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Mechanisms of neurulation: traditional viewpoint and recent advances

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Summary

In this review article, the traditional viewpoint of how neurulation occurs is evaluated in light of recent advances. This has led to the formulation of the following fundamentals: (1) neurulation, specifically neural plate shaping and bending, is a multifactorial process resulting from forces both intrinsic and extrinsic to the neural plate; (2) neurulation is driven by both changes in neurepithelial cell shape and other form-shaping events; and (3) forces for cell shape changes are generated by both the cytoskeleton and other factors. Several cell behaviors within the neural plate have been elucidated. Future challenges include identifying cell behaviors within non-neurepithelial tissues, determining how intrinsic and extrinsic cell behaviors are orchestrated into coordinated morphogenetic movements and elucidating the molecular mechanisms underlying such behaviors.

Key words: ectoderm, neural folds, neural groove, neural plate, neural tube, neurepithelium, neurulation, notochord.

Introduction

Neurulation, the process of neural tube formation, is a crucial moment in the prenatal life history of a developing organism. The entire precursor of the central nervous system is established during this multifarious process as an initially simple, generally single-layered tube. Failure of neurulation results in neural tube

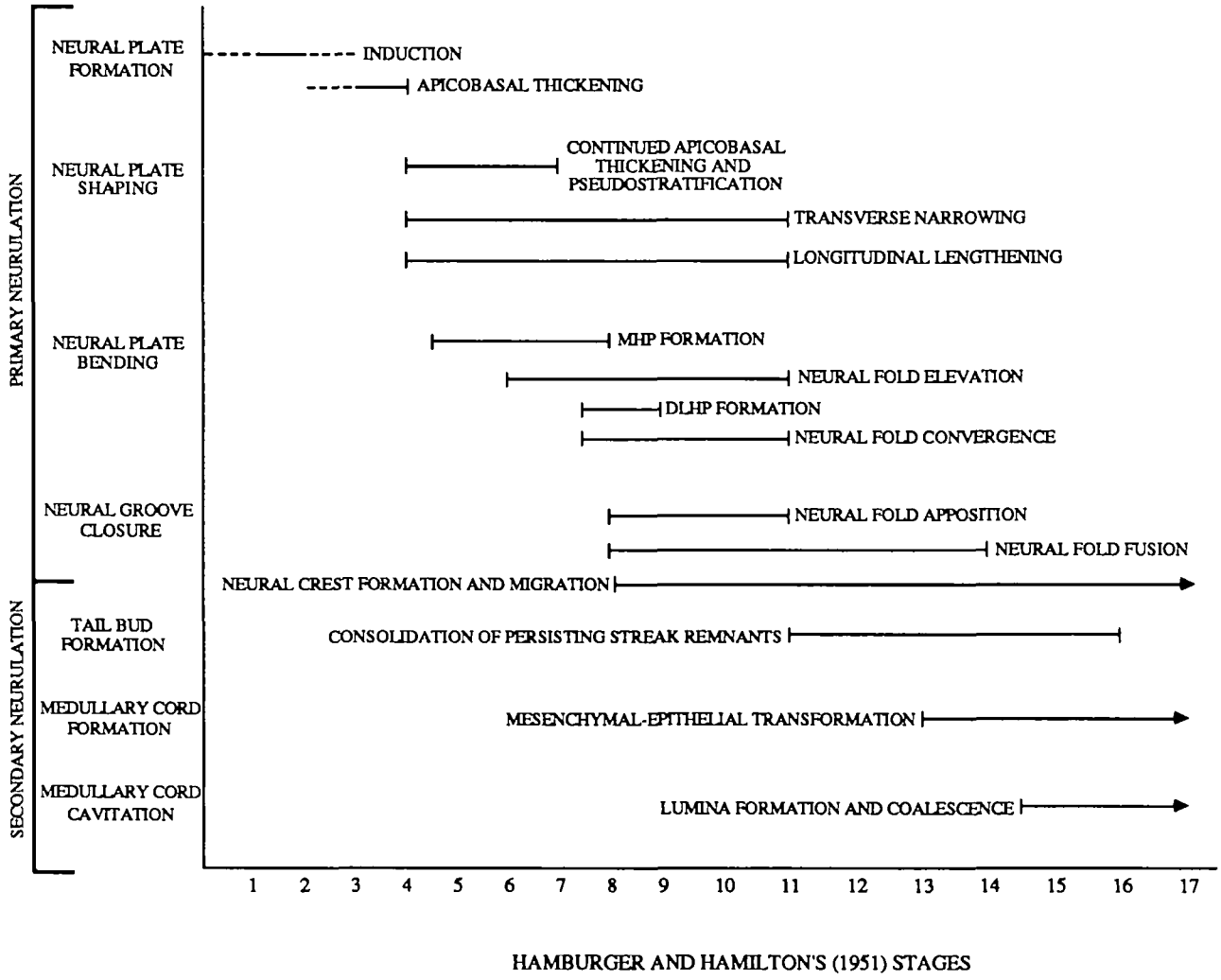


Fig. 1. Chart showing the major events of chick primary and secondary neurulation and the approximate stages at which they occur. Variation occurs from embryo to embryo and from craniocaudal level to level within the same embryo. Also, the exact timing of some events is unknown.

defects, major anomalies associated with substantial morbidity and mortality. Neurulation is spatially and temporally coupled with a number of other important morphogenetic activities, such as formation of the germ layers, heart, gut, body folds, neural crest and regional subdivisions of the mesoderm, but how these activities are integrated and the extent to which they are causally related are largely unknown.

In higher vertebrates, including humans, neurulation occurs in two phases: primary and secondary (Figs 1–3). During primary neurulation, the flat ectodermal neural plate is transformed into a neural tube. This process occurs in four recognizable stages although they overlap both spatially and temporally: (1) formation of the neural plate during which the ectoderm thickens as a consequence of induction; (2) shaping of the neural plate; (3) bending of the neural plate; and (4) closure of the neural groove (and its cranial and caudal ends, the neuropores), with formation of the roof plate of the neural tube, the neural crest and the overlying surface epithelium. After closure of the caudal neuropore, secondary neurulation occurs resulting in formation of the most caudal portion of the spinal cord (e.g. in birds, the lumbosacral and tail levels). Secondary neurulation, like primary neurulation, occurs in several overlapping stages (Fig. 1; reviewed by Schoenwolf and DeLongo, 1980; Schoenwolf, 1983; Schoenwolf *et al.* 1985). These stages will not be discussed here, but the fact that they exist points out that the embryo can use different morphogenetic processes to achieve the same endpoint: a neural tube (termed secondary neural tube), composed of pseudostratified columnar epithelium, neural crest (termed secondary neural crest) and overlying surface epithelium (Fig. 4).

In this article, we will evaluate the traditional viewpoint of how neurulation occurs in light of recent advances in the field; this will lead to the formulation of a contemporary viewpoint. We will focus on two aspects of primary neurulation, shaping of the neural plate and bending of the neural plate, because these two aspects

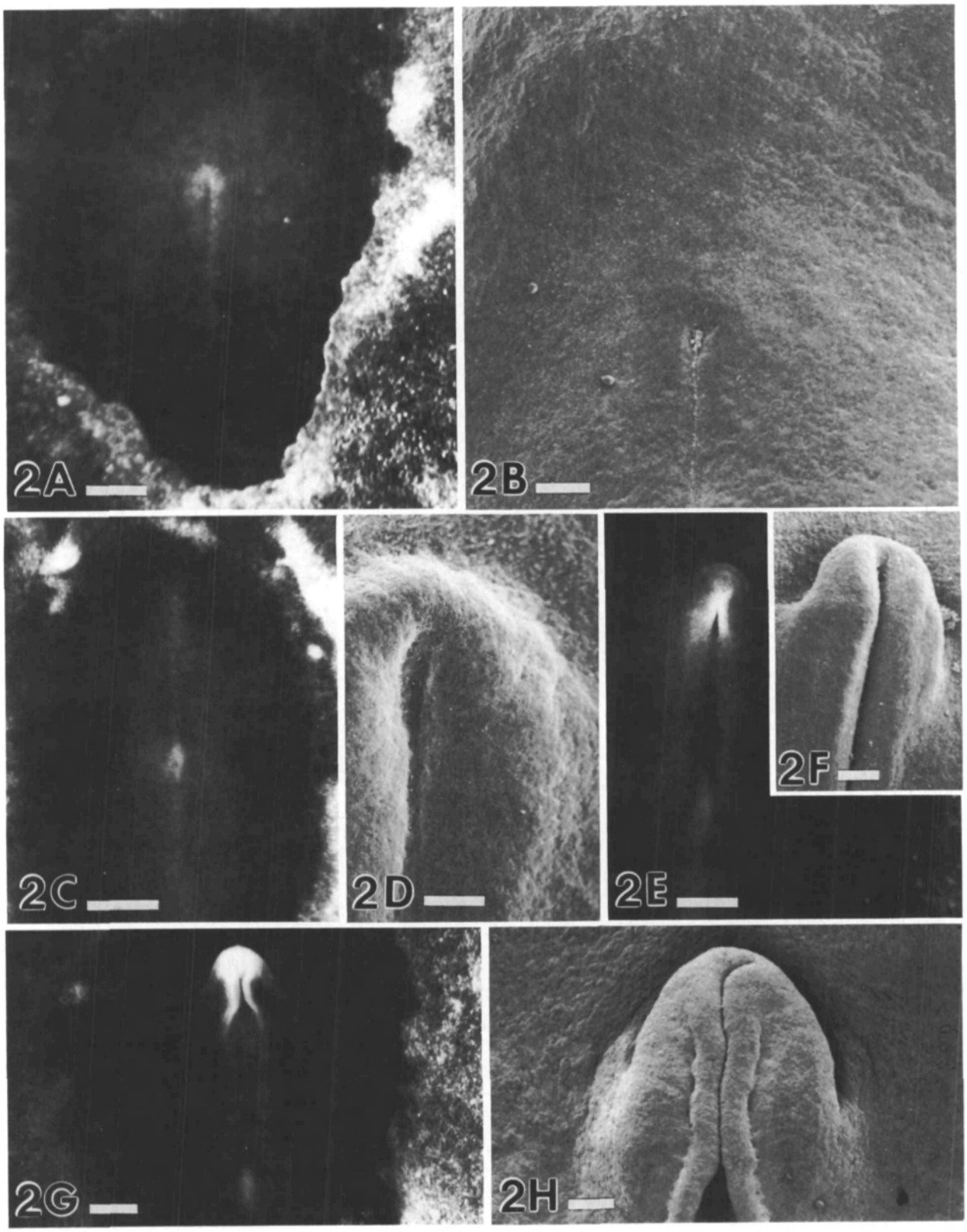


Fig. 2. Dorsal views of chick primary neurulation. Flat neural plate (stages 3–4) (A, light micrograph; B, scanning electron micrograph) and progressive stages during neural plate shaping and bending (stages 5–8) (C,E,G, light micrographs; D,F,H, scanning electron micrograph enlargements of cranial neuraxes from embryos similar to C,E,G, respectively). A,C,E,G, bars=400 μm; B,D,F,H, bars=100 μm.

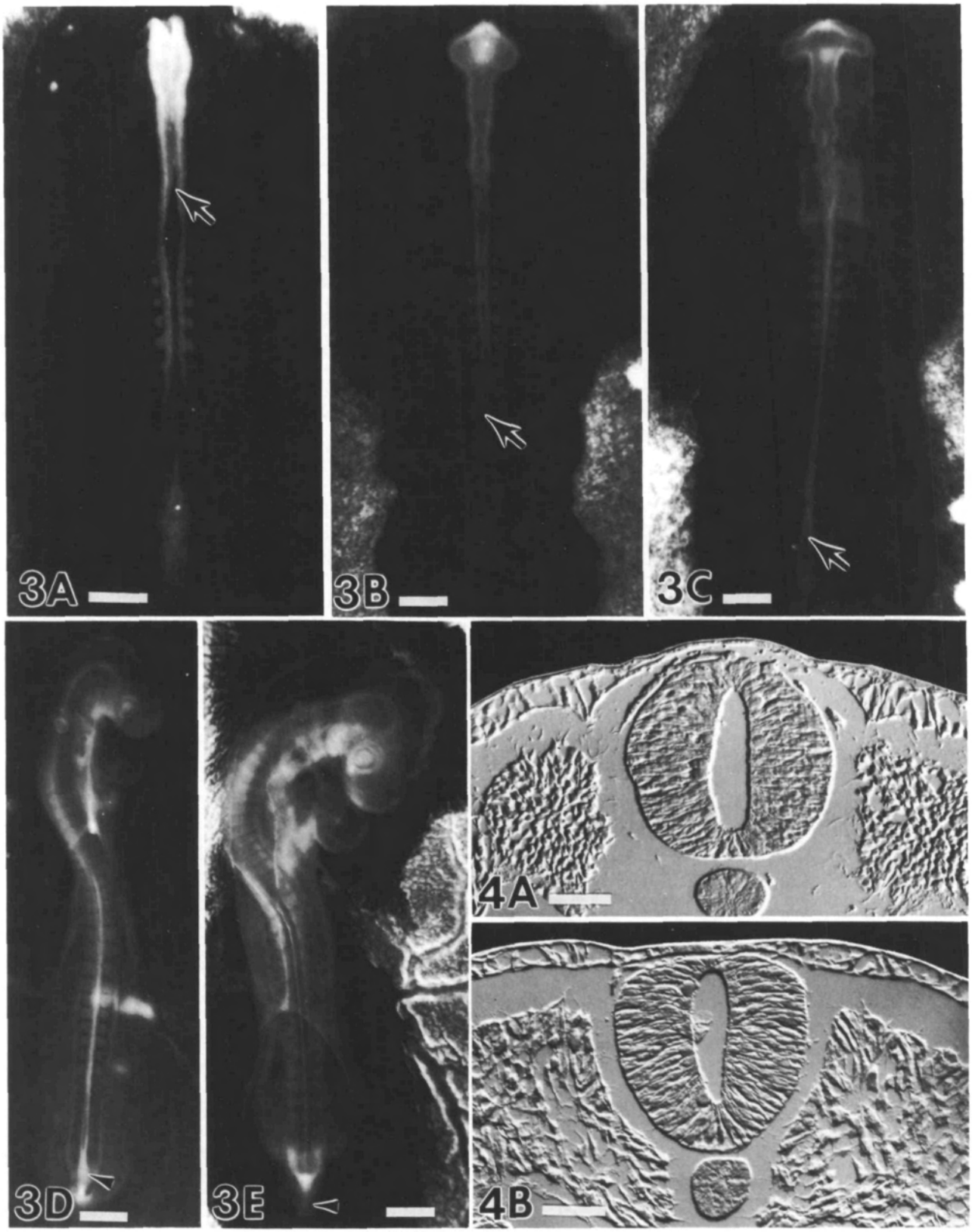


Fig. 3. Dorsal views of chick primary and secondary neurulation. (A–C) Light micrographs of progressive stages of neural groove closure (stages 9–11). Arrows indicate the caudal extent of the neural tube. (D,E) Light micrographs of embryos undergoing secondary neurulation following neural groove closure (stages 15, 17). Arrowheads indicate the tail bud. Bars=400 μm.

Fig. 4. Light micrographs of transverse plastic sections through the chick neural tube viewed with differential interference contrast optics. (A) Neural tube (spinal cord level; stage 14) formed by primary neurulation; (B) Neural tube (caudal most spinal cord level; stage 18) formed by secondary neurulation. Bars=40 μm.

have been studied in most detail. Our discussion will center on the chick embryo, because substantial descriptive and experimental data are available.

Traditional viewpoint[1]

How does neurulation occur? In this section, we will discuss the answer to this question according to the traditional viewpoint.

Neurulation has been viewed traditionally as an all-or-none process in which the neural plate either rolls up or does not. As a consequence of this view, neurulation and neural plate bending have often been equated, and the other cardinal events of neurulation, such as neural plate shaping, have been essentially ignored. Additionally, neurulation has been viewed as a process that (1) occurs independently of other concurrent morphogenetic events; (2) is driven by one or at the most a few 'motors'; and (3) occurs only one way, irrespective of the neuraxial level (e.g. brain *versus* spinal cord) and organism (e.g. amphibians, with more-or-less spherical embryos developing from the entire egg, *versus* birds and mammals, with flattened embryos developing from an essentially two-dimensional, discoidal blastoderm).

The three fundamentals of the traditional viewpoint

The traditional viewpoint consists of three fundamentals. The first fundamental is that all requisite forces for neurulation are intrinsic to the neurepithelium. This idea originated about one hundred years ago when Roux reported that the neural plate "when isolated could bend into a tube without external assistance" (Roux, 1895, cited by Weiss, 1939, on p. 498; although Weiss cited Roux's paper of 1895, Roux initially reported the results of this experiment in 1885). Other similar studies over the years have been viewed as providing evidence in support of Roux's original experiment (e.g. see Lewis, 1947; Karfunkel, 1974; Jacobson, 1981). Recent studies state that isolated chick neural plates can "form tubular structures closely resembling neural tubes of early chick embryos" (p. 60 of Lee and Nagele, 1988) and that amphibian (*Ambystoma mexicanum*) neural plates transplanted into epidermis produce "nodules of neural tissue with central lumens" (p. 44 of Moury and Jacobson, 1989).

