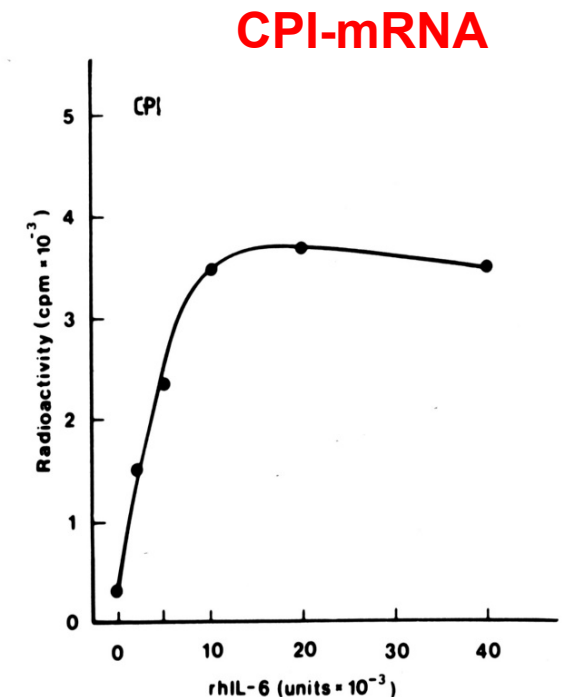
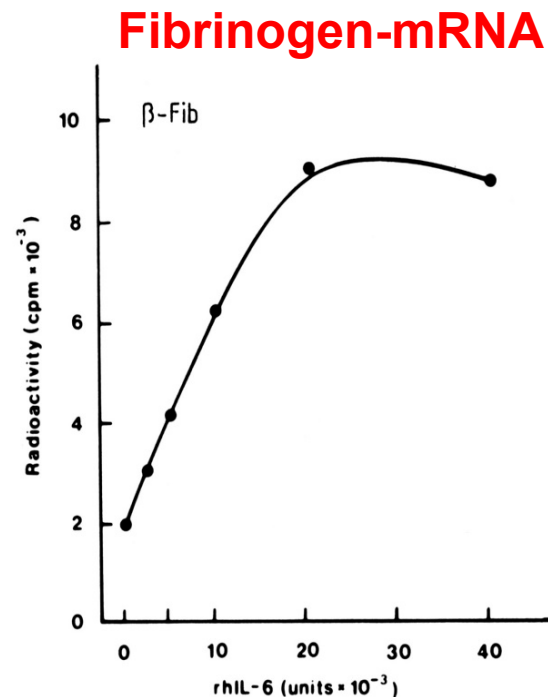
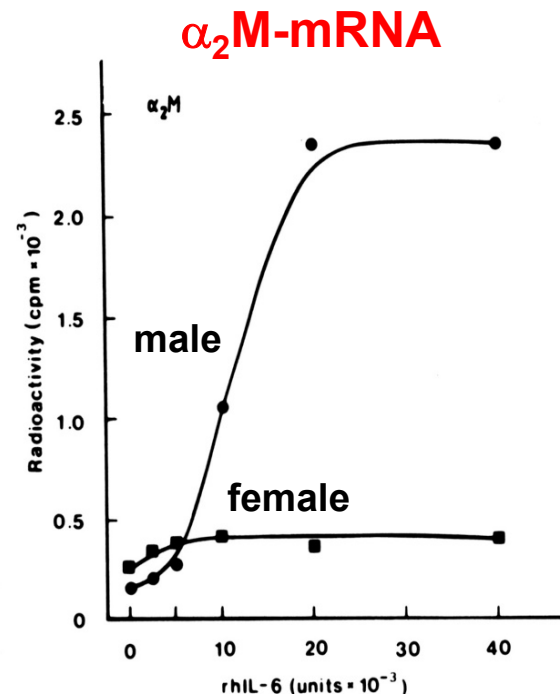
time dependence after rhIL-6

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)

418 citations
(07/2022)



rhIL-6 dose dependence

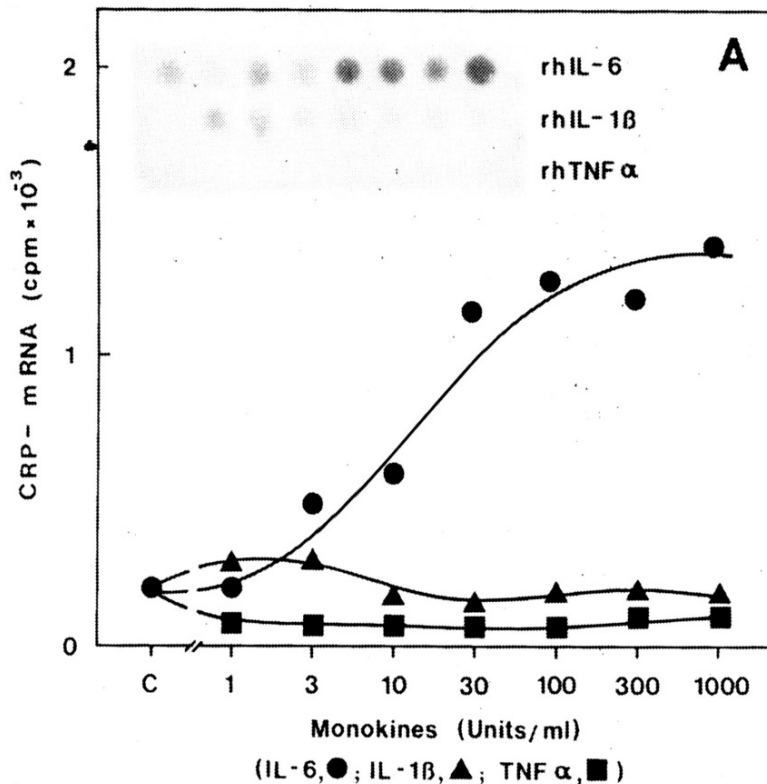
Interleukin-6 is the major regulator of acute phase protein synthesis in adult human hepatocytes

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

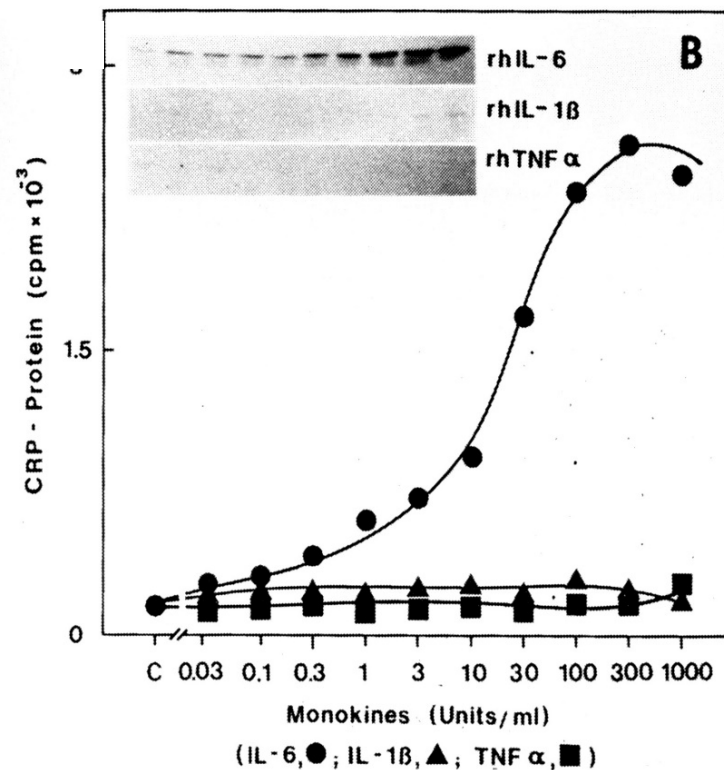
Biochemisches Institut, Universität Freiburg, Hermann-Herder-Str. 7, D-7800 Freiburg, FRG, ⁺ Centro de Investigación, Hospital La Fe, INSALUD, Avda. de Campanar, 21, E-46009 Valencia, Spain, [°] Medizinische Klinik der Universität Freiburg, Hugstetter Str. 55, D-7800 Freiburg, FRG and *Servicio de Cirugía,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

Received 12 October 1988

CRP- mRNA



CRP- protein

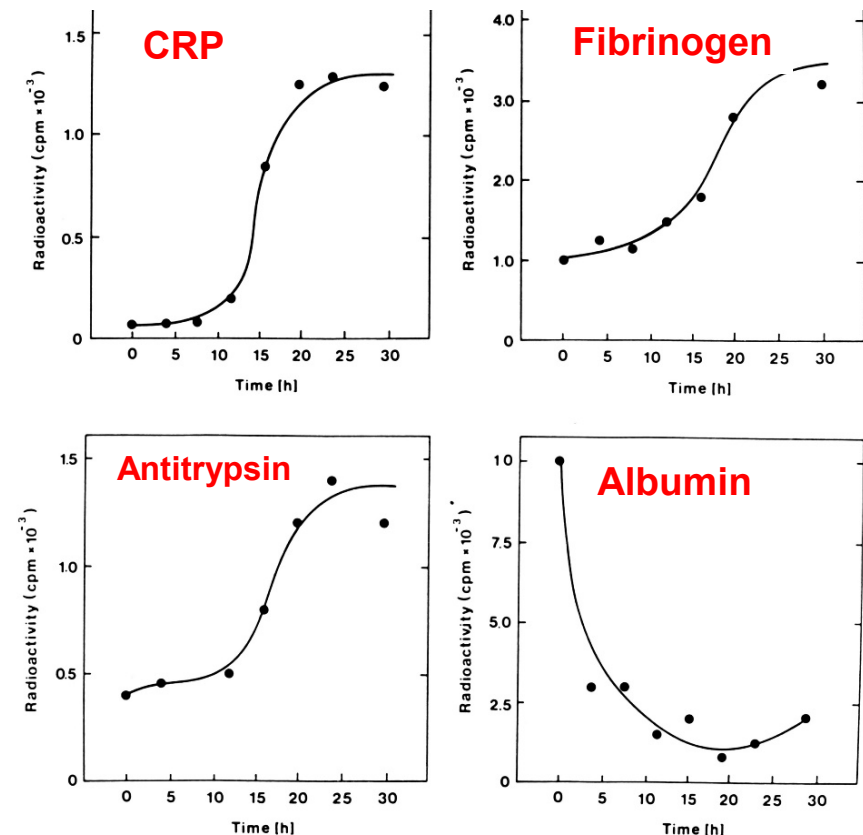
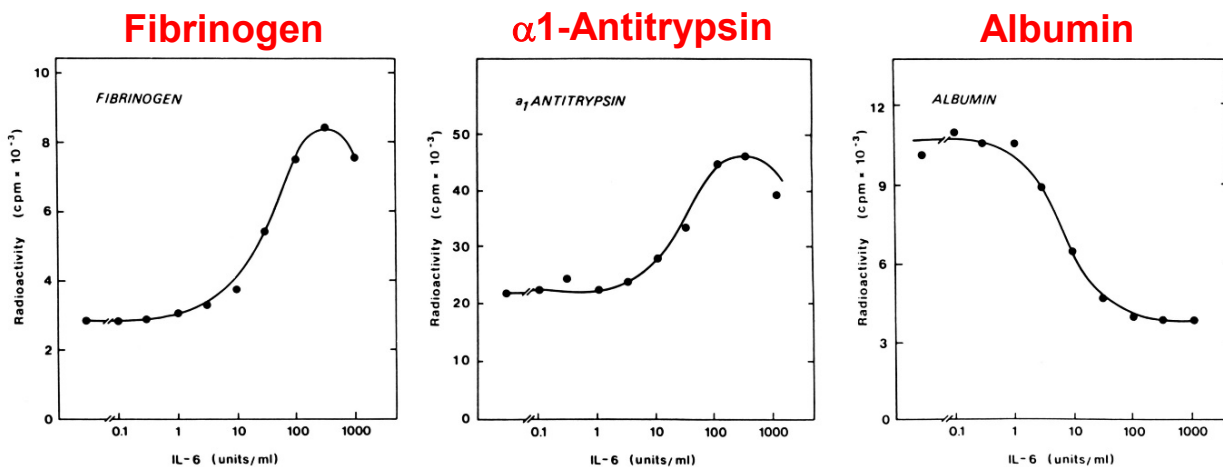


José Castell

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 TRULLENQUE³ AND PETER C. HEINRICH¹

Human hepatocytes



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 <i>in vivo</i>	+	+	+	+	+	+
Induction of ACTH secretion <i>in vitro</i>	-/+	+	+	+	nd	nd
Neural differentiation	+	+	+	+	+	+
Induction of bone loss/osteoclast formation	+	+	+	+	nd	nd
Induction of cardiac hypertrophy <i>in vitro</i>	-/+	+	+	+	-/+	+

Outline: Interleukin-6 signal transduction and its regulation

Part 1: Molecular mechanisms of IL-6 signal transduction

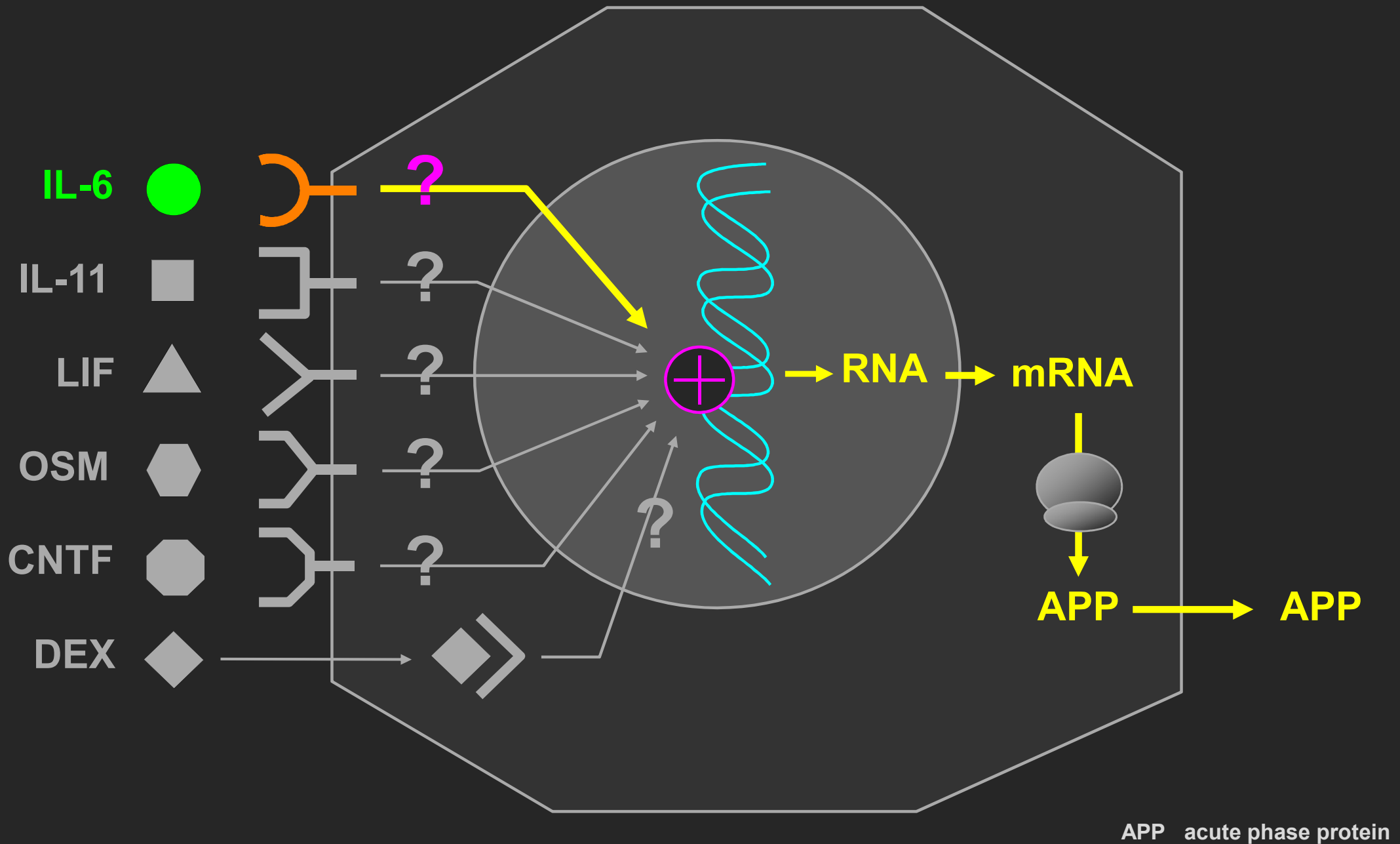
- **The acute phase response**
- **Identification of HSF as IL-6**
- **Structure and function of IL-6**
- **Acute phase protein synthesis induced by IL-6**
- **Molecular mechanism of IL-6 induced APP expression**
- **Formation of the IL-6-receptor complex**
- **Design of a highly potent IL-6 antagonist**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

Part 2: Regulation of IL-6 signal transduction

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

APP, acute phase protein

How does IL-6 induce APP expression?



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

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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^{*,‡}

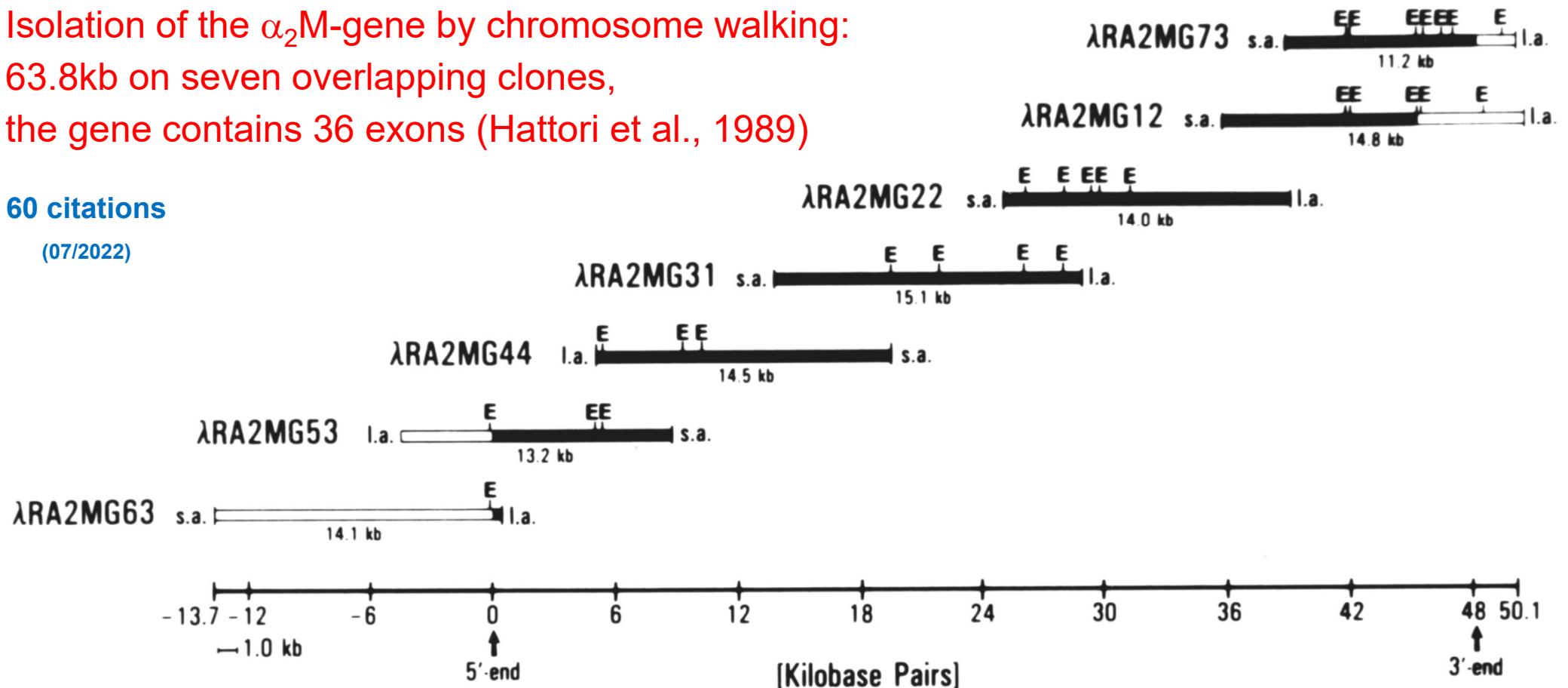
Department of Immunology, Research Institute of Scripps Clinic, La Jolla, California 92037, Department of Biochemistry, School of Medicine, Case Western Reserve University, Cleveland, Ohio 44106, and Institute of Biochemistry, University of Freiburg, Freiburg, West Germany

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

Isolation of the α_2 M-gene by chromosome walking:
63.8kb on seven overlapping clones,
the gene contains 36 exons (Hattori et al., 1989)

60 citations

(07/2022)



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

Dieter Kunz, René Zimmermann¹, Michael Heisig and Peter C. Heinrich^{1*}

75 citations

(07/2022)

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

Received September 28, 1988; Revised December 20, 1988; Accepted January 13, 1989

Acc. no. ⁺

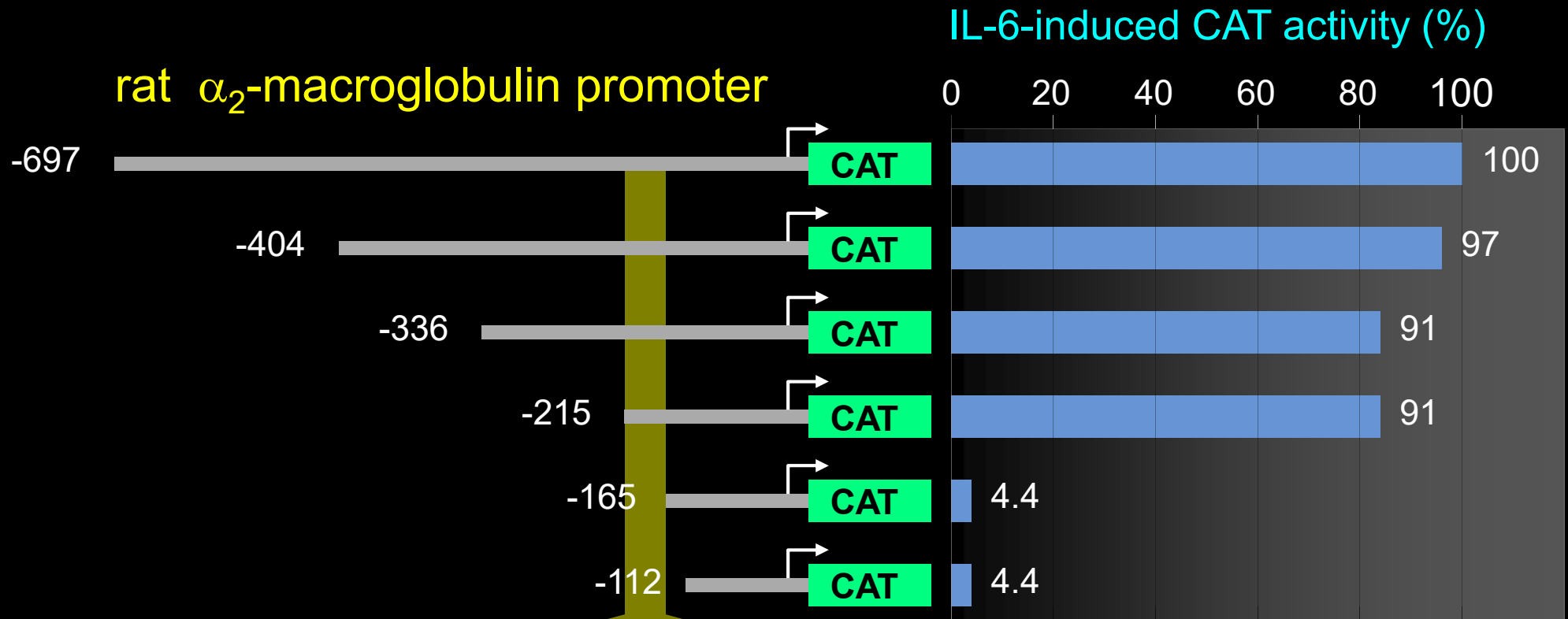


**Search for an acute phase response element (APRE)
in the α_2 -macroglobulin-promoter**

Dieter Kunz

René Zimmermann

Analysis of the α_2 -macroglobulin promoter



GTA**ACTGGAA**AGTCCTTAATCCTTCTGGGAAT

Acute-Phase Response Element (APRE)

1988 start in Aachen



Friedemann Horn

Promoter sequences of APP genes

T T C T G G G A A

rat α_2 M "core"

T A A C T G G A A

rat α_2 M "core-like"

T T A C G G G A A

human α_2 M

G T A C G G T A A

human α_2 M

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

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

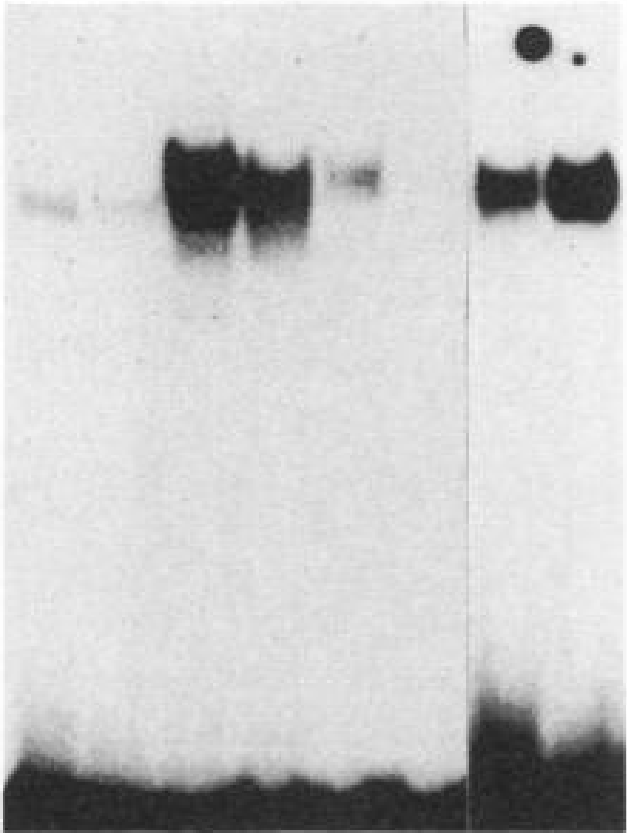
1993

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



probes: α_2 M CTGGGA

free
probe
→



time (h)	0	0.5	1	2	4	10	0.25	0.5
treatment	LPS						IL-6	

injection of LPS or IL-6 into rats

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

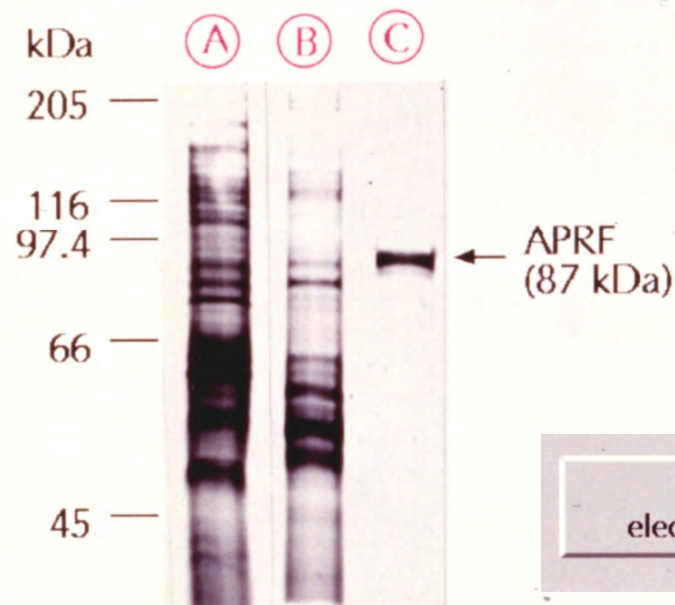
URSULA M. WEGENKA,¹ CLAUDIA LÜTTICKEN,¹ JAN BUSCHMANN,¹ JUPING YUAN,^{1†}
FRIEDRICH LOTTSPEICH,² WERNER MÜLLER-ESTERL,³ CHRIS SCHINDLER,⁴
ELKE ROEB,¹ PETER C. HEINRICH,^{1*} AND FRIEDEMANN HORN^{1*}

