Branching morphogenesis in a reaction-diffusion model

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I show that a class of reaction-diffusion models of vasculature growth developed in the mid 1970s is in fact a class of dendritic growth models. I then comment on the relevance of these models.

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Models and experiments in branching morphogenesis have been developed independently by different communities. In the context of solid-state science and phase transitions, models of branched growth have been developed, such as the diffusion limited aggregation model (DLA) [1], the phase-field model (PF) [2], and the Stefan problem of a moving boundary with the Gibbs-Thomson condition at the interface that can be solved using different methods [3]. These models, which are inspired by dendritic growth in metallurgy or in crystallogenesis, are all linked to each other in one limit or another. For example, the well-known DLA model of fractal growth is recovered in the limit of vanishingly small capillary length and anisotropy of a model of out-of-equilibrium crystal growth [3]. Many results are now well established, and the regimes of growth of dendrites are well known, especially in metallurgy [3–7].

In Biology, branching patterns are ubiquitous, and they have long fascinated naturalists and scholars [8]. Since branching patterns are one class of morphogenetic processes, it was tempting to apply to these patterns the reaction-diffusion (RD) models that were initially introduced by Turing [9], and that are well known in the contexts of animal fur coatings [10] or sea shell patterns [11]. While Turing patterns are generally stationary in time, with a well-defined wavelength (like zebra stripes or leopard spots), growing structures can also be modeled in long-scale gradients of morphogens. This idea was put forward in the mid 1970s by Meinhardt [12,13], who introduced a class of models of which the following is the canonical example:

$$\partial A/\partial t = cA^2S - \mu A + D_a \Delta A, \tag{1}$$

$$\partial S/\partial t = c_0 - cA^2S - gS - \varepsilon YS + D_s \Delta S, \tag{2}$$

$$\partial Y/\partial t = dA - eY + Y^2/(1+fY^2). \tag{3}$$

In this model, there is an autocatalytic production of a substance A, an activator of A, S, and a field Y. A and S are two diffusible coupled dynamical fields, but Y is a dynamical field that does not diffuse. We will be interested in the pattern of Y concentration. For the growth of elongated filaments of Y, the model requires that the diffusion constant of S is much larger than the diffusion constant of A. The work of Meinhardt was limited to the growth of such patterns [12,13]. However, dendritic patterns can also be obtained.

This is how patterning proceeds. In a medium of finite concentration S that spreads over large distances by simple diffusion, and above a threshold μ/cS, a small excess concentration of A is able to trigger autocatalytically a local peak of A, which propagates in the shape of a traveling wave. If, in the back of the traveling wave, the S concentration reverts to zero, then the A wave returns also to zero, and the wave profile is solitonlike. Y is a two-states dynamical system, a ''one or zero'' switch. Let us call ''a white pixel'' a point where Y is in the 1 value, and ''a black pixel'' otherwise. The Y switch falls from state 0 into state 1 in a zone of finite A concentration; as a consequence, a trail of white pixels follows the traveling wave. Now, the wave speed depends on the level of S, because the rate constant of the autocatalysis is proportional to S. Suppose the S field is not uniform, then, as appears in Eq. (1), A will tend to increase more quickly in the direction of higher values of S (prefactor of A^2). The wave speed is, in a crude approximation, proportional to $S^{1/2}$ [14]. In the back of the wave, the value of S is very small because the term $-\varepsilon YS$ in Eq. (2) removes S. This means that the white-Y pattern is a sink of S, and the magnitude of S, very close to the pattern, is proportional to the gradient of S.

In the end the process is the following: a traveling wave is used as a sensor of a diffusion field, and white pixels are deposited as the wave explores the diffusion field towards higher values. Though this is not rigorous, let us call $Y=0$ the ''liquid'' state, $Y=1$ the ''solid'' state, S the ''temperature,'' then we can state that the presence of a field A on the boundary allows transformation of the liquid into a solid in the direction of a high gradient of temperature, and the solid is maintained at a very low temperature. This is, in summary, analogous to growth in a diffusion field as described in Physics, but with a nonlinear kinetics with exponent $\frac{1}{2}$. It comes as no surprise that it gives branching patterns.

