the parathyroid tissue alone in *totally thyroidectomized* animals and therefore observed no effect. Again, hypercalcemic perfusion of thyroid tissue alone in dogs or sheep has consistently failed to elicit a hypocalcemic response (*1*, *2*), but the simultaneous perfusion of both thyroid and parathyroid in dogs, sheep, and goats (*1*, *2*, *4*, *6*) has consistently produced a drop in the calcium concentration of the plasma. Further, this hypothesis allows for the observation of Copp (*1*) that some extracts of parathyroid glands produced a momentary drop in the concentration of calcium in the plasma of intact dogs and explains their effect as that of extracted TCRF, mediated by the dog's own thyroid tissue.

Care recently found that both the hypercalcemic perfusion of parathyroid tissue alone in some surgically thyroidectomized sheep (*9*) and the hypercalcemic perfusion of surgically isolated thyroid tissue in two pigs (*8*) produced a drop in the plasma calcium. In neither case, however, was the elimination of the other tissue ascertained by the demonstration of subsequent deficiency states or by serial sections of the perfused tissue. In view of our hypothesis it seems desirable that any further perfusion experiments should completely rule out the presence of one or the other tissue in the system.

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Configuration of Inactive and Active Polysomes of the Developing Down Feather

Abstract. *Inactive four-ribosome polyribosomes, insensitive to ribonuclease, have the form of tight symmetrical squares and appear in feather cells during the early and intermediate periods of development. When, on the 13th day of incubation, the inactive polyribosomes become sensitive to ribonuclease and can then synthesize protein, the squares open up so that the four-ribosome polysomes are strung out in the configuration characteristic of functioning polysomes.*

Inactive ribonuclease-resistant polysomes which are converted to long-lived, functioning, ribonuclease-sensitive polysomes are found in chick down feathers during their differentiation (*1*). This conversion takes place during the critical transition period when "keratinization" of the feather begins around the 13th day of incubation of the egg. This is the time when disulfide bonds can be first detected histochemically; the feather first