251 citations

(07/2022)

Purification of APRF from rat liver

livers of 60 LPS-injected rats
↓
nuclear extracts
↓ (A)
heparin-Sepharose chromatography
↓ (B)
first DNA-affinity chromatography
↓
second DNA-affinity chromatography
↓ (C)
homogeneous APRF



SDS-polyacrylamide gel
electrophoresis (silver-stained)

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, Tadimitsu Kishimoto, Giovanna Barbieri, Sandra Pellegrini, Michael Sendtner, Peter C. Heinrich, and Friedemann Horn*

740 citations

(07/2022)



Claudia Lütticken



Ursula Wegenka



Juping Yuan



Friedemann Horn

RED-HOT RESEARCH PAPERS OF 1994

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

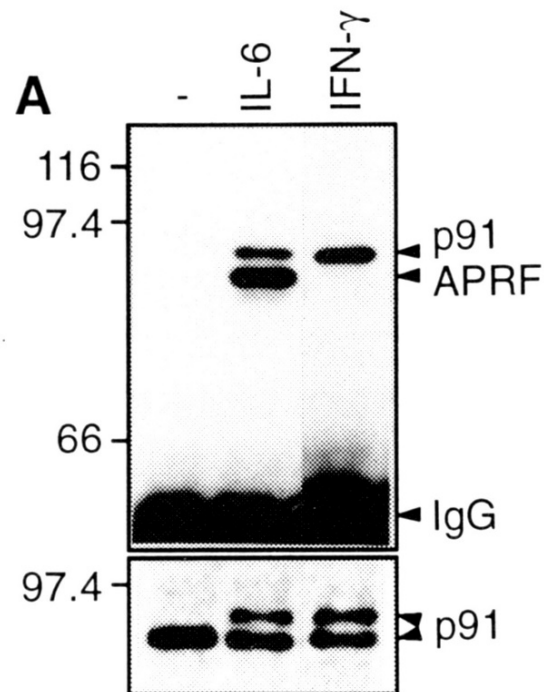
Association of Transcription Factor APRF and Protein Kinase Jak1 with the Interleukin-6 Signal Transducer gp 130

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

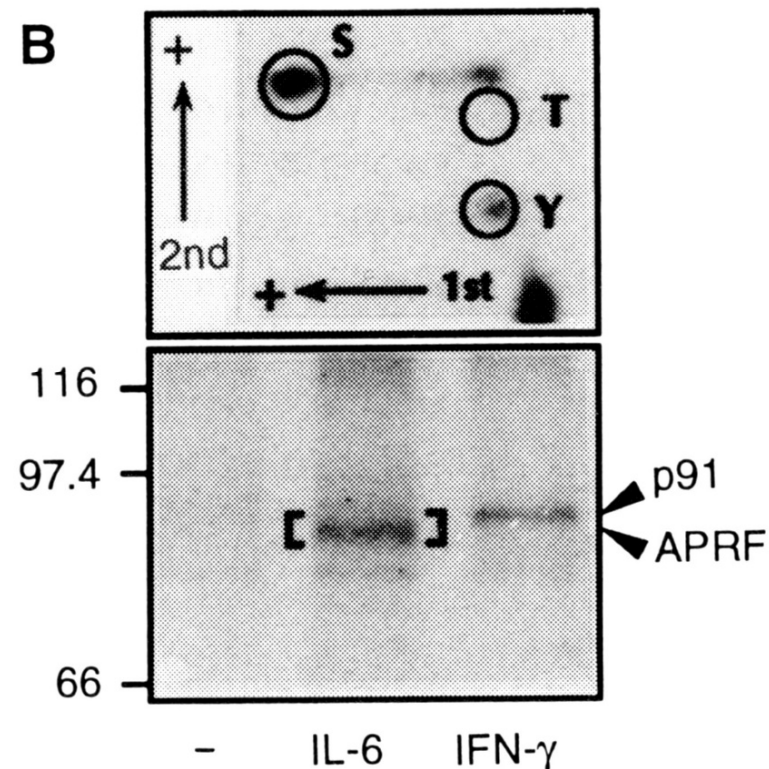
740 citations

(07/2022)

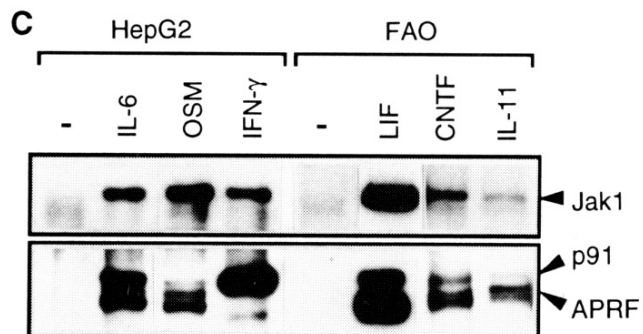
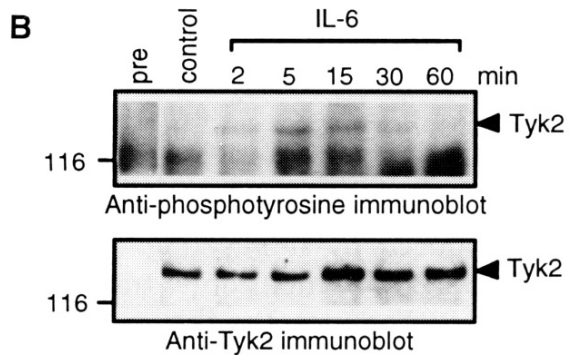
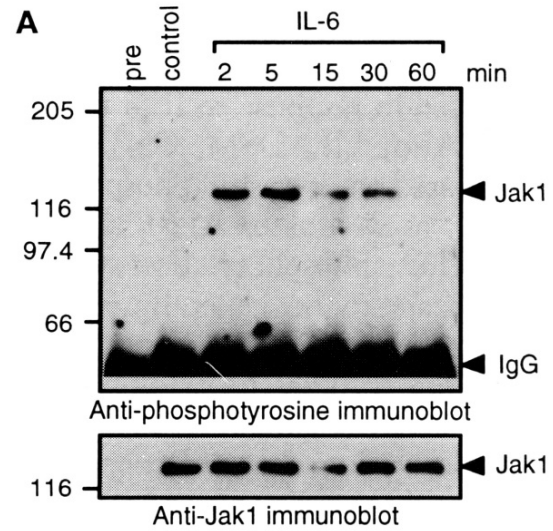
Y-P of APRF and ISGF-3 α



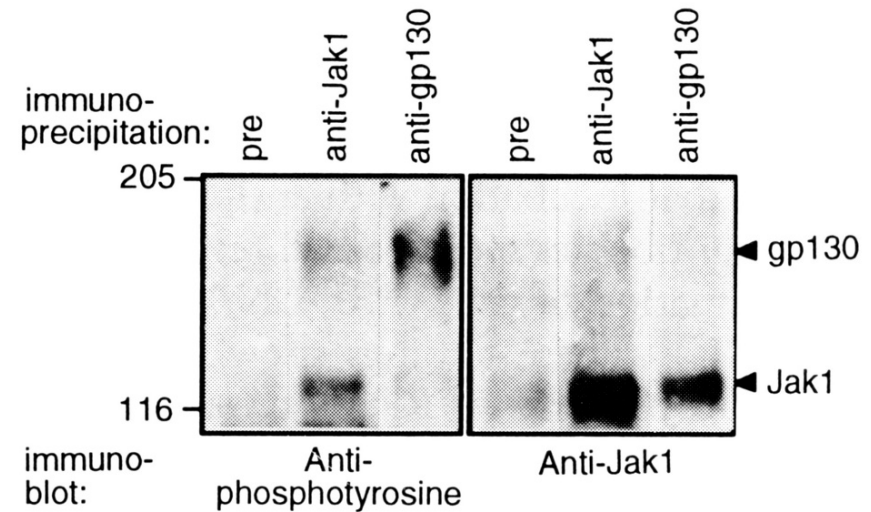
P-aa-analysis of APRF



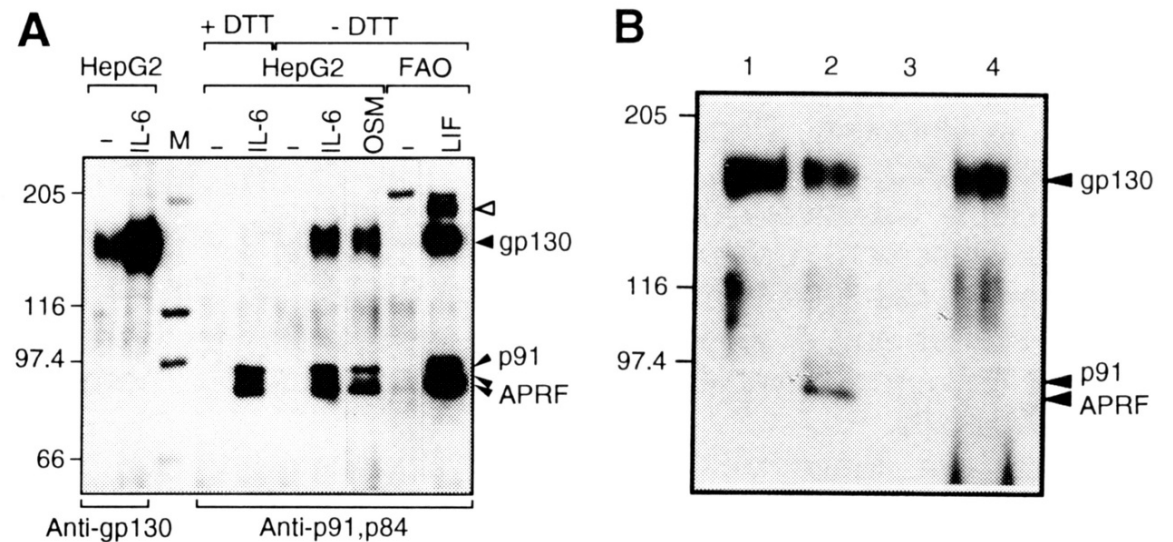
Y-P of Jak1 and Tyk2 after IL-6



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

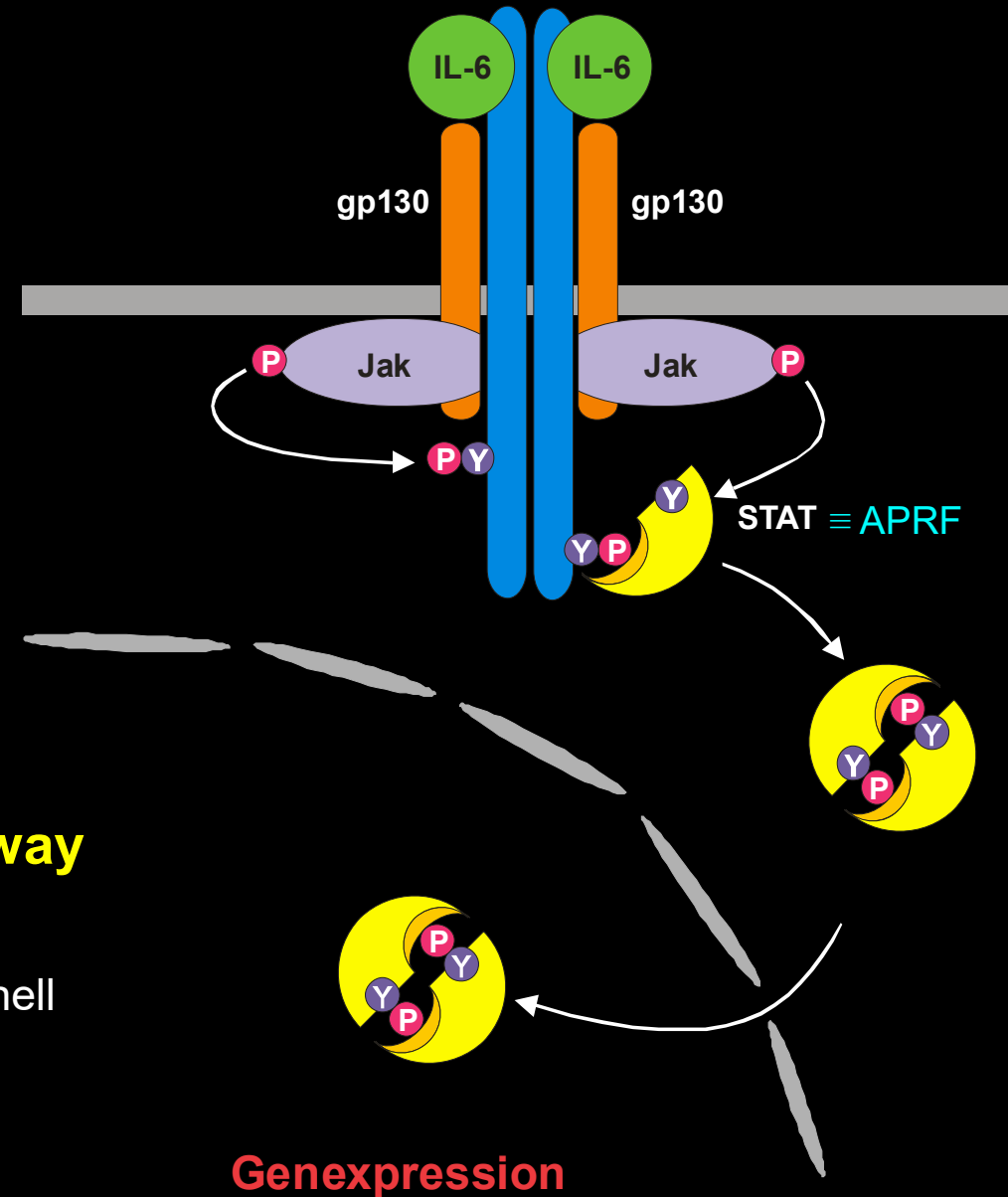
After IL-6 stimulation of HepG2 cells:

- 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.

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

Formation of the IL-6-receptor complex

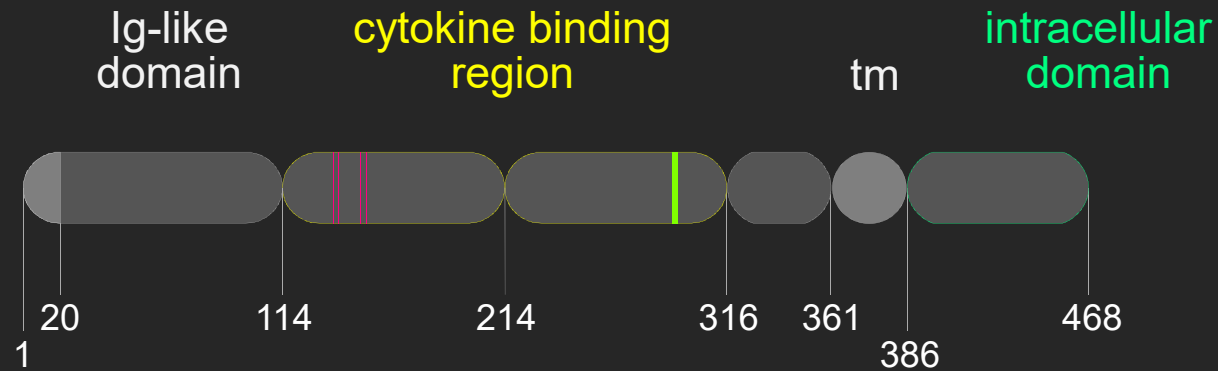
The **IL-6 receptor** consists of 2 subunits:

- 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.

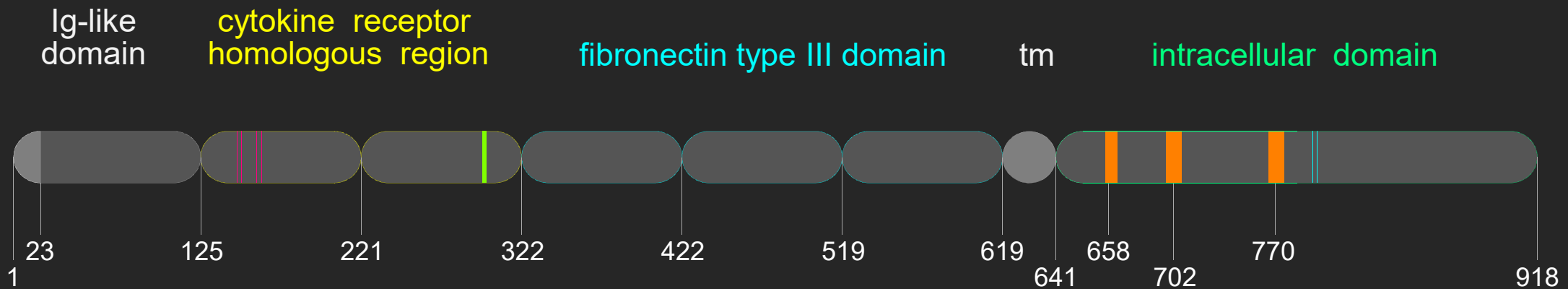
The IL-6R subunits gp80 and gp130

IL-6R α / gp80



Yamasaki et al. (1988) Sci 241, 825

IL-6R β / gp130



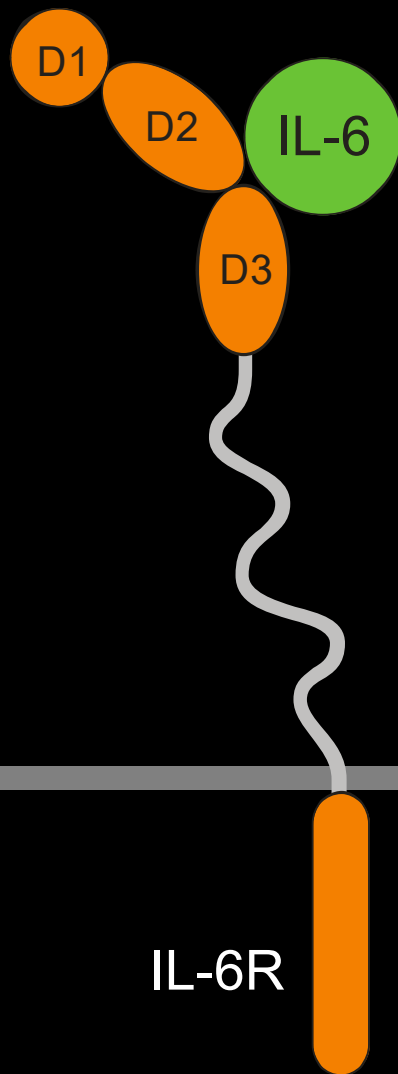
Hibi et al. (1990) Cell 63, 1149

tm, transmembrane region

Identification of the interaction sites of IL-6, IL-6R α 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



Krüttgen et al. (1990) FEBS Lett 262, 323-326

75 citations

Krüttgen et al. (1990) FEBS Lett 273, 95-98

68 citations

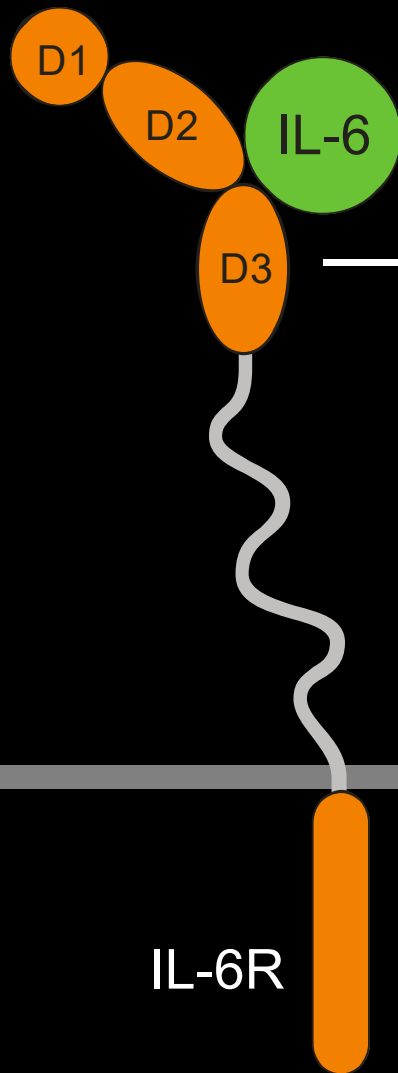
Lütticken et al. (1991) FEBS Lett 282, 265-267

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



gp80

D2-D3

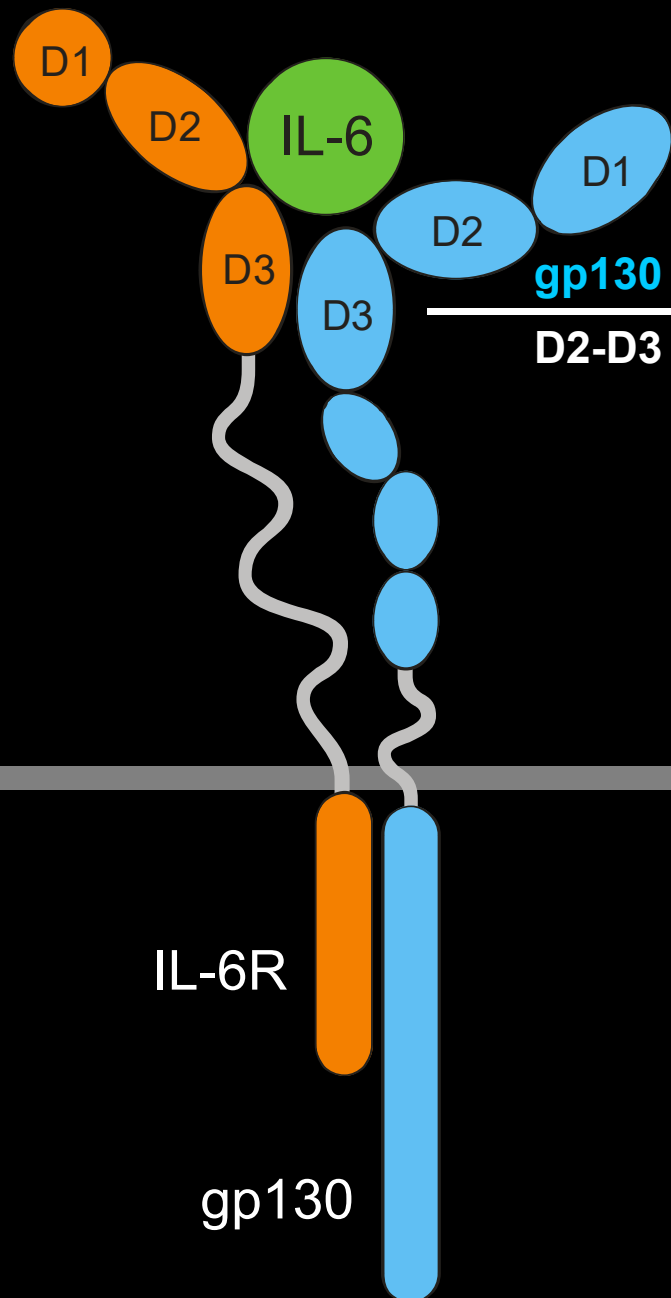
Weiergräber et al. (1996) FEBS Lett 379, 122-126

24 citations

Kalai et al. (1996) Eur J Biochem 238, 714-723

21 citations

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



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

21 citations

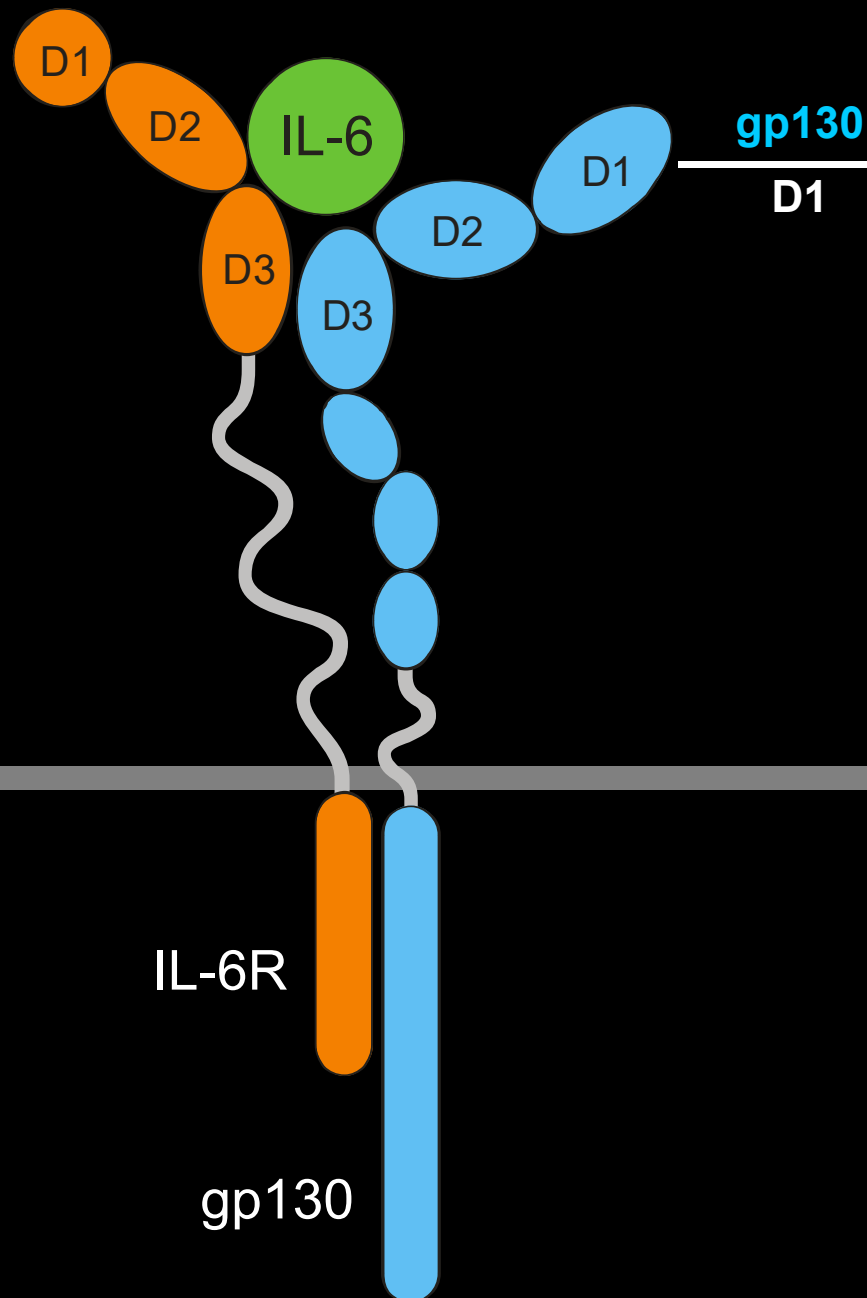
Müller-Newen et al. (1997) Eur J Biochem 247, 425-431

