Phosphatized Polar-lobe-forming Embryos from the Precambrian of Southwest China

The polar lobe formation is a symmetry-breaking process that occurs at the early stage of embryonic development and leads to blastomeres of unequal sizes in some extant spiralian animals, such as molluscs and few annelids. It leads to unique early cleavage morphologies that include trefoil, J-shaped, and five-lobed structures. Fossil embryos similar to modern lobe-forming embryos are collected from the Precambrian Doushantuo Formation phosphates, Weng'an, Guizhou Province, China. These embryos form a developmental sequence comparable to different developing stages in extant lobe-forming embryos. These data imply that lobe formation is an evolutionarily ancient process of embryonic specification.

The Neoproterozoic Doushantuo Formationin of southwest China has yielded the earliest known unambiguous fossil evidence of the metazoans. The fossil-bearing interval of the Doushantuo phosphates has been dated at around 580 Mya. Previous studies have presented fossil evidence of sponges, cnidarians and possible bilaterians in this formation. In addition, a large number of embryos display a variety of developmental patterns and different morphological types, implying that metazoans may have been diverse 40 million years before the Cambrian. In this study, we present fossil evidence of developing embryos in different developmental stages of lobe-forming embryos.

Lobe formation, which is observed in modern spiralians including many extant molluscs and some annelids, is a sequential process of dynamic change, which occurs by the protrusion and then absorption of a cytoplasmic lobe called a polar lobe (PL). The lobe protrudes from the vegetal pole of the embryo at each round of cytokinesis, leading to the formation of dumbbell, three-fold (trefoil), J-shaped, and five-lobed morphologies (Fig. 1). The PL superficially resembles a blastomere (CD cell), but is anucleate and forms by protruding and then absorbing from the blastomere by a deep constricted neck. PLs in fossil embryos can be recognized by the connecting necks and by the complementary relation between their size and that of the blastomere from which they arose.

All materials were collected from from the gray facies of the upper Doushantuo Formation at Wusi, Baisaikang, and Nanbao quarries, Weng'an county, Guizhou. Fossil embryos were isolated from the matrix by treating with 10% acetic acid, and observed and photographed with scanning electron microscopy. A few selected embryos with morphological characters like those of lobe-forming embryos common in many modern molluscs were examined by

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Authors

C.-W. Li and H.-J. Wu
National Tsing Hua University, Hsinchu, Taiwan

Y.-K. Hwu
Academia Sinica, Taipei, Taiwan

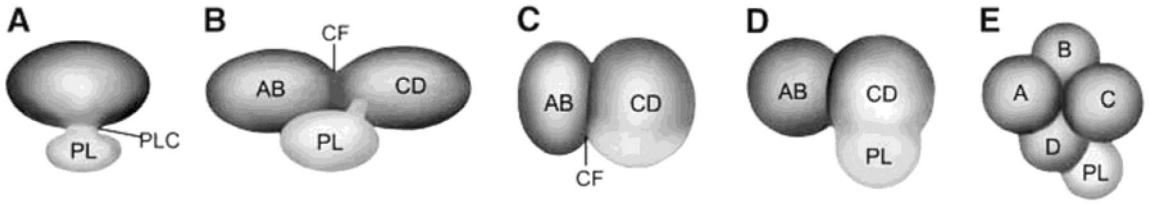

Fig. 1: Schematic diagrams showing the sequential process of dynamic changes in PL-forming embryos of some extant molluscs and annelids. (A) Egg with the first lobe; (B) trefoil stage; (C) two-cell stage; (D) J-shaped embryo; (E) five-lobed embryo. *(source: Science)*

Synchrotron Radiation micro-Computed Tomography (SR-μCT) at both the European Synchrotron Radiation Facility (ESRF) and the National Synchrotron Radiation Research Center (NSRRC).

A large number of specimens with structures identical to the trefoil stage are regularly spherical with smooth shells, as is characteristic of total cleavage. The diameter of the fossil eggs ranges from 0.2mm to 1.2mm. The embryos show variable arrangements with the blastomere and PL in loose contact in many specimens and in tight contact in others. Two different groups of embryos are recognized by the cleaved symmetry of embryos: equal division and unequal division. The CD cell (plus PL) in equal-division embryos is in the similar size to the AB cell (Fig. 2, A to K), whereas in unequal-division embryos it

