

# The neurotrophic factor neuroleukin is 90% homologous with phosphohexose isomerase

Mariella Chaput, Victor Claes, Daniel Portetelle, Isabelle Cludts, Alfredo Cravador, **Arsène Burny\***, H el ene Gras† & Andr e Tartar†

State Faculty of Agronomy, 5800 Gembloux and Department of Molecular Biology, University of Brussels, 1640, Rhode-St-Gen ese, Belgium.

† Chimie des Biomol cules, Institut Pasteur de Lille, 59019 Lille, France

Neuroleukin (NLK) is a protein of relative molecular mass (*M<sub>r</sub>*) 56,000 (56K) secreted by denervated rat muscle<sup>1</sup> and found in large amounts in muscle, brain, heart and kidneys<sup>2</sup>. The protein is a neurotrophic factor for spinal and sensory neurons<sup>2</sup> and a lymphokine product of lectin-stimulated T-cells<sup>3</sup>. It also induces immunoglobulin secretion by human mononuclear cells<sup>3</sup>. Molecular clones of NLK have been expressed in monkey COS cells and the product was shown to have the same biological and biochemical properties as the extracted protein. NLK is abundant in muscle, brain and kidney, but is active at concentrations of 10<sup>-9</sup> to 10<sup>-11</sup> M, similar to those for other polypeptide factors. We have cloned the gene for pig muscle phosphohexose isomerase (PHI) (EC 5.3.1.9) which catalyses the conversion of glucose-6-phosphate to fructose-6-phosphate, an obligatory step in glycolysis, and determined its amino-acid sequence. Surprisingly, it is 90% homologous to the sequence of mouse neuroleukin.

Several observations indicate that the protein sequence translated from our cloned putative PHI complementary DNA is that of mature pig PHI (Fig. 1). The C-terminus end has the same five residues as those determined by Achari *et al.*<sup>4</sup> by carboxypeptidase digestion. The first AUG codon, which we consider to be the start codon, is embedded in a canonical sequence for a eukaryotic translation initiation site (CC<sub>5</sub>CCAUGG)<sup>5</sup>. Pig PHI is resistant to Edman degradation, suggesting that the terminal α-amino group may be blocked. Pronase digestion showed this to be due to an N-terminal acetylalanine residue and this is consistent with the occurrence of alanine in position 1 and 2, but not elsewhere within the first 60 amino-acids of our derived sequence. Lastly, the amino-acid composition determined by Achari *et al.*<sup>4</sup> fits almost perfectly with the sequence translated in Fig. 1.

Comparison of the derived PHI amino-acid sequence with sequences in the NBRF (National Biomedical Research Foundation) data bank revealed its homology with mouse neuroleukin (Fig. 1). The two sequences have 90% homology, and both contain 558 residues and can be aligned without any requirement for insertions or deletions. The differences are mainly conservative replacements and probably reflect species and organ specificity. Of the four cysteine residues present in mouse NLK, only three are in pig PHI (residues 133, 333 and 404), the cysteine in position 330 being replaced by phenylalanine. This observation is of limited importance, however, as previous studies have shown that pig PHI contains no disulphide bonds, and that at least two of its sulphhydryl groups are unimportant for enzyme activity. Similarly no disulphide bond is involved in NLK activity. Three potential N-dependent glycosylation sites are found at positions 105, 129, and 249 in pig PHI and at positions 39, 91, and 129 in mouse NLK but neither molecule has been shown to be glycosylated. In contrast to the coding sequences, the untranslated RNA sequences at the 3' end of the pig PHI and mouse NLK RNAs are strikingly divergent.

