Species Differences in the Distribution of the Nonmuscle Myosin Heavy Chain IIB Inserted Isoform in the Brain

Authors: Hagiwara, Shin-ya, Takahashi, Masayuki, and Yamagishi, Akihiko

Source: Zoological Science, 18(1) : 43-49

Published By: Zoological Society of Japan

URL: https://doi.org/10.2108/zsj.18.43

ZOOLOGICAL SCIENCE **18**: 43–49 (2001)

Species Differences in the Distribution of the Nonmuscle Myosin Heavy Chain IIB Inserted Isoform in the Brain

Shin-ya Hagiwara, Masayuki Takahashi*
and Akihiko Yamagishi

Division of Biological Sciences, Graduate School of Science, Hokkaido University, Sapporo 060-0810, Japan

ABSTRACT—The alternatively spliced isoform of the nonmuscle myosin heavy chain IIB (MHC-IIB) with an insert of 21 amino acids near the actin-binding region, MHC-IIB(B2), is expressed specifically in the brain and spinal cord in Mammalia and Aves. We performed immunoblot analyses to elucidate the distribution of MHC-IIB(B2) in the brains of various animals. Nearly half of MHC-IIB existed as the B2 inserted isoform (MHC-IIB(B2)) in the cerebrum of the guinea-pig, rabbit and pig, while the non-B2 inserted isoform (MHC-IIB(ΔB2)) was the dominant form in the cerebrum of the rat, mouse and hamster. In the guinea-pig, the amount of MHC-IIB(B2) expressed in the cerebrum was low compared to MHC-IIB(ΔB2) during the first postnatal week, but it increased to comparable levels during postnatal development. In the rat, the amount of MHC-IIB(B2) protein in the cerebrum remains low compared to its expression elsewhere in the brain throughout life. Our results regarding the distribution of MHC-IIB(B2) in the adult brain lead us to classify species into two types; one type expresses significantly less MHC-IIB(B2) in the cerebrum than in other portions of the brain, and the second type expresses it at comparable levels throughout the brain. Based on these results, we hypothesize that MHC-IIB(B2) modulates the role of MHC-IIB(ΔB2) in the regulation of synaptic structure and function in the mature brain, and that the requirements for such function by MHC-IIB(B2) have shifted gradually from the cerebellum to the cerebrum with evolutionary increases in brain size.

INTRODUCTION

Myosin II is the most studied isoform in an expanding family of myosin motor proteins (Mermall *et al*., 1998; Sellers, 2000). It is a hexamer composed of a pair of heavy chains (200 kDa) and two pairs of light chains (20 and 17 kDa). The amino-terminal half of the heavy chain forms the head region, termed subfragment 1, containing both ATP and actin-binding sites. Two proteolytically susceptible portions are present in the head region of myosin II, and the proteolytic cleavage of the myosin heavy chain (MHC) produces fragments of 25 kDa, 50 kDa and 20 kDa (Balint *et al*., 1978).

All vertebrate cells, including muscle cells, contain a form of myosin II referred to as nonmuscle myosin II. Nonmuscle myosin II plays a role in cell motile processes such as cytokinesis, migration, and morphogenesis (Spudich *et al*., 1995). To date, two different isoforms of the nonmuscle MHC have been identified in vertebrate cells (Katsuragawa *et al*., 1989; Kawamoto and Adelstein, 1991). They are referred to as MHC-A and MHC-B (Kawamoto and Adelstein, 1991) or MHC-IIA and MHC-IIB. The entire cDNA sequence encoding both isoforms has been determined for the chicken (Shohet *et al*., 1989; Takahashi *et al*., 1992), human (Saez *et al*., 1990; Simons *et al*., 1991; Toothaker *et al*., 1991; Phillips *et al*., 1995), *Xenopus* (Bhatia-Dey *et al*., 1993, 1998) and the sequence of MHC-IIA alone for the rat (Choi *et al*., 1996). These two isoforms are expressed in a tissue-dependent manner. For example, the spleen and intestines are enriched in MHC-IIA, while the brain and testis are enriched in MHC-IIB (Katsuragawa *et al*., 1989; Kawamoto and Adelstein, 1991; Phillips *et al*., 1995; Takahashi *et al*., 1999).

Alternatively spliced isoforms of MHC-IIB, which include two cassettes of inserted amino acids at different locations in the head region, were discovered during the cloning of the cDNA encoding chicken brain MHC-IIB (Takahashi *et al*., 1992). These inserted isoforms are expressed in a tissue-dependent manner specific to the central nervous system (CNS). One insert of 10 amino acids was located at the 25/50-kDa domain junction close to the ATP binding region, and another insert of 21 amino acids was located at the 50/20-kDa domain junction close to the actin binding region. These inserts are referred to as B1 and B2, respectively.

