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*Original Article*

# Pattern formation in the epithelium of the oviduct of Japanese quail

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**ABSTRACT Cellular patterns of the oviduct epithelium from the Japanese quail were examined during maturation. The epithelium of a juvenile bird showed a jigsaw puzzle pattern consisting of a single, undifferentiated cell type. At the start of maturation, cells were rearranged into a pattern in which the length of boundaries between goblet type gland (G-) cells and ciliated (C-) cells (G-C boundaries) were maximized. At this stage, the surface area of G-cells was much smaller, but G-cells are more than 2 times more numerous than C-cells. Cells then gradually rearranged themselves into the checkerboard pattern through an increase in the proportion of C-cells and enlargement of the G-cells. At all times of maturation, the length of G-C boundaries was maximized. These observations strongly support the theory that the cellular pattern of the quail oviduct epithelium is spontaneously constructed by maximizing the length of boundaries between two different types of cells owing to the fact that theirs is the greatest adhesion capacity (Yamanaka and Honda, 1990).**

KEY WORDS: *pattern formation, cell adhesion property, monolayered epithelium*

## Introduction

Cell patterning caused by differential adhesion properties of cells is believed to play a main role in early morphogenesis during embryogenesis (Steinberg, 1962a, b, c, 1978). The idea of pattern formation by homophilic cell cohesion is widely accepted. Although cell adhesion properties were predicted for homophilic and heterophilic interaction, there has not been any actual instance reported to date for heterophilic cell adhesion.

We (Yamanaka and Honda, 1990) have shown that the oviduct epithelium of the adult Japanese quail showed a unique checkerboard pattern consisting of ciliated (C-) cells and goblet type gland (G-) cells. The pattern showed predominantly boundaries between G- and C-cells (G-C boundary) suggesting that G-C cells (heterophilic) adhesion is stronger than C-C or G-G cells adhesion (homophilic). We therefore proposed that the differential adhesion capacity of two different types of cells is the principal factor responsible for pattern formation in this tissue.

The epithelium develops from a juvenile epithelium composed of a single, undifferentiated cell type between 30 and 45 days after hatching when the animal undergoes sexual maturation, and the oviduct shows abrupt growth. During this period the immature oviduct develops into two different tissues. One of them is the tubular gland which is formed by invagination of the immature epithelium and locates beneath the maturated epithelium (Wrenn, 1971). Another is the oviduct epithelium composed of C- and G-cells, which covers the luminal surface of the oviduct and later shows the checkerboard pattern (Yamanaka and Honda, 1990).

Observation of the actual process of pattern formation during maturation is essential in order to evaluate our theory described in the preceding paper. Here I report various stages of pattern development in the oviduct epithelium, starting from a homogeneous, monolayered cell sheet culminating in the checkerboard pattern.

## Results and Discussion

### *The epithelium of a juvenile bird*

The surface pattern of an epithelium from a quail younger than 30-day-old does not show the polygonal pattern but is comprised of winding boundaries of undifferentiated cells (Fig. 1a). This pattern is conveniently described as the jig-saw puzzle pattern. The curvature of the boundaries is mild and differs from that of the typical interdigitating structure of opposed cell membranes observed at lower levels of the same cell (data not shown). The sinuous appearance of those boundaries at this stage might be relevant to the immature structure of the microfilament bundles because they are thinner than in matured cells (Fig. 1b). Cells are firmly attached to each other at the apical level constructing junctional complexes as in matured cells. A basal body of a cilium observed at the center of the cells is distinguished from the hair-like cilia of matured C-cells and pertains to the short cilium often observed in immature cells

