

CUTTING, PASTING AND PAINTING: EXPERIMENTAL EMBRYOLOGY AND NEURAL DEVELOPMENT

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The goal of experimental embryology seems rather simple: to manipulate embryos in systematic ways to elucidate mechanisms of development. Such manipulation involves variations in three main techniques — cutting, pasting and painting — and all three techniques have contributed enormously to the establishment of key principles of neural development.

DEVELOPMENT

Through the use of the techniques of experimental embryology, developed by such pioneering artisans as Wilhelm Roux, Hans Spemann, Ross Harrison, Frank Lillie and Viktor Hamburger, as well as their scientific ‘children’, ‘grandchildren’ and more distant ‘relatives’, key principles of neural development have been elucidated (TIMELINE). Although the goal of experimental embryology seems rather simple, embryos are tiny, delicate and surprisingly complex organisms that can easily be perturbed in many nonspecific ways, potentially confounding the interpretation of experimental results. Moreover, embryos are highly responsive to stress, and compensatory mechanisms that can replace whole anlagen (for example, the organizer or neural crest) are readily evoked. So, working with embryos requires the development of master micromanipulative skills, and a thorough understanding of appropriate embryonic anatomy, normal development and ‘embryo husbandry’.

This article briefly discusses the role of experimental embryology (and experimental embryologists) in discovering the principles of vertebrate neural development. After introducing the main strengths and weaknesses of the four vertebrate models, I will provide a historical account of the roles of cutting, pasting and painting, as well as modifications of these techniques that have led to new approaches, in elucidating the principles of neural development. I will define cutting, pasting and painting rather broadly to include not only tissue ablation, transplantation and marking, respectively,

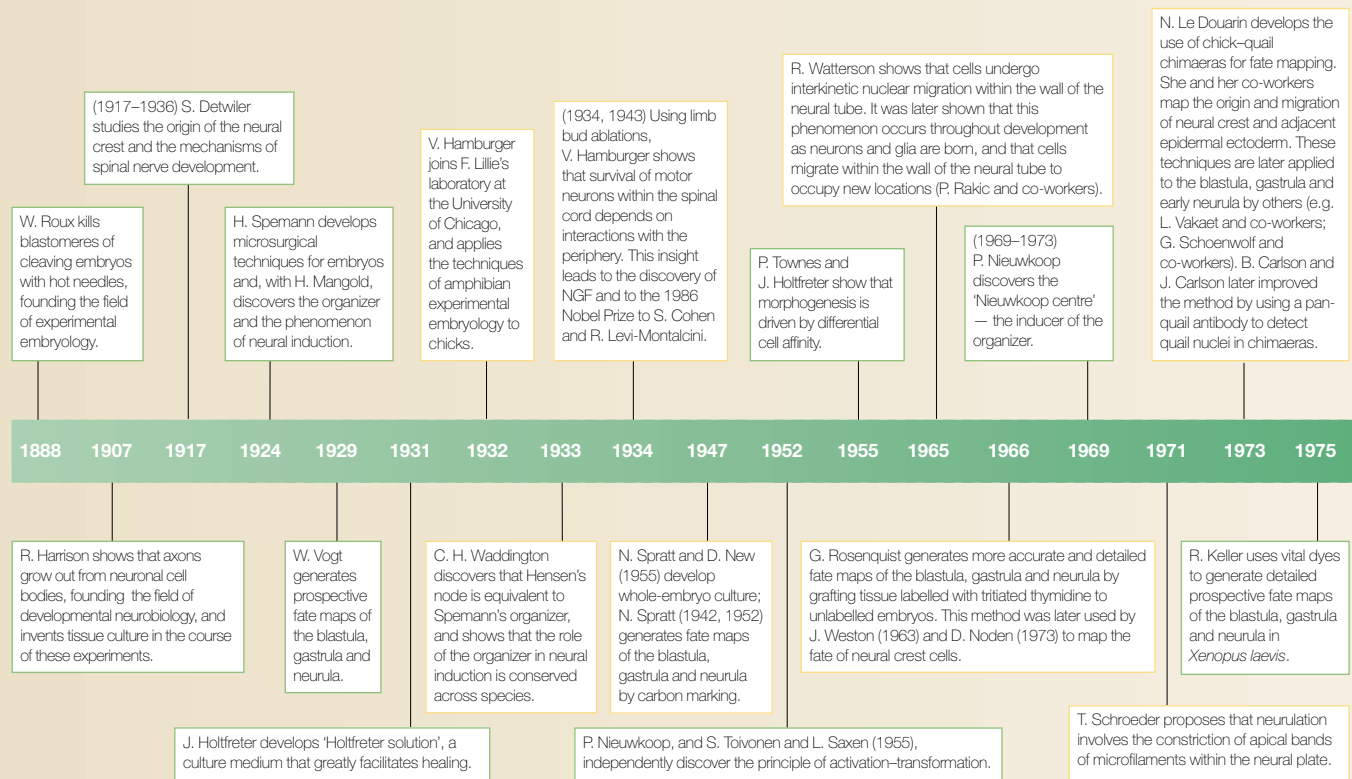
but also gene inactivation, the ectopic overexpression of genes and the expression of lineage markers, such as green fluorescent protein (GFP). I will focus on one central principle that has emerged from experimental embryology; namely, that the development of the nervous system requires a hierarchy of inductive interactions between neighbouring cells. The discovery of this principle occurred in conjunction with the discovery of the organizer¹ — the centre within the embryo that establishes the body plan — resulting in the award of the 1935 Nobel Prize to Hans Spemann.

