signed to test the effectiveness with which morph frequency pattern can be predicted. A model was developed (*10, 12*) in which England and Wales were represented by a grid of 360 square cells of linear dimension 22.5 km. Values for the minimum set of parameters required were obtained from the literature and from modeling the northern Wales cline (*11*). Mani (*12*) presented comparisons with the Kettlewell data for three transects. Figure 1c shows the complete surface, for comparison with Fig. 1a. The agreement is sufficiently close as to indicate that a set of assumptions derived from a small part of the area (the cline in the northwest) may be used to explain the morph frequency distribution over the whole of England and Wales, and that these assumptions need not include heterozygote advantage.

For the period from the 1970's to the present, fitness of *carbonaria* and *insularia* is assumed to have decreased linearly by an amount estimated from the change in recorded atmospheric sulfur dioxide on the Wirral (*15*). This produced a good match to the change in morph frequency at Caldy, and consequently the same change is assumed throughout the country. The result of the simulation projected to 1984 is shown in Fig. 1d. In this case also there is a satisfactory match to the observed data, with the plateau of high *carbonaria* frequency retreating to the northeast, although the predicted frequency in the London area is higher than the frequency observed. With the selective intensities involved in the model, such discrepancies are not surprising. The selection is associated with industrial pollution and is assumed to act through the agency of visual predation. Mikkola (*16, 17*) has pointed out, rightly, that more observations are needed before we can have a true picture of the role of visual selection, and that experiments to measure visual selection have design deficiencies. He questions whether the evidence even gives useful information on selective predation. The selective pressures assumed in these simulations are based on the visual selection experiments, however, and for the most part have a good predictive value. In our opinion, this suggests that the experiments do give a correct indication of the order and direction of the selective pressure involved.

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18. This survey could not have been made without the participation of Open University students. We are grateful to A. C. Arnold for help with the graphics.

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Expression of an Epidermal Antigen Used to Study Tissue Induction in the Early *Xenopus laevis* Embryo

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A monoclonal antibody (Epi 1) has been produced that recognizes an antigen expressed in epidermal cells of *Xenopus laevis* embryos. The Epi 1 antigen appears in embryonic epidermis at the end of gastrulation and is not expressed in nonepidermal structures derived from ectoderm (for example, neural tube or cement gland). The capacity to express the Epi 1 antigen is restricted to cells of the animal hemisphere prior to the midblastula stage of development (stage 8), and tissue interactions during gastrulation inhibit the expression of the Epi 1 antigen in neural ectoderm. This epidermal antigen will be a valuable marker for studies of ectodermal commitment.

THE FATES OF MANY EMBRYONIC cells are determined during the time between fertilization and gastrulation. In some tissues, such as the nervous system, cell fate is thought to be influenced by control signals arising in other parts of the embryo; these interactions are referred to as "induction" (*1–3*). Little is known about the molecular events underlying induction. This is due in part to the fact that the experimental analysis of this process relies on the appearance of morphological features appropriate to particular tissue types. Tissue differentiation may be far removed in time from the actual induction events and may involve complex patterns of cellular behavior that are not amenable to study by molecular techniques. One strategy is to identify molecules that are expressed in a tissue-specific fashion in the early embryo and to study the factors controlling the appearance of these molecules during development. We have produced a monoclonal antibody that distinguishes between epidermal ectoderm and neural ectoderm at the onset of neurulation. The antigen recognized by this antibody is restricted to epidermal ectoderm, and its expression in neural ectoderm appears to be inhibited by tissue interactions. This antibody may therefore be useful in molecular studies of neural induction.

