Characterization of the human nestin gene reveals a close evolutionary relationship to neurofilaments

JONAS DAHLSTRAND1, LYLE B. ZIMMERMAN2, RONALD D.G. McKay2 and URBAN LENDAHL1,*

1Department of Molecular Genetics, Medical Nobel Institute, Karolinska Institute, S-104 01 Stockholm, Sweden
2Department of Brain and Cognitive Sciences, E25-435, Massachusetts Institute of Technology, Cambridge, MA 02139, USA

*Author for correspondence

Summary

Multipotential stem cells in the neural tube give rise to the different neuronal cell types found in the brain. Abrupt changes in intermediate filament gene expression accompany this transition out of the precursor state: transcription of the intermediate filament nestin is replaced by that of the neurofilaments. In order to identify human neural precursor cells, and to learn more about the evolution of the intermediate filaments expressed in the central nervous system, we have isolated the human nestin gene. Despite considerable divergence between the human and rat nestin genes, in particular in the repetitive parts of the carboxy-terminal region, the positions of the introns are perfectly conserved. Two of the three intron positions are also shared by the neurofilaments, but not by other classes of intermediate filaments. This implies that nestin and the neurofilaments had a common ancestor after branching off from the other classes of intermediate filaments, and that nestin separated from the neurofilament branch before the different neurofilament genes diverged. The characterization of human nestin also facilitates the identification of human multipotential neural precursor cells. This will be of importance for central nervous system (CNS) tumor diagnosis and transplant-based clinical approaches to human neurodegenerative diseases.

Key words: intermediate filament, cytoskeleton, neurobiology, evolution.

Introduction

The mammalian brain presents a challenge to developmental biology: how is the large number of distinct neural and glial cell types produced and assembled into a functional nervous system? Understanding how these cell types are generated depends critically on the ability to identify the multipotential central nervous system (CNS) precursor cells (for review see McKay, 1989). In rodents, these precursor cells can be distinguished from their neuronal and glial progeny by the expression of the intermediate filament protein nestin, while the mature neurons and astrocytes express neurofilaments and GFAP (glial fibrillary acidic protein), respectively. Nestin was originally identified by the monoclonal antibody Rat.401 (Hockfield and McKay, 1985), and was shown to be expressed predominantly in CNS stem cells in the neural tube (Frederiksen and McKay, 1988). Analysis of the nestin gene in rat demonstrated that it encodes an intermediate filament protein of a novel, sixth class (Lendahl et al., 1990). Nestin expression has been used to identify rodent CNS stem cells in various areas of the developing nervous system and in immortalized CNS precursor cell lines (Frederiksen et al., 1988; Cattaneo and McKay, 1990; Redies et al., 1991; Renfranz et al., 1991; Valtz et al., 1991). Identification of the human neuroepithelial stem cell is important in neuroncology and in transplantation approaches to neurodegenerative diseases. The isolation of the human nestin gene will facilitate the characterization of this cell type.

Intermediate filaments undergo extensive remodelling during development, and various tissues express distinct sets of intermediate filament proteins. This is the result of strictly controlled temporal and spatial expression of approximately 40 different intermediate filament genes (Zehner, 1991). Common to all characterized intermediate filament proteins is a central approximately 300 amino acid long α-helical domain (Steinert and Roop, 1988). Based on expression patterns, sequence similarities and positions of introns, intermediate filaments are divided into six classes (Steinert and Liem, 1990). Within a class the amino acid identity is 50% or greater over the α-helical domain; between classes conservation is generally 20-30%. Class I and class II intermediate filaments are the basic and acidic keratins, respectively, which are expressed in epithelia. Class III consists of desmin, GFAP, peripherin and vimentin. Class IV are the neurofilaments and α-internexin; class V the nuclear lamins; and nestin comprises class VI intermediate filaments.

The coordinated changes of the intermediate filament proteins are particularly conspicuous in the tissues giving
rise to the CNS, in which all classes of cytoplasmic intermediate filaments are sequentially expressed. Preimplantation embryos express cytokeratins 8 and 18 (classes I and II, respectively); following neurulation multipotential CNS stem cells express nestin (class VI) and vimentin (class III). Finally, terminal neural differentiation involves down-regulation of nestin and induction of neurofilaments (class IV). A transient expression of α-internexin (class IV) occurs during CNS development, and GFAP (class II) is expressed in astrocytes (for references see Steinert and Liem, 1990). Peripherin (class III) is expressed by subsets of neurons, especially those with projections to the peripheral nervous system (Leonard et al., 1988; Thompson and Ziff, 1989).

