

# Differential Distribution of Myosin Isoforms among the Myofibrils of Individual Developing Muscle Fibers

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**Abstract.** Myosin was localized in situ in the posthatch chicken pectoralis using isoform-specific mAbs. The distribution among myofibrils was demonstrated by immunofluorescence and by immunogold EM. Fluorescein- or rhodamine-labeled antibody (12C5) specific for the head region (S1) of myosin was used as a marker to identify "embryonic" myosin. In longitudinal semithin frozen sections, a minority population of myofibrils stained intensely with 12C5. All other myofibrils in the same cell stained only weakly. Similarly, in Lowicryl-embedded ultrathin sections prepared for EM, a minority population reacted preferentially with gold-labeled 12C5. An antibody (5B4) specific for the rod portion of "neonatal" myosin reacted strongly with nearly all myofibrils, and this

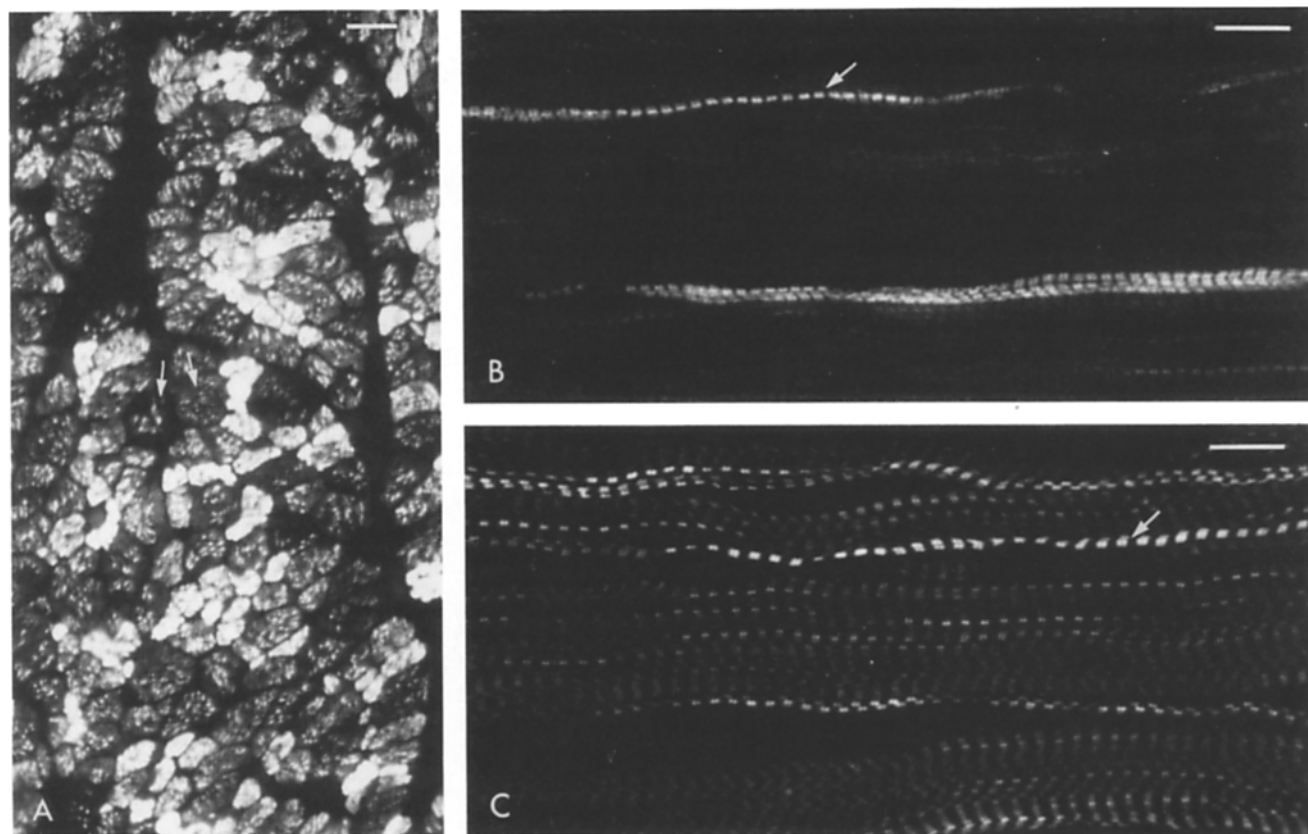
was evident by light and electron microscopy. A few of the fibrils that reacted strongly with 12C5 reacted weakly with 5B4. These observations demonstrate that an epitope reacting with 12C5 is more abundant in some myofibrils than in others within the same cell. Three categories of myofibrils can be identified by their relative proportions of embryonic and neonatal isoforms of myosin: in nearly all fibrils, a neonatal isoform predominates; in a minority population, embryonic and neonatal isoforms are both abundant; and in a few fibrils, an embryonic isoform predominates. It is concluded that there are distinct populations of myofibrils in which specific isoforms are segregated within an individual cell.

**D**URING normal skeletal muscle development, there are distinctive "fast" and "slow" isoforms of myosin that are expressed transiently. This has been demonstrated in a number of vertebrate muscles, but is especially well documented in the chicken pectoralis. Three general categories of fast heavy chain isoforms have been demonstrated in this muscle (Bader et al., 1982; Bandman et al., 1982; Lowey et al., 1983; Crow et al., 1983; Winkelmann et al., 1983). Because they make their appearance at specific developmental stages, they have been designated "embryonic," "neonatal," and "adult," according to the original observations on rat muscle cells (Whalen et al., 1981). However, they are not necessarily unique to each of these stages (Crow and Stockdale, 1986). As development progresses, different myosins appear in different populations of muscle fibers, giving rise to the "mosaic" pattern characteristic of most adult vertebrate muscles.

