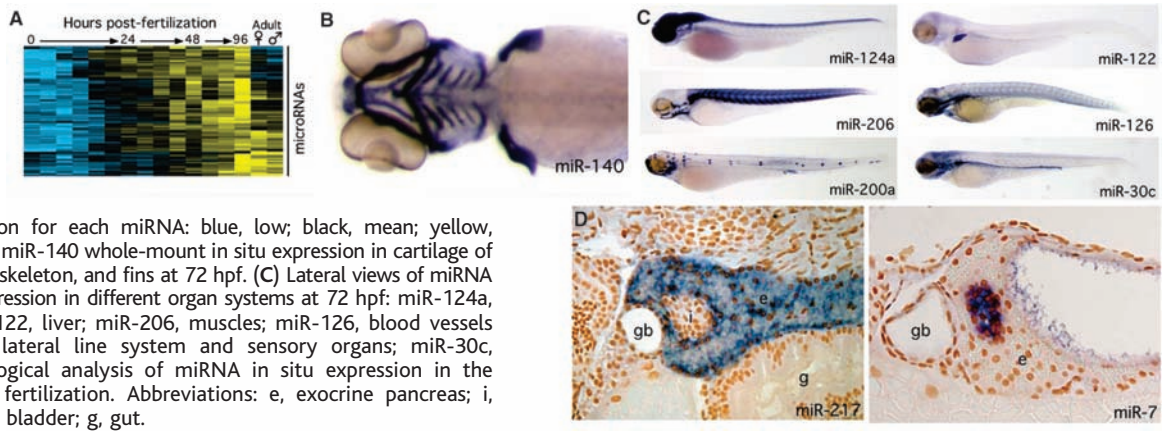

Fig. 1. miRNA expression in zebrafish embryonic development. (**A**) Microarray expression levels of 90 (of the 115) miRNAs during embryonic development. Colors indicate relative and mean-centered expression for each miRNA: blue, low; black, mean; yellow, high. (**B**) Ventral view of miR-140 whole-mount in situ expression in cartilage of pharyngeal arches, head skeleton, and fins at 72 hpf. (**C**) Lateral views of miRNA whole-mount in situ expression in different organ systems at 72 hpf: miR-124a, nervous systems; miR-122, liver; miR-206, muscles; miR-126, blood vessels and heart; miR-200a, lateral line system and sensory organs; miR-30c, pronephros. (**D**) Histological analysis of miRNA in situ expression in the pancreas 5 days after fertilization. Abbreviations: e, exocrine pancreas; i, pancreatic islet; gb, gall bladder; g, gut.

data for zebrafish and mammals (fig. S2 and table S3). Up to 77% of the in situ expression patterns were confirmed by at least one of the microarray data sets. In addition, miRNA in situ data showed patterns that cannot easily be detected by microarrays. For example, some miRNAs were expressed in hair cells of sensory epithelia (fig. S6).

In conclusion, we here describe the first comprehensive set of miRNA expression patterns in animal development. We found these patterns to be remarkably specific and diverse, which suggests highly specific and diverse roles for miRNAs. Most miRNAs are expressed in a tissue-specific manner during segmentation and later stages but were not detected during early development. Although we cannot exclude a role for undetectable early miRNAs, this observation indicates that most miRNAs may not be essential for tissue fate establishment but rather play crucial roles in differentiation or the maintenance of tissue identity.

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Ant Nestmate and Non-Nestmate Discrimination by a Chemosensory Sensillum

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In animal societies, chemical communication plays an important role in conflict and cooperation. For ants, cuticular hydrocarbon (CHC) blends produced by non-nestmates elicit overt aggression. We describe a sensory sensillum on the antennae of the carpenter ant *Camponotus japonicus* that functions in nestmate discrimination. This sensillum is multiporous and responds only to non-nestmate CHC blends. This suggests a role for a peripheral recognition mechanism in detecting colony-specific chemical signals.

