Cellular basis of morphogenesis

Computer analysis of cell density distributions in chick feather embryogenesis

S. A. Baranowitz 1 and P. F. A. Maderson 2, 1Department of Dermatology, Mount Sinai Hospital, 2Brooklyn College, Brooklyn, New York, U.S.A.

The relationship between cell density distributions and differentiation is of interest to workers in many different areas of developmental biology. Traditionally, such studies have required tedious counting of individual cells through a microscope eyepiece grid or on photographs. We have developed a rapid, reliable, and inexpensive microcomputer based system for counting cells in histological sections. The system employs an Apple IIe computer, Digisector video board (Micro Works, Del Mar, CA), Super Scan program (Magna Soft, La Canada, CA), Gibson Light Pen System (Gibson Laboratories, Laguna Hills, CA), and Telestar video camera (Bell and Howell). We have applied this to identify specific patterns of cell density in feathers. Our system is significantly different from other image analysis systems available because its low price (about $2500) makes it accessible to average laboratories, as opposed to the expensive image analysis systems which are available commercially or in a few special research laboratories. Normal feather germs, serial sectioned at 6 μ and stained using the cold hydrolysis Feulgen technique were photographed at 400 x using Kodak Tech Pan film and printed on Kodabromide paper (no. 5). One inch squares of the 4×6 inch print were exposed to the video camera. The correlation coefficient for the number of black pixels counted by the computer and the number of cells counted by hand from the photograph was >-98. Dramatic local increases in mesenchymal cell density in the area of the germ which protrudes distally were observed in the sagittal sections of all the specimens examined. (Supported by NIH Training Grant 5T32AM07376.)

Ciliary body morphogenesis: mechanisms of lateral cell detachment and epithelial folding

Jonathan B. L. Bard* and Allyson S. A. Ross, MRC Unit, Western General Hospital, Edinburgh EH4 2XU

The ciliary body of the avian eye forms when the anterior retina around the lens creases to give ~90 radial folds. This process starts on the 7th day of development and takes ~36 h. Two key mechanisms are responsible for morphogenesis: first and ~2 days prior to folding, the neural retina cells in the region of the presumptive ciliary body detach laterally from one another while maintaining links through junctional complexes at their bases; second, just prior to folding, the eye undergoes differential growth in that the eyeball swells but the pupillary ring around the lens does not. The mechanical stresses produced by this differential growth on a structurally weakened tissue cause the retinal epithelium to buckle and form radial folds.

The lateral detachment has been studied with ruthenium red (RR) which stains glycoproteins. The lateral surfaces of the neural-retina cells in the ciliary body region make few adhesions to one another at the time of folding, but there is RR staining where they do. Two days later, all cell surfaces stain with RR and have filopodia which adhere to their neighbours. Soon after, the surfaces join, leaving no evidence that they were ever detached. In sharp contrast, cells on either side of the ciliary body, those in the pupillary tip and the visual retina, maintain both lateral adhesions and RR staining before, during and after morphogenesis. The detachment phenomenon thus seems to be controlled by a glycoprotein whose synthesis is under temporal and spatial control.

The role of differential growth has been confirmed by its simulation in vitro. If eyes about to undergo morphogenesis are immersed in 50 % ethanol, they swell by about 10 %, show the differential enlargement seen in vivo and, within 15 m, form folds of the size expected after ~20 h of normal growth.


BARD, J. B. L. & Ross, A. S. A. The morphogenesis of the ciliary body of the avian eye. II: Differential enlargement causes an epithelium to buckle. Develop Biol. 92, 87-96.
Cellular basis of morphogenesis

Studies of growth pulsations in hydroid polyps

L. V. Belousov*, Ju. A. Labas, L. A. Badenko and A. G. Zaraiskii, Department of Embryology, Faculty of Biology, Moscow State University, Moscow 117234 USSR

Growth pulsations (GP) in several species of hydroid polyps have been studied by automatic recording, time-lapse cinematography, optical and transmission electron microscopy, and pharmacological analysis. A correlation between GP pattern and rudiment shape have been demonstrated, both in normal and chemically modified morphogenesis; the 'flattened' profiles of GP records corresponded to the transversely extended rudiments and the 'sharpened' profiles to the elongated rudiments. During each GP, a rudiment's extension is due to transverse cell rotations, proceeding in most cases in a proximo-distal succession, and accompanied with the increase in intercellular vesicle number. Rudiment retraction is correlated with the reverse cell rotations towards the initial orientation, rapid distalward sliding of external cell poles, decrease in intercellular vesicle number and with the formation of microfilament bundles oriented parallel to the cell's sliding direction. We suggest that GP are based upon the alternating activity of osmotic and contractile mechanisms. By cells swelling osmotically, an excessive mechanical pressure is created which shapes a growing rudiment.