The second fundamental of the traditional viewpoint is that neurulation is driven by changes in the shape of neurepithelial cells. This idea, which stems principally from theoretical ruminations and models (e.g. Rhumbler, 1902; Glaser, 1914; Spek, 1931; Lewis, 1947; Odell *et al.* 1981), was first articulated in the late 19th century by His: "Suppose we have a sheet of cells, the elements of which are as wide at their bases as they are at the free surface. If now, as a result of internal forces, the cells are all induced to become thicker (wider) at their bases and thinner (narrower) at their free ends, the result will be that the sheet bends and folds together to form a hollow structure" (His, 1894, translated by Jacobson, 1978, on p. 19; parenthetical words are ours). Cell 'wedging' has been documented in the neural plate of amphibians (*Xenopus laevis*: Schroeder, 1970; *Ambystoma mexicanum*: Brun and Garson, 1983), birds (chick: Schoenwolf and Franks, 1984) and mammals (rat: Moore *et al.* 1987). Thus, the view of neurulation that has persisted since the time of His is that neurepithelial cells change shape from column-like to wedge-like and that these changes drive bending.

The third fundamental of the traditional viewpoint is that forces for cell shape changes are generated by the cytoskeleton. This idea originated in the late 1960s and early 1970s with the application of transmission electron microscopy to embryonic tissues. Cloney (1966) suggested that tail absorption during ascidian larva metamorphosis is driven by forces emanating from contractile microfilaments and further postulated that the presumed apical narrowing of neurepithelial cells during their wedging is owing to the contraction of similar cytoplasmic filaments present in neurepithelial cell apices. Transmission electron microscope studies, beginning in 1967 (Baker and Schroeder) and continuing over the next twenty years (summarized by Karfunkel, 1974; Schoenwolf, 1982), have amply demonstrated the presence of circumferential microfilament bands in the apices of neurepithelial cells during neurulation. Immunocytochemistry and other similar procedures were used to demonstrate that such bands are composed of actin and are associated with a variety of contractile proteins (Nagele and Lee, 1978, 1980*a*; Sadler *et al.* 1982, 1986; Lee *et al.* 1983; Lash *et al.* 1985; Lee and Nagele, 1985). Finally, numerous experiments in amphibians, birds and mammals have shown that neurulation is inhibited when microfilament bands are depolymerized with cytochalasins (summarized by Schoenwolf *et al.* 1988).

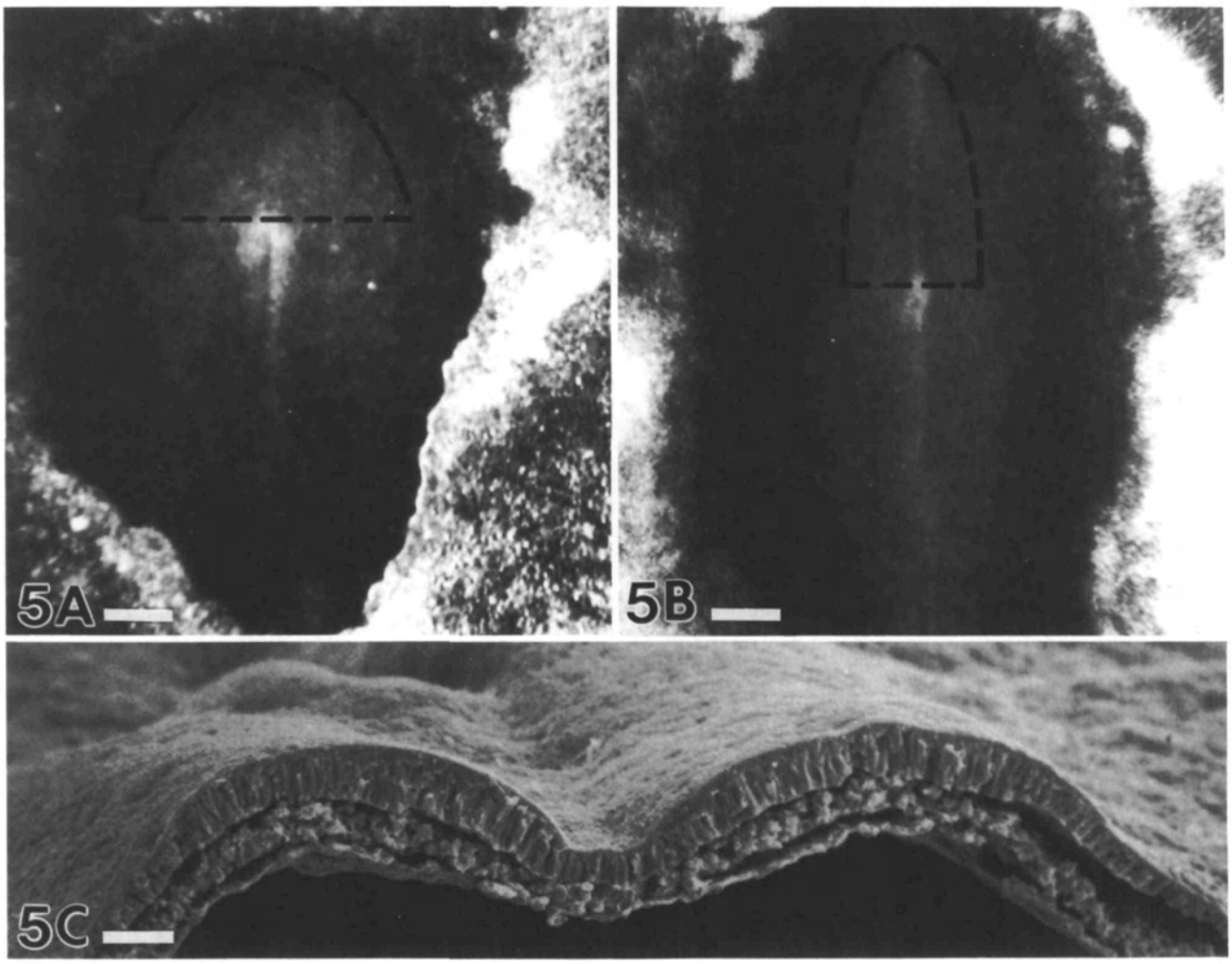
Contemporary viewpoint

Advances in recent years necessitate a change in the traditional viewpoint of neurulation. In this section, we will discuss the inadequacies of the traditional viewpoint and its evolution in light of new information, leading to what we will call the contemporary viewpoint. The contemporary viewpoint, like the traditional viewpoint, attempts to answer the question, *How does neurulation occur?* The answer to this question according to the contemporary viewpoint also consists of three fundamentals. Each fundamental concerns both neural plate shaping and bending.

Regional differences in neural plate shaping and bending

Neurulation has been viewed traditionally as a process in which the flat neural plate merely rolls up. However, if this were the case – that is, if *only* rolling up were required – then the neural plate, which, at the time of

[1] Textbooks are principally distillations of tradition. Thus, by the traditional viewpoint we mean the body of accepted facts, the dogma, appearing in most general textbooks of developmental biology.



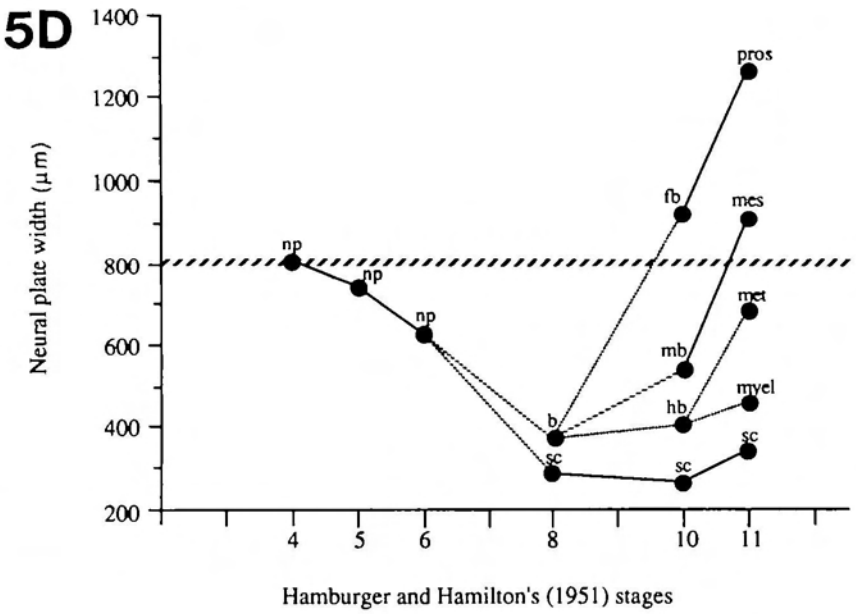


Fig. 5. Neural plate shaping in the chick. (A,B) Dorsal views of light micrographs before (A) and during (B) neural plate shaping (stages 3–4, 5–6). The approximate boundaries of the prenodal neural plate are outlined. Note that the neural plate narrows transversely and lengthens longitudinally. (C) Scanning electron micrograph of a slice through the narrowing neural plate from an embryo similar to B. Shaping of the neural plate also involves changes in neurepithelial cell height such that midline cells become shorter and lateral cells become taller. (D) Graph showing changes in the width of the neural plate during its shaping at various neuraxial levels (np, flat neural plate; b, brain; sc, spinal cord; fb, forebrain; mb, midbrain; hb, hindbrain; pros, prosencephalon; mes, mesencephalon; met, metencephalon; myel, myelencephalon). D is based on the data of Schoenwolf, 1985. A,B, bars=300 μm; C bar=40 μm.

its formation, is a relatively wide and short rudiment (Fig. 5A; Schoenwolf, 1985, his Tables 1, 2 and Fig. 8), would roll up into a vesicle rather than a longitudinal tube. Shaping of the neural plate, particularly narrowing in the transverse plane and lengthening in the longitudinal plane, changes its configuration so that subsequent bending creates a tube rather than a vesicle. Furthermore, according to the traditional viewpoint,

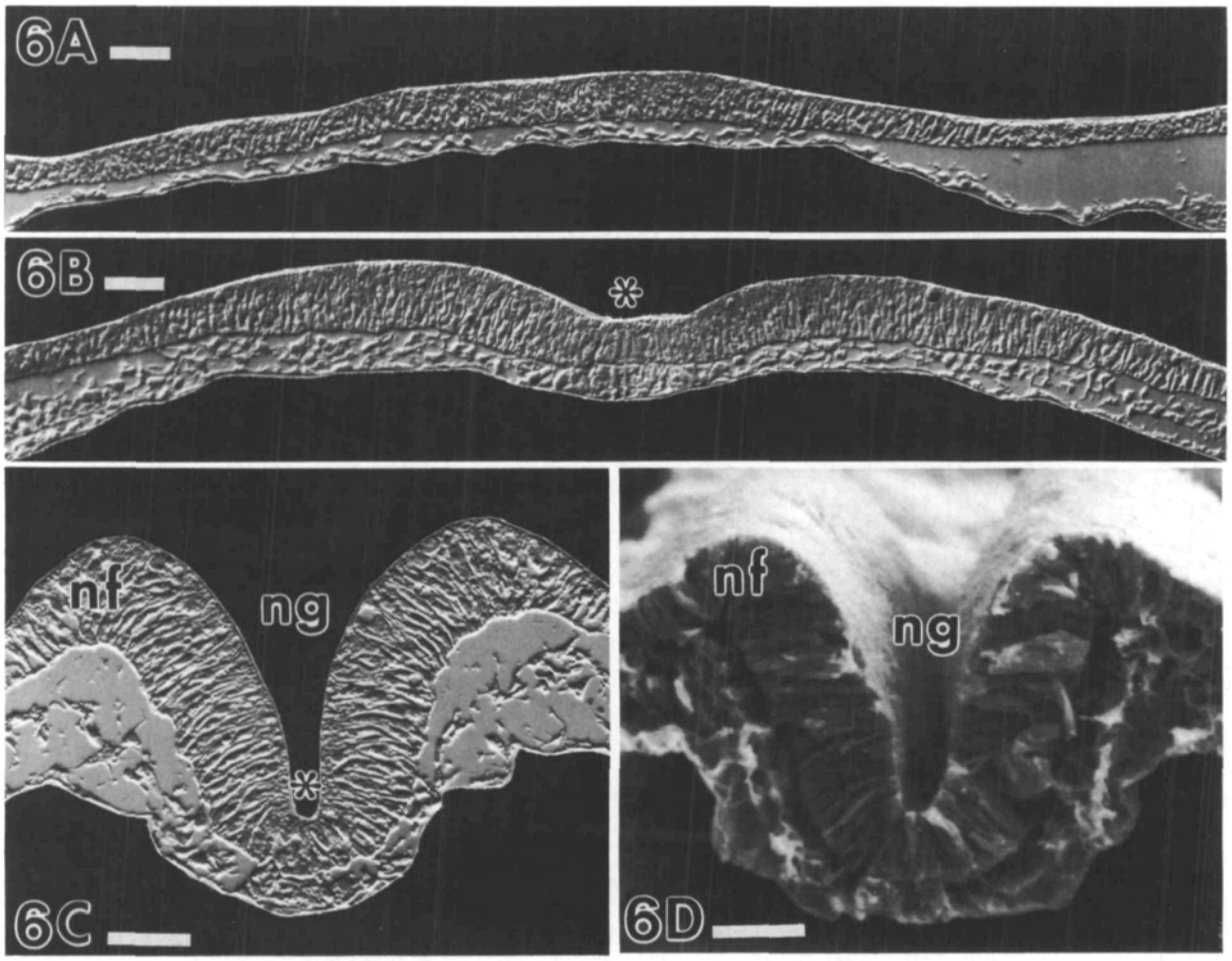


Fig. 6. Cross-sectional views of MHP (*) formation and *incipient* neural fold (nf) elevation at the future midbrain level of the chick. (A–C) Light micrographs of transverse plastic sections through progressively older embryos (stages 4–7); (D) Scanning electron micrograph similar to C. ng, neural groove. Bars=40 μm.

neurulation occurs only one way throughout the length of the neuraxis. However, recent studies have shown that there are regional differences in neural plate shaping and bending along the craniocaudal extent of the neuraxis and that multiple steps are involved at each level. For example, during shaping of the neural plate, the rostral part of the future brain region first narrows transversely and then widens, whereas the remainder of the neuraxis essentially only narrows (Fig. 5; Schoenwolf, 1985; Schoenwolf and Alvarez, 1989). Moreover, bending of the neural plate involves a series of morphogenetic events differing at various craniocaudal levels (Figs 6–8; Schoenwolf, 1982, 1985; Schoenwolf and Desmond, 1984; Schoenwolf and Franks, 1984). The inchoate neural plate is a flat epithelial sheet. As the axial mesoderm (i.e. prechordal plate mesoderm at the future forebrain level and notochord at the future midbrain, hindbrain and spinal cord levels) forms, it becomes anchored to the midline of the neural plate, which furrows simultaneously. This establishes a median hinge point (MHP) around which the more lateral neural plate on each side elevates, forming the incipient neural folds (i.e. the initial folds at the lateral margins of the neural plate; Fig. 6C,D) and the circumscribed neural groove (i.e. the V-shaped space extending the length of the bending neural plate; the midline furrow is the apex of the neural groove). As elevation occurs, the incipient neural folds transform into the definitive neural folds (i.e. bilateral ectodermal structures, each composed of an inner layer of neural plate firmly attached to an outer layer of surface epithelium; Fig. 7A,C,D). In the region of the future brain, bending is characterized further by the formation of paired dorsolateral hinge points (DLHPs). Each DLHP, like the MHP, is a localized area where the neural plate is anchored to adjacent tissue (the surface ectoderm of the neural folds) and furrowing occurs. In contrast to the MHP, furrowing of the DLHP does not occur along the entire extent of contact (i.e. it does not occur along the entire width of the neural fold). Rather, furrowing of the DLHP is restricted to the neural plate at the base of each neural fold, near the juncture where the two layers of the fold separate from one another. The lateral extremes of the optic vesicles are merely the expanded DLHPs present at the forebrain level (Fig. 7F,G). With formation of the DLHPs, the definitive neural folds undergo convergence, rotating around the DLHPs until they meet one another in the dorsal