Tissues with the highest NLK concentrations are also those

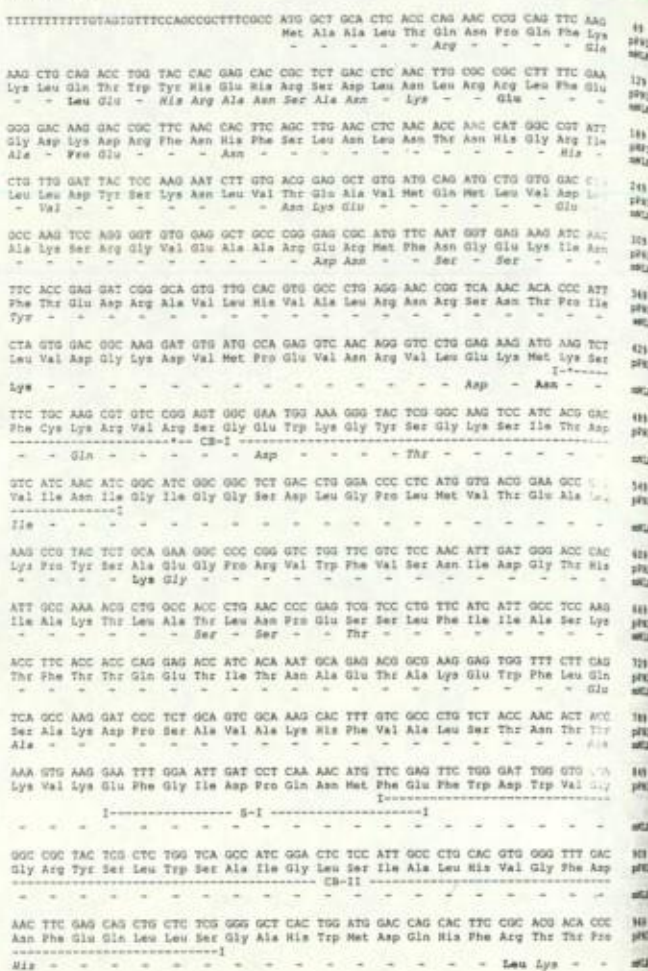


Fig. 1 DNA and translated amino-acid sequence of pig PHI (pPHI) compared to the mouse NLK (mNLK) sequence. Locations of sequenced peptides of pig PHI are underlined: CB-I to CB-V are cyanogen bromide fragments (Achari *et al.*<sup>4</sup>), S-I and S-II are VP staphylococcus protease fragments of our pig PHI preparation, CP is the C-terminus sequence (Achari *et al.*<sup>4</sup>). Protein sequences determined chemically or translated from the cDNA clone are in complete agreement. Positions that vary between pPHI and mNLK are indicated in italics for conservative changes and in dark letters for full changes. Alignment starts from the initiation ATG codons. **Methods.** Pig PHI was purified as described,<sup>8</sup> yielding 63 mg of enzyme from 390 g of tissue. After digestion with V8 protease, and separation by reversed-phase HPLC, two fragments were sequenced by gas phase sequencer analysis. Anti-PHI rabbit polyclonal antibody was obtained by three immunizations at two week intervals using 116 µg of protein emulsified in complete Freund's adjuvant. Mono-

with very high glycolytic activity where PHI is known to be important. PHI is, however, also present in serum and an increase in its activity in serum has been proposed as a marker in various diseases<sup>6</sup>. Release of PHI from muscle cells could create a concentration gradient and provide physiological significance to the observation that NLK promotes the survival of embryonic spinal neurons in cultures that probably include skeletal motor neurons.

Neuroleukin was also shown to be a lymphokine product of lectin-stimulated T-cells. It is not surprising that T-cell stimulation by three different lectins increased the level of PHI messenger RNA, probably because of increased energy requirement, but the lymphokine effect upon Ig release by B-cells is less expected. Possibly NLK itself acts in a lectin-like fashion and NLK has been shown to bind to a surface component of

\* To whom correspondence should be addressed.

