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Review

‘Superfast’ or masticatory myosin and the evolution of jaw-closing muscles of vertebrates

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Summary

There are four fibre types in mammalian limb muscles, each expressing a different myosin isoform that finely tunes fibre mechanics and energetics for locomotion. Functional demands on jaw-closer muscles are complex and varied, and jaw muscles show considerable phylogenetic plasticity, with a repertoire for myosin expression that includes limb, developmental, α-cardiac and masticatory myosins. Masticatory myosin is a phylogenetically ancient motor with distinct light chains and heavy chains. It confers high maximal muscle force and power. It is highly jaw-specific in expression and is found in several orders of eutherian and marsupial mammals including carnivores, chiropterans, primates, dasyurids and diprotodonts. In exceptional species among these orders, masticatory myosin is replaced by some other isoform. Masticatory myosin is also found in reptiles and fish. It is postulated that masticatory myosin diverged early during gnathostome evolution and is expressed in primitive mammals. During mammalian evolution, mastication of food became important, and in some taxa jaw closers replaced masticatory myosin with α-cardiac, developmental, slow or fast limb myosins to adapt to the variety of diets and eating habits. This occurred early in some taxa (rodents, ungulates) and later in others (macropods, lesser panda, humans). The cellular basis for the uniqueness of jaw-closing muscles lies in their developmental origin.

Key words: jaw muscle, fibre type, muscle contraction, mastication, myosin isoform, masticatory myosin, evolution, molecular phylogeny.

Molecular physiology of myosin

Myosin is the motor protein in the thick filament of striated muscles. Structurally, it is a hexamer consisting of a pair of heavy chains (MyHC) and two pairs of light chains (MyLC-1 and MyLC-2). Each myosin molecule consists of a superhelical rod formed by the C-terminal half of the MyHCs, with two globular heads attached at one end, formed by the amino terminals of the MyHCs and the MyLCs. During muscle contraction, myosin heads act as cross-bridges that cyclically interact with the actin molecules that form the thin filaments. This cyclic activity is coupled to the hydrolysis of ATP, generating the relative force and motion between filaments that underlie the sliding filament theory of muscle contraction.

Myosin controls the kinetics of energy transduction from ATP and, through it, the kinetic properties of muscles. The speed of contraction of a muscle is proportional to the ATPase activity of its myosin (Bárány, 1967). As the maximal stresses of fast and slow muscles are approximately the same, muscle power (force × velocity) is also dependent on myosin ATPase activity. High muscle speed and power may confer an evolutionary advantage to an organism, as in escaping from predators or chasing prey. However, skeletal muscles constitute some 45 % of the body mass of vertebrates and are the greatest consumers of energy in the body, the bulk of which is spent in cross-bridge cycling. The benefit of high muscle speed is balanced by the high energy cost and the need for high caloric intake. Muscles are sometimes used at low speed or to produce sustained tension. The energy cost for tension maintenance is then inversely related to speed and myosin ATPase activity. It is therefore advantageous for an organism to be able to use fast muscles when the occasion demands it and to use slower ones in less demanding situations. It is thus not surprising that 10 different striated muscle MyHC isoforms with different functional characteristics exist in the mammalian genome.

The expression of these MyHCs in adult limb, jaw-closer and extraocular muscles is shown in Table 1. Two major subclasses of vertebrate MyHCs are currently recognized: (i) the fast subclass, which includes IIA, IIX, IIB, embryonic, foetal and extraocular, and (ii) the cardiac subclass, which includes slow/β-cardiac and α-cardiac. The genes encoding these subclasses of MyHC are clustered in chromosomes 17 and 14, respectively, in the human genome (Schiaffino and

Table 1. *Mammalian myosin heavy chain (MyHC) isoforms and the repertoires for their expression in muscles of jaw-closer, extraocular and limb allotypes*

MyHC isoform	Jaw-closer	Extraocular	Limb
Masticatory	X		
α-Cardiac	X	X	
Slow/β-cardiac	X	X	X
Extraocular		X	
Foetal (perinatal)	X	X	
IIB	X	X	X
IIX	X	X	X
IIA	X	X	X
Embryonic		X	
Slow-tonic		X	

The MyHCs are grouped according to their subclass and chromosomal localization of their genes. The gene for masticatory MyHC is found in human chromosome 7, while clusters of cardiac and fast subclass genes are found in human chromosomes 14 and 17, respectively.

