

# Intercellular Communication Is Cell Cycle Modulated during Early *Xenopus laevis* Development

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**Abstract.** We investigated intercellular communication during the seventh and tenth cell cycles of *Xenopus laevis* development using microinjection of Lucifer yellow and FITC-dextran as well as freeze-fracture electron microscopy. We found that gap junction-mediated dye coupling visualized using Lucifer yellow was strongly cell cycle modulated in the tenth cell cycle. Cytoplasmic bridge-mediated dye coupling visualized

via FITC-dextran was also, of course, cell cycle modulated. The basis of cell cycle-modulated gap junctional coupling was investigated by measuring the abundance of morphologically detectable gap junctions through the tenth cell cycle. These proved to be six times more abundant at the beginning than at the end of this cell cycle.

PREVIOUS publications have reported electron microscopical observations of gap junctions (17) and also passage of low molecular weight fluorescent tracers (dye coupling) between nonsister cells (9, 10, 25) during early *Xenopus* development. Dye coupling is considered good evidence for functional gap junctions between cells in cases where it cannot be ascribed to cytoplasmic bridges remaining between sister cells after cell division. The recent dye coupling studies (9, 10, 25) indicate an interesting and complex spatial pattern of dye coupling (for example, more dye coupling dorsal than ventral in the animal cap of 16-, 32-, and 256-cell embryos) (9, 10, 25) as well as time-dependent changes in dye coupling in the animal cap (a decrease between the 64- and 128-cell stages and then an increase between 128- and 256-cell stages) (10, 25). These complex findings may help to explain why earlier studies of dye coupling delivered apparently conflicting results in amphibian and other embryos (3, 4, 9, 21). Another complication is reported here. It caught our attention that the spatial and temporal variations reported in dye coupling (9, 10, 25) resemble cell cycle variations in the animal cap of the early *Xenopus* embryo. The cell cycle length is constant until the tenth cell cycle and then begins to increase (5, 15, 18), just after the increase in dye coupling mentioned above. The cell cycle length then increases more at the dorsal than at the ventral side of the animal cap, showing a similar spatial pattern to the earlier dye coupling differences. These resemblances led us to wonder if a relationship exists between gap junctions and the cell cycle during early *Xenopus* development and we report an investigation of this point below. We found that dye coupling via gap junctions is, indeed, cell cycle modulated, being more frequent at the beginning of the cycle than at the end. Coupling between sister cells via cytoplasmic bridges is also, of course, similarly cell cycle modulated. There is

also a stage-dependent difference, both in the persistence of cytoplasmic bridges and in coupling via gap junctions through the cell cycle between the seventh cell cycle (64–128-cell stage) and the tenth cell cycle (512–1,024-cell stage). The cell cycle modulation in dye coupling in the tenth cell cycle is paralleled by a cell cycle modulation in the abundance of morphologically detectable gap junctions. These are more abundant at the beginning of this cell cycle than at the end.

## Materials and Methods

### Experimental Material

Fertilized eggs of *Xenopus* were obtained by mating animals that had been injected with human chorionic gonadotropin. The fertilized eggs or early embryos were dejellied using 2%  $\alpha$ -cysteine, adjusted to pH 7.8 with NaOH, then washed thoroughly, and transferred into Steinberg's solution.

### Dye Injection

The vitelline membrane was removed using watchmaker's forceps, and intact embryos were immobilized by transferring them to 1.6–1.8-mm-diameter holes in a perspex plate immersed in Steinberg's solution in a petri dish. Embryos, staged by counting cells in the animal cap, were then injected either with Lucifer yellow or with FITC-dextran, in one cell, in the center of the animal cap (as identified by counting cells in a transect across the animal cap). The injections were performed at a chosen time either in cell cycle 7 or in cell cycle 10 (see Results for further details). For Lucifer yellow injections, the impalements were performed using microelectrodes pulled from 1,500- $\mu$ m glass capillaries and having a tip impedance of 30–150 megohm (measured under the injection conditions; see below). The electrode tip was filled with a 2% solution of Lucifer yellow in 0.1 M KCl, and the rest of the electrode was filled with 0.1 M KCl. The Lucifer yellow was then injected electrophoretically by applying direct current pulses of  $5 \times 10^{-9}$  to  $1 \times 10^{-8}$  A (80% on, 20% off) for 1–1.5 min in the tenth cell cycle and for 2–3 min in the seventh cell cycle. This injection procedure was chosen to avoid cell damage. It had no effect on the resting membrane potential of injected cells. The Lucifer yellow concentration used (2%) is just soluble