All genes in the intermediate filament family probably originated from a lamin-like nuclear protein ancestor. Lamins are present in nucleated cells in a very broad range of species, ranging from mammals to molluscs (Dessev and Goldman, 1990), and probably even to yeast (Georgatos et al., 1990). Recently, an invertebrate cytoplasmic intermediate filament was identified (Dodemont et al., 1990), with an intron pattern very similar to that of lamins (Dodemont et al., 1990; Döring and Stick, 1990). The shared intron pattern suggests that the first cytoplasmic intermediate filament arose by loss of the nuclear localization signal and a nuclear membrane binding isoprenylation signal (Dodemont et al., 1990; Döring and Stick, 1990). The cytoplasmic intermediate filaments then separated into two groups: the classes I-III in one group and the neurofilaments (IV) in the other. The two groups have different splicing patterns, and it has been proposed that the neurofilament branch originated by retrotransposition of an intronless RNA intermediate and subsequent acquisition of the neurofilament-specific introns (Lewis and Cowan, 1986). An alternative view is that introns have been inserted separately into the classes I-III and neurofilament branches (Julien et al., 1987). Given the recent discovery of nestin it would be of interest to compare its evolution with other classes and to see to which of the two branches in the phylogenetic tree it belongs.

We here report the characterization of the sequence and genomic structure of the human nestin gene. Our data provide insight into the evolution of nestin, and also demonstrate that class VI intermediate filaments are closely related to the neurofilament branch. In addition, the expression of nestin in developing human brain will facilitate the identification of human neuroectodermal precursor cells for a variety of clinical uses.

Materials and methods

Southern blots

A 10 µg sample of high molecular weight genomic DNA from a number of species was digested with EcoRI, size-separated by electrophoresis in an agarose gel, and transferred to nylon membrane (Zoo blot, Clontech). The filter was hybridized with a 32P-labelled probe (Feinberg and Vogelstein, 1983), derived from the rat nestin gene, containing 680 bp of the first exon (Lendahl et al., 1990). Prehybridization was carried out in 6×SSC, 5×Denhardt’s, 0.5% SDS and 100 µg/ml salmon sperm DNA, at 65°C for 1 hour. Hybridization was carried out at the same conditions except for using 50 µg/ml salmon sperm DNA and hybridizing for 16 hours. The filters were washed for 2×30 minutes at 48°C in 2×SSC and 0.25% SDS. A parallel Zoo blot filter (Clontech) was hybridized with a human nestin probe, corresponding to a 750 bp fragment from the first exon of the human nestin gene, under the same conditions, but washed at high stringency: 0.1×SSC (1×SSC is 0.15 M NaCl, 0.015 M sodium citrate, pH 7.0), 0.25% SDS at 65°C for 2×30 minutes.

Cloning of the human nestin gene

A genomic library in the vector EMBL 3 made from partially Sau3A-digested human DNA (Clontech) was screened under the same conditions as described above for the low-stringency hybridization. A positive clone was purified and grown on a large scale (Maniatis et al., 1989). A restriction map was established and EcoRI fragments were subcloned in the plasmid vector Bluescript KS I (Stratagene). The sequence of all potentially coding regions was determined by dideoxy sequencing of truncated clones (Erase-a-base, Promega Biotech) with either 35S (Sequenase II, USB) or with fluorescent dideoxy nucleotides or primers on an automated sequencing machine, according to the manufacturer’s instructions (Applied Biosystems). The sequence for the coding parts of the human nestin gene will appear in the EMBL, GenBank and DDBJ Nucleotide Sequence Databases under the accession number X65964.

