REVIEW ARTICLE
Cytoskeletal coordination and intercellular signalling during metazoan embryogenesis

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SUMMARY
This article draws attention to certain recently discovered features of cell surface organization and cytoskeletal deployment that may be revealing a new basis for intercellular signalling during metazoan embryogenesis. It is a signal mode that could coordinate many aspects of 'Entwicklungsmechanik' by spatiotemporal integration of the cytoskeletal/motor network throughout developing tissues. Evidence that this is achieved by 'intercellular cytoskeletal/plasma membrane connecting systems' which coordinate the spatial organization of microtubules, microfilaments, and intermediate filaments in developing animal tissues is critically examined. It is argued that this system does operate but that it is not used to transmit positional information in embryonic fields. However, it probably responds to such information and might play an important part in establishing field boundaries during the very earliest stages of embryogenesis.

Certain aspects of cell surface organization in contemporary protozoans reveal ways in which the Protozoa could have been pre-adapted for the employment of cytoskeletal/cell surface signalling during the advent of multicellularity. In marked contrast, such signalling does not appear to be exploited during plant morphogenesis. The extent to which cytoskeletal organization might be coordinated in siser cells by transmission of spatial instructions during cell division in both animal and plant tissues is also considered.

INTRODUCTION
Specification of tissue shape and size is crucial for successful embryogenesis. Control of the growth and form of developing animal tissues involves the spatially coordinated shaping, orientation, polarization, locomotion, division and positioning of their member cells (Grant, 1978). It transpires that all these activities have a common feature. They are influenced by the spatial layout and mechanics of intracellular cytoskeletons (Wessels et al. 1971; Goldman, Pollard & Rosenbaum, 1976; Burgess & Schroeder, 1979). There is also evidence for cytoskeletal involvement during certain types of cell–cell adhesion (Phillips,
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Fig. 1. Portions of a glial cell process and an adjacent neuron, that is migrating along the process and is wrapped partly around it, showing the alignment of microtubules in these two cell types during foetal monkey brain-cortex development. Based on the micrographs and diagrams of Rakic (1972).

Jennings & Edwards, 1980) and matrix secretion (Ehrlich & Bornstein, 1972), but whether such participation in these two tissue-shape-related activities is widespread remains to be ascertained.

Since cytoskeletons play a large part in defining the shapes and positions of individual tissue cells during embryogenesis it follows that some intercellular spatial coordination of intracellular cytoskeletons may be required, and that some form of intercellular signalling additional to those currently recognized has evolved to effect it. These important possibilities and their main implications for cell and developmental biologists are pursued below.

Tissue cytoskeleton spatiomechanics

If cytoskeletal coordination contributes significantly to tissue shape control it should be possible to detect 'supracellular' cytoskeletal alignments and lattices that correlate spatially with tissue axes and topography. Several arrays that meet these criteria have been described. Some of them are difficult to account for unless cytoskeletal coordination is effected to at least a certain extent by intercellular signalling.

The alignment of actin and myosin filaments in adjacent striated muscle cells has long represented a striking instance of a cytoskeletal arrangement that is spatially coordinated throughout a tissue. Equally pronounced is the alignment of microtubules in adjacent cells during the elongation of closely juxtaposed myoblasts (Warren, 1974) and axons (Yamada, Spooner & Wessels, 1971) as muscles and nerve connexions, respectively, are formed. There are also instances of microtubule alignment in different cell types. For example, this occurs as
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Fig. 2. Schematic diagram showing a tube-shaped portion of epithelium, such as that which forms part of an ovarian follicle or the epidermis of a portion of a developing leg, in certain insects. The alignment of subsurface microtubules is indicated by the thin lines, and cell boundaries by the thicker lines.

neurons migrate along glial cell processes in the cerebral walls of foetal monkeys (Rakic, 1972) (Fig. 1). However, in all these cases alignment need not necessarily be effected by direct transmission of signals between the interiors of the cells in question. They may simply arise because adjacent cells, cell processes, and their cytoskeletons become aligned as a result of orientational responses to local environmental conditions. Alignments might be coordinated, for example, by chemotactic gradients or surface contact responses to previously oriented cells or matrix fibrils. A similar argument applies to cytoskeletal alignments in many epithelia; especially to those in which microtubules are aligned at right angles to the plane of the cell sheet (for example, Burnside, 1971). Such alignment could be set up as a consequence of apicobasal cell polarities induced by different conditions on opposite sides of epithelia.

