SPATIOTEMPORAL PATTERNING IN MODELS OF JUXTACRINE INTERCELLULAR SIGNALLING WITH FEEDBACK

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Abstract. Juxtacrine signalling is the class of intercellular communication mediated by ligands and receptors that are both anchored in the cell membrane. Two particularly well documented examples of such signalling pathways are the Delta-Notch and TGFα–EGF-R interactions. In this review, we discuss mathematical models for juxtacrine signalling, focussing on these two specific examples. We discuss the various model formulations that have been used, and consider gradient, travelling front, and spatial pattern type solutions. We show that juxtacrine mechanisms can explain a wide range of observed behaviours in each of these categories, in a manner that is genuinely different from that in traditional diffusion-based models for intercellular signalling.

1. Introduction to juxtacrine signalling. Signalling between cells (intercellular signalling) is an essential process in the development and maintenance of multicellular systems. The signals employed can take a variety of forms and act over a wide range of length scales. In general, signalling depends on the production of ligand (the mediator of the signal) by signalling cells and detection of this ligand by specific receptors expressed by receiving cells. Traditionally, intercellular signalling has been classified as either autocrine or paracrine. In autocrine signalling, a cell signals specifically to itself, whereas paracrine signalling involves signalling between distinct cells that are spatially separated and depends on secreted diffusible ligands such as growth factors and hormones.

Juxtacrine signalling is a distinct class of intercellular signalling that is mediated by ligands and receptors that are both anchored to the cell membrane (Massagué, 1990; Bosenberg & Massagué, 1993). Juxtacrine ligands can be either membrane-anchored precursors of soluble forms of the ligand (for example transforming growth factor α, TGFα) or purely membrane bound (for example, Delta). In the former case, the juxtacrine and soluble forms of the ligand can trigger qualitatively distinct responses in receiving cells due either to activation of distinct receptors (as for tumour necrosis factor—Grell *et al.*, 1995) or due to differences in ligand presentation to the receptor (as for certain ephrin-A ligands—Davis *et al.*, 1994).

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An immediate consequence of the membrane anchoring of juxtacrine ligands is that direct signalling can occur only between cells that are in intimate contact within a tissue (Fagotto & Gumbiner, 1996). In considering spatiotemporal patterning it is thus important to determine whether or not juxtacrine activation of receptor results in changes in the levels of expression of the juxtacrine ligand and/or receptor in the activated cell itself. If this is not the case (no feedback) then signals cannot be propagated over a range of more than one cell diameter (as is the case for Boss–Sevenless signalling in the developing *Drosophila* eye—Krämer *et al.*, 1991). However, if there is feedback between receptor activation and expression levels of ligand and/or receptor, then juxtacrine signalling provides an important mechanism for the long-range propagation of localised signals and the *de novo* generation of spatiotemporal pattern. It is the latter case that we are concerned with in this review.

Focussing on two particularly well documented biological examples, Delta–Notch and TGFα–EGF-R signalling, we will show that the implications of juxtacrine signalling with feedback depend crucially on whether binding of ligand to receptor up- or downregulates the further expression of ligand and receptor on the cell surface. For the Delta–Notch system, it has been well established that activation of the Notch pathway by Delta can lead to the downregulation of Delta activity, thus establishing a negative feedback within signalling cells that can act to amplify any small differences in levels of Notch pathway activity between neighbouring cells (reviewed in Simpson, 1997). However recent evidence suggests that this feedback regulation is context dependent, with experiments on *Drosophila* wing development showing upregulation of Notch and Delta expression by binding (see below). In the case of TGFα binding EGF-R, positive feedback is well established. Auto-induction of TGFα synthesis has been demonstrated in human keratinocytes (Coffey *et al.*, 1987), and EGF has been shown to stimulate production of EGF-R (Clark *et al.*, 1985; Earp *et al.*, 1986; Earp *et al.*, 1988; Kudlow *et al.*, 1986). Similar positive feedback loops have been documented in a number of other cell types (e.g. Zigmond, 1982).

1.1. Delta–Notch signalling. The Notch/Lin-12 family of transmembrane receptor proteins[1] mediate a wide range of cell-fate decisions during the development of flies, vertebrates and nematodes (reviewed in Artavanis-Tsakonas *et al.*, 1995; Fortini & Artavanis-Tsakonas, 1993; Kimble & Simpson, 1997; Lewis, 1996; Muskavitch, 1994). The receptors are characterised by the presence of multiple epidermal growth factor (EGF)-like repeats in the extracellular domain, and by a number of other conserved domains (reviewed in Greenwald, 1994). A number of ligands have been described that bind to the Notch/Lin-12 EGF-repeats and activate the intracellular Notch signalling pathway. These include Delta and Serrate in *Drosophila*, and their homologues in nematodes and vertebrates.

[1]The *Notch* mutation was named after the phenotype of heterozygous flies, which have little notches taken out of the wing margin.

To date, all the Notch-binding ligands that have been described are large transmembrane proteins with multiple EGF-like repeats and a conserved DSL motif in their extracellular domains (see Tax *et al.*, 1994; Simpson, 1995). The DSL motif is critical for binding to Notch. The transmembrane nature of Notch-binding ligands suggests that they act in a purely juxtacrine fashion. This is supported by a number of functional studies (for example, Heitzler *et al.*, 1996). However, recent studies have revealed that Delta can, in some instances, be proteolytically cleaved to yield a soluble secreted ligand (Klueg *et al.*, 1998; Qi *et al.*, 1999). There is as yet no direct evidence that secreted forms of Notch-binding ligands contribute to Notch signalling *in vivo*.

The binding of Delta-like ligands to Notch activates an intracellular signal transduction pathway that regulates the expression of tissue-specific target genes (for a recent review, see Weinmaster, 1998). If Delta-Notch signalling occurs between members of an initially equivalent group of cells, each of which can act both as a source and recipient of signalling, robust spatial patterning of cell fate can result. The process by which this happens, called lateral inhibition or lateral specification, appears to be the dominant mode of action of Delta–Notch signalling in development. Lateral inhibition has been studied most extensively in the contexts of the development of the central and peripheral nervous systems in *Drosophila* and of vulval development in *C. elegans* (Campos-Ortega, 1993; Goriley *et al.*, 1991; Heitzler & Simpson, 1991; Simpson, 1990; Wilkinson *et al.*, 1994). During the development of the central nervous system, Delta–Notch-mediated lateral inhibition acts to ensure that a reproducible proportion of a population of initially equipotential neural progenitor cells goes on to differentiate as neurons. Mutations in *Delta* and *Notch*, as well as in other genes that code for components of the signalling pathway, lead to a striking phenotype in which there is a massive overproduction of neurons at the expense of epidermis (Lehmann *et al.*, 1983); it is for this reason that genes involved in Delta–Notch-mediated lateral signalling are often referred to as neurogenic genes.

In at least some cases, Delta–Notch-mediated lateral specification depends on the inhibition of Delta activity in cells that are receiving Delta signalling from their neighbours. More specifically, it has been proposed that the level of expression of Delta in a cell is a decreasing function of the level of activity of the Notch signalling pathway in that cell (Heitzler & Simpson, 1991, 1993; Heitzler *et al.*, 1996). When this intracellular regulation operates in neighbouring cells, a feedback loop is formed between the cells; this feedback loop acts to amplify any small differences in the level of activity of the Notch pathway in neighbouring cells (Simpson, 1997; Sternberg, 1993). Since the level of activity of the Notch pathway within a cell is known to be a critical determinant of fate choice (elevated Notch activ-

ity inhibits differentiation and exit from the cell cycle in general—see, for example, Artavanis-Tsakonas *et al.*, 1995), the differences in Notch pathway activity in neighbouring cells can result in the cells adopting radically different fates. This mechanism can act within small populations of cells to single-out one cell for differentiation (such as in proneural clusters of five or six cells in the *Drosophila* neuroectoderm—Skeath & Carroll, 1992), or in large populations of cells to generate a fine-grained pattern of differentiated cells surrounded by inhibited cells (such as in the *Drosophila* endoderm—Tepass & Hartenstein, 1995).

