Development 105, 747–752 (1989)
Printed in Great Britain © The Company of Biologists Limited 1989

The correlation between patterns of dye transfer through gap junctions and future developmental fate in *Xenopus*: the consequences of u.v. irradiation and lithium treatment

D. J. NAGAJSKI[1], S. C. GUTHRIE[2], C. C. FORD[1] and A. E. WARNER[2]

[1]*School of Biological Sciences, University of Sussex, Falmer, Brighton, UK*
[2]*Department of Anatomy & Developmental Biology, University College London, Gower Street, London WC1E 6BT, UK*

Summary

The correlation between cell-to-cell communication through gap junctions at the 32-cell stage and the subsequent patterning of the embryonic axis has been examined in *Xenopus laevis* embryos. Disturbances of embryonic axis formation were generated by exposure to u.v. irradiation at the vegetal pole before 0·6 in the cell cycle, which generates embryos with dorsal axial deficiencies. Alternatively embryos were treated with 100 mM-lithium chloride between the 2-cell and 32-cell stage, which generates embryos with ventral axial deficiencies. The cell-to-cell transfer of Lucifer Yellow was used to monitor junctional permeability. Injections were made into animal hemisphere cells, lying in tiers 1 and 2 of the 32-cell embryo, whose position relative to the future dorsoventral axis of the embryo was determined on the basis of differences in pigmentation. The frequency of Lucifer Yellow transfer in the future dorsal half of the animal hemisphere was compared with that in the future ventral half for control (untreated), u.v.-irradiated and Li-treated embryos. Injected embryos were subsequently scored for axial development for comparison with dye transfer frequencies.

In control embryos at the 32-cell stage, Lucifer Yellow transfer was both more frequent and more extensive in future dorsal regions than in future ventral regions, as observed previously. In embryos that had been u.v. irradiated before 0·6 in the first cell cycle, Lucifer transfer was the same in both light and dark regions of the animal hemisphere and at the low level characteristic of future ventral regions in normal embryos. These embryos developed with massive reductions in dorsal axial structures. Embryos irradiated after 0·8 in the first cell cycle, when u.v. irradiation no longer inhibits the cytoplasmic movements initiated at fertilization, showed a normal dorsoventral difference in Lucifer Yellow transfer and developed with normal dorsoventral polarity. Embryos exposed to 100 mM-LiCl contained 1·4 mM-lithium at the end of the treatment period. The transfer of Lucifer Yellow between animal pole cells of 32-cell embryos previously treated with LiCl was the same in both light (dorsal) and dark (ventral) and at the high level characteristic of future dorsal regions in normal embryos. Such embryos subsequently developed with substantial ventral axis deficiencies. U.v.-irradiated embryos subsequently treated with lithium showed transfer rates in ventral regions equivalent to that in embryos treated with Li alone and developed with ventral axial deficiencies. We conclude that the frequency with which Lucifer Yellow transfers through gap junctions at the 32-cell stage indicates the future developmental fate of animal cells and can be used to predict the subsequent organization of the embryonic axis.

Key words: gap junction, Lucifer Yellow, *Xenopus*, amphibian embryo, cell–cell communication, lithium, u.v. irradiation, pattern formation, dorsal axial deficiency, ventral axial deficiency.

Introduction

The ability of cells in the early *Xenopus* embryo to exchange molecules through gap junctions is closely correlated with developmental stage and future developmental fate (Guthrie *et al.* 1988). Experiments using a variety of probes of junctional permeability showed at the 32-cell stage that the properties of gap junctions between cells lying in future dorsal and ventral regions were different and suggested that the selectivity of gap junctions varies according to cell location relative to the future polarity of the embryo. The difference in gap junctional properties was most strikingly demonstrated by cell-to-cell transfer of the probe Lucifer Yellow. At cleavage stages (up to 128-cells), the exchange of Lucifer Yellow was both most frequent and most extensive between cells lying in future dorsal regions. The difference between future dorsal and future ventral

cells was conspicuous at the 32- and 64-cell stages, when important developmental events relating to embryonic patterning are probably in progress (for discussion see Guthrie *et al.* 1988).