The expression of these alternatively spliced isoforms is

* Corresponding author: Tel. +81-11-706-3810;
FAX. +81-11-706-4909.
E-mail: takahash@sci.hokudai.ac.jp

regulated developmentally in the brains of the chicken, mouse and rat. The mRNA encoding the B1 inserted isoform (MHC-IIB(B1)) is expressed early in embryonic development, while expression of the mRNA encoding the B2 inserted isoform (MHC-IIB(B2)) begins just before birth in the developing chicken (Itoh and Adelstein, 1995). The heavier isoform of the MHC-IIB polypeptide begins to appear in the second postnatal week in the mouse cerebellum (Murakami *et al.*, 1993). Both the polypeptide and the mRNA of MHC-IIB(B2) are expressed with different timing in distinct regions during development of the rat brain (Takahashi *et al.*, 1999; Miyazaki *et al.*, 2000).

It has also been demonstrated that the distribution of MHC-IIB(B2) in the brain differs among some species. The expression of MHC-IIB(B2) in the cerebrum is low in the brains of adult chickens (Takahashi *et al.*, 1992) and rats (Murakami *et al.*, 1991; Takahashi *et al.*, 1999; Miyazaki *et al.*, 2000). In contrast, MHC-IIB(B2) is expressed in comparable levels to the non-B2 inserted isoform (MHC-IIB(ΔB2)) in the cerebrum of adult human and bovine brains (Kimura *et al.*, 1993; Itoh and Adelstein, 1995). This distinct difference has motivated us to compare the distribution of MHC-IIB(B2) in the adult brain among animals located in the neighborhood of the rat and bovine in phylogenetic classifications. We expected that the boundary of the difference exists somewhere between these two. For that purpose, six species were selected from laboratory animals belonging to Rodentia (rat, mouse, hamster and guinea-pig), Lagomorpha (rabbit) and Artiodactyla (pig).

Our results lead us to classify species into two types on the basis of the distribution of MHC-IIB(B2) in the adult brain; one type (chicken, rat, mouse and hamster) expresses significantly less MHC-IIB(B2) in the cerebrum than in other portions of brain, and the second type (guinea-pig, rabbit, pig, bovine and human) expresses it at comparable levels throughout the brain. The present study thus might provide a new index for the classification of species.

MATERIALS AND METHODS

Experimental Animals

Mouse (*Mus musculus*) strain ddY, golden hamster (*Mesocricetus auratus*) strain Syrian, guinea-pig (*Cavia porcellus*) strain Hartley, and rabbit (*Oryctolagus cuniculus*) strain Japanese White were obtained from Sankyo Labo Service Corporation (Tokyo, Japan). Pig (*Sus scrofa*) brain was obtained from the Ebetsu Livestock Breeding Corporation (Ebetsu, Japan). Rat (*Rattus norvegicus*) strain Wistar was generously donated by Dr. Etsuro Ito's laboratory (Hokkaido University).

SDS-Polyacrylamide Gel Electrophoresis and Immunoblotting

Tissues were homogenized in an extraction buffer (0.6 M NaCl, 5 mM EDTA, 5 mM EGTA, 14 mM 2-mercaptoethanol, 0.1 mM PMSF, and 40 mM MOPS, pH7.6) and centrifuged at 15,000 g for 60 min at 4°C. The resulting supernatants were used as tissue extracts. The tissue extracts were separated on an SDS-5% polyacrylamide gel

