Stability of Chicken Troponin T Expression in Cultured Muscle Cells

Authors: Mashima, Jun, Nakada, Kazuto, Miyazaki, Jun-Ichi, and Hirabayashi, Tamio

Source: Zoological Science, 14(1) : 109-114

Published By: Zoological Society of Japan

URL: https://doi.org/10.2108/zsj.14.109

ZOOLOGICAL SCIENCE 14: 109–114 (1997)

Stability of Chicken Troponin T Expression in Cultured Muscle Cells

Jun Mashima, Kazuto Nakada, Jun-Ichi Miyazaki and Tamio Hirabayashi*

Institute of Biological Sciences, University of Tsukuba, Ibaraki 305, Japan

ABSTRACT—Cells prepared from chicken skeletal muscles of early developmental stages were cultured to study their troponin T isoform expression, using antisera specific to fast- and slow-muscle-type isoforms, and compared with the cells from later stages described in the previous study (Mashima *et al.*, 1996). We found that cultured myogenic cells from chickens and chick embryos could be classified, as in the previous study, into two types, fast type and fast/slow type in which fast- and slow-muscle-type isoforms were coexpressed. Ratios of these two types of muscle cells varied depending on their origins and developmental stages, and fast/slow type cells were in the majority at early stages. Since two distinct populations of cells committed to myogenic cell lineages were supposed to give rise to the two types of myotubes, we investigated the intrinsic stability of troponin T expression of the cultured myogenic cells using the serial subcloning method. The results of clonal analysis suggested that the expression pattern of troponin T isoform in cultured muscle cells is stable and that myogenic cell lineages play an important role in giving rise to different muscle types.

INTRODUCTION

Recently, our understanding of the factors regulating muscle cell commitment and differentiation has been advanced, but little is known about the mechanisms dictating diversity among muscle fibers. The differences of fast and slow muscles in the contractile and biochemical properties are considered to be generated by the selective expression of genes coding for contractile protein isoforms in respective myofibers (Bandman, 1992; Cossu and Molinaro, 1987; Jolesz and Sreter, 1981; Schiaffino and Reggiani, 1996; Stockdale, 1992).

In fact, myosin heavy chain was frequently used as a marker of muscle fiber types (Cho *et al.*, 1993; DiMario *et al.*, 1993; Dusterhoft and Pette, 1993; Edom *et al.*, 1994; Feldman and Stockdale, 1991; Hartley *et al.*, 1992; Miller and Stockdale, 1986; Schafer *et al.*, 1987). Since different isoforms of myosin heavy chain are expressed in different muscle fibers and at different stages of development, myosin heavy chain is considered useful for studying myogenic cell lineage.

On the other hand, troponin T (TnT), one of the three subunits of troponin complex, has three classes of isoforms specific to different fiber types of striated muscle: fast-muscle-type (F-type) TnT, slow-muscle-type (S-type) TnT, and cardiac-muscle-type TnT (Bandman, 1992; Schiaffino and Reggiani, 1996). The three classes of isoforms are encoded by three different genes (Cooper and Ordahl, 1984; Smillie *et al.*, 1988; Yonemura *et al.*, 1996). Using TnT as a marker, we showed by tissue transplantation experiments that expression patterns of TnT isoforms seemed to be fixed in cell lineage (Yao *et al.*, 1992). Additionally, our *in vitro* analysis on the basis of TnT isoform expression showed that cultured myogenic cells from chickens and chick embryos were classified into two types, fast type and fast/slow type in which fast- and slow-muscle-type isoforms were coexpressed. No cells could be found which were stained only with anti-S-type TnT even in the muscle cells prepared from slow muscle, *anterior latissimus dorsi*, in which the fibers stained only with anti-S-type TnT were present. In this respect, the *in vitro* expression of TnT isoforms was different from the *in vivo* expression, and the composition of cell types of each muscle seemed to be determined differently during the developmental course. Most cultured cells from 11-day old embryos belonged to the fast/slow type, while those from 13-day old embryos contained fast/slow type and fast type cells at a ratio of 3:1. The ratios in the cultured cells prepared from embryos older than the 17th day of incubation seemed to be fixed depending on the cell sources (Mashima *et al.*, 1996).

