First Steps Towards a Comprehensive Model of Tissues
or
A Physicist Looks at Development

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Abstract: Subdividing embryological development into a set of simpler interacting processes described by effective energies and auxilliary diffusing fields, creates a framework which indicates the experimental parameters needed to model a particular developmental phenomenon. This model agrees quantitatively with simple cell sorting experiments and qualitatively with aggregation, mound formation and slug migration in *Dictyostelium discoideum*. Additional biological mechanisms are easy to include.

Introduction: To justify an intrusion into biology, physicist must bring humility and novel approachs. Our approaches must yield results meaningful to biologists or we reenact the old joke of the spherical cow.

Naively, we can look at embryological development as a problem in pattern formation. How does a fertilized (or even unfertilized) egg, give rise to the complex structure of an animal? Clearly, the question is arrogant and too hard: animals are much more complex spatially and temporally than even the most complex hydrodynamics. On the other hand, we can recognize, at least locally and over short times, processes that resemble pure physical or chemical phenomena.

Biologists traditionally decribe pathways, enumerating reactants and the interactions among them. This focus provides qualitative explanations, *e.g.* the expression of particular homeotic genes give a body segment its identity, a received signal leads to a particular kinase/phosphorylation cascade. The ultimate understanding of organisms will require this level of detail, but we still cannot model all of the reaction kinetic steps in even fairly simple processes because of the large number of rate constants each of which is a major experimental effort. (Though Arkin's paper shows that we are making rapid progress in this direction). More seriously, if even one layer of a detailed reaction kinetics pathway is missing, quantitative modeling becomes hopeless. Studies of cAMP reception pathways in *Dictyostelium discoideum* combined with those on the role of capping proteins in creating leading edges of actin polymerization and hence pseudopod formation [Hug *et al*, 1995] may form a nearly closed pathway for chemotaxis in *Dictyostelium*. If so, we will soon be able to develop true molecular kinetics models of cell motion. For the moment our goal is to develop a general framework which allows us to include reaction kinetics but does not require it.

Physicists look for general patterns of behavior that can be captured in universal, few parameter models which are independent of smaller scale processes. *E.g.* if we have a model that indistinguishably reproduces the cell motion in response to chemical gradients, we don't ask about internal cellular processes. This emphasis on quantitative response rather than pathway annoys biologists, but works well for phase transitions, astrophysics and turbulence. We can add reaction when pathways are fully characterized.

We begin by making the maximal simplifications that still have a hope to include the behaviors in question, then look for known mechanisms that mimic them since many categories of pattern formation like spiral wave oscillations occur in numerous different materials. Our experiments try to separate out individual mechanisms and our models try to reproduce the experiments quantitatively. When we have understood all processes individually, we recombine them and look for novel effects arising from interactions.

We can conveniently describe cell motion and differentiation by fields and what a physicist would call an **effective energy, *E***. Some of the fields are material, *e.g.* the local concentration of a diffusant, others, like an orientation vector field, are not. Similarly, the effective energy will be a mixture of true energies, like cell-cell adhesion, and terms that mimic energies, *e.g.* the response of a cell to a chemotactic gradient. The names are merely a shorthand for the mathematical structure. Given an effective energy we can calculate the resulting cell motion, since differences in energy produce forces, $\vec{F} = -\vec{\nabla} E$. Since we are in an extremely viscous regime, the velocity, not acceleration is proportional to force, with mass replaced by an effective cell mobility, $\vec{v} = m\vec{F}$:

$$\vec{v} = -m\vec{\nabla} E. \qquad (1)$$

Equation (1) implies that cells move in such a way that they minimize the total effective energy. Our cells generalize as well; we can treat additional materials, *e.g.* medium, substrate, extracellular matrix (**ECM**) simply as cells with special properties.

