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Gap junction assembly in the preimplantation mouse conceptus is independent of microtubules, microfilaments, cell flattening, and cytokinesis

(membrane channels/dye transfer/embryo aggregates/nocodazole/cytochalasins)

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ABSTRACT **Gap junctions first appear during compaction in the eight-cell stage of mouse development. Their assembly can be initiated in the near absence of transcription and protein synthesis from the four-cell stage, indicating the existence of preformed precursors. We have investigated the temporal control of this event, focusing on the possible involvement of the cytoskeleton, cell flattening, and cytokinesis. Embryos in various cleavage stages were treated with cytochalasins, to disrupt microfilaments and block cell flattening, cytokinesis, or both, or nocodazole, to promote microtubule depolymerization. To assess their capacity to initiate gap junction assembly after such treatments, the embryos were then aggregated with communication-competent, compacted embryos that had been labeled with carboxyfluorescein diacetate. Passage of the fluorescent dye, carboxyfluorescein, from labeled to unlabeled embryo was taken as evidence that interembryonic junction formation had occurred. The capacity to assemble gap junctions was acquired at the normal time by embryos prevented by cytochalasin treatment from undergoing cell flattening or any cytokinesis from fertilization onward. Likewise, treatment with nocodazole beginning in the four-cell or early eight-cell stage did not interfere with gap junction assembly. Neither drug affected the inability of four-cell embryos to assemble gap junctions prematurely. We conclude that intact microfilament or microtubule networks are not required for gap junction assembly in this system, nor do they restrain junctional precursors from assembling prematurely. Furthermore, the timing of gap junction assembly is not linked to cell flattening, cytokinesis, or cell number.**

One of the most striking features of embryogenesis in animals is the precise timing of developmental events: within a given species or strain, a particular biochemical or morphological change can be expected to occur at a precise time after fertilization (or, in some cases, ovulation) in virtually all individuals developing at that temperature. This regularity has allowed the construction of "normal tables" of development for a variety of animals. However, the basis for the temporal control of developmental events is at present only vaguely understood. Recent findings indicate that cell cycle parameters are important components of the timing mechanism governing embryogenesis, but even within a given system, there may not be a single "clock" that governs all events (1).

To explore the temporal regulation of morphogenetic events during mouse embryogenesis we have chosen to study the assembly of intercellular membrane channels (gap junctions). These channels are among the simplest of cellular organelles, consisting of pairs of hexagonal units, the connexons, aligned between adjacent, closely apposed cells and each providing a narrow pore through which inorganic ions and small metabolites can pass from cell to cell. The connexon pairs aggregate in the plane of the plasma membranes to form a plaque, which is the morphological entity seen in the electron microscope as the gap junction (2). The protein subunit of the connexon has recently been isolated and antibodies prepared against it (3, 4). Gap junctions are widely believed to play a significant role in developmental processes, although the nature of this role is yet to be defined (5). The most important consideration for our purposes is that the initiation of gap junction assembly is a precisely timed event in mouse embryogenesis (6). Gap junctions first appear in the eight-cell stage, beginning about 3 hr after completion of the third cleavage (7–11). Gap junction assembly is associated with the cell flattening that accompanies compaction, although the timing of these two processes is not strictly correlated (11). Experiments with inhibitors of transcription or protein synthesis have demonstrated that at least a limited number of junction channels can form in the absence of gene expression from the four-cell stage, implying the existence of a pool of gap junction precursors (10, 11). These findings remove gene expression from consideration as a direct agent in the timing of gap junction assembly during compaction.

