Mechanism of anteroposterior axis specification in vertebrates

Lessons from the amphibians

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Summary

Interest in the problem of anteroposterior specification has quickened because of our near understanding of the mechanism in *Drosophila* and because of the homology of Antennapedia-like homeobox gene expression patterns in *Drosophila* and vertebrates. But vertebrates differ from *Drosophila* because of morphogenetic movements and interactions between tissue layers, both intimately associated with anteroposterior specification. The purpose of this article is to review classical findings and to enquire how far these have been confirmed, refuted or extended by modern work.

The "pre-molecular" work suggests that there are several steps to the process:

(i) Formation of anteroposterior pattern in mesoderm during gastrulation with posterior dominance.

(ii) Regional specific induction of ectoderm to form neural plate.

(iii) Reciprocal interactions from neural plate to mesoderm.

(iv) Interactions within neural plate with posterior dominance.

Unfortunately, almost all the observable markers are in the CNS rather than in the mesoderm where the initial specification is thought to occur. This has meant that the specification of the mesoderm has been assayed indirectly by transplantation methods such as the Einsteckung.

New molecular markers now supplement morphological ones but they are still mainly in the CNS and not the mesoderm. A particular interest attaches to the genes of the Antp-like HOX clusters since these may not only be markers but actual coding factors for anteroposterior levels.

We have a new understanding of mesoderm induction based on the discovery of activins and fibroblast growth factors (FGFs) as candidate inducing factors. These factors have later consequences for anteroposterior pattern with activin tending to induce anterior, and FGF posterior structures.

Recent work on neural induction has implicated cAMP and protein kinase C (PKC) as elements of the signal transduction pathway and has provided new evidence for the importance of tangential neural induction. The regional specificity of neural induction has been reinvestigated using molecular markers and provides conclusions rather similar to the classical work.

Defects in the axial pattern may be produced by retinoic acid but it remains unclear whether its effects are truly coordinate ones or are concentrated in certain regions of high sensitivity.

In general the molecular studies have supported and reinforced the "pre-molecular ones". Important questions still remain:

(i) How much pattern is there in the mesoderm (how many states?)

(ii) How is this pattern generated by the invaginating organizer?

(iii) Is there one-to-one transmission of codings to the neural plate?

(iv) What is the nature of the interactions within the neural plate?

(v) Are the HOX cluster genes really the anteroposterior codings?

Introduction

All multicellular animals have some sort of anteroposterior polarity at some stage in their life cycle. In the case of the fruit fly *Drosophila* we now understand, in outline, how different body regions are specified along the anteroposterior axis and we know that the end result of this process is the activation of the homeobox genes of the Antennapedia (ANT-C) and Bithorax complexes (BX-C) at different body levels (reviews: Akam, 1987; Ingham, 1988; Slack, 1991). Immense excitement has resulted from the discovery that there are homeobox (HOX) clusters in the mouse and other vertebrates which show a definite evolutionary homology to the ANT-C/BX-C in *Drosophila* such that individual genes have retained their relative positions in the cluster, and also that their position in the cluster is directly related to the spatial domain of expression in the anteroposterior axis of the body (Duboule and Dollé, 1989; Graham et al., 1989). Because of this evolutionary homology of HOX cluster gene expression, high hopes have been generated of solving the problem of the anteroposterior axis, perhaps the greatest problem of embryology, in a single giant leap from *Drosophila* to Man.

But vertebrates differ from insects in three important ways. Firstly, in most insects, including *Drosophila*, the early events of regional specification occur while the embryo is a multinucleate syncytium, whereas in vertebrates most regional specification occurs in a multicellular embryo. Secondly, at about the time that the main regions of the body plan are specified, the *Drosophila* embryo consists of a simple ellipsoidal monolayer of cells. In vertebrates, extensive morphogenetic movements take place before the stage at which the body plan has become specified, which is around the end of gastrulation. Thirdly, induction is critical from the earliest stages in setting up regional identities in vertebrate embryos. In *Drosophila*, interactions between tissue layers do seem to take place but only at a later stage.

In this article we shall review what experimental embryology has to say about the problem of anteroposterior specification and then enquire to what extent the

questions have been answered by the application of molecular techniques over the last few years. The embryological work has overwhelmingly concentrated on amphibian embryos and so we have focused on them, only including occasional comparisons with mammalian data where it is available.

Results from experimental embryology

Possible commitments for anteroposterior-posterior levels at pre-gastrula stages

It is well known that various experimental manipulations carried out between fertilization and the beginning of gastrulation have effects on the anteroposterior axis; for example, irradiation of fertilized eggs with ultraviolet light causes dose-dependent reductions of the anterior end (Malacinski et al., 1975; Scharf and Gerhart, 1983). Because of this, some authors have suggested or implied that at least some anteroposterior codings become specified before gastrulation (eg Cooke, 1989). But of course early treatments may have late effects that are indirect and involve several intervening causal steps, so what is the real evidence bearing on this question?

Fate map

We start with the fate map of the blastula. In amphibian embryos, fate mapping studies have all shown that the notochord and adjacent part of the somites arise in normal development from the dorsal marginal zone, a region known as the *organizer* (Vogt, 1929; Pasteels, 1942; Keller, 1976; Dale and Slack, 1987). Although the older fate maps based on vital staining showed prospective regions for individual somites on the late blastula, it is now known from studies with injectable lineage labels that there is too much cell mixing during gastrulation for such small territories to remain coherent and distinct from one another (eg Dale and Slack, 1987). Particularly among the dorsal midline cells forming the notochord, there is a high degree of active cellular intercalation which provides the driving force for the extreme anteroposterior extension of this region (Wilson et al., 1989). So there cannot be a detailed topographic projection from regions in the blastula to particular anteroposterior levels in the later axis, and this necessarily means that there cannot be a detailed mosaic of regions committed to particular anteroposterior levels at the blastula stage. In the mouse a similar situation exists insofar as the anterior part of the primitive streak tends to populate the notochord and somites along the entire length of the body (Tam and Beddington, 1987; Lawson and Pedersen, 1987), and there seems to be even more cell mixing than in the amphibians.

However, even if there is too much mixing for a *detailed* topographic projection from pregastrula to postgastrula anteroposterior levels, it remains possible that there is *some* projection; for example, separate cohorts of cells destined to become head (prechordal mesoderm), trunk (notochord) and tail (tailbud blas-
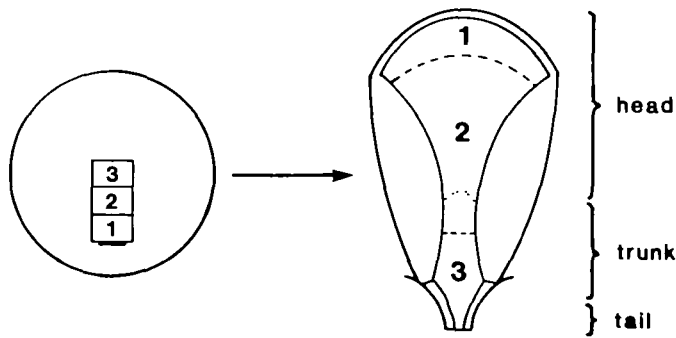
Fig. 1. Fate of the dorsal marginal zone in *Cynops*, after Okada and Hama (1945). An early gastrula is shown on the left and 1,2,3 represent three zones of tissue above the dorsal lip which were stained with vital dyes. On the right is shown the approximate projection of these zones onto the dorsal side of the neurula. The dotted line shows the anterior limit of the notochord.
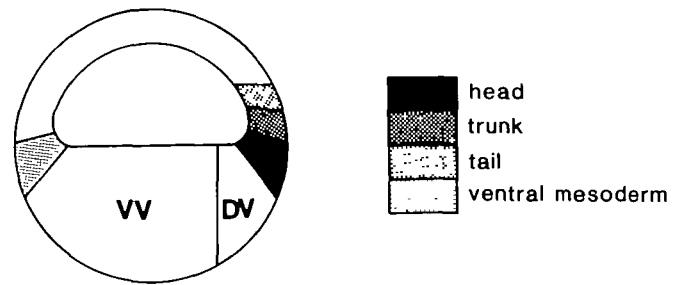

Fig. 2. Diagram showing how a potential specification of anteroposterior states would have to occur at the mesoderm induction stage. DV, dorsal mesoderm-inducing zone; VV, ventral mesoderm-inducing zone.
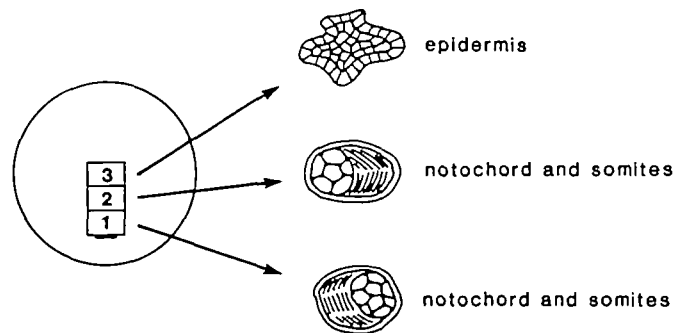

Fig. 3. Specification of dorsal marginal zone in *Cynops*, showing that it is not as expected from the model of Fig. 2. Explants were removed, wrapped in ectoderm and cultured in isolation until differentiation (see Hama et al 1985). Pieces 1 and 2 both produce mainly trunk-like structures (notochord and somites) while piece 3 produces mainly epidermis.

tema) might involute in sequence and thus be derived from territories arranged in a vegetal-to-animal sense in the blastula. The highest resolution studies addressing this question were carried out long ago with *Cynops* using vital stains (Okada and Hama, 1945). These also show the dramatic elongation of small dye marks over much of the axial length but the results do suggest that there is a projection of about "head-trunk-tail" resolution (Fig. 1). Whether this is also true for *Xenopus*, where the corresponding region is much smaller and lies internally, remains to be seen.