8 citations

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

27 citations

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



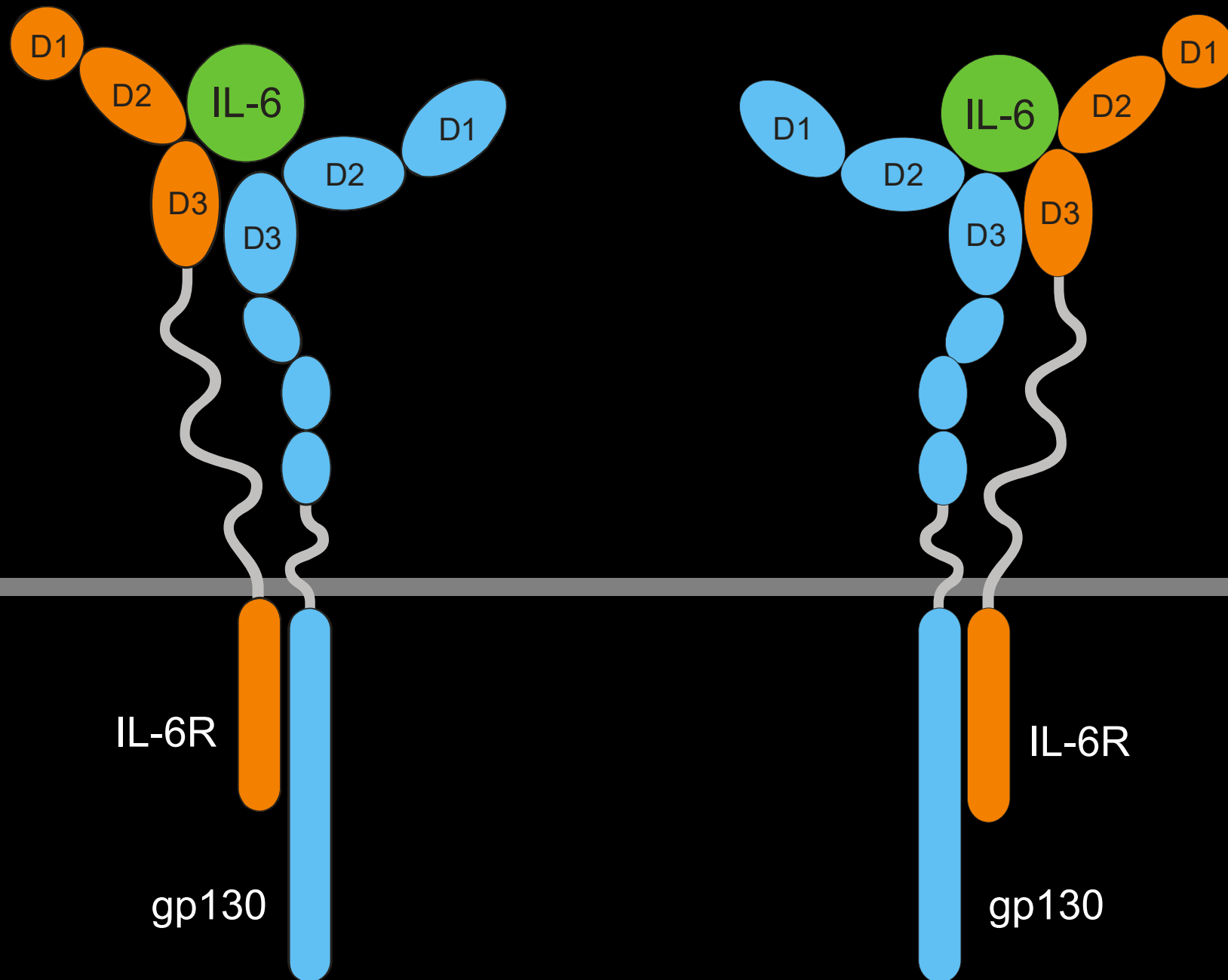
Kurth et al. (1999) J Immunol 162, 1480-1487

69 citations

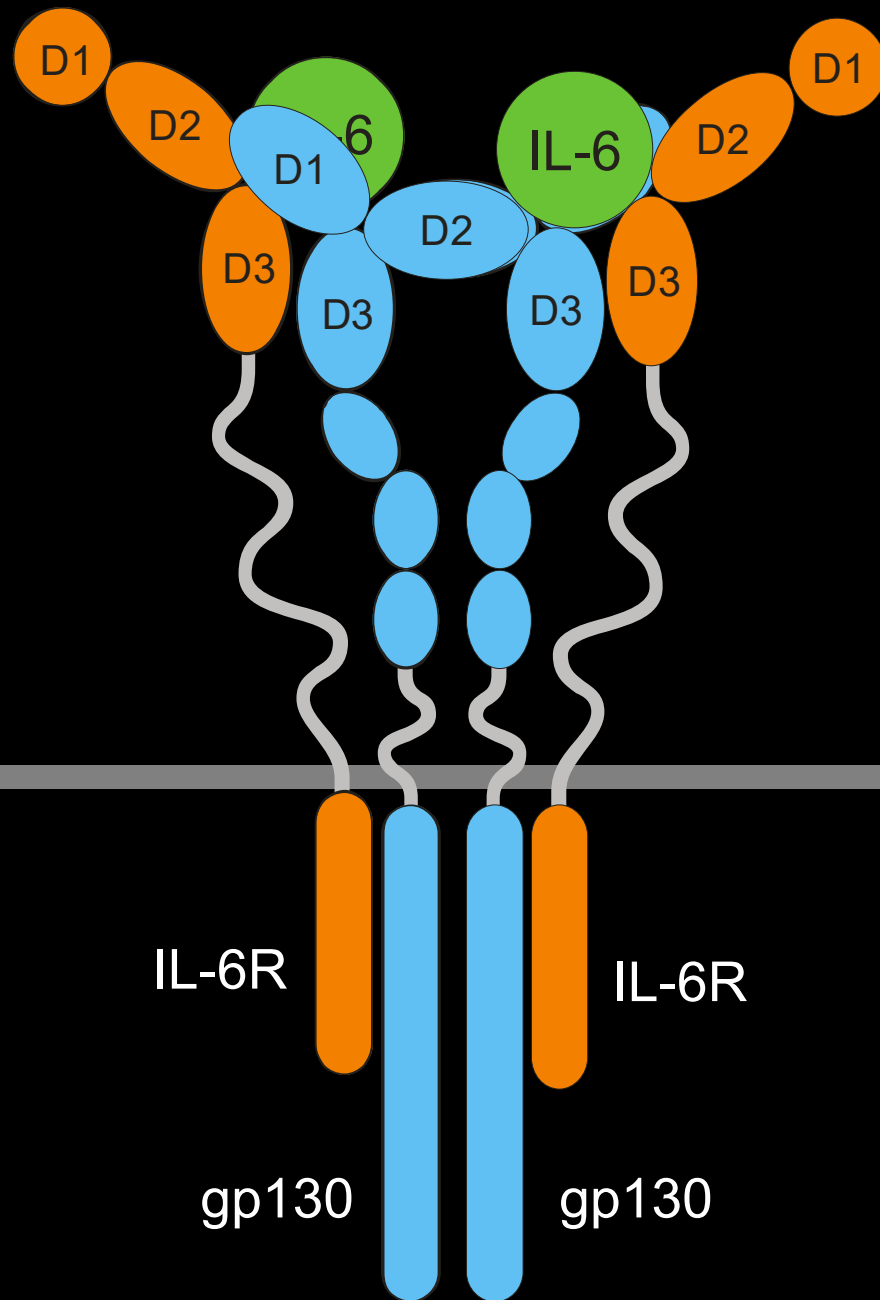
Pflanz et al. (2000) J Immunol 165, 7042-7049

30 citations

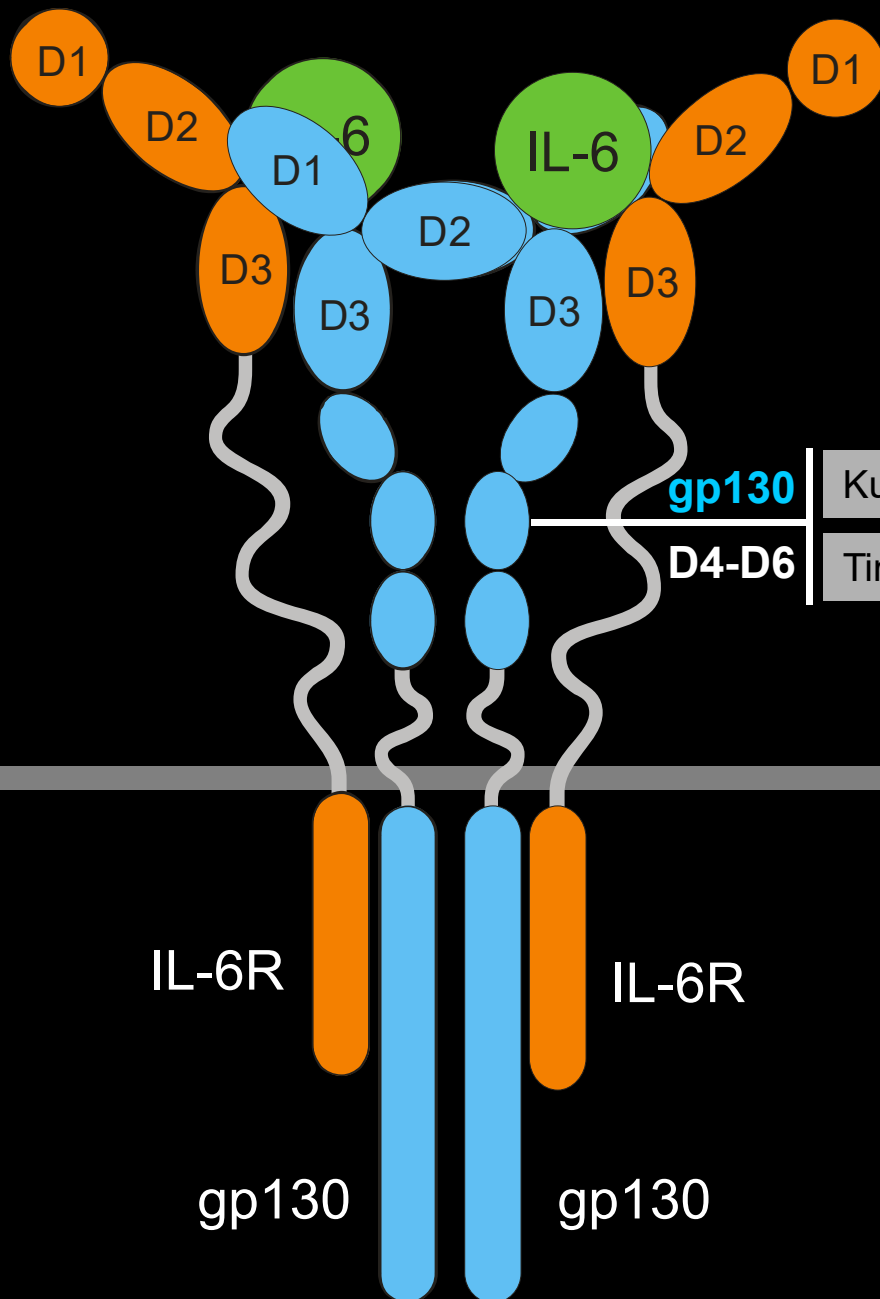
Structural assembly of the functional human IL-6 hexamer signaling complex



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



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



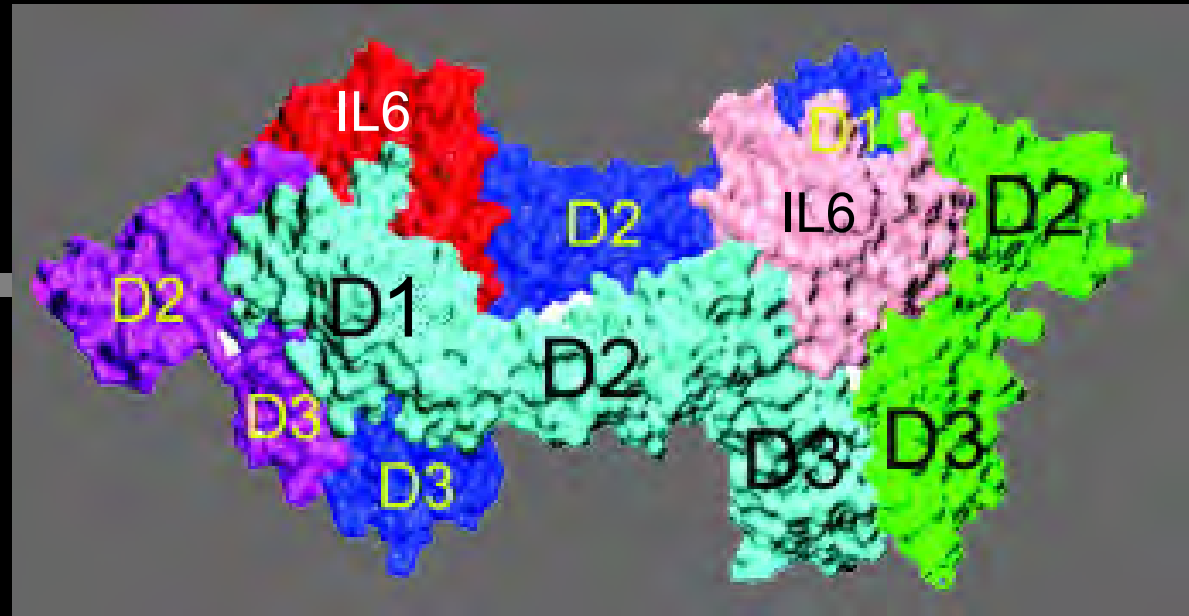
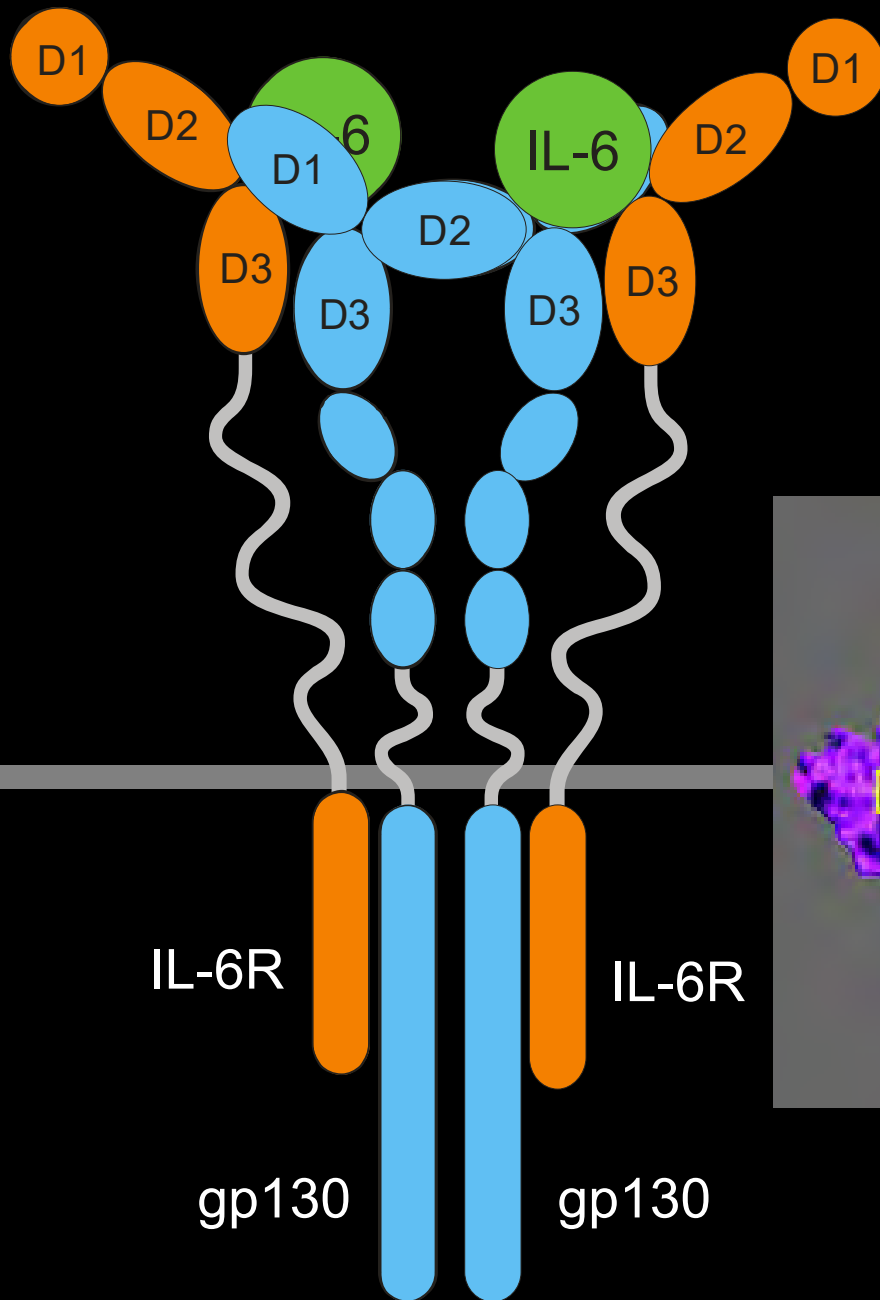
Kurth et al. (2000) J Immunol 164, 273-282

14 citations

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

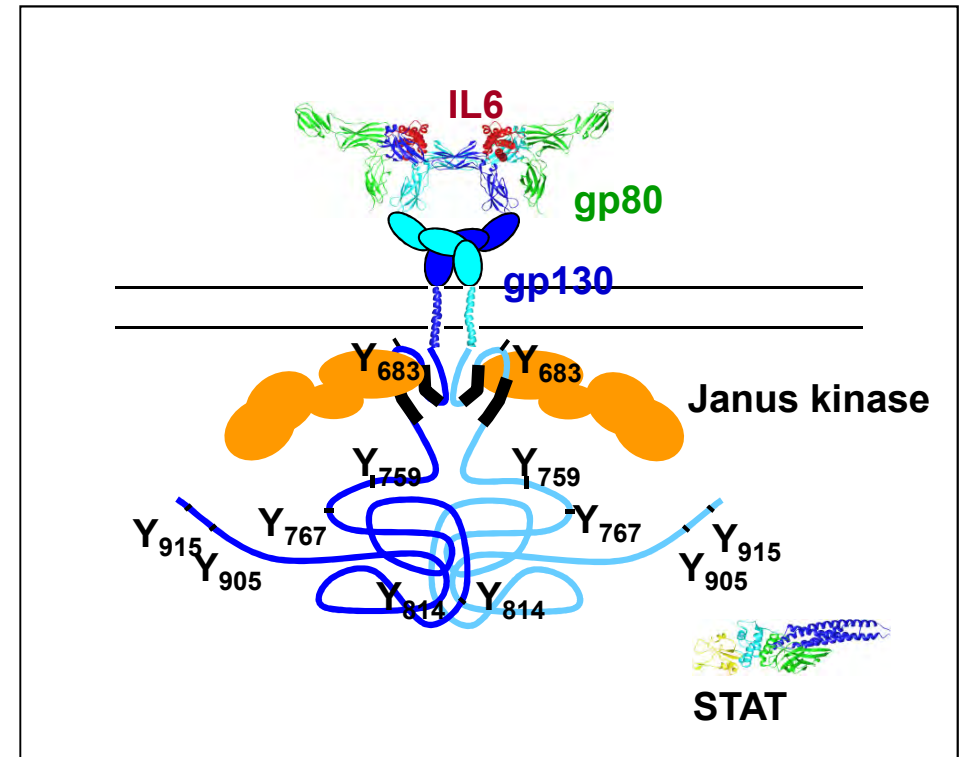
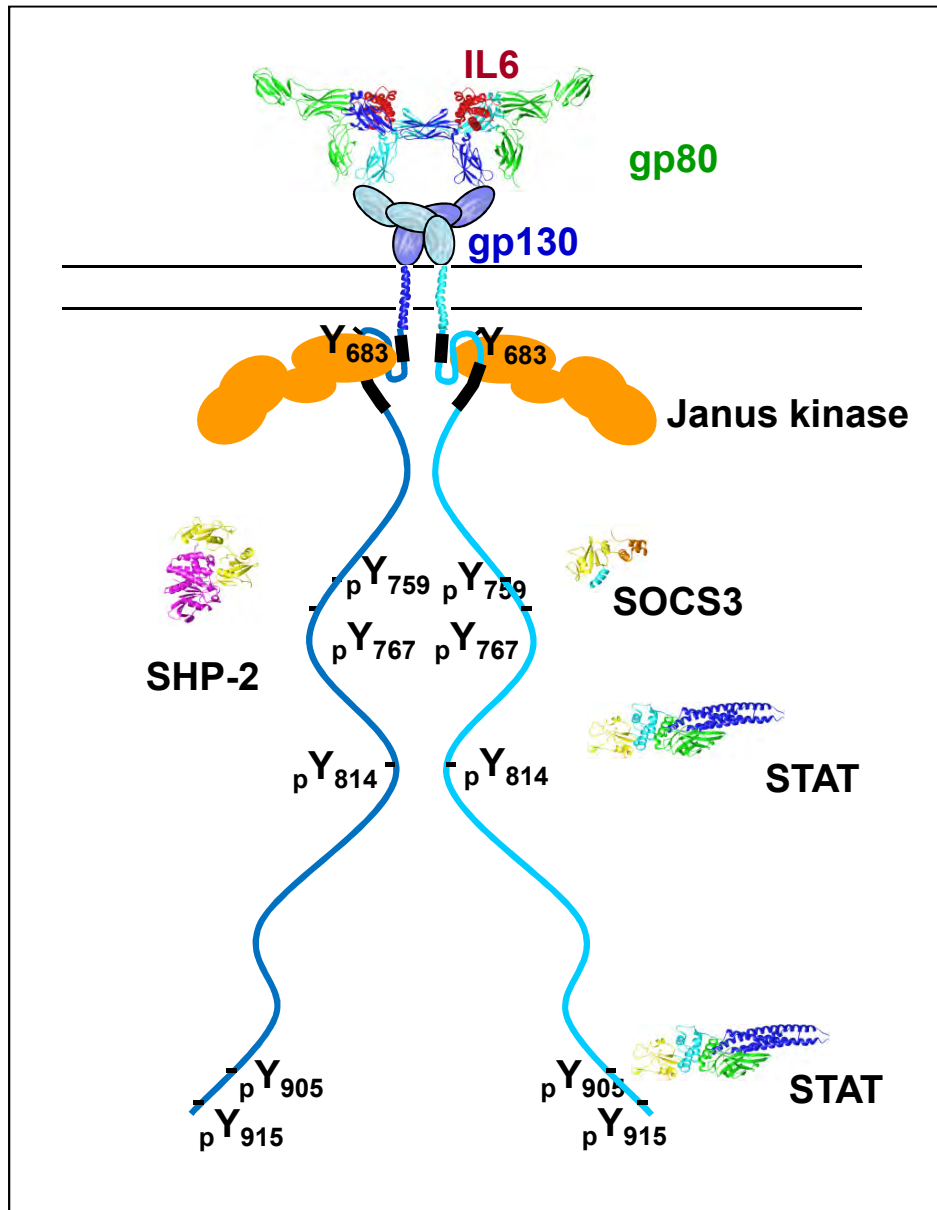
33 citations

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



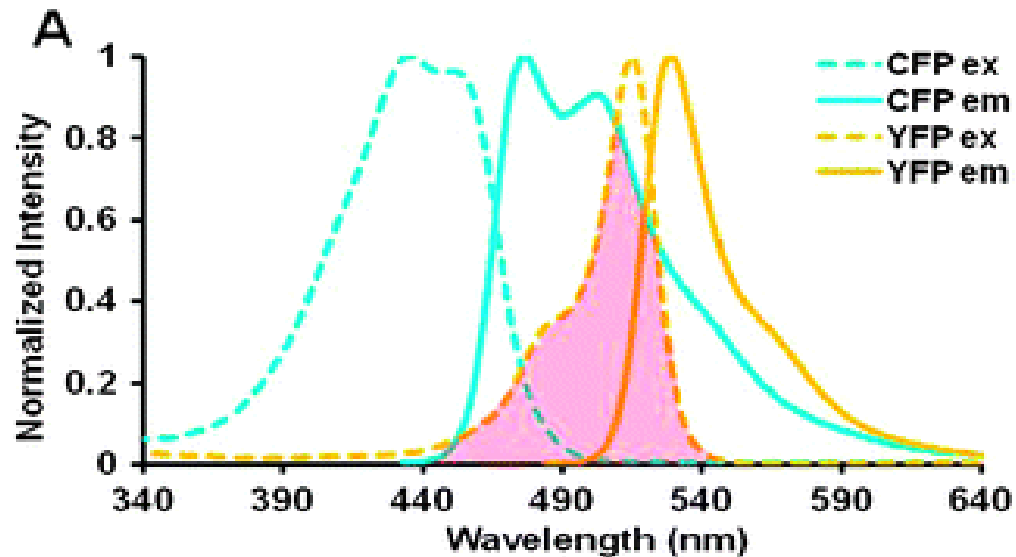
Are structural properties important for activation and modulation of cytokine signaling ?

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

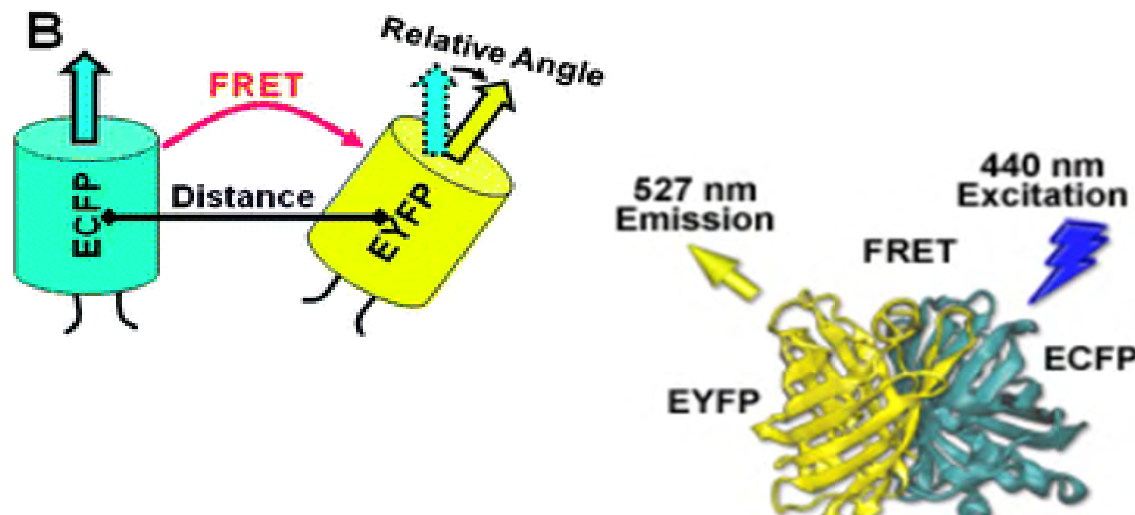
>>> We concluded that
gp130 is present as a preformed dimer on the cell membrane

protein-protein interaction → FRET



Förster/Fluorescence Resonance Energy Transfer

- Energy transfer between fluorophores based on dipole-dipole coupling not 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)

