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Dynamics of the control of body pattern in the development of *Xenopus laevis*

III. Timing and pattern after u.v. irradiation of the egg and after excision of presumptive head endo–mesoderm

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SUMMARY

A series of *Xenopus* egg batches has been exposed to doses of u.v. (2537A) light on the vegetal hemisphere at precleavage stages, calculated to result in a range of minimal axial deficiency syndromes in the developing larvae. At the time of onset of gastrulation in synchronously fertilized but non-irradiated batch members, each experimental group was regularly scanned so that small subsamples of embryos could be set aside as showing particular, progressive degrees of delay in onset of the visible gastrulation movements. Such sampling was found to have preselected embryos showing generally progressive degrees of pattern impairment at larval stages, and this observation was extended by histological examination of the anterior axial anatomy. Such examination was also made of the least abnormal-looking members of a series of larvae resulting from excision of the presumptive head endo–mesoderm, traditionally called 'the organizer', from stage-10 gastrulae (Cooke, 1975). The results support the notion that production of the most anterior endo–mesodermal pattern parts (and of their inductive capacities in giving rise to the brain pattern) occurs only in material whose timing, in the onset of gastrulation activity, is close to the normal onset time after fertilization. *Either* an early failure of the egg to generate a location with the 'position value' corresponding with this extreme of the pattern, *or* the much later excision of the region from a physiologically normal gastrula, results in a system of pattern formation permanently truncated at its apical (head and dorsal) end. There is no evidence for any dynamic, in the system ascribing position value, that will cause regulative restoration of this cellular state (the most extreme 'activation' for development) in response to its absence after precleavage stages. An earlier statement (Cooke, 1975) that this *could* occur was based upon inadequate analysis of larvae with an often misleading external anatomy.

The present results are discussed as supporting the overall view of the early *Xenopus* patterning system that has been developed in the previous two papers of the series.

INTRODUCTION

An overall conclusion from two previous papers in this series has been that a system of graded 'activation' is set up around the fertilized *Xenopus* egg, by very early cleavage stages, that can specify the regional developments which make up a normal mesodermal body pattern. The local level or value for activation on each meridian of the egg can act autonomously, setting both the time schedule for the

Key words: u.v. irradiation, dorsal lip, gastrulation, pattern formation, *Xenopus laevis*.

onset of new cell activities at gastrulation and the regional pattern contribution made by the mesoderm induced there. This early system must reside in states of some structure within the relatively vegetal material (Gerhart *et al.* 1981; Kirschner, Gerhart, Hara & Ubbels, 1980; Neff, Wakahara, Jurand & Malacinski, 1984). On the evidence from the patterns developed in early isolates, its dynamics are such that local intermediate levels of the information can be maintained without loss when the system is separated into two parts so as to sever possible communication between boundary regions. On the other hand, there appears to be little capacity to restore missing information by 'upgrading' material when a part is isolated so as to lack the 'apical' or most activated regions, as is seen for instance in the morphallactic regeneration of hydroids (e.g. Wolpert, Hornbruch & Clarke, 1974 – apical restoration). Large isolated parts from the early embryo can thus set up mosaic development of part patterns, but appear not to restore pattern towards wholeness. In particular, the results from isolated fragments that *just* exclude the presumptive dorsoanterior end of the system indicates that a partial plan may be preserved, but not augmented.

The positional information contained in *Xenopus* blastula stages thus departs from the behaviour predicted for dynamic (e.g. reaction–diffusion or source–sink organized) morphogen gradient systems, in its stability but lack of regulative capacity. It is as if the 'upper' or most activated boundary state were created only by special events at essentially precleavage stages, and cannot subsequently be achieved by any other means. Contact with relatively more activated material can upgrade the position value of other, less activated material even at much later stages (Spemann & Mangold, 1924; Smith & Slack, 1983). For the sake of consistent understanding, it now becomes important to examine closely certain other properties of this amphibian embryo documented in the literature. One of these offers strong supporting evidence that 'apical restoration' cannot occur. This is the progressive obliteration of the development of pattern parts by increasing doses of u.v. irradiation to the egg, starting with parts specified by the upper end of the system of activation (Scharf & Gerhart, 1980; Malacinski, Allis & Chung, 1974). Such embryos, apparently deprived of an upper subset of the levels of activation normally available to them, complete development with any partial set remaining, without a tendency to restore to completion or to any *particular* intermediate level despite the maximum opportunity (in terms of time) to make such adjustment. This paper confirms in a detailed anatomical fashion the previous observation (Malacinski, Brothers & Chung, 1977), that the degrees of incompleteness of pattern seen in individuals are predicted by variously delayed onsets in their gastrulation activity. Also in need of re-examination is the report by the present author (Cooke, 1973, 1975) that surgical excision of the presumptive head endo–mesoderm around the dorsal lip of the beginning gastrula (the classical 'organizer' region), can be followed by the development of a complete axial pattern. It is precisely the normal pattern contribution by this region that is the most sensitive to obliteration by early u.v. irradiation, and is thus presumed to depend upon the highest degree of activation created by the early spatial events.

Completion of pattern after late surgical ablation of these presumptive territories would therefore imply a rapid regulative replacement, by upgrading of surrounding tissue, that is inexplicable in relation to the early isolation results and the u.v. phenomenon. If such regulative ability were confirmed, moreover, it would represent the only known system of embryonic development clearly distinguishable from regeneration (Slack, 1980), where the embryo's pattern itself is susceptible to surgical investigation and where a true apical restoration phenomenon is known to occur.