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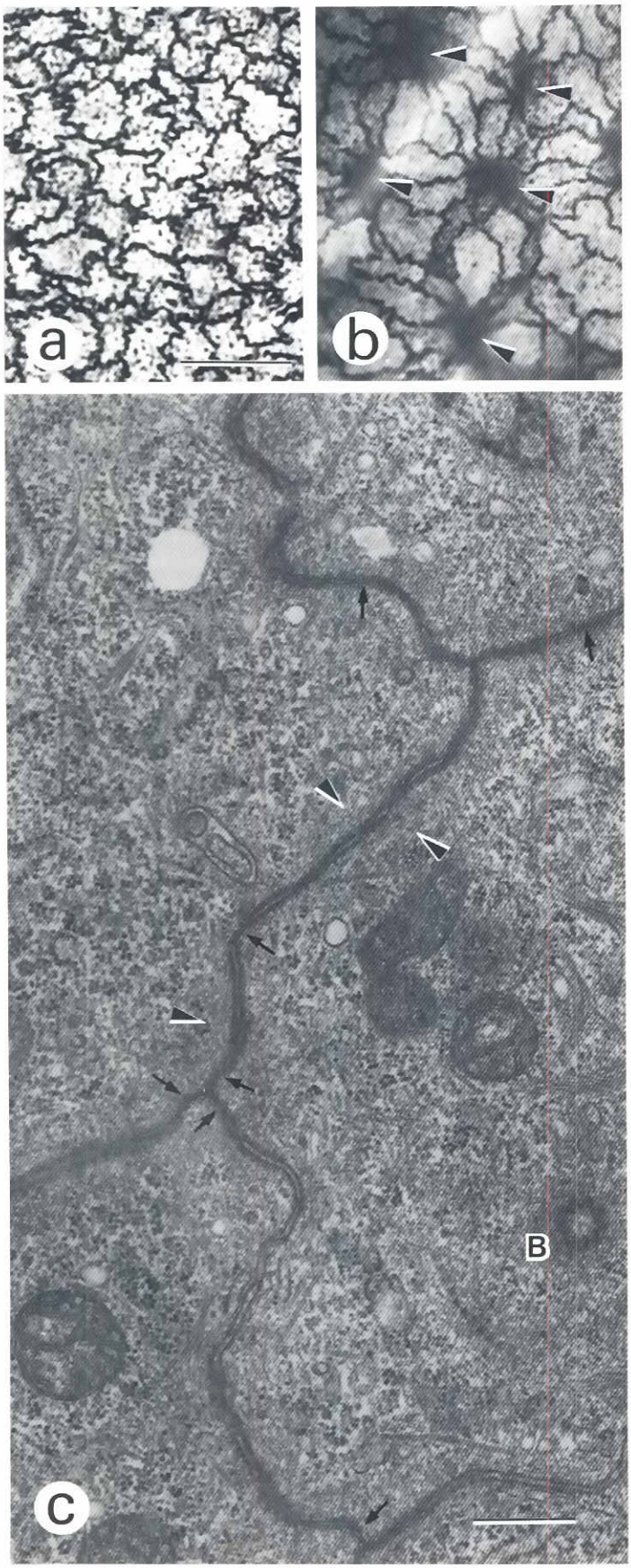


(Fig. 1c) (Chailley *et al.*, 1982). Neither C-cells, G-cells nor tubular gland cells are observed at this stage. The significant fact for pattern formation is that this monolayered epithelium consists of a homogeneous cell population.

***Cellular patterns in early stage of maturation***

After 30 days, when the sexual maturation of the animal takes place, the tubular gland starts differentiation by invagination of immature epithelium (Wrenn, 1971). Foci of invagination were very frequently observed at the beginning of this stage (Fig. 1b), but lessened in number as the tissue grew.

Immediately after the invagination of the tubular gland, the jig-saw puzzle pattern turned into a polygonal one with the simultaneous appearance of large cells similar in size to mature cells (Fig. 2a, c). The large cells were identified as differentiating C-cells by electron microscopy because of the large number of cilia and basal bodies (Fig. 2b, c). The rest of the cells, whose size is similar to undifferentiated cells were identified as differentiating G-cells by the numerous secretion granules in their cytoplasm (Fig. 2c). These G-cells still have one short cilium near the center of the apical cell surface but it is distinct from cilia of C-cells. This pattern was designated as the random polygonal pattern since large differentiating C-cells and small differentiating G-cells do not have any regularity in their shape and distribution. Neighboring C-cells, which are rarely observed in mature tissue (Yamanaka and Honda, 1990), are often observed at this stage. The pattern suggested that the cell differentiation into G- and C-cells is not regulated by neighboring cells as shown in neurogenesis of *Drosophila* (Campos-Ortega, 1985; Knust and Campos-Ortega, 1989) but possibly by direct hormonal action of estrogen and progesterone (Sandoz *et al.*, 1976) on individual cells. It is clear that cell differentiation is independent of cell arrangement in the tissue at this stage.