Figure 1 shows an original figure from Meinhardt's work [12]; it seems to bear only a vague resemblance to dendritic growth. Now, we have solved the system numerically on a grid, have explored the parameters, and found with no difficulty dendritic growth. Figure 2 shows the S, A, and Y fields for the parameters listed in the caption. Figures 3(a)–3(c) the A pattern for a set of values of D_a. The zone $Y=0$ is separated from the zone $Y=1$ by a traveling up-and-down wave (front and back) of A that behaves as a surface layer, with dependence of the growth speed on the absolute value of S and also on the local curvature, which is classical in trigger-wave problems [14]. Since, in the original model, the growth speed is proportional to $S^{1/2}$, it comes as no surprise that the wedge shape of the dendrites is more linear than parabolic. Figure 4 shows a dendrite obtained with a modified set of

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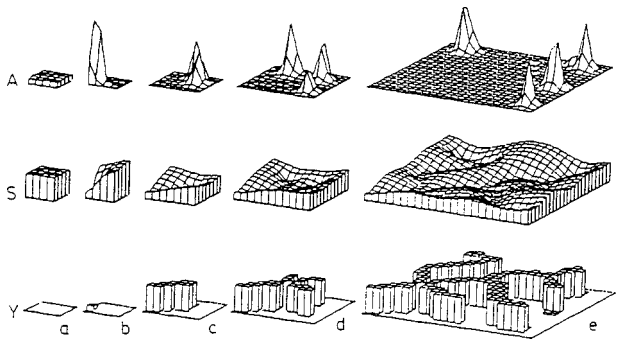

FIG. 1. Branching pattern obtained from a model analogous to Eqs. (1)–(3), reprint from the original work of Meinhardt [Differentiation (Berlin) **6**, 117 (1976), copyright Springer-Verlag] [12]. $c=0.008$; $\mu=0.04$; $D_a=0.0065$; $c_0=0.05$; $g=0$; $\varepsilon=0.25$; $D_s=0.18$; $d=0.000\,32$; $e=0.1$; $f=10$. Meinhardt does not give the system size. As we see the pattern does not really evoke dendritic growth. The nonlinear wave of A is restricted to very small regions of size ''1 pixel'' located right at the tips of the growing filaments. Trails of Y follow these spots of A, which climb up the gradients of S. A pattern evoking leaf venation is obtained. Meinhardt's solution, at his time, was restricted to very small sample sizes (one dot=1 grid point).

equations in which Eq. (1) bears a factor S^2A^2, instead of SA^2. A sharper dendrite is obtained. The fact that the dendrite in the ''S model'' is more space filling than in the ''S^2 model'' can also be ascribed to this exponent, as is known from the study of the DLA model, in its dielectric breakdown (DB) version [3,4].

Among the different patterns that can be obtained with this model, one finds the celebrated ''doublon'' morphology

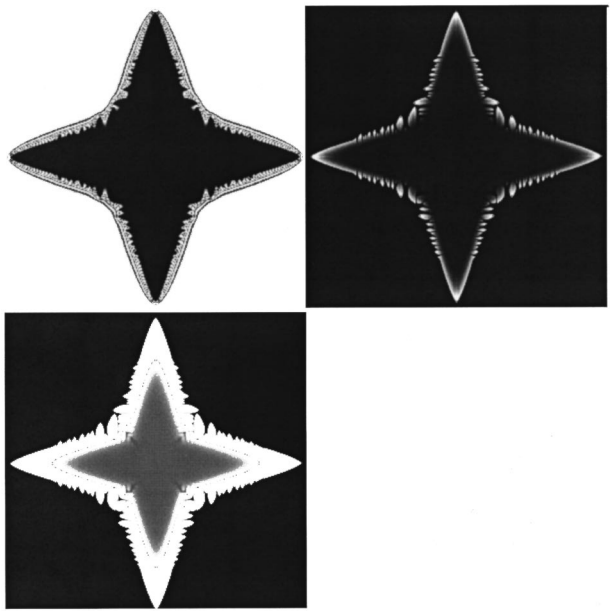

FIG. 2. A large dendritic pattern obtained, with the following set of parameters: $c=0.5$; $\mu=0.04$; $D_a=0.005$; $c_0=0$; $g=0$; $\varepsilon=0.2$; $D_s=0.5$; $d=0.1$; $e=0.1$; $f=9$. The grid size is 300×300, $dx=1$, and $dt=0.005$; the number of iterations is ca. 100 000. The initial value of S is 15, the initial values of A and Y are 0 everywhere except on a small cross of size 4, where the value of A is 0.1, and the value of Y is 1. Side branching occurs spontaneously, from numerical noise.