The fast subclass MyHCs are listed in the order in which their genes occur in the cluster.

Reggiani, 1996; Weiss et al., 1999). Masticatory MyHC (see below) and probably also the slow-tonic MyHC, which is yet to be cloned, are also distinct subclasses. The 10 MyHC isoforms cater for the wide range of functional demands on muscles in different parts of the body. These isoforms greatly help to optimize contractile functions of different organs while minimizing energy use.

Fibre types and their myosins in locomotory muscles

The muscles of the limb and trunk subserve locomotion and the maintenance of posture. Fibre types in mammalian locomotory muscles have been extensively studied and reviewed (Pette and Staron, 1990). They can be classified into two broad phenotypes, fast and slow. The slow fibres express a MyHC that is also found in the ventricle of the heart (Lompre et al., 1984), and is referred to as the slow/β-cardiac MyHC. Slow myosin has a low ATPase activity and produces tension economically. Slow fibres are endowed with a high mitochondrial content and a rich blood supply, enabling them to generate a steady supply of ATP and so to resist fatigue. Appropriately, slow muscles are used for low-speed locomotion and postural maintenance. The fast fibres can be further divided phenotypically into three subtypes termed IIa, IIx and IIb, which respectively express IIA, IIX and IIB MyHC. These fibres provide a range of muscle speed and power (IIa<IIx<IIb) (Bottinelli et al., 1991). The IIx and IIb fibres in most mammals can generate rapid bursts of ATP by glycolytic metabolism. These fibres are appropriate for short spurts of high speed and power, but they lack endurance.

Individual locomotory muscles in both eutherian (Lucas et al., 2000) and marsupial (Zhong et al., 2001) mammals are composed of some or all of these four basic fibre types in different proportions. These fibre types show physiological plasticity (Pette and Staron, 1997), fibres of one type can be converted to those of another type by neural and hormonal influences. Properties of locomotory fibres also vary among species; small animals compensate for their size by having faster muscles (Close, 1972; Rome et al., 1990). This is achieved largely by increasing the ATPase activity of each myosin isoform as body size decreases, but changes in fibre type profile also play a part. This is exemplified by the soleus muscle, which is composed almost entirely of slow fibres only in cats and rabbits, but acquires a large proportion of IIa fibres in small animals such as rodents. In very small mammals, e.g. shrews, slow fibres are completely replaced by fast ones (Savolainen and Vornanen, 1995). At the other end of the body size spectrum, the fastest IIb fibres are absent from carnivores (Snow et al., 1982; Lucas et al., 2000) and primates (Smerdu et al., 1994; Lucas et al., 2000). Thus, the four MyHCs found in locomotory muscles of mammals appear to be more than adequate for coping with the locomotory demands in diverse species. Fibre types in locomotory muscles show only a minimal degree of phylogenetic plasticity.

Jaw-closing muscles have a high degree of phylogenetic plasticity

Jaw-closing muscles provide the power behind the specialized bony apparatus designed for the procurement and mastication of food. The functional demands on these muscles depend largely on the lifestyle, diet and eating habits of the animal, being much more variable among species compared with locomotory demands. Hence, the characteristics of the four fibre types found in locomotory muscles may not always be appropriate for closing the jaw. In contrast to the relative constancy among species of fibre types in locomotory muscles, fibre types in jaw closers of different species are extremely divergent. With respect to their jaw-closer fibre type composition, mammals can be classified into two groups: (i) those with only fibre types found in locomotory muscles and (ii) those with additional fibre types expressing MyHCs not found in mature locomotory muscles or having only these new fibre types.