In this review, we shall focus on the generation of spatial patterns of cell fate by juxtacrine lateral signalling. However, the Delta–Notch signalling system is undoubtedly more versatile than this. In particular, recent data on the development of the *Drosophila* wing veins and margin suggest that in some instances Notch activation can lead to an upregulation of the expression of its ligands Delta and Serrate, thus generating a positive feedback loop between neighbouring cells (Huppert *et al.*, 1997; de Celis & Bray, 1997; Micchelli *et al.*, 1997; Panin *et al.*, 1997). Other evidence suggests that Notch activity can also upregulate expression of Notch itself (Christensen *et al.*, 1996; de Celis *et al.*, 1997; Heitzler *et al.*, 1996; Wilkinson *et al.*, 1994). These data tend to suggest that the Notch signalling pathway can also play a role in the generation of boundaries between two cell types, and in the functioning of these boundaries as organising centres. Recent results have also implicated the Notch pathway in vertebrate segmentation (reviewed in Jiang *et al.*, 1998; McGrew & Pourquié, 1998); however, the mode of action of Notch signalling in these systems is currently unclear.

1.2. TGFα–EGF-R signalling. TGFα is a member of the epidermal growth factor family, and is an important regulator of epithelial cell behaviour, in particular cell division—see Kumar *et al.* (1995) for a review. The name of the growth factor derives from its original identification in cultures of transformed cells (de Larco & Todaro, 1978), and TGFα production is thought to be elevated in a number of malignancies (Cohen *et al.*, 1994; Derynck *et al.*, 1987); this has been particularly well-studied in breast cancer (Ciardiello *et al.*, 1989). TGFα is now known to play an important role in untransformed epithelia (Bates *et al.*, 1990; Derynck, 1988), by binding to EGF-R, and thus promoting cell division. The TGFα–EGF-R interaction is one of the best studied examples of a cellular control loop (van de Vijver *et al.*, 1991); it operates via an extracellular pathway, as opposed to intracellular autocrine loops such as v-*sis*-PDGF-R (Bejcek *et al.*, 1989; Keating & Williams, 1988).

TGFα is synthesised in the cell as a 160 amino acid membrane-bound precursor, pro-TGFα. The 50 amino acid soluble form of TGFα is generated by two separate cleavages of this precursor—see Brachmann *et al.*(1989) and Massagué (1990) for details. Originally it was assumed TGFα activity was due to the soluble form of the growth factor, but in the last decade

it has emerged that pro-TGFα can also activate EGF-R (Brachmann *et al.*, 1989). Moreover, cleavage of pro-TGFα, which has a half-life of about 4 hours, is typically slower than the turnover rate of pro-TGFα, so that the membrane-bound precursor is in fact the dominant form of the growth factor (Massagué, 1990), and the TGFα–EGF-R control loop is now recognised as being a prime example of the juxtacrine signalling mechanism. A number of other soluble growth factors similarly derive from membrane-bound precursors that can themselves bind to receptors, making them candidates for juxtacrine receptor activation (Bosenberg & Massagué, 1993). For example, Tumour Necrosis Factor-α has a membrane bound precursor, and has been found to kill cells in a juxtacrine fashion (Perez *et al.*, 1990), and to mediate B-cell activation (Macchia *et al.*, 1993).

TGFα is of particular interest because of its role in epidermal wound healing (Martin *et al.*, 1992a; Schultz *et al.*, 1991). In adult mammals, such wounds heal by a combination of cell crawling at the wound edge, and enhanced proliferation further back—see Martin (1996) for review. Although this combined mechanism of healing was established many years ago (Winter, 1972), the underlying molecular details remain unclear. Growth factor regulation is known to be central to the wound healing process in general, with TGFα, keratinocyte growth factor, and epidermal growth factor all contributing to epidermal repair. TGFα is implicated as an important element of the process in humans, since normal human keratinocytes produce TGFα both *in vivo* and *in vitro* (Coffey *et al.*, 1987), and TGFα upregulates both migration and proliferation of keratinocytes in culture (Barrandon & Green, 1987). Moreover, Schultz *et al.* (1987) have shown that addition of exogenous TGFα accelerates epithelial wound healing. The realisation that TGFα communication is mainly juxtacrine raises a key question: Can such a nearest neighbour signalling mechanism account for the observed increase in cell proliferation many cell diameters away from from the wound edge? This question will be answered by our discussion of gradient-type solutions to juxtacrine models.

2. Mathematical modelling of juxtacrine signalling. We consider only juxtacrine signalling within either a one-dimensional line of epithelial cells or a two-dimensional epithelial sheet; these are much the most important cases in development, and also include signalling within the epidermis, such as occurs in response to wounding. The most natural way in which to model this system is to represent the cells individually, with the model variables being ligand and receptor levels for each of these cells; thus, mathematically, the model has the form of a large system of coupled ordinary differential equations.

2.1. Discrete formalism. In the case of the Delta–Notch interaction, Collier *et al.* (1996) use this approach, solving the equations

$$dN/dt = F(\langle D \rangle) - \mu N \tag{1a}$$

(1b) $$dD/dt = G(N) - \rho D$$

for each cell in a regular array. Here $N(t)$ and $D(t)$ represent the levels of activity of Notch and Delta on the cell, relative to a typical activity level, and the functions $F(.)$ and $G(.)$ represent the feedback control. Thus $F(.)$ is an increasing function, corresponding to the activation of Notch (the receptor) by binding with the ligand Delta on neighbouring cells, while $G(.)$ is decreasing, representing downregulation of Delta activity by binding. The notation $\langle . \rangle$ indicates an average over neighbouring cells. In a one dimensional line of cells, one can index the cells by a single integer j, so that $\langle D_j \rangle = (D_{j-1} + D_{j+1})/2$ (Figure 1). In the two-dimensional case, Collier *et al.* (1996) consider a hexagonal array of cells, indexed as described in Figure 1, so that

$$\langle D_{i,j} \rangle = \frac{1}{6} \left(D_{i-1,j-1} + D_{i,j-1} + D_{i-1,j} + D_{i+1,j} + D_{i,j+1} + D_{i+1,j+1} \right) .$$

It is important to stress that this type of local averaging is quite different from the more traditional diffusion mechanism of signalling. In particular, the above formula is quite different from a discrete representation of diffusion, which would involve the difference between concentrations on nearby cells, rather than their average (pattern formation in arrays of discrete cells coupled by diffusion is discussed in Othmer & Scrivens, 1971; Babloyantz, 1977).

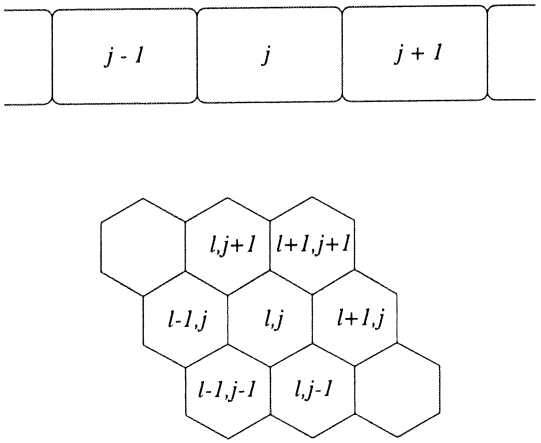

FIG. 1. *The labelling scheme used for cells in linear and two-dimensional arrays.*

For TGFα binding to EGF-R, Owen & Sherratt (1998) consider only a two-dimensional cell sheet, in which the cells are assumed to occupy a rectangular grid. In their model, free and bound receptors are included as explicit variables in the model, with binding represented by the kinetic scheme

illustrated in Figure 2. Their model thus consists of a series of coupled ordinary differential equations for the numbers of ligand molecules $a_{i,j}(t)$, unoccupied receptors $f_{i,j}(t)$, and occupied receptors $b_{i,j}(t)$, on the surface of cells in column i and row j. All ligand is assumed to be membrane-anchored. As discussed above, membrane-anchored ligand can also be cleaved to give a freely diffusing form; however, we neglect this complication in order to focus on juxtacrine signalling in isolation. Using the kinetic scheme discussed above, the model equations are:

(2a) $$da/dt = -k_a a \langle f \rangle + k_d \langle b \rangle - d_a a + P_a(b)$$

(2b) $$df/dt = -k_a \langle a \rangle f + k_d b - d_f f + P_f(b)$$

(2c) $$db/dt = k_a \langle a \rangle f - k_d b - k_i b$$

for each cell in the array.

