Differential expression of novH and CTGF in human glioma cell lines

L W Xin, C Martinerie, W Zumkeller, M Westphal, B Perbal

Abstract

Aims—(1) To investigate the expression in human derived glioblastoma cell lines of two structurally related genes, *novH* (nephroblastoma overexpressed gene) and CTGF (connective tissue growth factor), which encode putative insulin-like growth factor binding proteins of a novel type. (2) To investigate whether the same transcription factors regulate CTGF and *novH* expression.

Methods—Expression of *novH* and CTGF was analysed in 24 glioblastoma derived cell lines by northern blotting. The CTGF promoter region was characterised by nucleotide sequencing, RNase protection experiments, by transient transfections, and CAT assays.

Results—CTGF and *novH* mRNA levels differed in the glioma cell lines studied. *NovH* and CTGF genes were not co-expressed in all cell lines. The CTGF promoter region was highly conserved compared with the corresponding region in the mouse (FISP12) and exhibited *in vitro* transcriptional activity.

Conclusions—Although the coding regions of *novH* and CTGF are highly homologous, their promoter regions are substantially different, suggesting that these two genes may be regulated by different mechanisms. Considering that *novH* and CTGF are likely to be, respectively, negative and positive regulators of growth and that some glioma cell lines expressing *novH* are not tumorigenic, expression of these two genes might represent a key element in determining the stage of differentiation or the malignant potential, or both, of some tumour cell lines.

(J Clin Pathol: Mol Pathol 1996;49:M91-M97)

Keywords: *nov*, CTGF, IGF binding proteins, gliomas, brain tumours.

A new family of highly related genes likely to play a role in the control of cell proliferation have been characterised recently.¹ CEF10² gene expression was reported to be induced after production of active v-src in chicken embryo fibroblasts (CEF). CYR61 is the mouse orthologue of the CEF10 gene and was classified as immediate early on the basis of transcriptional activation in mouse NIH3T3 cells by serum, platelet derived growth factor (PDGF), fibroblast growth factor and TPA (12-O-tetradecanoylphorbol-13-acetate).³ FISP12

is also transcriptionally activated by serum in NIH3T3 cells.⁴ CTGF (connective tissue growth factor), the human orthologue gene of FISP12, maps to chromosome 6q23.1⁵ and exhibits mitogenic and chemotactic activities.⁶

The *nov* gene (nephroblastoma overexpressed gene) was originally identified as an integration site of myeloblastosis associated virus type 1 (MAV-1). *Nov* is expressed in normal fetal and newborn kidneys.^{1,7} Raised levels of *nov* mRNA were detected in all MAV-1 induced nephroblastomas. The level of *nov* mRNA in different tumours varied, but it was always significantly higher than in adult normal kidneys.¹ Avian nephroblastoma induced by MAV represents an animal model of Wilms tumour.⁷⁻⁹ The human *nov* gene (*novH*) maps to chromosome 8q24.1 and is highly conserved.^{5,10} Expression of *novH* is also altered in various histological types of Wilms tumours.¹⁰

Alignment of the primary structures¹¹ has suggested that members of this family of proteins share, in addition to a signal peptide and an insulin-like growth factor (IGF) binding domain (GCGCCXXC), three putative distinct modules: (1) an oligomeric complex forming domain (VWC); (2) a binding domain for soluble and matrix molecules (TSP1); and (3) a dimerisation domain (CT). Interestingly, members of this family are distinct from the previously described IGF binding proteins.¹²

Recent studies have revealed that *nov* is not an immediate early gene.⁷ Its expression is associated with quiescence and it is transcriptionally downregulated upon expression of p60^{v-src} in RSV infected CEF.^{7,13} We have previously shown that overexpression of the normal *nov* gene in CEF has an inhibitory effect on cell growth, whereas expression of an amino-terminal truncated form of *nov* results in the induction of morphological transformation.¹ These results indicate that, in contrast to CTGF, *nov* might be a negative regulator of growth and expression of the truncated form could result in oncogenic activation.

Expression of FISP12 has been detected in normal mouse adult brain by northern blotting⁴; *nov* is highly expressed in avian fetal and adult brain¹ and has been detected in human fetal brain by *in situ* hybridisation (Kocalkowski *et al*, manuscript in preparation).