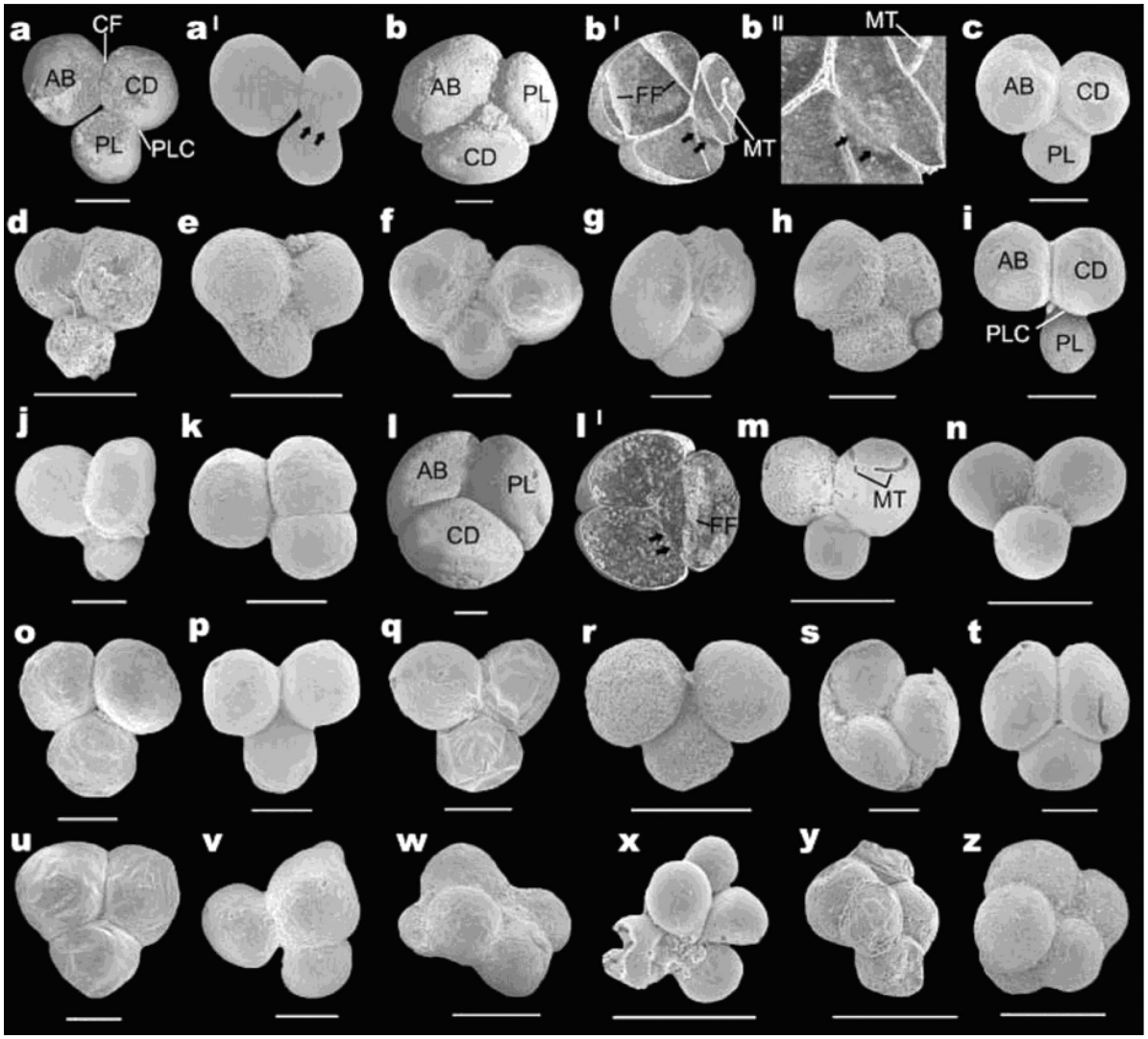

Fig. 2: Lobe-forming embryos from the Precambrian Doushantuo Formation. (A to K) Lobe-forming embryos with equal division. (L to V) Lobe-forming embryos with unequal division. (W to Z) Five-lobed stage of lobe-forming embryos. Panels A', B' and L' are dissecting images of A, B and L, respectively; B'' is a magnified image of B'. Images are SEM (C to K), (M to Z) or SR-μCT (A, B and L). Scale bar, 250 μm.

is typically twice as large as the AB cell (Fig. 2, L to V).

The three lobes are noticeably different in size in these specimens. SR- μCT examination of the trefoil fossils in equal (Fig. 2, A and B) and unequal division (Fig. 2L) suggests that three lobes are homologous with the CD cell, AB cell, and PL, respectively. Among the three lobes in the equal-division embryos, the second largest one is interpreted as the CD cell , separated completely from the likely AB cell (the largest lobe) by a thin membrane but is connected to the likely PL by a narrow neck. The smallest among the three parts of each embryo typically represents the PL. It can also be recognized by the presence of the polar-lobe constriction (PLC), which is relatively deeper than the cleavage furrow (CF)

When first cleavage is completed in modern lobe-forming embryos, the neck of the PL rapidly increases in diameter, leading to form a J-shaped embryo (Fig. 2, I to K and V) until the CD cell finally absorbs the PL. During second cleavage of modern lobe-forming embryos, the last phase of lobe protrusion leads to the formation of a five-lobed morphology (Fig. 2, W to Z), which is consisted of the first four blastomeres of equal size and a PL protruding from the D cell. The embryos are mostly nearly equal with the smallest one corresponding possibly to the PL, and are mostly un-compacted.

