

Hypophyseal Control of Genetic Expression during Chick Feather and Skin Differentiation

Abstract. *The normal sequence of molecular events which occurs during feather and skin differentiation in the chick embryo is interrupted by pituitary gland ablation. The characteristic pattern of development is reestablished in hypophysectomized embryos treated with pituitary extract and in hypophysectomized embryos in parabiotic union with their twins within a double-yolked egg. These results suggest that genetic expression in differentiating chick feather and skin after day 12 of incubation is regulated by hormones.*

The developing chick embryo has a characteristic age-dependent skin and feather polysome profile in sucrose gradients (1, 2). The pattern of polysome distribution remains constant until day 12 of incubation, when feather

keratinization begins. By day 15 when down feathers are obvious, the profiles show a decided shift from the monosome peak to the heavier polysome region. In addition to the changes in polysome profile, the morphology of a

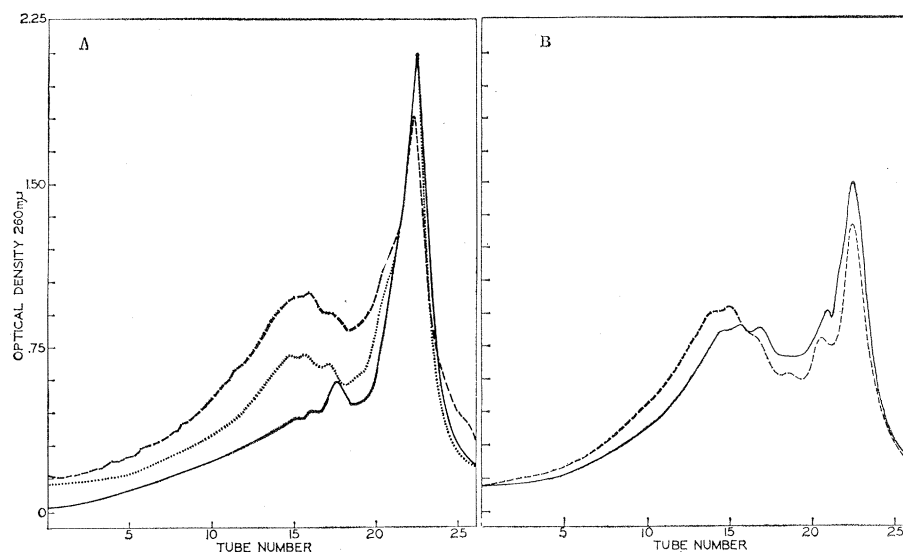


Fig. 1. (A) Optical density at 260 $m\mu$ in a Gilford continuous-flow spectrophotometer of 15-day-old embryo skin and feather extracts. The polysome pattern of the control is represented by the dashed line, that of the hypophysectomized 15-day-old embryo treated with pituitary extract by the dotted line, and that of an untreated, hypophysectomized 15-day-old embryo by the solid line. (B) Pattern of 15-day-old embryos from double-yolked eggs in which one embryo was hypophysectomized and the other left intact. The polysome pattern of the control is represented by the dashed line and that of its hypophysectomized twin by the solid line.

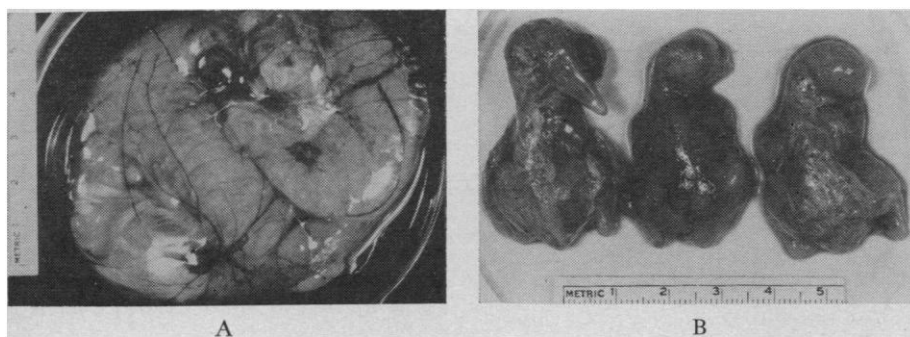


Fig. 2. (A) Photograph of a 15-day-old control (left) and its twin, hypophysectomized embryo (right) within their membranes after removal from a double-yolked egg. Note that the vascular anastomosis between the embryos is quite extensive and forms a natural parabiotic union. (B) The twin embryos removed from their membranes. On the left is the control; on the right, the operated twin. In the center is an untreated 15-day-old hypophysectomized bird from a single-yolk egg. Note the essential similarity of feather development of the twin embryos, whereas the untreated hypophysectomized bird has poorer feathering.