A membrane preparation obtained from late neurula (stages 19 to 22) *Xenopus laevis* embryos (*4*) was used to immunize mice for the production of monoclonal antibodies (*5*). Several antibodies demonstrating tissue-specific binding were isolated and characterized. One of these, referred to as Epi 1, recognizes an antigen expressed in epidermal tissue. Immunocytochemical preparations of embryos processed with the Epi 1 antibody exhibited intense staining in the ventral and lateral surface ectoderm, whereas the dorsal ectoderm overlying the archenteron lacked detectable staining (Fig. 1, A and D). The boundaries of the unstained region correspond to the lateral borders of the developing neural plate (Fig. 1B). Immunocytochemical and Western blot analysis revealed that this antigen appears in the embryo at the time of blastopore closure (stage 13) and persists in the epidermis through the feeding larva stage. At no time during embryonic or larval development does the Epi 1 antigen appear in the nervous system or other nonepidermal structures derived

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from ectoderm. For example, the anlage of the cement gland, first detectable at stage 15 (*4*), did not stain with Epi 1 (Fig. 1D), nor was there evidence for expression of the antigen in this structure at later developmental stages (Fig. 1, E through G). The lens and other ectodermal placodes were likewise unstained by the Epi 1 antibody.

The absence of the Epi 1 antigen from the neural plate, cement gland, and placodes suggests that its expression might be controlled by tissue interactions. Each of these structures is thought to develop in response to inductive signals from other embryonic tissues (*1, 6, 7*). In contrast, the early development of the epidermis appears to be relatively independent of such interactions (*8*). We carried out a series of tissue culture experiments in order to determine (i) whether the expression of the Epi 1 antigen could occur in presumptive epidermal ectoderm isolated from its normal tissue environment and (ii) whether tissue interactions control the expression of this antigen in neural structures.