***Cellular patterns in late stage of maturation***

The random polygonal pattern subsequently rearranged into a transitional pattern described below. Small G-cells were linked together and formed a network structure. C-cells became isolated individually by the network of G-cells (Fig. 3a). The total length of G-C boundaries was maximized by this rearrangement. The boundaries between two G-cells (G-G boundary) were observed between adjacent G-cells but the number of boundaries between two C-cells (C-C boundary) were few. The particular trait of this pattern – isolated C-cells and surrounding linked G-cells so as to maximize the G-C boundary – was consistently observed throughout the rest of maturation. This suggests that the different adhesive properties of the two cell types is acquired during differentiation and is responsible for this rearrangement.

At the beginning of this stage the ratio of G-cells to C-cells was

**Fig. 1. The jig-saw puzzle cellular pattern of an immature oviduct epithelium. (a)** *A cellular pattern of a 20-day-old animal revealed by silver impregnation.* **(b)** *A cellular pattern of a 35-day-old animal. Cells are elongated toward invagination foci but are still showing the jig-saw puzzle pattern. Arrows indicate foci of invagination of the tubular gland.* **(c)** *Electron micrograph at the epithelial surface. A basal body of a short cilium (B) is often observed but distinguished from cilia of the differentiated C-cell. Tight junctions are frequently observed (arrows) and presumed to be surrounding a cell. Microfilaments run along winding boundaries (arrowheads). Bar in (a), (b), 10μm; (c), 0.5 μm.*