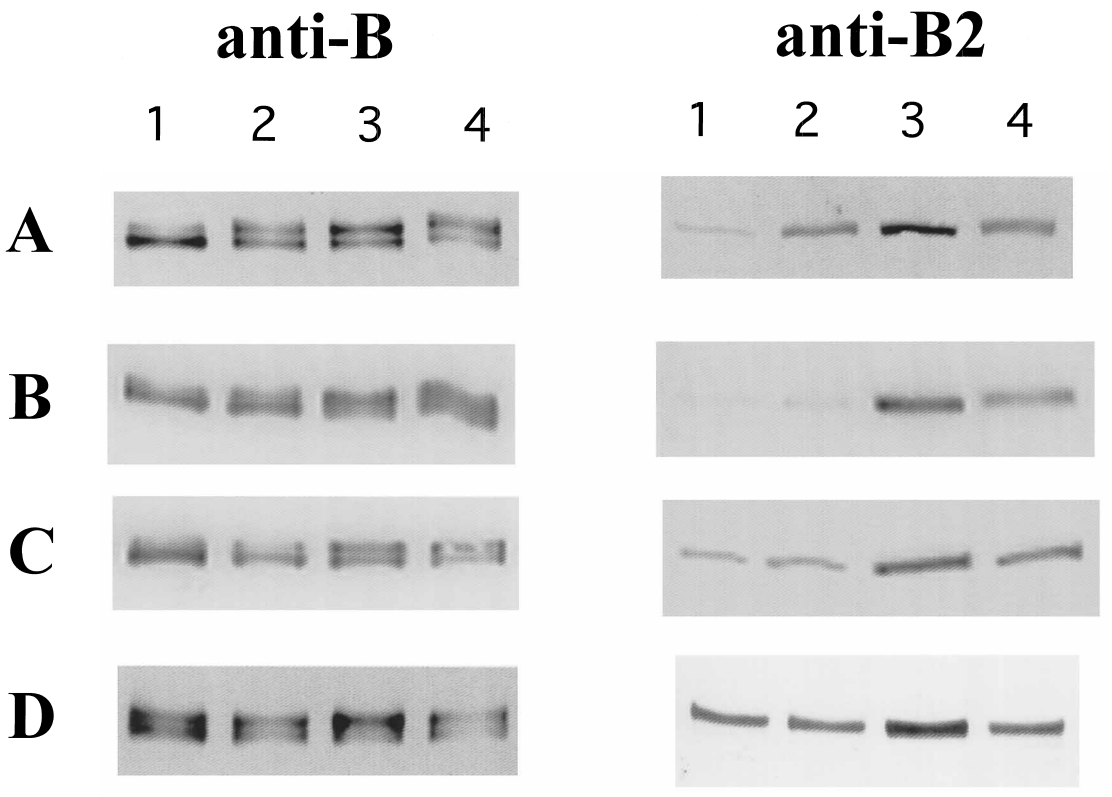

Fig. 1. Immunoblot analysis of extracts from the adult brain of various species in Rodentia probed with anti-B2 and anti-B antibodies. The extracts (75 μg/lane for anti-B2, 10 μg/lane for anti-B) were subjected to SDS-5% polyacrylamide gel electrophoresis followed by immunoblotting. A; rat (5-month-old), B; mouse (6-month-old), C; golden hamster (6-month-old), D; guinea-pig (10-month-old). *Lanes 1–4* are as follows: *1*, cerebrum; *2*, di- and mesencephalon; *3*, cerebellum; *4*, brain stem.

with 0.065% bisacrylamide using the buffer system of Laemmli (1970). The same amount of protein was loaded on each lane in each gel. The gel was electroblotted onto Immobilon-P (Millipore) and immunostained with antiserum. The antiserum was diluted as follows; anti-MHC-IIB 1 to 3000: anti-MHC-IIB(B2) 1 to 100. Following incubation with a second antibody coupled to horseradish peroxidase, the blots were developed using 4-chloro 1-naphthol as a substrate. Antiserum specific for MHC-IIB was generously donated by Dr. Robert S. Adelstein (NHLBI, NIH).

RESULTS

Expression of MHC-IIB(B2) in the adult brain of various species

In order to investigate the expression of MHC-IIB(B2) polypeptide in adult brain tissues from various animals, we performed immunoblot analyses using two different antibodies. Anti-MHC-IIB(B2) antibodies were generated against a peptide synthesized on the basis of the derived amino acid sequence of the rat B2 inserted residues (Takahashi *et al.*, 1999). We also used anti-MHC-IIB antibodies, which cross-reacted with MHC-IIB(B2) as well as non-inserted MHC-IIB (Mr = 200,000), as these antibodies recognize the carboxyl-terminal region of MHC-IIB isoforms. The anti-MHC-IIB(B2) antibodies cross-react only with the slower-migrating of the two bands recognized by the anti-MHC-IIB antibodies. Fig. 1. shows the immunoblots of brain extracts from different adult species of Rodentia. We analyzed extracts from four different regions of the brain, that is, cerebrum, di- and mesencephalon, cerebellum and brain stem. Fig. 1 demonstrates that the distribution of the expression of MHC-IIB(B2) in the brain is different among these species. In the rat, mouse and hamster, MHC-IIB(B2) was expressed in the cerebellum at a higher level than in other regions of the brain. In guinea-pig, a higher expression of MHC-IIB(B2) was also observed in the cerebellum. However, it was expressed in the cerebrum much more than in the other species. Almost half of the MHC-IIB existed as MHC-IIB(B2) in the guinea-pig cerebrum, while MHC-IIB(ΔB2) was the dominant form in the rat, mouse and hamster cerebrum. MHC-IIB(B2) was dominant in the guinea-pig cerebellum.

We next analyzed the expression of MHC-IIB(B2) in the rabbit and pig. These species have a relatively larger brain than rodents, enabling us to dissect the brain more precisely. Fig. 2. shows the immunoblots of brain extracts from the rabbit and pig. The results indicate that MHC-IIB(B2) is expressed at comparable levels throughout the brain in the rabbit and pig. Both the non-inserted and B2 inserted isoforms were expressed almost to the same extent throughout the brain in the rabbit (Fig. 2A). In contrast, the B2 inserted isoform was expressed more than the noninserted isoform, especially in the lower brain of the pig (Fig. 2B).

