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MOUSE TRANSGENIC LINES THAT SELECTIVELY LABEL TYPE I, TYPE IIA AND TYPES IIX+B SKELETAL MUSCLE FIBERS

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Abstract

Skeletal muscle fibers vary in contractile and metabolic properties. Four main fiber types are present in mammalian trunk and limb muscles; they are called I, IIA, IIX and IIB, ranging from slowest- to fastest-contracting. Individual muscles contain stereotyped proportions of two or more fiber types. Fiber type is determined by a combination of nerve-dependent and –independent influences, leading to formation of “homogeneous motor units” in which all branches of a single motor neuron form synapses on fibers of a single type. Fiber type composition of muscles can be altered in adulthood by multiple factors including exercise, denervation, hormones and aging. To facilitate analysis of muscle development, plasticity and innervation, we generated transgenic mouse lines in which Type I, Type IIA, and Type IIX+B fibers can be selectively labeled with distinguishable fluorophores. We demonstrate their use for motor unit reconstruction and live imaging of nerve-dependent alterations in fiber type.

Keywords

Denervation; fluorescent proteins; motor unit; myosin heavy chain; neuromuscular; parvalbumin; myosin heavy chain

Skeletal muscle fibers are diverse in their metabolic, electrical and contractile properties (Bertchold et al., 2000; Schiaffino and Reggiani, 1996). In most mammalian skeletal muscles, fibers are divided into 4 categories based primarily on the particular myosin heavy chain (MyHC) isoform they express (Pette and Staron, 2000). The slowest contracting fibers express MyHCI (*Myh7*), with successively faster contracting fibers expressing MyHCIIA (*Myh2*), MyHCIIIX (*Myh1*) and MyHCIIIB (*Myh4*) (Weiss et al., 1999). Individual muscles contain fibers of multiple types, with their proportions sub-serving each muscle's function. Thus, tonically active postural muscles are rich in slow fibers that are richly vascularized and highly dependent on oxidative metabolism (red muscles). In contrast, muscles used phasically, for example to run, are rich in fast fibers that depend largely on glycolytic metabolism (white muscles) (Bassel-Duby and Olson, 2006).

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Fiber type is determined during embryogenesis and early postnatal life by a variety of factors including intrinsic differences among muscle progenitor cells, morphogenetic signals and nerve-evoked activity (Wigmore and Evans, 2000). A prominent role of innervation is indicated by the observation that nearly all muscle fibers innervated by branches of a single motor neuron are of a single type, a phenomenon called "motor unit homogeneity" (Burke et al., 1971; 1973; reviewed in Burke, 1999). Indeed, even in adults, alterations in levels and patterns of nerve-evoked activity can reprogram muscle fibers, leading to alteration in fiber type (Gauthier et al., 1983; Ausoni et al., 1990; Gorza et al., 1988; Pette and Staron, 2000; Schiaffino et al., 2007). Thus, analysis of fiber type provides useful information not only about muscle function but also about its development, plasticity and innervation.

Many reagents are available for determination of muscle fiber type, most notably isoform-specific antibodies to MyHCs (Cho et al., 1994; Schiaffino et al., 1989; 1996). These labels suffer, however, from two limitations. First, they recognize intracellular antigens, and thus cannot be used to mark living cells. Second, epitopes recognized by most currently available fiber type-specific antibodies are highly sensitive to fixation, so the antibodies must be applied to unfixed tissue. Therefore fiber type analysis is generally restricted to cross-sections of fresh frozen skeletal muscles, making it difficult to analyze fiber type distribution in whole mounts, or to combine fiber type determination with other labeling methods.

To circumvent these limitations, we generated three transgenic mouse lines that can be used to label distinct subsets of muscle fibers -Types I, IIA and IIX/B- with fluorescent proteins. We show that these mice can be used for reconstruction of motor units in whole mounts and for analysis of fiber type plasticity in live animals.