The relative sizes of the PL and CD cells differ among the different embryos but our size analysis reveals a complementary relationship between the volumes of the PL with the CD cells at the trefoil stage (Fig.3). For the analysis in Fig. 3, we normalized the size data for the different embryos by plotting the volumetric ratios PL/AB versus PL/(PL+CD) for each embryo.

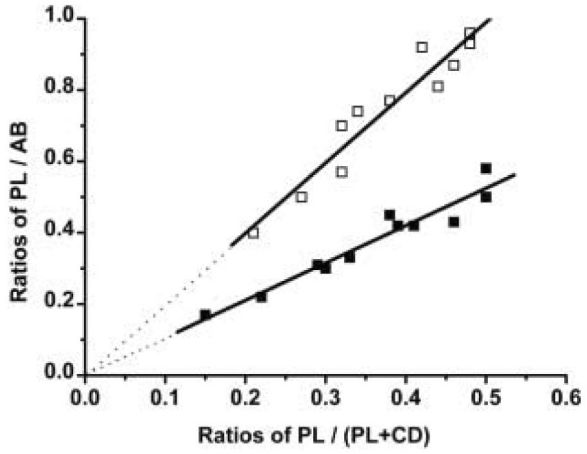

Fig. 3: Volumetric ratios PL/AB (y axis) plotted versus PL/(PL+CD) (x axis). The plots of open and solid squares were come from unequal-division and equal-division embryos, respectively. *(source: Science)*

The results show that the volumetric size of CD plus PL has a constant relationship to the AB volume. Two populations of fossil embryos, the equal and unequal embryo types referred to above, could be indentified in our study: those in which PL/AB=PL/(CD+PL), and those in which PL/AB=2PL/(CD+PL). The high correlation coefficients for two populations demonstrate that each is a statistically robust relation that could not have resulted from the random aggregation. The complementary relation of the PL with the CD volume supports directly the homology of the three lobes in these fossils with the PL, CD cell, and AB cell, respectively, of modern trefoil embryos. It also implies that the PL volume is dynamic, undergoing active protrusion and absorption.

In some extant spiralian animals, blastomere fates are established as early as the first cleavage, mediated by the formation of a PL, which segregates certain cytoplasmic and/or cortical components during early cleavage. Lobe formation and retraction is a symmetry-breaking device for the segregation of polar material to only one blastomere, an alternative to asymmetric cleavage. This mechanism is used today in disparate groups of molluscs including polyplacophorans, gastropods, scaphopods and bivalves, as well as a few types of annelids (*Chaetoplerus*). The PL appears also in a widely accepted basal group of spiralians, polyclad turbellarian flatworms (*Hoploplana inquilina*). In spiralian embryogenesis, the constituents of PL are required for specification of mesodermal structures and secondarily of other embryonic parts that are induced by mesoderm. That is, the blastomeres that receive the PL cytoplasm become mesoderm founder cells.

These fossil embryos may not be spiralians or spiralian ancestors, and indeed none of the later spiral cleavage forms in the same deposits. The most widespread and basal mode of embryogenesis in bilaterians, both in direct and indirect developing forms, operates by means of cleavage stage specification of blastomere. PL formation is only one of a great variety of mechanisms whose essential function is the asymmetric delivery of maternal components of regulatory significance to specific blastomeres.

In the case of the lobe-forming embryos studied here, the second cleavage lobe indicates that these embryos will specify one particular blastomere out of four that will be distinct in subsequent regulatory states. Only bilaterian embryos proceed in such a manner, but this is a typical bilaterian strategy of early development. Thus, these fossils imply that lobe formation is an ancient evolution-

ary device, and that the general strategy of precocious blastomere specification still used in most bilaterian groups was extant at least 40 million years before the Cambrian.

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Publications

J. Y. Chen, D. J. B ottjer, E. H. D avidson, S. Q. D ornbos, X. G ao, Y. H. Y ang, C. W . Li, G. L i, X. Q. W ang, D. C. X ian, H. J. W u, Y. K. Hwu , and P. Tafforeau, SCIENCE 312, 1644 (2006).

Contact E-mail

cwli@life.nthu.edu.tw