Change in expression of MHC-IIB(B2) during postnatal development of the guinea-pig

To characterize whether MHC-IIB(B2) is expressed at the same level throughout the brain in the developing guinea-pig,

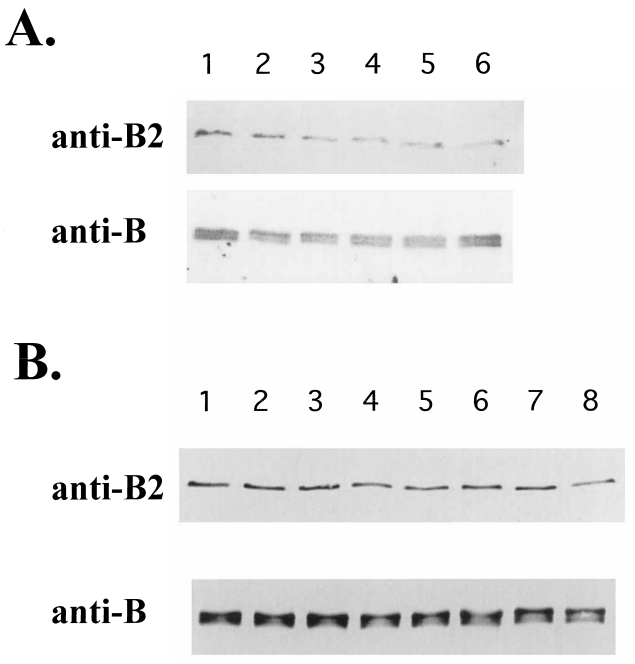

Fig. 2. Immunoblot analysis of extracts from the adult brain of the rabbit (A) and pig (B) probed with anti-B2 and anti-B antibodies. A, *Lanes 1–6* are as follows: *1*, cerebrum; *2*, callous body; *3*, diencephalon; *4*, mesencephalon; *5*, cerebellum; *6*, medulla. B, *Lanes 1–8* are as follows: *1*, cerebrum; *2*, callous body; *3*, thalamus; *4*, amygdala; *5*, mesencephalon; *6*, cerebellum; *7*, pons; *8*, medulla.

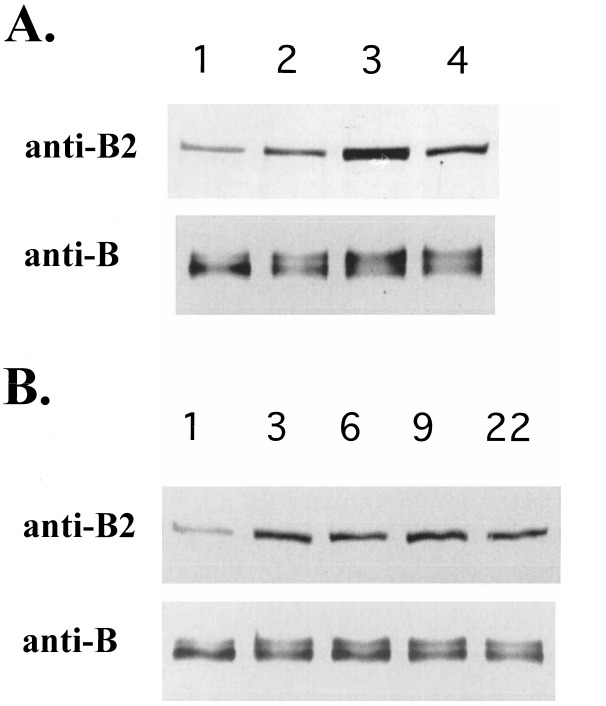

Fig. 3. Immunoblot analysis of extracts from the guinea-pig brain at the first postnatal week (A), and developing cerebrum (B) with anti-B2 and anti-B antibodies. A, *Lanes 1–4* are as follows: *1*, cerebrum; *2*, di- and mesencephalon; *3*, cerebellum; *4*, brain stem. B, The numbers above each set of immunoblots correspond to the weeks after birth.