We can begin our subdivision as shown in figure 1. Development results from the interaction of four main processes, cell division, cell migration, cell differentiation, and the formation of extra cellular structures. While cell division is inherently complex, the final result, two daughter cells, can be simulated easily if we neglect cell polarity. The key is to define a rate of cell growth. The secretion of ECM can be expressed naturally in our formalism. Differentiation involves two processes, temporal changes in cell properties and spatial changes leading to anisotropy in cell behavior, *e.g.* cell elongation. Both of these involve large numbers of experimental parameters, and we will initially restrict ourselves to cases in which cells are effectively **isotropic** and **static**, though we can relax the latter within the formalism. Differentiation is the most difficult biological process for a physicist. The information determining differentiation can be internal (*e.g.* cascades of gene expression, information carried in asymmetric components of cytoplasm during cell division e.g. selection of body axis after fertilization) or external (*e.g.* contact signaling or external diffusible morphogens). Cell migration is the easiest process to model, its causes subdivide into short and long range signaling mechanisms, the former

including differential adhesion, contact inhibition and haptotaxis, the later including chemotaxis, galvanotaxis and gravity.

Model: Our goal is to develop a set of standard terms describing individual processes, which can then be combined as necessary. This brief overview cannot show the details of implementing each mechanism. However, the references in each section point to full information. Similarly we will not discuss the many other approaches to tissue modelling of many types [Graner, 1993; Agarwal, 1995]

The minimal definition of a cell: Label each cell under consideration by a unique index σ. At any time, t, a cell, of type, τ, has a volume $v(\sigma,t)$. Since we know that cell volume is not absolutely fixed (*e.g.*, due to changes in osmotic pressure) we describe the cell volume in terms of an effective pressure or membrane elasticity, λ_σ, and a target volume $v_{\text{target}}(\sigma,t)$:

$$E_{\text{volume}} = \sum_{\text{all cells}} \lambda_\sigma \left(v(\sigma,t) - v_{\text{target}}(\sigma,t) \right)^2 . \tag{2}$$

Equation (2) has the form of an elastic volume constraint with membrane elasticity, λ [Weaire *et al.*. 1991]. If we neglect cell polarity and assume that growth of cells is simple and deterministic, both strong assumptions, we can model cell growth by setting:

$$v_{\text{target}}(\sigma,t) = v_0(\sigma) + g \int \left(\text{nutrient supply} * \text{cell cycle factors} \right) \mathrm{d}t . \tag{3}$$

To reduce the number of parameters we will usually assume that all the cells of a given type have the same λ and v_0, and that the growth rate of a cell is constant. Where cells are not growing or dividing, we simply take $v_{\text{target}}(t)=v(\tau)$. These simplifications are reasonable in homogeneous tissues and in plants, but are far from the true situation in early stage embryos.

Mitosis: Mitosis can occur either when the cell reaches a fixed type dependent volume, or when the ratio between cell surface area and cell volume reaches a critical value (since cell nutrient absorption is roughly proportional to surface area and metabolism is roughly proportional to volume). In yeast and in other simple organisms as well as in cancer cells, the growth rate depends mainly on the supply of nutrients. Cell division is triggered only after the cell reaches a critical size [Hayles *et al*., 1986]. As the cell enlarges in volume, the concentration of a diffusible chemical will fall and the decrese of concentration below a certain critical threshold could be the signal to flip the molecular swithch that triggers cell division. In the model, we create two daughter cells when this critical stage has been reached, with the plane of separation along the cells longest axis and new target areas $v_{\text{target}}/2$.

Contact between cells: Cells express surface adhesion molecules on their membranes, *e.g.* integrins, cadherins, N-Cams, some highly specific, others nonspecific. If we bring two cells together, pulling them apart requires work. Since they are only now being

measured (Engel), we neglect changes in binding strength due to diffusion of adhesion molecules into the contact area, local reorganization of adhesion molecules, *e.g.* into adhesive plaques, and changes in the number or adhesivity of the binding molecules induced by the adhesion itself. We can describe the net interaction between two cell membranes by an effective binding energy per unit area, $J_{\tau,\tau'}$, which depends on the types of the cells. We can measure $J_{\tau,\tau'}$ with laser tweezers [Sato-Maeda *et al.*, 1994], a shaker, a compression apparactus or a Taylor-Couette shear cylinder. The effective surface energy is:

$$E_{\text{Contact}} = \int_{\text{cell surface}} J_{\tau,\tau'}\,\mathrm{d}s. \tag{4}$$