Polymerase chain reaction

To establish exactly the intron/exon boundaries a fragment was amplified by the polymerase chain reaction (PCR) from approximately 5×106 pooled clones of a cDNA library from a 21-week-old fetal human brain. The primers were 5′ GGCTTGGAAACA-GAGTTGGA 3′ (corresponding to amino acid 240 to 246) and 5′ TGGGAGAAAGATCCAAGAC 3′ (amino acid 322 to 328, in reverse), and the PCR reaction was carried out for 30 cycles at 94°C for 1 minute, 52°C for 1 minute, and 72°C for 1 minute. The amplified fragment was directly sequenced as described above, using fluorescent dideoxynucleotides (Applied Biosystems). To confirm the length of the repetitive part of the carboxy-terminal region we amplified the region between the primers 5′ CTCGGCAAGAGAACCTAGAG 3′ (amino acid 659 to 664) and 5′ GACCGAGAGATCTAG 3′ (amino acid 791 to 797, in reverse), starting from 100 ng of total genomic human DNA or 10 pg of plasmid DNA from the positive human nestin clone. The PCR conditions were the same as above, except that 35 cycles were carried out.

Northern blots

A 1.25 µg sample of poly(A)+ RNA from human fetal brain (21 weeks after gestation), fetal liver and kidney, and from the cell line 293 (30 µg total RNA), was denatured and electrophoresed in a 1% agarose gel containing 2.2 M formaldehyde in 1×MOPS buffer (Maniatis et al., 1989) for 4 hours. The RNA was then transferred to Nylon membrane (Hybond-N, Amersham). The filter was prehybridized and hybridized (5×SSC, 5×Denhardt’s, 250 µg/ml salmon sperm DNA, 50% formamide, 50 mM sodium phosphate (pH 6.5), 0.5% SDS at 42°C for 16 hours) with 2×106 cts/min of one of two different 32P-labeled probes (Feinberg and Vogelstein, 1983) made from the α-helical region (ranging 800 bp starting from 100 nucleotides upstream from the transcription start) and from the carboxy-terminal region (corresponding to amino acid 375 to 1453) of the human nestin gene, respectively. After hybridization the filter was washed (final wash = 2×30 minutes at 65°C in 0.2×SSC, 0.2% SDS) and exposed to X-ray film at −70°C for 14 days with intensifying screens. The size of the hybridizing mRNA was determined by running RNA molecules of known size (BRL) in parallel.
**Computer analysis of DNA sequences**

The compiled sequences from the human and rat nestin genes were aligned and compared with help of the University of Wisconsin Genetics Computer Group program package (Devereux et al., 1984). The window size and stringency using the “Compare” program were 21 and 14.0, respectively.

**Results**

**Cloning of the human nestin gene**

To investigate the evolution of nestin, we first analysed DNA from a variety of species for sequences that cross-hybridized to a probe from the rat nestin gene. The probe was selected to represent most of the α-helical region, since this domain has been best conserved during evolution of other intermediate filaments. At moderately stringent hybridization and washing conditions, we identified cross-hybridizing sequences from all mammalian species analysed, including human, and possibly also from chick (Fig. 1a), but not from yeast (data not shown). The strongest hybridization signals were derived from rat and mouse. The simple band pattern suggested that mammals possess only one nestin gene, and that we did not detect more distantly related genes. When more stringent conditions were applied only bands in rat and mouse were identified (data not shown).

Having determined the hybridization and washing conditions that allowed the identification of a single band in human genomic DNA, we used the same conditions to screen a human genomic library for nestin-hybridizing clones. A genomic library rather than a cDNA library was chosen because we wanted to establish the intron pattern in addition to the coding sequence. One positive clone out of $1.5 \times 10^6$ contained the entire human nestin gene and was selected for further analysis. A restriction map of the clone was established and the various fragments subcloned into plasmid vectors (data not shown).

To verify that we had cloned the human gene most closely related to rat nestin, we hybridized one of the subcloned fragments of the α-helical region in the human gene to DNA from the same species as above. After hybridization and washing at high stringency, the strongest band was detected in man (Fig. 1b). The hybridizing band was of the same size as the band weakly hybridizing to the rat probe, proving that we had cloned the correct sequences. Weaker bands were also observed in African Green monkey, rabbit and chick.

