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ARTICLE

# *Alu*-splice cloning of human *Intersectin* (*ITSN*), a putative multivalent binding protein expressed in proliferating and differentiating neurons and overexpressed in Down syndrome

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**By *Alu*-splice PCR we have trapped two exons and subsequently identified the full length cDNA of a human gene, *Intersectin* (*ITSN*), which maps to chromosome 21q22.1 between markers D21S320 and D21S325. The gene has the potential to code for at least two different protein isoforms by alternative splicing (*ITSN-L* and *ITSN-S*). *Intersectin* exists with a high degree of similarity in flies, frogs and mammals, suggesting a conserved role in higher eukaryotes. Analysis of the expression pattern of human and mouse *Intersectin* detected mRNAs in all adult and foetal tissues tested, with the longer isoform present in brain. *In situ* hybridisation studies in the developing mouse brain showed *ITSN* expression in both proliferating and differentiating neurons. The genomic structure of *ITSN* was determined using the chromosome 21 sequences deposited in the public databases. The protein contains several known motifs which implicate ITSN in clathrin mediated endocytosis and synaptic vesicle recycling. The expression pattern of *Intersectin* in mouse brain, its presumed function and its overexpression in brains from Down syndrome patients, suggest that *Intersectin* may contribute in a gene dosage-dependent manner to some of the abnormalities of Down syndrome.**

**Keywords:** ***Intersectin*; chromosome 21q22; Down syndrome; endocytosis**

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## Introduction

Down syndrome (DS) or trisomy 21 is the most common chromosomal aneuploidy that comes to term in humans. With an incidence of 1 in 700 live births DS is the most frequent genetic cause of mental retardation.[1] DS affects every major organ system in the body and includes many abnormalities, such as an increased risk of childhood leukaemias, congenital heart and gastrointestinal tract defects, and characteristic cranofacial anomalies. DS individuals also develop Alzheimer-like amyloid plaques and neurofibrillary tangles by approximately the third decade of life, with a significant number of these patients developing presenile dementia.[2]

The most likely consequence of three copies of human chromosome 21 is the overexpression of the genes on this chromosome. Therefore, the DS phenotype presumably results from a perturbation of the interactions of these chromosome 21 gene products with their usual targets, due to the increased gene dosage. One of the major aims of DS research is to identify the roles of individual genes in specific pathologies that occur as part of the DS phenotype. The isolation and characterisation of these genes is the first step in understanding the biochemical and molecular bases for these pathologies and this information is crucial for the development of therapeutic strategies.

Human chromosome 21 is estimated to contain 600–1000 genes. International gene cloning efforts have led to the identification of numerous genes and transcriptional units[3–11] and, more recently, large scale sequencing (http://www-eri.uchsc.edu/chr21/eridna.html) has enabled the prediction of many more. However, even though 112 genes (GDB, 20 September 1998) have been assigned to chromosome 21, the functions of most of these genes remain largely unknown, as does their contribution, if any, to the DS phenotype.

With the aim of contributing to the transcriptional map of human chromosome 21 we developed a technique to isolate expressed sequences called *Alu*-splice PCR,[12] and trapped two exons of the gene that we report here called human *Intersectin* (*ITSN*). During the preparation of this manuscript the human *Intersectin* gene was published[13] and we concur with all aspects. The assembly of the partial clones we obtained by cDNA library screening and RACE gave rise to a continuous 6439 bp long cDNA with an open reading frame of 1721 amino acids, a 5'-UTR of 268 bp and a 3'-UTR of 1005 bp lacking a clear consensus polyadenylation signal. We also identified a shorter form of *ITSN* arising from an alternative splicing event which would encode the first 1220 amino acids of Intersectin. The 3'-UTR of this isoform was 1450 bp and had several consensus polyadenylation signals. We refer to the two different isoforms as *Intersectin* long (*ITSN-L*) and short (*ITSN-S*). *ITSN-L* was expressed specifically in brain (adult and foetal) and gave a transcript of about 11 kb, whilst *ITSN-S* was expressed in all the tissues tested and gave a predominant band of about 6 kb with a less abundant transcript of 5 kb in some tissues.