There is also some heterogeneity in the intracellular distribution of myosin isoforms. Different myosins may occupy separate locations in cultured chicken myotubes (Cerny and Bandman, 1986; Miller and Stockdale, 1986). In myotubes derived from the fusion of mouse and human myoblasts, a myosin heavy chain has been localized to the domains of the nuclei responsible for its synthesis (Pavlati et al., 1989). In adult rat muscle fibers, innervation by a "foreign" motoneuron can induce synthesis of a different myosin exclusively at the site of the newly formed endplates (Salviati et al., 1986).

Also, regional differences in myofibrillar ATPase are evident along the length of rabbit muscle fibers that have been chronically stimulated (Staron and Pette, 1987). It follows that, when there is a focal alteration of the myosin in a muscle fiber, some filaments will differ from nearby unaffected filaments. Recently, three categories of thick filaments were observed in whole-muscle homogenates prepared from the embryonic chicken pectoralis, each containing one or both of two myosin isoforms (Taylor and Bandman, 1989), but the differences were not demonstrated within individual cells. Studies on the nematode have shown that two different myosin isoforms occupy different sites along the thick filament (Miller et al., 1983), and this arrangement appears to be associated with two phases in the assembly of the thick filament (Epstein et al., 1985, 1986).

These observations contrast with recent in vitro studies of synthetic thick filaments that suggest that there is rapid exchange of myosin between filaments, and that unassembled myosin is in equilibrium with the filaments (Saad et al., 1986). This implies that there is a constant uniform turnover of myosin among existing thick filaments. This interpretation is supported by a recent study of myosin incorporation in cardiac muscle cells in vivo (Wenderoth and Eisenberg, 1987). Synthesis of the  $\alpha$ -myosin heavy chain can be switched off completely by propylthiouracil and then switched on again by thyroid hormone. Incorporation of the newly synthesized myosin heavy chain into the myofibril can therefore be fol-



**Figure 1.** Pectoralis, 7 d posthatch. (A) Transverse cryostat section, fluorescein-labeled anti-embryonic myosin, 12C5. (B) Longitudinal cryostat section, rhodamine-labeled 12C5. (C) Longitudinal ultracryomicrotome section, rhodamine-labeled 12C5. Some cells stain uniformly with 12C5, but the majority (arrows) show a particulate staining pattern (A). In longitudinal sections of the majority fiber, individual brightly-stained myofibrils (arrows) are evident among more numerous weakly stained myofibrils (B and C). The weak staining reflects small amounts of myosin rather than nonspecific binding of IgG to the A bands. Bars, 10  $\mu$ m.

lowed using a monoclonal antibody specific for this isoform as a marker. It was demonstrated, in ultrathin frozen sections, that between 24 and 96 h, there is uniform incorporation along the entire length of the thick filament, although there is initially a preferential addition at the ends of the filaments. This would be consistent with the incorporation of newly synthesized myosin into preexisting filaments during myofibrillogenesis.

In our studies of muscle development, we had observed a nonuniform distribution of myosin isoforms within individual muscle cells, which offered another system in which to examine the question of whether myosin is compartmentalized or rapidly exchanged. If the thick filaments or myofibrils of a single cell differ in their myosin composition, then rapid exchange *in situ* would seem unlikely. By using stage-specific mAbs to localize myosin *in situ*, we demonstrate here that, within individual muscle cells of the normally developing chick, there are distinct populations of myofibrils in which specific myosin isoforms are segregated.

## Materials and Methods

### Preparation of Muscle

Muscles were obtained from White Leghorn chickens. 1-mm strips of the homogeneous "white" region of the pectoralis major and the heterogeneous

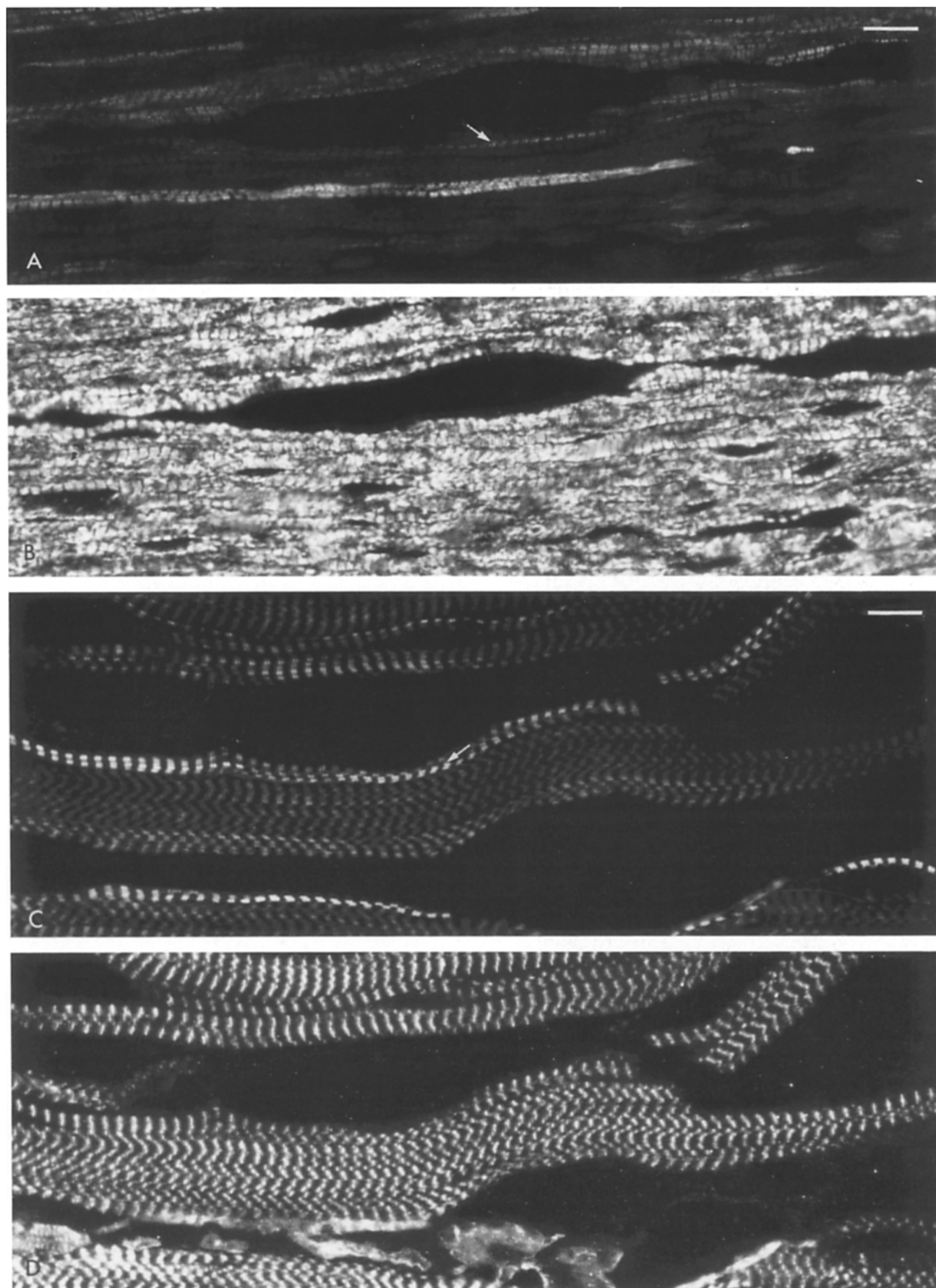
"red strip" (Gauthier and Lowey, 1977) were isolated from adult chickens (1–1.5-y) by blunt dissection, tied to wooden splints to prevent contraction, and either frozen or fixed as described below. Muscle from 7-d posthatch chicks was isolated from an area equivalent to the white region of the adult muscle and either fixed or frozen apposed to a specimen of adult red strip. The red strip served as a control for comparing the antibody response by the developing fibers with that of adult fibers that are known to exhibit either a positive or a negative reaction under identical conditions (Gauthier et al., 1982).