Certain insect epithelia provide particularly clear examples of microtubule alignment in the plane of a cell sheet (Fig. 2). Microtubules positioned close to the outer surfaces of ovarian follicle cells in certain insects are oriented in the plane of the follicle surface so that they curve around the spheroidal follicle and run at right angles with respect to its longitudinal (polar) axis (Tucker & Meats, 1976). A similar situation occurs during evagination of leg imaginal discs in Calliphora. Microtubules just below the outer surfaces of epidermal cells are oriented around the elongating leg (Figs. 2, 3, 4). Microfilamentous actin cables are also sometimes aligned in neighbouring cells and lie in the plane of a sheet of such cells. Reasonably extensive arrays of this sort have been demonstrated for cell groupings in tissue culture (Albrecht-Buehler, 1979; Lo & Gilula, 1980) (Fig. 5). Whether, like the microtubule arrays described above, such configurations are ever present in the cell layers and sheets of intact embryos and organisms remains to be ascertained. The fact that they are established in tissue culture is probably an indication that this is also the case.
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for some tissues in situ. It is an important issue in the present context because arrays of actin cables (or stress fibres as they are sometimes called) and microtubules are included in mechanochemical systems that alter cell shape by active contraction or tension transmission and by promoting the elongation of whole cells or cell processes. The supracellular alignment of these cytoskeletal arrays that run in the plane of an epithelium is such that they are especially favourably deployed to exert a major influence on spatial variations in the contraction, spreading, curvature, and thickness of a cell sheet or layer of cells and hence on the shaping of tissues that include sheets and layers of cells (i.e. most tissues). There is evidence that cytoskeletons really do provide intercellular motor networks for control of tissue shaping.

The positioning of filaments and microtubules in adjacent cells on either side of desmosomes (Figs. 6, 7) provide striking instances of direct structural coupling between the cytoskeletons of adjacent tissue cells. It is generally agreed that they probably transmit mechanical forces on an extensive intercellular basis to modify and/or maintain tissue shape in a variety of situations. These include desmosome/terminal web complexes in intestinal epithelia (Hull & Staehelin, 1979), alignment and attachment of cardiac muscle actin filaments on either side of desmosomal intercalated discs (Fawcett & McNutt, 1969), and a neurerec-todermal desmosome/microfilament-ring lattice that acts as a coordinated intercellular contractile system during neurulation (Karfunkel, 1974). Some of the ‘intercellularly-aligned’ insect follicle cell microtubules (Fig. 2) are attached to desmosomes where adjacent cells interdigitate (Fig. 7). Fine structural and experimental analyses indicate that they form part of a cytoskeletal system that transmits tension right around a follicle to promote anisometric epithelial expansion and elongation of the enclosed oocyte (Tucker & Meats, 1976; Went, 1978). The oocyte fails to elongate if enclosure by the follicle is prevented although other cytoskeletonally associated events such as nuclear division and migration, and blastoderm formation, sometimes apparently proceed normally.

If some form of intercellular signalling exists so that adjacent tissue cells can interact directly for the purposes of spatiotemporal modulation of cytoskeletal arrangement and mechanochemical activity this would represent a valuable facility for control and coordination of cell and tissue shaping during embryogenesis. Greater architectural complexity and versatility would presumably be possible than in a system that relied entirely on chemotactic gradients or

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Fig. 3. Transmission electron micrograph of a thin section grazing through the apical surface of an epidermal cell in the third tarsomere of a metathoracic leg in the dipteran Calliphora erythrocephala during pupal cuticle secretion. Dense cuticular material borders the surface profile of the cell. Most of the sub-surface microtubules (see Fig. 2) are oriented at right angles with respect to the longitudinal axis of the developing leg (the orientation of this axis is shown by the arrow). × 27000.

Fig. 4. As Fig. 3. × 32000.
the pre-existing orientation of cells and matrix fibrils for cytoskeletal coordination. At a cellular level tissue architecture is very complex and versatile. The fine structure and behaviour of certain regions where cells make contact in tissues and tissue cultures provide substantial indications that surface interactions between adjacent cells actually do make a major contribution to intercellular cytoskeletal coordination.

Cytoskeletons and cell surface contacts

Diffusible signals could not ensure that the two ‘halves’ of a cell junction are exactly positioned with respect to each other and groupings of microtubules (Fig. 7) or filament bundles (Fig. 6) in cell neighbours unless generated on a very highly localised basis. This must also be so in some cases of contact inhibition; specialized plasma membrane regions and attached groupings of filaments are rapidly (20 sec) established on either side of points where cell surfaces initially make contact (Heaysman & Pegrum, 1973). Indeed, cell shaping and locomotion are sensitive to the orientation of other cells and extracellular matrix fibrils during a whole range of other surface-contact-mediated phenomena such as contact-following and substrate-guidance (Rakic, 1972; Dunn & Ebendal, 1978; Lofberg, & Ahlfors, 1978). The cytoskeletal reorganizations that ensue are almost certainly induced by surface-bound signals rather than diffusible ones. The possibility that surface contact interactions could promote cytoskeletal alignments with respect to the orientation of pre-existing materials has already been considered. However, this possibility does not exclude another. Namely, one in which surface contact interactions are exploited as part of a mechanism for transmission of signals between cell interiors and the setting up of cytoskeletal alignments and lattices with new orientations. For example, some cells produce a variety of cell extensions such as filopodia and lamellipodia that effect what appear to be exploratory sorties in which the
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Fig. 6. Attachment desmosomes and associated intermediate filaments joining an outer epidermal cell (towards the left of the micrograph) to a basal cell in a newt tadpole (Triturus helveticus). × 72000.

