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Activating Mechanism of CNTF and Related Cytokines

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Abstract

Ciliary neurotrophic factor (CNTF) shares structural and functional properties with members of the hematopoietic cytokine family. It is composed of a four-helix bundle structure and shares the transmembrane signal transducing proteins, glycoprotein-130 (gp130) and leukemia inhibitory factor receptor (LIF-R). Structure–function analysis showed that the gp130-interactive proteins bind in a similar manner to that of growth hormone (sites I and II). In addition, gp130-interactive proteins and granulocyte colony-stimulating factor (G-CSF) utilize another binding site (site III) at the boundary between CD loop and helix D. CNTF triggers the association of receptor components, resulting in activation of a signal transduction cascade mediated by specific intracellular protein tyrosine kinases. The Janus kinase (JAK)/signal transducer and activator of transcription (STAT) and Ras/mitogen-activated protein kinase (MAPK) signaling pathways have been characterized in terms of gp130-interactive protein, and there should be other pathways and some crosstalk between them to enhance, prolong, or specify the signals.

Index Entries: Cytokine; CNTF; IL-6; LIF; gp130; four-helix bundle; structure–function; JAK kinase.

Introduction

Ciliary neurotrophic factor (CNTF), originally characterized as a survival promoting factor for ciliary ganglion neurons *(1)*, exerts neurotrophic activity on various neuronal cell types, including sensory and motor neurons in vitro and in vivo *(2,3)*. The effectiveness on motor neurons led to a clinical trial for amyotrophic lateral sclerosis (ALS) patients with CNTF *(4)*. However, these trials disclosed serious side effects resembling a generalized inflammatory reaction *(5)*.

CNTF is a member of a family of proteins that includes leukemia inhibitory factor (LIF), oncostatin M (OSM), cardiotrophin-1 (CT-1), interleukin-6 (IL-6), and interleukin-11 (IL-11). The family is characterized by their interaction with structurally related and partially common receptor subunits, as well as overlapping biological activities *(6–8)*. Moreover, CNTF shares with these proteins a four α-helix bundle

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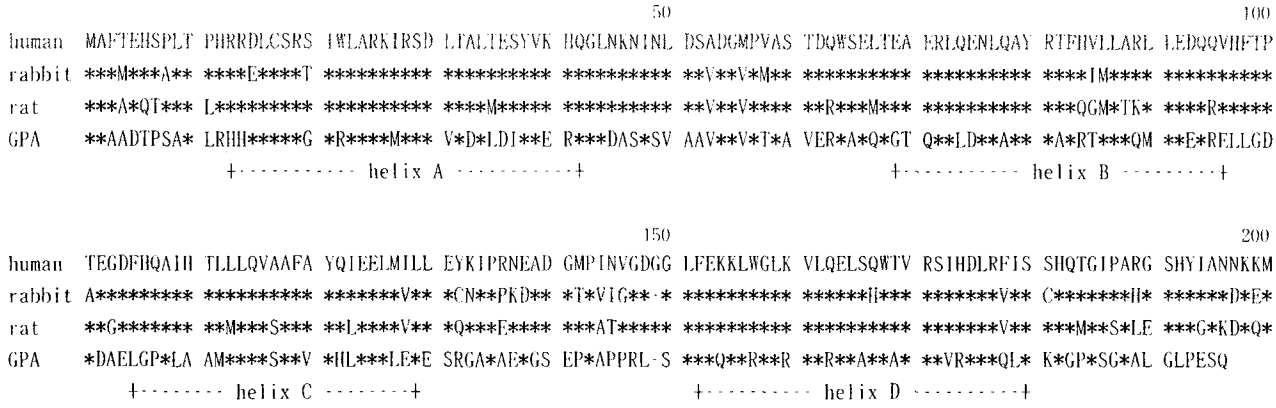

Fig. 1. Amino acid sequence comparison of CNTFs. Human *(12)*, rabbit *(10)*, and rat *(11)* CNTF and GPA (chicken CNTF) *(14)* sequences are shown by the one-letter code.

topology similar to that of human growth hormone (GH), granulocyte colony-stimulating factor (G-CSF), prolactin (PRL), and erythropoietin (EPO). CNTF exerts its biological effects through the activation of a multichain receptor complex *(7)*, consisting of a ligand-specific subunit (CNTF-Rα) and two structurally related transmembrane signal transducing proteins, glycoprotein-130 (gp130) and LIF receptor (LIF-R), resulting in the activation of a signal transduction cascade mediated by specific intracellular protein tyrosine kinases *(7)*. In this article we focus on the unity and diversity of CNTF and related cytokines (and growth factors), in terms of the ligand structure, receptor binding, and signal transduction.

Ligand Structure

Primary Structure

CNTF shows a very low sequence similarity to other known proteins, including related cytokines. Even the LIF, one of the most similar proteins, shares only 15% amino acid identity expected from the structure-based alignment *(9)*. Regarding species diversity, rabbit *(10)*, rat *(11)*, and human *(12,13)* CNTF proteins share about 80% identity (Fig. 1). Less conserved is the sequence of a chicken counterpart *(14)*, growth-promoting activity (GPA)/chicken CNTF, which is considerably less homologous (about 50%) with mammalian CNTFs (Fig. 1). Since the homology is much lower than that between avian and mammalian homologs of neurotrophins, such as brain derived neurotrophic-factor (BDNF) *(15)*, it has been still argued whether GPA is the chicken form of CNTF or whether there is a family of CNTF/GPA-like molecules *(16)*. A GPA-specific receptor (GPA-Rα) has been cloned that has 70% amino acid identity with human CNTF-Rα *(17)*, which value is relatively higher than that of ligands. GPA displays similar biological effects on neuronal cells, but it is more active on chick neurons than human CNTF and shows different receptor binding ability from that of human CNTF *(18)*. Another important difference is that GPA is a secreted protein that is released from isolated choroid cells in a biologically active form *(14)*. In contrast, mammalian CNTF is not secreted and is called a lesion factor, which is released from damaged cells to restore neuronal function after injury *(19)*. Neither GPA nor CNTF contains an N-terminal signal sequence for secretion. However, the first 57 amino acids of GPA are considered to act as an internal signal sequence for secretion *(20)*.

Tertiary Structure

CNTF is structurally and functionally related to members of a family of cytokines that include LIF, OSM, CT-1, IL-6, and IL-11 *(6–8,21)*. The tertiary structures of CNTF (Fig. 2A) *(9)* and LIF