Considering our previous data, two questions emerge. One is whether or not all muscle cells prepared from embryos earlier than the 11th day of incubation are of fast/slow type. The other is do the *in vitro* expression patterns of TnT remain stable or not, since it is not clear from our previous work that the cells committed to two myogenic lineages are stable in the *in vitro* system.

In this study, we investigate the expression of TnT isoforms in myogenic cells prepared from early stages of chick

* Corresponding author: Tel. +81-298-53-6649; FAX. +81-298-53-6649.

embryos by immunohistochemical techniques and we have confirmed that all cells prepared from 7-day old embryos are of fast/slow type. Furthermore, we have performed clonal analysis of cultured myogenic cells prepared from *pectoralis major* of 13-day old chick embryos and 1-day old chicks to show that the *in vitro* expression patterns of F-type and S-type TnTs are stable without any change of cell types detected in colonies repeatedly subcloned.

MATERIALS AND METHODS

Animals

White leghorn chickens (*Gallus domesticus* (L)) and their fertilized eggs were obtained from commercial sources.

Preparation of antisera

The anti-F-type TnT (guinea pig serum) and the anti-S-type TnT (rabbit serum) used in this study were described previously by Mashima *et al.* (1996) and Yao *et al.* (1992), respectively.

Myogenic cell isolation and purification

Myogenic cells were prepared from somites of 3.5-day old embryos, breast and lower leg muscles of 7-, 8- and 9-day old chick embryos, and *pectoralis major* and *gastrocnemius* of 11-, 13- and 17-day old chick embryos and 1-day old chicks by the method of Mashima *et al.* (1996) with some modifications as follows. The growth medium consisted of 37.5% Dulbecco's modified Eagle's minimum essential medium (DMEM), 37.5% Ham's F12, 20% horse serum, 5% chick embryo extract, and Gentamicin at 4.0 mg/liter of medium. The differentiation medium consisted of 47.5% DMEM, 47.5% Ham's F12, 4% horse serum, 1% chick embryo extract, and Gentamicin at 4.0 mg/liter of medium. The chick embryo extract was prepared essentially according to the method of Hauschka and Konigsberg (1966).

Cell culture for clonal analysis

All cultures at a clonal density were grown in an equal mixture of fresh and conditioned growth media. The conditioned growth medium was prepared by the method of White *et al.* (1975). The isolated cells were counted and plated at a clonal density (approximately 100 cells) on a collagen-coated 60 mm dish with 2 ml of the medium and cultured at 37°C in 5% CO_2. After 5 days in culture, subcloning of chicken myogenic cell colonies was carried out as described by Rutz and Hauschka (1982) with some modifications as follows. A muscle colony was located and marked on the dish. An open-ended stainless cylinder which had silicone grease applied to one rim was placed over the colony, so that the cylinder encircled the colony and formed a greased seal with the dish. Then, the colony was rinsed and dissociated with 0.05% trypsin. The dissociated cells from a single colony were divided into two groups: One group was plated on a new collagen-coated 60 mm dish again for the next colony formation, the other was in one well of a 24-well plate with 0.3 ml of the medium on a collagen-coated Celltight C-1 Celldesk (Sumilon). The latter cells adhering to Celldesks were used for immunocytochemical studies on the 10th day after plating. The former cells on the 60 mm dish were grown for 5 days to form the next colonies and the colonies were subcloned once more.

Primary colonies were those formed by muscle cells isolated directly from tissues; secondary colonies were formed from dissociated muscle cells of subcloned primary colonies, tertiary (3°) colonies designated #1.1 and #1.2 in Table 3, for example, were formed from secondary (2°) colonies designated #1 in Table 3, and quaternary (4°) colonies formed, for example, from the colony #1.1 were designated #1.1.1 and #1.1.2 in Table 3. Then TnT expression in the progeny of the colonies was investigated as mentioned above, so that the sequentially subcloned colonies were numbered to indicate their ancestry in Tables 3 and 4.