To mark Type I fibers we generated transgenic mice in which regulatory elements from the *Myh7* gene, encoding myosin heavy chain I (Rindt et al., 1993), drove expression of a cDNA encoding the enhanced cyan fluorescent protein (CFP). We call the lines "MyI-CFP." In one of four MyI-CFP lines tested, we observed high levels of reporter expression in the predominantly slow soleus muscle, with negligible levels in the adjacent, predominantly fast, plantaris muscle (Figure 1A; Talmadge et al., 2004). To assess specificity of expression in this line, we labeled cross sections of lower limb muscles from adult MyI-CFP mice with isoform-specific antibodies to MyHC (Figure 1B-E). All CFP-positive fibers were labeled with an antibody that specifically recognizes type I slow MyHC, and none were labeled with antibodies that recognize the fast type IIA, IIX and IIB MyHC (Figure 1F). The correspondence of CFP-positive to Type I-positive fibers was nearly perfect in all muscles examined in one month-old animals. Unexpectedly, at later ages, CFP expression declined in about half of the Type I fibers of some muscles (for example, tibialis anterior and extensor digitorum longus) but was maintained in others (for example, medial gastrocnemius and soleus; data not shown). It was our impression that transgene expression declined most in those muscles with the fewest Type I fibers. We have no explanation for this pattern.

To label intermediate Type IIA fibers, we inserted a red fluorescent protein (RFP) at the translational start site of the *Myh2* gene, encoding myosin heavy chain IIA, in a bacterial artificial chromosome (BAC). We then generated transgenic mice from the engineered BAC. Three lines were generated, which we call "MyIIA-RFP." In all three lines, RFP was readily detectable in approximately half of the muscle fibers in the soleus, which is known to contain ~50% Type IIA fibers, and some labeling was also evident in the neighboring fast plantaris muscle, which contains ~25% Type IIA fibers (Figure 2A; Talmadge et al, 2004). One line was characterized in detail. In cross sections of limb muscles from this line, nearly >90% RFP-positive muscle fibers were labeled with antibodies specific for Type IIA MyHC, but <5% of RFP-positive fibers were labeled with antibodies that mark Type I, IIX

and IIB fibers (Figure 2B–F). In this line, expression persisted in all and only Type IIA fibers into adulthood.

To mark fast muscle fibers, we took advantage of previous work showing that the calcium binding protein parvalbumin (PV), which is often studied as a marker of central neuronal subsets (e.g. Hippenmeyer et al., 2005), is also selectively expressed by Type IIX and IIB muscle fibers (Bertchtold et al., 2000; Ecob-Prince and Leberer, 1989; Klug et al., 1988). We obtained mice in which Cre recombinase had been inserted into the endogenous PV locus (Hippenmeyer et al., 2005) and mated them to mice in which broad expression of RFP was Cre-dependent (STOP-RFP; Madisen et al., 2010). In PV-Cre;STOP-RFP double transgenic mice, most fibers were RFP-positive in the fast plantaris muscle, but few fibers were RFP-positive in the slow soleus muscle (Figure 3A). Double-labeling with isoform-specific antibodies to MyHCs revealed that >90% of Type IIB and ~85% of type IIX fibers but few if any Type I or IIA fibers were RFP positive (Figure 3B–F). These patterns persisted into adulthood.

Figure 4 illustrates the use of these lines for analysis of fiber types in whole muscles. Because labeling is bright enough to be seen with a fluorescent dissecting microscope, it can be used to facilitate recognition of muscles in young animals (Figure 4A) or of small, fragile muscles in adults (Figure 4B). The lines can also be used to assess arrangement of fiber types within a muscle. Stereotyped arrangements of fiber types can be appreciated in sections of geometrically simple muscles (Bertchtold et al., 2000), but are more difficult to document in muscles with complex shapes. Because the fiber type-specific lines mark fibers in whole mounts they simplify such analysis, as shown for a MyI-CFP;MyIIA-RFP double transgenic mouse in Figure 4C.