Fig. 7. Part of the outer surface of an ovarian follicle in the gall midge Heteropeza pygmaea where two adjacent epithelial cells overlap, interdigitate, and are joined by spot desmosomes. Aligned microtubules (arrows) (see Fig. 2) run alongside the desmosomes and seem to be connected to them by strands of dense material. From Tucker & Meats (1976) with the permission of the Rockefeller University Press. × 63000.
Fig. 8. A region where two Calliphora tarsomeric epidermal cells interdigitate. Aligned sub-surface microtubules (compare Figs. 3, 4) extend into the interdigitation and run alongside it. × 145000.

Fig. 9. Tarsomeric epidermal cells in Calliphora. Basal portions of two cell bodies are situated to either side of the micrograph. As pupal cuticle secretion starts these cell bodies are closely juxtaposed at their apical extremities (not shown) and joined by several types of cell junctions. They also make contact at levels just above the basement membrane (arrow) via filopodial cell processes that extend across the spaces between the basal portions of cell bodies and overlap each other. × 55000.

Surfaces of neighbouring cells and substrates are contacted and examined (Gustafson & Wolpert, 1967; Albrecht-Buehler, 1976). Such behaviour may be an indication that cells and their cytoskeletons monitor, and respond to, materials bound to the surfaces of other cells as well as to noncellular substrata. Further indication that this is so is provided by the complex topography often present where the plasma membranes of adjacent cells apparently make close (10–20 nm) ‘contact’. This appears to be excessive if coupling via gap junctions and/or other modes of diffusible signal transmission can account for all forms of cell coordination.

Epithelial cell surface interdigitation is especially common (Figs. 7, 8) (Lawrence & Green, 1975; Schliwa, 1975; Tucker & Meats, 1976; Meier, 1978).
Filopodia and pseudopodia sometimes interconnect portions of cell bodies where they are separated by substantial extracellular spaces although such cells are closely juxtaposed at other levels (Figs. 9, 10, 11) (Baker, 1965). In some of these instances the extravagance of interdigitiation and ‘podial’ contact (Figs. 8, 9) is difficult to reconcile only with a need for exchange of diffusible signals, cell adhesion, and locomotory requirements. Their presence is reasonable if cytoskeletal organization near a plasma membrane region in one cell locally influences that in a closely contacted portion of an adjacent cell. If this is the case, it is to be expected that cells might locally increase the frequencies and areas of such contacts, and the opportunities for concentrating certain cytoskeletal combinations at particular surface loci. This is especially the case in situations like that found in the epithelium illustrated in Figs. 10 and 11, where it is clear from the shapes of individual cells that the cytoskeletal configurations controlling shape must differ at various levels in the cells but be more or less in register with each other through the thickness of the epithelium. There are numerous fine filopodial cell processes (arrow) that interconnect long widely spaced cell extensions. These extensions run between the closely packed cell bodies and the basal portions of the cells which are spread over the basement lamina. The filopodia may play a part in coordinating cytoskeletal arrangement and cell shape at different levels in the epithelium as well as increasing its mechanical integrity.

All this evidence for intercellular coordination of cytoskeletal organization, although circumstantial, strongly suggests that interactions between the cytoskeletons of adjacent cells might take place via their plasma membranes in regions of close cell contact. This possibility is particularly attractive because evidence for a range of structural associations between cytoskeletal and plasma membrane components has recently emerged that could, at least in theory, provide the molecular basis for such interactions.