With the experiments described in the present report, we have sought to examine the involvement of the cytoskeleton, cell flattening, and cell division (cytokinesis) in the assembly of gap junctions. Using the microtubule poison nocodazole, we investigated the role of microtubules in this morphogenetic event. We have also treated "embryos" (i.e., preimplantation concepti) with the microfilament poisons cytochalasins B and D, beginning at various times after fertilization, to prevent them from undergoing cell flattening in the eight-cell stage or cytokinesis in the first, second, or third cell cycle. The capacity of embryos to assemble gap junctions at the appropriate time after these various treatments was assessed by aggregating them with other embryos containing the fluorescent dye carboxyfluorescein and noting whether the dye was transmitted from the labeled to the unlabeled embryo (8). Our results make it clear that gap junction assembly does not require intact networks of microtubules or microfilaments and that the timing of gap junction assembly is not related to either cell flattening or cytokinesis but is strictly dependent on the developmental age of the zygote.

MATERIALS AND METHODS

Zygotes and preimplantation embryos were collected, using procedures described previously (10, 11), from superovulated, random-bred female mice (strain CD-1, Charles River Canada, St. Constant, PQ or Ha/ICR, West Seneca

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Abbreviations: CF, carboxyfluorescein; CFDA, CF diacetate; DAPI, 4′,6-diamidino-2-phenylindole.

Laboratories, Roswell Park Memorial Institute, Buffalo, NY) mated with CB6F1/J males (The Jackson Laboratory). Zonae pellucidae were removed by brief immersion in acidified Tyrode's solution (11). Embryos to be labeled with fluorescent dye were then transferred through four drops of Dulbecco's phosphate-buffered saline (PBS) containing 0.3% polyvinylpyrrolidone and carboxyfluorescein diacetate (CFDA; Molecular Probes, Junction City, OR) at 40 or 100 μg/ml. The embryos were kept in the last drop for 20 min. After several rinses, labeled and unlabeled embryos were aggregated without the use of lectins (10) and then returned to the incubator for 7–9 hr (see *Results*) before being checked for interembryonic dye transfer.

Nocodazole and cytochalasins (all from Sigma) were dissolved in dimethyl sulfoxide to make stock solutions (1 mg/ml), which were added to culture medium to give a final concentration of 3 μg/ml for nocodazole, 10 μg/ml for cytochalasin B, and 0.5 μg/ml for cytochalasin D. To test the long-term effects of these treatments on nuclear division, embryos were incubated for 30 min in medium containing the DNA-binding fluorescent dye 4',6-diamidino-2-phenylindole (DAPI) dihydrochloride (Polysciences, Warrington, PA) at 10 μg/ml and then rinsed several times (12).

RESULTS

Embryo Aggregation as an Assay for the Capacity to Assemble Gap Junctions. The aim of these experiments was to examine several parameters that could influence the timing of gap junction assembly in early mouse embryos. In our previous work we had used ionic coupling measurements as well as fluorescent dye microinjection to detect the presence of intercellular membrane channels (10, 11). Both of these techniques have practical limitations (discussed in ref. 6). The CFDA/embryo aggregation method introduced by Goodall and Johnson (8) provides a simpler means of testing the capacity of embryos to assemble membrane channels after various treatments. This method is more sensitive than dye injection, approaching the sensitivity of ionic coupling (9), and allows a greater number of embryos to be tested. We began by monitoring the time course of dye transfer between aggregated eight-cell embryos.

Our first experiments involved incubating embryos in CFDA at 40 μg/ml. This membrane-permeant, nonfluorescent molecule is readily taken up by cells, after which it is cleaved by esterases to yield the hydrophilic fluorescent dye carboxyfluorescein (CF), which remains trapped inside the cells (8). We aggregated early, uncompacted eight-cell embryos with each other, as well as compacted eight-cell embryos with each other; in each case, one member of the pair was labeled, the other not. As shown in Fig. 1, almost 20% of aggregates showed interembryonic dye transfer after only 3 hr, and 50–65% showed dye transfer after 7 hr. In subsequent experiments the concentration of CFDA was increased to 100 μg/ml to produce brighter fluorescence in the labeled embryo and thus improve the detectability of dye transfer. This resulted in an increase in the percent of 7-hr aggregates showing dye transfer to around 80% (Table 1; Fig. 2 *a* and *b*). Further incubation of aggregates did not significantly improve the extent of interembryonic dye transfer after about 9 hr; hence, in all subsequent experiments 7–9 hr was the standard time interval for the assay. Dye transfer never occurred between embryos that were not in physical contact, even though they were in close proximity. Furthermore, labeled embryos maintained their fluorescence intensity for many hours, indicating that nonjunctional permeability to CF is low. After being scored for dye transfer some aggregates were returned to the incubator for further development so that deleterious effects of the dye or handling procedures could be monitored. The vast majority of aggregates (mean ± SD = 0.94 ± 0.04, n = 27) went on to form blastocysts after another day in culture, indicating that the assay procedure had not compromised their viability.

