CELL REGULATION, Vol. 2, 819–826, October 1991

Developmental regulation of calmodulin gene expression in rat brain and skeletal muscle

Jacqueline Weinman,* Bruno Della Gaspera,* André Dautigny,† Danielle Pham Dinh,† Jian Wang,‡ Hiroshi Nojima,§ and Serge Weinman*

*UFR Biomédicale des Saints-Pères
Université René Descartes
75270 Paris, France
†Institut des Neurosciences
CNRS
Université Pierre et Marie Curie
75252 Paris, France
‡INSERM U75
UFR Necker-Enfants Malades
Université René Descartes
75730 Paris, France
§Research Institute for Microbial Diseases
Osaka University
Osaka 565, Japan

Three different calmodulin genes that encode the identical protein have been identified in the rat (Nojima, 1989); however, calmodulin gene expression at the various stages of tissue differentiation and maturation has not been previously determined. We have quantitated the content of mRNAs encoding calmodulin in the developing brain and skeletal muscle using RNA blot analysis with three specific cDNA probes. Our results show that five species of calmodulin mRNAs: 4.0 and 1.7 kb for CaM I, 1.4 kb for CaM II, and 2.3 and 0.8 kb for CaM III are detectable at all ages in the brain as well as in skeletal muscle but exhibit a tissue-specific developmental pattern of expression. The comparison of the temporal pattern of calmodulin gene expression with both mitotic activity, as demonstrated by cyclin A mRNA levels, and differentiation and maturation of specific brain or muscle regions is consistent with calmodulin involvement in development.

Introduction

Calmodulin is a highly conserved protein belonging to the EF-hand family of Ca^{2+}-binding proteins (Cheung, 1970; Kakiuchi *et al.,* 1970; Babu *et al.,* 1985). Calmodulin is found in all eucaryotic cells at varying concentrations (for review, see Klee and Vanaman, 1982). As a ubiquitous and major Ca^{2+}-receptor, calmodulin has been implicated in the regulation of a number of cellular processes, including rate of cell proliferation (reviewed in Rasmussen and Means, 1989), assembly and disassembly of microtubules (Marcum *et al.,* 1978), and activation or inhibition of a wide variety of enzymes (for review, see Cheung, 1980). It should be noted, however, that there is no unequivocal demonstration of the function of calmodulin in intact cells. In vertebrates, calmodulin is produced from multiple genes. As nucleotide divergence occurs throughout the coding region in the third base of a codon, the protein produced from different genes within an organism is identical (reviewed in Nojima, 1989). The molecular mechanisms by which calmodulin synthesis is modulated are unknown. It is noteworthy that calmodulin concentration is insensitive to hormones (for review, see Means *et al.,* 1982). As changes in specific gene expression are among the earliest and potentially most important events in protein synthesis regulation, a study of calmodulin transcripts might well afford a better understanding of the modulation of calmodulin synthesis.

We previously have analyzed the expression of calmodulin at the protein level from fetal to adult stages in the rat and have shown that its developmental pattern is unique from tissue to tissue (Rainteau *et al.,* 1988). In the rat, calmodulin is the product of at least three bona fide genes in the calmodulin multigene family: CaM I, CaM II, and CaM III, which encode identical proteins (Nojima, 1989). For a more complete understanding of calmodulin gene expression at the mRNA level during the different stages of rat tissue formation, we have carried out a Northern blot analysis of developing brain and skeletal muscle RNA. We have used three cDNA probes specific for CaM I, CaM II, and CaM III transcripts, respectively (Nojima, 1989). Because it is important to place this study in the context of cell divisions that occur during the same developmental period, we have determined simultaneously the cyclin A gene expression. Here we present the results for CaM I–III gene expression from fetal (14 d of gestation) to postnatal and young adult stages in the rat. Our data show that the three distinct

