

## Analysis of Golgi Apparatus Orientation in the Perichordal Region in the Chick Embryos

Mohammed Bahgat Hussein Mohammed

*Department of Zoology, Faculty of Science,  
University of Qatar, P.O. Box 2713, Doha, Qatar*

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**ABSTRACT.** The patterns of orientation of individual sclerotomal cells have been evaluated in the perichordal region of the chick embryo at stages 20, 23 and 26. Orientation was measured by determining the angular relationship between the Golgi-nucleus axis of each cell relative to the notochordal axis. These analyses showed that the mesenchyme cells are oriented predominantly toward the notochordal axis.

A distinct pattern of Golgi orientation has been observed during differentiation of the mesenchyme in the chick and mouse limbs (Ede *et al.* 1977, Holmes and Trelstad 1977, 1980) and the developing vertebral body (Trelstad 1977). This orientation is associated with centripetal movement of cells in a zigzag pattern to the centre of the chick limb mesenchyme aggregate (Ede *et al.* 1977).

The sclerotomal cells are arranged concentrically around the notochord to form the perichordal region during the precartilage condensation (Kardosha 1983). In this region, the chondrogenesis depends partially on induction by the notochord and the neural tube (Cooper 1965).

In this study the Golgi orientation has been used to determine perichordal cell orientation in relation to the notochord longitudinal axis in 3- to 5-day-old chick embryo. The Golgi activity in mucopolysaccharide synthesis has also been investigated during morphogenetic process in order to obtain better understanding of the chondrogenesis process in chick embryo.

### Material and Methods

Fertilized eggs of White Leghorn chickens were incubated at 38°C and staged by the criteria of Hamburger and Hamilton (1951). The trunk regions of two embryos at the level of the fore limbs at each stages 20, 23 and 26 were used (Fig. 1). The Golgi apparatus was impregnated with silver (Holmes and Trelstad 1977) before the specimens were processed for sectioning at 3  $\mu$ m. Serial sections, horizontal and transverse, were examined at a magnification of 1000x.

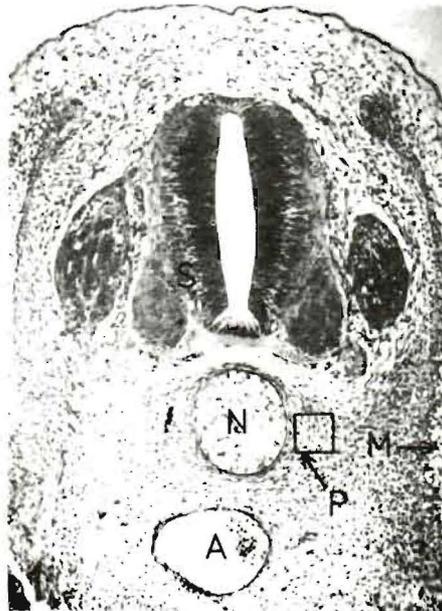


Fig. 1.

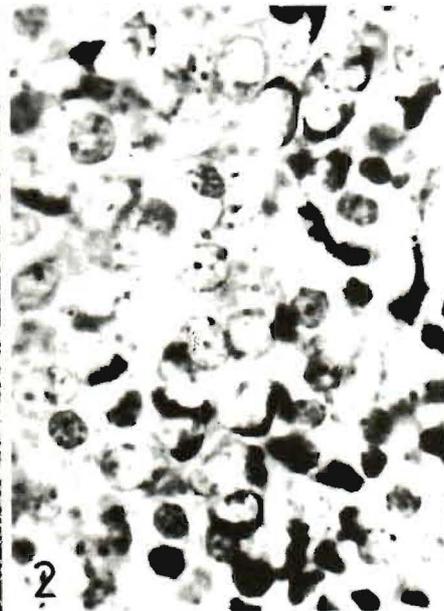
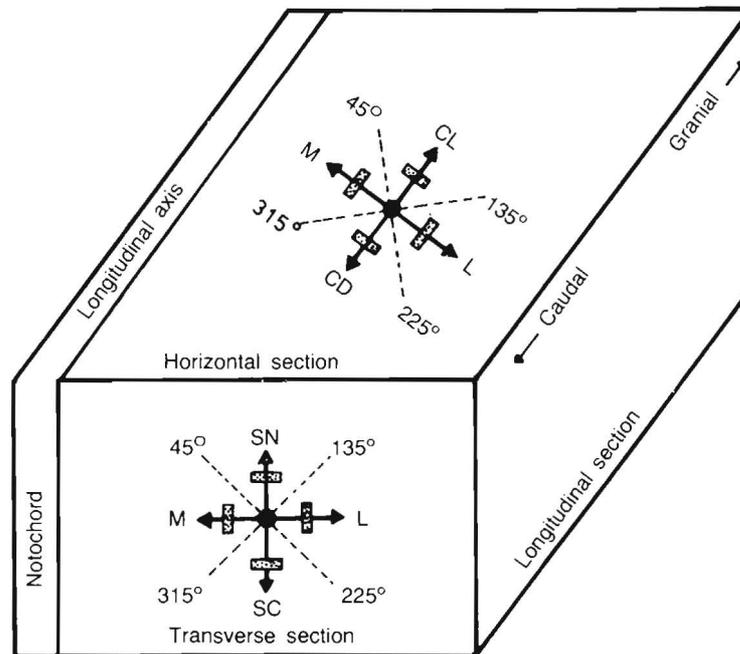