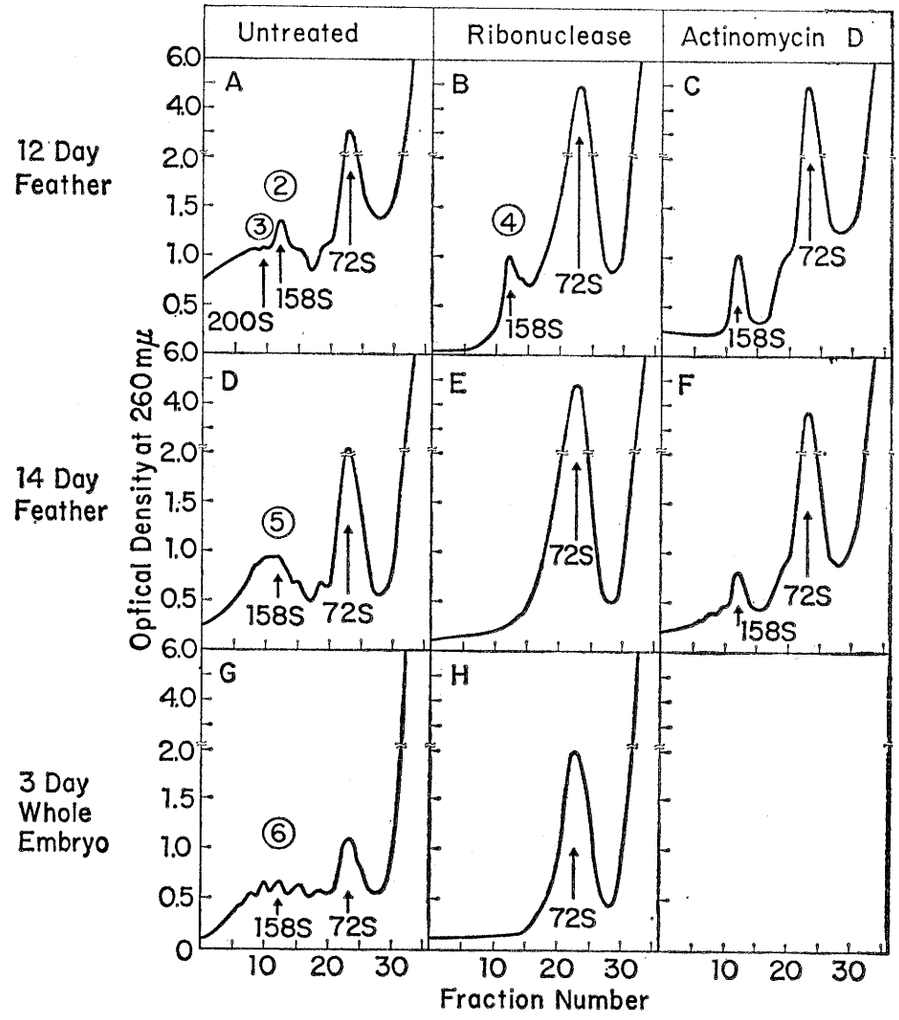

Fig. 1. Optical density at 260 mμ of cytoplasmic extracts of feathers or whole embryos as displayed in sucrose gradients. Numbers refer to electronmicrographs of the fraction. Where indicated, extracts were of feathers treated in vitro with 60 μg of actinomycin D per milliliter of Waymouth medium for 18 hours. Others were untreated, and some untreated extracts were incubated at 4°C for 30 minutes with ribonuclease (4 μg/ml). Extracts were layered on a linear sucrose (15 to 30 percent) gradient and centrifuged for 3 hours at 24,000 rev/min in an SW 25 rotor (model L Spinco). The optical density of gradients was read at 260 mμ in a Gilford continuous-flow spectrophotometer. Treatment of 14-day control feathers (*E*) or 3-day whole embryo (*H*) extracts with ribonuclease resulted in complete loss of optical density due to the polysomes whereas treatment of 12-day feathers dispersed all polysomes except those which sedimented at 158*S*.

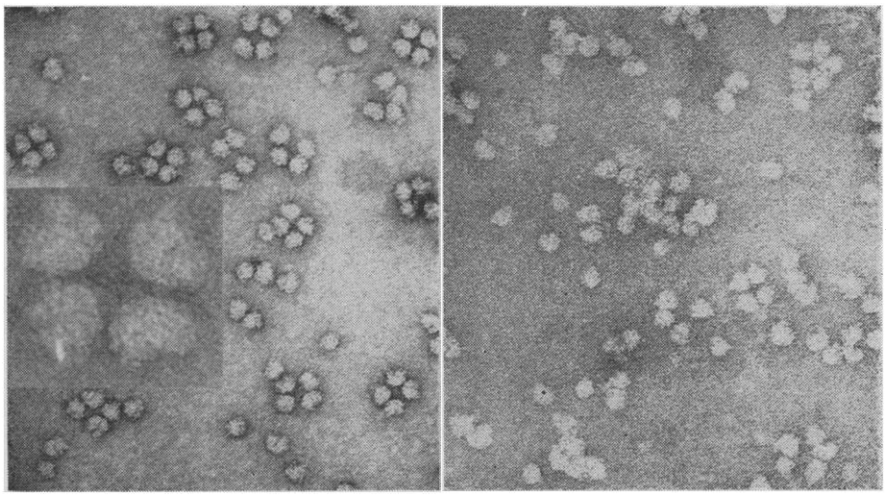

Fig. 2 (left). (see Fig. 1*A*) Polysomes from cytoplasmic extract of 12-day feathers taken from the region of four-ribosome polysomes sedimenting at 158*S*. They are predominantly ribonuclease-insensitive tight symmetrical squares (× 100,000). Insert: An "inactive" polysome enlarged (× 400,000). Fig. 3 (right). (see Fig. 1*A*) Large polysomes from the same cytoplasmic extract as that from which material described in Fig. 2 was taken. They sediment at about 200*S* and they consist mainly of chains of ribosomes (× 100,000).

gives the x-ray diffraction pattern characteristic of β-keratin (*2*), a new feather-specific antigen appears (*3*), the rate of incorporation of cystine into protein increases sharply, and the frequency of cell divisions reaches a maximum and begins to decrease (*4*). We now report the unusual configuration of the inactive polysomes and the change which occurs in this configuration when they are activated.

The experimental demonstration of these inactive and long-lived active polysomes has been reported (*1*, *5*). To summarize briefly: cytoplasmic extracts—from skin or feathers of chick embryos aged 5½ days to 13 days—when sedimented in a sucrose gradient give an unchanging, tissue-specific curve of optical density at 260 mμ, due to polysomes. The feature which dominates the optical-density profile of developing-feather extract and distinguishes it from that of other tissues is a peak at 158*S*, the region where four-ribosome polysomes sediment. About 70 percent of the material in this peak remains in 12-day feathers after the feathers are treated in vitro with actinomycin D (60 μg/ml) for 24 hours. All other polysomes break down during the 24-hour treatment. The same percentage of the material in this peak is also resistant to ribonuclease (4 μg/ml); all other classes of polysomes in the feather extract are sensitive to this amount of the enzyme. The peak of optical density of material sedimenting at 158*S* which remains after treatment of feathers with actinomycin D, or after treatment of the cytoplasmic extract with ribonuclease, has no radioactivity associated with it when feathers are pulse-labeled with radioactive protein precursors. This indication that no protein synthesis is associated with these polysomes is the criterion for designating the polysomes as nonfunctional. In feathers older than 13 days the polysomes resistant to treatment with actinomycin D also sediment at 158*S*, but they rapidly incorporate radioactive amino acids into protein and are sensitive to ribonuclease. In these respects they are equivalent to normal functional polysomes observed in many other cells.