Database searches revealed that the predicted ITSN polypeptide shared 81% amino acid identity with *Xenopus leavis* intersectin[14] (GenBank acc. no. AF032118). A partial sequence of the gene (SH3P17, GenBank acc. no. U61166) had also been reported in a screening of SH3 domain-containing proteins[15] and later on was mapped to chromosome 21.[16,17] ITSN was also similar to two *Drosophila* proteins, Dap160-1 and Dap160-2.[18] A number of functional domains are present in the protein. ITSN posesses two EH domains or Eps15 homology domains which are involved in protein–protein interactions and are frequently present in multiple copies.[19] Most of the EH-containing proteins, such as mammalian Eps15[20] and yeast End3p,[21] have been linked to endocytic pathways. The central part of the protein has five consecutive Src homology 3 (SH3) domains.[22] SH3 domains are commonly found in signal transduction and cytoskeletal proteins. They mediate protein–protein interactions by binding to proline rich peptides.[23]

The carboxy terminus specific to the long ITSN isoform contains three well described domains. The first matches a Dbl homology (DH) domain. DH domains are regions of about 180 amino acids which serve as the catalytic regions of guanine nucleotide exchange factors (GEF) for Rho-like GTPases.[24] As in all the other DH domain-containing proteins, the ITSN DH domain is followed by a pleckstrin homology (PH) domain. PH domains are conserved protein modules of about 100 amino acids.[25] They mediate membrane phosphoinositide interactions and in some cases they may function in protein–protein interactions. Many of the PH domain-containing proteins are involved in regulating intracellular signalling pathways or the cytoskeleton.[26] The most carboxy region of the long isoform has sequence homology with C2 domains.[27] The C2 domain is a calcium-binding motif which is widely distributed among eukaryotic proteins including lipid modifying

enzymes, protein kinases, GTPases and proteins involved in membrane trafficking. Thus, human *Intersectin* encodes a putative multi-modular protein with several conserved domains that suggest it is related to proteins involved in clathrin mediated endocytosis and synaptic vesicle recycling.

Here, in addition to the above, we report that *ITSN* is expressed early in mouse development and *in situ* hybridisation in mouse brains shows that *Intersectin* is expressed in many areas of the developing and adult brain. Moreover, the human *Intersectin* gene is overexpressed in DS and, as such, is a candidate for some of the clinical aspects of the pathogenesis of this chromosomal aneuploidy.

# Materials and Methods

### *cDNA Cloning and Sequencing*

The original clone 860g11-76 (GenBank acc. no. U84494) was isolated by *Alu*-splice PCR using the human chromosome 21 YAC 860G11 as template, the *Alu* primer *Sal*I-A33 (5'-CGCGTCGACCACTGCACTCCAGCCTGGGCG-3') and the splice primer *Not*I-5' spl (5'-CGCGCGGCCGCACWYACCW-3'), where W represents A or T and Y represents T or C), using the same conditions described in Fuentes *et al.*[12] PCR products were cloned in pBluescript SK (Stratagene, La Jolla, USA). This clone was used as a probe for isolating a full-length cDNA by a combined approach of cDNA library screening and RACE. Protein analysis for domain and pattern recognition was carried out using the following public programs: Profilescan (http://www.isrec.isb-sib.ch/software/PFSCAN_form.html), SMART[28] (http://coot.embl-heidelberg.de/SMART) and Motif (http://www.motif.genome.ad.jp).

### *Northern Blotting*

A northern blot containing poly $A^+$ RNA from mouse embryos of different developmental stages (Cat. No. 7763-1) was purchased from Clontech (Palo Alto, USA). Northern blots were prehybridised in a buffer containing 5 × SSPE, 10 × Denhardt's, 2% SDS and 100 μg/ml salmon sperm DNA for 3 h at 65°C. Hybridisation was in the same buffer at 65°C for 16 h with the probe indicated in the Results section. Blots were washed sequentially in 2 × SSC-0.1%SDS, 1 × SSC-0.1%SDS, 0.5 × SSC-0.1%SDS and 0.2 × SSC-0.1%SDS, at 60°C for 20 min each and exposed to X-ray film for 3 to 10 days at –70°C.