### Antibodies

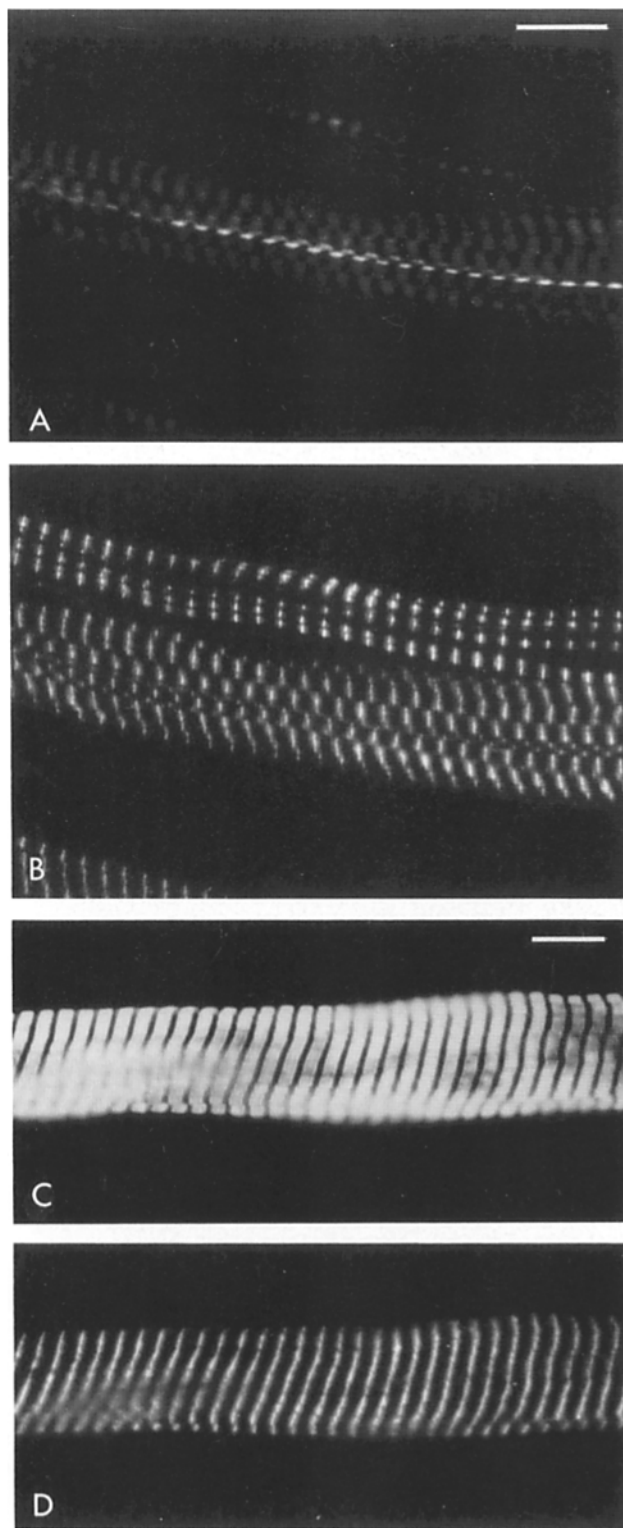
Stage-specific mAbs were a gift from Dr. Susan Lowey (Brandeis University). Antibodies against fast (white) myosin from the adult chicken pectoralis were prepared and assayed as described by Winklemann et al. (1983). Specificity was also demonstrated in sections of fast and slow fibers from the adult pectoralis red strip. Antibody (7C10) is specific for the fast myosin light chain, LC2, and it reacts with embryonic, posthatch, and adult myosin. Two antibodies (12C5 and 10H10) are specific for the amino-terminal 25-kD region of the heavy chain in the head (S1); they react with embryonic and adult myosin. An antibody (5B4) that is specific for neonatal myosin was prepared against myosin from the 19-d posthatch pectoralis; its epitope is located on the carboxy-terminal end of the rod (Lowey, S., personal communication). Another antibody (5C3) against the carboxy-terminal end of the rod is specific for adult myosin (Winklemann et al., 1983).

