

Models and Hypotheses

Morphogenesis of Lines and Nets

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Complex linear appearing structures and networks (e.g. blood vessels, leaf veins, nerves) are formed reproducibly during the development of nearly every organism, but the molecular mechanism leading to such patterns is still unknown. A model is proposed in which a few simple coupled biochemical reactions are able to generate such structures. Among undifferentiated cells, a local peak of differentiation-inducing substance (activator) is formed by autocatalysis and lateral inhibition. The activator peak triggers the differentiation of the cell at that location. Due to changes in metabolism, the differentiated cell repels the activator peak and drives it to a neighbouring cell which then also differentiates. The repulsion between the activator peak and the already differentiated cells forces the activator peak to move ahead of the tip of the extending filament. Long filaments of differentiated cells may be formed, which can split, branch laterally, reconnect with each other and grow towards specific target cells. Partial differential equations describing the mutual interaction of the substances involved were presented and solved with a computer. The resulting patterns show self-regulating properties and other features found in the leaf vascular system, the pattern of tracheae in insect epidermis, and other biological networks.

Introduction

Lines and branching networks of lines are etched into the anatomy of nearly every living organism. The axons and dendrites of nerve cells, the vascular system [1, 2], the veins of leaves [3] and insect wings [4], the tracheae of insect epidermis [5, 6] and the hyphae of fungi [7, 8], all testify to the ubiquity of branching patterns. The formation of these structures must be coded in the genes, but little is known about the mechanism. Self-assembly of subunits may be an attractive possibility in some cases, e.g. in the formation of microtubules. In this paper we call attention to another possibility: the genesis of networks by wandering concentration peaks of differentiation-inducing substances which leave behind trails of differentiated cells.

A brief synopsis of the mechanism would include the following sequence of events: firstly, a localized concentration peak of a differentiation-inducing substance (an activator) is formed. Secondly, the activator concentration serves as a local signal for cells to differentiate. Finally, the differentiation of these cells causes the acti-

vator peak to shift its position in a direction which is, in general, away from the already differentiated cells. Thus the peak wanders, leaving behind a trail of differentiated cells.

Branching can result from a binary fission of the activator peak: lateral branching can result from the origin of a new activator peak at the side of a line of already differentiated cells. This mechanism can also explain many other well-known features of "biological lines" including growth by localized elongation (tip growth), growing together of lines to form networks, and positioning of lines and networks within the tissue boundaries.

We have developed a theoretical model in which network morphogenesis is carried out by a simple system of coupled biochemical reactions. We assume a collection of contiguous cells and a number of substances which may act as substrates, inhibitors or activators in either their own or each other's production and breakdown reactions. Thus, the production and breakdown

rates in each cell are affected by the local concentration of the substances and, via diffusion, by the concentration in adjacent cells.

It is possible to describe such systems with sets of partial differential equations and determine their behaviour by solving the equations with a computer. The model is presented here both intuitively and in its mathematical form, accompanied by computer-generated illustrations of the model's behaviour. For computational simplicity, we have dealt only with patterns on a plane which is subdivided like a chessboard into square cells.

Formation of a Local Activator Peak

Gierer and Meinhardt [9–12] proposed a model for the formation of non-uniform distributions of substances within tissues. The model explains the formation of local concentration peaks within tissues that had shown initial uniform concentrations save for shallow gradients or noise variations. The model features an *activator*: substance A . Substance A is stimulatory to two local pro-

Table 1. Equations (1–8). Time dependence of the activator A , inhibitor H and the substances S and Y ; all these substances are space-dependent. Equation (1) means: the change of A per time unit (\dot{A}) is proportional to an autocatalytic term (A^2); the autocatalysis is slowed with increasing inhibitor concentration ($1/H$); A decays in a first order reaction ($-\mu A$) and diffuses ($D_a \Delta A$). Equations (1) and (2) (activator-inhibitor model) as well as Equations (3) and (4) (activator-depleted substance model) lead to the formation of local high activator concentration [9] by autocatalysis of A and the inhibitory effect of H or S . Equation (5) models the differentiation of the cells: under the influence of the activator, Y switches from low to high concentration, which leads, according to Equation (4) or Equation (8) to a depletion of S at this point. Depletion of S induces a directional drive of the activator peak away from high Y concentration, because S is necessary for the A -production [Eqs. (3) and (6)]. Equations (3–5) allow net formation with binary branching (Fig. 3), Equations (5–8) allow lateral branching in addition (Fig. 4–6)