Fig. 2.

**Fig. 1.** A section from the chick trunk region at stage 23 to show the position of the perichordal region in which Golgi orientation was done. (100x). A: aorta; N: notochord; M: myogenic region; P: perichordal region; S: spinal cord.

**Fig. 2.** Silver-impregnated section from perichordal region at stage 26. (1000X). The illustration shows the altered geometrical relations between nucleus and Golgi apparatus and the cells deformed in a polarized way.

In order to evaluate cell orientation in the silver-impregnated sections, composite photographs were prepared from ten successive sections of an area  $20 \times 20 \mu\text{m}^2$ , and the total number of cells in this area was calculated. Cells with a Golgi apparatus and those with none were counted. The nucleus-notochord axis was determined and considered to divide the cell into two halves. The cells were further divided into four quadrates by a line perpendicular to that axis. The angle formed by the Golgi-nucleus axis and the nucleus-notochord axis of each cell was determined, in a clockwise direction (Fig. 3). The qualitative judgment was made as to whether the orientation of the Golgi apparatus was medial (M = toward the notochord), lateral (L = toward the myogenic region), cranial or caudal (CL = CRAN; CD = CAUD, the chick embryo axis), subneural (SN = toward the spinal cord) and subchordal (SC = toward the aorta vessel).



**Fig. 3.** The manner of analysis is illustrated. Cell orientation in perichordal region was determined by the position of the Golgi apparatus relative to the notochordal axis. The angle made by the intersection of a line from the centre of the nucleus to the centre of the Golgi apparatus and a line from the centre of the nucleus to the notochord was measured. CD: caudal direction; CL: cranial; L: lateral region, myogenic region; M: medial; N: notochord; SC: subchordal; SN: subneural.

□ Golgi apparatus; ● : notochord.

The mucopolysaccharide distribution was demonstrated using the alcian blue stain/chlorantine fast red (0.01%) in pH 5.6 acetate buffer (Scott and Dorling 1965).

### Observations

The geometrical relations between nucleus and Golgi apparatus changed during the period of studied and the cells became deformed in a polarised way (Fig. 2), though there is no detectable homogeneity in the perichordal cells at every stage. Fig. 4 shows that the multipeak and significantly non-uniform patterns arise from the existence of six distinct populations of cells, in each of which the Golgi angle has a characteristic main direction. These six populations of directed cells are: medial (315°-45°), cranial (45°-145°), lateral (135°-225°), caudal (225°-315°), subchordal (225°-315°) and subneural (45°-135°). Perichordal cells were found to be oriented more toward the notochord *i.e.*, medially, than to any other directions ( $p < 0.05$ ) (Tables 1 and 2). The number of cells directed medially increased with