Another motivation for generating these lines was to facilitate labeling of motor units - a motor neuron and the entire cohort of fibers it innervates (Burke 1999). Until recently, motor units were generally labeled by a glycogen depletion method, in which a single motor axon is stimulated until contraction of the fibers it innervates depletes their glycogen stores. A histochemical stain for glycogen is then applied to muscle sections to identify depleted fibers (Burke et al., 1973; Gauthier et al., 1983). Limitations of this method include reliance on uniform depletion of glycogen stores in all stimulated fibers, the technical difficulty of isolating and stimulating a single axon, and the need to detect glycogen in frozen sections. To test the feasibility of reconstructing motor units in whole muscles with fluorescent reporter lines, we crossed MyI-CFP mice to previously described double transgenic mice (SV2A-CreER; Thy-STOP-YFP) in which slow motor neurons are preferentially labeled following administration of the CreER activating ligand, tamoxifen (Chakkalakal et al., 2010). We isolated muscles in which a single motor axon was YFP-positive and labeled all synaptic sites with alpha-bungarotoxin, which binds to neurotransmitter receptors in the postsynaptic membrane (Figure 5A). Those fibers on which the synaptic sites are innervated by the YFP-positive axon are members of the motor unit; YFP-negative sites are members of other (unmarked) motor units. High-power confocal analysis of the motor unit shown in Figure 5A revealed that the fibers comprising it were CFP-positive Type I fibers (Figure 5B–D).

We next asked whether the lines could be used to study alterations in fiber type composition in adult muscle, such as those that occur following denervation (Pette and Staron, 2000). We transected the sciatic nerve unilaterally in MyI-CFP; MyIIA-RFP double transgenic mice, then imaged denervated muscles of the lower hindlimb and intact contralateral muscles 10 days later. Consistent with results of a recent study (Agbulut et al., 2009), the number of Type I (CFP-positive) fibers changed little following denervation (Figure 6A,B, E, F). In contrast, we observed a substantial increase in the proportion of Type IIA (RFP-positive)

fibers following denervation (Figure 6C–F). Interestingly, increases were most prominent in specific muscles, such as the medial gastrocnemius (Figure 6C,D). The increases in RFP expression observed in denervated muscle reflected increased levels of endogenous Type IIA MyHC, as assessed by immunohistochemistry (Figure 6G,H; 90 and 85 % of RFP fibers were Type IIA antibody-positive in innervated and denervated muscles, respectively; $n = 3$, 150 fibers).

Finally, we used the upregulation of MyIIA-RFP following denervation to determine whether alterations in fiber type composition could be imaged in live mice. A mouse was anesthetized and the medial gastrocnemius muscle was exposed and imaged with fluorescence optics (Ngyuen et al., 2002; Pan et al., 2003). The sciatic nerve was then transected, the wound was closed, and the animal was returned to its cage. Ten days later, it was anesthetized and imaged again. Consistent with observations in fixed tissue, we observed a robust increase in RFP expression (Figure 6I,J).

In conclusion, we have generated and characterized three fluorescent transgenic lines capable of labeling distinct skeletal muscle fiber types with distinguishable reporters. These lines facilitate visualization of multiple fiber types and their innervation. We illustrated this potential with two sets of double transgenic mice - one in which Type I fibers are CFP-positive and Type IIA fibers are RFP-positive (Figures 4D and 6A–D), and another in which motor axons are YFP-positive and Type I fibers are CFP-positive (Figure 5). Type IIX and IIB fibers express Cre recombinase in PV-Cre mice, so although we labeled them with RFP here, they can in principle be labeled with any reporter, including YFP, which is readily distinguishable from RFP or CFP.

In conclusion, analyses of muscle fiber development, plasticity, motor unit composition and development, have been hampered by current labeling methods, which are difficult to apply to whole muscles and cannot be applied to live muscles. The lines we have described circumvent these limitations and thereby facilitate visualization of fiber type distribution in whole muscles (Figure 4); reconstruction of entire motor units (Figure 5); detection of alterations in fiber types by time lapse imaging in live mice (Figure 6); and analysis of the development of motor units and muscle fibers (J.V.C and J.R.S. in preparation).

METHODS

Animals

MyI-CFP mice—The coding sequence for the enhanced CFP was inserted downstream of ~5kb of putative regulatory sequences from the mouse myosin Type I heavy chain gene (Rindt et al., 1993). Transgenic mice were generated by pronuclear injection using standard methods. MyI-CFP mice have been sent to the Jackson Laboratory (Tg(Myh7-CFP)1Jrs/J; Stock #16922)