in 0.1 M KCl; LiCl was not used as the electrolyte to avoid side effects due to lithium ions. FITC-dextran was pressure injected, using a glass capillary, with a tip diameter of  $\sim 2 \mu\text{m}$  (accurately broken using a microforge [de Fontbrune, Paris, France]), mounted in a 10- $\mu\text{l}$  syringe (Unimetrics, Anaheim, CA). The tip of the syringe was filled with a 3% aqueous solution of FITC-dextran, and the rest of the capillary and syringe were filled with paraffin oil. Less than or equal to 6 nl of fluorophore was injected, via a fluid-filled coupling, using a micrometer device. Embryos were always kept in Steinberg's solution during injection. This has a low calcium concentration (0.3 mM) and should not cause closure of gap junctions due to an influx of external calcium ions. Fluorescence of Lucifer yellow or FITC-dextran was observed using a fluorescence microscope (Carl Zeiss, Inc., Oberkochen, FRG). The dye injection times were spread out over the cell cycle for sister cell coupling measurements because the abundant sister cell coupling early in the cycle made it easy to determine coupling levels by using a small number of injections. We concentrated on two time points, 0–2 ( $T_0$ ) and 10–12 min ( $T_{10}$ ), in the seventh cell cycle and three time points, 0–2 ( $T_0$ ), 10–12 ( $T_{10}$ ), and 20–22 min ( $T_{20}$ ), in the tenth cell cycle for the nonsister cell measurements because nonsister cell coupling levels were low so that more injections and long observation times were needed to quantitate this type of coupling at any given time in the cell cycle.

### ***Freeze-Fracture Electron Microscopy***

Embryos were fixed, stored, and dissected in a solution of 2.5% glutaraldehyde in 0.1 M sodium cacodylate buffer, pH 7.4, at room temperature. 20% glycerol, in the same buffer, was used as a cryoprotectant. The fixed embryos were dissected, using tungsten needles, to isolate a square piece of ectoderm,  $\pm 800 \mu\text{m}$  square, covering most of the animal cap. A 200- $\mu\text{m}$ -wide slice was then cut from the middle of the piece, and five such slices, from five embryos, were mounted in a pair of copper sandwich disks, in such a way that the freeze-fracture plane would be forced through intercellular membranes running perpendicular to the surface of the embryo and near to the middle of these slices (within  $\pm 200 \mu\text{m}$ ). This procedure ensured that the freeze-fracture replicas obtained were of intercellular membranes between cells close to the middle of the animal cap. There is no way of knowing whether the intercellular membranes examined were between sister and nonsister cells, between cells in the surface layer of the animal cap, or between cells in deeper layers. The specimens were frozen in Nitrogen Slush and fractured and replicated in a Cryofract 190; (Reichert Jung, Paris, France). Replicas collected on 700-mesh electron microscopy grids (Hex 700 TB cu; Bio-Rad Laboratories, Veenerdaal, The Netherlands) were examined with an electron microscope (CM 10; Philips Electron Optical, Eindhoven, The Netherlands). The grids were scanned systematically to identify pieces of membrane, and the membrane pieces were then scanned systematically at a higher magnification to count gap junctions (see Results).

## **Results**

### ***Dye Coupling Measurements through the Cell Cycle in Early *Xenopus* Embryos***

Lucifer yellow (450 mol wt) and FITC-dextran (39,000 mol wt) were injected into *Xenopus* embryonic cells at intervals during two identified cell cycles. Embryos were followed up until the beginning of the cell cycle in question (using cell counting). The beginning of the cell cycle was then identified (and defined) by the first appearance of the cleavage furrow in the mother of the cell to be injected, and dye was injected at a desired moment in this cell cycle. The injected cell was then observed for up to 15 min after injection to determine if dye was transferred to neighboring cells. These injection experiments were performed using cells at only one selected position in the embryo, the center of the animal cap. This position was chosen because it can be defined, very reliably, by counting cells along a transect across the animal cap, thus reducing variability in our results due to spatial differences in coupling within the embryo (9, 10, 25). We did not further distinguish whether the cells that we injected were just left, right, dorsal, or ventral of the exact center of the animal cap and can make no statements about the effects of such small