For a regular grid of square cells, the value of $\langle a \rangle$ for cell (i, j) is

$$\langle a \rangle = \frac{1}{4} \left(a_{i,j-1} + a_{i-1,j} + a_{i+1,j} + a_{i,j+1} \right)$$

and similarly for $\langle b \rangle$ and $\langle f \rangle$. Owen & Sherratt specialise to the case where all cells in each column i are equivalent, in which case the variables can be labelled by the single index j. $\langle a \rangle$ then reduces to

$$\langle a \rangle = \frac{1}{4} \left(a_{j-1} + 2a_j + a_{j+1} \right),$$

and similarly for $\langle b \rangle$ and $\langle f \rangle$.

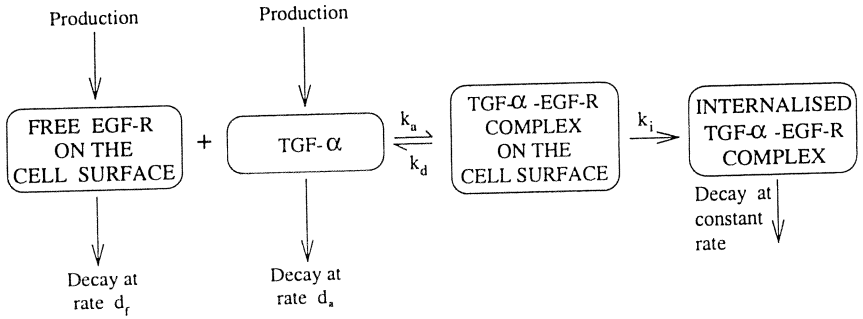

FIG. 2. *A schematic representation of the kinetic scheme used in the model for the binding of* TGFα *to* EGF-R. *The scheme is similar to that of Waters* et al. *(1990) for* EGF–EGF-R *interactions, and the parameters used are based on the values they determined from experiments on the binding of* EGF *to* EGF-R *on rat lung epithelial cells.*

The synthesis of new ligand and receptor by epithelial cells is a crucial aspect of the model. As explained above, we assume that this is controlled by a positive feedback to the level of occupied receptors on the cell surface.

Thus the production rates P_a of ligand and P_f of receptor are functions of the bound receptor number b. Our only assumption in general is that both of these production rates increase with b. In particular applications, the data available on production rates of ligand and receptors is typically extremely limited. However, the forms chosen for P_a and P_f can be specified to some extent because they must satisfy a number of conditions that relate them to quantities that are more easily measurable in experiments:

(i) In the absence of any ligand binding at the cell surface, there will be a background level of receptor expression, say r_0. This is a homogeneous steady state of the model, and so the equation for f in (2) gives

$$P_f(0) = d_f r_0. \tag{3a}$$

(ii) Normal equilibrium levels of free and bound receptors, f_e and b_e say, are often known in particular systems. Specifying f_e and b_e defines the normal steady state level of free ligand, a_e, implicitly through equation (2c), as well as the values of the feedback functions at the steady state, so that

$$a_e = \frac{(k_d+k_i)b_e}{k_a f_e}, \quad P_a(b_e) = k_i b_e + d_a a_e, \quad \text{and} \quad P_f(b_e) = k_i b_e + d_f f_e. \tag{3b}$$

(iii) In any system, there will be a maximum possible level of receptor expression, r_m say. This can be estimated experimentally by saturating cells with ligand. Such saturation means that the rate of internalisation of bound receptors must be equal to the rate of free receptor production, giving

$$P_f(r_m) = k_i r_m. \tag{3c}$$

Monk (1998) considers a less general form of juxtacrine signalling with positive feedback, in which only ligand production is explicitly enhanced by receptor activity. In addition to levels of activity of ligand and receptor, each cell has a variable competence to respond to signalling from its neighbours, which is in general a function of receptor activity in the cell. In a one-dimensional array of cells, indexed as in Figure 1, the equations for receptor, ligand and competence take the form

$$dr/dt = -\mu r + c\mathcal{R}(\langle l \rangle), \tag{4a}$$

$$dl/dt = -\rho l + \mathcal{T}(r), \tag{4b}$$

$$dc/dt = -\nu c + \mathcal{C}(r), \tag{4c}$$

where r, l and c represent receptor, ligand and cell competence, respectively. $\mathcal{R}$ and $\mathcal{T}$ are increasing functions encoding ligand–receptor binding and receptor–ligand intracellular feedback, while $\mathcal{C}$ is a decreasing (or constant) function encoding the influence of receptor activity on cell competence.

2.2. Continuous formalism. The above model frameworks involve a discrete regular representation of the cell population. In reality, cells in an epithelial sheet are not of course located in a regular grid, but rather have a range of shapes and sizes. Thus it is important to consider whether the assumption of regularity, which is implicit in the discrete models, is significant in the predictions of these models. One method of addressing this is to consider an alternative, spatially continuous framework, which does not involve this assumption. The approach of using different mathematical representations of the same phenomenon, in order to highlight the common features, is a valuable one that has been widely used in ecology (Hassell *et al.*, 1991; Savill & Hogeweg, 1997; Sherratt *et al.*, 1997). For simplicity, we restrict attention to the case of an epithelial sheet that is effectively homogeneous in one direction, so that the solutions of interest are one-dimensional. This is an important geometry for pattern formation, applying to striped patterns, and is also relevant to wound healing, where wound size is very large compared with a typical cell length, so that signal propagation away from a wound edge is effectively one-dimensional.

One benefit of using a continuous framework is that there is a very large body of previous theoretical work to call upon. In particular, a further development of our model would be to include the movement and proliferation of epidermal cells as they close the wound, and most of the previous models of wound healing have been of reaction-diffusion type. It would be relatively easy to combine a continuous model of juxtacrine signalling with such models. We have discussed previously the possibility of interactions between the epidermis and dermis, and continuous mechanochemical models including such interactions have been proposed for morphogenesis (Cruywagen *et al.*, 1992). Again, extensions of such models could include juxtacrine signalling mechanisms.

Our approach to formulating a continuous model of juxtacrine signalling is similar to that often used to model dispersal in ecological systems. In such models, the population after disperal, at a point x, is given by the sum over all y of the individuals that have moved from $(x - y)$ to x. A redistribution kernel specifies the probability of such movement as a function of y. Neubert *et al.* (1995) discuss the derivation of a number of redistribution kernels, and their pattern-forming potential in predator-prey models. In our case, we are not interested in redistribution as such, but in estimating the average contribution that spatially distributed bound receptors, which are the consequence of juxtacrine binding on the surfaces of randomly distributed cells, make to the TGFα and EGF-R production terms at each point in space. Our idea is to consider a cross section through the epidermis, parallel to the wound edge, with x representing the distance of this cross section from the edge. We expect that cells centred less than half a cell length from x have about an equal probability of contributing to the number of bound receptors at x, and cells centred between half and one cell length from x have a probability of contributing that decreases

with distance from x. This idea is illustrated in Figure 3, along with the piecewise linear weighting function that we will actually use in our model.