One way of determining whether these differences in gap junction properties are diagnostic of the prospective organization of the early embryo is to impose treatments that are known to disturb the patterning of the embryonic axis and examine whether junctional communication alters in a way that correlates with future developmental fate. This paper reports experiments in which gap junctional communication was assayed at the 32-cell stage in embryos that had been irradiated with u.v. light before first cleavage, a treatment known to enhance ventral structures at the expense of dorsal structures (Grant & Wacaster, 1972; Malacinski *et al.* 1977; Scharf & Gerhart, 1980; Cooke & Smith, 1987). Alternatively, embryos were treated with lithium chloride between the 2-cell and 32-cell stage, which has the converse effect, enhancing dorsal structures (Kao *et al.* 1986; Breckenridge *et al.* 1987; Kao & Elinson, 1988). The effects of u.v. irradiation and lithium treatment are manifested as changes of fate in animal hemisphere cells, destined to give rise to the mesoderm and ectoderm. We have therefore tested whether the properties of gap junctions between animal cells alter in response to these treatments. We have chosen Lucifer Yellow as the most appropriate indicator of alterations in junctional properties because gap junctions in dorsal and ventral regions display marked differences in their ability to transfer this particular probe (Guthrie *et al.* 1988).

Materials and methods

Eggs were stripped from female *Xenopus laevis* previously injected with 500 i.u. chorionic gonadotrophin (Chorulon, Intervet, Cambridge). The eggs were rinsed in 40% Barth X (Barth X (mM): 88 NaCl; 1 KCl; 0·4 $CaCl_2$; 0·3 $CaNO_3$; 0·8 $MgSO_4$; 2·4 $NaHCO_3$; pH 7·6; Gurdon *et al.* 1975) and fertilized in a minimum of this solution. After 15 min the eggs were dejellied in 2% cysteine–HCl at pH 7·9, rinsed thoroughly in 10% Barth and reared at 19–21 °C.

U.v. irradiation

Eggs were mounted on quartz slides (Horst Baumbach, Suffolk) in a minimum of 10% Barth solution. The vegetal pole was irradiated for 5 min at a total fluance rate of 54 ergs $mm^{-2}s^{-1}$ with a Mineralight UVS 54 (predominant emission 254 nm: Ultra-violet products, California) either before 0·6, or after 0·8, in the first cleavage cycle, taking fertilization as zero and first cleavage as 1·0. The embryos were scored for axial development using the index of axial deficiency (IAD) of Scharf & Gerhart (1980, 1983) when controls had reached stages 25, 32, 37 and 42 (Nieuwkoop & Faber, 1956).

Lithium treatment

Embryos were soaked in 0·1 M-LiCl in 10% Barth from the 2-cell to the 32-cell stage. They were then washed extensively with 10% Barth and reared in 10% Barth until scored for dye transfer or axis deficiencies. Lithium analyses using a flame photometer were carried out on samples of 5 eggs that had been washed three times with distilled, deionized water and then broken up by repeated freeze/thaw cycles.

The embryos were scored for lithium-induced disruption of axial development when controls had reached stages 25, 32, 37 and 42 using a scale from 0–5 where 0 corresponds to normal embryos and 5 to radial embryos.

This scale differs slightly from the scale 5–10 used by Kao & Elinson (1988). Our points 1 and 2 form category 6 on their scale, while their grades 9 and 10 are equivalent to point 5.

Severely affected embryos showed poor survival to the later stages of development.

Dye injection

Embryos at the 32-cell stage were placed in 50% Ringer's solution (60 mM-NaCl; 1·25 mM-KCl; 4 mM-$CaCl_2$; 10 mM-Tris: pH 7·4) with 5% Ficoll 400 (Sigma) for iontophoresis or 5% Ficoll in Barth X for pressure injection. Micropipettes were pulled from omega dot glass and the tips filled with 2–5% Lucifer Yellow.

For iontophoresis, the pipettes were back-filled with 100 mM-lithium chloride and inserted into the chosen blastomere while recording the membrane potential. The circuit was then switched over to current injection and rectangular, hyperpolarizing current pulses (100 nA, 30 ms at a frequency of 10 Hz) applied for about 5 min to expel the Lucifer. The circuit was then returned to the voltage-recording mode and the pipette withdrawn. The membrane potential and injected current were recorded with conventional electrophysiological techniques and displayed on an oscilloscope and pen recorder.