Based on this Differential Adhesion Hypothesis (DAH), Steinberg predictied that in an aggregate of two cell types, in which the heterotypic energy is greater than the homotypic, the lowest energy configuration has the more cohesive cell type in a sphere completely surrounded by the less cohesive cell type. This classic cell sorting is seen in *hydra* endoderm and ectoderm, and in embryonic chicken neural and pigmented retinal cells. If the heterotypic energy is less than the homotypic energy the lowest energy pattern is the checkerboard as seen in Japanese quail oviduct. If the cell-medium energy is less than the heterotypic energy but greater than the homotypic energy then the cells divide into discrete homotypic clusters (as seen in mixtures of *hydra viridiana* and *hydra vulgaris* cells in which it results in self-nonself identification).
Complications arise becuase the cell adhesion molecules may change both in quantity and identity, e.g. in *Dictyostelium*, at least four different adhesion molecules are significant at different stages of aggregation and mound formation (Bozzaro and Ponte, 1995). We model all these complex changes as variations in cell specific adhesivity

Membrane Fluctuations: How do cells move from their initial random positions to the lowest energy configuration? Do they have time to reach the lowest energy configuration? In mixtures of liquid droplets, the thermal fluctuations of the droplet surfaces cause diffusion (Brownian motion) leading to energy minimization. Cytoskeletally driven cell membrane ruffling of a few μm have no need to be thermal and the dynamics might depend sensitively on their fluctuation spectrum. In addition, we are neglecting cells like keratocytes as described by Anderson, which move in straight lines over long distances. Such highly correlated cell motion seems to depend on the presence of a substrate. In aggregates, cell motion is more random, in our ignorance of the actual spectrum, the simplest assumption is that the cell membrane behaves like an **effective temperature, *T*,** of about a million degrees. If we neglect all filopodia and other cytoskeletal structures. We can describe these fluctuations statistically using Monte Carlo Boltzman dynamics, where T defines the size of the typical fluctuation: if a proposed change in configuration produces a change in effective energy, ΔE, we make it with probabilty:

(5) $$P(\Delta E) = \begin{cases} 1 : \Delta E \le 0 \\ e^{-\Delta E / kT} : \Delta E > 0 \end{cases},$$

where, k ,is a constant converting T into units of energy.

One consequence of this dynamics is that if the energy fluctuates up and down greatly as cells rearrange, as it does in a random aggregate, then the cells will not be able to reach a globally optimal configuration if T is too small, *i.e.* the liquid freezes into a glass. Freezing, or partial cell sorting, occurs experimentally when cell membrane fluctuations are eliminated either with cytochalasin-B or by holding the cells at 4°C. In our model, low values of T, also lead to partial sorting. However, if two compact tissue fragments are brought into contact, when one surrounds the other, the contact energy decreases smoothly, so freezing should not occur. As predicted, experimental engulfment requires much smaller membrane fluctuations.

Chemotaxis: Chemotaxis requires an additional field to describe the local concentration of the chemotractant, $C(\vec{x})$, which diffuses in extracellular space (or, in the presence of gap junctions, through cells as well) with diffusion constant, d, decays at a rate, Γ, and is secreted or absorbed at the surface of cells in a complicated history dependent way, $s_C(\sigma, \vec{x}, t)$, which is difficult to measure and requires a detailed description of the internal processes of the cell. The equation for the field then is:

(6) $$\frac{\partial C(\vec{x})}{\partial t} = d\nabla^2 C(\vec{x}) - \Gamma C(\vec{x}) + \sum_{\sigma} s_C(\sigma, \vec{x}, t).$$

Fortunately, a few simple approximations to $s_C(\sigma, \vec{x}, t)$ work well for *Dictyostelium*.. [Levine *et al*., 1996; Hofer *et al.*, 1995] We then describe the cell's response to the chemical field by an effective chemical potential, $\mu(\sigma)$, which may be time dependent, *e.g.* in a refractory period μ will be nearly 0. μ>0 yields repulsion, and μ <0 attraction. The effective chemical energy is:

(7) $$E_{\text{Chemical}} = \sum_{\sigma} \int_{\text{Surface of } \sigma} \mu(\sigma) C(\vec{x}) \, \mathrm{d}s \, .$$

The cell executes a biased random walk, which averages to directed motion in the direction of the gradient:

(8) $$\vec{v}(\sigma) = -\mu(\sigma) \vec{\nabla} C(\vec{x}),$$

which has the same form as equation (1), justifying our energy treatment.