Cytoskeletal/plasma membrane interactions

The rather substantial cytoskeletal/desmosome complexes that occur in certain tissues (Figs. 6, 7) (Kelly, 1966; Lentz & Trinkaus, 1971; Burnside, 1971; Friedman, 1971) provided the first really tangible indications that intercellular cytoskeletal coordination might be effected via certain cell surface regions where cytoskeletal/plasma membrane associations occur. The diversity of associations now documented (Weihing, 1979) provides a considerable ‘vocabulary’ for the cytoskeletons of adjacent cells to potentially indulge in fairly detailed cell-surface-mediated ‘conversations’ concerning spatial coordination. For example, it is becoming apparent that microtubules, microfilaments and intermediate filaments can all probably become attached to certain cell junctions – most especially to desmosomes. In certain situations: microtubules appear to be connected to spot (Fig. 7) and belt desmosomes and to desmosome-like junctions, certain intermediate filaments (tonofilaments) to spot desmosomes.
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(Fig. 6), microfilaments to spot and belt desmosomes and intermediate junctions, and fine filaments of as yet unknown composition to tight junctions (Friedman, 1971; Burnside, 1971; Bernstein & Wollman, 1976; Tucker & Meats, 1976; Staehelin & Hull, 1978; Hull & Staehelin, 1979). However, it is also becoming apparent that microtubules and microfilaments may be joined to integral membrane proteins at many points on a cell’s surface and not just where plasma membranes form part of distinct cell junctions or specialized substrate attachment regions such as hemidesmosomes and focal adhesions. Indeed it has been argued that the plasma membrane should be regarded as an integral component of a cell’s cytoskeletal framework (Ben-Ze’ev, Duerr, Solomon & Penman, 1979) because a substantial proportion of this membrane appears to remain more or less intact, and connected to the underlying cytoskeleton, after certain cells have been subject to extraction with a non-ionic detergent. Some of the membrane proteins in question form the proximal portions of glycoprotein surface receptors. The evidence comes mainly from studies of surface receptor mobility and its spatial relationship to sub-plasmalemmal cytoskeletal deployment for certain cells, particularly fibroblasts and lymphocytes, in tissue culture (Nicholson, 1974; Ash, Louvard & Singer, 1977; Flanagan & Koch, 1978). For example, certain receptors diffuse in the plane of the membrane up to ten times more rapidly in directions that parallel sub-plasmalemmal stress fibres than they do at right angles to them (Smith, Clark & McConnell, 1979). Actin and tubulin, as well as receptors are sometimes transported into ‘caps’ when capping and receptor cross-linkage are induced by the application of multivalent ligands such as lectins and antibodies (Gabbiani, Chaponnier, Zumbe & Vassalli, 1977). Drugs that promote disassembly of microtubules and microfilaments influence receptor mobility in the plane of the plasma membrane in a variety of ways (depending on the system studied) which seem to indicate that these fibres anchor and/or propel surface receptors under certain conditions (Weiheing, 1979). Microfilaments may also interact with another type of cell surface glycoprotein, fibronectin, that influences cell spreading and adhesion (Yamada & Olden, 1978). In certain fibroblasts so exact is the alignment of sub-surface microfilaments with fibronectin filaments projecting from the

Fig. 10. Tarsomeric epidermal cells in Calliphora. As pupal cuticle secretion is completed much larger intercellular spaces than previously present intervene between extensively narrowed elongate portions of cells. Filopodia (arrow) interconnect cells at these levels although cell bodies make direct contact with those of their neighbours at their wider apices (towards the top of the figure) and where the basal portions of cells spread across the basement membrane (towards the bottom of the figure). Part of a haemocyte that is adhering to the inner haemocoelic surface of this membrane is also shown. Living material mounted in saline (King, Rubinson & Smith, 1956); interference contrast microscopy. ×1100.

Fig. 11. As Fig. 10 showing a more extensive set of filopodial interconnexions in an inter-tarsomeric region. ×1400.
plasma membrane’s outer surface that direct connexion between them by some transmembrane intermediary seems likely (Singer, 1979).

The precise ways in which cytoskeletal fibres connect to integral membrane proteins and the substantial aggregates of membrane-bound materials that make up the bulk of cell junctions and specialized substrate attachment regions remains to be ascertained. It is already clear that many, perhaps all, connexions to integral membrane proteins are not direct ones. Proteins such as ankyrin, and perhaps $\alpha$-actinin and vinculin, are included in molecular complexes that join actinoid microfilaments to the plasma membrane in certain cells (Lux, 1979; Geiger & Singer, 1979; Geiger, Tokuyasa, Dutton & Singer, 1980; Lloyd, 1980). Connexion of microtubules is sometimes effected by the bridges that join them to adjacent portions of plasma membranes (Fig. 12) (Roberts, 1974; Tucker & Meats, 1976; Dentler, Pratt & Stephens, 1980) and resemble intertubule arms and links. Bridges might attach to, and account for, the (mysteriously) substantial quantities of tubulin that is tightly bound to certain plasma membranes (Bhattacharyya & Wolf, 1976; Stephens, 1977; Matus, 1978; Weihing, 1979). Such bridging may be more common than is generally appreciated. Portions of microtubules are sometimes situated within a ‘bridge’s-length’ (up to about 40 nm) of the plasma membrane in many types of tissue cells. It is only in cases where bridges are closely concentrated together along most of the lengths of such tubule portions that they are likely to be detected. So far there is no evidence for connection of intermediate filaments to the plasma membrane except at desmosomes. However, lateral cross-bridge associations between microtubules and intermediate filaments (Yamada et al. 1971; Albertini & Anderson, 1977; Rice, Roslansky, Pascoe & Houghton, 1980) and microtubules and microfilaments (Griffith & Pollard, 1978) raises the possibility that intermediate filaments might interact with plasma membranes generally by virtue of connexion to other types of cytoskeletal fibres.
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Could some of these cytoskeletal/plasma membrane associations link the cytoskeletons of adjacent tissue cells and transmit signals for cytoskeletal coordination?