Rodents and ruminants are examples of the first group. The jaw closers of the rat have the four fibre types found in limb muscles (Sfondrini et al., 1996); those of the hedgehog are probably similar, since their fibres are histochemically similar to limb fibres (Lindman et al., 1986). Jaw-closer fibres of sheep and cattle are homogeneously slow (Mascarello et al., 1979; Kang et al., 1994). Among the second group of animals are rabbits, whose jaw closers contain α-cardiac fibres in addition to slow and IIa fibres (Bredman et al., 1991; English et al., 1999). Human jaw closers have fibres co-expressing α-cardiac and foetal MyHCs in addition to slow, IIa and IIx fibres (Korfage and Van Eijden, 2000), while fibres of jaw closers of kangaroos are homogeneously α-cardiac (Hoh et al., 2000). Of

considerable importance is the fact that jaw closers of carnivores and several other orders of mammal (see below) have fibres which express a highly jaw-specific 'superfast' or masticatory MyHC (Rowlerson et al., 1983b). As a final example of the extraordinary degree of phylogenetic plasticity, marsupial possums have jaw-closer fibres that express masticatory MyHC as well as α-cardiac fibres (J. F. Y. Hoh and L. H. D. Kang, unpublished observations).

Properties of masticatory myosin and fibre type

Rowlerson and coworkers first described an unusual isoform of myosin extracted from cat jaw-closing muscles (Rowlerson et al., 1981). Both the MyHC and the pair of MyLCs of this myosin are unique in structure. The actin-activated ATPase activity of this myosin is 2–3 times higher than that of limb fast myosin. In view of the Bárány relationship (Bárány, 1967) and the fact that the time course of the isometric twitch of cat jaw muscle was faster than that of the fast limb muscle (Taylor et al., 1973), the label 'superfast' was applied to this myosin and fibre type (Rowlerson et al., 1981). It will later emerge that this label is inappropriate, and we shall refer to this myosin as masticatory myosin. Masticatory MyHC and MyLCs have also been described in jaw closers of the dog (Shelton et al., 1985), where it is associated with susceptibility to the development of an autoimmune myositis affecting specifically jaw closers (Orvis and Cardinet, 1981). This condition is characterized by the presence of autoantibodies against masticatory myosin (Shelton et al., 1987). In a limited number of species, masticatory MyHC has been shown to be glycosylated (Kirkeby, 1996). The functional significance of this unusual post-translational modification of MyHC is currently obscure. In the species in which masticatory myosin is found in jaw closers, this myosin is sometimes, but not always, expressed in other muscles derived from the first branchial arch mesoderm, namely the tensor veli palatini and tensor tympani (Rowlerson et al., 1983b). It has never been observed in the jaw-opening anterior digastric muscle nor in limb, extraocular or laryngeal muscles.

Masticatory MyLC-2 (Qin et al., 1994) and MyHC (Qin et al., 2002) from cat jaw muscle have been cloned in this laboratory. Both genes show low homology (less than 70 % sequence identity) with known homologues in mammalian striated muscles. Analysis of nucleotide substitution rates between non-synonymous sites revealed that rates between cat masticatory MyHC and members of mammalian fast and cardiac subclasses are almost twice those between mammalian fast and cardiac isoforms themselves (Qin et al., 2002).

A phylogenetic tree comprising invertebrate and vertebrate MyHC sequences revealed that the masticatory MyHC gene was the first among vertebrate MyHC genes to diverge from other vertebrate MyHC genes. Next to diverge was the chicken ventricular MyHC. Subsequently, the two major subclasses of MyHC, cardiac and fast skeletal, diverged from each other (Qin et al., 2002). It is of interest that the mammalian slow/β-cardiac and α-cardiac genes group with the quail slow skeletal MyHC gene rather than with the chicken ventricular MyHC. Fluorescence *in situ* hybridization analysis revealed that the masticatory MyHC gene is present in the human genome and is located at 7q22, a site different from the locations of the fast skeletal and cardiac MyHC genes. These results show that masticatory MyHC is of very ancient origin and that this gene should be considered as a distinct subclass of vertebrate MyHC genes (Qin et al., 2002). In the light of these findings, it is inappropriate to call this myosin (and fibre type) IIM (Rowlerson et al., 1983a,b), with the connotation that it belongs to the fast subclass. The finding that the shark expresses masticatory MyHC (see below) suggests that this gene diverged more than 400×10^6 years ago.