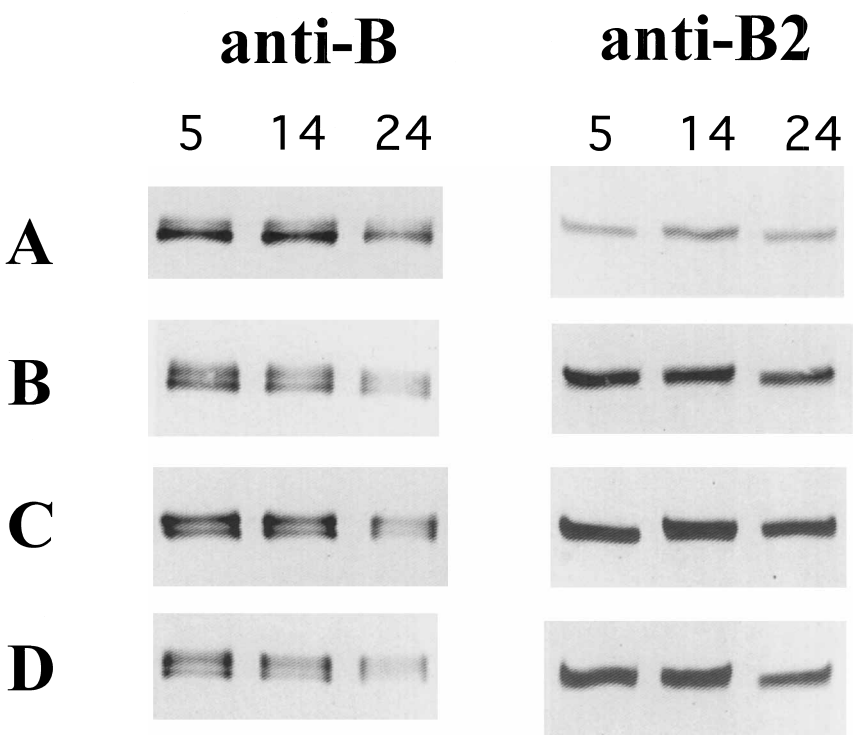

Fig. 4. Immunoblot analysis of extracts from aging rat cerebrum (A), di- and mesencephalon (B), cerebellum (C), brain stem (D) with anti-B2 and anti-B antibodies. The numbers above each set of immunoblots correspond to the months after birth.

selected brain tissues were dissected from guinea-pigs at various developmental stages after birth and were analyzed by immunoblotting. Fig. 3A. demonstrates that MHC-IIB(B2) is already expressed throughout the brain during the first postnatal week, and MHC-IIB(ΔB2) is the dominant form in cerebrum. Interestingly the expression pattern of MHC-IIB(B2) of the guinea-pig during the first postnatal week (Fig. 3A) was quite similar to that of the adult rat (Fig. 1A). The expression of MHC-IIB(B2) in the cerebrum increased to levels comparable to those of the adult during the third postnatal week (Fig. 3B). The expression of MHC-IIB(B2) in the di- and mesencephalon and the brain stem were found to increase gradually, while that in the cerebellum was unchanged during postnatal development (data not shown). These results indicate that even species expressing MHC-IIB(B2) almost to the same extent as MHC-IIB(ΔB2) in the adult cerebrum such as the guinea-pig, express limited amounts of MHC-IIB(B2) in the cerebrum during the early days of postnatal development.

Expression of MHC-IIB(B2) in the rat with increasing age

To investigate whether the expression pattern of MHC-IIB(B2) in the rat brain changes during aging, selected rat brain tissues were individually dissected from 5-month-old, 14-month-old and 24-month-old rats and were analyzed by immunoblotting. Fig. 4. demonstrates that the expression of MHC-IIB(B2) does not change during aging in any portion of the brain, except for a slight decrease in the 24-month-old rat. That is to say, the expression of MHC-IIB(B2) in the rat cerebrum never increases to levels comparable to that in the guinea-pig cerebrum during 5–24 months of age. This result suggests that differences in the distribution of MHC-IIB(B2) in the brain does not depend on differences in the aging process of individual species.

DISCUSSION

We have shown in the present study that the distribution of MHC-IIB(B2) in the brain apparently differs among species. The results have led us to classify species into two types on the basis of the distribution of MHC-IIB(B2) in the adult brain. One type expresses less MHC-IIB(B2) in the cerebrum than in other regions of the brain (referred to as type A), while the other type expresses it at comparable levels throughout the brain (referred to as type B). In other words, the noninserted isoform is the dominant form in the cerebrum of the type A species, while MHC-IIB(B2) is expressed at the same or even at greater levels than MHC-IIB(ΔB2) in the cerebrum of the type B species. According to this classification, the rat, mouse, hamster and chicken (Takahashi *et al.*, 1992) belong to type A, while the guinea-pig, rabbit, pig, and possibly the bovine and humans (Itoh and Adelstein, 1995) belong to type B. In the rat, the expression of MHC-IIB(B2) in the cerebrum never reached levels comparable to those of the guinea-pig throughout life, suggesting that the difference in the distribution of MHC-IIB(B2) in brain is not caused by differences in the aging process of individual species used for analysis.

In the rat (type A), MHC-IIB(B2) is not expressed at the protein level in the newborn brain and only becomes apparent at different times in different regions of the brain during postnatal development (Takahashi *et al.*, 1999). It first ap-

pears at postnatal day 10 (P10) in the cerebellum and increases markedly from P14. Its appearance in the cerebrum (P28) is later than in the cerebellum. It is also of note that the emergence of MHC-IIB(B2) in the cerebellum corresponds to the time when dendritic elongation and synaptogenesis occur actively in the cerebellum of the rat (Altman, 1972).