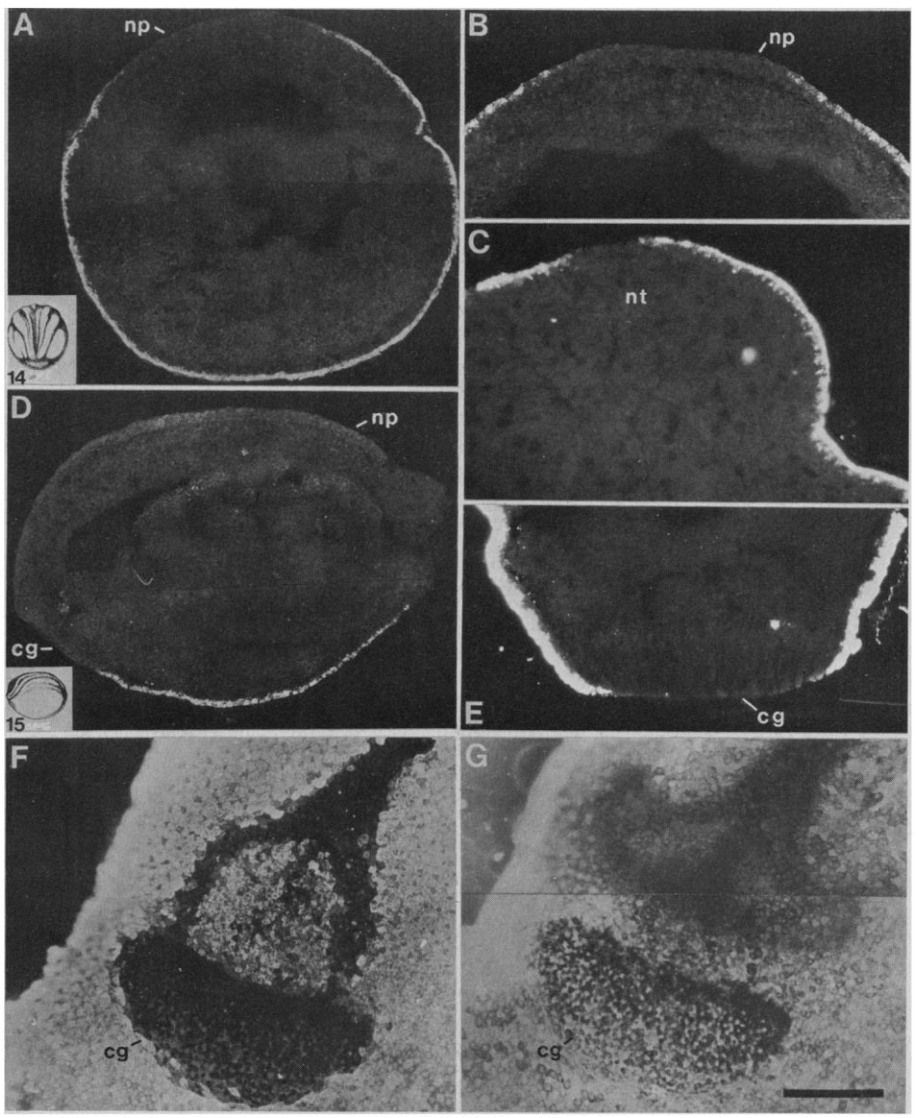


Fig. 1. Patterns of immunofluorescence in frozen sections of neurula-stage embryos stained with the Epi 1 antibody. (A) Transverse section of a stage-14 embryo, illustrating intense staining in the ventral and lateral surface ectoderm and the absence of staining in the dorsal ectoderm overlying the archenteron. Scale = 300 μm. (B) Transverse section of the dorsal region of a stage-15 embryo, showing the absence of staining in the developing neural plate (np). Scale = 240 μm. (C) Transverse section of the dorsal region of a stage-20 embryo during closure of the neural tube (nt). Scale = 150 μm. (D) Sagittal section of a stage-15 embryo. The anlage of the cement gland (cg) lies just ventral to the anterior edge of the neural plate and is unstained by the Epi 1 antibody, as is the region surrounding the closed blastopore. Scale = 300 μm. (E) Transverse section through the cement gland of a tail-bud stage (stage 29) embryo. Elongated epithelial cells within the cement gland lack detectable staining, whereas the surrounding epidermal cells stain intensely. Scale = 150 μm. (F) Whole mount preparation of the head region of an embryo at the end of neural tube closure (stage 20). The cement gland and the region over the closing neural tube are devoid of staining. Scale = 125 μm. (G) Bright-field photomicrograph of the embryo shown in (F), indicating the position of the developing cement gland. Scale = 125 μm.

Two regions can be distinguished within blastula-stage *Xenopus* embryos. Cells of the pigmented animal hemisphere give rise to ciliated epidermis when grown in isolation from their normal embryonic environment, whereas cells from the unpigmented vegetal hemisphere form endodermal structures (*9*). Figure 2 shows the results of an experiment in which clusters of cells were removed from the animal and vegetal hemispheres of a stage-7 (early blastula) embryo. Explants were processed for immunocytochemistry with the Epi 1 antibody after 24 hours in culture, at which point the tissue had reached a stage equivalent to an early tail-bud embryo. The surface cell layer of animal hemisphere explants stained intensely with Epi 1, but staining was absent from explants of vegetal hemisphere tissue. These results demonstrate that the capacity of cells to express the epidermal antigen is restricted to the animal hemisphere by the early blastula stage; thus the elements required for the localized appearance of the antigen are deployed before the midblastula stage (stage 8) and prior to the onset of embryonic transcription (*10*).

Fate maps of blastula-stage embryos have shown that animal hemisphere cells contribute to both neural and epidermal structures (*11*). In order to examine the capacity of these two cell types to express the Epi 1 antigen in culture, we isolated tissue during gastrulation, at which time the presumptive neural and epidermal ectoderm are localized in distinct areas of the embryo (*12*). Figure 3 shows the results of removing tissue during the early gastrula and midgastrula stages. Presumptive epidermis isolated at either stage stained with the Epi 1 antibody after 24 hours in culture (Fig. 3, B and D). In addition, presumptive neural ectoderm isolated prior to midgastrulation stained intensely with the Epi 1 antibody (Fig. 3A); after the midgastrula stage, this tissue progressively lost its capacity to express the epidermal antigen in culture (Fig. 3C). These findings are consistent with earlier reports which suggest that the commitment of ectodermal tissue to an epidermal or neural fate becomes fixed at the end of gastrulation (*13*).