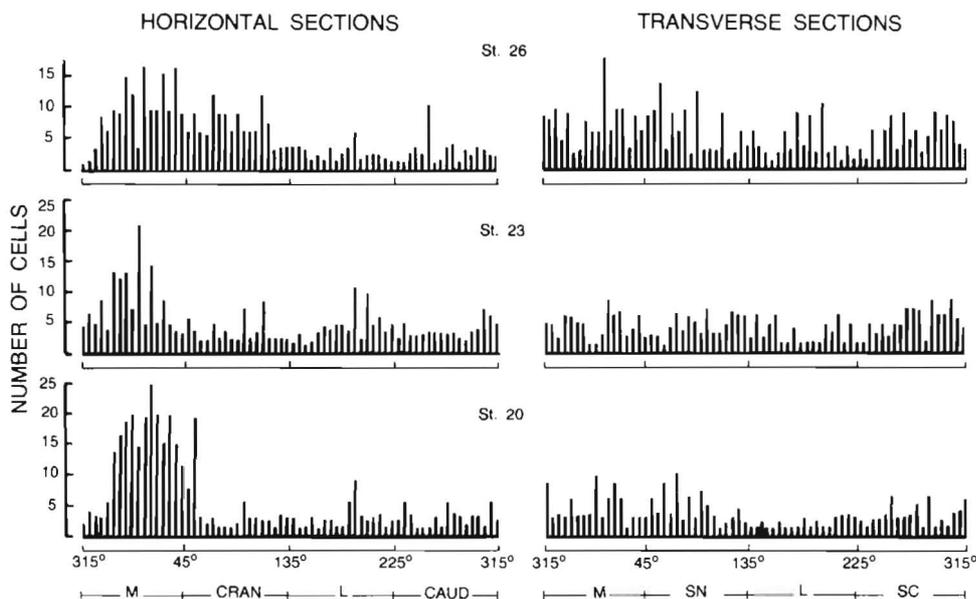
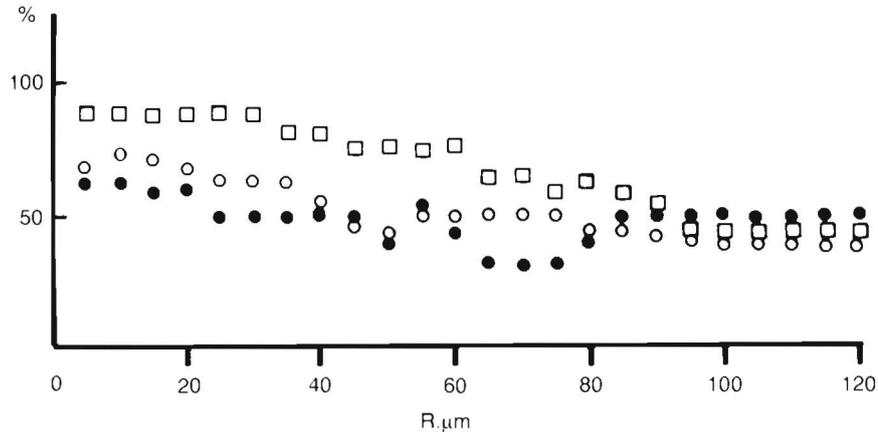


Fig. 4. Histograms of the orientation angles of perichordal cells in horizontal and transverse sections at stages 20, 23 and 26. Each bar represents the total number of cells with the same angle of Golgi orientation.

CAUD = caudal direction; CRAN = cranial direction.

advance of development between stage 20 and stage 26 ( $p < 0.05$ ). As the distance from the notochord increased *i.e.*, toward the myogenic regions, the number of cells with active Golgi apparatus became lower (Fig. 5).



**Fig. 5.** A comparison of change in Golgi density in various levels from the notochord (at 0  $\mu\text{m}$ ) to the myogenic region (at 80-100  $\mu\text{m}$ ) during stage (20) ( $\bullet$ ), stage 23 ( $\circ$ ) and stage 26 ( $\square$ ) of the chick embryos. Each point represents the average of Golgi density of ten consecutive sections. R  $\mu\text{m}$  = radial distance from notochord.

**Table 1.** The average cell orientation in the perichordal region at stages 20, 23 and 26 from horizontal sections.

Stage	Medial	Cranial	Lateral	Caudal
20	34.60%	30.24	15.18	19.98
23	39.48	27.41	15.40	17.71
26	53.33	18.48	14.26	13.93

**Table 2.** The average cell orientation in the perichordal region at stages 20, 23 and 26 from transverse sections.

Stage	Medial	Subneural	Lateral	Subchordal
20	32.21%	28.50	20.11	19.18
23	35.47	26.85	14.89	22.79
26	38.86	25.29	10.89	25.05

Mucopolysaccharides (mps) steadily increased during development from stage 20 to stage 26. The principal accumulation of extracellular mps occurred in the perichordal region with a high level at the notochord wall. At stage 26 a gradient of mps content was observed from the notochord to the myogenic region (Fig. 6).