$$\dot{A} = cA^2/H - \mu A + D_a \Delta A \quad (1)$$

$$\dot{H} = cA^2 - \nu H + D_h \Delta H \quad (2)$$

$$\dot{A} = cA^2S - \mu A + D_a \Delta A \quad (3)$$

$$\dot{S} = c_0 - cA^2S - \gamma S - \epsilon YS + D_s \Delta S \quad (4)$$

$$\dot{Y} = dA - \epsilon Y + Y^2/(1 + fY^2) \quad (5)$$

$$\dot{A} = cA^2S/H - \mu A + D_a \Delta A + \varrho_0 Y \quad (6)$$

$$\dot{H} = cA^2S - \nu H + D_h \Delta H + \varrho_1 Y \quad (7)$$

$$\dot{S} = c_0 - \gamma S - \epsilon YS + D_s \Delta S \quad (8)$$

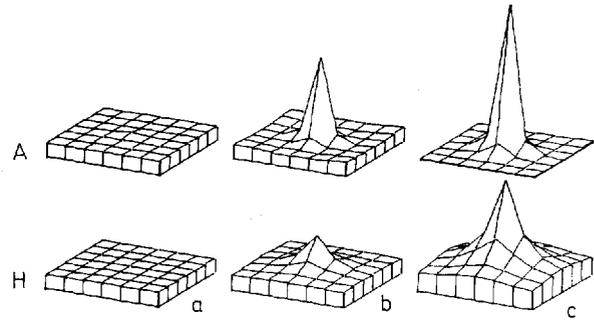


Fig. 1a–c. Activator A (upper row) and inhibitor H (lower row) distribution as function of time: (a) A small initial elevation of the activator concentration leads to the formation of a stable activator peak (b, c) [Eqs. (1) and (2)]

cesses: its own production (autocatalysis), and the production of its antagonist, the inhibitor substance H ; this inhibitor diffuses faster than the activator. Equations (1) and (2) (Table 1) embody these mutual interactions. A small initial elevation of the activator concentration within a single cell is amplified by the activator autocatalysis (Fig. 1). This cell then produces additional inhibitor, but most of the inhibitor rapidly diffuses to neighbouring cells. As a result, A continues to increase within the activated cells but its production is blocked in the outlying cells. This can lead to a pattern in which one or very few cells with high activator concentration are surrounded by a large group of cells in which the activator concentration is generally very low.

The role of the inhibitor can also be played by an activator substrate S , which is produced everywhere and consumed during activator production. Here, the inhibition is mediated by depletion of the substrate S by the activated cells. (Activator-depleted substance model, Eqs. (3) and (4), Fig. 3a, b). In either case, the diffusion range of inhibition must be greater than that of the activator to allow the formation of activator peaks. Thus S or H must diffuse faster than A .

The Activator Peak Migrates

One consequence of cell activation is to prevent activation of adjacent cells. Yet elaboration of a pattern as in filament elongation requires that the active state move to adjacent cells. For this, we arrange to turn off A production in the activated cells. Consequently, Inhibitor production (or S -depletion) will stop and, as seen below, neighbouring cells may become activated. To conserve the information inherent in the activator pattern, we further arrange that activated cells remember their original

