动 物 学 研 究 2009，Dec. 30(6):639-644 CN 53-1040/Q ISSN 0254-5853
Zoological Research DOI：10.3724/SP.J.1141.2009.06639

Physical Blocking Neural Tube Closure Affects Radial Intercalation and Neural Crest Midline-directed Migration in *Xenopus* Dorsal Explants

SHI Yu[1,2,#], ZHAO Shu-hua[1,2,#], MAO Bing-yu[1,*]

(1. *State Key Laboratory of Genetic Resources and Evolution, Kunming Institute of Zoology, the Chinese Academy of Sciences, Kunming* 650223, *China*; 2. *Graduate University of the Chinese Academy of Sciences, Beijing* 100049, *China*)

Abstract: Neural tube defects (NTDs) are severe congenital malformation diseases, which occur in 1 out of 1000 births in human. In *Xenopus*, several tissue movements are involved in the neural tube closure process. Immediately after the neural tube fusion, the neural crest cells get monopolar protrusion toward dorsal midline and migrate to form the roof of the neural tube. At the same time, radial intercalation takes place from the ventral neural tube and forces it to be single-layered. Here, we physically block the neural tube closure to test the cell movements and the following patterning in *Xenopus laevis* explants. The results show that the single-layered neural tube fails to form and the neural crest cells remain at the lateral regions in the explants with NTDs. However, the patterning of the neural tube is not affected as indicated by the normal expression of the preneural genes. These results indicate a requirement of the neural tube fusion for the radial intercalation and the dorsal midline directed neural crest migration, but not for the dorsal-ventral patterning of the neural tube.

Key words: *Xenopus laevis*; Neural tube defect; Neural tube closure; Neural crest; Morghogenesis; Intercalation; Dorsoventral patterning

机械阻断神经管的闭合影响爪蟾神经管辐射状切入及神经嵴细胞定向迁移

石 宇[1,2,#]，赵树华[1,2,#]，毛炳宇[1,*]

(1. 中国科学院昆明动物研究所 遗传资源与进化国家重点实验室，云南 昆明 650223；2. 中国科学院研究生院，北京 100049)

摘要：神经管闭合缺陷 (NTDs)是一种严重的先天畸形疾病，在新生儿中有千分之一的发病率。神经管融合前后，多种组织参与形态发生运动。神经管一经融合，神经嵴细胞就会向背侧中线方向产生单极突出并向此方向迁移形成神经管的顶部。与此同时，神经管从腹侧开始发生辐射状切入以实现单层化。在此，我们在非洲爪蟾的移植体中机械阻断神经管的闭合以检测其细胞运动及随后的图式形成。结果显示神经管闭合缺陷的移植体不能形成单层化的神经管，并且神经嵴细胞滞留在侧面区域不能向背侧中线迁移，而对神经前体标记基因的检测显示神经管的背腹图式形成并未受到影响。以上结果表明神经管的融合对于辐射状切入和神经嵴细胞向背侧中线方向的迁移过程是必需的，而对于神经管的沿背腹轴方向的图式形成是非必需的。

关键词：非洲爪蟾；神经管缺陷；神经管闭合；神经嵴；形态发生；细胞切入；背腹图式
中图分类号：Q593.2; R726.2; Q959.5; Q786 文献标志码：A 文章编号：0254-5853-(2009)06-0639-06

Neural tube defects (NTDs) are the most common malformations of the central nervous system causing fetal and infant morbidity and mortality. Approximately 1 out of every 1000 births occur with NTDs (Detrait et al,

Received date: 2009-05-31; Accepted date: 2009-06-08
Foundation items: This work was supported by grants from the National Natural Science Foundation of China (30425011; 30530380); the Innovation Project of the Chinese Academy of Sciences (KSCX2-YW-R-090)
收稿日期：2009-05-31；接受日期：2009-06-08
基金项目：国家杰出青年科学基金（30425011）；国家自然科学基金重点项目（30530380）；中国科学院知识创新工程重要方向项目（KSCX2-YW-R-090）
[*]通讯作者(Corresponding author), 博士生导师，E-mail: mao@mail.kiz.ac.cn
[#]共同第一作者（Authors contributed equally to the work）