The struggle to maintain order in societies has led social animals, including human beings, to evolve and develop various means of communication. Ants have developed a sophisticated chemical communication system that enables them to reject non-nestmate conspecifics and to accept nestmates (*1*, *2*). Many behavioral experiments have suggested that their aggressive behavior against non-nestmates is evoked by contact chemosensory detection of differences between colony-specific chemical signals (*3*–*8*). Despite this well-defined behavior, the sensory mechanism for nestmate and non-nestmate discrimination has been unclear. It is thought that a "neural template" of nestmate recognition cues is formed that represents a constantly changing, experience-derived memory (*9*, *10*). By comparing the chemosensory discriminators or "labels" of encountered individuals with the "template" previously acquired, ants decide between acceptance or aggression (*11*, *12*). For such a decision rule by "template-label matching," several models have been proposed (*13*–*15*). They are constructed on a threshold-response hypothesis (*2*) in which some neural mechanism in the brain sets a threshold of similarity between template and label, thus regulating aggression.

For the carpenter ant, *C. japonicus*, cuticular CHC blends consist of at least 18 compounds in colony-specific ratios (Fig. 1A). To investigate how these organisms discern nestmate from non-nestmate signals, we developed a bioassay whereby a glass bead was used as a surrogate ant. The aggressive behavior of worker ants toward encountered non-nestmates was mimicked by a glass bead inoculated with either cuticle extract or a CHC fraction derived from the non-nestmate body surface (Fig. 1B). No aggression was elicited in response to extract from the nestmate body surface. There was a significant difference (t test; $P < 0.001$) in ant aggression against nestmate and non-nestmate compounds. About 40% of the ants became ag-

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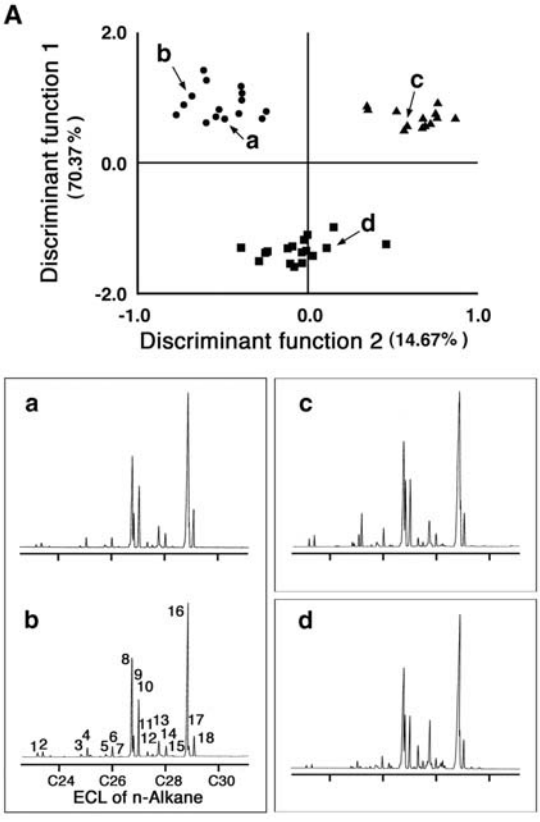
gressive in response to the non-CHC fractions, regardless of their origin (Fig. 1C). Thus, similar to other social insects (*16*), the colony-specific CHC blend was the most important recognition-discriminating factor in *C. japonicus*.

Behavioral observations of ants suggest that aggression depends on antennal contact with non-nestmate compounds. Electrophysiological recordings show that typical taste sensilla in antennae did not respond to CHCs, but one type of sensillum (Fig. 2A) did respond to contact stimulation with the non-nestmate CHC blend (Fig. 3A). (Because CHCs are highly lipophilic, we used 0.1% Triton X-100 to dissolve CHCs into stimulus solutions). Response to the non-nestmate CHC blend gradually adapted to the control level but usually lasted longer than 10 s. This type of sensillum responded neither to the CHC blend nor to crude extract of nestmates. This electrophysiological response was observed in both an antenna attached to a head capsule and an antenna separated from the head (Fig. 3B). More than 75% of the tested sensilla responded to the crude extract or the purified CHCs of non-nestmates (Fig. 3C). Only 12% responded to the crude extract of nestmates, and only 24% responded to the purified CHCs of nestmates. The non-nestmate–derived non-CHC fraction elicited a response in only 30% of the tested sensilla, whereas the nestmate-derived non-CHC fraction did not elicit any response. When a mixture of nestmate and non-nestmate CHCs were tested, impulses diminished faster than those induced by non-nestmate CHCs. Moreover, this type of sensillum responded to CHC blends of different ant species (4 of 4 to *Formica japonica*, 4 of 4 to *Lasius japonicus*, and 23 of 29 to *Camponotus obscuripes*).