Unfortunately, the final configuration for an aggregate composed of two cell types is the same whether they exhibit differential adhesion or differential chemotaxis. Only the kinetics differs. Experiments on slug phase *Dityostelium* suggest that the cell sorting that

occurs between prestalk and prespore cells is primarily chemotactic (Takeuchi *et al.* 1988; Traynor *et al.* 1992). But the quantitative experiments to check the kinetics have not been done.

Differentiation: Classic reaction-diffusion equation models of differentiation look at pairs of continuous fields rather than individual cells. However, their formalism carries over to our cellular model. If A and B are diffusing morphogens, which evolve like the diffusants in chemotaxis [Turing, 1952]:

$$\begin{aligned} \frac{\partial A(\vec{x})}{\partial t} &= d_A \nabla^2 A(\vec{x}) - \Gamma_A A(\vec{x}) + \mathrm{f}\left(A(\vec{x}), B(\vec{x})\right) \\ \frac{\partial B(\vec{x})}{\partial t} &= d_B \nabla^2 B(\vec{x}) - \Gamma_B B(\vec{x}) + g\left(A(\vec{x}), B(\vec{x})\right) \end{aligned} \quad . \tag{10}$$

A is excitatory if $\partial \mathrm{f}(\sigma, \vec{x}, t) / \partial A > 0$ and inhibitory if $\partial \mathrm{f}(\sigma, \vec{x}, t) / \partial A < 0$. Turing, [1952] showed that if A is excitatory and B inhibitory and B diffuses faster than A, $d_A < d_B$, then an initial uniform distribution of A and B is unstable and the concentration field will evolve into domains. Cells, instead of moving, change their parameters,*e.g.* λ, in response to A and B,, as a function, h, of the surface concentrations of the chemotractants:

$$\frac{\partial \lambda_\sigma}{\partial t} = \mathrm{h}\left(\oint_{\substack{\text{Surface of} \\ \sigma}} A \, \mathrm{d}^2 s, \ \oint_{\substack{\text{Surface of} \\ \sigma}} B \, \mathrm{d}^2 s, \ldots \right) . \tag{11}$$

If the cells differentiate primarily as a result of their history and not due to positional signals (as appears to be the case with prestalk/prespore selection in *Dictyostelium* (Takeuchi et al. 1988)), then the cell carries a set of order parameters with it which evolve according to a set of internally defined ordinary differential equations.

A gradient based on purely reaction diffusion mechanisms can be maintained only in small tissues ~ 1mm, otherwise the time required for diffusive exchange of molecules would be too long. In larger organisms and tissues, particular genes have to be activated in response to signals, setting off a gene cascade. This requires an autocatalytic activation of genes (Meinhardt H., 1978). A simple example of a such a biochemical switch is :

More complex interactions allow the space-dependent activation of several genes under the influence of a single gradient (Meinhardt H., 1978).

Cell Signalling: Cell signalling has been studied widely in *Dictyostelium* . Tang and Othmer (1995) have modelled cAMP response of these cells - adaptation to stimuli, amplification of extracellular cAmp and the time scale of response to stimuli, as well as travelling wave chemotaxis. In a mathematical model, the variation of a parameter related to biochemical propoerties of a cell can be used to simulate changes in the developmental stage of that cell. Such parameters can be introduced very naturally in the Potts model. Concentrations of chemicals or gene products can be assigned to each cell and the

evolution takes place at the level of a cell. _As opposed to continuum models one can describe the phenomenon in terms of cellular

Gravity: Gravity is usually too weak to be important, but may play a role in *Dictyostelium* slug formation. It is simply a potential energy depending on the local cell mass, $\rho(\vec{x})$, and the vertical height, z:

$$E_{\text{Gravitational}} = \int_{\text{Space}} \rho(\vec{x}) z \, \mathrm{d}^3 \vec{x} \,. \tag{9}$$

Extracellular Matrix: In many tissues non-cellular materials provide much of the mechanical stability, e.g. bone in vertebrates, mesoglea in *Hydra*, and the slime sheath in *dictyostelium*.. In some cases cell sorting requires the secretion of ECM compounds (e.g. sorting between chick embryo cardiac mesenchyme and myocytes, Armstrong, 1985). ECM is similar to a secreted signalling molecule except that the secreted material does not diffuse. Instead of treating it as an auxiliary field, we treat it as a new cell with the property that the target area is always fixed to its current area. If a cell secretes a unit of ECM, the target area is increased by one unit. Similarly if a cell absorbs it, the target area decreases by one. If the ECM is solid, then local concentrations of ECM cannot fluctuate or move, if liquid, it behaves exactly like a normal cell. As before, we need to define the interaction energy between each cell type and the ECM.