Mesoderm induction

Even if there is a limited or statistical fate map projection, this does not necessarily mean that there is any anteroposterior commitment at the early stage. We know that during the blastula stages, mesoderm-inducing factors (MIFs) are released from the vegetal cells and induce a belt of cells around the equator (the marginal zone) to a mesodermal character. This process is called *mesoderm induction* (reviewed Smith, 1989; Slack, 1991). Around most of the circumference the induced mesoderm is of ventral character but in a small region it is of dorsal character and this region is called the *organizer*. It has been proposed that the organizer-inducing signal consists of activin or something with similar properties to activin. If prospective regions for head, trunk and tail mesoderm had different states of specification in the blastula then this would presumably reflect the response to a vegetal-to-animal gradient of the activin-like factor such that head (closest the blastopore) was formed in response to high levels and tail (furthest from the blastopore) to low levels (Fig. 2).

Direct studies on the specification of the dorsal marginal zone do not show much difference in antero-posterior quality between regions normally forming head, trunk and tail. The best studies were done with *Cynops* (Hama et al., 1985; Fig. 3). When explants similar to the regions 1, 2, 3 of Fig. 1 are wrapped in ectoderm and cultured, pieces 1 and 2 form predominantly trunk structures, and piece 3 predominantly

epidermis. Piece 1 does not form heads as might be expected from the model of Fig. 2. In this series the implants and jackets were not labelled so we do not know what was formed by self-differentiation and what by induction. The difference between pieces 1 and 2 (mesodermalized) and 3 (still epidermal) is probably due to the fact that the response to the activin-like factor spreading up from the vegetal hemisphere has not yet reached its final frontier. This conclusion is supported by other studies in which it is shown that region 3 becomes mesodermalized as it approaches the lip (Kanéda and Hama, 1979; Kanéda, 1980, 1981). The general absence of head structures formed by any of the explants argues against a mechanism of the type shown in Fig. 2 and suggests that the formation of rudiments for different levels of the anteroposterior axis occurs after the blastula stage.

Formation of the anteroposterior mesodermal pattern during gastrulation

Methods

Unfortunately there are no histological markers of position along the anteroposterior axis of the mesoderm except for the difference between the prechordal region and the trunk. This means that it is necessary to make
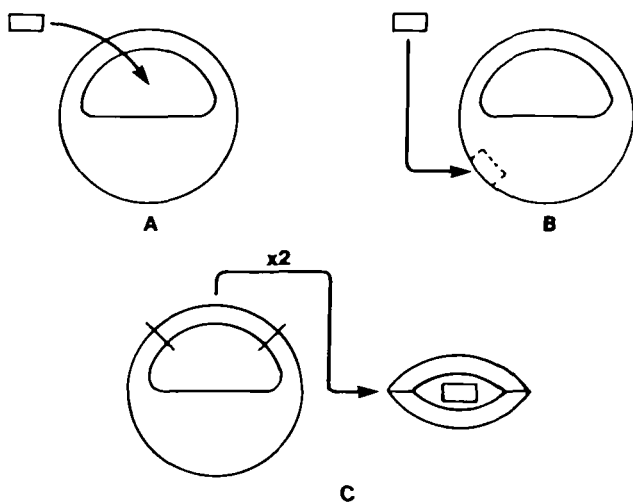
Fig. 4. Procedures for assaying anteroposterior character. (A) Einsteckung: The explant is grafted into the blastocoel of the host, is moved to the anteroventral region by the gastrulation movements, and may interact with host pharyngeal endoderm, ventral mesoderm and ventral ectoderm. (B) Surface graft: The explant is grafted to a particular position on the embryo surface, often the ventral marginal zone. (C) Sandwich: The explant is wrapped in early gastrula ectoderm.
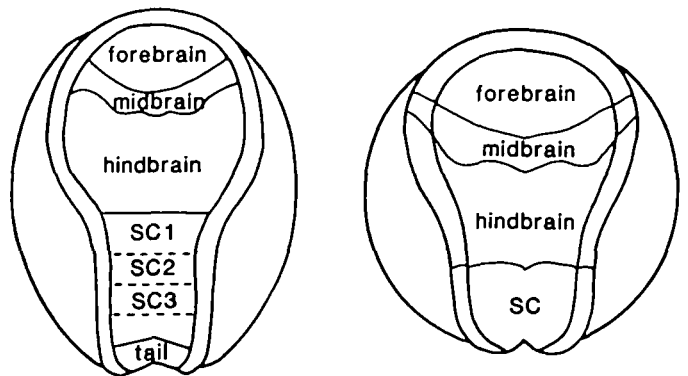

Fig. 5. (A) Fate map of the urodele open neural plate after Jacobson (1959). (B) Fate map for *Xenopus* open neural plate, after Eagleson and Harris (1990). SC, spinal cord.

use of structures induced by the mesoderm such as parts of the central nervous system. The validity of such results depends on an assumption of a one-to-one regional specificity of neural induction which we shall consider more carefully below.

The early (pre-Second World War) studies employed mainly the "Einsteckung" procedure introduced by O. Mangold, in which the test tissue is introduced into the blastocoel of an early gastrula through a hole in the ectoderm (Fig. 4A). The gastrulation movements carry the graft to a ventral position and bring it into contact with ventral ectoderm. Neural structures formed in this position are regarded as having been "induced" by the graft although in most cases there was no attempt to label the grafts and thus find the contribution of graft and host tissue to the structures. More seriously the graft may interact with the advancing pharyngeal endoderm, or with ventral mesoderm of the host gastrula. There may also be mechanical effects on the forming host axis leading to splitting, particularly at the anterior end. All these factors make Einsteckung experiments difficult to interpret. An improvement is to implant the graft in the surface of the host. This at least controls its position more accurately and hence the host regions with which it can interact (Fig. 4B). A better method still is to culture the test tissue in a jacket of gastrula ectoderm (Fig. 4C). This removes most of the uncertainties due to morphogenetic movements and to interactions with non-ectodermal parts of the host. It still requires the assumption of one-to-one neural induction, but could only be improved on by direct observation of anteroposterior character using molecular markers.

Neural induction

Because most of the observable anteroposterior pattern scored in experiments consists of neural or even placodal structures, it is essential to be aware of the potential for complexity in the results arising from neural induction itself. Fate maps of the neural plate are shown in Fig. 5. It should be noticed that the head is grossly overrepresented in relation to the trunk and tail and much of the posterior part of the body is formed post-neurulation by the expansion of the tailbud. In fact the most posterior part of the neural plate is not exclusively neural but contributes heavily to tail somites (Woodland and Jones, 1988).

Workers on neural induction have often scored their specimens into three classes: *archencephalic*, meaning forebrain-like and also containing derivatives of the cephalic placodes; *deuterencephalic*, or mid/hindbrain like; and *spinocaudal*, or containing simple neural tube, notochord, somites and tailfin. Spinocaudal inductions contain a lot of tissue conventionally classified as mesodermal. This has in the past led to confusion between the mesoderm induction which occurs in the blastula and a possible spinocaudal induction leading to the formation of the most posterior part of the neural plate. But the distinction between these two events should now be clear in both time, space and in the logic of developmental decision making. We shall return below to the question of whether they might be due to the same chemical inducing factor.

Neural induction has long been regarded as an *appositional induction*, that is a process involving communication between two separate tissue layers, although it has long been known that neural induction can also propagate within a sheet of competent ectoderm (homeogenetic induction: Mangold and Spemann, 1927). In normal development neural induction occurs in a sequential manner as gastrulation proceeds, the most anterior part of the invaginating tissue migrating under the entire prospective neural plate from posterior to anterior.