Transmembrane molecules and signal transmission

Four years ago Edelman (1977) suggested that surface receptors and associated cytoskeletal components (surface modulating assemblies) might be major transmembrane control elements responsible for coordinating cell movement, division, and interaction, if cell-associating molecules from different cells interact directly with each other. In view of more recent developments this is an attractive hypothesis for intercellular cytoskeletal coordination that merits more detailed attention.

Structural interactions between cytoskeletal fibres, surface receptors, and other components projecting from plasma membranes could provide a structurally contiguous intercellular signalling system for effecting cytoskeletal coordination if certain receptors of adjacent cells bind to each other on a specific complementary basis (Fig. 13). Intercellular cytoskeletal coordination could be achieved if: the affinity with which these receptors bind to each other depends on pre-existing connexions to cytoskeletal fibres (or the lack of them), cytoskeleton/receptor attachments are sensitive to the state of receptor–receptor binding, and cytoskeletal organization is influenced by association with receptors. Desmosomes, synapses, and other cytoskeletally associated cell junctions may simply be very compact, highly specialized examples of a more general, less readily detectable, intercellular coupling phenomenon.

This hypothesis is supported by a number of observations. It has been established for some cell types that the distribution of certain surface receptors is sensitive to the spatial state of the underlying cytoskeleton which, in turn, is modified when lectins and antibodies bind to surface receptors (Albertini & Anderson, 1977; Thom, Cox, Safford & Rees, 1979; Damsky, Wylie & Buck, 1979). Aggregated human blood platelets remain firmly attached to each other after extraction with the non-ionic detergent Triton X-100; there are indications that attachment is effected and maintained because transmembrane glycoproteins interconnect the cytoskeletons of adjacent platelets and that this mode of structural coupling survives the extraction procedure (Phillips et al. 1980). Cohesion of aggregating cellular slime mould amoebal cells is apparently facilitated by the binding of complementary surface-bound lectin-like molecules and receptor-like glycoproteins; a similar procedure may operate in certain vertebrate tissues (Newell, 1977; Barondes, 1978). There are also indications that binding of substrate-attached lectin-like molecules to surface receptors is part of a mechanism for effecting cytoskeletal reorganization during cell attachment to, and spreading on, certain substrata (Rees, Lloyd & Thom, 1977; Grinnell, 1978).

Studies of chemotaxis provide additional support for the notion that surface
Fig. 13. Schematic diagram summarizing spatial relationships and possible modes of interconnexion between cytoskeletal fibres and integral membrane glycoproteins (unshaded) where the plasma membranes of adjacent cells contact each other. The circular cross-sectional profiles of microtubules, intermediate filaments, and microfilaments (blocked in black) have been drawn to scale (diameters 24 nm, 10 nm, 6 nm, respectively), cross-bridges between them are stippled; molecules and molecular complexes that bridge them to membrane proteins are cross-banded. The glycoproteins represent complementary sets of surface receptors and junctional proteins that bind to each other at their distal extremities or are interconnected, perhaps in some cell junctions, via other macromolecular intermediates. This representation is not intended to exclude the possibility that a much greater range of receptors than indicated above may participate. For example, each member of a complementary pair of receptors might connect to a different type of cytoskeletal fibre. Several different species of receptors might connect to the same type of cytoskeletal fibre and modify its potential for connection to other cytoskeletal components and receptors in a correspondingly varied range of ways. Similarly, a greater variety of bridges than indicated above may be involved to join fibres and receptors together. This would also increase the range of structural interactions available for intercellular signalling.
receptors can mediate control of cytoskeletal deployment. Concentration gradients of peptides and cyclic AMP chemotactically induce polarised orientation and locomotion of leucocytes (Zigmond, 1977) and cellular slime mould amoebae (Newell, 1977), respectively. A certain amount of cytoskeletal re-orientation and repolarization is involved as these cells effect up-gradient directed locomotion (Malech, Root & Gallin, 1977; Spilberg, Mandell & Hoffstein, 1979). Binding of chemotactic agents to surface receptors is apparently the first step for signalling cytoskeletal reorganization (Zigmond, 1977; Williams, Snyderman, Pike & Lefkowitz, 1977; Hewitt, 1978). It would make good sense in terms of design and material economy if the same basic system monitors diffusible signals as well as bound signals presented to it by the surfaces of nearby cells and noncellular substrates.