Effects of exogenous mRNA on the postnodal explant of chick embryo

A. M. Bolzern*, M. Cigada Leonardi, F. De Bernadi., U. Fascio, S. Ranzi, A. Saita and C. Sotgia, Department of Biology, University of Milano, 20133 Milano, Italy

Postnodal explants of chick embryo, cut at the primitive streak stage, 0-6 mm behind the Hensen's node, showed modifications of the mesodermal cells after treatment with mRNA specifically coding for the myosin heavy chain (Bolzern et al., 1983). In the presence of mRNA extracted from chick and from other animals muscles (duck, rabbit, trout, crayfish) the mesodermal cells became elongated, spindle-shaped and joined at the bases. Moreover, with homospecific chick mRNA the elongated cells organized themselves around a myocoele, to form a true somite. We came to the conclusion that the mRNA is translated inside the mesodermal cells into myosin heavy chain, giving rise to the elongation and to the peculiar arrangement of the cells to form a somite.

Therefore we looked to see if the mRNA coding for the major microtubular protein, tubulin, can modify the shape of the cells of the postnodal explants. During cellular differentiation, the microtubules play a role in determining cell shape. The postnodal explants treated with purified tubulin mRNA extracted from chick embryo brain showed, after 20 h of culture, some characteristic protuberances, which appeared to consist of a series of aligned ectodermal cells, surrounding several mesodermal cells. In other cases the ectoderm looked thicker and the cells were slightly prismatic. At the ultrastructural level the elongated ectodermal cells showed many microtubules oriented in parallel with the major axis of the cell. Moreover the ectodermal cells at the base of the protuberances contained bundles of microtubules. The results show that the tubulin mRNA specifically modifies the shape of the ectodermal cells increasing their microtubules content.

Cellular basis of morphogenesis

Some key events in cell biology can be studied with a new cell model –
the molluscan apyrene sperm

J. A. Buckland-Nicks and F.-S. Chia, Department of Zoology, University of Alberta, Edmonton, Canada

Sperm polymorphism is a common phenomenon among prosobranch molluscs. The genesis of molluscan apyrene sperm involves progressive nuclear degradation, multiple ciliogenesis, secretion formation, and exocytosis. In some species, viable eupyrene sperm become attached to an apyrene 'carrier' sperm late in spermiogenesis forming a spermatozeugma. At the end of the breeding season, or during starvation, spermatozeugmata are phagocytized in the seminal vesicles. We have been studying these events in the two apyrene sperm of the prosobranch *Fusitriton oregonensis*, using a variety of techniques. Fine structural details have been observed in fixed tissues with light and electron microscopy. More recently we have isolated living immature sperm from the testis, using a simple squash technique. The progressive reduction of double stranded DNA during nuclear degradation was rendered visible with Hoescht's 33342 strain (λ max 365nm), viewed with a Nikon fluorescence microscope. Acridine orange (λ max 498nm) was used as an indicator of lysosome activity. Video enhanced contrast microscopy (Allen et al., 1981) was used to observe cellular organelles in living spermatozoa. Our results indicate that the molluscan apyrene sperm is a valuable model for studying cell-cell recognition phenomena, and such key events in cell morphogenesis as exocytosis, ciliogenesis, and depolymerization of DNA. Supported by an NSERC of Canada grant to F.-S. Chia.


Cell activation and movement during wound healing and regeneration in insects

G. L. Campbell, Department of Zoology, The University of Leicester, Leicester LE1 7RH

The major stages of wound healing following mechanical damage to the insect integument have been described previously by Wigglesworth (1937). They include activation of surrounding epidermal cells, migration of these cells, accumulation of haemocytes, spreading of cells over the wound, cell division and closure of the epidermal sheet.

This poster describes three of these stages, activation, migration and spreading, in greater detail. Activation has been studied using the transmission electron microscope. Ultrastructural changes undergone by the epidermal cells to achieve the activated state are highlighted and a comparison is made between activated and nonactivated cells. Cell movement has been investigated using pigmented and white mutant cells of *Oncopeltus fasciatus*. Chimeras of wild-type and mutant can be constructed and the behaviour of graft and host cells can thus be followed after wounding.

Cellular basis of morphogeneseis

Cartilage histogenesis in micromass cultures

Christopher P. Cottrill and Charles W. Archer. Department of Anatomy and Biology as Applied to Medicine, The Middlesex Hospital Medical School, Cleveland Street, London W1P 6DB. Institute of Orthopaedics, Royal National Orthopaedic Hospital, Brockley Hill, Stanmore, Middlesex

