Major Loss of Junctional Coupling during Mitosis in Early Mouse Embryos

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Abstract. Junctional coupling was assessed during the transition from the fourth to the fifth cell cycle of mouse embryogenesis by injection of the dye carboxyfluorescein and by measurement of electrical continuity between cells. Junctional coupling, which arises de novo in early 8-cell mouse embryos, subsequently becomes reduced towards the end of the cell cycle as the blastomeres enter into mitosis. Arrest of the cell cycle in metaphase by nocodazole, an inhibitor of tubulin polymerization, reveals that cell coupling becomes undetectable at mitosis. Junctional coupling then is resumed during interphase of the 16-cell stage. Nocodazole itself has no effect on junctional coupling in interphase cells, regardless of the extent of intercellular flattening, whereas taxol, a microtubule-stabilizing agent, does reduce the extent of coupling in interphase cells.

Most non-excitable cells can communicate with their neighbors by means of specialized structures called gap junctions which span their apposed membranes and exist in localized plaques that exhibit a characteristic gap of ~2 nm in cross-section (for reviews see Loewenstein, 1981; Perrachia, 1980; Spray, 1985). These junctions are impermeable to extracellular substances but, by virtue of the small, regularly sized channels that pass through them, allow both metabolic and physiological coupling of the cell interiors by diffusion of low molecular weight components. These structures perform a vital homeostatic role in many tissues but little is known of their regulation except that internal Ca²⁺ (Loewenstein, 1981) or pH (Spray and Bennett, 1985) appears to modulate their activity. External influences such as hormones (Lawrence et al., 1978; Radu et al., 1982) or direct innervation (Schneider-Picard et al., 1984) may modulate the activity of gap junctions but neither cell adhesion nor the extent of physical intercellular contact appears to be a critical regulatory factor (Goodall, 1985). Many studies have been performed to determine the extent of coupling in groups of cells but it is not known whether coupling is modulated according to the stage in the cell cycle. In particular the mitotic phase of the cell cycle involves changes in the levels (Keith et al., 1985; Poenie et al., 1985; Schantz, 1985) and organization (Silver et al., 1980) of internal Ca²⁺ that might be incompatible with the continued presence of free intercellular communication. Investigations on the behavior of coupling junctions during mitosis has yielded contradictory results. Electrical coupling was detected between mitotic and interphase mouse 3T3 fibroblasts (O'Lague et al., 1970) and gap junctions have been identified ultrastructurally between such stages among granulosa cells in the mouse ovary (Merk and McNutt, 1972). However, the loss of junctions in the regenerating rat liver has been shown to coincide with the peak in the mitotic index (Myer et al., 1981; Yee and Revel, 1978) and further work has revealed that this is due to degradation of gap junctions (Traub et al., 1983).

The mouse preimplantation embryo is a convenient system with which to resolve this problem because of the size and accessibility of its blastomeres. Gap junctions arise for the first time during the 8-cell stage (Ducibella et al., 1975; Lo and Gilula, 1979; Magnuson et al., 1977; Goodall and Johnson, 1982; Goodall and Johnson, 1984) at a time when extensive morphological changes are taking place resulting in cell polarization (Calarco and Epstein, 1973; Ducibella and Anderson, 1975; Ducibella et al., 1977; Handyside, 1980; Reeves, 1981; Reeves and Ziemek, 1981; Johnson and Maro, 1984; Maro et al., 1985) and extensive cell apposition (Lehtonen, 1980). Subsequently these cells round up as they approach the division to the 16-cell stage (Fig. 1, this paper; Lehtonen, 1980; Maro and Picketing, 1984).

This study was designed to show whether junctions are functional during the mitotic phase of the cell cycle by measuring the extent of passage of ionophoretically injected dye and electric current between blastomeres of known age. In the course of this study, we have investigated the effects on junctional coupling of two different microtubule inhibitors: nocodazole, which inhibits tubulin polymerization (Hoebbeke et al., 1976), and taxol, which stabilizes microtubules (Schiff et al., 1979). This has enabled us to block the cells in mitosis, thus avoiding the problems caused by the transience and asynchrony of division among the blastomeres of each em-
Embryos were cultured routinely under 5% CO₂ at 37°C in medium 16 (M16: after Whittingham, 1971). When embryos were manipulated under air, medium 2 (M2) (Whittingham, 1971) was used.