Cell Polarity: Our assumption of a uniform distribution of adhesion molecules is an oversimplification. In many cases, *e.g.* aggregating *Dictyostelium* or *myxobacteria* (in which the apical/basal cell ends are much more cohesive than the sides (Bozzaro and Ponte, 1995; Kuspa *et al.*, 1992) or in vertebrates (where surface cells in embryos or epithelial cells in adults both are non-adhesive on the apical/luminal surface, Roberson *et al.*, 1980), the cell's membrane adhesivity varies over the surface of the cell and may have local maxima at junctions. Such cell polarity is more difficult to treat in our framework. Two possible approaches are to create a physical cell membrane, by defining patches of membrane at the surface of each cell (computationally expensive), which allows locally variable properties, or assigning an orientation vector to each cell, which gives a cell axis and hence allows angular variation of parameters in compact form. The axes in turn must evolve, possibly using a flock interaction formalism [Sano *et al.* 1996].

Putting it All Together--Simulation: If we sum the effective energies in equations (2), (4), (7) and (8) we obtain:

$$E_{\text{Total}} = E_{\text{Volume}} + E_{\text{Contact}} + E_{\text{Chemical}} + E_{\text{Gravitational}} + E_{\text{Other}} \,. \tag{12}$$

To simulate, we simply superimpose a lattice on the cells, where the value at a lattice site is σ if the site lies in cell σ [Srolovitz *et al.*, 1984, Grest *et al.*, 1984]. We select a lattice site at random and propose to reassign it from cell σ to a cell σ' (usually a neighbor). We calculate the change in effective energy caused by the proposed reassignment and accept

it according to equation (5) [Graner and Glazier, 1992. Glazier and Graner, 1992]. Similarly we discretize any auxilliary fields and evolve them according to equations (6) or (10). The parameters of each cell evolve according to equations (3) and (11), and any other equations we may have defined for the internal states of cells.

Experimental Verification: How well does it work?

Extensive previous work has shown that this simulation method reproduces the evolution of fluids and foams subject to energy gradients [Glazier, Anderson and Grest, 1990, Jiang and Glazier, 1996].

The simple mitosis model (equations (2), (3), (4) and (5)) has been used by Mombach (1993) to quantitatively repoduce the observed cell arrangements in a variety of plant tissues. Dirk Drasdo has presented his approach to tumor growth using a reduced version of this formalism, while Elizabeth Stott of University of Bath is using the complete formalism to model tumor growth and angiogenesis as a function of nutrient supply.

The DAH component of the simulation (equations (2), (4) and (5)) has been verified in experiments using embryonic chicken cells [Mombach *et al.*, 1995]. In this case, if we begin with a random aggregate of neural and pigmented retinal cells, and use the best available values for cell volumes, fluctuation amplitudes and surface energies, we are able to quantitatively reproduce the evolution of the dark-light contact length. (figure). In addition, as we hypothesized, if we reduce the fluctuation temperature in the simulation, or suppress membrane fluctuations in the experiment (with cytochalasin-B) the sorting halts (freezes). Restoring the fluctuations leads to normal sorting.

While we have not yet been able to verify that the cell membrane really does undergo thermal fluctuations, we have shown that the spectrum of velocities of a single pigmented cell in a neural cell aggregate is identical to that of a simulated cell when the fluctuation amplitude is matched [Mombach and Glazier, 1996], *i.e.* the experimental cells execute a thermodynamic random walk. (figure).

