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Physical fields and cellular organisation: field-dependent mechanisms of morphogenesis

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The articles in this review series have considered several very different aspects of morphogenesis. As much as it was desirable to do so, however, we have not included a paper which deals specifically with the role of the gene during this process. To some extent this heinous crime is mitigated because reviews have recently appeared which comprehensively (and exclusively) cover the subject [1, 19]. Some of the other contributors to this volume have made reference to the genome as an important component of the developmental process and for the sake of completeness I shall also say a few words. Without any prior prompting of the other contributing authors, however, the prevailing theme which has emerged is that in order to solve problems in morphogenesis one must draw inspiration from physics and chemistry to complement biological intuition. And, indeed, this review synthesis was conceived principally with that aim in mind.

For the main part of my contribution, I would like to emphasise the likely influences of various types of physical fields on cellular organisation and to morphogenesis in particular. Thus in reply to Schatz's edict it is held that some of the hitherto obscure structures within eucaryotic cells may be chemical, dielectric, electrical, mechanical or osmotic, scalar or vector fields. The nature of physical fields (vector or scalar), however, is not readily appreciated within conventional cell biology and so to begin I shall briefly describe their nature and origin.

Scalar and vector fields

A physical field may be said to exist if a discrete value of a parameter may be identified over a given spatial region (unit) within the total area designated by the field. The parameter may be any quantity that can be measured. A scalar field represents a field of scalar quantities such as temperature or concentration (e.g. Ca^{2+} or electric charge). Or it may be a vector field whereby a field of vector quantities exist such as electric (or ionic) currents. A good example of both occurring together is illustrated by a standard weather map. The discrete values of temperature at each position on the map represent a scalar field whereas discrete wind velocities represent a vector field.

Similarly, in a living cell there may be concentration gradients of ions within the cell which would represent a scalar field of electrochemical potential or the individual currents which generate the concentration gradient may represent a vector field of currents. Another way of expressing this state of affairs which is more in line with the jargon of developmental biologists is by describing the fields as patterns of molecular species within cells. Pattern analysis is a major preoccupation of developmental biologists and there are some elements of this work which I shall now discuss.

The formation of spatial patterns within cells

Dynamic, apparently spontaneous, pattern formation is a characteristic of almost all eucaryotic cells. A rather nice introduction to the subject may be found in *Pattern Formation* [26]. This phenomenon is not exclusive to living systems, however, as even 'simple' chemical systems may exhibit it, of which the most celebrated example is the Belousov-Zhabotinski (B-Z) reaction. Although the reaction consists of rather simple component parts, such as an organic reductant (such as bromate) which is oxidised by a redox couple (such as Mn^{2+}/Mn^{3+}), nevertheless, it exhibits a very rich phenomenology [8].

In an effort to describe these systems Winfree [60] has elaborated the concept of an oxidising 'trigger wave' moving through an excitable reducing medium. The 'wave' and its effects are visualised by colour changes of an appropriate redox indicator or from the redox potential measured around a platinum electrode. The 'trigger wave'

moves unidirectionally through the medium oxidising bromide at the wave front but leaving a greater concentration behind it. The rate of propogation of the wave is determined by the bromide concentration; a sufficiently large concentration may stop the wave altogether. When such trigger waves meet they annihilate each other without any interference patterns or difraction effects. Most remarkable about these B-Z patterns, however, is their resemblance to those formed within living cells.

B-Z reactions, may be said to exhibit deterministic chaos or amplified fluctuations as well as other types of symmetry-breaking and are topics of intense experimental and theoretical study. Stimulated by Turing's prodigious achievement together with observations of chemical systems, much work has been done to develop mathematical frameworks upon which biological pattern formation or symmetry-breaking phenomena may be understood. Direct experimental support for this wealth of biological theory, however, leaves a lot to be desired. On the other hand Murray and Oster[28] both independently and together have consistently provided good mathematical models which can indeed describe dynamic bio-systems. In accordance with the spirit of these reviews, I prefer to direct the reader to original publications of this work[8, 13, 18, 28] and references therein rather than simply reiterating what Murray and Oster have so precisely done previously. And, I shall concentrate on an area hitherto not explored.

Traditionally developmental or cell biologists may use homogeneous or chaotic almost indiscriminately to describe the state of the eucaryotic cytoplasm, although the former may be the result of the latter. The question, in biological terms as to what constitutes a uniform or homogeneous condition, however, is not easily answered. Under some circumstances an apparently homogeneous chemical system or even one which exhibits a stable pattern (e.g. as with the B-Z reaction) with a very small perturbation may, respond, respectively by forming a pattern or relaxing to a new one. When the cytoplasm exhibits such behaviour the traditional response (perhaps also the correct one) would be to resort to the genome in order to provide an explanation[26]. I would like to offer an alternative by considering that the nature of development is a kind of stimulus-response coupling process which also calls upon the genome although not quite in the expected manner. First it is necessary to say a few words about chaos . . .

An homogeneous chemical system may exhibit a condition which is known as *chaos*. This means that there are deterministic time evolutions involving a sensitive dependence on the initial conditions from which periodic or non-periodic behaviour may be observed. From a mathematical point of view the deterministic time evolution corresponds to a differentiable dynamical system. Because of the sensitivity to the initial condition, a small perturbation may grow exponentially with time. The asymptotic evolution of the system may take place on a complicated set in phase space called a *strange attractor*. Work along these lines is found in many aspects of quantitative biology from such diverse areas as population behaviour to brain function. As far as I know, however, developmental processes in biology have not hitherto been considered from the point of view of Hamiltonian dynamics and the particular role of chaos therein. This is not surprising considering the large number of degrees of freedom that biological systems possess, although, we may make some simplifying assumptions. Unfortunately, space does not permit a complete exposition of this interesting area of physics, although, I would like to emphasise that several aspects of this work may be relevant to biological systems and should be explored.