MyIIA-RFP mice—A ~100kb genomic fragment containing the MHC-IIA gene and adjacent sequences was transferred from a P1-based artificial chromosome (561-O-14; Weiss et al., 1999) into a bacterial artificial chromosome (BAC) vector, pBeloBAC11, using bacterial homologous recombination-based gap repair (Liu et al., 2003). The loxP site in the pBeloBAC11 vector had previously been replaced with a gene for ampicillin resistance. In parallel, a Frt-Neo-Frt (FnF) cassette was inserted into the pDsRed2-N1 vector (BD Biosciences Clontech). The DsRed and Neo sequences were targeted to the translation start site of MHC-IIA in the BAC, using Neo to select recombinants. Finally, the FnF selection cassette was excised by induction of Flp recombinase in the bacteria (Lee et al., 2001). MyIIA-RFP mice have been sent to the Jackson Laboratory (Tg(Myh2-DsRed2)1Jrs/J; Stock #16921)

PV-Cre;STOP-RFP mice—PV-Cre and STOP-RFP knock-in mice were obtained from Jackson Laboratories. In the PV-Cre line, an internal ribosome entry site- (ires-) Cre recombinase cassette was targeted to the 3' untranslated region of the endogenous PV gene using homologous recombination in embryonic stem cells (Hippenmyer et al., 2005). In STOP-RFP mice, the CAGS regulatory elements, a lox-stop-lox cassette and a cDNA encoding tdTomato were targeted to the ROSA26 locus by homologous recombination (Madisen et al., 2010). Selective expression of PV in Types IIX and IIB fibers led to selective labeling of these fibers in PV-Cre;STOP-RFP double transgenics.

Mice that label slow motor neurons—SV2A-CreER and Thy1-STOP-YFP mice were described previously (Chakkalakal et al., 2010; Buffelli et al., 2003). In SV2A-CreER, a ligand activated Cre recombinase (CreER) was inserted at the translation initiation site of the SV2A gene in a BAC. In the Thy1-STOP-YFP line, neuron-specific expression of YFP under the control of regulatory elements of the Thy1 gene is dependent on excision of the STOP cassette by Cre. Consistent with selective expression of SV2A in slow (Type I and some Type IIA) motor neurons, administration of tamoxifen and excision of the STOP cassette leads to labeling of these neurons in SV2A-CreER; Thy1-STP-YFP double transgenics (Chakkalakal et al., 2010).

Immunohistochemistry

Antibodies—Primary antibodies used were: anti-MyHC I (A4840 from Developmental Studies Hybridoma Bank and NCLslow from Leica Microsystems/Novacastra Laboratories Ltd), anti-MyHC IIA (2F7 and SC-71 from Developmental Studies Hybridoma Bank), anti-pan MyHC except IIX (BF35 from Developmental Studies Hybridoma Bank), anti-MyHC IIB (BFF3 from Developmental Studies Hybridoma Bank), and anti-GFP (Millipore). Secondary antibodies and alpha-bungarotoxin conjugated to Alexa fluorophores were purchased from Invitrogen.

Cross-sections—Mice were perfused with the ester based fixative dimethylsulfaimidate (Sigma), followed by 2% ice-cold paraformaldehyde (PFA). Muscles were dissected, rinsed in phosphate buffered saline, pH7.4 (PBS), fixed again in dimethylsulfaimidate followed by 2% PFA, incubated in 30% sucrose overnight at 4°C embedded in Tissue Freezing Medium (Electron Microscopy Sciences), frozen in melting 2-methyl butane in liquid nitrogen, and cross-sectioned at 20µm in a cryostat. Sections were allowed to thaw for 5 minutes and subsequently blocked with 1% Normal Goat Serum, 4% Bovine Serum Albumin and 0.1% Triton-X 100 for 1hr. Sections were then stained with primary antibodies overnight at 4°C followed by incubation with Alexa-conjugated secondary antibodies for 1hr at room temperature, then mounted in Fluoro Gel (Electron Microscopy Sciences). Images were taken with a Zeiss Apotome microscope (25× or 40× objective), an Olympus Fluoview1000 confocal microscope (20× objective) or a Zeiss LSM Pascal confocal microscope (25× objective). Levels were adjusted in Photoshop (Adobe) and individual channels were combined to generate color images.

Whole-mounts—Mice were perfused with 4% PFA, then muscles were isolated, post-fixed in ice-cold 2% PFA for 30 minutes at 4°C, and blocked overnight in 1% Normal Goat Serum, 4% Bovine Serum Albumin, 0.1% Triton-X 100, 0.1 M glycine and 0.02% sodium azide. Muscles were then incubated for 1 day each with primary antibodies and Alexa-conjugated secondary antibody plus bungarotoxin. After washing, muscles were mounted in Vectashield and confocal stacks were obtained on a Fluoview1000 using a 20× objective. The stacks were examined and images processed using Imaris software (Bitplane).

