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REMOVAL AND RECONSTITUTION OF Z-LINE MATERIAL IN A STRIATED MUSCLE

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Many detailed structural studies of the Z line have been reported (14, 13, 9, 16). Huxley (13) infers that tropomyosin is the principal component of the Z line. Evidence that tropomyosin is not confined to the Z line alone has been presented (8) and was previously suggested by Hanson and Lowy (10). If tropomyosin is located in the Z line in crystalline form and also associated with thin filaments in another form, then it would be necessary for two structural forms of the protein to exist in the myofibril. Cohen and Longley (5) have shown that two forms of tropomyosin can be obtained.

Corsi and Perry (6) extracted both actin and tropomyosin and removed Z lines and I-band material. Several reports (2, 8, 20) have shown that Z lines can be removed by trypsin treatment. Ebashi and Kodama's (7) work indicated that both tropomyosin and troponin are sensitive to trypsin; however, these authors reported that 50% of the tropomyosin in myosin B was unaffected by their conditions of hydrolysis. This was explained by the hypothesis that tropomyosin bound to myosin B was more resistant to tryptic attack than "free" tropomyosin. Since tropomyosin in the myofibril is probably associated with another structural protein, it seems equally plausible that tropomyosin may exhibit a relatively higher resistance to hydrolysis by trypsin.

The evidence that tropomyosin is the main component of the Z line was, therefore, not conclusive. Also, it has recently been proposed that α-actinine is a component of the Z line (4). We have studied the involvement of tropomyosin in the Z line by selective extraction of the myofibril followed by recombination of various protein fractions under defined conditions of ionic strength.

It was reported (19) that tropomyosin depressed the Ca^{++}-activated ATPase activity of reconstituted actomyosin, and we employed this observation to assay the protein fractions for tropomyosin.

MATERIALS AND METHODS

Rabbit psoas muscle which had been glycerinated for at least 30 days in the usual buffered (pH 7.0) 50% glycerol was the starting material. The tissue was teased into thin bundles (40–70 μ) of myofibrils in 2 mM Tris pH 7.6, 1 mM dithiothreitol at 0°C. After 1 hr, the teased myofibrils were transferred to fresh Tris-dithiothreitol solution and stored under nitrogen. The supernatant was then removed (after 10 days) for fractionation, and the myofibrillar bundles were removed to an aliquot of the Tris solution for rinsing. The bundles were transferred after 10 min to a 2.5% glutaraldehyde solution or were used for recombination experiments.

The fractions used for the recombination studies were those obtained at 40% ammonium sulfate satu-

