CHAPTER 12

Physical Mechanisms of Branching Morphogenesis in Animals:

From Viscous Fingering to Cartilage Rings

Vincent Fleury, Tomoko Watanabe, Thi-Hanh Nguyen, Mathieu Unbekandt, David Warburton, Marcus Dejmek, Minh Binh Nguyen, Anke Lindner and Laurent Schwartz

Introduction*

From a physicist's point of view, and regardless of the genetic controls, the branching mechanisms of many organs and glands look similar. Most generally, an epithelium forms a pouch-like sheet which elongates and branches repeatedly. During the final steps of organogenesis, the mesenchyme is vascularized in a pattern greatly influenced by the branched epithelium so that main vessels go down (arteries) and up (veins) the main ducts towards distal branches where exchange with capillaries is performed over a very large total surface area. This principle of construction can produce a secretory or filtering or breathing organ and most glands and organs are built in this way. There is either a common phylogeny to all branching organs (see Chapter 1), or there is some simple building principle which implies easy construction and hence straightforward evolutionary convergence (Fig. 1).

In this chapter, we shall not consider how the vasculature connects to the branches; during the early stages of branching morphogenesis a full vasculature is absent in any case, although capillaries may exist. Vascular development is described in Chapter 6. We shall instead concentrate on epithelial ducts and will describe of how an organ develops in terms of a moving boundary between the pouch-like epithelium and the mesenchyme. In the introduction, we shall first present general principles of moving boundary problems and show why this description applies to branching morphogenesis. Then, after describing the simplest system of all, the penetration of one fluid into another (a phenomenon called viscous fingering), we shall go towards more complex systems including the invasion of one non-Newtonian fluid by another, the self-similar extension of membranes and the elasto-plastic growth of ducts and T-shaped ampullae. We will stress the fact that a peculiar region exists at the tips of the ducts, called the apical region, and discuss how it may form and how it finds its way in a physical or chemical "field". In a last part we will discuss briefly features which are linked to the directional nature of biological tissue. At the end of the chapter, we will reconcile physics and biology by listing all the genetic elements which a physical model requires, before being able to predict anything. At each step, we shall try to remain as close as possible to specific experimental examples or situations.

There are 23 generations of branching in a human lung. Attempts have been made to reconstruct the geometry of organs by directly measuring parameters such as branching angles,

* A glossary for this chapter begins on page 230.

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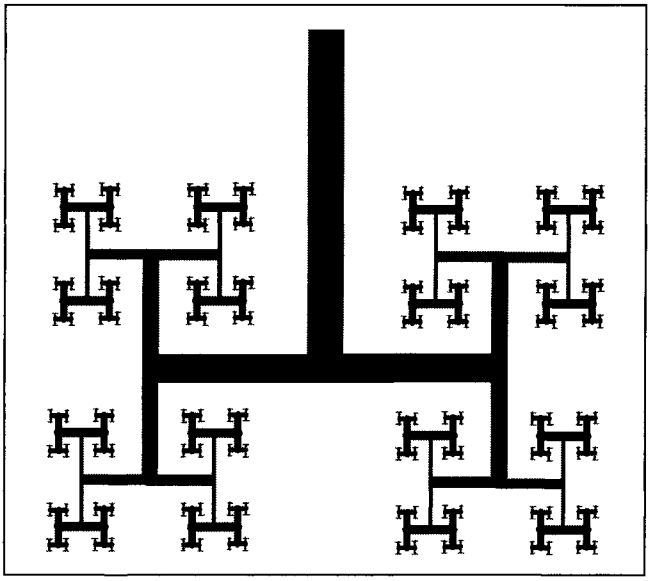

Figure 1. A fractal interative model of 'lung', inspired by a model by Mandelbrot.[2]

length and diameter ratios, etc. Although such numbers can be statistically significant, when one tries to actually re-build an organ by implementing the same rules iteratively, one fails to form a plausible organ, except for visual impressions. This is because, although a few branching events may be performed by simplistic iterative means, inspiring (or inspired by) fractal geometry, the reiteration of the rules generally leads to aberrant crossing of tubes and anatomically impossible geometries.

On another hand even if one does implement successfully a set of iterative rules to form animage of a plausible organ with many iterations, the rule cannot accomodate any anatomical modification such as the presence of a big vessel, of a bone, or of the heart, or the gentle compression by the organ capsule, by surrounding fascia or even the neighbouring organs. In order to be able to construct a plausible organ, on has to invoke many additional ad hoc rules (nine in this case).

Actually, the formation of branching organs, such as the kidney (see Chapter 8), shows that the branching events are very plastic, depending "on the context". By this it is meant that a growing tubule may experience dichotomous growth, budding, stopping, left or right turns, depending on the exact geometrical and physical situation which surrounds it; there is not a single outcome to the forward push of an epithelium. Branching may be spontaneous, or be caused by collision with the capsule, or by head-on collision of branches against each other, etc. The possibility of local adaptations is essential to the generation of such complex structures as organs, but at the scale of one actively growing duct, these adaptations are not trivial. Although an incredible number of branches form, the entire organ (e.g., kidney) keeps a bean-like shape gently surrounded by its capsule, and almost uniformly filled with branches. Therefore, although genetics may provide a set of tools for making branches iteratively, the precise positioning of branches is very dependent on the entire history and geometry of the growth, and on the spatial distribution of "fields" (diffusion, elastic, hydrodynamical...) around a given growing duct. The first few divisions may well seem very stereotypic, but reproducibility is lost after a few branching events, such that distal parts are more and more different between individuals, including identical twins, a classical observation, common to all branching organs and to the vasculature. In effect, the entire geometry and physical field around the branching pattern

provides an epigenetic context which will influence the exact pattern. Still, organs are produced with some constants: general shape of kidneys, lungs or livers, regular septa formation, sometimes perfect angular divisions of tubes, and even some stereotypy such as frequent 90° rotation of subsequent divisions, as in the kidney or lung. Later on, the vascularization process shows again enough plasticity to accomodate all the micro-events which have lead to one given organ growth. How all this is physically possible is the object of this chapter, which can be regarded as a discussion of what epigenetic cues actually are.

Moving Boundary Problems

The growth of a branched organ is characterized by a soft epithelium/basement membrane/mesenchyme interface which expands and forms a tree. This is most easily seen in GFP mice (Fig. 2).[3]

For a physicist, there exists a medium A (inside the lumen), which pushes a medium B (the mesenchyme), and the interface I between the two (epithelium+basement membrane) moves (Fig. 3).

There are many physical displacements and deformations during this process. The process by which medium A pushes towards medium B, with an interface whose growth speed depends on the physical and chemical content inside B, is known in physics as a 'moving boundary

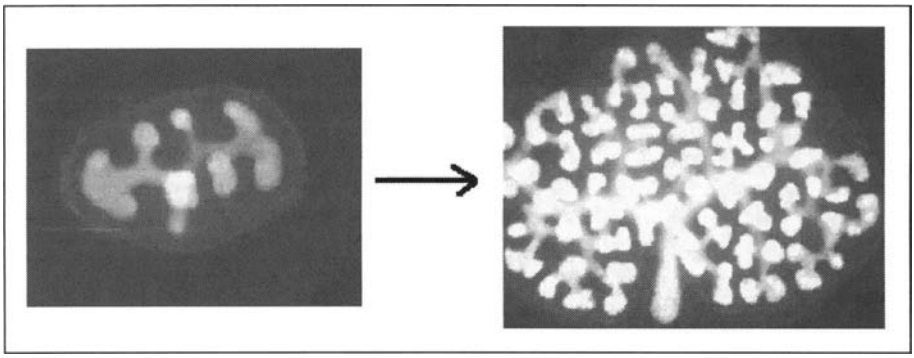

Figure 2. Optical microscope observation of a kidney growth of a GFP mouse, showing the epithelium develoment during the first two days.

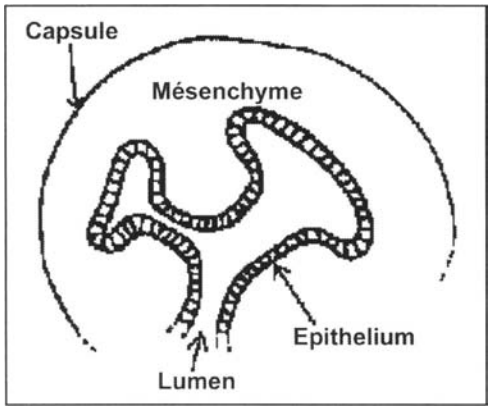

Figure 3. A simplistic view of the geometry of the problem.