2005). However, the genetic and cellular basis of NTD remains poorly understood. Vertebrate neurulation involves several tissue movements and cell shaping. In *Xenopus laevis*, the neural tissue, which is induced by Spemann organizer (Spemann and Mangold, 1924), consists of two layers of cells (Schroeder, 1970). The neural tissue thickens to form neural plate due to the elongation of the superficial cells. Then, the neural plate bends as cells constrict apically and become wedge-shaped. The medial migration of epidermis forces the bending further and pushes the neural folds into apposition. The apposed neural folds finally fuse to form a neural tube at the condition of the elongation of underlying notochord (Baker and Schroeder, 1967; Burnside, 1971; Schoenwolf and Smith, 1990; Schroeder, 1970; Schroeder, 1971).

After the neural tube closure, two major types of cell movements are involved in the neural tube shaping (Davidson and Keller, 1999). The neural crest cells migrate towards the dorsal midline through medially directed monopolar protrusions, which results in a single medial population of neural crest cells that form the roof of the neural tube. The radial intercalation of neural tube by protrusions both medially and laterally brings deep and superficial cells to form a single-cell-layered neural tube from ventral to the dorsal. In "giant sandwich" explants, cell elongation and wedging are induced without vertical interactions with underlying mesoderm. However, the neural tube fusion and radial intercalation fail in these explants, which can be induced by vertical interaction with mesoderm beyond the late gastrula stage (Poznanski et al, 1997). It is not clear whether the radial intercalation is initiated by the signals from the underlying mesoderm or the neural tube fusion.

In this study, we block the neural tube closure physically in the presence of underlying mesoderm to generate neural tube closure defect models in *Xenopus* explants and analyze the two types of cell movements. We show that the neural cells radial intercalation and the neural crest medially directed migration both fail in the explants with NTDs, indicating a requirement of fusion signals for these cell movements. We also find that the dorsoventral patterning of the open neural tube is not affected, indicating a non-necessity of neural tube fusion for the origination dorsal patterning signals.

1 Materials and Methods

1.1 The generation of neural tube closure defects explants

In vitro fertilization, embryo culture was carried out as described (Gawantka et al, 1995). The embryos at stage 13 were dissected horizontally into dorsal and ventral explants (Fig. 1A−C). The dorsal explants, including neural plate, lateral epidermis and underlying mesoderm, were cultured in the agar cavities full of Low-calcium Magnesium Ringer's (LCMR) (Stewart & Gerhart, 1990). Then, coverslips were erected just up the neural plate midlines to block the neural tube closure (Fig.1D, E).

1.2 DAPI and immunostaining analysis

The explants and control embryos were fixed at stage 25 by formaldehyde, embedded in paraffin, sectioned at 10 um. The sections were immunostained using anti-β-catenin (SANTA CRUZ) at a concentration of 1:200. The nuclei were stained with DAPI (sigma) at a concentration of 1 μg/ml. The stained sections were photographed by Leica *DM IRB* fluorescent microscope.

1.3 Whole-mount in situ hybridization and sections

Whole mount in situ hybridization (WMISH) of *Xenopus* embryos and explants was carried out as described (Gawantka et al, 1995). Stained embryos and explants were embedded in paraffin, sectioned at 30 μ m, and the sections were counter-stained with eosin.

2 Results

2.1 Blocking neural tube closure affects radial intercalation

To generate the neural tube closure defect model, the embryos of early neurula were cut into dorsal and ventral explants (Fig.1A−C). The dorsal explant includes neural tube, lateral epidermis as well as underlying mesoderm. The closure of the neural tube was blocked by a coverslip (Fig.1D, E).