We therefore decided to investigate expression of *novH* and CTGF in human glial tumours, the most common type of primary intracranial tumour.¹⁴ At present, prognosis for patients with malignant gliomas is extremely poor.

Laboratoire
d'Oncologie Virale et
Moléculaire, Institut
Curie-Recherche,
91405 Orsay Cedex,
France
L W Xin
C Martinerie
B Perbal

Department of
Paediatrics, University
Hospital Eppendorf,
Hamburg, Germany
W Zumkeller

Department of
Neurosurgery
M Westphal

UFR de Biochimie,
Université Paris, 7-
Denis Diderot, France
B Perbal

Correspondence to:
Dr B Perbal.

Accepted for publication
14 December 1995

Table 1 Patient data, tumour histology and grading

Cell line NCE	Sex	Age (years)	Histology	WHO grade	Number of passages in vitro
G-22	F	56	GM	4	219
G-28	M	66	GS	4	395
G-44	F	64	GM	4	340
G-55	M	65	AA	3	235
G-59	M	66	GM	4	290
G-60	F	68	AO	4	268
G-61	M	58	AA	4	327
G-62	F	4	GM	4	324
G-63	M	52	GM	4	244
G-84	F	47	AA	3	171
G-96	F	9	GM	4	323
G-111	F	47	GM	4	233
G-118	F	54	GM	4	146
G-120	M	59	GM	4	250
G-121	M	53	GM (recurrent)	4	208
G-122	M	50	GM	4	140
G-123	M	68	GM	4	240
G-140	M	59	GM	4	99
G-141	M	52	GM	4	141
G-142	M	58	GM	4	68
G-167	M	50	GM	4	13
G-168	M	71	GM	4	75
G-169	F	71	GM	4	74
G-195	M	59	GM (recurrent)	4	38

GM = glioblastoma multiforme, GS = gliosarcoma; AA = anaplastic astrocytoma; AO = anaplastic oligodendroglioma.

In an attempt to determine whether *novH* or CTGF, or both, play a role in the biology of these tumours, we compared the pattern of their expression in 24 cell lines, mainly established from human glioblastomas.¹⁵ We also characterised the CTGF promoter region and assessed its activity in vitro.

Methods

CELL CULTURE

HBL100-ras,¹⁶ HEL-1¹⁷ and 293 cells (ATCC CRL 1573) were grown in Eagle's minimum essential medium (MEM, Gibco/BRL) supplemented, respectively, with 10% newborn calf serum, 20% heat inactivated newborn calf serum and 10% heat inactivated horse serum.

Glioma cell lines were established from fresh tumour specimens,¹⁵ obtained from patients aged from four to 71 years. The tumours were classified according to WHO guidelines. The tumour cell lines analysed in this study (table 1) were derived either from tumours of WHO grade 3 or 4 and are referred to as high grade hereafter.¹⁸ The cell lines were maintained at 37°C in Earle's modification of MEM, containing 10% fetal calf serum in a 8% CO₂ humidified atmosphere.

GENOMIC CLONES AND PLASMID SUBCLONES

The structure of the pBH7 and pS6 clones which contain, respectively, the *novH* and the CTGF genes have been described previously.⁵

RNA PURIFICATION AND NORTHERN BLOTTING

Procedures for purifying RNA from cell cultures and northern blotting are described elsewhere.¹⁹ Northern blots were hybridised either to the 3.5 kilobase BglII-BamHI fragment from the pBH7 *novH* clone (probe pBH7/BB) or to the 700 base pair PstI fragment derived from the pS6 CTGF clone pS6 (probe pS6/PSP07) (fig 1A).