Indirect immunofluorescence microscopy

Immunocytochemistry was carried out as reported by Mashima *et al.* (1996). Pictures were taken with a combination of a Nikon Optiphot microscope and a Nikon FX-35WA camera.

RESULTS

TnT expression in cultured muscle cells prepared from chick embryos of early stages

Our previous studies showed that cultured myogenic cells from chickens and chick embryos were classified into two types with respect to TnT isoform expression, fast type and fast/slow type (Mashima *et al.*, 1996). Since the ratio of fast type to fast/slow type cells in culture is low at the earlier stages, it is interesting when the fast type cells appeared in the early development.

Myogenic cells were isolated from somites and breast and lower leg muscles of chickens of early stages, using differential trypsinization and cell adhesion techniques, cultured for 10 days, and double-immunostained with the anti-F-type TnT and anti-S-type TnT to examine which type of TnT isoforms was expressed in each cell.

Representative results were presented in Fig. 1. Cells prepared from breast muscles of 8-day old embryos were stained with the anti-F-type TnT and anti-S-type TnT (Fig. 1a-d). The cells stained with both the antisera were in the majority at these stages (Fig. 1a and b). However, some cells were stained only with the anti-F-type TnT (Fig. 1c and d).

The results of double staining of cultured muscle cells with the anti-F-type TnT and S-type TnT were summarized in Table 1. All cells from somites of 3.5-day old embryos and breast and lower leg muscles of 7-day old embryos (E7) were stained with both anti-F-type TnT and anti-S-type TnT ("Fast/Slow" in Table 1). Approximately 90% of cells prepared from breast and lower leg muscles of the 8- and 9-day old embryos (E8 and E9) were stained with both anti-F-type TnT and anti-S-type TnT, leaving small fractions (approximately 10%) stained only with the anti-F-type TnT ("Fast" in Table 1). These results suggested that all myogenic cells were fast/slow type until the 7th day of incubation and that fast type cells appeared on the 8th day of incubation. No cells were stained only with the anti-S-type TnT ("Slow" in Table 1).

Clonal analysis of chicken muscle cells

The changing ratio of fast type cells to fast/slow type cells depending on developmental stages and different muscles raises doubts on the stability of troponin T isoform expression in cultured cells, which was investigated by clonal analysis.

Firstly, clonal colonies of the myogenic cells prepared from breast and lower leg muscles at various stages were stained with anti-F-type and anti-S-type TnT sera. These muscle colonies were secondary ones subcloned from single primary colonies. So it was statistically expected that these colonies were the progeny of a single muscle cell. The results of this experiment were summarized in Table 2. All colonies from breast muscles of 9-day old embryos and lower leg muscles of 9-day old and 11-day old embryos were stained with both