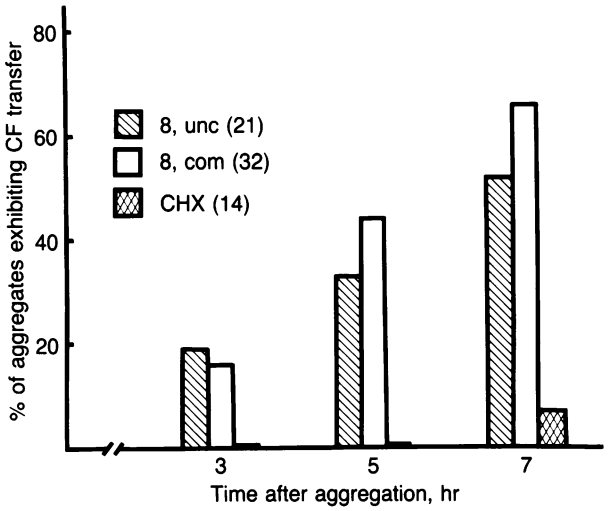

FIG. 1. Time course of CF transfer between aggregated eight-cell mouse embryos. Embryos had been incubated in CFDA at 40 μg/ml. Aggregates were scored for visible dye transfer at 3, 5, and 7 hr after aggregation. The three bars at each time point represent, from left to right, aggregates of uncompacted eight-cell embryos, eight-cell compacted embryos, and cycloheximide-treated compacted embryos, with the number of aggregates given in parentheses. Cycloheximide treatment (50 μg/ml) was begun 3 hr prior to zona removal and continued throughout the aggregation procedure.

One additional finding (Fig. 1) is that the assembly of membrane channels between aggregated embryos can be blocked by cycloheximide. Compacted eight-cell embryos were incubated in cycloheximide-containing medium (50 μg/ml) for 3 hr prior to zona removal, CFDA labeling, and aggregation, as well as during the 7-hr culture period after aggregation. This treatment almost completely abolished interembryonic dye transfer. The assembly of new membrane channels between embryos must therefore require new protein synthesis, a conclusion reached previously from ionic coupling measurements (10).

Involvement of Microfilaments and Cell Flattening in Gap Junction Assembly. The initiation of cell flattening, a component of compaction, immediately precedes the appearance of gap junctions in the eight-cell stage (11). Maintenance of cell flattening, however, is not required to maintain intercellular coupling via membrane channels once established (10, 13). Cell flattening and cytokinesis are microfilament-dependent processes; both are reversibly blocked by treatment with cytochalasins, which have been shown by several investigators to disrupt the normal distribution of actin in mouse blastomeres (14–16). Given these facts, we set out to determine if microfilament function is an essential component of gap junction assembly and whether extensive cell apposition brought about by cell flattening provides the trigger for this event. Early eight-cell embryos that had not yet shown any signs of cell flattening were held in medium containing cytochalasin B at 10 μg/ml for 1 hr after zona removal, then aggregated with CFDA-labeled, compacted eight-cell embryos. The aggregation and subsequent culture were carried out in the continued presence of the drug, which caused decompaction of the labeled embryos (Fig. 2*c*). The frequency of interembryonic dye transfer to embryos that had been prevented from undergoing cell flattening was not significantly different (according to paired t tests) from the frequency in the control groups, which consisted of aggregates of age-matched, untreated, compacted eight-cell embryos (Table 1, series A). Furthermore, the dye that was