NUCLEOTIDE SEQUENCING

Double stranded DNA sequencing was carried out by using the dideoxy-chain termination method²⁰ in the presence of (α -³⁵S) dATP and T7 polymerase (Pharmacia, St Quentin en Yvelines, France). Compressions were resolved using deaza 7-GTP. Sequence data treatments were carried out using the computer facilities at CITI2 in Paris.²¹

RNASE PROTECTION ANALYSES

The pEP subclone was linearised with EcoRV. The linearised subclone (1 µg), was transcribed for 45 minutes at 37°C in a mixture containing transcription buffer (Promega, Charbonnières, France), 10 mM DTT, 25 units RNasin (Promega), 50 mM UTP, 0.3 mM each of ATP, GTP, CTP, 40 µCi (800 Ci/mmol) ³²P [UTP], and 15 units T7 RNA polymerase (Promega). The DNA template was digested with RNase-free DNase (1 unit for 15 minutes at 37°C). The antisense riboprobe (3 ng) was hybridised with total RNA (15 µg) for 24 hours at 50°C in a buffer containing 80% formamide. RNA-RNA hybrids were then digested with RNaseA (40 µg/ml) and RNaseT1 (1000 U/ml) (Boehringer) and analysed in a denaturing 6% polyacrylamide-urea sequencing gel along with ³²P labelled size markers and a nucleotide sequence ladder.

CONSTRUCTION OF THE CHLORAMPHENICOL ACETYLTRANSFERASE (CAT) REPORTER PLASMID

The p605C-CAT was obtained by fusing a PCR generated fragment to the promoterless pCAT5 vector.²² Oligomer #151 (with a HindIII restriction site at its 5' end) and oligomer #152 (with a BglII restriction site at its 5' end) were used to amplify p605C-CAT. The integrity of the PCR generated fragments was checked by sequencing.

TRANSIENT TRANSFECTIONS AND CAT ASSAYS

Forty eight hours before transfection, 293 cells were seeded at a density of 5×10^5 cells per 6 cm dish. Cells were co-transfected with reporter plasmids (10 µg), carrier DNA (10 µg) and the reference RSV-β-galactosidase plasmid (2 µg) using the calcium-phosphate procedure. Plasmids pCAT5 and pRSV-CAT were used as negative and positive controls, respectively. Forty eight hours after transfection, cell extracts and CAT assays were carried out as described by Gorman *et al.*²³ CAT activities were quantitated using a phosphorimager. Cell cultures were transfected in duplicate with each reporter plasmid sample. At least two different preparations of each plasmid DNA were used in these transfection experiments.

Results

Northern blotting experiments carried out on 24 cell lines derived from gliomas (table 1) revealed that *novH* was expressed as a 2.5 kilobase mRNA (fig 1B), which is in agreement with results from earlier studies.¹⁰

Figure 1 Expression of *novH* and *CTGF* in glioma cell lines. **A**, Schematic representation of the *novH* and *CTGF* specific probes. The black boxes represent exons. H=HindIII; P=PstI; K=KpnI; Bg=BglII; Hc=HincII; B=BamHI; S=SacI. **B**, Samples containing total RNA (20 µg) were transferred to Nytran+ membranes (Schleicher and Schuell) and hybridised either with ³²P labelled *novH* or *CTGF* specific probes. The amount of RNA in each lane was normalised following hybridisation with a human GAPDH probe (Clontech).

Levels of *novH* mRNA varied from one type of glioma derived cell line to another and within the same type of tumour derived cells. Expression of *novH* could not be detected in G59, G167, G122, and G120 and barely detected in G169, G142, and G141 cell lines derived from glioblastoma multiforme.

Three different *CTGF* mRNAs species (2.5, 3.5 and 7.0 kilobases) were expressed (fig 1B). The most abundant, 2.5 kilobases, *CTGF* mRNA species has been described previously in HUVE cells.^{6,24} This mRNA species was expressed at various levels in all of the glioma cell lines studied. The 3.5 kilobase mRNA was

observed in a subset of these cell lines. There was no correlation between the expression of the 2.5 and 3.5 kilobase mRNA species. Expression of the 7.0 kilobase mRNA was detected in most of the glioma cell lines except G60, G141 and G120. No obvious correlation with the two other *CTGF* mRNA species could be established.

Both *novH* and *CTGF* genes were expressed at either high (for example, G22, G63, or G62) or low (for example, G60) levels in some glioma cell lines, whereas in others (for example, G59 and G140), expression of these two genes was found to be inversely correlated. These ob-

Figure 2 Alignment of CTGF and FISP12 promoter sequences. CTGF=upper nucleotide sequence. FISP12=lower nucleotide sequence. The putative *cis* regulatory elements for known transcription factors are indicated by horizontal brackets. Horizontal arrows indicate orientation of the oligonucleotides used in PCR. The transcription start sites for CTGF and FISP12 are indicated by vertical arrowheads. Numbers on the left refer to the nucleotide positions relative to the transcription start sites +1. The CTGF sequence has been deposited in the GenBank/EMBL data bank under accession number X92511.

servations suggested that control of the expression of these genes was not exclusive and they may be differentially regulated.