Scanning electron microscopy revealed that this type of sensillum is relatively thick (approximately 20 μm in length and 4 μm in diameter) and multiporous (Fig. 2B). Multiporous chemosensilla are generally assumed to be olfactory rather than contact chemosensory (*17*). However, in a few parasitic bees, a particular type of multiporous sensillum is presumed to act as a contact chemosensory organ for host discrimination (*18*). We found that ant sensilla that are sensitive to non-nestmate CHC blend also house a large number (about 200) of putative receptor neurons (Fig. 2C). Such a large number of receptor neurons might facilitate the resolution of small differences in the multiple-component chemical cues encountered by the sensillum and also might integrate the chemical information as a unit (*19*). A single sensillum responded to non-nestmate CHCs with different shapes of impulses (Fig. 3A), which suggests that several neurons in a sensillum could simultaneously respond to several compounds in the non-nestmate CHC blend.

The electrophysiological (Fig. 3) and behavioral (Fig. 1) results suggest that this sensillum might have a direct role in nestmate and non-nestmate discrimination in this ant species. There was not a full match between the behavioral (Fig. 1Cc) and electrophysiological (Fig. 3Cc) effects of the nestmate-derived non-CHC fraction to indicate the involvement of other types of sensilla. However, because this sensillum showed a response specificity for non-nestmate blends, even in isolated antenna preparations, the effect is generated peripherally and does not require feedback from the brain.

Analysis of the antennal extract of *C. japonicus* identified a 12-kD protein with an N-terminal amino acid sequence similar to that in members of the chemosensory protein (CSP) family (fig. S1A). Generally, receptor neurons in insect chemosensilla are surrounded by an aqueous lymphatic fluid. Thus, water-insoluble stimulants such as CHCs require water-soluble carriers to access receptor neurons (*20*). To obtain the complete sequence of the CSP, cDNA was derived from poly(A)-rich RNA isolated from the flagellum where CHC-sensitive sensilla were abundant. Reverse transcription polymerase chain reaction was used with degenerate primers and amplified a 580–base pair cDNA sequence (fig. S1B) consisting of 102 amino acid residues (fig. S2) (CjapCSP) (DNA Data Bank of Japan, accession number AB182637). CjapCSP showed 63% identity with two other *Hymenoptera* CSPs (LhumCSP and PdomCSP-1) and 30% to 40% identity with other CSPs (fig. S3). Histochemical staining with antiserum against recombinant CjapCSP (Fig. 3D) showed localization beneath the cuticle in the flagellum (Fig. 3Da) and also inside the CHC-sensitive antennal sensillum (Fig. 3Dc), although sensillar specificity in localization was not clear.