positional differences on dye coupling. Because of the design of these experiments (where we followed cells to be injected from the beginning of the cycle in which the injection was performed), it was possible to identify sister cells unambiguously, and we were thus able to distinguish between dye transfer to sister cells and to nonsister cells (below). The experiments were performed for two identified cell cycles: the seventh cell cycle (64–128 cells; Lucifer yellow and FITC-dextran injection) and the tenth cell cycle (512–1,024 cells; Lucifer yellow injection only). The animal cap of the *Xenopus* embryo begins to become multilayered from stage 7 (eighth cell cycle). For practical reasons, we have restricted our observations to dye transfer between cells in the surface layer of the animal cap, and our estimates of dye transfer in the tenth cell cycle thus concern a restricted subpopulation of cells and may also be underestimates (since dye transfer to inner layers of cells is ignored). Our findings (below) indicate that for these cells, at least, dye coupling via gap junctions and cytoplasmic bridges is cell cycle modulated.

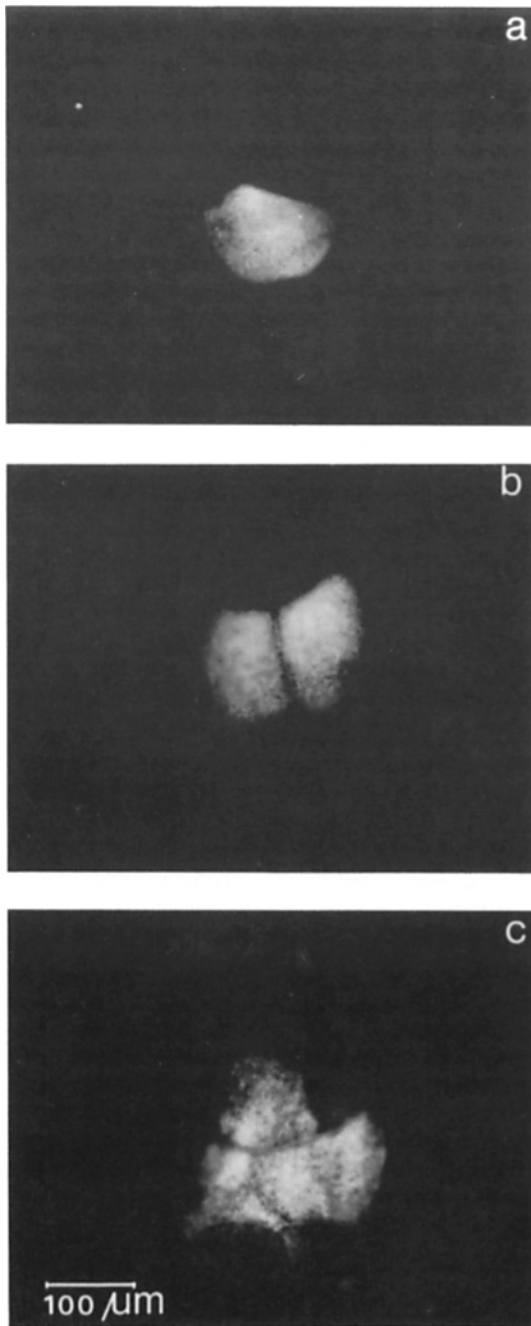
### ***Dye Transfer to Sister Cells is Cell Cycle Modulated***

Lucifer yellow injections performed at different times in the cell cycle gave one of three results. Either there was (a) no dye transfer (Fig. 1 a); (b) rapid, strong dye transfer to only one cell, which was always identifiable as the sister cell from the previous division (identifiable because all cells that were injected were followed from before this division; see above); or (c) rapid dye transfer to the sister cell and also weaker, slower, dye transfer to one or more other, nonsister cells (Fig. 1 c). In most cases of nonsister cell coupling, Lucifer yellow was transferred to one nonsister cell (see legend to Fig. 3). FITC-dextran was either not transferred or was transferred to a sister cell only (Fig. 1 b; see below).

Lucifer yellow transfer to sister cells was, of course, cell cycle modulated, both in the seventh and tenth cell cycles. We found that injecting cells between 0 and 10 min after division gave dye transfer to  $\sim 100\%$  of sister cells in both of these cell cycles, whereas injecting near the end of the cycle (after 10 min in the seventh and after 20 min in the tenth cell cycle) gave little or no dye transfer. Injecting between 10 and 20 min in the tenth cell cycle gave an intermediate coupling frequency (see Fig. 2, a and c). It is, of course, expected that sister cell coupling reflects the persistence of cytoplasmic bridges that connect sister cells after cell division and are present for the first part of the next cell cycle. There may, however, also be some sister cell coupling via gap junctions. This possibility was examined for the seventh cell cycle, where the cells can easily be injected via pressure injection, by comparing injection with FITC-dextran, which only passes cytoplasmic bridges and not gap junctions, with injection with Lucifer yellow, which passes both. FITC-dextran showed similar timing for transfer to sister cells as Lucifer yellow (compare Fig. 2, a with b), indicating that most, or all, of the Lucifer yellow transfer to sister cells during the seventh cell cycle occurs via cytoplasmic bridges. Gap junctional coupling can, however, be examined unambiguously by following Lucifer yellow transfer to nonsister cells, and we examined it in this way (below).