Masticatory fibres in the cat express other jaw-specific isoforms of myofibrillar proteins besides the unique MyHC and MyLCs. An isoform of α-tropomyosin different from those in limb muscles has been resolved in two-dimensional gels (Rowlerson et al., 1983a). This isoform differs in cyanogen bromide peptide map from the α-tropomyosins in fast and slow limb muscle fibres (Hoh et al., 1989). Unlike its equivalent in limb muscle fibres, the jaw-specific α-tropomyosin is not coexpressed with β-tropomyosin. There is also a jaw-specific isoform of myosin binding protein C (Hoh et al., 1993), which is immunochemically distinct from the fast and slow isoforms in limb muscles (Dhoot et al., 1985). Fibres of jaw closers in the cat (Hoh et al., 1991) and rat (Sfondrini et al., 1996) have been shown to have physiological plasticity.

Distribution of masticatory myosin expression in jaw muscles of vertebrates

Using a polyclonal antibody against masticatory myosin, Rowlerson and coworkers showed that the expression of this myosin was widespread among vertebrates, including five species of carnivore (Rowlerson et al., 1983b), six species of primate, including both the New World and Old World monkeys (Rowlerson et al., 1983b), two species of opossum (Sciote et al., 1995; Sciote and Rowlerson, 1998) and two species of reptile, the caiman and the terrapin (Rowlerson, 1994).

This laboratory has broadened the distribution of masticatory myosin expression by the use of a battery of 32 monoclonal antibodies against cat masticatory MyHC. Animals whose jaw closers were shown to react with the majority of these antibodies include six out of seven species of chiropteran (bats and flying foxes) examined (Kang et al., 1994), the Indopacific crocodile (Hoh et al., 2001), three species of dasyurid (marsupial carnivores), two species of diprotodont (ringtail and brushtail possums) and one species of shark (J. F. Y. Hoh and L. H. D. Kang, unpublished observations). Of the 32 monoclonal antibodies, 15–17 reacted specifically with marsupial jaw closers and 12–13 with jaw closers of the crocodile and the shark. Five monoclonal antibodies, including 2F4 described previously (Kang et al., 1994), reacted specifically with jaw closers of all species studied. The known distribution of masticatory MyHC expression among vertebrate species is summarized in Table 2.

Table 2. *Distribution of masticatory myosin in jaw-closers of vertebrates*

- Eutherian mammals
  - Carnivora (except lesser panda)
    - Cat (*Felis catus*)
    - Black panther (*Panthera pardus melas*)
    - Dog (*Canis familiaris*)
    - Fox (*Vulpes vulpes*)
    - Ferret (*Mustela foina*)
  - Primates (except man)
    - Marmoset monkey (*Calisthrix penicillata*)
    - Squirrel monkey (*Saimiri sciurens*)
    - Ceropithecus
    - Macaque
      - *Macaca fasciocolata*
      - *Macaca irus*
    - Chimpanzee (*Pan troglodytes*)
  - Chiroptera (except *Miniopterus schreibersii*)
    - Flying fox
      - *Pteropus scapulatus*
      - *Nyctimene robinsonii*
    - Microbat
      - *Macroderma gigas*
      - *Kerivoula papuaensis*
      - *Nictophilus gouldii*
- Marsupial mammals
  - Didelphimorphia
    - South American opossum (*Monodelphis domestica*)
    - American opossum (*Didelphys virginiana*)
  - Dasyuromorphia
    - Fat-tailed dunnart (*Sminthopsis crassicaudata*)
    - Brown antechinus (*Antechinus stuartii*)
    - Yellow-footed antechinus (*Antechinus flavipes*)
  - Diprotodontia (except macropodids)
    - Brushtail possum (*Trichosurus vulpecula*)
    - Ringtail possum (*Pseudocheirus peregrinus*)
- Reptiles
  - Crocodylia
    - Indopacific crocodile (*Crocodylus porosus*)
    - Caiman (*Caiman crocodylus*)
  - Chelonia
    - Terrapin
- Fish
  - Pleurotremata
    - Reef shark (*Carcharhinus limbustus*)

In some orders with members known to express masticatory myosin, exceptional species not expressing this myosin are also noted.

