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Research report

Quantitative analysis of neural plate thickness and cell density during gastrulation in the chick embryo

Lucía Rodríguez-Gallardo [a,*], Matías Hidalgo-Sánchez [a], Luisa Sánchez-Arrones [b], Lucía Prior [a], Luis Puelles [b]

[a] *Departamento de Biología Celular, Facultad de Ciencia, Universidad de Extremadura, Badajoz, Spain*
[b] *CEBERER grupo 736, Neurobiología, Facultad de Medicina, Universidad de Murcia, Spain*

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Abstract

We quantitatively analyzed the developing prospective neural and non-neural ectoderm during chicken gastrulation on semithin transverse sections. At stage PS8 (primitive streak stage 8 of Lopez-Sanchez et al. [C. Lopez-Sanchez, L. Puelles, V. Garcia-Martinez, L. Rodriguez-Gallardo, Morphological and molecular analysis of the early developing chick requires an expanded series of primitive streak stages, J. Morphol. 264 (2005) 105–116.], equivalent to stage HH4), the thickest area of the ectoderm agrees in extent with the fate-mapped neural plate we had reported previously. The thickness of the median ectoderm is constantly higher up to a distance of 250 μm from Hensen's node, and thickness decreases along a mediolateral gradient with a further drop at the prospective lateral border of the neural plate. A higher cell density of the developing ectoderm also coincided with the prospective neural plate. We observed that cell death does not play an important role in the spatial definition of the neural plate.

Keywords: Chick development; Hensen's node; Primitive streak; Ectoderm thickness; Cell death

1. Introduction

During early development, the embryo undergoes complex morphogenetic processes leading to gastrulation. The beginning of avian gastrulation becomes evident with the appearance of the primitive streak (PS) in the caudal portion of the area pellucida, at which locus cells delaminate and migrate into the subgerminal cavity to form the definitive germinal layers of the embryo. As development proceeds, the PS elongates rostrally towards the cranial pole, its rostral tip forming Hensen's node. We recently published a detailed chick neural plate fate map at stage 3d/4, interpreted relative to morphology observed at closed neural tube stages (HH9-11) [2,9]. The late gastrula/early neurula stage (HH3d/4; [11]) was selected, since this is the stage at which the neural plate attains full fate specification [1]. In this fate map the relevant epiblast area was sampled near to saturation with multiple overlapping grafts. We recently reexamined gastrulation in chick embryos from stages HH2 to HH6, using whole-embryo New culture and time-lapse video-microscopy, determining the organization of key median structures involved in gastrulation and obtaining an expanded series of primitive streak stages PS1-PS14 (HH2-HH6) [5]. A partial molecular specification map of the fate-mapped chick neural plate at PS8 (HH4; [8]) raised a number of points regarding the early delimitation of the neural plate from non-neural ectoderm. Both experimental and descriptive data gathered so far suggest the need of more detailed knowledge about the cellular structure of the neural plate primordium.

In the present study, we quantitatively analyzed study the spatio-temporal variation in thickness and cell density of the developing neural plate and surrounding non-neural ectoderm. We studied semithin transverse sections at several of the new (more discriminative) developmental stages (PS; [5]), in order to test the prediction that the emergent selective thickening (and possible higher cell density) of the earliest neural plate primordium is consistent in shape and extent with the fate-mapped contour. Cell death was also mapped in this material,

* Corresponding author. Tel.: +34 924 289411; fax: +34 924 289411.
E-mail address: lrguez@unex.es (L. Rodríguez-Gallardo).

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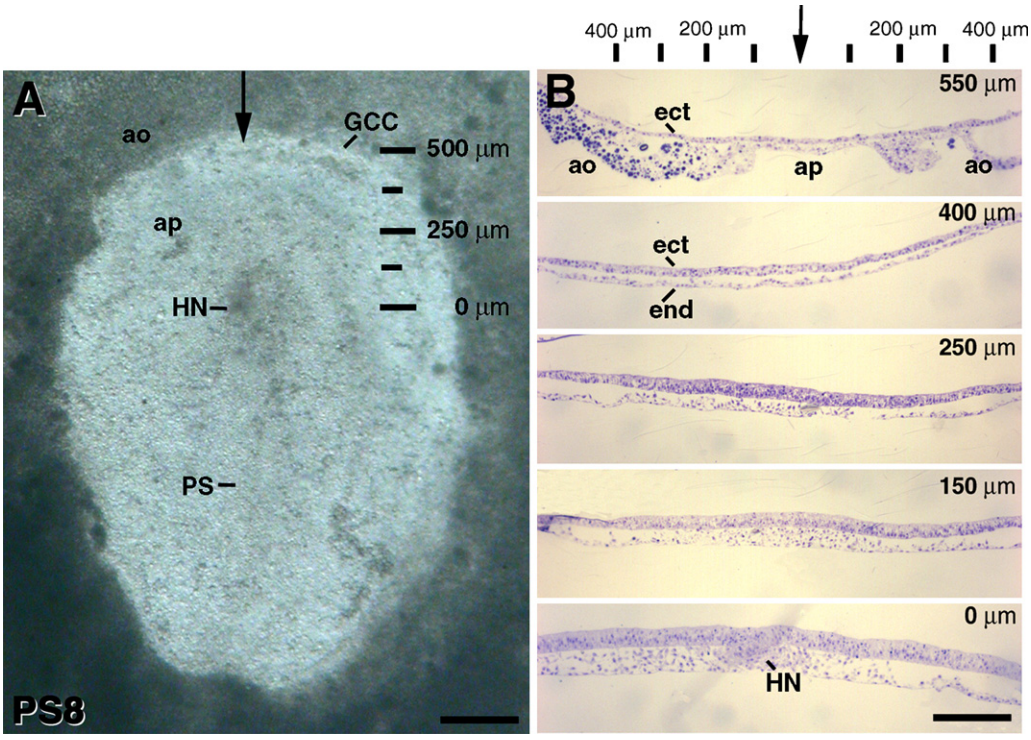