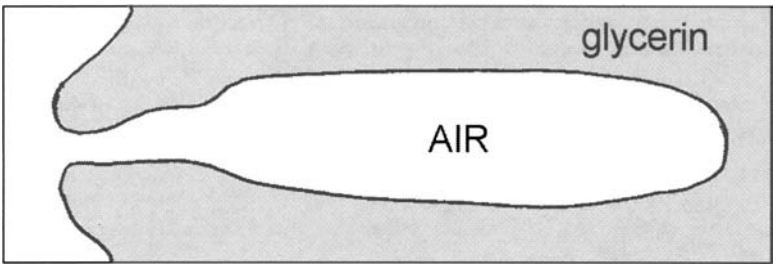

Figure 4. Drawing of a viscous finger in a channel. The white "bubble" is an area of air, pushing a viscous fluid (glycerin, in this experiment).[5]

problem', or 'free boundary problem'.[4] The simplest of all moving boundary problems is known as viscous fingering, and it shares several similarities with organ growth. Viscous fingering is the process by which a given fluid penetrates into a more viscous one.[5,6] It has been known for about three hundred years that, in a thin-cell apparatus (2 dimensions), the interface between such two liquids is unstable. In a small channel, such an interface will take the shape of a pouch or bubble, which is called a "viscous finger" (Fig. 4).

This shape is very reminiscent of a lung or kidney bud. In a large open medium (which is rarely the case in biology, all organs coming with a surrounding capsule), viscous fingers are unstable, and branch repeatedly to form very complex, self-organized trees. The interface may take the form of a self-similar tree (Fig. 5).

The equations of this process were derived by Saffman and Taylor,[5] who discovered the fingering solutions which occur in a channel in a thin-cell apparatus. Many similar duct-like solutions were found after them.[7-11]

The equations are:

$$\partial^2 P/\partial^2 x + \partial^2 P/\partial^2 y = 0 \qquad (1)$$

The Laplace equation shown above (where x and y are spatial coordinates) is just the conservation of flux of a non-compressible viscous fluid in a 2D thin cell. In addition there are boundary conditions.

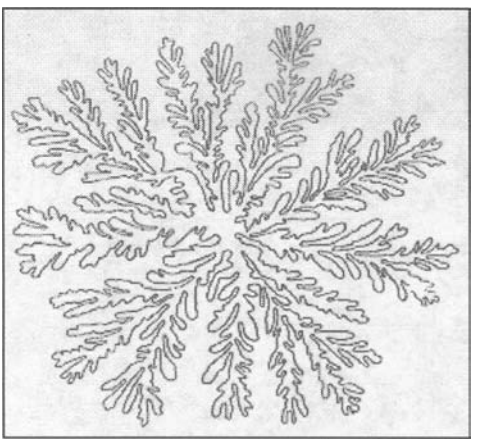

Figure 5. In an infinite isotropic medium, a bubble between air and oil spontaneously develops itself into a very complex branching pattern (courtesy of Yves Couder).

$P = P_1$ inside (some high pressure inside the lumen)
$P = P_2$ on the capsule or in the surrounding medium (some low pressure far away)

$$P_{exterior} - P_{interior} = \gamma/R \text{ at the interface} \quad (2)$$

(γ is the surface tension, R the radius of curvature)
zero flux on the boundaries (walls of a channel, for example)

And an additional equation for how the interface moves, generally called 'the kinetics' of the interface, is

$$v = -k\,\mathrm{grad}P \quad (3)$$

(speed proportional to pressure gradient along the interface).

More recently, it has been shown that repeated viscous fingering and branching indeed leads to the structures known as "dendrites", as observed by Scheuchzer[12,13] three hundred years ago (Fig. 6).

The origin of the fingering and branching processes is rooted in the Mullins-Sekerka instability. This instability is described as follows. First, we have a pressure field outside the pattern, in a viscous fluid such as oil, that satisfies the Laplace equation because of fluid incompressibility and the thin-cell geometry.[5] Then, there is the other fluid, such as air, pushing. The kinetics of

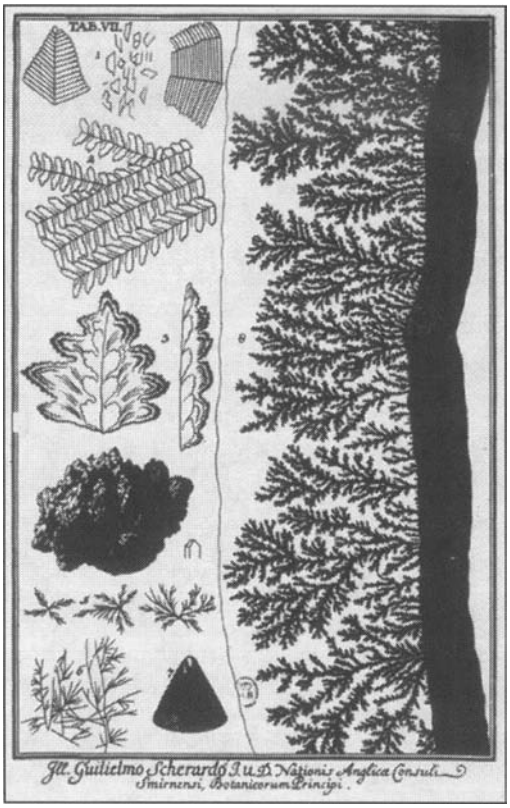

Figure 6. The branching of viscous fingers which leads to self-similar dendrites was already observed and described three hundred years ago.

the interface are very simple: by definition the interface is the last fluid surface, before the air; and it is well known that it moves (in a thin cell) with a speed just proportional to the pressure gradient (this is identical to the flow of water in pipes: the speed of a cylinder of water passing in a pipe is directly proportional to the pressure difference between the pressures at either ends of the water). The mathematics of the Laplace equation have the following consequence: as soon as a protrusion bulges forward, this protrusion encounters a sharper gradient of pressure, so it tends to grow faster and to increase in size. Similar instabilities arise with gradients of diffusible molecules, instead of a pressure gradient,[14,15] and also in electric fields. This is known in physics as the point effect, and it has the same origin as the attraction of lightning towards lightning rods (sharp protrusions tend to generate higher fluxes). The same point effect is observed in the growth of bacteria colonies in Petri dishes: since they grow quicker in the directions of high fluxes of nutrients, and since they use up the nutrient, they self-organize in a branching pattern which has elongated "pseudopods", "processes" or simply "dendrites".[16]

This sort of growth has been identified in many areas of research: metallurgy, electrochemistry, bacteria growth, fungi growth, clay or plaster erosion, vessel formation, etc.[14] It is generally called Diffusion Limited growth, after the seminal work of Witten and Sander.[18]

Intuitively, the reason why the interface is unstable is the following: in order to grow forward, the interface needs to push the medium B facing it. However, the more of B it has to push, the slower the speed, as for water flowing through pipes. When a bulge starts forming, it has less B to push, and it leaves more of B to its sides. Then, the bulge can grow faster, while its sides grow more slowly, and the instability generates a bulge exponentially. However, capillarity tends to limit the curvature of the interface. Capillary forces are tensions which are developed in a fluid interface as a direct consequence of **making** such a piece of interface. The "interfacial" energy per element of surface area dA of the interface is γdA; this interfacial energy is just the binding energy of the "molecules" or "elements" of the interface. In a simple 2D picture, it is exactly equal to the work of the capillary force or tension γ per unit length ($\gamma dx\ dy = \gamma dA$) along the contour. By definition, the tension exerts itself tangentially in the interface, just as if the surface were a piece of elastic. The equilibrium of the pressure inside, the pressure outside, and the capillary force along a curved contour, gives a relationship between the pressure drop across the interface, the radius of curvature and the interfacial energy:

$$P_{ext} - P_{int} = \gamma/R$$

Consider a flat or slightly curved interface. In order to bulge out, an interface not only has to push the material ahead, but it has to bend the interface more. Suppose a pressure P_{int} (with respect to the external pressure far away) is used to push **and** bend the interface. Part of this pressure is dedicated to bending the interface, and only the remaining fraction of this pressure is left to serve the purpose of pushing the material ahead. The pressure drop across an interface is all the bigger as it is more curved. So, bending the interface requires sacrificing a fraction of the available pressure, and this sacrificed fraction is all the bigger when the growing bud is sharper. So, an interface which "wants" to bulge out has to make a compromise between making a sharp bud, and being able to push ahead the rest of the material. The compromise between the two gives a width criterion for the size of the "fingers" of A penetrating into B: in an **infinite** thin cell, where the interface would be completely free to develop, the typical width of "a duct" would be

$$L = 1/12[b^2\gamma/\mu V]^{1/2}$$

where b is the cell thickness, γ the surface tension, V the speed of growth, and μ the viscosity. This width is obtained by what is known as a linear stability analysis, a technical tool very much used in the field of moving boundary problems.[4] It corresponds to a ratio between viscous forces and capillary forces. This is the size of a bulge strong enough to push the viscous fluid: smaller bulges cannot push at all, and larger bulges rapidly break off into smaller bulges which have this size.