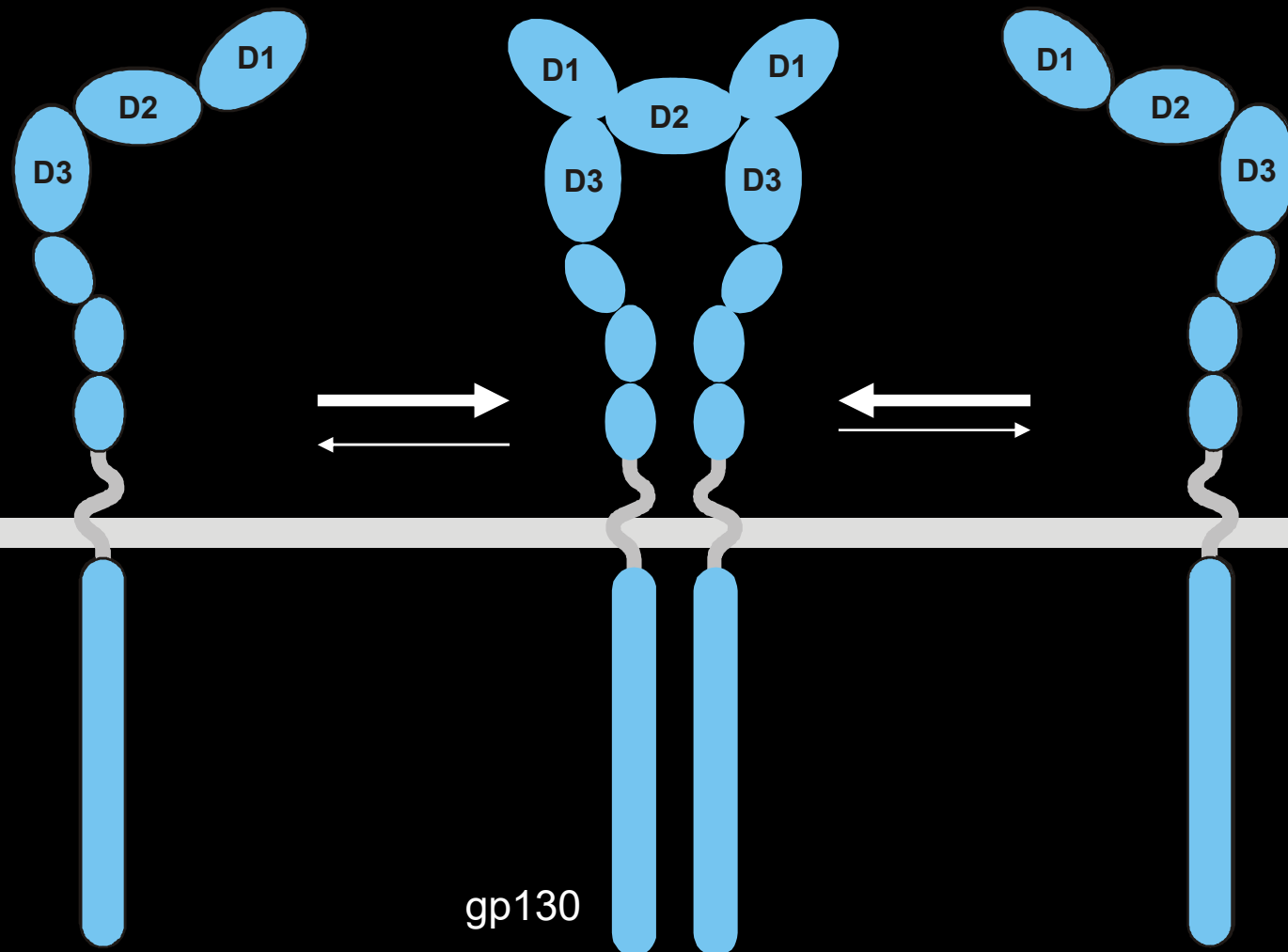
Summary

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

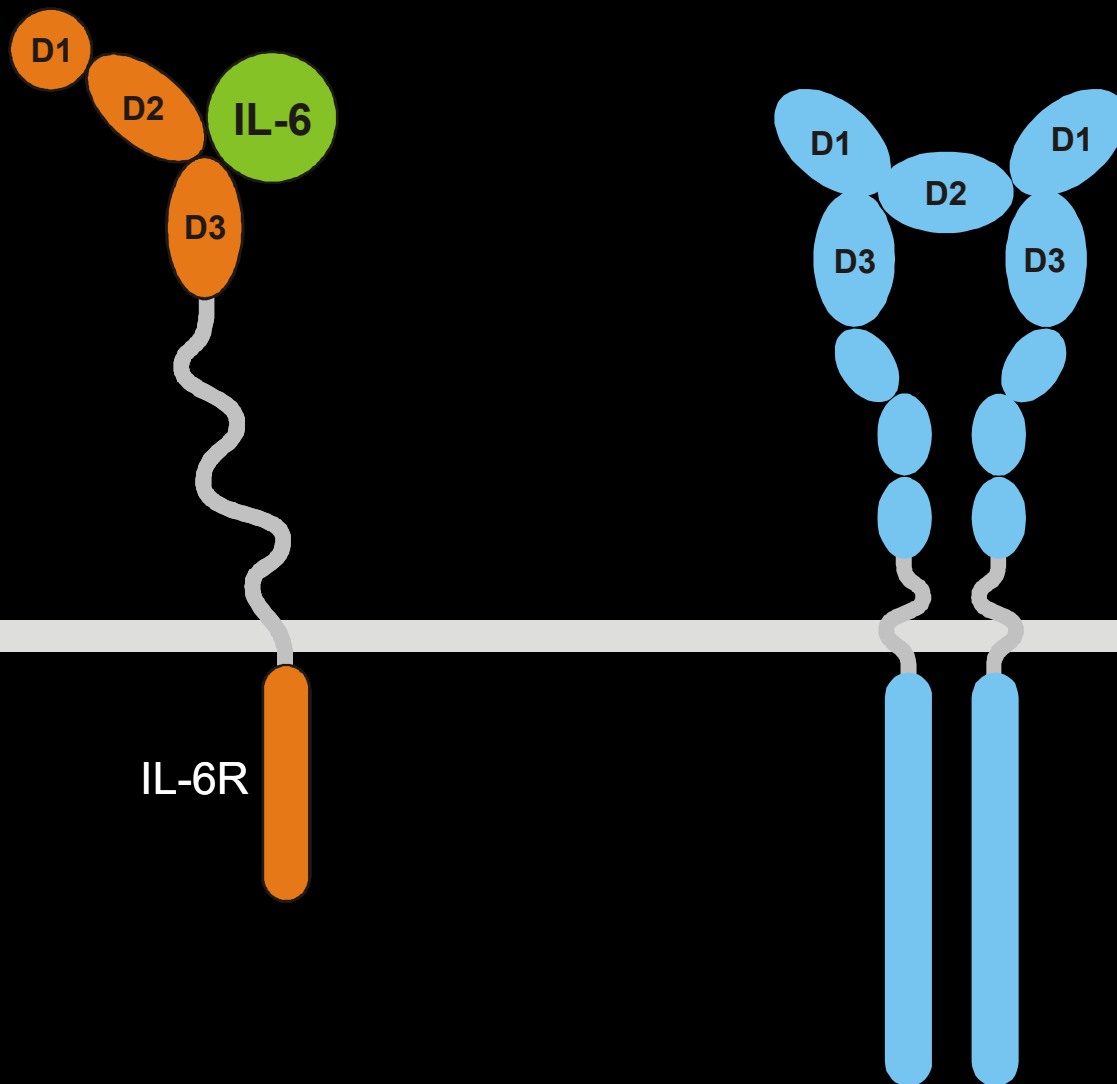
approach that detects stable interactions we show that fluorescence complementation of gp130 constructs tagged with matching 'halves' of fluorescent proteins increases upon IL-6 stimulation. Taken together, these findings suggest that transient gp130 homodimers on the plasma membrane are stabilized by 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

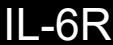
gp130 is present as a preformed dimer



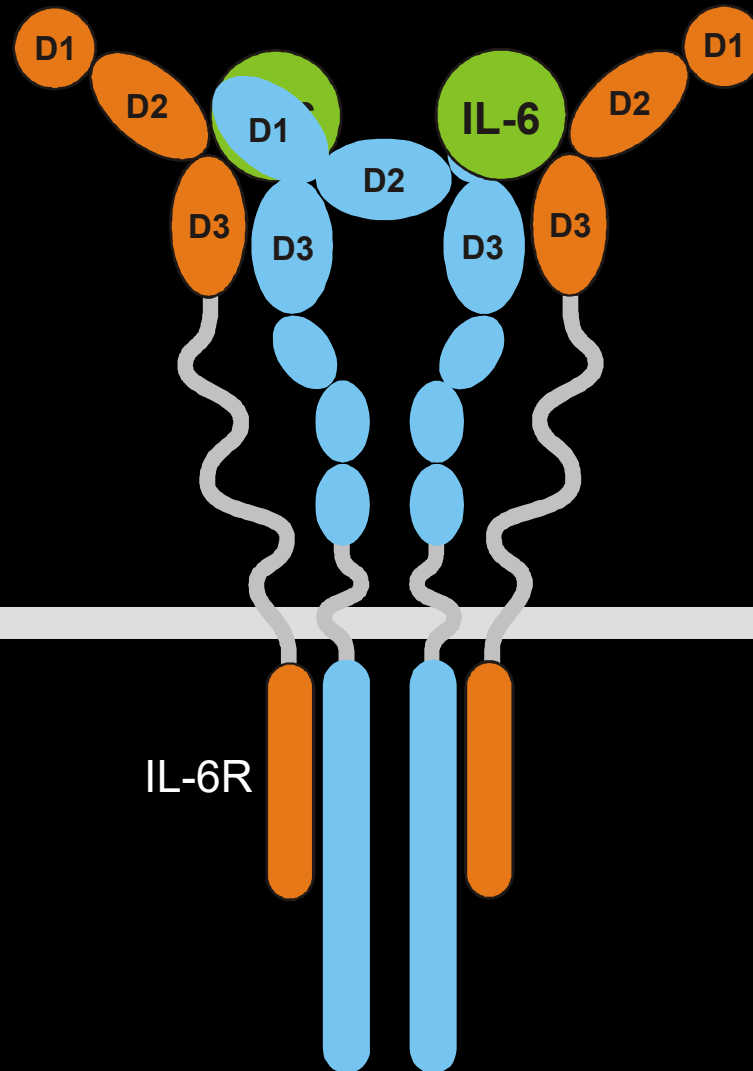
gp130 is present as a preformed dimer



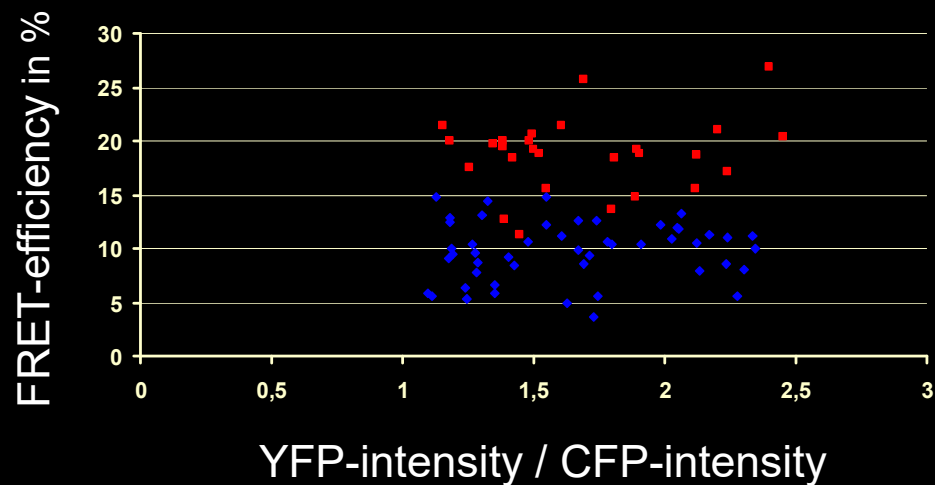
gp130 is present as a preformed dimer



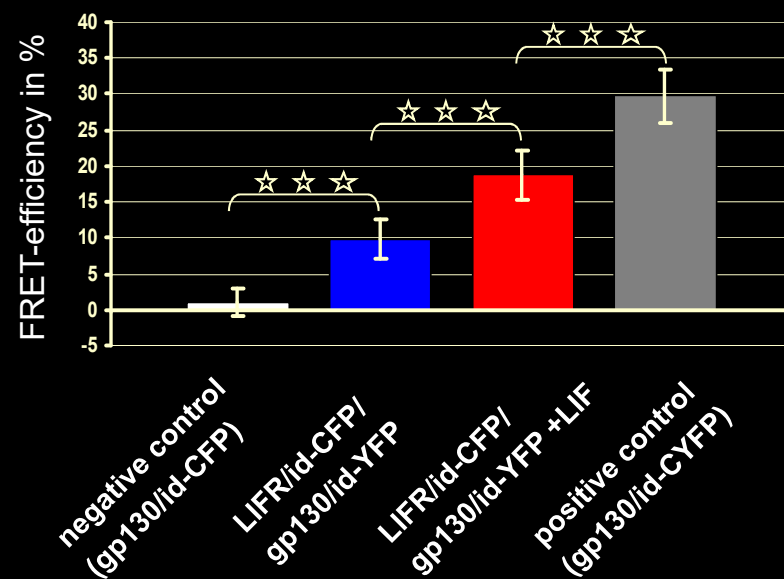
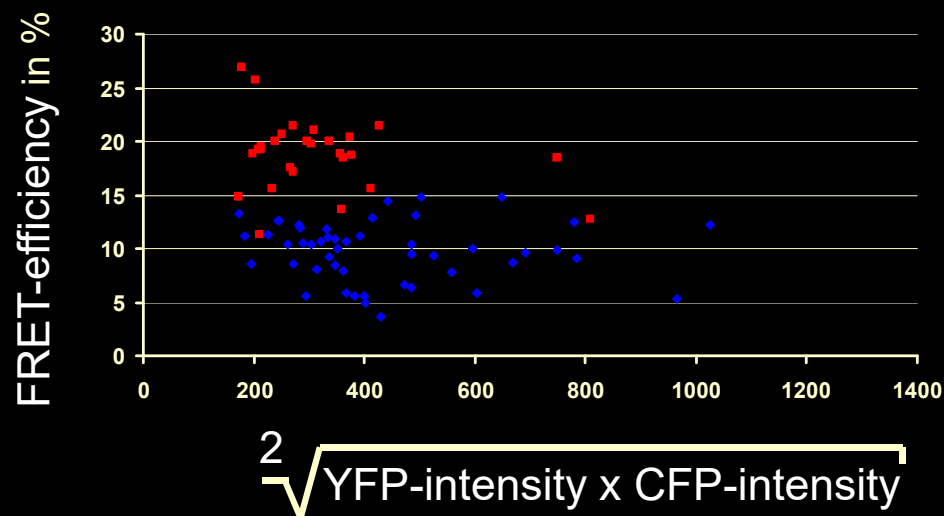
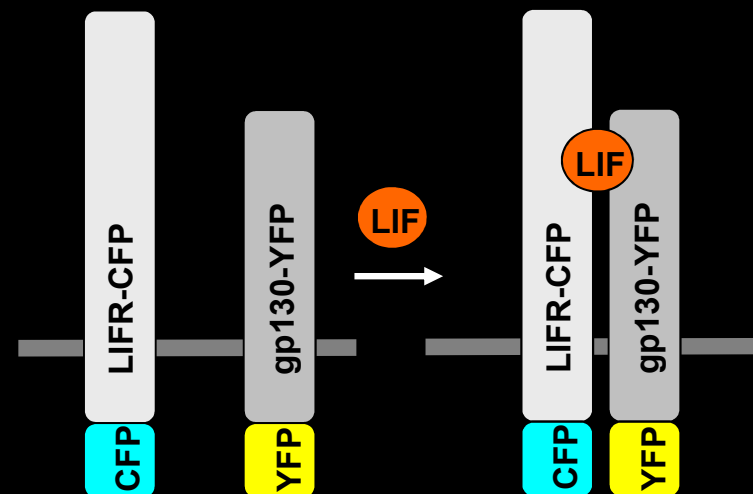
gp130 is present as a preformed dimer



Detection of ligand-induced LIFR/gp130 heterodimerization by FRET



■ + LIF
◆ unstimulated



Outline: Interleukin-6 signal transduction and its regulation

Part 1: Molecular mechanisms of IL-6 signal transduction

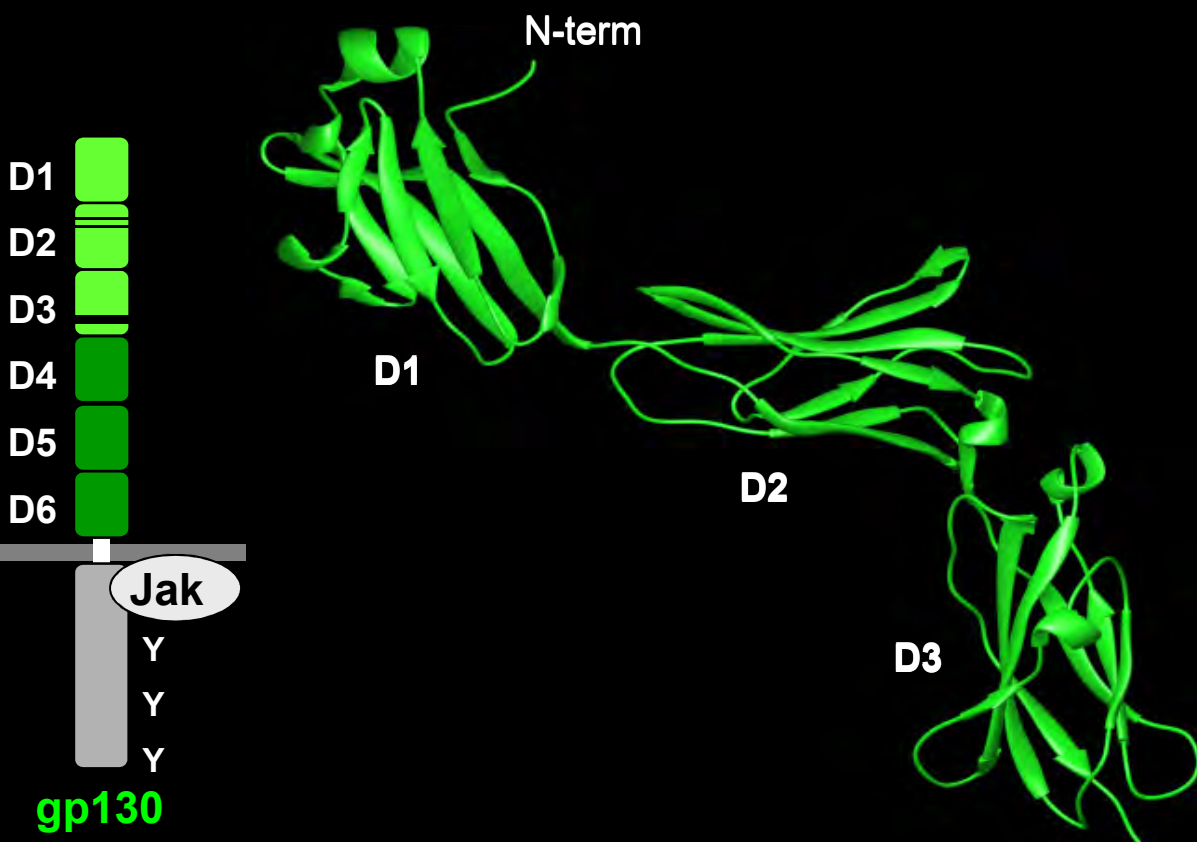
- **The acute phase response**
- **Identification of HSF as IL-6**
- **Structure and function of IL-6**
- **Acute phase protein synthesis induced by IL-6**
- **Molecular mechanism of IL-6 induced APP expression**
- **Formation of the IL-6-receptor complex**
- **Design of a highly potent IL-6 antagonist**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

Part 2: Regulation of IL-6 signal transduction

Design of a highly potent IL-6 antagonist

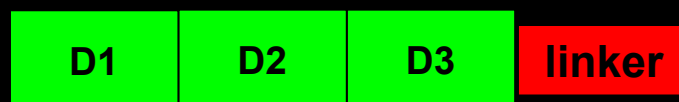
Human IL-6 Receptor Fusion Protein (hIL-6RFP)

gp130



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

gp130



N-term

D1

D2

D3

D1

D2

D3

D4

D5

D6

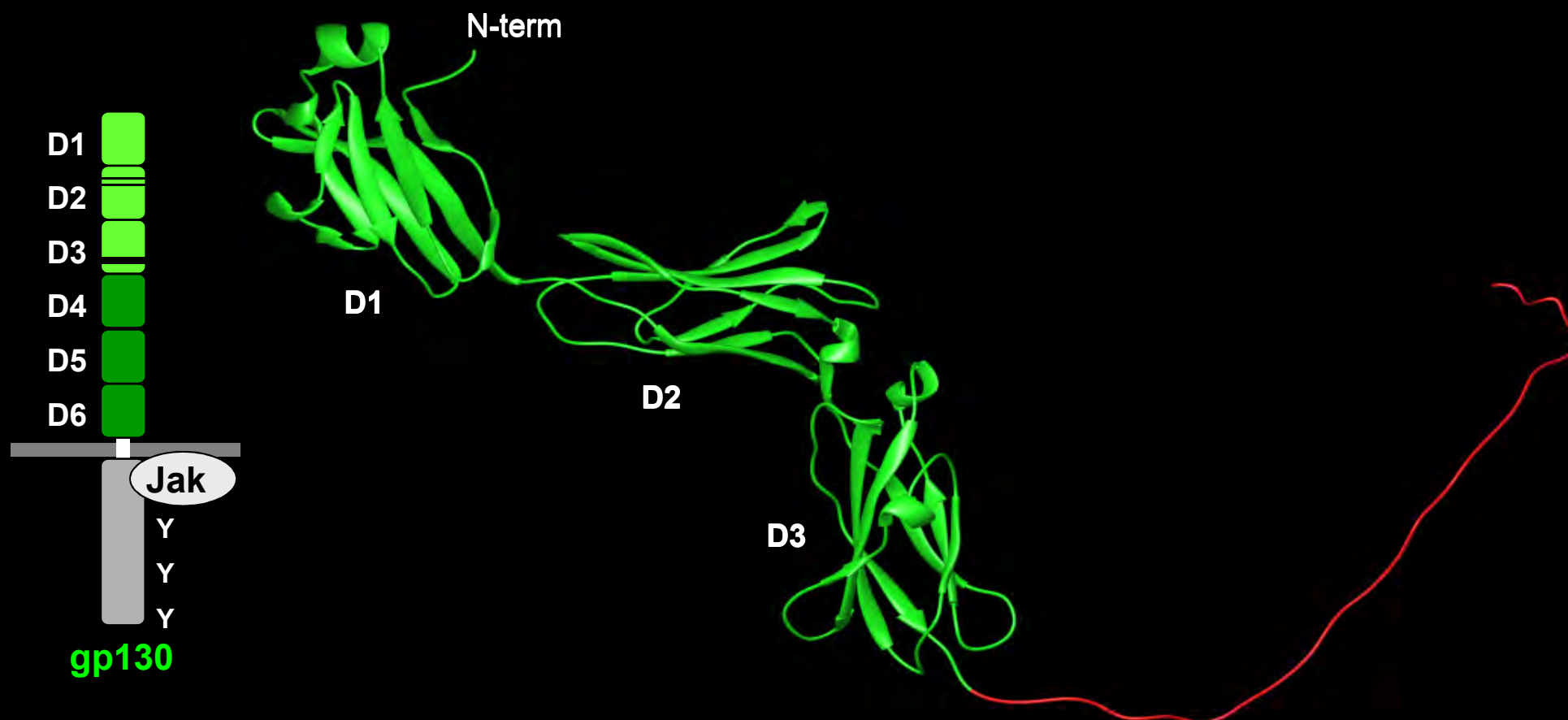
Jak

Y

Y

Y

gp130



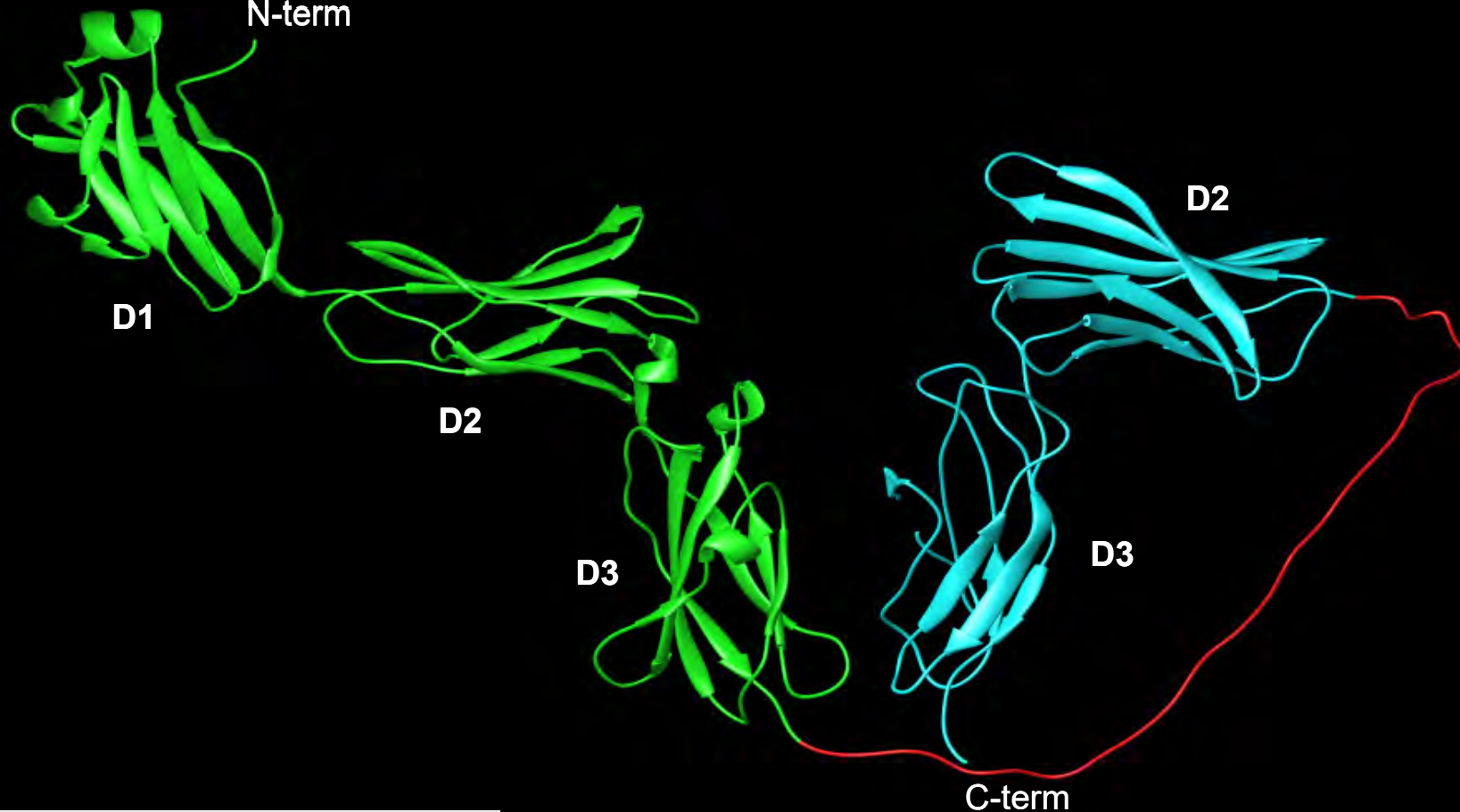
Human IL-6 Receptor Fusion Protein (hIL-6RFP)