Composition of medium 16 is as follows: 94.70 mM NaCl, 4.78 mM KCl, 1.19 mM MgSO₄, 1.19 mM KH₂PO₄, 1.71 mM CaCl₂, 25.00 mM NaHCO₃, 23.3 mM sodium lactate, 0.33 mM sodium pyruvate, 5.56 mM glucose, 100 U/ml penicillin, 50 µg/ml streptomycin sulfate, 0.001% phenol red, pH 7.4.

Composition of medium 2 is as for M16 except that (a) NaHCO₃ was reduced to 4.00 mM and (b) HEPES was added to a concentration of 21.00 mM.

**Collection of Embryos**

MF1 female mice (laboratory bred, OLAC derived, 3–5 wk old) were superovulated by injection of 5 IU of pregnant mare’s serum gonadotrophin (Intervet Laboratories Ltd, Cambridge, UK) and human chorionic gonadotrophin (In-tervet) 48 h apart. The females were paired overnight with HC-CFLP males (Hacking & Churchill, Alconbury, UK) and inspected for vaginal plugs the next day.

Embryos were recovered as a mixture of 2- and 4-cell stages by flushing the oviducts at 48 h after injection of human chorionic gonadotrophin with M2 containing 4 mg/ml bovine serum albumin (M2 + BSA). Embryos were then cultured overnight in M16 containing 4 mg/ml bovine serum albumin (M16 + BSA). Staged embryos were obtained by selecting newly formed 8-cell embryos at hourly intervals between 65 and 70 h after injection of human chorionic gonadotrophin. Blastomeres flattened upon one another 5–7 h after the division from the 4-cell stage (Fig. 1). Embryos that were to be assayed for junctional coupling as they approached mitosis were cultured for 10–12 h after division to the 8-cell stage and inspected for rounding up of blastomeres as they decompacted at entry into mitosis (Fig. 1). After mitosis, the 16-cell blastomeres flattened upon one another during the first hour of the following cell cycle (Fig. 1).

In the rest of this communication we will refer to time after division as being the time elapsed since the division to the 8-cell stage.

**Treatment of Embryos**

To test the effects of a microtubule inhibitors on junctional coupling, groups of embryos were cultured from division to 8-cells in 24 µM taxol (National Institutes of Health, lot T-4-112), 10 µM nocodazole (Aldrich Chemical Co., Milwaukee, WI) or control medium (M16 + BSA). All three categories were scored for coupling during interphase over the period 8–10 h after division to the 8-cell stage. At that time control embryos are compacted, nonmitotic, and show extensive junctional coupling (Goodall and Johnson, 1984; Maro and Pickering, 1984).

A further population of embryos was cultured in M16 + BSA for 8 h from division and then cultured further in nocodazole (10 µM) in order to arrest them in mitosis. As this event approached, the blastomeres rounded up in the normal way but did not proceed further and the embryos were assayed for coupling between 10 and 14 h after division. To ensure that no irreversible changes had occurred to the embryos as a result of this treatment, some of them were removed from nocodazole after a mitotic arrest and cultured in M16 + BSA alone for a further 4–6 h. During this period, the blastomeres divided, flattened upon one another again, and were then assayed for junctional coupling. After removal of the nocodazole block, embryos were able to reach the blastocyst stage in culture.

As a control for the possibility that nocodazole itself might prevent coupling between blastomeres only when they were well-rounded up and had only focal contact with their neighbors, embryos were cultured from division to 8-cell in a mixture of nocodazole (10 µM) and ECCD-1 (1:50 dilution), a monoclonal antibody preventing surface adhesion between cells but known not to prevent the onset of cell coupling (Shirayoshi et al., 1983; Goodall, 1986; Johnson et al., 1986). These interphase embryos were assayed for coupling when age-matched controls were fully compacted, ~8 h after division.