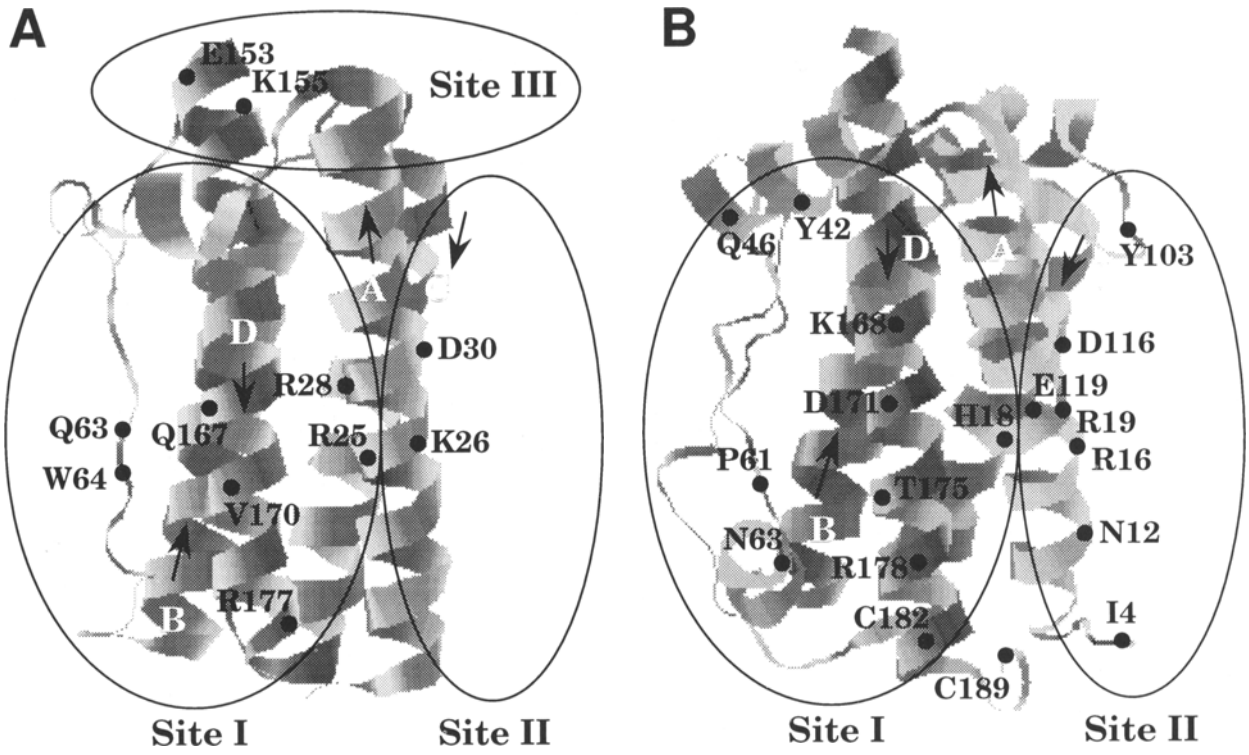

Fig. 2. Tertiary structural model and schematic receptor binding sites of CNTF **(A)** and GH **(B)**. CNTF uses the extra site (site III) for receptor recognition. Detailed data for receptor binding site and residues are summarized in Table 1.

(23) have been solved by X-ray crystallography, confirming the prediction that they belong to the long-chain helical cytokine superfamily, including GH and GCSF *(21)*. That is, they have highly similar tertiary structures, although there is little or no primary sequence homology. The common structural motif of these proteins is a core of four helices, A, B, C, and D, arranged in the "up-up-down-down" topology seen in long- and short-chain helical cytokines *(21)*.

Furthermore, the four-helix bundle structure is common to other functionally diverse proteins with different topologies from the above hematopoietic cytokines, an "up-down-up-down" topology in cytochromes C' and b562, an "up-down-down-up" topology in ferritin superfamily, and others *(24)*. The four-helix bundle would be a simple and stable form with buried hydrophobic interaction of the amphiphilic helix and the interaction of helical dipole *(24)*. Therefore, many proteins would use the structure by convergent evolution. In an "up-up-down-down" topology protein like CNTF, additively, the interligand interactions between helix A/D and helix B/C would be important for structural stability, considering that antiparallel helices are more stable than parallel helices *(25)*.

A structural comparison of CNTF and the other long-chain helical cytokines, such as LIF *(23)*, GH *(22)*, and G-CSF *(26)*, which have been determined by X-ray crystallography, has revealed that CNTF has similar packing arrangements and interhelical angles to other long-chain helical cytokines *(9)*, similar to those described above. However, minor structural differences between CNTF and LIF have also been reported *(9)*. The helix D of CNTF is comparable in length with that of G-CSF (and GH) and is about two helix turns longer than that of LIF, and helix A shows great variation in those proteins (CNTF and LIF). CNTF can be super-

imposed best with G-CSF. CNTF and LIF share the same signal transducing proteins, LIF-R and gp130, but LIF solely binds LIF-R, whereas CNTF does not *(27)*. The binding of CNTF to the other ligand-specific subunit, CNTF-Rα, is considered to be essential for subsequent LIF-R and gp130 binding *(28)*. The cause of these differences has not yet been identified. Tertiary and/or primary structural differences, that is, the minor tertiary difference described above and/or the variation of residues that are critical for the binding to respective receptor components, should produce them.

Receptor Binding

Receptor Binding Region on Ligand (Structure–Function Relationship Analysis)

Structure–function relationship analyses based on physical studies, computer modeling, and mutagenesis have helped researchers understand how CNTF and its related cytokines bind. The regions of human GH that interact with its homodimeric receptor were revealed unambiguously by X-ray crystallography of the GH/GH-receptor complex *(22)*. The primary receptor binding site of GH (site I) includes surface residues of helix D, residues in the loop between helices A and B (AB loop), including the segment of the minihelix present in this loop, and some residues of helix A. The secondary receptor binding site (site II) comprises surface residues at the N-terminus of helix A, in helix C, and in the loop between helices B and C (BC loop). The detailed structural data of GH has provided a paradigm for the proposed receptor binding sites of other cytokines *(29,30)*. Accordingly, receptor binding complex models of CNTF *(31)*, LIF *(23)*, G-CSF *(26)*, and IL-6 *(32)* like that of GH have been proposed. The results obtained from the structure–function relationship analyses of long-chain group four-helix bundle proteins are summarized in Table 1. Two receptor binding sites, site I and site II, seem to be common to all of them. The overall receptor binding mode would be similar in all long-chain group four-helix bundle proteins.

Crosslinking studies suggest that CNTF makes direct contact with all the receptor components on the cell surface *(68,69)*. This indicates that there should be at least three receptor binding sites on the CNTF ligand that participate in the binding of each component. The localization of part of the CNTF-Rα epitope to an accessible surface constituting helix D (Q167 *[43]*), the AB loop (Q63 and W64 *[31,39]*), and helix A (R25 and R28 *[31]*) is at an equivalent position to site I of GH *(22)*. Identification of the epitope for gp130 is difficult because of the lack of conserved surface residues between gp130-interactive cytokine sequences. Mutagenesis data for CNTF suggest that K23 and D30 on helix A contribute to the gp130 receptor epitope *(31)*, as do the equivalent residues Y31 and G35 of IL-6 *(29)*. Both pairs of residues are located at a region similar to site II of GH *(22)*.

In addition to site I and site II, the boundary region between the CD loop and the helix D so-called D1 cap region *(70)*, site III in Table 1, is involved in the receptor recognition and biological activity of CNTF *(44)*. The involvement of this region in receptor recognition has also been suggested for IL-6 *(51)*, LIF *(23)*, and G-CSF *(61)*. However, GH does not utilize site III for receptor recognition in the crystal structure of the GH/GH-receptor complex *(22)* and neither do PRL and EPO. That is, a site III receptor binding site might be common only to gp130-interactive protein and G-CSF. The D1 motif consisting of ϕ-F/W-E/Q-K/R-K/R-ϕ-*X*-G (ϕ = hydrophobic residue, *X* = any residue) was proposed as the consensus sequence in the D1 cap region *(70)*. The D1 motif consensus sequence is well conserved only in LIF-R-interactive proteins, such as CNTF, LIF, OSM, and CT-1 among the gp130-interactive proteins (Table 2). The corresponding residues of IL-6 and IL-11, both of which are gp130-interactive proteins, but do not interact with LIF-R, have less homology with the D1 motif consensus sequence. This led to the proposal that site III is recognized by LIF-R in CNTF, LIF, OSM, CT-1, and gp130 (second gp130) in IL-6 and IL-11. In

Table 1
Summary of the Receptor Binding Regions Predicted
from Structure–Function Relationship Analysis of Long-Chain Four-Helix Bundle Proteins

		Site I			Site II		Site III
Protein, total AA	Helix part	1, Helix D	2, AB loop	3, Helix A	1, Helix A	2, BC loop and helix C	1, Boundary between CD loop and helix D
CNTF (200)	A(13–42), B(69–96) C(105–129), D(152–180)	Q167, V170, R177 177–182	Q63, W64 (Q74)[a]	R25, R28	K26, D30 15–23		E153, K155 152–157
LIF (184)	A(20–48), B(84–103) C(112–134), D(155–181)	V177				H112, S113 (103–130)[c]	150–160
OSM (196)	A(11–32), B(78–99) C(105–131), D(157–184)	183–190	44–47		22–36		
CT-1 (203)	A(30–47), B(89–111) C(119–141), D(174–198)						
IL-6 (184)	A(19–48), B(80–101) C(109–131), D(157–184)	F173–S176, R179, R182 173–184	(77–96)[a]		Y31, G35 30–35 (41–56)[b]	S118, V121	W157, Q159, T162, H164 153–165
IL-11 (177)	A(16–34), B(69–90) C(102–125), D(150–177)	H153, D164, W165, R168, 170–177	K41 M58	(P13, E16, L17)[d] 13–34	(R25, T31, R32, L34)[d] 13–34	K98	
G-CSF (175)	A(11–40), B(72–92) C(101–125), D(144–171)	165–174	L50, H53, G56 S63, S67, Q68		S13 35–47 (1–5)		A144 139–163
GH (191)	A(9–34), B(72–92) C(106–128), D(155–184)	K168, D171, T175, R178, C182, C189	Y42, Q46 P61, N63	H18	N12, R16, R19, (I4)	D116, E119 Y103	—
PRL (199)	A(18–43), B(77–97) C(112–134), D(163–193)	171–178	46–52 62–66		12–19		—
EPO (166)	A(4–28), B(58–82) C(89–113), D(136–160)	N147, G151 154–159			R14	R103 102–106	—