We detected the radial intercalation at stage 25 by staining the nuclei using DAPI and the cell membranes using anti-β-catenin, which is membrane associated. In the wild type stage 25 embryos, the radial intercalation took place to form single-layered neural tubes at the ventral and medium levels (Fig.2A−D). The neural tubes of dorsal explants fused normally without coverslip blocking, suggesting that the removed ventral explants were not required for the neural tube closure. In these explants, the single-layered neural tubes formed just as in the wild types (Fig.2E−H). However, when the closure of the neural tube was blocked by the coverslip, the formation of single layer failed (Fig.2I−L) and the neural tube remained in two layers of cells in the ventral part (Fig.2J). These results indicate a requirement of neural

tube closure for the radial intercalation in the neural tube.

2.2 Blocking neural tube closure affects the location of neural crest cells at dorsal midline

In *Xenopus* wild type embryo, the neural crest cells arise at the lateral of neural plate. After neural tube fusion, the neural crest cells get monopolar protrusions directed toward the midline and migrate medially to form the roof of the neural tube (Fig.3A) (Davidson & Keller, 1999). We traced the neural crest cells by *in situ* hybridization using the probe of *Slug*, a neural crest marker (Mayor et al, 1995). We found that the neural crest cells arose normally throughout the anterior-posterior axis (Fig.3B), but these cells remained the lateral location in the neural tube closure blocked explants. In the control explants with normal neural tube fusion, the neural crest cells arrived at the dorsal midline successfully (Fig.3C, E). These results suggest that the fusion of neural tube is required for the migration of neural crest cells toward dorsal midline. Once the neural crest cells approach the midline, they lose their protrusions and stay until they start to emigrate from the tube (Davidson & Keller, 1999). We then tested if the emigration of the neural crest cells was affected in the explants with NTDs which failed their dorsal midline location of neural crest cells. We found that the neural crest cells emigrated normally along the lateral of the neural folds (Fig.3E) like in the control (Fig.3E),

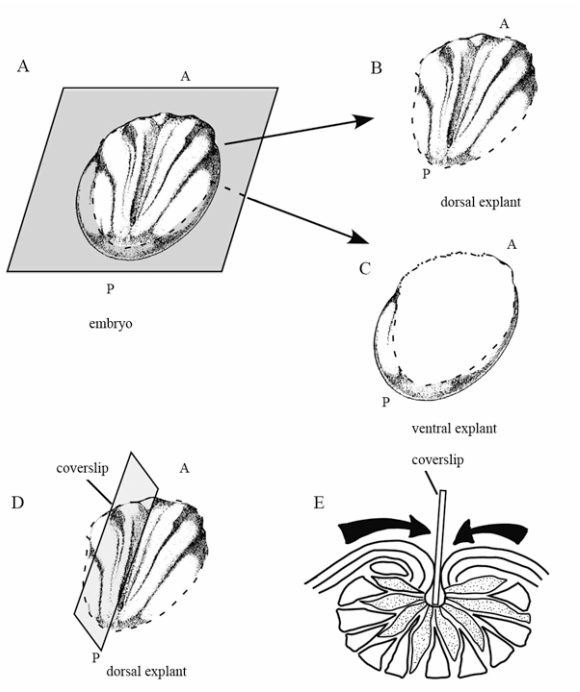

Fig. 1 Illustrations of the generation of explants of NTDs

(A) The embryo (dorsal view) at stage 13 is dissected horizontally into dorsal and ventral explants. The dorsal explant (B) includes neural plate, lateral epidermis and underlying mesoderm, while the ventral one (C) consists of ventral endoderm and epidermis. (D) The neural tube closure is blocked by a vertically set coverslip with the bottom adjacent to the neural plate midline. (E) The delineation of the neural tube closure blocked explant. Modified from Poznanski et al (1997). A: anterior; P: posterior.