### ***Dye Transfer to Nonsister Cells Is Cell Cycle Modulated***

Lucifer yellow was transferred not only to sister cells but also

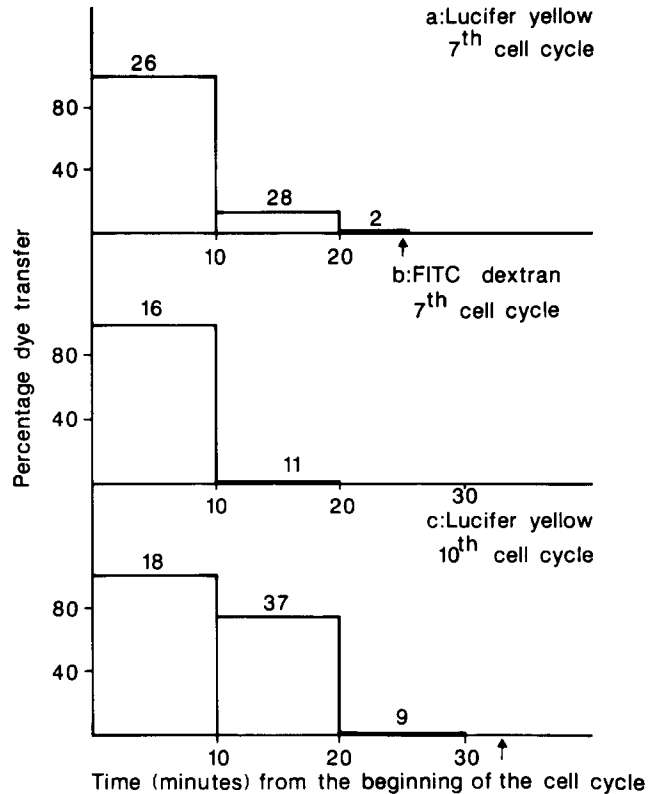


**Figure 1.** Examples of results obtained in the dye injection experiments. (a) No dye transfer. Lucifer yellow was injected at 20 min after cell division in the tenth cell cycle. (b) Transfer to a sister cell only. FITC-dextran was injected at 0 min after cell division in the tenth cell cycle. (c) Transfer to a sister cell and to a nonsister cell. Lucifer yellow was injected at 0 min after cell division in the tenth cell cycle.

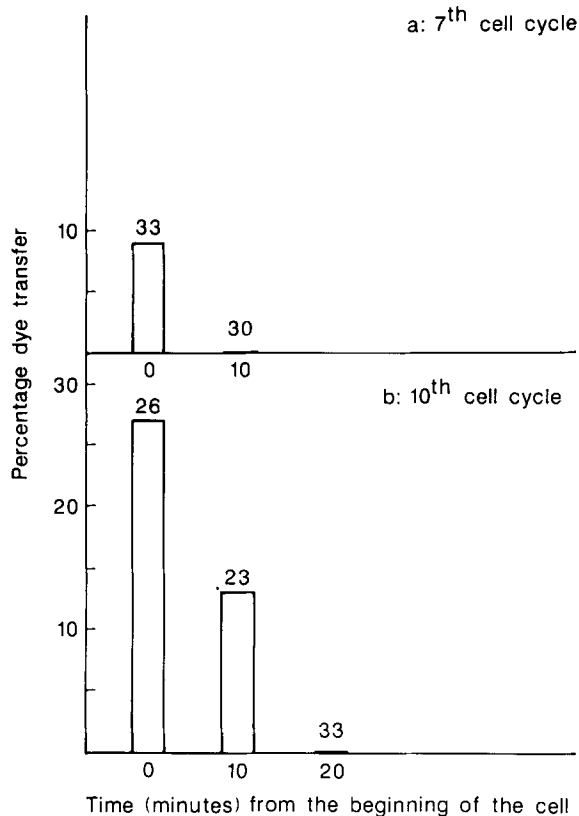
to nonsister cells (above and as also observed by others [9, 10, 25]). Nonsister cell coupling was never observed after FITC-dextran injection (see above), and, since we also observed that sister cell coupling ceases completely before the end of each cell cycle (Fig. 2, a and c), we do not think that nonsister cell coupling can be accounted for by the persistence of cytoplasmic bridges for more than one cell cycle. It almost certainly reflects dye transfer via gap junctions. This

conclusion is also supported by the fact that a cell cycle modulation of nonsister cell coupling during the tenth cell cycle correlates with a cell cycle modulation in the density of morphologically detectable gap junctions (see below).