This paper reports a specific investigation of the two related phenomena of early u.v. damage to, and late surgical interference with, the anterodorsal extreme of pattern. Detailed monitoring of the time course of visible gastrulation and histological assessment of pattern have been carried out. The results confirm that individual degrees of pattern erosion from the apical end after exposure of eggs to low doses of u.v. are strongly correlated with progressive lateness of their earliest gastrulation activity in relation to the control schedule (see Malacinski *et al.* 1977). The earlier conclusion that apical restoration of pattern could follow surgical excision in *Xenopus* gastrulae is shown almost certainly to have been mistaken, because it was based upon inadequate examination of larval structure. The sequence of partial notochords in mildly u.v.-impaired embryos, at their anterior ends, supplements information coming from certain early isolates (Papers I and II of the series Cooke & Webber, 1985*a*,*b*). They are evidence that in the early embryo, regionalized coding by body position value is more fundamental than coding for specific differentiated states.

MATERIALS AND METHODS

Ultraviolet irradiation

The procedures and apparatus for ultraviolet irradiation (2537A) were as used by Scharf & Gerhart (1980, 1984). Eggs artificially fertilized with testis suspension (see general preparation of material in Paper I) were allowed to undergo their spontaneous rotation in response to gravity when the fertilization membrane lifted (fert. + 20–25 min at 20 °C). They were then de-jellied (2·0 % cysteine HCl brought to pH 7·9 with NaOH), without agitation except to free them from their capsules by a few passenges through a wide-mouth pipette after 10 min in the solution. After quickly rinsing in 20 % Ringer, they were allowed to fall free through a 3-cm layer of the same medium (for spontaneous orientation in gravity) onto the quartz-bottomed compartment of a chamber overlying the u.v. source. After a few minutes all eggs not showing a normal orientation of animal/vegetal pigment pattern in gravity were pipetted off, and the remainder exposed to u.v. light on the vegetal hemisphere, the aim being to complete irradiation before the midpoint of the precleavage interval (i.e. by fert. + 40–45 min at 20 °C). The dose/time was that calculated empirically, with the individual source used, to produce a population of mainly low-grade (u.v. 1 and 2, Scharf & Gerhart, 1984) pattern deficits, with a fair proportion of externally normal-looking (u.v. 0) larvae. Control eggs from each batch had fallen onto the u.v.-opaque bottomed compartment of the same apparatus, and then been selected for normal orientation in the same way.

Monitoring of gastrulation schedules

As reported in previous papers a sample of, say, 30 control eggs will commence visible gastrulation within about 15 min if kept together. Each sample of experimental eggs and controls

after u.v. irradiation was placed at 15 °C in 20 % Ringer overnight, beginning at the 64-cell stage. The following morning, embryos could be placed inverted within their membranes on a grid of wells under 5 % Ficoll (Sigma type 400) in 20 % Ringer at 20 °C. Individuals with abnormal or incomplete cleavage patterns, particularly in the vegetal hemisphere, were rejected. Under these conditions the onset and spread of gastrulation activity (beginning around 15 h after fertilization) could be monitored at 10 min intervals.

Surgical excision of the organizer region

Embryos whose treatment had resembled that for controls in the u.v. series, but without passage through the irradiation chamber, were demembranated manually in 66 % Ringer (pH 7·3) at stage 10 (Nieuwkoop & Faber, 1967), the incipient appearance of the dorsal lip. Within the following half-hour they were subjected to an operation which resembled as closely as possible that previously performed on beginning gastrulae by the author (Cooke, 1973, 1975). A small plug of cells beneath and including the area just animal and vegetal to the line of pigment gathering and 'bottle-cell' formation of the stage-10 lip was removed with tungsten needles and a small hair loop. Care was taken to excise right through to the blastocoel, to suck out debris, and not to let loose internal cells at the inner (presumptive anterior) end of the plug fall into and across the blastocoel cavity. Operations were carried out in groups of around 20, and examples delayed beyond 40 min in wound closure because of undue disruption of surrounding cellular contacts were rejected. The medium was restored to 20 % ionic strength for completion of gastrulation and subsequent development.

Examination of the stage-30 larval pattern

Controls, operated gastrulae and subsamples of the u.v.-irradiated populations segregated out as showing particular delays in gastrulation were all fixed at the late tailbud larval stage 30. Their external morphologies were then recorded, and representative samples fixed for histological analysis at this, the standard stage used for such examination (see other papers in this series). For fixation, embedding and staining see previous work (Cooke, 1981, 1983). Horizontal sectioning facilitated comparison of proportions in the complex head mesodermal and brain pattern.

RESULTS

The u.v. syndrome

As described previously in this series of papers, the time course of dorsal lip formation and then progression to give a ventrally complete, annular blastopore is remarkably consistent across carefully treated control embryos of a batch. Increasing familiarity with the relations between early experiences of eggs after fertilization, their subsequent gastrulation and their final larval patterns, fosters an impression that excessive early manipulations make eggs more variable as to gastrulation patterns. It is clear that a certain such extra variability is compatible with production of an array of larval patterns that is not detectably different from normal. But beyond these relatively narrow limits, deviant gastrulation schedules in individuals seem to predict deviant pattern in later development. The present study is in effect part of an attempt to investigate more objectively this intimate impression. Accordingly, the early treatment of all eggs (see Materials and Methods) has been chosen to minimize abnormal relationships with gravity, and deformation, during the precleavage period. Thus as regards u.v. treatment, all eggs should have received an identical dose to very similar hemispheres of their subsurface structure, while in very similar orientations to gravity. Deviations from

normal development *and* differences among individuals should be due to individual responses to the damaging effect of u.v. on the apparatus.