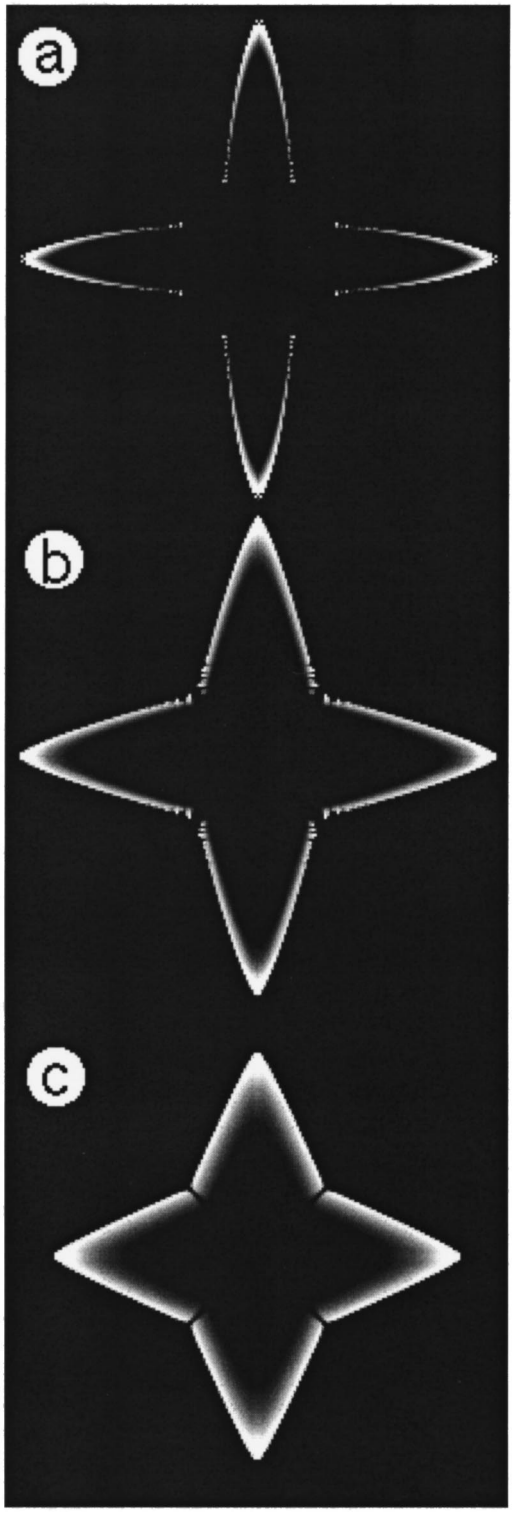

FIG. 3. Patterns of A for the same set of parameters as in Fig. 2, except for the size (200×200) and the diffusion constant of A: (a) 0.002; (b) 0.005; (c) 0.01.

[6], which is composed of twin tips growing together (Fig. 5). Finally, by introducing noise in the system, one can destroy the anisotropic dendrite and produce a transition to tip-splitting morphologies (Fig. 6) [15]. A more detailed study of this system will be presented in a forthcoming publication [16].

A number of straightforward remarks and consequences

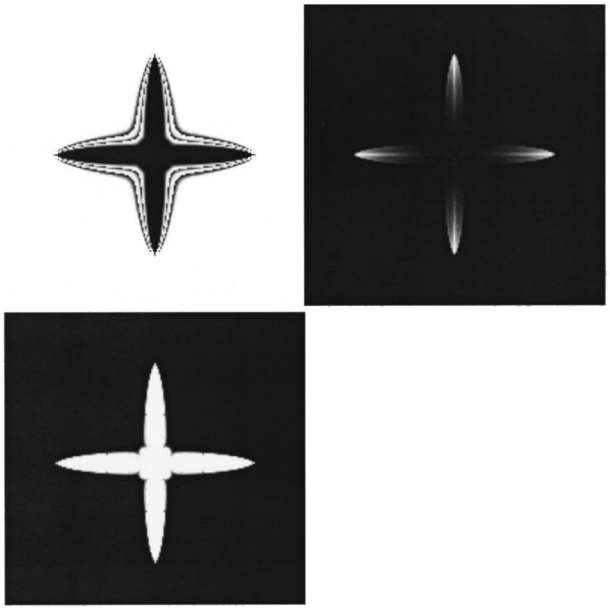

FIG. 4. Dendritic pattern obtained with the ''S^2-model.'' The growth speed is then proportional to S. This situation is closer to dendritic growth in the Stefan problem, and it gives rise to much sharper dendrites than in the ''S model.'' This is linked to the nonlinear autocatalytic term in Eq. (1) (see text).

