International Journal of Multidisciplinary Research And Studies

Available online at https://ijmras.com/

INTERNATIONAL JOURNAL OF MULTIDISCIPLINARY RESEARCH AND STUDIES
ISSN: 2640 7272
Volume:03; Issue:05 (2020)

FORMATION OF ALL PRE-THORACIC VERTEBRAE AND ASSOCIATED TISSUES IN ANAMNIOTES AND AMNIOTES

PRABHU JEE
M.Phil., Roll No.: 150664 Session-2015-16
Department of Zoology, B.R.A. Bihar University, Muzaffarpur, India
E-mail: prabhuji5689@gmail.com

ABSTRACT

The structure of the skull, the number of apertures in the skull, the presence of particular bones in the skull, and the structure of the limbs are some of the morphological and molecular criteria that are used in the categorization of amniotes. The sauropsids and the synapsids are the two most important subdivisions of the amniotes. To have a complete knowledge of the variety of vertebrate life on Earth as well as its history, it is vital to have a firm grasp on the taxonomy and development of amniotes. Both the bottlenose dolphin and the African elephant have been subjected to comparative anatomical research, which has found both similarities and differences between the two species. For instance, people of both species have hearts that are divided into four chambers and are warm-blooded, sometimes known as endothermic. Nonetheless, as a result of their adaptations to their respective surroundings, their skeletal structures are strikingly unlike to one another. While the large and sturdy skull of the elephant is able to carry the weight of its enormous tusks, the delicate and streamlined skull of the dolphin reduces the amount of resistance it experiences when swimming. In a similar fashion, the teeth of an elephant are built for crushing tough plants, but the teeth of a dolphin are adapted for capturing and holding onto prey

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Keywords: Tissues, Anamniotes , Amniotes , Pre-Thoracic, Vertebrae

INTRODUCTION

Anamniotes And Amniotes

There are two primary classes of vertebrates, known as anamniotes and amniotes, which are distinguished from one another by the embryonic growth and anatomy of their offspring. In this chapter, we will provide a definition for each of these two categories, as well as a detailed description of each.

Anamniotes are a category of vertebrates that do not have an amniotic membrane present during embryonic development. Anamniotes are also known as agnathans. Their eggs are often placed in water or on damp ground, and they are either aquatic or semi-aquatic in their lifestyle. Agnatha, Chondrichthyes, and Osteichthyes are the three primary classes of vertebrates that belong to the order Anamniotes.

The hagfish and the lamprey are both members of the Agnatha family, which is a group of jawless fish. These fish do not possess actual bones but rather have a skeleton made of cartilage. They consume other fish by using tooth-like features on their mouths and jaws to scrape away at the flesh of their prey. This is an unusual mode of eating. Agnatha are additionally distinguished by the absence of paired fins and scales.

The Chondrichthyes are a class of cartilaginous fish that consists of sharks, rays, and skates, among other related species. The fish in question have a skeleton that is composed of cartilage rather than bone. In addition to this, they have many rows of teeth, all of which are continually replaced during the course of their lives. The skin of chondrichthyes is rough and leathery, and it is covered in microscopic scales that resemble teeth and are termed dermal denticles. Chondrichthyes have paired fins.

The Osteichthyes are a group of bony fish that contain both lobe-finned fish and ray-finned fish in their classification. The skeleton of this fish is formed of bone, and each of its fins is coupled with a ray of bony cartilage. They are also equipped with a swim bladder, which gives

them the ability to regulate their level of buoyancy. The Osteichthyes have scales that, for the purpose of protection, are coated in a coating of mucus..

Protective barrier against desiccation and mechanical damage

The amniotic egg, which is a characteristic that is unique to amniotes, possesses a number of modifications that make it possible for embryonic development to take place on land. The amniotic membrane, which encases and protects the embryo, acts as a barrier that prevents the embryo from becoming dry and being damaged by mechanical forces. Moreover, the eggshell is permeable to gases, which enables the free circulation of oxygen and carbon dioxide within the egg.