The four vertebrate models

Although developmental biologists use a broad range of animal and plant species for their studies, experimental embryologists have mainly used four vertebrate models to elucidate the principles of neural development. Historically, the field of experimental embryology has tended to move from ‘lower’ vertebrate models (presumed to be simpler and easier to manipulate) towards ‘higher’ vertebrate models, with the ultimate aim of understanding normal and abnormal development of the human embryo and preventing birth defects. Experimental embryology began in the nineteenth century with freely available amphibians collected from local ponds. During the early part of the twentieth century, a warm-blooded organism, the chick, was added to the repertoire of experimental organisms. Owing to their agricultural importance, fertile chick eggs are readily and inexpensively available throughout most of the world.

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Timeline | **Key events in experimental embryology that led to the elucidation of the principles of neural development**

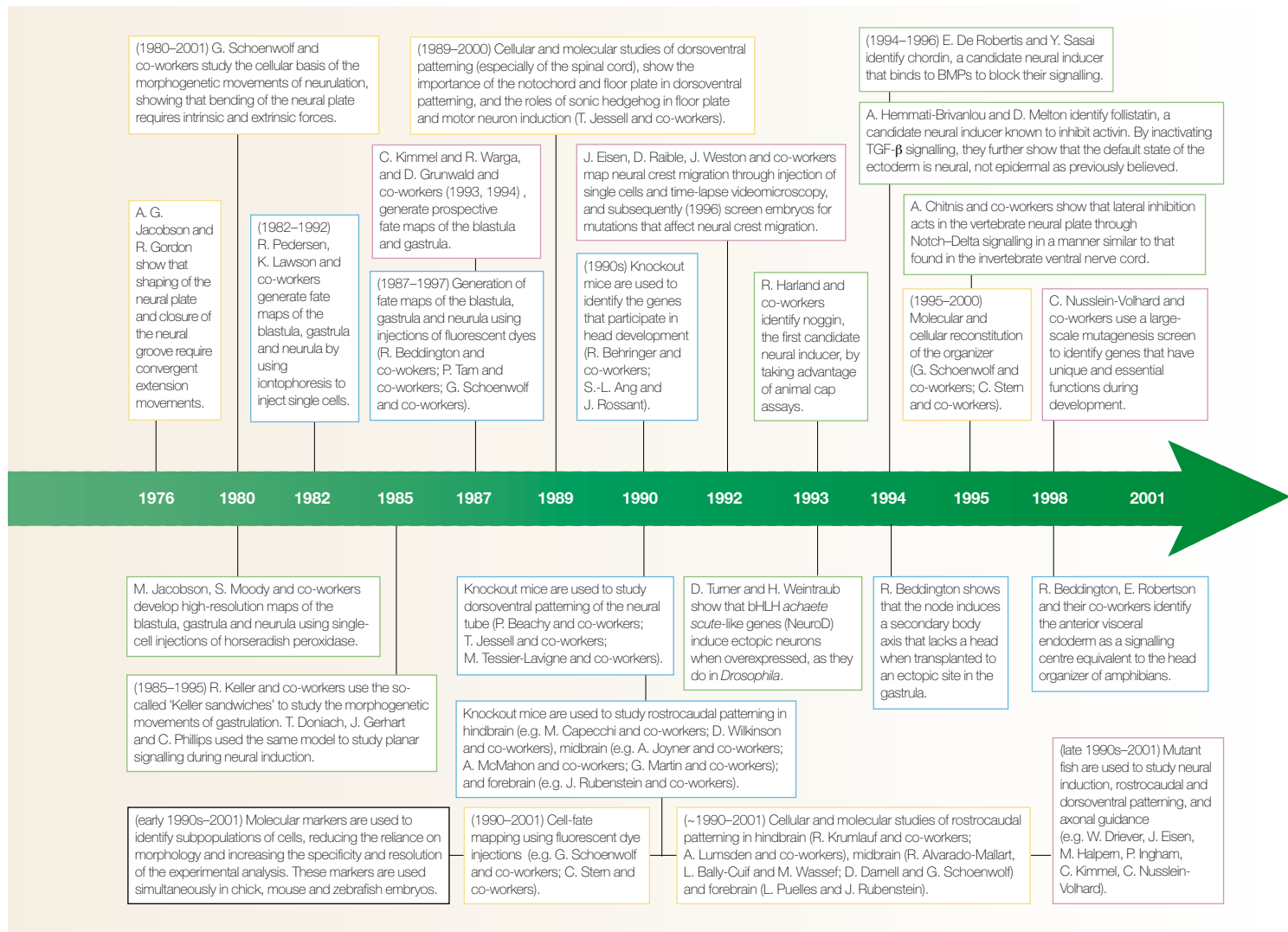


Text boxes are outlined in colour according to the vertebrate model studied: green, amphibians; yellow, chick; blue, mouse; purple, zebrafish; black, all four models. bHLH, basic helix–loop–helix; BMP, brain morphogenetic protein; NGF, nerve growth factor; TGF- β , transforming growth factor- β .

Also around this time, the mouse became a favoured genetic organism. However, experimental embryologists were slow to adopt the mouse embryo as a model system, presumably because mouse embryos develop within the maternal uterus, and are therefore less accessible than those of amphibians and birds. Moreover, it was also believed that their 'inside-out' development, with the endoderm on the outside and the ectoderm on the inside, in contrast to that of amphibians, chicks, zebrafish and most mammals, was too confusing. So, over the past 150 years or so, the techniques of cutting, pasting and painting, which were thoroughly applied to amphibian embryos, were largely repeated on chick embryos and have only recently begun to be applied to mouse embryos. During the latter part of the twentieth century, the main advantages of the zebrafish were recognized by experimental embryologists; namely, their transparency and the ease with which mutations in developmental genes can be generated, allowing the creation of a wealth of mutant embryos to study. The techniques of experimental embryology are now being adapted to the zebrafish embryo, and studies on mouse and zebrafish are occurring essentially in parallel.