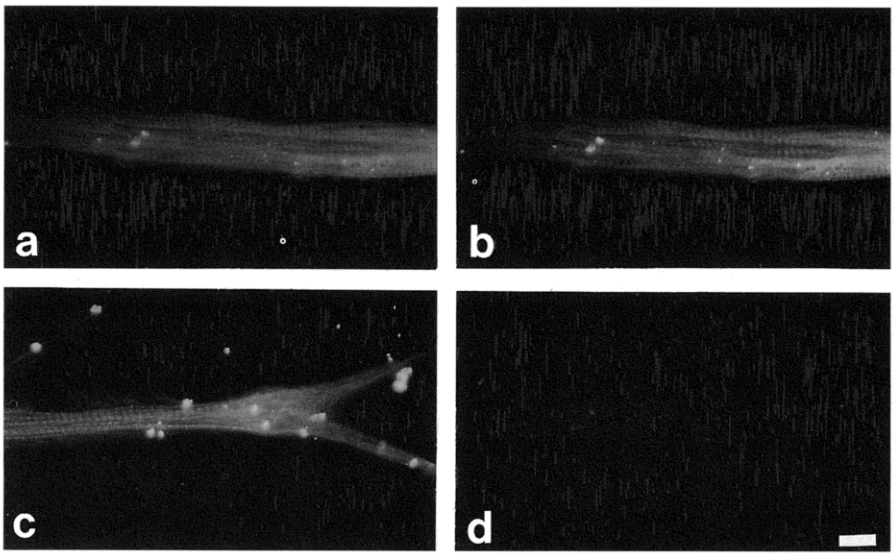

Fig. 1. Double immunofluorescence analysis of troponin T in cultured muscle cells. Myoblasts were prepared from breast muscle of 8-day old embryos and cultured for 9 days. Cells were fixed and processed for double immunofluorescence as described in MATERIALS AND METHODS. Rhodamine (**a** and **c**) and fluorescein (**b** and **d**) fluorescence represents expression of fast-muscle-type troponin T and slow-muscle-type troponin T, respectively, in the same myotubes. Bar = 10 μm.

Table 1. Reactivity of cultured muscle cells with antisera

Source of cells		Percentage (numbers) of cells		
Muscle	Stage	Fast	Fast/Slow	Slow
Somite	E3.5	0 (0)	100 (128)	0 (0)
Breast	E7	0 (0)	100 (262)	0 (0)
	E8	3 (6)	97 (166)	0 (0)
	E9	11 (6)	89 (48)	0 (0)
Lower leg	E7	0 (0)	100 (227)	0 (0)
	E8	4 (9)	96 (210)	0 (0)
	E9	14 (9)	87 (57)	0 (0)

The results from double immunofluorescence analysis of cultured cells prepared from somites and breast and lower leg muscles are summarized. The cells stained with the antisera were counted. Fast, only anti-fast-muscle-type troponin T positive; Fast/Slow, both anti-fast- and anti-slow-muscle-type troponin T positive; Slow, only anti-slow-muscle-type troponin T positive; E3.5~9, 3.5~9-day old embryos.

Table 2. Reactivity of cultured muscle cell colonies with antisera

Source of cells		Numbers of colonies		
Muscle	Stage	Fast	Fast/Slow	Slow
Breast	E9	0	28	0
	E11	15	25	0
	E13	21	36	0
	E17	53	0	0
Lower leg	E9	0	19	0
	E11	0	18	0
	E13	14	25	0
	E17	42	27	0

The results from double immunofluorescence analysis of cultured colonies prepared from breast and lower leg muscles are summarized. Each colony was subcloned from a primary colony, so these secondary colonies were statistically expected to be the progenies of single myoblasts. The colonies stained with the antisera were counted. There were no colonies in which fast and fast/slow types of cells intermingled. Fast, only anti-fast-muscle-type troponin T positive; Fast/Slow, both anti-fast- and anti-slow-muscle-type troponin T positive; Slow, only anti-slow-muscle-type troponin T positive; E9~17, 9~17-day old embryos.

anti-F-type TnT and anti-S-type TnT. There were both fast type and fast/slow type colonies from breast muscles of 11-day old and 13-day old embryos and lower leg muscles of 13-day old and 17-day old embryos. All colonies from breast muscles of 17-day old embryos were stained only with anti-F-type TnT. No colonies were stained only with anti-S-type TnT ("Slow" in Table 2).

Since the colonies were uniform with respect to reactivity with the antisera and no colonies were intermingled with fast and fast/slow types of cells, it was strongly suggested that TnT expression of the cultured myogenic cells was intrinsically stable.

