Embryological evidence for a possible polyphyletic origin of the recent amphibians¹

By P. D. NIEUWKOOP² AND LIEN A. SUTASURYA³

From the Hubrecht Laboratory, Utrecht, and the Department of Biology, Bandung

SUMMARY

The markedly different mode of mesoderm formation in anuran and urodelan amphibians (which is related to the early double-layered nature of the anuran blastula wall in contrast to its single-layered nature in the urodeles), but particularly the fundamentally different place and mode of origin of the primordial germ cells in the two groups of amphibians, strongly pleads in favour of a very ancient bifurcation in the phylogenetic history of the two groups, even suggesting a polyphyletic origin from different ancestral fishes.

INTRODUCTION

Although Haeckel’s biogenetic law (1891, 1894) – according to which animals pass through the same stages in their embryonic development as they did in their phylogenetic history – is no longer considered as having general validity, it cannot be denied that related species show a nearly identical embryonic development up to a relatively advanced stage, after which their development begins to diverge. This is the consequence of the fact that related species have a large number of genes in common. Animals which are not so closely related generally diverge in their embryonic development at an earlier stage. Finally, the development of species which are hardly related at all differs from the very beginning or shows a very short common phase only, after which each follows its own separate course. (It should, however, be realized that these considerations cannot simply be generalized, since at any stage of development the embryo represents a functional unit which vitally depends upon its environment. Unrelated forms may therefore be subject to similar environmental conditions leading to common features in their development.) In our opinion, the converse is therefore also true: the earlier and the more pronounced the differences in embryonic development of the two species or two animal groups, the wider they are apart genetically. Accepting the theory of evolution for the

¹ Dedicated in personal friendship to Prof. Dr Etienne Wolff on the occasion of his retirement.
² Author’s address: Hubrecht Laboratory, Utrecht, Holland.
³ Author’s address: Department of Biology, I.T.B., Bandung, Indonesia.
explanation of the diversity of forms in the animal and plant kingdoms, this would mean that forms which differ strongly in development must have developed along different evolutionary pathways from an early phase of their phylogenetic history. We would like to review some features in the development of the two groups of the living infraclasses of amphibians, namely the anurans (belonging to the Salientia) and the urodeles (belonging to the Caudata) in the light of the above considerations. We must leave the rather unknown order of the Caecilians out of consideration for lack of relevant information.

**OBSERVATIONS: DISCUSSION**

*Comparison of the embryonic development of anuran and urodelan amphibians*

It is evident that the anuran and urodelan amphibians differ anatomically in many respects. This becomes particularly evident during metamorphosis, when the anuran tadpole changes into a tailless frog or toad, whereas the urodelan larva hardly changes in general appearance. It is, however, not our intention to discuss here the anatomical features of representatives of the two groups, but to focus attention upon their early development.

When comparing the maps of presumptive organ rudiments in early embryos of the two groups (see the anlage maps of *Triturus* and *Bombina*, Vogt (1929)) there is, among other things, a striking difference in the width of the marginal zone. At the early blastula stage (ca. stage 8 Harrison) the marginal zone comprises a sector of about 60° on the dorsal side in *Triturus* (cf. Fig. 1d), but of only 45° in *Bombina* (cf. Fig. 1c). The presumptive neural area is also markedly larger in *Triturus* than in *Bombina*: 65° as against 35°. Whereas the future anterior end of the brain coincides with the original animal pole of the *Triturus* egg, it lies 35° away from the animal pole in the *Bombina* blastula; consequently in the *Bombina* larva the original animal pole corresponds to a point somewhere in the cranio-ventral epidermis of the heart region. These differences, however, only concern the relative dimensions of the organ anlagen. Although they are quite pronounced, it is not self-evident that they are of a fundamental nature.