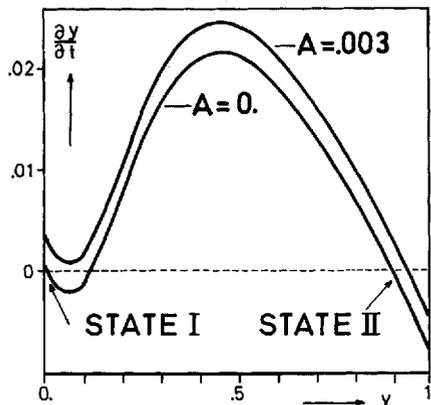


Fig. 2. A model for switching behaviour (differentiation) of a cell: A high activator concentration induces an irreversible transition from one state of differentiation (state I) to another (II). We accomplish this as follows: a substance Y is produced proportionally to the activator concentration, and broken down by simple first-order process. In addition, Y has a positive feedback on its own production which saturates at high concentration [Eq. (5)]. In the absence of the activator ($A = 0$), only two stable states are possible: at low Y -concentration, the change of Y per time unit (\dot{Y} or $\delta Y/\delta t$) is negative, Y will decrease to zero (state I), or, above a critical level of Y , \dot{Y} is positive and increases until the upper stable state II is reached. If A is above a critical level, Y is positive even at low Y -concentration, and only the state II is stable. Once the state II is reached, there is no way back to state I; under the influence of the activator, the cell has been irreversibly switched to the “differentiated” state II. The details of the mechanism are not critical for the model proposed as long as the irreversible transition from one state of differentiation to another occurs when the activator exceeds a critical concentration [Eq. (5); $d = 1$, $e = 0.1$, $f = 10$]

high activity by undergoing an irreversible change (Fig. 2 and Eq. (5)). We call this change “differentiation”. The cessation of activator production can be in fact a simple consequence of this change.

When an isolated activator peak is formed, then its component cell or cells will become differentiated, lose their high activation, and cease the inhibitor production (or S -Depletion). Activator which has spread from the original activator peak into adjacent cells will thus become autocatalytic, and activator production will begin in all of the immediate neighbours of the newly differentiated cells. Activator production thus rises in a ring of cells surrounding the first cell. Seen intuitively, all cells in the ring try to enhance their own activator production and suppress that of the neighbouring cells. In the course of this competition, small asymmetries, even random fluctuations, will be decisive: as the whole amplification proceeds, the ring is left with only one activated cell. This newly activated cell will then ultimately become differentiated.

We now have two differentiated cells in a row. The line formed by these two cells will be extended if new activator peaks tend to form at the ends of the line and not at the sides. This will happen if differentiated cells produce H (or consume S) at a moderate rate. Neighbours at the sides of the line, which are near two differentiated cells, will thus experience higher H (or lower S) than neighbours at the ends, which are adjacent to only one differentiated cell. The neighbours at the end of the line will thus win the competition to become activated, and subsequently will themselves differentiate. This mechanism can produce a filament of any arbitrary length. The inhibitor (or depleted substance S) has two different functions: it is the antagonist of the activator, producing lateral inhibition necessary for the activator peak to form; and it provides a “repulsive force” which drives the activator peak away from the differentiated cells.

One interesting consequence of this model is the positioning of the filament of differentiated cells away from the edges of the developing field. The growing tip avoids the edges of a field as long as other space is available, because the inhibitor (or substance S) cannot diffuse past the margins of the field. Thus the inhibitor concentration is higher (or S -concentration lower) on the side of the filament tip facing an edge than on the side facing away from the edge; thus growth in the direction away from the edges is favoured.

In small fields, this effect can be seen very early and can in fact fix the initial direction of filament growth by specifying the location of the second activated cell. Thus, the filament tends to grow down the long axis or along a diagonal in a small field. This effect can be seen in Figures 3c and 4.

Formation of Branches

1. Binary Branches. During the extension of a filament of differentiated cells, the growing point can split. The model can explain such binary branching: it occurs when two activator peaks form within the “active ring” surrounding a newly differentiated cell; and its frequency increases with distance of the growing tip from other filaments or from the boundaries of the field. In general terms, branching is favoured by increasing “free space” into which the inhibitor can diffuse, or from which the substance S can be obtained.

2. Lateral Branches. Biological filaments commonly make lateral branches long after their own formation. In order to achieve this behaviour in the model, it is neces-