Nonsister cell coupling was followed, as described above, after injecting cells with Lucifer yellow at different times



**Figure 2.** Lucifer yellow and FITC-dextran transfer to sister cells during the cell cycle. (a) Lucifer yellow was injected at intervals during the seventh cell cycle. The figure shows the frequencies of Lucifer yellow transfer to sister cells from cells injected between 0 and 10, between 10 and 20, and >20 min after the beginning of this cell cycle. Lucifer yellow transfer to sister cells was significantly less frequent from cells injected between 10 and 20 min than from cells injected between 0 and 10 min after the beginning of the cycle ( $\chi^2$  test,  $P < 0.005$ ) and it disappeared by the end of this cell cycle. (b) FITC-dextran was injected at intervals during the seventh cell cycle. The figure shows the frequencies of FITC-dextran transfer to sister cells from cells injected between 0 and 10 and between 10 and 20 min after the beginning of this cell cycle. FITC-dextran was transferred frequently from cells injected between 0 and 10 min but never from cells injected between 10 and 20 min. The difference is significant ( $\chi^2$  test,  $P < 0.005$ ). (c) Lucifer yellow was injected at intervals during the tenth cell cycle. The figure shows the frequencies of Lucifer yellow transfer to sister cells from cells injected between 0 and 10, 10 and 20, and 20 and 30 min after the beginning of this cell cycle. Lucifer yellow was transferred significantly less frequently from cells injected between 20 and 30 min than from cells injected between 0 and 10 or between 10 and 20 min ( $\chi^2$  tests,  $P < 0.005$ ). The numbers above the histogram bars indicate the number of embryos injected with dye for each measurement. The arrows indicate the mean duration of the cell cycle in each case. The cell cycle length was  $25.2 \pm 1.5$  min (9 measurements) for the seventh cell cycle and  $32.8 \pm 2.1$  min (11 measurements) for the tenth cell cycle.



**Figure 3.** Lucifer yellow transfer to nonsister cells during the cell cycle. (a) Lucifer yellow was injected at  $T_0$  (0–2 min) and at  $T_{10}$  (10–12 min) after the beginning of the seventh cell cycle. The figure shows the frequencies of dye transfer to nonsister cells from cells injected at these times. This was not detectable after injection at  $T_{10}$  and detectable after injection at  $T_0$ . The difference between the transfer frequencies at  $T_0$  and  $T_{10}$  is just not significant ( $\chi^2$  test,  $0.05 < P < 0.10$ ). (b) Lucifer yellow was injected at  $T_0$  (0–2 min),  $T_{10}$  (10–12 min), and  $T_{20}$  (20–22 min) after the beginning of the tenth cell cycle. The figure shows the frequencies of dye transfer to nonsister cells, as above. Lucifer yellow transfer to nonsister cells is significantly less frequent for cells injected at  $T_{20}$  than for cells injected at  $T_0$  ( $\chi^2$  test,  $P > 0.005$ ). The numbers above the histogram bars show the number of embryos injected with Lucifer yellow for each measurement. Injected cells were observed for 15 min after injection and were scored as positive if they showed nonsister cell coupling within this time. Dye was transferred to only one nonsister cell in all cases except for two of the injections (7.6%) at  $T_0$  in the tenth cell cycle, where dye was clearly transferred to at least two nonsister cells within the 15-min observation period.