Despite this progression of selected model organisms, *Xenopus* and chick embryos remain the workhorses for experimental embryologists, owing to the comparative ease with which these two classes of vertebrate embryos can be manipulated.

Each of the four vertebrate models has its own unique strengths and weaknesses. Although experimental embryologists near the turn of the twentieth century used frogs (especially *Rana*) and salamanders (especially *Ambystoma*) for their studies, in recent years, the South African clawed toad, *Xenopus laevis*, has become the main amphibian species that is used to study embryogenesis. Amphibian embryos have several attributes that make them desirable for experimental embryology. Adults can be maintained and bred in the laboratory, and eggs can be collected throughout the year and in large numbers. Embryos develop externally (outside the mother) in simple salt solution (that is, pond water), and they can be readily viewed and manipulated throughout development. Embryos are relatively large, containing copious yolk. Individual cells can be injected with lineage tracers and followed throughout development to generate high-resolution prospective fate



maps (FIG. 1). Furthermore, genes of interest, including DOMINANT-NEGATIVE constructs, can be overexpressed by injecting RNA into the desired cells, resulting in spatially targeted gene mis-expression. *Xenopus* embryos are extremely receptive to experimental manipulation. Cells and tissues can be ablated or transplanted easily, and the techniques of experimental embryology are well established in this organism²⁻⁷. Recently, it has become possible, using *Xenopus tropicalis*, to establish transgenic animals^{7,8}. In addition, *X. tropicalis*, unlike *X. laevis*, has a diploid genome and a relatively short life cycle, offering the promise of a powerful system for mutagenesis and FORWARD-GENETIC SCREENS, which have been carried out so successfully in zebrafish (see below). On the negative side, amphibian eggs are opaque, obscuring internal developmental events.

Chick embryos, like *Xenopus* embryos, can be obtained throughout the year and in large numbers. Embryos develop within a shell that can be breached to study development *in ovo*, or embryos can be removed from their shell and readily cultured^{6,9-11}. Also like those of *Xenopus*, chick embryos have the disadvantage of being opaque (even though most of the yolk is

sequestered to the extra-embryonic region), and conventional genetic approaches are difficult. However, a chief advantage of chick embryos over *Xenopus* embryos is that true growth of cells and tissues accompanies morphogenesis, as in human embryos. Chick embryos, like *Xenopus* embryos, can be readily manipulated^{2,6,12}, although the small size of their cells precludes reliable single-cell injections. However, prospective fate maps have been generated for many stages of development (FIG. 1), either by injecting vital fluorescent dyes extracellularly^{13,14}, or by transplanting quail donor cells into chick hosts and using the naturally occurring quail nucleolar marker (or a pan-quail nuclear antibody) to identify donor cells in the resulting chimaeras^{15,16} (for a thorough discussion of how this technique, developed by Nicole Le Douarin, has been applied to the study of neural crest cells, see REF. 17). Recently, viral vectors have been used to transfect cells¹⁸, and a new technique for temporally and spatially targeted ELECTROPORATION of cells in whole embryos has been developed^{19,20}.

Mouse embryos share many of the strengths and weaknesses of chick embryos as model systems. As stated above, they develop within the uterus, but there are

DOMINANT NEGATIVE
A mutant molecule capable of forming a heteromeric complex with the normal molecule, knocking out the activity of the entire complex.

FORWARD-GENETIC SCREEN
A genetic analysis that proceeds from phenotype to genotype by positional cloning or candidate-gene analysis.

ELECTROPORATION
The transient generation of pores in a cell membrane by exposing the cell to a high field strength electrical pulse.

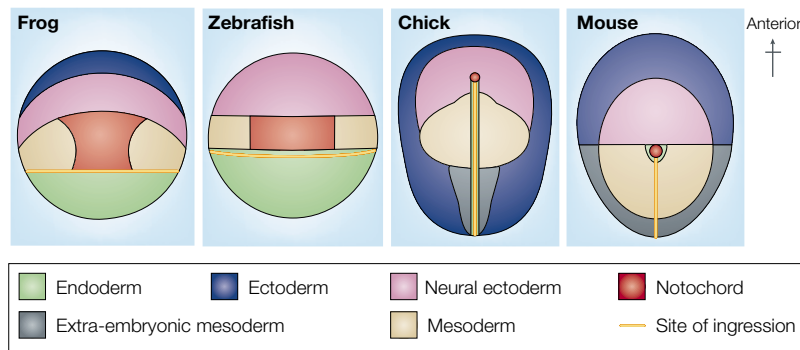


Figure 1 | **Prospective fate maps of gastrula stages of the four vertebrate models.** Modified with permission from REF. 66 © 1998 Current Biology Ltd.

excellent techniques for whole-embryo culture^{21,22}. As in the case of chick embryos, true growth accompanies morphogenesis, cells are small and embryos are opaque. Although more difficult to manipulate than chick embryos, and more costly and available in smaller numbers, cutting, pasting and painting are increasingly being applied to mouse embryos as they develop in culture^{22–24}. Prospective fate maps have been generated for many stages of development (FIG. 1), either by labelling single cells using IONTOPHORESIS²⁵ or by injecting vital fluorescent dyes extracellularly²⁶. The main strength of the mouse embryo is the richness of the available molecular genetic data. Genes can be overexpressed by transgenesis and underexpressed (knocked out) through homologous recombination, and conditional mutagenesis is becoming routine using the CRE/LOXP recombination system.