### Immunofluorescence

For semithin sections (0.5–1.0  $\mu$ m), tied strips of muscle were fixed in 3% paraformaldehyde in 0.1 M phosphate buffer (pH 7.4) at room temperature for 30 min, then removed from splints and fixed an additional 15 min, and



**Figure 2.** Pectoralis, 7 d posthatch. (A and B) Cryostat section double-stained with rhodamine-labeled anti-embryonic myosin, 12C5 (A) and fluorescein-labeled anti-neonatal myosin, 5B4 (B). (C and D) Ultracryomicrotome section double-stained with rhodamine-labeled 12C5 (C) and fluorescein-labeled 5B4 (D). Some myofibrils (arrows) react strongly with 12C5 (A and C), but nearly all myofibrils react with 5B4 (B and D). One myofibril reacts strongly with 12C5 (C, lower right) but weakly with 5B4 (D, lower right). 12C5 stains the entire A band except for the central bare zone, whereas staining with 5B4 is most intense at the bare zone. This is more obvious in ultracryomicrotome (D) than in cryostat sections (B). See also Fig. 3 B. Bar, 10  $\mu$ m.



**Figure 3.** Ultracyromicrotome sections. (A and B) 7-d posthatch pectoralis double-stained with mAbs 12C5 (A) and 5B4 (B). (C and D) Adult red strip double-stained with 12C5 (C) and 5B4 (D). In the posthatch muscle, a single myofibril is stained strongly with 12C5 (A), whereas all myofibrils react with 5B4 (B). Staining with 5B4 is most intense at a narrow stripe in the center of the A band and less intense laterally (B). In the adult muscle, unlike the posthatch muscle, all myofibrils within the fiber react with 12C5 (C) as well as with 5B4 (D). 12C5 stains the entire width of the A band (C), whereas 5B4 stains the center of the A band preferentially (D), similar to the posthatch muscle (B). Bar, 10  $\mu$ m.

rinsed in three changes of phosphate buffer, 15 min each, at 4°C. The washed strips were infused with 1M sucrose in phosphate buffer at 4°C for 1 h, followed by 2.3 M sucrose at 4°C for 1.5 h. 1  $\times$  1.5 mm blocks were mounted on aluminum specimen carriers (model 9701950; Reichert-Jung S. A., Paris), frozen directly in liquid nitrogen for 20 sec, and then stored in liquid nitrogen. Sections were cut dry, using glass knives, on an ultramicrotome (Ultracut E; Reichert-Jung S. A.) fitted with an FC4D cryokit at -40°C, retrieved in a drop of 2.3 M sucrose, and mounted on glass slides (see Tokuyasu et al., 1984). They were stored overnight in 1% goat serum in PBS at 4°C.

For cryostat sections, unfixed tied strips of muscle were frozen in isopentane cooled to -160°C with liquid nitrogen. Sections were cut at 2–4  $\mu$ m in a Harris refrigerated cryostat at -20°C.

Sections were incubated with unlabeled monoclonal antibodies (0.01 mg/ml), and then reacted with fluorescein- or rhodamine-labeled goat anti-mouse immunoglobulin (0.1 mg/ml protein) (Cooper Biomedical, Inc., Malvern, PA) as described previously (Gauthier and Lowey, 1979). For double labeling, sections were reacted sequentially. They were first exposed to an unlabeled mAb followed by fluorescein-labeled goat anti-mouse IgG. The second monoclonal antibody was labeled directly with rhodamine. In control sections, nonimmune mouse serum ( $\sim$ 0.01 and 0.05 mg/ml protein) was substituted for the primary antibody. To examine the effect of fixation on the staining pattern, unfixed cryostat sections were compared with serial sections fixed with paraformaldehyde or with paraformaldehyde and glutaraldehyde. After fixation, the response to both 12C5 and 5B4 was diminished overall, but the contrast between stained and unstained regions was enhanced, especially after paraformaldehyde and glutaraldehyde. The sections were examined with a Zeiss fluorescence microscope equipped with an epi-illumination system and a mercury HBO 100 W/2 lamp, and photographed using Kodak Technical Pan 35-mm film.