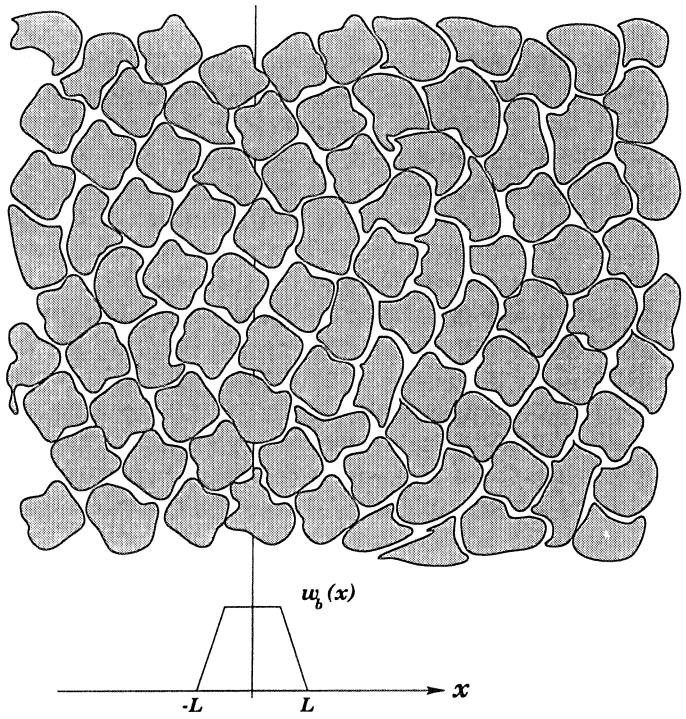

FIG. 3. *A schematic representation of the continuous representation of juxtacrine signalling. When the cells are considered to be randomly distributed with varying shapes, the model variables can be considered as averages given by some spatial weighting kernel. The particular piecewise linear kernel that we use in our numerical simulations is illustrated.*

The ideas discussed above give rise to a model in which equations (2) apply at every point in a continuous spatial domain, with local averages defined using a kernel $\omega(.)$:

$$\langle b\rangle(x,t) = \int_{-\infty}^{\infty} \omega(s)\, b(x+s,t)\, ds \tag{5}$$

(and similarly for $\langle a\rangle$ and $\langle f\rangle$). For mathematical and computational convenience, we use a piecewise linear kernel, which gives equal weights within half a cell length, and then decreases linearly to a zero weight at one cell length:

$$\omega_b(x) = \begin{cases} 0 & x < -L \\ \dfrac{4}{3L^2}(L+x) & -L < x < -\dfrac{L}{2} \\ \dfrac{2}{3L} & \text{if } \dfrac{L}{2} < x < \dfrac{L}{2} \\ \dfrac{4}{3L^2}(L-x) & \dfrac{L}{2} < x < L \\ 0 & x > L \end{cases} \tag{6}$$

where L is a typical cell length.

3. Types of solution. The nature of the spatiotemporal pattern that is generated by juxtacrine signalling with feedback clearly depends on the nature of the feedback between cells, on the initial and boundary conditions for ligand and receptor, and on the geometry of the cellular array. However, in general, three classes of solution can be distinguished. In all the cases considered here, the systems possess at least one spatially homogeneous steady state. If a localised perturbation is made to such a steady state (represented by a boundary condition fixed at a value different from the steady state), then stable spatial gradient and propagating front solutions can be generated. Under appropriate conditions, perturbation of the homogeneous steady state can also result in stable spatially-periodic patterns.

3.1. Spatial gradients. In the case of positive feedback of receptor activation on ligand production (models (2) and (4)), it is of interest to consider the effect of a localised disturbance on a stable homogeneous steady state. For TGFα binding to EGF-R, such a situation could represent the effect of an epidermal wound on surrounding unwounded cells (Owen & Sherratt, 1998); in a developmental context, it could represent the spread of an inductive signal within a population of cells (Monk, 1998). To investigate this situation, homogeneous steady state initial conditions are used, together with a boundary condition on one margin of the array that is fixed at a value distinct from that at the homogeneous steady state. To restrict attention to this single boundary, the boundary conditions on all other boundaries are taken to be either zero flux or fixed at the homogeneous steady state level.

Because of the anomalous boundary condition, the initial homogeneous steady state is no longer a solution of the model equations. Owen & Sherratt consider a non-zero homogeneous steady state of the model described by (2) with a zero ligand boundary condition at one margin of a two-dimensional array of cells. In the final steady solution, the levels of ligand and receptor grade smoothly from their fixed boundary values to their homogeneous steady state levels over a range of several cell diameters

(Figure 4). Since juxtacrine ligands can mediate signalling only between immediately neighbouring cells, it is not immediately obvious whether or not the spatial range of these gradient solutions is bounded above. Considering both discrete and continuous forms of the TGFα model, Owen *et al.*(1999) have shown that in principle there is not an upper bound on spatial range. As the strength of the positive feedback between activated receptor and ligand production increases, the range of the gradient solution increases without bound (Figure 5). However, as the spatial range of solutions increases, linear analysis suggests that the rate at which the solutions approach steady state decreases (Owen & Sherratt, 1998), and so there may be an upper bound to the spatial range over which a signal can be propagated in a realistic time.

Monk (1998) considers the effect of a non-zero fixed boundary condition on the zero homogeneous steady state of the model described by (4). In this case, the absence of feedback between activated receptor and receptor levels has the effect of setting an upper bound on the range over which a gradient solution can be generated (where range is defined as the maximum distance from the fixed boundary at which a given proportion of the receptors on a cell are bound by ligand). Typical solutions are shown in Figure 6. As for the TGFα model, the range is an increasing function of the strength of feedback between the cells; furthermore, for a given strength of feedback, the range is an increasing function of the magnitude of the fixed boundary condition. However, there are two effects that impose a strict upper bound on the spatial range of the gradient solutions in this case. Firstly, as the strength of feedback between the cells is increased, other non-zero homogeneous steady states can come into existence. When this occurs, the fixed boundary condition can initiate a propagating front solution which dominates over gradient solutions (see below). Secondly, in cases where gradient solutions are stable, it is not possible to increase their range indefinitely by increasing the magnitude of the fixed boundary condition. The reason for this is that each of the cells has a fixed number of receptors; once the receptors in the cell neighbouring the boundary have been saturated, there is no possibility of increasing the ligand output from this cell (see Figure 7). The differing results of models (2) and (4) demonstrate that the presence or absence of feedback control of the amount of receptor expressed by the cells can have a significant impact on the properties of gradient solutions.

3.2. Travelling fronts. As mentioned in the previous section, a localised perturbation of a homogeneous steady state can also generate travelling front solutions (Monk, 1998). For suitable forms of binding and feedback functions in (4), in addition to a stable zero homogeneous steady state, there exists a stable non-zero homogeneous steady state in which levels of bound receptor are high, together with an intermediate unstable homogeneous steady state. When this is the case, the intermediate steady

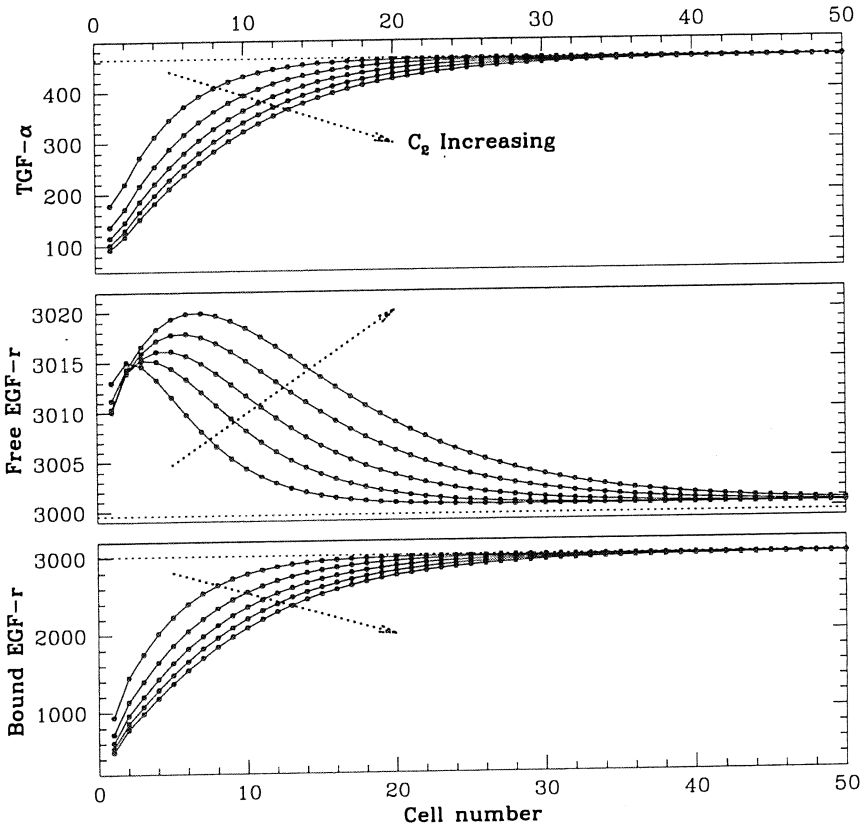

FIG. 4. *Numerically calculated solutions of the model (2). The solutions are shown after 166.7 hours (10000 minutes) of evolution with the wounded boundary condition ($a = f = b = 0$ at cell 0), for C_2 increasing from* 10000 *to* 50000 *at intervals of* 10000. *The distance of propagation of the wound-induced perturbation clearly increases as the parameter C_2, which measures the strength of feedback in* TGFα *production, increases. The other parameters are* $k_a = 0.0003\text{molecules}^{-1}\text{min}^{-1}, k_d = 0.12\text{min}^{-1}, k_i = 0.019\text{min}^{-1}, d_a = 0.006\text{min}^{-1}, d_f = 0.03\text{min}^{-1}, f_e = 3000, b_e = 3000, r_0 = 3000, r_m = 25500$. *For details, see Owen & Sherratt, 1998.*