Accurate assessments of the distances separating the plasma membranes of neighbouring cells in regions of close contact are essential for elucidating whether the mechanism for cytoskeletal coordination proposed above (Fig. 13) could operate. Unfortunately, this information is not available because of uncertainty of the extent to which plasma membrane separation is altered during preparation of cell aggregates and tissues for electron microscopy. The plasma membranes of adjacent cells often appear to approach to within 10–20 nm of each other even in regions that do not contribute to obvious junctional specializations (Fig. 12) (Heaysman & Pegrum, 1973; Radice, 1980). If these values represent the situation in vivo then the surface receptors of one cell could bind directly to those of a neighbour, because it is not unreasonable to suppose that receptors project for 5–10 nm from the outer lipid bilayer surfaces of plasma membranes (Fig. 13).

The scheme suggested here (Fig. 13) is an extreme representation of one of a range of related ways in which intercellular cytoskeletal coordination may be mediated by membrane proteins. It does not exclude the possibility that coordination is also promoted by local changes in membrane permeability and ion levels induced by receptor interactions (Wang, Heggeness & Singer, 1978), although these would provide less stringent spatial information than structurally contiguous coupling (Fig. 13) of cytoskeletal fibres across cell boundaries.

Signal transduction and the assembly and repositioning of cytoskeletal components

Although structural interactions between cytoskeletal fibres, surface receptors, and cell junctions might locally influence fibre orientation and positioning (and determine whether fibres elongate, shorten, or break down completely), some of the cytoskeletal remodelling required for spatial coordination will involve the assembly of new components. Could interactions between the plasma membranes of adjacent tissues cells promote such assembly?

Actinoid microfilament assembly appears to be nucleated at sites attached to plasma membranes in some situations (Gordon & Bushnell, 1979; Tilney, 1979). It is not yet clear if, or how, this is influenced by plasma-membrane
Fig. 14. Schematic diagrams showing the two possible distributions indicated by immunofluorescent studies (Weber & Osborn, 1979) for microtubules in certain cells assuming for simplicity that only one central pericentriolar MTOC (stippling) is present. (a) Proximal portions of all microtubules remain associated with the MTOC that nucleated their assembly as their distal portions elongate and ramify around the cell surface. (b) Some tubules do not maintain this association, or alternatively, were nucleated at the cell surface.

organization. However, in terms of proximity these sites are favourably located for interaction with surface receptors that might promote or inhibit site formation and nucleating activities. Little is known about the initiation of intermediate filament assembly, although it is suspected that assembly of some types may be nucleated by sites attached to desmosomes (Lazarides, 1980). On the other hand, if cell surface modulation of microtubule initiation occurs, it must often be of a very indirect nature. This is because in many tissue cell types assembly of most, perhaps all, microtubules is nucleated by microtubule-organizing centres (MTOCs). These MTOCs are situated several microns from the nearest cell surface region and are commonly in the centre of the cell close to the nucleus (Schliwa, 1978; Tucker, 1979; Raff, 1979). When microtubules grow out from these MTOCs some of them extend towards and
then run closely beneath the cell surface (Weber & Osborn, 1979; Brinkley, Fistel, Marcum & Pardue, 1980). However, it is not yet clear whether they all retain an association with an MTOC (Fig. 14a) (Warren, 1974), or if some of them detach from the MTOC that nucleated their assembly and migrate out to the cell surface (Fig. 14b). Nor can the possibility that the assembly of some subsurface microtubules is nucleated by sites attached to the plasma membrane be ruled out. The important point is that the spatial details of subsurface microtubule arrangement are unlikely to be organized by centrally positioned MTOCs. Arrangement more probably depends on conditions, such as the deployment of other cytoskeletal elements and receptors, at the cell surface. Hence, plasma-membrane interactions could play a considerable part in coordinating the microtubule layout in adjacent cells even if they have little direct impact on the production of new microtubules.

However, if one considers that subsurface microtubule arrangement may be sensitive to the deployment of other plasma-membrane-associated fibres such as microfilaments, then indications that microtubules are sometimes involved in defining the positions and orientations of nearby microfilament bundles and intermediate filaments (Lloyd, Smith, Woods & Rees, 1977; Wang & Goldman, 1978; Berlin, Caron & Oliver, 1979) cannot be ignored. They raise the question of what organizes what, and whether there is an organizational hierarchy? Microtubules, actin cables, and bundles of intermediate filaments often exhibit distinctly different overall layouts within individual cells and sometimes display a variety of orientations with respect to each other in different regions of the same cell (Lazarides, 1978; Weber & Osborn, 1979; Blose, 1979; Henderson & Weber, 1979). Thus there is no evidence for any species of ‘master fibre’ that dictates the arrangement of all others in its vicinity under all physiological conditions. It seems more likely that all the main cytoskeletal fibre types interact reciprocally, perhaps by lateral cross-bridge associations, in ways that are sensitive to a wide spectrum of local physicochemical conditions and structural associations. Hence, surface-receptor-induced alterations in the organization of any one type might less directly influence that of others in its vicinity.