It is of considerable interest that, among several orders of mammals shown to express masticatory myosin, there are species that deviate from this phenotype. Thus, among carnivores, masticatory myosin was not expressed in the lesser panda (Rowlerson et al., 1983b), which is no longer carnivorous. Among primates, humans do not express masticatory myosin (Rowlerson et al., 1983b) although they have the gene, possibly associated with the fact that the human diet has softened since the consumption of cooked food during recent human evolution. Among microbats, *Miniopterus schreibersii* is exceptional in expressing limb fast myosin rather than masticatory myosin (Kang et al., 1994). Among marsupial mammals, jaw closers in possums contain a mixture of masticatory and α-cardiac fibres, but those in macropods, which belong to the same order (Diprotodontia), have 100 % α-cardiac fibres with no trace of masticatory MyHC or MyLC expression (Hoh et al., 2000). These known deviations from masticatory MyHC expression can be viewed as examples of recent evolutionary adaptations of jaw muscles in response to changes in diet or feeding pattern, and attest to the phylogenetic plasticity of jaw-closing muscles.

Mechanical properties of fibres expressing masticatory myosin

The isometric twitch contraction time of cat masticatory fibres is 11–13 ms, a value that is half that for cat limb fast muscle (Taylor et al., 1973), suggesting that masticatory fibres may be faster than limb fast fibres. This observation indicates that the sarcoplasmic reticulum of masticatory fibres can rapidly sequester Ca^{2+}, but does not necessarily imply rapid cross-bridge kinetics. To study cross-bridge kinetics more directly, Kato and coworkers used skinned fibres and showed that, when subjected to rapid length stretches, masticatory fibres had a much more rapid tension recovery phase compared with fibres from the jaw-opening anterior digastric muscle (Kato et al., 1985). The mean value of the time constant during the phase of tension recovery was 58.5 ms in masticatory fibres and 362.6 ms in digastric fibres. The anterior digastric muscle is known to contain slow and two types of fast fibre (Rowlerson et al., 1983b). The data of Kato and coworkers are difficult to interpret vis-à-vis the question of whether jaw fibres are faster than limb fast fibres because they did not determine the fibre type of the digastric fibres used. However, these authors made the important incidental finding that masticatory fibres produced 65 % more stress (force per unit cross-sectional area) during maximal activation (Kato et al., 1985). Subsequent work from the same laboratory confirmed the higher maximal stress of masticatory fibres and showed, further, that the rate of ATP consumption and tension cost were higher in masticatory fibres than in digastric fibres (Saeki et al., 1987).

The kinetics of cross-bridge cycling during isometric contraction can be studied by imposing small-amplitude length perturbations over a range of frequencies (Rossmanith, 1986), such analysis yielding a parameter, f_{min}, the frequency at which the dynamic stiffness of the active fibre is a minimum. The value of this parameter is related to the kinetics of cycling of cross-bridges (Rossmanith and Tjokorda, 1998) and is a useful index of fibre kinetics. For example, analysis of the three types

of fibre from rabbit fast limb muscle gave f_{min} values in the range 10–26 Hz, while extraocular muscle fibres gave values ranging from 4 to 33 Hz. This result reflects the wider diversity of MyHCs expressed in this muscle, which includes embryonic, foetal and extraocular MyHCs not found in limb fibres (Li et al., 2000). Using this method of analysis, f_{min} of skinned cat masticatory and limb fast (IIa, IIx) fibres were both in the range 10–13 Hz, and the mean values for the two types of fibre were not significantly different (Z. B. Li, J. F. Y. Hoh and G. H. Rossmanith, unpublished observations). In terms of a three-state model of cross-bridge function, this observation implies that the power stroke and detachment rates of cross-bridges in masticatory and limb fast fibres do not differ (Rossmanith and Tjokorda, 1998). These results do not support the notion that masticatory fibres are faster than limb fast fibres.

Recently, the maximal velocity of shortening (V_{max}) of dog masticatory fibres has been compared with values for fast and slow limb fibres in the same animal (P. Reiser, personal communication). The V_{max} of masticatory fibres was found to lie midway between the values for limb fast and slow fibres. Thus, mechanical analyses of single masticatory fibres by various methods have surprisingly provided no evidence that masticatory fibres are faster than limb fast fibres, as expected on the basis of the Bárány (1967) relationship between myosin ATPase and muscle speed. It is no longer justified to refer to masticatory myosin and fibre type as 'superfast'. A truly superfast muscle has been described: the swim bladder muscle of the toad fish (Rome et al., 1999). This muscle develops a high speed of contraction at the cost of low force production and does not cross-react with anti-masticatory MyHC antibodies. In contrast, masticatory muscle is a moderately fast muscle capable of developing high force at the expense of high ATPase activity and tension cost. The moderate speed and high force characteristics make jaw closers powerful in animals that express masticatory myosin. These characteristics are very appropriate in carnivores, to cater for their predatory lifestyle, and in frugivores (flying foxes) and certain folivores (possums, opposums and primates), for the mastication of tough vegetable matter.