The amniotic egg has developed into a number of different forms in reptiles throughout the course of their evolutionary history. These forms include hard-shelled eggs, soft-shelled eggs, and leathery eggs. Both the composition of the eggshell and the organisation of the amniotic membrane are modified to correspond with the conditions of the habitat in which the reptile lives. For instance, in order to protect themselves from becoming dehydrated, the eggshells of reptiles that inhabit arid settings may be thicker, and their amniotic membranes may be more complex.

On the other hand, mammals have developed a one-of-a-kind adaption in order to facilitate embryonic development on land. They have created a unique foetal membrane that is referred to as the placenta. This membrane enables the transfer of nutrients, gases, and waste products from the mother to the developing foetus. The foetal membrane, the uterine lining, and specialised tissues known as chorionic villi are all components of the placenta. Together, these make up the placenta's complex structure.

The creation of an internal skeleton, which offers support and protection for the body, is another another adaptation that amniotes have made in order to survive on land. Amniotes have a backbone or vertebral column that is made up of individual vertebrae as part of their skeletal system. The vertebrae are connected to one another by intervertebral discs, which enable the body to be mobile and flexible.

The skull of an amniote is likewise substantially changed, and it has a number of changes that improve the animal's ability to eat and perceive its environment. The jaws of reptiles and

mammals are usually hinged and may move freely, which enables them to employ a diverse array of eating tactics. In amniotes, the sense organs, such as the eyes, hearing, and olfactory system, are likewise highly developed and suited to the particular requirements of the creature..

OBJECTIVES OF THE STUDY

1. To study on Protective barrier against desiccation and mechanical damage
2. To study on Anamniotes And Amniotes

RESEARCH METHOD

Zebrafish Embryo Culture And Live-Imaging

Adult zebrafish were purchased from ZFIN and kept in a tank with synthetic "fish water" (Westerfield, 1995). Brine shrimp, either fresh, frozen, or lyophilized, were provided on a consistent basis to the animals (Artemia salina). In order to achieve the highest possible fertility in the fish, they were kept in a circadian cycle consisting of 14 hours of light and 10 hours of darkness at a temperature of 28 degrees Celsius throughout the experiment. Moreover, the fish were only allowed to mate once per week at most. Embryos from several broods were collected on separate days from natural spawning in mating boxes equipped with perforated bottoms. The purpose of these boxes was to collect eggs and protect them from being eaten by adults. The collected embryos were put in "embryo medium" (Westerfield, 1995), and after that, they were kept in an incubator at a temperature of 28 degrees Celsius so that they could develop normally. We picked embryos for imaging using a stereoscope, and according to Kimmel et al. (1995), we determined their phases of development based on the amount of epiboly that was finished and the amount of time that had passed from fertilisation at 28.5 degrees Celsius. Chosen embryos were removed from their chorion by hand and then mounted on 0.3 percent low-melting agarose (LMA) in embryo media for the purpose of immobilisation (see Figure 1). This concentration was selected because it offered the best possible balance between the amount of time it took for the LMA to polymerize and the amount of freedom of movement that was required for optimal embryo development. Following dechorionation, the embryos were embedded in LMA by transferring them to a Falcon 15ml tube that contained melted LMA. This step took place after the embryos had been decapsulated. After that, a drop of the solution that contained each embryo was transferred to Petri plates of 35 mm in diameter, and

the polymerization process was allowed to continue at room temperature. After that, freshly prepared embryo media was poured into the Petri dish until it reached the very top, and a coverslip was placed on top (see figure 1 for a schematic view of the mounted embryo). This kept the embryo from being dehydrated as well as preventing condensation from forming on the coverslip. The temperature in the room was maintained at 28.5 degrees Celsius..

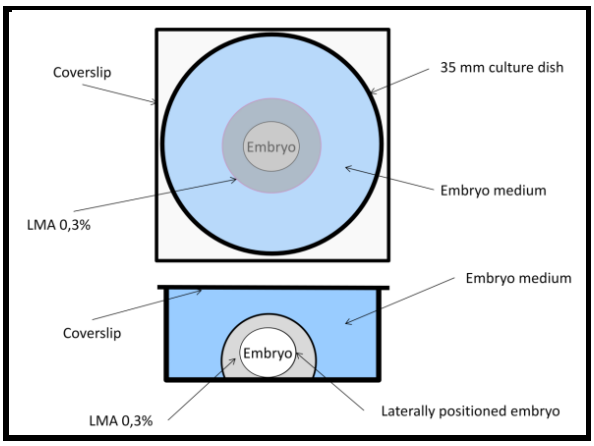

Figure 1 (Top and side views) A diagrammatic representation of the culture chamber that was used to scan zebrafish embryos)