A much more fundamental difference between anuran and urodelan development concerns the position of the marginal zone, i.e. the presumptive endomesodermal anlage, in the two groups. In the anuran blastula/early gastrula the marginal zone is exclusively internally located (cf. Fig. 1c), in contrast to its external position in the urodelan blastula (cf. Fig. 1d). This difference was not clearly understood by Vogt (1929), but was extensively described by Nieuwkoop and Florschütz (1950) and recently verified by Keller (1975) by means of careful vital staining experiments. The internal position of the presumptive chordal and prechordal mesoderm in the anuran blastula (see also Pasteels (1949) for the position of the prechordal endo-mesoderm) is clearly
Polyphyletic origin of recent amphibians

Fig. 1. Diagrammatic representation of mesoderm formation and origin of PGCs in the anuran and urodelan amphibians. (a) Location of the germinal cytoplasm in the vicinity of the vegetative pole of the anuran egg. (b) Absence of germinal cytoplasm in the urodelan egg. (c) Location of the PGCs in the floor of the blastocoeel, and formation of the relatively narrow, exclusively internal marginal zone in the anuran blastula. (d) Formation of the more extensive and externally situated marginal zone, and origin of the PGCs from the latero-ventral mesoderm in the urodelan blastula. (e) Migration of the endodermal PGCs towards the future gonadal anlagen in the young anuran larva. (f) Migration of the mesodermal PGCs towards the future gonadal anlagen in the young urodelan larva.
related to the double-layered nature of the blastocoelic wall, in contrast to the essentially single-layered nature of the urodelan blastula wall. In the anuran blastula the outer, unicellular layer gives rise to the ectodermal epithelial layer of the epidermis and the ependymal layer of the central nervous system, as well as to the endodermal inner lining of the archenteron, while the inner, pluricellular layer comprises the presumptive sensorial layer of the epidermis, the bulk of the future nervous system, the entire chordal and prechordal mesoderm, and the bulk of the endoderm (cf. Fig. 1c). In the urodelan blastula there is no sharp delimitation between an outer and an inner layer and both the presumptive nervous system and the mesoderm are represented in the outer surface (cf. Fig. 1d). A segregation of the ectoneuroderm into an epithelial and a sensorial epidermal layer and into the corresponding layers of the C.N.S. only occurs much later in the urodelan embryo. This developmental difference between the two groups is quite fundamental. In the anuran embryo the double-layered character arises very early in development, namely between the 32-cell stage (stage 6 N. and F.), which is still single-layered, and the early blastula (stage 7 N. and F.), which is already essentially double-layered due to radial cell divisions starting in the equatorial region (stage 6½ N. and F.) and spreading rapidly over the animal hemisphere (Nieuwkoop & Faber, 1967). A further thinning of the outer, epithelial layer by local radial divisions continues, up to at least the late blastula stage. The double-layered character of the anuran embryo certainly represents a very early divergence in the development of the two groups.

We now come to a still more fundamental difference between the two groups of amphibians, namely the origin and development of the primordial germ cells (PGCs).

In the anuran Rana temporaria the development of the PGCs was studied in detail by Bounoure in the nineteen-thirties (see Bounoure, 1939). He found that the PGCs are characterized by a special cytoplasmic component, the so-called 'germinal cytoplasm', which according to Mahowald & Hensen (1971), Williams & Smith (1971) and Czolowska (1972) consists of an accumulation of mitochondria, 'germinal granules' with a particular ultrastructure, and polyribosomes. This germinal cytoplasm can be traced back to the fertilized egg, where it is situated under the plasmalemma in the vicinity of the vegetative pole in the form of small islands (cf. Fig. 1a). It could even be found in a more fragmentary form in the mature oocyte (Czolowska, 1969). As a consequence of the original location of the germinal cytoplasm in the vicinity of the vegetative pole the PGCs are of endodermal origin in the anurans. According to Whittington & Dixon (1975), in Xenopus the germinal cytoplasm during cleavage becomes distributed over a restricted number of blastomeres and often passes into only one of the daughter cells of a dividing blastomere. They could show that only those blastomeres which receive (sufficient?) germinal cytoplasm will develop into PGCs. During cleavage and blastula formation they move towards
the vicinity of the blastocoel (cf. Fig. 1c) and are subsequently found in the caudal portion of the invaginated yolk mass, from where they migrate – probably by amoeboid movement – towards the mid-dorsal endoderm, from there into the dorsal mesentery, and finally into the germ ridges (cf. Fig. 1e). During neurulation the germinal cytoplasm becomes displaced within the blastomeres from an originally peripheral to a juxtanuclear position. Blackler (1970) could show that the PGCs represent the only cellular elements which give rise to the next generation. In the anurans, therefore, the continuity of the germ line throughout the entire life of the individual could be clearly demonstrated; the PGCs being set apart as specific elements from the somatic cells at a very early stage of development and being characterized by the presence of germinal cytoplasm.