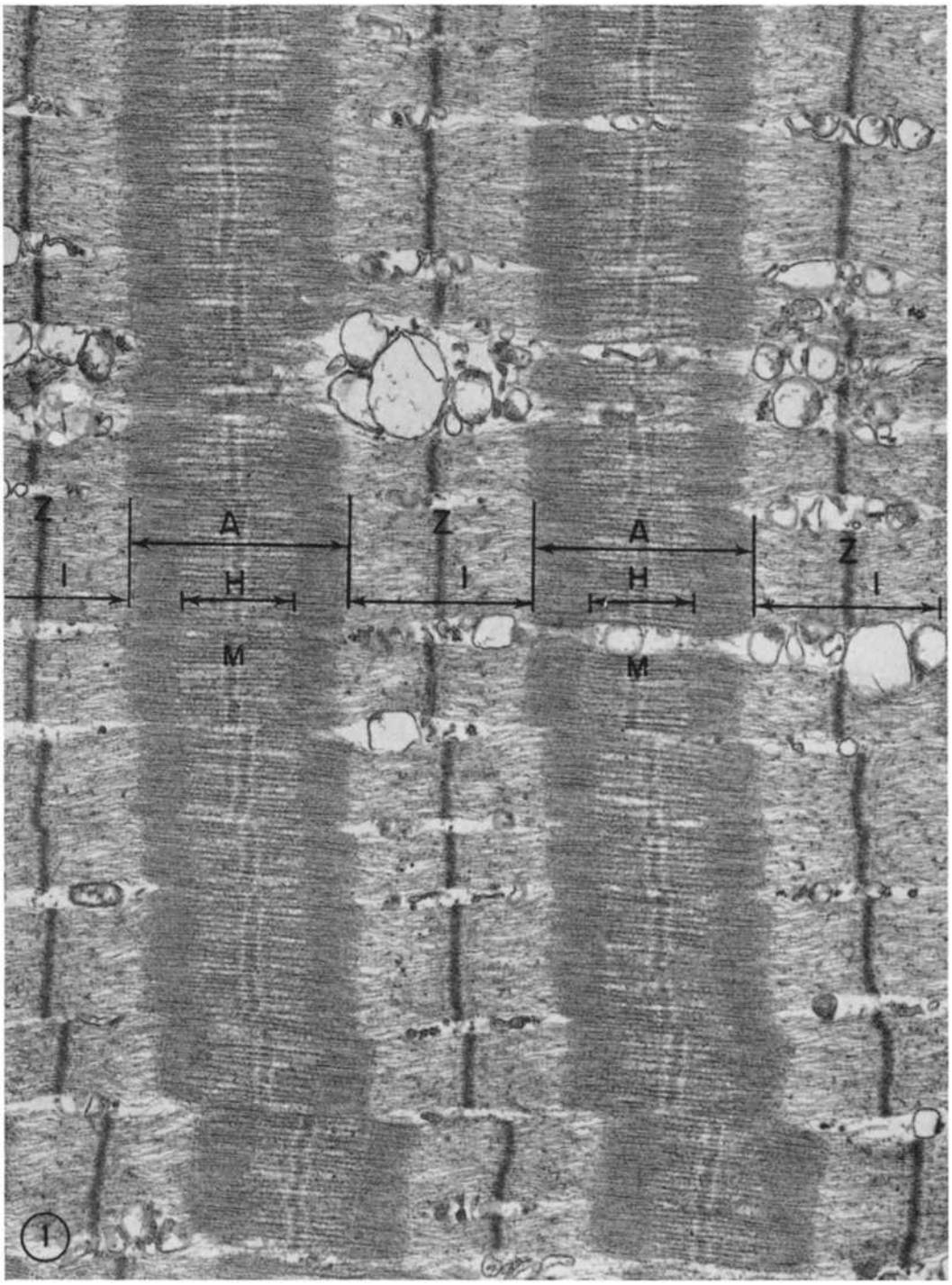

FIGURE 1 Glycerinated rabbit psoas muscle prior to extraction. All samples were fixed with glutaraldehyde and postfixed with osmium tetroxide. Sections were stained with uranyl acetate and lead citrate. *A*, A band; *I*, I band; *H*, H zone; *M*, M line; *Z*, Z line. × 26,000.