The borders between helices and loops are determined from crystallographic data of CNTF *(9)*, LIF *(23)*, G-CSF *(26)*, and GH *(22)*, and secondary structure predictions for OSM *(33)*, CT-1 *(34)*, IL-6 *(35)*, IL-11 *(36)*, PRL *(37)*, and EPO *(38)*. Structure–function relationship analyses of CNTF *(31,39–44)*, LIF *(23,45)*, OSM *(46–48)*, IL-6 *(32,35,49–58)*, IL-11 *(36,59)*, G-CSF *(26,60–61)*, GH *(31,62–63)*, PRL *(37,63–65)*, and EPO *(38,66–67)* are summarized.

[a]The residue (Q74 of CNTF) and the region (77–95 of IL-6) would not locate on the site I regions (helix D/AB loop/helix A) in the model based on GH and GH-receptor complex, but would be on helix B. However, they are involved in the respective α-receptor binding *(31,49)*.

[b]The region (41–56 of IL-6) would not locate on the site II regions (helix A/helix C/BC loop), but would locate on the AB loop. However, it is considered to participate in gp130 binding *(49)*.

[c]The region (103–130 of LIF) effectively binds to LIF-R *(45)*. It is spatially close to the boundary between the CD loop and helix D *(23)*.

[d]It has not been identified whether residues 13–34 of IL-11 are in site I or site II *(59)*. Some residues would constitute part of site I, and others may be in site II. They were classified solely on our prediction.

Table 2
Amino Acid Sequence Comparison Among gp130 Interactive Proteins

D1 motif consensus sequence		ϕ	F/W	E/Q	K/R	K/R	ϕ	X	G	Residue ranges
gp130- and LIF-R-interactive proteins										
CNTF	Human	L	**F**	**E**	**K**	**K**	L	W	**G**	151–158
(GPA)	Rat	L	**F**	**E**	**K**	**K**	L	W	**G**	151–158
	Rabbit	L	**F**	**E**	**K**	**K**	L	W	**G**	150–157
	Chicken	L	**F**	**E**	Q	**K**	L	R	**G**	150–157
LIF	Human	V	**F**	**Q**	**K**	**K**	K	L	**G**	154–161
	Mouse	A	**F**	**Q**	**R**	**K**	K	L	**G**	154–161
	Rat	A	**F**	**Q**	**R**	**K**	K	L	**G**	154–161
OSM	Human	A	**F**	**Q**	**R**	**K**	L	E	**G**	159–166
	Simian	V	**F**	**Q**	**R**	**K**	L	E	**G**	159–166
	Bovine	T	**F**	**Q**	**R**	**K**	L	R	N	147–154
CT-1	Mouse	I	**F**	S	A	**K**	V	L	**G**	169–176
gp130-interactive, but LIF-R-noninteractive proteins										
IL-6	Human	Q	**W**	L	Q	D	M	T	T	156–163
	Mouse	E	**W**	L	**R**	T	K	T	I	156–163
	Rat	E	**W**	L	**R**	T	K	T	I	156–163
	Bovine	E	**W**	V	**K**	N	A	K	I	152–159
	Sheep	E	**W**	V	**K**	N	A	K	V	152–159
	Pig	E	**W**	M	**K**	N	T	K	I	156–163
	Dog	E	C	V	**K**	H	T	T	I	156–163
IL-11	Human	G	G	I	**R**	A	A	H	A	148–155
	Monkey	G	G	I	**R**	A	A	H	A	148–155

ϕ: Hydrophobic amino acid; X: any amino acid residue. Boldface show the amino acids that are homologous to D1 motif.

fact, the CD loop region of LIF is involved in LIF-R binding *(45)*. Owczarek et al. have also shown that the BC loop and helix C region (103–130) of LIF are involved in LIF-R binding *(45)*. Some part of the BC loop and helix C, which is especially close to the D1 cap region, might be included in site III. In addition, the D1 motif is not conserved in GH, PRL, and EPO, but it is in G-CSF *(44,70)*, supporting the notion that only G-CSF among gp130 uninteractive long-chain four-helix bundle proteins can use site III for receptor recognition.

Receptor Binding Scheme

CNTF exerts biological activities through the activation of a multichain receptor complex, consisting of a ligand-specific subunit (CNTF-Rα), which is linked to the cell membrane by a glycosyl-phosphatidyl-inositol (GPI) anchor *(68)* and two structurally related transmembrane signal transducing proteins, gp130 and LIF-R *(7)*. CNTF and its related proteins, LIF, OSM, CT-1, IL-6, and IL-11, all share the signal-transducing receptor component, gp130, which was initially identified as the signal transducer for IL-6 (Fig. 3) *(6,8,71)*. LIF-R is shared among CNTF, LIF, OSM, and CT-1 and forms high-affinity binding sites for LIF and OSM in combination with gp130 *(72)*. Accordingly, these cytokines show overlapping biological activities. They are considered to be activated by the ligand-dependent formation of either homo- and heterodimeric receptor molecules *(73)* or higher-order complexes *(74)*. CNTF and IL-6 induce disulfide-linked heterodimers of gp130 with LIF-R *(28)* and disulfide-linked homodimers of gp130 *(75)*, respectively.

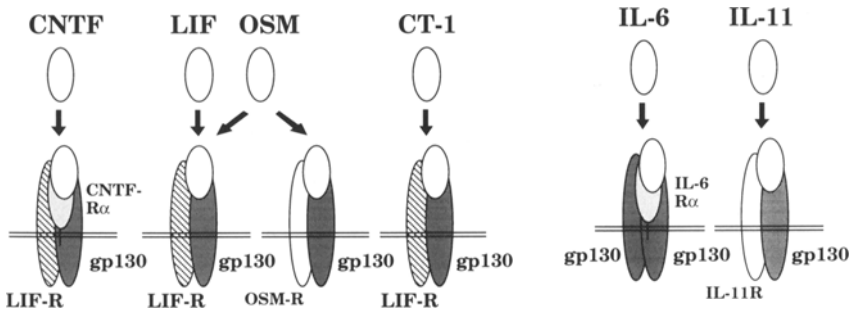

Fig. 3. Gp130 signal-transducing system. All the constituent proteins share gp130, whereas CNTF, LIF, OSM, and CT-1 among them share LIF-R to transduce signals.

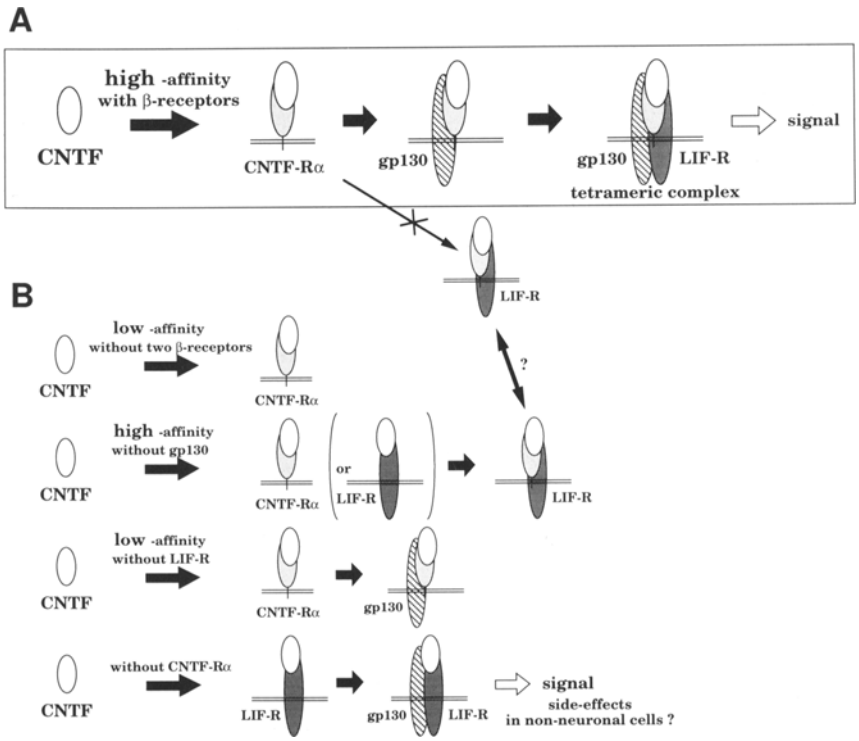

Fig. 4. Receptor binding scheme of CNTF. **(A)** Sequential binding under physiological conditions. CNTF binding to CNTF-Rα induces the next association of gp130 and LIF-R. An inactive intermediate, consisting of CNTF, CNTF-Rα, and gp130, has been proposed *(28)*. **(B)** Receptor binding under artificial conditions. CNTF shows high-affinity receptor binding without gp130 *(27)* and transduces signals without CNTF-Rα *(27)*.