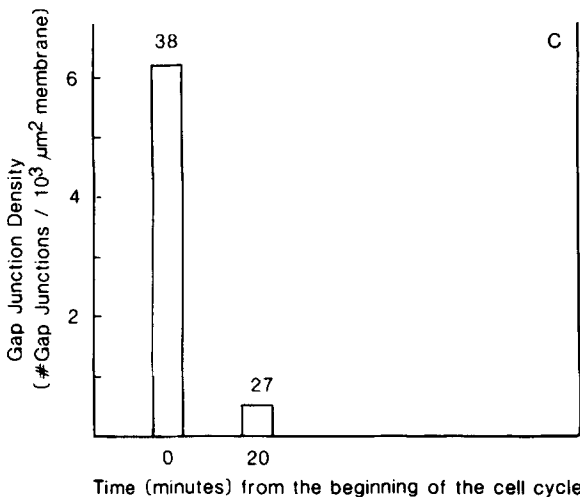
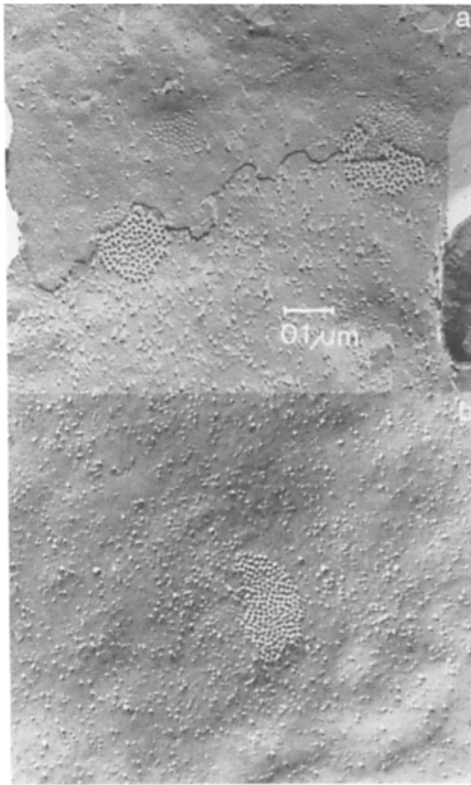
during the seventh and tenth cell cycles, as described in Materials and Methods. In these experiments, the injected cells had to be followed for 15 min to detect the weak dye transfer to nonsister cells. The results are shown in Fig. 3, which shows that nonsister cell coupling occurs less frequently in cells injected late in the tenth cell cycle (at  $T_{10}$  and  $T_{20}$ ) than in cells injected early in this cell cycle (at  $T_0$ ). There may also be a similar difference between injection at  $T_0$  and  $T_{10}$  in the seventh cell cycle, but nonsister cell coupling is infrequent in this cycle and the difference observed was just not significant ( $\chi^2$  test,  $P < 0.10$ ). We conclude that dye coupling via gap junctions is cell cycle modulated, in *Xenopus* animal cap cells, during the tenth cell cycle of development.

### The Abundance of Gap Junctions in the Plasma Membrane Is Cell Cycle Modulated

The cell cycle modulation of gap junctional dye coupling reported above could either reflect modulation of the size or abundance of gap junctions or else modulation of the permeability of existing gap junctional channels (e.g., via pH,  $Ca^{++}$ , or cAMP). The first possibility was tested by using freeze-fracture electron microscopy to measure the sizes and abundance of gap junctions in the plasma membrane. We examined intercellular membranes close to the center of the animal cap after fixing embryos near the beginning of the tenth cell cycle (0–2 min after division;  $T_0$ ) or near the end of it (20–22 min after division;  $T_{20}$ ). 20 embryos were fixed at  $T_0$  and 20 at  $T_{20}$  in the tenth cell cycle. These embryos were then processed, as described in Materials and Methods, to obtain freeze-fracture replicas from intercellular plasma membranes running roughly perpendicular to the surface of the embryo and close to the center of the animal cap. We could not establish any further details of these replicas (whether they were from membranes in the surface layer or in deeper layers of the animal cap nor whether they were from membranes between sister cells or between nonsister cells). The freeze-fracture replicas were collected on grids which were first screened systematically at low magnification (2,000–5,000 $\times$ ) to find pieces of membrane; this procedure yielded between 1 and 15 membrane fragments, in 18 out of 20 grids from the  $T_0$  embryos and in 17 out of 20 grids from the  $T_{20}$  embryos. The membrane fragments were then screened systematically at high magnification ( $\sim 30,000\times$ ) to identify gap junctions, which were identified as clusters of  $>20$  intramembranous particles (IMPs), showing a typical hexagonal lattice arrangement (see Fig. 4, a and b). The gap junctions were then counted and summed for each class of embryos, and the areas of the membrane fragments were also measured and summed for each class. This enabled calculation of a mean gap junction density (number of gap junctions per 1,000  $\mu m^2$  membrane) for the  $T_0$  and  $T_{20}$  membrane fragments (Fig. 4 b). The sizes of the gap junctions were also measured by counting the number of IMPs per gap junction.