The failure of masticatory myosin to comply with the Bárány relationship requires some comment. Bárány's (1967) correlation between myosin ATPase activities and muscle speeds was derived from data on limb fast and slow myosins of animals of various sizes. It is likely that the slope of the Bárány relationship is MyHC-isoform-specific. The unique functional characteristics of masticatory fibres may be associated with unusual combinations of rate constants in different parts of the cross-bridge cycle. A very rapid cross-bridge attachment rate coupled with a moderate detachment rate may help to explain the high maximal stress in these fibres.

Structure and function of mammalian cardiac myosins

As α- and slow/β-cardiac MyHC genes are expressed in masticatory muscles, their structure, function and evolution are briefly dealt with here. The ventricles of eutherian (Hoh et al., 1978, 1979) and marsupial (Hoh et al., 2000) mammals express two cardiac MyHC genes, α-cardiac and slow/β-cardiac MyHC, the products of which associate to form the heavy chain cores of the three ventricular isomyosins: V_1 (αα), V_2 (αβ) and V_3 (ββ). These isoforms have identical pairs of ventricular MyLCs. Ventricular isomyosins differ in enzyme kinetic properties, V_1 having a twofold higher actin-activated myosin Mg^{2+}-ATPase activity relative to V_3 (Pope et al., 1980). Ventricular muscles containing different myosin isoforms also differ in a variety of kinetic properties: compared with V_3 muscle, V_1 muscle liberates twice as much heat during isometric contraction (Loiselle et al., 1982), shortens twice as fast (Cappelli et al., 1989), consumes twice as much ATP during contraction (Rossmanith et al., 1995) and has twofold higher cross-bridge kinetics as measured by f_{min} (Rossmanith et al., 1986). Thus, V_1 muscle is more powerful, while V_3 muscle has a lower tension cost. The importance of these isoforms in the heart lies in the fact that isoform expression can be changed during the lifetime of an animal by hormonal and other influences, with consequent changes in cardiac function. For example, in the hyperthyroid state, V_1 expression is increased while V_3 expression is inhibited. This increases cardiac contractility, thereby helping to raise the cardiac output needed to support the thermogenic effect of thyroid hormone.

Expression of cardiac myosins in mammalian jaw muscles

The slow/β-cardiac MyHC is expressed in slow fibres, which occur commonly as a minor component of jaw closers whatever the predominant fibre type may be: limb fast, α-cardiac or masticatory. Such common occurrence of slow fibres across species may have a postural function in holding the lower jaw up against gravity. Sheep and cattle are unusual in that all their jaw muscle fibres are of the slow type (Mascarello et al., 1979; Kang et al., 1994). The jaw closers in these animals execute tens of thousands of chews per day for grazing and rumination. As these slow fibres have a low tension cost and high fatigue-resistance, they are highly suited to the task. In the cat, the expression of slow/β-cardiac MyHC is associated with masticatory MyLCs rather than slow MyLCs (Sciote et al., 1995). This may account for the apparent immunohistochemical difference between jaw and limb slow fibres (Hoh et al., 1991). This unusual combination of MyHC and MyLCs has a powerful effect on mechanical properties, raising f_{min} to a value between those for slow and fast fibres (Z. B. Li, J. F. Y. Hoh and G. H. Rossmanith, unpublished observations).