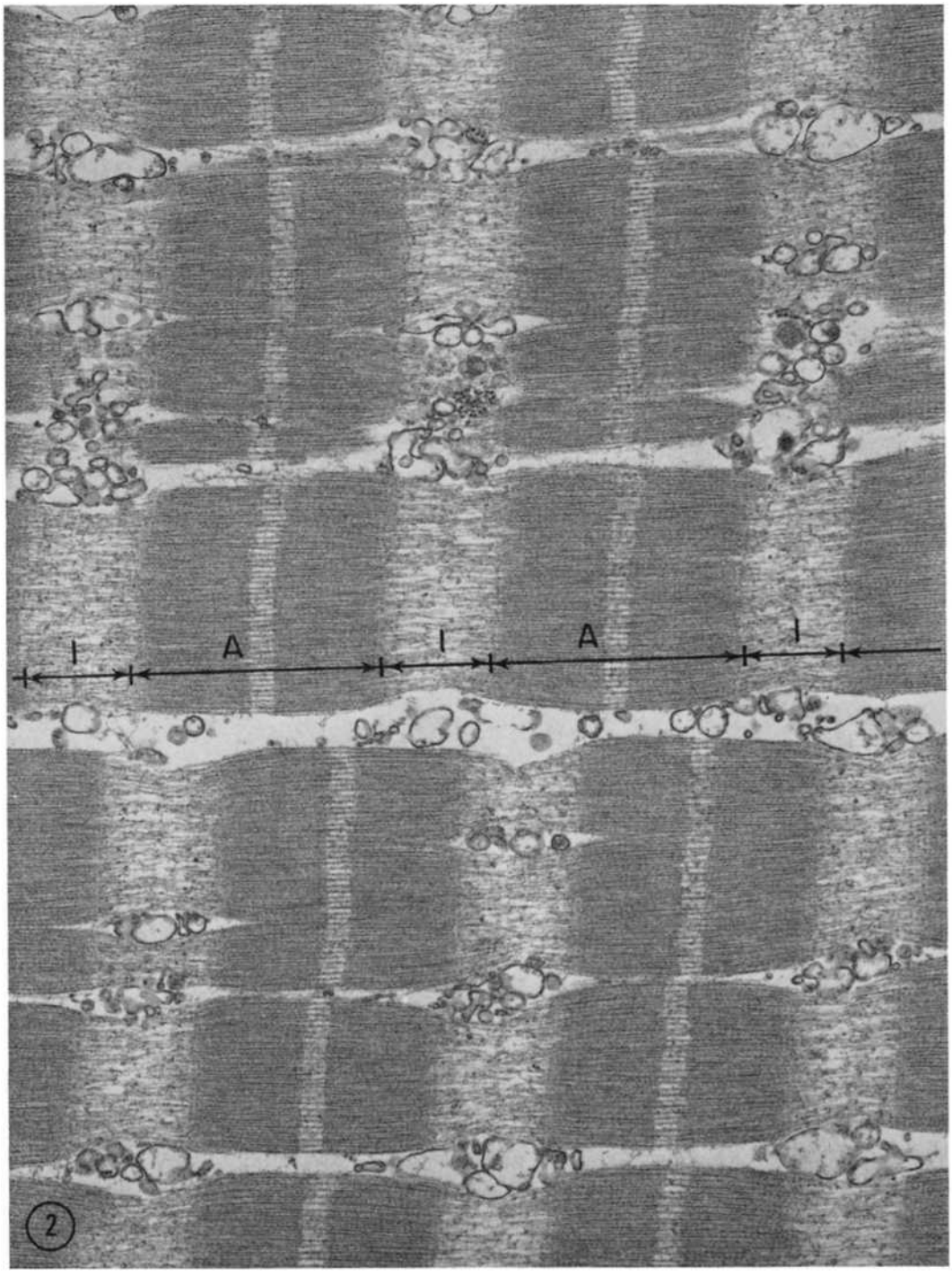

FIGURE 2 Typical morphology of teased glycerinated rabbit psoas fibers extracted for 10 days at 0°C with 2 mM Tris, 1 mM dithiothreitol, pH 7.6. Note absence of Z-line material and removal of most of the M-line material. *A*, A band; *I*, I band. × 26,000.