Table 1. CFDA/embryo aggregation assay for assembly of interembryonic membrane channels after various treatments

Series	Type of aggregate*	No. of trials	No. of aggregates	Fraction showing dye transfer
A	8/fc + 8/fc	10	92	0.78 ± 0.07
	8/fc + 8/uc(CB)	10	115	0.69 ± 0.12
B	8/fc + 8/fc	15	197	0.78 ± 0.05
	8/fc + 4(CB)	6	68	0.76 ± 0.05
	8/fc + 2(CB)	5	70	0.69 ± 0.01†
	8/fc + 1(CB)	6	126	0.78 ± 0.03
	4(CB) + 4(CB)	6	53	0.73 ± 0.15
	4(CB) + 2(CB)	5	72	0.70 ± 0.06
	4(CB) + 1(CB)	6	127	0.75 ± 0.04
	2(CB) + 2(CB)	4	67	0.64 ± 0.01†
	2(CB) + 1(CB)	6	122	0.68 ± 0.03†
	1(CB) + 1(CB)	6	120	0.63 ± 0.03†
C	8/fc + 8/fc	2	24	0.80
	8/fc + 4(CB)	2	48	0
D	8/fc + 8/fc	12	236	0.76 ± 0.04
	8/fc + 8/uc(NOC)	4	44	0.79 ± 0.03
	8/fc + 4(NOC)	5	103	0.72 ± 0.03
	4(NOC) + 4(NOC)	5	98	0.70 ± 0.02
E	8/fc + 8/fc	2	14	0.79
	8/fc + 4(NOC)	2	14	0

Results are presented as mean ± SD.

*The CFDA-labeled embryo in each pair is printed first; 8, 4, 2, and 1 refer to cleavage stage (no. of cells); fc, fully compacted; uc, uncompacted; (CB), cytochalasin B-treated; (NOC), nocodazole-treated. In series A, B, and D both embryos of each pair were the age of compacted eight-cell embryos. In series C and E the drug-arrested four-cell embryos were aggregated immediately, without waiting for untreated embryos from the same batch to cleave and compact.

†Significantly different from control aggregates ($P < 0.05$).

transferred was always seen to permeate the entire recipient embryo, including those blastomeres that were not in direct contact with the labeled donor. This indicates that intraembryonic, as well as interembryonic, membrane channels had formed in the presence of cytochalasin and the absence of cell flattening. We conclude that an intact microfilament network is not required for gap junction assembly and that this morphogenetic event is not triggered by cell flattening.

Involvement of Cytokinesis in the Timing of Gap Junction Assembly. Since intercellular membrane channels can form in the presence of cytochalasin, this drug can be used to interrupt cytokinesis in order to test the role of this cell cycle parameter in the timing of junction assembly. These experiments were very similar to those described in the previous section except that cytochalasin treatment was begun earlier, so that various cleavage cycles were blocked in addition to blocking cell flattening. We also tested aggregates in which both embryos had been treated with the drug prior to aggregation. Some of these experiments necessitated culturing zygotes through the first cleavage and beyond; we were fortunate to have discovered by chance that zygotes from CD-1 or Ha/ICR females developing in our culture system do not usually suffer a two-cell block. It remains to be determined why our experience with this notorious obstacle should be so different from that of others; possibly some component used in our medium is responsible.