**Preparation of Embryos for Coupling Studies**

Falcon plastic dishes (35 mm) were prepared by cutting out slots on opposite sides to enable horizontal entry of microelectrodes. A solution of polystyrene latex (Sigma Chemical Co., St. Louis, MO; 0.2 ml; 1 mg/ml in H₂O) was spread over the dish surface and dried at 37°C and the dishes were stored subsequently over dessicant. Immediately before use, 1 ml of a solution containing 0.1 mg/ml concanavalin A (Miles Laboratories Inc., Elkhart, IN) in phosphate-buffered saline (PBS) was spread over the dish, left for 5–10 min, washed with two rinses of distilled water followed by two rinses of M2 and finally with M2 containing the appropriate agent(s) when necessary (nocodazole, taxol, ECCD-1). Between 5 and 10 embryos were then pipetted onto the center of the dish where they remained adherent. The protein-free M2 was finally replaced by M2 + BSA containing the appropriate agent. The preparation was maintained at 28°C on the water-heated stage of a Leitz Ortholux microscope.

**Investigation of Junctional Coupling**

**Microscopy.** Bright-field illumination (×160) was used for placement of electrodes while the output from a 2 W Argon ion laser (Spectra-Physics, St Albans, UK) was routed into an incident light illuminator (vertical-POI, Leitz, Wetzlar, FRG) with an appropriate chromatic beam splitter for transmission of fluorescent emission. A Zeiss 50 filter enabled precise cut off of transmission below 500 nm. The laser output was maintained at 200 mW for the duration of the photographic exposures. Photographs were taken on Kodak Tri-X film with a Leica M3 camera.

**Electrophysiology.** Carboxyfluorescein (Eastman Kodak, Rochester, NY) was first injected ionophoretically into a single blastomeres of each embryo for Table I. Resting Potentials in Groups of Embryos during the Various Experimental Conditions

<table>
<thead>
<tr>
<th>Treatment</th>
<th>Number of embryos</th>
<th>Membrane potentials (mV)</th>
<th>p value*</th>
</tr>
</thead>
<tbody>
<tr>
<td>Interphase embryos</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Control</td>
<td>30</td>
<td>-10/-30</td>
<td>-18.7 + 4.9</td>
</tr>
<tr>
<td>Nocodazole</td>
<td>20</td>
<td>-10/-35</td>
<td>-18.2 + 7.7</td>
</tr>
<tr>
<td>Taxol</td>
<td>24</td>
<td>-10/-25</td>
<td>-15.3 + 4.5</td>
</tr>
<tr>
<td>ECCD-1</td>
<td>16</td>
<td>-12/-28</td>
<td>-18.3 + 4.5</td>
</tr>
<tr>
<td>ECCD-1 + Nocodazole</td>
<td>34</td>
<td>-10/-30</td>
<td>-17.8 + 6.0</td>
</tr>
<tr>
<td>Recovery from block</td>
<td>11</td>
<td>-12/-30</td>
<td>-19.2 + 5.5</td>
</tr>
<tr>
<td>Mitotic embryos</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Control</td>
<td>30</td>
<td>-10/-30</td>
<td>-17.8 + 5.1</td>
</tr>
<tr>
<td>Nocodazole block</td>
<td>25</td>
<td>-10/-24</td>
<td>-15.3 + 3.8</td>
</tr>
</tbody>
</table>

* To assess the statistical significance of the observed differences, the non-parametric Mann-Whitney U test was used. All experimental groups were compared to the interphase control embryos except the nocodazole block group which was compared to the control mitotic embryos.