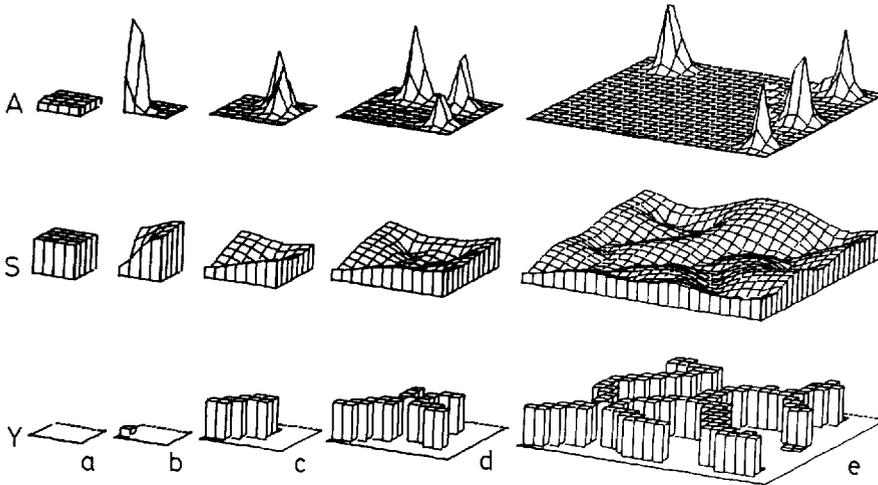


Fig. 3a–e. Generation of dichotomously branching filaments in a growing field [activator-depleted substance model, Eqs. (3–5)]: (a) A small deviation from the equilibrium between activator A and the activator substrate S leads (b) to a local high A -concentration and to a depletion of S . The high A -concentration triggers cell differentiation (high Y -concentration). (c) The location of the high activator concentration will be shifted if the system grows [12]. (d, e) During this shifting, fission of the activator peak is possible. The result is a binary (dichotomous) branch in the differentiated structure. Due to the depletion of S along the differentiated structure, no lateral branches can occur. Equations (3–5) are used with $c = 0.008$, $\mu = 0.04$, $D_a = 0.0065$, $\gamma = 0$, $D_s = 0.18$, $c_0 = 0.05$, $\epsilon = 0.25$, $d = 0.00032$, $e = 0.1$, $f = 10$. (a) (b) (c) (d) (e): pattern after 80, 300, 1200, 2400 and 6000 iterations (relative time units), growth is simulated by addition of new cells at two edges every 300 iterations

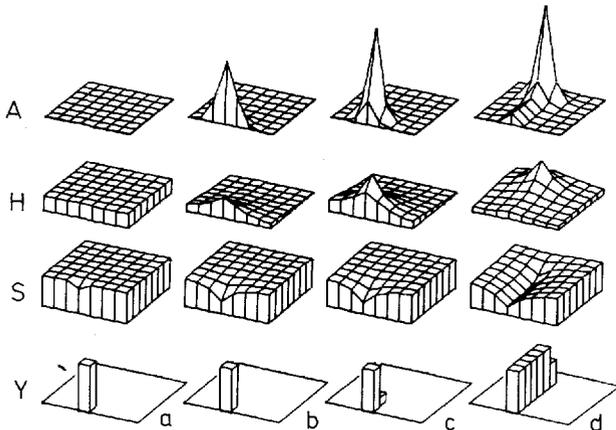


Fig. 4a–d. Development of a filament of differentiated cells: (a) An initially differentiated cell (high Y -concentration, *bottom row*) triggers (b) an activator peak and simultaneously depletes the substance S . The activator peak migrates (c) toward higher S -concentration. A filament is obtained (d) by the repetition of the following steps: local high activator concentration, differentiation, depletion of S , shift of the activator peak to a neighbouring cell. Equations (5–8) with $d = 0.0013$, $e = 0.1$, $f = 10$, $c = 0.004$ with 5% random fluctuation from cell to cell, $\mu = 0.12$, $\varrho_0 = 0.03$, $D_a = 0.02$, $\nu = 0.04$, $D_h = 0.18$, $\varrho_1 = 0.0003$, $c_0 = 0.02$, $\gamma = 0.02$, $\epsilon = 0.2$, $D_s = 0.06$

sary for a new activator peak to be formed at the side of an existing filament. However, in the model as developed so far, differentiated cells are assumed to be inhibi-

tory. This provides the directional drive for forming straight lines, but concomitantly suppresses potential new activator peaks.