We have recently suggested that the morphology of 3-day micromass cultures taken from various proximo-distal levels of the chick wing bud might indicate the heterogeneity of cell states within such populations (Archer et al., 1984). For example, a micromass culture of cells taken from beneath the apical ectodermal ridge (progress zone) gives rise to a uniform sheet of closely packed cells. This, we have suggested, indicates the homogeneity of cell state within the population. Indeed, by application of anti-type II collagen antibodies, we can now show that all cells in such a culture differentiate into chondrocytes. In contrast, a micromass culture of proximal limb bud mesenchyme gives rise to chondrogenic nodules with large areas of non-chondrogenic internodular tissue. The hypothesis that the formation of chondrogenic nodules is a consequence of cell sorting within a heterogeneous population can be tested by deliberately making a progress zone population heterogeneous by adding known non-chondrogenic cells and preparing micromass cultures. As the proportion of non-chondrogenic cells in such mixtures increases, the cultures assume a more nodular morphology. An unexpected finding of these experiments was that while undifferentiated potential chondroblasts normally aggregate in culture, this is not an essential prerequisite for their differentiation in vitro. This is in contrast to currently held ideas (Solursh et al., 1982). To investigate further the process of nodule formation we have repeated the mixing experiments using mature chondrocytes. These cells do not aggregate to form nodules, an event which therefore appears to be a characteristic only of undifferentiated prospective chondroblasts.


Tissue morphogenesis and cell/extracellular matrix interaction during teleost development

P. J. Dane and J. B. Tucker; Zoology Department, St Andrews University, St Andrews, Fife, KY16 9TS

Ultrastructural studies of caudal fin development in Brachidionio rerio reveal several new features of teleost fin morphogenesis and cell/extracellular matrix interactions. Well defined changes in the shape of individual cells play an important role during fin and epidermal shape generation due to the small number of cells that are initially involved in fin fold morphogenesis.

Fin fold formation is preceded by the formation of filopodia and gap junctions. Within the epidermis filopodia and gap junctions are spatially confined to the site of the prospective fin fold. The epithelial cells of the prospective fin fold become wedge shaped as the fin fold is established. A distinctive row of 'apical cleft cells' is positioned along the crest of the epidermal fold. These 'apical cleft cells' cap the sub-epidermal space which lies within the fin fold. Two main types of oriented extracellular fibres assemble within the sub-epidermal space. Previously undescribed cross fibres span the sub-epidermal space perpendicular to dorso-ventrally oriented actinotrichia.

Networks of intermediate filaments assemble within the epidermal cells of early fins. These networks are located close to cell surfaces flanking the sub-epidermal space. Bundles of intermediate filaments align with extracellular actinotrichia. Intermediate filaments, sub-epidermal space cross fibres and actinotrichia may provide an effective system for cytoskeletal/extracellular matrix co-ordination during teleost fin fold morphogenesis.
Cellular basis of morphogenesis

Direct demonstration of a spatial pattern of cell-basement membrane adhesions in chick skin during feather primordium development

Duncan Davidson, MRC Clinical and Population Cytogenetics Research Unit, Western General Hospital, Edinburgh EH4 2XU

A simple method is described which provides a direct measure of the adhesion between dermis and epidermis during the development of feather primordia in chick dorsal skin in culture. The epidermis was peeled from the dermis and the surfaces so exposed were examined under the scanning electron microscope: regions of strong adhesion between the tissues were revealed as areas where their separation was incomplete. The results show that soon after primordia become morphologically distinct, cells from the surface of dermal condensations form adhesions to the basement membrane which are stronger than those between dermis and epidermis in interplumar skin. These adhesions may help to hold the epidermis and dermis together during the outgrowth of the primordium.

Correlation between cellular and nuclear stereological parameters of the chick embryo neuroepithelium

P. de Paz and J. M. Villar, Departamento de Anatomia y Embriologia, Facultad de Veterinaria, Universidad de León, León, Spain

The ultrastructure of the neuroepithelial cells in chick embryos was the subject of a large number of assays in order to explain a basic factor of the neurulation mechanism, namely, cellular differentiation into neuroepithelium. A very objective approach to such ultrastructure is a cytometric study by means of stereological methods (Weibel and Boldender, 1973, Williams, 1977) which has permitted us to measure the following parameters: Vc (cellular volume), CFc (coefficient of cell form), Vn (nuclear volume), CFn (coefficient of nuclear form) and Sn (nuclear surface) from a sample of 70 cellular profiles of neuroepithelium at Hamilton and Hamburger stages 5, 6, 7 and 8.

The correlation analysis between these parameters shows significance (P < 0.05) for three cases: Vc/Vn; Vc/Sn; Vn/Sn. The values obtained for the 5, 6, 7 and 8 stages were: 0.89, 0.85 and 0.93; 0.95, 0.96 and 0.96; 0.92, 0.91 and 0.97; 0.89, 0.87 and 0.99 respectively. For the remaining cases the parameters showed no correlation. Therefore, since only Vc, Vn and Sn show a high positive correlation, we deduce a remarkable structural dependence both between cellular and nuclear size, and with nuclear surface, in this critical period of the chick embryo neuroepithelium development.


Cellular basis of morphogenesis

Acceleration of fusion in mouse palates by in vitro exposure to excess g
Jackie C. Duke, Liliana Janer, and Marian M. Campbell, Dental Science Institute, U.T. Dental Branch, P.O. Box 20068, Houston, Texas 77225, U.S.A.

