Multiple Synapsin I Messenger RNAs Are Differentially Regulated during Neuronal Development

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Abstract. Synapsin I is a neuron-specific protein consisting of two isoforms Ia and Ib. It is thought to play a role in the regulation of neurotransmitter release. In this study the structure and expression of two classes of synapsin I mRNA have been examined. The two mRNA classes have molecular sizes of 3.4 and 4.5 kb, respectively. Both classes translate into synapsin I polypeptides and display a high degree of base sequence homology. Utilizing an oligonucleotide-directed RNase H assay we have shown that both mRNA classes have a common start site of transcription and differ from one another toward their 3' ends. The expression of the two synapsin I mRNA classes is differentially regulated during the development of the rat brain and cerebellum. In the cerebellum the 4.5-kb transcript is expressed until postnatal day 7, after which it decreases to an undetectable level. The 3.4-kb mRNA is found throughout cerebellar development and in the adult. This suggests that the 3.4-kb mRNA class consists of messages which can encode both synapsin I polypeptides. Using quantitative Northern blot analysis a peak in the expression of this mRNA was observed at postnatal day 20. The maximum expression of the 3.4-kb class coincides with the period of synaptogenesis in the cerebellum. In addition to the developmental time course of synapsin mRNA expression a description of its spatial distribution throughout the cerebellum was performed using in situ hybridization histochemistry. From postnatal day 15 onwards, with a maximum at postnatal day 20, synapsin mRNA was localized in the internal granule cell layer of the cerebellum. On a cellular level, the granule cells, but not the neighboring Purkinje cells, express high levels of synapsin mRNA. These observations implicate developmentally coordinated differential RNA splicing in the regulation of neuron-specific gene expression and substantiate the correlation of synapsin gene expression with the period of synaptogenic differentiation of neurons.

The nervous system offers an interesting system for the study of gene expression. It consists of a complicated network of highly specialized cell types whose development requires the precise regulation of the expression of subsets of genes. The phosphoprotein synapsin I, which is specifically expressed in neurons, is a good model for the investigation of neuron-specific gene expression. It is composed of two similar polypeptides of 74 and 78 kD, called synapsin Ia and Ib, respectively (Ueda and Greengard, 1977). Immunocytochemical studies have localized synapsin to the presynaptic terminal of virtually all neurons, where it is associated with the cytoplasmic surface of small synaptic vesicles (DeCamilli et al., 1983a, b; Navone et al., 1984). The protein is phosphorylated by both cAMP-dependent and Ca\(^{2+}\)/calmodulin-dependent protein kinases, and its state of phosphorylation is altered by conditions that affect neuronal activity (for a review, see Nestler and Greengard, 1984). Nerve cell depolarization has been shown to induce the phosphorylation of synapsin (Nairn et al., 1985). In vitro this phosphorylation leads to a reduction of the affinity between synapsin and synaptic vesicle membranes (Huttner et al., 1983; Schiebler et al., 1986). These and other data have led to the proposal that synapsin may play a role in neurotransmitter release. This hypothesis has been supported recently by the experiments of Llinas et al. (1985), in which the injection of synapsin or the Ca\(^{2+}\)/calmodulin-dependent protein kinase into the squid giant axon caused a change in postsynaptic potential.

Additional insight into the functional role of synapsin in the nerve terminal has come from studies that suggest that synapsin may interact with neuronal cytoskeletal elements. Baines and Bennett (1985) demonstrated that synapsin is immunologically related to the erythrocyte cytoskeletal protein 4.1 and is also a spectrin-binding protein. More recently synapsin has been shown to contain a potential actin-binding site (McCaffery and DeGennaro, 1986) and to bundle F-actin in a phosphorylation-dependent manner in vitro (Bähler and Greengard, 1987).