Secondly, we performed serial subclonal analysis to get a more precise idea of the stability of the commitment of TnT expression of cultured muscle cells. The cells isolated from *pectoralis major* of 13-day old embryos and 1-day old chicks were cultured at a clonal density and incubated for 5 days to form primary muscle colonies. Secondary muscle colonies were formed from subcloned myogenic cells isolated from single primary muscle colonies, tertiary muscle colonies were from cells of single secondary muscle colonies, in this subcloning procedure. At each stage of subcloning, a part of

those colonies was analyzed by double immunofluorescence staining with anti-F-type and anti-S-type TnT sera to determine the type of TnT expressed in each colony. The results of serial subclonal analysis are summarized in Tables 3 and 4. This sequential clonal analysis was a painstaking experiment since numbers of colony to be analyzed increased exponentially in every generation. With the limited capacity of our culture facility we could follow 14 tertiary colonies as summarized in Tables 3 and 4. The important results were that, without exception all of the fibers in a colony formed from a single muscle cell were of the same type and that no change of the immunoreaction was observed in muscle colonies formed from a myogenic cell progeny repeatedly subcloned.

Colonies from *pectoralis major* of 13-day old embryos were stained with the antisera and the representative results were presented in Fig. 2a-d. One colony, a progeny of the colony designated #4.1.1 in Table 3, was stained with both anti-F-type TnT and anti-S-type TnT (Fig. 2a and b), and the other, a progeny of the colony designated #8.2.1 in Table 3, was stained only with the anti-F-type TnT (Fig. 2c and d).

DISCUSSION

TnT expression in cultured muscle cells from chick embryos of early stages

We could confirm that our previous observation was also true for chick embryos of early stages. All cells from 3.5- and 7-day old embryos were stained with both anti-F-type TnT and anti-S-type TnT ("Fast/Slow" in Table 1). Most cells (approximately 90%) from breast and lower leg muscles of the 8- and 9-day old embryos were stained with both anti-F-type TnT and anti-S-type TnT, but small fractions (approximately 10%) were stained only with the anti-F-type TnT ("Fast" in Table 1). These results suggested that all myogenic cells were fast/slow type until the 7th day of incubation and that fast type cells appeared at the 8th day of

Table 3. Effect of sequential subcloning on the troponin T expression in muscle colonies from *pectoralis major* of 13-day old chick embryos

3°	Numbers of colonies			4°	Numbers of colonies			5°	Numbers of colonies		
	F	F/S	S		F	F/S	S		F	F/S	S
#1	0	3	0	#1.1	0	3	0	#1.1.1	–	–	–
								#1.1.2	0	2	0
				#1.2	0	4	0	#1.2.1	0	3	0
								#1.2.2	0	1	0
#2	0	2	0	#2.1	0	2	0	#2.1.1	–	–	–
								#2.1.2	–	–	–
				#2.2	0	6	0	#2.2.1	–	–	–
								#2.2.2	–	–	–
#3	0	2	0	#3.1	0	1	0				
				#3.2	0	4	0				
#4	0	8	0	#4.1	0	8	0	#4.1.1	0	10	0
								#4.1.2	–	–	–
				#4.2	0	5	0	#4.2.1	0	2	0
								#4.2.2	–	–	–
#5	0	2	0	#5.1	0	1	0	#5.1.1	–	–	–
								#5.1.2	–	–	–
				#5.2	0	1	0	#5.2.1	–	–	–
								#5.2.2	–	–	–
#6	0	3	0	#6.1	0	1	0				
				#6.2	0	1	0				
#7	18	0	0	#7.1	11	0	0				
				#7.2	8	0	0				
#8	17	0	0	#8.1	6	0	0	#8.1.1	3	0	0
				#8.2	7	0	0	#8.2.1	5	0	0
#9	–	–	–	#9.1	1	0	0				
				#9.2	5	0	0				
#10	21	0	0	#10.1	5	0	0	#10.1.1	–	–	–
				#10.2	8	0	0	#10.2.1	–	–	–

The results from double immunofluorescence analysis of the sequentially subcloned colonies prepared from *pectoralis major* of 13-day old embryos are summarized. Myogenic cells were dissociated from secondary muscle colonies designated #1~10. Tertiary (3°) colonies (#1.1~10.2) formed by progeny of each secondary colony were likewise subcloned to form 4° colonies (#1.1.1~10.2.1), and the troponin T expression of the myotubes of the colonies in the remaining 3° subclones was determined by double immunofluorescence analysis with anti-fast-muscle-type and anti-slow-muscle-type troponin T. Sequential subcloning and numbering to indicate ancestry were repeated until 4° or 5° subclones were analyzed. F, only anti-fast-muscle-type troponin T positive; F/S, both anti-fast- and anti-slow-muscle-type troponin T positive; S, only anti-slow-muscle-type troponin T positive; –, no muscle colonies were formed.