Figure 1. Schematic representation of the morphology of mouse embryos between the late 4-cell stage and the early 16-cell stage. Time scale is in hours following division to the 8-cell stage (corresponding to the age of the 8-cell embryo).
Figure 2. (a-c) Control compacted 8-cell embryo (8–10 h old) showing (a) extensive cell apposition, (b) ionic coupling, and (c) complete transmission of injected carboxyfluorescein over the 10-min injection period. (d–f) Mitotic 8-cell embryo (10–12 h old) showing (d) decompacted mitotic blastomeres, (e) a residual degree of ionic coupling, and (f) presence of injected carboxyfluorescein in only one cell. (g–i) Nocodazole-arrested mitotic 8-cell embryo (12 h old) showing (g) absence of intercellular flattening (h), ionic coupling, and (i) carboxyfluorescein transmission. All bright-field and fluorescence photographs are at the same magnification. Bar in a, 50 μm. The oscilloscope traces (b, e, and h) indicate (upper trace) hyperpolarizing current pulse; (middle trace) combined signal from electrode and injected cell (this was limited at the lower end of its excursion and a period of 10 min and the embryos were inspected for further spread of the dye. If little or no dye spread occurred, the extent of electrical coupling was then measured by insertion of one current-injecting electrode and placement of a recording electrode in at least four other blastomeres. This procedure ensured that blastomeres coupled by persistent mid-bodies alone (which allow up to no more than four cells to be coupled [Goodall and Johnson, 1984]) would not be classified as showing gap junctional coupling. Control compacted embryos could be assayed only by the extent of carboxyfluorescein spread since the cell boundary could not be seen well enough for multiple impalement studies.

Electrodes were pulled from 1.5 mm, fiber-loaded glass tubing (GC 150F-15; Clarke Electro-medical Instruments, Pangbourne, UK) on a horizontal

Figure 3. Diagrams showing the extent of dye and electrical coupling in populations of compacted interphase 8-cell embryos (upper histograms), 8–10 h old; mitotic 8-cell embryos (center histograms), 10–12 h old; and nocodazole-arrested mitotic 8-cell embryos (lower histograms), 12–14 h old.

was used only for inspection of the resting potential in the cell); (lower trace) perceived signal from remote cell. Horizontal bar, 0.5 s; vertical bar, 5 nA (current signal) or 10 mV.
Figure 4. (a-f) Taxol-treated (24 μM, 8–10 h) 8-cell embryos (8–10 h old) showing absence of intercellular flattening (a and d). Junctional coupling is absent in one of them (b and c) while ionic coupling (e) and transmission of injected carboxyfluorescein in four blastomeres (f) over the 10-min injection period can be observed in the other. (g–h) Nocodazole-treated (10 μM, 8–10 h) 8-cell embryo (8–10 h old) showing (g) extensive cell apposition and (h) carboxyfluorescein transmission. All bright-field and fluorescence photographs are at the same magnification. Bar in a, 50 μm. The oscilloscope traces (b and e) indicate (upper trace) hyperpolarizing current pulse; (middle trace) combined signal from electrode and injected cell; (lower trace) perceived signal from remote cell. Horizontal bar, 0.5 s; vertical bar, 5 nA (current signal) or 10 mV.

Figure 5. Diagrams showing the extent of dye and electrical coupling in populations of 8-cell embryos, 8–10 h old. One group was cultured in control medium (upper histogram), while the experimental groups were treated with 24 μM taxol (center histograms) or 10 μM nocodazole (lower histogram).

pipette puller (Campden Instruments, London, UK) to a resistance of 70–90 Megohms when filled with 0.2 M KCl. For dye injection, the tips were back-filled with a solution of 5 mg/ml carboxyfluorescein that had been dissolved at pH 10 and then adjusted to pH 7.4 in 0.2 M KCl (Goodall and Johnson, 1984) while the shaft was filled with 0.2 M KCl. Recording electrodes were filled with 0.2 M KCl. These electrodes were mounted on silver/silver chloride wires on preamplifiers that were themselves held by Leitz micromanipulators.