Time-lapse images were acquired with a Zeiss Stereo LUMAR stereoscope beginning at the shield stage (6 hours post fertilisation) (n=2) or at the 75% epiboly stage (8 hours post fertilisation) (n=2) (Kimmel et al., 1995) and continuing until approximately the 15 somite stage (16,5 hours post fertilisation). The zebrafish embryo has produced the tissues that, in the adult, would give birth to the whole of the head as well as the pre-caudal vertebrae by the time it reaches the 15 somite stage. A total of nine embryos were videotaped, but only three of them were chosen for further examination since their development was normal and they kept the lateral orientation (Fig 2) that was required for the calculations of axis elongation. The starting stage was chosen because it is the first stage in which the dorso-ventral polarity can be identified. This makes it possible to orient the embryo in a sagittal plane, which in turn makes it easier to measure the AP axis. The starting stage was also chosen because it is the stage at which the embryo is most likely to survive (see movie 1 in annex). We decided to halt the time-lapses at the fifteen somite stage because at that moment, the embryo's tail had reached its head and there were physical limits within the LMA that needed to be overcome in order to permit

appropriate embryo growth. The amount of time that passed in between each time point was determined to be 5 minutes..

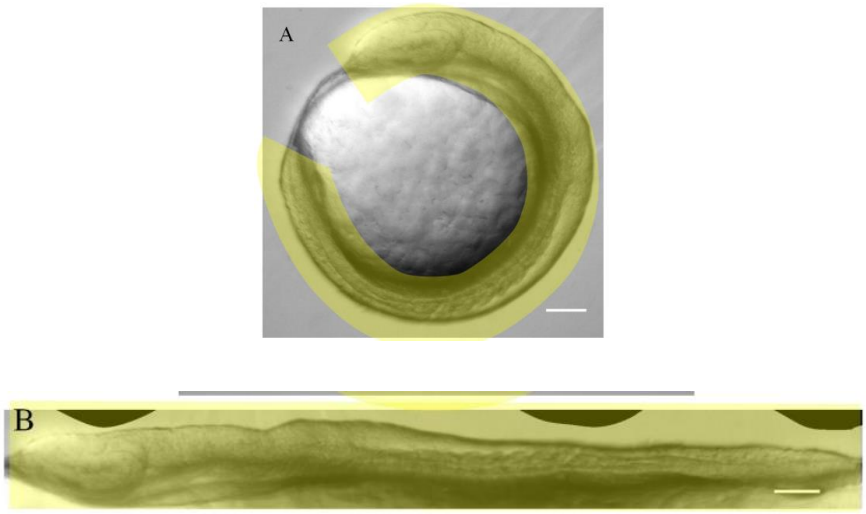

Figure 2 An illustration of how to position and "straighten" digital photographs of zebrafish embryos may be seen here. A) The picture in its original state, prior to any "straightening," with the region of interest highlighted in yellow. B) The final picture, which may be used to take linear measurements of the embryo's axis extension.

Embryo Cultivation and Live Imaging of Chick Embryos

In an incubator set to a temperature of 38 degrees Fahrenheit, fertilised eggs from a chicken (Gallus gallus) were inserted. In accordance with Hamburger and Hamilton's research, eggs were windowed, and embryos were staged (Hamburger and Hamilton, 1951). Embryos that were between stages 3 and 6 were collected on a variety of days and transplanted into the "New" culture system (New, 1955) for the purposes of further culture and observation, as well as the capture of time-lapse images (Figure 4). On the other hand, we utilised plastic rings instead of glass rings. These rings had an inner diameter of 2.50 centimetres and an outside diameter of 2.80 centimetres and were cut from 50 millilitre Falcon tubes. These rings had the perfect diameter, which enabled sufficient expansion of the region opaca and proper fitting of the vitelline layer (Stern & Bachvarova, 1997).