Surgery

Mice were anesthetized with a ketamine and xylazine and an incision was made to expose the medial gastrocnemius muscle. The leg was imaged with a 10× objective on a Zeiss Apotome microscope. The sciatic nerve was then transected at the interface of the hamstring and femur, the incisions were sutured and the mice were returned to their cages. Ten days later, the denervated inner portion of the peripheral medial gastrocnemius muscle was re-examined as described above. Animal procedures were in compliance with the NIH Guide for the Care and Use of Laboratory Animals and approved by the Animal and Care and Use Program at Harvard University.

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REFERENCES

- Agbulut O, Vignaud A, Hourde C, Mouisel E, Fougereousse F, Butler-Browne GS, Ferry A. Slow myosin heavy chain expression in the absence of muscle activity. *Am J Physiol Cell Physiol*. 2009; 296:C205–C214. [PubMed: 18945940]
- Ausoni S, Gorza L, Schiaffino S, Gundersen K, Lomo T. Expression of myosin heavy chain isoforms in stimulated fast and slow rat muscles. *J Neurosci*. 1990; 10:153–160. [PubMed: 2405110]
- Bassel-Duby R, Olson EN. Signaling pathways in skeletal muscle remodeling. *Annu Rev Biochem*. 2006; 75:19–37. [PubMed: 16756483]
- Berchtold MW, Brinkmeier H, Muntener M. Calcium ion in skeletal muscle: its crucial role for muscle function, plasticity, and disease. *Physiol Rev*. 2000; 80:1215–1265. [PubMed: 10893434]
- Buffelli M, Burgess RW, Feng G, Lobe CG, Lichtman JW, Sanes JR. Genetic evidence that relative synaptic efficacy biases the outcome of synaptic competition. *Nature*. 2003; 424:430–434. [PubMed: 12879071]
- Burke RE. Revisiting the notion of 'motor unit types'. *Prog Brain Res*. 1999; 123:167–175. [PubMed: 10635714]
- Burke RE, Levine DN, Tsairis P, Zajac FE 3rd. Physiological types and histochemical profiles in motor units of the cat gastrocnemius. *J Physiol*. 1973; 234:723–748. [PubMed: 4148752]
- Burke RE, Levine DN, Zajac FE 3rd. Mammalian motor units: physiological-histochemical correlation in three types in cat gastrocnemius. *Science*. 1971; 174:709–712. [PubMed: 4107849]
- Chakkalakal JV, Nishimune H, Ruas JL, Spiegelman BM, Sanes JR. Retrograde influence of muscle fibers on their innervation revealed by a novel marker for slow motoneurons. *Development*. 2010; 137:3489–3499. [PubMed: 20843861]
- Cho M, Hughes SM, Karsch-Mizrachi I, Travis M, Leinwand LA, Blau HM. Fast myosin heavy chains expressed in secondary mammalian muscle fibers at the time of their inception. *J Cell Sci*. 1994; 107(Pt 9):2361–2371. [PubMed: 7531198]
- Ecob-Prince MS, Leberer E. Parvalbumin in mouse muscle in vivo and in vitro. *Differentiation*. 1989; 40:10–16. [PubMed: 2526037]
- Gauthier GF, Burke RE, Lowey S, Hobbs AW. Myosin isozymes in normal and cross-reinnervated cat skeletal muscle fibers. *J Cell Biol*. 1983; 97:756–771. [PubMed: 6885917]
- Gorza L, Gundersen K, Lomo T, Schiaffino S, Westgaard RH. Slow-to-fast transformation of denervated soleus muscles by chronic high-frequency stimulation in the rat. *J Physiol*. 1988; 402:627–649. [PubMed: 3236251]
- Hippenmeyer S, Vrieseling E, Sigrist M, Portmann T, Laengle C, Ladle DR, Arber S. A developmental switch in the response of DRG neurons to ETS transcription factor signaling. *PLoS Biol*. 2005; 3:e159. [PubMed: 15836427]