### *RNA Isolation and Semiquantitative RT-PCR*

Total RNA was extracted from prosencephalon of 18–22 weeks' gestation DS and non-DS foetal abortusses using the RNeasy Kit (Qiagen GmbH, Gilden, Germany). In both cases, the RNA was a mixture from two individuals. Tissue was obtained in accordance with institutional guidelines and for all the cases the post-mortem period was less than 12 h. cDNA was synthesised by reverse transcription of 3 μg of total RNA using the Ready-to-Go kit (Pharmacia Biotec, Uppsala, Sweden) and random hexamers as primers, according to manufacturer's recommendations. One μl of the cDNA first strand synthesis was used as a template in a PCR reaction with human *ITSN-L*-specific primers 6 (5'-TTCTCCCCGGACATCTTCTT-3') and 7B (5'-TCAGAGGGCTCTGGAACTGCT-3') and *glyceraldehyde phosphate dehydrogenase* (*GAPDH*) primers[29] using the following cycle conditions: 95°C for 30 s, 59°C for 30 s and 74°C for 50 s, for 16, 20, 23 and 27 cycles. Controls were performed under the same conditions with 25 amplification cycles. Products were run on a 2% agarose gel, stained with ethidium bromide and photographed.

### *RNA* in situ *Hybridisation*

*In situ* hybridisation was performed on free-floating sections essentially as described.[30] Embryos (stage E16) and postnatal OF1 mice (Iffa Credo, Lyon, France) were used in this study. Embryos and adults were transcardially perfused with 4% paraformaldehyde in phosphate buffered saline (PBS) and their brains were postfixed. After cryoprotection with 30% sucrose, brains were frozen on dry ice and cut in coronal sections (thickness: 60 μm E16 and 30 μm adult). Sections were collected in a cryoprotective solution (30% glycerol-30% ethylenglycol-40% PBS) and stored at –80°C until use.

The mouse IMAGE clone 482370 was used to synthesise the riboprobes.[31] Sense and antisense riboprobes were generated on a linear fragment of the clone obtained by PCR using M13 primers. The DIG RNA labelling kit (Boehringer Mannheim GmbH, Mannheim, Germany) was used with either T3 or T7 RNA polymerase.

# Results

## *Structure of* ITSN

Two forms of human *Intersectin* were isolated using the *Alu*-splice clone 860g11-76 (accession no. U84494).[12] The sequences of the two forms, *ITSN-L* and *ITSN-S* are deposited in GenBank with accession nos. AF114487 and AF114488, respectively and are identical to the sequences reported by Guipponi and colleages.[13] The *ITSN* cDNA sequence was contained within the genomic sequences present in the GenBank database entries AP000048, AP000049 and AP000050 derived from chromosome 21 and deposited by Sakaki and co-workers. Comparison of these sequences with the *ITSN* cDNA allowed us to elucidate its genomic organisation. *ITSN* consists of at least 41 exons (Table 1), spans more than 233 kb of genomic DNA between markers D21S320 and D21S325 and is transcribed in the 21cen– > qter direction. All the introns have canonical splice donor and acceptor sites (Table 1). The genomic structure enabled us to confirm that the generation of the long isoform is the result of an alternative splicing event in which exon 29, which provides the stop codon for the short isoform when joined to exon 28, is skipped. Sequence analysis of human *Intersectin* ESTs, as well as *SH3P17*, which

variously lack exons 19, 24 and 25, revealed that other alternatively spliced mRNAs may exist.