So the shape of the growing "duct" is basically related to the growth speed, the surface tension and material parameters of the fluids. There is no actual "sensing" of the size: the size is

only the consequence of some physical dynamics, with given parameters, which themselves are genetically determined. It is, of course, extremely difficult to imagine a way of measuring such parameters in vivo, in a developping human embryo, and to our knowledge, such measurements have never been performed.

If the thin-cell apparatus is lined by **walls** (and we may think of the capsule of an organ as forming such a wall), but infinite in the forward direction, the finger occupies **one half** of the channel. This comes from a subtle stabilizing effect of the walls,[19] the duct is lead naturally towards the middle between the two walls, where it grows stably.

To return to organ growth: **IF** the push of the epithelium were a simple pressure driven push towards the mesenchyme, **IF** the mesenchyme and the fluid were simple viscous fluids with a simple surface tension at the interface and **IF** the capsule were very flat, and infinite and narrow, then a single lung or kidney duct would look like Figure 4, and be described by the model given above (Equs. 1-3). In this spirit, the growth of a duct is almost entirely epigenetic, and quite easy: all pouches which grow by osmotic push should be able to generate finger-like structures.

Although a single field does the job, we can conceptually divide it into two contributions (this distinction will be needed in what follows): first, the duct has a specialized "apical" region where growth is promoted—or made easier—by the presence of a higher field. This apical region is not maintained by a specific cellular bio-chemistry but is the result of the self-organization of the field. Second, the tip of the duct either bifurcates or is driven gently towards the center of a channel, not by cellular bio-chemistry but, again, by the fields around the duct, which self-organize in such a way as to generate either stable ducts, or branching patterns, depending on boundary conditions. So this is the simplest example of a completely physical process for branching morphogenesis, it may have existed in very primitive organisms, and it is observed in some simple biological contexts.

However, the reality is not so simple, in that not only the epithelium but the mesenchyme also grows. The capsule is not infinite and itself expands. The tissue does not behave like a simple fluid, it has an elastic component and it adapts by growth and cell reorganization on a longer time scale, in a plastic way. Also, the problem is not a simple 2D problem in a thin cell, it is more of a 3D problem. Finally, the living tissue is made of several cell types, and they tend to form an orientational order. This is especially true of the fibroblasts in the mesenchyme, which are most generally described as elongated cells which lay down collagen in an orderly fashion.[20-22]

This is apparent in the final shape of lungs, for example, which exhibit a very conspicuous orientational structure, known as cartilage rings, which obviously play a role in the mechanical equilibrium of the tubes.[22]

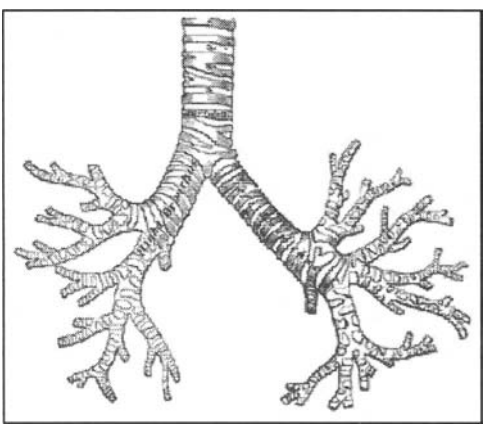

Figure 7. Classical picture of cartilage rings in a lung.

No model is at present able to incorporate in a comprehensive way all the features mentioned in this list. However, for each of these facts, a partial description can be reached.

3D Aspect

In three dimensions, an interface between fluids is much less unstable, because the viscous force acting on the sides of a bulge and preventing the rest of the interface catching up is smaller. In simple terms, the drag on a 3D cylinder is much less than the drag between two plates, as in Figure 4. When, say, air is pushed into a viscous material in a 3D cylinder, a finger of air propagates leaving part of the viscous material on the wall. The air bubble, pouch or finger occupies approximately 90% of the width. In other words, the apical region is wider in 3D than in 2D, if this active region is determined by self-organization of fluid streamlines. (Note that in many cases, the capsule of organs has some sort of a lenticular shape, which is somewhere in between a 2D and a 3D geometry, an additional difficulty). An experimental demonstration is shown in Figure 8.

It resembles the penetration of epithelium into mesenchyme. It is interesting to note that, eventually, there remains only 10% of supporting material around such a "duct". This figure is similar to the one observed, for example, in a lung, whose supporting material occupies approximately 10% of the volume.[25]

However, in 3D the interface between fluids is much more stable and, although some kind of "finger" forms, it tends not to tip-split dichotomously spontaneously. So, it is unlikely that branching proceeds via a Mullins-Sekerka-type of instability, in 3D, unless this instability is driven by a field of **diffusible molecules**, instead of a pressure field. (The deep root of this 3D instability comes from the fact that the diffusion equation is the same in 2D and 3D, and

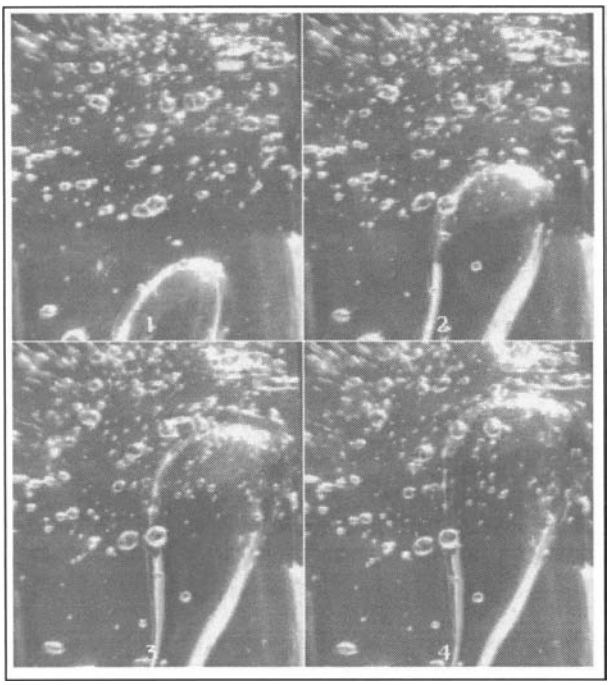

Figure 8. A viscous finger, in 3D. The experiment is much more difficult to perform, because of gravity, and of bubbles which get trapped in the gel during preparation.

generates a point effect in all geometries. The viscous fingering equations are not the same in 2D and in 3D; they amount to a **diffusion equation**—Equ. 1—only in 2D; i.e. in 3D the point effect is much weaker than in 2D).

Finite Capsule

The capsule is a finite bag surrounding the mesenchyme forming the boundary of the organ. It is not always present in in vitro experiments, but in some of these instances the surrounding gel or the mesenchyme-medium interface may act as a virtual boundary, with similar conclusions as explained here. As the kidney duct (for example) extends, it forms very conspicuous T-shaped patterns, upon collision with the limit of the tissue (Fig. 9). But, as stated before, it may aswell simply make a right turn (Fig. 10).

It would be daring to suggest a very complex position information in the form of diffusible molecules, which would lead to such a T-shaped dichotomy, or to just a turn. The T-shape is very common in physics of fluids, including fingering experiments, when an air bubble or pouch collides with a "wall". This is a mechanism of branching which is different from the Mullins-Sekerka instability. Figure 11 shows a typical numerical simulation of this kind of event.[26] It describes a bubble rising through a sink and "hitting" the ceiling.