Receptor association triggering CNTF results in the tyrosine phosphorylation of receptor subunits and the activation of intracellular signaling components. The key event for signal initiation is considered to be the dimerization of the signal-transducing proteins, gp130 and LIF-R. A sequential binding model (Fig. 4A) has been proposed for the binding scheme of CNTF *(28)*. In this model, CNTF first binds to CNTF-Rα, engages gp130 to form an inactive complex, and then finally recruits LIF-R, resulting in the heterodimerization of the signal transducers and the initiation of signaling. That is, the binding with CNTF-Rα introduces

the oligomerization of gp130 and LIF-R. An intermediate complex consisting of CNTF, CNTF-Rα, and gp130, but not LIF-R, was predicted because CNTF triggered the association of CNTF-Rα and gp130 even in the absence of LIF-R and did not associate with LIF-R without gp130 *(28)*. A similar sequence of events would be considered to lead to the homodimerization of gp130 and signaling for IL-6 *(76)*.

Under many experimental conditions, CNTF and its related cytokines bind their receptors in a biphasic manner, both high and low affinity *(27,77,78)*. The initial receptor (α-receptor) that shows low-affinity binding is CNTF-Rα for CNTF, IL-6Rα for IL-6, and LIF-R for LIF *(28,72)*. Gp130 is considered to be the affinity converter to high affinity for IL-6, LIF, and OSM *(72)*, and gp130 and/or LIF-R would be the one for CNTF. CNTF bound with high affinity in the presence of CNTF-Rα and LIF-R without gp130, and not in the presence of CNTF-Rα and gp130 without LIF-R (Fig. 4B) *(27)*. This is somewhat contrary to the finding that the CNTF-induced association of CNTF-Rα and LIF-R was undetectable in the absence of gp130 *(28)*. The question remains whether gp130 or LIF-R is the main contributor to the high-affinity binding of CNTF. Moreover, CNTF can transduce signals with complexes of LIF-R and gp130 without CNTF-Rα *(27)*. A receptor-binding scheme of CNTF other than the sequential binding model, such as the initial binding to LIF-R, would need to be considered under some artificial conditions. However, under physiological conditions, most of the signal would be transduced via the sequential binding scheme, and the other schemes might be artificial because CNTF acts in nervous and muscular systems in which CNTF-Rα is expressed. However, the receptor binding of CNTF without CNTF-Rα should be considered because this could cause side effects by systemic administration in clinical trials of CNTF.

Stoichiometry

Several cytokines are associated as dimers either with covalent or noncovalent interactions with quite different arrangements *(21)*. If ligands act as monomers, they should present distinct surfaces to each receptor, or if as dimers, they should present identical and symmetry-related binding surfaces *(21)*. Judging from the monomeric nature at physiological concentrations below 40 μ*M* and the asymmetric binding surface of CNTF predicted from the structure–function analysis (Table 1), CNTF would act as a monomer under physiological conditions, even though it dimerizes at concentrations above 40 μ*M* and its crystal structure reveals a dimer *(9)*. CNTF binds CNTF-Rα with a 1:1 stoichiometry *(79)*. These results indicate that CNTF transduces signals through a tetrameric complex consisting of CNTF, CNTF-Rα, gp130, and LIF-R (Fig. 4). However, Ward et al. have found by studying the formation of IL-6 complexes with the soluble extracellular domains of IL-6Rα and gp130 that IL-6 binds IL-6Rα in a stoichiometric ratio of 1:1, and that the high-affinity ternary complex is a hexamer consisting of two molecules each of IL-6, IL-6Rα, and gp130 *(80)*. This model of the complex *(53,80)* might be applicable to CNTF. However, the stoichiometry of the active receptor complex of CNTF has not been absolutely defined yet and remains to be investigated.

Signal Transduction

Multiple-Signaling Activation

The dimerization of gp130 and LIF-R by CNTF results in the activation of various signal-transduction pathways that can also be triggered by other cytokines (Fig. 5) *(6,84,85)*. The CNTF signaling can be mediated by at least two distinct pathways. One is the Janus kinase (JAK) and signal transducer and activator of transcription (STAT) pathway, and another is the Ras and mitogen-activated protein kinase (MAPK) pathway *(86–88)*. Phospholipase Cγ(PLCγ) and phosphatidylinositol 3-kinase (PI3K)-p110 can also be activated by CNTF with a direct and an indirect binding to gp130 (and LIF-R), respectively *(88)*. Moreover, it was reported that hematopoietic cell tyrosine

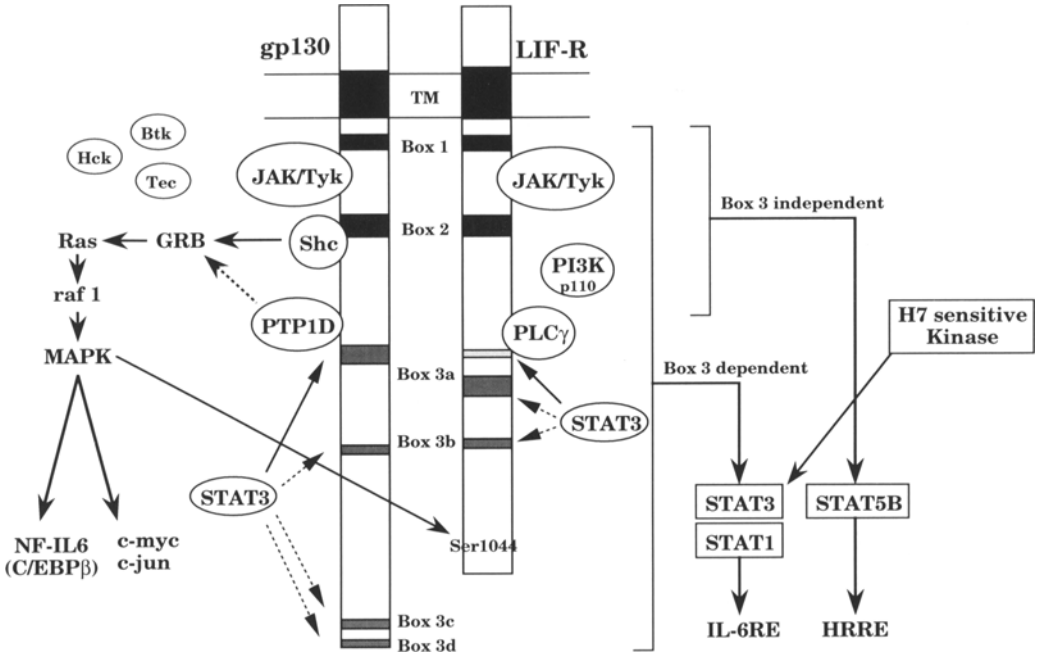

Fig. 5. Signal transduction of CNTF and related cytokines. Conserved Box 1, 2, and 3a motifs are ^{651}-IWPNVPDP, ^{693}-VVEIEANDKKP, and ^{761}-TVVHSGYRHQ in gp130 and ^{869}-FYPDIPNP, ^{910}-VLETRSAFPKI, and ^{995}-PVGGAGYKPQ in LIF-R, respectively *(81)*. *YXXQ* (*X* means any residue) motif sequences, which are recognizable by STAT3 *(82)*, are ^{767}-YRPQ ^{814}-YFKQ, ^{905}-YLPQ, and ^{915}-YMPQ in gp130 and ^{981}-YQPQ, ^{1001}-YKPQ, and ^{1028}-YRPQ in LIF-R. Both LIF-R and gp130 associate with the SH2 domain of PLCγ, a potential consensus binding site for the PLCγ SH2 domain, which is found in the C-terminal region (^{974}YIDV) of LIF-R *(83)*.

kinase (Hck) *(89)*, Bruton's tyrosine kinase (Btk), and tyrosine kinase expressed in hepatocellular carcinoma (Tec) *(90)* are activated via gp130 signaling following a direct association with gp130 in embryonic stem (ES), B-lineage, and hematopoietic lineage (and hepatic) cells, respectively. The signaling is likely to be very complex.