Regional specificity of neural induction

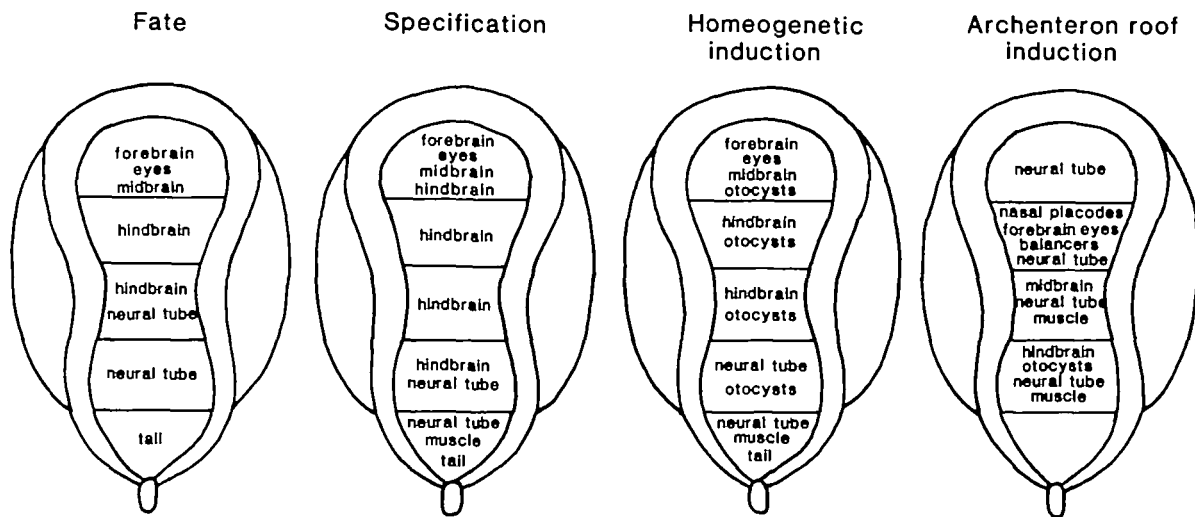The first studies indicating the regional specificity of

Fig. 6. Comparison of normal fate, specification (ie self- differentiation behaviour of explants), homeogenetic inducing capacity (ie what is induced by each level from gastrula ectoderm) and inductions by the archenteron roof, based on the data of ter Horst (1948). In this diagram structures occurring with a frequency of >40% are shown. "neural tube" represents the three original categories "neural", "neural tube" and "spinal cord".

neural induction date back to the 1930s, using *Triturus*. They employed either archenteron roof explants, presumed to be the normal inducer of the neural plate, or explants of the neural plate itself.

The best study is that of ter Horst (1948) who compared the specification of each level of the neural plate of *Triturus* embryos with its homeogenetic inductive capacity and also with the inductive capity of the archenteron roof from the same level (Fig. 6). She implanted the archenteron roof or neural plate pieces in jackets of ectoderm from early gastrulae and stained one member of the combination with Nile Blue. The vital staining meant that she could gain at least some idea which parts of the resulting structures were derived from the ectoderm and which from the implant. The neural plate pieces give homeogenetic inductions appropriate to the fate map; brain, eyes and nasal placodes from the most anterior fifth, hindbrain from the second and third fifth, and tails from the most posterior fifth. There is also considerable induction of otocysts by all levels. On the other hand, with the archenteron roof pieces the maximal induction of brains, eyes and otocysts was by the second piece, and maximum induction of hindbrain by the fourth piece (Fig. 6). The archenteron roof thus seemed rather more anterior in character than its own normal fate, or the normal fate of its overlying ectoderm, would suggest. As we shall see, the reason for this is probably that the induced tissue also receives signals through the plane of the ectoderm. Another study by Sala (1955) using the axolotl gives similar results, as does the original Einsteckung study of Mangold (1933).

The conclusion from these studies is that there are indeed regional differences in the inductive character of different anteroposterior levels of the archenteron roof. To some extent these correspond to normal fates, but

there tends to be a anterior shift in character relative to fate.

Reciprocal effects

Although the discussion of neural induction is normally predicated on the assumption that the signals pass in one direction, from the archenteron roof to the ectoderm, this may be an oversimplification since there are several papers describing reciprocal effects. Kato and Okada (1956) claimed that early dorsal lips from *Cynops* would only produce notochord and somites if combined with gastrula ectoderm which itself formed florid neural structures. Muchmore (1958) showed that coculture of prospective *Ambystoma* somite with neural plate could greatly boost the amount of muscle formed. Nieuwkoop and Weijer (1978) found that twice as much notochord was formed by posterior archenteron roof cultured with gastrula ectoderm in which florid neural induction occurred, rather than with neurula epidermis, in which it did not.

Specification of anteroposterior codings

The acquisition of distinct anteroposterior character appears to be progressively determined during gastrulation. A recent experiment on *Xenopus* which supports this idea involves the inhibition of gastrulation movements at different times by the injection of polysulphonated compounds such as suramin or trypan blue (Gerhart et al., 1989). These agents cause truncations from the anterior end of the embryo the extent of which is dependent on the time of injection. Injections at the onset of gastrulation result in a massive deletion of the axis all the way from the head to tail whereas injections at the mid-gastrula stage result in deletions of only the most anterior structures and injections into the late gastrula have little effect. These observations suggest
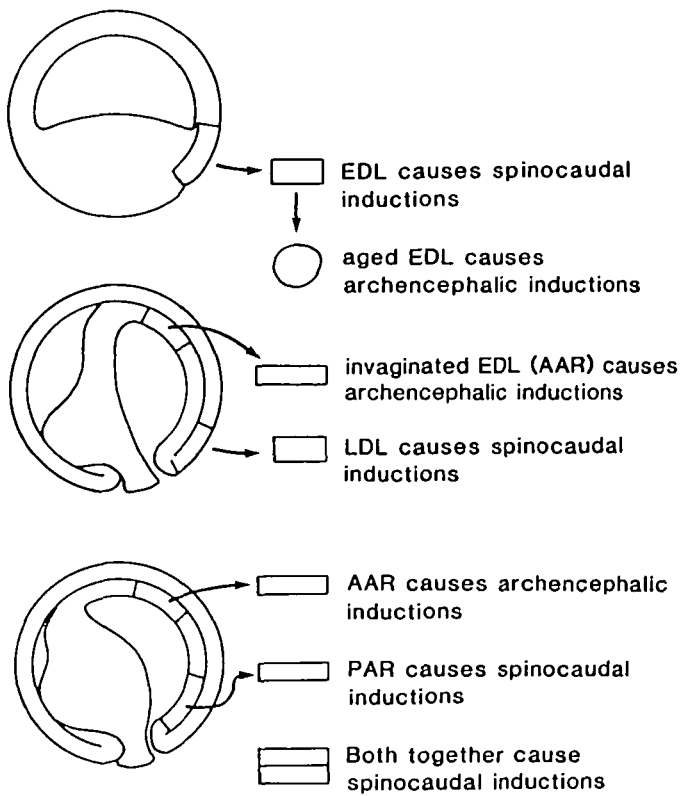
Fig. 7. Summary of the results of experiments by Okada and Takaya (1942a,b). EDL, early dorsal lip; LDL, late dorsal lip; AAR, anterior archenteron roof; PAR, posterior archenteron roof.

that axis determination happens progressively during gastrulation such that the length of time of gastrulation and/or the extent of axial elongation determines the ability to produce succesively more anterior structures.

Most of the earlier experiments on anteroposterior specification were carred out on *Cynops*. The crucial observation, made by Okada and Takaya (1942a,b) was that the inducing effect of the dorsal lip region changed on invagination (Fig. 7A,B). Early dorsal lip. tissue from the surface, grafted to the ventral side of another embryo, induced trunk/tails, while the same tissue if taken after invagination induced heads. The change in inducing capacity also occurred during *in vitro* culture of the tissue for 12-24 hours. Several of the original experiments were repeated with similar results by Suzuki et al. (1984). When the inductive capacity of explants of archenteron roof were examined (Fig. 7C), there was the same sort of regional specificity noted above, although these studies are of lower resolution than those of ter Horst or Sala. But significantly, when both anterior and posterior archenteron roof were used together as the inducer, the inductions were of trunk/tail character. The same result was obtained when recently invaginated material from early and late gastrulae were used in combination as an inducer. Although as cephalized creatures our natural inclination is to suppose that the *head* should be the dominant

region, or "high point" of the axis, these results suggest that it is actually the *posterior* which is dominant.

This surprising conclusion is also supported by experiments of Hall (1937) using *Triturus* in which early and late dorsal lips were interchanged. If a late lip was removed and replaced with an early lip, the resulting embryos were fairly normal, ie the early lip both formed and induced posterior structures. If a late lip was substituted for an early lip then the anterior end of the resulting embryos very much resembled an ectopic tail. In both series of experiments the juxtaposition of anterior with posterior leads to the posterior remaining posterior and to the anterior becoming posterior. In other words *posterior dominates over anterior*. The main problem with both series is that they did not use a cell label and so could not reliably distinguish graft from host tissue. Repeating these critical experiments with a lineage label must now be regarded as an important priority.

Posterior dominance within the neural plate

Posterior dominance in the neural plate is most clearly shown in a series of experiments using urodeles in which folds of gastrula ectoderm were implanted vertically into the open neural plate of a host embryo (Nieuwkoop, 1952a,b,c; Fig. 8). Neural induction proceeds up the folds; the structure induced at the base is the same as that formed by the surrounding neural plate, while those formed further up the fold are successively more anterior in character. This suggests that the anteroposterior series of structures formed by the neural plate are coded in the early neurula by some gradient-like set of states which can propagate in a decremental way through the ectoderm and does not require the immediate proximity of the archenteron roof. A rather similar set of data has recently been produced from a series in which prospective forebrain instead of gastrula ectoderm was used for the folds (Nieuwkoop and Albers, 1990).