Four batches of about 80 embryos each were subjected to u.v. irradiation, and investigated in comparison with samples of about 30 synchronous controls each. Gastrulation progress was monitored every 10 min, and embryos sorted into subsamples according to their deviation from the control schedule in various ways. Attention was paid particularly to individuals showing only a small but appreciable delay in the onset of lip formation, and to those with a very belated onset and a radially synchronous blastopore formation. Table 1 shows the overall spectrum of morphogenesis in each population as judged by external appearance of larvae, and also the samples kept for histological examination at stage 28–30 after having shown particular small deviations from control gastrulation schedule. According to the most recent 'index of axis deficiency' of Scharf & Gerhart (1984), which I attempt to use comparably in this work:

u.v. 0 = Normal in all *externally* visible respects.
u.v. 1 = Reduced forehead; eyes smaller than normal and sometimes joined.
u.v. 2 = Eyes fused or cyclopic, but at least some retinal pigment visible.
u.v. 3 = No retinal pigment; otic vesicles or single vesicle still visible.
u.v. 4 = No otic vesicle present; somites present in trunk or portion thereof.
u.v. 5 = No somites present; trace of tail mesenchyme occasionally seen but mesoderm close to radially symmetrical.

It is seen that batch one resulted in larvae nearly half of which were externally normal looking (u.v. 0 or 0/1), and in no u.v. 4s or 5s. Yet none of these embryos commenced gastrulating until over half of the controls had begun, and most gastrulation onsets in the experimentals occurred during the 10 min immediately after the *last* of the control onsets. In the other three batches normal-looking larval bodies were a minority, and *no* experimental had begun stage 10 before the last of the control group, i.e. within less than 15 min after the mean time of control onset. But as Table 2 shows, with two slight exceptions the earliest-beginning 8% of experimentals in *each* batch produced larvae of externally and internally normal body pattern. Again with two exceptions, all embryos delayed any further in commencing lip onset, had significant deficit in internal patterning even if classified externally as u.v. 0 or 0/1. Gastrulae delayed more than 50 min behind control schedule in commencing activity, invariably then showed little or no progression of lip character and timing around the marginal zone. A shallow, radially symmetrical lip appearance was thus generated close to the time of control stage 11, or in instances from batches 2–4 only, significantly after that time. All such examples developed as u.v. 4/5s or 5s, and on internal examination showed a small somite-like ridge, at most, as their only deviation from radial lateral plate/blood island pattern. Our concern in this paper is with the question of how direct the relationship is, between various of the *smaller* delays to gastrulation activity and any restricted pattern deficits that histology can reveal.

It would appear that u.v. irradiation in the precleavage period causes a certain component of retardation in gastrulation schedule in *all* embryos of a batch, and

that this may vary with dose, and sensitivity of the particular egg batch. Thus not only is there a statistically measurable delay among embryos going on to form perfect patterns, but if this factor is large in a batch, then even the formation of 'lip' of lateral or posterior character occurs somewhat later than the comparable activity in controls. But beyond this is a further, highly individually variable component of delay. It appears that specifically, delay of more than some 25–30 min with respect to control onset at the dorsal lip is strongly associated with development of apically incomplete patterns. The loss of head pattern in the more delayed sample of Table 2 is highly significant. There are only two counter-examples in each of two samples of 20 embryos being compared, even though these differ in mean time of onset of dorsal lip formation by only some 15 min at a temperature where pregastrular development would occupy about 11 h.

Fig. 1 shows details from various examples of the syndrome of anterior pattern loss among the individuals delayed by 25–30 min in Table 2. In such individuals the new cell activity, when it did appear, still spread with a noticeable rate gradient (i.e. as a wavefront) from anterodorsal to posterior around the marginal tissue, to complete a blastoporal ring with or soon after controls. It is found that the general correlation of reduced anterior morphology with u.v.-induced gastrulation delay previously observed (Malacinski *et al.* 1977) holds true even on the time scale of

Table 1. *Spectrum of morphological results, and samples for histological examination, in four batches of u.v.-irradiated eggs*

	$u.v._0$ or $_{0/1}$	$u.v._{1+2}$	$u.v._3$	$u.v._{4+5}$	Total exp.	Controls
Batch I	34	26	18	0	78	30 normal
Kept:	7 first-gastrulating exps., all within control schedule, all grade $u.v._0$			7 gastrulating 25–30 min after mean control schedule, 3 $u.v._{0/1}$, 4 $u.v._{1/2}$		
Batch II	12	29	28	12	81	2/30 as $u.v._1$
Kept:	5 first-gastrulating exps., all within 20 min of control mean schedule, all grade $u.v._0$			5 next-gastrulating exps., i.e. 25–30 mins after mean control schedule, 4 $u.v._{0/1}$, 1 $u.v._1$		
	4 gastrulating 50+ min after control schedule, all $u.v._{4/5}$					
Batch III	15	23	31	10	79	30 normal
Kept:	5 first-gastrulating exps. (as batch II), all grade $u.v._0$			5 next-gastrulating exps. (as batch II), all grade $u.v._0$ or $u.v._{0/1}$		
	4 gastrulating 50+ min after control schedule, all $u.v._{4/5}$					
Batch IV	7	21	27	20	75	1/28 as $u.v._1$
Kept:	3 first-gastrulating exps. (as batches II + III), all grade $u.v._0$			3 next-gastrulating exps. (as batches II + III), all grade $u.v._0$ or $u.v._{0/1}$		
	5 gastrulating 50+ min after control schedule, all $u.v._{4/5}$					