In the guinea-pig (type B), MHC-IIB(B2) is already expressed at the first postnatal week throughout the brain. However, that distribution is similar to that of the adult brain of the type A species; that is, much less MHC-IIB(B2) is expressed in the cerebrum than in other portions of the brain during the early stages of postnatal development. The expression of MHC-IIB(B2) increases to levels comparable to MHC-IIB(ΔB2) in the cerebrum during postnatal development. In contrast, MHC-IIB(ΔB2) is the dominant form in the cerebrum throughout the life of the rat, which is a type A species. These results suggest that the differences in MHC-IIB(B2) distribution in the brain between type A and B species occur during postnatal development. The expression of MHC-IIB(B2) throughout the brain during the early postnatal stages of the guinea-pig might be related to the high degree of neurological maturity at birth.

The inserted amino acids B1 and B2 are located at 25/50-kDa and 50/20-kDa domain junctions, respectively. These portions were first identified as two protease-sensitive regions in the myosin head (Balint *et al*., 1978). These regions have not been resolved in the crystal structures of the chicken skeletal myosin II subfragment 1, indicating that they might be present as disordered surface loops (Rayment *et al*., 1993). The regions at the junction of the 25/50-kDa fragments and at that of the 50/20-kDa fragments are termed loop 1 and loop 2, respectively (Spudich, 1994). Loops 1 and 2 are located close to the ATP binding and actin binding regions, respectively. It has recently been demonstrated that the sequences of these loop regions appear to be more constrained than those of the rest of the myosin molecule in a comparison of myosins known to be kinetically or developmentally similar (Goodson *et al*., 1999). These loop regions could define the kinetic characteristics specific to each myosin isoform (Uyeda *et al*., 1994; Spudich, 1994; Goodson *et al*., 1999). We recently observed that the B2 insertion reduces motor activity with a reduction in the maximal actin-activated ATPase activity and a decrease in the affinity for actin using chimeric myosin II expressed in *Dictyostelium* (Takahashi *et al*., in press). The presence of the B2 insertion might reduce the enzymatic and/or motor activity of the MHC-IIB molecule by changing the interaction with actin in CNS tissue.

Immunohistochemical studies have demonstrated that MHC-IIB is expressed in most neuronal cells (Murakami and Elzinga, 1992; Miller *et al*., 1992). In particular, the cell bodies of Purkinje cells and their dendrites in the molecular layer were stained dramatically in the rat cerebellum, while the cell bodies and apical dendrites of pyramidal neurons were stained in the rat cerebral cortex. We have recently demonstrated that MHC-IIB(B2) is localized at cell bodies and dendrites of Purkinje cells in the rat cerebellum using antibodies specific to the B2 insert (Miyazaki *et al*., 2000). We have also demonstrated that MHC-IIB(ΔB2) is highly expressed in neurons of the cerebral cortex and hippocampus as well as the cerebellum of the rat by in situ hybridization (Miyazaki *et al*., 2000).

As to the roles of the myosin II molecule in neurons, three hypotheses have been proposed. First, myosin II could play an important role in the motility of the growth cones (Miller *et al*., 1992; Smith, 1988; Cheng *et al*., 1992; Rochlin *et al*., 1995; Mummert and Schengrund, 1997; Wylie *et al*., 1998). Second, it could be involved in neurotransmitter release (Mochida *et al*., 1994). Third, it may be involved in the process of synaptic plasticity (Miller *et al*., 1992; Morales and Fifkova, 1989). It is reasonable to suppose that MHC-IIB isoforms participate in such processes, as they are dominant isoforms in the brain. Actually, a knockout mouse study has demonstrated that MHC-IIB is required for normal development of the brain as well as the heart (Tullio *et al*., 1997).

MHC-IIB(B2) is not expressed in *Xenopus* tissues, whereas MHC-IIB(B1) is constitutively expressed in all *Xenopus* tissues (Bhatia-Dey *et al*., 1993), suggesting that MHC-IIB(B2) might have started to be expressed in the brain, except for the forebrain, after the amphibian period. Thereafter, the distribution of this inserted isoform may have extended to the cerebrum sometime after the appearance of mammals. Interestingly, in humans, MHC-IIB(B2) is expressed to almost half the extent in the cerebrum as the type B species, but is expressed only in limited amounts in the cerebellum (Itoh and Adelstein, 1995).

With these results taken together, we hypothesize the following regarding the role of MHC-IIB isoforms in the brain: (I) MHC-IIB(ΔB2) plays an active role in the regulation of synaptic structure and function, and (II) MHC-IIB(B2) modulates the role of MHC-IIB(ΔB2). Combining these hypotheses with the species differences of distribution of both MHC-IIB(ΔB2) and MHC-IIB(B2) in the adult brain, we come to the following conclusion regarding the evolutionary development of these isoforms. At the beginning of the appearance of brain tissue, MHC-IIB(ΔB2) may have begun to play a role (I) in the neuron. Accompanying the development of both the size and function of the brain, MHC-IIB(B2) may have become necessary in the lower brain, particularly in the cerebellum. With further developments in brain size in mammals, the requirement for MHC-IIB(B2) function (II) may then have been extended and gradually shifted from the cerebellum to the cerebrum.