These transplantations and fold experiments clearly show that posterior dominates over anterior in the neural plate as it does in the mesoderm. This might suggest that the regional specification of the neural plate could be achieved by a single posterior-to-anterior gradient, but Nieuwkoop and his colleagues have always favoured a two-step process consisting of an
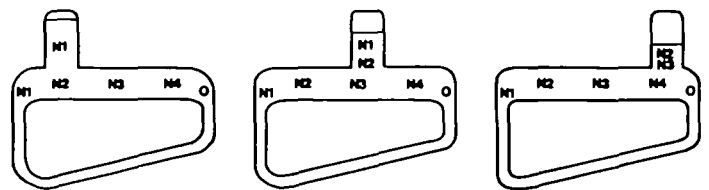

Fig. 8. Nieuwkoop's (1952a-c) "fold" experiments, in which a fold of gastrula ectoderm is implanted into the open neural plate. Anterior lies to the left and the numbers N1-N4 and O represent territories forming different neural structures (see also Fig. 9). The anterior inductions contain few structures but are large, while the posterior ones contain more structures but are smaller.

initial *activation* and a later *transformation*. Activation means a simple induction of neural tissue, and without further stimulus this will lead to forebrain. Transformation represents the further stimulus required to induce more posterior structures and, because of the number of such structures, it would have to represent a complex signal of a graded character, or some graded set of states with the high point or maximum number of active components present at the posterior end. The original reasons for postulating the existence of two separate steps were rather complex and depend on considerations which may no longer be valid today. But two of them still seem reasonable: firstly, the volume of induced tissue in the fold experiments decreases from anterior to posterior while the degree of posteriorization increases. This behaviour would not be expected from a single posterior-to-anterior gradient in which the size of inductions should correlate with the number of structures produced (see Slack, 1991, Chapter 3). Secondly, the spread of the neural induction into mediolaterally oriented ectodermal grafts which are in contact with the archenteron roof does not result in a posterior-anterior set of structures but simply a lateral extension of the appropriate AP level, as it does during normal development (Albers, 1987).

A comparable "two-signal" model for neural induction was been produced by Yamada (1950, 1990). Here the activating signal is called "dorsalization" (unfortunately this could be confused with dorsalization of the mesoderm by the organizer) and the transforming signal is called "caudalizing". Saxen and Toivonen also produced a two-signal model which was based on the effects of heterologous inducers on gastrula ectoderm (Toivonen and Saxen, 1955; Saxen and Toivonen, 1961).

How many territories?

We have seen that there is good evidence for at least some regional specificity in the archenteron roof. The results of Nieuwkoop's fold experiments and ter Horst's jacket experiments also show the potential for substantial regional specification by interactions within the neural plate. (This is really the "tangential induction" much stressed recently and discussed further below). So what is the balance of these processes in normal development? It might be that the archenteron roof contains as few as two specificities, representing roughly the activating and transforming principles of Nieuwkoop. The activating principle might be all over and the transforming principle just in the extreme posterior. The appositional induction would then induce the neural plate as a whole and also initiate a posteriorizing centre within the neural plate from which would propagate a graded signal (or set of states) towards the anterior. All the resulting pattern within the neural plate would depend on this signal. At the other extreme it might be that there is an appositional induction of all the territories in the neural plate one-to-one by corresponding territories in the archenteron roof. The tangential interactions within the neural plate would not then be a part of normal development, but

only demonstrable in experimental circumstances in which the two tissue layers had been separated. We do not know which of these models is nearer the truth and indeed the most likely possibility is that it is some mixture of the two: a certain complexity of pattern in the archenteron roof would be communicated by appositional induction and then this would become elaborated to a more complex pattern by interactions within the neural plate.

Preliminary conclusions

The results reviewed thus far, although some are quite recent in time, represent a tradition of work stemming from the late 1920s and can fairly be described as "premolecular". We shall now briefly summarise these results as a model and below we shall ask how well it can stand up to recent molecular data.

The essential features of the model are:

(i) Anteroposterior specification occurs during gastrulation, not before.

(ii) Anteroposterior codings are both transmitted from the mesoderm to the neural plate and within the plane of the neural plate.

(iii) Both in the mesoderm and in the neural plate, posterior dominates over anterior.

This model is illustrated in Fig. 9. The organizer is formed at the blastula stage in response to a "DV" signal from the dorsovegetal blastomeres. It initially has a posterior specification but the first cohorts of cells to invaginate (or involute, in the *Xenopus* deep marginal zone), acquire an extreme anterior specification, and progressively later cohorts acquire progressively less anterior codings. We do not know whether this change occurs in response to an environmental change, such as exposure to the blastocoelic fluid, or occurs autonomously with time. We also do not know to what extent interactions within the forming archenteron roof are necessary to establish the territories in the correct sequence or how many such territories there are. As the endo-mesodermal axis is formed, the surrounding ectoderm begins to become regionalized in response to signals emitted from it. The neural plate is formed as two or more territories appropriate to the AP codings of the underlying mesoderm. Further pattern complexity arises through a gradient-like signal emitted from the posterior and transmitted in the plane of the neural plate. The neural crest is probably also formed as part of this process. The epidermis becomes subdivided into head and trunk level, the head level embodying a competence to form the various sensory placodes of the head (otic, nasal, lens, lateral line) in response to later induction from appropriate parts of the neuraxis.

The molecular era

Molecular markers
Non-homeobox markers

Along the axial mesoderm there are no qualitative histological differences except for the prechordal/notochord boundary. In the experiments described in the
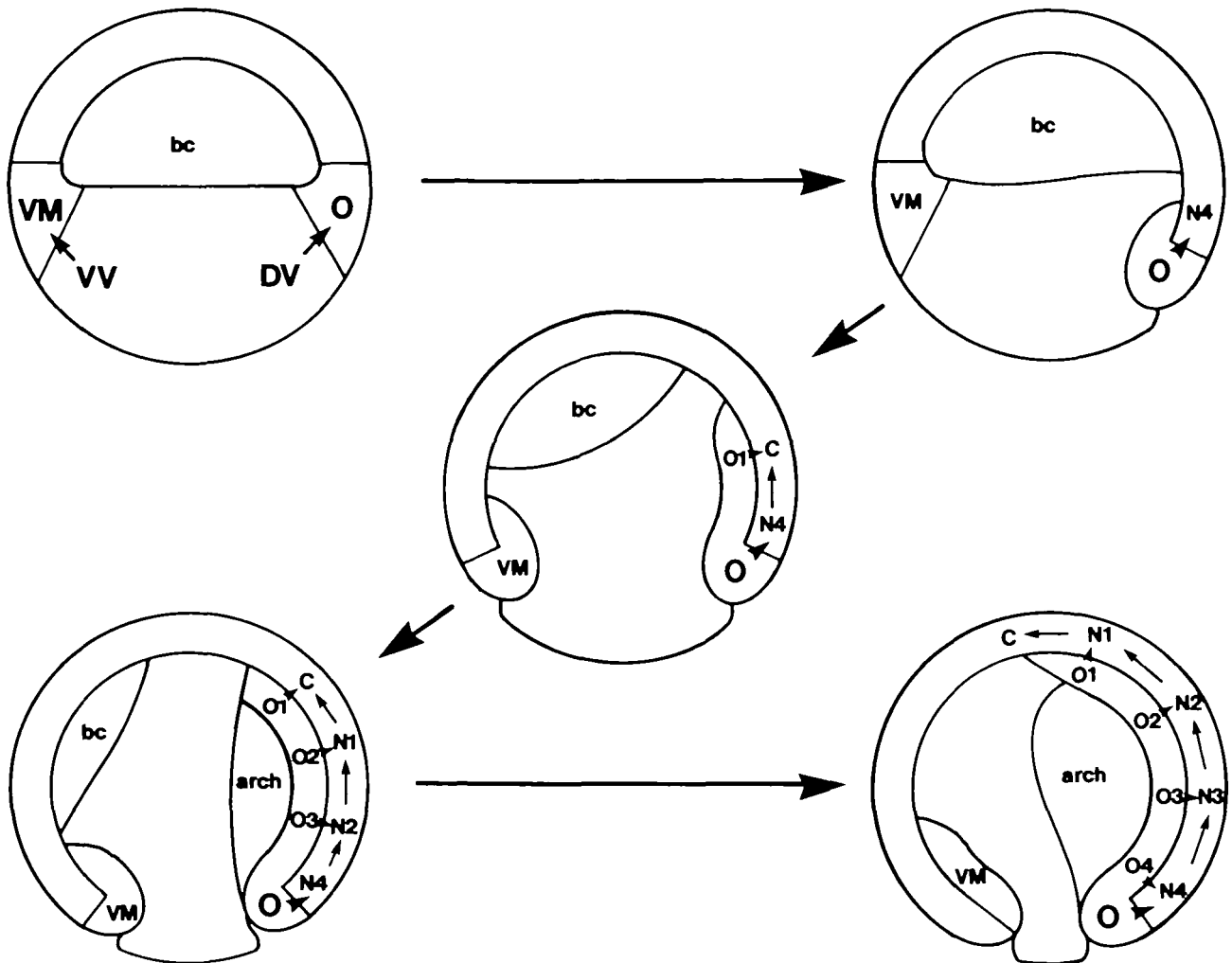
Fig. 9. Summary of mechanism of anteroposterior specification based on "pre-molecular" work. Arrows represent inductions. DV, dorsovegetal territory; VV, ventrovegetal; O, organizer; VM, ventral mesoderm; C, cement gland territory; O1-O4, territories in dorsal mesoderm; N1- N4, territories in neural plate; bc, blastocoel; arch, archenteron.

previous section, the assessment of anteroposterior coding was necessarily based on markers in ectodermal structures. The forebrain, midbrain, hindbrain and spinal cord are distinguished on the basis of thickness of different parts of the tube and the disposition of the fibre tracts (Fig. 10). Other commonly used markers are the eye, the otocyst, cement gland (anura) and balancers (urodeles). This method of scoring the results presents several problems. Firstly, since the visible markers are neural or epidermal they depend on inductive processes after the initial anteroposterior specification in the mesoderm. Secondly, the differentiation markers appear quite late after the events of anteroposterior specification themselves; in the case of urodeles several days of culture may be required. Thirdly, the more subtle distinctions, for example between different parts of the brain, take some practice to make reliably, especially if the resulting tissue is disorganized.