More complex processes have been qualitatively verified in *Dictyostelium*. Levine has shown that the chemotactic mechanism presented here qualitatively reproduces the patterns of aggregating *Dictyostelium* amoebae. Jiang has shown that the peculiar tipped shape and cell sorting of *Dictyostelium* mound formation is due to the competition between chemotaxis and differential adhesion. (figure). Finally, Saville and Hogeweg have used equation (12) to simulate qualitatively the entire aggregation process from single amoeba through migrating slug.

The continum reaction-diffusion equations (10) and (11) have been succesful in describing a large number of differentiation phenomena, *e.g.* head formation in *hydra*, but have not yet been simulated in conjunction with equation (12).

Remaining Problems: The main problem with modeling biological processes is the vast number of parameters that must be experimentally determined to have any hope of other than accidental agreement. The particular task is to determine which mechanisms combine in any developmental processes and their relative rates.The energy formalism provides insight into which parameters are important, *e.g.* cell-cell adhesivities, rate of cell motion in response to specified chemotactic gradients, cell membrane amplitude fluctuations, *etc*... In addition, our understanding of basic thermodynamic processes like phase separation and freezing gives us confidence that even if our parameters are somewhat inaccurate the qualitative behavior of the model will be correct. We should not observe phenomena wildly divergent from experiment. This robustness allows us the chance to begin with rough qualitative models and gradually refine them into quantitative models as our experimental measurements improve. In this sense, though we are still far from being able to reproduce even such basic phenomena as gastrulation, we have made a true step towards a comprehensive model of cellular patterns.

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References

Agarwal, P, *J. theor. Biol.* **176** (1995) 79-89.
Anderson M. P., Srolovitz D. J., Grest G. S. and Sahni P. S., *Acta. Metall.* **32** (1984) 783-791.
Armstrong P. B.and Parenti D., *J. Cell. Biol.* **55** (1972) 542-553.
Armstrong, P. B., *Expe. Biol. and Med.* **10** (1985) 222-230.
Armstrong P. B., *Crit. Rev. Biochem. and Mol. Biol.* **24** (1989) 119-149.
Bozzaro S. and Ponte, E., *Experientia* **51** (1995) 1175-1188.
Dan-Sohkawa M., Yamanaka H., and Watanabe K., *J. Embryol. exp. Morph.* **94** (1986) 47-60.
Evans E., in Physical Basis of Cell-Cell Adhesion, P. Bongrand ed. (CRC Press, Boca Raton, 1988) Chapters 4 & 7.
Fristrom D., *Tissue & Cell* **20** (1988) 645-690.
Foty R. A., Forgacs, G., Pfleger, C. M. and Steinberg M. S. , *Phys. Rev. Lett.* **72** (1994) 2298-2301
Garrod D. R. and Steinberg M. S., *Nature* **244** (1973) 568-569.
Gerisch, G., Albrecht, R. Heizen, C. and Hodgkinson, S., Maniak, M., *Current Biology* **5** (1995) 1280-1285.
Gierer A. and Meinhardt H., *Kybernetik*, **12** (1972) 30-39.
Gierer A., Berking S., Bode H., David C. N., Flick K., Hansmann G., Schaller H. and Trenkner E., *Nature New Biol.* **239** (1972) 98-101.
X Glazier J. A. and Graner F., *Phys. Rev. E* **47** (1992), 2128-2154.
Glazier J. A., The Evolution of Cellular Patterns, Ph.D. dissertation, (University of Chicago, Chicago, 1989).
X Glazier J. A., Anderson M. P. and Grest G. S., *Phil. Mag. B* **62** (1990), 615.
X Graner F. and Glazier J. A., *Phys. Rev. Lett.* **69** (1992) 2013-2016 .
Graner F. and Sawada Y., *J. theor. Biol.* **164** (1993) 477-506.