## *Expression of Mouse* Intersectin *mRNA*

Searches of mouse EST databases identified several putative murine *Intersectin* homologue clones. One of these, IMAGE clone 482378 (GenBank acc. no. AA063751) was used as a hybridisation probe with a foetal mouse northern blot containing four mouse embryo developmental stages (Figure 1) to define the time course of expression during development. As in the human northerns, the probe detected bands of 5 kb, 6 kb and 11 kb, suggesting a high degree of conservation and demonstrating that the brain-specific splicing event is conserved in the mouse. For the 5 kb and 6 kb transcripts, expression was first detected at day 11 of mouse foetal development, whilst the 11 kb transcript was first visible by northern at day 17. Since the RNA on the blot is derived from the whole mouse embryo, the relative amount of the brain-specific transcript is high.

**Table 1** Exon–intron boundaries of human *ITSN*

| *Exon* | *Size (bp)* | *5′ splice donor*[a] | *Intron* | *Size (bp)* | *3′ splice applicator*[a] |
|---|---|---|---|---|---|
| –1 | >236 | TGAG**gt**gagg | –1 | >62184[b] | attttcacttac**ag**GCGT |
| 1 | 60 | GGTG**gt**aagt | 1 | 2321 | tctttggatttt**ag**GCAG |
| 2 | 93 | ACTG**gt**aatc | 2 | 1317 | tgtttaatttac**ag**GTGA |
| 3 | 64 | TATG**gt**aagt | 3 | 12392 | ctgttggtttgc**ag**GGCA |
| 4 | 161 | TTTG**gt**aagt | 4 | 14938 | tttttcttcat**ag**GTAT |
| 5 | 180 | CCTG**gt**atgt | 5 | 1487 | atttatttttac**ag**CAGC |
| 6 | 97 | CCAG**gt**aagt | 6 | 3390 | tcttttccccac**ag**TGTC |
| 7 | 101 | ACAG**gt**attt | 7 | 6524 | tggttcctgttt**ag**GTCC |
| 8 | 64 | TATG**gt**aagg | 8 | 3888 | tttttgttgaat**ag**GAAT |
| 9 | 138 | TTAG**gt**aagg | 9 | 1700 | ccccgcaattgc**ag**AAGA |
| 10 | 116 | CCTG**gt**aagg | 10 | 4232 | ttccttgtctga**ag**TAAC |
| 11 | 236 | AGAG**gt**aagc | 11 | 2405 | tgtaaatgtcac**ag**GCTG |
| 12 | 150 | TCTA**gt**gagt | 12 | 89 | ttcatttgttca**ag**AATG |
| 13 | 141 | ACAG**gt**gagg | 13 | 6352 | tccttccttccc**ag**GAAT |
| 14 | 88 | CACA**gt**aggt | 14 | 445 | tttttctaaaat**ag**GAGA |
| 15 | 140 | GAAG**gt**aact | 15 | 12207 | tcggttaattat**ag**GAAC |
| 16 | 128 | AAAG**gt**gagt | 16 | 2910 | gtgtttgttggc**ag**ACGA |
| 17 | 230 | GCAG**gt**atta | 17 | 2199 | ttttttgtaaac**ag**AAAA |
| 18 | 122 | CATG**gt**aaga | 18 | 2500 | ctttcctggtgg**ag**GTTA |
| 19 | 15 | ATGG**gt**aagt | 19 | 8530 | gactttttccac**ag**GTGG |
| 20 | 248 | CCAG**gt**acgt | 20 | 2690 | gcttttccctcc**ag**GTGG |
| 21 | 160 | CCAG**gt**aaag | 21 | 4194 | ttattccacaca**ag**GGTG |
| 22 | 206 | CAAG**gt**attt | 22 | 768 | ttccattattgc**ag**CATG |
| 23 | 83 | GAAG**gt**gagg | 23 | 4163 | tttctccccacc**ag**AATT |
| 24 | 167 | AGAG**gt**aaac | 24 | 3164 | tgctctgtttta**ag**GGCT |
| 25 | 46 | CCTG**gt**aagt | 25 | 2760 | atcttgtttttc**ag**AAAT |
| 26 | 122 | GCAA**gt**cagt | 26 | 4561 | tattttcttact**ag**GCAC |
| 27 | 118 | GCAG**gt**aagg | 27 | 2016 | cctgtctcctgc**ag**TGTG |
| 28 | 192 | CAAT**gt**aagt | 28 | 354 | cctcctttttct**ag**GAAT |
| 29 | >1453 | – | 29 | 18246 | gttgtgttttcc**ag**GGTG |
| 30 | 122 | AGAG**gt**aagg | 30 | 1828 | ttctgcatctgt**ag**ATTT |
| 31 | 107 | TAAA**gt**aagc | 31 | 6358 | gtgtttccgtgc**ag**AGCG |
| 32 | 184 | CAAA**gt**aagg | 32 | 1898 | acatctcatttc**ag**AGAT |
| 33 | 99 | AAAT**gt**aagt | 33 | 8022 | attctcctgtgt**ag**ATCC |
| 34 | 168 | TGAG**gt**agcc | 34 | 6721 | ttttcctttctc**ag**CAAC |
| 35 | 213 | AACA**gt**aagt | 35 | 1094 | gtgttactttcc**ag**CCTA |
| 36 | 122 | AAAG**gt**gaga | 36 | 1369 | tttttctgtcca**ag**GACT |
| 37 | 83 | CTGG**gt**aatg | 37 | 315 | tcctgccgtcat**ag**TCCG |
| 38 | 84 | CATG**gt**aagg | 38 | 764 | tttgttccctcc**ag**GAAA |
| 39 | 174 | GATG**gt**gagt | 39 | 1691 | tgttctcttttc**ag**ATTT |
| 40 | >1154 | – | | – | |