The α-cardiac MyHC is expressed in fibres of jaw closers in the rabbit (Bredman et al., 1991; English et al., 1999), in four species of kangaroo (Hoh et al., 2000) and weakly in humans (Bredman et al., 1991). In the rabbit, α-cardiac fibres constitute approximately one-third of the fibre population, the rest being slow/β-cardiac and IIa fibres. Mechanical analysis of single α-cardiac fibres of this muscle revealed that the maximal speed of shortening of these fibres lies between those

of fast IIa fibres and the slow/β-cardiac fibres in the same muscle (Sciote and Kentish, 1996). In kangaroos, 100 % of jaw closer fibres are α-cardiac, and this stands in sharp contrast to the homogeneously slow fibres in eutherian grazers, which feed essentially on the same diet. In common with sheep and cattle, kangaroos are also foregut fermenters, but they are not ruminants and, unlike them, have a large, simple stomach (Dawson, 1995). A difference in kinetic property of jaw fibres between eutherian and marsupial grazers is expected because α-cardiac MyHC is associated with a higher rate of cross-bridge cycling compared with β-cardiac MyHC. The appropriateness of α-cardiac fibres in kangaroos may lie in the fact that, with their presumed higher speed and power, α-cardiac fibres ensure rapid comminution of food into fine particles necessary for efficient fermentation prior to passage through the buccal cavity.

Evolution of mammalian α- and β-cardiac MyHC genes

The genes for α- and β-cardiac MyHC in eutherian mammals are structurally very similar and are in tandem in the mammalian genome (Schiaffino and Reggiani, 1996), suggesting that one of these genes evolved by duplication from the other during phylogeny. The finding that these genes for cardiac MyHC are also present in marsupial mammals (Hoh et al., 2000) suggests that this duplication occurred prior to the divergence of marsupials from eutherians, at least some 130×10^6 years ago. Which gene came first? From which ancestral gene did they originate?

It is well established that the gene for β-cardiac MyHC in eutherians is expressed in slow skeletal muscle fibres (Lompre et al., 1984), and this gene is thus more appropriately referred to as the gene for slow/β-cardiac MyHC. The recent finding that β-cardiac MyHC is also expressed in marsupial slow skeletal muscle fibres (Hoh et al., 2000) suggests that the expression of this MyHC gene in skeletal muscle must predate the divergence of these two subclasses of mammal. This raises the intriguing question as to whether the gene for β-cardiac MyHC was originally a skeletal muscle gene which evolved a mechanism for expression in cardiac muscle, or *vice versa*.

Features of the phylogenetic tree of vertebrate MyHCs referred to above (Qin et al., 2002) are helpful here. The tree suggests that mammalian α- and β-cardiac MyHC genes and the quail slow skeletal MyHC gene shared a common ancestral gene. Further, the mammalian slow/β-cardiac MyHC gene is more closely related to quail slow skeletal MyHC than to chicken ventricular MyHC. A likely scenario for the evolution of these MyHCs is that mammalian slow/β-cardiac MyHC evolved from an ancestral slow limb MyHC and, further, that it duplicated in the course of mammalian evolution to give rise to the α-cardiac MyHC gene. These genes subsequently evolved cardiac-chamber-specific expression (α-cardiac in the atrium, α-cardiac and slow/β-cardiac in the ventricle), and thyroid-sensitivity in the ventricle. The α-cardiac MyHC acquired faster kinetics, presumably driven by the evolutionary advantages of being better able to cope with the thermogenic effect of thyroid hormones.

Having thyroid-sensitive cardiac myosin genes permits individual mammals in their lifetime to modify their cardiac function to cope with the metabolic demands of a low ambient temperature. This ability enables mammals to extend their habitats to higher latitudes and altitudes than would otherwise be possible. In contrast to the evolution of masticatory MyHC (see below), the infrequent expression of α-cardiac MyHC in jaw muscles of mammals is unlikely to have played a significant role in driving the evolution of this gene.

Evolution of mastication and jaw-closing muscles

Different vertebrates use jaws in diverse ways to deal with food. Carnivorous lower vertebrates use them to catch and hold prey, which they swallow whole, or large pieces thereof. Their teeth are sharp for piercing and holding, and jaw movements are limited simply to opening and closing. Food is not masticated, and the resulting slow digestion is tolerable in these animals with a low metabolic rate. In mammals, metabolic rate is high, and food has to be chewed to greatly increase digestive efficiency. Different taxa have evolved diverse anatomical features in the teeth, jaw and surrounding head structures to allow prolonged chewing of many different types of food. To optimize function and minimize energy expenditure, the muscles that power these structures must also adapt to the changing pattern of use. The phylogeny of masticatory MyHC and the pattern of expression of MyHCs in the jaw closers of vertebrates suggest the following scenario for their evolution.