### Immunogold Electron Microscopy

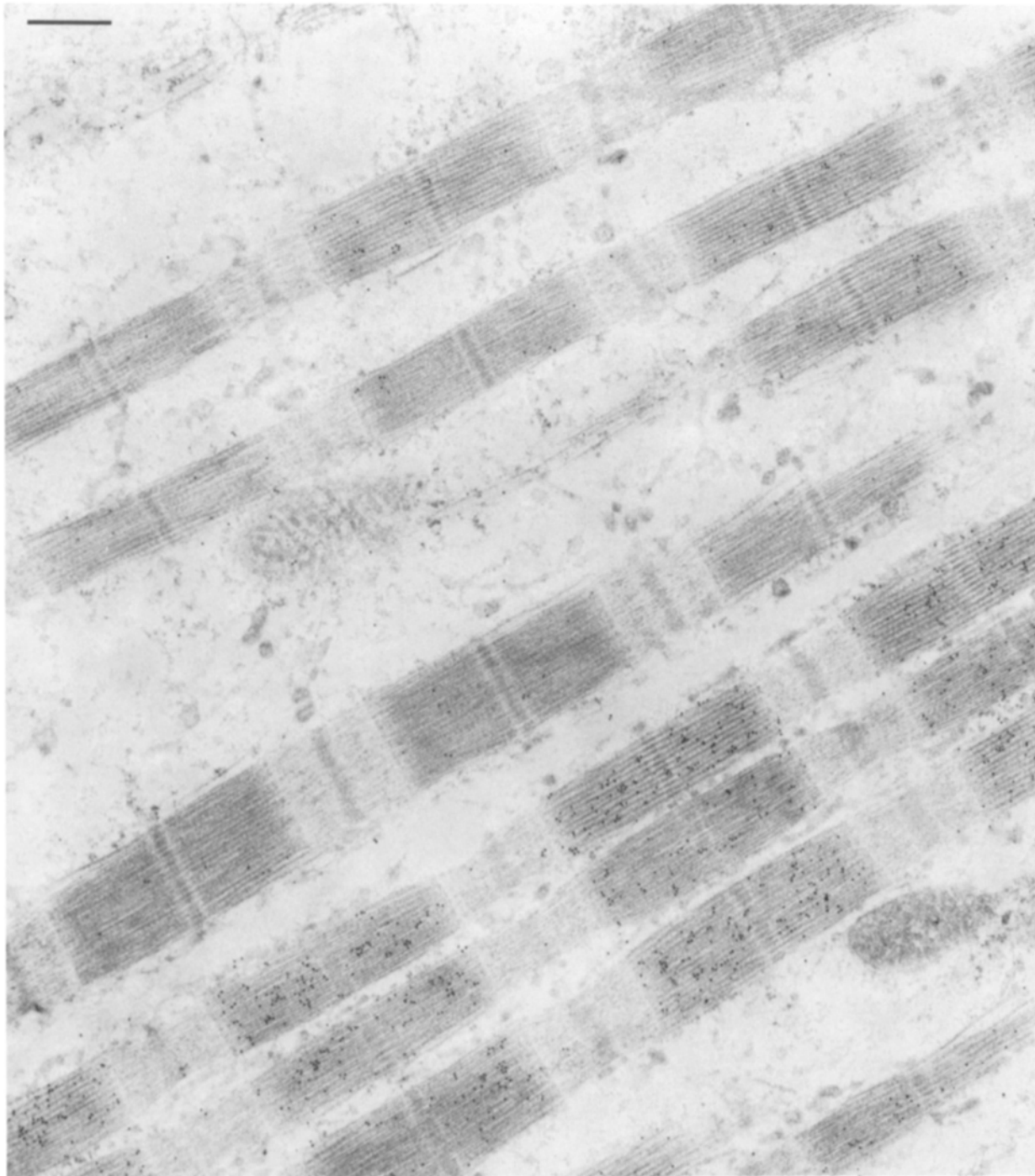
Muscle specimens were embedded in Lowicryl at low temperature and stained with gold-labeled antibody using adaptations of procedures described by others (Bendayan, 1983; Altman et al., 1984). Tied strips of muscle were fixed in 2% paraformaldehyde and 0.1% glutaraldehyde in 0.1 M sodium phosphate buffer (pH 7.4) at 4°C for 35 min, then cut into 1-mm<sup>2</sup> blocks and fixed for an additional 15 min. They were rinsed and treated with phosphate-buffered 0.5 M ammonium chloride at 4°C, then rinsed and dehydrated in increasing concentrations of dimethylformamide. They were infiltrated, while rotating, in a series of increasing proportions of Lowicryl K4M (Polysciences Inc., Warrington, PA) to DMF at 4°C, and embedded in Lowicryl using inverted BEEM capsules with tips removed. The capsules were placed under vacuum (650 mmHg) for 5 min, and covered with parafilm. Blocks were polymerized at 4°C using 15-W UV lamps (GE F15T8-BLB) at 10 cm from the specimens for 50 min, and then kept at room temperature under standard cold fluorescent lamps for 2–3 d.

Sections (60–90 nm) were cut on an ultramicrotome (model MT2-B; Sorvall, Inc., Norwalk, CT) and mounted on uncoated nickel grids. They were stained with primary antibody (0.01 mg/ml) for 2 h and then with 10-nm gold-conjugated goat anti-mouse IgG (0.03 mg/ml protein) (Janssen Life Sciences Products, Beerse, Belgium) for 1 h, then stained with uranyl acetate and lead citrate, and examined with a JEOL 100-CX electron microscope.

### Results

#### Localization of Myosin by Immunofluorescence

**Distribution of Embryonic Myosin among Myofibrils.** mAbs were used to localize myosin in situ in the pectoralis muscle ("white" region) of 7-d posthatch chicks. In 4- $\mu$ m transverse cryostat sections, some muscle fibers reacted strongly and uniformly with a fluorescein-labeled mAb (12C5) specific for the amino-terminal 25-kD region of the myosin heavy chain in the head (S1), but many fibers exhibited a particulate staining pattern. Intensely stained "dots" (transverse sections of myofibrils) were interspersed with "unstained" or weakly stained myofibrils (Fig. 1 A). The same stained and unstained sites were evident in sequential sections and at different planes of focus, indicating that they did not reflect sectioning