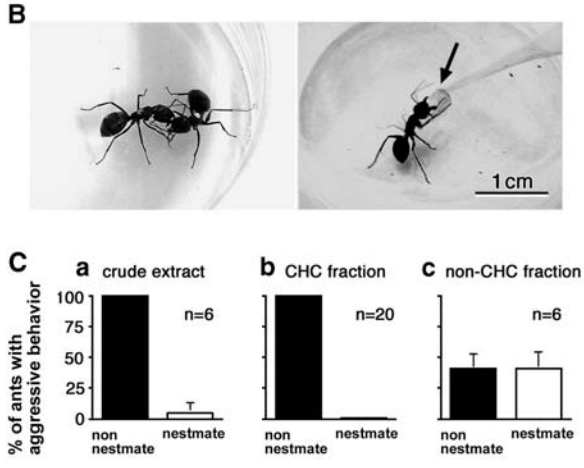


Fig. 1. Ant CHC profiles and behavioral responses. (**A**) (Top) Discriminant analysis of individual CHC profiles of three different colonies according to Dietemann *et al.* (*22*). (Bottom) CHC profiles of individuals belonging to each colony are indicated by the same symbols forming a cluster, which suggests similarity among them; (a and b) CHC profiles of different individuals within a colony; (c and d) CHC profiles of individuals in different colonies. Peak 1: tricosene; 2: *n*-tricosane; 3: pentacosene; 4: *n*-pentacosane; 5: hexacosene; 6: *n*-hexacosane; 7: 5,7,12- and 7,9,12-trimethylpentacosane; 8: 9-heptacosene; 9: 7-heptacosene; 10: *n*-heptacosane; 11: 13-methylheptacosane; 12: 5-methylheptacosane; 13: 7,15-dimethylheptacosane; 14: *n*-octacosane; 15: 5,7,12-trimethylheptacosane; 16: 9-nonacosene; 17: 7-nonacosene; 18: *n*-nonacosane. (**B**) Photographs of *C. japonicus* encountering a non-nestmate ant and a glass bead (arrow) covered with the CHC blend derived from non-nestmates. (**C**) The ratio of ants showing aggressive behavior to crude extract, CHC fraction, and non-CHC fraction from the non-nestmates (closed columns) or the nestmates (open columns). Twenty ants of one colony were tested for the stimuli derived from the same or a different colony. Reciprocal tests converting the colonies for the test ant and the test stimulus were also carried out, and data were statistically evaluated in a, b, and c, using 6, 20, and 6 colonies, respectively.

To determine whether CjapCSP is a carrier protein for *C. japonicus* CHCs, an aqueous buffer solution was added to CHCs (Fig. 4). Buffer solution alone did not dissolve CHCs, as determined by gas chromatography. However, buffer containing 10 or 100 μM CjapCSP did dissolve CHCs (0.9 ± 0.5 or 2 ± 0.8 μg/ml CHCs) at the given ratio. Buffer containing 100 μM bovine serum albumin (BSA) dissolved very little CHC (0.1 ± 0.2 μg/ml) in comparison. This was observed for CHC blends derived from three different colonies. When CjapCSP was used to dissolve CHCs in the stimulus solution, electrophysiologically recorded responses from CHC-sensitive sensilla indicated that only non-nestmate CHCs were stimulative (Fig. 3A). Thus, with CjapCSP as a carrier, lipohilic CHCs might be transferred to stimulate receptor cells.

Our data suggest that specialized sensilla in the antenna appear to have almost all-or-none sensitivity to non-nestmate or nestmate CHC blends. To discriminate between non-nestmates and nestmates by their CHC patterns of multiple components, a sensillum would be expected to have multiple receptor neurons. In accordance with olfactory coding by receptor neurons, each receptor neuron in the sensillum might express a different receptor molecule. Each CHC component might interact with more than one receptor molecule; therefore, each CHC component might activate several neurons. The receptor neurons must simultaneously be desensitized to stimulation by nestmate CHC blends. Desensitization of receptor neurons can occur by down-regulation of receptor molecules, by inhibition among receptor neurons or at the receptor molecule level, or even by sensory adaptation of receptor neurons. In any case, when a receptor neuron is desensitized to a stimulant, it shows an increased electrophysiological threshold (*21*). Even if all of the receptor neurons in a sensillum are desensitized to nestmate CHCs, some neurons can still respond to non-nestmate CHC components, concentrations of which are higher than the increased threshold levels. However, it was difficult to precisely evaluate the desensitization property of every receptor neuron because temporal and kinetic analysis of impulses to the multiple CHC components was not possible. If enough of the pure compounds that constitute the nestmate CHC blend could be obtained, more precise behavioral and electrophysiological analyses would be allowed. It also remains possible that ants obtain chemo-

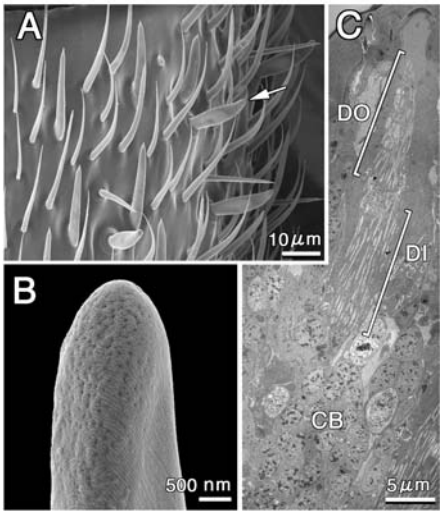


Fig. 2. CHC-sensitive sensillum and CPS localization. (**A**) Scanning electron micrograph (SEM) of the antennal surface at low magnification. The non-nestmate-CHC-sensitive sensillum is indicated by an arrow. (**B**) SEM of the tip of the non-nestmate-CHC-sensitive sensillum at high magnification. (**C**) Transmission electron micrograph of a longitudinal section beneath the cuticular apparatus of non-nestmate-CHC-sensitive sensillum. DO, dendritic outer segments; DI, dendritic inner segments; CB, cell bodies of the receptor neurons.