To determine whether the same transcription factors regulate *novH* and CTGF expression, we analysed the organisation of the CTGF promoter. The nucleotide sequence of the 688

base pair EcoR1-Pst1 fragment has been determined (fig 2) and aligned to the corresponding region of the CTGF mouse homologue, FISP12.⁴ A comparison of the nucleotide sequence (fig 2) revealed 77% (476/619 nucleotides) homology in this region.

Computer analysis of the CTGF promoter

Figure 3 Determination of CTGF transcription start sites by RNase protection assay. Schematic structure of the CTGF genomic riboprobe used. Total RNA (15 µg) from HEL-1, HBL100-ras and 293 cells was hybridised to riboprobe EP. Controls with the riboprobes alone (no RNA) were used to check RNase digestion efficiency. Samples (tRNA) containing riboprobes and yeast tRNA (10 µg) were included in each reaction to control for hybridisation specificity. PBR322 digested either with *HinfI* or *HaeIII* and *TaqI* was end labelled with T4 polynucleotide kinase and used as molecular weight markers (MW). Nt = nucleotides

sequence revealed putative binding sites for general and enhancer transcription factors, such as TATA (at -412 and -141), SP1 (at -153, -123, and -46), and AP2 (at -127, +16). The putative CTGF promoter region also contains consensus AP1 binding sequences (at -401 and -90) and a SRE motif (at -491) overlapping an ETS-1 consensus sequence in the opposite orientation. Three M-CAT motifs found in the promoter of several muscle specific genes^{25,26} are also present in the CTGF promoter region.

Several of these binding sites for general and enhancer and transcription factors are also conserved in similar positions in the mouse FISP12 promoter region (fig 2), although no SRE motifs have been found. An AP1-like consensus sequence has been described at a similar position (-390).⁴ The FISP12 promoter contains one additional SP1 site (+134) and a putative ETS-1 consensus sequence (+195).

Comparison of the CTGF/FISP12 and *novH* promoter regions^{27A} did not reveal any structural similarities, suggesting that the expression of these genes is controlled by different mechanisms.

Several putative transcription initiation sites were identified by RNase mapping with the antisense riboprobe EP (fig 3) (735 nucleotides). Two of these sites correspond to the major protected fragments (146–148 nucleotides) obtained on isolation of total RNA from HEL-1, 293 and HBL100-ras cells. Only one of these sites corresponded to the 5' end of the CTGF cDNA isolated from HBL100-ras cells. The potential minor site detected in HEL-1 and 293 mRNAs gave rise to a protected fragment 253 nucleotides long. This site is identical with the transcription start site of FISP12.⁴

The other 131–132 base pair fragments are likely to originate from minor transcripts expressed in these cells. In HBL100-ras cells the EP riboprobe protects two transcripts initiated, respectively, 24 and 200 nucleotides downstream from the TATA box located at position -412 (figs 2 and 3). None of these protected fragments has been detected in reactions involving yeast tRNA or the riboprobe alone. From these results, we conclude that the CTGF gene might be expressed through several alternative promoters depending on the origin of cell the gene is being expressed in.

To confirm that the CTGF promoter region is transcriptionally active in vitro, the -519, +85 region was subcloned upstream of the CAT reporter gene. The 293 cells were transfected with p605C-CAT construct, and the CAT activity measured. Positive (pRSV-CAT) and negative (pCAT5) controls were cultured in parallel. As shown in fig 4, the CAT activity driven by the p605C-CAT construct was in the same range as that driven by the positive control, indicating that the CTGF 5' flanking sequences (-519, +85) indeed represent a potential functional promoter.

Discussion

We report that both *novH* and CTGF are expressed in glioblastoma derived cell lines. The level of expression of these genes differed from one cell line to another. Several different CTGF transcripts were also identified. Among them, the 3.5 and 7.0 kilobase mRNAs were not detected in HUVE cells^{6,24}; however, these mRNA species were detected in other tissues and cell lines (unpublished data). Although the structure of the 3.5 kilobase mRNA species is not known as yet, it could result from alternative splicing events. Inasmuch as CTGF transcription can be initiated at several sites in different cells lines, expression of the 2.5 and 3.5 kilobase mRNAs might be under the control of alternative promoters. Further studies will reveal whether these mRNAs encode the same or different proteins.