This problem can be solved by assuming that two different inhibitory systems operate at once, one providing the directional drive, and the other acting as the direct antagonist of the activator. This can be accomplished by employing both an inhibiting substance and a depleted substrate for the two inhibition mechanisms. We have done this in Equations (5–8); the resultant filament pattern is illustrated in Figures 4–6. Here S has been made mainly responsible for the drive: it is broken down massively by the activated cells [ϵYS , Eq. (8)], but its lateral inhibition function is slight, since the consumption of S by the activated cells is negligible. The inhibitor H , on the other hand, is made mainly by cells with a high concentration of activator and it serves to mediate the lateral inhibition. Due to a small constitutive activator production of the differentiated cells [$\varrho_0 Y$ in Eq. (6)], filaments generated by the Equations (5–8) will spontaneously form lateral branches at a critical distance behind the growing tip (Figs. 5 and 6). This distance depends upon the diffusion range of the inhibitor produced by the growing tip and upon the density of other filaments in the area. In the absence of other constraints, such as edges of the field or proximity of other filaments, such branches will grow out at right angles,

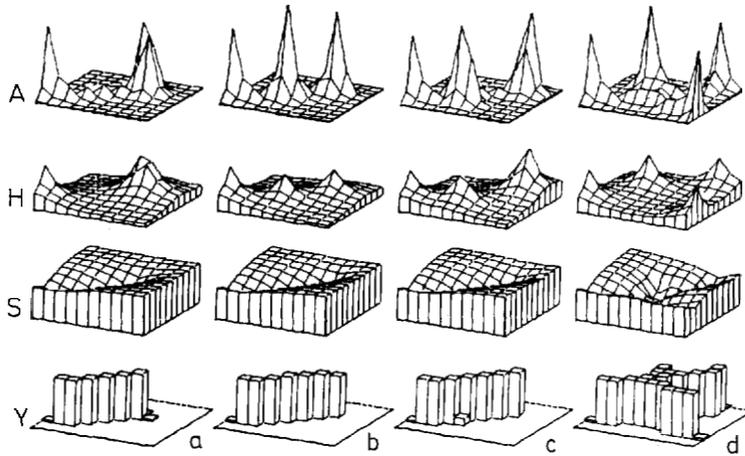


Fig. 5a-d. A new activator peak is formed at a critical distance behind the growing point (a, b), initiating a lateral branch (c), which elongates. When the tip of this branch has attained a sufficient distance from the original filament (d), a new branch can be formed