cDNA clones complementary to synapsin mRNA have
been isolated and used to identify two synapsin mRNA species (Kilimann and De Gennaro, 1985), to determine the primary structure of synapsin (McCaffery and De Gennaro, 1986), and to localize the gene encoding synapsin to the human X chromosome (Yang-Feng et al., 1986). We report here the application of these clones as probes to study the molecular nature of the mRNAs encoding synapsin and the regulation of their expression during neuronal development. The relationship between multiple synapsin mRNA species has been investigated by the application of a technique involving oligonucleotide-directed cleavage of the mRNAs by RNase H. The rat cerebellum was chosen as a model system for the developmental study because it matures postnatally and has simple cellular architecture. We have used quantitative Northern blot analysis to assay changes in synapsin gene expression during the development of the cerebellum. In addition, in situ hybridization histochemistry was applied to provide further insight into the temporal and spatial appearance of the synapsin mRNA in individual neurons.

**Materials and Methods**

**Isolation of RNA**

Total RNA was isolated from brain and cerebella of rats of various ages using a guanidine/cesium chloride method (Gliniak et al., 1974). The cerebella of newborn pups were dissected under the microscope to avoid any contamination with other brain tissue. The tissue was homogenized in 6 M guanidine isothiocyanate and centrifuged at 20°C, 35,000 rpm through a 5.7 M CsCl cushion. The resulting RNA pellet was resuspended in 10 mM Tris/HCl, pH 7.5, 5 mM sodium citrate, and 1% SDS. The RNA was further purified by butanol/chloroform extraction and ethanol precipitation. The protocol of Donis-Keller (1979) was modified and applied for the RNase H digestion. The RNA was precipitated after the addition of 2 μl of 3 M sodium acetate (pH 5.5) and 65 μl of 95% ethanol by cooling in a dry ice-ethanol bath for 15 min. The precipitate was recovered by centrifugation at 12,000 rpm and 4°C. The RNA pellets were dried briefly under vacuum, denatured by glyoxalation, and further analyzed by agarose gel electrophoresis and blotting as described by Thomas (1980). Sizes of the resulting RNA fragments were determined by comparison with the migration of ethidium bromide-stained RNA size standards (Bethesda Research Laboratories, Gaithersburg, MD).

**Quantitative Northern Blot Analysis**

RNA was denatured by glyoxalation, electrophoresed on a 1% agarose gel, and blotted on nitrocellulose as described by Thomas (1980). Each gel contained a serial dilution of purified 28S and 18S ribosomal RNA from calf liver (Pharmacia Fine Chemicals) applied as standards adjacent to cerebellar RNA samples. After hybridization with the synapsin-specific probe and autoradiography, the blots were subsequently hybridized with an 18S ribosomal RNA-specific cDNA probe (Grummt et al., 1979) which had been labeled with [32P]dCTP by nick-translation. The autoradiogram of the RNA dilution series was scanned densitometrically (model DU-8 spectrophotometer, Beckman Instruments, Inc., Palo Alto, CA), and the peak areas were plotted vs. the amount of RNA loaded, to give a standard curve. The actual amount of total cerebellar RNA loaded in each lane could be determined by comparing the densitometrically obtained value of 18S cerebellar RNA with this standard. The signal obtained with the synapsin-specific probe was then corrected relative to the amount of total RNA loaded in each lane. Exposure times of the film were chosen to be within the linear range for densitometric scanning.

To confirm the reliability of the quantification based on hybridization with a 18S rRNA-specific probe, a second method using ethidium bromide was applied (Eiden et al., 1984; Biguet et al., 1986). Northern gels were run in duplicate with serial dilutions of rRNA standards (see above) and cerebellar total RNA samples. One-half of the gel was stained with ethidium bromide (30 μg/ml) for 30 min and washed extensively with 40 mM N-morpholinopropane sulfonic acid (MOPS), pH 7.0, 10 mM sodium acetate, 1 mM EDTA for several hours. The other duplicate of the gel was used for Northern transfer. The negative images of UV-illuminated photographs of the ethidium bromide-stained gel were densitometrically scanned. The areas of the peaks from 28S and 18S rRNA bands of the cerebellar samples were compared with those of the serial dilution of standard rRNA. The actual amount of RNA loaded in the cerebellar samples could thus be calculated and used to standardize the synapsin specific signal.