Presumptive neural ectoderm isolated from its normal environment prior to midgastrulation is capable of expressing an epidermal antigen. It is thus possible that tissue interactions may inhibit the expression of this antigen during normal development. To test this possibility, we combined presumptive epidermis from early gastrula-stage embryos (stage 10½) with explants of the

dorsal lip region from stage-10 embryos; the latter cells constitute the classical "neural organizer" region described by Spemann and Mangold (*14*). After 24 hours in culture, epidermal explants combined with dorsal lip tissue stained faintly with the Epi 1 antibody (Fig. 4), but the immunofluorescence in these cultures was much less intense than in control explants of epidermis cultured alone (Fig. 3, B and D). This apparent reduction in the expression of the Epi 1 antigen occurred in the absence of detectable neural differentiation, although a thickening of the epidermal layer was evident.

These experiments demonstrate the feasibility of producing antibodies to tissue-specific molecules that appear within the early embryo and of using these antibodies in studies of tissue induction. Preliminary characterization of the Epi 1 antigen by Western blot analysis indicates that it is a high molecular weight glycoprotein (approximately 300,000 daltons) and is therefore not one of the cytokeratins, which are expressed in epidermis during the neurula stages (*15*). Other tissue-specific molecules, including muscle-specific actin (*16*) and an epidermal glycoprotein (*17*), make their first appearance in the embryo during neurulation. However, there is considerable evidence that the capacity of cells to express these tissue-specific markers may be determined much earlier, during the first few cleavages after fertilization (*18*).

Our findings indicate that the capacity to express the Epi 1 antigen is restricted to a population of cells within the animal hemisphere of the early blastula-stage embryo. In addition, it appears that tissue interactions occurring during gastrulation suppress the appearance of this antigen in the developing neural ectoderm. Thus, induction of neural structures in the embryonic ectoderm may involve, in part, the inhibition of the epidermal phenotype; presumably, other positive