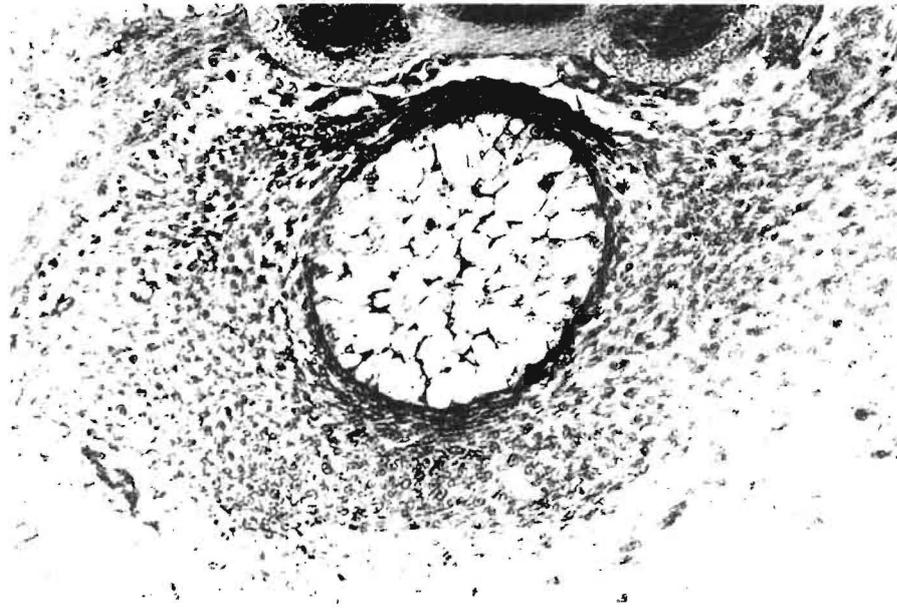


Fig. 6. Transverse section at stage 26 to show the gradient in mucopolysaccharide (mps) contents from the notochord to the myogenic region (150X).

### Discussion

This study indicated that as development proceeded, perichordal cells became directed toward the notochord and that this is associated with a gradient of mps content toward the myogenic region. These findings are similar to those of Ede *et al.* (1977) and Holmes and Trelstad (1980) on precartilaginous condensation in the chick and mouse limb buds; the chondrocytes with Golgi apparatus are oriented predominantly toward the centre of the cartilaginous aggregates. In the present study six cell populations have been observed during perichordal cell orientation. These results suggest that three different mechanisms may promote this pattern of cell orientation: (1) the effect of notochord, (2) perichordal blood supply, and (3) the extracellular matrix effect.

Perichordal cells were found to be oriented more toward the notochord than any other directions. Possibly this pattern of Golgi orientation was derived from the notochord induction. Cooper (1965) and Zillikin (1967) have shown that the vertebral chondrogenesis involves a direct inductive effect of the notochord and the neural tube. The sclerotomal cells become stabilized as cartilage after they surround the notochord and begin to be surrounded by metachromatic matrix (Searls 1973).

With regard to mps active secretion, the matrix may affect cell orientation in the perichordal region of the chick embryo. In limb development, Holmes and Trelstad (1977, 1980) also observed a polypeptide gradient affecting cell orientation during limb chondrogenesis. In the present study the mps contents are associated with Golgi apparatus than those toward the myogenic region. Ede *et al.* (1977) suggested that cells with highly activated Golgi apparatus move centripetally and those with less active Golgi move peripherally during precartilag condensation *in vitro*. Involvement of sclerotomal cell movement in chondrogenesis has been postulated by Strudel (1971) and Minor (1973).

In the chick limb, the pattern of vascular perfusion sets up two different environments *i.e.*, myogenic and chondrogenic regions (Caplan and Koutroupas 1973). The exogenous chondromucoprotein stimulates mps synthesis by cartilage cells *in vitro*. (Nevo and Dorfman 1972). Similarly, in the perichordal region, diffusible substances derived from the aorta may play a role in cell orientation and the mps distribution.

The results of the present investigation provide basic background data essential for further studies on the role of notochord, blood supply and extracellular matrix on chondrogenesis in chick embryo because the events involved in the development of the limb chondrogenesis, however, are quite different from those observed during perichordal formation.

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## تحليل اتجاهية جهاز جولجي في المنطقة الحولحبلية في أجنة الدجاج

محمد بهجت حسين محمد

قسم علم الحيوان، كلية العلوم، جامعة قطر  
الدوحة ص. ب: ٢٧١٣ - قطر

يقوم البحث على قياس الزوايا الإتجاهية للخلايا الهيكلية في المنطقة الحولحبلية للجهاز المحوري لأجنة الدجاج عند المراحل ٢٠، ٢٣، ٢٦. بناءً على العلاقة الزاوية بين محوري جهاز جولجي والنواة والمحور الطولي للحبل الظهري. وأظهرت النتائج ست مجموعات إتجاهية مختلفة للخلايا الهيكلية - والنسبة العالية من الخلايا تتجه ناحية المحور الطولي للحبل الظهري وتتناقص في الإتجاهات الأخرى - واتضح أن هناك دوراً ما تلعبه المادة الخلالية وأيضاً الحبل الظهري والأورطي في تحديد إتجاهية الخلايا في المنطقة الحولحبلية.