Some embryos were nurtured in an incubator designed for cell culture, which was maintained at a temperature of 38 degrees Celsius with a humidity level of 100 percent. An Olympus C-

4040ZOOM camera attached to the eyepiece of a Nikon SMZ645 stereoscopic microscope was used to capture images of these embryos at the beginning and conclusion of the culture phase.

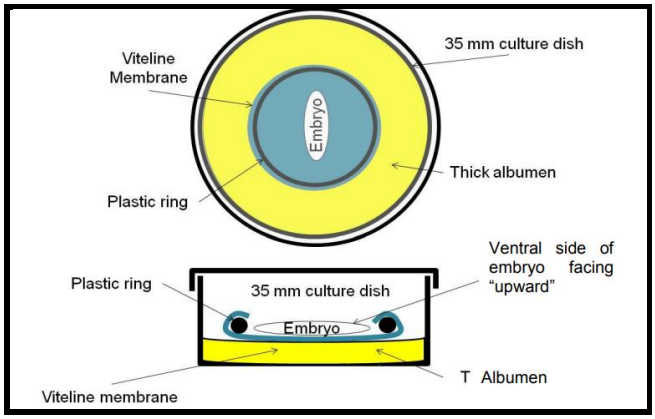

Figure 3 A diagrammatic representation of the culture chamber that was used to take pictures of the chick embryos. Top and side views.

A fully automated Zeiss Stereo LUMAR stereoscope equipped with a Cooled CCD Axiocam was used to capture time-lapse images of other embryos. The camera was programmed to take a picture every six minutes over a period of 14 to 24 hours (see movie 2 in annex). Because chick embryos develop at a more gradual rate than zebrafish embryos, we decided to use this particular gap between time periods. In order to keep the appropriate environmental conditions, we fabricated a custom incubation chamber (Figure 4), which consisted of a small round box (Figure 4A), whose interior contained a "donut-shaped" reservoir filled with water (to maintain a humid environment), and an inner chamber where the Petri dish containing the embryo in "New" culture was placed. Figure 4 shows the incubation chamber in its assembled state. After that, an episcopic vision of the embryo was made possible by covering the box with a lid that had a big no. 0 coverslip attached to it (Figure 4B). The temperature within the chamber was kept constant by placing a terrarium heater (shown in Figure 4C) underneath the microscope stage and another heater on top of the incubation chamber. Both of these methods were used in conjunction with one another (Figure 4B). This not only maintained a constant temperature within the chamber, but it also prevented condensation from forming on the lid, which would have rendered it difficult to observe the embryo..

DATA ANALYSIS

Mechanics of Axis Elongation During Zebrafish Embryo Development

The dynamics of axis extension in zebrafish demonstrate that there are two different periods of embryonic growth between the shield stage (6 hours post fertilisation) and the 15 somite stage (16.5 hours post fertilisation). The shield stage is the beginning of the first phase, and it lasts until about the fourth somite stage. The second phase begins at approximately the fourth somite stage and lasts until at least the fifteenth somite stage (Figure 9A). We displayed the average lengths and the related standard deviations for each "synchronised" time-point (see Materials and Methods), which allowed us to determine that the transition point between the two phases occurs at the 4 somite stage. Figure 9B shows the results of this analysis (the time-point with the highest standard deviation during the period of 4-5h since the beginning of the culture). We divided the data into two sets (one with measurements up to the 4 somite stage, and another with measurements from the 4 somite stage onwards) and plotted the numbers against a time series so that we could do a more in-depth analysis of the growth rates of each phase (Figure 9C). The initial phase of growth is unmistakably linear, which means that the embryo expands at a pace that is always the same. In order to demonstrate this, we computed the matching trendline, and the linear model proved to be the most accurately adjusted option, with an R2 value that was more than.99. 310.78 micrometres per hour was the average elongation rate (AER). Nevertheless, during the second phase, the best corrected trendline was logarithmic, and the AER was significantly lower, averaging 90.68 micrometres per second.