[a]Nucleotides from exon (u.c.); from intron (l.c.). Conserved splice donor and acceptor sites are in bold.
[b]The exact size of intron –1 is unknown as the genomic sequence deposited for this region is incomplete

To analyse further the pattern of *Intersectin* expression in the developing and adult mouse brain, we performed *in situ* hybridisation studies (Figure 2). At embryonic day 16 (E16), *Intersectin* mRNA was widely distributed in different regions of the brain. For instance, in the forebrain, high expression levels were detected in the neocortex, hippocampus, septum and striatum (Figure 2A). Expression was observed both in the proliferative layers (eg ventricular zone in the cerebral cortex), as well as in the differentiating fields where postmitotic neurons are undergoing maturation (eg the cortical plate in the cerebral cortex). *Intersectin* mRNA expression was also detected in other areas, such as in discrete nuclei of the thalamus, mesencephalon, and rhombencephalon (Figure 2B). The gene was also expressed in the developing cerebellum and spinal cord (data not shown). These observations show that *Intersectin* is expressed in both proliferating and differentiating neurons.

In the adult murine brain, *Intersectin* was expressed in many neuronal populations in the forebrain, midbrain and hindbrain. In the cerebral cortex, for instance, transcripts were detected in all the layers of the neocortex as well as in the pyramidal neurons and granule cells of the hippocampus and the dentate gyrus, respectively (Figure 2C). Expression was also observed in the olfactory bulb, striatum, thalamus, brain stem and cerebellum, where the Purkinje cells were labelled (Figure 2D). Taking into account all these data, we can conclude that *Intersectin* is widely expressed in both the developing and adult brain.

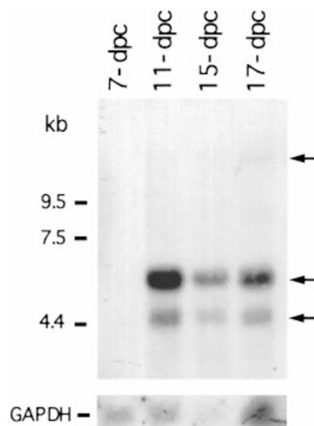


**Figure 1** *Expression analysis of mouse* Intersectin *by northern blot hybridisation. Northern blot containing 2 μg of poly $A^+$ from mouse embryos of 7 dpc, 11 dpc, 15 dpc and 17 dpc was hybridised with IMAGE clone 482370. A* GAPDH *control hybridisation is shown below*