For pressure injection, calibrated pipettes were connected to a micrometer syringe *via* plastic tubing and the whole system filled with light paraffin. The pipette was inserted into a blastomere and pressure applied to the tip. Approximately 2 nl of Lucifer Yellow was expelled, determined by measuring the movement of the meniscus between the Lucifer and the paraffin with an eyepiece graticule. The injections were made into cells designated as lying in the future dorsal or ventral half of the animal hemisphere on the basis of pigment distribution, dorsal cells being more lightly pigmented than ventral cells.

After injection, the embryos were left for 5–10 min and then scored for the presence or absence of dye transfer to neighbouring cells on a Zeiss microscope equipped for epifluorescence (filter set 05). Transfer into the sister cell of the previous cleavage was ignored as possibly occurring *via* cytoplasmic bridges (See Guthrie *et al.* 1988).

Measures to avoid bias

To ensure that observer bias was not a factor in these experiments, the injection site was chosen without knowledge of the treatment group and transfer scored by an observer who knew neither the treatment group nor the site of injection. At the end of the experiment, transfer scores were used to predict both treatment and site of injection for comparison with eventual fate.

Results

Table 1 summarizes the results of Lucifer Yellow transfer between blastomeres lying in the future dorsal or future ventral halves of the animal hemisphere in control embryos at the 32-cell stage for all the experiments. Blastomeres were assigned to the future dorsal or future ventral half of the animal hemisphere on the basis of pigment distribution and blastomere size. Cells

Table 1. *The transfer of Lucifer Yellow through gap junctions between cells lying in future dorsal or ventral regions of control embryos at the 32-cell stage*

	Transfer (n)	No transfer (n)	Transfer (%)	IAD
A All embryos				
Dorsal	90	43	68	
Ventral	45	56	45	
B Survivors				
Dorsal	68	32	68	0·72
Ventral	35	49	42	0·55

Transfer through gap junctions was scored when Lucifer Yellow was found in one or more cells in addition to the injected cell, excluding the sister cell of the previous cleavage.

The index of axis deficiency (IAD) gives the average score of embryos surviving to at least stage 25 calculated using the Scharf & Gerhart (1980) scale indicating dorsal axis deficiency. The survival rate of injected embryos was close to that observed in uninjected controls. The IAD score for Lucifer-injected controls reflects some embryos with minor abnormalities, which were not strictly within the u.v. Syndrome, but were given a score of 1·0 to indicate that the embryos were not entirely normal. Uninjected controls showed an equivalent level of minor abnormality.

Table 2. *The transfer of Lucifer Yellow through gap junctions in embryos that had been u.v. irradiated before first cleavage*

	Transfer (n)	No transfer (n)	Transfer (%)	IAD
A All embryos				
(i) Irradiation before 0·6 in the first cell cycle				
Light (dorsal)	30	54	36	
Dark (ventral)	24	31	44	
(ii) Irradiation after 0·8 in the first cell cycle				
Dorsal	41	24	61	
B Survivors				
(i) U.v. before 0·6				
Light (dorsal)	18	38	32	3·96
Dark (ventral)	11	24	31	4·2
(ii) U.v. after 0·8				
Dorsal	30	13	70	1·07

U.v. irradiation before 0·6 in the first cell cycle induces substantial dorsal axial deficiency. After 0·8 in the first cell cycle the embryos are no longer sensitive.

Transfer of Lucifer Yellow through gap junctions scored positive when Lucifer was present in one or more cells other than the injected cell, excluding the sister cell of the previous cleavage.

in both tier 1 and tier 2 were injected. The transfer of Lucifer Yellow was assessed without knowledge of the site of injection.

The frequency of dye transfer between cells lying in the future dorsal half to their neighbours through gap junctions was significantly greater than that between ventral cells ($P<0{\cdot}001$; χ^2 test). There was no difference between the transfer rate of all embryos injected (A) and those that survived to a sufficiently advanced stage to be scored for axis deficiency (B). The difference in the frequency of transfer observed here confirms the observations of Guthrie *et al.* (1988). In their experiments the comparable transfer frequencies were 60 % in the dorsal half ($n = 128$) and 24 % in the ventral half ($n = 128$).

The accuracy of assignment of cells to future dorsal or future ventral regions of the animal hemisphere was estimated by determining the location of Lucifer staining after the embryonic axis had formed. The failure rate was low; in only 2 out of 21 cases so assessed (an inaccuracy of 10 %) did the initial assignment prove to be incorrect.