As the 7.0 kilobase mRNA species is not detected with all CTGF derived probes (Martinierie *et al*, unpublished data), it might be

Figure 4 Activity of the CTGF promoter construct. 293 cells were transfected with p605C-CAT (10 µg). Plasmids pRSV-CAT and pCAT5 (10 µg) were used, respectively, as positive and negative controls. CAT assays were carried out as described in the methods section. CAT activities were quantitated relative to the control reaction with the pRSV-CAT vector. A representative autoradiogram and the average of relative CAT activities with standard errors are shown.

either an alternatively spliced transcript or originate from another CTGF related gene.

The differential pattern of *novH* and CTGF expression seen in some glioma cell lines has also been observed in other series of human tumours and cell lines²⁷ (unpublished data). These observations strongly suggest that expression of *novH* and CTGF is controlled by different transcription factors. The lack of homology between *novH* and CTGF promoter regions supports this hypothesis. However, we cannot rule out the possibility that post-transcriptional regulation could account for at least part of the differential expression observed. It is of worth noting that *nov* mRNA species do not contain the 3' proximal TTATTAT motif that renders transcripts unstable,²⁸ while CTGF mRNA species do.⁶

A growing body of evidence suggests that *novH* is a negative regulator of cell growth.^{17 13} CTGF, however, has mitogenic and chemotactic properties.⁶ It is therefore conceivable that a balance between expression of the *novH* and CTGF genes is required to modulate the proliferation state of cells. The *nov* product is associated with the extracellular matrix²⁷ and the CTGF/FISP12 proteins are secreted into the medium.⁴⁶ Thus, taking into account that CTGF competes with PDGF BB for binding sites on the NIH3T3 cell surface,⁶ it is possible that *nov* also interacts directly with an as yet unidentified cell receptor.

Characterisation of *nov* and CTGF as putative IGF binding proteins of a novel type suggests that signal transduction induced by *nov* and CTGF could be mediated through their interactions with IGF proteins and their receptors. Interactions between *nov*/CTGF and IGF could either act positively or negatively, by increasing the local concentration of IGF, allowing activation of IGF-I receptor or by trapping IGF and regulating its release and availability. It is worth noting that IGF-I and II are involved in the regulation of brain development²⁹ and are thought to play a crucial

role in the proliferation of brain tumours, particularly gliomas.^{30 31}

The glioma cell lines used in this study have been extensively analysed for expression of many growth factors and their receptors,^{32–35} and for the production of oncogenes and tumour suppressors.^{36 37} Their karyotypes have also been established and their tumorigenicity assessed in nude mice.¹⁸ Interestingly, five of the *novH* expressing cell lines (G44, G22, G61, G121, and G96) were not tumorigenic in nude mice.¹⁸ Conversely, the G59 cell line in which no *novH* mRNA species could be detected was tumorigenic. This apparent correlation between a lack of *novH* expression and tumorigenicity would support a role for *nov* as a negative regulator of growth. However, the G55 and G28 cell lines expressed *novH* and were tumorigenic. It will be interesting to establish whether *novH* protein can be detected in these cell lines. The tumorigenicity of the other cell lines has yet to be evaluated.¹⁸ No correlation between CTGF and tumorigenicity could be established.

Ongoing experiments should allow us to determine whether modulation of the expression of *novH* and CTGF can be used to alter the growth or tumorigenicity, or both, of these glioma cell lines. Conversely, use of antisense mRNAs and *nov* antibodies will allow us to study the effect of inhibition of *nov* and CTGF expression on cell growth.

Future studies are proposed to elucidate whether *novH* and CTGF represent specific markers of tumoral differentiation or progression, or both, and whether they can be used in targeted gene therapy.

This work has been supported by grants to B Perbal from Association pour la Recherche contre le Cancer (ARC, "marqueurs tumoraux"), Ligue Nationale Contre le Cancer (Comités du Cher, de Paris), Fondation pour la Recherche Médicale, Fondation Franco-Chinoise (PRA), CNRS and Institut Curie.