state defines a threshold of receptor activation and the nature of the solution depends on the magnitude of the fixed boundary condition. For values that fail to raise the level of receptor activation above the threshold, spatially-graded solutions result; in contrast, if the threshold is surpassed, then a travelling front connecting the two stable steady states is initiated (Figure 8). The front propagates away from the fixed boundary at a constant speed, maintaining an invariant profile, and leaving a homogeneous level of receptor activation in its wake. For biologically-realistic parame-

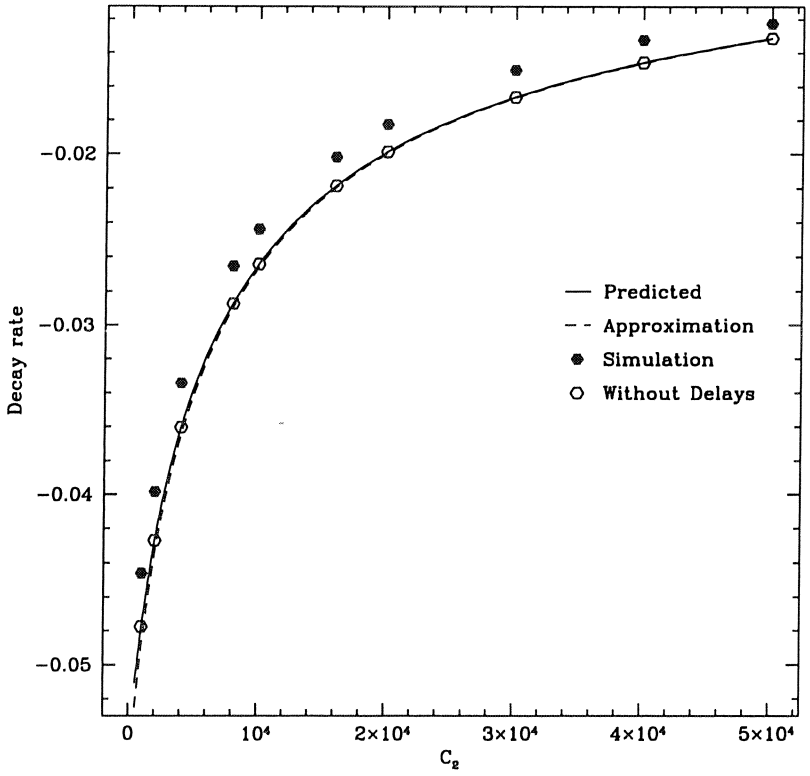

FIG. 5. *Predicted spatial decay rate of gradient solutions as C_2 varies, for the steady state of the model (2), with the wounded boundary condition $a = f = b = 0$ at cell 0. The points represent decay rates calculated from simulation data* 166.7 *hours (*10000 *minutes) after wounding. Here the spatial decay rate is calculated as $(1/L) \cdot \ln[(a_{j+1} - a_e)/(a_j - a_e)]$, which estimates the quantity λ in a solution of the form $a_j - a_e \propto \exp(\lambda L j)$. Thus a less negative growth rate corresponds to a greater signal range. The solid line indicates the decay rate predicted by linear analysis, and the dashed line shows the values given by a lowest order approximation to the decay rate. The other parameters are as in Figure 4.*

ters, the speed of such a front can be great enough to allow propagation over hundreds of cell diameters in the space of a few hours.

3.3. Spatial patterns. Fine-grained patterns are a ubiquitous feature of epithelia in early animal development, and juxtacrine signalling is a natural candidate for the generation of these patterns. Numerical simulations of the mathematical models described in §2 show that both the negative and positive feedback models do indeed support permanent spatial patterns for appropriate parameter values. However, the type of pattern depends crucially on feedback regulation.

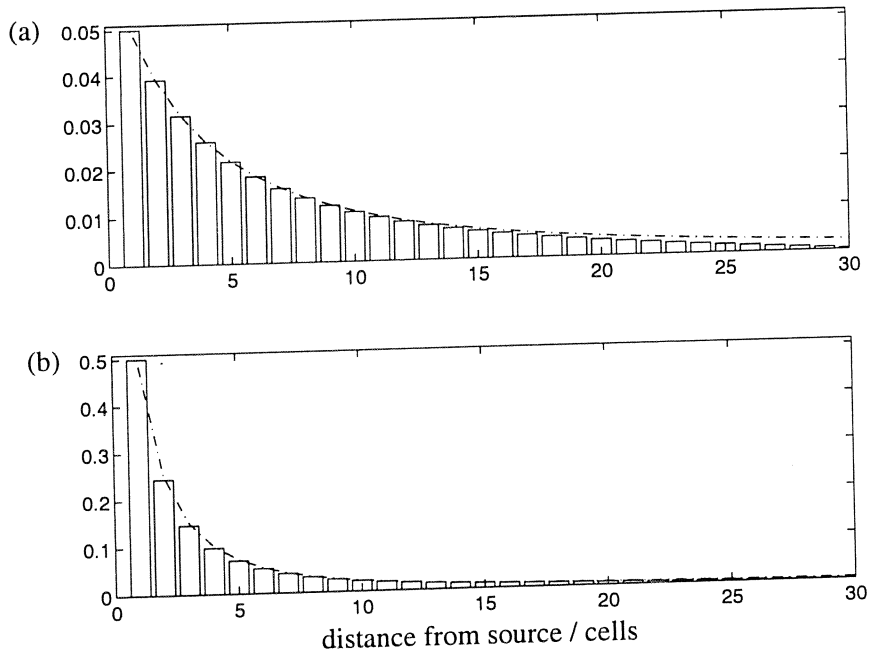

FIG. 6. *Examples of steady state spatial gradients of cell activation resulting from a fixed signal source (cell 1). The strength of signal source in (b) is 10-times greater than that in (a), illustrating the fact that the qualitative shape of the gradient is dependent on the strength of the signal source. Histograms show the results of numerical simulation of the model described by (4); continuous curves show corresponding analytical approximations to these solutions (in (b), the agreement between the two solutions is such that they are indistinguishable at distances of more than about 5 cells from the source). Responsive cells had random initial levels of activation between 0 and 0.01 and $c_i(0) = 1$. $\mu = \rho = \nu = 1$, $C(\xi) = 1$, $\mathcal{T}(\xi) = \xi$, $\mathcal{R}(\xi) = \xi/(1 + \xi)$.*

In the case of negative feedback, which is represented in model (1), patterns form provided that the feedback functions $F(.)$ and $G(.)$ are sufficiently strong: specifically, stability analysis shows that the condition for patterning is $F'(D^*)G'(N^*) < -\mu\rho$, where $D = D^*$, $N = N^*$ is the unique homogeneous steady state. In the case of a one-dimensional line of cells, the patterns have a characteristic and highly reproducible form, consisting of alternating high and low levels of Notch and Delta activity (Figure 9). The key property of a wavelength of two cells is independent of parameters, and arises because of the lateral inhibition intrinsic in the model. A cell with higher Notch activity than its neighbours has lower Delta activity, because of negative feedback. This in turn reduces Notch activity in the neighbouring cells, leading to increased Delta activity in these cells (negative feedback again) and hence further increase in Notch activity. Thus the feedback regulation enables a regular, alternating pattern to be sustained.