The frequency of dye transfer in our experiments is higher than those reported by Guthrie *et al.* (1988), particularly for ventral injections. However, in the thirteen experiments contributing to the data presented here there was considerable variability between experiments in the absolute levels of Lucifer Yellow transfer observed in both dorsal and ventral regions. This was, in part, the consequence of the different methods of injection. In general, pressure injection gave higher levels of transfer and this probably accounts for the relatively high average frequency of transfer in ventral regions, since most of these results were obtained by pressure injection. Pressure injection of dorsal cells occasionally yielded transfer rates as high as 90 %. However, transfer frequencies of 80 % were observed also after iontophoretic injection into dorsal regions, suggesting that the major source of variability lay between batches. This suggestion is reinforced by the observation that in one batch, where ventral levels of transfer were unusually high, many embryos subsequently developed with reduced tails (see below).

Dye transfer after u.v. irradiation

Table 2 shows the frequency of dye transfer in light (dorsal) and dark (ventral) regions in embryos irradiated before 0·6 in the first cell cycle, the period when the cytoplasmic movements that foreshadow normal axial development can be disrupted by vegetal irradiation. Most embryos developed little or no dorsal axial structures.

Throughout the animal hemisphere dye transfer was low and transfer in light (dorsal) regions was not significantly different from transfer in dark (ventral) regions ($P>0{\cdot}3$, χ^2), or from transfer levels in ventral controls (u.v. light (dorsal) *vs.* control ventral: $P>0{\cdot}2$; u.v. dark (ventral) *vs.* control ventral: $P>0{\cdot}9$; χ^2 test). However, both were significantly different from dorsal control values ($P<0{\cdot}01$ for both). Embryos that survived to stages late enough to be scored for axis deficiency (Table 2B) showed equivalently low transfer rates irrespective of the site of injection. Thus u.v. irradiation sufficient to induce a massive reduction in dorsal axial structures prevents also the development of a dorsoventral difference in Lucifer Yellow transfer frequency, with the transfer level throughout the animal hemisphere being characteristic of future ventral regions.

In Table 2, the terms light (dorsal) and dark (ventral) refer to the pigment differences used for choice of injection site. In u.v.-irradiated embryos, the distinction between dorsal and ventral regions on the basis of

Table 3. *The transfer of Lucifer Yellow in embryos treated with lithium*

	Transfer (n)	No transfer (n)	Transfer (%)	Li-score
A All embryos				
Dorsal	17	4	81	
Ventral	48	21	70	
B Survivors				
Dorsal	15	1	94	3·7
Ventral	27	14	66	4·1
C Unaffected by Li treatment				
Ventral (all injections)	3	11	21	
Ventral (survivors)	3	9	25	0·75
D Lithium treatment following u.v. irradiation				
All injections				
Light (dorsal)	6	0	100	
Dark (ventral)	41	28	59	
Survivors				
Light (dorsal)	4	0	100	4·0
Dark (ventral)	17	9*	65	3·7

*3 embryos that failed to transfer were not affected by the lithium treatment. Li-score assessed on the basis of the scale given in the Methods section.

Note: The alteration in transfer rates between all embryos and those surviving to stages late enough to be scored (compare A and B) is discussed in the text.

pigment difference is less secure than in controls because of the inhibition of the cytoplasmic movements that normally generate them. However, even if it is assumed that animal blastomeres in u.v.-irradiated embryos were injected randomly, the combined data shows transfer levels typical of ventral controls. Choosing blastomeres in irradiated embryos on the basis of pigment density did not yield any differences in transfer frequency although in controls it clearly did (Table 1).

Table 2 shows also results from embryos irradiated late in the cell cycle, after the u.v.-sensitive period. Average transfer frequencies were not significantly different from those observed in untreated controls (dorsal u.v. (0·8) *vs.* dorsal control $P > 0{\cdot}5$). Thus reduced levels of dye transfer are not an automatic consequence of u.v. irradiation but occur only in those embryos destined to develop with dorsal axial deficiencies.