The results can be summarized succinctly: continuous treatment with cytochalasin B beginning in any cleavage cycle after fertilization does not prevent embryos from acquiring the capacity to assemble membrane channels at the appropriate time, although the fraction of aggregates showing dye transfer was slightly reduced in certain groups (Table 1, series B and Fig. 2 *d* and *e*). As with the previous experiments, dye passing into a recipient embryo always permeated the entire embryo. We also found that it is not necessary for one of the members of the aggregate to remain untreated prior to aggregation. Even cleavage-blocked one-cell zygotes can be aggregated when they have reached the age of compacted controls, and membrane channels will appear, allowing dye to pass between them. It was predominately these groups, in which both embryos had been cytochalasin treated from the one- or two-cell stage, that exhibited a significant reduction in the frequency of dye transfer; we believe this reflects our inability to recognize at such early stages those embryos (about 10% of the population) that are destined to become arrested before the eight-cell stage. Using the DAPI stain for DNA, we verified that cleavage-blocked embryos become binucleate (Fig. 2 *f* and *g*), as reported by others (14, 15, 17, 18). Thus, although cytokinesis was prevented, at least one more nuclear multiplication cycle occurred regardless of the cell number at the start of cytochalasin treatment. Aggregates that, after scoring for dye transfer, were transferred to fresh medium lacking cytochalasin underwent immediate cell flattening and resumed cleaving. These experiments were repeated, with a smaller number of trials, using cytochalasin D at 0.5 μg/ml, with the same results (not shown).

A point that must be emphasized is that successful dye transfer between cleavage-blocked embryos occurred only when both embryos in the aggregate were the same age as compacted eight-cell embryos: four-cell embryos (60 hr after human chorionic gonadotropin) treated with cytochalasin for 4 hr, then aggregated immediately with compacted eight-cell embryos (without waiting for controls of the same batch to cleave and compact), were not capable of assembling interembryonic membrane channels (Table 1, series C). This result confirms earlier failures to elicit premature gap junction assembly by blastomere or embryo aggregation (8, 10), and it indicates that the inability of four-cell stage blastomeres to assemble functional gap junctions despite the presence of junctional precursors is not due to constraints on gap junction assembly imposed by the microfilament network.

Involvement of Microtubules in Gap Junction Assembly. Since the assembly of interembryonic membrane channels