**In Situ Hybridization Histochemistry**

Cerebella of young rats of various ages were fixed by immersion in Bouin's (picric acid, formaldehyde, acetic acid) solution for 4-12 h depending on the size of the tissue, cleared with 70% ethanol, dehydrated, and embedded in paraffin. Sections of 10 μm were cut, deparaffinized in xylene, and rehydrated. Pretreatment of the tissue was performed as follows: PBS (10 mM, pH 7.5) rinse twice for 5 min; 0.2 M HCl for 10 min at room temperature; PBS rinse twice for 3 min; 1 μg/ml proteinase K (Boehringer Mannheim Diagnostics, Houston, TX) in 20 mM Tris/HCl, pH 7.5, and 2 mM CaCl2 at 37°C for 15 min; PBS with 2 mg/ml glycine twice for 3 min; postfixation with 4% buffered paraformaldehyde for 5 min at room temperature; and a final wash in PBS/glycine twice for 3 min. To reduce nonspecific binding, slices were acetylated according to Hagauchi et al. (1978) for 10 min at room temperature, washed in H2O, and dehydrated. Control sections were incubated for 30 min at 37°C with 100 μg/ml RNase A (Boehringer Mannheim Diagnostics) in 0.5 ml NaCl and 10 mM Tris/HCl, pH 7.5. Prehybridization (4-6 h) and hybridization (overnight) were carried out in the following buffer: 50% recrystallized deionized formamide, 2x SSC, 1 mM EDTA, 5x Denhardt's, 0.1 M DTT, 0.5 mg/ml salmon sperm DNA, 0.5 mg/ml heparin, 50 μg/ml RNA from baker's yeast, and 5 μM unlabeled d-thio-UTP at 45°C in a moist chamber. Hybridization was performed in a volume of 8 μl containing 100,000 cpm of 35S-labeled single-stranded cRNA probe (see below). During the hybridization the slides were covered with siliconized, heat-treated cover slips. After careful removal of coverslips, the slides were first washed with 2x SSC for 2 h at 50°C and then with 0.2x SSC at 50°C under radioactivity was no longer detectable with a Geiger counter. Sections were then treated with RNase A 10 μg/ml at 37°C for 30 min. Slices were dehydrated through a graded series of ethanol washes containing 0.3 M ammonium acetate, dipped in Kodak NTB-2 photoemulsion (diluted 1:2 in H2O), exposed for 4-7 d, and finally developed in Kodak D 19 for 2 min. As a last step the sections were counterstained with thionin.

**Preparation of Single-stranded RNA Probes for In Situ Hybridization**

Single-stranded RNA probes were produced by transcription from DNA.
templates derived by insertion of the 5E2 fragment of synapsin cDNA into the plasmid vector pSP65 (Promega Biotec, Madison, WI). The two clones used contained the 5E2 fragment in opposite orientations with respect to the SP6 promoter. Transcription with SP6 polymerase yielded transcripts that were sense (identical to mRNA) and anti-sense (complementary to mRNA). Transcription reactions were performed with Bgl II (anti-sense)- and Acc I (sense)-linearized plasmids in the presence of [35S]UTP, which yielded probes of 132 and 129 nucleotides, respectively. The specific activity of the [35S]-labeled RNA probes ranged from 1 to 3 x 10^6 cpm/μg template.