Li-treated embryos

Lithium uptake

The concentration of lithium in the embryos at the end of the treatment period (2-cell to 32-cell) was determined in samples of five embryos from six batches, and gave an average of 1·4 mM (taking the embryo volume as 1·33 μl), closely similar to the average uptake after 2 h exposure to 100 mM-LiCl found previously (Breckenridge *et al.* 1987). This concentration of lithium would not be expected to produce a maximal lithium score, as proved to be the case (see Table 3), although the severity of the ventral axial deficiencies was greater than expected from the results of Breckenridge *et al.* (1987) (see their Fig. 2). The very considerable variability in sensitivity to lithium treatment from batch to batch (see Breckenridge *et al.* 1987) probably accounts for the difference. For comparison, lithium uptake was determined also in embryos treated according to the schedule of Kao *et al.* (1986). These embryos contained on average 1·2 mM-Li at the end of the treatment period (6 min in 0·3 M-LiCl), supporting the suggestion (Breckenridge *et al.* 1987) that the very high ionic strength of the treatment solution induces a massive increase in the permeability of the outer membranes of the embryo.

Dye transfer

The results of Lucifer Yellow transfer assays in embryos that had been exposed to 100 mM-LiCl from the 2-cell to the 32-cell stages ($1\frac{1}{2}$–2 h) is shown in Table 3. The lithium scores indicate that many embryos developed with radial dorsal structures. Since lithium treatment is carried out after the cytoplasmic movements that generate pigment asymmetry have occurred, we presume that our choice of dorsal and ventral regions on the basis of this difference was as accurate as in control embryos.

Assays carried out immediately after lithium treatment showed that transfer levels in future dorsal regions were not significantly different from those observed in dorsal controls ($P > 0{\cdot}2$). However, the transfer rate in presumptive ventral regions was significantly higher than in ventral controls ($P < 0{\cdot}01$) and at the same level as in dorsal controls ($P > 0{\cdot}7$). Thus lithium treatment abolishes the dorsoventral difference in transfer frequency found in controls, setting the whole of the animal hemisphere at the level appropriate for presumptive dorsal regions.

Among survivors, the transfer rate for dorsal injections was higher than for survivors of injections into ventral regions (Table 3). Superficially this suggests that transfer rates are elevated throughout the animal blastomeres but that a dorsoventral difference is maintained in lithium-treated embryos. However, of all the experiments performed, this was the only group in which there was a change in transfer rate when only survivors were considered. It was largely the consequence of the small number of dorsal injections in the lithium-treated group (21, Table 3) and the fact that of the four embryos failing to transfer, three happened to die, taking the dorsal transfer rate after lithium treatment from 81 % to 94 %. We therefore consider that lithium treatment effectively eliminates the dorsoventral difference, the conclusion drawn from consideration of all injections. Certainly the dye transfer rates in the presumptive ventral region were elevated to those typical of dorsal controls, whether all embryos or just survivors are considered.

The importance of batch-to-batch variability, both in control levels of transfer and in the response to lithium treatment, was brought out by one experiment, set out separately in Table 3C. As on all other occasions, the injection site was chosen, and transfer scored, without knowledge of the treatment group. In this particular experiment, the lithium-treated, ventrally injected group was incorrectly deduced to be a ventral control,

on the basis of the transfer frequency. We predicted that this batch would prove to be insensitive to lithium treatment. Subsequent development confirmed this prediction (Table 3C). This experiment therefore provides strong independent evidence for a close link between transfer frequency in ventral regions and the subsequent degree of dorsoanterior enhancement.

Lithium treatment of u.v. irradiated embryos

Kao *et al.* (1986) showed that the dorsal axis deficiency characteristic of embryos irradiated with u.v. light during the sensitive period of the first cell cycle could be converted to dorsoanterior enhancement by subsequent lithium treatment. Table 3D summarizes two experiments carried out to test whether Lucifer Yellow transfer in such embryos was consistent with their predicted fate. In light (dorsal) regions the number injected was too small to allow conclusions. But in dark (ventral) regions the dye transfer frequency was significantly different from that observed in u.v. irradiated embryos that were not subsequently Li-treated ($P < 0{\cdot}01$), and not significantly different from ventral regions of embryos treated with Li alone ($P > 0{\cdot}99$). Thus Li treatment not only converts the radial ventral embryo that normally results from u.v. irradiation into the radial dorsal, but also brings the frequency of Lucifer Yellow transfer up to the high level observed in ventral regions of embryos treated with Li alone.

Discussion

The main conclusion of this paper is that the generation of axial deficiencies in the *Xenopus* embryo is preceded by alterations in the permeability of gap junctions that are apparent at the 32-cell stage and predict the future dorsoventral axis of the embryo.