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1029	CGG GAG AAG AAC GCC CCC GTC CTG CTG GCT CTG CTG GGT ATC TGG TAC ATC AAC TTC TTT	pPHI
	Leu Glu Lys An Asn Pro Val Leu Leu Ala Leu Leu Gly Ile Trp Tyr Ile An Phe Phe	nHLK
	--- Cys Tyr ---	
1089	GGT TGT GAG ACG GAC GCC ATG CTG CCA TAT GAC CAG TAC CTG CAC GCG TTT GCT GGC TAC	pPHI
	Gly Cys Glu Thr His Ala Met Leu Pro Tyr Asp Gln Tyr Leu His Arg Phe Ala Ala Tyr	nHLK
	--- Leu --- Met ---	
1149	CTC CAG CAG GGT GAC ATG GAG TCC AAC GGG AAG TAC ATC ACC AAG TCC GGC ACC GGT GTG	pPHI
	Thr Glu Glu Gly Asp Met Glu Ser Asn Gly Lys Tyr Ile Thr Lys Ser Gly Thr Arg Val	nHLK
	--- Ala ---	
1209	GAC CAC CAG ACC GCG CCC ATT GTG TGG GGG GAG CCA GGG ACC AAT GGC CAG GAT GGC TTC	pPHI
	Asp His Thr Thr Gly Pro Ile Val Trp Gly Glu Pro Gly Thr Asn Gly Gln His Ala Phe	nHLK
1249	TAC CAG CTG ATC CAC CAA GGT ACC AAG ATG ATA CCG TGT GAC TTC CTT ATC CCG GTC CAG	pPHI
	Leu Ile His Glu Gln Gly Thr Lys Met Ile Pro Cys Asp Phe Leu Ile Pro Val Gln	nHLK
	--- ---	
1289	ACA CAG CAC CCG ATA CCG AAG GGT TTS CAT CAC AAC ATG CTC CTG CCG AAC TTC TTS GCC	pPHI
	Thr Glu His Pro Ile Arg Lys Gly Leu His His Lys Ile Leu Leu Ala Asn Phe Leu Ala	nHLK
	--- CB-III ---	
1389	CAG ACT GAG GGC CTA ATG AAG GGG AAG TGG ACG GAA GAG GCG CCG AAG GAG CTG CAG GGC	pPHI
	Glu Thr Glu Ala Leu Met Lys Gly Lys Ser Thr Glu Glu Ala Arg Lys Glu Leu Glu Ala	nHLK
	--- ---	
1449	GCT GGG AAG AGT CCA GAG GAC TTT GAG AAA CTG CTG CCG CAC AAG GTC TTT GAA GGA AAT	pPHI
	Ala Gly Lys Ser Pro Glu Asp Phe Glu Lys Leu Leu Thr Pro Phe Ile Leu Gly Ala Leu Ile	nHLK
	--- CB-IV --- B-II ---	
	--- Leu ---	
1509	CGC CCC ACC AAC TCT ATT GTG TTC ACC AAG CTC ACG CCG TTC ATC CTT GGA GCG TTG ATT	pPHI
	Arg Pro Thr An Ser Ile Val Phe Thr Lys Leu Thr Pro Phe Ile Leu Gly Ala Leu Ile	nHLK
	--- ---	
1569	GCC ATG TAC GAG CAC AAG ATC TTC GTC CAG GGC GTC ATC TGG GAC ATC AAC AGC TTT GAC	pPHI
	Ala Met Tyr Glu His Lys Ile Phe Val Gln Gly Val Ile Trp Asp Ile An Ser Phe Asp	nHLK
	--- Ile Met ---	
1629	CAG TGG GGA GTG GAG CTG GGA AAG CAG CTG GCT AAG AAA ATT GAA CCG GAG CTT GAT GGC	pPHI
	Glu Trp Gly Val Glu Leu Gly Lys Glu Leu Ala Lys Lys Ile Glu Pro Glu Leu Asp Gly	nHLK
	--- CB-V --- Glu ---	
1689	AAC ACC CCA GTS ACT TCT CAT GAT TCT TCC ACC AAT GGG CTG ATC AAC TTC ATC AAG CAG	pPHI
	Ser Ser Pro Val Thr Ser His Asp Ser Ser Thr An Gly Leu Ile An Phe Ile Lys Glu	nHLK
	--- Ala --- Ser ---	
1749	GAG CGT GAG GGC AGA AGC CAA TAAACTGGTGGCCACTGCACTGCCACTGTGACTGGTCTCTGTGCTCCCT	pPHI
	Glu Arg Glu Ala Arg Ser Gln	nHLK
	--- CP ---	
1839	GTCCGACAGTCTGACGTGATGGTCCGCCCTCTGGTTTTGGGTTTTGGACATAGACCTTGTGGGGAACTGGTGC	
1919	TGGAAATGGCCAGCCCTGCCCTGCATGATTCATGCCGCCCTGTGTTTTAAAGTTGGCTGAAGTGTTCGGTGCAGCTGAAT	
1999	TTCTGACCCATGTTCTCATGTTTCATACCCAGGTGAGAAAATAAAGATGCCATAAGGGAGAAAAA	
2029	AAAAAAAAAAAAAAAAAAAAAAAAAAAAA	

To the best of our knowledge, this is the first example of a protein molecule being endowed with glycolytic activity and trophic activity. We cannot, however, exclude the possibility that the latter function is mediated by peptide fragments of the native molecule generated by extracellular processing.

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● Gurneys' reply—page 456.