JAK/STAT Pathway

Members of cytokine receptor family contain conserved box 1 and box 2 motifs within a membrane-proximal cytoplasmic domain *(91,92)*. In CNTF signaling, they are essential for the activation and association of the JAK family, JAK1, JAK2, and Tyk2, in distinct combinations in various cells *(86,87)*. These in turn may tyrosine phosphorylate the transcription factors STAT3 *(86,93)*, STAT1 *(94)*, and STAT5B *(95)*, after which phosphorylated STAT proteins dimerize, translocate to the nucleus, and bind to specific DNA sequences *(96)*, IL-6 response element (IL-6RE), hematopoietin receptor response element (HRRE), and sis-inducible element (SIE). Moreover, gp130, LIF-R, and G-CSF receptor have another conserved box 3 motif on the distal side from boxes 1 and 2 *(81,97)*, in which Stahl et al. identified the specific tyrosine-based STAT3 binding motif, *YXXQ* (*X* means any residue), in gp130 and LIF-R *(82)*. The motif is different from those in STAT3 (YLKT) and STAT1 (YIKT), being implicated in mediating the homo- or hetero-dimerization of STATs *(96)*. Therefore, they discussed that STAT3 might recognize the *YXXQ* motif with a lower affinity than that to the dimerization motifs, thus favoring rapid release from the receptor and subsequent STAT dimerization *(82)*. In addition, phosphotyrosine phosphatase (PTP1D) (also called Syp or SH-PTP2), which may couple platelet-derived growth factor (PDGF) receptor activation to the

Ras signaling pathway *(98,99)*, binds ^{759}YSTV on gp130, but not on LIF-R *(82)*.

Two separate transcription signals have been identified in the JAK/STAT pathway. One is directed to the IL-6RE and is box 3 dependent, whereas the other is directed to the HRRE and is independent of the box 3 motif *(100,101)*. The former is transduced via STAT3 and STAT1 *(95,101)*, and the latter via STAT5B *(95)*. That is, gp130 uses a separate mechanism to activate STAT, STAT1, and STAT5B with distinct requirements for cytoplasmic receptor domains to regulate the expression of different genes. However, both pathways are dependent on the action of JAKs, indicating there should be other STAT binding sites, especially for STAT5B. Therefore, it has been suggested that STATs directly interact with kinase or connector molecules *(95)*. The gp130-interactive cytokines, including CNTF, induce two distinct forms of STAT3 differing from the Ser phosphorylation involving H7-sensitive kinase compared with STAT1 *(102–104)*. Serine phosphorylation is constitutive, is increased by signaling through gp130, and appears to enhance or to be required for the formation of stable STAT3–STAT3–DNA complexes *(105)*. Additively, G-CSF, which utilizes a receptor highly related to gp130, also induces these two forms of STAT3 *(102)*. An H7-sensitive kinase that phosphorylates Ser residue of STAT3 has not yet been identified. If it is MAPK or other MAPK-related kinases, the phenomenon might be crosstalk between the JAK/STAT and Ras/MAPK pathways.

Ras/MAPK Pathway

Gp130 engages the MAPK cascade via Shc, Grb2, Raf1, and MAPKK (MEK1) *(88,106)*. Although protein kinase C (PKC) can activate Ras and MAPK in lymphocytes, gp130 does not induce PKC activity *(107,108)*. In addition to the above cascade, MAPK phosphorylates Ser1044 of LIF-R *(109)*. However, the substitution of Ser1044 to Ala failed to alter the magnitude of cytokine-mediated gene regulation *(109)*. The role of the Ser phosphorylation of LIF-R has not been identified, but signals may be prolonged or modified by this process.

Multiple Regulation of Transcription

Dual regulation of both JAK/STAT and Ras/MAPK pathways by gp130-related cytokines has been characterized in some promoter genes, for example, a vasoactive intestinal polypeptide (VIP) promoter, in which CNTF stimulation is transferred through a STAT binding site and a CCAAT/enhancer binding protein (C/EBP)-related site *(110,111)*. Moreover, JunB is regulated by IL-6 in a biphasic fashion, namely, early and delayed expression *(112)*. The early expression is H7-sensitive, whereas the delayed required protein synthesis is dependent on tyrosine kinase *(112)*. Such complex regulation would be required in order to prolong activity or to show specificity.

Perspective

The clinical trials of CNTF for ALS-patients disclosed side effects, consisting of dry cough, fever, and weight loss, resembling a generalized inflammatory reaction *(5,113,114)*. Similar side effects, as well as acute phase protein expression *(115)*, pyrogenic fever *(116)*, and cachexia *(117)*, have also been seen in experimental animals. If these problems can be overcome, CNTF should be a useful therapeutic agent. Therefore, modified CNTF molecules (next generation) with beneficial properties, such as increased biological activity and potentially reduced side effects, have been constructed *(39,43,44)*. Since much of the side effects of CNTF can be explained by its structural and functional similarity to the members of gp130-interactive cytokines, there is a rationale in increasing the binding ability to specific CNTF-Rα through such mutations as Q63R *(39)* and Q167A *(43)* to design agents with reduced side effects. Furthermore, combinations with the listed mutations (Table 3) would produce a more potent agent than a single modification, since the activity increase itself would permit

Table 3
Modified Mutant Proteins Having Some Beneficial Properties

Protein	Mutation substitution and/or deletion	Reference
With agonistic feature		
CNTF	Q63R[a]	*39*
	Q167A[a]	*43*
	E153R, E153Y	*44*
	D2–14, D173–200	*40*
	D1–14, D182–200	*42*
IL-6	F171L	*54*
	Q160A/D161A, T164A/H165A	*50*
	S177R, S177R/Q175I/Q183A	*55*
	V121Y/F125V	*35*
OSM	Δ87–90	*33*
	ΔA191–196	*46*
G-CSF	T1A/L3T/G4P/P5R/C17S	*60*
	C17A[b]	*118*
GH	C181S/Δ184–191	*119*
EPO	R143A, S146A, N147A	*38*
With antagonistic feature		
CNTF	K155A	*44*
IL-6	Q160E/T163P	*51*
	W158R/T163P	*50*
	Y31D/G35F	*35*
	Y31D/G35F/S118R/V121D,	*32,35*
	Y31D/G35F/S118R/V121D/S177R/Q175I/Q183A	*32*

[a](Owing to) increased selectivity to CNTF-Rα receptor.
[b](Owing to) protein stabilization.

the dose of CNTF administrated to be reduced, which would solve the problem of insolubility at neutral pH. Of course, because excessive modification would produce a neutralizing antibody, modification should be minimized. Since CNTF triggers gliosis *(120)*, CNTF antagonists, such as K155A mutant *(44)*, might be useful as an inhibitor of gliosis. More potent antagonists that bind CNTF-Rα with high affinity but do not show any survival activity (as designed for IL-6 antagonists *[32,52]*) could be constructed by combining substitutions, such as K155A, Q63R, Q167A, and others. The designs for constructing mutant proteins, such as those for CNTF and IL-6, could also be applied to the other gp130-interactive cytokines, LIF, OSM, CT-1, and IL-11.