Zebrafish embryos offer the advantage of being transparent, so internal developmental events can easily be observed. Large numbers of embryos can be quickly and inexpensively generated. Although technically more difficult than in the other vertebrate models, owing to the internal pressure of the embryo and its small size, cells can be ablated or transplanted to ectopic sites²⁷ (see [The Zebrafish Book](#) online). Prospective fate maps have been generated by injecting single cells (FIG. 1), although cell lineages are indeterminate at early blastula stages^{28–30}.

RNA can be injected into single cells for overexpression studies, and MORPHOLINO antisense oligonucleotides can be injected to knock down gene expression³¹. As mentioned above, the main strength of the zebrafish model has been its use in forward-genetic screens after chemical mutagenesis, and large numbers of mutations have been collected that affect virtually every developmental event. Discovering the developmental roles of genes that underlie phenotypes resulting from mutations has been hampered by the fact that the zebrafish genome seems to have undergone an extra duplication during evolution, compared with that of *X. tropicalis*, the chick or the mouse.

Before the onset of the modern molecular era and the realization that developmental mechanisms are highly conserved across diverse organisms, it was widely believed that mammalian embryos, and by default the mouse embryo, were the best organisms to study to gain an understanding of human development. It is now clear that an understanding of human development can be gleaned from the study of much more amenable and experimentally suitable model organisms, including invertebrate models such as *Drosophila melanogaster* and *Caenorhabditis elegans*. So, the fields of embryology and developmental biology, which began with morphologically based comparative embryology, have returned to a comparative approach, although molecular and mechanistic comparisons have largely supplanted morphological comparisons.

Techniques of experimental embryology

Experimental embryology is founded on three main techniques: cutting, pasting and painting (see BOX 1 for a summary of the tools used in these experiments). Although first attempted well over 100 years ago, these three approaches and their molecular variations remain the mainstay of the field today. At the tissue and cellular level, ‘cutting’ involves either ablation — the removal and discarding of a tissue, a group of cells or single cells to test whether they are required for a particular developmental event (FIG. 2a) — or the isolation of tissues and cells for further testing. In the latter case,

IONTOPHORESIS

The introduction of a substance into a cell by ion transfer, using electrodes to apply an electrical potential to the membrane.

CRE/LOXP

A site-specific recombination system derived from *Escherichia coli* bacteriophage P1. Two short DNA sequences (*loxP* sites) are engineered to flank the target DNA. Activation of the Cre-recombinase enzyme catalyses recombination between the *loxP* sites, leading to excision of the intervening sequence.

MORPHOLINO

An antisense oligonucleotide that acts specifically to block the initiation of translation.

Box 1 | Tools of the trade

Experimental embryologists have used some of the most unusual tools to experimentally manipulate small, delicate embryos. The chief tool, used by all experimental embryologists, is the surgical (dissecting or stereo-) microscope, which provides sufficient magnification and depth of field to see and manipulate embryos. A second tool, of extreme importance for understanding the internal anatomy of opaque and complex embryos, is the microtome, which was first used for embryonic tissues by Wilhelm His. The use of the microtome, combined with optimized processing and embedding techniques, allows embryos to be serially sectioned and examined in detail.

Some of the more exotic tools used by experimental embryologists include loops of baby hair, eyebrow hairs, burnt-out light bulb filaments, glass and tungsten needles, sharpened sewing needles, orthodontia wire, cactus spines, coverslip slivers, microspatulas and micropipettes. These are supplemented with watchmaker’s forceps and the finest tools of the medical surgeons, such as iridectomy knives. However, because most tools must be fabricated owing to their small size, experimental embryologists must be skilled toolmakers as well as skilled microsurgions; indeed, as a general axiom, it can be said that a microsurgion is only as good as his or her ability to design and make fine (and often ingenious) microsurgical tools that precisely match the desired task.

With the merger of experimental embryology and cell and molecular biology, the tools of the experimental embryologists now include all those of the cell/molecular biologist. So, the toolbox of the experimental embryologist continues to expand as the number and range of tools becomes almost unlimited.