Consider a simple system with a spatial periodicity (or a regular motion, i.e. an ergodic system); typically both chaos and regular motion are exhibited depending upon the initial conditions. The nature of the regular motion will be the same as that of traditional integrable systems so that it exhibits a quasiperiodicity with a discrete set of frequencies v_j; the motion is confined to a region of N-dimensional space in the 2-N dimensional phase space.

$$\sum_{J=i}^{N} v_j n_j \qquad (1)$$

Chaotic motion occurs when a local exponential divergence of trajectories is accompanied by global confinement in phase space, which stretches and then folds, etc. etc. It is this repeated folding that is described as chaotic. The stretching, however, is compensated for by shrinkage so that the area in the phase space is preserved (Liouville's theorem follows when this involves volume). In dissipative systems there is no compensation and the chaotic motion differs. Both are not well characterised but are observed to be exponentially unstable (as with the B-Z system) and interestingly far away from the regular (smooth) region, the chaotic motion may resemble a diffusion process.

Studies of these properties have involved the use of area-preserving maps which are phase plots obtained by subjecting the regular motion of a system in one dimension to a sequence of perturbations at unit time intervals. The interpretation of this technique (also known as Henon mapping) requires the use of KAM theory which was developed over the period 1954–1963 and named after the mathematicians A. N. Kolmogorov, V. I. Arnold and J. Moser[16]. KAM theory explains how a small periodic perturbation disturbs a stable dynamical system and whether it may lead to instability[16]. One of the most interesting properties of the theorem, however, is the prediction that the perturbed system may retain its overall stability but microscopic bands of instability become manifest. These bands correspond to 'resonances' between the original stable macroscopic system and the disturbance. If the intensity (or frequency) of the perturbations increases then the resonance bands will widen and exist as independent 'islands' of stability. Further

increase of the amplitude of the perturbation may lead to the eventual domination of the system by the resonance (islands). The resonance bands also possess fine structures of smaller bands with resonance instabilities, which themselves possess second and third order hyperfine structures, ad infinitum . . . (i.e. they exhibit fractal geometry).

The resonance bands exhibit two types of behaviour; stability or smoothness (which I will refer to as islands, so-called because on area-preserving maps thats what they look like) and chaos. If the system settles upon the island regime, then there is relative stability with chaos between the islands. Depending upon the position of the system: close to the centre of the island it may exhibit cyclic regular motion or towards the edges the motion may still be regular but will not be exactly reproducible (i.e. the fringes of the system are evolving). The fate of the system, therefore, depends upon the disposition, number density and size of the respective regions of chaos and islands. It is also possible that very minor perturbations may upset a large otherwise stable system.

KAM theory has been directed towards understanding the solar system with success, the Kirkwood gaps in the asteroid belt, for example, are thought to be due to resonances. Similarly, it has found a use in atomic physics. In fact any system in which dynamic stability is an important parameter may be addressed by the theory. The limiting factor, as always, with such work is the number of degrees of freedom that the system possesses. Clearly it will be a long time before KAM theory may be utilised to totally model the complex systems found in biology. Current work in KAM theory is centered around the structure of the resonance bands; Birkhoff, in particular, has developed a representation of these bands using a code which he terms 'symbolic dynamics'.

Now, consider a relevant biological application: during the course of cellular development, patterns (whatever their nature) may form within a cytoplasm or syncitium apparently without any observable cause or direction (in other words the mechanism is unknown); the segmentation patterns of *Drosophila* are a nice example of this. The very early events in these processes may be referred to as cytodifferentiation and prelude morphogenesis. The distinction between them, however, is purely for convenience as it could be argued that cytodifferentiation is merely morphogenesis over a smaller spatial interval. Although, the developmental biologists have good reason for this discrimination (see Stern and Cannings), it is not strictly necessary for my purposes, and I shall refer to them both indiscriminately. Explanations of the mechanism of pattern-formation (morphogenetic) phenomena are rich and varied [26]; some are referred to in the articles by Goodwin, Nuccitelli, and Stern and Canning. But, there is still no generally accepted mechanism which causally describes morphogenesis although it seems that the molecular geneticists have at least a framework with which to assess the origin of say the segmented germ band of *Drosophila* [1]. Consequently, there are vigorous efforts to describe development from the genetic point of view. Gene-based explanations, however, still require the ad hoc assumption that there is an underlying periodicity of unknown nature and origin which must interact with the gene in order to account for the developmental process [19]. Thus KAM theory might be utilised to account for the hidden periodicity and therefore, the 'spontaneous' morphogenesis of undifferentiated cytoplasm (or regions of the cytoplasm) to something akin to the early segmental (germ band) pattern of the *Drosophila* embryo. An appropriate mechanism based on KAM theory to describe the origin of stable periodic structures (cytoplasmic or otherwise) which may interact with genes and, therefore, complement genetic explanations of development might be advanced as follows: the cytoplasm is regarded to be in communication with the genome, whether there is a single copy or multiple copies as in a syncitium. Similarly the cellular environment is in contact with the cytoplasm, as shown in figure 1. The system is also considered to be dynamic which means that there is molecular movement (i.e. due to Brownian motion) and a flux of matter, the total amount of which may be constant but its chemical form may be altered.