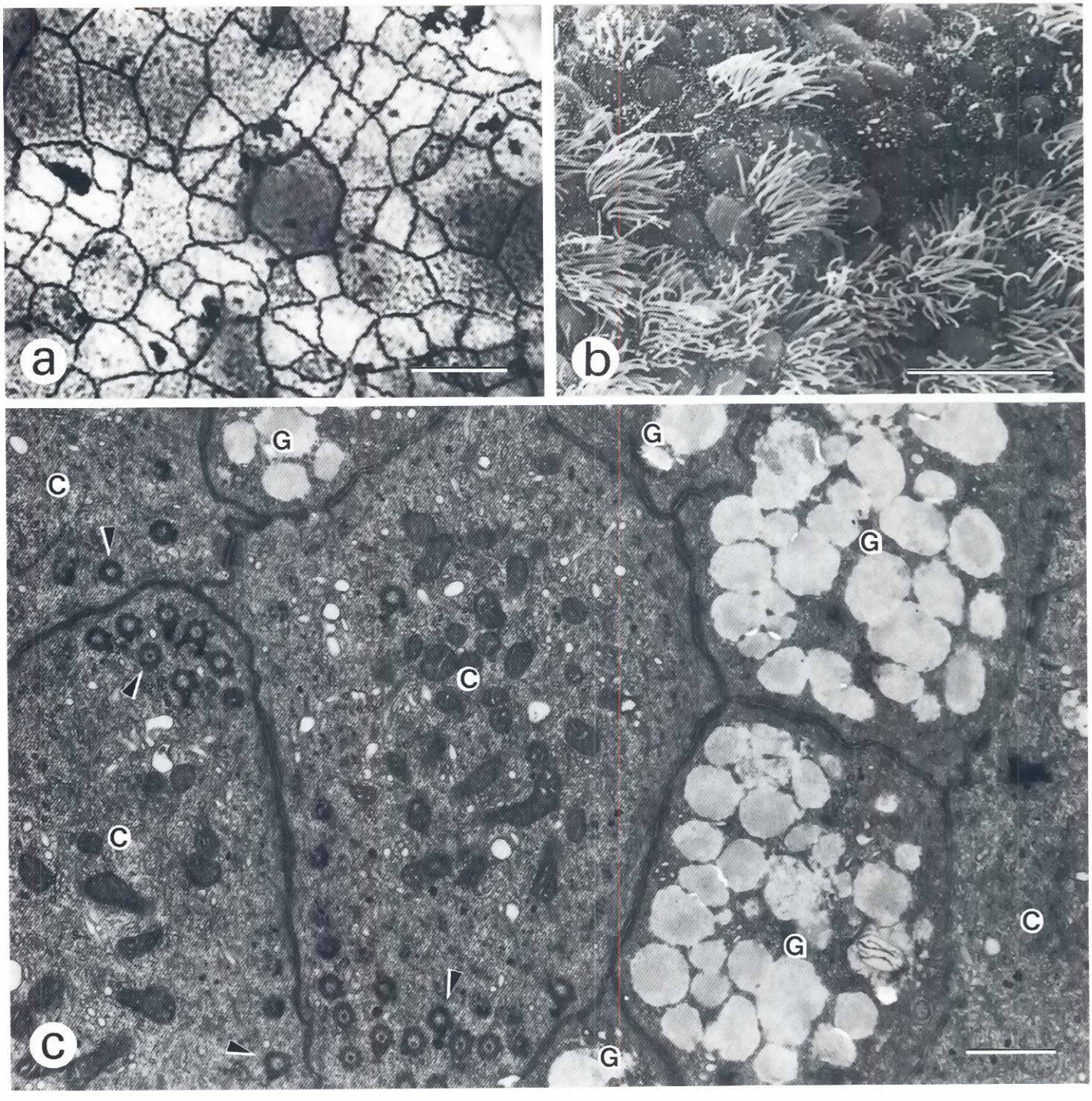


**Fig. 2. The random polygonal pattern of a maturating oviduct epithelium. (a)** *A cellular pattern of a 40-day-old animal by silver impregnation. Cells are becoming polygonal but their boundaries are still slightly wavy.* **(b)** *Differentiation of C-cells shown by elongating cilia is visualized by scanning electron microscopy. Small G-cells can be identified by their round surface with a short cilium at the center.* **(c)** *A transmission electron micrograph of a tangential section at the surface of the epithelium. Large cells in (a) are identified as differentiating C-cells by the basal bodies of their hair-like cilia (arrowheads). Small cells in (a) are identified as differentiating G-cells by their secreting granules (G). These G-cells still have a short cilium at their center as jig-saw puzzle cells. Larger numbers of microfilaments run along cell boundaries (arrows) compared to the previous jig-saw stage. Bar in (a) and (b), 10μm; (c), 1μm.*

greater than three to one. This value gradually decreased as maturation progressed. When it became about two a transient pattern was exhibited as shown in Fig. 3b. This pattern can be approximated to a star pattern comprised of a small triangle and a large hexagon (Fig. 5). The star pattern is one of the typical geometrical patterns that maximize boundaries between two diffe-