are worth explaining here. First, the two states of Y do not correspond to a thermodynamic phase transition. Indeed, in the classical Ginzburg-Landau two-well system that appears, for example, in the phase-field formalism [2,17], the phase

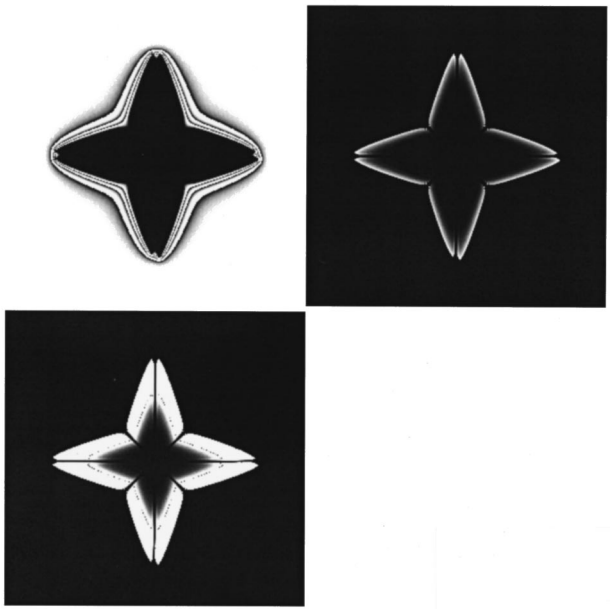

FIG. 5. A doublon dendrite, obtained in the ''S model,'' with the following parameters: $c=0.1$; $\mu=0.04$; $D_a=0.0001$; $c_0=0$; $g=0$; $\varepsilon=0.2$; $D_s=0.1$; $d=0.1$; $e=0.1$; $f=9$. The grid size is 300×300, $dx=0.3$, and $dt=0.0006$; the number of iterations is ca. 100 000. The initial value of S is 15. To produce a doublon it is necessary to start from a little circle of nonzero A and Y, instead of a little cross. So, the initial values of A and Y are 0 everywhere except on a small circle of size 4, where the value of A is 0.1, and the value of Y is 1.

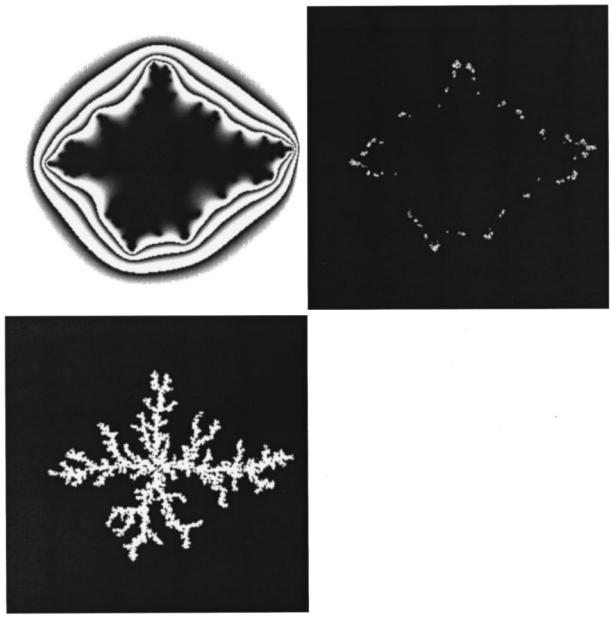

FIG. 6. (a)–(c) A branching pattern obtained with an additional 150% noise on the diffusion of A. With such a noise, the anisotropy of the lattice is overwhelmed, and a tip-splitting pattern emerges (note that in the ''S model,'' the kinetics is not DLA-like but closer to a dielectric breakdown [4] with an η coefficient equal to $\frac{1}{2}$). The parameters are $c=0.1$; $\mu=0.04$; $D_a=0.002$; $c_0=0$; $g=0$; $\varepsilon=0.2$; $D_s=0.5$; $d=0.1$; $e=0.1$; $f=9$. The initial value of S is 5. The grid size is 300×300, $dx=1.0$, and $dt=0.03$, the number of iterations is 140 000.