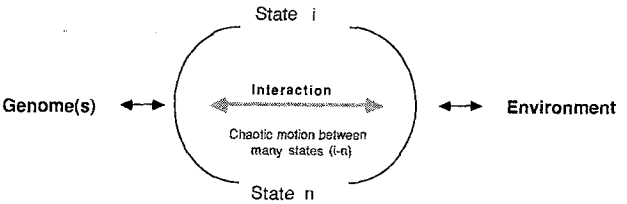

Figure 1. A schematic representation of some possible interactions between a genome (or several in the case of a syncitium), the cytoplasm and the environment of the cell. The cytoplasm exhibits and may exist in many different (i–n) but not independent states during a time evolution.

Now, on the basis of the KAM theory, the time average structure of the cytoplasm may exhibit stable dynamic structures (regions of smoothness or islands, i.e. order) in equilibrium with regions of chaos (no structure) schematically shown in figure 1. Any islands of regularity (order) will be microscopic and probably also unobservable. During, the time evolution of the system, it is likely that the genome(s) is also active, resulting in protein synthesis. Whether this be regulated or random, catalytic or structural proteins are introduced into the system. This represents a perturbation of the cytoplasm. According to KAM these perturbations may elicit changes in the disposition of both islands and chaos within the cytoplasm. The resultant status of the cytoplasm may, therefore, be either much more ordered, i.e. with islands dominating the system or alternatively, a more chaotic regime may ensue. In the case of the former, a macroscopic dynamic pattern would occur which, if it were stable (or stabilised), would in effect be (cyto-)differentiation (or e.g. segmentation). If development were to occur along these lines then the role of the genome becomes much more

subtle. It is even conceivable that the (specified-homeotic?) gene products may be produced randomly and cytodifferentiation could still occur. Of course the genes must eventually be switched on and off but one could imagine that the cytoplasm may influence them by a (simple?) physical mechanism (equivalent to +/− feedback) in order to control its activity, at least in the early stages of development.

Obviously to do KAM theory justice one should be more explicit both mathematically and biologically. I avoided the former because I wished to make it more accessible to biologists and the latter due to lack of space but this short account indicates that further exploration would be a fruitful enterprise. Preliminary work in this area looks encouraging and will appear presently.

Physical fields in the cell

Once the primary developmental events have taken place and the cell has begun to cytodifferentiate, then more specific mechanisms may take over to consolidate and elaborate cell structures and attain the 'required' (observed) morphology [26]. This is likely to involve the establishment (which may be the primary morphogenetic event), maintenance and metamorphosis of physical fields. The following, therefore, is a catalogue (albeit rather brief) of those macroscopic fields which are anticipated to play important roles in cellular organisation and morphogenesis.

Electrochemical fields

Electrochemical (ionic) currents and spatial potential differences along cell membranes have been identified in many biological systems. Much of this work is reviewed by Rich Nuccitelli, so I shall only mention it where I wish to make a specific comment. The most abundantly documented effects of ionic potentials and currents on biological structures, however, are those which concern *transmembrane* traffic. As Rich Nuccitelli mentions this in passing, I shall discuss them in more detail.

The effects of a transmembrane electric field on membrane organisation

In physical terms according to the fluid mosaic model [47], biological membranes represent a single hydrophobic, smectic mesophase of the order of 4 nm across which separates two bulk aqueous phases. Despite the success of this model it has received criticism and many have suggested that some elaboration is necessary [5, 23, 27, 32, 36].

Under many circumstances an electrochemical potential difference exists across biological membranes. The potential difference across the inner-membrane of rat liver mitochondria for example is around 200 mvolts [33]. Similarly the plasma membrane potential of Acetabularia acetabulum, is about − 170 mvolts [43]. Membrane potentials close to 200 mvolts, therefore, are quite common in biological systems. They are equivalent to a DC electric field of approximately $5 \cdot 10^8$ volts/m. In macroscopic terms this is an enormous field, which would result in sparking in a vacuum. In microscopic terms (i.e. at the atomic or molecular level) such fields are not altogether unusual and we should not be mislead by their magnitude. In terms of the effects of these fields on a given biological function, therefore, one must decide whether it is the microscopic or macroscopic properties of the membrane which are relevant.

Estimates of the field strength across membranes include the assumption that they are homogeneous (or at least symmetrical) and linear. Although, there are many indications that both assumptions are not entirely justified [55]. Large fields, for example, do not affect the average fluidity state of the membrane to any great extent but they may affect the motion of the phospholipid headgroup region [35]. Similarly, the ionic conductivity of phospholipid membranes bears a non-linear (exponential or hyperbolic sine) relationship with the transmembrane electric field [34]. We have shown that both biological and artificial membranes exhibit a linear relationship between conductivity (G_m) and voltage till about 130–150 mvolts is reached. Around this voltage the G_m begins to increase exponentially until at voltages of 250–700 mvolts, the membrane ceases to represent a permeability barrier and is said to have undergone dielectric breakdown. This is a stochastic process, for depending upon the duration of the high voltage the membrane may return to its former, relatively, impermeable condition or it may be permanently denatured. Dielectric breakdown is a complex phenomenon(a) and occurs under several, not altogether well-defined, conditions with various types of membrane. Its functional significance may be nothing more than determining the maximum voltage which may occur across membranes or judging by work on electrofusion it may have a role to play in fusion/secretion or have some pathological significance [61].

Surprisingly not much work has been done on the effects of membrane potentials on the physical state of membranes. We have had indications that the membrane potential exerts its greatest (only?) influence within a few debye lengths of the phosphate head-group [35]. This may be related to the role a transmembrane electrochemical potential difference plays in membrane biogenesis. Schatz and co-workers have demonstrated [11] that the insertion of enzyme precursors into the inner-mitochondrial membrane require the assistance of a protonmotive force ($\Delta p [= \Delta \mu_{H^+}/F]$). Furthermore, other studies (Ohta, O'Shea and Schatz, unpublished observations) indicated that there was an exponential or hyperbolic dependence of protein insertion with respect to the electric field.