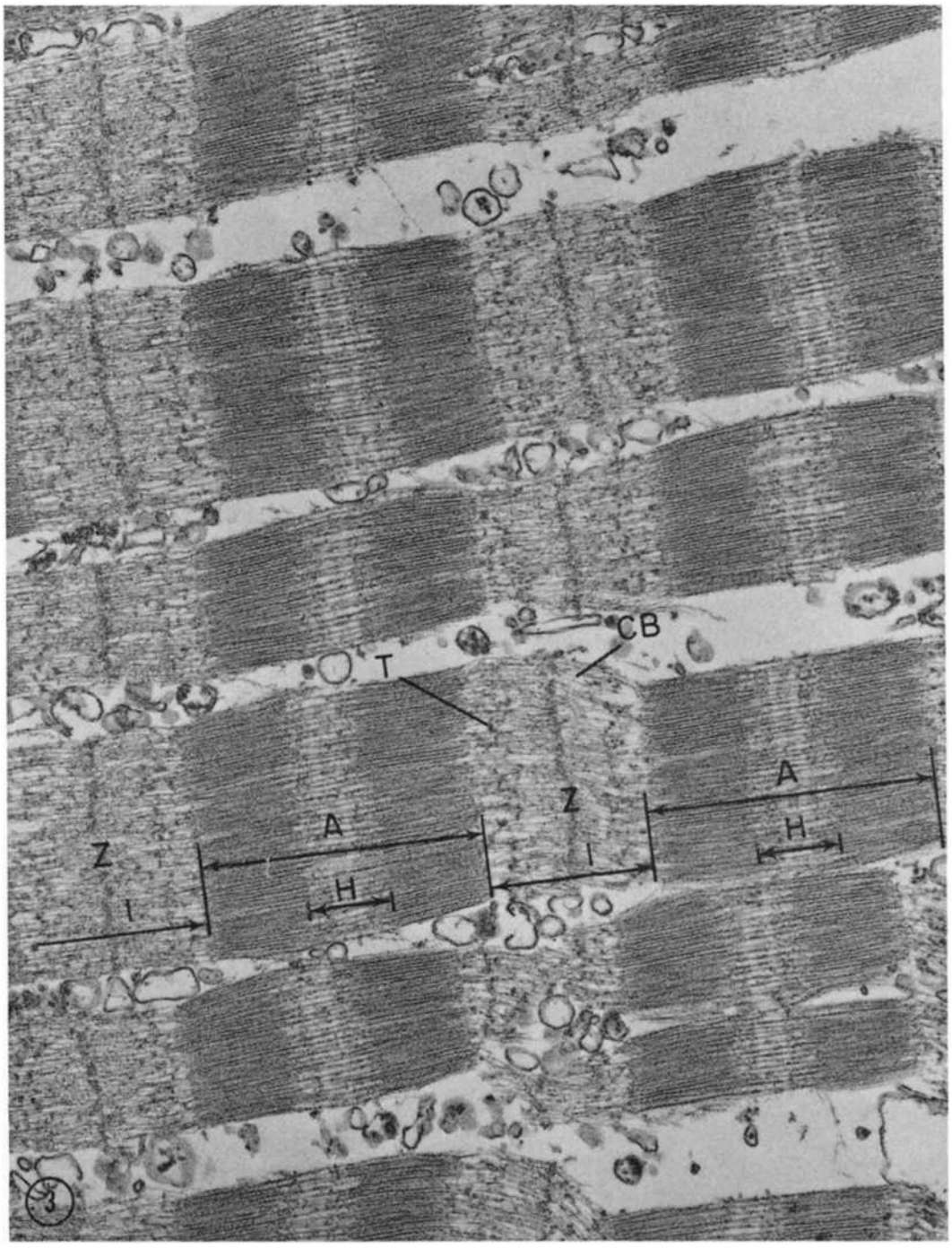

FIGURE 3 Replacement of Z-line material with an ammonium sulfate fraction (3.5 mg/ml) obtained at 40% saturation from the 10-day extract of psoas fibers. Prior to recombination, no Z lines were observed (see Fig. 2). Conditions during the 65-hour recombination period were 0.1 M KCl, 2 mM Tris, 1 mM dithiothreitol, pH 7.6, 0°C. *T*, tufts; *CB*, cross-bridges; *A*, A band; *I*, I band; *H*, H zone; *Z*, Z line. × 29,000.

ration from either the extract of the teased myofibrils after 8–10 days or from a Bailey extract (3) of natural actomyosin (11).[1] The final concentration of the fraction to be tested under recombination conditions was 3.5–4 mg/ml.

The tissue was fixed in 2.5% glutaraldehyde buffered with 0.02 M phosphate for 2 hr and was rinsed in the same phosphate buffer. While in the buffer, the teased bundles were cut into 1-mm lengths and transferred to 1% osmium tetroxide (Palade's fixative, 15). Dehydration in graded acetone preceded embedding in an Epon-Araldite mixture, as described by Anderson and Ellis (1) and modified to include 10% DER 732. Sections were stained with 2% uranyl acetate in methanol and poststained with lead citrate (17). A Philips 200 or a JEM 7 electron microscope was operated at 80 kv with a 30-μ objective aperture.

RESULTS AND COMMENTS

The usual morphology of glycerinated rabbit psoas prior to extraction is shown in Fig. 1. All the customarily observed bands of normal striated muscle are seen. These results and previous studies (12) on glycerinated skeletal muscle indicate that, while glycerination removes much of the soluble components, little, if any, removal of structural protein occurs.

Extraction for 8–12 days with 2 mM Tris pH 7.6, 1 mM dithiothreitol at 0°C results in removal of Z lines and most of the M-line material, as shown in Fig. 2. M-line material is removed more quickly by this method than Z-line material. The A band has not been disrupted but, when viewed obliquely, micrographs of these sections show that all that remains of the M line is one distinct line at each border of the M line. When extraction time is prolonged up to 21 days, there is a reduction in the number of thin filaments in the I band, and the remaining bridges which appear to form the single line at each edge of the M line are removed. These findings are substantially in agreement with those of Samosudova (18). Therefore, by controlling the time of extraction, it is possible to extract the M and Z lines and cause little other structural alteration.