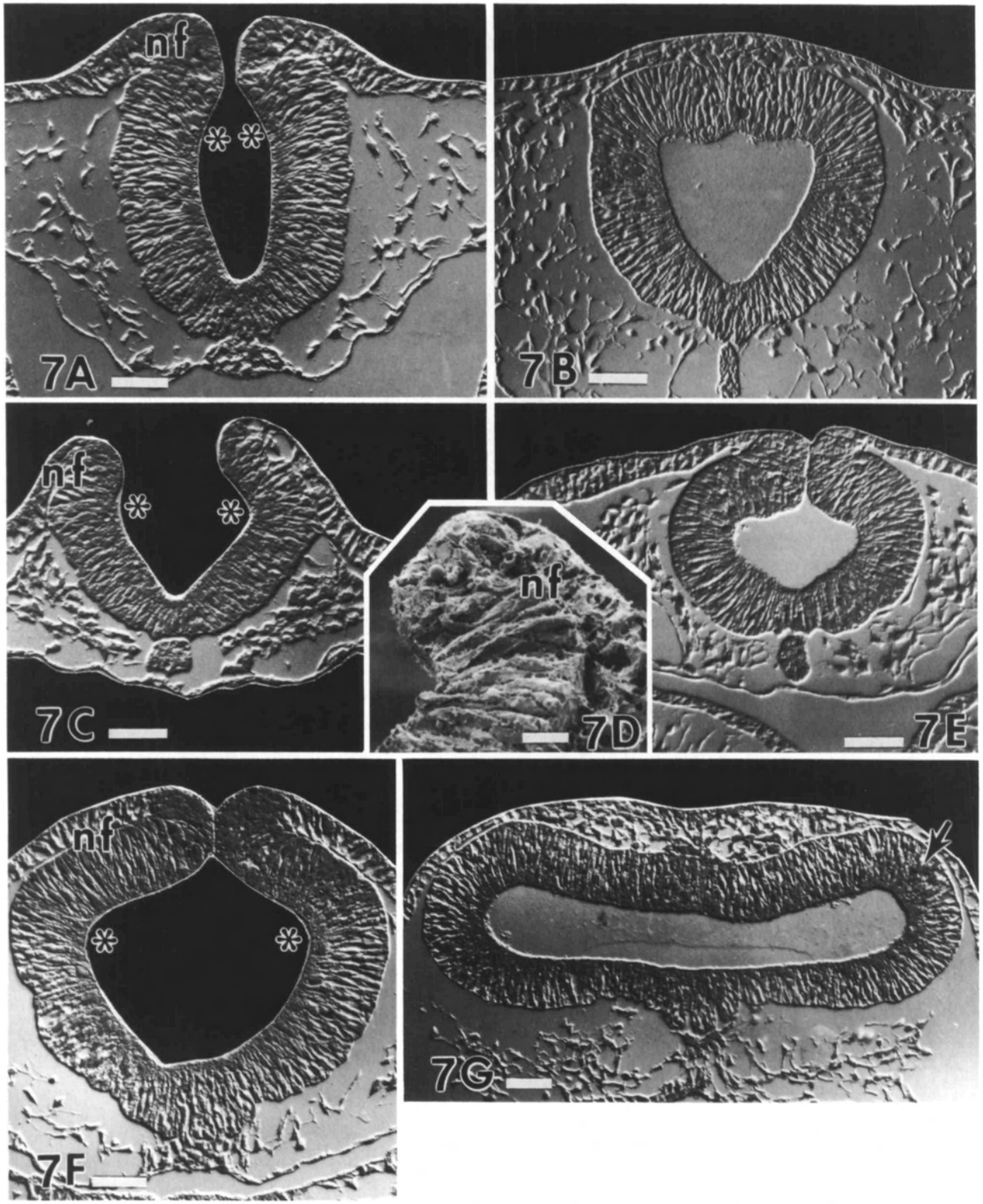


Fig. 7. Cross-sectional views of DLHP (*) formation, *definitive* neural fold (nf) convergence and neural groove closure in the chick. (A,B) Light micrographs of plastic transverse sections through the future midbrain level at stages 8 and 10; (C–E) Future hindbrain level (C, E, light micrographs of plastic transverse sections at stages 8 and 10; D, scanning electron micrograph of neural fold similar to C); (F,G) Light micrographs of plastic transverse sections through the future forebrain level at stages 9 and 10. Arrow, optic vesicle. A–C, E–G, bars=40 μm; D, bar=10 μm.

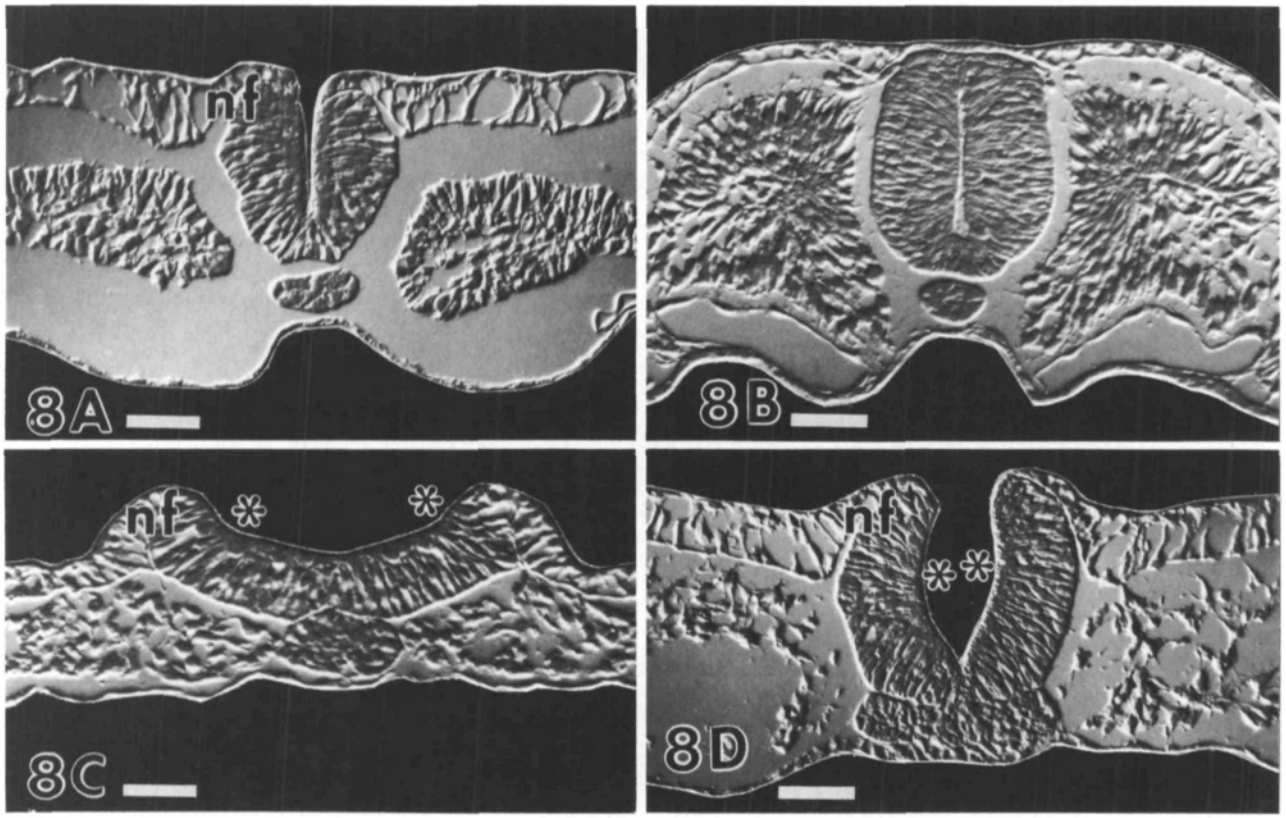


Fig. 8. Light micrographs of transverse plastic sections through the future spinal cord (A,B) and sinus rhomboidalis (C,D) levels of the chick (A,C, stage 10; B,D, stage 11). Note that true DLHPs do not form at the spinal cord level (i.e. furrowing of the dorsolateral neural plate fails to occur); in contrast, true DLHPs (*) form at the sinus rhomboidalis level and do so prior to neural fold elevation. nf, definitive neural folds. Bars=40 μm.

midline. In the region of the future spinal cord, bending of the neural plate occurs similarly, except that true DLHPs do not form (i.e. anchoring of the neural plate occurs as the definitive neural folds form, but furrowing does not occur; Fig. 8A). Thus, as neural folds elevate, each lateral half of the neural plate comes into midline apposition with its counterpart, resulting in temporary occlusion of the neural tube lumen (Fig. 8B). Finally, bending of the neural plate at the sinus rhomboidalis level (i.e. region of the closing caudal neuropore), like that at future brain levels but unlike that at more cranial spinal cord levels, involves the formation of both the MHP and paired DLHPs around which the neural folds elevate and converge, respectively (Fig. 8C,D). In summary, bending of the neural plate involves two major morphogenetic events: hinge point formation (i.e. neural plate anchoring and furrowing) and folding (i.e. neural fold elevation and convergence). The traditional viewpoint ignores both the regional differences that exist in neural plate shaping and bending as well as the fact that these processes involve multiple steps. These differences are integral features of the contemporary viewpoint.

Contemporary viewpoint: First fundamental

The first fundamental of the contemporary viewpoint is that forces for neurulation are both intrinsic and extrinsic to the neurepithelium. This view is not new; it was clearly stated 50 years ago by Weiss: "...medullary (neural) plate and non-neural (surface) ectoderm are both taking an active, coöperative, part in the movements of neurulation. While capable of manifesting their individual abilities independently, they normally do team work by assisting and reinforcing each other..." (p. 500 of Weiss, 1939; parenthetical words are ours). In contrast, the traditional viewpoint espouses that only intrinsic forces are required. What is the evidence for extrinsic forces in neurulation and why have they been largely disregarded?

Traditional disregard for extrinsic forces in neurulation

There are likely two major reasons why extrinsic forces have been disregarded by the traditional viewpoint. First, the view that a complex morphogenetic process such as neurulation occurs exclusively as a result of intrinsic forces is attractive because it tremendously simplifies its conceptualization and facilitates a reductionist approach to its analysis. Thus, there has been a strong preference for this simpler explanation. Second, the original neural plate isolation experiment carried out by Roux in 1885, which has taken on legendary status because it was one of the first embryology experiments, has been considered by many investi-

gators to have established conclusively that forces for neurulation are intrinsic to the neurepithelium. However, we question whether Roux's experiment truly demonstrates the presence of intrinsic forces and rules out a role for extrinsic forces. From Roux's experiment alone, it is impossible to draw a conclusion about the location of neurulation forces because he failed to provide sufficient documentation. Consequently, it is unknown whether the neural plate was isolated totally or only partially from surrounding tissues, how the isolated plate was maintained after its removal from the embryo, how much neurulation had occurred prior to its isolation, how much time was required for it to roll up (i.e. whether it rolled up according to the normal time course) and whether it underwent shaping as well as bending to form a normal neural tube with the typical cross-sectional morphology.

Some studies have tried to address the inadequacies of Roux's (1885) study and have been interpreted as providing support for the traditional viewpoint. However, such studies still have failed to demonstrate the location of neurulation forces. In one type of study, the prospective neural plate was isolated from stage 13 newt (*Taricha torosa*) embryos and placed on a bed of neutral agar (Jacobson and Gordon, 1976). The neural plate isolates rolled up into tube-like structures unless they were weighted down. Bending of the neural plate in newt embryos typically does not occur until stage 15 (i.e. about 12.5 h after stage 13; Burnside and Jacobson, 1968) and normally is preceded by shaping. Shaping (i.e. longitudinal lengthening) occurred only when the notochord was left attached to the neural plate isolate. Similarly, transverse strips of stage 15 newt neural plate were isolated and cultured on agar (Jacobson, 1981). Such strips either rolled up or remained flat, and those that rolled up did so in the wrong direction (i.e. apical surface outermost and basal surface innermost). It has been our experience, as well as that of others (e.g. Burnside, 1972; Vanroelen *et al.* 1982; Stern *et al.* 1985), that isolated pieces of epithelium in culture *quickly* roll up into tube- or vesicle-like structures, depending on their initial shape, and they consistently do so in the wrong direction, regardless of the particular type of epithelium cultured (e.g. endoderm also rolls up). Based on these results, it is clear that the rolling up of cultured neural plate isolates does not mimic the normal events of neurulation occurring *in vivo*. The isolates roll up substantially sooner, they fail to undergo lengthening prior to bending and, in some experiments, they remain flat or even roll up in the wrong direction.

In another type of study on the spherical neurulae of amphibians, an incision was made in the surface ectoderm immediately lateral to the neural fold on one side of the embryo, and it was reported that a morphologically intact neural tube formed even though the slit gaped widely (Lewis, 1947; Karfunkel, 1974; Jacobson and Gordon, 1976). It was concluded that the surface ectoderm "is under a considerable tension which is uniform in every direction' and, therefore, could 'not possibly be 'pushing' on the neural folds to augment neurulation movements" (p. 215 of Jacobson and Gordon, 1976). Does the evidence truly warrant such a conclusion? From examining the available illustrations (Figs 31, 32 of Jacobson and Gordon, 1976), it seems that the incisions were made considerably deeper than just through the surface layer. Thus, the observed gaping likely resulted from the extrusion of deep cells into the external milieu rather than from tension restricted to the surface. It was also reported that within 1 h after making the slits, the gaping wounds had healed and closed or were in the process of doing so. This observation contradicts the conclusion that the surface ectoderm is under considerable tension because it seems highly unlikely that healing movements could overcome such tension, especially in such a short period of time. Moreover, closure of the large round gape would not be expected to occur if all forces for shaping and bending are intrinsic to the neurepithelium because the neurepithelium, in isolation from more lateral 'restraining' tissue, should narrow and bend earlier than normally and thereby pull away from the wound; this, in conjunction with the tension on the surface ectoderm, would be expected to produce an even larger gape. It has been reported that "neurulation proceeds faster" when the surface ectoderm is slit just lateral to the neural fold (p. 253 of Karfunkel, 1974). However, the observation that the 'freed' neural fold is displaced medially cannot be taken as evidence of faster neurulation because wound gaping alone would be expected to cause such a displacement, especially in view of the observation that "the direction of slit makes no difference" (p. 214 of Jacobson and Gordon, 1976). Finally, it is likely that the surface ectoderm, owing to its rapid healing, was intact during most, if not all, of the period of neural plate shaping and bending and could have played a role in these processes. It is also possible that the intact neural fold on the side opposite the incision rotated past the midline (conceivably as a result of the unopposition of forces generated by tissues lateral to the intact neural fold) and fused with the flat neural plate on the side of the incision to form the neural tube. Thus, with such a paradigm it is not possible to distinguish between intrinsic and extrinsic neurulation forces.

In the final type of study, again in amphibians, a piece of neural plate was transplanted into lateral or ventral surface ectoderm where it formed an ectopic neural tube-like nodule (Boerema, 1929; Moury and Jacobson, 1989). On the surface, this type of experiment seems to be ideal for determining the origin of neurulation forces. However, it is well established that small pieces of tissue transplanted to the flank of an embryo can organize new morphogenetic movements that are atypical for this region and, in the most dramatic cases, can result in an entire ectopic embryo (reviewed by Spemann, 1938). The dorsal lip of the blastopore is one example of such a tissue. When transplanted to the flank, the dorsal blastoporal lip induces a neural plate which integrates with surrounding tissues to form normal axial and paraxial structures, some of which are composed of both host and donor cells. As thoroughly discussed in a recent review of the contributions of

Spemann to the field of Experimental Embryology, it is clear, based on the outcome of such transplants, that "there is undoubtedly a participation of regulatory forces which tend toward wholeness" (Spemann, 1931, as cited by Hamburger, 1988, on p. 59) so that the transplant can "create an integrated whole embryo out of heteroplastic parts" (p. 82 of Hamburger, 1988). Thus, not only can small pieces of transplanted tissue undergo regulation, but they can also complement themselves by "assimilative induction" of host material (i.e. appositional growth by incorporation of already existing but previously indifferent elements of the host). Because of these abilities, it is possible in the studies in which pieces of neural plate were transplanted into surface ectoderm that the neural plate in its new location organized the surrounding surface ectoderm (and, perhaps, underlying tissues) to undergo movements similar to those that occur normally and that this, in turn, generated forces adequate to cause the neural plate transplants to form neural tube-like structures. Therefore, this paradigm, like the aforementioned paradigms, fails to identify the origin(s) of neurulation forces.