When we now turn our attention towards the urodeles, Humphrey (1925, 1927, 1928, 1929) already demonstrated that in an advanced tail-bud stage of *Ambystoma* the PGCs are situated in the lateral mesoderm in the vicinity of the Wolffian ducts, and move from there towards the genital ridges after the formation of the coelomic cavities (cf. Fig. 1f). By means of extirpation and transplantation of portions of the endoderm or of the entire endoderm at gastrula to neurula stages, Nieuwkoop (1947) could demonstrate that in the urodeles the PGCs do not originate from the endoderm (cf. Fig. 1b). Removal of the presumptive lateral plate mesoderm at an early neurula stage led to completely sterile larvae (Nieuwkoop, 1947). In hetero- and xenoplastic chimaerae made by transplantation of the latero-ventral marginal zone at a mid-gastrula stage, PGCs of both species were found in the genital ridges, and there was a clear correlation between the regional chimaeric composition of the lateral plates and the regional distribution of PGCs of the two species. In our opinion, these facts irrefutably prove the mesodermal origin of the PGCs in the urodeles (cf. Fig. 1d). Smith (1964), using eggs of genetically different white and black axolotls, could show that the PGCs which originate from the latero-ventral mesoderm actually give rise to the next generation. In contrast to these observations the Japanese authors Amanuma (1957), Asayama (1950, 1961) and Asayama & Amanuma (1957) came to a different conclusion on the basis of extirpations of lateral mesoderm. However, Capuron (1972) could demonstrate that their extirpated material did not correspond topographically with the actual position of the PGCs at the stages of operation. Kotani (1957) was the first to claim that in the urodeles the PGCs originate from the ectoderm. This observation was recently corroborated by induction experiments made by Kocher-Becker and Tiedemann (1971). Very recently Miss Sutasurya could demonstrate that the PGCs in the urodeles exclusively originate from the animal, ectodermal moiety of the blastula. They are not formed from specific cellular elements set aside at the beginning of development, but actually originate from common, totipotent ectodermal cells under an inductive influence exerted by the ventral endodermal yolk mass. They apparently constitute one of the characteristic cellular elements.
of the induced ventro-caudal mesoderm, just as, for example, the pigment cells represent a characteristic element of the neural crest; for the PGCs can arise from any portion of the animal, ectodermal moiety of the blastula. However, in the blastula the competence for PGC formation (like that for mesoderm induction in general) decreases from the equator towards the animal pole. In normal development PGCs are only formed in the ventral and latero-ventral marginal zone (cf. Fig. 1d). This is because their formation elsewhere is suppressed by the dorsal type of mesoderm induction leading to notochord and somite differentiation in the dorsal and lateral marginal zone (Sutasurya & Nieuwkoop, 1974). As soon as the mesodermal competence is lost, PGCs can no longer be induced. Smith's (1964) negative results after transplantation of early gastrula ectoderm into the latero-ventral marginal zone are probably due to the fact that the inducing capacity of the endoderm had faded out by the time the operations were carried out (the mid-gastrula stage) (See Boterenbrood & Nieuwkoop, 1973).