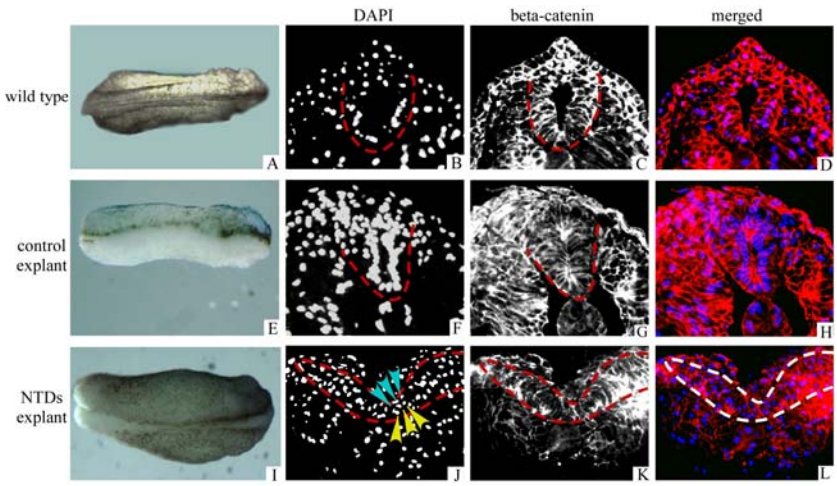

Fig. 2 The single-layered neural tubes fail to form in the explants with NTDs

A, E, I, dorsal views of a stage 25 embryo (A, anterior to the right), a normal dorsal explant (E) and an explant in which the neural tube closure was physically blocked using a coverslip. B, F, J, DAPI staining of nuclei on traverse sections of a control embryo (B), a normal explant (F) and a blocked explant (J), showing the single layered structure of the neural tube fails to form in the blocked explant (J). C, G, K, anti- β -catenin staining showing the cell shapes. D, H, L, merged pictures of B and C, F and G, J and K respectively. The neural tubes or neural folds are outlined by red broken oval lines in B, C, F, G, J, K and white one in L. The arrowheads in J show the superficial (blue) and deep layer cells (yellow) of the neural folds in the explant with NTD.

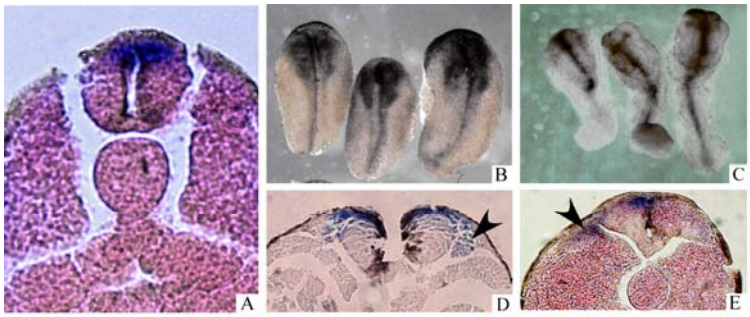

Fig. 3 The neural crest cells fail to locate at the dorsal midline in the explants with NTDs

(A) The location of neural crest cells in wild type embryos at stage 25 showed by *Slug*. The expression of Slug remains at the lateral in the dorsal explants with NTDs (B) and the section of the explants (D). In the control explant, the neural tube closure is normal (C) and *Slug* is expressed in the midline (E). Arrowheads in D and E show the neural crest cells that have emigrated away from the midline.

indicating that different signals are involved in the medially directed migration and the emigration of the neural crest cells.

2.3 The dorsoventral patterning is not affected in the neural tube closure blocked explants

During neurulation of vertebrate embryo, a complex program of gene regulation defines different classes of neurons within the neural tube. The different neurons are defined by transcription factors under the control of the ventral and dorsal signaling centers (Caspary & Anderson, 2003; Helms & Johnson, 2003; Novitch et al, 2003). The dorsal center is the roof plate whose ablation leads to dorsal pattern disturbing (Lee et al, 2000). In the explants whose neural tube fusion is blocked, the roof of the neural tube can not form. We tested the neural patterning by markers located at different dorsal-ventral levels. These marker genes include dorsally expressed Pax3 (Bang et al, 1999; Bang et al, 1997), medially and ventrally expressed *Nkx6.2* (Zhao et al, 2007) and the ventrally expressed *Shh* (Ekker et al, 1995). The expression patterns of all three markers were not affected in the neural tube closure blocked explants (Fig.4), indicating a non-necessary of neural tube fusion for the dorsal patterning signals.