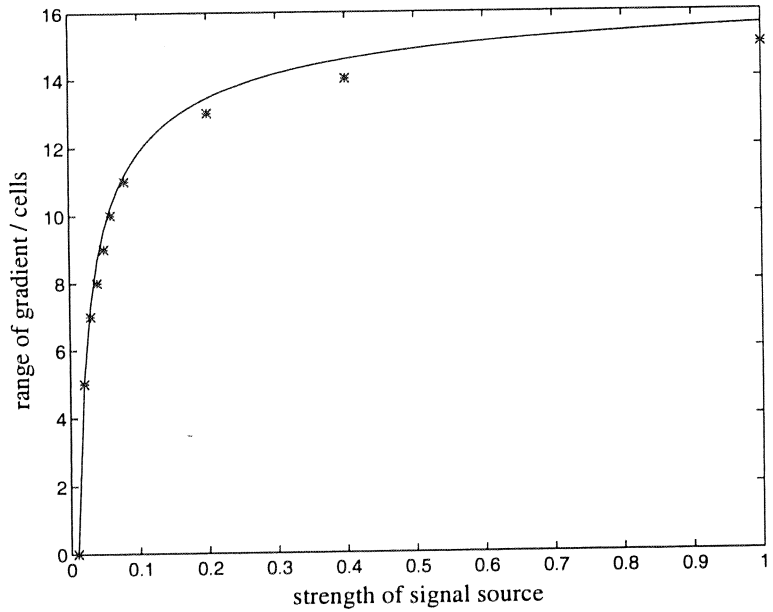

FIG. 7. *Distance over which a level of cell activation of at least 0.01 (i.e. 1% receptor saturation) can be attained at steady state as a function of the strength of signal source in the model described by (4). The continuous curve shows an analytical approximation, while stars show results of corresponding computer simulations. Parameters and functional forms as in Figure 6.*

In numerical solutions, the only significant difference between simulations is the presence of defects, where two adjacent cells have high levels of Notch activity — these are a consequence of the particular perturbations applied initially to the uniform steady state. However, solutions in which two adjacent cells have low levels of Notch activity are never seen, in agreement with experiment.

In the two-dimensional hexagonal grid of cells, the model (1) again predicts spatial pattern formation. Again, the possible patterns are all fine-grained, with scattered cells with low Notch activity surrounded by cells with high Notch activity, but a number of different pattern configurations are in principle possible (Figure 10). Numerical simulations show that the boundary conditions exert a major influence on the patterns formed, at least on relatively small domains, and thus Collier *et al.* (1996) considered patterns formed on 6×6 or 7×7 arrays of hexagonal cells with periodic boundary conditions (Figure 11), enabling consideration of potential periodic patterns on an infinite domain. On a 6×6 array, patterns with periods 1,2,3 or 6 in i and j are in principle possible. It is the period 3 pattern (with slight defects) that emerges in the simulations in Figures 11(a) and (b);

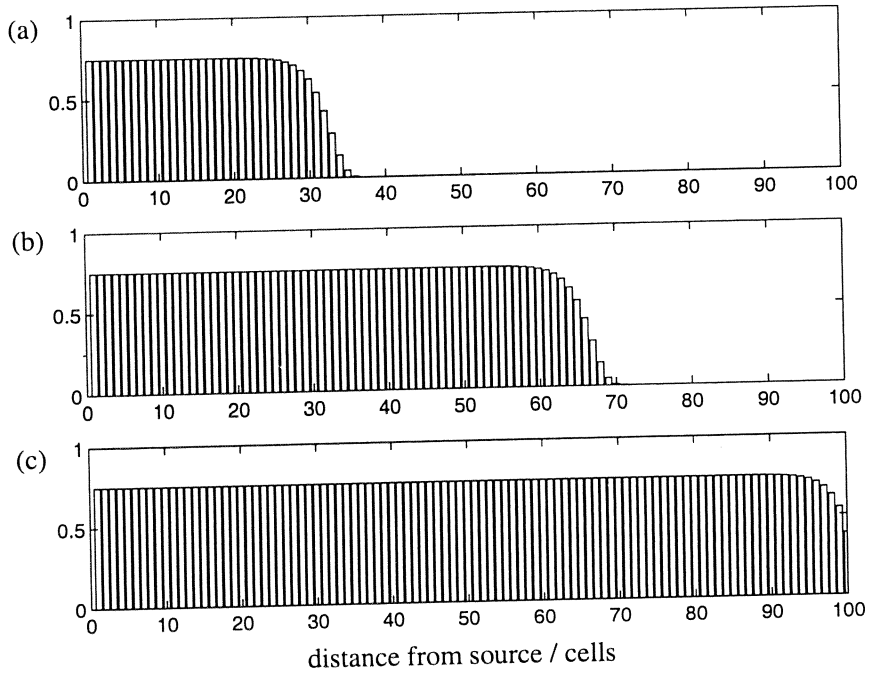

FIG. 8. *A typical travelling front of cell activation generated by (4). The responsive field is fixed at 100 cells by setting the level of activation of cell 101 to zero.*

however in the 7×7 array, a pattern with period 7 (as in Figure 10c) can be achieved by using initial conditions that are biased towards this pattern.

In the case of positive feedback in ligand and receptor production, juxtacrine signalling is again able to produce spatial patterns, as shown by numerical simulations of (2). Mathematical conditions for patterning can be derived by linear analysis (Wearing, Owen & Sherratt, 2000). Patterns form when feedback is relatively low for ligand and moderate for receptors, with alternative behaviours being a stable uniform state (when feedbacks are weak) and uncontrolled upregulation in ligand and receptors throughout the domain (when feedbacks are strong). Figure 12 illustrates such a pattern forming when the homogeneous steady state is perturbed locally at one edge of a large sheet of cells; for simplicity we assume that the perturbation is such that a one-dimensional (striped) pattern results.

Intuitively, spatial patterning in this system arises via spatially localised positive feedback. The weak feedback in ligand expression, which is a key ingredient, means that ligand levels remain relatively constant despite large variations in receptor expression. This means that both increases and decreases in receptor levels, away from homogeneous equilibrium levels, are self-reinforcing. This enables a wide range of spatial patterns, with clear division of cells into high and low receptor occupancy.