**Structure of the human nestin gene**

After subcloning the human nestin gene into plasmid vectors, we determined the nucleotide sequence of all coding regions and parts of the introns (Fig. 2). To establish the boundaries of the introns, we used the polymerase chain reaction (PCR) to amplify a fragment made from human fetal brain cDNA using two primers located in the first and fourth exon, respectively. The resulting 270 base pair (bp) long fragment was then sequenced directly in its entirety, and intron positions precisely located by comparison with the genomic sequence. Putative initiation and stop codons

---

**Fig. 1.** Analysis of the nestin gene in different species. (a) Analysis of EcoRI-digested DNA from the indicated species, probed with the α-helical region of the rat nestin gene at moderate stringency. (b) A similar blot to that in (a), probed with parts of the α-helical region of the human nestin gene at high stringency. A schematic representation of the rat (a) and human (b) nestin genes is shown below, with the α-helical region in black, and the region used as probe shown striped.
Fig. 2. The predicted amino acid sequence of the human nestin gene. The amino acid sequence in one letter code for human (upper), and, for comparison, for the rat nestin gene (lower). A vertical line indicates identity between the two sequences, a dot in the sequence shows that an amino acid is missing. The sequences have been aligned to optimize similarity. The 11 amino acid repeats have been underlined. Introns positions are denoted with an arrowhead above the sequence, and the α-helical region is boxed.
Table 1. A comparison of evolutionary rates for the various classes of intermediate filaments

<table>
<thead>
<tr>
<th>Intermediate filament</th>
<th>α-Helical region</th>
<th>C-terminal</th>
</tr>
</thead>
<tbody>
<tr>
<td>K18</td>
<td>90</td>
<td>87</td>
</tr>
<tr>
<td>K8</td>
<td>94</td>
<td>77</td>
</tr>
<tr>
<td>GFAP</td>
<td>93</td>
<td>96</td>
</tr>
<tr>
<td>NFH</td>
<td>97</td>
<td>74</td>
</tr>
<tr>
<td>NFM</td>
<td>99</td>
<td>84</td>
</tr>
<tr>
<td>NFL</td>
<td>98</td>
<td>89</td>
</tr>
<tr>
<td>Lam C</td>
<td>99</td>
<td>92</td>
</tr>
<tr>
<td>Nestin</td>
<td>82</td>
<td>55</td>
</tr>
</tbody>
</table>

The degree of amino acid identity between man and mouse (for nestin, man and rat) was compared for the α-helical domain and the carboxy terminus. The values denote amino acid identity after maximizing the alignment of the corresponding genes, using the Gap program. mouse: K18 (Ichinose et al., 1988); K8 (Morita et al., 1988); GFAP (Balcarek and Cowan, 1985); NFH (Julien et al., 1988); NFM (Levy et al., 1987); NFL (Lewis and Cowan, 1986); lamin C (Riedel and Werner, 1989); nestin (rat) (Lendahl et al., 1990); man: K18 (Kulesh and Oshima, 1988); K8 (Krauss and Franke, 1990); GFAP (Reves et al., 1989); NFH (Lees et al., 1988); NFM (Myers et al., 1987); NFL (Julien et al., 1987); lamin C (Fisher et al., 1986).

Expression of the human nestin gene
Poly(A)+ RNA from a brain of a 21-week-old human fetus was analysed with respect to nestin expression by Northern blot analysis, using probes derived from the human nestin gene. In the fetal brain RNA we observed a single hybridizing band, approximately 6 kb in length (Fig. 5). In addition, we analysed RNA from fetal liver and kidney of the same age. Liver did not contain detectable amounts, whereas very low levels of nestin hybridizing RNA was detected in kidney (data not shown). Finally, RNA from the immortalized human embryonic kidney cell line 293 (Graham et al., 1977) did not contain detectable levels of nestin RNA (data not shown).
**Discussion**

The cells that become the mammalian CNS express all five different classes of cytoplasmic intermediate filaments in a strict temporal and spatial order, beginning with the cytokeratins (classes I and II) expressed by early embryonic cells, through nestin and vimentin (classes VI and III) in the mitotic neuroepithelium, to the neurofilaments and GFAP (classes IV and III) expressed by differentiated neurons and astrocytes. In this carefully staged pathway of cytoskeletal gene expression, nestin represents multipotent neuroectodermal stem cells and neurofilaments terminally differentiated neurons. To understand further how this complexity has arisen during the evolution of intermediate filament genes, to study the intragenic evolution of class VI intermediate filaments, and to identify human neuronal stem cells, we have analyzed the expression, structure and genomic organization of the human nestin gene.