- Joliet V, Martinerie C, Dambrine G, Plassiart G, Brisac J, Crochet J, *et al*. Proviral rearrangements and over-expression of a new cellular gene (*nov*) in myeloblastosis-associated virus type 1-induced nephroblastomas. *Mol Cell Biol* 1992;12:10–21.
- Simmons DL, Levy DB, Yannoni Y, Erikson RL. Identification of a phorbol ester-repressive v-src-inducible gene. *Proc Natl Acad Sci USA* 1989;86:1178–82.
- O'Brien TP, Yang GP, Sanders L, Lau LF. Expression of *cyr-61*, a growth factor inducible immediate-early gene. *Mol Cell Biol* 1990;10:3569–77.
- Ryseck RP, MacDonald-Bravo H, Mattei MG, Bravo R. Structure, mapping and expression of FISP-12, a growth factor-inducible gene encoding a secreted cysteine-rich protein. *Cell Growth Differ* 1991;2:225–33.
- Martinier C, Viegas-Pequignot E, Guenard I, Dutrillaux B, Nguyen VC, Bernheim A, *et al*. Physical mapping of human loci homologous to the chicken *nov* proto-oncogene. *Oncogene* 1992;7:2529–34.
- Bradham DM, Igarashi A, Potter RL, Grotendorst GR. Connective tissue growth factor: a cysteine-rich mitogen secreted by human vascular endothelial cells is related to src-induced immediate early gene product CEF-10. *J Cell Biol* 1991;114:1285–94.
- Perbal B. Contribution of MAV-1 induced chicken nephroblastoma to the study of genes involved in human Wilms' tumor development. *Critical Reviews in Oncogenesis* 1994; 5:589–613.
- Heine U, DeThé G, Ishiguro H, Sommer JR, Beard D, Beard JW. Multiplicity of cell response to the BAI strain A (myeloblastosis) avian tumor virus. II. Nephroblastoma (Wilms' Tumor): ultrastructure. *J Natl Cancer Inst* 1962; 29:41–61.
- Ishiguro H, Beard D, Sommer JR, Heine U, DeThé G, Beard JW. Multiplicity of cell response to the BAI strain A (myeloblastosis) avian tumor virus. I. Nephroblastoma (Wilms' Tumor): gross and microscopic pathology. *J Natl Cancer Inst* 1962;29:1–38.
- Martinier C, Huff V, Joubert I, Badzioch M, Saunders G,