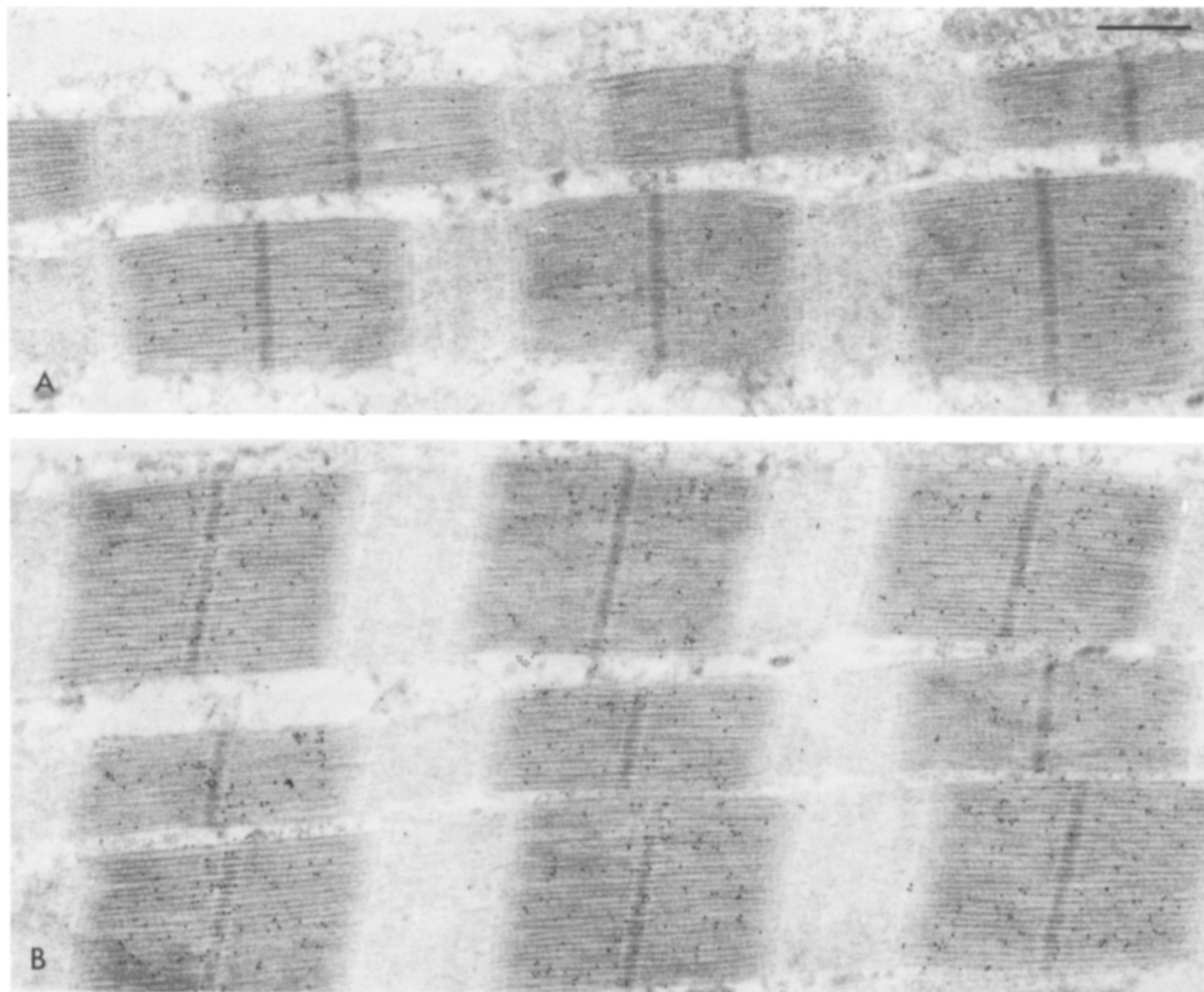


**Figure 4.** Pectoralis, 7 d posthatch. Electron micrograph showing response to gold-labeled anti-embryonic myosin, 12C5. Specificity is demonstrated by the absence of 10-nm gold particles from the I bands. 12C5 discriminates between two types of myofibrils: in myofibrils at the lower right, gold particles are localized to the A bands except for the central bare zone. In myofibrils at the upper left only a few gold particles are present in the A bands. Bar, 0.5  $\mu$ m.

at different positions along the length of the sarcomere. In 2- $\mu$ m longitudinal cryostat sections (Fig. 1 *B*) and in 0.75- $\mu$ m (semithin) ultracyromicrotome sections (Fig. 1 *C*), single myofibrils exhibited bright immunofluorescence, and they were contrasted by the surrounding more numerous weakly stained myofibrils. The transverse banding of the weakly stained sites indicated that they did not reflect an absence of myofibrils (Figs. 1, *B* and *C*). This was verified by double

staining with fluorescein-labeled antibody against the LC2 light chain of myosin (7C10) and rhodamine-labeled 12C5 (not illustrated). All myofibrils reacted with 7C10, whereas only certain of the same myofibrils reacted strongly with 12C5. In some muscle fibers, all myofibrils reacted strongly with 12C5 in longitudinal as well as transverse sections (Fig. 1 *A*), which indicates that differences in staining among fibrils are not related to the thickness of the portion of the