The absolute levels of Lucifer Yellow transfer through gap junctions were influenced by the method of injection of dye and there was substantial variability from batch to batch. However, provided most embryos in the batch were destined to form a normal embryonic axis, a relative difference between Lucifer Yellow transfer in dorsal and ventral regions of the animal hemisphere was always present, as reported in Guthrie (1984) and Guthrie *et al.* (1988). But the variability shows that conclusions about individual batches can only be drawn if a sufficient number of measurements (15–20) are made in both dorsal and ventral regions.

Evidence for a link between gap junction properties and future embryonic polarity was provided by experiments on u.v.-irradiated embryos and lithium-treated embryos. We examined gap junction properties between cells in the animal hemisphere, destined to form the mesoderm and ectoderm derivatives that reflect the altered polarity of the embryo. However, it is likely that vegetal pole cells were affected also since the dorsoventral difference in Lucifer Yellow transfer through gap junctions is present in the vegetal pole at the 32-cell stage (Guthrie *et al.* 1988).

Guthrie *et al.* (1988) concluded that the difference in permeability sequence of gap junctions in dorsal and ventral regions at the 32-cell stage was unlikely to reflect numbers of gap junctions or open gap junctional channels, and showed that Lucifer Yellow transfer is a sensitive indicator of the 'dorsality' or 'ventrality' of gap junctions. Two pieces of evidence suggest that the changes in transfer frequency associated with u.v. irradiation and lithium treatment reflect an alteration in junctional selectivity rather than absolute permeability. First, junctional transfer of Lucifer Yellow was altered substantially only in the region with an altered fate. Second, methylamine, a weak base that increases intracellular pH and might not be expected to affect relative permeability, raises junctional permeability, but has no effect on axis formation (Guthrie *et al.* 1988).

Mesoderm arises at least partly as the result of an inductive signal(s) emanating from cells of the vegetal pole and acting on adjacent animal pole cells. Evidence that gap junctions may be involved in the patterning of the mesoderm has come from experiments showing that inhibiting gap junctional communication, by the injection of gap junction antibodies into vegetal cells (Warner, Guthrie & Gilula, quoted in Warner, 1986), generates embryos that are superficially very similar to those produced by u.v. irradiation. However, embryos injected with gap junction antibody always contain dorsal mesoderm derivatives such as notochord and muscle unlike u.v.-irradiated embryos. In animal cap explants, the induction of muscle-specific mRNAs does not require functional gap junctions to be formed between vegetal and animal pole cells (Warner & Gurdon, 1987) suggesting that the situation in explants may be different from that in whole embryos. 'Dorsal' and 'ventral' gap junctions may arise as part of, or in response to, the signal inducing dorsal mesoderm. Since the dorsoventral difference in Lucifer Yellow transfer is apparent at the 16-cell stage (Guthrie *et al.* 1988), both signal production and response may be very early events. The difference in gap junction properties cannot arise from asymmetric distribution of gap junctional components since lithium induces the production of dorsal gap junctions whether or not the cytoplasmic movements of the first cell cycle occur. Furthermore, the transplantation of normal, dorsal vegetal pole cells into u.v.-irradiated embryos at the 32- or 64-cell stages can restore formation of the embryonic axis (Gimlich & Gerhart, 1984), showing that the inductive signal(s) can still be effective at later stages. The dorsoventral difference in gap junctions at the 256-cell stage in normal embryos (Guthrie *et al.* 1988) may reflect a continuing action of the signal.

Lithium treatment induces a physiological response that is apparent at the 32-cell stage and signals a permanent change in developmental fate of ventral cells. Intracellular lithium reaches a level sufficient to begin altering cell fate during the 8-cell stage (see Breckenridge *et al.* 1987). Thus the properties of gap junctions must be modulated rapidly (within 2 cleavage cycles) by a mechanism that operates well beyond the first cleavage cycle. This could be achieved by modification of gap junction protein within already estab-

lished junctions, or by the insertion of new protein.

In normal development, the permeability properties of gap junctions between cells with a dorsoanterior fate is probably the consequence of an event initiated during the first cell cycle and could be an early indicator of a response to an inductive signal released from dorsal vegetal pole cells. Altogether the results reinforce suggestions that communication through gap junctions may be intimately linked with the processes involved in embryonic patterning (e.g. Warner & Lawrence, 1982; Warner *et al.* 1984; Fraser *et al.* 1987), although definition of the underlying mechanisms will require a more complete understanding of both the way in which the alterations in gap junction properties are effected and the identity of the molecules that are transferred through gap junctions during establishment of the embryonic axis.