It suggests that the T-shape dichotomous events are driven by the mechanics of the collision of the epithelium-basement membrane interface against the capsule, or the surrounding culture medium. That the epithelium should not succeed in reaching the boundary itself is a straightforward consequence of the visco-elastic nature of the mesenchyme. The important issue in this last sentence is that the distance of "collision" is linked to a material property of the tissue, and not to a distance fixed by some threshold in a gradient of molecules. In this view, the cells shape the ducts by participating in the dynamical process which generates them and by modifying the set of physical parameters. If there are specific genetically-regulated pathways for a shape, it is a matter of how the physical parameters of the problem are modified by biochemistry, in addition to possible inhibition/activation pathways.

In many instances, the T-shaped dichotomous event is the result of a collision between ducts that grow towards each other, instead of towards the limit or boundary surrounding the tissue (Fig. 12). The concomitant deformation of the ducts is straightforward in this sort of models (symmetry of boundary conditions).

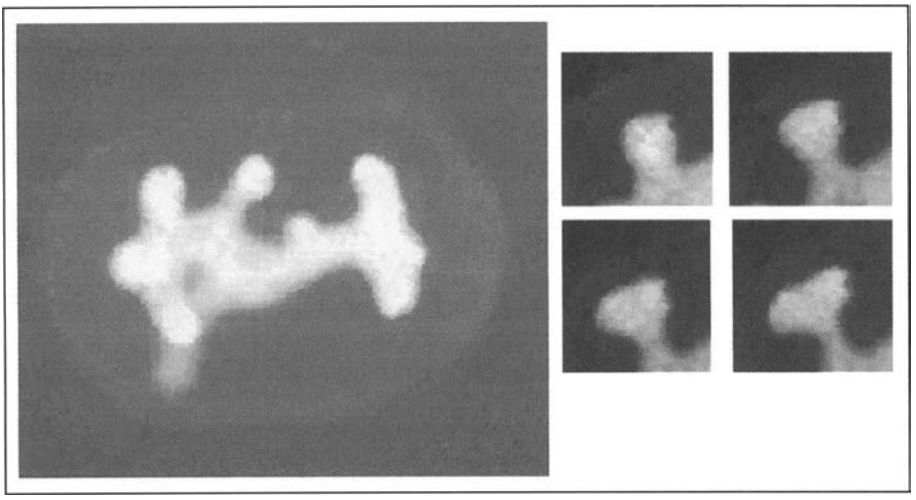

Figure 9. "Collision" of the ducts against the capsule induces a T-shape dichotomy.

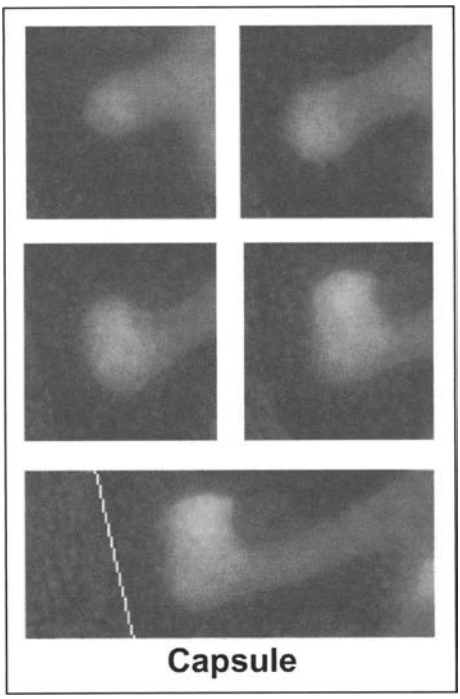

Figure 10. Close view of a right turn of a duct which gets close to the boundary and makes a right turn.

There is not one specific pathway for head-on collision and one for collision on the capsule. Many seemingly different events are just the same physics with different boundary conditions.

Non-Newtonian Fluids

Viscous fingering instability, and the formation of "ducts" or "fingers" between two immiscible fluids has been described mainly for Newtonian fluids inside a channel geometry. Newtonian fluids have a uniform constant viscosity; for example, flow of Newtonian fluids in pipes is directly proportional to gradients of pressure. It is very unlikely that the mesenchyme should be such a fluid, because the dry weight of mesenchyme is 50% collagen (in the adult). It is well known that gelatin or collagen suspensions in high concentrations have shear-thinning (or "pseudo-plastic") properties: they flow more easily under shear stress, and

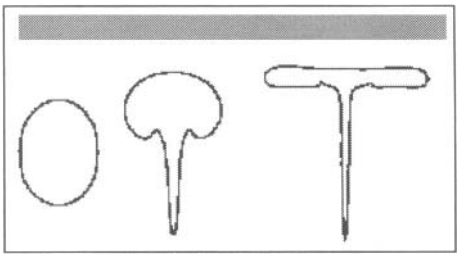

Figure 11. Collision of a rising bubble against a "ceiling" (after Pozrikidis[26]). The bubble never touches the "ceiling". Note that this image has cylindrical symmetry.

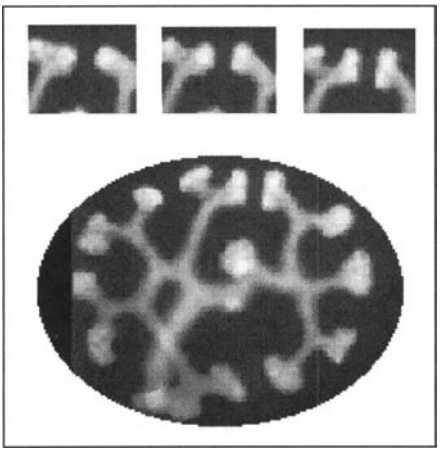

Figure 12. T-shaped dichotomies are also observed when ducts "collide" head on.

they exhibit a threshold. This means that below a certain threshold of shear, called the yield stress, the material behaves like a solid gel, although quite elastic (Fig. 13). Above that threshold, the material behaves like a fluid and flows all the more easily as it is more sheared. It has long been known that viscous fingers also form in shear-thinning materials, with finger widths much smaller than in Newtonian fluids.[27] Recent work has shown that the Mullins-Sekerka instability also exists for non-Newtonian fluids.[28,29] In this case, the width criterion is different, although it is still obtained by writing the ratio of capillary vs viscous forces :

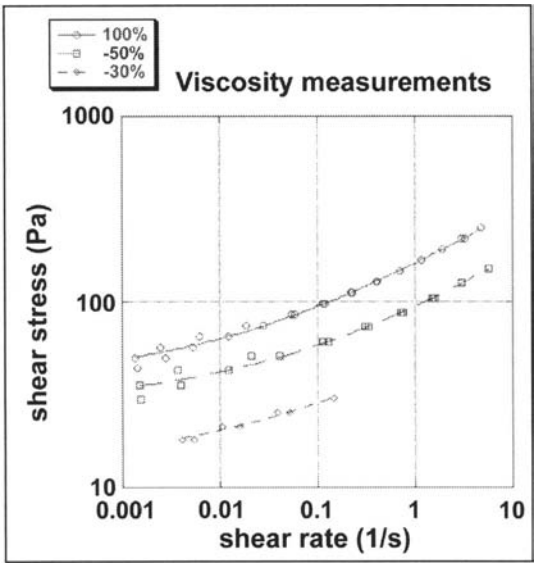

Figure 13. Top. Typical physical behaviour of gel-like materials. Below some threshold, they behave like elastic solids : the shear *rate* is zero, although there is stress. Above a threshold, they flow, and the shear rate increases with stress. As expected, the threshold (yield stress) depends on gel dilution. The more diluted the collagen, the lower the threshold.