According to Kimmel and colleagues' (1995) findings, the length of the zebrafish embryo, which he defines to be the "embryo's longest linear dimension," does not expand until the 14 somite stage. According to the findings of our study, there is a genuine elongation that occurs before the 14 somite stage, and this elongation occurs in two phases. We estimate a growth rate for the first phase (310,78 m) that is actually higher than the one determined by Kimmel (1995) for the period between 16 hpf (14 somites) and 32 hpf (prim-stage), which was of 125 m/h. This is the case when comparing the values of the elongation rates analysed here and the ones proposed by (Kimmel et al., 1995)..

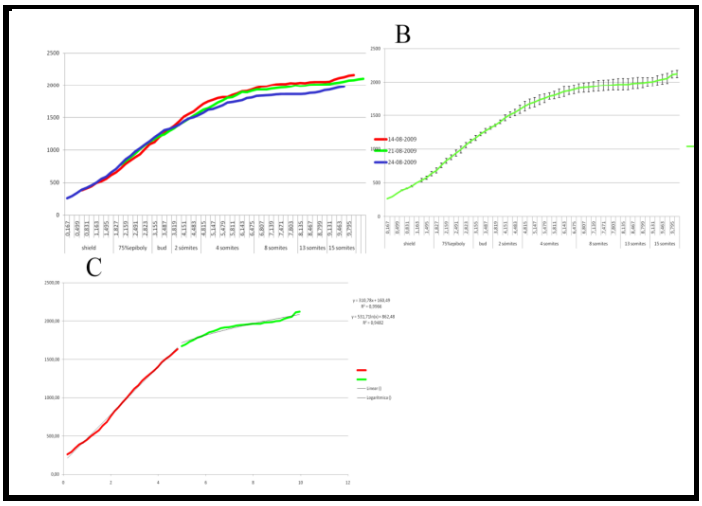

Figure 4. Plots illustrating the expansion of the embryonic axis in zebrafish embryos The time and/or synchronised phases are denoted in hours, and the length is denoted in micrometres. A) Illustrates how each of the three embryos under study developed over time. B) Displays the averages as well as the standard deviations for each time point that has been "synchronised." C) Plots showing the two periods of development independently, together with their respective trendlines and R2 values.

Table 1 Lengths of zebrafish embryos at each developmental stage, together with their associated standard deviations. A value of 0 indicates that there was only one embryo measured at that stage.

Stage (Kimmel 1995)	Shield	75%epiboly	Bud	2 sómites	4 somites	8 somites	13 somites	15 somites
Embryo length (µm)	262,29	681,98	1163,12	1352,29	1634,9	1908,71	1964,03	2003,46
Standard deviation	±0	±39,3	±36,96	±17,96	±71,46	±68,81	±91,95	±64,88

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The Mechanics of Axis Elongation During Chick Embryo Development

After the completion of our investigation into the dynamics of embryo axis elongation in zebrafish, we turned our attention to the dynamics of embryo axis elongation in chicks between stages HH4 and HH11. At the beginning, we tried to have this analysis cover a longer period of time [at least until stage HH13 (19 somites)], so that we could analyse the formation of all

of the pre-thoracic vertebrae and accompanying tissues. Because of the restrictions that come along with the "New" culture

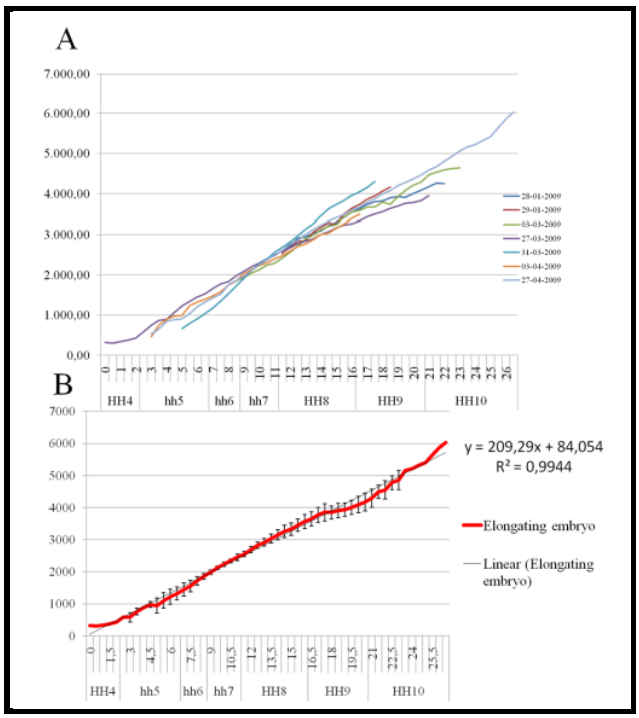

Figure 5A) The length of each of the seven time-lapse videos that were created is represented below along with its respective measurement. XX represents the hours and phases of HH. YY is the length of the embryo in micrometres. B) Displays the average embryo length as well as the standard deviations for each "synchronised" time point at which embryo length was assessed. It is calculated that the AER is 209.29 m/h.