With the evolution of gnathostomes from agnathous fish approximately 400×10^6 years ago, duplicates of pre-existing MyHC, MyLC-1, MyLC-2 and other myofibrillar genes became jaw-specific in expression and subsequently diverged as the masticatory MyHC, MyLCs and other jaw-specific genes, driven by the survival advantage of powerful jaw closure. The carnivorous lower vertebrates, including the reptilian ancestors of mammals, advantageously expressed masticatory myosin genes in jaw muscles. Early mammals, both marsupial and eutherian, continued to express masticatory myosin, this feature representing a primitive or undifferentiated phenotype. During the mammalian radiation into their various ecological niches that followed the demise of the dinosaurs approximately 65×10^6 years ago, mastication of food became progressively more important, and rapid evolutionary changes in the masticatory apparatus, including changes in muscle fibre types, took place. Early during mammalian radiation, some taxa (carnivores, chiropterans, primates, most marsupial orders) retained masticatory myosin expression where high force and power in jaw closers remained functionally advantageous to their life style. Others (rodents, ungulates, rabbits) replaced masticatory myosin with functionally more appropriate isoforms normally expressed in limb muscles or the heart. With further diversification and adaptation to diet and feeding habits in more recent times, the ancestors of certain members of mammalian

orders previously expressing masticatory myosin (lesser panda, *Miniopterus schreibersii*, kangaroos and humans) also deviated by expressing limb, developmental or cardiac myosins in their jaw closers.

What makes jaw-closing muscles so unique?

What make jaw-closing muscles so different from limb muscles with respect to their phylogenetic plasticity and the types of myofibrillar proteins they express? A likely answer is that they are derived from a different lineage of myoblasts. Jaw muscles are derived from presomitic mesoderm, which forms the first branchial arch musculature (Noden, 1983), whereas limb and trunk muscles are derived from somites. Muscle transplantation experiments in the cat showed that satellite cells in jaw muscles are preprogrammed to express masticatory myosin even when reinnervated by a limb fast muscle nerve (Hoh and Hughes, 1988). Jaw muscles are said to belong to a distinct allotype from limb muscles (Hoh et al., 1993).

Skeletal muscle myogenesis is controlled by the sequential expression of the *MyoD* family of genes (Rudnicki and Jaenisch, 1995). The same genes are used to control myogenesis of craniofacial muscles and somitic muscles. These genes induce in jaw closers the expression of embryonic and foetal myosins during development (Hoh et al., 1988; Shelton et al., 1988; Hoh and Hughes, 1989) and regeneration (Hoh and Hughes, 1988), in common with limb muscles, but the mature fibre phenotypes are distinct and species-specific. There presumably are allotype-specific muscle determination genes regulating the expression of *MyoD* family members. This suggestion is strongly supported by the work of Tajbakhsh and coworkers, who showed that, in *Pax*-3/*Myf*-5 double-mutant mice, limb and trunk muscles were absent while craniofacial muscles developed normally (Tajbakhsh et al., 1997). This clearly shows that *Pax*-3 is specific for myogenesis within the limb/trunk allotype, not in craniofacial allotypes where, presumably, some other genes regulate the *MyoD* family of genes. A Hox gene called *engrailed* is specifically expressed in jaw muscle precursor cells (Hatta et al., 1990), but it is not known whether *engrailed* is in the pathway for the determination of the jaw muscle allotype.

Jaw closers present interesting questions in muscle phenotype control. What *trans*-acting factors are involved in the regulation of masticatory MyHC, MyLCs and other masticatory fibre-specific myofibrillar genes? Are these factors also involved in the regulation of α-cardiac MyHC and limb myofibrillar proteins in jaw closers? Are limb-like fibres in jaw closers regulated by the same regulatory pathways as limb fibres? Identification of *cis*-acting elements of myofibrillar genes and *trans*-acting factors involved in the determination and differentiation of jaw-closer muscles in different species will greatly advance our understanding of the special role these muscles play in evolutionary biology.

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