Hybridization with [3H]Polyuridilate

To estimate the poly(A)^+ RNA content of the cerebellar neurons with respect to increasing age, hybridization with [3H]polyuridilate ([3H]poly(U)) was performed. The protocol of Griffin et al. (1985) was applied with slight variations. After fixation and pretreatment the sections were hybridized at 20°C overnight in 10 μl of hybridization solution (10 mM Tris/HCl, pH 7.6, 200 mM NaCl, 5 mM MgCl2, 25% formamide, 500 μg/ml salmon sperm DNA, 100 μg/ml RNA) containing 40,000 cpm [3H]poly(U), 3 Ci/mmol (New England Nuclear, Boston, MA). Washing was done in 50 mM Tris/HCl, pH 7.6, 10 mM KCl, 1 mM MgCl2 at 50°C for 1 h and at room temperature for 6 h. The sections were then further processed as described above.

Results

Two mRNA Classes Encode Synapsin

Previous experiments had demonstrated the existence of two synapsin mRNA classes in 10-d-old rat brain, 4.5 and 5.8 kb in length (Kilimann and DeGennaro, 1985). Using more accurate RNA standards (RNA ladder, Bethesda Research Laboratories) we now estimate the size of the two mRNA classes to be 3.4 and 4.5 kb (data not shown). The two transcripts will be designated by these newly determined sizes throughout the rest of this paper.

Several lines of experimental evidence demonstrate that both mRNA classes encode synapsin polypeptides. When mRNA is purified by immunoabsorption of rat brain polypeptides with synapsin-specific antibodies, both the 3.4- and 4.5-kb mRNAs are isolated. This could only occur if both RNA classes were associated with polypeptides bearing nascent synapsin polypeptides. Furthermore, in vitro translation reactions programmed by the immunoabsorbed mRNAs direct the synthesis of both synapsin polypeptides. Furthermore, in vitro translation reactions programmed by the immunoabsorbed mRNAs direct the synthesis of both synapsin polypeptides. Furthermore, in vitro translation reactions programmed by the immunoabsorbed mRNAs direct the synthesis of both synapsin polypeptides.

Structural Analysis of Synapsin mRNAs by Oligonucleotide-directed RNase H Cleavage

We have employed specific oligonucleotide-directed RNase H digestion, followed by RNA blot analysis of the resulting products, to address more carefully the characteristics of the two synapsin mRNA classes. An oligonucleotide complementary to synapsin mRNA was deduced from the synapsin cDNA pSyn5 sequence (bases 478-497, arrow in Fig. 2). We synthesized this oligonucleotide, hybridized it to synapsin mRNA present in the total poly(A)^+ RNA of 10-d-old rat brain, and cleaved the resulting hybrids with the RNA-DNA hybrid-specific endonuclease RNase H, as described in Materials and Methods. The resulting RNA fragments were denatured, displayed by agarose gel electrophoresis, and blotted onto nitrocellulose. These filters were hybridized with probes specific for the 3' (E3 probe) or 5' (E2 probe) products of the RNase digestion. Fig. 2 presents the results of this study. Fig. 2 A shows the mRNA digestion products detected by the 5'-specific probe. Lane I represents a control reaction containing RNA and RNase H but no oligonucleotide. It indicates the presence of both synapsin mRNA classes in the starting material. Lanes 2 and 3 show the products after digestion with RNase H in the presence of oligonucleotide with two different amounts of poly(A)^+ RNA in the digestion reactions. The band detected by the probe is ~800 bases in length. This experiment suggests that the 3.4- and 4.5-kb synapsin mRNAs have a common start site for transcription 800 bases upstream from the oligonucleotide binding site. This conclusion is supported by the mRNA digestion products detected by the 3'-specific probe, shown in Fig. 2 B. Lane I is a control lane indicating the presence of both synapsin mRNA classes. Lanes 2 and 3 show the fragments produced by the oligonucleotide directed RNase H cleavage. The 3'-specific probe detects two digestion products 2.6 and 3.2 kb.