gp130

IL-6R α



N-term



D1

D2

D3

D4

D5

D6

D2

D3

D1

D2

D3

Jak

Y

Y

Y

gp130

IL-6R α

C-term

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

gp130

IL-6R α



N-term

IL-6

D1

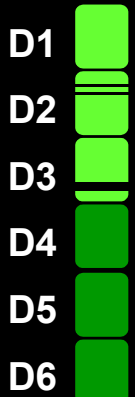
D2

D3

D2

D3

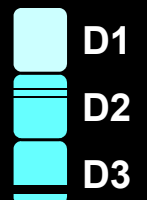
C-term



Jak

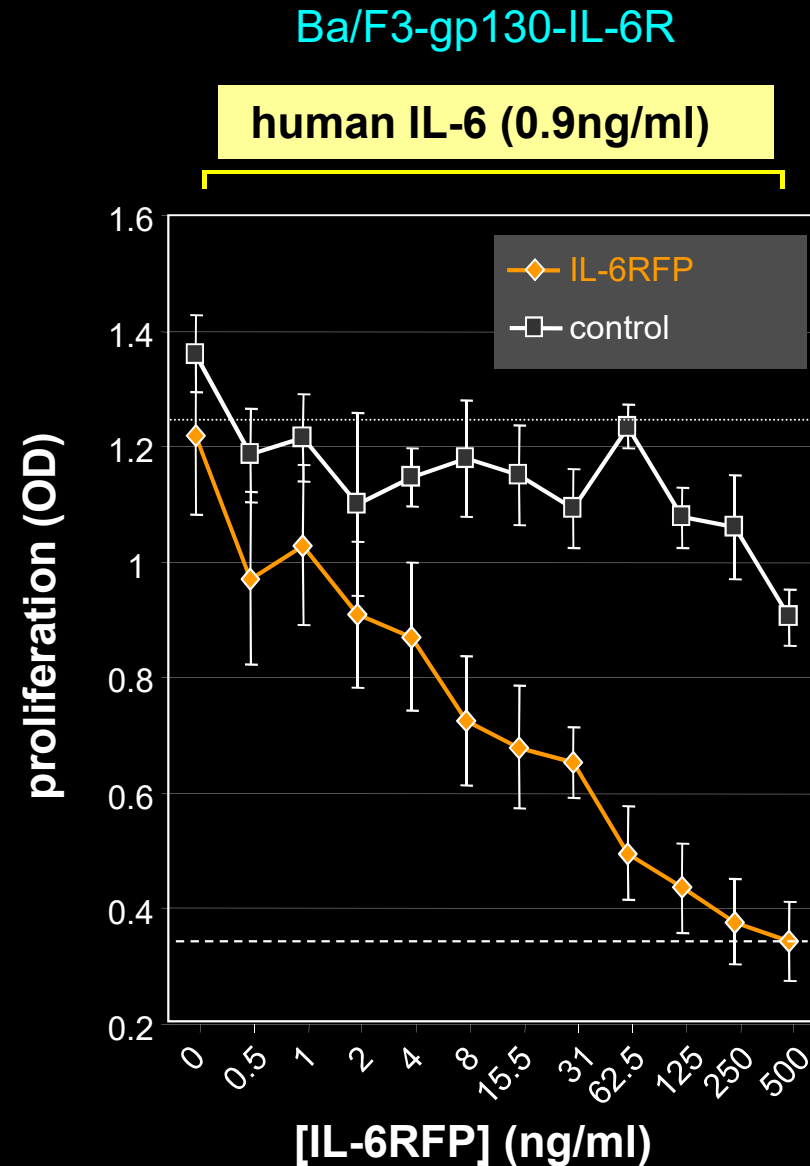
Y
Y
Y

gp130

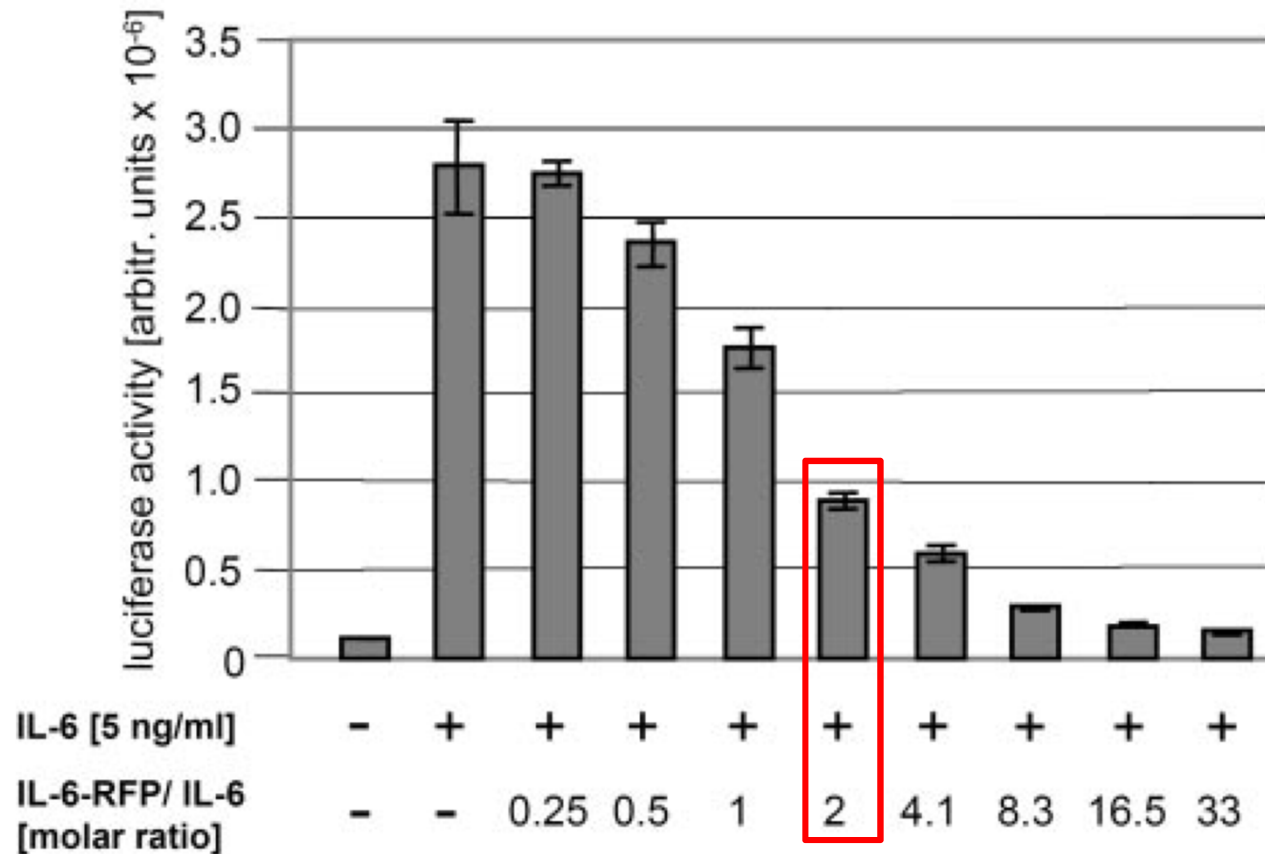


IL-6R α

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



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



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

29 citations
(07/2022)

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



Cécile Ancey



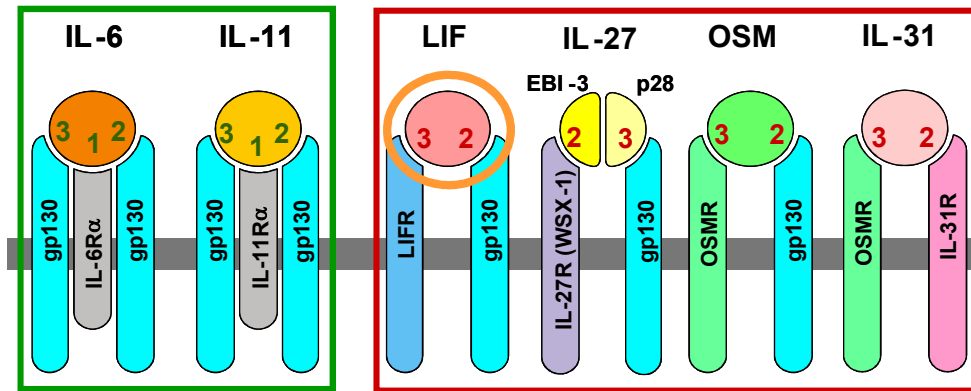
Andrea Küster



Serge Haan

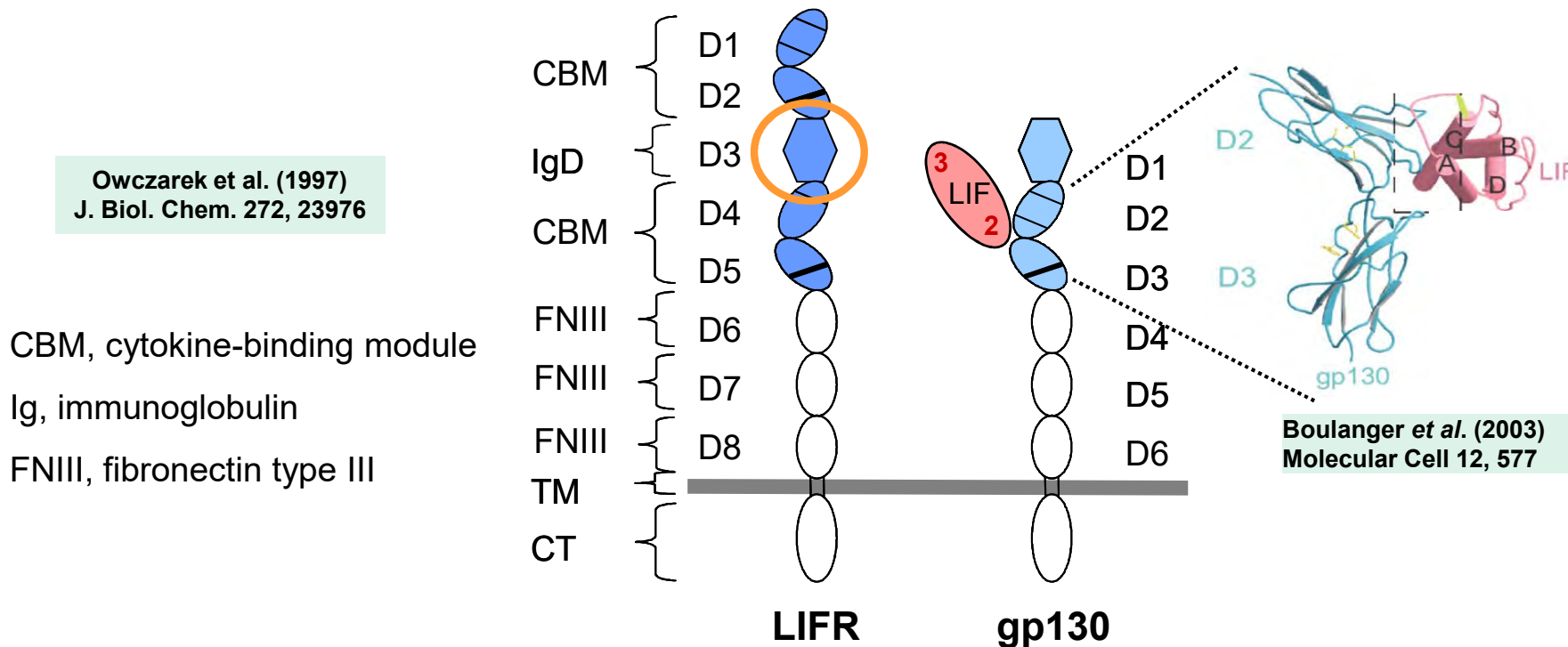
This inhibitor strategy was also applicable to other cytokines that signal *via* heteromeric receptor complexes, e.g. LIF-R and OSM-R

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)



Outline: Interleukin-6 signal transduction and its regulation

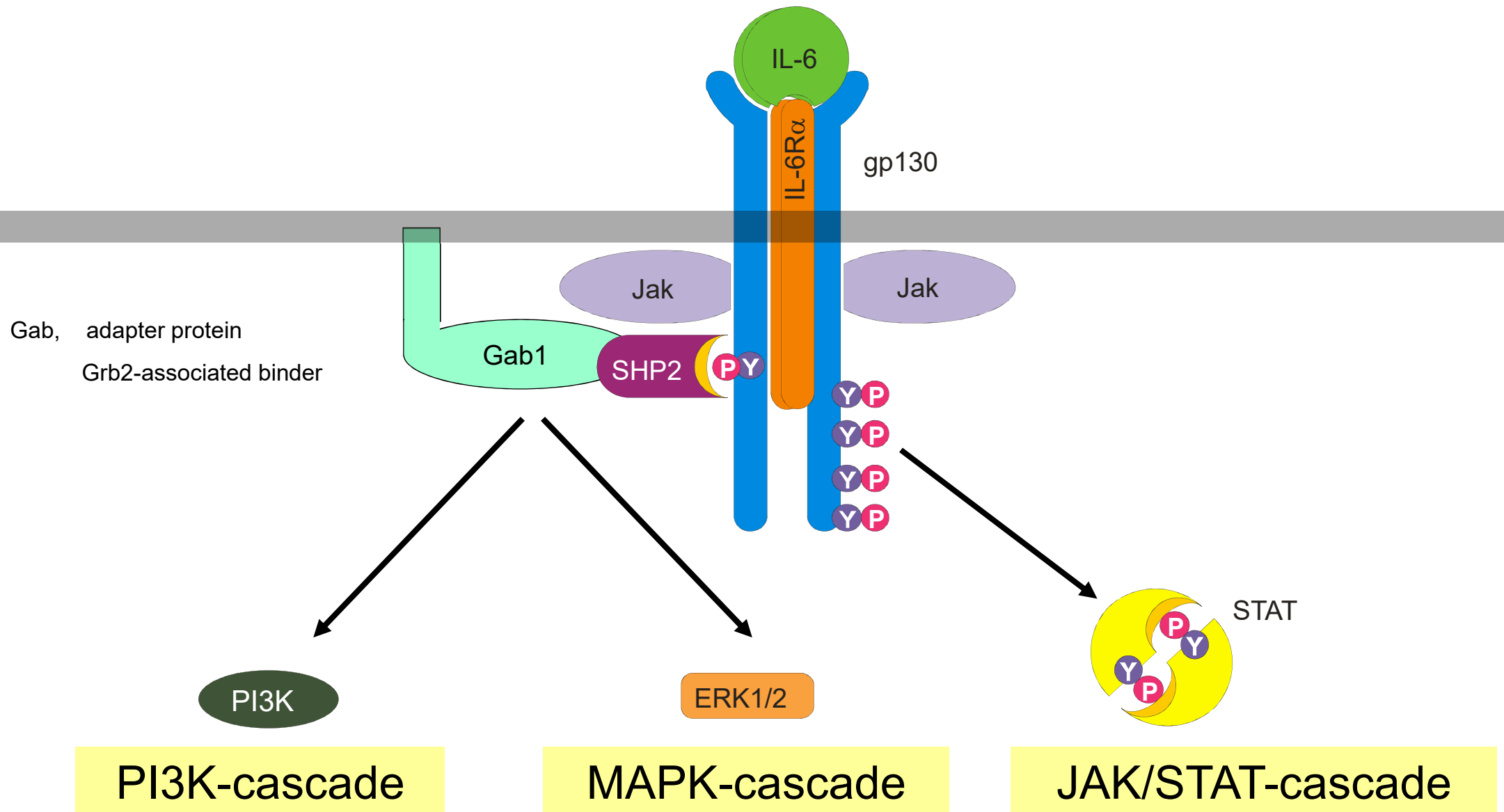
Part 1: Molecular mechanisms of IL-6 signal transduction

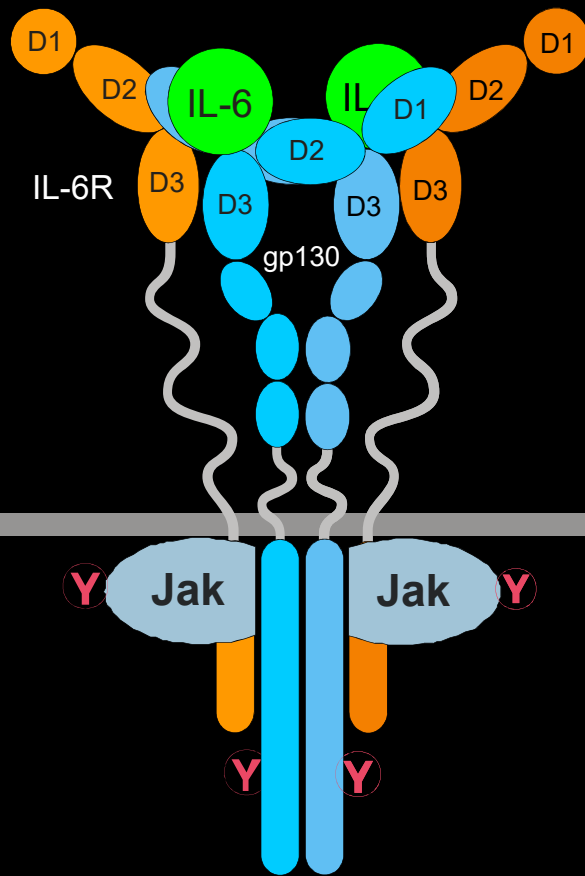
- **The acute phase response**
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- **Structure and function of IL-6**
- **Acute phase protein synthesis induced by IL-6**
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- **Molecular mechanisms of IL-6 signal transduction**
- **Nuclear translocation of STAT3-YFP**

Part 2: Regulation of IL-6 signal transduction

Molecular mechanism of IL-6 signal transduction

IL-6-initiated signaling pathways



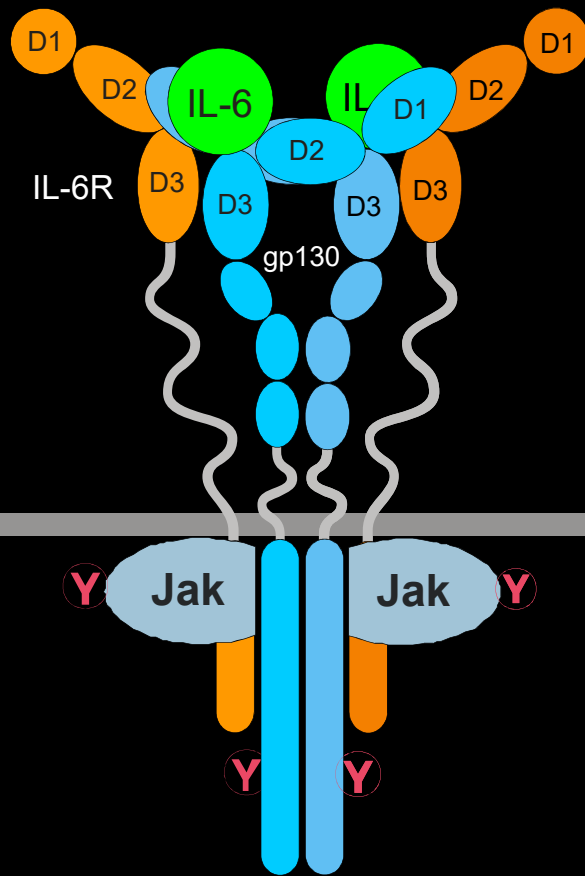


Lütticken et al. (1994) Science 263, 89-92

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

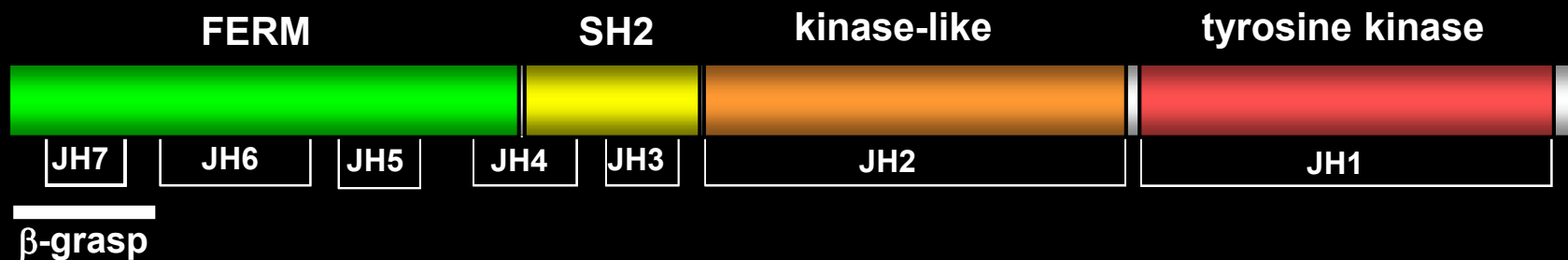
517 citations (07/2022)

Janus

Roman god of “doors and gates”

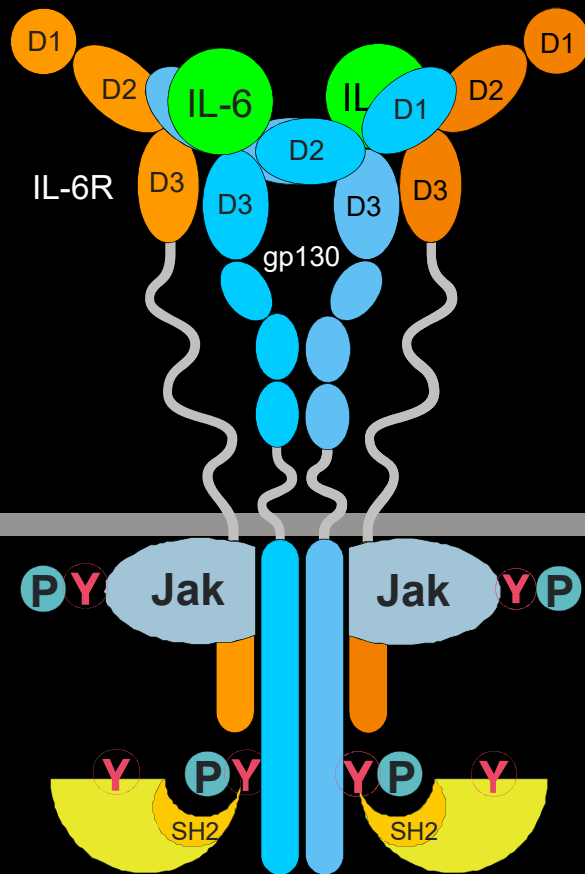


Jak = Janus kinase









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

691 citations (2018)

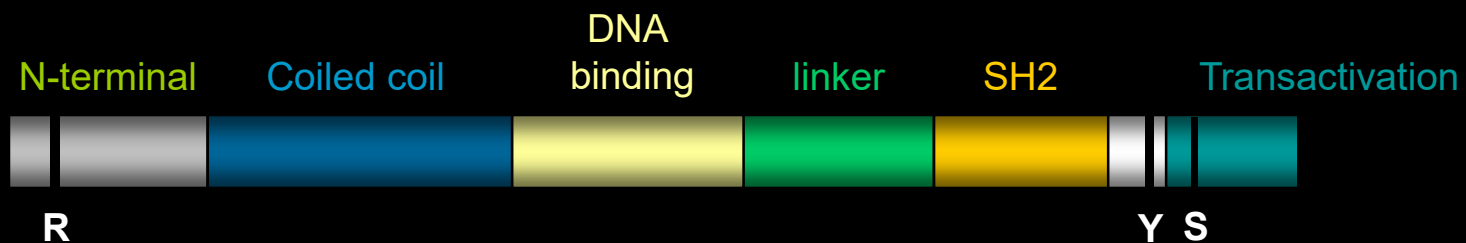
Wegenka et al. (1993) Mol Cell Biol 13, 276-288

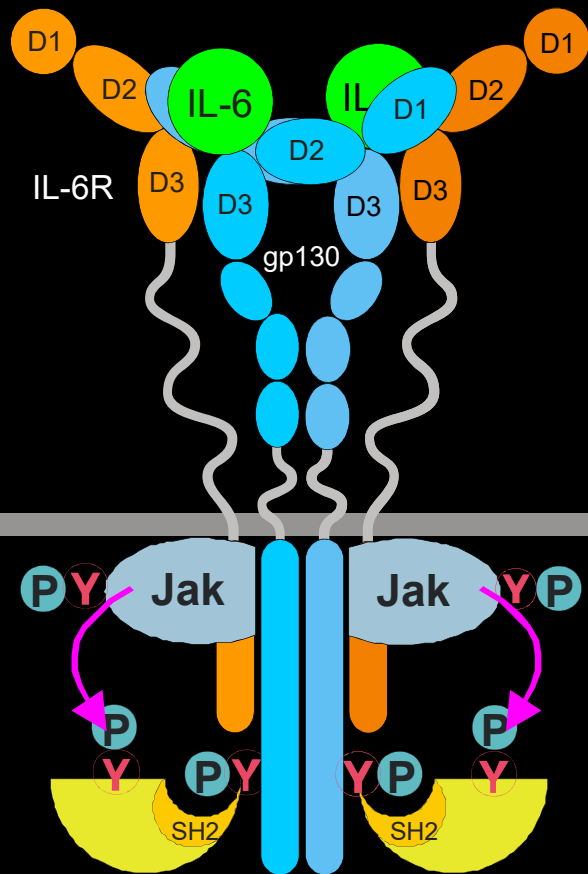
487 citations (2018)

STAT1 / STAT3

STAT1 / STAT3

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





STAT1 / STAT3

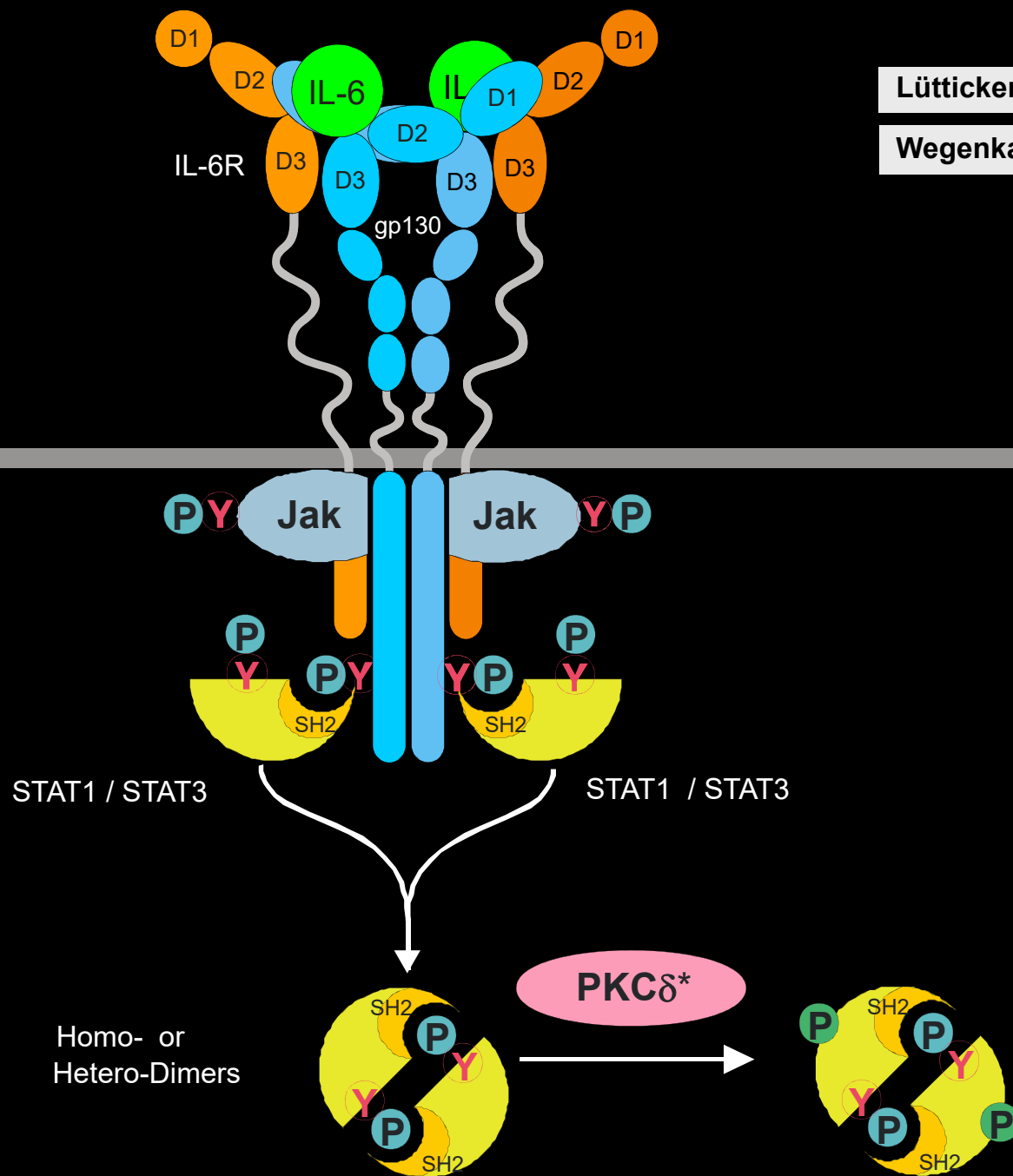
STAT1 / STAT3

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

740 citations (07/2022)

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517 citations (07/2022)