### *Analysis of* ITSN *Expression in Down Syndrome*

We compared the level of expression of *ITSN* in brains from DS and non-DS foetuses. We carried out a semiquantitative RT-PCR on RNA prepared from prosencephalon. We used *GADPH* primers[29] as control primers, which produced a band of 496 bp, and *ITSN*-specific primers, which produced a band of 748 bp from the brain-specific long isoform (Figure 3). The results clearly show that *ITSN-L* is overexpressed in the brains of DS foetuses compared with controls.

## Discussion

We have reported the identification and isolation of a novel chromosome 21 gene, *Intersectin (ITSN)*, so named because of its great similarity (81% amino acid identity) to *Xenopus intersectin*. The human gene codes for at least two transcripts, the larger being the product of a brain-specific splicing event. The two proteins differ in the length of their carboxy termini, and thus the larger isoform contains extra functional domains. Two of the exons of human Intersectin were identified by *Alu*-splice PCR, which we have demonstrated is a powerful method for the isolation of exons from large genomic clones.[12,32]

*Xenopus* intersectin interacts via its SH3 domains with dynamin (a GTPase) and synaptojanin 1, proteins identified as components of the recycling machinery of synaptic vesicles in nerve terminals.[33] It also binds two mouse proteins, Ibp1 and Ibp2 through its EH domain.[14] Ibp1 is the murine homologue of human epsin,[34] a protein concentrated in presynaptic nerve terminals which binds the α-adaptin subunit of AP-2 and the clathrin-coat-associated protein Eps15. Human Intersectin showed a great similarity to two *Drosophila* proteins Dap 160-1 and Dap 160-2.[18] Dap 160 was isolated as a dynamin associated protein and identified as a membrane protein present in resting endocytic hot spots in *Drosophila* nerve terminals.[18] Thus, it is possible that Dap 160, *Xenopus* intersectin and human Intersectin are members of a family of proteins which may serve as scaffolding proteins required for clathrin-mediated endocytosis.

In humans, a long, brain-specific isoform of *ITSN* exists. This isoform is also present in the mouse, suggesting an essential role for this species in the brain throughout development and adulthood. This long isoform of human *Intersectin* has the extra capacity to encode DH, PH and C2 motifs. The DH (or Dbl)