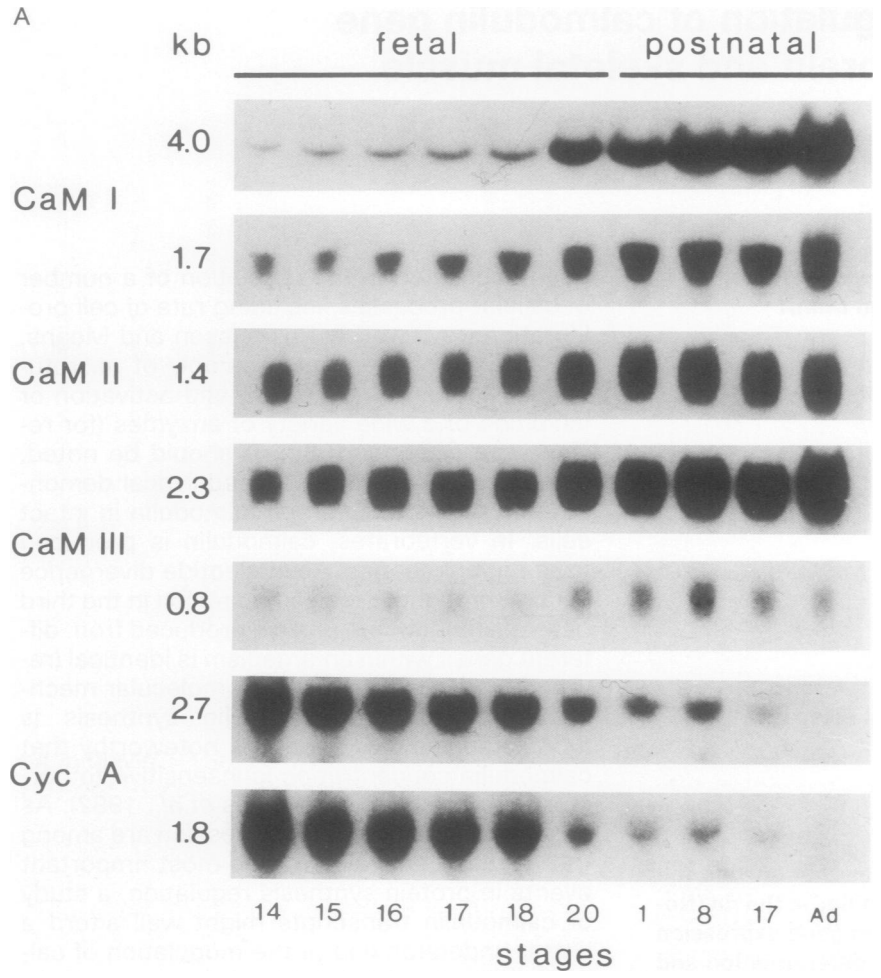

Figure 1. **Calmodulin gene expression in rat brain at different stages of development.** (A) Total RNA analysis on Northern blot by sequential hybridization to cDNA probes for CaM I–III and cyclin A. The size of the transcripts is given in kb that were inferred from RNA ladder. (B) Quantitative analysis of Northern blots shown in (A). For accurate quantitation of calmodulin and cyclin A mRNA levels, autoradiographs were scanned and normalized to the 18S ribosomal RNA loading. Units of absorbance are arbitrary. For each probe, relative mRNA abundance was normalized to the peak level. For CaM I, CaM III, and cyclin A, the peak level was the sum of the two mRNA species. □, CaM I: 4.0 (left) and 1.7 (right) kb mRNAs; □, CaM II: 1.4 kb mRNA; ▩, CaM III: 2.3 (left) and 0.8 (right) mRNAs; ■, cyclin A: 2.7 (left) and 1.8 (right) kb mRNAs. All procedures are given in Materials and methods.

calmodulin genes in the rat genome are transcribed in a manner that is tissue-specific. Our results also demonstrate that the different mRNAs for each calmodulin gene have distinct patterns of accumulation during development in relationship with the tissular calmodulin protein concentration and the changes in calmodulin-binding protein content that occur during tissue formation and differentiation.

Results

Calmodulin gene expression during development

To determine the effect of development on calmodulin gene expression, total tissular RNA was isolated from rat brain and skeletal muscle at different stages of fetal and postnatal life. The levels of specific transcripts for CaM I–III and cyclin A genes were determined by Northern blot analysis and densitometric measurements.

Brain. Five species of calmodulin mRNAs, 4.0 and 1.7 kb for CaM I, 1.4 kb for CaM II, and 2.3 and 0.8 kb for CaM III, are present in brain RNA throughout all of the periods of fetal and postnatal life (Figure 1, A and B). The two mRNA species for CaM I and CaM III correspond to the utilization of alternative polyadenylation signals (Nojima, 1989). By day 14 of gestation, they all accumulate progressively reaching peak levels at 8 d of age. A slight, reproducible decrease is observed for CaM II and CaM III mRNAs at 17 d of age, followed by an increase of CaM I and CaM II mRNAs at adult stage. A major change in the relative temporal expression of the two CaM I mRNA species occurs during brain development. In the earliest stages, from 14 to 18 d of gestation, mRNA of 1.7 kb is the more abundant of the two CaM I mRNA species. By 20 d of gestation, mRNA of 4.0 kb becomes predominant and accounts for two-thirds of the total CaM I mRNA. Throughout fetal and postnatal development, the ratio of the levels of the two mRNA species of CaM III of 2.3 and 0.8 kb remains approximately constant at 9:1. The levels of the two cyclin A mRNA species