Table 4. Effect of sequential subcloning on the troponin T expression in muscle colonies from *pectoralis major* of 1-day old chicks

3°	Numbers of colonies			4°	Numbers of colonies			5°	Numbers of colonies		
	F	F/S	S		F	F/S	S		F	F/S	S
#1	16	0	0	#1.1	3	0	0	#1.1.1	3	0	0
								#1.1.2	–	–	–
				#1.2	5	0	0	#1.2.1	–	–	–
#2	12	0	0	#2.1	2	0	0	#2.1.1	4	0	0
								#2.1.2	–	–	–
								#2.1.3	3	0	0
								#2.1.4	9	0	0
								#2.1.5	5	0	0
				#2.2	2	0	0				
#3	20	0	0	#3.1	6	0	0	#3.1.1	–	–	–
				#3.2	1	0	0	#3.2.1	–	–	–
#4	19	0	0	#4.1	5	0	0				
				#4.2	2	0	0				

The results from double immunofluorescence analysis of the sequentially subcloned colonies prepared from *pectoralis major* of 1-day old chicks are summarized. Myogenic cells were dissociated from secondary muscle colonies designated #1~4. Tertiary (3°) colonies (#1.1~4.2) formed by progeny of each secondary colony were likewise subcloned to form 4° colonies (#1.1.1~3.2.1), and the troponin T expression of the myotubes in the remaining 3° subclones was determined by double immunofluorescence analysis with anti-fast-muscle-type and anti-slow-muscle-type troponin T. Sequential subcloning and numbering to indicate ancestry were repeated until 4° or 5° subclones were analyzed. F, only anti-fast-muscle-type troponin T positive; F/S, both anti-fast- and anti-slow-muscle-type troponin T positive; S, only anti-slow-muscle-type troponin T positive; –, no muscle colonies were formed.