Evidence of intrinsic forces in neural plate shaping and extrinsic forces in bending

The origins of neurulation forces have been demonstrated directly in a recent study (Schoenwolf, 1988). Chick embryos at Hamburger and Hamilton's (1951) stages 3 and 4 were placed in whole-embryo culture, and unilateral or bilateral longitudinal cuts were made through the entire thickness of the blastoderm at the approximate boundaries between the prospective surface ectoderm and neural plate. When the flat neural plate was separated bilaterally from adjacent non-neurepithelial tissues, the forebrain level of the neuraxis typically formed a closed vesicle often with optic vesicles. In contrast, more caudal levels of the neuraxis underwent normal shaping (i.e. thickening, narrowing and lengthening) and MHP formation (with midline furrowing) but failed to undergo further bending (i.e. elevation and convergence). When the neural plate was separated unilaterally, shaping occurred normally. In contrast, in some cases bending resulted in a closed neural tube, whereas in others the *intact neural fold* elevated and converged to the dorsal midline while the neural plate on the operated side remained flat. In those cases in which a closed neural tube formed, observations suggested that caudal to the forebrain level, the neural fold on the *intact* side had rotated *past the midline* and fused with the essentially flat neural plate on the operated side. These results provide evidence that bending of the neural plate is an autonomous event at the future forebrain level of the neuraxis (also see Fig. 1 of Lee and Nagele, 1988, which provides supporting evidence that the chick *forebrain* level can neurulate autonomously), but that it requires forces generated by lateral tissues at more caudal levels. The fact that the forebrain neurulates after microsurgical manipulation argues strongly against the possibility that the methods *per se* damage the blastoderms to the extent that they are incapable of neurulating, providing an internal control for this experiment. An additional control comes from another microsurgical study in which blastoderms were subjected to transection and most events of neurulation still occurred (Schoenwolf *et al.* 1989). Microsurgical experiments, therefore, provide direct evidence in support of a role for extrinsic forces (from lateral tissues) in bending. In contrast, the same experiments suggest that shaping is largely or exclusively the result of intrinsic forces, although they do not rule out a potential role for extrinsic forces generated by tissues (i.e. mesoderm and endoderm) *underlying* the neural plate (the latter possibility seems unlikely as discussed by Schoenwolf, 1988).

Additional but less direct evidence of extrinsic neurulation forces has appeared over the years. For example, evidence that "the presumptive epidermis (surface ectoderm) *independently* of the neural material (neural plate) has the power to move up over the entire embryo" (p. 20 of Jacobson and Jacobson, 1973; parenthetical words and italics are ours; see also Jacobson, 1962) has been provided for amphibians (*Ambystoma mexicanum*) by a series of microsurgical experiments. In one series, the entire neural plate, either with or without the neural folds, was extirpated. In those embryos in which the neural folds were left intact, the neural folds came together in the dorsal midline and underwent normal fusion, even though the neural plate was absent. In embryos in which the neural folds were excised along with the neural plate, the lateral surface ectoderm expanded medially, moving up over the wound, and fused across the dorsal midline on schedule. Although histological documentation was not provided and atypical tissue movements may have been generated by wound healing, this experiment at the very least suggests that coordinated, neurulation-like movements still continue in the complete absence of the neural plate. This implicates a role for surface ectodermal expansion (and perhaps expansion of the underlying tissue) as an extrinsic force in neurulation.

Other experiments on *Ambystoma mexicanum* have also led to the conclusion that "the *epidermis* contributes to raising and closing of the neural folds" (p. 275 of Brun and Garson, 1983; italics are ours). Embryos were treated with colchicine or nocodazole to cause neurepithelial cells to round up and thereby prevent them from becoming wedge-shaped, effectively inactivating forces generated by the neural plate while leaving this structure *in situ*. Despite this, formation, mediad migration and apposition of the neural folds still occurred. Again this implicates the involvement of forces extrinsic to the neural plate in neurulation.

Contemporary viewpoint: Second fundamental

The second fundamental of the contemporary viewpoint is that neurulation is driven by both changes in neurepithelial cell shape and other form-shaping events. Because extrinsic forces are essential for normal neurulation, factors other than neurepithelial cell shape changes must also be involved. What are the roles of

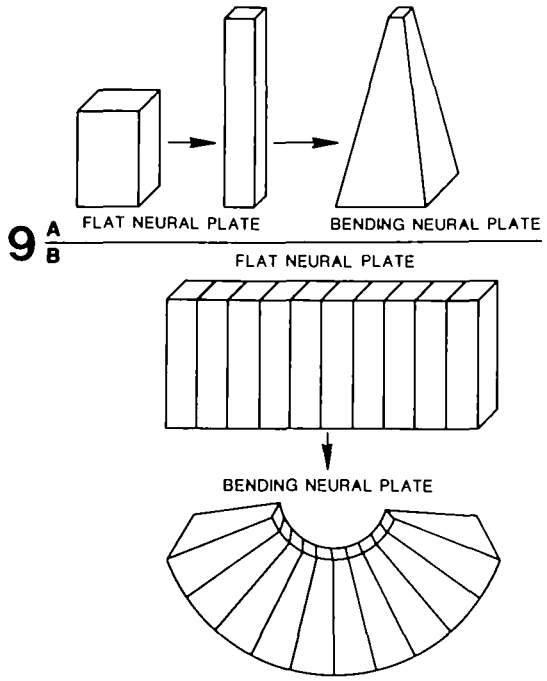


Fig. 9. Traditional view of neurepithelial cell shape changes during neural plate shaping and bending (A) and their effects on the neural plate (B). To visualize the effects of cell wedging on the neural plate three-dimensionally, first consider B to be a cross-sectional view. Cell wedging would result in bending in the transverse plane. Next, consider B to be a sagittal view. Cell wedging would result in an identical bending in the longitudinal plane, which, in conjunction with concomitant bending in the transverse plane, would result in three-dimensional, rather than two-dimensional, bending.

cell shape changes in neural plate shaping and bending and what other events are involved in these processes?

Traditionally, cells of the *flat* neural plate have been viewed as being column-like, whereas those of the *bending* neural plate have been viewed as being wedge-like. According to this view, neural plate shaping results solely from neurepithelial cells becoming taller (i.e. increasing their heights or undergoing 'palisading') and neural plate bending results solely from the elongated cells becoming wedge-shaped (Fig. 9). However, when considered three-dimensionally, this elementary view breaks down. First, simple (i.e. isotropic and without change in cell volume) elongation of the cells of a flat sheet would narrow the sheet in the transverse plane, but it would also shorten the sheet in the longitudinal plane. Neural plate shaping involves not only apicobasal thickening and transverse narrowing but also longitudinal *lengthening*. Second, simple wedging of the cells of a flat sheet would bend that sheet uniformly in three dimensions, not just in the transverse plane as usually diagrammed. Thus, the sheet would form a vesicle rather than a tube.

Role of neurepithelial cell elongation in neural plate shaping

The view that neurepithelial cells become taller during neural plate shaping is well documented for amphibians, birds and mammals (summarized by Schoenwolf and Powers, 1987), and modeling studies support the hypothesis that cell elongation acts in transverse narrowing of the neural plate during its shaping (Jacobson and Gordon, 1976; Schoenwolf, 1985). However, there has been only one direct test of this hypothesis (Schoenwolf and Powers, 1987). Traditionally, forces for neurepithelial cell elongation, like forces for neurepithelial cell wedging, have been attributed to the cytoskeleton; in particular, to paraxial microtubules (i.e. microtubules oriented parallel to the apicobasal axis of cells). Thus, neurepithelial cell height was compared to the width of the neural plate before microtubule depolymerization, after depolymerization and following subsequent repolymerization (in the chick embryo, in which this experiment was done, neurepithelial cell height is equivalent to neural plate thickness because the neurepithelium consists of a single layer of cells). Such comparisons revealed a direct correlation between the heights of neurepithelial cells and neural plate width: as neurepithelial cell height decreased, reducing the apicobasal thickness of the neural plate, neural plate width increased and as neurepithelial cell height subsequently increased, neural plate width decreased (Fig. 10). These results suggest a causal relationship between neurepithelial cell elongation and transverse narrowing of the neural plate, namely, that change in cell shape from low columnar to high columnar results in a corresponding decrease in cell diameter (a necessary condition if cell volume remains constant) and in neural plate width (change in cell diameter also affects neural plate length as discussed by Schoenwolf and Powers, 1987). Therefore, these results support the traditional viewpoint that changes in neurepithelial cell shape drive at least some aspects of neural plate shaping.

Roles of other form-shaping events in neural plate shaping

Neurepithelial cell elongation can account for only about 15% of the 50% reduction in neural plate width that normally occurs during shaping (estimates obtained from the data of Schoenwolf, 1985; see Schoenwolf and Alvarez, 1989, for calculations). The remaining 35% must be driven by other forces. Furthermore, besides the thickening and narrowing of the neural plate that occur during its shaping, there is also a rapid longitudinal lengthening. What events act in neural plate shaping to cause this lengthening and the remaining narrowing? Experiments on the chick neural plate suggest that in addition to neurepithelial cell elongation, two other events act in neural plate shaping, both of which generate intrinsic forces: neurepithelial cell rearrangement and cell division.

Cell rearrangement. Cell rearrangement or intercalation acts in both neural plate narrowing and lengthening. Through the interdigitation of adjacent tiers of neurepithelial cells, so that the lateral edges of the

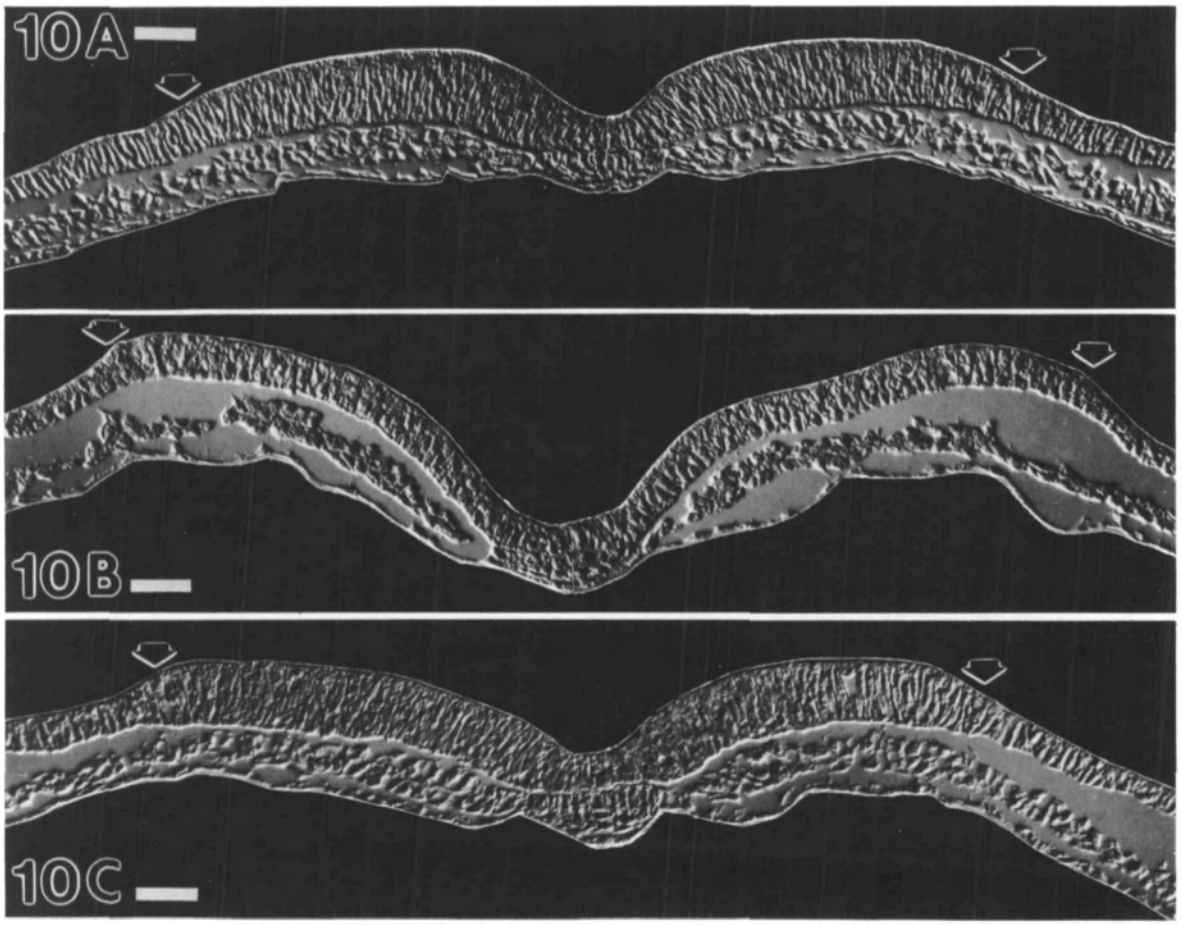


Fig. 10. Light micrographs of transverse plastic sections showing the effect of microtubule depolymerization and repolymerization on chick (stage 7) neural plate height and width. (A) Control embryo; (B) cold-treated embryo lacking paraxial microtubules; (C) cold-treated embryo subsequently reincubated for 3 h to allow paraxial microtubules to repolymerize. Arrows indicate the approximate lateral extent of the neural plate. Note that neural plate height and width are inversely correlated. Bar=40 μm.

neural plate move medially, the neural plate undergoes concomitant narrowing and lengthening (Fig. 11). Such coordinated movements resemble the well-documented convergent extension occurring during amphibian gastrulation (Keller *et al.* 1985). Cell rearrangement has been reported to occur in the amphibian neural plate (Jacobson and Gordon, 1976). Recently, it was demonstrated that cell rearrangement also occurs in the avian neural plate during shaping (Schoenwolf and Alvarez, 1989). Circular plugs of quail neural plate were transplanted homotopically and isochronically to chick embryos and displacement of cells within the grafted plug were followed over time. The plug narrowed transversely and lengthened longitudinally concomitant with neural plate narrowing and lengthening. Analysis of the number of quail cells spanning the width and length of the plug revealed that the number of cells within its width decreased, while the number within its length increased, corresponding to an average of 2 complete rounds of rearrangement. This provides direct evidence for a role for cell rearrangement in both neural plate narrowing and lengthening.

Cell division. Another event involved in neural plate shaping is cell division. For amphibian embryos, in which little cell division and no cell growth occur during neurulation (Gillette, 1944), it is likely that cell rearrangement is the major factor underlying neural plate lengthening (Schroeder, 1971; Jacobson and Gordon, 1976). However, in higher vertebrates, substantial cell division occurs within the neural plate during neurulation (Langman *et al.* 1966; Tuckett and Morriss-Kay, 1985; Smith and Schoenwolf, 1987, 1988). Thus, it would be expected that neural plate lengthening in birds and mammals also involves cell division.

In the studies most germane to the present investigation, the flat neural plate of the chick (i.e. at stage 4) was shown to have a cell cycle length of about 8 h (Smith and Schoenwolf, 1987, 1988). Cell cycle of prospective MHP cells (including both the S and non-S portions, but not its M phase) increases to about 12 h as the notochord forms and establishes apposition with their bases, but it remains unaltered in L cells. Thus, over a 24 h period, neural plate cells would be expected to undergo between 2–3 rounds of cell division,

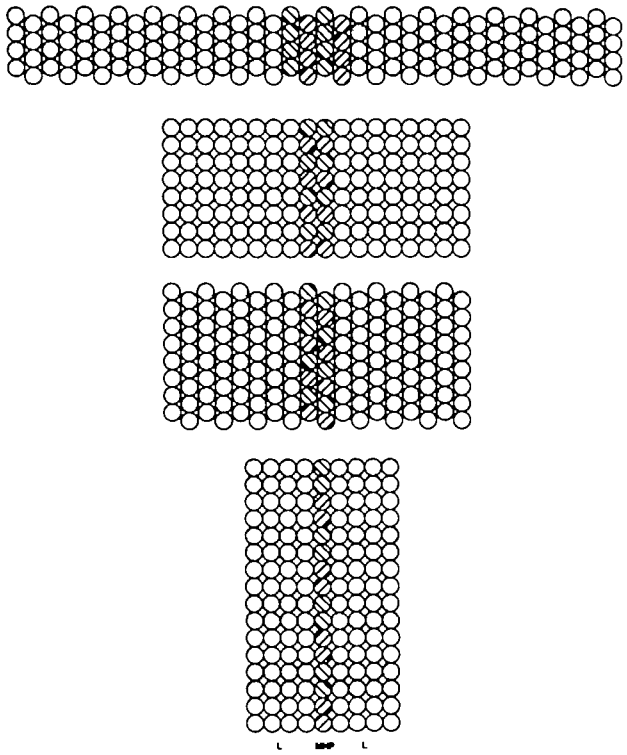


Fig. 11. Model of neurepithelial cell rearrangement (with cells viewed from their apices) during neural plate shaping. Cell rearrangement has been restricted in the model so that MHP cells interdigitate only with MHP cells and L cells interdigitate only with L cells.

although this value may be somewhat high because cell cycle length generally increases with advance in developmental age. Therefore, a single population of cells (e.g. L cells of the midbrain) could have a longer cycle at the closed neural tube stage (stages 10–11) than at flat (stages 4–6) or bending (stages 7–9) neural plate stages.