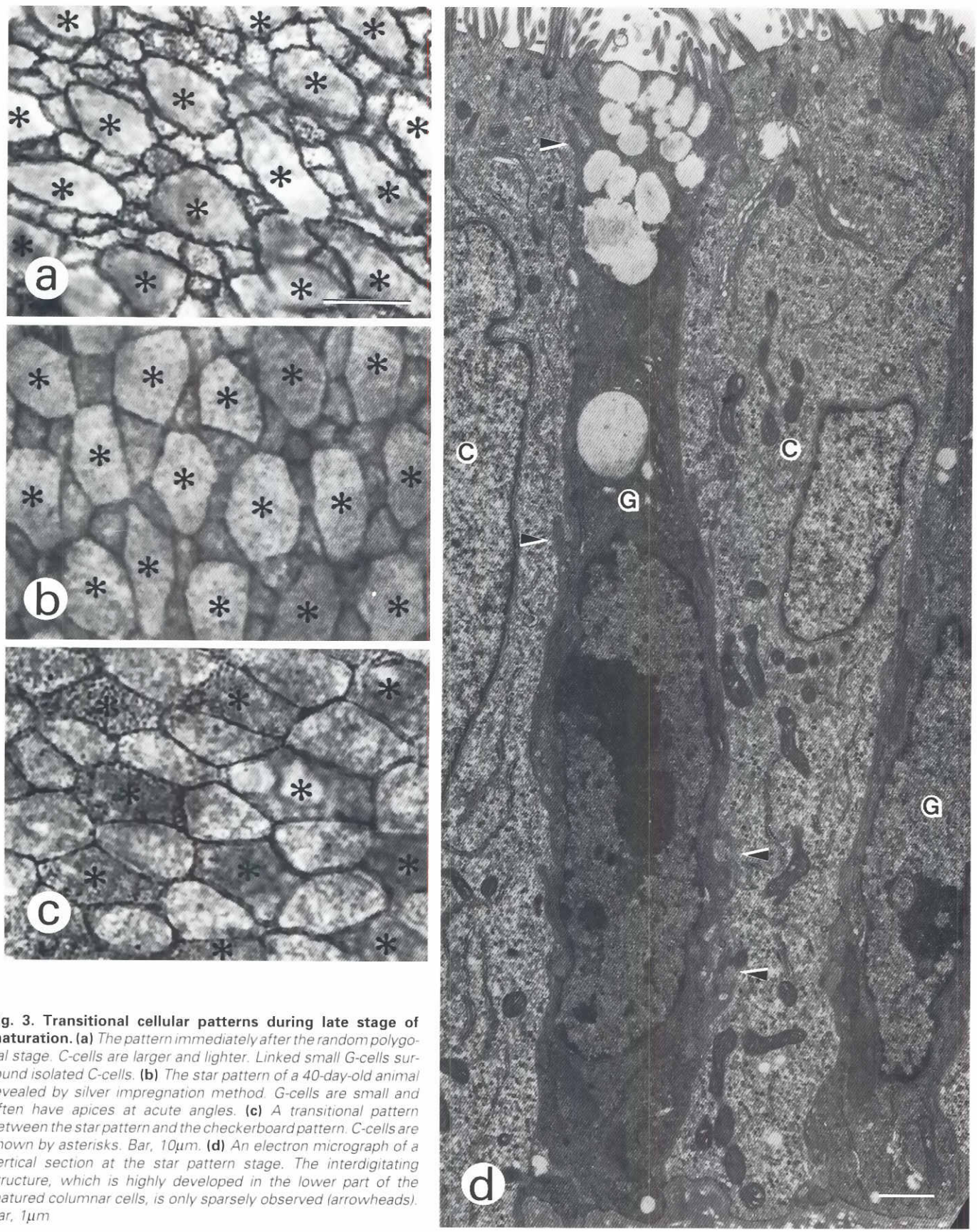


**Fig. 3. Transitional cellular patterns during late stage of maturation. (a)** *The pattern immediately after the random polygonal stage. C-cells are larger and lighter. Linked small G-cells surround isolated C-cells.* **(b)** *The star pattern of a 40-day-old animal revealed by silver impregnation method. G-cells are small and often have apices at acute angles.* **(c)** *A transitional pattern between the star pattern and the checkerboard pattern. C-cells are shown by asterisks. Bar, 10μm.* **(d)** *An electron micrograph of a vertical section at the star pattern stage. The interdigitating structure, which is highly developed in the lower part of the matured columnar cells, is only sparsely observed (arrowheads). Bar, 1μm*