For each egg batch, the first row in table indicates spectrum of morphological results in terms of 'u.v. grades' (see text). The subsamples kept for histological analysis (see Table 2), with records of precise onset times of visible gastrulation and of external u.v. grade, are indicated beneath.

these minutes-long delays, associated with restricted pattern deficits. Various internal deficits in the morphology of the head region are partially correlated in individuals, but each shown to varying extents. Most such larvae are externally normal or very near normal, but diminution of ventral (gill) head architecture and of cement gland size, reduced architecture and thickness of brain and eyecup walls, and attenuation or loss of anterior notochord distinguish them internally. The structure of all but two of the twenty embryos representing the fastest-gastrulating 8% of each u.v. sample in Table 1 (thus with the 'baseline' level of u.v. retardation of some 15 min), is indistinguishable from controls. The exceptions are mild cases of even the minimal syndrome, showing thinning of the walls and hypomorphosis of the forebrain and eyes, and in one case slenderness of the anterior notochord.

The onset of epidermal ciliary beat and the tailfin and cement gland histogenesis are at most two hours behind control schedule in the low-grade u.v. siblings. It is thus unlikely that the very small delay incurred by treatment at gastrulation (fractions of an hour), later becomes so extended as to prejudice comparison of brain anatomies between synchronously fertilized embryos. The larval brain in the minimally u.v.-affected development is characterized by a tendency toward equalization of volume and rostrocaudal extent in fore-, mid- and hindbrain territories,

Table 2. *Internal patterns in comparison with controls, for larvae selected by precise delay in gastrulation*

	Diminution of ventral (gill) region + cement gland	Reduced architecture/size of forebrain region	Attenuation and loss of antr. NC
Batch I			
minimal delay	1/7	1/7	0/7
	i.e. one shows deficiency syndrome		
delay 25–30 min	5/7	5/7	5/7
	i.e. two are without deficiency		
Batch II			
minimal delay	0/5	0/5	0/5
delay 25–30 min	4/5	3/5	4/5
	i.e. none are without deficiency		
Batch III			
minimal delay	0/5	0/5	0/5
delay 25–30 min	2/5	3/5	5/5
Batch IV			
minimal delay	1/3	0/3	1/3
	i.e. one shows deficiency syndrome		
delay 25–30 min	3/3	2/3	2/3

Numbers of embryos exhibiting some pattern deficiency or numbers lacking any deficiency, in each subsample, are indicated only where this cannot be deduced from the numbers showing each aspect of the syndrome.

as well as diminution of eyecup size, coupled with thinning of the walls in the normal centres of neuroblast density. This suggests that fewer ectodermal cells had been recruited into the original neural plate, presumably because of a reduced field of induction emanating from the anterior mesoderm. The extent of such reduction does not appear to be well correlated, in individuals, with that of reduction in ventral head structure or anterior notochord, though all three abnormalities are typically detectable. In particular a qualitatively complete brain, eye and gill pattern (leading to external classification as normal, u.v. 0) can accompany considerable loss of anterior notochord with bridging across of somite segments (Fig. 1F).

The pattern of notochord diminution and loss is striking. The normal notochord extends as a rod, with little or no tapering right up to the cranial flexure behind the forebrain base (Fig. 1A). Individuals with a definite but minimal u.v. syndrome can show only a slightly abnormal anterior tapering but normal extent, or else reduction to a slender intermittent cell population or complete absence in a sector of the axial pattern varying from the first few somites only, up to the anterior two thirds of the somite completement. But except for local gaps in very thin regions, loss is always progressive from anterior towards posterior end of the pattern (see also Youn & Malacinski, 1981). Neither in this nor in any other procedure causing partial body patterns (Papers I and II), has any individual been seen with a reduced notochord cell population that makes an anterior, partial rod, or a complete one that is abnormally thin and few celled throughout. The limiting instances, seen in presumed oblique egg fragments isolated from 2- or 4-cell stages, show a small, posteriorly confined sector of relatively normal looking notochord in the tail.