1. Abbreviations used in this paper: PN, postnatal day; poly(U), polyuridilate.
3.7 kb in length. This result indicates that the difference between the two synapsin mRNA classes occurs to the 3′ side of the oligonucleotide binding site. A summary of these conclusions appears in diagrammatic form at the bottom of Fig. 2. Note that the lengths of the observed RNA fragments sum to the expected mature RNA lengths. We believe that the relatively weak hybridization signal given by the 800-base RNA fragment is the result of poor retention of this fragment by the nitrocellulose filter during RNA transfer. The results reported above have been confirmed in several experiments and with the use of additional oligonucleotides which hybridize to alternate sites in the synapsin mRNAs (data not shown).

**Synapsin mRNA Expression Is Differentially Regulated during Neuronal Development**

Northern blot analysis of synapsin mRNA expression during the development of the rat cerebellum was carried out using total RNA extracted from rat cerebella of various ages. The blots were hybridized with the 32P-labeled E2 probe. Our data show that the two synapsin transcripts are differentially regulated during the development of the rat cerebellum (Fig. 3, A and B). The larger 4.5-kb transcript is easily detectable in cerebellar RNA at birth and through postnatal day 7 (PN 7); it then decreases rapidly to almost undetectable levels through PN 10 and 15, and in the adult. The smaller mRNA species (3.4 kb), however, is expressed throughout cerebellar development and in the adult. This phenomenon is not restricted to the cerebellum. Differential regulation of the two mRNA species can also be seen by comparing RNA from 10-d-old and adult rat brain minus cerebellum (Fig. 3 B). Furthermore, the 4.5-kb transcript is not detected in RNA from adult rabbit cerebellum (Fig. 3 B). These data indicate that differential regulation of the synapsin mRNAs occurs during development in both the rat brain and cerebellum and possibly in the cerebellum of at least one other species, the rabbit.

We next carried out a quantitative analysis of the 3.4- and 4.5-kb synapsin mRNA classes throughout postnatal development of the rat cerebellum. The mRNA levels were quantified by densitometric scanning of autoradiograms like those shown in Figs. 3 and 4. The 4.5-kb transcript is not visible in Fig. 4 because of the short exposure time chosen to ensure the linearity of the autoradiographic signal for the 3.5-kb mRNA. A graphic representation of the standardized data
Figure 4. Quantitative Northern blot analysis of synapsin mRNA during rat cerebellar development. (A) Northern blot of total RNA from cerebella of various ages hybridized with 32P-labeled 5E2 probe. Lane 5, newborn; lane 6, PN 5; lane 7, PN 10; lane 8, PN 20; lane 9, PN 30; lane 10, adult. (Exposure time: 3 d at -70°C.)

(B) The same blot as in A was subsequently hybridized with a nick-translated 18S rRNA cDNA probe. Lanes 1-4, serial dilution of standard calf liver rRNA: 2, 1, 0.5, and 0.25 µg. Lanes 5-10, the same as in A. (Exposure time 3 h, at -70°C.)

The developmental time course of synapsin mRNA expression described by in situ hybridization corresponds well with the result of the Northern blot analysis described above. It confirms that the peak of synapsin gene expression is correlated with the synaptogenesis of cerebellar granule cells. Synapsin mRNA in young cerebella (PN 0 and PN 5), no synapsin specific signal was observed (Figs. 6, A and B, and 7 B). At this stage of development the layered structure of the cerebellum has not yet formed and the undifferentiated granule cells still reside in the external granule cell layer. From PN 15 onward, however, synapsin gene expression was detectable in the internal granule cell layer, but not in the external granule cell layer (Fig. 6 C). The synapsin-specific signal increased sharply at PN 20 in the granule cell layer (Fig. 6, E and F). In control experiments in which an RNase A pretreatment of the sections was performed, the signal could be abolished (not shown). Control sections hybridized with the sense strand probe show a few grains homogeneously distributed over all layers of the cerebellum and confirm the specificity of the synapsin cRNA hybridization (Fig. 6 D).

