THE IN VIVO AGGREGATION OF CHICK EMBRYO RIBOSOMES IN RESPONSE TO LOW TEMPERATURE[1]

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Ribosomes which have been isolated from the cell can aggregate in vitro under a number of conditions, such as extremes of pH and salt concentration. (Hamilton and Peterman, 1959; Tissieres, et al 1959; and others). In vivo ribosomal aggregates apparently are mostly polysomes with the ribosomes held together by the messenger RNA which they are translating. An aggregate of four ribosomes which fit into neither of these categories was isolated from chick embryo feathers (Humphreys, et al 1964). The number of these curious aggregates in a homogenate was unaffected by any of the conditions which artificially cause aggregation of extracted monomer ribosomes. Nor did these aggregates break down in response to small quantities of ribonuclease or participate in protein synthesis in the way characteristic of polysomes. Because the aggregates appeared to increase in the feather during morphogensis and then to disappear during an important stage in the keratinization of the feather, it was hypothesized that these aggregates were special polysomes involved in the storage of messenger RNA for the developmental regulation of protein synthesis. This hypothesis was further supported by the first appearance of long-lived messenger RNA when these unusual aggregates disappeared as though the latter were yielding the messenger RNA. This original hypothesis and correlation now appear incorrect.

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A number of experiments indicated that the ribosomes of these unusual aggregates did not contain messenger RNA in any form (Humphreys, unpublished data). No messenger can be demonstrated either physically or by stimulation in in vitro protein synthesizing systems under numerous conditions. However, the true nature of these aggregates was first suspected by Byers (1966) when his electron micrographs revealed sheets of four-ribosome aggregates in chick embryo tissue which had been cooled to 0°C for other purposes. Our experiments quickly confirmed that the four-ribosome aggregates were formed in the cells of the chick embryo feather in response to the low temperatures used in our experiments during the dissection of the feathers. The physical or chemical nature of this cold induced aggregation and the reasons for its disappearance during development are unknown.

MATERIALS AND METHODS

Feathers were collected from embryos of 12 days incubation. Procedures for isolation of polysomes from feathers dissected at 0°C have been previously described (Humphreys, et al, 1964). The dissection of feathers at 0°C usually took from one to three hours.

Embryos from which feathers were to be dissected at 37°C were washed in Weymouth medium with two per cent fetal calf serum at 37°C. The feathers were removed in the same medium and were held at 37°C until all feathers were collected. This dissection was always completed within one hour. The feathers were then washed two more times in the medium, centrifuged, resuspended in hypotonic buffer at 0°C as previously described. All subsequent operations were at 0°C as previously described.

RESULTS

The size distribution of polysomes obtained when a cytoplasmic extract of 12 day chick feathers dissected at 0°C was centrifuged on a sucrose gradient is shown on Figure 1a. The most striking feature of the polysome gradient is the dominant peak of four-ribosome polysomes. The distinctiveness of this peak of

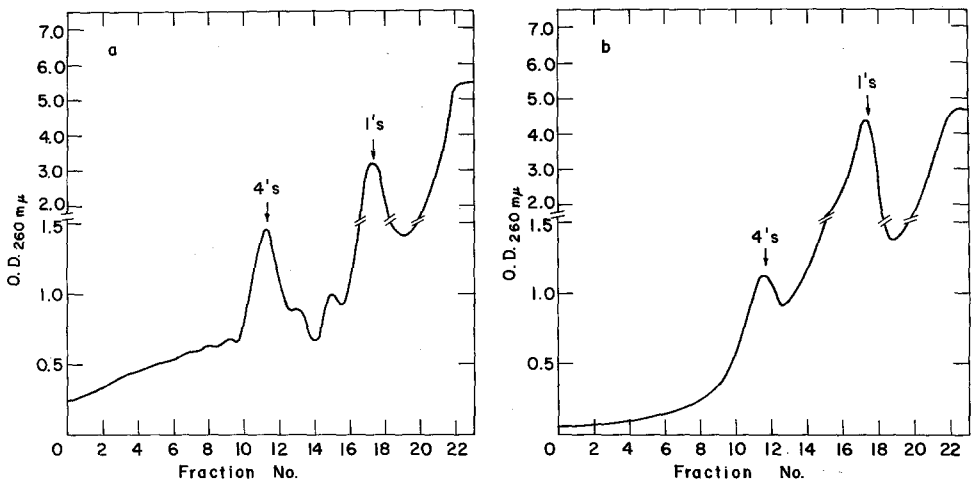

Figure 1. Cytoplasmic extracts prepared from embryo feathers dissected at 0°C and sedimented for 3 hours at 24,000 rpm on 15 to 30% linear sucrose gradients (a) no ribonuclease (b) 0.1 ugm ribonuclease per ml.

four-ribosome polysomes was enhanced greatly if the extract was treated with 0.1 μgm ribonuclease. This treatment dissociated all the polysomes except the majority of the four-ribosome polysomes (Figure 1b). Previous studies show that this ribonuclease-resistant peak does not synthesize protein although it is composed of aggregates of four-ribosomes (Humphreys, et al, 1964; Bell, et al, 1965).

