APPEARANCE OF ACTIN IN EMBRYOS OF BUFO REGULARIS REUSS
STUDIED BY IMMUNOFLUORESCENCE

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ABSTRACT
Synthesis of actin was studied using indirect immunofluorescence technique in embryos of Bufo regularis. The first reaction was ascertained at the stage with closed neurul tube as in the case of myosin. It therefore seems that actin appears at the same time and under identical chains of events, as does myosin. The data in the literature reporting that the appearance of actin preceded that of myosin could no be confirmed.

INTRODUCTION
In the previous study (Abo-Egla, 1984) used the indirect immunofluorescence technique to detect myosin in the embryonic stages of Bufo regularis. Both myosin and muscle specific proteins which were not characterized in detail, seemed to appear in c with morphological establishment of myotomes in cells which probably were seen between embryos of Bufo regularis and Xenopus laevis whereas in Bufo regularis myotomal cells fuse into myotubes almost immediately after the first appearance of the positive reaction indicating the presence of myosin in cells, in Xenopus laevis no formation of myotubes in the classical sense was observed until the stage of swimming larva, the elements of myotomes having been found to be mononuclear according to Ishimori, Yoshio, 1986, Bagni et al., 1990 and Burchfield et al., 1986. In the present paper, we are dealing with another contractile protein of muscle,
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the actin, in order to extend our knowledge on the terminal differentiation of the muscle cell of Amphibia in vivo.

Direct observations on the development of actin in Amphibia are almost missing the only findings are those of Ranzi and Citterio (1955) who by means of the precipitin test found a positive reaction at gastrula stage of Rana esculenta and those of Ogawa (1958, 1962, b) who using the same technique detected actin specificity in Triturus pyrrhogaster at the stage with several well-developed somites (stage 24 according to the normal table of Okada and Ichikawa which is comparable with Harrison's stage 24 to 25).

MATERIAL AND METHODS

Preparation of actin:

Actin was prepared by means of a technique which excluded contamination by tropomyosin in the following way. Actomyosin was first prepared from muscles according to the method of Kahlbrock Nass (1962) by extraction with 0.5 M KCl at pH 7.0 and was reprecipitated twice. The last precipitate was denatured with 10 volumes of acetone at room temperature collected, washed with acetone dried and stored in dessicator. Before use actin was extracted with approximately 10 volumes of distilled water at pH 7.5 (NaHCO₃). After centrifugation the pH was adjusted to 4.8 (30% acetic acid) and the precipitated actin collected by centrifugation. The precipitate was mixed with an equal volume of distilled water and after adding a small amount of NaHCO₃ the actin was dissolved. Eventual precipitates were removed by centrifugation. The solution prepared in this way was used for immunization and testing.
Identification of actin and control of its purity:

Actin preparation showed a single zone in electrophoresis in 8% acryl-amide gel in a continuous buffer system of 0.0192M glycine +0.0025M Tris (pH 8.9). When the usual systems of higher ionic strength were used, polymerization of actin took place and several zones appeared in electrophoresis.

Our actin preparation reacted with myosin of Bufo regularis forming the actomyosin complex detectable by a considerable rise in viscosity which ceased after the addition of ATP to make up a final concentration of 0.005M.

Preparation of antiserum:

An equivalent of 30-40 mg of actin was given subcutaneously to rabbits weighing 4-5 kg at weekly intervals for 3 months. One week after the last injection rabbits were bled serum collected, absorbed with frog kidney extract (0.15M NaCl tested for its specificity and stored at -25 °C.

Preparation of embryonic material:

Embryos of Bufo regularis were obtained after inducing ovulation and artificial insemination in the usual way. Eggs and embryos according to the normal table of Sedra and Michael (1961) were kept at 18°C staged according to Kopsch (1952) and fixed in cold (0-2°C) absolute ethyl alcohol. Further preparation of embryos the technique of immunofluorescence and control experiments were essentially the same as described previously (Abo-Egla, 1984).
RESULTS

Specificity of antiserum:
The antiserum gave only one precipitin line in the Ouchterlony (1949a) test in reaction both with actin preparation and muscle extract (0.15M NaCl or 0.5M KCl, pH 7.4). Myosin (0.5M KCl) gave no reaction. The antiserum was therefore, considered highly specific.