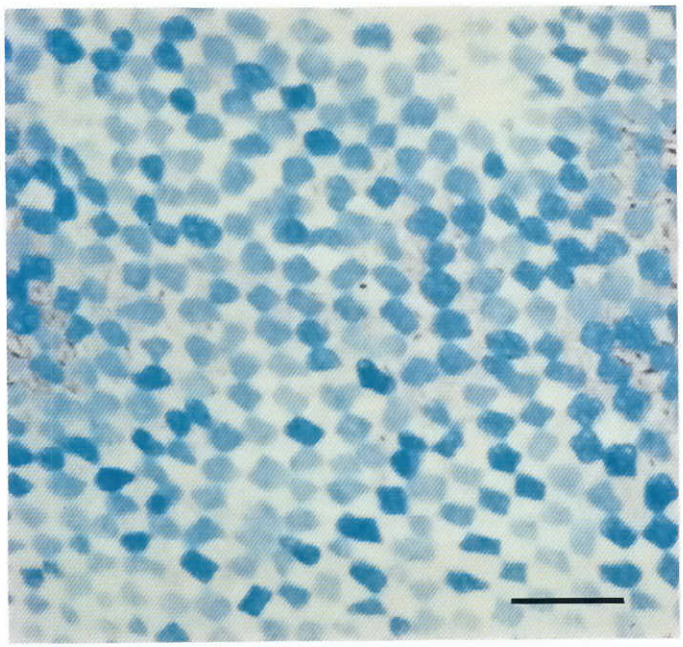

**Fig. 4. Cellular pattern of the oviduct epithelium of the Japanese quail in albumen-secreting (magnum) region**. *Only G-cells are stained with Alcian blue. Bar, 10μm.*

rent types of polygons. The star pattern in the actual tissue has some G-G boundaries and maintains the trait of isolated C-cells surrounded by linked G-cells. The small number of G-G boundaries indicates the secondary adhesion capacity of the G-G boundary (Yamanaka and Honda, 1990). Thus the major morphogenetic factor in pattern formation of the oviduct epithelium is concluded to be the predominant formation of G-C boundaries due to their preferred adhesion. An electron micrograph at this stage showed smaller quantities of the interdigitating structure compared to matured cells. This suggested the possibility of cell motility during rearrangement.

Another transitional pattern, which follows Fig. 3b, is shown in Fig. 3c.

### *The pattern in matured tissue*

Enlargment of the G-cells and a faster increase of the C-population were the only morphogenetic processes to occur thereafter. When the oviduct finished its maturation at about 40 or 45 days after hatching, the ratio of G-cells to C-cells was close to equal. The checkerboard pattern was frequently observed (Fig. 4). The checkerboard pattern of actual tissue is also slightly different from an ideal checkerboard pattern because the apical surface area of the G-cell is slightly larger than that of the C-cell (Fig. 5). The presence of short G-G boundaries demonstrates the secondary adhesion preference of the G-cells. The particular trait of this tissue – isolated C-cells with surrounding linked G-cells – is the same as in the star pattern.

The length of the oviduct increases about 1.5-fold during this period, which provides a 2.3-fold increase of the inner surface area, assuming growth in a similar shape. C-cells in the checkerboard pattern were shown to be two times more numerous compared to the corresponding area at the star pattern stage (Honda *et al.*, 1986). The increase of the inner surface area of the oviduct during the transition from the star pattern to the checkerboard pattern could be explained by one more cell division of every C-cell and a 5-6-fold enlargement of the surface area of G-cells. Transdifferentiation of either type of cells is unlikely because intermediate cells were not seen in my extensive survey. The overall process of pattern formation and maturation of the oviduct is summarized in Fig. 5.

We have proposed in the preceding paper (Yamanaka and Honda, 1990) that adhesion capacity between two different types of cells is the principal factor in pattern formation of oviduct epithelium in the Japanese quail. In this paper I studied maturation of the cellular pattern and successfully obtained strong support for our theory.

Families of proteins called cadherins and CAMs were identified as specific adhesion molecules on the cell surface (Edelmann, 1985, 1986; Takeichi, 1988). They appear in a time-dependent manner during morphogenetic processes in development (Nose and Takeichi, 1986; Ogou *et al.*, 1982) and are considered to play an important role in cell sorting for homogenous tissue formation (Takeichi *et al.*, 1985). However, most of the cell adhesion molecules known so far are homophilic. There may be a pair of heterophilic cell adhesion molecules in oviduct epithelium, because it consists of two types of cells individually mixed. They should be expressed concomitantly with other phenotypes in the differentiation of the G- and C-cells.