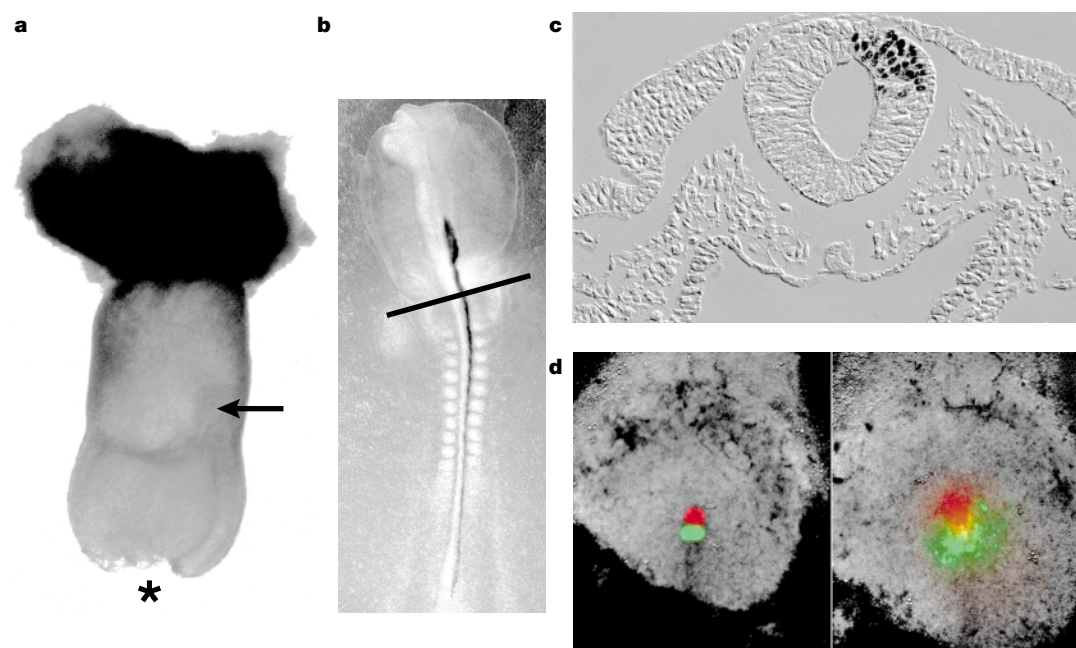


Figure 2 | Cutting, pasting and painting experiments in birds and mammals. a | Cutting experiments. Lateral view of a 7-day mouse embryo immediately after ablation of the node (an asterisk marks the site of extirpation). An arrow marks the allantois at the caudal end of the embryo. Collected by the author in the laboratory of P. Tam. **b,c** | Pasting experiments. Whole mount (**b**) and cross-section (**c**; at the level shown by the line in the whole mount) of quail–chick transplantation chimaeras about 24 h after transplanting a plug of quail epiblast cells to the chick prospective lateral neural plate, adjacent to Hensen’s node. Quail cells have been labelled with a pan-quail antibody. Collected by S. Yuan in the author’s laboratory. **d** | Painting experiments. Cells at two levels of the rostral primitive streak were microinjected with dyes that fluoresce as different colours. Time zero, left panel; 6 h later, right panel. Collected by A. Lawson in the author’s laboratory.

tissues/cells can be maintained *in vitro* in explant culture, frequently in collagen gels, to test their state of specification at a particular time in development. A tissue/cell is said to be specified for a particular developmental fate if it can realize that fate when placed in a neutral environment.

In addition to being placed in explant culture, isolated tissues can serve as donor cells, which are then transferred to host embryos. So, ‘pasting’ involves transplanting tissues or cells from donor to host embryos (FIG. 2b), and can be done between embryos at the same stage of development (isochronic transplants) or at different stages of development (heterochronic transplants). Moreover, tissues or cells can be transplanted from the donor to an identical site in the host (homotopic transplants) or they can be transplanted to an ectopic site (heterotopic transplants). Cells can be transplanted to host embryos for a variety of reasons. For example, a suspected inducer tissue can be transplanted to naive tissue in a host embryo that is presumed to be competent; that is, capable of responding to an inductive signal. Alternatively, cells can be transplanted to an ectopic site in a host embryo to test their state of commitment at a particular time in development. A tissue or cell is said to be committed for a particular developmental fate if it can realize that fate when placed in an environment that is likely to contain conflicting inductive signals.

For transplantation studies, donor cells typically carry an endogenous marker, such as the quail nucleolar

marker, to distinguish them from host cells. Chick cells lack a nucleolar marker, so quail nuclei can be readily identified in quail–chick transplantation chimaeras. Alternatively, cells can be labelled with an exogenous marker, such as the fluorescent dye DiI. So, ‘painting’ involves the use of labelled cells for the purpose of tracking cell movements and fates during further development (FIG. 2c).

The molecular equivalents of these cutting, pasting and painting experiments involve the under- and over-expression of labelled molecules, either RNAs (or complementary DNAs) or proteins (FIG. 3). Knocking down or knocking out the expression of a molecule is the equivalent of cutting. This results in the loss or ablation of the function of the molecule. This can be done in mouse embryos by knocking out a gene using targeted homologous recombination, in *Xenopus* or zebrafish embryos by microinjecting antisense oligonucleotides or RNAi (double-stranded/interfering RNA) into individual cells, or in chick embryos by electroporating antisense oligonucleotides into groups of cells.

The ectopic expression of a molecule is the equivalent of pasting. This can be done by generating transgenic mice or mice containing a gene that has been knocked in. Similarly, it can be done in *Xenopus* and zebrafish embryos by microinjecting single cells with full-length constructs, or in chick embryos by electroporating full-length constructs into groups of cells. In chick embryos, growth factors are often overexpressed by implanting chromatography beads that are coated with the particular