- Klug GA, Leberer E, Leisner E, Simoneau JA, Pette D. Relationship between parvalbumin content and the speed of relaxation in chronically stimulated rabbit fast-twitch muscle. *Pflugers Arch.* 1988; 411:126–131. [PubMed: 3357751]
- Lee EC, Yu D, Martinez de Velasco J, Tessarollo L, Swing DA, Court DL, Jenkins NA, Copeland NG. A highly efficient *Escherichia coli*-based chromosome engineering system adapted for recombinogenic targeting and subcloning of BAC DNA. *Genomics.* 2001; 73:56–65. [PubMed: 11352566]
- Madisen L, Zwingman TA, Sunkin SM, Oh SW, Zariwala HA, Gu H, Ng LL, Palmiter RD, Hawrylycz MJ, Jones AR, et al. A robust and high-throughput Cre reporting and characterization system for the whole mouse brain. *Nat Neurosci.* 2010; 13:133–140. [PubMed: 20023653]
- Nguyen QT, Sanes JR, Lichtman JW. Pre-existing pathways promote precise projection patterns. *Nat Neurosci.* 2002; 5:861–867. [PubMed: 12172551]
- Pan YA, Misgeld T, Lichtman JW, Sanes JR. Effects of neurotoxic and neuroprotective agents on peripheral nerve regeneration assayed by time-lapse imaging in vivo. *J Neurosci.* 2003; 23:11479–11488. [PubMed: 14673013]
- Pette D, Staron RS. Myosin isoforms, muscle fiber types, and transitions. *Microsc Res Tech.* 2000; 50:500–509. [PubMed: 10998639]
- Rindt H, Gulick J, Knotts S, Neumann J, Robbins J. In vivo analysis of the murine beta-myosin heavy chain gene promoter. *J Biol Chem.* 1993; 268:5332–5338. [PubMed: 8444907]
- Schiaffino S, Gorza L, Sartore S, Saggin L, Ausoni S, Vianello M, Gundersen K, Lomo T. Three myosin heavy chain isoforms in type 2 skeletal muscle fibres. *J Muscle Res Cell Motil.* 1989; 10:197–205. [PubMed: 2547831]
- Schiaffino S, Reggiani C. Molecular diversity of myofibrillar proteins: gene regulation and functional significance. *Physiol Rev.* 1996; 76:371–423. [PubMed: 8618961]
- Schiaffino S, Sandri M, Murgia M. Activity-dependent signaling pathways controlling muscle diversity and plasticity. *Physiology (Bethesda).* 2007; 22:269–278. [PubMed: 17699880]
- Talmadge RJ, Otis JS, Rittler MR, Garcia ND, Spencer SR, Lees SJ, Naya FJ. Calcineurin activation influences muscle phenotype in a muscle-specific fashion. *BMC Cell Biol.* 2004; 5:28. [PubMed: 15282035]
- Weiss A, McDonough D, Wertman B, Acakpo-Satchivi L, Montgomery K, Kucherlapati R, Leinwand L, Krauter K. Organization of human and mouse skeletal myosin heavy chain gene clusters is highly conserved. *Proc Natl Acad Sci U S A.* 1999; 96:2958–2963. [PubMed: 10077619]
- Wigmore PM, Evans DJ. Molecular and cellular mechanisms involved in the generation of fiber diversity during myogenesis. *Int Rev Cytol.* 2002; 216:175–232. [PubMed: 12049208]

Figure 1. MyI-CFP transgene is selectively expressed in Type I muscle fibers

A–E) Cross-section of lower leg from adult MyI-CFP mouse. A) Region containing portions of the plantaris and soleus muscles. Many fibers are CFP-positive in the Type I-rich soleus, but few are positive in the Type I-poor plantaris. B–E) Sections were doubly-stained with antibodies that recognize MyHC I (B), MyHC IIA (C), all adult MyHCs except MyHC IIX (D) or MyHC IIB (E). Left, CFP; center, anti-myosin; right, merge. Note that in C, the antibody-negative fibers are IIX. F) Fiber type-specific labeling by the MyI-CFP transgene, based on quantification of images such as those in B–E. Graph shows data pooled from 3 animals. Scale bar, 200 μ m for A, 25 μ m for B–E.