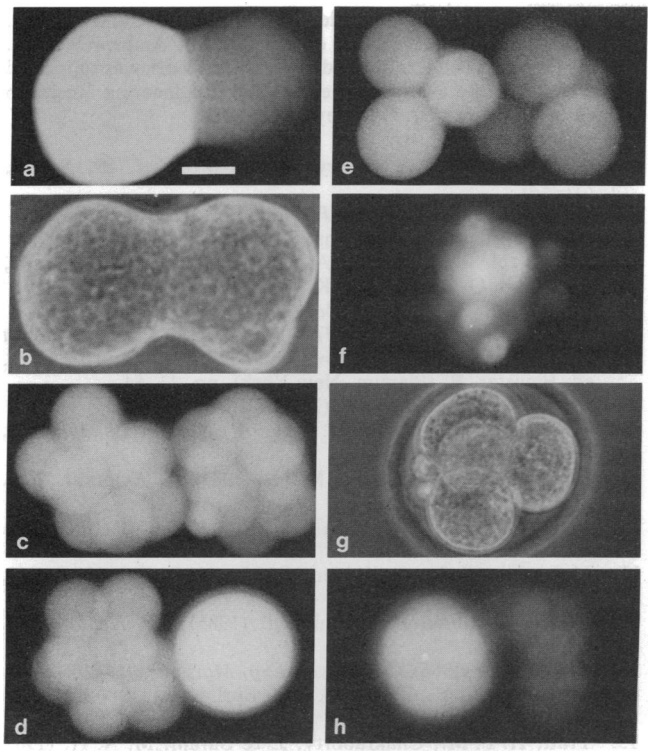

FIG. 2. Interembryonic dye transfer or nuclear number after various treatments. Fluorescence and phase-contrast images of a control aggregate (compacted eight-cell) are shown in *a* and *b*, respectively; the scale bar indicates 20 μm and applies to all parts of the figure. In *c* the recipient embryo (on the right) was prevented by cytochalasin treatment from initiating cell flattening, whereas the donor embryo has decompacted due to the continued presence of the drug. Embryos prevented from undergoing cytokinesis after fertilization were also able to pass dye to age-matched, communication-competent recipients (*d*). Part *e* illustrates dye transfer from one cytochalasin-blocked four-cell embryo to another, both being chronologically the same age as compacted eight-cell embryos. In *f* and *g*, respectively, are shown fluorescence and phase-contrast images of a cytochalasin-treated four-cell embryo stained with DAPI to reveal two nuclei in each blastomere. In *h* the recipient embryo had been treated since the four-cell stage with nocodazole but was chronologically the age of compacted controls at the time of aggregation.

presumably involves the transport and insertion of newly synthesized components into previously unapposed membrane regions, we wanted to test the involvement of microtubules in this process. We treated embryos with nocodazole, a drug that has been shown by immunocytochemistry to be very effective in promoting microtubule depolymerization in mouse embryos (19). In addition to treating uncompacted eight-cell embryos, we treated some embryos in the four-cell stage, so that prevention of the third cleavage could provide visual proof that the drug was indeed working. As with the cytochalasin experiments, the drug treatment was continued until age-matched controls had reached the compacted eight-cell stage, at which point the aggregation step was carried out; nocodazole was retained in the culture medium until the aggregates were scored for dye transfer. As expected, nocodazole treatment arrested cell division (confirmed by DAPI staining) but did not reverse cell flattening. The treatment had no significant effect on the capacity of embryos to assemble either interembryonic or intraembryonic membrane channels (Table 1, series D, and Fig. 2*h*). We conclude that an intact network of microtubules is not required for gap junction assembly. Nor do microtubules exercise a restraint on premature assembly: attempts to elicit interembryonic dye transfer to cleavage-blocked four-cell embryos prior to the time when age-matched controls had begun to compact met with failure (Table 1, series E). In this experiment, four-cell embryos (52–60 hr after human chorionic gonadotropin) were treated with nocodazole for 4 hr prior to aggregation with compacted controls. It appears that microtubules have no involvement in the initiation of gap junction assembly in this system.

DISCUSSION

The initiation of gap junction assembly is a precisely timed event in preimplantation mouse development (6), and as such is a useful model for exploring both the control of morphogenesis at the cellular level and the means by which intercellular membrane channels are assembled. We had demonstrated previously that this event, which takes place in the mid-eight-cell stage, can occur on schedule even when transcription or protein synthesis has been blocked from the four-cell stage (10, 11). Under these circumstances, however, the level of intercellular coupling achieved is well below normal, indicating that the store of preformed gap junction components present in four-cell embryos is limited and is normally augmented by new synthesis (11). It is this new synthesis that presumably provides the connexon subunits that are used to assemble interembryonic membrane channels when previously unapposed cell surfaces are brought together by embryo aggregation.

The CFDA/embryo aggregation method provides a simple means of assessing the capacity of embryos to assemble gap junctions after various types of manipulation. In untreated embryos this capacity develops a few hours after completion of the third cleavage, which is just when gap junctions actually appear (8, 9, 11). This correspondence suggests that embryos utilize the capacity to assemble gap junctions as soon as that capacity is acquired. It is not clear, however, whether interembryonic junction assembly proceeds by the same mechanism that governs the assembly of membrane channels between blastomeres within an embryo. Whereas gap junction assembly within embryos can be initiated in the presence of cycloheximide, interembryonic junction assembly cannot be (ref. 10 and Fig. 1). We interpret this to mean that the assembly of gap junctions between embryos involves the insertion of newly synthesized components into the plasma membrane rather than the mobilization of connexons from elsewhere. The initiation of gap junction assembly within embryos could involve both of these mechanisms.