For all these reasons, molecular markers for different anteroposterior levels are highly desirable. In principle

they may be direct, expressed early, and objective. They can be made quantitative, in Northern blots or probe protection assays, or combined with conventional histology by *in situ* hybridization. Unfortunately it is still the case that the recently introduced molecular markers are predominantly neural or epidermal, so we still cannot observe directly the anteroposterior coding of mesoderm which is probably the leading tissue layer in formation of the anteroposterior pattern.

The disposition of several molecular markers is shown in Fig. 11. Three pan-neural markers have been described: N-CAM (Kintner and Melton, 1987) is expressed from gastrulation onwards. XIF-6, a homologue of mammalian neurofilament-M, (Sharpe, 1988; Sharpe and Gurdon, 1990) is expressed from the time of neural tube closure. A glycoprotein identified by a monoclonal antibody 2G9, is expressed from about the same stage (Jones and Woodland, 1989). XIF3 (Sharpe et al., 1989) is an intermediate filament gene homologous to mammalian peripherin. It is expressed at a low level from the onset of gastrulation in the whole animal
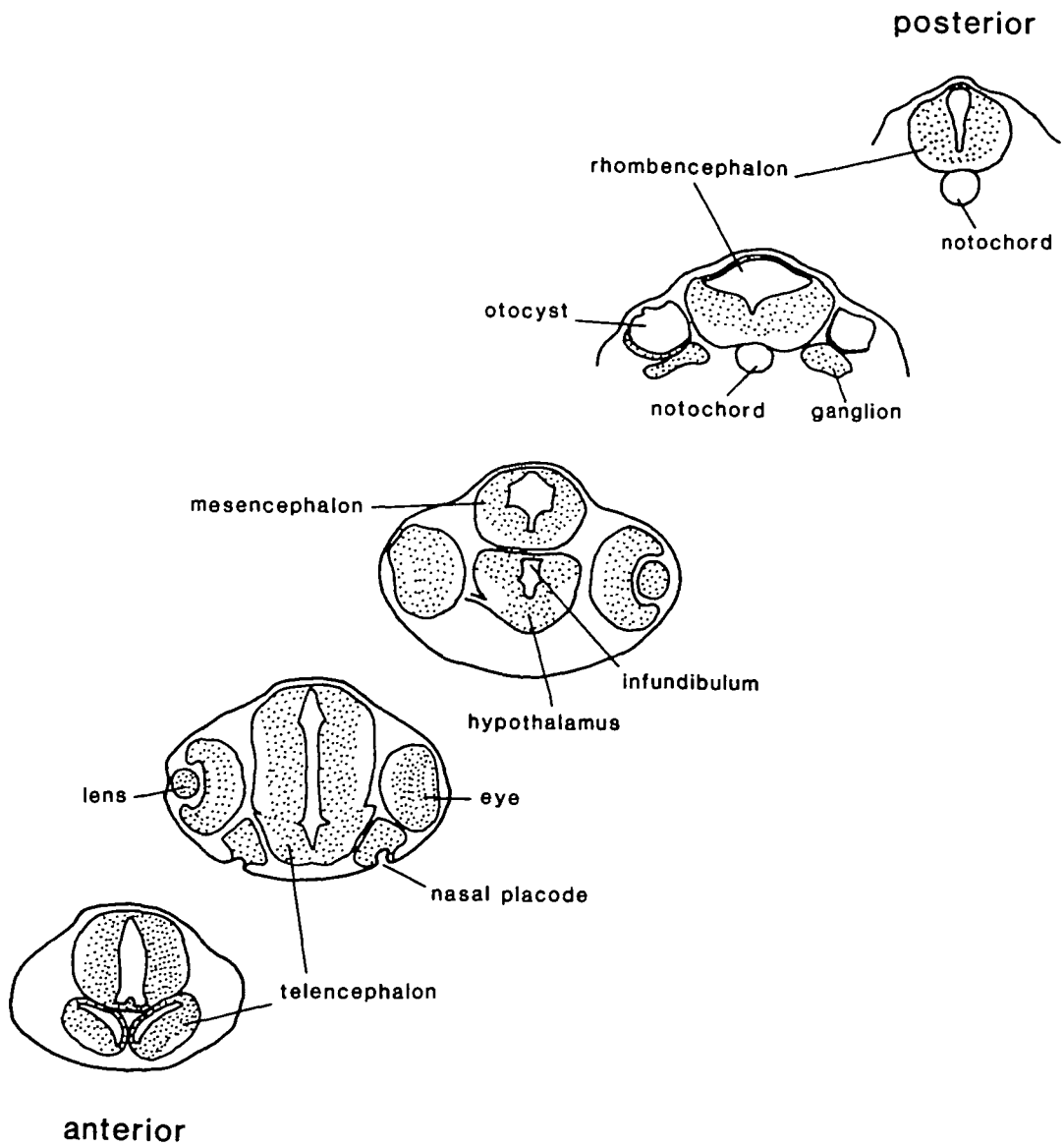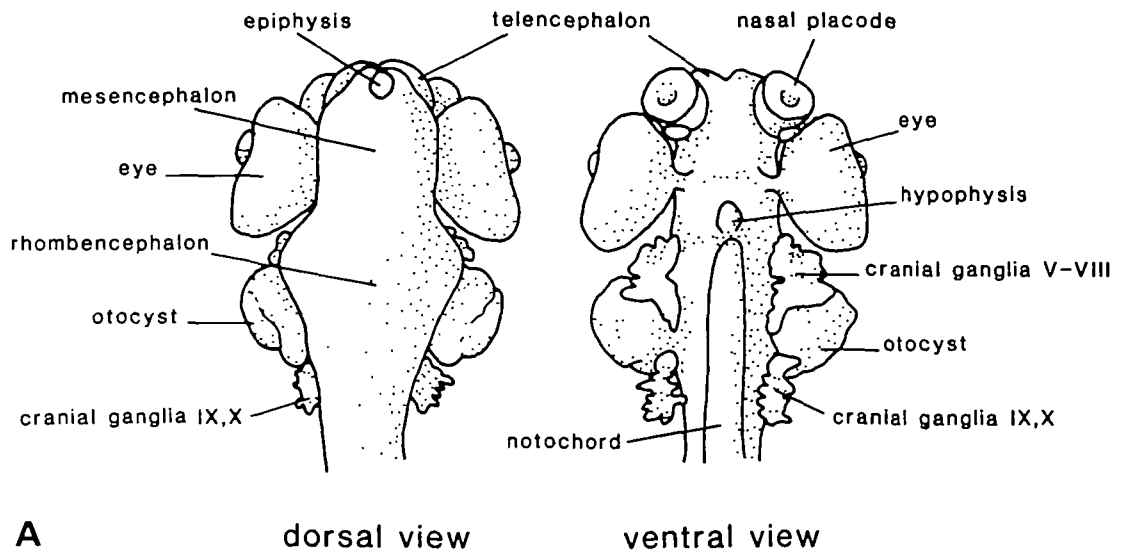
Fig. 10. Structures in urodele CNS commonly used to score specimens with respect to anteroposterior level. (A) Whole brains. (B) Transverse sections. Adapted from von Woellwarth (1952).

XCG-1, XAG-1⊂⊐

N-CAM, XIF-6, NF-3, 2G9

XIF-3 ⊏

Xhox-3(late) ⊏

En-2 ⊏⊐

XlHbox-1 ⊏⊐
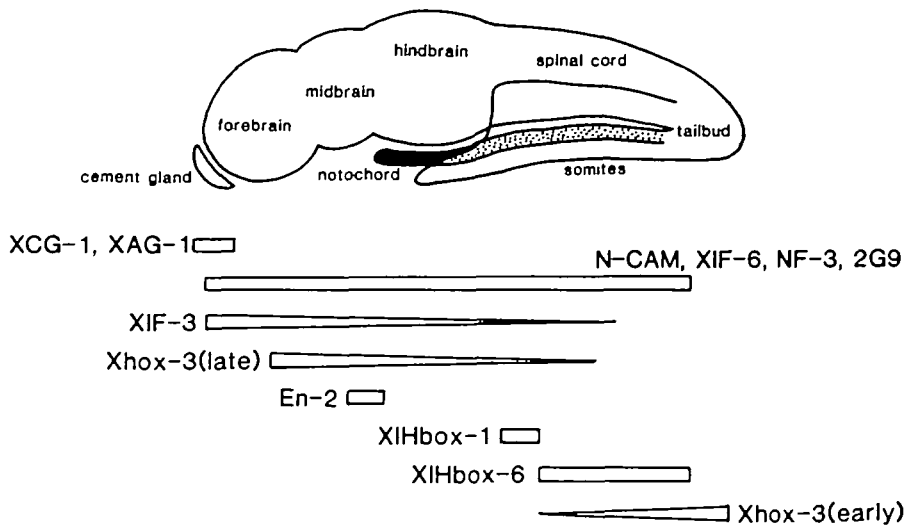
XlHbox-6 ⊏

⊐ Xhox-3(early)

Fig. 11. Approximate levels of expression of molecular markers used to study anteroposterior specification in *Xenopus*. The proportions of the axial levels shown are about what they would be at the time of neural tube closure although the markers are not all necessarily expressed at this stage.

hemisphere and becomes enriched in various neural structures: the cranial ganglia, the cranial neural crest, the hindbrain and motor nuclei along the neural tube. Quantitatively this expression pattern means that it predominates in the anterior. Other neural-specific antibodies have been described by Itoh and Kubota (1989). Numerous pan-epidermal markers have been described (eg Slack, 1985; Akers et al., 1986; London et al., 1988) and a number of markers specifically for the cement gland (Jamrich and Sato, 1989; Sive et al., 1989).