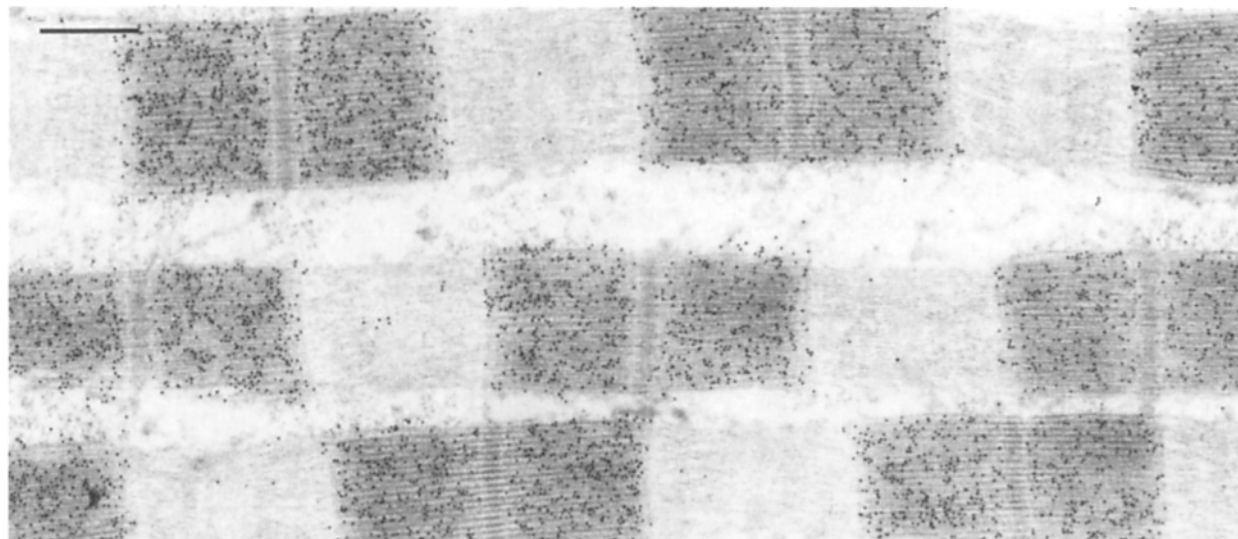
**Figure 5.** Pectoralis, 7 d posthatch. (A) Anti-embryonic myosin 12C5; (B) anti-neonatal myosin, 5B4. In A, 12C5 reacts more strongly with the lower than with the upper myofibril. Gold particles are excluded from the I bands and from the central bare zone. In B, all myofibrils react with 5B4. Gold particles are present in the central bare zone as well as in the lateral portions of the A band. Bar, 0.5  $\mu$ m.

fibril included in the section. Also, in a population of fast fibers in the adult pectoralis "red strip," all myofibrils reacted strongly with 12C5 (Fig. 3 C). Double staining, moreover, ensures that different responses to two antibodies are not related to the slight differences in thickness or plane of section that can occur in serial sections. A second antibody against the amino-terminal 25-kD region of Sl(10H10) exhibited the same selective staining pattern among myofibrils as that observed with 12C5. After double staining with 12C5 and 10H10, the same myofibrils reacted with both antibodies. Immunofluorescence was always confined to the A bands (see Figs. 1, B and C and 2 A), except for an unstained central region corresponding to the pseudo H or bare zone.

To determine whether the weak fluorescence in the majority population of myofibrils exposed to 12C5 was specific, control sections were treated with nonimmune mouse serum followed by rhodamine-labeled goat antimouse serum. There was some A-band staining in all myofibrils, but this was barely perceptible, even at a protein concentration five times that of 12C5. Hence the weak staining by 12C5 represents a small amount of myosin with that epitope rather than nonspecific binding of the IgG to the A bands.

Immunochemical analysis has shown previously that the anti-S1 antibody (12C5) reacts with both adult and embryonic myosin, but not with neonatal myosin (Winkelman et al., 1983). To confirm that the response to 12C5 described here is due to embryonic myosin, longitudinal semithin sections of posthatch pectoralis were reacted with an antibody (5C3), which is specific for adult myosin (Winkelman et al., 1983). The fibers were completely unreactive, except for a rare weakly stained fibril, indicating that the isoform that reacts with 12C5 at 7 d after hatching is most likely embryonic myosin.

**Distribution of Neonatal Myosin among Myofibrils.** An antibody (5B4) that is specific for the carboxy-terminal end of the neonatal myosin rod reacted strongly with nearly all myofibrils in the 7-d posthatch pectoralis (Fig. 2, B and D, and 3 B), in contrast to the differential staining by 12C5 of only certain of the same myofibrils (Figs. 2, A and C, and 3 A). A few myofibrils stained less intensely with 5B4 (Fig. 2 D) than with 12C5 (Fig. 2 C), although they were difficult to detect. Therefore, two developmental isoforms of myosin have different distributions among the myofibrils of the posthatch pectoralis. A neonatal myosin is the predominant



**Figure 6.** Pectoralis, 7 d posthatch. Antibody (7C10) against the myosin light chain, LC2. All myofibrils react strongly with 7C10. Numerous gold particles occur at the A bands except for the central bare zone. I bands are unreactive. Bar, 0.5  $\mu$ m.

form in most of the myofibrils; both neonatal and embryonic isoforms are abundant in a minority population; and in a few myofibrils, an embryonic isoform is predominant.

**Localization within the Myofibril.** The antibody (12C5) against embryonic myosin stained the entire A band except for the central bare zone (Fig. 1, *B* and *C*), whereas the antibody (5B4) against the neonatal isoform stained the bare zone intensely and the lateral regions only weakly (Figs. 2 *D* and 3 *B*). This pattern was more obvious in ultracryomicrotome sections (Figs. 2 *D* and 3 *B*) than in cryostat sections (Fig. 2 *B*). The enhanced contrast between stained and unstained regions in the ultracryomicrotome sections is most likely an effect of fixation, as aldehyde-fixed cryostat sections exhibited the same staining pattern (see Materials and Methods).

The differential staining of the central bare zone by the neonatal antibody, 5B4, is not unique to developing fibrils or to the neonatal antibody. The same pattern was exhibited when fast fibers in the adult pectoralis "red strip" were reacted with 5B4 (Fig. 3 *D*) and also when the adult "white" pectoralis was reacted with the adult-specific antibody, 5C3 which, like 5B4, is against the carboxy-terminal end of the myosin rod. In the assembled thick filament, the most reactive region might be expected to reside in the bare zone, where the absence of heads might increase accessibility to the antibody. The epitope that reacts with 12C5 is located in the head region (S1), which accounts for the lateral staining of the A band and the absence of staining at the bare zone.

### Ultrastructural Localization of Myosin

We have shown, by immunofluorescence, that embryonic and neonatal myosins occur in different populations of myofibrils within individual muscle cells. The same preferential distribution was evident when the cells were examined in situ by EM, confirming the validity of the observations with the light microscope.