X Graner F., *J. theor. Biol.* **164** (1993) 455-476.
X Grest. G. S., Srolovitz D. J. and Anderson M. P., *Phys. Rev. Lett.* **52** (1984) 1321-1324.
Hayles J. and Nurse P., *J. Cell Sci., Suppl.* **4** (1986), 155-170.
Hofer T., Sherratt J. A. and Maini P. K., *Proc. R. Soc. Lond. B* **259**, (1995), 249-257
Holtfreter J., *J. Morph.* **80** (1947) 25-55.
Holtfreter J., *Rev. Can. Biol.* **3** (1944) 220-250.
Honda H., Yamanaka H. and Eguchi G., *J. Embryol. Exp. Morph.* **98** (1986) 1-19.
Hug C., Jay P. I., Reddy I., McNally J. G., Bridgman P. C., Elson E. L. and Cooper J. A., *Cell* **81** (1995), 591-600
X Jiang Y. and Glazier J. A., *Phil. Mag. Lett.* **74** (1996), 119-128.
Kuspa, A, Plamann, L., and Kaiser, D. *J. Bacteriol.* **174** (1992) 7360- 7369.
Keller R. and Hardin J., *J. Cell Sci. Suppl.* **8s** (1987) 369-393.
Keller R., *Zool. Science* **4** (1987) 763-779.
Koch A. J. and Meinhardt H., *Rev. Mod. Phys.* **66** (1994), 1481-1507.
Lewis F. T., *Anat. Rec.* **38** (1928) 341-362.
Levine H., Aranson I., Tsimring L. and Truong T.V., *Proc. Nat. Acad. Sci. USA* **93**, (1996) 6382-6386
Matzke E. B., *Proc. Natl. Acad. Sci. (USA)* **31** (1945) 281-289.
McClay D. R. and Ettensohn C. A., *A. Rev. Cell Biol.* **3**, (1987) 319-345 .
Mombach J. C. M.,
X J. C. M. Mombach, J. A. Glazier, R. C. Raphael and M. Zajac, "Quantitative Comparison between Differential Adhesion Models and Cell Sorting in the presence and Absence of Fluctuations," *Physical Review Letters* **75**, 2244-2247 (1995).
X Mombach J. C. M. and Glazier J. A., *Phys. Rev. Lett.* **76** (1996), 3032-3035.
Nicol A.and Garrod D. R., *J. Cell Sci.* **38** (1979) 249-266.
Nicol A.and Garrod D. R., *J. Cell Sci.* **54** (1982) 357-372.
Noda K., *Zool. Mag.* **80** (1971) 99-101.
Phillips H. and Davis G., *Am. Zool.* **18** (1978) 81-93.
Roberson, M., Armstrong, J., and Armstrong, P., *J. Cell Sci.* **44** (1980) 19-31.
X Sato-Maeda M., Uchida M., Graner F. and Tashiro H., *Devel. Bio.* **162** (1994) 77-84.
X Srolovitz D. J., Anderson M.P., Sahni P. S. and Grest G. S., *Acta Metall.* **32** (1984) 793-802.
Steinberg M. S., *Science* **141** (1963) 401-408.
Steinberg M. S. and Garrod D. R., *J. Cell Sci.* **18** (1975) 385-397 .
Steinberg M. S. and Wiseman, *J. Cell Biol.* **55** (1972) 606-615.
Steinberg M. S., *J. Exp. Zool.* 1**73** (1970) 395-434.
Steinberg M. S., *J. Theor. Biol.* **55** (1975) 431-443 and references therein. See also references in Graner and Sawada (1993).
Takeichi M., *Science* **251** (1991) 1451.
Takeuchi, I., Kakutani, T. and Tasaka, M., *Developmental Genetics* **9** (1988) 607-614.
Tang Y. and Othmer H. G., *Phil. Trans. R. Soc. Lond. B* **349** (1995), 179-195.
Tashiro, H., Uchida, M. and Sato-Maeda, M., *Optical Eng.* **32** (1993) 2812-2817.
Thomas W. A. and Yancey J., *Development* **103** (1988) 37-48.
Thompson D'A. W., On Growth and Form, 2nd edition, (Cambridge University Press, Cambridge 1942).
Townes P. and Holtfreter J., *J. exp. Zool.* **128** (1955) 53-120.

Traynor, D, Kessin, R. H., and Williams, J. G., *Proc. Natl. Acad. Sci. USA* **89** (1992) 8303-8307.
X Turing A. M., *Phil. Trans. Roy. Soc. Lond.* **B237** (1952) 37-72 .
X Weaire D., Bolton F., Molho P. and Glazier J. A., *J. Phys. Condens. Matter* **3** (1991) 2101-2114.
Wilson H., *J. Exp. Zool.* **5**, (1907) 245-258.