four-unit ribosome polysome with 158S sedimentation characteristics is changed (3). Prior to day 13, the 158S peak consists primarily of this four-unit polysome with its unique symmetrical tetrad form, whereas, after day 13 the polysomes comprising the 158S peak are mainly linear in form (3).

Studies of hypophysectomized embryos indicate that, until day 13 of incubation, the pituitary gland has little apparent effect on the distribution or morphology of the skin and feather polysomes. However, polysome patterns of the 15-day-old hypophysectomized embryos differ markedly from those of their controls. Tetrad-shaped polysomes, characteristic of the 12-day embryos but largely absent from the 15-day control embryos, are retained in operated birds at 15 days (2).

In order to determine the effect of hormonal replacement on chick embryo skin and feather polysomes, fertilized White Leghorn chicken eggs were incubated (37.4° to 38.6°C) until the 12 to 14 somite stage was attained; the eggs were then opened and the hypophyseal anlagen, which were identifiable as thickened ectodermal plates, were surgically removed (4). These embryos were referred to as "hypophysectomized." Control eggs were also opened and handled in the same manner, except for the removal of the placode. The eggs were sealed with transparent tape and returned to the incubator. On day 11 a phosphate buffer (pH 7.46) extract of pituitary glands was injected into the air sacs of some of the eggs containing hypophysectomized embryos. The extract contained the equivalent of either two pituitaries from 77-day-old chickens or three pituitaries from 21-day-old chickens. These injections were repeated on incubation days 13 and 14. Both the eggs containing unoperated controls and those with untreated hypophysectomized embryos were injected with phosphate buffer. In experiments on double-yolked eggs, one embryo from each egg was hypophysectomized and the other left intact. The expectation was that a natural parabiotic union would result, or that hormonal replacement would occur by diffusion.

On day 15 of incubation all embryos were removed from their eggs; the skin and feathers were collected into cold, hypotonic buffer and were allowed to stand for 30 minutes to facilitate homogenization. After homogenization, the polysomes were isolated in a pellet

(5) without the use of deoxycholate. The pellet was resuspended and then centrifuged at 0°C in a linear sucrose gradient (15 to 30 percent) at 25,000 rev/min in the SW-25.1 rotor of a Spinco L-2 preparative centrifuge. Optical densities were read at 260 $m\mu$ in a Gilford recording spectrophotometer.

Polysome profiles of cytoplasmic skin and feather extracts of 15-day-old hypophysectomized embryos which had been treated with pituitary extract are shown in Fig. 1A. The patterns of untreated hypophysectomized birds are very similar to those of either control or operated embryos at 12 days of incubation. In contrast, skin and feathers of the treated 15-day embryos yield intermediate patterns, approaching those of the 15-day control birds in which a marked shift from the monosome region to the heavier polysome region of the gradient profile occurs.

When one embryo of a double-yolked egg is hypophysectomized, vascular anastomoses between the two embryos are extensive and apparently allow hormonal replacement to occur (Fig. 2A). The pattern of feathering of the control is similar to that of the operated birds (Fig. 2B). This similarity in feather development is also reflected in the polysomes; there is complete differentiation of the polysome pattern in the hypophysectomized embryo (Fig. 1B).

In the normal course of events, maturation of down feathers is completed by a rapid deposition of the structural protein keratin between day 13 and day 17. The underlying molecular events are reflected by changes in the polysome distribution and morphology (1). It has been proposed that these molecular events are under endocrine control (2), since extirpation of the pituitary gland prevents the normal gross anatomical and intracellular changes. That there is an increased quantity of heavier polysomes and, by inference, of messenger-RNA after hormonal treatment of hypophysectomized embryos indicates that differentiation of skin and feathers is hormonally controlled. Whether new species of messenger-RNA are synthesized when pituitary hormones are replaced is not known.