The effects of a transcellular current and voltage on cellular organisation

In addition to a transmembrane electrical potential difference many cells (and tissues) exhibit a transcellular electrical potential difference [22, 30]. The latter may originate due to an asymmetric disposition of the ionic sources and sinks which generate and maintain the former [37]. Jaffe [22] and Nuccitelli have suggested that this electrical polarity may have a major developmental significance. Although, Stern has argued that such claims are excessive [49]. It must be conceded, however, that a considerable body of evidence has been amassed which is at least consistent with their promulgation [30]. Whilst I intend to make some comment on this proposition, for the moment I shall merely mention some of the mechanisms of how a transcellular field may effect cellular morphogenesis.

Lateral electrophoresis

Jaffe [21] has pointed out that an electric field parallel to the surface of a cell should redistribute charged macromolecules which are free to move laterally along the plasma membrane. In agreement with this Poo and co-workers [39] demonstrated that a DC electric field applied parallel to the cell membrane does indeed redistribute lectin and acetylcholine receptors which have an initial random disposition. Once the imposed field was negated, the lectin receptors subsequently reattained their random regime. The acetylcholine receptors, however, remain concentrated, despite the absence of the field.

Electro-osmosis

With the imposition of an electric field parallel to a charged cell surface an electro-osmotic flow of fluid may be produced which may then drive charged macromolecules along the cell surface [25]. The direction of movement of the macromolecules was suggested to depend upon the respective zeta potentials (ζ) of the cell surface and the macromolecule. As an example of this phenomenon McLaughlin and Poo [25] demonstrated the migration of native and modified concanavalin A (Con A) receptors along the plasma membrane surface of embryonic muscle cells of *Xenopus*.

Electrostatic fields

The influence of the electrostatic environment has now become recognised as one of the more important parameters for stabilising protein structure [56] and also for catalysis. Scalar fields of electric potential due to static charges located around the active site have been proscribed to be important in the catalytic mechanism of certain enzymes [42, 57]. The site-directed mutagenesis studies of Fersht and Russell [42], in particular, have elegantly demonstrated the importance of the local field.

The electrostatic environment of the entire enzyme may also influence catalytic efficacy. Ricard [40] has recently reviewed the role of this extra-enzyme environment on the control of the catalytic activity of enzymes associated with cell expansion located within plant cell walls. Ricard's proposition is essentially phenomenologically based but has nonetheless proved useful for understanding the biochemical component of this particular growth process. We have also been concerned with the environment provided by plant cell walls and have treated it as a 'reticulated charged lattice' (O'Shea and Ridge 1987, submitted for publication). Our analysis is rather more formal than Ricard's approach and makes a number of interesting predictions some of which I will reveal below. Other effects of electrostatic fields include the possibility that large cellular or extra-cellular environments may be created by movements of polyelectrolyte sheets which have some role to play in development.

Dielectric fields and hydrophobic interactions

Hydrophobic interactions play a central role in micelle formation, biological membrane structure and in stabilising the conformation of proteins. It was formerly believed that as the interactions were so strong, some kind of bond was associated with it; now of course, it is realised that they involve the configurational rearrangement of water molecules as two hydrophobic species come into proximity. Israelachvili and Pashley [20] determined the attractive component of the 'hydrophobic interaction' to be about an order of magnitude stronger than the van der Waals dispersion force and the range of the interaction to be about the same. The hydrophobic 'influence', therefore, is exerted over a much longer range than the vectorial co-valent bond.

Whilst the 'hydrophobic effect' is well documented [51] and its function in stabilising macromolecular structures relatively well understood, its role in the overall or long-range organisation of the whole cell is rather more obscure. Whilst it is clear that a cell is equipped with a macromolecular cytoskeletal network [44] some parts of which are hydrophobic, the role of the accompanying 'dielectric field' has received scant attention. This field may extend about the skeleton with a radius of something like 10–20 nm [20]. It could, therefore, represent a considerable proportion of the cell volume.

As far as I know there have been no estimates of the area of hydrophobic or charged surfaces within a cell. Any such analysis would at the present state of knowledge be purely speculative. It is worth considering a simple case such that if two identical macromolecular hydrophobic patches were nearer than say 20 nm then a symmetrical 2-dimensional (2-D) dielectric field would exist. If the hydrophobic regions were of macroscopic dimensions (e.g. $> 100\ nm^2$ and also not identical) then a significant 3-D, non-linear dielectric field would exist. Such fields would be effectively made up of water molecules with rotational correlation coefficients unlike that of bulk water as a result they may be identified by

NMR spectroscopy (or in the future with NMR imaging; see Lohman and Ratcliffe). NMR and dielectric-relaxation studies have indicated that about 5% of the total cell water is tightly bound and about the same percentage is loosely bound. On this basis one might conclude that water plays a relatively minor role in cell architecture. Clegg et al.[3] have pointed out, however, that studies of cell-water are based on the average rotational correlation times of single H_2O molecules and no provision is made for the long-term collective dynamics of long-range effects. With this last point in mind, Watterson[58,59] has recently introduced a model which accommodates the pedagogical 'flickering cluster' concept of liquid structure but in addition, suggests that ordered clusters do not appear and disappear randomly, rather they travel as a wave. From Wattersons analysis, I calculate that under fairly dilute conditions, a single cluster wavelength will be about 3.3 nm and may contain something like 1331 water molecules with a volume of 36 nm^3 which means the molecular mass will be more than 20 Kd. Watterson also proposed that solutes influenced the structure of the cluster. If the 'solute' was an extended 2-D surface, however, it would force a nodal plane in the wave motion. Similarly an extended 1-D 'solvent' filament could be formed next to a solute-filament (e.g. such as an actin filament) parallel to its axis. In other words standing waves of water clusters may provide a means of stabilising supra-macromolecular structures within cells. It is conceivable that filaments of water molecules may form a scaffolding network at least as complex as the cytoskeleton. If Watterson's analysis can be corroborated and developed then it is potentially as important in understanding the nature of cellular structure as Watson and Cricks efforts were in understanding biological inheritance.