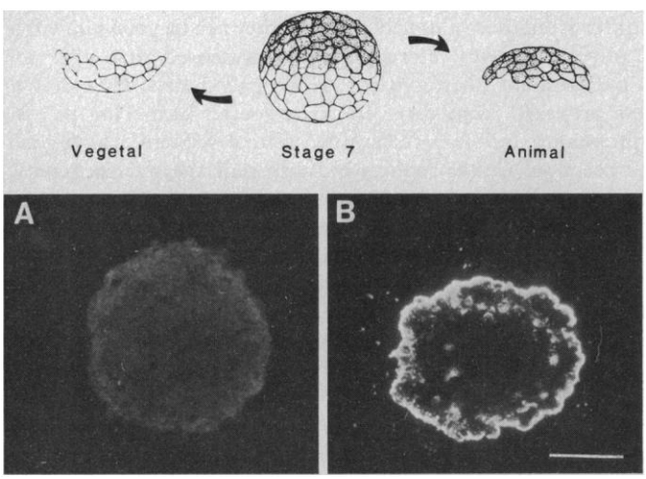
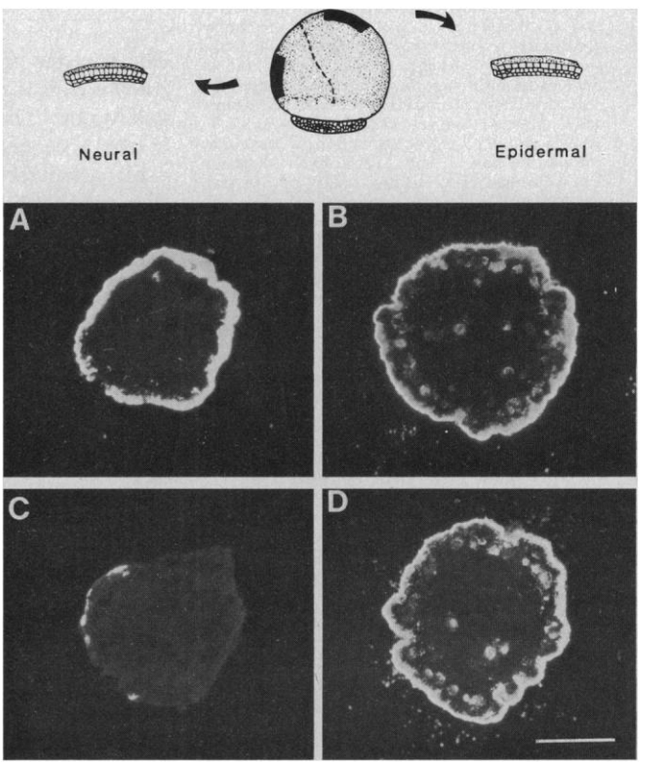
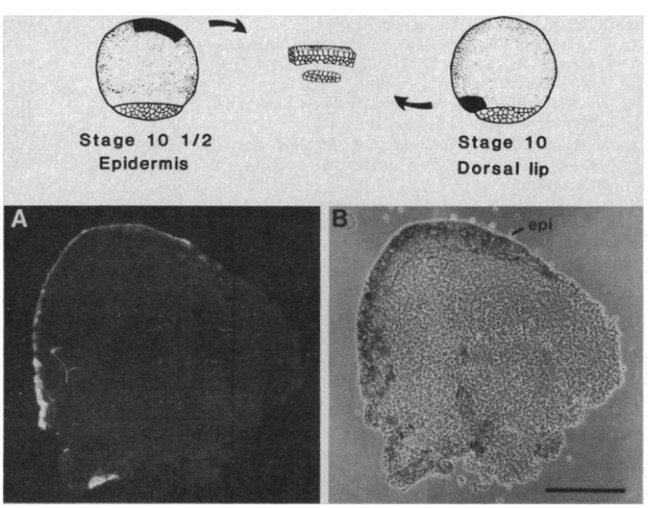


Fig. 2 (top left). Immunocytochemical staining of frozen sections of explants removed from the vegetal (A) and animal (B) regions of a stage-7 (early blastula) embryo. Explants were maintained in full strength Niu-Twitty solution (*19*) for 24 hours prior to processing for immunocytochemistry with the Epi 1 antibody. Tissue derived from the animal hemisphere exhibits intense staining with the Epi 1 antibody, but tissue from the vegetal hemisphere does not. Explants processed at earlier (16 hours) and later (30 hours) times showed similar results. Scale = 100 μm. Fig. 3 (top right). Immunocytochemical staining of explants removed from the presumptive neural (A and C) and epidermal (B and D) ectoderm at two different stages of gastrulation. Epidermal explants removed at either the early gastrula (stage 10½) (B) or midgastrula (stage 11) (D) stages express the Epi 1 antigen after 24 hours in culture. In contrast, explants of neural ectoderm show intense staining only if removed prior to the midgastrula stage (A); there is a progressive reduction in staining with Epi 1 as neural explants are isolated at stages after midgastrulation (C). Scale = 100 μm. Fig. 4 (bottom right). Inhibition of Epi 1 expression by tissue interactions. Immunofluorescence (A) and phase-contrast (B) photomicrographs of a section through an explant of presumptive epidermis removed from an embryo at the early gastrula stage (stage 10½) and combined in culture with an explant of the dorsal lip region of a stage-10 embryo (time in culture, 24 hours). The original epidermal explant can be identified by the presence of heavy pigmentation in (B) (epi); staining with the Epi 1 antibody is present but is much less intense than in epidermal explants cultured in the absence of the dorsal lip (Fig. 3, B and D). Immunofluorescence is restricted to the apical region of the epidermis in epidermal-dorsal lip cultures, whereas in control cultures fluorescence is evident throughout the epidermal layer. Scale = 100 μm.





control events activate developmental programs appropriate to neural tissue. The production of monoclonal antibodies that recognize other early-appearing markers of neural and epidermal development should facilitate the analysis of the events underlying ectodermal commitment.