$Y=1$ and the phase $Y=0$ are one equilibrium phase and one metastable phase. Across the boundary, when going from the liquid towards the solid, the ''atoms'' fall from one metastable well into the stable well. In the case presented here, the two phases are both equilibrium phases; however, across the interface, the presence of an A peak locally raises one well. Hence the stable $Y=0$ phase is destablized locally at the interface and turns into another *stable* state when crossing the boundary, by crossing only a transient unstable region. It is the interface only that is actually out of equilibrium, and follows the gradients of S.

We should underline that the model presented here allows modeling of a three-media system, composed of an inner dendrite, an outer driving field, and a surface skin represented by the A field. In this respect the model is distinct from PF, because a specific field (A) carries the surface properties. By adjusting the parameters of the A field, one can reproduce specific features of the interface.

As the simulations show, in this model, the dendritic morphology is favored by the lattice anisotropy (an artifact introduced by the use of a discretized numerical scheme); this is the analog of a well-known effect in dendritic growth [17,18]. This anisotropy induces a transition from dichotomous branching (''tip splitting,'' in physics) to side branching (''budding'' or ''monochotomy'' in biology), in a model of growth that, in its continuous mathematical form, does not contain preferred growth directions. Therefore, some conclusions derived by Meinhardt [12] are questionable. This author obtained dichotomous branching in his model because the initial seed for the growth was put at 45° of the lattice, and because noise was introduced in the system. But the

model, in fact, is not restricted to dichotomous growth. It may induce a regular ''venation'' if an anisotropy is incorporated. Meinhardt has invoked an additional set of equations (action of an inhibitor) in the model, in order to produce straight filaments with side branches, but as shown here, this is not truly necessary. Also, the fact that the pattern is space filling in Ref. [12] can be ascribed to the presence of a source term $c_0 \neq 0$, and to the fact that the exponent of the surface kinetics is smaller than 1.

Now we turn to the relevance of this sort of model for vasculature growth. We believe it very unlikely that this model explains the large scale structure of the venation in the animal realm for the following reasons.

First, the filamentary structures, either in this model or in the more complex version with inhibitors, are only obtained in the limit of very small ''capillary length,'' a rather special case. Also, one finds hardly a continuity of functioning branching patterns in these models when varying the parameters. More specifically, there is, to our knowledge, no evidence of the existence of any taxon or mutant having a ''dendriticlike'' vasculature *in lieu* of the usual filamentary (possible regular) vasculature. There neither exists, to our knowledge, any obvious evolutionary continuity between nonvascularized and vascularized tissues that would give a hint of how the vascular trees could have evolved from a reaction-diffusion growth process of this sort, which requires a rather complex relationship between different equations and the corresponding parameters.

Second, though this may read surprising at first glance, vascular systems do not form by a mechanism of ''growth.'' A model exhibiting the growth of a branching tree growing like the ones shown in Figs. 1–4 simply does not correspond to the reality of the growth of any vascular tree in animals. The vascular tree in animals forms in two stages. First a ''primary plexus'' composed of very thin capillaries forms. These capillaries appear by percolation of small blood islands (angioblasts) that are generated randomly [19]. After enough blood islands have appeared, a primary array of these smallest vessels is formed (the ''plexus''), which consists of a mass of very small capillaries embedded in the tissue to vascularize. Most generally, these blood islands that give rise to the first segments of capillaries, form locally, at random spots, in a process akin to bond percolation, and do not actually ''grow'' across the tissue. In some instances, there does exist growth of capillaries emerging from existing vessels (a mechanism called ''sprouting'') that contributes to the formation of a random plexus, in a process which, in this instance, is akin to percolation in a gradient. Percolation without sprouting is general in embryogenesis, while sprouting occurs more favorable in adult animals (in a wound healing, for example). There is no sprouting at all, for example, in the formation of the arterial tree of the chick embryo [20].

As long as the capillary plexus does not percolate, there is no flow and no branching vascular network. Once percolation is possible, and flow occurs, the vascular tree will form by replacement of a subset of the small vessels by larger vessels (the so-called ''pruning'' of the plexus, or ''maturation'') in a mechanism that we now describe.