Interestingly, both types of species existed even among the order Rodentia in the present study; that is, the guinea-pig alone belonged to the type B classification. Interestingly, phylogenetic analyses based on molecular data have suggested that the guinea-pig belongs to a new order (Graur *et al*., 1991; D'Erchia *et al*., 1996; Hoyle, 1999). The difference in the distribution of MHC-IIB(B2) in the present study supports this view, suggesting that this difference may provide a new index for the classification of species.

ACKNOWLEDGMENTS

We are grateful to Dr. Robert S. Adelstein (NHLBI, NIH) for his

helpful discussion and critical reading of the manuscript. This work was supported by a Grant-in-Aid from the Ministry of Education, Science, Sports and Culture of Japan, and a Special Grant-in-Aid for the Promotion of Education and Science in Hokkaido University provided by the Ministry of Education, Science, Sports and Culture. This work was also supported by The Akiyama Foundation.

REFERENCES

Altman J (1972) Postnatal development of the cerebellar cortex in the rat. II. Phases in the maturation of Purkinje cells and of the molecular layer. J Comp Neurol 145: 399–464.

Balint M, Wolf L, Tarcsafalvi A, Gergely J, Sreter FA (1978) Location of SH-1 and SH-2 in the heavy chain segment of heavy meromyosin. Arch Biochem Biophys 190: 793–799

Bhatia-Dey N, Adelstein RS, Dawid IB (1993) Cloning of the cDNA encoding a myosin heavy chain B isoform of *Xenopus* nonmuscle myosin with an insert in the head region. Proc Natl Acad Sci U S A 90: 2856–2859

Bhatia-Dey N, Taira M, Conti MA, Nooruddin H, Adelstein RS (1998) Differential expression of non-muscle myosin heavy chain genes during *Xenopus* embryogenesis. Mech Dev 78: 33–36

Cheng TPO, Murakami N, Elzinga M (1992) Localization of myosin-IIB at the leading-edge of growth cones from rat dorsal-root ganglionic cells. FEBS Lett 311: 91–94

Choi OH, Park C-S, Itoh K, Adelstein RS, Beaven MA (1996) Cloning of the cDNA encoding rat myosin heavy chain-A and evidence for the absence of myosin heavy chain-B in cultured rat mast (RBL-2H3) cells. J Muscle Res Cell Motil 17: 69–77

D'Erchia AM, Gissi C, Pesole G, Saccone C, Arnason U (1996) The guinea-pig is not a rodent. Nature 381: 597–600

Goodson HV, Warrick HM, Spudich JA (1999) Specialized conservation of surface loops of myosin: Evidence that loops are involved in determining functional characteristics. J Mol Biol 287: 173–185

Graur D, Hide WA, Li WH (1991) Is the guinea-pig a rodent? Nature 351: 649–652

Hoyle CH (1999) Neuropeptide families and their receptors: evolutionary perspectives. Brain Res 848: 1–25

Itoh K, Adelstein RS (1995) Neuronal cell expression of inserted isoforms of vertebrate nonmuscle myosin heavy chain II-B. J Biol Chem 270: 14533–14540

Katsuragawa Y, Yanagisawa M, Inoue A, Masaki T (1989) Two distinct nonmuscle myosin-heavy-chain mRNAs are differentially expressed in various chicken tissues: Identification of a novel gene family of vertebrate non-sarcomeric myosin heavy chains. Eur J Biochem 184: 611–616

Kawamoto S, Adelstein RS (1991) Chicken nonmuscle myosin heavy chains: Differential expression of two mRNAs and evidence for two different polypeptides. J Cell Biol 112: 915–924

Kimura A, Nakashima S, Uda T, Ikeda H, Yasuda S, Tsuji T, Matsumura S (1993) Heavy-chain isoforms of non-muscle myosin in human tissues. Eur J Biochem 213: 5–10

Laemmli UK (1970) Cleavage of structural proteins during the assembly of the head of bacteriophage T4. Nature 227: 680–685

Mermall V, Post PL, Moosker MS (1998) Unconventional myosins in cell movement, membrane traffic, and signal transduction. Science 279: 527–533

Miller M, Bower E, Levitt P, Li D, Chantler PD (1992) Myosin II distribution in neurons is consistent with a role in growth cone motility but not synaptic vesicle mobilization. Neuron 8: 25–44

Mochida S, Kobayashi H, Matsuda Y, Yuda Y, Muramoto K, Nonomura Y (1994) Myosin II is involved in transmitter release at synapses formed between rat sympathetic neurons in culture. Neuron 13: 1131–1142

Morales M, Fifkova E (1989) *In situ* localization of myosin and actin in dendritic spines with the immunogold technique. J Comp Neurol 279: 666–674