**Fetal human brain expresses nestin**

The developing brain of a 21-week-old human fetus expresses nestin as a single 6 kb mRNA species. Expression in early human CNS is in accordance with previous data from rat (Frederiksen and McKay, 1988; Cattaneo and McKay, 1990; Lendahl et al., 1990; Renfranz et al., 1991; Valtz et al., 1991). The level of nestin mRNA in the 21-week-old fetal brain is relatively low compared to levels recorded during rat CNS development (Lendahl et al., 1990). However, in a 21-week-old human fetus CNS development is relatively advanced in most major brain areas, implying that the number of actively proliferating stem cells has declined at this stage (Friede, 1989). This view is also supported by results obtained by anti-nestin immunohistochemistry on developing human CNS (Tohyama et al., 1992). An expression pattern very similar to that in rat was observed, with maximal nestin immunoreactivity correlated to the proliferative state in spinal cord, telencephalon and cerebellum. The transient expression in neuroectodermally derived cells suggests that nestin may be a useful marker for multipotential neural stem cells in man as well as rodents.

The absence of detectable amounts of nestin RNA in the...
non-neuroectodermal 293 cell line is interesting in the light of nestin expression in tumors. Previous data indicate that nestin expression is observed in immortalized cell lines of neuroectodermal origin (Frederiksen et al., 1988; Redies et al., 1991; Valtz et al., 1991). Lack of expression in the 293 cell line suggests that nestin expression is not automatically associated with immortalization, but may occur only in tumors or immortalized cell lines of neuroectodermal origin. This notion receives support from experiments by Redies et al. (1991), in which cell lines immortalized by the oncogene SV40T from early CNS, but not from epithelia, expressed nestin. Similarly, only primary CNS tumors, but not metastasizing tumors, express nestin in tumor cells (Dahlstrand et al., unpublished). These various lines of evidence suggest a strong correlation between nestin expression and the proliferative CNS stem cell state, which may make nestin a useful tool for the diagnosis of various CNS tumors (Dahlstrand et al. unpublished; Tohyama et al., 1992).

An improved characterization of human CNS stem cells may also be important in therapeutic approaches to neurodegenerative diseases. Progress has been made in recent years with transplantation of fetal brain tissue in the treatment of Parkinson’s disease (for review, see Björklund, 1991). However, the numbers of embryonic cells available currently limit the application of this technology. This problem has been addressed in rodents in two ways: nestin positive CNS precursor cells can be expanded in primary culture (Cattaneo and McKay, 1990; Reynolds et al., 1992) and cell lines derived from the embryonic brain can show extensive differentiation following transplantation (Renz et al., 1991; Snyder et al., 1992). The isolation of the human nestin gene is a useful step towards the application of similar strategies in humans.

Nestin evolves faster than other intermediate filaments

On the basis of the hybridization experiments, a single nestin gene appears to be conserved in all mammalian species analysed and possibly in birds. The detailed characterization of the nestin gene from a second species, man, allows an examination of evolutionary rates in various parts of the nestin gene and a comparison with other intermediate filaments. It is apparent that the α-helical domain and the carboxy-terminal region evolve at quite different rates. The amino acid sequence in the α-helical region is 82% identical in rat and man, but only 55% identical in the carboxy-terminal region. The relatively slower evolution in the α-helical region is in accordance with observations from the other five classes (Table 1), suggesting that the same principal mode of selection operates also in class VI intermediate filaments. The rate of change, however, is considerably higher in nestin for both the α-helical and carboxy-terminal regions than in the other five classes, where conservation between rodent and man is between 90 and 99% in the α-helical domain and between 74 and 92% in the carboxy-terminal region (Table 1).