Mechanical fields

The mechanical properties of non-contractile tissues have, until fairly recently, been considered a curiosity rather than an integral part of cellular function. Nevertheless, the cell is a complex 3-D body containing scaffolding (*contractile*) structures in the form of the cytoskeleton and the extracellular matrix. Moreover, the cytoskeleton appears to be linked to the extracellular matrix via a transmembrane structure known as 'integrin'[7]. The latter appears to be a family of homologous glycoprotein structures of diverse cellular origins. Their functional role appears equally diverse; they are involved in haemostasis, cell-matrix interactions and lymphocyte killing and help.

Now, associated with these macromolecular structures, as I have briefly mentioned above, there must be certain physical fields. The properties (elastic-tensile?) of some of these supra-molecular assemblies, therefore, may well depend upon their respective positions within a Ca^{2+} field. The cytoskeleton may interact with the ambient aqueous environment through the medium of these fields, thus its local properties may be modulated by small charged or uncharged molecules in the cytoplasm: Ca^{2+} binding to E–F hand-like structures in non-muscle α-actinin has been shown to affect the rate and extent of cytoskeleton synthesis[44,45]. Furthermore, the elastic modulus of a charged elastic surface (such as the cytoskeleton) may be influenced by the surface potential, which is itself a function of the ambient ionic strength[4].

The visco-elastic properties of cellular components may be influenced in several ways: by varying the rate of their synthesis, i.e. because it would influence the mechanical strain on the existing structures; the presence of absence of Ca^{2+} in the cytoplasm may influence both the elastic modulus of the structure and the rate of synthesis, as in the case of the cytoskeleton[13,14,44]. An interesting example of this may be found in David Deranleau's article whereby, cytoskeletal effects are suggested to be involved in the cell shape changes promoted by stimulation and Ca^{2+} movements.

Multicellular assemblies may also exert stresses and strains upon each other and cell migration is the basis of many theories of morphogenesis[53]. It appears that cell-adhesion presumably via the extracellular matrix to the substratum are the most likely candidates for cell-cell contact. The cells apparently sort into layers, then bend and buckle to prelude tissue formation. Some of these mechanisms as well as the specific role of cell migration have been reviewed by Trinkhaus[53]. Similarly, many of these properties have been assembled by Oster and coworkers[28] into a convincing theory of the morphogenetic role of mechanical forces.

Fields of water potential

The presence of a cell wall means that plant cells have an extra degree of freedom in that they may exhibit regions of varying osmotic pressure due to a limited ability to swell. This means that mechanical pressure may be exerted and may be important in the growth process. Green[17,18] has incorporated some of these ideas into an elegant model of phyllotactic pattern formation at the apical meristem of plants. Animal cells may also employ water potential gradients (resulting from ion transport) to exert Maxwell pressure[15]. This latter possibility has not been explored at the cellular level as much as it deserves but it might be an interesting line for future research.

Magnetic fields

I would like to reiterate Steve Swithenby's anathema to any suggestion that biologically generated macroscopic magnetic fields (or any other ill-defined field i.e. so-called 'auras') are causal features of biological development or are instrumental agents in physiological processes. This is not to say that magnetic fields which result from biological phenomena do not exist. Indeed, as ion cur-

rents are a basic feature of living cells, magnetic fields will exist (provided destructive interference does not occur). These magnetic fields are too small to elicit any biologically useful action. They are of sufficient magnitude, however, to be measured by the SQUID magnetometer (see Swithenby).

The solid state cell

So far, I have described (albeit briefly) how some macroscopic physical fields may exist alongside the now well-established supra-molecular ('quinternary/hexaternary') chemical structures (e.g. cytoskeleton, cell wall, organelles, etc.) of the eucaryotic cell. These fields may result from the supra-molecular structures themselves or be generated by other processes. This view of the whole cell seems to have emerged with complementary perspectives in metabolic biochemistry [9]. For, although the enzymic components of intermediary metabolism have been identified and relatively well characterised for some time, the traditional reductionist experimental strategy of enzymologists has failed to reveal the organised systemic behaviour of metabolic pathways.

Physically unconnected soluble enzymes, such as those in the glycolytic sequence, exhibiting one specific activity in vitro and another in vivo, initially proved to be enigmatic. The in vivo activity of citrate synthase deduced from oxygen electrode studies is greater, for example, than may be accounted for from its known kinetic constants. An attractive solution to this paradox incorporated the recent ideas of supramolecular structures or coupled behaviour for it was proposed that citrate synthase must receive oxaloacetate directly from the malate dehydrogenase [9, 48]. Thus Freidrich [9], for instance, suggested that metabolic sequences may exist as a 'fluid' 3-D 'jigsaw puzzle' and exhibit what might be called dynamic compartmentation. These assemblies appear to be preferred as they comply with and facilitate the strict energy economy of the cell. Recent quantitative assessments of metabolic pathways indicate the wisdom of considering the pathway 'holistically' (i.e. in terms of system or control theory [54]).

Contemporary views of the systemic organisation of biological catalysts together with the other more well-defined architectural features such as the cytoskeleton, the extracellular matrix, integrin, that it exhibits collective dynamics [10] and the possibility that 'dynamic chaos' is a feature of the cytoplasm must indicate that the cell is a 'kicking-screaming stochastic machine' and is far more complex than has hitherto been suspected. In fact it seems that the cell possesses many of the attributes of a solid-state device with a long-range internal organisation rather than just a biochemist's bag.