Mice lacking LIF-R die shortly after birth *(121,122)*, unlike those lacking CNTF *(123)* or LIF *(124,125)*, which are viable. A CNTF-Rα deletion causes developmental defects more serious than those produced by a deletion of CNTF *(126)*. Additively, a CNTF null mutation in humans appears to cause no neurological defects *(127)*. Accordingly, it was suggested that another unidentified cytokine(s) might be a novel ligand for LIF-R and/or CNTF-Rα *(122)*. To clone such novel ligands, if they exist, probes corresponding to the identified receptor binding regions (Table 1) might be used, especially the D1 motif, which is homologous to LIF-R interactive proteins *(44,70)*.

STAT3 is directly phosphorylated by the JAKs and translocated in the nucleus within minutes following CNTF stimulation, where it is rapidly dephosphorylated *(86,102)*. MAPK is also activated and then inactivated within approximately half an hour *(128)*. However,

metabolic and functional activities, such as differentiation, proliferation, and cell-survival promoting, were revealed after several hours and/or days. Therefore, the mechanism between the early activation and the following responses should be resolved. There should be a lot of crosstalk between signaling pathways, such as Ser phosphorylation of STAT3 by the H7-sensitive kinase *(102,105)*. The different signaling pathways respectively involved in the immediate and delayed transcription of JunB mRNA *(112)* might explain this. In addition, cytokines, including CNTF, induce multifunctional responses. If there is a difference in signaling between each response, antagonistic mutant proteins of CNTF *(44)* and IL-6 *(32,35,50–52)* might give a useful clue for identifying unique pathways corresponding to a specific function. The difference might be at a magnitude and/or in a time-course for the signals. Regardless, future efforts to unravel the activating mechanism of cytokines in addition to these findings will provide valuable information directed toward the further clinical application of cytokines themselves, their modified proteins, or low-molecular-mass mimics and/or specific signaling modifiers.

References

1. Varon S., Manthorpe M., and Adler R. (1979) *Brain Res.* **173,** 29–45.
2. Acheson A. and Lindsay R. M. (1994) *Semin. Neurosci.* **6,** 507–515.
3. Richardson P. M. (1994) *Pharmacol. Ther.* **63,** 187–198.
4. Brooks B. R., Sanjak M., Mitsumoto H., Szirony K, Neville H., Ringel S., Brinkmann J., Pestronk A., Florence J., Cedarbaum J. M., Charatan M., Stambler N., Wittes J., and Brittain E. (1993) *Neurology* **43,** A416.
5. Barinaga M. (1994) *Science* **264,** 772–774.
6. Kishimoto T., Taga T., and Akira S. (1994) *Cell* **76,** 253–262.
7. Stahl N. and Yancopoulos G. D. (1994) *J. Neurobiol.* **25,** 1454–1466.
8. Pennica D., Shaw K. J., Swanson T. A., Moore M. W., Shelton D. L., Zioncheck K. A., Rosenthal A., Taga T., Paoni N. F., and Wood W. I. (1995) *J. Biol. Chem.* **270,** 10,915–10,922.
9. McDonald N. Q., Panayotatos N., and Hendrickson W. A. (1995) *EMBO J.* **14,** 2689–2699.
10. Lin L.-F. H., Mismer D., Lile J. D., Armes L. G., Butler E. T. III, Vannice J. L., and Collins F. (1989) *Science* **246,** 1023–1025.
11. Stöckli K. A., Lottspeich F., Sendtner M., Masiakowski P., Carroll P., Götz R., Lindholm D., and Thoenen H. (1989) *Nature* **342,** 920–923.
12. Masiakowski P., Liu H., Radziejewski C., Lottspeich F., Oberthuer W., Wong V., Lindsay R. M., Furth M. E., and Panayotatos N. (1991) *J. Neurochem.* **57,** 1003–1012.
13. Negro A., Tolosano E., Skaper S. D., Martini I., Callegaro L., Silengo L., Fiorini F., and Altruda F. (1991) *Eur. J. Biochem.* **201,** 289–294.
14. Leung D. W., Parent A. S., Cachianes G., Lee A. L., Nikolics K., Esch F., Coulombe J. N., Blacher R. W., Eckenstein F. P., and Nishi R. (1992) *Neuron* **8,** 1045–1053.
15. Barde Y.-A. (1994) *J. Neurobiol.* **25,** 1329–1333.
16. Nishi R. (1994) *J. Neurobiol.* **25,** 612–619.
17. Heller S., Finn T. P., Huber J., Nishi R., Geiben M., Puschel A. W., and Rohrer H. (1995) *Development* **121,** 2681–2693.
18. Heller S., Huber J., Finn T. P., Nishi R., and Rohrer H. (1993) *NeuroReport* **5,** 357–360.
19. Adler R. (1993) *Curr. Opinion Neurobiol.* **3,** 785–789.
20. Reiness C. G., Sweeney S., and Nishi R. (1995) *Soc. Neurosci.* **21,** 1047.
21. Sprang S. R. and Bazan J. F. (1993) *Curr. Opin. Struct. Biol.* **3,** 815–827.
22. DeVos A. M., Ultsch M., and Kossiakoff A. A. (1992) *Science* **255,** 306–312.
23. Robinson R. C., Grey L. M., Staunton D., Vankelecom H., Vernallis A. B., Moreau J. F., Stuart D. J., Heath J. K., and Jones F. Y. (1994) *Cell* **77,** 1101–1116.
24. Kamtekar S. and Hecht M. H. (1995) *FASEB J.* **9,** 1013–1022.
25. Robinson C. R. and Sligar S. G. (1993) *Protein Sci.* **2,** 826–837.
26. Lovejoy B., Cascio D., and Eisenberg D. (1993) *J. Mol. Biol.* **234,** 640–653.
27. Gearing D. P., Ziegler S. F., Comeau M. R., Friend D., Thoma B., Cosman D., Park L., and Mosley B. (1994) *Proc. Natl. Acad. Sci. USA* **91,** 1119–1123.
28. Davis S., Aldrich T. H., Stahl N., Pan L., Taga T., Kishimoto T., Ip N. Y., and Yancopoulos G. D. (1993) *Science* **260,** 1806–1808.