A “Neurolog” system (Digitimer, Welwin Garden City, UK), comprising an NL303 period generator, NL403 delay width module, and two NI02 preamplifier/injection modules, was used in these experiments. Dye and current injection were performed using 0.5-s hyperpolarizing current pulses with 0.5-s intervals, at a magnitude of 2 nA. Electrode entry into each cell was achieved by using a brief pulse of maximum capacitance compensation after the electrodes had been placed on the cell surface. Embryos were used that showed a maintained minimum resting potential of ~10 mV. Few significant differences in resting potential were observed between the various experimental and control groups (Table I). Only the interphase taxol-treated embryos and those blocked in mitosis with nocodazole showed significant depolarization in resting potential compared with control embryos indicating that artifactual loss of the signal through leakage past the electrode was unlikely. Moreover, similar small inter-experiment variations were observed when groups of control embryos were compared. No attempt was made to balance out that component of the signal from the injected cell due to the electrode resistance both due to the limitations of the chosen system (see Goodall and Johnson, 1984) and also, but more important, because rapid identification of routes of coupling was required in each sample. The circuit was completed with two 2% Agar/0.2 M KCl Ag/
Results

As compacted 8-cell embryos began to decompact, just before mitosis (Maro and Pickering, 1984), the rounding up of their blastomeres was associated with a great reduction in the ability of carboxyfluorescein to pass between blastomeres (Fig. 2, a, c, d, and f and Fig. 3). However, the blastomeres were still coupled by gap junctions as shown by the extent of electrical coupling (Fig. 2, b and e and Fig. 3). As blastomeres began division to 16 cells, the cell outline became irregular and dye was never seen to progress beyond the mitotic sister while electrical continuity was just discernible between some blastomeres only. In particular, within six transitional embryos where 1/8 and 1/16 blastomeres could be identified unambiguously and impaled, 1/8 blastomeres allowed electrical continuity with other 1/8 blastomeres in 3 out of 3 cases, but showed coupling with 1/16 blastomeres in only 2 out of 6 cases, while 1/16 blastomeres showed electrical coupling with each other in 3 out of 5 cases. These results indicated that there was some change in the extent of cell coupling as the cell entered mitosis; however, it was not possible to judge unequivocally whether complete uncoupling was systematic since (a) division is both transitory and asynchronous within cell entered mitosis; however, it was not possible to judge unequivocally whether complete uncoupling was systematic since (a) division is both transitory and asynchronous within a single embryo and (b) we cannot rule out the existence of persisting midbodies that would couple some 1/16 blastomeres and 1/8 blastomeres as well as other 1/16 blastomeres.

One way to overcome that problem was to block the cells in mitosis. This can be achieved by use of microtubule inhibitors such as nocodazole, which induces a loss of microtubules by inhibiting tubulin polymerization (Hoebeke et al., 1976), or taxol, which stabilizes microtubules (Schiff et al., 1979). In both cases, the rounded mitotic 8-cell blastomeres do not divide and can be impaled with relative ease.

First it was essential to investigate whether either of these drugs exerted a direct uncoupling effect on junctions independent of any effect they might have via mitotic arrest. Therefore, early 8-cell embryos were placed in the drugs, cultured to the late 8-cell stage (but not as far as mitosis), and junctional coupling was assessed. Taxol proved not to be a suitable drug, since it reduced the spread of the dye in most embryos and prevented the onset of extensive electrical coupling in more than half of them (Fig. 4, a–e and Fig. 5). In addition taxol caused a reduction in the rate and number of embryos showing complete cell flattening (Fig. 4, a and d and Fig. 6; Maro and Pickering, 1984). In contrast, nocodazole allowed dye coupling among interphase blastomeres to the same extent as controls and flattening was normal (Fig. 4, g and h and Fig. 5).

Compacted embryos were therefore treated with nocodazole and allowed to enter mitosis. They began to decompact and their blastomeres rounded up at entry into mitosis but they did not proceed further, remaining arrested in metaphase with condensed chromosomes and lacking nuclear membranes. Under these conditions, not only was the spread of the injected dye restricted to the mitotic sister cell still connected by the mid-body from the previous division (from the 4- to the 8-cell stage), but also the extent of electrical coupling was now restricted almost completely (Fig. 2, g–i and Fig. 3).