Homeobox expression patterns

In *Drosophila* the activation of the homeobox genes of the Antennapedia and Bithorax complexes is the last step in a long and complex process of anteroposterior specification which starts with the maternal factors *bicoid* and *nanos* and works through the zygotic gap and pair-rule genes (reviewed Slack, 1991). Other insects, such as *Tribolium*, have a single HOM gene cluster containing genes homologous to both the ANT-C and BX-C (Beeman et al., 1989). It is thought that this represents the primordial condition with the complex having become split in *Drosophila*. As is well known, the order of the genes on the chromosome is the same as the order of expression in the embryo, with the genes centromere-proximal in the cluster being expressed in the anterior of the germ band and the genes centromere-distal in the cluster being expressed posterior.

We now know that vertebrates possess clusters of homeobox genes (HOX clusters) showing a distinct homology to the ANT-C/BX-C of *Drosophila* (or HOM complex of other insects) and that for each cluster there is also the correlation between gene position and anteroposterior level of expression such that the anterior limit of expression in the neural tube correlates with the order of the genes from the 3' to the 5' end of the cluster (Duboule and Dollé, 1989; Graham et al., 1989). The different HOX clusters in vertebrates appear to have arisen from the primordial HOM cluster by gene duplication, and the corresponding members of different clusters have been called *paralogs*. There are

also a number of genes outside the HOX clusters which contain recognisable homeoboxes with a lower degree of amino acid identity and presumably diverse functions. A recent review (Shashikant et al., 1991) provides extensive discussion on the molecular biology and expression patterns of the homeobox genes, particularly in the mouse, and this material will not be repeated here.

A number of homeobox genes have been cloned from *Xenopus* over the last few years and those published to date are collected in Table 1, which also lists their relationship to the mouse genes. In general the 3'-anterior: 5'-posterior correlation seems to hold up for *Xenopus*, although the expression data is somewhat incomplete and some genes are known to have have different expression patterns at different developmental stages.

Are homeobox genes coding factors?

From the discovery of the first homeobox gene outside *Drosophila* (Carrasco et al., 1984), there has been a strong presumption that the HOX clusters represent a kind of biological Rosetta stone that would enable us to read the epigenetic code of the vertebrates (Slack, 1984). Evidence for this view has steadily accumulated (De Robertis et al., 1989, 1991). The expression patterns (Shashikant et al., 1991) are, to a first approximation, what would be expected if genes were turned on at different concentrations of a posterior-to-anterior morphogen gradient. So each gene has a sharp anterior expression limit and its domain of expression extends from there to the posterior end. Expression of the same gene is frequently found in both mesoderm and neural tube suggesting an association with anteroposterior level rather than tissue type.

More direct evidence comes from experiments in which homeobox gene expression has been caused to occur ectopically or has been reduced below normal. Ectopic expression in *Xenopus* is achieved by injection of a synthetic mRNA prepared from a vector bearing a phage RNA polymerase promoter and, usually, various features to optimise stability and translational efficiency

Table 1.

Name	Synonyms	putative mouse homologue	Normal expression pattern	References
HOX cluster genes (listed approximately in anteroposterior sequence of expression domains)				
Xhox.lab		1.6	Up late gastrula in ectoderm and mesoderm.	Sive and Cheng, 1991
XhoxlA		2.6	Up from early gastrula. Trunk-tail axis, mainly somites	Harvey et al., 1986
XlHbox 4	Xhox 1B Xhox 2.1	2.1	Up from late neurula, peak in tailbud stage. Stripe in hindbrain.	Harvey et al., 1986 Fritz and De Robertis, 1988 Jegahan and De Robertis, 1990
XlHbox 5		3.4	Up late neurula, down tailbud stage.	Fritz and De Robertis, 1988
XlHbox 1	AC1, Xeb1	3.3	Up late gastrula, spinal cord, and post. mesoderm. In tadpole, cervical spinal cord and adjacent mesoderm. Two proteins.	Carrasco and Malacinski, 1987 Oliver et al., 1988
XlHbox 2	MM3	2.3	Ovary. Up early gastrula.	Müller et al., 1984 Fritz and De Robertis, 1988
XlHbox 7		2.4	Up neurula, band in spinal cord.	Fritz and De Robertis, 1988
XlHbox 6		2.5	Up early neurula, post. neural plate.	Sharpe et al., 1987 Wright et al., 1990
XlHbox 3	Xhox 36		Up gastrula, peaks late neurula, post. ecto and mesoderm.	Condie and Harland, 1987 Fritz and De Robertis, 1988
Other homeogenes (listed approximately in temporal order of expression)				
Mix			Up late blastula in endo and mesoderm.	Rosa (1989)
Goosecoid			Up late blastula, in dorsal lip.	Blumberg et al., 1991
Xhox 3		Evx	Up early gastrula, posterior mesoderm. In tailbud, ant. neural crest and ventrolat. cells from midbrain-ant.sp.cord.	Ruiz i Altaba and Melton, 1989a Ruiz i Altaba, 1990
Xhox 7.1		7.1	Up gastrula in dorsal mesoderm of whole AP axis, later in neural tube, neural crest, lateral plate.	Su et al., 1991
En 2		En 2	Up early neurula, stripe at mid-hind brain junction.	Hemmati Brivanlou and Harland, 1989 Hemmati Brivanlou et al., 1991
XlHbox 8			Up tailbud stage as band in endoderm level of somite 3-5.	Wright et al., 1988

(Moon and Christian, 1989). Overexpression of Xhox 1A, normally expressed in the somites, causes a disruption of somite segmentation (Harvey and Melton, 1988). Overexpression of Xhox-3, normally expressed early on in the posterior, causes a loss of anterior parts (Ruiz i Altaba and Melton, 1989c). Overexpression of XlHbox 1, initially expressed in the posterior and later in the cervical region of the spinal cord, also disrupts segmentation and CNS pattern (Wright et al., 1989). There are also some relevant data on this question from the mouse. Overexpression of Hox 1.1, normally expressed in the thoracic region spinal cord and somites, causes malformations in the hindbrain region (Kessel et al., 1990). This dominant posteriorization would be expected if the region modified normally differed only in that it lacked Hox 1.1 expression. Several groups are currently attempting to ablate the activity of HOX genes by homologous recombination of inactive gene copies into ES cells followed by the creation of germ line chimeras, and hence a strain of heterozygous mice from which the homozygote embryos may be produced by mating. An early example concerns Hox 1.5 (Chisaka and Capecchi, 1991) which is normally expressed in the neural tube anterior to the otocysts and in the prevertebral somites. The homozygous nulls have defects in the branchial arch region. A cheaper but less direct way of altering HOX gene expression in mouse embryos is to administer retinoic acid to the mothers at 7-8 days of incubation. This causes ectopic (anterior) expression of some HOX genes and repression of others and also leads to a variety of vertebral transformations (Kessel and Gruss, 1991). Of course in this case it cannot be guaranteed that the retinoic acid is actually acting via the HOX genes.

A more sophisticated variant of the overexpression experiment is to overexpress in one embryo and use this as the donor of a graft into a normal embryo. Cho et al. (1991) have overexpressed XlHbox-6 (normally posterior) in blastulae and grafted animal caps into the blastocoel of normal hosts by the Einsteckung procedure (Fig. 4A). This produces ectopic tails in which most of the mesoderm is graft derived and most of the neural structure and fin is host derived. Although there are many objections to the Einsteckung procedure which have been listed above, it is quite clear that in this experiment the XlHbox-6 mRNA has a dramatic biological activity, and there is at least a *prima facie* case that, directly or indirectly, it confers posterior character on the graft.

Ablation of a *Xenopus* homeobox gene has been attempted by Wright et al. (Wright et al., 1989) using an antibody to XlHbox 1. This does appear to transform the cervical cord into a more hindbrain-like structure, the predicted result of the ablation of a gene whose function was to distinguish the hindbrain from the anterior cord. Here, however, the uncertainty is whether the antibody actually inhibits the biochemical

activity of the gene product, since there is no independent assay for this.

At present, the evidence from *Xenopus* and from the mouse that HOX cluster genes really code for anteroposterior levels is suggestive and encouraging, although the case remains not fully proven.

Reinvestigation of problems using molecular methods

Mesodermal induction and axial patterning

The problem of the anteroposterior axis intersects with that of mesoderm induction because in amphibian embryos the gastrulation movements cause the anteroposterior and dorsoventral axes to be intimately associated with each other, and not independent as they seem to be in *Drosophila*.