Schoenwolf and Alvarez (1989) compared the number of quail cells within grafts at 8, 12, 18 and 24 h postgrafting with the number of cells originally grafted and found that between 2 and 3 cycles of cell division occurred during the period of neural plate lengthening. Their results, in conjunction with those from a morphometric analysis of the shaping and bending neural plate (Schoenwolf, 1985), suggest that the *direction* of cell division (i.e. whether division is oriented to insert daughter cells into the longitudinal or transverse axis) differs among various craniocaudal levels of the neuraxis. Portions of quail grafts that contribute to the forebrain (and to the lateral midbrain) widen, whereas those that contribute to spinal cord exhibit marked transverse narrowing. The intact neural plate as a whole exhibits a similar expansion and narrowing, as revealed by reconstructions of the shapes of neural plates from serial sections at various stages (Fig. 8 of Schoenwolf, 1985). Based on differences in the numbers of quail cells per transverse sectional level between the brain and spinal cord levels of the neural plate, it seems likely that cell division is directed mainly within the transverse plane in the brain and within the longitudinal plane in the spinal cord. This would explain why 2 cycles of cell division and 2 rounds of cell rearrangement result in only 3 rounds of craniocaudal extension rather than 4 and why cell number doubles but graft length remains virtually constant in grafts contributing principally to the forebrain. Modeling studies using these values reveal that a neural plate having approximately the normal size and shape can result if all forces for shaping are intrinsic to the plate and if just three forces are involved: neurepithelial cell elongation, rearrangement and division (Fig. 15 of Schoenwolf and Alvarez, 1989).

Role of neurepithelial cell wedging in neural plate bending

The traditional viewpoint that bending of the neural plate merely involves a transformation in cell shape from column-like to wedge-like is a gross oversimplification for two major reasons. First, neurepithelial cells do not have regular polygonal shapes. Rather, scanning electron microscopy and light microscopy of horseradish peroxidase-filled neurepithelial cells have revealed that neurepithelial cells of both the flat and bending neural plate have irregular shapes that can be grouped into four categories (Fig. 12; Schoenwolf, 1982; Schoenwolf and Franks, 1984; Smith and Schoenwolf, 1988; Schoenwolf and Sheard, 1989, 1990): (1) spindle-shaped cells have a bulbous waist and both apically and basally tapering processes; (2) wedge-shaped cells have a bulbous base and an apically tapering process; (3) inverted wedge-shaped cells have a bulbous apex and a basally tapering process; and (4) globular cells are spherical cells that reside at the apex of the neural plate and are in the M-phase of the cell cycle. (True column-like cells are rarely present. Instead, as cube-like cells elongate during neural plate formation, they form low columnar cells, which are, in reality, more spindle-like than column-like. As low columnar cells become high columnar during neural plate shaping, they retain their spindle-like configurations and lose all semblance of column-ness.) Comparing the greatest diameter of neurepithelial cells from scanning electron micrographs with the diameter of neurepithelial cell nuclei from light micrographs of a plastic sections revealed that neurepithelial cell nuclei reside within the widest portion of each neurepithelial cell; this finding has been supported by examining the shapes of neurepithelial cells filled with horseradish peroxidase. Collectively, our observations suggest that the shape of a neurepithelial cell is directly correlated with the position of its nucleus.

Second, not all neurepithelial cells undergo wedging during neural plate bending. Neurepithelial cells of the flat neural plate have a uniform height and about three-fourths of these cells are spindle-shaped, whereas about one-fourth are wedge-shaped, with spindle- and wedge-shaped cells intermixed throughout the neurepithelium (Schoenwolf and Franks, 1984; Schoenwolf, 1985). As the notochord forms and the overlying neural plate becomes anchored to it, neurepithelial cells within the resulting MHP become strikingly different from those

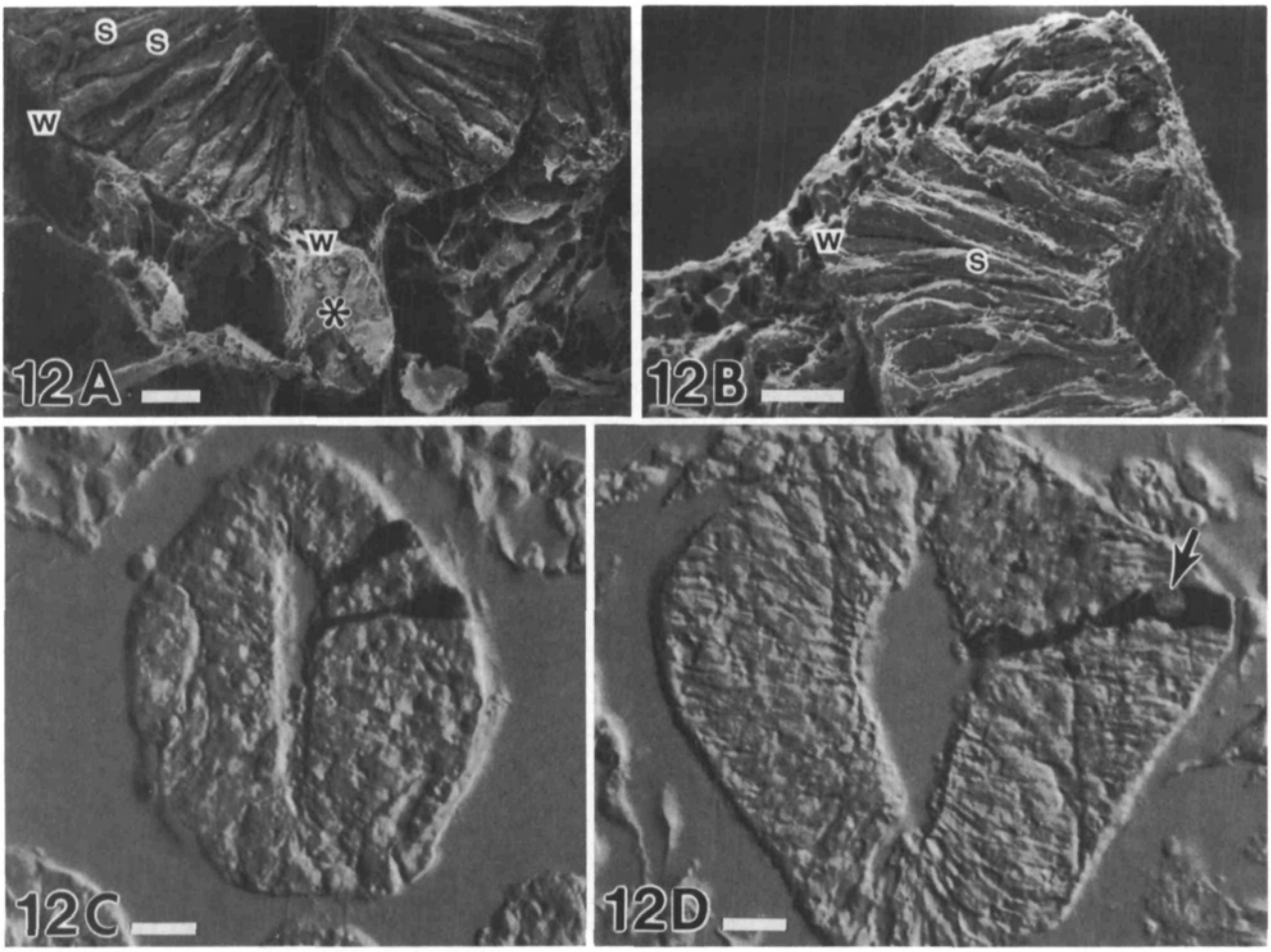


Fig. 12. Micrographs showing chick neurepithelial cell shapes. (A,B) Scanning electron micrographs of transverse slices through the median (A) and dorsolateral (B) hinge points at the future hindbrain level (stage 8); asterisk, notochord; w, s, wedge- and spindle-shaped cells, respectively. (C,D) Light micrographs of transverse paraffin sections through the spinal cord (C) and hindbrain (D) levels (stage 11). Three wedge-shaped neurepithelial cells (2 in C, 1 in D) were filled with horseradish peroxidase by microinjecting the flat neural plate; embryos were collected 24 h later. In D, the basal position of the nucleus is especially evident (arrow). Bars=10 μm.

in adjacent, paired lateral areas of the neural plate (L) in four principle ways (Figs 12A, 13). (1) Most MHP cells transform from spindle-shaped to wedge-shaped, such that more than 70% of the cells in the MHP are wedge-shaped when bending is completed (Schoenwolf and Franks, 1984). Cell wedging within the hinge points begins concomitantly with furrowing, continues with elevation and convergence and occurs synchronously with bending at all neuraxial levels. In contrast, most L cells remain spindle-shaped during bending. (2) MHP cells become about one-half as tall as L cells. This difference in height results from both a 1.5-fold decrease in the mean height of MHP cells and a 1.3-fold increase in the mean height of L cells (Schoenwolf, 1985). (3) The average cell cycle length of MHP cells increases (Smith and Schoenwolf, 1987). (4) MHP cells come to line a furrow, whereas L cells do not (Schoenwolf, 1982). These differences between MHP and L cells occur consistently and are detectable only after the notochord has formed and the overlying neural plate has become anchored to it. Similarly, neurepithelial cells within the DLHPs become different from L cells as the paired dorsolateral areas of the neural plate become anchored to the surface ectoderm (Figs 12B, 13). Thus, during neural plate shaping and bending, neurepithelial cells undergo a series of cell shape transformations. Characteristically, neurepithelial cells throughout the neural plate change from low columnar to high columnar (i.e. tall spindle) during shaping, and then those within the hinge points change from spindle-like to wedge-like during bending. However, because the shape of a neurepithelial cell is related to the position of its nucleus and neurepithelial cell nuclei undergo interkinetic migration, all neurepithelial cells continually undergo cell shape changes throughout neurulation. That is, as they traverse the cell cycle, neurepithelial cells sequentially assume the four characteristic cell shapes described above. Although this results in a constant turnover of neurepithelial cells of a particular shape, it has been demonstrated that there is a signifi-

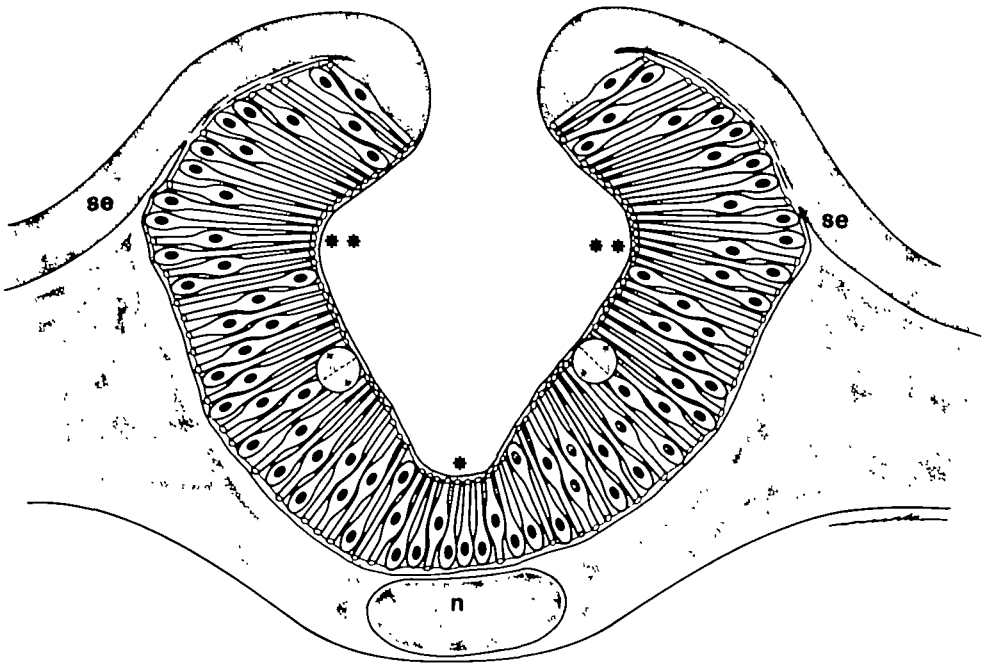


Fig. 13. Schematic representation of a transverse section through the future hindbrain level of a stage 8 chick embryo during bending, illustrating the characteristics of neurepithelial cells in the MHP (asterisk), DLHPs (double asterisks) and lateral neural plate *between* the hinge points; n, notochord; se, surface ectoderm. Note the differences in cell shape in these three regions of the neurepithelium.

cantly greater proportion of wedge-shaped cells in the MHP and DLHPs than in L at any one time during bending (Schoenwolf and Franks, 1984).

MHP cell wedging: an active event. The fact that changes in neurepithelial cell shape occur during neural plate shaping and bending is interesting, but for such changes to drive neurulation, as put forth by the traditional viewpoint, they must be active rather than passive, occurring independently of forces generated by neural plate shaping (e.g. transverse narrowing) and neural plate bending (e.g. neural fold elevation and convergence). Although cell wedging is viewed traditionally as being an active event, actual evidence in support of this was provided only recently (Schoenwolf, 1988). Chick embryos at Hamburger and Hamilton's (1951) stages 3 and 4 (i.e. prior to formation of the notochord beneath the midline of the neural plate and, therefore, prior to the appearance of the MHP) were placed in whole-embryo culture. Unilateral or bilateral longitudinal cuts were made through the entire thickness of the blastoderm at the approximate boundaries between the prospective MHP and L to separate these regions from one another and to determine whether midline neurepithelial cells still become wedge-shaped in the absence of lateral tissues. As a notochord formed beneath the narrow, isolated midline strip of neural plate, most overlying neurepithelial cells became wedge-shaped and furrowing occurred. These results provide direct evidence that cell wedging within the MHP occurs independently of forces generated by neural plate (i.e. L) narrowing and neural fold elevation and convergence, suggesting an active rather than a passive event.

MHP cell wedging and neural plate furrowing. Active cell wedging within the hinge points could play at least two roles in neural plate bending. First, cell wedging could drive bending of the neural plate by providing forces adequate to elevate and converge the neural folds; this is in accordance with the traditional viewpoint. This possibility seems unlikely, however, because very few cells actually become wedge-shaped during bending (i.e. roughly 10% of the cells in the neural plate are wedge-shaped at any one time during bending; calculated from values in Schoenwolf and Franks, 1984) and the few cells that do become wedge-shaped are localized, residing mainly within the hinge points, rather than being spread uniformly throughout the neural plate. Second, active cell wedging within the hinge points could drive neural plate furrowing, an initial event of bending that precedes folding, thereby localizing bending to restricted sites. By establishing the sites of bending, cell wedging would also play a major role in generating the characteristic cross-sectional morphology of the neural tube (i.e. thin roof and floor plates, thick lateral walls and a slit-like lumen extending dorsoventrally from the roof plate to the floor plate).

Evidence in favor of the latter role for cell wedging is provided by recent studies on chick neural plate. First, cells within the MHP begin to transform from spindle-shaped to wedge-shaped and furrowing occurs within this region about 4 h prior to the onset of neural fold elevation (Schoenwolf and Franks, 1984). Thus, cell wedging and furrowing within the MHP occur concomitantly and precede neural fold elevation. Additionally, cell wedging and furrowing still occur concomitantly within the MHP when L and the flanking, elevating neural folds have been removed (Schoenwolf, 1988). The best explanation for this is that formation of the midline furrow is caused by active cell wedging. It could be argued instead that cell wedging is a consequence of midline furrowing since these two events occur simultaneously. However, furrowing still occurs when all

possible pushing forces from lateral tissues (which could have caused furrowing to occur passively) are eliminated in the midline neural plate isolates. Thus, the most likely possibility is that cell wedging causes midline furrowing. Second, the sites at which bending occurs are correlated both spatially and temporally with cell wedging. Cell wedging and furrowing are restricted to the hinge points in control embryos, and elevation and convergence occur around the hinge points (Schoenwolf and Franks, 1984). When prospective lateral neural plate is removed only on one side, leaving the prospective MHP and adjacent lateral neural plate intact (Schoenwolf, 1988), neurepithelial cells become wedge-shaped and a distinct furrow forms in the *original* midline (i.e. the area underlain by notochord), which is now off center with respect to the neural plate isolate. Nevertheless, the lateral neural plate on the intact side still elevates around this furrow rather than around the new 'midline' (i.e. centermost plane) of the neural plate. This suggests that cell wedging and furrowing within the hinge points determine the sites at which bending occurs. Third, neural plate was separated from lateral tissues (i.e. surface ectoderm and underlying lateral plate mesoderm and endoderm) prior to bending (Schoenwolf, 1988). In the absence of nonneural lateral tissues, cell wedging and furrowing occurred within the MHP, but the intact neural folds failed to elevate and converge caudal to the forebrain level. This suggests that rather than driving elevation and convergence of the neural folds, cell wedging within the MHP acts only in furrowing. Fourth, notochordless chick embryos were generated by removing Hensen's node to determine whether midline neurepithelial cells still developed typical MHP characteristics (Smith and Schoenwolf, 1989). In the absence of the notochord, such characteristics did not develop. Midline neurepithelial cells were as tall as or taller than L cells; wedge- and spindle-shaped cells appeared to be intermixed throughout the neurepithelium, with most cells being spindle-shaped, and a midline furrow was absent. In some cases in which midline neurepithelial cells failed to develop typical MHP characteristics, the neural folds elevated but failed to converge and fuse in the dorsal midline, and the neural groove displayed a broad U-shape rather than the typical V-shape observed in control embryos during the period of neural fold elevation (Fig. 14A). In others, the neural folds elevated, converged and fused, forming a closed neural tube with an abnormally thick ventral midline region and a small eccentric lumen (Fig. 14B). These results demonstrate that elevation and convergence of the chick neural folds can occur in the absence of both the notochord and wedging of MHP cells. (Neural tube formation can also occur in the absence of cell wedging in amphibians; Brun and Garson, 1983.) This provides strong evidence that wedging of MHP cells drives furrowing and is necessary to establish the characteristic cross-sectional morphology of the floor plate of the neural groove and tube, but it is not the driving force behind neural fold elevation and convergence. Hence, other forces must be effecting these processes.