Fig. 3 shows results of recombination experiments in which the fraction of the extracted Z-line and M-line material obtained at 40% ammonium sulfate saturation was made 0.1 M in KCl and then allowed to interact with previously extracted, teased bundles of psoas. Two striking features are apparent. First, there is a significant amount of material bound to the Z-line region of the extracted myofibril. At higher magnification, fine structure resembling that described by Knappeis and Carlsen (14) in normal Z lines is beginning to return, and beading similar to that seen in F-actin is visible along the thin filaments. Second, there are cross-bridges between adjacent thin filaments and tufts of material resembling the restored Z-line material bound in the I band. Alternatively, if one uses a 40% ammonium sulfate fraction of an extract of natural actomyosin,[1] Z lines are also restored but only tufts are observed on the thin filaments in the I band. In view of these experiments, it seems possible, by altering ionic conditions, to replace material in the Z-line region and, concomitantly, to observe cross-bridges or tufts in the I band. If one uses exhaustively extracted bundles of myofibrils which exhibit marked I-band damage for recombination experiments, Z lines are not restored but tufting is very prominent on the remaining thin filaments. Z-line formation, therefore, seems to occur when I filaments of adjacent sarcomeres are close enough to permit binding to each filament. This would indicate that both restoration of Z lines and tufting are dependent on availability of binding sites on the thin filaments.

The fractions obtained at 40% ammonium sulfate saturation were the most efficient in the restoration of Z lines. These two fractions were deficient in tropomyosin, as ascertained by their inability to inhibit the Ca^{++}-activated ATPase activity of reconstituted actomyosin, and exhibited no EGTA [ethylene glycol bis (β-aminoethyl ether)-*N*,*N*′-tetraacetic acid]–sensitizing factor activity for which tropomyosin is essential (7, 11). On the other hand, the fraction obtained between 40 and 60% ammonium sulfate saturation from the extract of natural actomyosin[1] which is known to contain tropomyosin had greatly reduced ability to reconstitute Z lines.

In summary, both Z-line and M-line material can be removed from glycerinated rabbit psoas muscle, and, by adjustment of ionic conditions, certain protein fractions restore Z lines and cause deposition of material in the I band. Experiments are presently underway to characterize more completely the extracted Z-line and M-line material.

[1] Mueller, H., and D. J. Hartshorne. Data in preparation.

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REFERENCES

1. ANDERSON, W. A., and R. A. ELLIS. 1965. *J. Protozool.* **12**:483.
2. ASHLEY, C. A., K. R. PORTER, D. E. PHILPOTT, and G. M. HOSS. 1951. *J. Exptl. Med.* **94**:9.
3. BAILEY, K. 1948. *Biochem. J.* **43**:271.
4. BRISKEY, E. J., K. SERAYDARIAN, and W. F. H. M. MOMMAERTS. 1967. *Biochim. Biopnys. Acta.* **133**:424.
5. COHEN, C., and W. LONGLEY. 1966. *Science.* **152**: 794.
6. CORSI, A., and S. V. PERRY. 1958. *Biochem. J.* **68**: 12.
7. EBASHI, S., and A. KODAMA. 1966. *J. Biochem.* **60**: 733.
8. ENDO, M., Y. NONOMURA, T. MASAKI, I. OHTSUKI, and S. EBASHI. 1966. *J. Biochem.* **60**:605.
9. FRANZINI-ARMSTRONG, C., and K. R. PORTER. 1964. *Z. Zellforsch. Mikroskop. Anat.* **61**:661.
10. HANSON, J., and J. LOWY. 1964. *Proc. Roy. Soc. (London) Ser. B.* **160**:449.
11. HARTSHORNE, D. J., and H. MUELLER. 1967. *J. Biol. Chem.* **242**:3089.
12. HUXLEY, H. E. 1957. *J. Biophys. Biochem. Cytol.* **3**: 631.
13. HUXLEY, H. E. 1963. *J. Mol. Biol.* **7**:281.
14. KNAPPEIS, G. G., and F. CARLSEN. 1962. *J. Cell Biol.* **13**:323.
15. PALADE, G. E. 1952. *J. Exptl. Med.* **95**:285.
16. REEDY, M. K. 1964. *Proc. Roy. Soc. (London) Ser. B.* **160**:458.
17. REYNOLDS, E. S. 1963. *J. Cell Biol.* **17**:208.
18. SAMOSUDOVA, N. V. 1966. Proceedings 6th International Congress on Electron Microscopy. Vol. 2. Maruzen Co., Ltd, Tokyo, Japan. 691.
19. SCHAUB, M. C., D. J. HARTSHORNE, and S. V. PERRY. 1967. *Nature.* **215**:635.
20. STROMER, M. H., D. E. GOLL, and L. E. ROTH. 1967. *J. Cell Biol.* **34**:431.