Unfortunately space does not permit a more comprehensive description of the role of physical fields in each structure, process or function carried out by the eucaryotic cell. So rather than concentrating on the morphogenesis of a given cell (Deranleau), tissue (Stern and Canning) or organism (Lohman and Ratcliffe), I would like to consider one relatively simple cellular structure in some detail. I have chosen the plant cell wall as it seems to me, that the chemical nature is known in some detail and it is a rather nice example of how the morphogenetic process could be intimately linked with other more routine cellular processes. To illustrate this I shall consider some of the physical properties of the cell wall, paying particular attention to the effects of electrochemical fields which may then give us some other clues of the 'ground rules' of morphogenesis (stimulus-response coupling?).

The reticulated charged lattice model of the plant cell wall

The plant cell wall is effectively continuous around the cell or indeed around the whole plant and may justifiably be regarded as a single giant macromolecule or an extracellular organelle. They are composed of a complex array of polysaccharides, microfibrils, structural proteins and enzymes [50]. Owing to the presence of polyuronic acid groups and other charged species [41] we regard the wall, as a complex 3-D charged lattice. Under virtually all circumstances cells are bathed in an electrolyte which will presumably be in some form of equilibrium with the cell wall and the cell. We have attempted to identify the electrostatic nature of the cell wall, so as a first approximation we have treated the wall as a reticulated lattice of charged surfaces in quasi-equilibrium with a bulk phase aqueous electrolyte. An appropriate equation (eq. 2) relating the surface charge density, the electrostatic potential and the electrolyte may be written as follows:

$$\sigma = [2\,\varepsilon_r\,\varepsilon_o\,kT\,\Sigma_i\,C_{ni(\infty)}\exp(-Zn\,F\,\psi_{(d)}/RT)]^{1/2} \qquad (2)$$

where:

σ = The surface charge density of the cell wall lattice

ε_r = The dielectric constant of the ambient electrolyte solution

ε_o = The permittivity of free space

$\psi_{(d)}$ = The potential at distance (d) from a surface within the cell wall lattice ie. $\psi_{(0)}$ = The surface potential, $\psi_{(10\text{ nm})}$ = The potential 10 nm from the surface K, T, Z etc. have their usual meanings

The formal derivation and the inherent assumptions of this analysis may be found in O'Shea and Ridge (1988, and references therein, submitted for publication). Eq. 2 may be applied as long as the surface is planar (Poisson Equation) and the charge density is uniform for a given surface.

The statistical tendencies for the counter-ions and co-ions to diffuse away and toward the charged cell wall lattice, respectively, are expressed by the Boltzmann relation. It is clear, therefore, that the anion and cation concentrations near the surfaces of the charged lattice representing the plant cell wall are not expected to be

equivalent. The electrostatic attraction of the counter-ions and the repulsion of the co-ions to the charged surface is described by Poisson's equation which is the different form of Gauss' law and, for a single point charge, would reduce to Coulombs law. The combination of these two expressions results in the Poisson-Boltzmann equation which may be solved with appropriate boundary conditions to yield the potential at any distance from a cell wall surface. Eq. 2, therefore, also expresses the quantitative dependence of the potential with distance ($\psi_{(d)}$) from any surface within the cell wall, on the surface charge density and the salt concentration and valency of the bulk phase electrolyte.

On the basis of the known molecular components[41, 50] and some assumptions for the wall architecture we may obtain a value for the average surface charge density (σ) of a given surface within a cell wall of $4.8 \cdot 10^{-4}$ C $\cdot$ cm^{-2} which is equivalent to a charge density of about 1 electronic charge/3 nm^2. This is roughly equivalent to the figure quoted by Demarty et al.[6]. With this value for σ, it is shown in figure 2 that we may estimate the average potential at all the surfaces ($\psi_{(0)}$) within the cell wall, to be $-$ 60 mV. Furthermore, the potential at any distance from the charged surface of the cell may be calculated and varies in the manner shown in figure 2.

The point in figure 2, where the potential falls to 1/e (at D) of the value at the surface is known as the Debye length (i.e. 1/D). This is dependent upon such factors as temperature, ionic strength and the valency of the supporting electrolyte: if we consider, for example, a charge density of 1 electronic charge/3 nm^2, the resultant Debye length may be about 1 nm in 10^{-1} M (1 +:1 $-$) electrolyte (such as KCl) and about 10 nm in 10^{-3} M at room temperature. At a distance from the lattice surface of several Debye lengths (fig. 2), the potential will be the same as that in the bulk solution. This is quite different from the relationship predicted by the Donnan models of Sentenac and Grignon[46] and Demarty et al.[6] which anticipates a discontinuous (step-wise) potential change from the cell wall surface to the bulk phase solution.

In dynamic physical systems the total free energy will always be at a minimum. There may also be local equilibrium even though the system could be very far from thermodynamic equilibrium[24]. In terms of ions binding freely to a charged surface, therefore, any potential difference between the surfaces within the cell wall and the bulk phase solution must be minimised. Thus appropriately charged ions would bind to the surfaces within the cell wall minimising the potential difference between the bulk phase solution and the charged surfaces. Thermodynamic equilibrium will not be reached but a dynamic quasi-equilibrium condition (steady-state) will exist. This means that the ionic concentrations will be quite different at the cell wall surface from that in the free solution.