29. Savino R., Lahm A., Salvati A. L., Ciapponi L., Sporeno E., Altamura S., Paonessa G., Toniatti C., and Ciliberto G. (1994) *EMBO J.* **13,** 1357–1367.
30. Goodall G. J., Bagley C. J., Vadas M. A., and Lopez A. F. (1993) *Growth Factors* **8,** 87–97.
31. Panayotatos N., Radziejewska E., Acheson A., Somogyi R., Thadani A., Hendrickson W. A., and McDonald N. Q. (1995) *J. Biol. Chem.* **270,** 14,007–14,014.
32. Lahm A., Savino R., Salvati A. L., Cabibbo A., Ciapponi L., Demartis A., Toniatti C., Paonessa G., Altamura S., and Ciliberto G. (1995) *Ann. NY Acad. Sci.* **762,** 136–151.
33. Rose T. M. and Bruce A. G. (1991) *Proc. Natl. Acad. Sci. USA* **88,** 8641–8645.
34. Pennica D., King K. L., Shaw K. J., Luis E., Rullamas J., Luoh S.-M., Darbonne W. C., Knutzon D. S., Yen R., Chien K. R., Baker J. B., and Wood W. I. (1995) *J. Biol. Chem.* **92,** 1142–1146.
35. Savino R., Lahm A., Salvati A. L., Ciapponi L., Sporeno E., Altamura S., Paonessa G., Toniatti C., and Ciliberto G. (1994) *EMBO J.* **13,** 1357–1367.
36. Czupryn M. J., McCoy J. M., and Scoble H. A. (1995) *J. Biol. Chem.* **270,** 978–985.
37. Goffin V., Struman I., Goormaghtigh E., and Martial J. A. (1993) *Eur. J. Biochem.* **214,** 483–490.
38. Wen D., Boissel J.-P., Showers M., Ruch B. C., and Bunn F. (1994) *J. Biol. Chem.* **269,** 22,839–22,846.
39. Panayotatos N., Radziejewska E., Acheson A., Pearsall D., Thadani A., and Wong V. (1993) *J. Biol. Chem.* **268,** 19,000–19,003.
40. Negro A., Corsa V., Corona G., Grandi C., Skaper S. D., and Callegaro L. (1994) *Neurochem. Res.* **19,** 223–227.
41. Krüttgen A., Grötzinger J., Kurapkat G., Weis J., Simon R., Thier M., Schröder M., Heinrich P., Wollmer A., Comeau M., Müllberg J., and Rose-John S. (1995) *Biochem. J.* **309,** 215–220.
42. Thier M., Simon R., Krüttgen A., Rose-John S., Heinrich P. C., Schröder J. M., and Weis J. (1995) *J. Neurosci. Res.* **40,** 826–835.
43. Saggio I., Gloaguen I., Poiana G., and Laufer R. (1995) *EMBO J.* **14,** 3045–3054.
44. Inoue M., Nakayama C., Kikuchi K., Kimura T., Ishige Y., Ito A., Kanaoka M., and Noguchi H. (1995) *Proc. Natl. Acad. Sci. USA* **92,** 8579–8583.
45. Owczarek C. M., Layton M. J., Metcalf D., Lock P., Willson T. A., Gough N. M., and Nicola N. A. (1993) *EMBO J.* **12,** 3487–3495.
46. Linslay P. S., Kallestad J., Ochs V., and Neubauer M. (1990) *Mol. Cell. Biol.* **10,** 1882–1890.
47. Kallestad J. C., Shoyab M., and Linsley P. S. (1991) *J. Biol. Chem.* **266,** 8940–8945.
48. Radka S. F., Kallestad J. C., Linslay P. S., and Shoyab M. (1994) *Cytokine* **6,** 55–60.
49. Ehlers M., Grötzinger J., de Hon F. D., Müllberg J., Brakenhoff J. P. J., Liu J., Wollmer A., and Rose-John S. (1994) *J. Immunol.* **153,** 1744–1753.
50. de Hon F. D., ten Boekel E., Herrman J., Clement C., Ehlers M., Taga T., Yasukawa K., Ohsugi Y., Kishimoto T., Rose-John S., Wijdenes J., Kastelein R., Aarden L. A., and Brakenhoff J. P. J. (1995) *Cytokine* **7,** 398–407.
51. Brakenhoff J. P. J., de Hon F. D., Fontaine V., ten Boekel E., Schooltink H., Rose-John S., Heinrich P. C., Content J., and Aarden L. A. (1994) *J. Biol. Chem.* **269,** 86–93.
52. Savino R., Ciapponi L., Lahm A., Demartis A., Cabibbo A., Toniatti C., Delmastro P., Altamura S., and Ciliberto G. (1994) *EMBO J.* **13,** 5863–5870.
53. Paonessa G., Graziani R., DeSerio A., Savino R., Ciapponi L., Lahm A., Salvati A. L., Toniatti C., and Ciliberto G. (1995) *EMBO J.* **14,** 1942–1951.
54. Leebeek F. W. G., Kariya K., Schwabe M., and Fowlkes D. M. (1992) *J. Biol. Chem.* **267,** 14,832–14,838.
55. Savino R., Lahm A., Giargio M., Cabibbo A., Tramontano A., and Ciliberto G. (1993) *Proc. Natl. Acad. Sci. USA* **90,** 4067–4071.
56. Yasueda H., Miyasaka Y., Shimamura T., and Matsui H. (1992) *Biochem. Biophys. Res. Commun.* **187,** 18–25.
57. Li X., Rock F., Chong P., Cockle S., Keating A., Ziltener H., and Klein M. (1993) *J. Biol. Chem.* **268,** 22,377–22,384.
58. Arcone R., Fontaine V., Coto I., Content J., and Ciliberto G. (1991) *FEBS Lett.* **288,** 197–200.
59. Czupryn M., Bennett F., Dube J., Grant K., Scoble H., Sookdeo H., and McCoy J. M. (1995) *Ann. NY Acad. Sci.* **762,** 152–164.
60. Kuga T., Komatsu Y., Yamasaki M., Sekine S., Miyaji H., Nishi T., Sato M., Yokoo Y., Asano M., Okabe M., Morimoto M., and Itoh S. (1989) *Biochem. Biophys. Res. Commun.* **159,** 103–111.
61. Layton J. E., Morstyn G., Fabri L. J., Reid G. E., Burgess A. W., Simpson R. J., and Nice E. C. (1991) *J. Biol. Chem.* **266,** 23,815–23,823.
62. Cunningham B. C. and Wells J. A. (1989) *Science* **244,** 1081–1085.

63. Cunningham B. C., Jhurani P., Ng P., and Wells J. A. (1989) *Science* **243,** 1330–1336.
64. Goffin V., Norman M., and Martial J. A. (1992) *Mol. Endocrinol* **6,** 1381–1392.
65. Cunningham B. C., Henner D. J., and Wells J. A. (1990) *Science* **247,** 1461–1465.
66. Bittorf T., Jaster R., and Brock J. (1993) *FEBS Lett.* **336,** 133–136.
67. Wen D., Boissel J.-P., Tracy T. E., Gruninger R. H., Mulcahy L. S., Crelusniak J., Goodman M., and Bunn F. (1993) *Blood* **82,** 1507–1516.
68. Davis S., Aldrich T. H., Valenzuela D. M., Wong V., Furth M. E., Squinto S. P., and Yancopoulos G. D. (1991) *Science* **253,** 59–63.
69. Stahl N., Davis S., Wong V., Taga T., Kishimoto T., Ip N. Y., and Yancopoulos G. D. (1993) *J. Biol. Chem.* **268,** 7628–7631.
70. Bazan J. F. (1991) *Neuron* **7,** 197–208.
71. Ip N. Y. and Yancopoulos G. D. (1992) *Prog. Growth Factors Res.* **4,** 139–155.
72. Gearing D. P., Comeau M. R., Friend D. J., Gimpel S. D., Thut C. J., McGourty J., Brasher K. K., King J. A., Gills S., Mosley B., Ziegler S. F., and Cosman D. (1992) *Science* **255,** 1434–1437.
73. Young P. R. (1992) *Curr. Opinion Biotechnol.* **3,** 408–421.
74. Stahl N. and Yancopoulos G. D. (1993) *Cell* **74,** 587–590.
75. Murakami M., Hibi M., Nakagawa N., Nakagawa T., Yasukawa K., Yamanishi K., Taga T., and Kishimoto T. (1993) *Science* **260,** 1808–1810.
76. Taga T., Hibi M., Hirata Y., Yamasaki K., Yasukawa K., Matsuda T., Hirano T., and Kishimoto T. (1989) *Cell* **58,** 573–581.
77. Huber J., Dittrich F., and Phelan P. (1993) *Eur. J. Biochem.* **218,** 1031–1039.
78. Godard A., Heymann D., Raher S., Anegon I., Peyrat M.-A., Mauff B. L., Mouray E., Gregoire M., Virdee K., Soulillou J.-P., Moreau J.-F., and Jacques Y. (1992) *J. Biol. Chem.* **267,** 3214–3222.
79. Panayotatos N., Everdeen D., Liten A., Somogyi R., and Acheson A. (1994) *Biochemistry* **33,** 5813–5818.
80. Ward L. D., Howlett G. J., Discolo G., Yasukawa K., Hammacher A., Moritz R. L., and Simpson R. J. (1994) *J. Biol. Chem.* **269,** 23,286–23,289.
81. Baumann H., Symes A. J., Comeau M. R., Morella K. K., Wang Y., Friend D., Ziegler S. F., Fink J. S., and Gearing D. P. (1994) *Mol. Cell. Biol.* **14,** 138–146.
82. Stahl N., Farruggella T. J., Boulton T. G., Zhong Z., Darnell J. E. Jr., and Yancopoulos G. D. (1995) *Science* **267,** 1349–1353.
83. Songyang Z., Shoelson S. E., Chaudhuri M., Gish G., Pawson T., Haser W. G., King F., Roberts T., Ratnofsky S., Lechleider R. J., Neel B. G., Birge R. B., Fajardo J. E., Chau M. M., Hanafusa H., Schaffhausen B., and Cantley L. C. (1993) *Cell* **72,** 767–778.
84. Ihle J. N. (1995) *Nature* **377,** 591–594.
85. Hirano T., Matsuda T., and Nakajima K. (1994) *Stem Cells* **12,** 262–277.
86. Lütticken C., Wegenka U. M., Yuan J., Buschmann J., Schindler C., Ziemiecki A., Harpur A. G., Wilks A. F., Yasukawa K., Taga T., Kishimoto T., Barbieri G., Pellegrini S., Sendtner M., Heinrich P. C., and Horn F. (1994) *Science* **263,** 89–92.
87. Stahl N., Boulton T. G., Farruggella T., Ip N. Y., Davis S., Witthuhn B. A., Quelle F. W., Silvennoinen O., Barbieri G., Pellegrini S., Ihle J. N., and Yancopoulos G. D. (1994) *Science* **263,** 92–95.
88. Boulton T. G., Stahl N., and Yancopoulos G. D. (1994) *J. Biol. Chem.* **269,** 11,648–11,655.
89. Ernst M., Gearing D. P., and Dunn A. R. (1994) *EMBO J.* **13,** 1574–1584.
90. Matsuda T., Takahashi-Tezuka M., Fukuda T., Okuyama Y., Fujitani Y., Tsukada S., Mano H., Hirai H., Witte O. N., and Hirano T. (1995) *Blood* **85,** 627–633.
91. Murakami M., Narazaki M., Hibi M., Yawata H., Yasukawa K., Hamaguchi M., Taga T., and Kishimoto T. (1991) *Proc. Natl. Acad. Sci. USA* **88,** 11,349–11,353.
92. Fukunaga R., Ishizuka-Ikeda E., Pan C.-X., Seto Y., and Nagata S. (1991) *EMBO J.* **10,** 2855–2865.
93. Zhong Z., Wen Z., and Darnell J. E. Jr. (1994) *Science* **264,** 95–98.
94. Sadowski H. B., Shuai K., Darnell J. E. Jr., and Gilman M. Z. (1993) *Science* **261,** 1739–1744.
95. Lai C.-F., Ripperger J., Morella K. K., Wang Y., Gearing D. P., Horseman N. D., Campos S. P., Fey G. H., and Baumann H. (1995) *J. Biol. Chem.* **270,** 23,254–23,257.
96. Shuai K., Horvath C. M., Huang L. H. T., Qureshi S. A., Cowburn D., and Darnell J. E. Jr. (1994) *Cell* **76,** 821–828.
97. Ziegler S. F., Bird T. A., Morella K. K., Mosley B., Gearing D. P., and Baumann H. (1993) *Mol. Cell. Biol.* **13,** 2384–2390.