Despite the existence of a pool of gap junction precursors in four-cell embryos, all attempts to evoke premature gap junction assembly by aggregation of two- or four-cell embryos or blastomeres with communication-competent eight-cell embryos or blastomeres have failed (8–10). Thus the initiation of gap junction assembly is somehow restrained before the onset of cell flattening in the eight-cell stage. We thought it reasonable that cytoskeletal elements might exert such a restraining influence. Both microtubules and microfilaments have been implicated in such a role, since treatment of somatic tissues with either colchicine or cytochalasin has been shown to promote the proliferation and expansion of gap junctions (20, 21). However, neither cytochalasin nor nocodazole could confer competence for gap junction assembly on four-cell embryos, leading us to conclude that these elements do not exert restraint to control the timing of this event in mouse embryos. This may be because the junctional precursors present in four-cell embryos do not reside in the plasma membrane and hence do not become more mobile when cytoskeletal interactions with the membrane are disrupted (21). Nor are microtubule or microfilament networks required for gap junction assembly when this process is triggered to begin (Table 1, series A, B, and D). Since both microtubules and microfilaments are involved in generating the polarized distribution of endosomes that develops in

eight-cell mouse blastomeres (22), one might have expected these drugs to interfere with the delivery of newly synthesized connexon subunits to apposed cell surfaces. In this respect our results are comparable to those of Salas *et al.* (23), who found that targeting of viral proteins to specific plasma membrane domains in MDCK cells does not depend on intact microtubules or actin microfilaments. Another interesting finding from our experiments with nocodazole is that, unlike late eight-cell blastomeres, which uncouple from one another when blocked in mitosis (24), four-cell embryos prevented from undergoing mitosis can initiate gap junction assembly after reaching the proper age. Thus, the initiation of gap junction assembly must be independent of the preceding mitosis, whereas the maintenance of intercellular coupling via existing gap junctions later on in that and succeeding cell cycles is directly dependent on the mitotic cycle.

What does trigger the initiation of gap junction assembly in the eight-cell stage? The most obvious candidate, the cell shape change (cell flattening) that accompanies compaction and results in extensive cell apposition, has been ruled out by our cytochalasin experiments as well as by the recent work of Goodall (13). He treated early eight-cell embryos with a monoclonal antibody that blocks components of the cell–cell adhesion system, thus preventing cell flattening; intercellular coupling was established nonetheless. Furthermore, both our results and those of Goodall (13) demonstrate that maintenance of extensive cell apposition is not a requirement for continuous coupling via gap junctions once established. Having ruled out transcription, protein synthesis (10, 11), microtubules, microfilaments, and cell flattening as direct agents in the timing of gap junction assembly in the eight-cell stage, we must turn our attention to cell cycle events. Cytokinesis, mitosis, and DNA replication are the most obvious cyclic events in early embryos that could contribute to a developmental timing mechanism, perhaps by linking morphogenetic events to cell number, number of nuclear divisions, or DNA-to-cytoplasm ratio. Our experiments have eliminated cytokinesis or cell number as controlling the timing of gap junction assembly, just as these parameters have been ruled out as influencing the timing of compaction and cavitation (15, 25–27). On the other hand, DNA replication continues in embryos continuously exposed to cytochalasin (27), and recent evidence suggests that this process may play a critical role in the timing of events associated with compaction: DNA replication in the two-cell stage, but not in the four- or eight-cell stage, must occur at the normal time in order for changes in cell shape and polarity to occur (28). In addition, DNA replication or the DNA-to-cytoplasm ratio has been clearly implicated in the timing of developmental events in early embryos of ascidians and *Xenopus* (29–31). By providing the precursors and enzymes for DNA synthesis in the early cleavages and by controlling cyclic biochemical events (32) that govern the timing of DNA replication, the cytoplasm of the activated egg could control the timing of preimplantation development, a suggestion consistent with recent nuclear transplantation results (33).