Blastomeres arrested in mitosis were not flattened. To confirm that the uncoupling was not due merely to the reduction in cell apposition, embryos were cultured in the monoclonal antibody ECCD-1 which inhibits and reverses flattening in 8-cell embryos without modifying cell coupling (Fig. 7, a and b and Fig. 8). Finally, to show that nocodazole was not exerting an effect on junctional communication only in fully rounded up cells, interphase embryos were exposed to both nocodazole and ECCD-1. Under these conditions no reduction in the extent of either dye or ionic coupling was seen (Fig. 7, c–e and Fig. 8).

When embryos blocked in a mitotic stage by nocodazole were removed from the drug and cultured further in its absence, they resumed cell division and flattened. Such embryos were assayed for junctional coupling 5–6 h after removal of the drug. The results could not be put on a rigorously quantitative basis as they could for the 8-cell stage since it was impossible to count accurately those cells that were or were not labeled at this stage. However, of 15 embryos injected, eight (54%) showed dye spread through the entire embryo within the 10-min injection period (Fig. 8, f and g), five (33%) showed dye spread throughout the entire embryo without modifying cell coupling (Fig. 7, a and b and Fig. 8). Finally, to show that nocodazole did not exert an effect on junctional communication only in fully rounded up cells, interphase embryos were exposed to both nocodazole and ECCD-1. Under these conditions no reduction in the extent of either dye or ionic coupling was seen (Fig. 7, c–e and Fig. 8).

Discussion

It is clear from the data presented here that as 8-cell embryos prepare for cell division to the 16-cell stage and round up, they reduce the transmission capabilities of their junctions. Thus the ability of blastomeres to transmit carboxyfluorescein is reduced even though ionic coupling persists. This "down regulation" does not result simply from reduction in contact area due to the rounding up of blastomeres, as use of the monoclonal antibody ECCD-1 (Shirayoshi et al., 1983) reduces apposition between interphase cell to a comparable degree and yet does not prevent the spread of carboxyfluorescein in the 10-min assay period. Indeed ECCD-1 does not even prevent the formation of junctions at the early 8-cell stage, possibly the phase of blastomere junctional activity most vulnerable to external manipulation (Goodall, 1985). While it was very difficult to identify stages of mitosis in

Figure 6. Effect of 24 μM taxol on intercellular flattening in 8-cell mouse embryos. The results are expressed as the percentage of embryos showing flattening versus time in hours since division to the 8-cell stage.
Figure 7. (a and b) 8-cell embryo cultured for 8–10 h in the presence of monoclonal antibody ECCD-1 showing (a) absence of intercellular flattening and (b) complete transmission of injected carboxyfluorescein over the 10-min injection period. (c–e) 8-cell embryo cultured for 8–10 h in the presence of ECCD-1 monoclonal antibody and 10 μM nocodazole showing absence of intercellular flattening, (d) presence of ionic coupling, and (e) complete transmission of injected carboxyfluorescein. (f–g) 16-cell embryo arrested at mitosis of the 8-cell stage by 10 μM nocodazole and allowed to overcome the block by removal of the drug for 5 h showing (f) intercellular flattening and (g) carboxyfluorescein transmission. All bright-field and fluorescence photographs are at the same magnification. Bar in a, 50 μm.

The oscilloscope traces (b, e, and h) indicate (upper trace) hyperpolarizing current pulse (0.5 s every s); (middle trace) combined signal from electrode and injected cell; (lower trace) perceived signal from remote cell. Horizontal bar, 0.5 s; vertical bar, 5 nA (current signal) or 10 mV.