Previous studies have shown that excess gravity (2-6 g) is able to suppress morphogenesis in embryonic mouse limbs developing in vitro by promoting early differentiation (Duke, 1983). The present study reports the effects of exposure to excess gravity on development of the embryonic mouse palate. Palatal shelves from 13- to 14-day embryos were excised and cultured in contiguous pairs. Experimental cultures were exposed to 2-6 g in a culture centrifuge; controls were in the same incubator. After 24 h, palates were prepared for light or electron microscopy. Scoring of paraffin sections according to the stage of fusion seen in the medial epithelial edges (MEE) showed that palates exposed to cells of both palatal types had numerous mitochondria and ribosomes, short sections of slightly expanded RER and Golgi vesicles. Control MEE had tightly apposed cell membranes and numerous desmosomes; in centrifuged MEE, desmosomes had been removed and there was much intercellular space. Nuclear membranes were intact in control MEE, but showed marked deterioration in MEE of centrifuged palates. Few lysosomes and no necrosis were seen in control MEE; centrifuged MEE had numerous lysosomes as well as necrotic cells. Basal lamina were intact in controls, but interrupted in centrifuged palates. The results confirm the hypothesis that gravitational increases speed up the differentiative process.


Development of the rat allantois
S. K. L. Ellington, Physiological Laboratory, Downing Street, Cambridge CB2 3EG

This study is divided into two parts. In the first, the morphology of the developing allantois is described, and in the second, interactions between the allantois and other extra-embryonic tissues are investigated.

The morphological development of the allantois of the rat embryo has been studied from its first appearance as a cluster of cells near the caudal end of the primitive streak, through its growth across the extra-embryonic coelom and until its fusion with the chorion and the development of a functional vascular system. The study was based on transmission and scanning electron microscopy, light microscopy and histochemical techniques. Embryo culture in medium containing 0-1 % colchicine followed by light microscopy served to elucidate the pattern of cell division in the allantois.

Development and growth of the allantois is extremely fast; in the first 18 h after its initial appearance there is a rapid morphological differentiation; followed, in the next few hours, by cytodifferentiation and development of the vascular system. Dividing cells occur throughout the allantois at all stages but the mitotic index, initially very high, falls rapidly as the distal tip of the allantois approaches the chorion. The morphology suggests that the extension could be caused by hydrostatic pressure within the allantois.

Interactions between the allantois and other extra-embryonic tissues were investigated by microsurgery at 9½ or 10½ days post coitum followed by embryo culture for 24 or 48 h. Operations included: removal of the allantois; cutting the allantois and wedging the severed end between the amnion and the visceral yolk sac; removal of the ectoplacental cone and the chorion; and the removal of the ectoplacental cone alone. The following conclusions were drawn from these experiments:-
1. The direction of growth of the allantois, through the extra-embryonic coelom, is unaffected by the presence of the chorion and the ectoplacental cone.
2. The allantois can fuse with the yolk sac mesoderm (rather than the mesodermal layer of the chorion) and the vascular networks in the allantois and yolk sac may become confluent.
3. Maintained contact between the allantois and chorion is necessary for the normal development of the chorion.
Cellular basis of morphogenesis
Morphogenesis of the frog tail
Tom Elsdale*, MRC Unit, Western General Hospital, Edinburgh EH4 2XU

The bulk of the frog tail-bud consists of pre-somatic mesoderm. A rapid 8-fold extension of the bud occurs during the formation of the embryonic tail. Neither the notochord nor nerve chord are required for extension. Evidence will be presented demonstrating that the tail skin controls the extension of the somitic mesoderm and hence of the bud as a whole. The extension of the pre-somatic mesoderm takes place within a limited zone of extension that occupies the middle third of the bud. Within the zone, we picture an orderly unfolding of the prospective somitic tissue. A well-marked peak of mitotic activity in the somitic mesoderm coincides with this zone. However, evidence will be presented indicating that tail development is not driven by mitosis. The crucial importance of the control of small-ion transit and water entry in tail morphogenesis will be illustrated by reference to the oedematous and hypertonicity syndromes.

Lipovitellin can account for the inhibited state of the yolk-stored trypsin of Artemia
Begoña Ezquieta and Carmen G. Vallejo, Departamento de Enzimología, Instituto de Investigaciones Biomédicas del CSIC, Facultad de Medicina, U.A.M. Madrid-34, Spain

The gastrula of the crustacean Artemia can enter cryptobiosis under unfavourable environmental conditions. The dry gastrula (cysts) remain viable for years and resume development when placed in suitable conditions. Development proceeds up to the larval stage in the absence of cell division, although intense morphogenetic changes are produced.