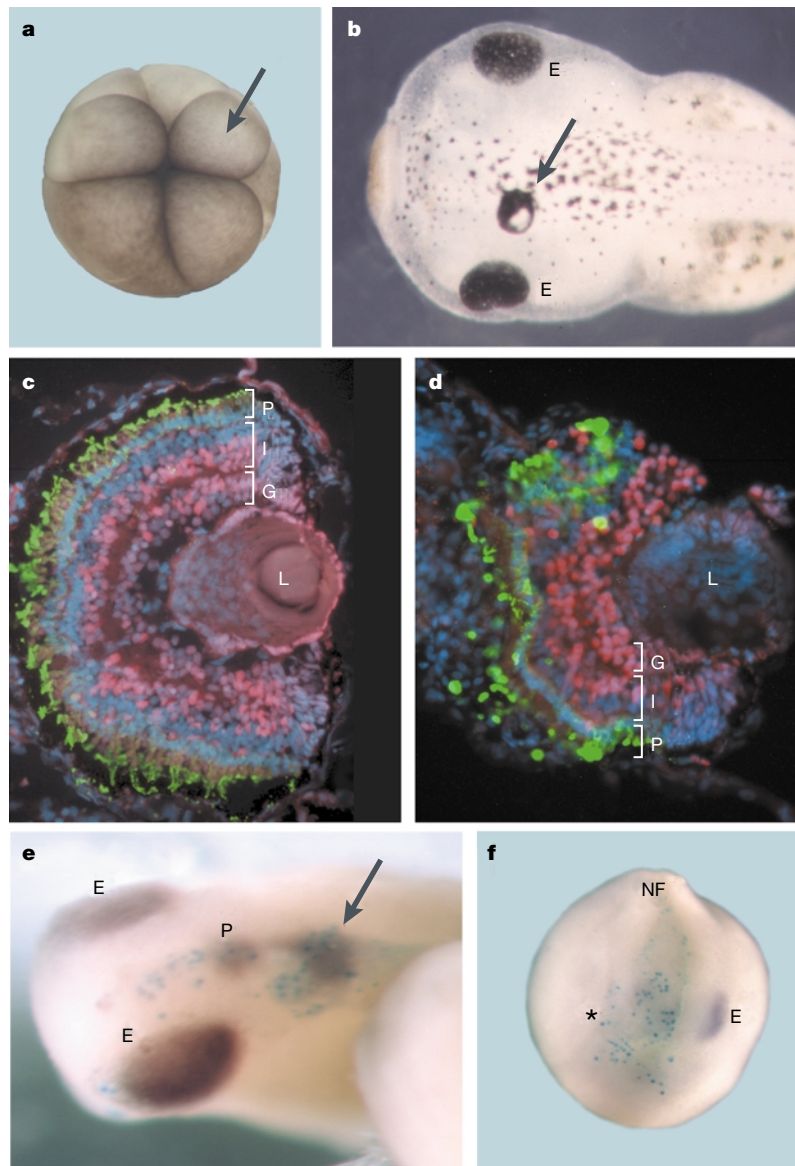


Figure 3 | The molecular equivalent of cutting, pasting and painting experiments.
a | A *Xenopus* embryo at the eight-cell stage. RNA is microinjected into a dorsal animal blastomere (arrow). **b** | Injection of RNA encoding the *Xenopus* receptor *frizzled 3* at the eight-cell stage (molecular equivalent of pasting) causes the formation of an ectopic eye (arrow), shown here in an embryo at developmental stage 45 (E, eye). **c** | Immunolabelling of a section through a normal eye from an embryo at stage 42. Anti-*Pax6* (paired box 6) antibodies (red) label ganglion cells in the ganglion cell layer (G) and amacrine cells in the inner nuclear layer (I), whereas anti-rhodopsin antibodies (green) label rod photoreceptors in the photoreceptor layer (P). L indicates the lens, which is nonspecifically labelled. **d** | Immunolabelling of a section through an ectopic eye using the same antibodies as in **c**. The retina of the ectopic eye has a similar laminar organization to that found in the normal eye. **e** | Injection of RNA encoding *Xenopus frizzled 3* at the eight-cell stage (molecular equivalent of pasting) causes the ectopic expression of the retinal homeobox gene *Rx* (arrow), shown here by *in situ* hybridization of an embryo at stage 28 (dorsal view; E, labelling of the endogenous eyes; P, labelling of the pineal gland). Co-injection of RNA encoding β -galactosidase allows the identification of tissue derived from the injected blastomere (sky blue; molecular equivalent of painting). **f** | Injection of RNA encoding a dominant-negative form of *frizzled 3*, consisting of the soluble extracellular ligand-binding region (molecular equivalent of cutting), prevents the expression of *Rx* on the injected side (asterisk) in a stage-18 embryo (rostral view). This effect correlates with suppression of eye development on the injected side (NF, fusing neural folds). The injected side is marked by co-expression of β -galactosidase. Photograph in part **a** courtesy of T. Van Raay in the M. Vetter laboratory; parts **b–f** reproduced with permission from REF. 67 © 2001 National Academy of Sciences, USA.

growth factor, or by transplanting COS cells that express the growth factor of interest. Furthermore, in mouse and chick embryos, viruses (typically replication-competent retroviruses, such as the RCAS virus) can be used to overexpress genes of interest.

The molecular equivalent of painting is the tagging of mis-expressed molecules with markers. For example, proteins can be labelled with an epitope tag that can be detected with an antibody, or by including GFP in the vector as part of the overexpressed construct. An alternative and new approach is to use *Cre/loxP* excision under the control of a tissue-specific promoter/enhancer to express a reporter gene in a particular domain of cells and their descendants, providing a molecular fate map.

Principles of neural development

The central principle of vertebrate neural development is that the nervous system and associated structures, such as the nose, eyes, ears and (in some organisms) lateral line, arise through a series of inductive interactions between neighbouring cells. In a seminal review, Antoine Jacobson summarized the traditional experimental embryological approaches on which this principle was founded³².

The use of cutting, pasting and painting by experimental embryologists has revealed several key principles of neural development that are related to the central principle. For example, through the use of painting (that is, marking techniques), prospective fate maps have been generated, showing that gastrulating embryos of different classes of vertebrate, regardless of the structure of their egg and the amount of yolk they contain, have prospective organ rudiments arranged in a similar manner around their site of internalization (the blastopore or primitive streak; FIG. 1). Moreover, these maps show that, in all classes of vertebrate, the prospective neural plate — the rudiment of the adult central nervous system and a major contributor to the peripheral nervous system through the neural crest, which forms at its lateral edges — arises in close proximity to the organizer (the embryonic shield of fish, the dorsal lip of the blastopore of amphibians and reptiles, Hensen's node of birds and the node of mammals). These experiments, as well as those discussed subsequently, rely on the availability of accurate staging criteria, and detailed stage series exist for all model vertebrates (zebrafish, see The Zebrafish Book online; *X. laevis*, REF. 33; chick, REFS 34,35; mouse, REFS 36,37). In addition, experiments rely on a thorough understanding of embryonic anatomy, which is available in several detailed atlases (zebrafish, see The Zebrafish Book online; *X. laevis*, REF. 38; chick, REFS 39,40; mouse, REFS 36,41,42; see also REFS 43,44).