The seminiferous epithelium of the rat is another instance of the approximate star pattern (Nagano and Suzuki, 1983). It can be considered to be based on the same mechanisms as in the quail oviduct. In the gills of deep sea fish, arteries and veins are aligned in order to exchange the heat of the blood stream. A transverse

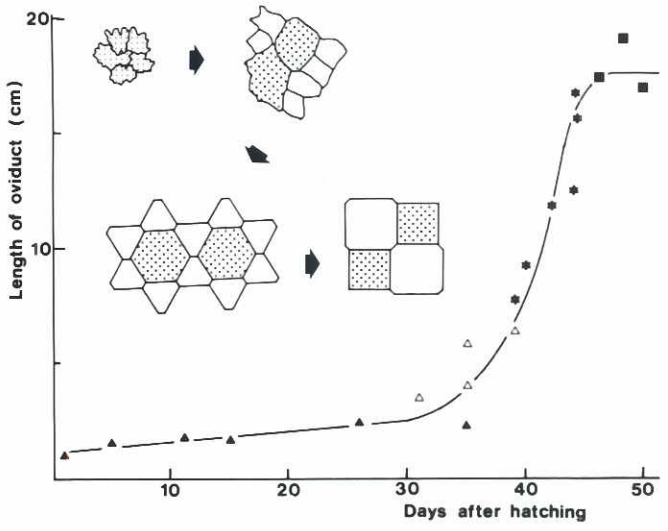


**Fig. 5. Transition of the cellular pattern of the oviduct epithelium after hatching.** *Lengths of oviducts except infundibulum and uterus are shown by the ordinate. An oviduct whose epithelium shows the jig-saw puzzle pattern is indicated by (▲); the random polygonal pattern (Δ); the pattern comprised of large isolated C-cells surrounded by small G-cells including the star pattern (★); the checkerboard pattern (■). C-cells are indicated by dots. Undifferentiated cells are indicated by smaller dots.*

section from these tissues shows a checkerboard or star pattern to maximized the efficiency of heat exchange (Scholander, 1969). Although this is not an instance of cellular patterning, similar mechanisms employing different adhesive strengths between cells of the outer surface of artery and vein might be in operation.

## Materials and Methods

### *Quail oviduct*

Fertilized Japanese quail (*Coturnix coturnix japonica*) eggs were purchase from Nihon-Uzura Co. Ltd. (Toyohashi, Japan). The eggs were incubated and hatched in a humidified incubator (Koitotron 2HNZ, Koito Kogyo Co. Ltd. Shizuoka) at 37°C. The thickest part of the albumen-secreting region of the oviduct was isolated from female animals up to 50 days after hatching. As for young animals prior to sexual maturation, the mid part of the oviduct which is expected to develop into the albumen-secreting region and the isthmus was employed.

### *Silver impregnation*

Silver impregnation for staining of cell boundaries was carried out according to the preceding paper (Yamanaka and Honda, 1990).

### *Histological examination*

The oviduct was fixed in 10% neutral formalin. Six μm-thick paraffin sections were prepared and stained with Alcian-Blue Hematoxylin (Yamanaka and Eguchi, 1981).

### *Scanning electron microscopy*

The oviduct was dissected into cubes of $1mm^3$ bearing the epithelium and washed with Dulbecco's phosphate buffered saline (PBS), processed routinely (Yamanaka and Eguchi, 1981) and observed with JSM-F7 scanning electron microscopy (JEOL, Tokyo).

### *Transmission electron microscopy*

The oviduct was dissected and processed as reported earlier (Yamanaka and Eguchi, 1981). Tangential and vertical ultra-thin sections against the surface plane of the oviduct epithelium were prepared and observed with a JEM-100C transmission electron microscope (JEOL, Tokyo) after staining with lead citrate and uranyl acetate.

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