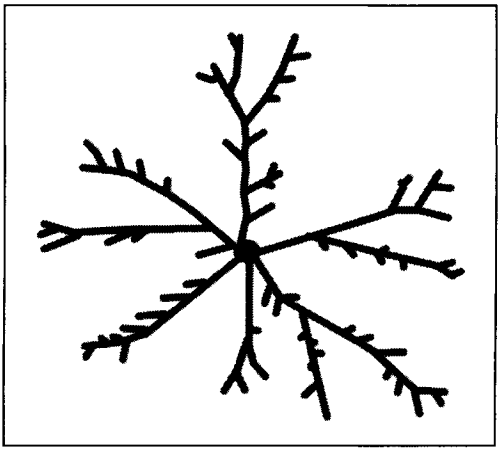

Figure 14. Drawing of radial fingering pattern with non-Newtonian fluids with shear-thinning properties : the fingers are much thinner and look like filaments.[30]

$$L = 2\pi[3\gamma b/(2\sigma)]^{1/2} \quad (4)$$

(in the Newtonian case: $L = 1/12[b^2\gamma/\mu V]^{1/2}$)

where σ is the stress, and γ the shear rate. γ is now a function of shear **rate** such that $\sigma = \sigma_{yield} + \alpha\gamma^n$. This last identity describes the non-Newtonian behaviour of the material: below the yield stress σ_{yield}, the deformation is static, there is no shear **rate**. Above the yield stress, there is a rate of shear, as for a liquid: the material moves. Again, in vivo the value of the yield stress is completely determined by the chemical content of the tissue (especially, collagen density and bridging), therefore giving a leverage for morphogenesis of elongated filaments.

The important issue to remember is that the stress is more important at the tip, and hence the material is more fluid, and likely to be pushed ahead, in the region of the tip, than outside the region of tip (see Fig. 14). The consequence is the formation of more elongated duct-like stuctures.

So, we may say that there exists a spontaneous "apical region" around ducts although this does not require any specific metabolic activity, because living material tends naturally to be softer at tips. In this view, the apical region is special in two respects: the pushing field is higher there (as for viscous fingering processes), but in addition the effect of the push is enhanced by the fact that, in presence of a high field, the material properties are changed (it is softer, more fluid). In 3D, the situation is analogous to the 2D situation, and in fact, such images as Figure 7, were taken with a non-Newtonian fluid (hair gel, which contains a lot of collagen). Again, the Mullins-Sekerka instability with non-Newtonian fluids is much weaker in 3D. It is clear that playing with the material properties, as cells do, will greatly modify the morphogenesis.

Elastic Properties

As mentioned above, it is uncertain whether the living material of an organ behaves like a fluid. It might behave like an elastic material with a threshold, since (1) it contains a lot of collagen (2) the shear regimes are very small (the growth is slow), so possibly below the thresholds for fluid flow. Therefore, the penetration of the epithelium might be treated with the laws of mechanics instead of fluids. From a fundamental point of view, the laws of mechanics are different in fluids and in solids. When a force is applied to a fluid, it moves. When a force is applied to a solid, it deforms. In the simplest hypothesis—the linear elasticity and small deformation—a supposed small push of the epithelium simply generates a small deformation, which

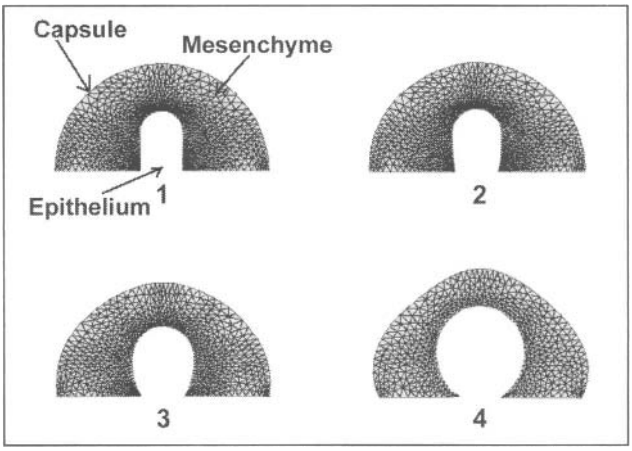

Figure 15. Simple elasto-plastic growth of a "bud" which generates a round pouch.

is static. If the force is removed, the shape resumes its original reference configuration, unless there is some plasticity. If we wish to develop a "finger" or duct into a solid, we need to consider a plastic material, which adapts to the new stress and deformed shape, in such a way that the deformation is absorbed, and the energy (energy = stress.deformation) is dissipated. If we remove the pushing force, the bud-like shape remains. Therefore, we may, in the simplest view, consider a small forward motion of a duct into the mesenchyme at a very small "flow" rate, as an elastic step, followed by a plastic step: the mesenchyme is first deformed, the deformation-stress relationship implies a high stress in the cells, then cell rearrangements, divisions and metabolism diminish the individual cell deformation so that the stress is reduced. The reduction of stress is both linked to morphology changes and to material changes (number of cell layers, shape and volume of cells etc.). This is described by Figure 15. In this picture, the deformation rate is proportional to the elastic deformation, with a proportionality constant which fixes the time scale for long time permanent adaptation. The growth and deformation continues, until some stress set point is reached. In this view, the mechanical state of the cell is a very important component of its mitotic rate and activity.

By so doing, we can, perform step-by-step, an elasto-plastic growth of a bubble inside a medium which itself expands. The simplest 2D simulation shows that it simply swells the entire pattern and does not develop a duct (Fig. 15).

This is also the case in 3D. In order for a tip to grow and form a tube, in this sort of elastic problem, one needs a more localized zone of deformation in the region of the tip. There could also exist mechanical instabilities, like buckling. It is a fact, however, that many human diseases are associated to excess of "ballooning" of the organs (polycystic kidney, emphysema, etc.).

The introduction of a special localized apical zone leads to what is known as "tip growth". In this class of models, the special zone at the tip is not self-organized by the field, but genetically predetermined. A recent elegant self-similar solution of this problem was proposed by Goriely and Tabor.[31] It is assumed that the elastic properties of the material depend, for "genetic" reasons, on the distance to the tip, so that the tip is softer than the shaft between a distance L from the apex and the said apex. This increase in softness may be produced, for example, by membrane degradation enzymes. In this case a tubular membrane or duct forming a "tip" growing at a constant speed, with a constant profile can be generated. This solution was derived in order to describe the uniform growth of an algal tip, but we may think that it applies to branching morphogenesis in a scenario where the mesenchyme is totally absent. Also, there exist many branching patterns, especially in invertebrates, which may be described by similar models.

The profile is known as a self-similar profile, i.e. although the tip elongates, the profile does not change. This problem is different from viscous fingering, in that the active apical region is due to a localized variation of material properties (while in viscous fingering the deformation localizes itself spontaneously, by self-organization of a diffusive field, either chemicals or pressure). In these models, something emanates from the tips and diffuses down the duct to a region where the round cap joins the trunk. So there is an activated tip, which is limited to a narrow region, and which pushes itself forward. The shape and growth speed is determined by the mechanical push (essentially the osmotic balance across the membrane), the rates of material production to accomodate the elongation, and the dimensions of the apical zone. This problem has a flavour of viscous fingering with a non-Newtonian fluid, in that a softer region is localized at the tip.

Reaction-Diffusion

The softer region which induces buds may have a more complex shape than just a spot at the tip. In this spirit, essential ingredients of morphogenesis of tubular structures may be brought into play by inhibition/activation genetic pathways. It has been suggested recently by molecular biological approaches that a complex interplay of activation-inhibition reactions gives rise to distribution of spots of mitotic activity, corresponding to the regions where ducts elongate. This is known as a "prepattern" model. This word means that although the model does not actually treat the true problem of how the boundary extends, it claims that the buds extend in specific regions on the surface, in some spatio-temporal regularity, which is determined by a couple of chemical reactions, in the spirit of Alan Turing's "leopard spots" models.[32] These models have also been put forward in models of plant growth.[33] In the case of lung growth, Warburton et al.[34] have reviewed the data concerning the complex interplay of biomolecules (peptides, FGFs, sprouty etc.) around a duct. Many genes are expressed in the lung, belonging to different pathways (shh, bmp, fgf, ...). It is known that several of these molecules are activators or inhibitors of each other. In the first place, FGFs act as chemoattractants of epithelium. A ridge of FGF10 exists close to the buds, which seems to provide the biological incentive for growth and elongation. This comprises certainly, albeit indirectly, the mechanical incentive. Disrupting fgf10 expression causes profound abnormalities. Coupling of this global incentive for growth with inhibitors, such as sprouty, may be the cause for regular and stereotypic branching.