This work was supported by grants from The Wellcome Trust and The Royal Society to A.E.W. D.J.N. was in receipt of a SERC Studentship and S.C.G. held a Beit Memorial Fellowship. We thank W. W. Stewart for a gift of Lucifer Yellow.

References

BLACK, S. D. & GERHART, J. C. (1986). High frequency twinning of *Xenopus laevis* embryos from eggs centrifuged before first cleavage. *Devl Biol.* **116**, 228–240.

BRECKENRIDGE, L. J., WARREN, R. L. & WARNER, A. E. (1987). Lithium inhibits morphogenesis of the nervous system, but not neuronal differentiation in *Xenopus laevis*. *Development* **99**, 353–370.

COOKE, J. & SMITH, J. C. (1987). The mid-blastula cell cycle transition and the character of mesoderm in the UV-induced non-axial *Xenopus* development. *Development* **99**, 197–210.

FRASER, S. E., GREEN, C. R., BODE, H. & GILULA, N. B. (1987). Selective disruption of gap junctional communication interferes with a patterning process in Hydra. *Science* **237**, 49–55.

GIMLICH, R. L. & GERHART, J. C. (1984). Early cellular interactions promote embryonic axis formation in *Xenopus laevis*. *Devl Biol.* **104**, 117–130.

GRANT, P. & WACASTER, J. F. (1972). The amphibian grey crescent – a site of developmental information? *Devl Biol.* **28**, 454–471.

GURDON, J. B., LASKEY, R. A. & REEVES, O. R. (1975). The developmental capacity of nuclei transplanted from keratinized skin cells of adult frogs. *J. Embryol. exp. Morph.* **34**, 93–112.

GUTHRIE, S., TURIN, L. & WARNER, A. E. (1988). Patterns of junctional communication during development of the early amphibian embryo. *Development* **103**, 769–783.

GUTHRIE, S. C. (1984). Patterns of junctional communication in the early amphibian embryo. *Nature, Lond.* **311**, 149–151.

KAO, K. R. & ELINSON, R. P. (1988). The entire mesodermal mantle behaves as Spemann's Organizer in Dorsoanterior enhanced *Xenopus laevis* embryos. *Devl Biol.* **127**, 64–77.

KAO, K. R., MASIU, Y. & ELINSON, R. P. (1986). Lithium induced respecification of pattern in *Xenopus laevis* embryos. *Nature, Lond.* **322**, 371–373.

MALACINSKI, G. M., BROTHERS, A. J. & CHUNG, H-M. (1977). Destruction of components of the neural induction system of the amphibian egg with ultraviolet irradiation. *Devl Biol.* **56**, 24–39.

NIEUWKOOP, P. D. & FABER, J. (1956). *Normal table of* Xenopus laevis *(Daudin)*. Amsterdam: North-Holland Publishing Company.

SCHARF, S. R. & GERHART, J. C. (1980). Determination of the dorsal-ventral axis in eggs of *Xenopus laevis*: complete rescue of U.V. impaired eggs by oblique orientation before first cleavage. *Devl Biol.* **79**, 181–198.

SCHARF, S. R. & GERHART, J. C. (1983). Axis determination in eggs of *Xenopus laevis*: A critical period before first cleavage identified by the common effects of cold, pressure and ultraviolet irradiation. *Devl Biol.* **99**, 75–87.

WARNER, A. E. (1986). The pattern of communication through gap junctions during formation of the embryonic axis. In *Somites in Developing Embryos* (ed. R. Bellairs, D. A. Ede & J. W. Lash), pp. 91–104. New York: Plenum Press.

WARNER, A. E. & LAWRENCE, P. A. (1982). Permeability of gap junctions at the segmental border in insect epidermis. *Cell* **28**, 243–252.

WARNER, A. E., GUTHRIE, S. C. & GILULA, N. B. (1984) Antibodies to gap junctional protein selectively disrupt junctional communication in the early amphibian embryo. *Nature, Lond.* **311**, 127–131.

WARNER, A. E. & GURDON, J. B. (1987). Functional gap junctions are not required for muscle gene activation by induction in *Xenopus* embryos. *J. Cell Biol.* **104**, 557–564.

(Accepted 5 January 1989)