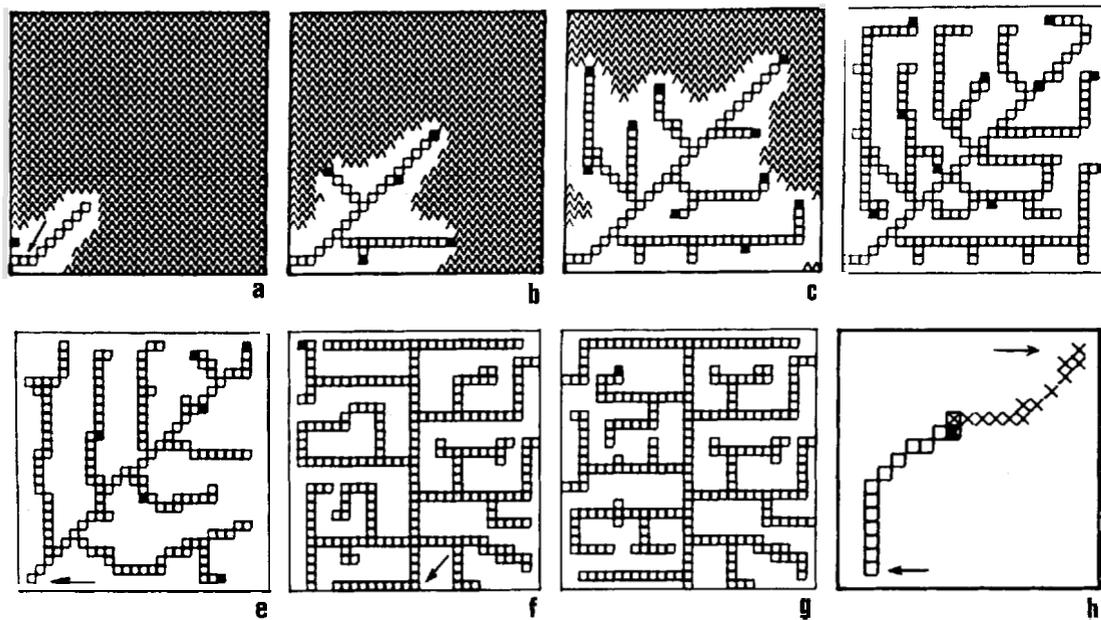


Fig. 6a-h. Formation of net-like structures of differentiated cells (\square , \times differentiated cell, \blacksquare activated cell, wavy high S concentration, \leftarrow initially differentiated cell). (a-d) Since the activator peak is repelled by the edges of the field, the filament grows diagonally. Lateral branches tend to grow out at 90° , but are deflected by the margins or by previous lateral branches. Reconnections are possible. (e) Result with higher basic activator production ρ_0 ; a cloud of inhibitor surrounding each filament prevents reconnection. (f) Result with smaller activator diffusion range; 90° branches dominant. (g) Result after removal and regeneration of left half of the network produced in Figure 6f. The regenerated half is similar but not identical to the original. (h) Two different filament types, which employ different inhibitors and each produce an activator substrate specific for the other type, can actively find each other

since their growing tips are attracted by high S -concentration, and a valley of S -concentration is centered on the differentiated filament (Fig. 4).

Branches themselves will form further branches when their growing tips become sufficiently remote (Fig. 6a-g). This continues through several generations. The ultimate density that the network attains can be regulated by the degree of S -depletion or by a small constitu-

tive inhibitor production by the differentiated cells. This creates a "background inhibition" which is proportional to the local net density and can prevent branch formation and elongation when this density exceeds a critical value. If a part of a "confluent" network is destroyed, new branches grow in to repair the damage [6]. The model thus shows self-regulatory properties and accommodates pattern regeneration (Fig. 6f, g).

Finding Specific Cells

In the model, a growing filament can "home in" on a particular target cell if the target cell produces the substrate S : this generates a gradient of S which the growing filament can follow. If the distance between the growing tip and the target is large, homing may be inaccurate since the gradient is shallow, and the direction of filament growth may be influenced by other factors such as edges. With diminishing distance, however, the accuracy of directional growth improves (Fig. 7). The growing points of two different types of filaments will actively find each other, if the two filament types employ different inhibitors and each elaborates an activator substrate specific for the other type (Fig. 6h).

An externally formed S -gradient can cause a larger number of filaments of the *same* type to grow parallel to each other in a particular direction: all filaments follow the S -gradient and maintain their distance from one other by mutual repulsion.

Comparison with Leaf Vascularization

Avery [3] has provided a careful analysis of the development of the tobacco leaf. The young primordium has the shape of a tall thin cone. At a length of about 1 mm, the first vascular structure appears, called the midrib; it runs down the long axis of the conical leaf primordium. The genesis of such a structure, in the correct position and with the correct orientation, is explained by our model as a consequence of the inhibitory effect of the edges (Fig. 3c). After further growth, the midrib sprouts perpendicular branches. The factor which immediately

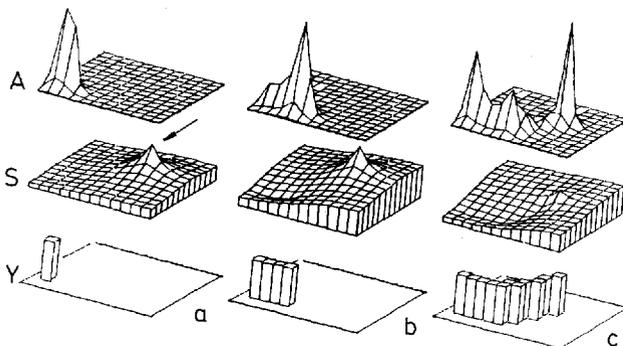


Fig. 7a-c. A filament grows toward a particular target cell: (a) The target (arrow) produces the substance S . (a, b) The activator peak migrates up the S -gradient to the target cell, leaving behind a trail of differentiated cells. (c) The precision of directional growth improves with diminishing distance between the filament tip and the target. Elongation of the filament ceases if the target is reached

controls the outgrowth of branches does not appear to be the distance from the growing tip, as we see in our routine calculations; instead, branches appear to grow out only after the leaf primordium, initially very narrow, attains a critical breadth. This behaviour is to be expected in a system with regulated net density and is consistent with the model. At a length of about 2.3 mm, the marginal growth in the tobacco leaf halts in favour of intercalary growth. We have made no attempts to simulate intercalary growth; but the self-regulatory property of the model predicts that new branches grow into the enlarging spaces between existing veins in such situations. It is clear that this is exactly what occurs during the growth of leaves. Avery [3] showed that veins are formed from cells of the middle mesophyll in a process that involves both cell divisions and cell differentiation at the tip of the growing vascular strand. One might thus imagine that the activator serves as a signal for increased mitotic activity as well as for cell differentiation. The steep activator gradient in the vicinity of the filament tip could even orientate the cell divisions.