Figure 2. MyIIA-RFP transgene is selectively expressed in Type IIA muscle fibers

A–E) Cross-section of lower leg from adult MyIIA-RFP mouse. A) Region containing portions of the plantaris and soleus muscles. Incidence of RFP-positive fibers mirrors known composition of these two muscles (Talmadge et al., 2004). B–E) Sections were doubly-stained with antibodies that recognize MyHC I (B), MyHC IIA (C), all adult MyHCs except MyHC IIX (D) or MyHC IIB (E). Left, RFP; center, anti-myosin; right, merge. F) Fiber type-specific labeling by the MyIIA-RFP transgene, based on quantification of images such as those in B–E. Graph shows data pooled from 3 animals. Scale bar, 200 μ m for A, 25 μ m for B–E.

Figure 3. RFP is selectively expressed in Type IIX and IIB muscle fibers of PV-Cre;STOP-RFP double transgenic mice

A–E) Cross-section of lower leg from adult PV-Cre;STOP-RFP double transgenic mouse MyI-CFP mouse. A) Region containing portions of the plantaris and soleus muscles. Many fibers are RFP-positive in the predominantly fast plantaris, but few are positive in the slow soleus. B–E) Sections were doubly-stained with antibodies that recognize MyHC I (B), MyHC IIA (C), all adult MyHCs except MyHC IIX (D) or MyHC IIB (E). Left, RFP; center, anti-myosin; right, merge. F) Fiber type-specific labeling by the PV-Cre;STOP-RFP transgene, based on quantification of images such as those in B–E. Graph shows data pooled from 3 animals. Scale bar, 200 μ m for A, 25 μ m for B–E.

Figure 4. Muscles from MyI-CFP mice

A) Whole muscles from lower hindlimb of MyI-CFP mice. GN, gastrocnemius; SOL, soleus; EDL, extensor digitorum longus. These muscles are mixed, Type I-rich and Type I-poor, respectively. B) Muscles in the neck of a MyI-CFP mouse. Bright muscle in center is spinotrapezius. C) Type I (blue) and Type IIA (red) fibers in the spinotrapezius of a MyI-CFP;MyIIA-RFP double transgenic mouse. Scale bar is 0.5mm in A, 20 μ m

Figure 5. Motor unit in transgenic mice that express YFP in slow motor neurons and CFP in Type I muscle fibers

Forelimb muscle from MyI-CFP; SVCreER; Thy1-STOP-YFP triple transgenic mouse. Tamoxifen was administered at P8 and P10 to selectively label slow motor neurons with YFP (yellow). Following fixation, muscles were incubated with rhodamine-bungarotoxin (red) to mark acetylcholine receptors concentrated in the postsynaptic membrane of neuromuscular junction. A) Reconstruction of the sole labeled motor axon in this muscle. B–D) Three neuromuscular junctions from the motor unit in A, shown at higher magnification. In each case, the synapse is on a Type I, CFP-positive (blue) fiber. Left panels, YFP plus bungarotoxin; right panels, YFP plus CFP plus bungarotoxin. Bungarotoxin labels the

postsynaptic membrane, both at synaptic sites innervated by the YFP-positive axon and at those innervated by unlabeled axons. Scale bar, 50 μ m for A, 25 μ m for B–D.

Figure 6. Altered fiber type composition following denervation imaged in MyI-CFP and MyIIA-RFP mouse

A–D) Cross-sections of denervated (A,C) and contralateral control muscles (B,D) from lower hindlimbs of MyI-CFP (A,B) and MyIIA-RFP (C,D) mice. Denervation leads to a dramatic increase in RFP but not CFP expression. E,F) Sections from denervated (E) and contralateral control (F) muscles of a MyI-CFP; MyIIA-RFP double transgenic mouse. G,H) Cross-sections of denervated (G) and contralateral control muscles (H) from MyIIA-RFP mouse stained with anti-MyHC IIA to show that increased transgene expression following denervation reflects increased numbers of MyIIA-positive fibers. Left, RFP; center, antibody; right, merge. I,J) Portion of the peripheral medial gastrocnemius of a MyIIA-RFP

mouse imaged prior to (G) and 10 days following denervation (H). Scale bars, 400 μ m for A–D; 25 μ m for E,F; 50 μ m for G,H; 20 μ m for I,J.