Appearance of actin specificity:
At stage 58 and 59 (3 and 8 somites) very faint fluorescence was observed in the very proximal myotomes in a small number of myotomic elements. The fluorescence was very weak, detectable at a higher magnification and was localized in the cytoplasm in a narrow zone adjacent to the cellular membrane. At stage 50 (15 segregated somites), the fluorescence could be detected in the proximal 7-8 myotomes and apart from its being still more pronounced in the periphery of (already formed) myotubes, a diffuse positive reaction appeared in the entire cytoplasm. At the same time, fluorescing unstriated very thin filaments could be found in the proximal myotomes.

Striated fibrils appeared at stage 61 (approximately 20 segregated somites, 12 showing fluorescence). With progressing development the fluorescence gradually became stronger reaching its maximum at stage 64. Beginning with stage 61 the fluorescing material became almost strictly localized in the confined areas in the cytoplasm of myotubes, no diffuse fluorescence could be observed figs. (1-6).
DISCUSSION

Comparison of the observations made in the present experiments with those of the development of myosin.

Abo-Egla (1984) revealed a considerable difference in the intensity of fluorescence at all stages examined. In no case did it attain the brightness which was usual beginning with stage 61, when anti-myosin was used. The possible explanation is that the amount of actin represents less than half of the amount of myosin present in the myofibril, although differences of an immunochemical nature (e.g. different degree of antigenicity) should be kept in mind. There is however, no proper basis for more concrete speculations. In the majority of the cases fluorescence was also localized in areas in the cytoplasm of myotubes and no diffuse fluorescence was seen. In the longitudinal sections, the striation of myofibrils remined strongly the pictures obtained by Pepe (1966) after absorption of anti-actin antiserum with F-actin or tropomyosin, when fluorescence was compared with that after application of anti-myosin antiserum also testricion in the amount of fluorescing material could be seen which was based on the molecular arrangement of the contractile proteins in the myofibril.

In our experiments, the positive reaction indicating the presence of actin was first seen at stage 58 and 59. It was very faint but distinct at a high magnification. Any unspecific staining could be excluded as no similar fluorescence could be found in other tissues or in control preparations. It seems, therefore, that actin appears at the same time and under identical morphological chains of events as does myosin Abo-Egla (1984).
The observation of Ranzi and Citterio (1955) who found specificities both for actin and myosin of gastrula, is difficult to compare with our results obtained by a different technique. It is however, also inconsistent with contemporary conceptions to expect terminal differentiation at such an early stage. A direct comparison with the results of Ogawa (1958, 1962, b) who worked with embryos of Triturus, is also hardly possible to make, as it is not entirely clear at what stage he has found actin specificity, 132 hours of development at 18°C (at 20±1°C according to Ogawa, 1958) represents, according to the normal table of Okada and Ichikawa (1947) stage 24 which is roughly comparable with Harrison's stage 24-25. At this stage several myotomes are already segregated and a relatively late appearance of actin specificity in his experiments can be explained by the differences in the sensitivity of his techniques. On the other hand, our experiments on urodela (Hurychova et al., 1973, in preparation) seem to show a relatively late appearance of both actin and myosin in his group approximately at Harrison's stage 29-30. We have not observed the appearance of actin prior to that of myosin. We should of course consider the electron microscopic observations (e.g. Allen and Pepe, 1965, Obinata et al., 1966, Przybylski and Blumberg, 1966). Suggesting that the synthesis of actin precedes that of myosin in the chick embryo.
Fig. 1: Longitudinal section of the front part of stage 59 embryo showing faint fluorescence in the peripheral zone of the myotomal elements.

Fig. 2: Cross section of stage 60 embryo, fluorescence in the peripheral zone of myotomal elements. 
N = Nervous, Ch = chorda
Fig. 3: Cross section of stage 62 embryo

Fig. 4: Cross section of stage 64 embryo.
Fig. 5: Longitudinal section of stage 63 embryo showing both unstriated and striated filaments in distal myotomes.

Fig. 6: Longitudinal section of stage 64 embryo showing striated myofibrils.
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دراسة ظهور الاكتئب في أجنة الصدمة الصغرية الرطبة

باستخدام طريقة التلورسنت الغير مباشرة أمكن دراسة ظهور الاكتئب في أجنة الصدمة الصغرية الرطبة ابتداءً من المرحلة اليرقية 68، 59، 61، 62، 63، 64، والتفاعل الأول بينها عند المرحلة والتي يتم عندها تحلق الأنبوبية المصيرة مثل ما حدث في المييسين.

بناءً عليه يكون ظهور الاكتئب في نفس الوقت في سلاسل مشابهة والنتائج التي خرج بها البحث تدل على أن ظهور الاكتئب يسبق المييسين.