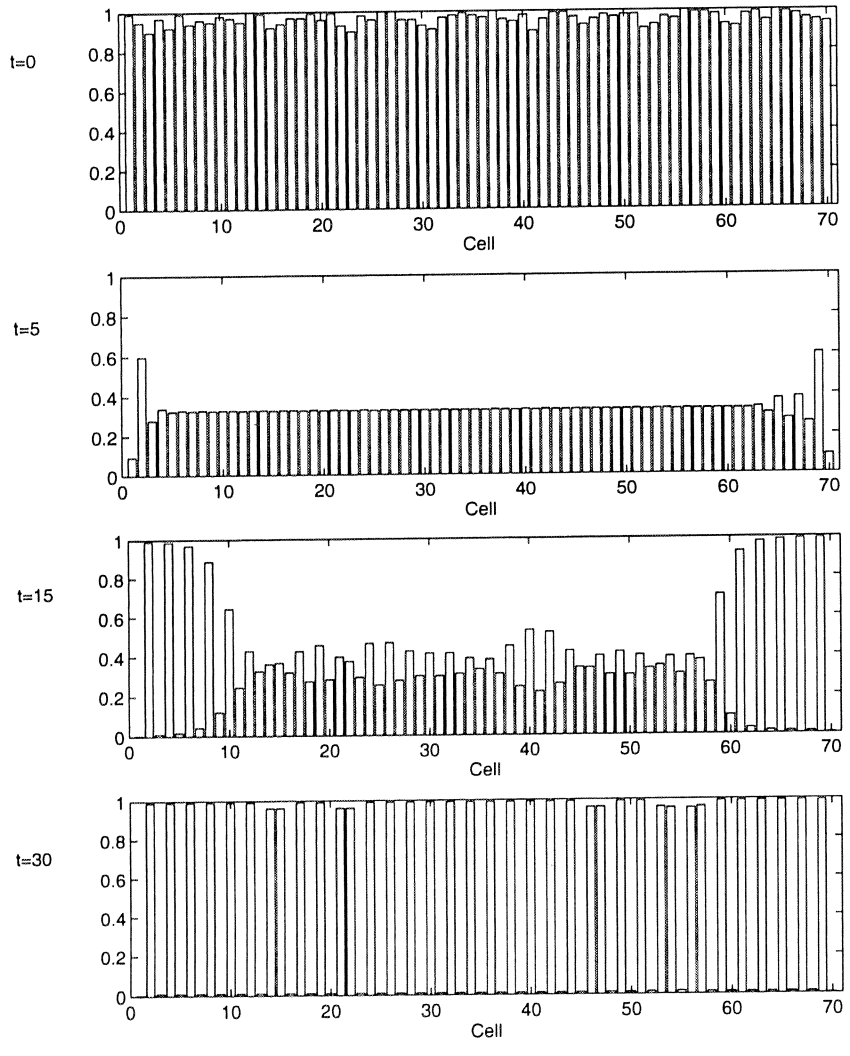

FIG. 9. *Time evolution of the level of Notch activity in a line of 70 cells starting from an initial near-homogeneous state. By $t = 30$, the levels of Notch and Delta activity have almost reached equilibrium. Initial conditions for n_j are shown in the top panel ($t = 0$), and $d_j(0) = 1$ for $1 \leq j \leq 70$.*

4. Further development of juxtacrine models. The models we have discussed here represent a first step in the investigation of the properties of juxtacrine signalling systems. The complexity of the models has been kept to a minimum in order to exhibit most clearly the properties of this type of signalling system, and to avoid where possible including any assumptions that cannot be experimentally justified. However, there are many extensions of the models that should be considered. Although

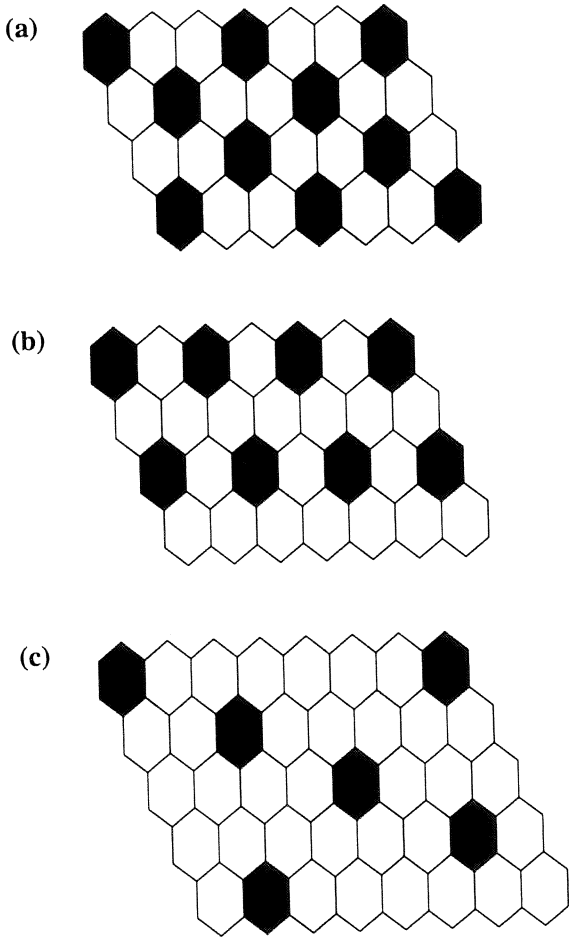

FIG. 10. *Examples of possible periodic steady-state patterns of Notch activity in (unbounded) two-dimensional arrays of hexagonal cells. Shaded cells have low Notch activity, while white cells have high Notch activity.*

the discrete and continuous models outlined above are equally suited to describing simple juxtacrine systems, they each have features that make them more or less suitable when considering more complex systems. For example, in order to investigate the effects of juxtacrine signalling in the context of cell proliferation and movement, it would be most effective to employ a continuous formalism. In contrast, a discrete formalism would be more suited to an investigation of the effects of internal cellular dynamics on patterning by juxtacrine signalling.

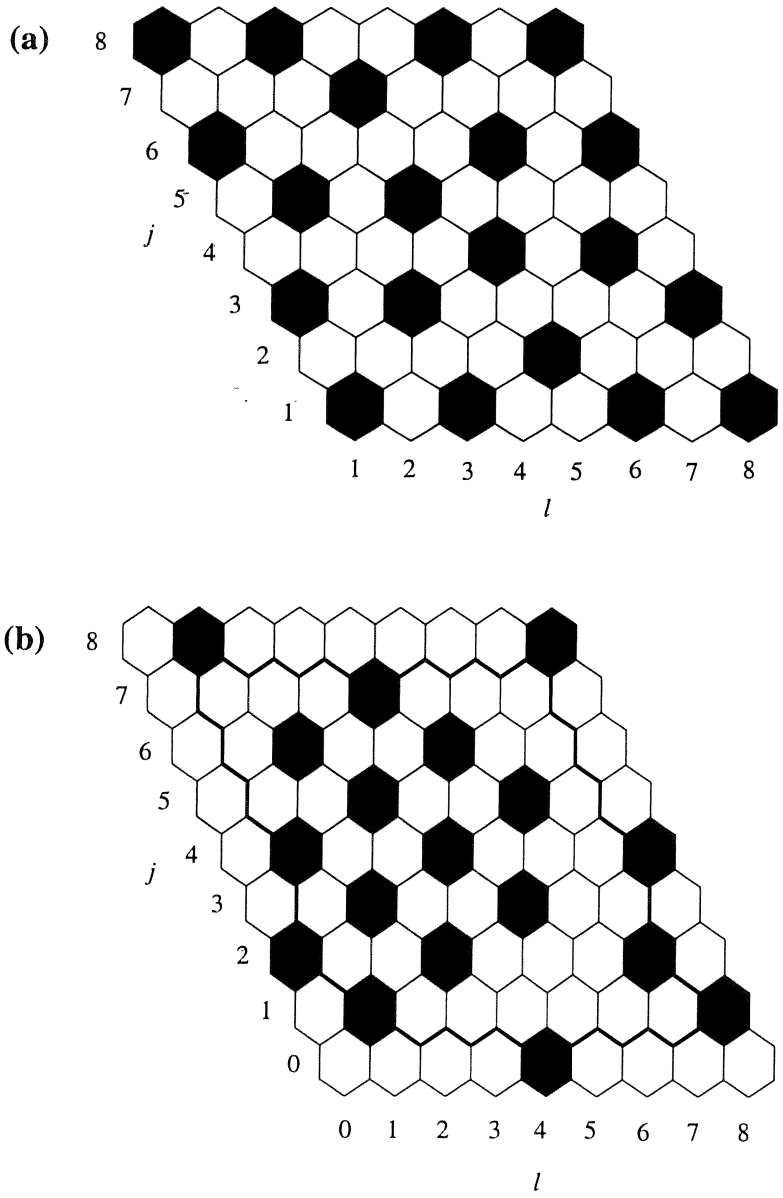

FIG. 11. *Typical steady state patterns of Notch activity in an 8 × 8 (a) and 7 × 7 (b) array of hexagonal cells starting from near-homogeneity. Shading as in Fig. 10. Boundary conditions: (a) $d_{lj} = 0$ for $l, j = 0$ or 9; (b) periodic. Initial conditions: $n_{lj}(0)$ and $d_{lj}(0)$ have arbitrary values between 0.95 and 1.0.*