If the feathers were dissected from chick embryos in Tyrode's solution which had been maintained at 37°C during the operations, nearly all ribosomes extracted from these feathers were monomers; there were virtually no polysomes. This was taken to indicate that polysomes broke down during incubation of the feathers in Tyrode's at 37°C.

If, instead, the feathers were dissected at 37°C in a complete nutrient medium with 2% serum, a considerable portion of ribosomes were in aggregates (Figure 2a). However, in this case the peak of four-ribosome aggregates did not predominate. Moreover, when the extract was treated with ribonuclease all ribosomes became monomers (Figure 2b). The ribonuclease-resistant peak of in-

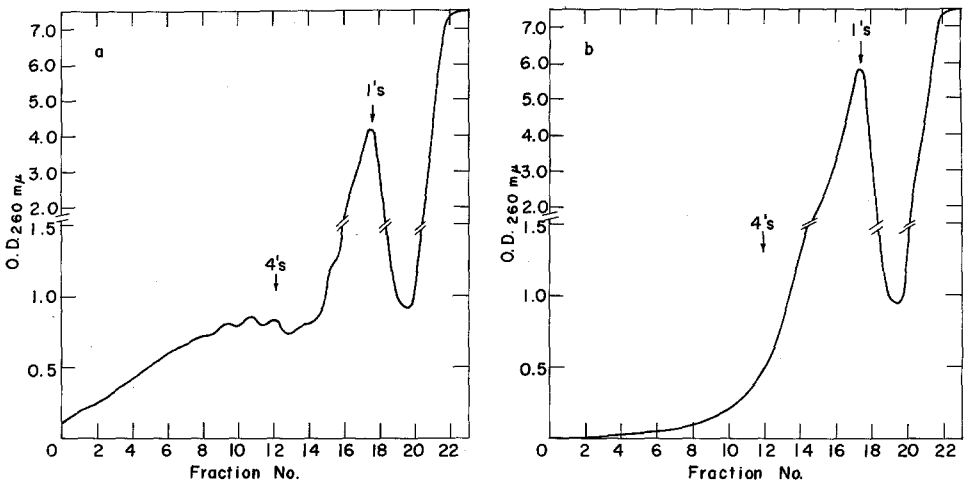

Figure 2. Cytoplasmic extracts prepared from chick embryo feathers dissected at 37°C and sedimented for 3 hours at 24,000 rpm on 15 to 30% linear sucrose gradients. (a) no ribonuclease (b) 0.1 ugm ribonuclease per ml.

active ribosomal aggregates, thus, did not appear in these feathers which had been dissected in 37°C. If graphs of an extract from feathers dissected at 37°C and at 0°C were compared quantitatively it could be easily seen that the presence of ribonuclease-resistant, four-ribosome aggregates was associated with a decrease in the single ribosomes only. This indicated that the ribonuclease-resistant structures were aggregates of single ribosomes induced by low temperatures.

Since the aggregation of the ribosomes at low temperature might be an inherent characteristic of chick embryo ribosomes, it was of interest whether the aggregates could be induced in vitro by low temperature after homogenization. No aggregates formed in homogenates of feathers dissected at 37°C even if they were held for two hours at 0°C. Chick embryo ribosomes maintained at 0°C in numerous other solutions containing up to 0.01 M magnesium ions, 0.1 M sodium ions, 0.1 M potassium ions, 0.1 M ammonium ions, and tris buffer in various combinations and concentrations at pH 7.4 have never been observed

to form aggregates of four ribosomes.

DISCUSSION

These data show that the ribonuclease-resistent aggregates of four ribosomes appearing in chick feathers (Humphreys, et al, 1964; Bell, et al, 1965), are formed *in vivo* in response to low temperature. The apparent tissue specificity of the aggregation noted earlier (Humphreys, et al, 1964) was probably due to the rapidity with which the tissues other than feather could be dissected. This conclusion has been affirmed by electron microscopy which shows that the aggregates are not present in normal chick tissues, but appear rapidly in several other tissues when cooled (Byers, 1966). The basic mechanisms causing this aggregation or its physiological function in the chick embryo, which normally experiences some cooling in development, are obscure.

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