Then, we wanted to examine the dynamics of the elongation of the various parts of the embryo to determine whether or not they develop linearly as well, or whether or not certain parts grow more quickly than others at the phases that were investigated. The length of these sections was measured and shown in a bar graph (Figure 11) to indicate the average embryo lengths over time. For further information on the materials and methods used, see above. The lengths of the three different sections of the embryo are listed in Table II, together with the accompanying standard deviations in the time period that was examined, and the average embryonic ratios (AERs) for each section..

Discussion

Both the physiology and reproductive physiology of amniotes and anamniote vertebrates are very different from one another. The development of the cleidoic egg set them on a course that ultimately led to their ability to successfully reproduce on land. Without running a significant risk of drying out, the embryos are able to develop, continue growing, and exchange gases with the surrounding environment thanks to this. There is a possibility that the development of the extraembryonic membranes of amniotes places a limitation on the smallest possible size of amniote eggs or embryos. This limitation is presumably not present in our anamniote relatives, and as a result, the eggs of anamniotes are often quite a bit smaller, even in species with huge bodies.

The next step in the evolution of the amniotes was the development of endotherms (twice), shelled ectotherms (once), and a massive lepidosaur/squamate radiation that maintained the primordial position of ectothermy without a shell. Endotherms, in contrast to other reptiles, developed sophisticated and time-consuming methods of thermal and nutritional care for their young, as well as frequently intricate social structures. Viviparity has undergone several rounds of evolution, and it can present itself in two quite different ways among mammals. All of these factors, in addition to others, almost definitely had an impact on the reproductive and life cycle features of amniote species. Remarkably, however, our assumptions, which stated that these big transitions will result in considerable quantitative differences between clades, were largely disproved by the data..

CONCLUSION

During the studies in which the notochord was removed, we also discovered that a cluster of cells had developed near the caudal end of the remaining piece of the cranial notochord. Using multi-photon time-lapses imaging of chick embryos expressing GFP, recent work from our group in chick embryos demonstrated a distinct relocation of notochord cells relative to the PSM and somitic mesoderm (Martins et al., 2009), unpublished data. Similar "shearing motions" have also been recorded in Xenopus embryos (Wilson 1989); the authors noticed a substantial flow of notochord in relation to the surrounding mesoderm in explant cultures of dorsal mesoderm from neurula stage embryos. They hypothesise that this is because of the various regional patterns of extension, and as a result, towards the back of the embryo, the

rearrangements of the mesoderm make it possible for it to keep up with the moving notochord. More recently, analogous motions have been detected in zebrafish embryos (Glickman, 2003), and the authors also rationalise this fact with a significantly higher expansion in the notochord compared to that of the somitic mesoderm. These discoveries led researchers to hypothesise that the "faster" pace of extension of the notochord could be the primary factor responsible for the embryo organizer's migration towards the caudal end of the embryo. Our findings make it very evident that this is not the case in the embryos of chicks. It has been demonstrated beyond a reasonable doubt that the early extension of anamniotes embryos, such as those of fish and amphibians, is driven by epiboly movements of tissues that have not yet been internalised. This means that the forces that produce embryo axis extension are generated outside of the embryo itself. In amniotes, such as the embryos of chicks, the forces that give the early embryo its characteristically elongated form must originate from tissues within the embryo itself. This is seen in the diagram below. We have demonstrated that the majority of these pressures must originate from other types of tissue besides the notochord. These forces must have developed in amniotes in order to allow the embryo to stretch on its own, without the "added" force provided by increasing extraembryonic tissues, as is still seen in the majority of anamniotes. This phenomenon is still observed in amniotes..

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