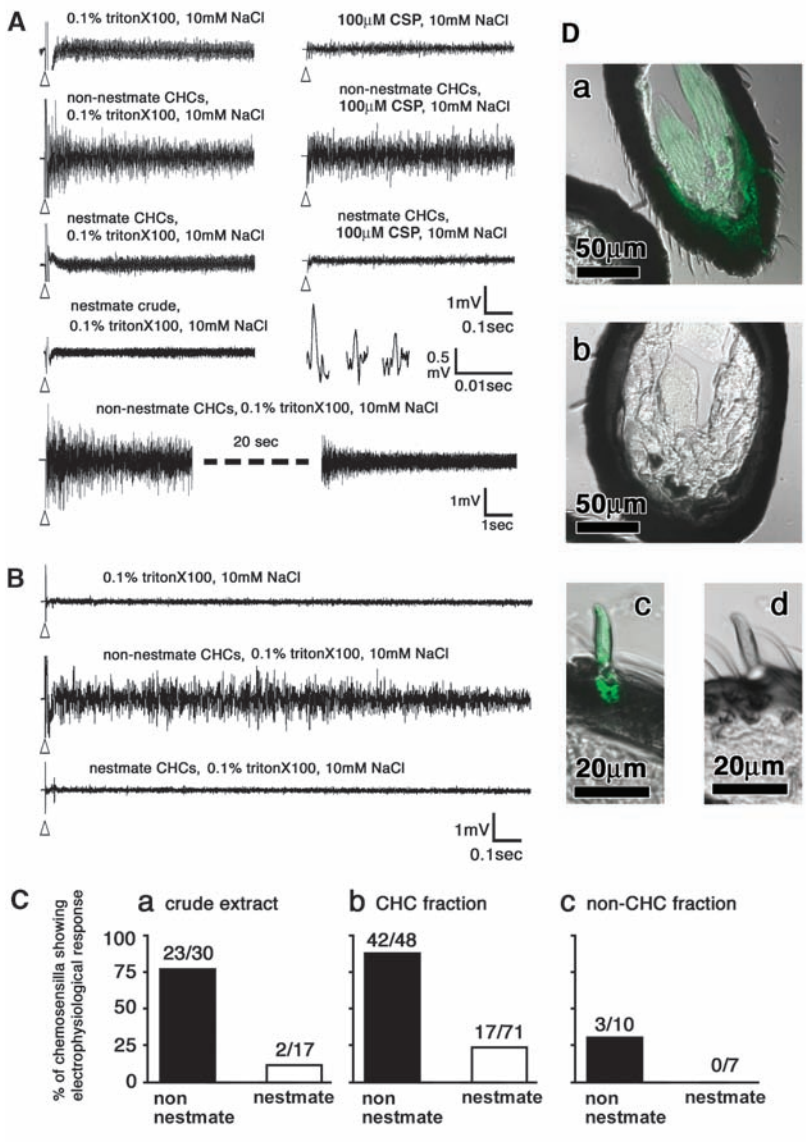


Fig. 3. Sensillum responses to CHCs. (**A**) (Left, top to bottom) Responses of the identified sensillum in a head-attached antenna preparation to indicated mixtures containing crude extract, CHCs, or CSP. A long response recording (20 s in the middle of the impulse recording is omitted) shows adaptation to non-nestmate CHCs dissolved in 10 mM NaCl containing 0.1% Triton X-100. Three distinguishable units of impulses are shown. Arrowheads indicate beginning of stimulation. The ants used for electrophysiological experiments were randomly chosen from seven colonies, and reciprocal tests converting the colonies for the test ant and the test stimulus were carried out. (**B**) Responses in an antenna preparation separated from the head to the indicated mixtures. (**C**) The ratio of sensilla in which impulses were observed in response to crude extract, CHC fraction, and non-CHC fraction derived from non-nestmates (closed columns) or nestmates (open columns). Fractions represent ratio of number of responding sensilla to number of tested sensilla. (**D**) Cross sections of flagellum (a) and scapus (b) stained with antiserum to CjapCSP. Longitudinal sections of the CHC-sensitive sensillum stained with antiserum to CjapCSP (c) and preimmune serum (d) as control. The photomicrographs are superimposed phase-contrast images and fluorescent images in which CjapCSP localization is indicated by green fluorescence.