Mummert CM, Schengrund C-L (1997) Nonmuscle myosin heavy chain B is recognized by a monoclonal antibody that inhibits GM-1 enhanced neuritogenesis. J Neurochem 68: 596–600

Murakami N, Mehta P, Elzinga M (1991) Studies on the distribution of cellular myosin II isoforms with antibodies to isoform-specific synthetic peptides. FEBS Lett 278: 23–25

Murakami N, Elzinga M (1992) Immunohistochemical studies on the distribution of cellular myosin II isoforms in brain and aorta. Cell Motil Cytoskel 22: 281–295

Murakami N, Trenkner E, Elzinga M (1993) Changes in expression of nonmuscle myosin heavy chain isoforms during muscle and nonmuscle tissue development. Dev Biol 157: 19–27

Miyazaki T, Watanabe M, Yamagishi A, Takahashi M (2000) B2 exon splicing of nonmuscle myosin heavy chain IIB is differently regulated in developing and adult rat brain. Neurosci Res 37: 299–306

Phillips CL, Yamakawa K, Adelstein RS (1995) Cloning of the cDNA encoding human nonmuscle myosin heavy chain-B and analysis of human tissues with isoform-specific antibodies. J Muscle Res Cell Motil 16: 379–389

Rayment I, Rypniewski WR, Schmidt-Bäse K, Smith R, Tomchick DR, Benning MW, Winkelmann DA, Wesenberg G, Holden HM (1993) Three- dimensional structure of myosin subfragment-1: A molecular motor. Science 261: 50–58

Rochlin MW, Itoh K, Adelstein RS, Bridgman PC (1995) Localization of myosin IIA and B isoforms in cultured neurons. J Cell Sci 108: 3661–3670

Saez CG, Myers JC, Shows TB, Leinwand LA (1990) Human nonmuscle myosin heavy chain mRNA: Generation of diversity through alternative polyadenylation. Proc Natl Acad Sci U S A 87: 1164–1168

Sellers JR (2000) Myosins: A diverse superfamily. Biochim Biophys Acta 1496: 3–22

Shohet RV, Conti MA, Kawamoto S, Preston YA, Brill DA, Adelstein RS (1989) Cloning of the cDNA encoding the myosin heavy chain of a vertebrate cellular myosin. Proc Natl. Acad Sci. U S A 86: 7726–7730

Simons M, Wang M, McBride OW, Kawamoto S, Yamakawa K, Gdula D, Adelstein RS, Weir L (1991) Human nonmuscle myosin heavy chains are encoded by two genes located on different chromosomes. Circ Res 69: 530–539

Smith SJ (1988) Neuronal cytomechanics: The actin-based motility of growth cones. Science 242: 708–715

Spudich JA (1994) How molecular motors work. Nature 372: 515–518

Spudich JA, Finer J, Simmons B, Ruppel K, Patterson B, Uyeda T (1995) Myosin structure and function. Cold Spring Harbor Symp Quant Biol 60: 783–791

Takahashi M, Kawamoto S, Adelstein RS (1992) Evidence for inserted sequences in the head region of nonmuscle myosin specific to the nervous system: Cloning of the cDNA encoding the myosin heavy chain-B isoform of vertebrate nonmuscle myosin. J Biol Chem 267: 17864–17871

Takahashi M, Hirano T, Uchida K, Yamagishi A (1999) Developmentally regulated expression of a nonmuscle myosin heavy chain IIB inserted isoform in rat brain. Biochem Biophys Res Commun 259: 29–33

Takahashi M, Takahashi K, Hiratsuka Y, Uchida K, Yamagishi A, Uyeda TQP, Yazawa M (2001) Functional characterization of vertebrate nonmuscle myosin IIB isoforms using *Dictyostelium* chimeric myosin II. J Biol Chem: in press.

Toothaker LE, Gonalez DA, Tung N, Lemons RS, Le Beau MM, Arnaout MA, Clayton LK, Tenen TG (1991) Cellular myosin heavy chain in human leukocytes: Isolation of 5' cDNA clones, characterization of the protein, chromosomal localization, and upregulation during myeloid differentiation. Blood 78: 1826–1833

Tullio AN, Accili A, Ferrans VJ, Yu Z, Takeda K, Grinberg A, Westphal H, Preston YA, Adelstein RS (1997) Nonmuscle myosin II-B is required for normal development of the mouse heart. Proc Natl Acad Sci U S A 94: 12407–12412

Uyeda TQP, Ruppel KM, Spudich JA (1994) Enzymatic activities correlate with chimaeric substitutions at the actin-binding face of myosin. Nature 368: 567–569

Wylie SR, Wu P-J, Patel H, Chantler PD (1998) A conventional myosin motor drives neurite outgrowth. Proc Natl Acad Sci U S A 95: 12967–12972

(Received September 8, 2000 / Accepted October 24, 2000)