From this it must be concluded that some very fundamental differences in PGC formation exist between anuran and urodelan amphibians. The PGCs not only have an entirely different place, but also an entirely different mode of origin in the two groups. On the other hand, it must be realized that in the anurans, where the germinal cytoplasm can already be found in the mature oocyte, the PGCs may nevertheless not fully segregate from somatic cell material before an advanced gastrula stage, while in the urodeles the induction of the latero-ventral mesoderm is not accomplished before a mid-gastrula stage.

Several important questions arise at this stage. When are the PGCs actually formed in the urodeles? Although in the urodelan larva the PGCs are histologically quite similar to those of the anuran larva, it is not known either whether in the former the PGCs are also characterized by germinal cytoplasm. If so, when and how does this arise? If not, what characterizes the PGCs in the urodeles, at what stage of development are they actually formed, and when do they acquire their characteristic features?

The phylogenetic origin of the anuran and urodelan amphibians

The pronounced differences in embryonic development of the anuran and urodelan amphibians, exemplified among other things by the differences in mesoderm formation, but above all by the fundamental differences in PGC formation in the two groups, in our opinion strongly suggests that the two groups of amphibians are actually only remotely related. These fundamental differences can only be understood if the bifurcation in the evolution of the two groups is placed as far back as possible in the phylogenetic history of the vertebrates.

Colbert in his monograph (1955) places the origin of both anurans and urodeles back in the Carboniferous period, possibly from different ancestors. In the second (1969) edition, however, he suggests on comparative-anatomical
Polyphyletic origin of recent amphibians

grounds that the two infraclasses *Salientia* and *Caudata* belong to the same subclass of the *Lissamphibia* and have a common origin. We must say that the arguments for the latter statement are not very convincing. He actually mentions that not only is the origin of the anurans not yet known – the first fossil records of the anuran ancestors being found in the lower Trias, namely the genus *Triadobatrachus* from Madagascar – but the origin of the urodèles is still more uncertain – the first urodelan fossils being known only from Cretaceous sediments. He further suggests that the teleosts have developed in the Devonian and Carboniferous periods from holostean and chondrostean ancestors, which in their turn have probably originated from the first, jawless vertebrates in the Ordovician period. The ancestors of the reptilians go back in history to the early Carboniferous vertebrates from which the amphibians may also have originated.

In her recent book Barbara J. Stahl (1974) extensively discusses the still controversial opinions on the origin of the modern Amphibia. Whereas, for example, Jarvik and Schmalhausen strongly defend a diphyletic origin of the recent Amphibia, Parsons & Williams and Thomson support a monophyletic origin; the sharply opposed opinions being mainly due to the scarcity of palaeontological evidence. It is, however, striking that in the extensive literature on the phylogeny of the vertebrates embryological evidence is hardly taken into consideration.

When one realizes that in the teleosts the PGCs are of endodermal origin and are at later stages characterized by germinal cytoplasm (see Satoh, 1974, in *Oryzias*) numerous questions arise. Do the teleosts actually show a similar formation of the PGCs as the anuran amphibians? Do the chondrosteans and the holosteans actually have the same type of PGC formation as the teleosts? Are there perhaps other fishes, e.g. the cartilaginous Chondrichthyes or the jawless Agnatha (modern Cyclostomata), which show a urodelan type of PGC formation? Are the two types of PGC formation found in anuran and urodelan amphibians also found in different subclasses of the reptilians? etc.

Assuming that the origin of the PGCs represents a very fundamental character in vertebrate development, a further analysis of the origin of the PGCs in the various groups may yield some further insight into the still mysterious phylogeny of the vertebrates. Our present, though limited knowledge of PGC formation in the two groups of amphibians in our opinion points towards a polyphyletic origin of the two groups from different ancestral fishes rather than towards an ancient, but common origin of the tetrapods.
REFERENCES


Polyphyletic origin of recent amphibians


(Received 5 September 1975)