Proteases have been proposed to play an important role in differentiation but the available data are scarce. We have found in cysts a trypsin-like proteinase mainly particulate and located in the yolk granules. In larvae, the trypsin activity is mainly soluble. The trypsin activity in the dry or cultured embryo appears very inhibited both with respect to reaction time and amount of extract. The total activity in the embryo could not be determined due to its inhibited state. The possibility that the increased trypsin activity detected in the larvae resulted from the activation of the enzyme present in the embryo was considered. Artemia is very rich in yolk, representing ≥ 80% of the total cyst protein. The major component of the Artemia yolk is lipovitellin (M₆ 660 K). The effect of lipovitellin on the larval enzyme was investigated. a) Lipovitellin was an uncompetitive inhibitor with respect to the exogenous protein substrate. A 65% inhibition of the larval enzyme was found with 75 µg of lipovitellin when assayed with 1 mg protein substrate at 17 °C. If the temperature assay was 37 °C, the inhibition dropped to only 20%. This phenomenon has probably a physiological meaning since the optimum temperature for cysts culture is 30-37 °C. Lipovitellin was not inhibitory when small synthetic substrats were used. b) Lipovitellin was a substrate of the larval trypsin. The affinity constant decreased but the maximal activity increased unexpectedly when the assay temperature was shifted from 17 °C to 37 °C. A control with bovine pancreas trypsin showed that lipovitellin was a substrate but not an inhibitor. The yolk origin of the larval trypsin, made evident by its capability of reacting with antilipovitellin antibodies, may explain the different behaviour of both trypsins versus lipovitellin. c) Lipovitellin contained endogenous trypsin activity supporting the above described location of the enzyme.

The dual character of lipovitellin as inhibitor and substrate could explain how the enzyme is inhibited and progressively activated during incubation and development. The fact that trypsin in Artemia appears so finely regulated during the embryonic and larval stages suggests an important role during differentiation.
Cellular basis of morphogenesis

Imaginal disc morphogenesis (evagination) in Drosophila

Dianne Fristrom*, University of California, Berkeley, CA 94720, California, U.S.A.

The insect moulting hormone, 20-hydroxy ecdysone, triggers the evagination of imaginal discs from concentrically folded ‘discs’ to elongated appendages both in vivo and in vitro. Initial studies indicated that this dramatic change in shape results largely from the rearrangement of cells within the single-cell layered epithelium.

Cell rearrangement occurs without disrupting the structural and functional contiguity of the epithelial sheet and extensive intercellular junctions (zonulae adherentes, septate and gap) persist as cells exchange neighbors. This apparently paradoxical situation has been resolved in part with respect to the septate junctions in discs. Freeze fracture and T.E.M. studies show that individual septa are confined to areas of contact between two adjacent cells and do not encircle cells as was previously thought. Thus, the septate junctions of each cell are distributed in a number of discrete domains equal to the number of neighboring cells. This arrangement allows for the septa in each domain to adjust rapidly to changes in the extent of contact between pairs of cells facilitating cell rearrangement or changes in cell shape.

Recent work has focused on identifying genes and proteins that might be involved in mediating evagination by identifying RNAs and proteins that appear in response to ecdysone. For example, a set of high molecular weight acidic proteins are synthesized in response to hormone and appear at the onset of evagination. In addition, a number of clones containing ecdysone response genes have been isolated. Further work is required to establish which, if any, of these ecdysone responsive molecules plays a direct role in morphogenesis.

Coordination of morphogenesis: nerves, muscles, skin and skeleton in the chick wing bud

J. H. Lewis*, A. A. Khan, G. J. Swanson, P. B. Martin and C. P. Cottrill, Department of Anatomy, King’s College, London WC2R 2LS and Department of Anatomy and Biology, Middlesex Hospital Medical School, London W1P 6DB

The limb bud of a vertebrate is assembled from a number of initially separate sets of cells – distinct lineages for connective tissue, muscle, epidermis, nerves and endothelium – each destined to form an intricately patterned component of the complete limb. By extirpation and transplantation experiments, it is possible to investigate systematically what degree of autonomy each component has in its own patterning, and what part it plays in controlling the patterning of the others. The connective tissue cells are found to have the dominant role: they carry the positional information that defines not only the form of the skeleton, but also the patterning of the skin, musculature and nerves. The skeleton develops normally even in limbs devoid of muscle cells or of innervation, or where a large patch of the surface of the bud has been denuded of epidermis by ultraviolet irradiation; whereas foreign muscle cells, foreign epidermis and foreign nerves in a developing limb form patterns appropriate to the connective tissue framework. The nerve pattern is especially precise and intricate. Its three levels of structure – the main trunks, the branches to individual muscles or patches of skin, and the twigs of the terminal arborization in the target region – correspond to three different levels of control. The detailed pattern of twigs is randomly variable, while that of the trunks and branches is strictly determinate. The formation of a branch is conditional on the presence of its specific target tissue: branches to muscle fail to form if the limb bud has been deprived of muscle cells, and branches to skin fail to form where the surface of the bud has been denuded of epidermis. But in each of these cases, the nerve trunks are present and follow almost normal routes. These routes may be defined by chemical markers in the connective tissue or by its mechanical properties. Detailed mapping of the pattern of growth of the limb bud reveals that (1) the direction of outgrowth of the nerve trunks generally coincides with the principle axis of elongation of the limb; (2) in the neighbourhood of joints, where growth of the connective tissue is minimal and has no clearly preferred direction, nerve trunks generally change direction and give rise to many divergent branches; and (3) at least one nerve trunk follows a route where rapid longitudinal expansion is accompanied by singularly marked lateral contraction of the connective tissue.
212 Cellular basis of morphogenesis