Fig. 1. (A) A whole-mount blastoderm preparation at stage PS8, seen from its apical surface in New culture. (B) Transverse semithin sections of a similar specimen taken at different anteroposterior levels from the germinal cell crescent (GCC) to Hensen's node; note increment of ectoderm thickness at about 250 μm from the node. The arrows in (A and B) point to the midline. Abbreviations: ao, area opaca; ap, area pellucida; ect, ectoderm; end, endoderm; GCC, germinal cell crescent; PS, primitive streak. Scale bars represent 600 μm in (A) and 250 μm in (B).

to determine its possible role in the development of neural plate morphology.

2. Material and methods

Fertilized chicken eggs were obtained from a supplier and incubated at 38 °C in a forced-draft, humidified incubator until the embryos reached appropriate stages for transfer to New culture [6]. A group of gastrulating embryos were fixed overnight in 2.5% glutaraldehyde in 0.1 M, pH 7.2, cacodylate buffer at 4 °C. The specimens were then rinsed in buffer solution, postfixed in 1% osmium tetroxide, dehydrated in a graded series of ethanol and propylene oxide, and embedded in Spurr resin. Serial semithin sections were cut with a LKB Ultratome III and stained with 1% toluidine blue.

For quantitative analysis, the thickness of the ectoderm was measured on digital microphotographic images obtained from the semithin sections. Hensen's node and the epibast midline were considered as caudal and medial reference landmarks, respectively. We determined the ectoderm thickness from Hensen's node on both sides of the epiblast midline, which was considered as the 0 μm point (Fig. 1). We also measured cell density in each semithin section (as cell nuclei per 100 μm).

Gastrulating embryos were hybridized *in situ* with chick *Sox3* probes [8,9]. Embryos were fixed in 4% paraformaldehyde overnight, and then processed for whole-mount *in situ* hybridization [7]. After whole-mount ISH the embryos were sectioned in a cryostat [10]. Sections 20 μm thick were obtained in the transverse plane. Selected slides from these series were processed for TUNEL staining [3].

3. Results and discussion

During gastrulation, the PS increases in length relative to that of the area pellucida (PS/AP) between PS2 (HH3 or stage 3a) and PS8 (HH4; [5]). Our results showed that at stage PS2, when the PS/AP is 40–45% maximal length, the ectoderm was slightly thicker near Hensen's node (in a stretch approximately 50 μm from Hensen's node, $n = 5$; not shown). At PS4 (50–55% maximal PS/AP), the pattern of ectoderm thickness was not much different from that described at PS2 ($n = 6$; not shown). At stage PS6 (60–65% maximal PS/AP) the ectoderm was consistently thicker along the midline up to about 200 μm from HN (Fig. 2A and B). At PS8 (HH4; maximal PS/AP), when the primitive streak halts its rostral progression and covers two-thirds of the area pellucida, the maximal thickness of the median ectoderm was constant in a domain extending 250 μm from Hensen's node ($n = 5$; Fig. 2E and G). More laterally, prospective spinal and medullary areas of the neural plate show the greatest overall thickness (Fig. 2E) and there is a decrease in this variable both laterally (into prospective neural crest) and rostrally (into prospective dorsal midbrain and forebrain; Fig. 2E and G). Thickness drops down more significantly into the non-neural etoderm surrounding the neural plate. A singularity is represented by the prospective adenohypophysial rudiment, mapped rostrally in front of the neural plate [2], which shows at PS8 an intermediate thickness between that in the median neural plate and the more peripheral non-neural ectoderm (Fig. 2E and G). These measurements are roughly consistent in length and timing with the prospective neural plate domain fate-mapped at PS8 (compare yellow contour in Fig. 2D; [2]).

The ectodermal cell density measurements showed a gradient decreasing progressively away from Hensen's node during