Cytoskeletal coordination in embryonic fields

There is a tantalising possibility that cytoskeletal arrays (and the cytoskeletal/plasma membrane interactions considered above) also influence tissue shaping by providing a coordinate system that assigns positional values in embryonic fields. The need for coordinate systems has often been considered but the material basis involved remains elusive (Wolpert, 1978). Cytoskeletal arrays have much to offer as potential candidates but also exhibit features incompatible with this role. Neither their suitability, nor their drawbacks, have been emphasised before.

An intercellular signalling system that supplies positional information in
an embryonic field needs to transmit positional signals at velocities of up to at least 4 \( \mu \text{m min}^{-1} \) (Wolpert, 1971). Elongation rates of up to 19 \( \mu \text{m min}^{-1} \) (Ockleford & Tucker, 1973) and 180 \( \mu \text{m min}^{-1} \) (Tilney, Hatano, Ishikawa & Mooseker, 1973) occur in vivo during microtubule and microfilament bundle assembly, respectively. During contact inhibition specialized plasma membrane contact regions are established and filaments become oriented on either side of them at points where cells meet each other within 60 sec of contact being made (Heaysman & Pegrum, 1973). Hence, some changes in cytoskeletal/cell surface organization proceed fast enough for them to transmit positional information. Perhaps such modulations emanate from field boundaries to generate patterns of scalar variation in some cytoskeletal component(s) that assigns positional values? There are indications that the organization of a cell's cytoskeleton and surface receptors is sensitive to, and influences, intracellular levels of cyclic AMP in some instances (Glennie, Stevenson, Stevenson & Virji, 1979; Kennedy & Insel, 1979; Dedman, Brinkley & Means, 1979). Hence spatial modulation of cytoskeletal/plasma-membrane organization might (via levels of cyclic nucleotides and enzyme activity) generate corresponding variations in gene expression for cells in different field regions. Furthermore, both microtubules and microfilaments have a polarized molecular structure (Begg, Rodewald & Rebhun, 1978; Bergen & Borisy, 1980) and contribute to cytoskeletal complexes that exhibit polarized mechanochemical activities (Kersey, Hepler, Palevitz & Wessels, 1976; Tucker, 1978). Thus, cytoskeletal modulation might also establish cell and tissue polarities within a field.

However, cytoskeletal organization is sensitive to local conditions. These include intricate microenvironmental details, such as the surface topography of adjacent cells and substrata, that are often presumably of purely parochial significance so far as the assignation of positional values is concerned. The cytoskeletal/plasma-membrane interactions considered above apparently form part of a sensitive signalling system that enables a cell's cytoskeleton to be modified subtly and appropriately in response to minor fluctuations in the cell's immediate surroundings. As in government, conflicts between global and local interests are bound to arise. It is important that cytoskeletal modifications in response to certain local conditions should not blur the overall positional 'field of view'. Furthermore, in some cases of cell differentiation, such as during the production of a bristle or a scale by an insect epidermal cell, assembly of a radically different cytoskeletal array from that found in surrounding cells that are not so committed occurs (Greenstein, 1972). All these involvements presumably put severe limitations on the extent to which cytoskeletons could be exploited for transmitting positional information. So will the marked cytoskeletal reorganisations that take place during cell division.

These arguments do not, however, exclude the possibility that cytoskeletal/cell surface organization in oocytes, zygotes, and early blastomeric stages may have important spatial consequences for the generation of positional informa-
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(Quatrano, Brawley & Hogsett, 1979; Zalokar, 1979). It may set up spatial differences that subsequently provide a basis for the location of field boundary regions. For this role, spatial responses to local conditions, such as contact with adjacent cells and tissues, could be positively advantageous during specification of embryonic axes. So would an ability to regulate in, for example, response to the changes in surface contact consequent on the death or loss of an adjacent blastomere.

If cytoskeletons do not transmit positional information then they must surely be sensitive to it and provide the embryo with a supracellular motor network that plays an important part in the interpretation of this information. This would most obviously be the case during gastrulation, neurulation, and the performance of other major morphogenetic movements. It would also facilitate control of finer details during tissue construction; for example, spatially coordinated cell shape modulations that promote cuticular ripple patterns in *Rhodnius* (Lawrence, Crick & Munro, 1972; Wigglesworth, 1973) and a cell elongation pattern in newt neural plate (Jacobson & Gordon, 1976).

**Multicellularity and signal evolution**

Did the ‘first metazoans’ exploit intercellular cytoskeletal coordination from ‘the beginning’? Is it a facility that is employed in plant tissues?