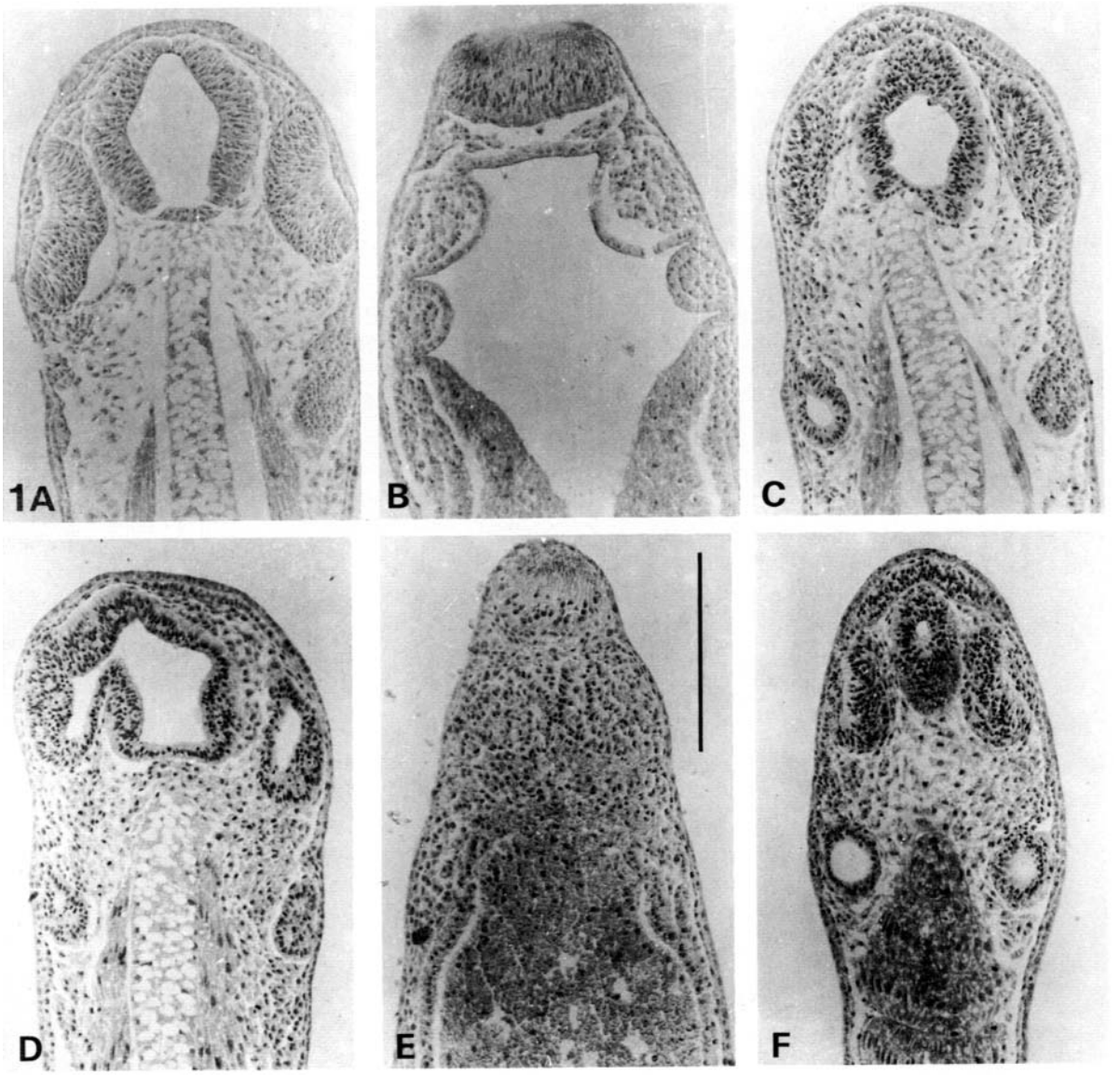
No minimal u.v. example lacked otic vesicles in its CNS pattern, or normal-looking pronephric structures, and the postotic segment number in somite material was normal in relation to controls. This is evidence of the relatively fine-grained or progressive way in which the u.v. effect on precleavage stages erodes the capacity for subsequent pattern development, as if it were truncating the

Fig. 1. The syndrome of anterior pattern deficiencies after low-grade u.v. impairment of the precleavage movements. Horizontal sections of the anterior regions of axial patterns in larvae. (A) The normal appearance at notochord level, to show the anterior limit of notochord below the forebrain, and the size and thickness of CNS wall and retinal rudiments. (B) The normal appearance at the level of cement gland and gill structure. Note wide expansion of the pharyngeal cavity and the deep indentations between bars of gill (neural crest) mesenchyme. (C) Small, foreshortened and thin-walled brain with reduced eye rudiments, in an embryo having begun gastrulation 25 min after mean control onset. (D) Brain of reduced proportions and morphology (note relative closeness of ear and eye rudiments) in a further embryo as above. (E) Severe underdevelopment of ventral head mesodermal, endodermal and crest-derived (gill) mesenchymal pattern, accompanied by a very small cement gland area. The embryo had begun gastrulation 25–30 min after mean control onset. (F) Severe hypomorphosis of brain (though all parts present), accompanied by loss of anterior notochord and bridging of somite material down to five segments behind ear vesicle level. Embryo had begun gastrulation 25 min after mean control onset, and ventral head pattern was slightly less reduced than shown in (E). Scale bar equals 0·5 mm approx.

normal fate map from one end (although the behaviour within the complex head pattern is apparently more capricious). Results in Paper I of this series suggest that pronephros and the capacity for otic vesicle induction represent a level of 'activation' for development achieved between one third and halfway across the egg from the centre of dorsalization.

Restoration of pattern after organizer excision

Four batches of operations were carried out, and operated and control embryos were kept overnight between healed gastrula and tailbud stages at each of two



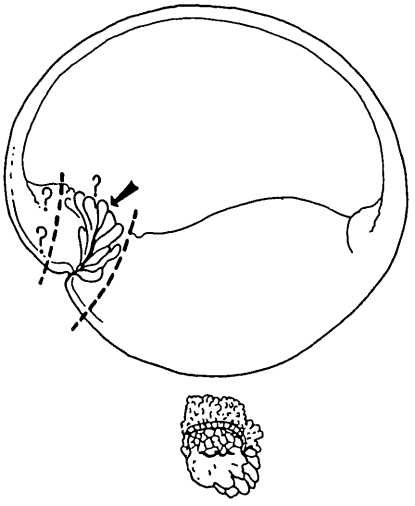


Fig. 2. The operation removing head mesoderm/endoderm territory. A sagittal section of the stage-10 embryo is shown, with heavy dashed lines indicating the extent of removal aimed at. The heavy arrow indicates the deep foregut endodermal cells which probably induce cement gland formation in ectoderm. Question marks indicate areas of variability in precise extent of removal, centred on foregut endoderm, the first-involuted mesoderm (prospective prechordal area) and the mesoderm next to involute (anterior notochord anlage). The excised region is shown with the external surface facing, showing the earliest blastoporal lip and the contrast of cell size between the mesodermal and endodermal inner cells. For regions and structure of the gastrula, see Keller (1976).