The normal fate map of *Xenopus* shows that the entire dorsal midline arises from the dorsal marginal zone. However, quantitatively there is a preferential contribution of cells from the dorsal marginal zone to the anterior of the later embryo and from the ventral marginal zone to the posterior. In fact the dorsal lip region forms both dorsal and ventral sides of the anterior. Tissue from the ventral marginal zone does not extend more than half way up the body from the blastopore in the ventral midline but makes a substantial contribution to the somites of the posterior trunk and tail (eg Dale and Slack, 1987). Because of this normal projection of tissues during gastrulation, treatments that alter the proportions of the marginal zone circumference devoted to dorsal and ventral type mesoderm have consequential effects on the anteroposterior axis. So eggs treated by irradiation of the vegetal hemisphere with ultraviolet light, which reduces the proportion of organizer tissue, later come to develop with anterior deficiencies (Malacinski et al., 1975; Scharf and Gerhart, 1983). Conversely, embryos treated with lithium at cleavage stages, which increases the proportion or organizer tissue, later come to have posterior defects (Kao et al., 1986; Kao and Elinson, 1988). The morphological findings have been reinforced by study of molecular markers: thus UV embryos express elevated levels of posterior markers such as Xhox 3 (Ruiz i Altaba and Melton, 1989a) whereas lithium embryos express elevated levels of anterior markers such as En-2 and XIF 3 (Hemmati Brivanlou and Harland, 1989; Sharpe et al., 1989).

The use of molecular markers that are normally expressed in restricted domains along the anteroposterior axis has suggested that the cytokines provisionally identified as mediating mesoderm induction can infuence patterning of the anteroposterior axis. Animal caps treated with activin tend to behave in an anterior way at later stages while those treated with FGF tend to behave in a posterior way. For example, animal caps treated with FGF display increased expression of two posterior markers, Xhox 3 and XlHbox 6 (Cho and De Robertis, 1990; Ruiz i Altaba and Melton, 1989b), whereas activin-treated caps show increased expression of a more anterior marker, XlHbox 1 (Cho and De Robertis, 1990). The most dramatic demonstration of anterior and posterior properties conferred by cytokine treatment involves Einsteckung experiments with treated animal caps (Ruiz i Altaba and Melton, 1989b). In these experiments activin-treated animal caps contribute to and induce secondary heads while FGF-treated caps contribute to and induce tails.

If we accept, as shown above, that there is not a specification of territories for particular anteroposterior values in the blastula, then how can these results be explained? Perhaps the way to think of the treated animal cap is as a kind of "engine" for making body parts. The cap is not homogeneous and we know that only the exposed, formerly blastocoelic, layer of cells initially responds to the cytokines (Darlington, 1989). After the cap rolls up the induced cells are in a clump at one end of the ball with uninduced cells elsewhere. Activin-induced caps undergo a much more pronounced and earlier elongation than FGF caps, so it may perhaps be the secondary interactions occuring in differently shaped domains which bring about the anteroposterior differences. As far as Einsteckung experiments are concerned, there is plenty of scope for further interactions to occur between the implanted cap and the ventral mesoderm, the pharyngeal endoderm and the ventral epidermis.

Neural induction

The textbook picture of neural induction is of an appositional induction between the invaginating prospective archenteron roof and the overlying ectoderm, and it is tacitly assumed in anteroposterior studies that there is a one-to-one regional specificity of the induction, each structure in the neural plate being induced by a corresponding territory in the mesoderm. Three issues that have received particular attention recently are the nature of the neural inducing signals, the existence of a neural "bias" in the dorsal part of the animal hemisphere, and the importance of "tangential" as opposed to appositional neural induction.

Neural inducing factors

Although it is widely known that urodele gastrula ectoderm can be provoked to forming neuroepithelium by a variety of stimuli, this is not the case for *Xenopus*. Neural structures are often induced by the activin group of inducing factors but this is always in association with axial mesoderm and is assumed to be secondary, resulting from neural induction within the explant. So there are still no candidates for the extracellular mediators of neural induction. It is however thought that the process is mediated within the responding cells by an activation of protein kinase C (PKC) together with an elevation of cyclic AMP. A limited degree of neural induction in dorsal gastrula ectoderm can be provoked by phorbol esters (Davids et al., 1987) and this is correlated with an activation of PKC (Otte et al., 1988). A more effective response can be obtained by a combination of phorbol ester with cAMP (Otte et al., 1989) suggesting that neural induction may involve the activation of more than one second message pathway.

The cement gland is a structure derived from the ectoderm just anterior to the neural plate. It appears to

be induced at the same time as the neural plate since the first molecular markers appear at stage 12 (Jamrich and Sato, 1989; Sive et al., 1989). It has been known for some time that the cement gland, but not neuroepithelium, can be induced in ectoderm explants by treatment with ammonium chloride (NH_4Cl; Picard, 1975). Presumably this works by raising the intracellular pH, an event which, like activation of PKC or elevation of cAMP, is a familiar second message response in cell biology.

Dorsal bias

In the past it has often been assumed, without very good evidence, that the competence of the animal hemisphere of the blastula/gastrula is uniform. We now know that at least in *Xenopus* there is some difference between dorsal and ventral regions of the animal hemisphere. This is shown by greater induction of N-CAM and XlHbox-6 in dorsal compared with ventral ectoderm of gastrulae using the same dorsal mesoderm as inducer (Sharpe et al., 1987). Conversely the epidermal marker Epi 1 is expressed better in isolated ventral compared with dorsal ectoderm (London et al., 1988; Savage and Phillips, 1989). Similar results have also been obtained for mesoderm induction: activin-treated dorsal ectoderm from blastulae forms dorso-anterior structures whereas similarly treated ventral ectoderm does not (Ruiz i Altaba and Jessell, 1991; Sokol and Melton, 1991). The molecular basis for these differences in competence are not yet known, although it has been shown that different isozymes of PKC predominate in the two regions (Otte et al., 1991).

These results do show that the dorsal ectoderm has some predisposition to form axial mesoderm or neural plate but they do not show that there is any anteroposterior specificity within the sensitive region. For the reasons discussed above we would not think this likely. It must also be remembered that the pre-disposition of ventral ectoderm in favour of epidermal differentiation can be over-ridden in certain experimental manipulations, and this tissue can be caused to form either axial mesoderm or neural plate.

Tangential induction

An issue that has been raised in a rather sharp way by the use of molecular markers is the possible contribution of "tangential" neural induction to the formation of the neural plate. Insofar as this involves a spread of the induced state through the ectoderm this concept resembles the older one of "homeogenetic induction", now known to occur in *Xenopus* (Grunz, 1990; Itoh and Kubota, 1991). But "tangential induction" also implies that the initial signal from the mesoderm is passed in the plane of the tissue before gastrulation rather than between the archenteron roof and the ectoderm during gastrulation. The classical experiments with urodeles suggested that this process was not of significant importance in normal development. For example the explantation studies on *Cynops*, shown in Fig. 7, do not show neural specification occurring in dorsal ectoderm before it has been underlaid by archenteron roof. The

original exogastrula experiments of Holtfreter likewise showed that the ectodermal part of the exogastrula was entirely epidermal rather than neural (Holtfreter, 1933). However, explants from the dorsal animal region of *Xenopus* blastulae can form neural tissue in the absence of mesoderm (JS unpublished) and also the expression of the epidermal marker Epi 1 is partially suppressed in such explants (London et al., 1988). This suggests that the situation in *Xenopus* may be different.

The proposal that neural induction can arise through the influence of tangential signals that spread through the plane of the ectoderm results from several types of study. In *Xenopus* exogastrulae there does seem to be some neural development in the ectodermal sac. It has been shown that the pan-neural markers N-CAM and NF-3, and the anterior neuronal marker Xhox 3 (not to be confused with its incarnation as a posterior mesodermal marker at earlier stages!) are expressed (Kintner and Melton, 1987; Dixon and Kintner, 1989; Ruiz i Altaba, 1990). Another way of looking at the putative tangential signal is to dissect explants consisting of early gastrula ectoderm with dorsal marginal zone attached, culture them with blastocoelic surfaces apposed to stop them rolling into balls ("Keller sandwiches": Keller et al., 1985) and then assay for neural markers. Experiments of this type have shown that N-CAM and NF-3 are expressed, suggesting that the involution of dorsal mesoderm is not required for neural induction (Dixon and Kintner, 1989). These results suggest that we should take seriously the idea that neural induction does proceed to some extent tangentially in *Xenopus* and probably that neural patterning depends both on signals from the archenteron roof and on those in the plane of the neural plate.

Tangential induction presumably occurs in response to a signal from the newly induced axial mesoderm. It is unlikely to be the same as the mesoderm-inducing signal from the vegetal cells because we would then have to suppose that neuralization represented the response to a weak (just above threshold) dorsal-mesoderm-inducing signal, and this is at variance with all the *in vitro* studies using cytokines.

Regional specificity of neural induction

We have seen above that there is good evidence for a degree of regional specificity in neural induction. This conclusion has been generally supported by studies using molecular markers. For example Hemmati Brivanlou et al. (1990) showed that anterior notochord could induce expression of the En-2 protein (midbrain-hindbrain boundary) more effectively than posterior notochord while both regions induced N-CAM to a similar extent. As we shall see below it is significant that *some* En-2 is induced by the posterior notochord. In this study UV-irradiated embryos were used as ectoderm donors to remove the neural bias normally found on the dorsal side.