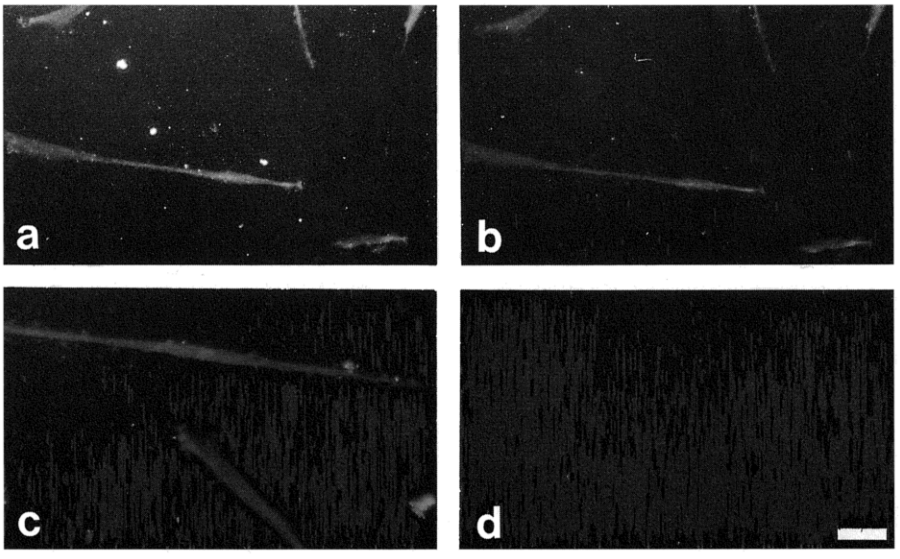

Fig. 2. Double immunofluorescence analysis of troponin T in cultured muscle cell colonies. As described in Table 3, myogenic cells isolated from individual primary muscle colonies prepared from *pectoralis major* of 13-day old chick embryos were subjected to serial subcloning and colony formation. A portion of 5° muscle colonies composed entirely of fast/slow myotube (#4.1.1 in Table 3) is shown in **a** and **b**. A portion of 5° colonies composed entirely of fast myotube (#8.2.1 in Table 3) is shown in **c** and **d**. Cells were fixed and processed for double immunofluorescence analysis as described in MATERIALS AND METHODS. Rhodamine (**a** and **c**) and fluorescein (**b** and **d**) fluorescence represents expression of fast-muscle-type troponin T and slow-muscle-type troponin T, respectively, in the same colonies. Bar = 50 μm.

incubation. Since the transition from primary myogenesis to secondary myogenesis occurs during days 6~8 *in ovo* (Miller and Stockdale, 1986), we think that the developmental change in the ratio of fast type cells to fast/slow type cells reflects the phase transition from the primary myogenesis to the secondary one.

The origin of fast type cells is not clear. These results and our previous study showed that most muscle cells were in a lineage of fast/slow type during early development (~11-day old embryo) and the ratio of fast type cells to fast/slow

type cells increased in the later developmental period. We suppose that this developmental change reflects the emergence of new subpopulations caused by extracellular cues such as nerve control and hormonal signals.

Stability of chicken TnT expression in vitro

The results in Table 2 were in good agreement with those in our previous study (Mashima *et al.*, 1996). Because of the small number of secondary colonies selected from the primary ones, ratios of fast type to fast/slow type clonal colonies did not closely reflect the ratios in original tissues and stages, but the ratios showed similar tendencies as those in Table 1 and our previous results: Most muscle cells are of fast/slow type at the early stage of development, and the ratios in the cultured cells prepared from embryos older than the 17th day of incubation seemed to be fixed depending on the cell sources.

The results of serial subclonal analysis in Tables 3 and 4 suggested that TnT expression of the cultured muscle cells was intrinsically stable, since there were no change of the cell types in colonies formed from a muscle cell progeny repeatedly subcloned. Studies on the expression of myosin heavy chain in myotubes derived from clonal cultures of chicken and rat muscle were consistent with the existence of two lineages of satellite cells that serve as the origin of slow and fast fibers (Cossu and Molinaro, 1987; Dusterhoft and Pette, 1993; Feldman and Stockdale, 1991; Hartley *et al.*, 1992; Hughes and Blau, 1992). On the other hand, slow and fast cell lineages were not revealed from similar studies in satellite cells of human postnatal muscle (Cho *et al.*, 1993; Edom *et al.*, 1994), but fusing or nonfusing muscle-colony-forming myoblasts were found in the human fetal limb (Hauschka, 1974). Serial subclonal study of quail muscle showed that the initial cell progeny of an individual fetal myogenic progenitor cell were of a fast cell lineage, whereas later progeny were a fast/slow cell lineage on the basis of myosin heavy chain expression (Schafer *et al.*, 1987). The clonal progeny of human satellite cell were found to be heterogeneous, using desmin and actins as markers (Baroffio *et al.*, 1995). The apparent contradiction among these different studies including this report may be due to the use of different markers, but it may also reflect differences in species and developmental stages.

In conclusion, only two cell lineages, fast and fast/slow types, were found in chicken skeletal muscle in respect to TnT isoform expression, which reflects the developmental stages and origins of the muscle cells and is intrinsically stable and reproducible, so that this protein can be a good marker of myogenic cell lineage, together with myosin heavy chain.