- Strong L, et al. Structural analysis of the human *nov* proto-oncogene and expression in Wilms tumors. *Oncogene* 1994; 9:2729–32.
- 11 Bork P. The modular architecture of a new family of growth regulators related to connective tissue growth factors. *FEBS Lett* 1993;327:125–30.
- 12 Kiefer MC, Ioh RS, Bauer DM, Zapf J. Molecular cloning of a new human insulin-like growth factor binding protein. *Biochem Biophys Res Commun* 1991;176:219–25.
- 13 Scholz G, Martinerie C, Perbal B, Hanafusa H. Transcriptional downregulation of the *nov* proto-oncogene in fibroblasts transformed by p60^{v-src}. *Mol Cell Biol* 1996;16:481–6.
- 14 Russell DS, Rubinstein LJ. *Pathology of tumors of the nervous system*. 5th edn. Baltimore: Williams and Wilkins, 1989: 449–52.
- 15 Westphal M, Hansel M, Brunken M, Köning A, Köppen JA, Herrmann HD. Initiation of primary cell cultures from human intracranial tumors on extracellular matrix from bovine corneal endothelial cells. *Exp Cell Biol* 1987;55: 152–63.
- 16 Lebeau J, Le Chalony C, Prosperi MT, Goubin G. Constitutive overexpression of a 89 kDa heat shock protein gene in the HBL100 human mammary cell line converted to a tumorigenic phenotype by the EJ/T24 Harvey-ras oncogene. *Oncogene* 1991;6:1125–32.
- 17 Azzarone B, Chaponnier C, Krief P, Mareel M, Suarez HG, Maceira-Coelho A. Human fibroblasts from cancer patients: lifespan and transformed phenotype in vitro and role of mesenchyme in vivo. *Mutation Res* 1988;199:313–25.
- 18 Westphal M, Hansel M, Hamel W, Kunzmann R, Hölzel F. Karyotype analyses of 20 human glioma cell lines. *Acta Neurochir (Wien)* 1994;126:17–26.
- 19 Perbal B. *A practical guide to molecular cloning*. New York: John Wiley, 1988.
- 20 Sanger F, Nicklen S, Coulson AR. DNA sequencing with chain terminating inhibitors. *Proc Natl Acad Sci USA* 1977; 74:5463–7.
- 21 Dessen P, Fondrat C, Valencien C, Mugnier G. *A French service for access to biomolecular sequence databases*. Cabios, 1990.
- 22 Stein B, Rahmsdorf HJ, Steffen A, Liftin M, Herrlich P. UV-induced DNA damage is an intermediate step in UV-induced expression of human immunodeficiency virus type 1, collagenase, c-fos and metallothionein. *Mol Cell Biol* 1989;9:5169–81.
- 23 Gorman CM, Moffat LF, Howard BH. Recombinant genomes which express chloramphenicol acetyl transferase in mammalian cells. *Mol Cell Biol* 1982;2:1044–51.
- 24 Igarashi A, Okochi H, Bradham DM, Grotendorst GR. Regulation of connective tissue growth factor gene expression in human skin fibroblasts and during wound repair. *Mol Biol Cell* 1993;4:637–45.
- 25 Mar JH, Ordahl CP. A conserved CATTCTCT motif is required for skeletal muscle-specific activity of the cardiac troponin T gene promoter. *Proc Natl Acad Sci USA* 1988; 85:6404–8.
- 26 Mar JH, Ordahl CP. M-CAT binding factor, a novel trans-acting factor governing muscle-specific transcription. *Mol Cell Biol* 1990;10:4271–83.
- 27 Perbal B. Caractérisation et expression du proto-oncogène *nov* humain dans les tumeurs de Wilms. *Bull Cancer* 1994b; 81:957–61.
- 27A Martinerie C, Chevalier G, Rauscher F J III, Perbal B. Regulation of *nov* by WT1: a potential role for *nov* in nephrogenesis. *Oncogene* (in press).
- 28 Shaw G, Kamen R. A conserved AU sequence from the 3' untranslated region of GM-CSF mRNA mediates selective mRNA degradation. *Cell* 1986;46:659–67.
- 29 Sara VR, Carlsson-Skewir C. The role of insulin-like growth factors in the regulation of brain development. *Prog Brain Res* 1988;73:87–99.
- 30 McMorris FA, Smith TM, De Salvo S, Furlanetto RW. Insulin-like growth factor I/Somatostatin C: A potent inducer of oligodendrocyte development. *Proc Natl Acad Sci USA* 1986;83:822–6.
- 31 Recio-Pinto E, Rechler MM, Ischii DN. Effects of insulin, insulin-like growth factor II, and nerve growth factor on neurite formation and survival in cultured sympathetic and sensory neurons. *J Neurosci* 1986;6:1211–19.
- 32 Hamel W, Westphal M, Shepard HM. Proto-oncogene expression in human glioma derived cell lines. *Int J Oncol* 1992;1:673–82.
- 33 Hamel W, Westphal M, Szonyi E, Escandon E, Nicolies K. Neurotrophin gene expression by cell lines derived from human gliomas. *J Neurosci Res* 1993a;34:147–57.
- 34 Westphal M, Herrmann HD. Growth factor biology and oncogene activation in human gliomas and their implications for specific therapeutic concepts. *Neurosurgery* 1989;25:681–94.
- 35 Westphal M, Hamel W, Anker L, Herrmann HD. Analysis of growth regulatory pathways in human neuro-oncology. *Molecular Diagnostics of Cancer* 1993;151–67.
- 36 Hamel W, Westphal M, Shepard HM. Loss in expression of the retinoblastoma gene product in human gliomas associated with advanced disease. *J Neurooncol* 1993b;16: 159–65.
- 37 Anker L, Ohgaki H, Ludeke BI, Herrmann HD, Kleihues P, Westphal M. p53 protein accumulation and gene mutations in human glioma cell lines. *Int J Cancer* 1993;55: 982–7.