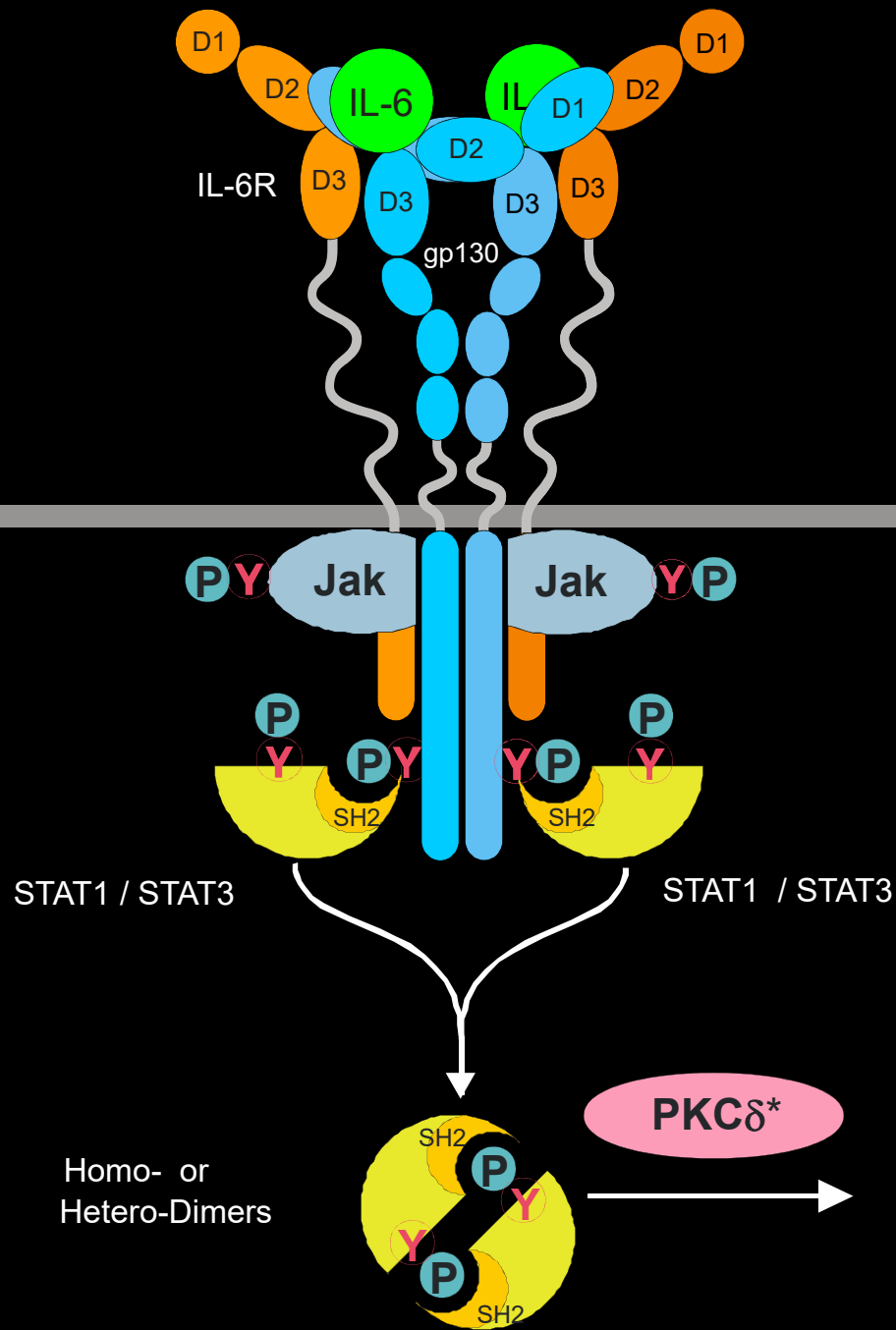
Lütticken et al. (1994) Science 263, 89-92

740 citations (07/2022)

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

517 citations (07/2022)

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



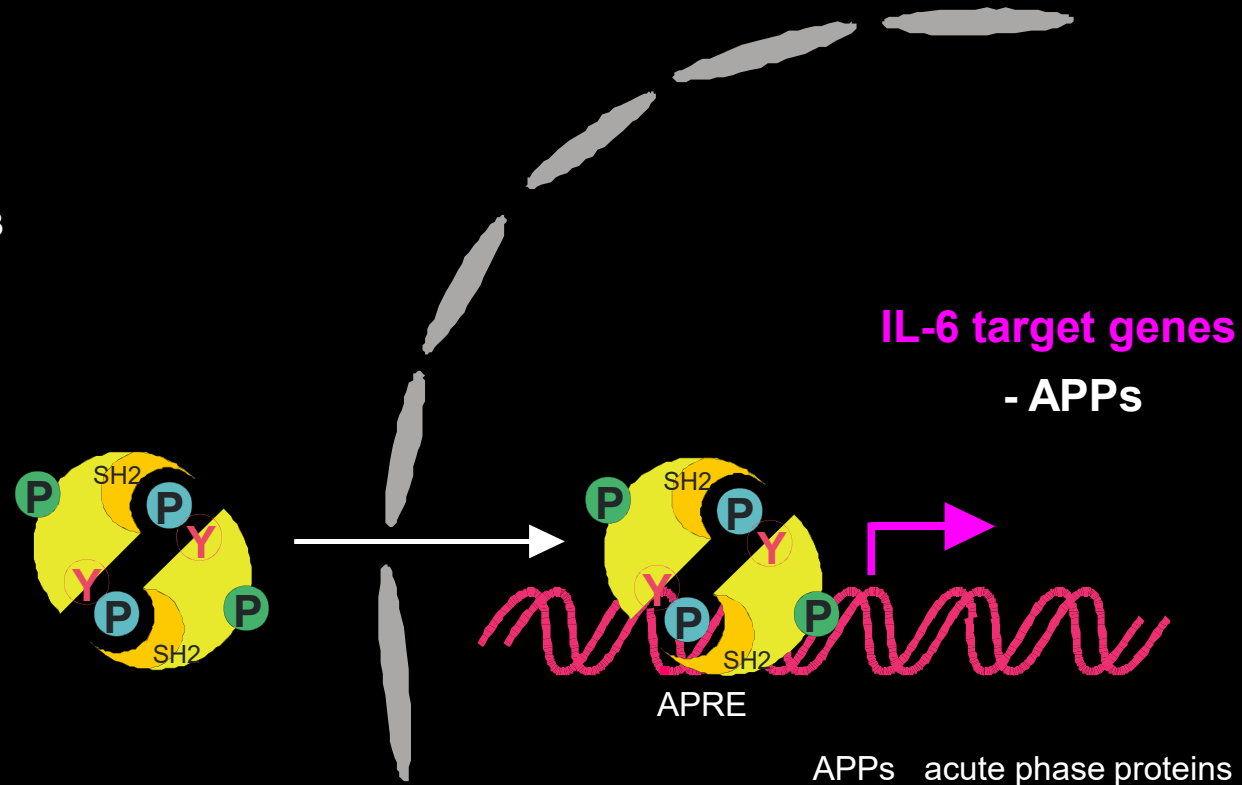
Lütticken et al. (1994) Science 263, 89-92

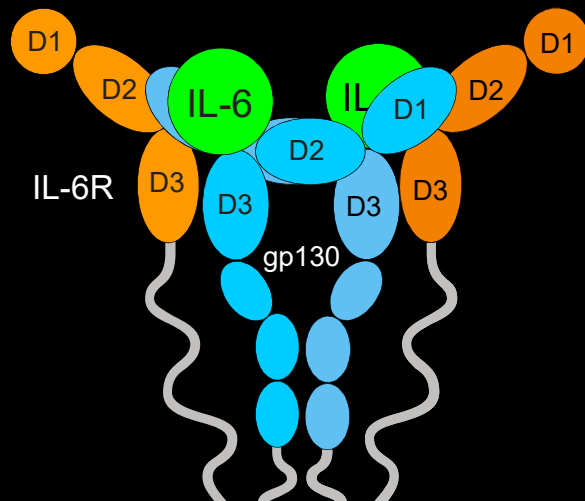
740 citations (07/2022)

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

517 citations (07/2022)

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



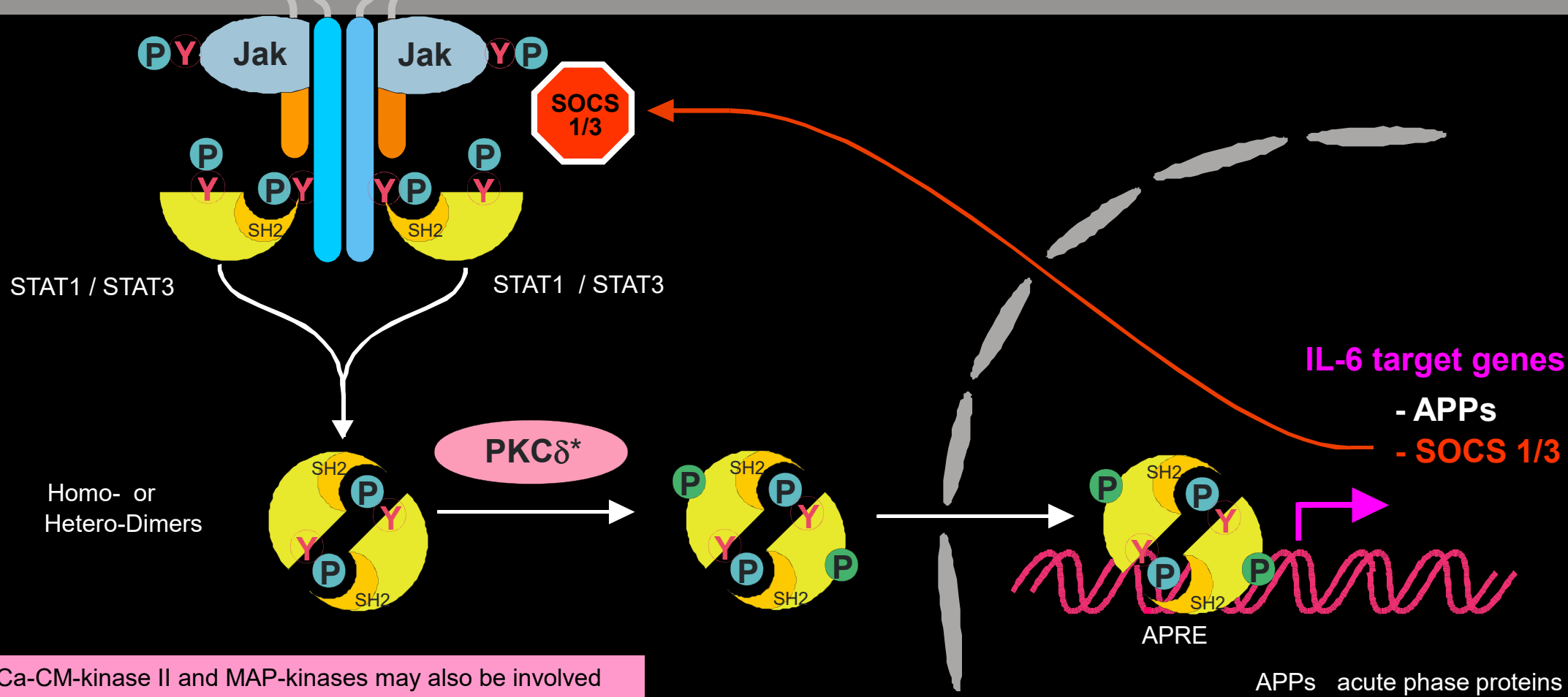


Lütticken et al. (1994) Science 263, 89-92

740 citations (07/2022)

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

517 citations (07/2022)



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

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

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 IFN γ

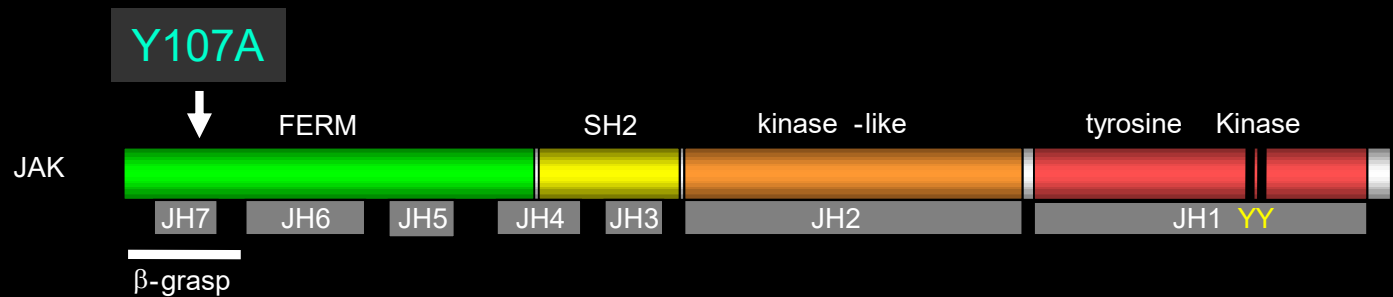
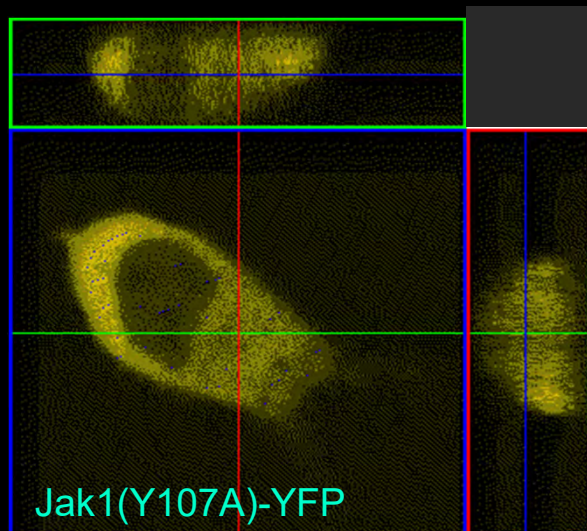
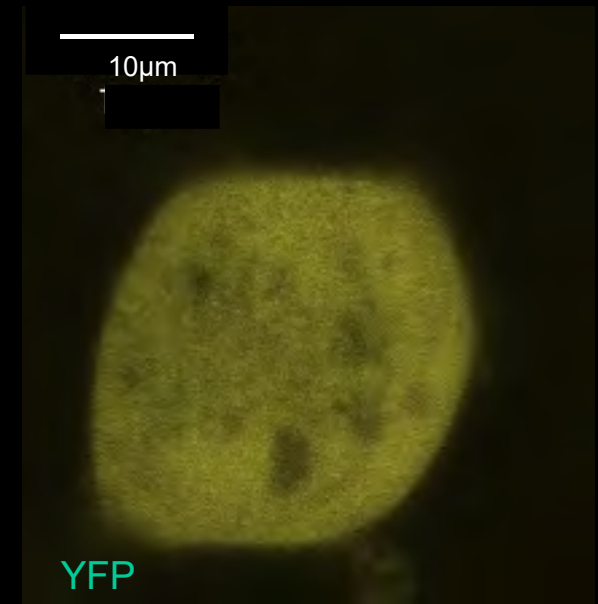
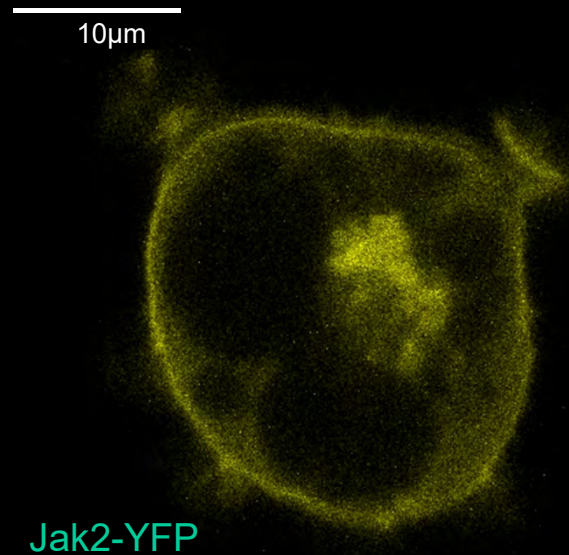
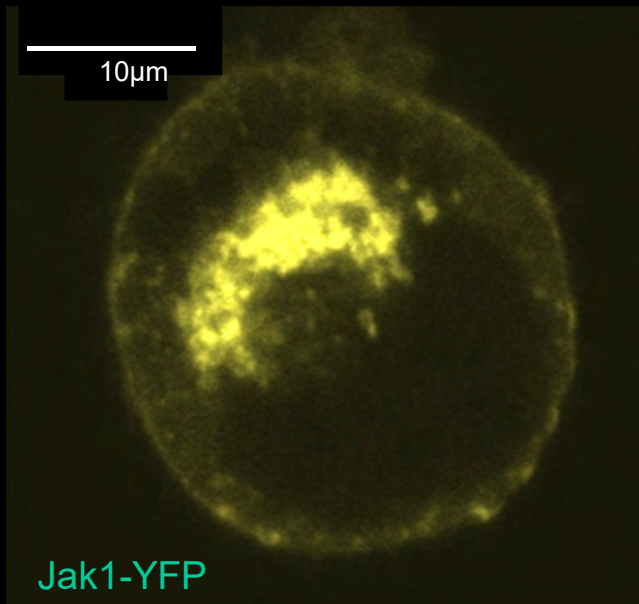
Parganas et al., Neubauer et al. (1998) Cell

Interestingly, the heterodimeric LIFR binds at least 3 of the 4 JAK family members, JAK1, JAK2, and TYK2.

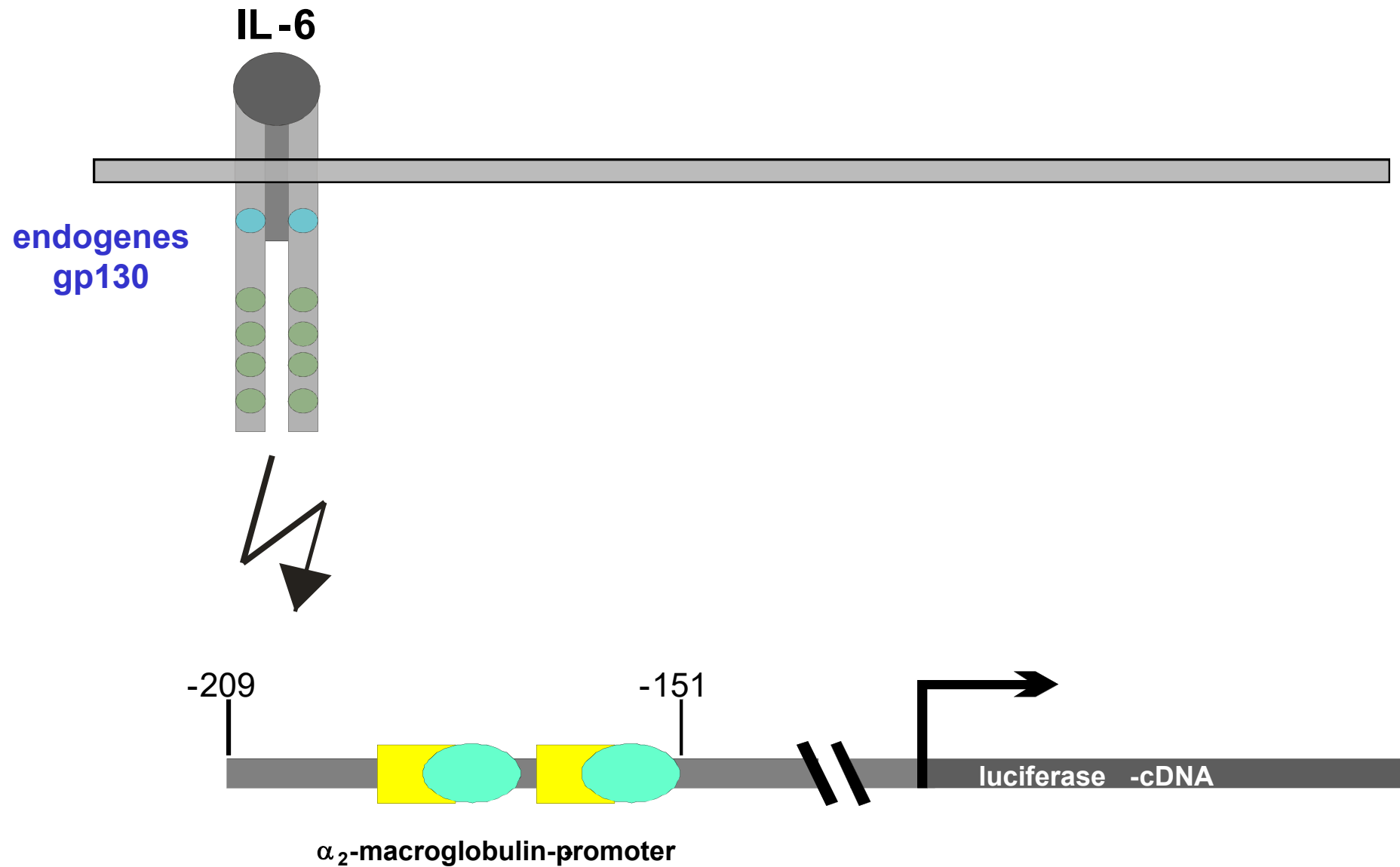
In the absence of JAK1, but not JAK2 or TYK2 LIF signaling is abrogated, exactly like in the case of IL-6 receptor gp130. This indicates that JAK1 has a dominant role.

gp130 - Jak interaction

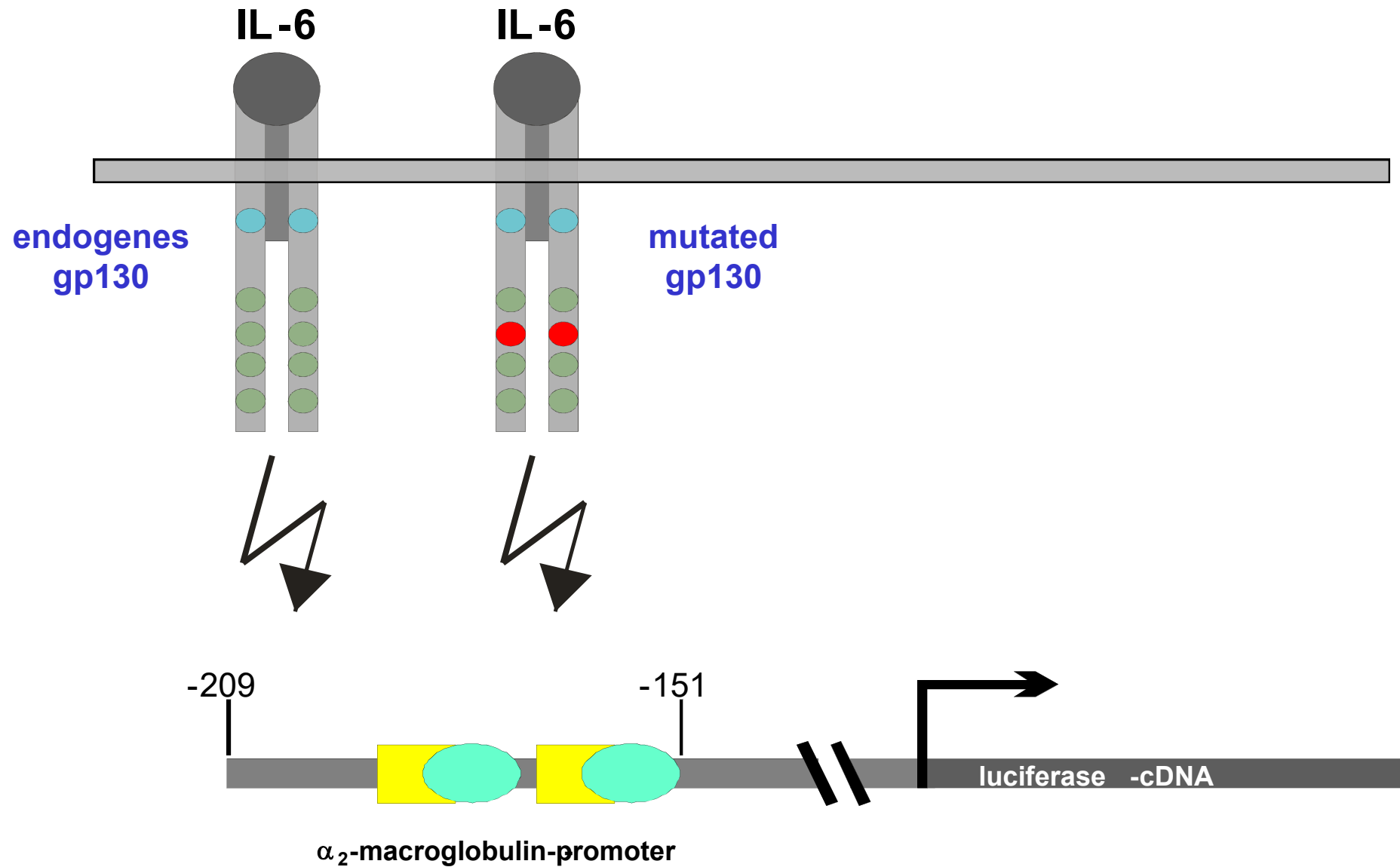
Jak1 and Jak2 are membrane-associated kinases



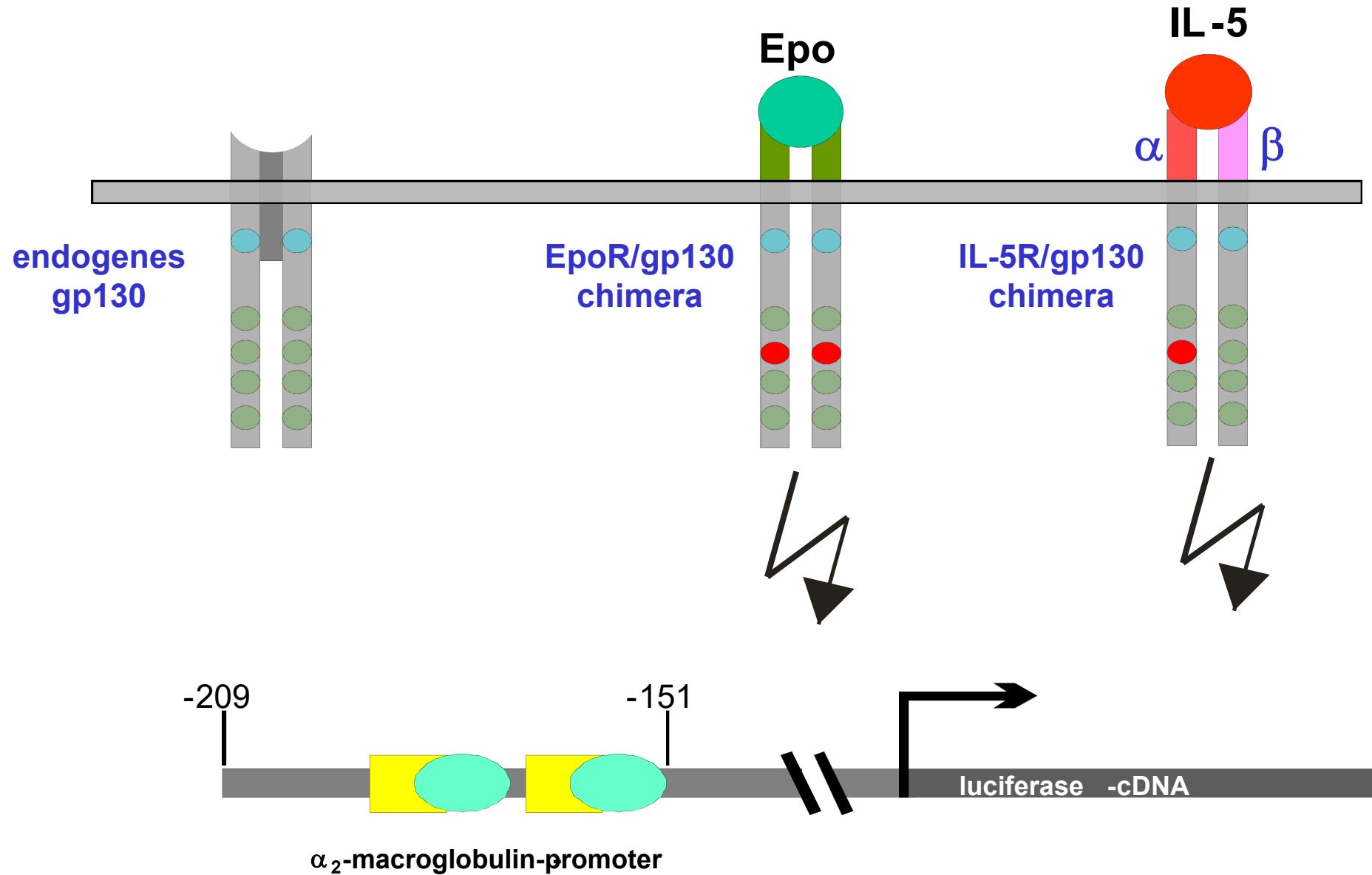
Using chimeric receptor systems

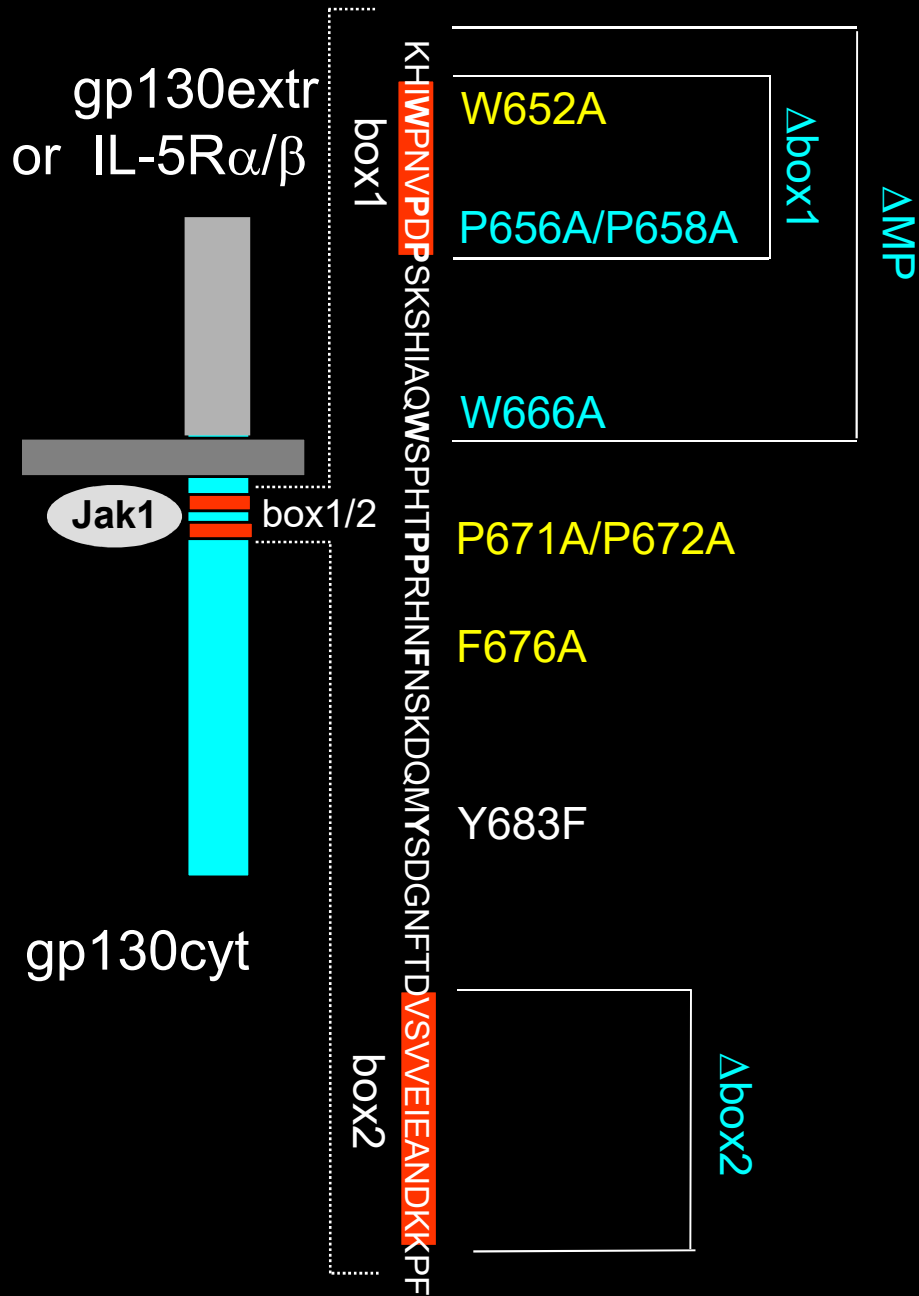


Using chimeric receptor systems



Using chimeric receptor systems





Mutational analysis of the membrane-proximal region of gp130

Δbox1	}	no Jak association
P656A/P658A		
ΔMP		
W666A		
Δbox2	}	normal Jak association, signal transduction impaired
W652A		
P671A/P672A		
F676A		
Y683F		no effect