The developmental time course of synapsin mRNA expression described by in situ hybridization corresponds well with the result of the Northern blot analysis described above. It confirms that the peak of synapsin gene expression is correlated with the synaptogenesis of cerebellar granule cells. Synapsin mRNA in young cerebella (PN 0, 5, and 10), demonstrated by Northern blot analysis, was not visualized in situ. This suggests that it lies below our present level of detection.

We exploited the potential for higher resolution offered by 35S-labeled probes to define the cell type expressing synapsin mRNA. High-magnification photomicrographs revealed heavy accumulation of silver grains over granule cells and only a small number of grains over Purkinje cells (Fig. 7 D). Thus the expression of synapsin mRNA was demonstrated specifically in the population of granule cells undergoing a particular phase of differentiation, i.e., synaptogenesis.

To show that the variation in synapsin mRNA expression was not due to selective loss of poly(A)+ RNA from particular cell types during the processing of the tissue, hybridization experiments were carried out using [3H]poly(U). This method has been applied previously to quantify total poly(A)+ mRNA in tissue sections. It was found that the abundance of autoradiographic grains from [3H]poly(U)/poly(A)+ RNA hybrids is directly proportional to the total poly(A)+ RNA in cells (Angerer and Angerer, 1981; Hecht et al., 1992).
Figure 6. Hybridization of single-stranded 35S-labeled synapsin cRNA probe to 10-μm sections from the rat cerebellum. (A and F) Phase-contrast photomicrographs. (B-E) Dark field photomicrographs; silver grains of the photoemulsion appear white. (A and B) Section from PN 5 cerebellum; homogenous distribution of silver grains over the tissue reflecting only background hybridization. (C) PN 15 cerebellum showing accumulation of grains over the GL but not the EGL. (D) Hybridization with control synapsin cRNA (same sequence as mRNA) to PN 15 cerebellum; background hybridization only. (E) PN 20 cerebellum; high accumulation of silver grains over GL. (F) Phase-contrast picture corresponding to E. GL, granule cell layer; ML, molecular layer; EGL, external granule cell layer. Exposure time: 6 d. Bars, 25 μm.
When we applied this technique to cerebellar slices of different ages, poly(A)$^+$ RNA was detectable in high amounts in the various cell types of the cerebellum at all stages studied. (Data for two of these are shown in Fig. 7, A and C). This result further demonstrates the specificity of the synapsin mRNA hybridization.

**Discussion**

**Nature of the Mature Synapsin mRNA Species**

Two synapsin mRNA classes have been described in RNA prepared from 10-d-old rat brain (Kilimann and DeGennaro, 1985), both of which actively encode synapsin protein in vivo, as described in Results. In this report we have further confirmed the sequence homology between them using RNA blot hybridizations with synapsin cDNA probes (Fig. 1).

The experiments described in Fig. 2 demonstrate that both mRNA classes have the same transcription start site, and that the difference between the messages occurs to the 3' side of the oligonucleotide used in the RNase H study. The following conclusions can be drawn from this data. The synapsin-specific cDNA pSyn5 represents a near full-length copy of the 3.4-kb synapsin mRNA, lacking only about 300 bases of 5' untranslated sequence. At the level of analysis performed, all of the sequences contained in pSyn5 seem also to be present in the 4.5 kb mRNA.

The recent localization of the synapsin gene to the human and murine X chromosome (Yang-Feng et al., 1986) and Southern blot data of rat genomic DNA (Carroll, D. P., and...
L. J. DeGennaro, unpublished observations) provide strong evidence for the existence of a single copy of the synapsin gene in the haploid genome. Preliminary data derived from the analysis of the rat synapsin gene suggest that additional sequences occur in the 3' untranslated region of the 4.5 kb mRNA (Carroll, D. P., C. A. Haas, and L. J. DeGennaro, unpublished observations). Therefore, the origin of the two classes of mature transcripts from this gene can now be explained. We conclude that the 4.5-kb mRNA is produced by alternate splicing of the primary synapsin gene transcript, to include additional sequences totaling ~1,000 bases. This means that the developmentally coordinated decrease in the expression of the 4.5-kb RNA that we have described results from an alteration in the processing of the primary transcript of the synapsin gene. The biological function of the alteration in splicing is unclear. The possibility that the alternate mRNA classes represent fetal and adult forms, or that they are expressed in different neuronal cell types, awaits investigation with hybridization probes specific for each class.