This analysis showed that gap junctions were present in the plasma membrane at a sixfold higher mean density at  $T_0$  (six gap junctions per 1,000  $\mu m^2$ ) than at  $T_{20}$  (one gap junction per 1,000  $\mu m^2$ ) in the tenth cell cycle of *Xenopus* development (Fig. 4 b). There was no significant difference between the mean size of the gap junctions in the  $T_0$  membranes ( $149 \pm 104$  IMPs per gap junction) compared with the mean size of those in the  $T_{20}$  membranes ( $113 \pm 46$  IMPs per gap junction). As stated in Materials and Methods, there were limitations to the precision with which we were able to locate the plasma membranes used for this analysis, but we do know that these membranes, just like those examined for dye coupling (above), are intercellular membranes between cells close to the middle of the animal cap, examined at precisely timed moments during the tenth cell cycle. These data thus suggest that the cell cycle-modulated dye coupling that we observed in the tenth cell cycle of *Xenopus* development is due to, or is contributed to, by cell cycle modulation of the abundance of gap junctions. The numbers of gap junctions per unit area and the fractional surface areas covered by gap junctions in our study are slightly lower than were found in a

1. Abbreviation used in this paper: IMP, intramembranous particle.



**Figure 4.** Freeze-fracture analysis of the abundance of gap junctions during the tenth cell cycle. (a) Photo of a freeze-fracture replica of membrane from an embryo fixed at  $T_0$  in the cell cycle. The replica shows the E face as well as the P face of the membrane, with gap junctions on both faces. (b) A freeze-fracture replica of membrane from an embryo fixed at  $T_{20}$  min in the cell cycle, showing a gap junction on the P face. (c) Mean densities of gap junctions in freeze-fractured membranes from embryos fixed at  $T_0$  and  $T_{20}$  in the cell cycle. Freeze-fractured membranes were scanned for gap junctions as described in the text. The data are based on 38 gap junctions observed in 17 replica grids with a total membrane area of  $5,893 \mu\text{m}^2$  for  $T_0$  and on 27 gap junctions observed in 18 replica grids with a total membrane area of  $20,825 \mu\text{m}^2$  for  $T_{20}$ . The  $T_{20}$  density is significantly lower than the  $T_0$  density ( $\chi^2$  test,  $P < 0.005$ ). The numbers of IMPs in the  $T_0$  and  $T_{20}$  gap junctions were also counted. These were  $149 \pm 104$  and  $113 \pm 46$ , respectively. On the basis that gap junctions contain 104 junctional particles/

previous study of Chinese hamster cells (27) and much lower than in a previous study of Novikoff hepatoma cells (20).

## Discussion

The first main conclusion from this investigation is that gap junction-dependent dye coupling is strongly cell cycle modulated, in surface animal pole cells of the *Xenopus* embryo, during the tenth cell cycle of development. Coupling is available at the beginning but not at the end of this cell cycle. Our observations admit the possibility that gap junction-dependent dye coupling is also cell cycle modulated in the *Xenopus* seventh cell cycle.

The gap junction-dependent dye coupling frequencies that we observed overlap with, but are generally lower than, analogous frequencies measured by others in 16–256-cell *Xenopus* embryos, in recent studies using Lucifer yellow (9, 10, 25). Direct comparisons are impossible, however, because we injected cells at a hitherto untested position (the middle of the animal cap) and because one of the stages that we examined (the tenth cell cycle) had never previously been examined for Lucifer yellow coupling.

Our conclusion (above) applies to dye transfer among one particular subpopulation of embryonic cells, surface cells in the middle of the animal cap in the tenth cell cycle *Xenopus* embryo. Whether cell cycle modulation is a general feature of dye coupling remains to be determined from analysis of other systems.

Our second main conclusion is that cell cycle modulation of gap junctional dye coupling in the tenth cell cycle correlates with a modulation in the abundance of morphologically detectable gap junctions. Gap junctions are six times more abundant (and cover a 6.4 times bigger fractional area) in *Xenopus* animal pole intercellular membranes fixed near to the beginning of the tenth cell cycle than in similar membranes fixed near to the end of this cell cycle. It is thus probable that the cell cycle-modulated dye coupling that we observed is due to, or contributed to, by cell cycle-modulated abundance of gap junctions.