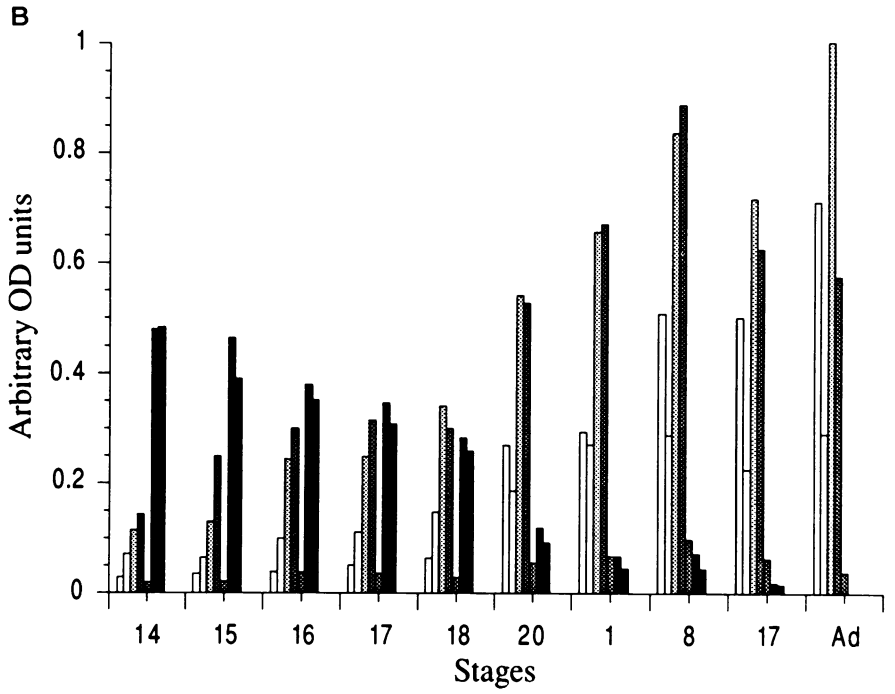

Figure 1. **(Continued)**

of 2.7 and 1.8 kb decline gradually from 14 d of gestation to 17 d of age, becoming indetectable at adult stage (Figure 1, A and B).

Skeletal muscle. Five calmodulin mRNAs are also detectable in the developing skeletal muscle by 14 d of gestation (Figure 2, A and B). The overall expression of the three calmodulin genes remains approximately constant during fetal development. In the perinatal period, CaM II and CaM III gene expression declines abruptly, CaM II and CaM III mRNAs becoming significantly lower. After birth, a gradual increase of CaM I and CaM III expressions is observed. During fetal and early postnatal development, the two CaM I mRNAs of 4.0 and 1.7 kb are expressed at constant levels, the ratio of which remains approximately constant at 1:2. The species of 1.7 kb is predominant. By 17 d of age, a major change in the temporal distribution of CaM I transcripts appears. The levels of the two CaM I mRNAs increase independently so that their ratio is reversed at adult stage. The species of 4.0 kb becomes the more abundant of the two. The level of CaM II mRNA of 1.4 kb tends to decline from 1 d of age to adult stage. By 15 d of gestation, two CaM III mRNAs of 2.3 and 0.8 kb are present at constant levels throughout the fetal period, the species of 2.3 kb being largely the more abundant. After birth, as early as 1 d of age, a change in the temporal expression of the CaM III gene occurs. Although CaM III transcripts decline, they remain present at lower levels. As development proceeds, CaM III gene expression resumes, and the levels of the two CaM III mRNAs increase. At adult stage, they are similar to those observed during the fetal period. The levels of the two cyclin A mRNAs species of 2.7 and 1.8 kb decrease abruptly after birth to reach a low level at 8 d of age and become almost indetectable in the 17-d-old rat (Figure 2, A and B).

Tissue specificity of calmodulin gene expression

To compare the expression of the three calmodulin genes in different tissues at the end of development, Northern blot analysis of young adult rat brain and skeletal muscle RNA was performed under the same experimental conditions (Figure 3). CaM I probe detects two mRNA species of 4.0 and 1.7 kb. Both are expressed at high levels in the brain. They are less abundant in the skeletal muscle. CaM II probe hybridizes with only one mRNA species of 1.4 kb. The intensity of this hybridization signal is much greater in the brain than that in the muscle. Using CaM III probe, an mRNA species of 2.3 kb and a faintly hybridizing species of 0.8 kb are seen in the two tissues. Both are more abundant in RNA from brain than that in RNA from skeletal muscle.

As the three calmodulin probes do not have the same lengths nor affinities, our experiments do not allow a direct comparison of the expres-