The electrochemical potentials of specific ions and thus the ionic concentration in the regions close to surfaces within the cell wall may be calculated from the Boltz-

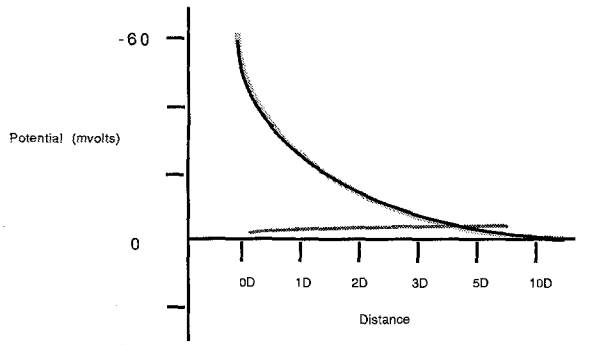

Figure 2. The profile of electrostatic potential (——) with distance from the plant cell wall surface based on eq. 2. The units of distance are in debye lengths (d) see text.
Also shown are the profiles of cationic (:::::) and anionic (≡≡≡) concentrations. The cation and anion concentrations at a cell wall surface is about 1.0 M and 10 mM, respectively.

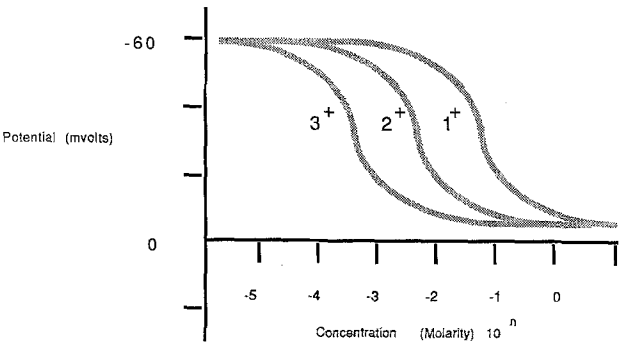

Figure 3. The relationship between the cell wall surface potential and the bulk phase cation concentration for ions of different valency, e.g. lanthanum, calcium and potassium.

mann relation. Thus taking the deduced value for the surface potential of $-$ 60 mV with a bulk phase electrolyte concentration of 10^{-1} M, then the concentration of cations at the wall surface would be 1.0 M and the concentration of anions would be 10^{-2} M (also shown in fig. 2). Alternative ion-binding models[6, 46] do not predict this behaviour but are still nonetheless useful as first approximations.

The surface potential (and hence $\psi_{(d)}$, under conditions of quasi-equilibrium) varies with the ionic concentration and valency as shown in fig. 3. The analysis also predicts at least an order of magnitude difference between the effects of a monovalent cation and a divalent (and a divalent and a trivalent, etc.) on the surface potential over equivalent concentration ranges. This relationship is counter-intuitive to that expected from the concept of ionic strength, from which it would be expected that divalent cations would exert an electrostatic effect only four times that of a monovalent cation. Presumably Demarty and co-workers[6] and Sentenac and Grignon[46] on the basis of their Donnan approach would also anticipate only a four-fold selection of divalents over monovalents. The concept of ionic strength, however, is based on the

elementary Debye-Huckel theory for weak electrolytes which is unreliable at the high ionic strengths of physiological systems. In terms of ion-binding to the cell wall, our model anticipates a much greater (10–100-fold) selection for divalent cations over monovalent cations and a similar preference for trivalent cations (such as lanthanum) over divalents.

In addition to the valency preference of binding, the model also predicts that there would be a hierarchy of selection for ions of similar valency. Thus not all divalent cations would exhibit the same binding properties, under many circumstances a five-fold greater selection for Ca^{2+} over Mg^{2+} is predicted. Our analysis, therefore, provides an alternative explanation to those advanced by Sentenac and Grignon[46] and Demarty et al.[6] for the apparent selectivity of ion binding observed with cell walls.

The ionic ratios and concentrations in the region between a charged surface and a bulk phase electrolyte solution may be quite different from those in the bulk phase proper (fig. 2). This interfacial region, therefore, represents a complex independent phase. Furthermore, the organisation of this layer(s) and its depth may also vary with factors such as the supporting electrolyte concentration, valency and temperature. The influence (principally electrostatic) of the interfacial region may be minimised by increasing the ionic strength of the environment. Divalent cations, such as Ca^{2+} are particularly useful for screening (for anionic surfaces) the surface charge[52]. In fact, if the surface charge is screened sufficiently then fusion may occur.

Although relatively little is known about the precise structure of the interfacial region we may be certain that it bears little resemblance to a free liquid[12]. By assuming that the cell wall lattice is a uniformly charged surface we can predict that the structure of the interfacial layer is similar to that thought to exist around an electrode surface[12]. Thus, ions close to a surface within the cell wall will be stripped of their outer hydration shells and those located on the surface may be completely naked. Similarly the water structure will be unlike that of liquid water and may more resemble that of ice. Both the dielectric constant of the interfacial region and the viscosity may also vary dramatically from that of aqueous electrolytes. It may be expected, therefore, that diffusion coefficients of both solute and solvent would be significantly different to that in the bulk phase.

Under some circumstances, a bulk phase solution between two adjacent surfaces may not exist at all. Thus if two charged surfaces were to become sufficiently close such that their interfacial regions met then there would be no bulk phase solution. All the water between the two surfaces would be 'structured' with a lower dielectric constant (ca 2–30) than that of free water (ca 80). The respective wall surfaces could come no nearer, however, because they would repel each other electrostatically. Perhaps one of the most interesting consequences of the presence of this interfacial region, therefore, is that it may determine the minimum distance that two similarly charged surfaces within a cell wall may approach one another. From a morphogenetic point of view this prediction may be very important, for we now have a simple physical mechanism whereby the microfibril spacing within the cell wall may be defined by the electrostatic environment of the cell wall. Moreover, this environment is under the influence of both the cell and the ambient medium of the plant.