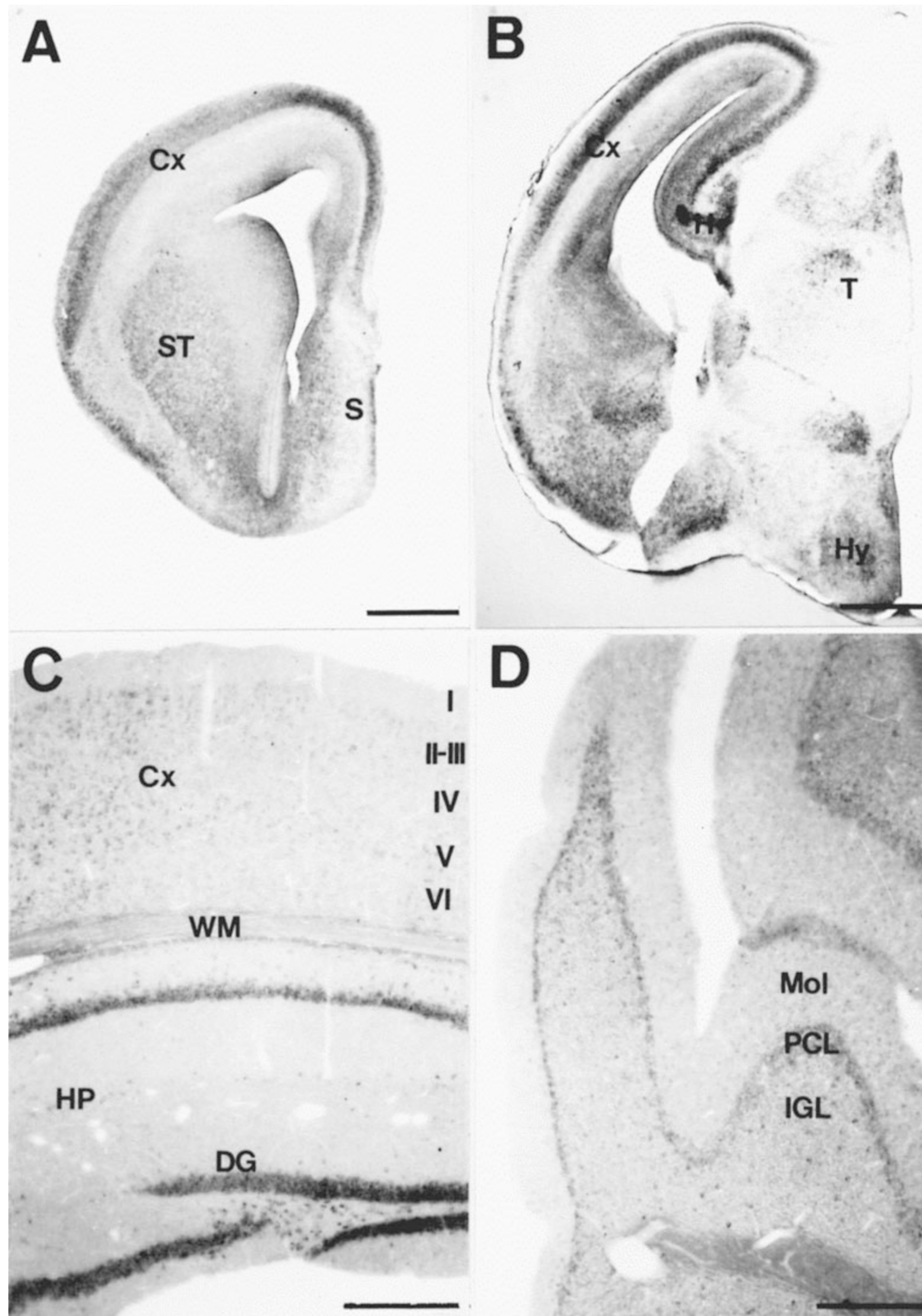


**Figure 2** In situ *hybridisation analysis. Low-power views illustrating the main features of* Intersectin *mRNA distribution in coronal sections at E16 (A, B) and in the adult central nervous system (C, D).* (**A**) *At rostral telencephalic levels* Intersectin *was expressed along the cerebral cortex (Cx), septum (S) and striatum (ST) at E16.* (**B**) *At E16 caudal telencephalic levels* Intersectin *mRNA was found in the cerebral cortex, hippocampus (H) and in some discrete thalamic (T) and hypothalamic (Hy) nuclei.* (**C**) *In the adult cerebral cortex and hippocampus (HP)* Intersectin *mRNA is widely distributed throughout the different layers.* (**D**) Intersectin *signal is detected in the Purkinje cells (PCL) and along the internal granular layer (IGL) in the adult cerebellum. DG, dentate gyrus; Mol, molecular layer; WM, white matter; I-VI, cortical layers. Scale bar A, B: 500 μm; C, D:300 μm*

domain is the region in guanine–nucleotide exchange factors that catalyses an exchange of GTP for GDP on Rho-like G proteins.[35] Interestingly, Rho and Rac have been shown to function in regulating endocytosis.[36] C2 domains are regulatory sequence motifs that occur widely in nature and although they were initially described as calcium-binding motifs, they have also been shown to bind phosphoinositides.[27] Synaptotagmin I, a synaptic vesicle protein involved in calcium regulation of endocytosis, contains two C2 domains, one of which acts as a calcium sensor.[37] Although it is difficult to speculate about how these domains may come together in a functional Intersectin protein, their unifying feature is that they are all found in proteins involved in membrane trafficking.