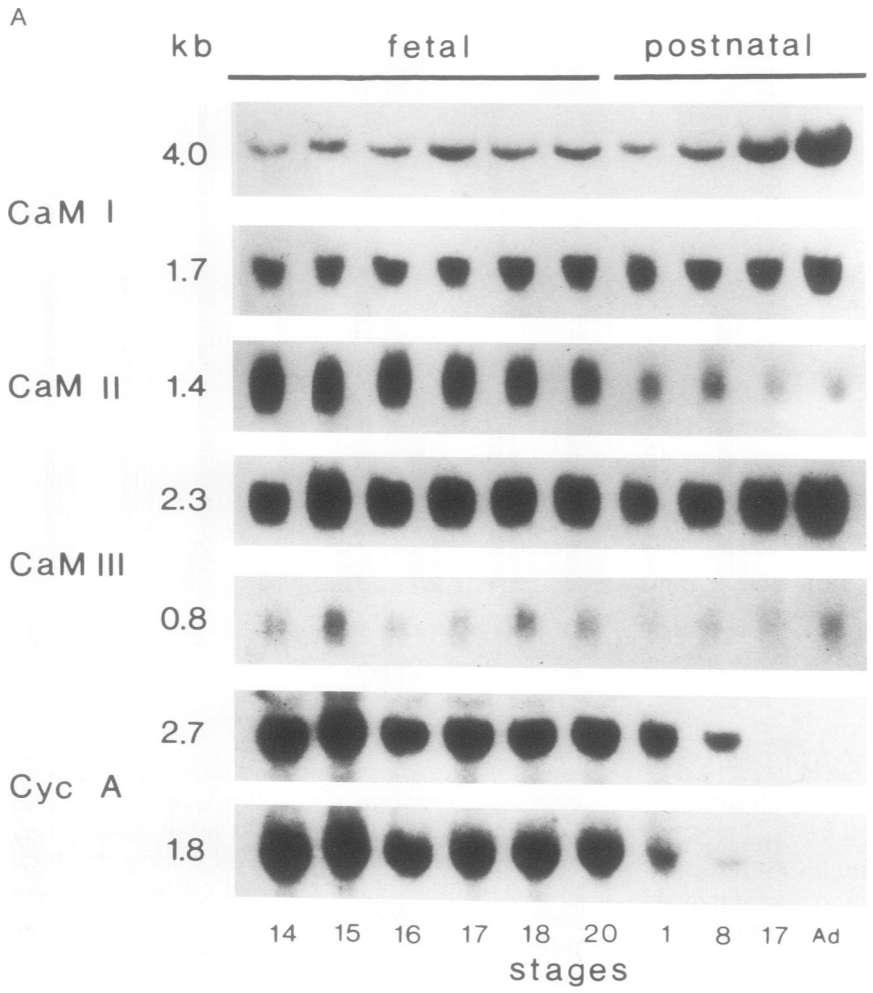

***Figure 2.* Calmodulin gene expression in rat skeletal muscle at different stages of development.** The total RNA was analyzed as described in Figure 1.

sion of the three calmodulin genes in a given tissue. Nevertheless, judging from the differences in spot radioactivity (Table 1), it appears that the CaM I gene is expressed in the brain preferentially to the CaM II and CaM III genes as two mRNA species of 4.0 and 1.7 kb. In skeletal muscle, the four mRNA species of 4.0 and 1.7 kb (CaM I), 1.4 kb (CaM II), and 2.3 kb (CaM III) are present at a similar level.

Discussion

This is the first detailed analysis of calmodulin gene expression during fetal and postnatal development in the rat brain and skeletal muscle at the mRNA level. Our results demonstrate that each gene examined has a unique and tissue-specific pattern of expression. These data can be compared with the previously reported ontogenic changes in tissue calmodulin concentration. Moreover, they must be examined in the context of the events that occur during cell proliferation and differentiation.

Brain. The continuous increase in the level of the five calmodulin mRNAs observed in brain during the late fetal and early postnatal development followed by a slight decrease at 17 d of age, as well as a second rise at adult stage, correlates well with the changes in calmodulin protein content we have reported previously (Rainteau *et al.,* 1988). Indeed, the concentration of calmodulin in the brain progressively increases during development, reaching a peak at 12–14 d of age, declines slightly at 15–25 d, and increases again to reach its highest levels in adulthood. Our results are also in agreement with the temporal accumulation of calmodulin protein (Caceres *et al.,* 1983) and calmodulin mRNAs (Cimino *et al.,* 1990; Messer *et al.,* 1990; Roberts-Lewis *et al.,* 1990) in different brain areas, such as hippocampus, cerebral cortex, thalamic nuclei, and cerebellum, as determined by immunocytochemical methods or in situ hybridization.

Several reports (Chafouleas *et al.,* 1982; Gallien *et al.,* 1984; Rainteau *et al.,* 1987; Rasmus-