REFERENCES

Bandman E (1992) Contractile protein isoforms in muscle development. Dev Biol 154: 273–283

Baroffio A, Bochaton-Piallat M-L, Gabbiani G, Bader CR (1995) Heterogeneity in the progeny of single human muscle satellite cells. Differentiation 59: 259–268

Cho M, Webster SG, Blau HM (1993) Evidence for myoblast-extrinsic regulation of slow myosin heavy chain expression during muscle fiber formation in embryonic development. J Cell Biol 121: 795–810

Cooper TA, Ordahl CP (1984) A single troponin T gene regulated by different programs in cardiac and skeletal muscle development. Science 226: 979–982

Cossu G, Molinaro M (1987) Cell heterogeneity in the myogenic lineage. Cur Top in Dev Biol 23: 185–208

DiMario JX, Fernyak SE, Stockdale FE (1993) Myoblasts transferred to the limbs of embryos are committed to specific fibre fates. Nature 362: 165–167

Dusterhoft S, Pette D (1993) Satellite cells from slow rat muscle express slow myosin under appropriate culture conditions. Differentiation 53: 25–33

Edom F, Mouly V, Barbet JP, Fiszman MY, Butler-Browne GS (1994) Clones of human satellite cells can express *in vitro* both fast and slow myosin heavy chains. Dev Biol 164: 219–229

Feldman JL, Stockdale FE (1991) Skeletal muscle satellite cell diversity: Satellite cells form fibers of different types in cell culture. Dev Biol 143: 320–334

Hartley RS, Bandman E, Yablonka-Reuveni Z (1992) Skeletal muscle satellite cells appear during late chicken embryogenesis. Dev Biol 153: 206–216

Hauschka SD (1974) Clonal analysis of vertebrate myogenesis III. Developmental changes in the muscle-colony-forming cells of the human fetal limb. Dev Biol 37: 345–368

Hauschka SD, Konigsberg IR (1966) The influence of collagen on the development of muscle clones. Proc Natl Acad Sci USA 55: 119–126

Hughes SM, Blau HM (1992) Muscle fiber pattern is independent of cell lineage in postnatal rodent development. Cell 68: 659–671

Jolesz F, Sreter FA (1981) Development, innervation, and activity-pattern induced changes in skeletal muscle. Ann Rev Physiol 43: 531–552

Mashima J, Nakada K, Yao Y, Miyazaki J-I, Hirabayshi T (1996) Expression of chicken troponin T isoforms in cultured muscle cells. Zool Sci 13: 571–576

Miller JB, Stockdale FE (1986) Developmental regulation of the multiple myogenic cell lineages of the avian embryo. J Cell Biol 103: 2197–2208

Rutz R, Hauschka S (1982) Clonal analysis of vertebrate myogenesis VII. Heritability of muscle colony type through sequential subclonal passages *in vitro*. Dev Biol 91: 103–110

Schafer DA, Miller JB, Stockdale FE (1987) Cell diversification within the myogenic lineage: *In vitro* generation of two types of myoblasts from a single myogenic progenitor cell. Cell 48: 659–670

Schiaffino S, Reggiani C (1996) Molecular diversity of myofibrillar proteins: Gene regulation and functional significance. Physiol Rev 76: 371–423

Smillie LB, Golosinska K, Reinach FC (1988) Sequences of complete cDNAs encoding four variants of chicken skeletal muscle troponin T. J Biol Chem 263: 18816–18820

Stockdale FE (1992) Myogenic cell lineages. Dev Biol 154: 284–298

White NK, Bonner PH, Nelson DR, Hauschka SD (1975) Clonal analysis of vertebrate myogenesis IV. Medium-dependent classification of colony-forming cells. Dev Biol 44: 346–361

Yao Y, Nakamura M, Miyazaki J-I, Kirinoki M, Hirabayashi T (1992) Expression pattern of skeletal muscle troponin T isoforms is fixed in cell lineage. Dev Biol 151: 531–540

Yonemura I, Watanabe T, Kirinoki M, Miyazaki J-I, Hirabayashi T (1996) Cloning of chicken slow muscle troponin T and its sequence comparison with that of human. Biochem Biophys Res Commun 226: 200–205

(Received October 4, 1996 / Accepted November 27, 1996)