The sixfold difference between the gap junction densities at  $T_0$  and  $T_{20}$  is greater than could be accounted for by "dilution" of gap junction-bearing membrane via insertion of new membrane during the cell cycle. These data suggest that gap junctions are assembled early and disassembled late in the cell cycle. Our freeze-fracture replicas showed no other phenomena that are associated with or expected from assembly or disassembly of gap junctions (no strings of IMPs [11, 22], partly formed gap junctions, nor a significant size difference between the gap junctions seen at  $T_0$  and  $T_{20}$ ). However, we were only able to examine a small sample of gap junctions, and these were sampled only at two times during the cell cycle. A thorough investigation of this point is required.

Our findings above focus attention on modulated abun-

$\mu\text{m}^2$  (20), the data indicate that  $0.5662 \mu\text{m}^2$  of membrane (0.0096% of the total) is covered by gap junctions at  $T_0$  and that  $0.305 \mu\text{m}^2$  of membrane (0.0015% of the total) is covered with gap junctions at  $T_{20}$ .

dance of gap junctions as a factor leading to cell cycle–modulated dye coupling. It is not unlikely, however, that other factors are also important. For example, rounding up of cells during early cleavage may break gap junctional channels. It is also known that intracellular pH, which shows cell cycle oscillations in many cells including early *Xenopus* cells (1, 26), influences the permeability of gap junctions (10) and can regulate their disassembly (28). It should be noted, however, that the intracellular pH oscillations measured in early *Xenopus* cells (26) are small compared with the intracellular pH shifts that have been shown to affect gap junctional dye coupling (10).

Our conclusions about gap-dependent dye coupling (above) were based on measurements of dye coupling between nonsister cells. It is therefore certain that new gap junctions are inserted into preexisting plasma membranes early in the cell cycle, not just into the new membrane that forms between sister cells during cytokinesis. We do not know whether or not there is a complex temporal sequence of events that regulates the insertion of gap junctions into intercellular membranes—e.g., whether gap junctions are inserted specifically into new intercellular membrane between cousin cells—in the second cell cycle after the membrane was formed. We sometimes observed nonsister cell dye coupling from each of a pair of sister cells to one neighbor, such that the quartet of labeled cells had mirror image symmetry, suggesting that gap junction–mediated dye transfer can occur preferentially to cousins. A recent publication also reports that nonsister cell coupling at the 64-cell stage occurs preferentially within sectors of four cousin cells, which are descendants of single blastomeres at the 16-cell stage (10). We have evidence against the idea that such nonsister cell coupling is due to persistence of cytoplasmic bridges for more than one cell cycle (see Results).

Making our measurements of Lucifer yellow coupling to sister cells through the seventh and tenth cell cycles made us aware of a problem in interpreting dye coupling experiments in early embryos. We used these data to calculate an average frequency for dye coupling in each of these cycles. This was ~50% in each case. The coupled period is longer in the tenth than in the seventh cell cycle, but the tenth cell cycle is also proportionally longer than the seventh cell cycle. These values contrasted strongly with preliminary data, obtained in an earlier experiment, which seemed to show a considerable increase in sister cell coupling between the same developmental stages. The explanation for the disparity is that we, like others, staged *Xenopus* embryos on the basis of cell number. In the early experiment, we collected small batches of synchronous embryos as they reached a desired developmental stage (64 cells, the beginning of the seventh cell cycle, or 512 cells, the beginning of the tenth cell cycle). We then positioned the batch of embryos and injected them, the whole procedure taking ~10–15 min. Embryos were thus always injected ~10–15 min after the beginning of the cell cycle and had more chance of being injected with the 15–20-min coupled period in the tenth cell cycle than in the 10–15-min coupled period in the seventh cell cycle. We note that gap junctional coupling is also subject to this artifact and that a recent publication reports a stage-dependent increase in gap junctional coupling between the seventh and eighth cell cycles (10).

Our measurements above were made using cells at only

one position in the embryo (the middle of the animal cap) but they may be relevant for understanding the development of patterns of intercellular communication during embryogenesis. It caught our attention that the spatial differences that arise in dye coupling in the *Xenopus* embryo (9, 10, 25) correlate to some extent with the onset of spatial differences in the cell cycle in the early embryo (5, 15, 16, 18). Similar correlations also exist between spatial coupling differences and cell cycle changes in the other embryos (1, 2, 6, 7, 8, 12, 13, 14, 19, 23, 24), and it may be worth investigating whether such correlations reflect causality: i.e., whether local cell cycle changes lead to uncoupling between different regions of the embryo or vice versa.

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