In effect, localized "spots" of sprouty exist and split concomitantly in the region of formation of the new tips. This model may explain the origin of splitting and of budding, somewhere outside the apical region, down the duct, and mathematical models are in progress to incorporate as much molecular detail as necessary. However, at this stage, it is unclear whether gene expression is the cause or a consequence of physical forces, or even whether this question even has any meaning. Although spots of active areas have indeed been identified, they are generally found in regions which are morphologically singular: convexities, edges, corners, centers of symmetry, apices etc.

In general terms, there are problems with Turing models. First of all, they require a complex interplay of molecules, and the generation of "spots" can be achieved only if activators and inhibitors have very different diffusion constants. This implies that they have probably very different sizes, and hence correspond to very different biochemical kinetics. The second problem is that in order to maintain the structure, molecules must be produced continuously. Although the pattern seems static and stable, it corresponds to a constant flux of molecules, which is not very favorable in terms of metabolism. Immunochemistry visualizes concentrations, and not fluxes. Thirdly, in Turing's models, when something like a spot is seen to be displaced, say, from left to right, by ten microns, it can only do so by being dissolved at the left, and recreated at the right, which is not coherent with what is generally observed: tips or branching points actually move. In addition, in Turing models, when a tissue of type A (say mesenchyme) eventually occupies a region formally of type B (say epithelium), it can do so only by transformation of B cells into A cells. It is likely that the description of the problem is more in terms of a moving boundary, which should incorporate inhibited or activated regions along the surface, which modify

locally the material constants (softness/hardness) and the distribution of forces (push-pull). For example: if shear and bending induce a mitotic activity of different cell types, gene expression will be naturally localized in different specific regions, giving a possible impression of one gene inhibiting the other. The next paragraph addresses the question of how active regions may move.

Filament Navigation in a Field

Be it duct growth by elastic deformation of an apical zone, or by viscous fingering,, these phenomena also share a similarity with a problem studied long ago by Meinhardt.[35] Meinhardt describes the growth of filaments which have an active localized tip and which "chemoattract" themselves forward.

In this model, there is an autocatalytic production of a substance, say A (for activator) there is a field S of morphogen, and a field Y which describes the cell states. This is how patterning proceeds. In a medium of finite concentration of morphogen S, a small excess of A is able to trigger autocatalytically a local peak of A. This peak propagates in the shape of a travelling wave. If, in the back of the wave, the concentration of S returns to zero, then the wave of A also returns to zero, because in the absence of S, the concentration of A goes down to zero exponentially. Y is a two-state switch of cellular activity, which turns cells from a state 1 to a state 2 irreversibly. In the state 2, S is consumed, so, there is a diffusion field of S between a low level close to the pattern and sources of S "far away". Since the exponential increase of A depends on the magnitude of S, we find a moving front of A which navigates inside a landscape of S, with a speed depending locally on gradients of S, close to the wave. Since this landscape corresponds to the diffusion of S, it has a "point effect" wired in. The model generates filaments which branch randomly, in response to noise (random obstacles or random noise generated by the mitotic activity, for example).

In this class of models **diffusing molecules** are responsible for forward growth of an active tip, instead of **mechanical features**, but, to be honnest, the field of S introduced by Meinhardt could just as well be a pressure field, so similar are the equations. The model neglects, of course, the elasticity of the tissue. The equations corresponding to what has been described above contain a set of activator-inhibitor equations which maintain a localized, but moving, active region. The reader will easily identify in Equ. 5 the term cA^2S, which induces an increase of A wherever S and A are non-zero, the term -mA, which makes A go down to zero in the absence of S, and the term -eYS, which makes S be consumed in the state where Y = 1

$$\partial A/\partial t = cA^2S - mA + D_a\Delta A \qquad (5)$$

$$\partial S/\partial t = c - cA^2S - gS - eYS + D_s\Delta S \qquad (6)$$

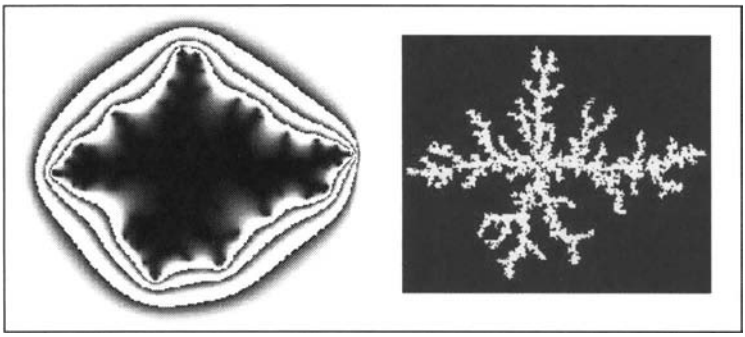

Figure 16. Navigation of filaments in a diffusible landscape of a morphogen S. This model is in its spirit very close to fractal viscous fingering. It requires noise at the moving tips of the filaments to induce dichotomous branching.

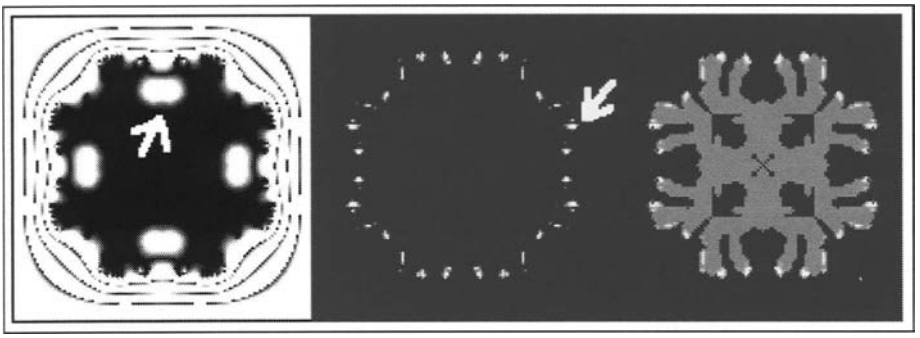

Figure 17. A self-organized growth of filaments, which avoid self-organized obstacles. The result is a set of branches surrounding obstacles, and filling space uniformly (pay attention to the regular distribution of active spots along the perimeter). The arrow in the left image points to large spots of inhibitors (there exist smaller ones at the curently branching tips) and the arrow in the middle points to moving spots of activators.

The two-state dynamical switch necessary to model the jump of cells from one state (state 0) to the other state (state 1) is given by :

$$\partial Y/\partial t = dA - eY + Y/(1 + fY^2) \quad (7)$$

Although these equations may look complex, they are conceptually very simple,[33] and we recommend to the reader to make the effort of reading some technical explanations about them.[33,35,36]

It is simple to incorporate in these models a feedback of the surrounding "tissue", which inhibits the growth when some threshold of S is reached. In such a case, one can form a more regular pattern which grows by avoiding self-organized obstacles (Fig. 17).

The image is typical of what a reaction diffusion process can give, in 2D, with active regions avoiding their inhibitors. The arrows point to the pools of inhibitor (in the left) and to the pools of activators (in the middle). These spots of chemicals form all by themselves, and the genetically programmed parameters are the parameters in the equations above. However, although such models may contain the correct molecular interplay, they are unable to provide realistic images, because they do not incorporate the mechanics of the tissue which "fingers".

Therefore, Meinhardt's model of wave propagation in a landscape by chemical reactions is weaker than the moving boundary models. It requires a different, additional set of concepts to explain why the filaments are actually tubes, how the cap is constructed, how the surrounding medium is pushed away, how the tubes slowly reorganize to make branching points at 120°, etc. Especially, the formation of ducts is generally **not** a matter of mesenchyme cells (fibroblasts) being turned into epithelial cells. There is a true displacement of all cells, which push themselves forward. This is absent in Meinhardt's models. In moving boundary models these features come all together by the laws of deformable bodies or of fluid flow.