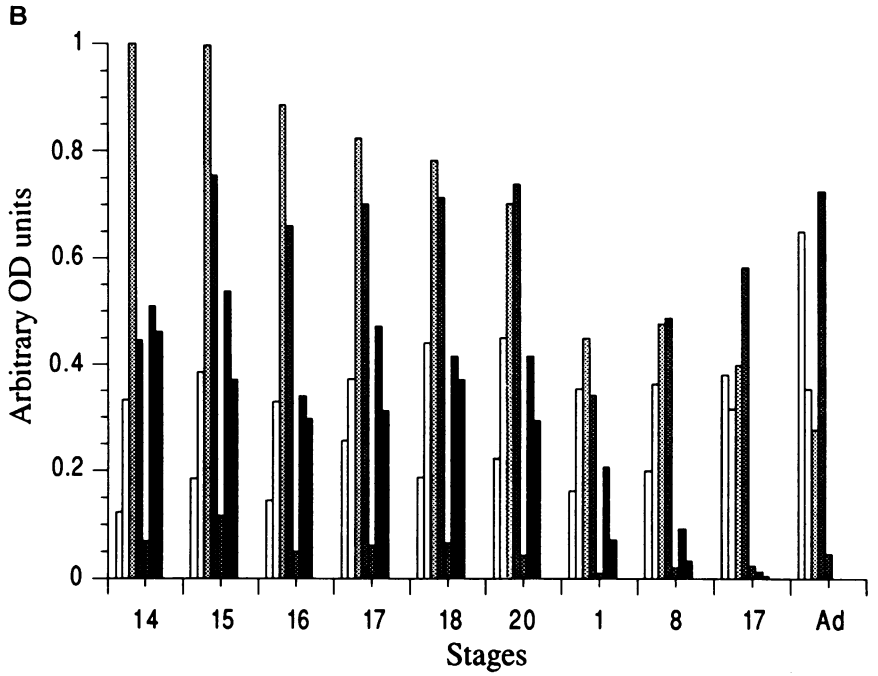

***Figure 2.* (Continued)**

sen and Means, 1989) have demonstrated that calmodulin plays important roles in cell division. The relatively high concentration of calmodulin mRNAs present in brain as early as 14 d of gestation is consistent with the intense mitotic activity in neuronal tissues, as demonstrated by the elevated levels of cyclin A transcripts. Our results are also in agreement with the progressive enrichment in calmodulin-binding proteins during rat brain development. Works by Hyman and Pfenninger (1985) and Seto-Ohshima *et al.* (1987) have suggested that calmodulin and calmodulin-dependent enzymes are involved in nerve growth cone function during the early development of the central nervous system. In particular, the calcium-binding protein neuromodulin is highly concentrated in developing axonal growth cones (Meiri *et al.*, 1986). Its level begins to decline 2 d after birth. Thus, our data correlate well with the rapid growth of neurons

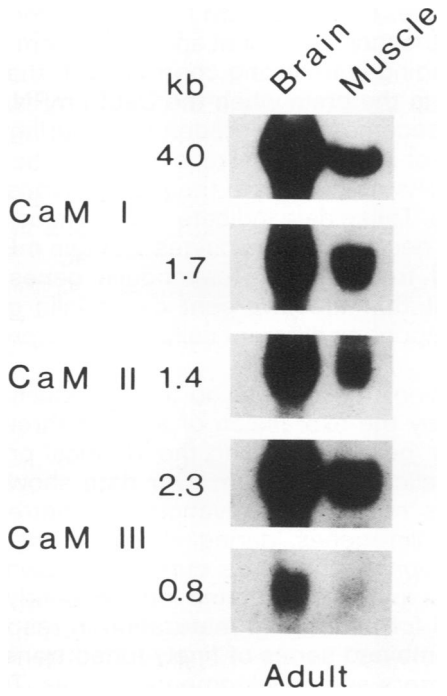

***Figure 3.* Tissue specificity of calmodulin gene expression in adult rat brain and skeletal muscle.** Exposure time of the Northern blots: 16 h.

Table 1. Comparison of the radioactivity of spots[a] corresponding to the various calmodulin gene transcripts in adult rat brain and skeletal muscle

	CaM I (kb)		CaM II (kb)	CaM III (kb)	
	4.0	1.7	1.4	2.3	0.8
Brain	1283	761	1361	1108	121
Muscle	211	216	220	328	40

[a] Spots were quantitated by excising regions of interest and measuring radioactivity that was expressed as cpm. Values are the means of two experiments; there was <10% variability between samples at each point.

that occurs at the early stages of brain development. Developmental changes in brain CaM-kinase II, a multifunctional enzyme, have been well documented. Goldenring *et al.* (1984) have identified CaM-kinase II as the major postsynaptic density protein. Very little of this calmodulin-dependent enzyme can be detected before 5 d of age, and the peak expression occurs between 5 and 20 d of age. Kelly *et al.* (1987) have described a similar pattern of development for CaM-kinase II in brain synaptic junctions. Work by Tallant and Cheung (1983) has established that calcineurin, identified as the major neural calmodulin-dependent protein phosphatase (for review, see Stewart and Cohen, 1988) was not detectable before 5 d of age and that calcineurin tripled between 8 and 20 d of age. Billingsley *et al.* (1990) have reported that the expression of calmodulin-dependent cyclic nucleotide phosphodiesterase in rat brain increases dramatically during postnatal days 7–20. It must be noted that many other calmodulin-sensitive proteins are expressed in rat brain. Among them are the three isoforms of the plasma membrane Ca^{2+}-transporting ATPase (Greeb and Shull, 1989) and the enzymes associated with brain actomyosin (Larson *et al.*, 1990).

Muscle. The pattern of calmodulin mRNA accumulation in rat skeletal muscle is in close correlation with the sharp decline in calmodulin protein concentration between 20 d of gestation and birth followed by an increase from birth over the first 2–3 wk, as previously described (Rainteau *et al.*, 1988).

As in the developing brain, the high levels of calmodulin mRNAs and calmodulin protein present in the fetal skeletal muscle are consistent with intense myoblast proliferation, as demonstrated by the elevated contents of cyclin A transcripts. Because the role of Ca^{2+} in regulating myoblast fusion in culture is well known, another possible function for calmodulin in fetal muscle may be the control of formation of primary and secondary myotubes by fetal days 16 and 18, respectively (Harris *et al.*, 1989). This hypothesis is reinforced by the fact that a sharp decline in calmodulin expression is observed at birth when the fiber number no longer increases. Nevertheless, further experiments will be required to substantiate this suggestion. Phosphorylase kinase, a crucial glycogenolytic regulatory enzyme, is strictly calmodulin-dependent. Work by Chamberlain *et al.* (1987) has shown that the level of the catalytic γ-subunit of mouse muscle phosphorylase kinase mRNAs increases dramatically from neonate to adult. Nothing is known about the developmental changes in the concentration of the other skeletal muscle calmodulin-binding proteins. However, in view of our results, it may be hypothesized that the expression of enzymes such as myosin light chain kinase, glycogen synthase kinase, calmodulin-dependent protein kinase (Tuana and MacLennan, 1988), protein phosphatase-2B (for review, see Stewart and Cohen, 1988), and plasma membrane Ca^{2+}-transporting ATPases 1 and 3 (Greeb and Shull, 1989), found in mammalian adult skeletal muscle, increases progressively during the postnatal period.