98. Li W., Nishimura R., Kashishian A., Batzer A. G., Kim W. J. H., Cooper J. A., and Schlessinger J. (1994) *Mol. Cell. Biol.* **14,** 509–517.
99. Bennet A. M., Tang T. L., Sugimoto S., Walsh C. T., and Neel B. G. (1994) *Proc. Natl. Acad. Sci. USA* **91,** 7335–7339.
100. Baumann H., Gearing D., and Ziegller S. F. (1994) *J. Biol. Chem.* **269,** 16,297–16,304.
101. Lai C.-F., Ripperger J., Morella K. K., Wang Y., Gearing D. P., Fey G. H., and Baumann H. (1995) *J. Biol. Chem.* **270,** 14,847–14,850.
102. Boulton T. G., Zhong Z., Wen Z., Darnell J. E. Jr., Stahl N., and Yancopoulos G. D. (1995) *Proc. Natl. Acad. Sci. USA* **92,** 6915–6919.
103. Lütticken C., Coffer P., Yuan J., Schwartz C., Caldenhoven E., Schindler C., Kruijer W., Heinrich P. C., and Horn F. (1995) *FEBS Lett.* **360,** 137–143.
104. Schwarzschild M. A., Dauer W. T., Lewis S. E., Hamill L. K., Fink J. S., and Hyman S. E. (1994) *J. Neurochem.* **63,** 1246–1254.
105. Zhang X., Blenis J., Li H.-C., Schindler C., and Chen-Kiang S. (1995) *Science* **267,** 1990–1994.
106. Kumar G., Gupta S., Wang S., and Nel A. E. (1994) *J. Immunol.* **153,** 4436–4447.
107. Daeipour M., Amaral M. C., Kumar G., and Nel A. E. (1993) *J. Immunol.* **150,** 4743–4753.
108. Downward J., Graves J. D., and Cantrell D. (1992) *Immunol. Today* **13,** 89–92.
109. Schiemann W. P., Graves L. M., Baumann H., Morella K. K., Gearing D. P., Nielsen M. D., Krebs E. G., and Nathanson N. M. (1995) *Proc. Natl. Acad. Sci. USA* **92,** 5361–5365.
110. Symes A., Lewis S., Corpus L., Rajan P., Hyman S. E., and Fink J. S. (1994) *Mol. Endocrinol.* **8,** 1750–1763.
111. Symes A. J., Rajan P., Corpus L., and Fink S. (1995) *J. Biol. Chem.* **270,** 8068–8075.
112. Rezzonico R., Ponzio G., Loubat A., Lallemand D., Proudfoot A., and Rossi B. (1995) *J. Biol. Chem.* **270,** 1261–1268.
113. Cederbaum J. M., Chapman C., Charatan M., Stambler N., Andrew L., Zhan C., Radka S., Morrisey D., Lakings D., Brooks B. R., Sanjak M., Pestronk A., Florence J., Mitsumoto H., Szirony K., Bittle L., Riingel S., Neville H., and Brinkmann J. (1995) *Clin. Neuropharmacol.* **18,** 500–514.
114. Cederbaum J. M., Chapman C., Charatan M., Stambler N., Andrew L., Zhan C., Radka S., Morrisey D., Lakings D., Brooks B. R., Sanjak M., Pestronk A., Florence J., Mitsumoto H., Szirony K., Bittle L., Neville H., Ringel S., Brinkmann J., and Wittes J. (1995) *Clin. Neuropharmacol.* **18,** 515–532.
115. Dittrich F., Thoenen H., and Sendtner M. (1994) *Ann. Neurol.* **35,** 151–163.
116. Shapiro L., Zhang X. X., Rupp R. G., Wolff S. M., and Dinarello C. A. (1993) *Proc. Natl. Acad. Sci. USA* **90,** 8614–8618.
117. Henderson J. T., Seniuk N. A., Richardson P. M., Gauldie J., and Roder J. C. (1994) *J. Clin. Invest.* **93,** 2632–2638.
118. Ishikawa M., Iijima H., Satake-Ishikawa R., Tsumura H., Iwamatsu A., Kodaya T., Shimada Y., Fukamachi H., Kobayashi K., Matsuki S., and Asano K. (1992) *Cell Struct. Funct.* **17,** 61–65.
119. Rowlinson S. W., Barnard R., Bastiras S., Robins A. J., Brinkworth R., and Waters M. (1995) *J. Biol. Chem.* **270,** 16,833–16,839.
120. Winter C. G., Saotome Y., Levison S. W., and Hirsh D. (1995) *Proc. Natl. Acad. Sci. USA* **92,** 5865–5869.
121. Ware C. B., Horowitz M. C., Renshaw B. R., Hunt J. S., Liggitt D., Koblar S. A., Gliniak B. C., McKenna H. J., Papayannopoulou T., Thoma B., Cheng L., Donovan P. J., Peschon J. J., Bartlett P. F., Willis C. R., Wright B. D., Carpenter M. K., Davison B. L., and Gearing D. P. (1995) *Development* **121,** 1283–1299.
122. Li M., Sendtner M., and Smith A. (1995) *Nature* **378,** 724–727.
123. Masu Y., Wolf E., Holtmann B., Sendtner M., Brem G., and Thoenen H. (1993) *Nature* **365,** 27–32.
124. Stewart C. L., Kasper P., Brunet L. J., Bhatt H., Gadi I., Kontgen F., and Abbondanzo S. J. (1992) *Nature* **359,** 76–79.
125. Escary J. L., Perreau J., Dumenil D., Ezine S., and Brulet P. (1993) *Nature* **363,** 361–364.
126. DeChiara T. M., Vejsada R., Poueyrnirou W. T., Acheson A., Suri C., Conover J. C., Friedman B., McClain J., Pan L., Stahl N., Ip N. Y., Kato A., and Yancopoulos G. D. (1995) *Cell* **83,** 313–322.
127. Takahashi R., Yokoji H., Misawa H., Hayashi M., Hu J., and Deguchi T. (1994) *Nature Genet.* **7,** 79–84.
128. Yin T. and Yang Y.-C. (1994) *J. Biol. Chem.* **269,** 3731–3738.