Early chick embryonic cells can form clones in agarose cultures
Eduardo Mitrani, Department of Zoology, Embryology Section, The Hebrew University of Jerusalem, Jerusalem 91904, Israel

Stage XIII occurs only about 4–6 h prior to primitive streak formation. At this stage the chick blastoderm is composed of two layers of cells: the epiblast from which all embryonic structures will develop and the hypoblast which is necessary for inducing an embryonic axis. Early chick embryonic cells prior to the formation of the primitive streak, have been cultured in a two layer soft-agarose system. Single, primary cells when grown in this system were capable of producing colonies ranging in size from 30 to 100 cells. The plating efficiency varied between 1 and 5 % and the colonies remained viable for about two weeks. The ability of cells to form colonies in soft agar is one of the properties that characterizes transformed or malignant cell lines. We believe this is the first report of normal, non passaged cells which show anchorage independent growth properties by forming colonies in a standard agarose culture in the absence of additional factors. The importance of being able to use normal monoclonal embryonic cell populations in studying early developmental processes is also discussed.

Cell polarity and tissue polarity in the insect epithelium
K. Nübler-Jung*, R. Bonitz, V. Grau and M. Sonnenschein, Institut für Biologie I (Zoologie), Alberstrasse 21a, D-7800 Freiburg, West Germany

We want to understand how polarity can be expressed within a cell and how individual cell polarities can be integrated into tissue polarity. We use the insect epithelium since here epidermal cells often express an asymmetry perpendicular to their apico-basal axes, that is, they appear polarized tangentially with respect to the epithelial surface. This tangential cell polarity is revealed by asymmetric cuticular protrusions such as denticles originating from single cells. The denticles normally point to the posterior of the animal. We consider overall polarity in the epidermis to result from the tangential polarities in all cells being uniformly oriented.

In order to test whether microtubular components of the cytoskeleton are involved in the expression and/or orientation of cuticular protrusions we injected colchicine into the larval abdomen of the cotton bug Dysdercus. The resulting anomalies in the adult cuticle indicate that colchicine can disturb – directly or indirectly – the tangential orientation of individual cuticular protrusions without, however, preventing the protrusions from being formed altogether. Thus, tangential polarity appears to reside in the individual cell as an autonomous property that can be oriented independent of the polarity orientation of neighbouring cells and should therefore not require a supracellular orienting system, like a gradient, for being established.

We have also tested whether an oriented migration of epidermal cells interfered with the expression or orientation of tangential cell polarity as would be expected if both were directed by the same factors. Migration was triggered by burning which causes the peripheral cells to migrate centripetally into the wound. The orientation patterns in the cuticle secreted during cell migration correlate with the orientation of cell migration, but other orienting factors complicate the results. The orientation of tangential polarity in epidermal cells is thus influenced by the direction of cell migration, and therefore epidermal cells might utilize the structures used for orienting cell migration also to orient their bristles and denticles.
Cellular basis of morphogenesis

Intestinal crypts in mouse aggregation chimaeras are each composed of epithelial cells of a single parental type

Bruce Ponder*1, Gunter Schmidt 1, Maureen Wilkinson 1, Marilyn Monk 2 and Maureen Wood 3. 1Institute of Cancer Research, Sutton, Surrey. 2MRC Mammalian Development Unit, London. 3MRC Laboratories, Carshalton, Surrey

Mouse intestinal crypts become morphologically recognisable about the time of birth. The mechanism of crypt formation is unclear, and no information exists about crypt progenitor cells. Using H2 antigens and a carbohydrate polymorphism recognised by Dolichos biflorus agglutinin as markers of mosaicism, we have examined multiple histological sections of small intestine and colon from 94 blocks of 29 mouse embryo aggregation chimaeras of five different strain combinations (CBA/Ca Lac «•• C57BL/6J Lac; C57BL/10ScSn Lac-cc «•• C57B.10A; RIII/Lac-ro «•• C57BL/6J Lac; DDK «•• C57BL/6J Lac; C3H/Bi «•• DDK) from 4 to 36 weeks of age. The sections examined sampled in excess of 10^6 crypts. To date, individual crypts have consistently contained epithelial cells of only one parental type: we have not observed a single ‘mixed’ crypt. The proportion of mixed crypts to be expected if each is formed by two or more progenitor cells depends on the patch size in the epithelium at the time of crypt formation, and is likely to vary between chimaeras with different proportions of each parental type. On ‘worst case’ assumptions based on observed patch sizes in sheets of adult intestinal epithelium, we estimate that the overall proportion would be at least 1 %, probably substantially higher. The absence of mixed crypts therefore implies either that the crypt epithelium is derived developmentally from a single progenitor cell, or that several progenitor cells are involved, but that in the chimaeras examined, cooperation in crypt formation can occur only between cells of the same strain. Analysis of individual colonic crypts dissected from female mice mosaic for the X-linked alleles Pgtk-la and Pgtk-lb has so far shown no mixed crypts in 127 examined. This suggests that the results are probably not attributable to strain differences in the chimaeric system, and that each intestinal crypt arises from a single progenitor cell.