To determine whether inactive polysomes are structurally different from those which are actively synthesizing protein, samples of polysome fractions from various extracts were prepared for electron microscopy (*6*).

Polysomes taken from two zones of a sucrose density gradient of an untreated cytoplasmic extract of 12-day feathers are shown in Figs. 2 and 3.

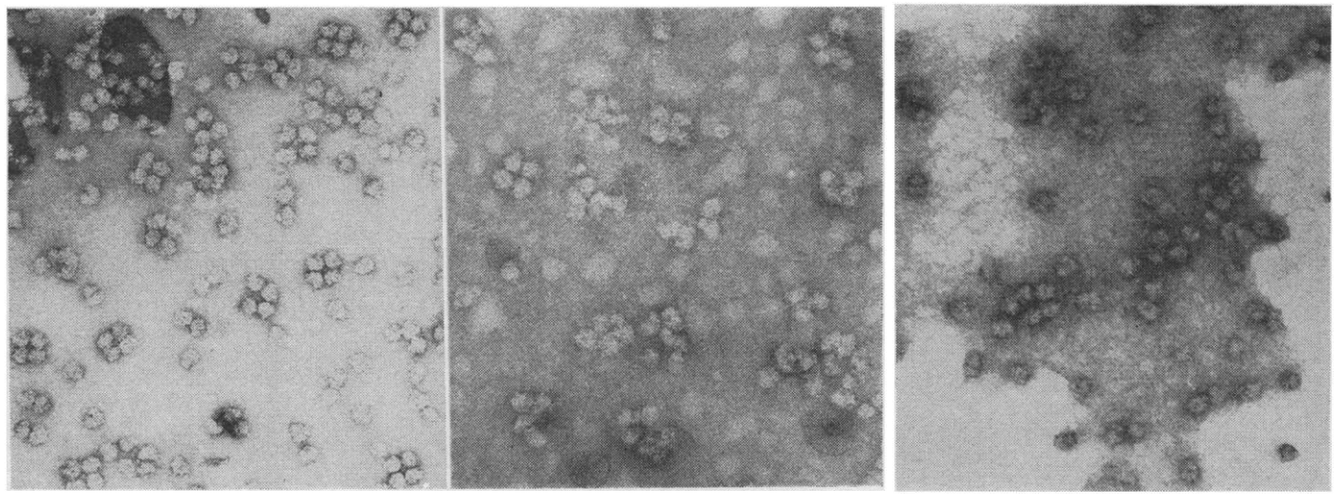

Fig. 4 (left). (see Fig. 1*B*) Polysomes sedimenting at 158*S* from 12-day embryonic-feather extract treated with 4 μg/ml ribonuclease. These polysomes are unaffected by this enzyme, which dissociates all other polysomes (× 100,000). Fig. 5 (center). (see Fig. 1*D*) Polysomes from 14-day feather extract which sediment at 158*S*. Although some closed squares are present, the majority are now open chains (× 100,000). Fig. 6 (right). (see Fig. 1*G*) Polysomes from a control gradient of cytoplasmic extract from 3-day whole embryo. The polysomes which sediment at 158*S* are made up of open chains of ribosomes (× 100,000).

The polysomes from the 200*S* region of the gradient (Fig. 3) are predominantly strung out in chains. Most of the ribosome units sedimenting at 158*S* are arranged in symmetrical tight squares with only some being strung out in chains, and the number strung out is consistent with the number in this peak which are sensitive to ribonuclease.

Polysomes from the 158*S* peak, from 12-day feathers, after treatment with ribonuclease (Fig. 4) are mostly tight squares. Electron micrographs of samples from the 158*S* region of a gradient of 14-day-feather extract reveal that the four-ribosome polysomes are no longer mainly tight symmetrical squares but are now largely open figures (Fig. 5). A sample from the 158*S* peak of a gradient of cytoplasmic extract from 3-day whole embryos also shows polysomes of principally open configuration (Fig. 6).