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17. J. M. W. Slack has described a high molecular weight glycoprotein ("epimucin") that appears in the epidermis of axolotl embryos during neurulation [*J. Embryol. Exp. Morphol.* **80**, 289 (1984); *Cell* **41**, 237 (1985)]. Like the Epi 1 antigen, epimucin is absent from developing neural structures, and its expression is modified by tissue interactions. Our preliminary characterization of the Epi 1 antigen indicates a higher molecular weight than that reported for epimucin (170,000 daltons). Since the Epi 1 antibody does not cross-react with axolotl tissue, it has not been possible to determine whether the *Xenopus* antigen and epimucin are structurally related.
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20. This report is dedicated to the memory of Geoffrey Goldin, a valued colleague who will be greatly missed. We thank R. Keller, C. Mason, D. Stopak, and F. Wilt for their helpful commentary. We also thank P. Sargent for providing some of the embryos used in these studies. This work was supported by NIH grant HD 04708 to N.K.W. and NSF grant PCM 83-09262 to F.W.

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Molecular Basis for the Auxin-Independent Phenotype of Crown Gall Tumor Tissues

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The transfer of specific Ti (tumor-inducing) plasmid sequences, the T-DNA, from *Agrobacterium tumefaciens* to a wide range of plants results in the formation of crown gall tumors. These tissues differ from most plant cells in that they can be grown in vitro in the absence of added phytohormones. Here, data are presented that offer an explanation for the auxin-independent phenotype of crown gall tissues. It is shown that crude cell-free extracts prepared from three bacterial species harboring pTiA6 gene 1 could convert L-tryptophan to indole-3-acetamide; control extracts lacking gene 1 could not carry out the reaction. Other reports indicate that the pTiA6 gene 2 product can convert indole-3-acetamide to indole-3–acetic acid, a naturally occurring auxin of plants. It is concluded that the auxin-independent phenotype of crown gall tissue involves the introduction of Ti plasmid sequences encoding a two-step pathway for auxin synthesis.

CROWN GALLS ARE TUMORS THAT occur on a wide range of dicotyledonous plants as well as a few gymnosperms and monocots (*1*). They are caused by the soil bacterium *Agrobacterium tumefaciens*. The virulence mechanism responsible for this disease, the transfer of genetic information from bacterium to plant, is novel among described prokaryotic-eukaryotic host-pathogen interactions. All virulent strains of *A. tumefaciens* harbor a Ti (tumor-inducing) plasmid, a specific portion of which, the T-DNA, becomes integrated into the plant genome. Subsequent expression of the T-DNA "oncogenes" causes changes in the normal regulated metabolism of auxins and cytokinins, two classes of phytohormones that have key roles in controlling plant cell proliferation and development. Whereas normal plant cells generally require the addition of exogenous auxin and cytokinin for propagation in vitro, axenic cultures of crown gall tissues can be grown indefinitely without them (*2*). This discovery was made some 40 years ago, and since then a fundamental goal of crown gall research has been to understand the

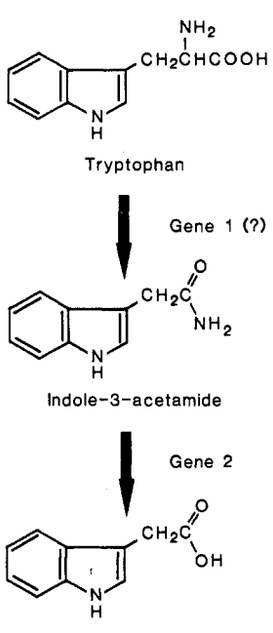

Fig. 1. Pathway for auxin synthesis in crown gall tumor tissues.

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