Indeed, our data show that the temporal profiles of expression of the calmodulin genes in brain and skeletal muscle correlate with the changes that occur in the levels of calmodulin protein during ontogeny. Moreover, these data support the concept that calmodulin levels affect essentially the rate of cell proliferation in the earliest stages of development and reflect the rate of anatomical and biochemical changes in the cell during periods of tissue differentiation and maturation. Our findings can be compared with those of Means and co-workers concerning calmodulin gene expression during rat germ cell differentiation (Slaughter *et al.*, 1987, 1989; Slaughter and Means, 1989). During spermatogenesis, the level of each calmodulin mRNA can be described by a unique developmental pattern. It is of interest to note that the mRNAs of lower size, 1.7 kb for CaM I and 0.8 kb for CaM III, are the more abundant species in germ cells. This finding is in strong contrast with that observed in the brain when the CaM I mRNAs of 4.0 kb become largely predominant during maturation or in the skeletal muscle where the CaM III mRNAs of 2.3 kb are the more abundant at all ages. These data indicate that germ cells, as well as neurons and myocytes, contain mRNAs derived from multiple calmodulin genes and suggest that the divergent calmodulin genes may respond to different cellular regulatory signals.

Defining the role played in calmodulin synthesis by the expression of at least three calmodulin genes, encoding the identical protein is a challenging problem. Our data show that there is no simple activation of a battery of calmodulin genes during development. The expression of the three currently known calmodulin genes is regulated independently during cell formation and maturation in response to a combined series of finely tuned transcription factors and developmental signals. This is particularly well exemplified by the changes in CaM III gene expression occurring during early

postnatal muscle development. The data from our study would also suggest that the transcripts of different sizes expressed by the same gene may play a role in the regulation of calmodulin synthesis. In this regard, the changes in CaM I mRNA ratio that occur during the early development of the brain and the late development of the muscle are highly evocative. It is worthy of note that CaM I mRNA is highly enriched in the species of 4.0 kb at these stages. Its 3′ noncoding sequence might well be involved in mRNA turnover rate.

Understanding how different regulatory influences are integrated at the molecular level to produce tissue-specific patterns of activation of different genes encoding the identical protein is a challenge that will require much more work. As genes may either respond to different regulatory signals or respond to the same signal differently, the elucidation of the promoter structure could afford a better understanding of calmodulin gene capacity in responding to regulatory factors.

Materials and methods

Materials

Sprague-Dawley timed pregnant rats (Charles River, France) were used as a source of all prenatal, postnatal, and young adult tissues. Fetal tissues were collected by anesthetizing the dam with sodium pentobarbital (45 mg/kg i.p.) and isolating the fetal rat pups by cesarean section at 14, 15, 16, 17, 18, and 20 d gestation. Pups from the same litter (8 pups per dam) were killed by means of guillotine at 1, 8, and 17 d after birth. Brain, including cerebellum, and dorsal and posterior limb muscles, referred to as skeletal muscle, were dissected from fetuses or pups and immediately frozen and stored in liquid nitrogen. All chemicals were of the highest purity available and purchased from Life Technologies (Gaithersburg, MD), Sigma Chemical (St. Louis, MO), Fluka (Buchs, Switzerland), and Merck (Darmstadt, Germany). Restriction enzyme *Bam*H1 and *Eco*R1 and T_4 nucleotide kinase were obtained from Boehringer (Mannheim, Germany). ATP and [α^{32}P]dCTP was from New England Nuclear (Dupont de Nemours, Les Ulis, France). Random primer extension kit, Hybond-N membranes, and MP Hyperfilms were obtained from Amersham (Amersham, UK).