Veins in leaves may reconnect with one other. In the model however, differentiated cells repel a moving activator peak, so that reconnections do not normally occur. In fact, examination of a leaf discloses that reconnections are more the exception than the rule: most of the finer veins end blindly, often turning away from existing veins at the tip. This suggests that, in nature, differentiated cells are indeed repellent, and that reconnections are made by a mechanism which can only occasionally overcome this repulsion. Such a mechanism is available in the model without further elaboration: recall that two growing filament tips which are close together show a strong mutual repulsion, since both produce inhibitor. In contrast, the repulsion of a growing tip by an existing filament results mainly from the depletion of S and this repulsion is weak. A consequence of this is that a growing tip can be deflected by a second tip onto a straight course towards an existing filament. The growing tip will then reconnect; or, if the network is already near its final density and the background inhibition is high, the tip may cease to grow entirely, leaving a blind end.

In the model, strong deflections of this sort are rare during the morphogenesis of the first veins: the midrib and the primary lateral branches. After a network of filaments has been laid down and the leaf switches from marginal to intercalary growth, however, growing tips may arise near to one another on neighbouring filaments. These tips interact strongly and are likely to make reconnections with other older filaments. The model thus suggests an explanation for the fact that the

main lateral branches of a leaf vascular system are straight and non-reconnecting, while the higher order branches may show strong irregular curves and frequent anastomoses. It should be mentioned that this type of reconnection is only possible in a two-dimensional array of cells. In three dimensions, the growing vein has the possibility of circumventing the existing vein by passing over or under. To obtain closed loops in three dimensions, two nets consisting of different cell types, e.g. veins and arteries, are necessary, allowing repulsion between cells of the same type but attraction between cells of different type.

In many leaves, the main lateral branches make an acute angle with the midrib. In other cases the angle is close to 90°. Both behaviours can be reproduced by the model. Branches at 90° are produced when the inhibitory effect of the parent filament is the main factor influencing the direction of growth of the branch; the branch tip seeks to escape from the parent as quickly as possible. Systems of acute angle branches may be formed if the first branch produced by a filament is deflected by the edge of the field; in this case, the first branch will then deflect the second, and so forth (Fig. 6a–e).

The leaf vascular system in higher plants generally shows profuse lateral branching. In lower plants [13] and in the Ginkgo, only dichotomous branching is found. The model suggests a reason why dichotomous branching should be the primitive condition, while lateral branching is “advanced”: dichotomous branching can occur in a simple system involving only three substances (Fig. 3), while four substances are necessary for lateral branching. Thus the model provides a basis for a hypothesis on plant evolution.

Other Types of Network Formation

The fibres of the nervous system, the tracheae [5, 6], or the hyphae of fungi [7, 8] are formed, not by successive accretion of existing cells to the filaments, but by the local extension of small portions of single cells. Network formation by single cells can probably be described by a model broadly similar to the one proposed here, but in which activator peaks are formed within the margin of

the growing filament (perhaps in its membrane) instead of outside the filament. Such marginal activator peaks could then set mechanical forces in motion that produce local growth of the cell. For instance, an activator peak could cause pseudopod extension in a growing nerve [14] or tracheole [5, 6], or cause local cell softening in a fungal hyphae [15]. Filament growth of this sort can be orientated by external gradients if the activator peak is mobile on the cell surface. As we have shown [11], the mobility of an activator peak is greatly increased if the peak repeatedly decays and reforms. Such oscillations occur when the half-life of the activator is shorter than that of the inhibitor. The extension and retraction of pseudopodia during nerve growth [14] could derive from such periodic formation of activator peaks.

A really complete explanation of network development would have to embrace marginal and intercalary growth, the possible involvement of many cell types, and the feedback of the net's function onto its maintenance or decay. Inclusion of these features remains for future work. But the model presented here shows that the interaction of three or four substances is sufficient to account for organized growth, branching and reconnection in network morphogenesis.

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