Figure 8. Diagrams showing the extent of dye and electrical coupling in populations of 8-cell embryos: ECCD-1 treated embryos, 8–10 h old (upper histogram) and embryos treated with both ECCD-1 and 10 μM nocodazole, 8–10 h old (lower histograms).

individual blastomeres within a dividing embryo, the use of nocodazole-induced mitotic arrest of the embryos confirmed the absence of detectable junctions during prolonged metaphase. The restoration of coupling after removal of nocodazole and the completion of cell division to the 16-cell stage indicated that no irreversible change induced by the drug has occurred. We were able to exclude the possibility that the loss of junctional activity was due to nocodazole itself, either alone or in conjunction with reduced cell apposition (ECCD-1 being present), by the absence of effect of the drug on junctional coupling in interphase embryos. Moreover, it is clear that in both mitosis and ECCD-1 treatment, areas of intercellular contact still persist since the embryos remain enclosed within the zona pellucida. Since ECCD-1 inhibits the only adhesion system functional at this stage, it seems that a simple apposition of cell membranes, without any maintained adhesion, is sufficient to allow the persistence of cell coupling. It appears likely therefore from our results that a transient down regulation and subsequent inhibition of junctional coupling occurs during normal mitosis. Reduction and disappearance of junctional coupling could occur via the dissemination of junctional particles (Lane and Swales, 1980), the degradation of the junctional protein (Traub et al., 1983), or the closure of...
the junctional channels themselves, possibly due to changes in the levels of intracellular Ca++ or pH. Though little is known of pH changes during mitosis, there is conflicting evidence relating to the levels of free Ca++ at this stage: in cultured epithelial cells, the concentration of free Ca++ appears to fall during mitosis (Keith et al., 1985) while in the first cell division of sea urchin development, free Ca++ levels show several peaks including one at the metaphase/anaphase transition (Poenie et al., 1985). In addition, increased free Ca++ levels have been observed during cytokinesis in embryonic cells of *Oryzias latipes* (Schantz, 1985). Since elevated Ca++ levels are known to be able to turn off gap junctions (Rose and Loewenstein, 1976), then such a mechanism may be important here, particularly as Ca++ seems to be involved also in the regulation of spindle assembly and dynamics. Not only does Ca++ destabilize microtubules, but vesicles with a high Ca++ content have been shown to localize around the spindle fibers during mitosis (Silver et al., 1980). Such a localization of Ca++ makes global measurement of its concentration difficult to interpret, since the local concentration of the ion in the junction area is difficult to assess.

During mitosis, and particularly during the transitions between interphase and M phase, the microtubule network is reorganized completely. In 8-cell blastomeres, the interphase microtubule network is restricted mainly to the cell cortex and to the perinuclear area, while the mitotic spindle is located centrally (Maro and Pickering, 1984). Nevertheless, it is unlikely that microtubules are involved in the regulation of junctional coupling during mitosis. The use of the two microtubule inhibitors, nocodazole and taxol, allowed us to examine this possibility. Nocodazole did not inhibit de novo formation of junctions, while taxol, which was capable of reducing the extent of coupling, was only totally effective in some embryos. Moreover, taxol has rather pervasive effects on the many changes in cell organization that occur during compaction of the 8-cell embryo. Taxol prevents the extensive cytoplasmic reorganization and polarization (Johnson and Maro, 1985), and modulates both the surface reorganization (Maro and Pickering, 1984; Johnson and Maro, 1985) and intercellular flattening (our data and Maro and Pickering, 1984). Prevention of cell flattening at this stage does not, in itself, prevent cell coupling (our data and Goodall, 1986). It is possible that stabilization of the microtubule network by taxol reduces the mobility of some cell surface components leading to a reduction in the ability of gap junctions to form. This is a corollary of the observation that disruption of the cytoskeleton promotes the assembly of gap junctions (Tadvalkar and Pinto da Silva, 1983), which itself is consistent with the inability of nocodazole to affect the onset of junctional coupling in the present study.

In conclusion, because of the large size of the blastomeres, the easy identification of mitotic cells and the long duration of the cell cycle, the early mouse embryo has allowed a detailed study of the functional organization of junctional communication during the cell cycle. At least in this system, we have shown that cells become uncoupled during mitosis by a process that is independent of intercellular flattening and of the levels of polymerized tubulin.

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