If a given duct is constructed mechanically by a localized apical region (not self-organized by the epigenetic field), the duct is still, not growing alone, in vacuo as is for example the beautiful solution of Goriely and Tabor.[31] The duct has to find his way across a mesenchyme. In the simplest picture, a free duct moves forward in a straight fashion, while a confined duct, inside a capsule or surrounded by a stiffer external medium, either moves up the gradients of a diffusible molecule (for example, if the duct produces degradation enzymes which soften the mesenchyme, then it grows in the directions of the gradient of it), or, otherwise navigates following the stress. This is because an additional force due to the surrounding tissue, breaks the symmetry of the duct,[31] and the duct grows laterally in response to forces in the mesenchyme (see Equ. 5 of ref. 31). In the simplest case, the growth amounts to elongation of a duct that follows a localized active region and navigates inside a diffusive or mechanical field (the

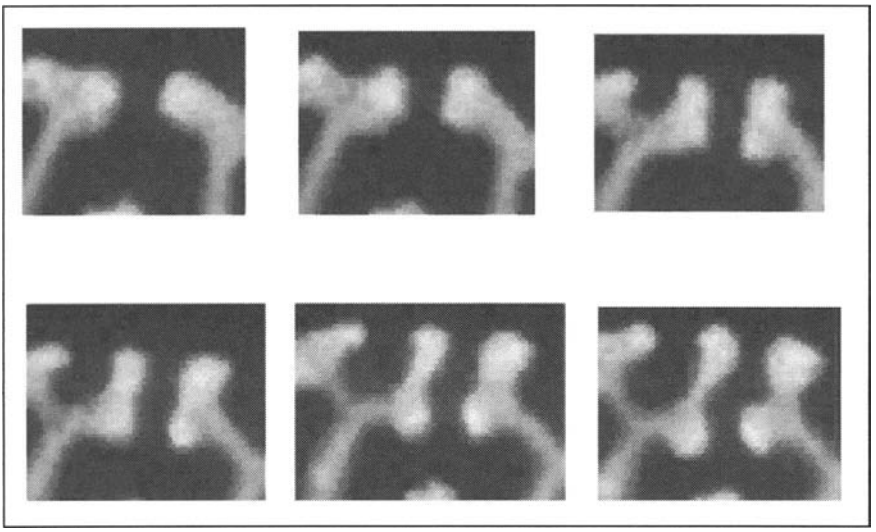

Figure 18. In situ observation of head on collision of ampullae.

pressure, if the capsule is a flat bag). Regardless of the mechanical detail, this is analogous to what Meinhardt has described.

However, although the pressure in 2D (inside a flat capsule) is a diffusible quantity, it makes a huge difference if the actual field is a mechanical field and not a chemical field, because the geometric parameters of the shape are "recorded" for good in material properties and not in levels of concentration of molecular fields produced by the cells, which are more variable quantities. In particular, the diffusion of activators across the tissue is formally replaced by the propagation of a mechanical stress in the tissue, which is much more robust.[36] In addition, many features are readily explained. For example, a mechanical field induces a subtle symmetry breaking of duct collision. As seen in Figure 18, it is observed that when ducts collide "head on" (instead of against the capsule) and branch, they tend to slowly shift sideways, in order that the branching points form an S shape, instead of remaining parallel.

This slow shift is easily accounted for by a mechanical buckling. Indeed, when a collision occurs head on, the region of contact between the two Ts is akin to a flat sheet, composed of several layers (epithelium, basement membrane, mesenchyme, basement membrane, epithelium). Such a composite material folds or buckles as a consequence of uniaxial tangent stress, a configuration with a broken symmetry (shift of the branching points) being more favorable (see below).

Active Growth of the Mesenchyme

So far, we neglected the active growth of the mesenchyme. This was taken into account by Meinhardt[35] as a mere homothetic dilation of the tissue, a good start for that time, although it is not quite realistic. It is obvious from inspection of experimental data that the epithelium/basement-membrane interface does not grow forward everywhere. In some regions it recedes (Fig. 19). The two basic regions where it recedes are the trunk of the ducts, and the top of the T shape. The recession of the tissue in the older parts of the ducts (Fig. 19) amounts to a strangling of the ducts.

So branching morphogenesis is not just a matter of the apical region being softer, but also of the trunk being contracted. This strangling has several consequences. First, the tube diameter narrows. Second the tubes become straighter and apparently more rigid, and third, the strangling pushes forward the region of the ampulla. In the region of the ampulla, at times when it

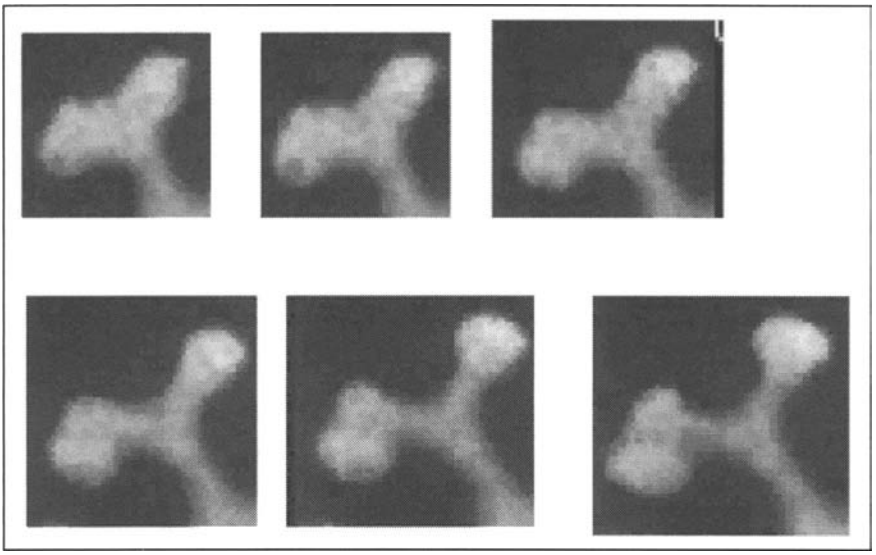

Figure 19. In situ observation of epithelium recession or strangling.

collides with the capsule and has a T shape, one observes a push of the mesenchyme which transforms the T shape ampulla into a 3-fold branching region (Fig. 20) with a saddle point.

It is clear that the mechanics of the transformation of the T into a branching point are the same as the mechanics of the contraction. They even are often concomitant. The difference lies only in the geometrical situation: either the push acts on a cylinder, or the push acts on a T-shaped ampulla. Therefore, we have to incorporate active deformation, growth and push of the mesenchyme towards the epithelium, a feature completely absent of Newtonian or non-Newtonian fluids, or of models of tip-growth. It is a well known fact that mechanical pushes of boundaries induces regular polygonal motives. This is very well known in the context

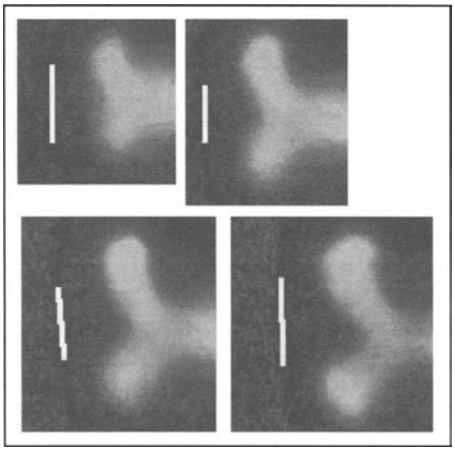

Figure 20. The formation of an interconnection of 3 tubes requires a recession of the T-shape, with respect to the capsule. Indeed, the mesenchyme between the capsule and the tip grows *against* the epithelium (the white bar is the border of the capsule).

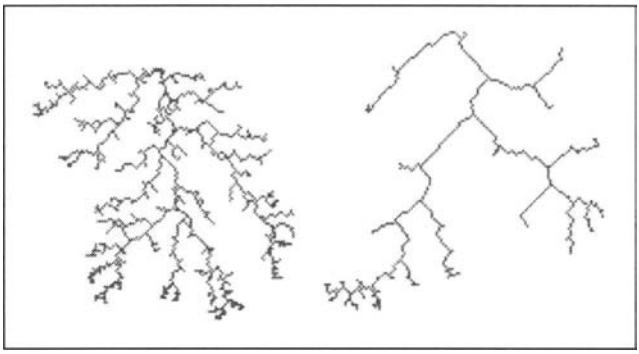

Figure 21. Transformation of a random tree into a more regular tree by push of the domains in between branches (2D).

of Dirichlet domains, and it has been suggested for long that such mechanical pushes may explain the regular ordering of spots, hairs, scales, in the animal realm. More recently, it has been shown that such mechanical pushes explain in part the regular ordering of vessels. Figure 21 explains the logic, on a dendritic tree in 2D.