RNA isolation and Northern blot analysis

Total RNA was isolated by extraction of tissues in guanidine-thiocyanate buffer and collected on a $CsCl_2$-gradient (Chirgwin *et al.,* 1979), using a TL-100 tabletop ultracentrifuge (Beckman, Palo Alto, CA). After isolation, RNA was ethanol precipitated. Quantitation and purity were assessed by absorbance at 260 and 280 nm. For Northern analysis, 17-μg RNA samples were denaturated in glyoxal and dimethyl sulfoxide and separated on a 1.2% agarose gel. Equivalent loading of RNA samples was confirmed by ethidium bromide staining of the ribosomal bands. RNA was transferred by overnight capillary blotting onto Hybond-N membranes. The membranes were baked (80°C, 2 h), prehybridized, and hybridized with ^{32}P-labeled probes. To control the amount of total RNA actually bound in each lane, the membranes were first hybridized with a 24-mer oligonucleotide complementary to a sequence of the rat 18 S ribosomal RNA (Mercadier and Dubus, 1991). This probe was labeled at the 5′ end in the presence of [γ^{32}P]ATP (6000 Ci/mmol) and T_4 nucleotide kinase. Then a 1.8-kb *Bam*H1 fragment of pRCM1, a 1.5-kb *Bam*H1 fragment of pRCM3, and a 0.8-kb *Bam*H1 fragment of pRCM4 were used as CaM I, CaM II, and CaM III probes, respectively (Nojima, 1989). Finally, a 1.7-kb *Eco*R1 fragment of a Blue-script vector was used as cyclin A probe (Wang *et al.,* 1990). The last four probes were labeled with [α^{32}P]dCTP (10^6 cpm/ml) by random primer extension (Feinberg and Vogelstein, 1983). Hybridization conditions were 6× SSC, 5× Denhardt's solution, 0.5% sodium dodecyl sulfate (SDS), 50% formamide, 100 μg/ml denatured salmon sperm DNA. Hybridization was for 20 h at 42°C. Hybridized blots were washed at high stringency (0.1× SSC, 0.1% SDS at 50°C) and then exposed to MP Hyperfilms for various lengths of time, at −70°C, using two intensifying screens. Removal of the probes was ensured by autoradiography before rehybridization with successive probes. Hybridization signals were quantitated by scanning densitometry at 550 nm using a Shimadzu CS 9000 densitometer (Shimadzu Inc., Tokyo, Japan) linked to an automatic integrator. Only bands in the linear response of the film were used in quantitation. All Northern analyses shown in this manuscript were performed at least twice using two or more independent preparations of RNA from tissues from different animals. Similar patterns of hybridization were observed, and trends and ratios were the same in each case.

Acknowledgments

We are grateful to Mr. Killion for his help in preparing the manuscript, to Ms. Turquois for typing the manuscript, and to Ms. Touret and Mr. Lemoine for their help in photography.

Received: March 25, 1991.
Revised and accepted: July 19, 1991.

References

Babu, Y.S., Sack, J.S., Greenhough, T.J., Bugg, C.E., Means, A.R., and Cook, W.J. (1985). Three-dimensional structure of calmodulin. Nature (Lond.) *315,* 37–40.

Billingsley, M.L., Polli, J.W., Balaban, C.D., and Kincaid, R.L. (1990). Developmental expression of calmodulin-dependent cyclic nucleotide phosphodiesterase in rat brain. Dev. Brain Res. *53,* 253–263.

Caceres, A., Bender, P., Snavely, L., Rebhun, L.I., and Steward, O. (1983). Distribution and subcellular localization of calmodulin in adult and developing brain tissue. Neuroscience *10,* 449–462.

Chafouleas, J.G., Bolton, W.E., Hidaka, H., Boyd, A.E., III, and Means, A.R. (1982). Calmodulin and the cell cycle: involvement in regulation of cell-cycle progression. Cell *28,* 41–50.

Chamberlain, J.S., VanTuinen, P., Reeves, A.A., Philip, B.A., and Caskey, C.T. (1987). Isolation of cDNA clones for the catalytic γ subunit of mouse muscle phosphorylase kinase: expression of mRNA in normal and mutant Phk mice. Proc. Natl. Acad. Sci. USA *84,* 2886–2890.

Cheung, W.Y. (1970). Cyclic 3′,5′-nucleotide phosphodiesterase: demonstration of an activator. Biochem. Biophys. Res. Commun. *38,* 533–538.

Cheung, W.Y. (1980). Calmodulin plays a pivotal role in cellular regulation. Science *207,* 19–27.

Chirgwin, J.M., Przybyla, A.P., MacDonald, R.J., and Rutter, W.J. (1979). Isolation of biologically active ribonucleic acid from sources enriched in ribonuclease. Biochemistry *18,* 5294–5299.

Cimino, M., Chen, J.F., and Weiss, B. (1990). Ontogenetic development of calmodulin mRNA in rat brain using *in situ* hybridization histochemistry. Dev. Brain Res. *54,* 43–49.

Feinberg, A.P., and Vogelstein, B. (1983). A technique for radiolabelling DNA restriction endonuclease fragments to high specific activity. Anal. Biochem. *132,* 6–13.

Gallien, C.L., Weinman, J., Rainteau, D., and Weinman, S. (1984). Changes in calmodulin level after fertilization and during first cleavage in the egg of the urodelan amphibian Pleurodeles waltlii. Exp. Cell Res. *155,* 397–405.

Goldenring, J.R., MacGuire, J.S., Jr., and DeLorenzo, R.J. (1984). Identification of the major postsynaptic density protein as homologous with the major calmodulin binding subunit of a calmodulin-dependent protein kinase. J. Neurochem. *42,* 1077–1084.

Greeb, J., and Shull, G.E. (1989). Molecular cloning of a third isoform of the calmodulin-sensitive plasma membrane Ca^{2+}-transporting ATPase that is expressed predominantly in brain and skeletal muscle. J. Biol. Chem. *264,* 18569–18576.

Harris, A.J., Fitzsimons, R.B., and McEwan, J.C. (1989). Neural control of the sequence of expression of myosin heavy chain isoforms in foetal mammalian muscles. Development (Camb.) *107,* 751–769.

Hyman, C., and Pfenninger, K.H. (1985). Intracellular regulators of neuronal sprouting: calmodulin-binding proteins of nerve growth cones. J. Cell Biol. *101,* 1153–1160.