Sharpe and Gurdon (1990) have carried out a rather comprehensive study employing the three markers XIF-6 (pan-neural) XIF-3 (anterior) and XlHbox-6 (posterior). As we would expect from the classical work,

neural specification, measured by synthesis of XIF-6 in explants, occurs progressively from posterior to anterior during gastrulation. The competence of ectoderm to respond to dorsal mesoderm is lost by stage 14 (early neurula) for both anterior and posterior markers. When combinations are made of ectoderm with anterior or posterior archenteron roof XIF-3 is expressed in both cases. XlHbox-6 is only expressed in the combination containing the posterior mesoderm. The expression of *both* XIF-3 and XlHbox-6 in the posterior combination is reminiscent of Nieuwkoop's fold experiments and represents support for the idea of posterior dominance.

As we have seen above, the induction of the cement gland is a process which seems to be allied to neural induction in general, although it differs in being mimicked by NH_4Cl. A study by Sive et al. (1989) showed that there was a transient specification of prospective neural plate to become cement gland. In other words, when explants were taken at different stages of gastrulation, the early region which self-differentiated cement gland later came to lie posterior to the position of the cement gland. Further, when combinations of gastrula ectoderm were made with different parts of the archenteron roof, the region most active at inducing cement gland lay somewhat posterior to the final cement gland position. This is exactly the effect observed, but not really discussed, by Mangold (1933), ter Horst (1948) and indeed also by Sharpe and Gurdon (1990).

To summarise: inductions in archenteron roof:ectoderm combinations are more anterior than would be expected from the normal fate of the mesoderm used. This is consistent with the idea of posterior dominance because a given level is able to induce structures more anterior to itself but not those of its own level or more posterior. It also implies the need for an additional stimulus, perhaps the tangential signal, to achieve the degree of posteriorization observed for each level in normal development.

Retinoic acid

If whole blastulae or gastrulae are incubated in retinoic acid then the resulting embryos are found to be truncated at their anterior ends (Durston et al., 1989; Sive et al., 1990; Ruiz i Altaba and Jessell, 1991). At high doses ($10^{-5}M$) forebrain- and midbrain-derived structures are lost and the hindbrain is disrupted, while at low doses ($10^{-7}M$) there is just a slight anterior reduction. The period of sensitivity extends from the egg to the neurula, and exposure as short as 30 minutes at the blastula stage can produce truncations. As expected, the expression of a number of anterior specific genes is abolished in the most severely truncated embryos (cement gland genes XCG-1 and XAG-1, anterior ectodermal gene XA-1 and the anterior neural homeobox gene En-2) (Sive et al., 1990).

Retinoic acid can also affect homeobox gene expression in human embryonal carcinoma cells such that it activates genes in the HOX-2 complex in a dose-dependent manner (eg Simeone et al., 1990). Low

concentrations of retinoic acid are sufficient to activate genes 3' (anterior) in the complex whereas high concentrations are required to activate the 5' (posterior) genes. Given that *Xenopus* embryos are known to contain endogenous retinoic acid (at a concentration of $1.5 \times 10^{-7}M$) (Durston et al., 1989) then these results might suggest that *Xenopus* embryos contain an endogenous gradient of retinoic acid from posterior to anterior which controls the body plan by activating HOX genes in a sequential manner at different anteroposterior levels. The addition of exogenous retinoic acid would then lead to the activation of posterior genes at more anterior positions than normal, resulting in the observed transformations. Although this model (Green, 1990) has an attractive simplicity, there are a number of problems. As far as the effects on HOX genes is concerned, the *Xenopus* data is not entirely consistent with the model. For example, why does the expression of XlHbox-6, which is more posterior than XlHbox-1, increase sixfold on treatment of whole embryos with retinoic acid whereas the expression of XlHbox-1 remains unaffected (Cho and De Robertis, 1990)? Why does XlHbox-3 (trunk-tail) remain constant while XlHbox-6 (trunk-tail) and XIF-3 (head) increase (Sive et al., 1990)? Why is XlHbox-6 not turned on at all in anterior explants (Sharpe, 1991) ?

The effects on the forebrain and rostral midbrain are not found in other vertebrates and at least some of this is probably due to a perturbation of gastrulation movements. Although the anteroposterior extent of the invaginated mesoderm is approximately normal, there is a persistence of the blastocoel and failure of the archenteron to expand fully (Sive et al., 1989). However there is also an apparently direct posteriorization of the neural structures in ectoderm-mesoderm combinations based on a reduction of eyes (Durston et al., 1989) and of anterior molecular markers (Sive et al., 1989). Retinoic acid can suppress anterior differentiation in hitherto neuralized ectoderm dissected from a mid-gastrula embryo (Durston et al., 1989) so this effect is probably a direct one on the neurectoderm rather than via the inducing capacity of the mesoderm. However additional effects on the mesoderm cannot be excluded (Ruiz i Altaba and Jessell, 1991; Sive and Cheng, 1991). In the anterior hindbrain, a detailed neuroanatomical study of treated embryos revealed a compression and disruption of structures rather than a coordinate posterior transformation (Papalopulu et al., 1991).

All the studies reveal defects in the tail as well as the head but these are not made much of. UV irradiation of fertilized eggs also reduces the tail but this is understandable in terms of reduction of the amount of organizer tissue, destined as it is to form the dorsal midline of the whole body. In the case of retinoic acid the tail reductions can arise from quite late treatments (stage 16-18: Ruiz i Altaba and Jessell, 1991) which suggest more of a specific toxic effect.

Ellinger-Ziegelbauer and Dreyer (1991) have shown that in the neurula at least one retinoic acid receptor (RAR gamma) is preferentially localized at both

anterior and posterior ends of the body: in the anterior, it is expressed mainly in the prospective pharyngeal endoderm/prechordal mesoderm, and in the posterior in both the mesoderm and the overlying neural plate. The expression patterns of other retinoic acid receptors have not yet been published, but are under active investigation in several laboratories.

So at present it seems likely that the effects of retinoic acid are quite complex. There may be some shift of epigenetic coding, particularly in the forebrain and midbrain, but there is also some disruption of morphogenetic movements and some toxicity which contribute to the overall morphological syndrome.

Conclusions

This review will have shown that specification of the anteroposterior axis in vertebrates is a very complex process and probably occurs in a number of steps, each of which involves different inductive signals and responses. This should not really be surprising: *Drosophila* is very complicated and whether or not elements of the mechanism are really homologous, vertebrates are not really going to be any simpler than *Drosophila*!

The pre-molecular work does provide us with a lot of data. Unfortunately nearly all of it refers to urodeles such as *Triturus* and *Cynops* and how many of the conclusions hold up for *Xenopus* remains to be established, particularly on the critical issues of the acquisition of anteroposterior coding on invagination and of posterior dominance. *Xenopus* has now become the world standard amphibian embryo and if urodele species are to retain their usefulness then someone will have to generate a set of molecular markers for them.

However, if we can legitimately pool the premolecular and the molecular, then we are left with a picture not dissimilar from that already summarised above and shown in Fig. 9. It looks as though some sort of anteroposterior pattern is set up in the mesoderm during gastrulation and that the posterior end is dominant over the anterior. How complex this mesodermal pattern is in terms of number of distinct territories we do not know, although it must be at least two. The pattern is then transmitted to the ectoderm in a neural induction process probably also involving reciprocal effects of neural plate on archenteron roof. Further interactions probably occur within the neural plate, also with posterior dominance to give the final pattern.

The main innovations of the molecular era have been threefold: the introduction of molecular markers, the discovery of the HOX cluster genes, and the introduction of pure substances which can modify commitment in a defined way (FGF, activin, retinoic acid). Molecular markers are a good idea in principle, but have so far not really extended the precision of what we can observe by conventional histology. This is mainly because too many of the markers are in the CNS and derivatives of the epidermis and too few in the mesoderm. However we can now at least think about the possibility of observing formerly cryptic subdiv-

isions in the mesoderm and observing them soon after they are set up. This is progress.

After some years of uncertainty, the HOX cluster genes look like they really do represent the Rosetta stone of regional specification. Further study of their structure, expression and regulation will undoubtedly advance our understanding of anteroposterior specification. The fact that more HOX genes are on in the posterior than in the anterior provides a simple molecular correlate with the principle of posterior dominance, deduced from the embryological experiments. But vertebrates and insects have evolved a long way apart and even if the same overall system of anteroposterior codings has been preserved, it does not currently look as though precise homologies can be drawn between particular segments or structures in insects and particular structures in vertebrates.

The effects of mesoderm-inducing factors and of retinoic acid are rather baffling at present. If activin really mimics the induction of the organizer then this should be capable of forming the whole of the axis rather than just the head. If posterior dominates over anterior then why do the highest doses of activin induce heads? The FGFs induce ventral rather than posterior structures *in vitro* and it probably needs the complex environment provided by the Einsteckung protocol for FGF-treated caps to produce high yields of tails. The effects of retinoic acid seem to be predominantly, but perhaps not entirely, on the ectoderm rather than the mesoderm. The simple model of a posterior-to-anterior gradient turning on the homeobox genes in 5' to 3' sequence is immensely appealing but the details of the data do not really bear it out.

There is still some way to go before we understand vertebrates as well as we do *Drosophila*. But once again the problem is in the forefront of scientific endeavour, and the tools are available for significantly extending our view derived from the pre-molecular era.

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