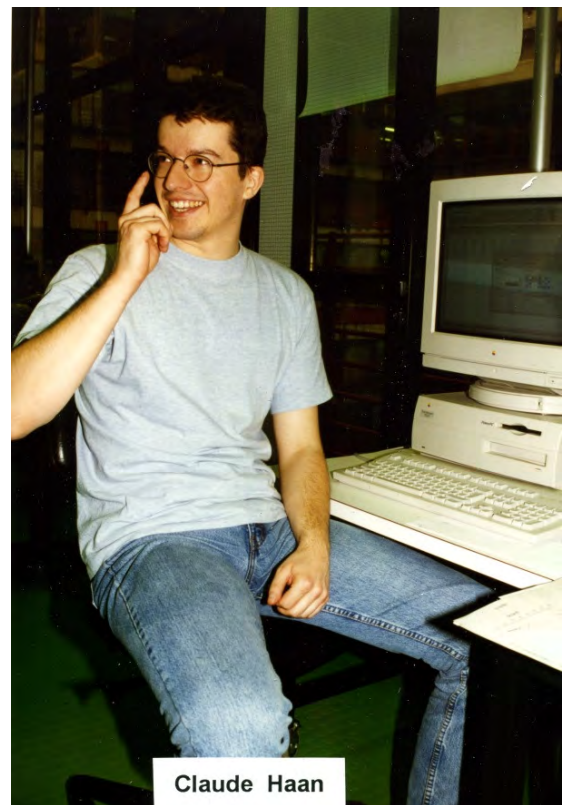
Mapping of a Region within the N Terminus of Jak1 Involved in Cytokine Receptor Interaction*

Received for publication, July 2, 2001

Published, JBC Papers in Press, July 23, 2001, DOI 10.1074/jbc.M106135200

51 citations
(07/2018)

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



Claude Haan



Heike Hermanns

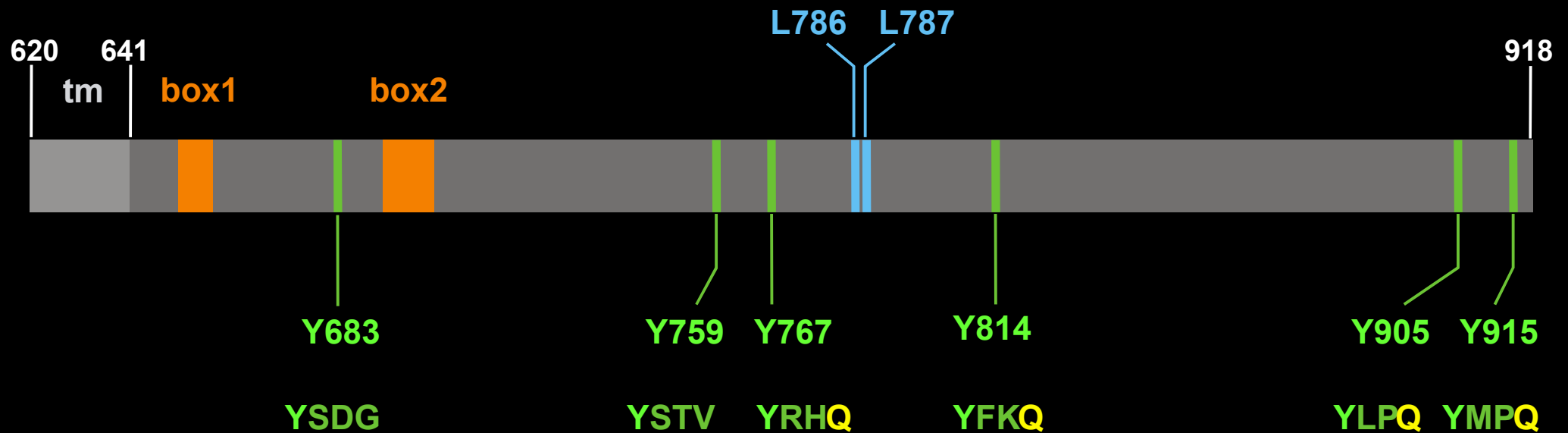


Joachim Grötzinger



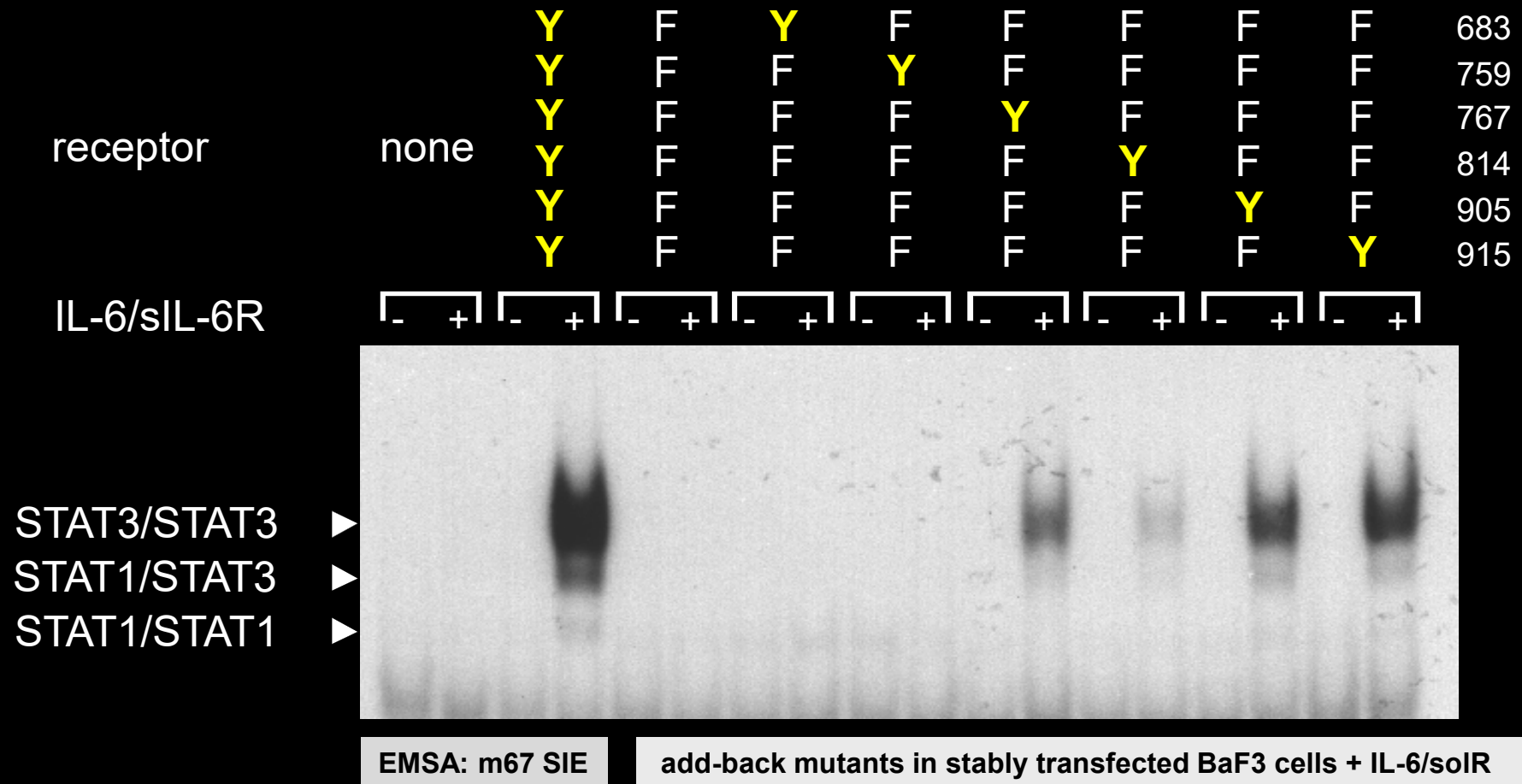
Iris Behrmann

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

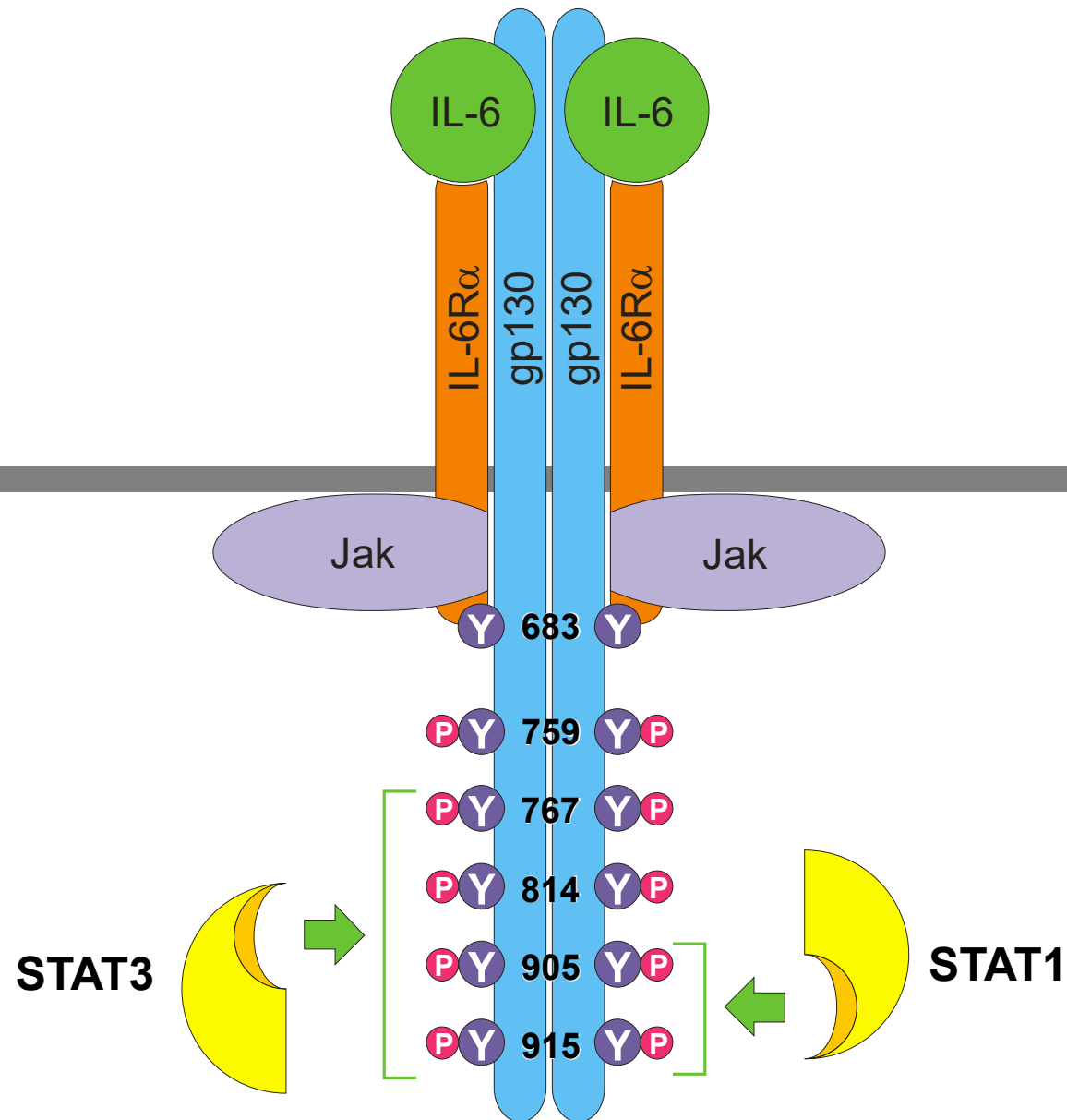


intracellular domain of gp130

Activation of STAT3 via individual tyrosine motifs in gp130



STAT3 and STAT1 recruitment sites in gp130



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)

Claudia Gerhartz‡, Birgit Heesel‡, Jürgen Sasse‡, Ulrike Hemmann‡, Christiane Landgraf§, Jens Schneider-Mergener§, Friedemann Horn‡, Peter C. Heinrich‡, and Lutz Graeve‡¶

From the ‡Institute of Biochemistry, Rheinisch-Westfälische Technische Hochschule Aachen, 52057 Aachen, Germany and the §Institute of Medical Immunology, Universitätsklinikum Charité, 10098 Berlin, Germany



The Cytoplasmic Tyrosine Motifs in Full-Length Glycoprotein 130 Have Different Roles in IL-6 Signal Transduction¹

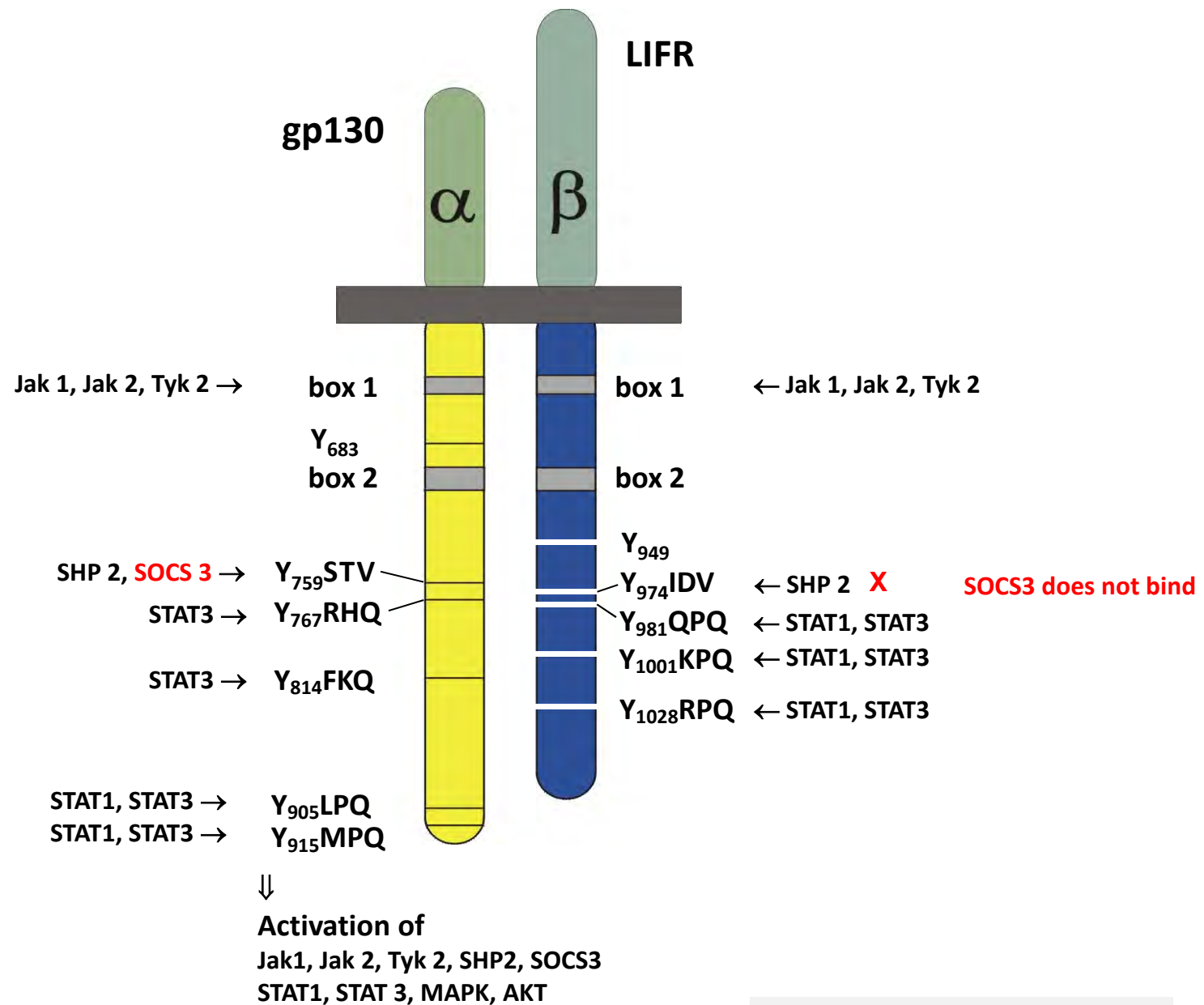
70 citations (07/2022)

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



Outline: Interleukin-6 signal transduction and its regulation

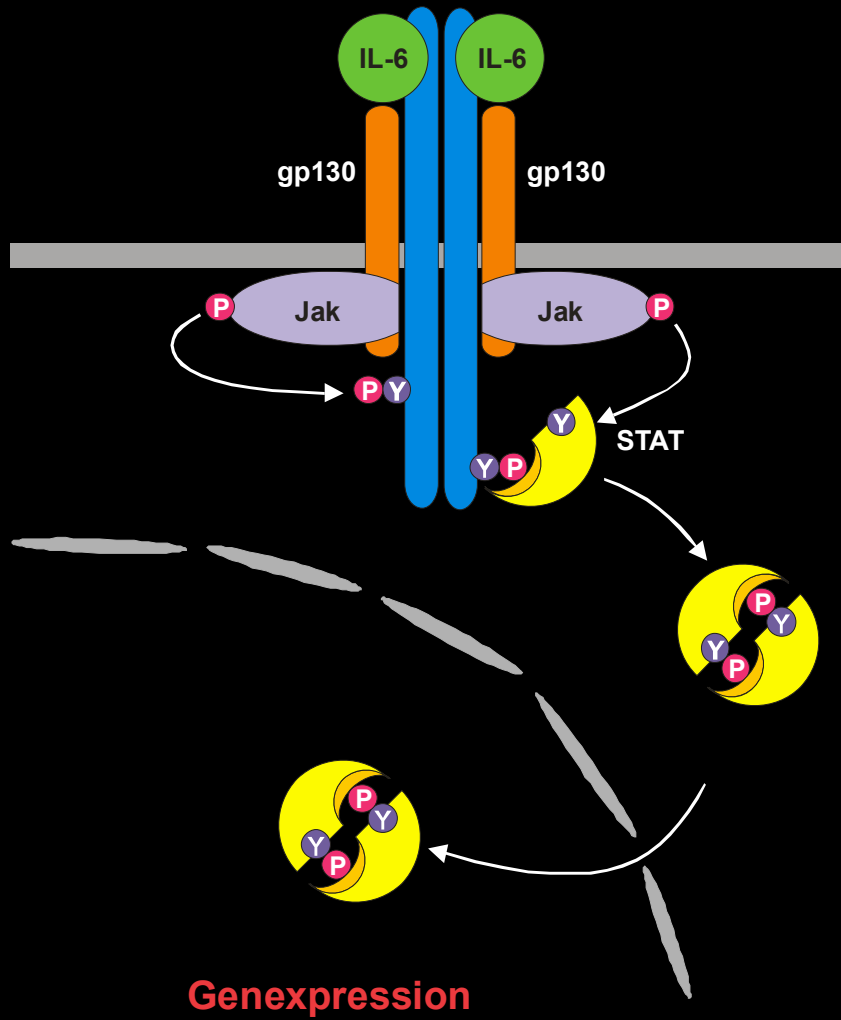
Part 1: Molecular mechanisms of IL-6 signal transduction

- **The acute phase response**
- **Identification of HSF as IL-6**
- **Structure and function of IL-6**
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- **Formation of the IL-6-receptor complex**
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- **Nuclear translocation of STAT3-YFP**

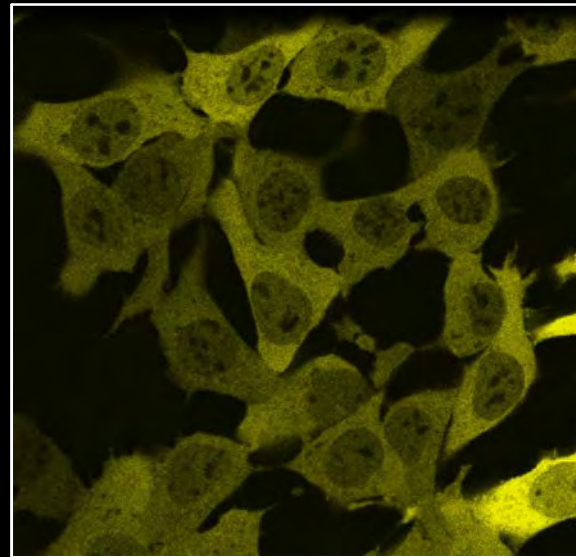
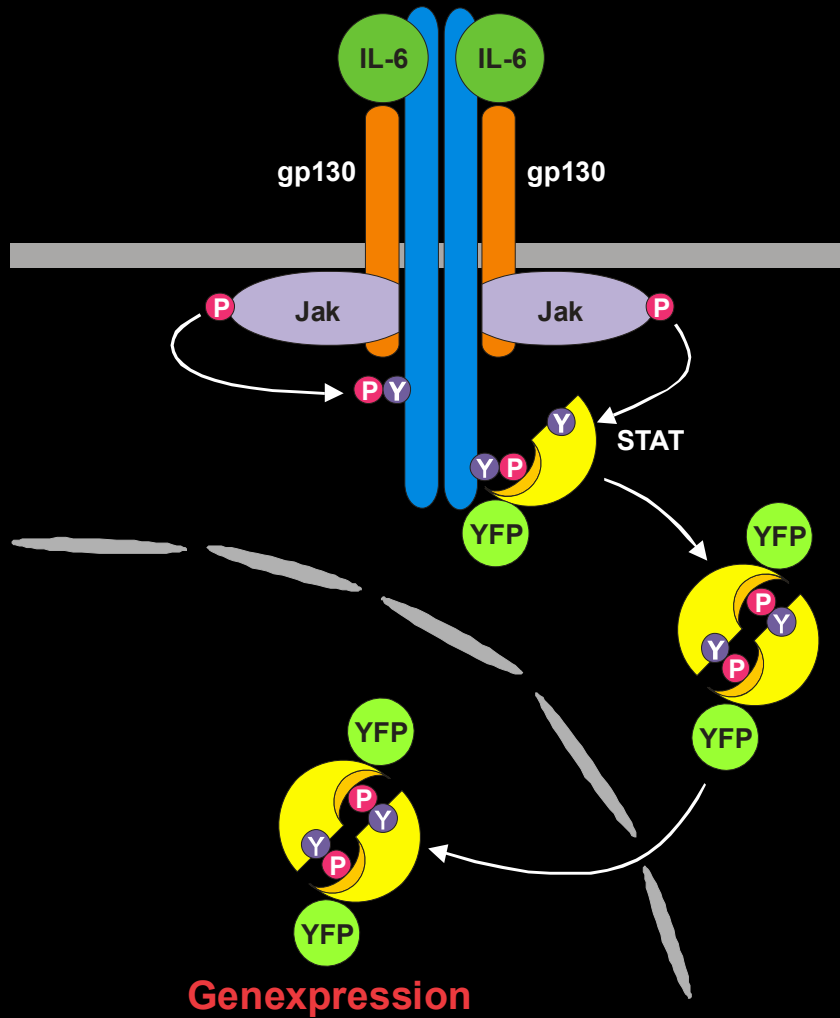
Part 2: Regulation of IL-6 signal transduction