temperatures (15 and 22°C). These temperatures led to a two-fold difference in the rate of morphological development, but no evidence was seen that the spectrum of results obtained was affected by this difference in intervening rates. The earlier report was confirmed that after the excision, the remaining schedule of lip spread, closure of the blastopore and neurulation was unaffected (Cooke, 1975). Morphogenesis at this stage resembles a locally controlled, mechanically autonomous series of events, appearing smoothly integrated but having in fact been preprogrammed throughout the mesoderm. Fig. 2 shows the version of the excision operation carried out for this work. As more attention was paid to features that might have led to the variability in outcome previously seen, it was noted that the most difficult detail to keep constant was the extent of removal of the deep-lying (juxta-blastocoelic) yolky cells at the inner end of the excised region. These are the presumptive ventral foregut tissue and that ultimately inducing cement gland differentiation in head ectoderm (see arrows of figure). This observation is consistent with the variable outcome when such excised regions are used as a succession of grafts to the ventral marginal zone of hosts. Some of the second axial patterns then produced are complete, in that they display ventral head morphogenesis and cement gland induction as well as brain parts, while others lack the former structures.

Fig. 3 displays the external appearance of a sample of the range of results in the present study, which observed 74 larvae after such operations. This is essentially similar to the series for the u.v. syndrome but without the general retardation which accompanies the latter treatment, and which becomes noticeable at the high doses that cause great loss of head pattern. The tails of very head-deficient u.v. larvae are thus short and ill-differentiated even though morphologically complete, an effect not seen in the present embryos.

The morphologies vary from the externally normal to that lacking all head structure. This does not necessarily imply varying degrees of success in the acquisition of new pattern information in regions round the excised territories, nor varying loss of pre-existing information in those regions. The parts actually excised

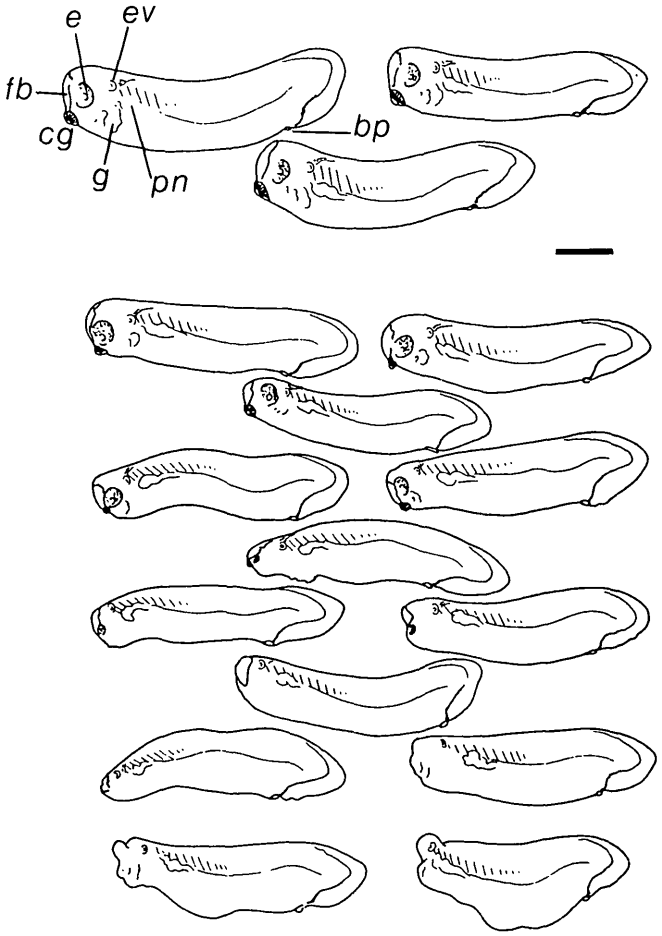


Fig. 3. The external body form after removal of head mesoderm/endoderm. *Camera lucida* drawings of three typical control and thirteen operated larvae, shown in order of increasing apparent pattern deficiency. In the sample of patterns actually obtained in the work, larvae retaining appreciable eyecup and cement gland were a smaller minority (<25 %) than suggested here. Patterns with well developed neural head structure but no gill and cement gland are not seen. *fb*, forebrain; *ev*, hindbrain level and ear vesicle; *cg*, cement gland; *g*, gill structure; *e*, eyecup; *pn*, pronephros; *bp*, proctodaeum (original blastopore). Scale bar equals 1 mm approx.

almost certainly vary enough to account for the results. The region surrounding and underlying the dorsal blastoporal lip, particularly on its animal pole (marginal zone) side, is one where a) many presumptive territories are compressed into a small tissue extent, and b) the extension movements after internal mesoderm migration lay out a great deal of pattern in the head-to-tail sense, using tissue that was somehow compacted in depth and width at stage 10 (Keller, 1976). The neurectodermal induced pattern is not itself derived from tissue that was excised in this operation, but the completeness of induced central nervous system and accessory structures is at least influenced by the completeness or otherwise of pattern in the mesoderm after gastrulation (Nieuwkoop *et al.* 1952; Saxen & Toivonen, 1961). The purpose of the present work was to ascertain whether there is *ever* a true restoration of the anterior pattern in mesoderm after excisions of precisely that region which normally makes or induces all anterior head structures, and which can organize a second *whole* version of the axial pattern when used as a graft (Cooke, 1972).