3 Discussion

Several types of cell movements are involved in the neural tube shaping after its fusion. Whether these movements require the signals from the fusion is poorly understood. We block the neural tube closure physically in the dorsal explants with underlying mesoderm to investigate the relationship between neural tube fusion and the subsequent cell movements.

During neurulation, the neural tube fusion is of great importance for the following neural patterning and differentiation. NTDs are the most common malformations of the central nervous system causing fetal and infant morbidity and mortality. We use *Xenopus laevis* embryos to generate NTDs model by physically blocking the neural tube closure (Fig.1). In the explants with NTDs, the single-layered neural tubes failed to form as the radial intercalations were not initiated (Fig.2). The similar results were reported by Poznanski and colleagues in the giant explants without underlying mesoderm (Poznanski et al, 1997). These results indicate a requirement of the fusion signals for the radial intercalation and the single-layered neural tube formation. The neural tube fusion involves several cell morphogenesis processes (Davidson & Keller, 1999). The fusing lips of the neural folds are superficial layer of the epidermis, and the deep layer cells are restored later. At this time, the dorsal cells of the neural tube are still far lateral. Thus, the initial signals by the fusion might come from the overlying epidermis. However, the radial intercalation initiates from the ventral neural tube, indicating that the ventral cells get the signals first. The contradiction between the ventral initiated radial intercalation and its requirement of the dorsal fusion signals remains to be studied.

The dorsal midline location of neural crest cells, another cell morphogenesis initiating after neural tube fusion, was prevented by the blockage of the neural tube closure, too (Fig.3D). In wild type embryo, the neural crest cells get their monopolar protrusions toward the dorsal midline immediately after the neural tube fusion and migrate to form the roof of the neural plate, stay still until emigrate along the neural tube (Davidson & Keller, 1999). Our results showed that the emigration of the neural crest cells took place normally in the neural tube fusion blocked explants (Fig.3D). However, the migration pathways were disrupted in the experimental group (Fig.3B), which were supposed to migrate to the

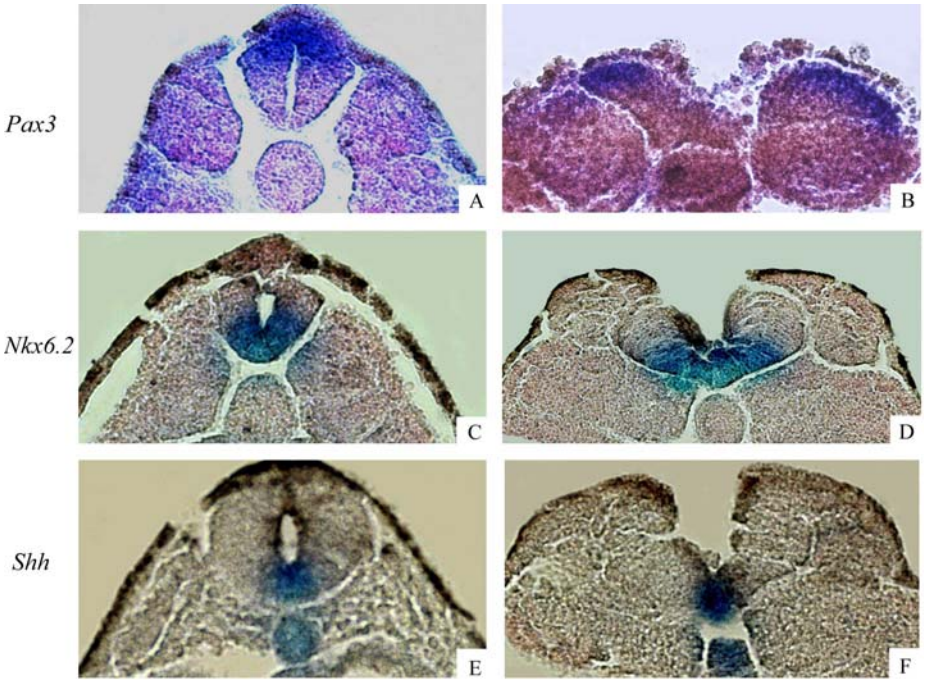

Fig. 4 The dorsoventral patterning of the neural tube is not affected by the NTDs