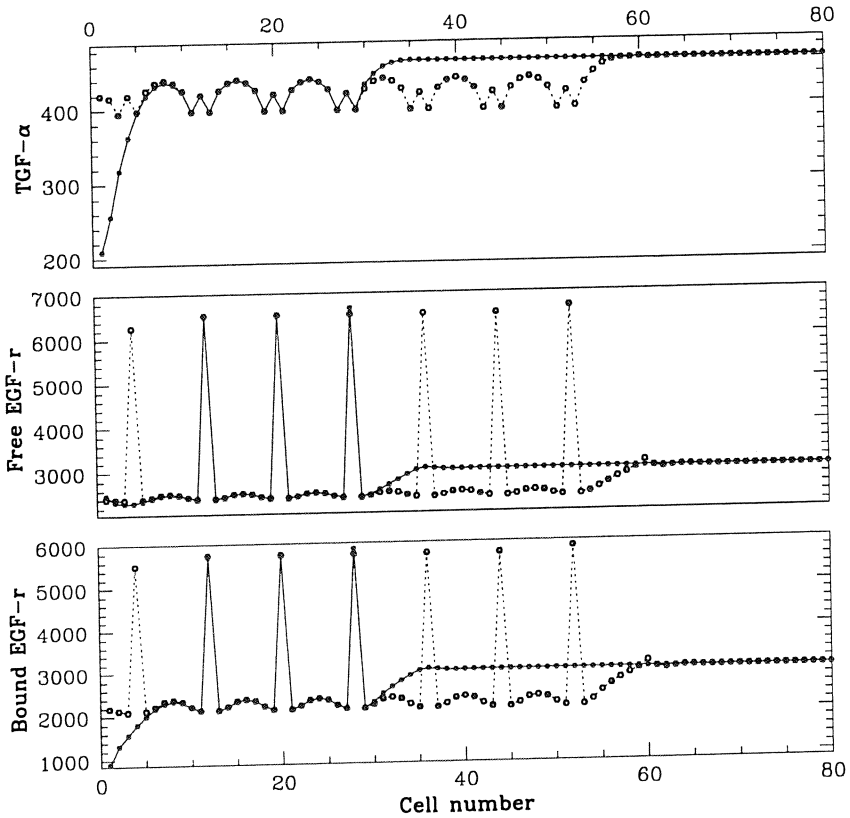

FIG. 12. *Numerical simulation of the model (2), specified with equations (1), and with $m = 1.0$ and $n = 3.0$. Linear analysis predicts pattern formation in this case. The solid points and lines indicate the solution after 500 hours of evolution with a wounded boundary condition ($a = f = b = 0$ at cell 0), and the open points and dotted lines indicate the solution a further 500 hours after the reintroduction of the healed boundary condition (values of a, f and b the same at cell 0 and cell 1). Interestingly the patterned solution continues to persist and spread, despite the completion of healing at the wound edge. Thus, this figure shows the early spread of a spatial pattern which would eventually spread to cover the entire domain. This simulation has no relevance to wound healing, but periodic patterns with a wavelength of a few cells are observed in a range of processes in early vertebrate development (see Lewis, 1998, for review). The other parameters are $k_a = 0.0003\text{molecules}^{-1}\text{min}^{-1}, k_d = 0.12\text{min}^{-1}, k_i = 0.019\text{min}^{-1}, d_a = 0.006\text{min}^{-1}, d_f = 0.03\text{min}^{-1}, f_e = 3000, b_e = 3000, r_0 = 3000, r_m = 25500, C_2 = 8000$.*

An important consideration that has been neglected in the models outlined here is the time taken for the operation of intracellular feedback. It is necessary to have at least an estimate of the time taken for a change in the level of receptor activity in a cell to have an effect on the levels of ligand and free receptor in that cell. For TGFα signalling, it is known that

changes in the number of bound EGF-R receptors in a cell result in changes in the level of transcription of the genes coding for ligand and receptor. For Delta–Notch signalling, it is known that such transcriptional control can occur, though it is not clear to what extent it is important in lateral signalling. If feedback is mediated by control of the rates of transcription of ligand and receptor, then the delay between a change in the level of receptor activity and a concomitant change in the rate of appearance of mature ligand and receptor in the cell membrane can reasonably be assumed to depend on their molecular weight. EGF-R and TGFα have molecular weights of 170kD and 18kD respectively (Ciardiello *et al.*, 1989; Kumar *et al.*, 1995). Notch and Delta are significantly larger, having molecular weights of around 300kD and 100kD respectively (Fehon *et al.*, 1990).

For all of these proteins, with the possible exception of TGFα, transcription alone would be expected to take a significant time. For example, the region of genomic DNA corresponding to *Delta* in *Drosophila* is approximately 25kb in length (Kopczynski *et al.*, 1988; Vässin *et al.*, 1987). Assuming a transcriptional rate of 1kb per minute, production of the primary mRNA transcript for *Delta* would take around 25 minutes. When mRNA processing, translation, protein processing and transport to the membrane are taken into account, it is likely that there would be a lag of at least 30 minutes in a Notch–Delta feedback mediated by transcriptional regulation. Thus the delays in regulation of the production of ligands and in particular receptors are likely to be significant in some applications. In this review we have neglected this complication, but models in which delays are included are discussed elsewhere (Owen & Sherratt, in preparation; Monk, in preparation).

The models we have discussed here are all based on the assumption that cells signal with equal strength to each of their immediate neighbours. However, it is not uncommon for epithelial cells to be overtly polarised within the plane of the epithelium (termed planar polarisation—see Eaton, 1997). In *Drosophila*, a genetic pathway centred on the transmembrane receptor Frizzled and the intracellular protein Dishevelled has been shown to be involved in the generation and coordination of polarisation in a wide range of epithelia during development (reviewed in Shulman *et al.*, 1998). While the precise mechanisms underlying planar polarisation remain unclear, the non-uniform activation of the Frizzled signalling pathway within epithelial cells has recently been implicated as an important step in the process (Axelrod *et al.*, 1998).

In order to investigate the effects of planar polarisation on patterning mediated by juxtacrine signalling, the models described here can be generalised to take into account spatially non-uniform activity of ligand and receptors in the cell membrane. This is most easily achieved in the discrete formalism, at least for simple forms of non-uniformity. Generalisations of this type are particularly relevant to the study of spatial patterning by lateral signalling. There is growing evidence for non-trivial interactions between the Notch and Wingless pathways, mediated by Dishevelled (Axelrod *et al.*, 1996; Martinez Arias, 1998), and it has been suggested that such an interaction could modulate the periodicity of the fine-grained patterns generated by Delta–Notch signalling (for example, in the *Drosophila* stomatogastric nervous system—González-Gaitán & Jäckle, 1995). Models including spatially non-uniform signalling within the cell membrane are discussed elsewhere (Monk, in preparation).

5. Discussion. The models we have described in this review are based on the basic biological premise that cells signal to their immediate neighbours via a transmembrane signalling system. We have shown that such models can account for the generation of a range of stable and propagating patterns within populations of interacting cells. The best-studied theoretical model for spatial pattern formation in biological systems is the Turing mechanism (Turing, 1952; for a recent review, see Maini *et al.*, 1997), in which diffusion-driven instability sets up a prepattern in two or more reacting chemical morphogens; this is translated into a discrete pattern of distinct cell fates via a threshold level of one of the morphogens. More generally, models based on the Turing mechanism belong to a class of models for spatial pattern formation that are based on the idea of a localised self-activating process balanced by a longer-range inhibitory process (Oster, 1988).

The juxtacrine mechanisms that we have described here are quite different to previous models based on local activation and long-range inhibition, both in their mathematical form and in their biological interpretation. In juxtacrine models, all interactions are local: Feedback depends on biochemical events restricted to the intracellular environment, while signalling between cells is restricted to immediate neighbours. They thus constitute a new and quite distinct class of mechanism for the generation of spatial patterns in biological systems.

In their spatially discrete form, juxtacrine signalling models bear some resemblance to cellular automata models (see, for example, Ermentrout & Edelstein-Keshet, 1993). However, the simple discrete rules that dictate the behaviour of conventional cellular automata (Wolfram, 1994) are replaced in juxtacrine models by functions based on well-characterised biochemical interactions (between membrane-anchored ligand and receptors). Consequently, the patterns generated lend themselves very naturally to biological interpretation: Bound receptor activity is the clear biological determinant of cell fate. Since this activity is either very high or very low in all the patterns we have observed, there is no requirement for the imposition of a particular (and arbitrary) threshold level of the type required in local activation and long-range inhibition models.

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