The use of cutting (ablation) has revealed that the organizer is required for the formation of the nervous system, and the use of pasting has shown that ectopic organizers induce secondary systems, consisting of neural plate and neural crest, which are derived from (that is, induced from) host cells⁵. By varying the stage of development at which tissues are transplanted to host embryos or explanted to tissue culture, the stages during which cells are capable of responding to inductive signals

— that is, their competence — has been established, as has the timing of neural specification and commitment⁴⁵. Moreover, such experiments have shown the important principle of activation-transformation^{46,47}; that is, that the formation of the neural plate involves an activation (neuralization) of the ectoderm, followed by its transformation (regionalization). The use of painting has shown that embryos of all classes of vertebrate undergo similar morphogenetic movements during GASTRULATION and NEURULATION, and the use of cutting and pasting has shown that gastrulation movements are largely autonomous to the organizer and associated structures⁴⁸, whereas neurulation movements require an interplay between the neural plate and adjacent rudiments^{49,50}.

Further studies using these same techniques of experimental embryology, especially when combined with the use of molecular markers, have shown that various levels of the neural tube interact with adjacent structures to induce sensory systems. For example, the forebrain interacts with the overlying epidermal ectoderm to induce the lens, whereas the hindbrain, along with the adjacent mesoderm, interacts with the epidermal ectoderm to induce the inner ear^{51,52}. Moreover, these studies have shown that the mesoderm (and probably the endoderm as well) interacts with the nascent neural tube, resulting in its regional specialization in the dorsoventral and rostrocaudal planes^{53,54}. They have also shown that interactions occur at the future neuroectodermal and epidermal interface of the early neural plate and groove, resulting in formation of the neural folds and neural crest^{55,56}.

In addition to characterizing cell interactions that lead to patterning, experimental embryology is being used to identify the molecular candidates that mediate these interactions. For example, the roles of *Shh* (sonic hedgehog; produced by the NOTOCHORD and FLOOR PLATE of the neural tube) in dorsoventral patterning of the neural tube⁵⁷, and of *Fgf8* (fibroblast growth factor 8; produced by the ISTHMUS) in organizing the midbrain–hindbrain boundary⁵⁸, have been particularly well studied. Finally, it is worth pointing out that, traditionally, experimental embryologists have first identified cell interactions, on the basis of tissue ablations and transplantations, and then the relevant molecules, through the overexpression of selected candidate proteins or gene knockouts or knock downs. However, this sequence can sometimes be reversed. As an important example, the identification of localized gene expression in a restricted region of the endoderm of the mouse embryo — the anterior visceral endoderm — led to the ablation of this tissue and, ultimately, to the identification of its crucial role in forebrain patterning⁵⁹.

Experimental embryology and neural induction

The techniques of experimental embryology have contributed to the elucidation of several principles of vertebrate neural development. Here, because of space limitations, I will outline only the crucial series of experiments that led to the discovery of neural induction, and recent modifications of experimental embryological techniques that have been used to begin to work out its molecular mechanisms.

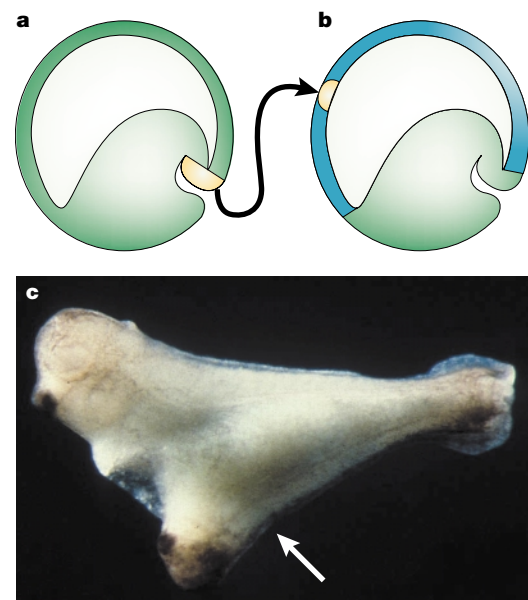


Figure 4 | The organizer experiment. a,b | In the original experiment, the dorsal lip of the blastopore of an unpigmented gastrula of the amphibian *Triturus cristatus* (**a**) was transplanted to the ventral side of a pigmented gastrula of the amphibian *Triturus taeniatus* (**b**). A secondary neuraxis formed ventrally. **c** | A side view of a tadpole showing a secondary embryo (arrow) obtained when the dorsal lip of the blastopore of a *Xenopus* embryo was transplanted to the ventral side of a host *Xenopus* embryo. Part **c** of the figure was obtained from experiments described in REF. 60.

In 1924, Hans Spemann and his doctoral student, Hilde Mangold, demonstrated that transplantation of the dorsal lip of the blastopore to the ventral side of an amphibian gastrula resulted in the formation of a secondary body axis consisting of a composite of host and donor tissues¹ (FIG. 4). This simple experiment revealed the principle of neural induction and galvanized several generations of experimental embryologists, and subsequently biochemists and molecular biologists, to determine its underlying mechanisms⁶⁰. Before the molecular era, most work focused on elucidating the range of tissues and stages of development over which neural induction could occur, defining the timing of competence of the ectoderm to form a nervous system, as well as its specification and commitment. As various tissues, and ultimately various chemicals and proteins (initially, the so-called ‘abnormal inducers’, and recently, endogenously expressed growth factors), were examined for their ability to cause neural induction, two approaches formed the basis of experimental analyses. For a particular tissue or molecule to be considered a candidate inducer, it was essential to show: first, that it was sufficient to cause a naive but competent tissue (frequently amphibian gastrula animal cap ectoderm) to form nervous system — typically defined morphologically as a thickened structure reminiscent of the early neural plate, or molecularly by the expression of early neural plate markers, such as *Sox2* (sex-determining region Y (SRY) box 2); and second, that it was necessary for the prospective neural tissue to form neural plate.