## Mouse glucose-6-phosphate isomerase and neuroleukin have identical 3' sequences

Pelin Faik, James I. H. Walker, Alison A. M. Redmill & Michael J. Morgan

Wellcome Research Laboratory for Molecular Genetics, United Medical and Dental Schools of Guy's and St. Thomas' Hospitals, London SE1 9R7, UK

Neuroleukin is a neurotrophic factor of relative molecular mass ( $M_r$ ) 56,000 (56K) found in skeletal muscle, brain, heart and kidneys which supports the survival of embryonic spinal neurones, skeletal motor neurones and sensory neurones<sup>1</sup>. Neuroleukin is also a lymphokine product of lectin-stimulated T cells and induces immunoglobulin secretion by cultured human peripheral blood mononuclear cells<sup>2</sup>. Mouse neuroleukin has been cloned, the complete nucleotide sequence has been determined<sup>3</sup> and its complementary DNA has been transiently expressed in monkey COS-1 cells. The serum-free supernatant of the transfected, but not of control mock-transfected, cells was shown to mimic the properties of neuroleukin isolated from mouse salivary glands. In our work on the molecular genetics of carbohydrate metabolism<sup>3</sup> we have recently isolated a mouse glucose-6-phosphate isomerase (or phosphoglucose isomerase, PGI) cDNA clone using the yeast PGI gene (PGI 1)<sup>4</sup> as a probe. We report here that there is complete sequence identity between the 759 nucleotides at the 3' end of this clone (coding and non-coding) and the sequence of mouse neuroleukin.

We screened a mouse cDNA library with the PGI 1 gene of *Saccharomyces cerevisiae*,<sup>4</sup> and isolated a recombinant containing a 3.7 kilobase (kb) insert which we sub-cloned into M13 for DNA sequence analysis. Comparison with the GenBank sequence data bank revealed a 100% sequence identity between the 759 nucleotides at the 3' end of the mouse PGI which we sequenced and bases 1,164-1,922 of mouse neuroleukin. The sequence of mouse PGI also shows 87% homology with the coding region of human neuroleukin; however, there is a significant loss of homology (down to 60%) in the 3' non-coding region. We have sequenced nearly 70% of the yeast gene (unpublished data): it has a homology of 61% with both mouse PGI and mouse neuroleukin. Further confirmation that neuroleukin and PGI are identical (or closely related) comes from a comparison of published peptide sequence data obtained from

clonal antibodies were obtained using Balb C mice immunized with two doses of 56 µg of the same PHI preparation. Poly(A<sup>+</sup>) RNA isolated from pig skeletal muscle<sup>9</sup> was used as a template to synthesize oligo(dT)-primed cDNA<sup>10</sup>. The cDNA was ligated into the expression vector Agt11 (Stratagene) and screened without amplification with <sup>125</sup>I protein A-labelled<sup>11</sup> rabbit polyclonal, or a mixture of mouse monoclonal anti-PHI antibodies. Four of nine positive clones had inserts of about two kilobases (kb). They were tested for PHI sequence homology by hybridization with an oligonucleotide probe<sup>12</sup> corresponding to amino acids 1-7 of peptide CB-I-A (Achari *et al.*<sup>3</sup>, amino acids 263-269 in our sequence). One of the four clones was subcloned in the EcoRI site of Bluescribe Vector (Stratagene) for DNA sequence determination by the Sanger dideoxynucleotide chain termination method on double-stranded DNA, using a modified T7 DNA polymerase (Sequenase).

the sensory neuron<sup>7</sup>. As PHI is able to recognize phosphorylated glucose and fructose, it could also recognize sugar-containing molecules at the cell surface. It is perhaps pertinent that active PHI is a dimer and that monomers bind the substrate but are inactive.

Recently, the gp120 envelope glycoproteins of human immunodeficiency virus 1 (HIV-1) and simian immunodeficiency virus (SIV) were found to inhibit neuron growth in the presence of NLK<sup>7</sup>. Sequence homology has been identified between NLK (residues 403-447) and a conserved domain of HIV-1 gp120 (residues 238-282). It was postulated that this might be important for this inhibitory property of gp120, and that interactions between gp120 and NLK might play a role in the pathogenesis of AIDS (acquired immune deficiency syndrome)-related dementia.

PHI (pPHI) locations of CB-V are S-II are variation, CP encodes determinant, K are indicators for full ons. ng 63 mg of rotease, and e sequenced, nal antibody ervals using vant. Mon-

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