**Embryonic Myosin.** Ultrathin sections of Lowicryl-embedded muscle were reacted with gold-labeled 12C5 and ex-

amined with the electron microscope. There was a differential staining pattern, similar to that observed with the light microscope. Overall, the concentration of gold particles was low, and this was most likely an inhibitory effect of the fixation process (see Materials and Methods). The degree of specificity, however, was high; gold particles were excluded from the I band and from the central bare zone. Some myofibrils were stained more heavily than others (Figs. 4 and 5 *A*). Hence 12C5 discriminates between two types of myofibrils, the more reactive being in the minority, which is consistent with the light microscopic observations. There was no relationship between the staining pattern and the position of the myofibril within the cell. In addition, all fibrils in sections serial to those reacted with 12C5, reacted uniformly and strongly with 7C10 (Fig. 6), as expected from the light microscopic observations. Again, specificity was demonstrated by the unreactive I bands and central bare zones. The differential response to 12C5 was validated, moreover, by the observation that all myofibrils in the fast fibers of the adult pectoralis "red strip" reacted with 12C5 (not illustrated).

**Neonatal Myosin.** The reaction of ultrathin sections of post-hatch muscle fibers with gold-labeled 5B4 was low overall, consistent with the diminished immunofluorescence in cryostat sections that had been fixed with paraformaldehyde and glutaraldehyde. There was a uniform positive response to 5B4 by all myofibrils (Fig. 5 *B*), and specificity was high, as demonstrated by the absence of gold particles from the I bands. Gold particles were present at the bare zone as well as in the more lateral portions of the A band, in contrast to 12C5-stained fibrils, where they were generally absent from the bare zone. However, there was no concentration of gold particles at the central bare zone, as might have been anticipated from the light microscopic observations. The difference may be related to the procedure. Additional antigenic sites lateral to the bare zone might be exposed by ultrathin sectioning, making the differential between central and lateral regions less obvious. The staining with gold-labeled 5B4 is probably a more accurate representation of the location of antigenic sites.

It is well established that myosins differ from cell to cell, even within a single muscle. Certain isoforms may be intrinsic to specific categories of myoblasts from their origin (Butler et al., 1982; Miller and Stockdale, 1986; Schafer et al., 1987), or their expression may be induced, at later stages of development, by external regulatory factors such as neuronal activity. The motor unit is one manifestation of such an influence. The isoform composition of each category of muscle cell is influenced by the type of nerve supply, and is associated with specific functional properties (see Gauthier, 1987). In this study, we have shown a similar heterogeneous pattern of isoforms at the subcellular level. Certain isoforms are more abundant in some myofibrils than in others within the same muscle cell. However, whereas individual cells are separated by plasma membranes, there are no structural barriers between myofibrils, apart from the discontinuous membranes of the sarcoplasmic reticulum. Spatial segregation of myosins among myofibrils would therefore have to be maintained by other factors in the surrounding sarcoplasm, for example, a mechanism involving the local distribution of specific mRNAs (see below). Unlike adult muscle fibers, developing muscle cells contain abundant ribosomes. They occur as polysomes and individual ribosomes located at the periphery of the cell, between myofibrils, and in close association with individual thick filaments (Przybylski and Blumberg, 1966; Gauthier and Schaeffer, 1974; Gauthier, G. F., unpublished observations).

The "compartmentalization" of different myosins observed in this study contrasts with *in vitro* studies of synthetic thick filaments from both smooth and skeletal muscle, which show that rapid exchange takes place between filaments and monomeric myosin in the medium (Saad et al., 1986; Trybus and Lowey, 1987). Similarly, *in vivo* studies of thyroid-induced incorporation of myosin indicate that there is uniform exchange along the sarcomere in cardiac muscle cells (Wenderoth and Eisenberg, 1987). Experiments with microinjected fluorescein-labeled myosin have shown that it can be readily exchanged along the full length of the A band in living chick myotubes (Mittal et al., 1987). However, although initial incorporation into the myofibril is rapid (within 10–15 min), further exchange is slow (Johnson et al., 1988). In native filaments isolated from whole-muscle homogenates of the embryonic pectoralis, there are differences in myosin isoform composition. It was shown that a small population of filaments contain only an embryonic myosin, while the remainder contain either a neonatal isoform or both isoforms together (Taylor and Bandman, 1989). However, it was not shown whether the different filaments originated from the same cell. We have demonstrated that there are differences in myosin composition among the myofibrils of the pectoralis after hatching. The differences are shown, moreover, within the same cell *in situ*.

The spatial separation of different myosins implies that assembly of the myofibril does not involve rapid exchange with a myosin "pool." The preferential localization of different isoforms could be indicative of local synthesis, and this would be compatible with the presence of abundant ribosomes among and within developing myofibrils (see above). Specific mRNAs for cytoskeletal proteins have been detected in nonmuscle cells by *in situ* hybridization, and their distribution suggests that they are closely associated with the respec-

tive proteins (Lawrence and Singer, 1986; Singer et al., 1989). Moreover, *in vitro* experiments with cultured chick muscle cells indicate that the association between myosin heavy chains and myofilaments occurs during translation (Isaacs and Fulton, 1987). Also, in muscles that have been induced to synthesize a new myosin isoform, the native protein appears on polysomes before being detected in the cytosol (Gagnon et al., 1989). These observations provide a strong indication that assembly of the thick filament takes place at the site of myosin synthesis. Myosin monomers would, accordingly, be assembled directly into filaments at these sites, an arrangement that would be compatible with the *de novo* formation of new filaments.

In conclusion, we have demonstrated, in the posthatch chicken pectoralis, that different isoforms of myosin are segregated into different populations of myofibrils within the same cell. The observations suggest that there is little or no rapid exchange between newly synthesized myosin monomer and preexisting filaments, and that new filaments may be formed directly at the sites of myosin synthesis during assembly of the myofibril.

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