Nuclear translocation of STAT3 -YFP

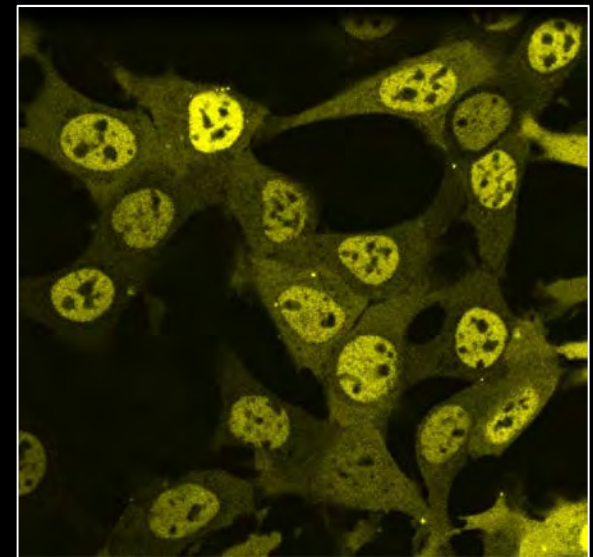
Nuclear translocation of STAT 3



Nuclear translocation of STAT3-YFP



0 min



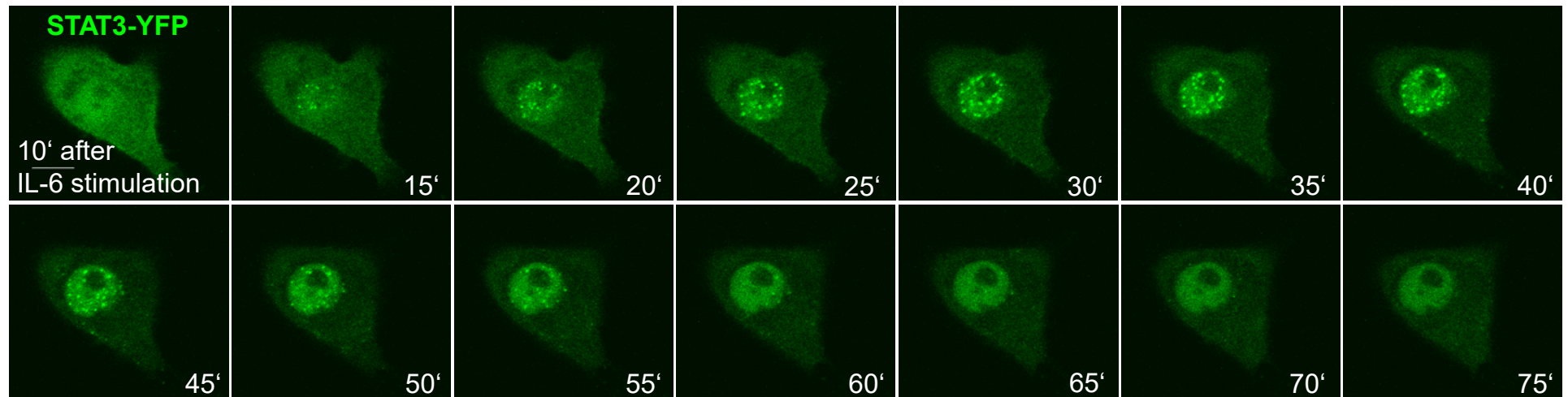
60 min

Lap time 60 mins

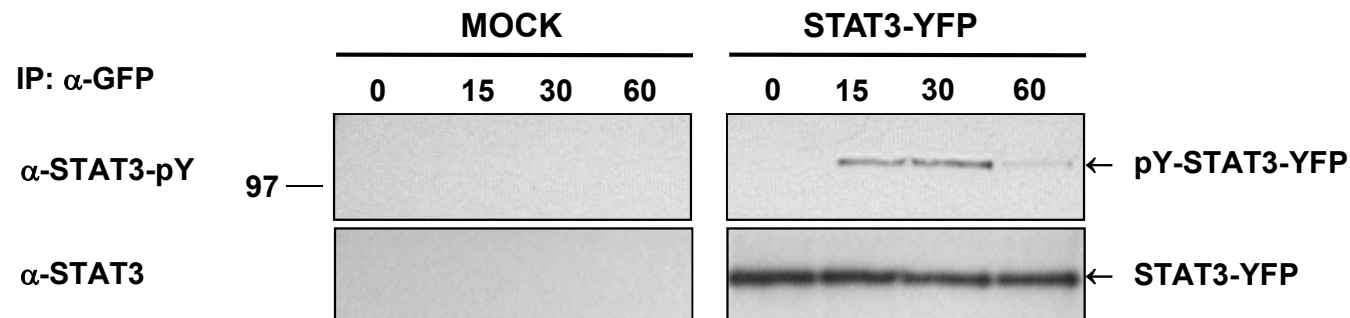
HepG2 cells

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

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

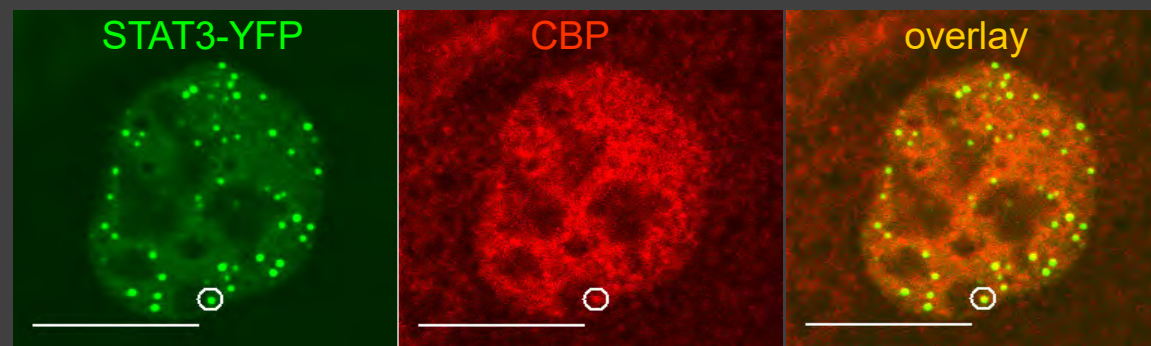
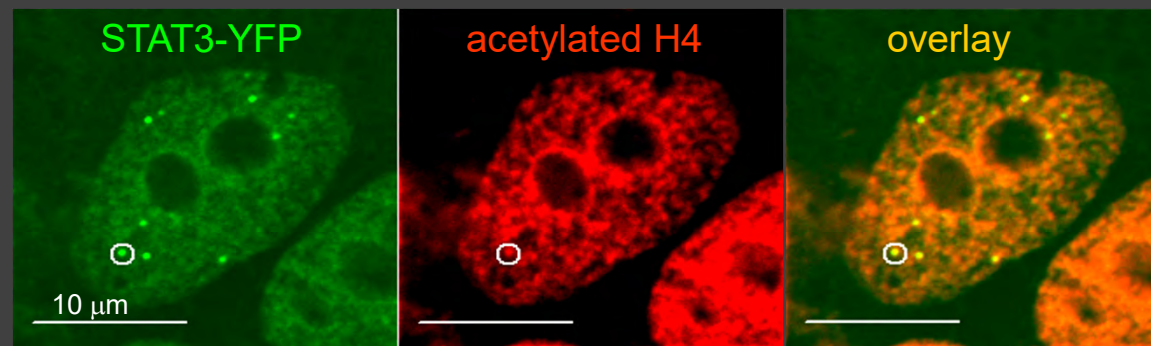


- nuclear body formation correlates with tyrosine phosphorylation of STAT3



STAT3 nuclear bodies are associated with transcriptionally active chromatin

- immunofluorescence:



CBP, CREB binding protein marker for transcriptional active chromatin

no co-localization with PML-bodies

STAT3 is enriched in nuclear bodies

Andreas Herrmann¹, Ulrike Sommer¹, Albert L. Pranada¹, Bernd Giese¹, Andrea Küster¹, Serge Haan¹, W. Becker², Peter C. Heinrich¹ and Gerhard Müller-Newen^{1,*}

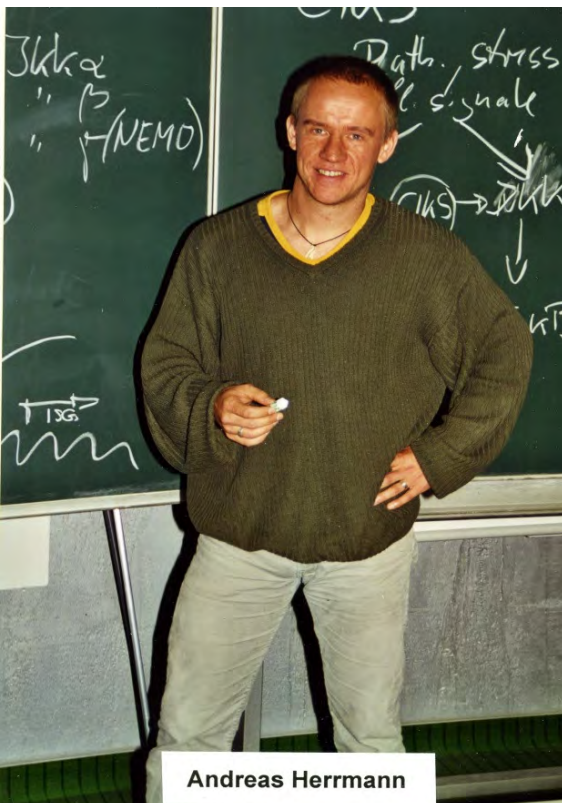
¹Institut für Biochemie, ² Institut für Pharmakologie und Toxikologie, Universitätsklinikum RWTH Aachen, Pauwelsstraße 30, 52057 Aachen, Germany

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

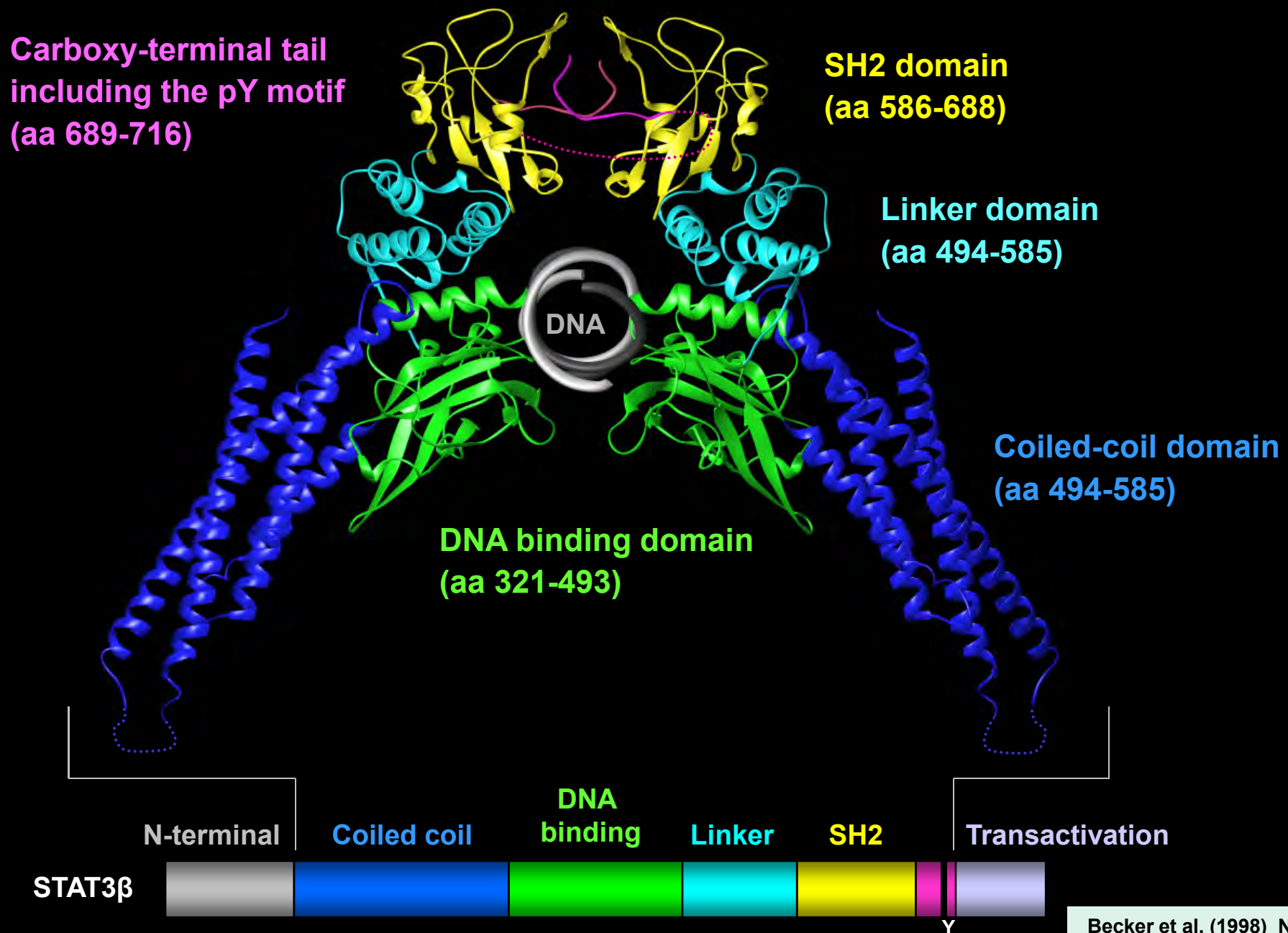
Accepted 13 August 2003

Journal of Cell Science 116, 339-349 © 2003 The Company of Biologists Ltd

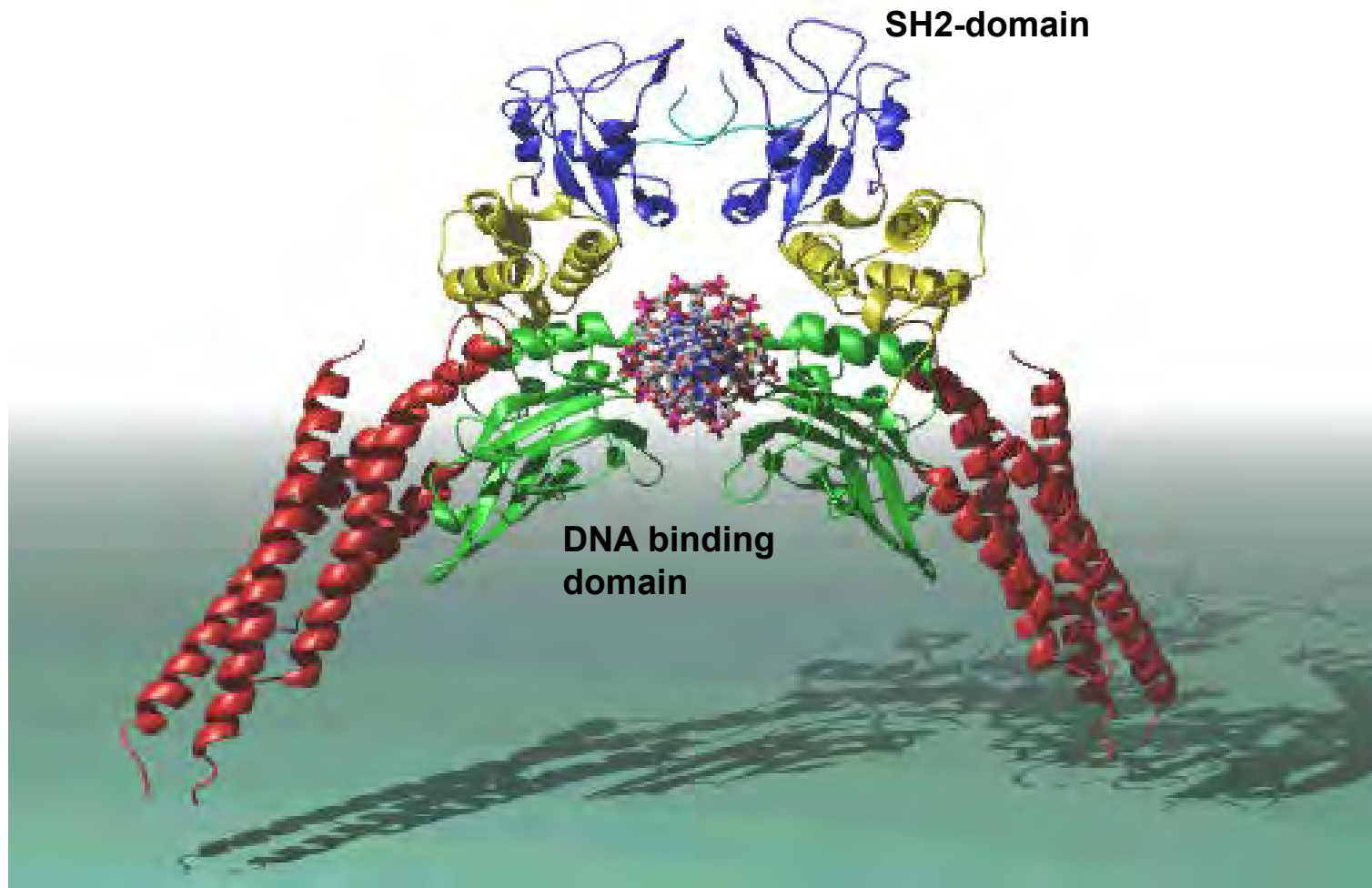
doi:10.1242/jcs.00833



STAT3 β homodimer bound to DNA



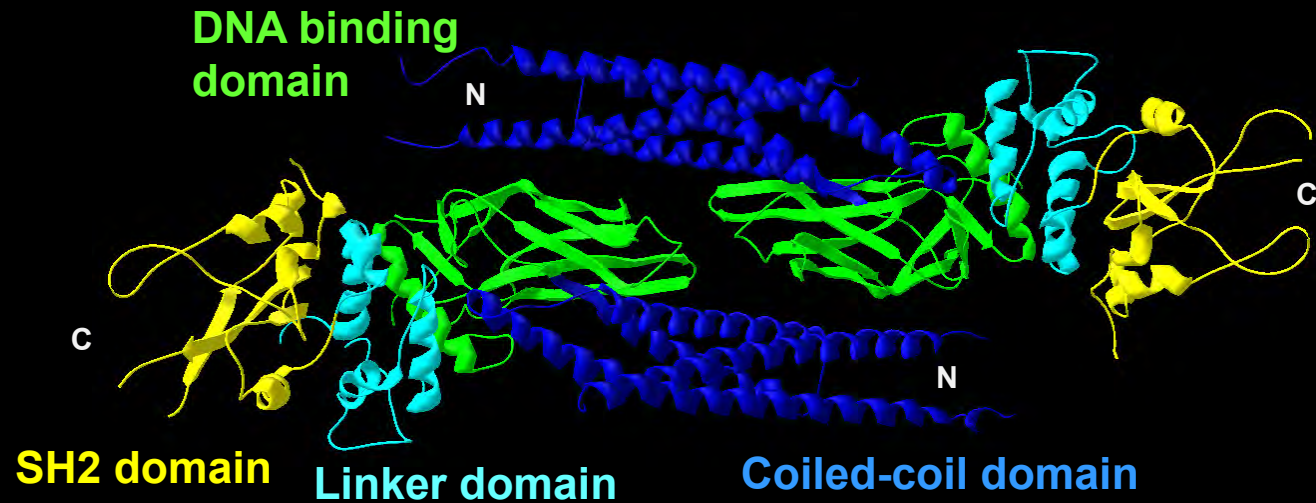
STAT3- β homodimer bound to DNA



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

STAT factors undergo extensive conformational changes upon activation

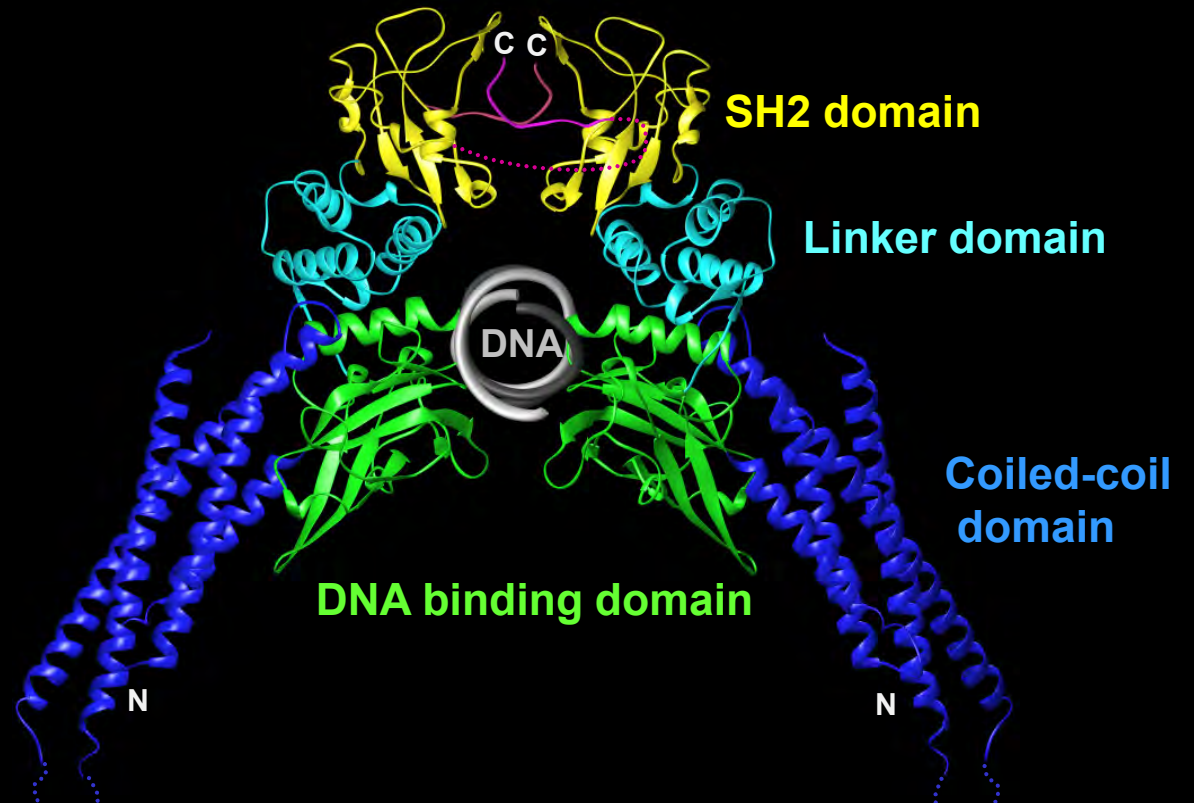
non-phosphorylated STAT-dimer (anti-parallel orientation)



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)



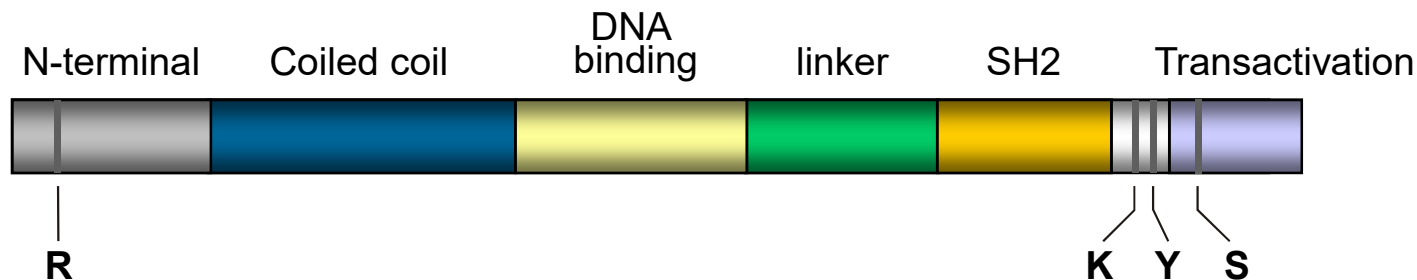
STAT1: Chen et al. 1998, Cell 93, 827

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 monoclo-

nal 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)

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Citations 155

(07/2022)

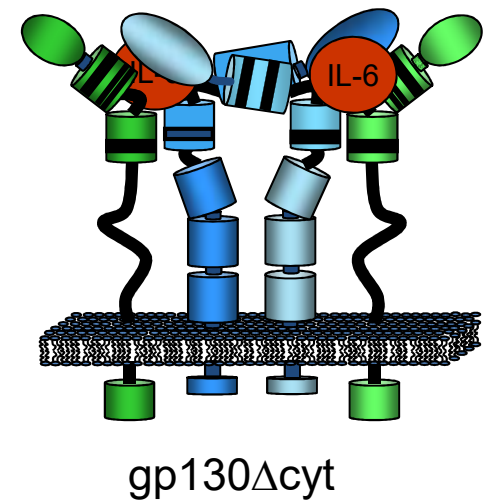
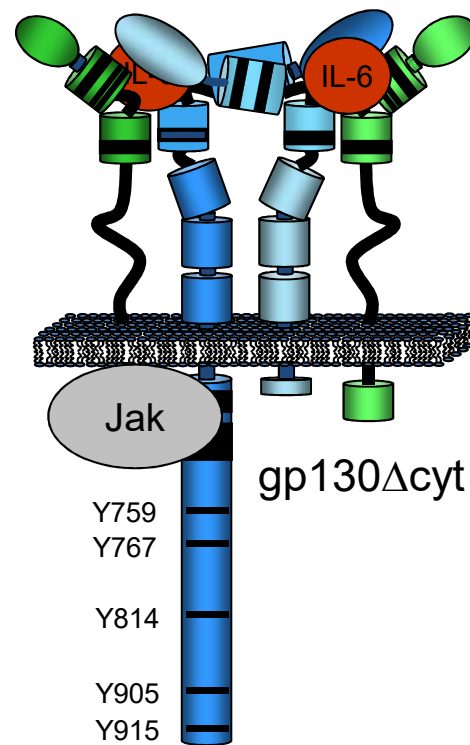
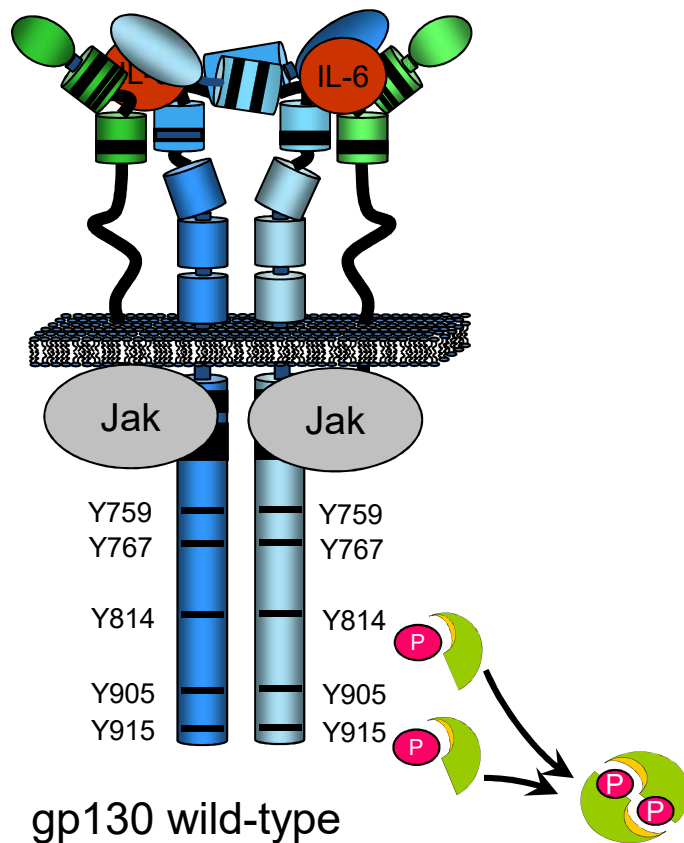
In tumour cells constitutive STAT3 activation can be accomplished through different signaling mechanisms

- 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.

Inhibition of gp130 signaling in breast cancer cells blocks constitutive activation of STAT3 and inhibits malignancy

Selander et al. (2004) Cancer Research 64, 6924-6933



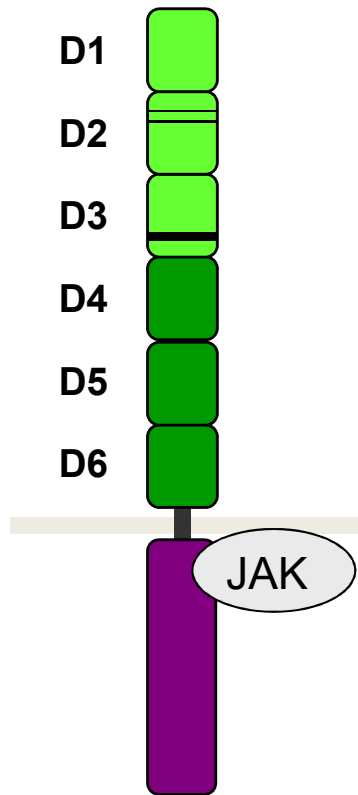
gp130 Δ cyt acts as a dominant-negative gp130 protein

Inhibition of gp130 Signaling in Breast Cancer Blocks Constitutive Activation of Stat3 and Inhibits *in Vivo* Malignancy

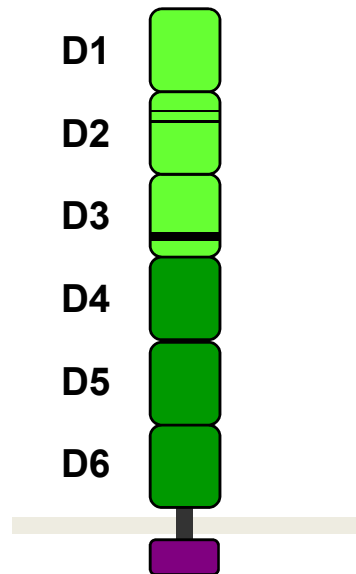
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75 citations
(07/2022)

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Wild-type gp130



gp130Δcyt = dn-gp130

Gp130Δcyt acts as a dominant-negative gp130 protein