It is well known, from the histological point of view, that vasculatures are made of tubular segments of varying diameter, such as main vessels, secondary, and tertiary vessels, etc. down to the venules and arterioles, and further down to the capillaries. In the models presented in this article, one is forced to suppose that the segments of the vasculature adopt different diameters in a secondary process. However, increasing experimental [19–24] and theoretical [21] evidence shows that the *flow* in the vasculature is the essential feature of the formation of the large scale features of the vascular system. If flow is interrupted in a vessel, the vessel regresses, when anastomosis is formed, small vessels enlarge in regions of higher flux [19–21], etc. If one invokes the known feedback of the flow, first on the capillaries and, later on, on the vessels, then one finds a simple and attractive way of forming a vascular system that allows both formation of the branching vascular tree in one given being (ontogeny), and progressive, continuous, evolution of the vascular system among different species (phylogeny).

At the start, in a very primitive being, or in the early embryo, the body is spanned by the random array of holes or of small segments of vessels (the capillaries). The body is ''porous,'' so to speak, and fluid flow is possible either spontaneously or by motile contractions of the body (as in small existing primitive invertebrates [25]). Then, as evolution proceeds, or as development moves forward, a feedback of the fluid circulation acts on the pore structure, enlarging the lumen wherever the shear stress is large. It is also a well-established fact that very primitive cells have shear sensors on their membranes. Then, as the loop (shear sensing $\Rightarrow$enlargement of the vessel) becomes better and better, a more sophisticated branching pattern will be formed, which will be more and more robust as the evolution proceeds, and the size of each vessel will depend on the local flux. Still, at every geological time, or at each stage of the embryogenesis, a functioning vasculature will be present. We have shown [21] that the progressive replacement of the small capillaries by the action of shear flow on the endothelium is indeed identical to dendritic growth, but in its dielectric breakdown (DB) version [4], across a lattice of small tubes, some of which become eventually larger vessels.

In the end, sensitivity to shear stress explains both the formation of the vascular tree after formation of the primary plexus, *and* the fact that the branches of the vascular tree have a varying diameter. The segments of vessel that form the vasculature do not actually ''grow,'' they were already there, in the primary plexus. The formation of the tree across the capillaries is more a *selection* phenomenon, than a *growth* phenomenon.

In summary, we have shown that a class of RD models, imagined long before DLA, DB, or PF models by Meinhardt [12,13] is in fact very close to dendritic growth. The simplest of all models, on which all other models are built, is *almost identical* to dendritic growth, at least in the limit of small capillary length. However, while the process proposed in these models might be relevant for the formation of leaf venation or insect trachea, it is certainly irrelevant for true animal vasculature maturation into a tree because (1) the primary plexus forms generally by bond percolation, (2) the maturation into a tree is not actually a growth process, (3) the reaction-diffusion model does not take into account the fluid transport function, which is the very reason for vasculature maturation into a tree, and (4) the size of the vascular tree is too large.

The role of the long-range morphogen is played by pressure (via the shear stress), and it makes diffusible molecules unnecessary. Even if a diffusion limited aggregation (or a dielectric breakdown) model of maturation based on pressure and shear, and a reaction-diffusion model of tree growth are formally very close, it makes a huge difference from the point of view of biomechanics and of robustness of morphogenesis to process locally the information transported by the flow, or to secrete long-range diffusible morphogens. Indeed, Turing models require construction and control of several coupled fields, while mechanical fields, like the pressure field, are always present. If nature uses the pressure field as ''morphogen'' instead of a self-produced diffusible field, the morphogenetic process is much more economical, simple, and prone to appear spontaneously.

Several intriguing questions are raised by this work. First, since the branching model presented by Meinhardt is a development of Turing's RD morphogenesis, and since the actual vasculature maturation process is in fact linked to mechanical stress, could it be possible, in return, that such structures as leopards spots, zebra stripes, and the like may be produced by mechanical stresses playing the role of the long-range inhibitor, and not a diffusible morphogen? In that case, the spots' pattern would be akin to a ''strain figure,'' classic in mechanics experiments, and not to a ''Turing patter.'' A mechanism of this sort has already been proposed for chondrogenic condensations [26].

The second question lies in the apparent coincidence of vegetal and animal vasculature, at the present stage of evolution. Could the receptors of shear stress be the same, and the vegetal and animal vasculatures have the same phylogeny? Finally, the class of models proposed by Meinhardt provides an interesting alternative to PF models, which may help in understanding pattern formation in nonthermodynamical contexts, where dendriticlike structures are formed. One such context may be meristem growth [27].

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