We proposed earlier (Kilimann and DeGennaro, 1985) that each of the mRNA classes might encode one of the two synapsin polypeptides Ia and Ib. Our Northern blot analysis, however, clearly demonstrates differential regulation of the two synapsin mRNA classes during the development of rat cerebellum and brain such that only the 3.4-kb transcript is detectable by 120 nucleotides. The existence of synapsin mRNA vari- ety (DeCamilli et al., 1983; Mason, 1986). The specificity of the hybridization probe was demonstrated by RNase A treatment of the tissue prior to hybridization; by RNase H cleavage assay, quantitative Northern blots and in situ hybridization histochemistry has allowed the detailed analysis of the nature of the mature messenger RNAs encoding the neuron-specific protein synapsin and the description of changes in their expression at the cellular level during development. The results have yielded the intriguing finding that the expression of the gene for synapsin, reflected by steady-state levels of mature synapsin mRNA, is regulated in

**Correlation of Synapsin Gene Expression and Granule Cell Synaptogenesis**

The localization of synapsin mRNA during development by in situ hybridization provides additional insight into the temporal and spatial appearance of synapsin mRNA in relation to the state of differentiation of a particular neuronal cell type (Figs. 6 and 7). As a model for a developing neuron we chose the granule cell of the cerebellum. We localized the synapsin mRNA from PN 15 onward in the internal granule cell layer of the cerebellum. This coincides with the maximum level of synapsin mRNA demonstrated by quantitative Northern blot analysis. The lower levels of expression of synapsin mRNA present in the cerebellum from birth until PN 10 could not be visualized in situ, indicating the limit of detection. Using 35S-labeled probes synapsin mRNA localization could be extended to the cellular level. Thereby it was possible to define the cell population responsible for the strong increase in synapsin mRNA expression during development as the granule cells of the internal granule cell layer. The accumulation of silver grains over the cell bodies of these neurons reflects synapsin mRNA production during the period of synaptogenesis of the parallel fibers on the Purkinje cell dendrites. In other cell populations of the cerebellum, such as the granular matrix of external granule cells, the interneurons of the molecular layer or the Purkinje cells, synapsin mRNA levels were below the limit of detection. These findings are also in good agreement with the immunocytochemical localization of the synapsin protein in the molecular layer of the cerebellum during the same period of development (DeCamilli et al., 1983; Mason, 1986). The specificity of the in situ hybridization procedure has been demonstrated by RNase A treatment of the tissue prior to hybridization; by the use of the nonhybridizing synapsin cRNA as a negative control and by demonstrating the poly(A)+ RNA content by hybridization with a 3H-labeled poly(U)-probe.

In summary, the application of an oligonucleotide directed RNase H cleavage assay, quantitative Northern blots and in situ hybridization histochemistry has allowed the detailed analysis of the nature of the mature messenger RNAs encoding the neuron-specific protein synapsin and the description of changes in their expression at the cellular level during development. The results have yielded the intriguing finding that the expression of the gene for synapsin, reflected by steady-state levels of mature synapsin mRNA, is regulated in

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at least two ways. First, a developmentally coordinated decrease in the production of one of two synapsin mRNA classes appears to be the consequence of a change in splicing of the primary transcript of the gene. Secondly, the overall level of expression of the synapsin gene is further regulated in a fashion coordinate with the major period of synaptogenesis. Finally, a direct demonstration of the presence of the brain with a probe which identifies the cell bodies of neurons in the process of synaptogenesis.

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