Another experiment in our laboratory (Smith and Schoenwolf, 1990) has provided additional evidence that MHP cell wedging drives furrowing but not folding, as well as further direct evidence of extrinsic forces in neurulation. MHP cells were extirpated shortly after their formation and prior to elevation of the neural folds (Fig. 15A). In some cases, the midline neurepithelial cells and underlying notochord were completely absent for long craniocaudal expanses, although embryos exhibited an essentially normal gross morphology (Fig. 15B). In the absence of midline neurepithelial cells and underlying notochord, the neural folds still formed and underwent full elevation as well as some convergence (Fig. 15C). In such cases, however, neural fold fusion failed to occur. These results, in conjunction with those from the aforementioned studies published previously by our laboratory, provide strong evidence that wedging of MHP cells does not drive elevation and convergence of the neural folds. Furthermore, they demonstrate extrinsic forces in neural plate bending because the two isolated halves of neural plate, which were oriented horizontally at the time of node removal, could not have become oriented vertically in the absence of such forces.

Roles of other form-shaping events in neural plate bending

We have considered the role of neurepithelial cell wedging (an intrinsic force) in neural plate bending. Because extrinsic forces also act in bending and intrinsic forces other than those generated by cell wedging likely exist, it becomes necessary to determine the nature of these forces and their underlying mechanisms. Over the years, many different mechanisms have been implicated in bending (reviewed by Schroeder, 1970; Burnside, 1973; Karfunkel, 1974; Jacobson, 1980, 1981; Schoenwolf, 1982, 1988; Trinkaus, 1984; Gordon, 1985; Jacobson *et al.* 1986; Martins-Green, 1988). Although, on the surface, these mechanisms or form-shaping events appear both numerous and diverse, it is possible to place them into five groups: (1) migration and/or expansion of the surface ectoderm toward the dorsal midline; (2) neurepithelial cell packing owing to localized changes in cell surface adhesivity and/or to differential growth of the neural plate, resulting from rapid proliferation and/or enlargement of its cells combined with lateral limitations on neural plate expansion by adjacent tissues; (3) longitudinal stretching of the neural plate, resulting in transverse buckling; (4) hinge point formation; and (5) expansion of the tissue underlying both L and the adjacent surface ectoderm (i.e. mesoderm, endoderm and associated extracellular matrix). Below, we will discuss only the latter three groups. The first group has already been discussed above. Likewise, the second group has already been discussed thoroughly in the general context of epithelial morphogenesis (Ettensohn, 1985; Fristrom, 1988). Although little information currently exists to support a role for this possibility in neural plate bending, it has not been ruled out and deserves further study.

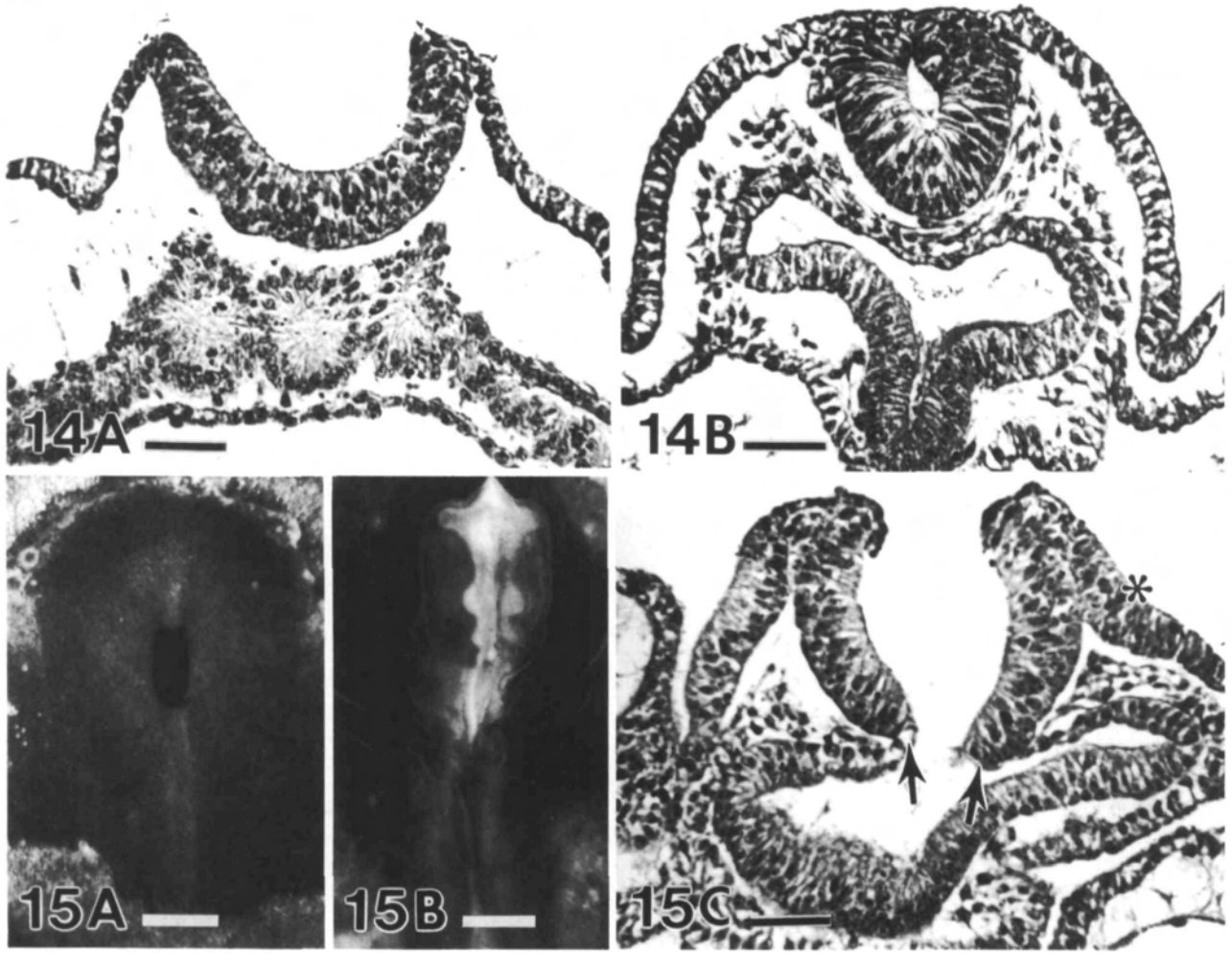


Fig. 14. Light micrographs of transverse paraffin sections showing the results in the chick of Hensen's node removal at stage 4; embryos were collected 24 h later. Note that the notochord has failed to form and that neural fold elevation (A) and neural groove closure (B) occurred in the absence of typical MHP formation. Three somites underlie the neurepithelium in A. Bars=40 μm.

Fig. 15. Micrographs showing the results of MHP cell removal at stage 5; embryos were collected 24 h later. (A) Light micrograph of a dorsal view at the time of excision; (B) light micrograph of a dorsal view 24 h later; (C) light micrograph of a transverse paraffin section. Note that although the MHP and notochord are absent, neural fold elevation and some convergence have occurred. Arrows, continuity between neural ectoderm and foregut endoderm, which likely serves to anchor the medial edges of the neural plate; asterisk, otic placode. A,B, bars=500 μm; C, bar=40 μm.

Longitudinal stretching. Longitudinal stretching could act in neural plate bending. It has been postulated that such stretching creates longitudinal tensions within the neural plate and, because of the viscoelastic nature of the plate, transverse buckling (i.e. bending) occurs (Jacobson and Gordon, 1976; Jacobson, 1978, 1984; Gordon and Jacobson, 1978; Gordon, 1985). According to the authors of this proposal:

> "If an elastic sheet is distorted by being stretched along a line, the sheet buckles out of the plane. Ridges rise on each side of a valley that runs along the line of stretch...If stretching is continued, the ridges roll toward the midline of the valley and may meet to form a tube..."

(p. 18 of Jacobson, 1978). They proposed that the neural plate rolls up into a tube by the same mechanism. This postulate, which has been called the transverse buckling, Poisson buckling or Eulerian buckling model, depends on two untested assumptions: the neural plate is a viscoelastic sheet and longitudinal lengthening is caused by stretching. It is well documented that longitudinal lengthening of the neurepithelium occurs during neurulation, but such lengthening need not be the result of stretching. In fact, sufficient cell rearrangement and division occur within the chick neural plate to account for its lengthening without requiring substantial stretching (Schoenwolf and Alvarez, 1989). Moreover, assuming that longitudinal stretching forces exist, it is unknown how they are generated. A logical possibility is that such forces result from elongation of the notochord by virtue of its attachment to the midline neural plate, but experiments have not supported this idea (Malacinski and Youn,

1981, 1982; Youn and Malacinski, 1981; Smith and Schoenwolf, 1989). Alternatively, it has been proposed that stretching forces are generated by cell rearrangements within the MHP (i.e. notoplate of Jacobson and co-workers) (Jacobson *et al.* 1986). Such cells do rearrange, but whether they generate longitudinal stretching forces by this behavior remains to be determined. Nevertheless, recent evidence is at least compatible with the idea that longitudinal tension plays a role in neurulation, specifically in neural groove closure (Schoenwolf *et al.* 1989). During normal development, the cranial end of the primitive streak moves caudally, while the caudal end of the neural plate lengthens longitudinally in its wake. Thus, young chick blastoderms were transected to isolate the flat neural plate from streak regression, a movement that could conceivably generate longitudinal tension. The isolated neural plate underwent normal shaping and bending, but neural groove closure failed to occur. This suggests that longitudinal tension generated by primitive streak regression acts only in the latter events of neurulation.

Hinge point formation. Another form-shaping event that could act during neural plate bending is hinge point formation. Hinge point formation consists of a number of events, one of which is the anchoring of the neural plate to adjacent tissues. How this anchoring is accomplished is largely unknown. Transmission electron microscopy reveals that the apposed tissues are separated by a narrow interface bordered by basal laminae (Fig. 16; unpublished observations). This space contains extracellular matrix (ECM) and lacks cell processes, indicating that anchoring is not accomplished by direct cell contact. Immunocytochemical studies have

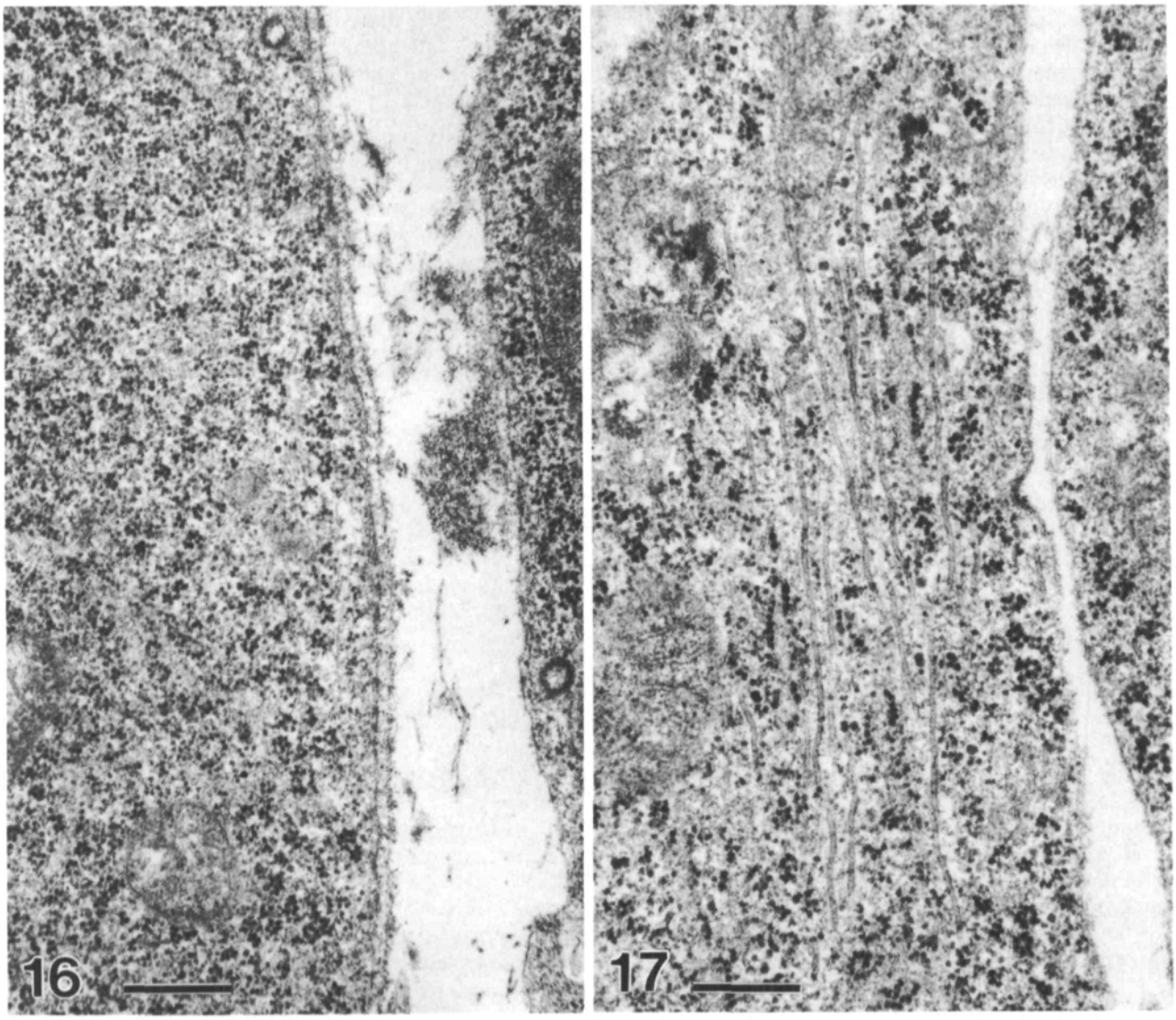


Fig. 16. Transmission electron micrograph showing the surface ectodermal (right) and neural plate (left) interface of a DLHP at the future hindbrain level (stage 9) of the chick. Note the absence of spanning cell processes and the presence of basal laminae and intervening ECM. The interface between notochord and MHP cells appears similar. Bar=0.5 μm.
Fig. 17. Transmission electron micrograph of chick MHP cells (stage 7). Note the abundant paraxial microtubules. Similar paraxial microtubules are found in both L and DLHP cells. Bar=0.5 μm.

revealed that the interface is rich in a number of glycoproteins, including collagen IV, fibronectin, laminin, tenascin and entactin (Tuckett and Morriss-Kay, 1986; Martins-Green, 1988; Riou *et al.* 1988), but whether any of these components mediates anchoring is unknown. Anchoring likely stabilizes the neurepithelium during its shaping and bending. Such stabilization would be requisite for extrinsic forces to play a major role in neural plate bending. For example, forces generated by the expansion of lateral tissues could cause the neural plate to bend either inwardly or outwardly in the absence of stabilization. In the presence of stabilization, however, only inwardly directed bending can occur. Although anchoring is likely involved, it is unlikely to be the only event controlling the direction of bending. The localized changes in neurepithelial cell height and transformations in neurepithelial cell shape from spindle-like to wedge-like (and concomitant furrowing) that occur during hinge point formation also play important roles. But are these roles obligatory or only facilitatory? Two recent experiments provide evidence that formation of the MHP has only a facilitatory role in neural plate bending. First, as discussed above, when the MHP is prevented from forming by extirpating the notochordal rudiment (i.e. Hensen's node), bending of the neural plate can still occur (Smith and Schoenwolf, 1989). (In the absence of the notochord, midline neurepithelial cells become underlain by somites or masses of mesodermal cells, which likely serve to anchor them. Likewise, in the complete absence of midline neurepithelial cells and underlying notochord, e.g. Fig. 15C, the ventromedial edges of the two isolated halves of neural plate become attached to endoderm, which likely serves to anchor them.) Second, also as discussed above, when the neural plate is isolated from lateral tissues, the MHP still forms and furrows, but the neural folds fail to elevate and converge (Schoenwolf, 1988). Thus, MHP formation is neither absolutely required for bending nor sufficient by itself to cause bending.