It can be seen that the external morphologies tend *either* to include a cement gland of near-normal size and then a rather normal head appearance (a minority of the results), *or* to have a very small or absent cement gland induction and then very reduced or absent head structures. Accordingly, the ten most normal-looking larvae of the former category and the ten least severe of the latter type, from the entire series, were examined histologically in relation to controls. Most of the ten with well-developed cement glands were externally classifiable as normal, but in fact only two such larvae preserved this near-normal appearance in horizontal section, showing only slight thinning of the walls of the brain and of the ventral head mesoderm and neural crest-derived structures (Figs 4A and 4B). The remainder showed loss of anterior notochord with somite bridging, and sometimes hypomorphosis of diencephalon and eye regions as if the prechordal inductive territory were reduced (Fig. 4C). All ten which lacked a properly formed ventral head and cement gland showed more severe deficits in internal head structure, including endodermal, mesodermal and neural systems (Fig. 4D–F). The notochords of such apically incomplete bodies tended to reach near the anterior extreme of the remaining pattern however, even though the normal marker of the anterior notochord limit, the flexure beneath the forebrain, was absent. There was thus less evidence for loss of notochord territory at operation, in this version of the outcome, than was the case for the externally more normal-looking outcome.

DISCUSSION

The external progression of the mechanical activities of gastrulation is an imperfect monitor of the progress of development in the deep-lying mesodermal cylinder, and the degree of regularity of the visible progress, once started, also varies somewhat between egg-batches. The data on gastrulation schedules nevertheless do much to supplement the evidence, gained from watching isolates