Metazoans presumably evolved from protozoans. Contemporary protozoans indulge in intercellular cytoskeletal/cell surface signalling and highly specific cell surface recognition procedures, especially during feeding (Ockleford & Tucker, 1973; Tucker, 1978) and mating (Preer, 1969). In trypanosomes the flagellar membrane is attached to plasma membrane regions alongside the cell body by desmosome-like junctions (Vickerman, 1969). Furthermore, the arrangement of plasma-membrane-associated cytoskeletal components in ciliates appears to be sensitive to positional information that may have a similar basis to that which is transmitted in the embryonic fields of metazoans (Frankel, 1974; Lynn & Tucker, 1976). Hence, there are plenty of indications that the pro-metazoan protozoans could have been well equipped to deal with new aspects of intercellular cytoskeletal coordination that emerged with the advent of multicellularity.

Although plant cell shaping is cytoskeletally coordinated (Marchant, 1979; Gunning & Hardham, 1979; Lloyd, Slabas, Powell & Lowe, 1980) there are no grounds for supposing that structurally and intercellularly contiguous cytoskeletal/surface receptor complexes have evolved to effect coordination in plant tissues. Outgrowth of motile cell extensions, cell migration, morphogenetic movements, actively contractile cell shortening, and hence a requirement for spatial coordination of the cytoskeletal activities associated with such phenomena, are all lacking during plant tissue development. The entire range of cell junctions that are so common in metazoan tissues are also lacking. Plant tissue cells do not have the opportunity of exercising the dynamic...
repertoire of surface contacts that is available in metazoans. In most cell surface regions the plasma membranes of adjacent plant cells are separated by layers of rigid extracellular wall material. Although the plasma membranes and cytoplasm of neighbouring cells are usually continuous with each other via the sometimes numerous plasmodesmata that traverse cell walls, there are as yet no indications that plasmodesmata are structurally associated with cytoskeletal fibres (Gunning & Robards, 1976). Thus a dearth of cytoskeletal/plasma membrane interactions for intercellular coordination may sunder the plant kingdom from the animal kingdom as markedly as any of the other more obvious distinctions.

There is a way of effecting intercellular cytoskeletal coordination that is perhaps common to both animal and plant tissues. It is distinct from the plasma-membrane-associated procedures considered so far. It is mainly confined to coordination between cells of close clonal relation (most especially sister cells) and is accomplished by the transmission of spatial instructions from one cell generation to another during cell division. Indications that this is so are as follows. It has been argued that cytoplasmic inheritance of specialized cortical zones might provide a basis for specification of cortical microtubule arrangement in certain plant tissues (Gunning, Hardham & Hughes, 1978). Examinations of certain fibroblasts and neuroblastoma cells in tissue culture have revealed that sister cells are often more or less identical twins, or alternatively are sometimes mirror images of each other, in terms of cell shaping. For fibroblasts this is also the case in terms of actin cable arrangement and patterns of cell migration. Sister cells apparently inherit very similar sets of spatial instructions concerning cytoskeletal deployment from a parent cell in these instances (Albrecht-Buehler, 1977; Solomon, 1979). The coordination of cytoskeletal layout in adjacent cells that results could play an important role during tissue morphogenesis. For example, Solomon (1979) and Bate & Grunwald (1981) have pointed out that it may help to set up certain mirror-image axon configurations.

How might spatial instructions for cytoskeletal organization be transmitted during metazoan tissue cell division? Pericentriolar MTOCs (Fig. 14) may be involved (Solomon, 1980a). In many cases each sister cell receives one pericentriolar MTOC from the parent cell. These MTOCs replicate once prior to cell division and are evenly segregated and cytoplasmically inherited during division. Furthermore, there are indications that pericentriolar MTOCs exert some control over the numbers and lengths of microtubules that grow out from their surfaces (see Solomon, 1980b). Perhaps, in addition, a pericentriolar MTOC can be switched into one of several modes and each provides a different set of spatial instructions for control of microtubule orientation and/or the extent to which microtubules elongate as they grow out in particular directions. Some basal-body-associated MTOCs certainly do control microtubule orientation (see Tucker, 1979). Hence pericentriolar MTOCs might provide spatial
programmes that influence cell shaping and locomotion via their impact on cytoskeletal arrangements. No doubt, analyses of cortical basal body positioning and cytoskeletal assembly in certain ‘doublet’ ciliates that exhibit mirror-image cell-surface patterns will continue to be illuminating in this context (Jerka-Dziadosz & Frankel, 1979; Grimes, McKenna, Goldsmith-Spoegler & Knaupp, 1980). A very sophisticated level of intercellular cytoskeletal coordination is displayed during metazoan embryogenesis. It is worth considering whether its evolution has been facilitated because inherited central control by pericentriolar MTOCs has been successfully integrated with cortical plasma-membrane-mediated control.

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