Rescue of irradiated planarians by injection of purified neoblasts: evidence for the role of neoblasts as stem-cells and against the role of cell dedifferentiation during regeneration

E. Saló*, C. Auladell and J. Baguña, Departament Genètica, Universitat de Barcelona, Diagonal 645, Barcelona-28, Spain

Two main theories have been put forward to explain the origin of blastema cells during planarian regeneration: 1) the 'neoblast theory' (Wolff, 1964; Bronsted, 1969) where the blastema is formed by the accumulation of undifferentiated parenchymal cells (neoblasts); and 2) the 'dedifferentiation theory' (Hay, 1968; Coward, 1969; Chandebois, 1976) which states that the blastema is formed mainly by dedifferentiation of differentiated cells near the wound. Recently, Gremigni et al., (1980; 1982), using karyological mosaics which allow distinction between somatic and germinal cell lines, suggest the existence of 'trans-differentiation' during regeneration.

To clarify this problem, we have tested the regenerative and stem-cell capabilities of purified neoblasts and differentiated cells when introduced into an irradiated organism (host). Neoblast and differentiated cells were purified by serial filtration through nylon meshes and discontinuous density gradients respectively. The purity of both fractions was over 95 %.

The results show that when total cells and purified neoblasts are introduced into the irradiated host, mitotic activity recovers leading to a long survival, and blastema formation does occur. Instead, injection of differentiated cells does not lead to mitotic recovery and blastema formation. It is concluded, that, under these experimental conditions, dedifferentiation does not play any role in organismal recovery and regeneration, and that neoblasts appear to be true stem-cells and the only cell type leading to blastema formation.
Cellular basis of morphogenesis

Zebrafish epiboly: wheeling movement of deep cells at the blastodisc rim

K. Sander*, K. Dollmetsch and H. Vollmar, Biologisches Institut I (Zoologie), Albertstr. 21a, D-77800 Freiburg, West Germany

Blastodisc epiboly and the question of its homology to amphibian gastrulation have received new interest by the demonstration of Ballard (1973) that no invagination whatsoever occurs at the disc rim. Our finding that incomplete epiboly is followed by bipartite axiation or rachischisis anterior in the zebrafish (Baumann and Sander, 1984) led us to time-lapse filming of the deep cells. Using medium magnification Nomarski optics we found that initially all deep cells near the blastodisc rim follow the margin of the enveloping layer. However, on approaching the equator of the globular yolk mass, the blastodisc rim retards or even briefly reverses its progress while individual deep cells, perhaps one-third of those located near the rim, reverse their direction and head straight for the animal pole. These cells move at about 12 \( \mu \text{m/h} \), that is, much faster than before, and appear larger and more ameboid. This mass exodus is very conspicuous in the running time-lapse film. It continues on a lower scale until nearly the end of epiboly. By changing the level of focus we established that the reversing cells travel directly underneath the enveloping layer, and on top of the main mass of deep cells. Such a movement could have misled the early proponents of epiblast invagination (see discussion in Ballard, 1973) but of course the reversing cells should move below the deep cell mass if this were a remnant of gastrulation movements. None of our stage 15 film scenes revealed a rim sector void of reversing cells. It thus seems that ‘evaginal wheeling’ of part of the deep cell population occurs all around the blastodisc margin and even over the incipient germ shield (which was appearing out of focus in some of the scenes). How far reversing and non-reversing deep cells derive from the two differently staining classes of blastomeres (Dasgupta and Singh, 1981) remains to be shown. The reversing cells sooner or later stop moving singly and join a mass streaming related to deep cell convergence, but the vicinity of the animal pole remains free of these cells throughout. Convergence is thus seen to occur far above the germ ring, and this may provide an explanation for the incipient rhombencephalic duplications not caused by incomplete epiboly as such (Baumann and Sander, 1984).


Blastema size and cockroach leg regeneration

P. J. J. Shelton and P. R. Truby, Department of Zoology, The University of Leicester, Leicester LE1 7RH

Leg amputation in Periplaneta americana is followed by the dedifferentiation of stump tissue to form a blastema (Truby, 1983). The present experiments were designed (i) to investigate blastema size in animals of different sizes after the metathoracic leg had been amputated at the same level (the preformed breakage plane at the trochanter-femur joint), and (ii) to investigate blastema size in animals of the same size, where the metathoracic leg had been amputated at different proximo-distal levels. When the leg is amputated at the trochanter-femur breakage plane, we found that the stump dedifferentiated to the same anatomical level in the coxa, irrespective of animal size. The largest late instar P. americana nymph is seven times larger than the 1st instar nymph. The blastema in large animals is larger by a similar factor. Thus the pattern of the regenerated limb can be specified over blastemas of different size and cell number. Blastema size was also measured in 2nd instar animals (all the same size) after amputation at the following levels along the proximo-distal axis: coxa-femur joint, proximal tibia, mid-tibia, distal tibia and tibia-tarsus joint. For a given size of animal, it was found that the size of the blastema increases with the amount of limb amputated.