Considering, for the moment, cell expansion (i.e. morphogenesis) of say, an aquatic plant, the microfibril spacing and thus the overall architecture of new wall may be defined by several parameters: i) the rate of synthesis of the microfibril components, ii) the rate of assembly of the microfibrils, iii) the ionic nature of the existing cell wall where the new material is to be located, iv) the ionic nature of the medium surrounding the plant, v) the activity of the local plasma membrane ion transporting devices (iv and v would exert their influence upon iii and its possible that i and ii are also influenced by iii).

So far, I have only described the electrostatic environment represented by the plant cell wall. There are, of course, a number of other physical properties of the wall which may be related to morphogenesis. Wall plasticity (and to a lesser extent elasticity), for example, has some role to play in morphogenesis[18]. Its worth remembering however, that the ionic status has a considerable influence on the elastic-plastic properties of the cell wall.

My speculations about the physical nature of the plant cell wall have so far tended to be limited to items of morphogenetic relevance but equally important are the possible effects that the cell wall may have on other cellular functions. There are two related mechanisms by which a cell wall with the described properties could influence ion transport at the plasma membrane. Firstly, the cell wall lattice together with the associated interfacial regions represents a significant binding site for ions. In addition, if the distance between adjacent surfaces (fig. 2) is within about 15 Debye lengths then the passage of water and ions through these regions will be seriously hindered. On account of both these effects the cell wall, perhaps not surprisingly may represent a significant barrier to the flow of ions and water. It is likely, therefore, that the concentration of ions immediately adjacent to the plasma membrane, may not be the same as that in the supporting electrolyte. The activity of the plasma membrane ion transporters would be affected accordingly. In other words the area adjacent to the plasma membrane represents a micro-environment and may be modified by the cell. From a morphogenetic point of view, the plasma membrane ion transporting devices may influence the ionic status of this environment and thereby change the physical properties of the cell wall. This is particularly interesting as de novo cell wall synthesis takes place close to the plasma membrane and so the new wall architecture may be under the direct control of the cell.

The ionic status of a cell wall with the properties described above, may be modified both transversally and longitudinally by ion transport mechanisms as described by Jaffe[22], Nuccitelli[30] and ourselves[37]. There is almost infinite scope therefore, for metamorphosis of plant cell walls to take place even with the relatively simple mechanisms I have mentioned. The cell wall is surely more complicated than this but at least we believe we know what happens in the simplest case.

The second means by which the properties of the cell wall may affect plasma membrane ion transport is through electrostatic or dielectric effects of the cell wall upon the membrane transport protein. It has been demonstrated that surface charges (and surface pH) may significantly affect the activity of an enzyme (including a membranous ion-transport protein). This work was recently reviewed by Russell and Fersht[42] who identified several criteria (such as surface charge density) as a means of assessing the effects of surface phenomena on enzyme function. Thus the K_{cat}/K_m ratio may well be a function of the electrostatic environment. This has been recently discussed in terms of cell wall located enzymes by Ricard[40]. There are several lines of evidence which are consistent with this possibility, some of which are reviewed by Ricard[40] as well as some of the points I have raised.

In fact the respective activities and diffusion profiles of wall-located enzymes and their substrates and products predicted by Ricard[40] bear a striking resemblance to chemical reactions which take place on electrode surfaces[12]. And it is from the observation and the subsequent explanation of these latter phenomena that modern theories of surface chemistry and interfacial physics allow us to make predictions of the properties of charged matter such as cell walls. Similarly at the membrane level, the thylakoid stacking/unstacking phenomenon has been demonstrated to be a function of the surface charge density[2]. This latter parameter is under the influence of the chloroplast and represents a beautiful example of how morphogenesis (i.e. metamorphosis) is directly related to the requirements of the cell; namely the control of light harvesting efficiency.

Last words

Whilst the overall process of cellular development appears irreversible, the elemental processes may well be reversible. This assumption is analogous to those used in quantitative assessments of multicomponent dynamic systems with non-equilibrium thermodynamics (NET). Thus it is possible to identify and rigorously characterise each element of the process, consider it from an equilibrium (reversible thermodynamics) point of view, and then assemble all the individual information in order to understand the properties of the system[24]. This strategy fails to reveal any overall collective properties of the system. The work of Frohlich[10], in particular, illustrates what is possible if the whole system is considered (see also the KAM analysis above). Similarly, the relatively new formalism of Network thermodynamics[38] is a more versatile thermodynamics-based tool. Although, some would argue that you have to put more into it than you get out. I personally believe that once sufficient experimental data has been accumulated the value of Frohlich's approach[10] will become evident and prove absolutely necessary to satisfactorily describe dynamic biosystems.

As with the NET analogy I would like to emphasise that morphogenesis cannot be understood simply from 'knowing what proteins the genome codes for'. It is not enough to know only the properties of the elements which compose the set; one must consider the properties of the set as well as its relationships with other sets. Some of the contributing authors have utilised reductionism where it is appropriate but all have recognised that spacio-temporal 'holistic' information must be included. The static physical properties of the system must be investigated as well as the dynamic properties of matter; all these are necessary before we may feel confident that we understand the physical nature of a living system. In this review series we have tried to identify the bare bones of what is necessary. There is still a long way to go, but through multi-disciplinary ventures, progress will be less tortuous.

Acknowledgments. I would like to express my sincere gratitude to Drs Irene Ridge and Joy Manners for much help and good company during many stages of this work. I would also like to thank Drs Joan Mason, Alistair Matheson and Irene Ridge for help in assessing some of the articles in this review series. And the S.E.R.C., the O.U. Research fund, the Royal Society, I.C.I., the Emil-barel Stiftung and Sandoz AG for financial assistance towards some of my research mentioned in this paper.

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