Thus, keratinization of feathers requires the presence of pituitary hormones. Hypophyseal control of feather differentiation might be exerted by one

or a combination of the following: increased rate of synthesis, rate of translation, or enhanced stability of messenger-RNA already present in skin and feathers. A final possibility is that pituitary hormones operate via cistron depression which allows the synthesis of entirely new species of messenger-RNA.

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References and Notes

1. T. Humphreys, S. Penman, E. Bell, *Biochem. Biophys. Res. Commun.* **17**, 618 (1964).
2. M. B. Yatvin, *Science* **151**, 1001 (1966).
3. E. Bell, T. Humphreys, H. S. Slayter, C. E. Hall, *ibid.* **148**, 1739 (1965).
4. N. W. Fugo and E. Witschi, *Lav. Biol. Biedr. Rak.* **8**, 73 (1938).
5. F. O. Wettstein, T. Staehelin, H. Noll, *Nature* **197**, 430 (1963).
6. Supported by NIH grant CA 08318-01, center grant CA 06295-04, and by American Cancer Society grant T-66G. I thank R. L. Engerman for suggestions, R. W. Haller for providing the chicken pituitaries, and N. V. Brown, C. Crouch, and A. Cameron for technical assistance.

3 May 1966

Lipid Films as Transducers for Detection of Antigen-Antibody and Enzyme-Substrate Reactions

Abstract. *The transverse electrical impedance of thin lipid films separating two aqueous saline solutions containing a small concentration of antibodies or enzymes decreases markedly and reversibly after immunological and enzymatic reactions involving such protein molecules, which presumably are adsorbed to the lipid-water interface.*

Development of techniques to form stable phospholipid films between two aqueous phases (1) offers an enticing opportunity to experiment with artificial systems which resemble, after a fashion, the structure of cell membranes—that is, thin lipid leaflets coated by protein molecules adsorbed to the lipid-water interface.

The work we now summarize was undertaken to find out whether reac-

tions involving such affixed protein molecules would influence the electrical impedance of the lipid phase. This possibility was suggested by a number of published observations showing that immunological and enzymatic reactions, in which the layers of protein coating participate, often result in large changes in membrane permeability.

It is known, for instance, that specific antisera, in the presence of com-

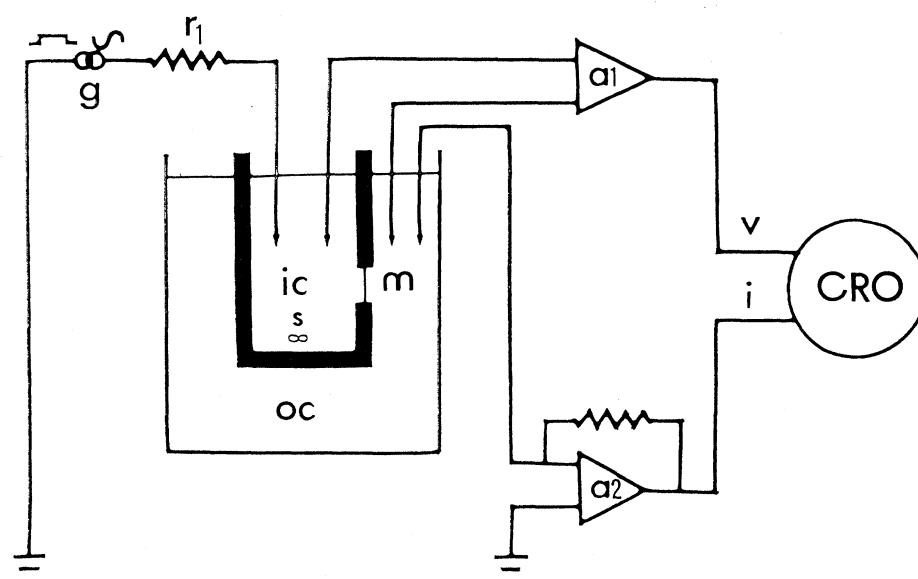


Fig. 1. Experimental arrangement. The current (i) is made to flow from the generator through a resistor (r_1), up to 250 Mohm, across the lipid film (m) separating the inner (ic) from the outer (oc) compartments (s , stirring device). The current electrode in the outer compartment is connected to the summing point of an operational amplifier (a_2). The potential changes (v) across film m are differentially recorded with help of a high-input-impedance amplifier (a_1). Both potential v and current i are displayed on a dual-beam cathode-ray oscilloscope (CRO).