The expression of *Pax3* (A, B), *Nkx6.2* (C, D), and *Shh* (E, F) shows no significant difference along D-V axis between the wild types (A, C, E) and the explants with NTDs (B, D, F).

third, fourth and sixth pharyngeal arches at the head level in normal embryos (Snider et al, 2007). This disruption may be due to the derangement of the structure in the explants with NTDs.

The roof plate of vertebrate neural tube, expressing secreted factors such as Wnt and BMP, acts as a dorsal signaling center for the neural tube dorsal patterning and neural cell differentiation (Lee et al, 2000; Liem Jr et al, 1997; Liem Jr et al, 1995; Millonig et al, 2000). The ablation of the roof plate in mice disrupts dorsoventral patterning and leads to deficiency of the dorsal interneuron subtypes (Lee et al, 2000). We tested the dorsoventral patterning in the explants with NTDs by preneural patterning markers (Fig. 4). All the markers we detected did not show noticeable difference compared with the wild types. These results suggest that the dorsal signals can still arise even if the roof of the neural tube fails to form.

References:

Baker PC, Schroeder TE. 1967. Cytoplasmic filaments and morphogenetic movement in the amphibian neural tube [J]. *Dev Biol*, **15**(5): 432.

Bang AG, Papalopulu N, Goulding MD, Kintner C. 1999. Expression of Pax-3 in the lateral neural plate is dependent on a Wnt-mediated signal from posterior nonaxial mesoderm [J]. *Dev Biol*, **212**(2): 366-380.

Bang AG, Papalopulu N, Kintner C, Goulding MD. 1997. Expression of Pax-3 is initiated in the early neural plate by posteriorizing signals produced by the organizer and by posterior non-axial mesoderm [J]. *Development*, **124**(10): 2075-2085.

Burnside B. 1971. Microtubules and microfilaments in newt neuralation [J]. *Dev Biol*, **26**(3): 416.

Caspary T, Anderson KV. 2003. Patterning cell types in the dorsal spinal cord: what the mouse mutants say [J]. *Nat Rev Neurosci*, **4**(4): 289-297.

Davidson LA, Keller RE. 1999. Neural tube closure in *Xenopus laevis* involves medial migration, directed protrusive activity, cell intercalation and convergent extension [J]. *Development*, **126**(20): 4547-4556.

Detrait ER, George TM, Etchevers HC, Gilbert JR, Vekemans M, Speer MC. 2005. Human neural tube defects: developmental biology, epidemiology, and genetics [J]. *Neurotoxicol Teratol*, **27**(3): 515-524.

Ekker SC, McGrew LL, Lai CJ, Lee JJ, Von Kessler DP, Moon RT, Beachy PA. 1995. Distinct expression and shared activities of members of the hedgehog gene family of *Xenopus laevis* [J]. *Development*, **121**(8): 2337-2347.

Gawantka V, Delius H, Hirschfeld K, Blumenstock C, Niehrs C. 1995. Antagonizing the Spemann organizer: role of the homeobox gene Xvent-1 [J]. *EMBO J*, **14**(24): 6268.

Helms AW, Johnson JE. 2003. Specification of dorsal spinal cord interneurons [J]. *Curr Opin Neurobiol*, **13**(1): 42-49.

Lee KJ, Dietrich P, Jessell TM. 2000. Genetic ablation reveals that the roof plate is essential for dorsal interneuron specification [J]. *Nature*, **403**: 734-740.

Liem Jr KF, Tremml G, Jessell TM. 1997. A role for the roof plate and its resident TGF-β-related proteins in neuronal patterning in the dorsal spinal cord [J]. *Cell*, **91**(1): 127-138.