Cellular basis of morphogenesis

Calcium and neurulation in mammalian embryos

M. Stanisstreet*, M. Smedley and D. P. Moore, Department of Zoology, University of Liverpool, Liverpool L69 3BX

Neurulation is accompanied by changes in the shapes of the cells of the neural ectoderm and microfilaments appear in neural cells at the time when they are changing shape. Thus it is reasonable to assume that neurulation is effected, at least in part, by co-ordinated changes in cell shape brought about by microfilament contraction. Since in non-embryonic systems the contraction of microfilaments is initiated by changes in the level of intracellular free calcium, calcium is implicated as being important in mammalian neurulation.

In the present experiments rat embryos at 10.5 days of gestation, as a stage when the neural folds are elevated but not fused, have been cultured in various media, and then examined by scanning electron microscopy. Embryos cultured in medium without calcium show rapid collapse of the elevated neural folds. Addition of calcium, but not magnesium, to the Ca++ and Mg++-free saline prevents the collapse of the folds. Control experiments, in which embryos were deliberately dissociated, shows that the collapse of the folds is not due to cell dissociation. Culture of embryos in which the neural folds had collapsed in serum shows that the folds can re-elevate.

Culture of embryos in mannose produces neural tube defects after 48 h. In the present experiments embryos have been cultured in mannose, but examined for defects at times before the completion of neurulation, to attempt to observe the first indications of neural tube defects.

Thus successful neurulation requires the continued presence of extracellular calcium and the correct energy pathways within the cell. Perturbation to either could lead to neural tube defects in mammalian embryos, including perhaps humans.

Morphogenesis of the vertebrate chondrocranium

Peter Thorogood*, Department of Biology, Medical and Biological Sciences Building, University of Southampton, Southampton SO9 3TU

The developing vertebrate skull is composed of both chondrogenic and osteogenic cells. The greater proportion of these are derived from the cranial neural crest and a smaller contribution comes from the primary mesoderm. The differentiation of avian crest cells into cartilage and bone is the result of interactions with epithelia in the developing craniofacial complex, either during migration(1) or after migration has been completed(2). Using transfilter culture techniques, we have previously shown that the mechanism of interaction leading to chondrogenic differentiation of crest cells is not mediated by freely diffusible factors or mobile matrix components. The degree of close association necessary between the two cell populations indicates that the interaction is mediated either by plasmalemmal cell-cell contact or by crest cell contact with non-diffusible matrix components associated with the basal surface of the epithelium, in which case the basal lamina is implicated(3).

Ultrastructural investigation of the interface in vivo revealed that the basal lamina is intact and that cell-cell contact does not occur(4). Immunocytochemical analysis of the composition of the matrix at the in vivo interface demonstrated that although laminin, fibronectin, type I and type IV collagen were present throughout, type II collagen was only detectable during the interaction and not after its completion(4). Subsequent investigation of the spatial and temporal expression of type II collagen revealed that it is present at the basal surfaces of a number of ectodermal and neurectodermal epithelia from the earliest stages of head development and prior to the completion of crest migration(5). Its spatial pattern maps precisely with the subsequent chondrocranial pattern. Thus it appears that this particular epithelio-mesenchymal interaction has not only a cytodifferentiative role, but a morphogenetic one too.

Patterns of cell division during the formation of lateral outgrowths in the leg of the cockroach

P. R. Truby, Department of Zoology, The University of Leicester, Leicester LE1 7RH

When the antero-posterior axis of a leg is reversed at a graft by exchanging a left leg for a right leg at the mid-tibia level, regeneration occurs in the region of the graft/host junction. This results in the formation of a pair of lateral supernumerary legs. In these experiments the patterns of cell division which take place during supernumerary leg formation were observed in sections of regenerating legs of the cockroach Leucophaea maderae. Early patterns of cell division resemble those seen in controls in which no axis reversal had been carried out during grafting, and these cell divisions correspond to the process of wound healing. Later, a large area of the epidermis proximal to the graft/host junction and on the internal face of the leg becomes activated and shows a rapid rate of cell division. This area forms two outgrowths which grow into the supernumerary legs by cell division throughout their epidermis.

These results suggest that, as for leg regeneration (Truby, 1983), the formation of supernumerary legs involves dedifferentiation of the epidermis in the region of the graft to form a blastema, and is not due to local cell division at the point of maximum pattern discontinuity.