GASTRULATION

The process by which the embryo becomes regionalized into three layers: ectoderm, mesoderm and endoderm.

NEURULATION

A morphogenetic process during which the progenitors of the nervous system segregate from the ectoderm as a dorsal, hollow nerve cord.

NOTOCHORD

A rod-like structure of mesodermal origin that is found in vertebrate embryos. It participates in the differentiation of the ventral neural tube and in the specification of motor neurons.

FLOOR PLATE

The ventral cells of the neural tube that lie in the midline.

ISTHMUS

A narrow section of the neural tube, which separates the midbrain from the hindbrain.

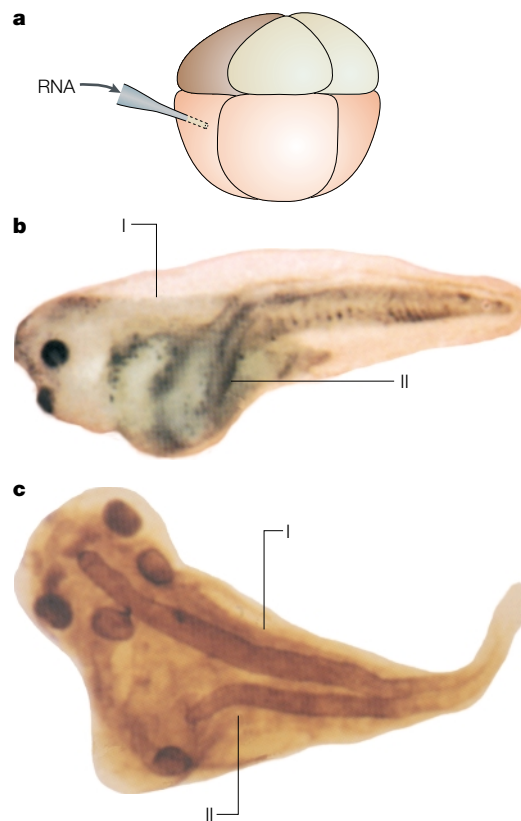


Figure 5 | **The molecular equivalent of the organizer experiment. a–c** | Injection of chordin messenger RNA into a ventrovegetal blastomere of an eight-cell *Xenopus* embryo (a) results in the formation of an ectopic axis, shown here in lateral (b) and dorsal (c) views. I and II indicate, respectively, the primary and secondary axes. Modified with permission from REF. 63 © 1994 Elsevier Science.

The molecular equivalent of the experimental embryologist's cut (demonstrating necessity) and paste (demonstrating sufficiency) experiments is: first, to knock out or knock down the expression of a protein (for example, using antisense), or to block its signalling by its receptors (for example, using dominant-negative constructs), to examine whether a particular protein is sufficient; and second, to overexpress the full-length protein adjacent to competent tissue, thereby showing whether a particular protein is necessary. Experiments in *Xenopus* using dominant-negative constructs have shown that, in the absence of functional neural induction signals from the organizer, the prospective neural plate forms epidermis⁶¹. So, the mechanism of neural induction is to

inhibit the formation of epidermis, revealing that the default state of the ectoderm is neural plate, not epidermis as had long been assumed. Moreover, the molecular equivalent of the Spemann and Mangold¹ experiment — the overexpression of proteins secreted by the organizer (FIG. 5) — has shown that inhibition of both BMP (bone morphogenetic protein) and Wnt (wingless-type MMTV integration site) signalling, by molecules such as **chordin**, **noggin** and **follistatin** (secreted inhibitors of BMP signalling), and **Frzb** (frizzled-related protein) and **cerberus** (both secreted Wnt antagonists), underlies neural induction⁶². These results have been confirmed, in essence, in other vertebrate models, taking advantage of their particular strengths; for example, the ability to knock out genes in mice or the availability of mutations that affect neural induction in zebrafish.

Interestingly, similar molecular mechanisms underlie the induction of the nervous system in invertebrates. For example, formation of the nerve cord in *Drosophila* involves the inhibition of Dpp (Decapentaplegic) signalling by a secreted protein known as Sog (Short gastrulation). Dpp is a member of the transforming growth factor- β (TGF- β) superfamily, homologous to BMP2/4. The homologue of Sog in vertebrates is chordin, one of the molecules secreted by the vertebrate organizer that inhibits BMP signalling, leading to neural induction⁶³. It has been proposed that, during evolution, the dorsoventral axis of the embryo has become inverted, so that the ventral region of *Drosophila* (which forms the ventral nerve cord) is equivalent to the dorsal region of *Xenopus* (which forms the neural plate)⁶⁴. However, in addition to inversion of the axes, there are other explanations for the presence of homologous signalling events. For example, such signalling events might have had an ancient role in specifying neural and epidermal ectoderm, which functions independently of the specification of the dorsoventral axis⁶⁵.

Conclusion

The techniques of experimental embryology, used in a traditional fashion to identify tissues and cells that are involved in inductive signalling, or in combination with molecular genetic approaches to identify associated signalling molecules, their receptors and downstream effectors, have been instrumental in discovering the key principles that govern the development of the nervous system. The continued use of such techniques during the twenty-first century promises to teach us much more about the nervous system, providing an understanding not only of its ontogenesis, but also of its phylogenesis.

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organizer, as viewed by a pioneer of experimental embryology and developmental neurobiology.
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