In contrast to MHP formation, DLHP formation seems to be playing a more active role in bending, specifically in neural fold formation and convergence. Like MHP formation, formation of paired DLHPs involves anchoring of the neurepithelium to adjacent tissues, changes in cell shape and furrowing. The definitive neural folds form and converge concomitantly with these events. The fact that these processes occur simultaneously suggests that they might be causally related. In support of this, when DLHP cell wedging and furrowing are inhibited, convergence of the neural folds fails to occur (Schoenwolf *et al.* 1988; discussed in more detail below); however, it is unknown whether convergence is driven by changes in cell shape or by extrinsic forces. Moreover, the mechanism(s) underlying formation of the definitive neural folds is unknown. Two ideas have appeared recently. Although they are highly speculative and little data currently exist to support them, they are intriguing possibilities well worthy of further study.

The first idea is that the definitive neural folds form owing to a progressive delamination of neural and surface ectoderm that results in the formation of an interface between these two layers (Fig. 11E–H of Martins-Green, 1988). This delamination is accompanied by the deposition of ECM molecules within the resulting interectodermal space and the subsequent formation of paired basal laminae (one associated with neural plate and another associated with surface ectoderm) between the two ectodermal layers. In addition, the basal lamina originally underlying the unsubdivided epithelium remains intact and ultimately bridges the zone of delamination, thereby connecting the neural plate and surface ectoderm. During neural fold convergence, the interectodermal spaces on each side extend dorsomedially and then coalesce, concomitant with neural fold fusion, to span the dorsal midline. Thus, the coalescence of these spaces results in the separation of the surface ectoderm from the newly formed roof of the neural tube and creates a space for neural crest cell migration. Further separation of the surface ectoderm and roof of the neural tube as well as alteration of the intervening ECM occur during migration of neural crest cells into the cell-free space (Bolender *et al.* 1980; Brauer *et al.* 1985).

The second idea is that the definitive neural folds form as a result of local interactions between neural plate and surface ectoderm (Jacobson *et al.* 1986; Moury and Jacobson, 1989). It has been proposed that neural plate cells crawl beneath the surface ectodermal cells and generate a "rolling moment...which forces up the plate edge to form the neural fold" (p. 35 of Jacobson *et al.* 1986) and that the continuation of this process results in neural fold convergence. Such crawling is speculated to occur by means of "cortical tractoring" (i.e. "a time-averaged motion of cortical cytoplasm which flows from the basal and lateral surfaces to the apical region"; p. 19 of Jacobson *et al.* 1986). Besides neural fold formation, cortical tractoring has also been hypothesized to explain neurepithelial cell elongation and wedging; however, observational and experimental verification of this interesting hypothesis are currently lacking.

Expansion of deep tissues. The final form-shaping event that could act during neural plate bending consists of forces generated by the expansion of the tissue underlying both L and the adjacent surface ectoderm. Observations of living embryos by means of time-lapse microscopy (e.g. Vakaet, 1970; Keller, 1978) and fixed embryos at various stages (Schoenwolf and Watterson, 1989) have revealed that coordinated morphogenetic movements occur throughout all three germ layers during neurulation. The orchestration of such movements has led to the hypothesis that tissues underlying the neural plate and surface ectoderm play a role in neural plate morphogenesis. For example, the paraxial mesoderm undergoes mediolateral condensation and dorsoventral elongation during neural plate shaping and bending and has been proposed to play a role in these processes (Schroeder, 1970). Similarly, ECM, particularly hyaluronate, underlies the neurepithelium

and has been proposed to play a role in neural fold elevation (Morriss and Solursh, 1978*a*,*b*). Some investigations, namely, one using β-D-xyloside to inhibit chondroitin sulphate-proteoglycan synthesis (Morriss-Kay and Crutch, 1982), three using *Streptomyces* hyaluronidase to degrade hyaluronate (Anderson and Meier, 1982; Schoenwolf and Fisher, 1983; Smits-van Prooije *et al.* 1986) and one using heparitinase to degrade heparan sulphate proteoglycan (Tuckett and Morriss-Kay, 1989), have provided evidence in support of the hypothesis; yet two other investigations, namely, one using chondroitinase ABC (Morriss-Kay and Tuckett, 1989) and the other using *Streptomyces* hyaluronidase (Morriss-Kay *et al.* 1986), have not. Neurulation was delayed in the latter two investigations but, in contrast to the other studies, neural tube formation eventually occurred. The reason for this discrepancy is unclear. Nevertheless, the fact that neurulation was delayed or inhibited in all studies suggests a role (albeit perhaps only permissive) for the ECM in neurulation. Further support for a role for the ECM in neurulation comes from a recent study on the mouse mutant, curly tail, which exhibits neural tube defects at the caudal neuropore level. In affected mutant embryos, [^{3}H]hyaluronate accumulates in reduced amounts specifically in the region of the caudal neuropore (Copp and Bernfield, 1988).

Although the perturbation studies just discussed suggest a role for the ECM in neurulation, its precise role is undefined. In the aforementioned studies on the chick, the neural folds formed and elevated after treatment with hyaluronidase, but the later events of neurulation (i.e. neural fold convergence and fusion) were inhibited. This result was surprising in view of the suggestion that inflation of the ECM beneath the neural folds might produce forces sufficient to drive bending. Instead, these results suggest that the ECM plays a more subtle role in neurulation, which, perhaps, may be crucial only within the region of the neural fold. Not only would this explain the prevention of neural fold convergence and fusion in some studies, but it would also explain the delay reported in others. These results, in conjunction with the hypothesis that the ECM might be playing a role in the separation of the neural and surface ectoderm as proposed in the delamination model for definitive neural fold formation, convergence and fusion (Martins-Green, 1988), warrant further experiments to clarify the role of ECM in neurulation.

Contemporary viewpoint: Third fundamental

The third fundamental of the contemporary viewpoint is that forces for cell shape changes are generated both by the cytoskeleton and by other factors. Traditionally, the cytoskeleton has been viewed as being solely responsible for the changes in neurepithelial cell shape that occur during neural plate shaping and bending.

Role of microtubules in neurepithelial cell elongation

The view that cell elongation during neural plate shaping is generated by the cytoskeleton of neurepithelial cells is based on a simple and rational mechanism that was initially suggested to explain elongation of lens placode cells (Byers and Porter, 1964). By means of transmission electron microscopy, it was demonstrated that elongated lens placode cells typically contain paraxial microtubules and it was proposed that these microtubules cause elongation. Subsequent studies revealed abundant paraxial microtubules in elongated neurepithelial cells of amphibians (Waddington and Perry, 1966; Baker and Schroeder, 1967; Schroeder, 1970; Burnside, 1971, 1973; Karfunkel, 1971), birds (Lyser, 1968; Messier, 1969; Handel and Roth, 1971; Karfunkel, 1972; Nagele and Lee, 1979, 1980*b*; Schoenwolf and DeLongo, 1980; Schoenwolf and Powers, 1987) and mammals (Herman and Kauffman, 1966; Wilson and Finta, 1980*a*,*b*; Schoenwolf, 1984) as well as in blastoporal cells (Perry and Waddington, 1966) and flask cells of the primitive streak (Granholm and Baker, 1970). When embryos were treated with agents that disrupt microtubules (e.g. colchicine or its analogs), neurepithelial cells rounded up (amphibians: Karfunkel, 1971; Burnside, 1973; Löfberg and Jacobson, 1974; birds: Karfunkel, 1972; mammals: Ferm, 1963; O'Shea, 1981). Thus, it was concluded that neurepithelial cell elongation is mediated largely, if not exclusively, by paraxial microtubules. However, other interpretations are possible. For example, the presence of spherical neurepithelial cells after treatment with colchicine could be the result of direct inhibition of cell elongation, collapse of previously elongated cells or arresting of cells in metaphase (the phase of the cell cycle during which all cells are normally spherical). In addition, one study in the chick reported that rather than rounding up, neurepithelial cells remained elongated in the absence of paraxial microtubules (Handel and Roth, 1971).

The role of paraxial microtubules in elongation of chick neurepithelial cells was elucidated only recently (Schoenwolf and Powers, 1987). Depolymerization of microtubules, achieved by cold, colchicine, or nocodazole treatment, and subsequent measurement of neurepithelial cells, revealed that the heights of these cells were reduced by roughly 25 % when their microtubules were depolymerized, although most cells still remained considerably elongated, and that complete rounding up of neurepithelial cells occurred only when cells entered metaphase, where they arrested. Collectively, these results suggest that elongation of neurepithelial cells (and, therefore, apicobasal thickening of the neural plate) is generated by both paraxial microtubules and other factors. Furthermore, MHP cells, which become *shorter* rather than taller during neural plate shaping and bending, are replete with paraxial microtubules (Fig. 17; unpublished observations). Thus, other factors in addition to paraxial microtubules must be involved in regulating neurepithelial cell height. What are these other factors? Two have been proposed: cortical tractoring (Jacobson *et al.* 1986) and localized changes in intercellular adhesion (Gustafson and Wolpert, 1962, 1967; reviewed in detail by Ettensohn, 1985). However, direct evidence in support of these possibilities is currently lacking.

Role of apical microfilament bands in neurepithelial cell wedging

The view that the cytoskeleton causes neurepithelial cell wedging is also based on a simple and rational mechanism (Baker and Schroeder, 1967). By means of transmission electron microscopy, it was demonstrated that circumferential microfilament bands were present in the apices of *Hyla regilla* and *Xenopus laevis* neurepithelial cells during bending. It was proposed that these microfilaments contract gradually and simultaneously in a purse-string-like fashion, constricting the apices of neurepithelial cells and thereby transforming these cells from columns to wedges. This, in turn, was assumed to generate forces adequate to cause bending of the neural plate. Subsequent studies revealed microfilaments of similar arrangement in neurepithelial cells of other amphibians (Burnside, 1971; Schroeder, 1973), birds (Karfunkel, 1972; Schroeder, 1973; Camatini and Ranzi, 1976; Nagele and Lee, 1980*a*) and mammals (Freeman, 1972; Morriss and New, 1979; Wilson and Finta, 1980*a*) as well as in cells of many other developing systems in several species, including the thyroid rudiment, lens vesicle, optic vesicle, optic cup, pancreatic buds, oviductal glands and salivary glands (reviewed by Wessells *et al.* 1971; Wessells, 1977; Trinkaus, 1984). These microfilaments were considered to be contractile, producing the major force for bending. Subsequent experimental studies, particularly those on the neural plate, provided evidence in support of the hypothesis that apical microfilaments are capable of contraction. Such studies reported that (1) circular bands of apical microfilaments appear increasingly dense during bending of the neural plate, suggesting a possible 'sliding filament' action (Burnside, 1971); (2) apical microfilaments bind heavy meromyosin, suggesting they have actin-like properties (Nagele and Lee, 1980*a*); (3) immunologically active forms of actin, myosin and other contractile proteins are localized in the apices of neurepithelial cells (Nagele and Lee, 1978; Sadler *et al.* 1982, 1986; Lee *et al.* 1983; Lash *et al.* 1985; Lee and Nagele, 1985); (4) Ca^{2+}, a regulator of microfilament contractility, is located in coated vesicles in the apices of neurepithelial cells (Nagele *et al.* 1981) and is released during neurulation (Moran, 1976); and (5) bending of the neural plate is reversibly inhibited by papaverine, a smooth muscle relaxant that prevents release of bound Ca^{2+} (Moran and Rice, 1976), and is accelerated by ionophore A23187, an antibiotic that promotes Ca^{2+} transport and release (Moran and Rice, 1976; Lee *et al.* 1977). Other studies showed that treating embryos with agents that disrupt microfilaments (e.g. vinblastine sulfate or cytochalasin) results invariably in neural tube defects (amphibians: Karfunkel, 1971; Burnside, 1973; birds: Karfunkel, 1972; Linville and Shepard, 1972; Messier and Auclair, 1974; Lee and Kalmus, 1976; mammals: Wiley, 1980; Morriss-Kay, 1981; O'Shea, 1981). The results of these studies have been regarded as evidence that bending of the neural plate is caused by microfilament-mediated cell wedging, but none of these studies actually demonstrated that microfilament depolymerization prevents neurepithelial cell wedging. Moreover, the effect of microfilament depolymerization on stages of neural plate bending (e.g. furrowing *versus* folding) was not considered.

The role of microfilaments in neurepithelial cell wedging and neural plate bending has been reexamined recently (Schoenwolf *et al.* 1988). Although exposure to cytochalasin D results invariably in disruption of apical microfilaments and the formation of neural tube defects, MHP cells consistently become wedge-shaped on schedule, the midline furrow forms and elevation of the neural folds around the MHP (i.e. bending) still occurs (Fig. 18). This demonstrates that apical microfilaments are not required for wedging of MHP cells, midline furrowing and neural fold elevation during bending of the chick neural plate. In contrast, the effects of cytochalasin D on DLHP cells and neural fold convergence were variable. In about one-third of the embryos treated with cytochalasin D, the DLHPs formed and furrowed, many of the DLHP cells became wedge-shaped and convergence of the neural folds occurred even though apical microfilaments were absent. In the remainder of the treated embryos, however, formation and furrowing of the DLHPs and convergence of the neural folds around these hinge points were inhibited, and spindle-shaped cells were more common than wedge-shaped cells in the paired dorsolateral areas of the neurepithelium. These results seem to suggest that apical microfilaments act in wedging of DLHP cells, which, in turn, is required for furrowing of the DLHPs and neural fold convergence. However, because of the variable adverse effects of cytochalasin D on the DLHPs and on the shapes of cells within these hinge points, it is unclear whether these effects were mediated directly by the depolymerization of apical microfilaments or indirectly by some other mechanism (discussed in detail by Schoenwolf *et al.* 1988). Nevertheless, it is clear from this study that several events involved in neural plate bending, including wedging of MHP cells, can occur independently of apical microfilaments.

Role of cell cycle alteration and basal expansion in neurepithelial cell wedging

Theoretically, neurepithelial cells could become wedge-shaped by apical narrowing, basal expansion or a combination of these events. Basal expansion resulting from increased water uptake by the bases of neurepithelial cells was thought initially to be the mechanism responsible for neurepithelial cell wedging (Glaser, 1914). However, attempts to provide evidence in support of this 'basal swelling by hydration' hypothesis were unsuccessful (Glaser, 1914, 1916; Brown *et al.* 1941; Gillette, 1944), and, as a consequence, the concept of basal expansion has been disregarded traditionally. Attention became focused on apical narrowing with the discovery of circumferential bands of microfilaments in the apices of neurepithelial cells during bending.