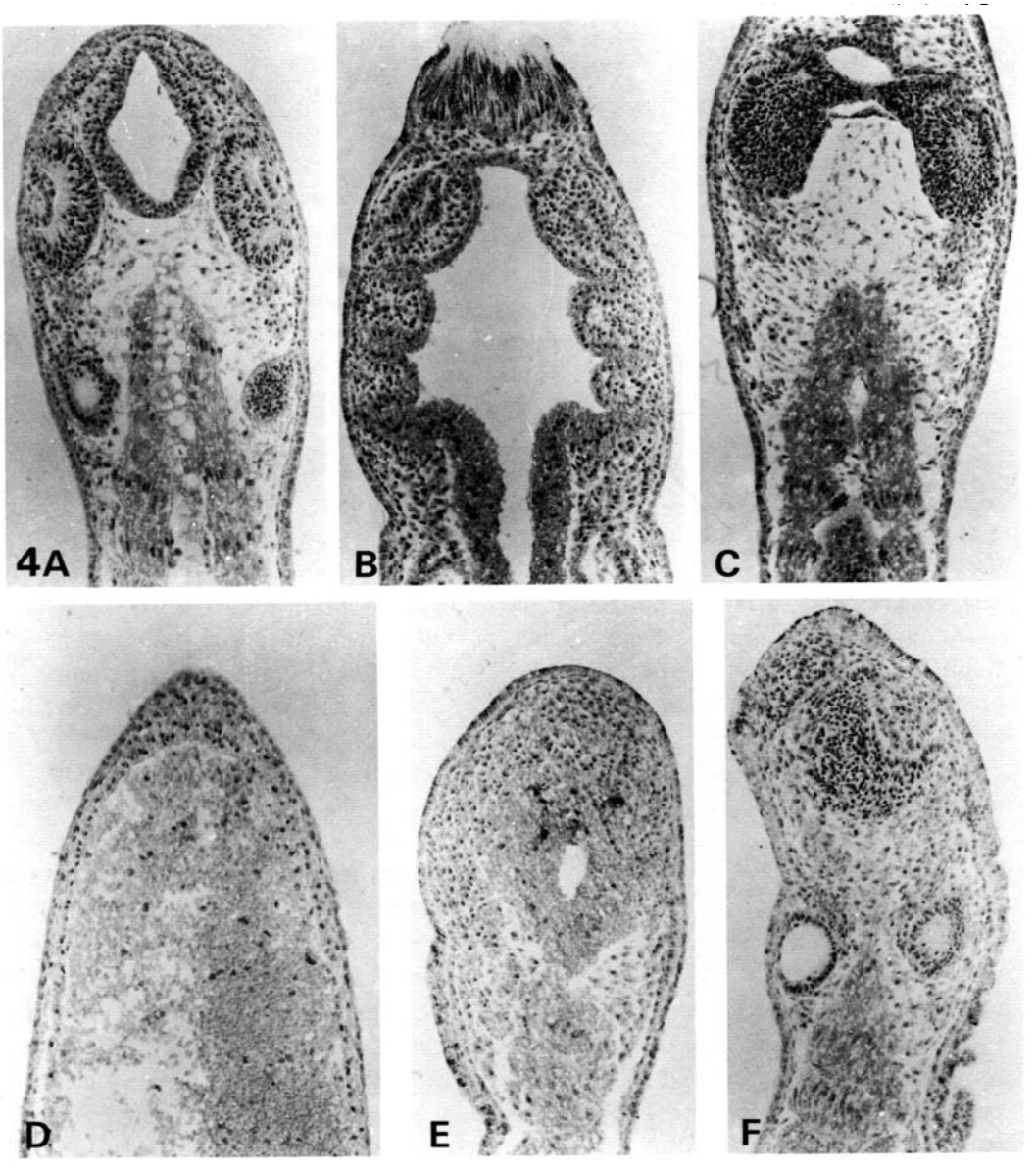


Fig. 4. The syndrome of anterior pattern deficiencies after removal of head mesoderm/endoderm. Horizontal sections of the anterior regions of axial patterns in larvae. For the normal appearance see Fig. 1A,B. (A,B) Notochord and gill levels respectively, of one of the extreme minority class of embryos showing near-normal structure after the operation. Note slight reduction in architecture and wall thickness of structures, in relation to Fig. 1A,B. (C) Extreme hypomorphosis of brain pattern, and loss of anterior notochord with reduced somite structure; one of the major classes of result from the operation. (D,E) total loss of gill and anterior endoderm pattern, and (F) total loss of anterior brain pattern; the second major class of result from the operation.

and whole embryos, that the rate of development occurring on each meridian of the egg material is an intimate correlate of its having been specified to form a particular region of the body plan. A certain small delay in schedule of development probably follows the irradiation in every embryo, but such delay is only followed by pattern loss if it exceeds some 25 min in terms of gastrulation onset in the most rapidly developing (dorsoanterior) extreme of the field. Beyond this, increasing lateness in activity of the fastest-developing region left to the individual embryo is broadly accompanied by the development of mesodermal pattern more truncated in dorsal and anterior aspects. These results also add to the evidence that the early positional system of the egg can maintain stable partial versions of the normal gradation of states, lacking various different amounts of the normal 'upper' end (see Papers I and II of this series). This behaviour, while currently difficult to comprehend in molecular or physiological terms, is distinctively different from the dynamic behaviour predicted for a morphogen gradient of specification, controlled by reaction–diffusion mechanisms.

The results of surgical deletions in the region of the normal 'apex' of egg activation and of pattern remain less decisive than had been hoped for, even though the development of a qualitatively complete pattern is very infrequent (2/74). The territories excised from embryos in fate-map terms are probably very variable. Labelled grafting experiments by the author indicate that the plug of tissue, as excised and then *grafted homotopically* into a matched excision site in a host, contributes variably to head endo–mesoderm and to an anterior domain of notochord varying from a few cells only to more than half the latter's extent. All excisions from the present series had been of closely comparable size, if not of precise composition. It is therefore of interest that there is a tendency to exclusivity within the results, for deficiency within two regions of mes–endodermal head pattern. Embryos markedly deficient in ventral and endodermal head structure show little evidence of notochord loss, whereas those in which the former territories have been spared have often an appreciable sector of anterior notochord missing. In many larvae a superficial impression of regulation is due to the undoubted ability of the neural induction process to compensate, in the pattern of the CNS, for considerable incompleteness in the pattern of the inducing mesoderm. Thus embryos with very deficient head and notochord mesodermal patterns can have qualitatively complete brains and spinal cords (see Fig. 1F). It could be that the mes–endodermal head pattern at gastrulation exists as a set of subfields, arising out of what was simply the apical end of the activation system in the egg and early blastula, and that each subfield shows internal regulative capacity against loss of a certain proportion of its cells. On this view, the occasional embryo in a population subjected to the intrinsically variable excision operation might retain sufficient cells in all subfields to produce qualitatively normal pattern. In all others, one or more subfields would have been rendered so deficient in material as to fail to produce their part of the overall plan. The present results, based on internal anatomy, are consistent with the idea that genuine restoration of complete head pattern cannot be achieved after excision of the

corresponding territories at stage 10, whereas the earlier report (Cooke, 1973, 1975), based only on external criteria, was that this happened frequently.

In the less spatially differentiated egg or morula stages, deprivation of the upper end of the system by u.v. impairment or by a physical separation (Papers I and II) passing at more than a certain narrow angle to the sagittal plane for pattern, prevents development of any of the head territories from taking place. The rarity of complete pattern after gastrular apical excisions is thus consistent with all other data on apically incomplete *Xenopus* embryos in indicating that true restoration of information deleted from the 'upper' end of a system of position values cannot take place.

Taken together, the results in this paper confirm the view that the capacity to develop the most anterior and dorsal parts of the *Xenopus* body plan can arise only in material where some stimulus (activation), given during the precleavage period, reached a certain threshold of intensity. This situation is normally developed in only one region of the egg. Subsequent rescue to give complete development, in cases where the initial movements of egg plasm have been in some way impaired, can only be achieved by the grafting in of material that did achieve the requisite activation levels, followed by slow interactions during blastula/gastrula stages that upgrade the surrounding host tissues to enable their harmonious participation in complete pattern (Gimlich & Gerhart, 1984). This situation appears to contrast with equivalent stages of e.g. bird development. Here, establishment of the embryonic axis itself in the blastoderm is preceded by a more or less prolonged phase during which the precise location and even number of 'organizer' or head-producing centres is indeterminate within the multicellular material (Spratt & Haas, 1960; Mitrani, Shimoni & Eyal-Giladi, 1983). The normal single development of such a centre is thus, in this case, under the dynamic control of an intercellular communication system.

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