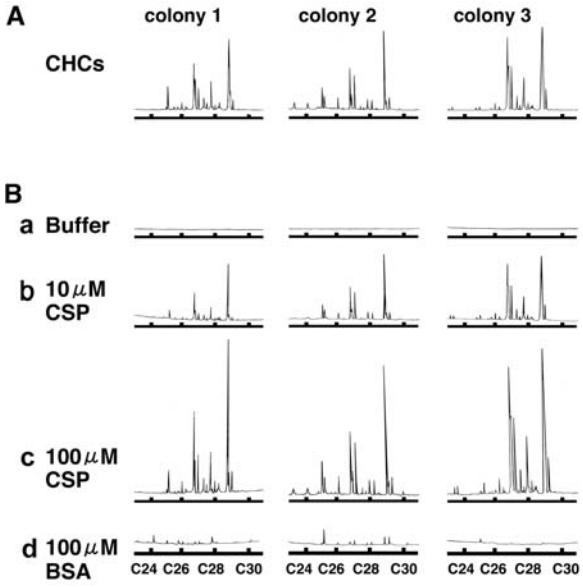


Fig. 4. CSP dissolves CHCs. (**A**) Gas chromatograms for the original CHC profiles from colonies 1 to 3. (**B**) (a, b, and c) CHC profiles dissolved in the buffer with 0, 10, and 100 μM CjapCSP, respectively; (d) CHC profiles dissolved in the buffer with 100 μM BSA as control. $n = 10$ for each assay using colony-specific CHC blend; the chromatograms are an average drawing of them.

sensory information from nestmate CHCs through other types of sensilla.

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22. Materials and methods are available as supporting material on *Science* Online.

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Bone Marrow Stromal Cells Generate Muscle Cells and Repair Muscle Degeneration

Mari Dezawa,[1]* Hiroto Ishikawa,[1] Yutaka Itokazu,[1] Tomoyuki Yoshihara,[1] Mikio Hoshino,[2] Shin-ichi Takeda,[3] Chizuka Ide,[1] Yo-ichi Nabeshima[2]

Bone marrow stromal cells (MSCs) have great potential as therapeutic agents. We report a method for inducing skeletal muscle lineage cells from human and rat general adherent MSCs with an efficiency of 89%. Induced cells differentiated into muscle fibers upon transplantation into degenerated muscles of rats and mdx-nude mice. The induced population contained Pax7-positive cells that contributed to subsequent regeneration of muscle upon repetitive damage without additional transplantation of cells. These MSCs represent a more ready supply of myogenic cells than do the rare myogenic stem cells normally found in muscle and bone marrow.

Cell transplantation therapy offers hope for the treatment of intractable muscle degenerative disorders. Embryonic stem (ES) cells and stem cells derived from muscle have been considered as candidates for transplantation therapy (*1*–*7*). Although they have great potential, they face limitations inherent in procurement from fetal tissue, including problems relating to histocompatibility and ethical concerns. Although muscle stem cells and satellite cells can be isolated from adult and prenatal tissues (*2*, *4*–*6*), the number of cells that can be harvested may be limited. Bone marrow is another source of myogenic stem cells (*3*, *8*); however, because the stem cell population is very small, the problem of inadequate tissue supply for therapeutic scale again arises.

Because bone marrow stromal cells (MSCs) are easy to isolate and expand rapidly from patients without leading to major ethical and technical problems, they have great potential as therapeutic agents. However, despite their potential for use in cell transplantation therapy, practical application to human muscle degenerative diseases depends on the ability to control their differentiation into functional skeletal muscle cells with high efficiency and purity. Recently we reported that efficient induction of neurons, without glial differentiation, from human and rat MSCs could be achieved by Notch1 intracellular domain (NICD) gene transfer and administration of certain trophic factors (*9*). Further addition of glial cell line–derived neurotrophic factor (GDNF) effectively induced dopamine-producing cells and resulted in functional recovery when those cells were grafted into the brains of Parkinson's disease model rats (*9*). Here we report a method to systematically and efficiently induce skeletal muscle lineage cells

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