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1. Satoh, N. (1985) *J. Embryol. Exp. Morphol.* **89,** Suppl., 257–270.
2. Loewenstein, W. R. (1981) *Physiol. Rev.* **61,** 829–913.
3. Hertzberg, E. L. & Skibbens, R. V. (1984) *Cell* **39,** 61–69.
4. Dermietzel, R., Leibstein, A., Frixen, U., Janssen-Timmen, U., Traub, O. & Willecke, K. (1984) *EMBO J.* **3,** 2261–2270.
5. Caveney, S. (1985) *Annu. Rev. Physiol.* **47,** 319–335.
6. Kidder, G. M. (1987) in *The Mammalian Preimplantation Embryo: Regulation of Growth and Differentiation In Vitro*, ed. Bavister, B. (Plenum, New York), pp. 43–64.
7. Lo, C. W. & Gilula, N. B. (1979) *Cell* **18,** 399–409.
8. Goodall, H. & Johnson, M. H. (1982) *Nature (London)* **295,** 524–526.
9. Goodall, H. & Johnson, M. H. (1984) *J. Embryol. Exp. Morphol.* **79,** 53–76.
10. McLachlin, J. R., Caveney, S. & Kidder, G. M. (1983) *Dev. Biol.* **98,** 155–164.
11. McLachlin, J. R. & Kidder, G. M. (1986) *Dev. Biol.* **117,** 146–155.
12. Park, C., Kimler, B. & Smith, T. (1985) *Exp. Hematol.* **13,** 1039–1043.
13. Goodall, H. (1986) *J. Embryol. Exp. Morphol.* **91,** 283–296.
14. Surani, M. A. H., Barton, S. C. & Burling, A. (1980) *Exp. Cell Res.* **125,** 275–286.
15. Pratt, H. P. M., Chakraborty, J. & Surani, M. A. H. (1981) *Cell* **26,** 279–292.
16. Johnson, M. H. & Maro, B. (1984) *J. Embryol. Exp. Morphol.* **82,** 97–117.
17. Petzoldt, U., Burki, K., Illmensee, G. R. & Illmensee, K. (1983) *Rouxs Arch. Dev. Biol.* **192,** 138–144.
18. Petzoldt, U. (1984) *Cell. Differ.* **15,** 163–167.
19. Maro, B. & Pickering, S. J. (1984) *J. Embryol. Exp. Morphol.* **84,** 217–232.
20. Rassat, J., Robenek, H. & Themenn, H. (1982) *Cell Tissue Res.* **223,** 187–200.
21. Tadvalkar, G. & Pinto da Silva, P. (1983) *J. Cell Biol.* **96,** 1279–1287.
22. Fleming, T. P., Cannon, P. M. & Pickering, S. J. (1986) *Dev. Biol.* **113,** 406–419.
23. Salas, P. J. I., Misek, D. E., Vega-Salas, D. E., Gundersen, D., Cereijido, M. & Rodriguez-Boulan, E. (1986) *J. Cell Biol.* **102,** 1853–1867.
24. Goodall, H. & Maro, B. (1986) *J. Cell Biol.* **102,** 568–575.
25. Kimber, S. J. & Surani, M. A. H. (1981) *J. Embryol. Exp. Morphol.* **61,** 331–345.
26. Watson, A. & Kidder, G. M. (1986) *J. Cell Biol.* **103,** 88a (abstr.).
27. Petzoldt, U. (1986) *Dev. Biol.* **113,** 512–516.
28. Smith, R. K. W. & Johnson, M. H. (1985) *J. Embryol. Exp. Morphol.* **89,** 133–148.
29. Satoh, N. & Ikegami, S. (1981) *J. Embryol. Exp. Morphol.* **61,** 1–13.
30. Newport, J. & Kirschner, M. (1982) *Cell* **30,** 675–686.
31. Newport, J. & Kirschner, M. (1982) *Cell* **30,** 687–696.
32. Howlett, S. K. (1986) *Cell* **45,** 387–396.
33. Surani, M. A. H., Barton, S. C. & Norris, M. L. (1986) *Cell* **45,** 127–136.