Another comparison of inactive with active polysomes can be made by examining samples from gradients of 12- and 14-day extracts from feathers treated with actinomycin D. Whereas both resulting profiles show that the material in the peaks (O.D.$_{260m\mu}$) sediments at 158*S*, only the four-ribosome polysomes of the 14-day extract have nascent protein as judged by counts of radioactivity associated with them. Study of electronmicrographs of samples from sucrose density gradients (Fig. 1, *C* and *F*) make it clear that inactive polysomes are arranged in tight symmetrical squares whereas active ones are not. All of the foregoing results illustrated with micrographs of negatively stained preparations were also confirmed in positively stained preparations.

Thus the inactive polysomes which are "stockpiled" to be activated when the feather begins to keratinize and convert its substance into a hard insoluble proteinaceous complex have a unique symmetrical structure. How this configuration is related to their nonfunctional state or their resistance to ribonuclease is not clear. It was suggested earlier (*1*) that the messenger RNA (mRNA) of these nonfunctional four-ribosome aggregates was specifically protected from ribonuclease. Protection may be bestowed by the tight-clustered arrangement of ribosomes. Calculations (*7*) suggest that normally the configuration of polysomal ribosomes is helical. Departure from the helix might result in a polysome whose mRNA is inaccessible for translation. It remains to be determined exactly how the configuration of the square polysome is related to its failure to make protein. It is clear, however, that as translation begins the square unfolds.

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Growth of Rats Fed on Opaque-2 Maize

Abstract. *Six weanling male rats were fed a diet containing 90 percent* opaque-2 *maize for 28 days. The average gain in weight was 97 grams. In control rats fed on a standard hybrid maize the average gain was 27 grams. This confirms results of a previous feeding test on rats and demonstrates the superior quality of the proteins in* opaque-2 *mutant endosperm.*

We reported in 1964 that the *opaque-2* mutant gene markedly changes the protein and amino acid composition of maize endosperm (*1*). Similar changes are not observed in the embryo (*2*). Based on chemical composition, the proteins of *opaque-2* endosperms should be of greater food value than those of normal endosperms. Sufficient amounts of *opaque-2* maize were harvested in the fall of 1964 to permit two feeding tests with rats. Results of the first test are described elsewhere (*3*); the results of the second test are reported here.

The *opaque-2* maize was from a back-cross progeny and the whole seed contained 1.69 percent nitrogen. The plants were self-pollinated in order to exclude pollen from normal plants, since *opaque-2* is a recessive gene. The normal or nonopaque maize was a standard hybrid, Indiana 453, grown in a yield trial in which pollination was not controlled. The whole seed contained 1.68 percent nitrogen. Both types of maize were ground in a Labconco burr mill at the finest setting before they were analyzed or incorporated in diets. Amino acid analyses (*1*) were made on ground samples of whole seed which had been defatted (*4*). For each 16 g of nitrogen, the defatted *opaque-2* maize contained 4.7 g of lysine and the standard hybrid, 2.8 g of lysine (Table 1). The lysine values, which were higher than those reported previously (*1*), reflect the presence of the embryo, which contains approximately 6 g of lysine for each 16 g of nitrogen. With the exception of histidine, the differences in amino acid composition were similar to those observed previously (*1*) in mutant and normal endosperms from the same ear of maize.

Groups of six weanling male rats (Wistar strain), each weighing 40 to 57 g, were kept in individual wire-mesh cages, and given unrestricted access to one of the following diets: diet A, 90 percent *opaque-2* maize, 5 percent corn oil, 4 percent Hawk-Oser salt mixture No. 3 (*5*), and 1 percent Vitamin Fortification Mixture (*6*); diet B, the same as diet A except that Indiana hybrid 453 replaced *opaque-2* maize; diet C, 10 percent casein, domestic (*7*), 75 percent corn starch, 10 percent corn oil, 4 percent Hawk-Oser salt mixture No. 3, and 1 percent Vitamin

Table 1. Amino acids in *opaque-2* and normal defatted corn (expressed as grams per 100 g of protein). Values for tryptophan were not available.

Amino acid	*Opaque-2*	Normal corn
Lysine	4.7	2.8
Histidine	3.0	3.0
Ammonia	2.7	3.4
Arginine	6.5	4.8
Aspartic acid	10.6	6.7
Glutamic acid	17.9	20.8
Threonine	3.9	3.6
Serine	4.9	4.8
Proline	8.1	10.0
Glycine	4.8	3.8
Alanine	6.9	7.9
Valine	5.5	5.0
Cystine	1.4	1.2
Methionine	1.9	2.0
Isoleucine	3.8	4.0
Leucine	9.8	13.9
Tyrosine	3.6	4.0
Phenylalanine	4.8	5.2