Liem Jr KF, Tremml G, Roelink H, Jessell TM. 1995. Dorsal differentiation of neural plate cells induced by BMP-mediated signals from epidermal ectoderm [J]. *Cell*, **82**(6): 969.

Mayor R, Morgan R, Sargent MG. 1995. Induction of the prospective neural crest of Xenopus [J]. *Development*, **121**(3): 767-777.

Millonig JH, Millen KJ, Hatten ME. 2000. The mouse Dreher gene Lmx1a controls formation of the roof plate in the vertebrate CNS [J]. *Nature*, **403**(6771): 764-769.

Novitch BG, Wichterle H, Jessell TM, Sockanathan S. 2003. A requirement for retinoic acid-mediated transcriptional activation in ventral neural patterning and motor neuron specification [J]. *Neuron*, **40**(1): 81-95.

Poznanski A, Minsuk S, Stathopoulos D, Keller R. 1997. Epithelial cell wedging and neural trough formation are induced planarly in Xenopus, without persistent vertical interactions with mesoderm [J]. *Dev Biol*, **189**(2): 256-269.

Schoenwolf GC, Smith JL. 1990. Mechanisms of neurulation: Traditional viewpoint and recent advances [J]. *Development*, **109**(2): 243-270.

Schroeder TE. 1970. Neurulation in *Xenopus laevis*: An analysis and model based upon light and electron microscopy [J]. *Development*, **23**(2): 427.

Schroeder TE. 1971. Mechanisms of morphogenesis: the embryonic neural tube [J]. *Int J Neurosci*, **2**(4): 183-197.

Snider P, Olaopa M, Firulli AB, Conway SJ. 2007. Cardiovascular development and the colonizing cardiac neural crest lineage [J]. *Sci World J*, **7**: 1090-1113.

Spemann H, Mangold H. 1924. Uber induktion von embryonalanlagen durch implantation artfremder organisatoren [J]. *Wilhelm Roux Arch Entw Mech Org*, **100**: 599-638.

Stewart RM, Gerhart JC. 1990. The anterior extent of dorsal development of the *Xenopus* embryonic axis depends on the quantity of organizer in the late blastula [J]. *Development*, **109**(2): 363-372.

Zhao S, Jiang H, Wang W, Mao B. 2007. Cloning and developmental expression of the *Xenopus Nkx6* genes [J]. *Dev Genes Evol*, **217**(6): 477-483.

（上接第 632 页）

本书运用拟人的写作手法，恰当地描述了猿猴生动而拟人的行为特点。从猩猩社会中的孤独压抑、长臂猿求爱的甜美忠贞、狮尾狒狒二雄的委曲求全到黑猩猩雄性间的残酷屠杀，作者邀请读者们一起进入神秘的猿猴社会，体会他们的喜怒哀乐。

作为高级科普读物，本书适合于对野生动物社会生态和人类起源感兴趣的读者群，有利于读者们了解神秘的猿猴世界和人类社会起源的背景。书中介绍的灵长类生态研究和进化理论有利于推进国内的灵长类学研究，也对国内古人类学学者、文化人类学学者、社会学学者、动物学学者等的研究有一定的参考价值。书中每章后面均设有思考题和推荐阅读文献，适合作为高等院校各相关专业灵长类课程的专业课教材。

《灵长类的社会进化》订购回单（寄回出版社）

书　　名	单价	订购册数	金额
《灵长类的社会进化》	45.00 元		
订书单位：		联系人：	
详细地址：		邮政编码：	
E-mail：		电话：	

欢迎来电订购。购书 100 元以上免邮资，批量订购折扣另议。

地址：广州市新港西路 135 号中山大学出版社　　邮政编码：510275

联系人：高丽萍、龚明娟　　联系电话：（020）84111901，84111998

邮局汇款：收款人栏写“中山大学出版社发行部”，附言栏请注明书名和数量。谢谢！

中山大学出版社发行部

2009 年 10 月