Kakiuchi, S., Yamazaki, R., and Nakajima, H. (1970). Properties of a heat-stable phosphodiesterase activating factor isolated from brain extract. Studies on cyclic 3′,5′-nucleotide phosphodiesterase. II. Proc. Jpn. Acad. *46,* 587–592.

Kelly, P.T., Shields, S., Conway, D., Yip, R., and Burgin, K. (1987). Developmental changes in calmodulin-kinase II activity at brain synaptic junctions: alterations in holoenzyme composition. J. Neurochem. *49,* 1927–1939.

Klee, C.B., and Vanaman, T.C. (1982). Calmodulin. In: Advances in Protein Chemistry, vol. 35, New York: Academic Press, 213–303.

Larson, R.E., Espindola, F.S., and Espreafico, E.M. (1990). Calmodulin-binding proteins and calcium/calmodulin-regulated enzyme activities associated with brain actomyosin. J. Neurochem. *54,* 1288–1294.

Marcum, J.M., Dedman, J.R., Brinkley, B.R., and Means, A.R. (1978). Control of microtubule assembly-disassembly by calcium-dependent regulator protein. Proc. Natl. Acad. Sci. USA *75,* 3771–3775.

Means, A.R., Tash, J.S., and Chafouleas, J.G. (1982). Physiological implications of the presence, distribution, and regulation of calmodulin in eukaryotic cells. Physiol. Rev. *62,* 1–39.

Meiri, K.F., Pfenninger, K.H., and Willard, M.B. (1986). Growth associated protein, GAP-43, a polypeptide that is induced when neurons extend axons, is a component of growth cones and corresponds to pp46, a major polypeptide of a subcellular fraction enriched in growth cones. Proc. Natl. Acad. Sci. USA *83,* 3537–3541.

Mercadier, J.-J., and Dubus, I. (1991). Assay of atrial natriuretic factor messenger ribonucleic acid. In: Methods in Neurosciences, vol. 5, ed. P. Michael Conn, New York: Academic Press, 22–34.

Messer, A., Plummer-Siegard, J., and Eisenberg, B. (1990). Staggerer mutant mouse Purkinje cells do not contain detectable calmodulin mRNA. J. Neurochem. *55,* 293–302.

Nojima, H. (1989). Structural organization of multiple rat calmodulin genes. J. Mol. Biol. *208,* 269–282.

Rainteau, D., Sharif, A., Bourrillon, R., and Weinman, S. (1987). Calmodulin in lymphocyte mitogenic stimulation and in lymphoid cell line growth. Exp. Cell Res. *168,* 546–554.

Rainteau, D.P., Weinman, S.J., Kabaktchis, C.A.M., Smith, V.L., Kaetzel, M.A., Dedman, J.R., and Weinman, J.S. (1988). The expression of the 35- and 67-kDa calcimedins is dependent on thyroid hormone. J. Biol. Chem. *263,* 12844–12848.

Rasmussen, C.D., and Means, A.R. (1989). Calmodulin is required for cell-cycle progression during G_1 and mitosis. EMBO J. *8,* 73–82.

Roberts-Lewis, J.M., Cimino, M., Krause, R.G., II, Tyrrell, D.F., Jr., Davis, L.G., Weiss, B., and Lewis, M.E. (1990). Anatomical localization of calmodulin mRNA in the rat brain with cloned cDNA and synthetic oligonucleotide probes. Synapse (NY) *5,* 247–254.

Seto-Ohshima, A., Yamazaki, Y., Kawamura, N., Kitajima, S., Sano, M., and Mizutani, A. (1987). The early expression of immunoreactivity for calmodulin in the nervous system of mouse embryos. Histochemistry *86,* 337–343.

Slaughter, G.R., and Means, A.R. (1989). Analysis of expression of multiple genes encoding calmodulin during spermatogenesis. Mol. Endocrinol. *3,* 1569–1578.

Slaughter, G.R., Meistrich, M.L., and Means, A.R. (1989). Expression of RNAs for calmodulin, actins, and tubulin in rat testis cells. Biol. Reprod. *40,* 395–405.

Slaughter, G.R., Needleman, D.S., and Means, A.R. (1987). Developmental regulation of calmodulin, actin, and tubulin mRNAs during rat testis differentiation. Biol. Reprod. *37,* 1247–1258.

Stewart, A.A., and Cohen, P. (1988). Protein-phosphatase-2B from rabbit skeletal muscle: a Ca^{2+}-dependent, calmodulin-stimulated enzyme. Methods Enzymol. *159,* 409–416.

Tallant, E.A., and Cheung, W.Y. (1983). Calmodulin-dependent protein phosphatase: a developmental study. Biochemistry *22,* 3630–3635.

Tuana, B.S., and MacLennan, D.H. (1988). Isolation of the calmodulin-dependent protein kinase system from rabbit skeletal muscle sarcoplasmic reticulum. FEBS Lett. *235,* 219–223.

Wang, J., Chenivesse, X., Henglein, B., Brechot, C. (1990). Hepatitis B virus integration in a cyclin A gene in a hepatocellular carcinoma. Nature (Lond.) *343,* 555–557.