It is now clear that basal expansion occurs as neurepithelial cells transform from spindle-shaped to

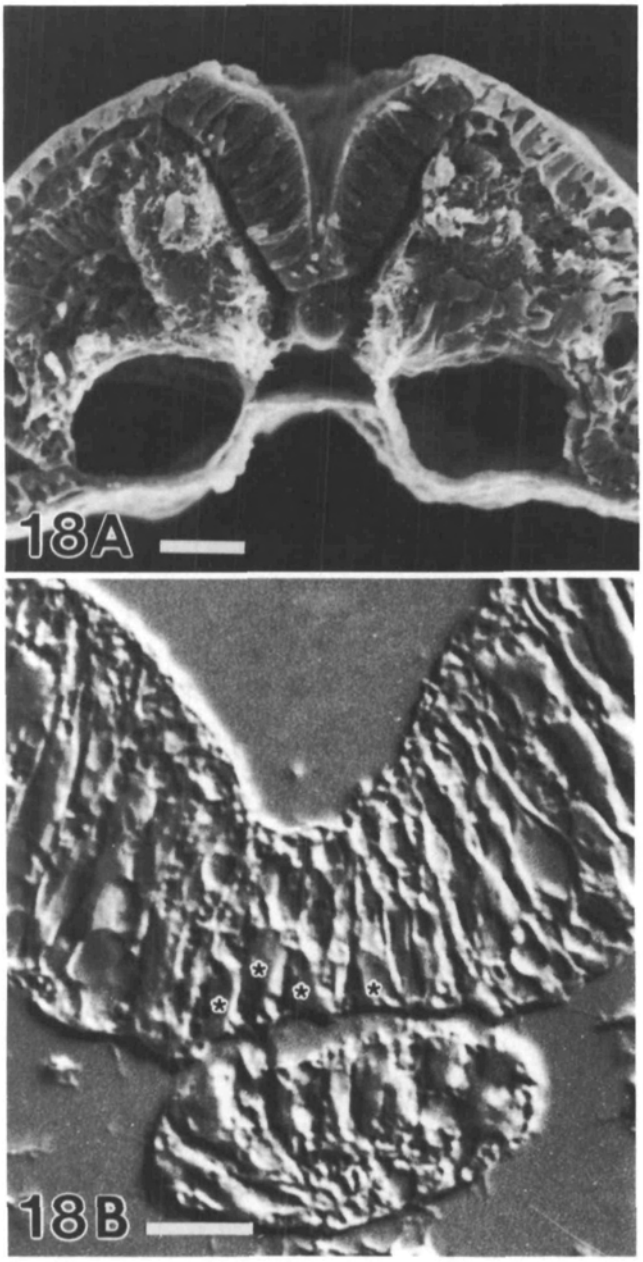
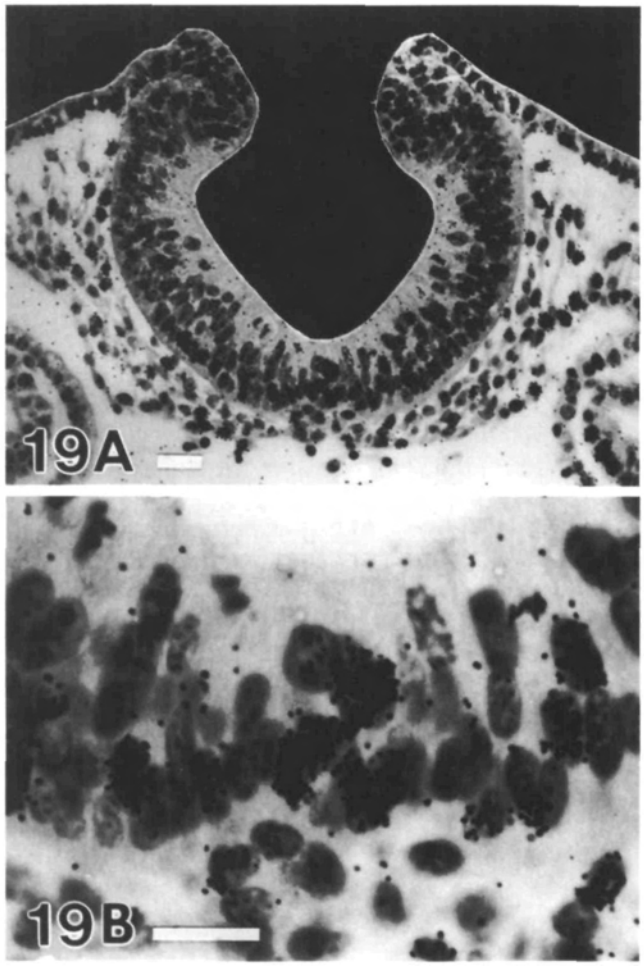


Fig. 18. Micrographs showing the effects of cytochalasin D treatment in the chick. (A) Scanning electron micrograph of a transverse slice through the spinal cord showing elevated neural folds (embryo treated at stage 9 and collected 24 h later); (B) light micrograph of a transverse plastic section through the midbrain showing that MHP cells have become wedge-shaped as indicated by the basal position of their nuclei (asterisks). The embryo was treated at stage 4 and collected 8 h later. A, bar=40 μm; B, bar=10 μm.

wedge-shaped during bending (Schoenwolf and Franks, 1984). The evidence for this derives from two observations. (1) Interkinetic migration of neurepithelial cell nuclei results in varied nuclear position during the cell cycle (Sauer, 1935; Sauer and Chittenden, 1959; Fujita, 1962; Martin and Langman, 1965; Watterson, 1965; Langman *et al.* 1966). Neurepithelial cell nuclei divide at the apex of the neural plate. Daughter nuclei then migrate toward and reside at the base of the neural plate. Eventually, they return to the apex again and repeat the cell cycle. (2) The nucleus resides within the widest portion of each neurepithelial cell, which tapers apically and/or basally to a slender cord-like process (Fig. 13; Schoenwolf and Franks, 1984). For example, a spindle-shaped neurepithelial cell has an expanded 'waist,' which contains the nucleus, and a much narrower apex and base, whereas a wedge-shaped neurepithelial cell has an expanded base, which contains the nucleus, and a much narrower apex and waist. Consequently, as nuclei migrate from the apex of the neural plate to the base and then back to the apex again to undergo division, neurepithelial cells sequentially transform from inverted wedge-shaped to spindle-shaped to wedge-shaped and then the transition is reversed. To generate wedge-shaped cells by basal expansion, presumably all that is necessary is to move the nucleus to the base of each cell and keep it there.

Evidence from two recent studies suggests that regulation of the cell cycle may contribute to neurepithelial cell wedging. First, it was shown that the generation time of MHP cells is lengthened as these cells become wedge-shaped during bending of the neural plate (Smith and Schoenwolf, 1987). Second, it was demonstrated that both the DNA synthetic (S) phase and non-DNA synthetic (non-S) portion of the cell cycle are significantly longer in the MHP than in L, whereas the mitotic (M) phase is significantly shorter in the MHP than in L during stages of neural plate bending (Smith and Schoenwolf, 1988). This study also revealed that wedge-, spindle- and inverted wedge-shaped cells within the MHP can be in either the S phase or non-S portion of the cell cycle (Fig. 19). Thus, DNA synthesis is not restricted to the base of the neural plate during bending as previously reported (Fujita, 1962; Martin and Langman, 1965; Langman *et al.* 1966) and nuclear position and cell cycle phases are not coupled totally. Finally, this study showed that all MHP cells are proliferative during stages when bending of the neural plate is occurring, suggesting that they do not become wedge-shaped by withdrawing from the cell cycle.

Based on the results of Smith and Schoenwolf (1987, 1988), a model was presented to explain cell wedging in the MHP produced by alteration of the cell cycle (Fig. 20; Smith and Schoenwolf, 1988). According to this model, neurepithelial cell nuclei in the MHP and L reside at the apex of the neural plate during the M phase of the cell cycle and either at the apex (i.e. cells are inverted wedge-shaped) or more centrally within the neurepithelium (i.e. cells are spindle-shaped) during the transition between the G_1 and S phases and during the lattermost part of the G_2 phase. In contrast, MHP and L cell nuclei reside at the base of the neural plate (i.e. cells are wedge-shaped) during the S phase, during the transition between the S and G_2 phases and during most of the G_2 phase. Based on this model, it is predicted that MHP cell nuclei reside basally (and thus MHP cells are wedge-shaped) during the prolonged part of the S phase and during most of the G_2 phase,



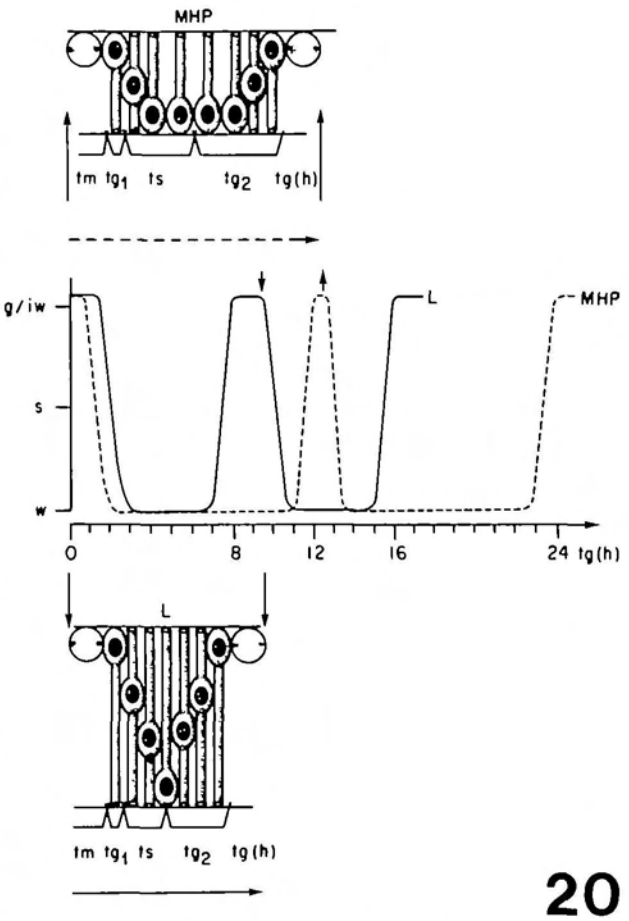


Fig. 19. Autoradiograph of a transverse paraffin section (A, low power; B, high power) through the future hindbrain level of a stage 8 chick embryo exposed to [^{3}H]thymidine for 5 min. The presence of silver grains overlying a nucleus indicates that the cell is in the DNA synthetic (S) phase of the cell cycle. Also, the position of neurepithelial cell nuclei is indicative of neurepithelial cell shape, such that wedge-shaped cells have basally located nuclei, spindle-shaped cells have centrally located nuclei and inverted wedge-shaped cells have apically located nuclei. A, bar=40 μm; B, bar=10 μm.

Fig. 20. Model illustrating how alteration of the cell cycle of neurepithelial cells might generate cell wedging within the hinge points; iw, inverted wedge-shaped cell; g, globular cell; s, spindle-shaped cell; w, wedge-shaped cell; MHP, median hinge point; L, lateral areas of neurepithelium between the MHP and DLHPs; tg, total generation time of cells; tm, tg_1, ts, tg_2, duration of M, G_1, S and G_2 phases of cell cycle, respectively. Curved lines indicate cell cycles.

which is predicted to be the phase of the non-S portion of the cell cycle that is prolonged in the MHP. This generates more wedge-shaped cells in the MHP compared to L because there is more time for cells to remain wedge-shaped. Although these studies do not demonstrate definitively that alteration of the cell cycle in the MHP causes cell wedging in this region, the proposed model provides a plausible and testable explanation of how this process could occur, warranting further study. Finally, although these studies suggest that wedging of MHP cells results from basal expansion owing to nuclear migration during the cell cycle, the mechanism(s) underlying interkinetic nuclear migration is currently unknown.

Smith and Schoenwolf (1989) demonstrated that midline cells in the chick neural plate fail to develop typical MHP characteristics (i.e. they fail to become wedge-shaped, decrease their heights, and line a furrow) in the absence of the notochord and that transplanted notochords are capable of inducing typical MHP characteristics in L cells. This strongly suggests that the notochord plays an inductive role in the formation of MHP characteristics. Based on these and other results (Smith and Schoenwolf, 1987, 1988), it is likely that the notochord, through inductive interactions, alters the cell cycle of neurepithelial cells in the MHP by lengthening the phases during which their nuclei reside at the base of the neural plate and that this, in turn, causes these cells to become wedge-shaped. In support of this, it has been reported recently that the mitotic density in L cells induced to form MHP characteristics by a transplanted notochord is less than that in the corresponding L cells on the side opposite the notochordal transplant, suggesting that the transplanted notochord is capable of altering the cell cycle through its inductive effect (van Straaten *et al.* 1988).

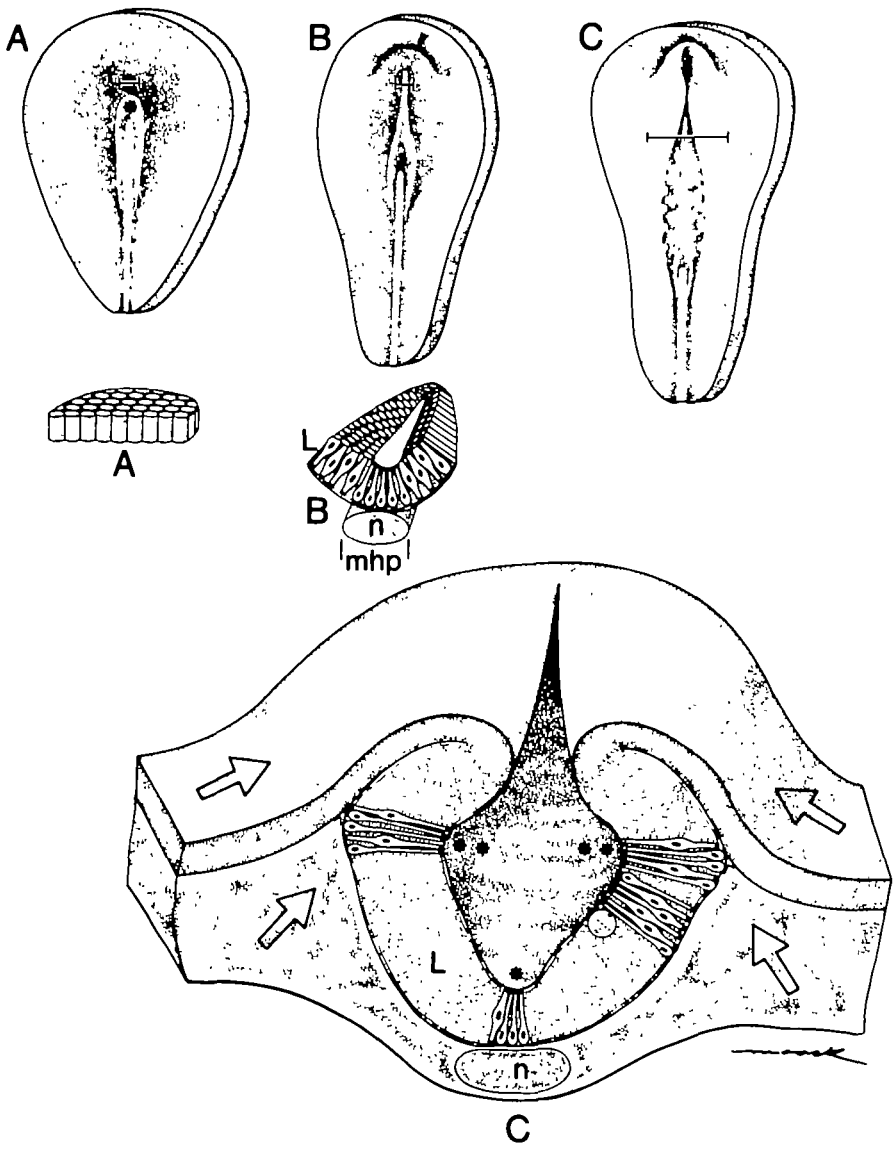


Fig. 21. Schematic diagram showing stages of primary neurulation in the chick. At stages 4 (A) and 6 (B), during which neural plate shaping is the predominant event, both whole mounts of entire blastoderms and cross-sectional views of neural plate discs are shown. The neural plate is indicated by heavy stippling, the surface ectoderm by light stippling and the head fold by an arrowhead. MHP cells derive from the disc-shaped area (shaded) rostral to Hensen's node (*) from which the cross-sectional view is taken (Schoenwolf *et al.* 1989). From this region they extend down the length of the midline where they are flanked by L cells. During neural plate shaping, the neural plate thickens apicobasally, narrows transversely and lengthens longitudinally (cf. stages 4 and 6). In addition, neural plate bending begins during its shaping. This process involves the formation of a MHP, during which the neural plate becomes anchored to the notochord (n) and midline neural plate cells decrease their heights, increase their cell cycle lengths and become wedge-shaped, resulting in neural plate furrowing (cf. stages 4 and 6). Evidence suggests that forces for these processes are generated intrinsic to the neural plate. Such forces include microtubule-mediated cell elongation, cell division and cell rearrangement. Between stages 6 and 8 (C), bending of the neural plate (shown in both a whole mount and cross-sectional view) is the predominant event. This process involves continued formation of the MHP, formation of paired DLHPs (during which the neural plate becomes anchored to surface ectoderm and dorsolateral neural plate cells increase their heights and become wedge-shaped, resulting in neural plate furrowing), neural fold elevation around the MHP (*) and neural fold convergence around the DLHPs (**). Neural groove closure occurs as a result of these events. Evidence suggests that forces for these processes are generated both intrinsic and extrinsic to the neural plate. Extrinsic forces (bold arrows) are provided by surrounding non-neurepithelial tissues. Candidates for such forces are the surface ectoderm, mesoderm (paraxial, intermediate and lateral plate) and gut endoderm as well as their associated ECM. Intrinsic forces include cell wedging, a process that is restricted to the MHP and DLHPs.

Conclusions

We have discussed the inadequacies of the traditional viewpoint of neurulation and have formulated a contemporary viewpoint to supersede the traditional one. The contemporary viewpoint takes into account that neurulation is a multifactorial process requiring the cooperation of both intrinsic and extrinsic forces. Within the neural plate, these forces are generated by common cell behaviors, such as cell division, cell rearrangement and changes in cell shape, which collectively result in coordinated morphogenetic movements. Similar cell behaviors likely generate forces outside the neural plate, but owing to the traditional focus on intrinsic forces, the behaviors that occur outside this structure have been largely ignored. Also ignored has been the problem of how intrinsic and extrinsic forces are coordinated so that a nervous system of proper size, shape and position develops and is integrated as part of a normal embryo. Finally, many questions remain regarding the molecular mechanisms underlying the characterized cell behaviors.

We conclude with a model that directs attention to forces likely acting in neural plate shaping and bending (Fig. 21). The model emphasizes the multifactorial nature of neurulation and reveals that much still needs to be learned about this process. Thus, many exciting challenges remain for future investigations.

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