Conspicuous among the differences between the rat and human nestin genes are a number of size variations in the carboxy-terminal region. The most dramatic changes have taken place in a region between amino acids 657 and 862. Interestingly, this region is also the most repetitive in the nestin gene. In the rat gene, 41 copies of an 11 amino acid repeat, S/PLEK/EEN/DQES/PLR, are located here. In the human gene, a virtually identical SLEK/EENQEXLR motif can be discerned 18 times in the same region, i.e. the human gene is 23 copies shorter. The correlation of size variation with the most repetitive regions is striking, and similar observations have been made in other repetitive genes, for example in the involucrin gene (Eckert and Green, 1986), or for myogenic dystrophy (Harley et al., 1992), where in the latter case the length difference also is related to the severity of the disease. Slipping during replication (Jones and Kafatos, 1982) and unequal crossing over (Smith, 1976) are two mechanisms known to generate size differences in repetitive DNA, and it is conceivable that these mechanisms may operate also in the carboxy-terminal region of the nestin gene. The repetitive regions in nestin may thus be part of why nestin evolves fast. It is interesting to note that the similarly repetitive regions in neurofilaments also are subject to size fluctuations (Julien et al., 1988; Lees et al., 1988).

Nestin and neurofilaments are closely related evolutionarily

The common denominators shared by the intermediate filament gene family include the α-helical region and the association of the proteins with filamentous structures in vivo. Furthermore, similarities within a class are high, in general upwards of 50% amino acid identity in the α-helical region (Steinert and Liem, 1990). However, the various classes have diverged sufficiently that evolutionary relationships between classes have been difficult to establish based on direct sequence comparisons. Instead, intron patterns have been more informative, and the current view of intermediate filament evolution holds that the ancestral gene was lamin-like and encoded a nuclear protein. A recent comparison between Xenopus lamin and an invertebrate cytoplasmic intermediate filament demonstrated a conserved intron pattern and suggested that the transition from nuclear to cytoplasmic intermediate filaments occurred by loss of nuclear localization and isoprenylation signals (Dodemont et al., 1990; Döring and Stick, 1990). Class I, II and III intermediate filament are most likely the direct descendents of this first cytoplasmic intermediate filament, since they share some of the intron positions. Neurofilaments, in contrast, represent a more distantly related class, with a completely different splice pattern. According to one hypothesis they are derived by retrotransposition through an intronless RNA intermediate, and have later acquired new introns, distinct from the ones in classes I to III (Lewis and Cowan, 1986). Alternatively, the original ancestor of the entire gene family could have been intronless and the two main branches, classes I, II and III versus the neurofilaments, the consequence of separate introduction of introns (Julien et al., 1987; Steinert and Roop, 1988). In light of the nuclear to cytoplasmic transition (Dodemont et al., 1990; Döring and Stick, 1990) the former hypothesis appears more likely.

We find that the human nestin gene, and also the rat gene, have two of the three intron positions in common with the three neurofilament genes. In contrast, none of the intron positions is identical to intron positions found in classes I,
II and III. This places nestin in the same evolutionary branch as the neurofilaments. Given that the degree of similari-
y in the α-helical region is approximately 50% among
the neurofilament genes and only 20% compared to nestin,
it is reasonable to assume that nestin branched off before
the split into the three different neurofilament genes, but
after the originally intronless post-retrotransposition ances-
tor had acquired the common two introns. The branching
was most likely the result of a gene duplication at this stage
to form nestin and the ancestor of the three neurofilament
genes. Later, further duplications probably gave rise to the
individual neurofilament genes. The common intron posi-
tions in nestin and neurofilaments renew the question of
classification of intermediate filaments, and it has in fact
been suggested that nestin should be assigned to the class
IV intermediate filaments (Weber et al., 1991). We, how-
ever, believe that the distinctly lower degree of sequence
similarity between nestin and neurofilaments, compared to
among different neurofilaments (20% versus 50%), and the
unusually short amino-terminal and long carboxy-terminal
region in nestin still speak in favor of nestin as a class VI
intermediate filament. In addition, an amphibian gene,
expressed in neuronal growth cones and developing axons,
and which encodes a 200 kDa protein with approximately
45% amino acid identity to nestin in the α-helical region,
has recently been characterized (Hemmati-Brivanlou, A.,
Mann, R. W. and Harland, R. M., personal communica-
tion). This suggests that a duplication has also occurred to
form a nestin subfamily.