The primary function of the synaptic vesicle is neurotransmitter release, or exocytosis. After exocytosis, the components of the synaptic vesicle membrane are selectively recovered by endocytosis and recycled within the nerve terminal to generate new synaptic vesicles. This local recycling pathway allows neurons to maintain a constant supply of synaptic vesicles and this recycling capacity is essential for the consistent release of neurotransmitter. The process of docking, fusion and endocytosis are not restricted to the nerve terminal, they are elements of membrane-trafficking pathways in all eukaryotic cells. However, in non-neuronal cells

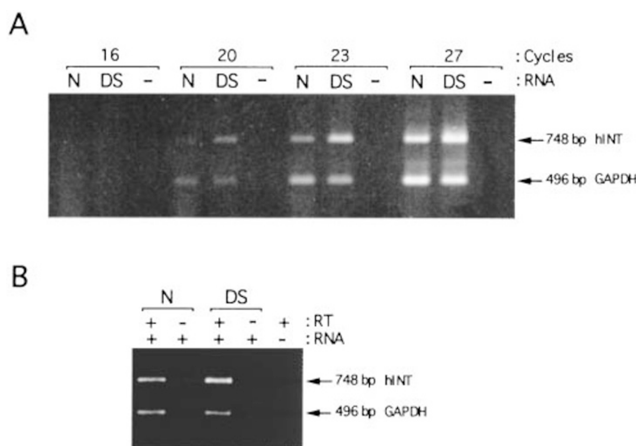


**Figure 3** *Comparison of the RNA expression of* ITSN1-L *in human brains from DS and non-DS foetuses.* (**A**) *Semiquantitative RT-PCR reactions were performed using as template total RNA from the brains of two Down syndrome foetuses (DS) and two non-Down syndrome foetuses (N) for the indicated number of cycles. ITSN primers produce a 748 bp band and GAPDH control primers produce a 496 bp band. In lanes 3, 6, 9 and 12, controls without RNA were included.* (**B**) *Controls of the RT-PCR reactions. A set of control reactions on total RNA from the brains of DS and non-DS foetuses amplified with and without reverse transcriptase*

most of these events are constitutive.[38] Therefore, additional mechanisms must account for the special temporal, spatial and calcium-sensitive properties of transmitter release and recycling, and the speed at which they occur. Consistent with the ubiquitous expression of the short *Intersectin* isoform, we propose that *ITSN-S* is involved in the generalised endocytic pathways of all cells and that the long isoform, *ITSN-L*, has been endowed with the more specialised properties required for brain-specific synaptic vesicle recycling.

Genes which show expression during development or, more particularly, in the developing brain may be of special importance in DS. Moreover, processes involving proteins with the capacity to interact with a myriad of other gene products may be more susceptible to the effects of dosage imbalance. *Intersectin* is expressed in the human foetus and in the developing mouse embryo. The long isoform was clearly overexpressed in the developing brains of foetuses with DS. At present, we can only speculate on the significance of this. Abnormal synaptic parameters have been reported in DS brains, including changes that could lead to a reduced efficiency of synaptic transmission.[39,40] In the brain of the Ts65Dn mouse (a mouse model for DS) impaired cAMP production, alterations in central noradrenergic transmission and reduced LTP (long term potentiation) have been found.[41–43] Both LTP and cAMP are associated with enhanced synaptic transmission which involves either increased transmitter release or increased receptivity to released transmitter.[38] If *Intersectin* is involved in synaptic transmission, then it is feasible that the abnormal stoichiometry of such a multi-modular protein may lead to a decrease in transmission efficiency and have detrimental consequences in Down syndrome.

Although during the preparation of this manuscript the human *Intersectin* gene was published,[13] we report additional information, specifically, the genomic structure of the human gene, a preliminary mouse *Intersectin* expression profile, including *in situ* hybridisation data, and the overexpression of *Intersectin* in DS.

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