The intermediate filaments are characterized by their
diversity and cell type-specific expression. This large family
of genes can be divided into two branches by intron posi-
tion. It is interesting that nestin and neurofilaments are evo-
lutionarily related by this criterion and are expressed
sequentially during the development of the nervous system.
The function of nestin is not known, but comparison of the
rat and human sequences identifies conserved structural
motifs that may be functionally significant. The function
of intermediate filaments can now be approached by trans-
genic technology, as demonstrated for keratins (Vassar et
al., 1991). Transgenic mice can also be used to study the
regulation of nestin expression (Zimmerman et al., unpub-
lished), and identification of nestin’s regulatory DNA ele-
ments may allow analysis of transacting factors that func-
tion specifically in neural stem cells. The cloning of the
human nestin gene will promote the analysis of this impor-
tant cell type in man.

We are grateful to Dr. Christer Höög for introducing us to the
fluorescent sequencing techniques and to Erik Nilsson and Pre-
drag Petrovic for excellent technical assistance. The work was
supported by the Swedish Cancer Society, the Swedish Medical
Research Council, Margaret and Axel Ax:son Johnsons Stiftelse,
Knut and Alice Wallenbergs Stiftelse, Magn. Bergvalls Stiftelse,
Stiftelsen Lars Hiertas minne, Karolinska institutets fonder (J.D.,
U.L.) and the NIH and Pew Foundation (L.Z., R.M.).

References

fibrilary acidic protein gene: implications for the evolution of the


Cattaneo, C. and McKay, R. (1990). Proliferation and differentiation of
neuronal stem cells regulated by nerve growth factor. Nature 347, 762-
765.

intermediate filament protein α-intermixin and functional analysis of its

major component of the nuclear lamina during embryonic development of
the surf clam. Int. J. Develop. Biol. 34, 267-274.

of sequence analysis programs for the VAX. Nucl. Acids Res. 12, 387-
395.

invertebrate gene encoding cytoplastic intermediate filament (IF)
proteins: implications for the origin and the diversification of IF proteins.
EMBO J. 9, 4083-4094.

Döring, V. and Stuck, R. (1990). Gene structure of nuclear lamin LIII of
Xenopus laevis; a model for the evolution of IF proteins from a lamin-like
ancestor. EMBO J. 9, 4073-4081.


DNA restriction endonuclease fragments to high specific activity. Anal.
Biochem. 132, 6-13.

Fisher, D. Z., Chaudhary, N. and Blobel, G. (1986). cDNA sequencing of
nuclear lamin A and C reveals primary and secondary structure homology
to intermediate filaments. Proc. Natl. Acad. Sci. USA 83, 6450-
6454.

Immortalization of precursor cells from the mammalian CNS. Neuron 1,
439-448.

Frederiksen, K. and McKay, R. (1988). Proliferation and differentiation of


Characteristics of a human cell line transformed by DNA from human

Harley, H. G., Brook, J. D., Rundle, S. A., Crow, S., Reardon, W.,
Expansion of unstable DNA region and phenotype variation in myotonic

the developing mammalian nervous system. J. Neurosci. 5, 3310-3328.

Ichinohe, Y., Morita, T., Zhao, X., Stumpf-Hausongeran, S., Tondella, M.
Nucleotide sequence and structure of the mouse cytokeratin endo B gene.
Gene 70, 85-95.

family: Evolutionary diversification of duplicated DNA. J. Mol. Evol. 19,
87-103.

Julien, J.-P., Côte, F., Beaudet, L., Sidky, M., Flavell, D., Grosvedl, F.

Julien, J.-P., Grosvedl, F., Yazdanbakh, K., Flavell, D., Meijer, D. and
(NF-L): a unique exon-intron organization in the intermediate filament


gene and its expression in nonepithelial mouse cells. Mol. Cell. Biol. 8,
1540-1550.

Lees, J. F., Shnedins, P. S., Skuntz, S. F., Carden, M. J. and Lazzarini,
neurofilament subunit (NF-H) and the gene encoding it. EMBO J. 7, 1947-
1955.

cells express a new class of intermediate filament protein. Cell 60, 585-
595.


(Received 29 May 1992 - Accepted 3 July 1992)

Note added in proof
The work described as by Dahlstrand et al. (unpublished results) has now been accepted for publication: Dahlstrand, J., Collins, V. P. and Lendahl, U. Cancer Res. (in press).