A dendritic growth is performed in the regime where fractal dichotomous dendritic branching exists. But a push of the interstitial tissue is added, which acts only outside the apical region. The mechanical push is self-organized. Pressure particles are released which move randomly until they touch the tree. There, the tree is displaced by one step, in the direction opposite to where the push came from. The global result is a progressive straightening of the strands, and formation of more regular angles, which accomodate the difference between the left-right pushes on each strand. The same can be performed in 3D, it gives such results as Figure 22.

A very important point of mechanical push by the mesenchyme is the correlated deformation of neighboring tubes. Indeed, when a mass of tissue pushes, by the principle of action-and-reaction, the surrounding tubes are pushed in a correlated manner. This is to say that the shape of a tube is not something per se, the shape of all tubes surrounding a given mass of mesenchyme is under the influence of that mass, considered as one "ball". This is easily seen, again, using GFP mice, where one clearly sees the push of "balls" of mesenchyme which gener-

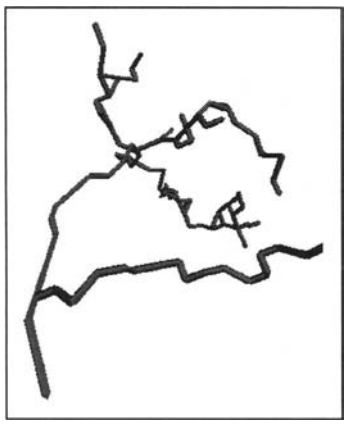

Figure 22. A random tree transformed into a more regular tree by push of the domains in between branches (3D).

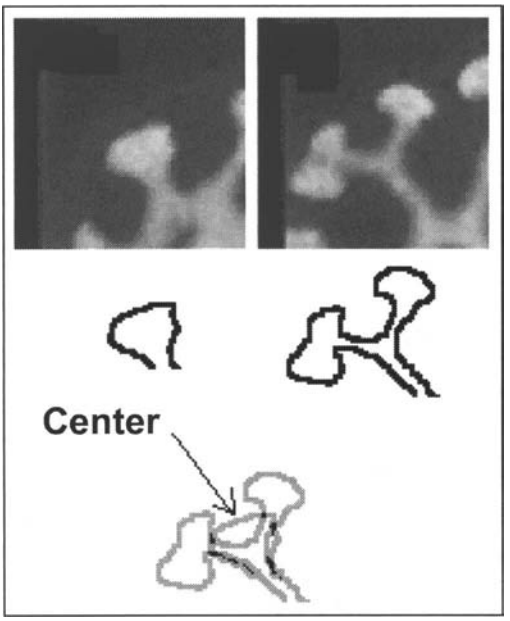

Figure 23. Image analysis of a sequence of growth. The 2 images on top show two stages of an initial T. The contours are extracted by thresholding the imlage (same threshold). Then the contours are superimposed. The result shows that the ampulla has receded, and has been pushed away from a region now occupied by mesenchyme. However, the middle of the diagonal of the gulf formed by the branches after splitting of the T, lies at the top and middle of the initial T. This shows that the position acquired by the two branches are not independent, they find themselves on either sides of a center of push, whose position can be traced back to the "ignition" point of the epithelium recession.

ate tubes eventually find themselves along the edges of polyhedrons. So, one cannot predict the position of one branch in isolation. A very pictorial evidence of the mesenchyme push is provided by the following image analysis. One starts from the film of a dichotomy event. Then the film image (grayscale) is thresholded, and the contours are extracted. Then the contours of the last and first image are superimposed on the first image. A very striking feature appears: the middle of the T of the initial T-shape is the starting point of a recession of the epithelium, as if it were pushed by the mesenchyme, but this point remains the middle of the diagonal of the "gulf" eventually formed by the two branches on either sides of the invisible ball of mesenchyme. So, there is a **correlated** motion of the epithelium on either sides, which gets away from this center at the same pace on the branch to the left, and the branch to the right, by the principle of action-reaction (it is not always the case that the situation is a symmetrical as here). In the Figure 23, the "context" of one branch is the presence of the other branch and of the tissue in between.

Now, in such images as Figures 20 and 23, the 2D projection is somewhat misleading, in that the region of the branching point where 3 tubes are well identified is not flat (contrary to what a projection shows) it has the shape of an hyperboloid (saddle) because of the topology of the interconnection of three tubes. If we state that tubes eventually follow polygonal motives along edges of mesenchyme polyhedrons, we need to explain the origin of the vertices (bifurcation points) and explain how they form, starting from a T-shape undergoing such a push by a mesenchyme. It is rather difficult to imagine a Reaction-Diffusion process à la Turing generating such hyperbolic shapes (saddle). However, mechanical push generates hyperboloids or saddle-shapes very simply. It is a general fact in mechanics that a tangential stress in a flat disk

generates a saddle-shape spontaneously.[38,39] It does not matter whether the stress is centripetal (contraction) or centrifugal (dilation). This is why potato-crisps have a saddle shape (the tangential stress inside the crisp comes from dessication by frying). Therefore, if we consider the flat T in contact with the mesenchyme, a small radial growth of the mesenchyme facing the epithelium will spontaneously turn the disk surface forming the top of the T into a saddle, and hence favor the transformation of the T into a Y, by breaking the symmetry of the disk. This description completes the T-shape dichotomy of the Figure 11 above (the drop colliding against a ceiling) which cannot be complete since it has cylindrical symmetry.

Intuitively, the reason why this flat disk is unstable is not the same as the fingering instability. In this case, the instability comes from the fact that when a force is exerted tangentially in a flat object (foil, sheet, plate) the object tends to buckle hyperbolically. Why is it so? When a force is exerted tangentially, the flat object deforms, and stores elastic energy. This elastic energy is the product of the deformation by the force, summed over the entire object. One could imagine that the object deforms uniformly (it shrinks, or dilates). This is not so, because a uniform deformation, multiplied by a uniform force and integrated over the surface, gives a bigger energy than a buckled configuration in which the deformation is indeed larger, but the integral of the product (force.deformation) is smaller. During the process of buckling, the object always tries to minimize the energy, so it adopts the saddle shape. This is exactly how potato crisps buckle, and similar models have been proposed for brain convolutional development.[40,41] This mechanism of transformation of the T-shaped tip into a Y-saddle point cannot be described by a purely 2D model.

There exist more complex deformation modes, which may lead to more complex branching, and which have been invoked in the case of plant growth.[42,43] A bizarre prediction of this model is that if the organs were grown more rapidly, they would exhibit a phyllotactic order (branches budding along spirals), like plants.[44,45] Such branching patterns, to our knowledge, are not observed in the animal kingdom. Only trifurcation events are also observed in branching morphogenesis.

However,the saddle buckling mechanism gives a very simple explanation to the growth of buds at right angles, outside the apical region, down the trunk. Indeed, when the saddle buckling of the T-shaped ampulla occurs, two points lying at either ends of one axis of the saddle grow forward and become the branches emanating from the T (Fig. 24 below), while the two other points lag behind and are driven down the existing trunk (Fig. 25). It is known that the mesenchyme carries pools of Fgf 10, which favor forward growth of the epithelium.

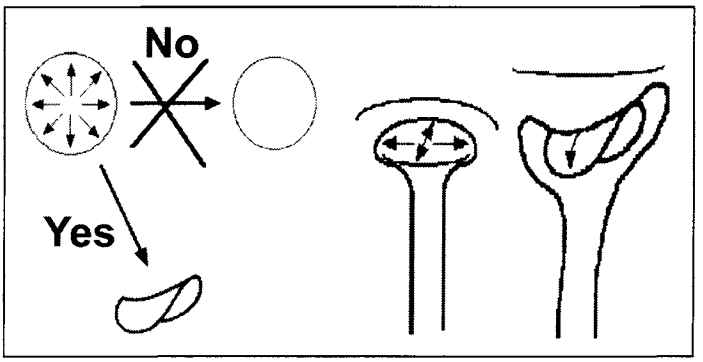

Figure 24. Left) When a disk (for example the T-shape seen from top) undergoes a uniaxial stress (the mesenchyme facing it increases or dilates radially), it is deformed spontaneously into a saddle (symmetry breaking). This explains naturally how a flat disk-like ampulla squeezed against a growing mesenchyme transforms itself into a bifurcation point. Right) Pictorial representation of buckling at a T-shaped interface. For clarity, the saddle region has been overlayed.

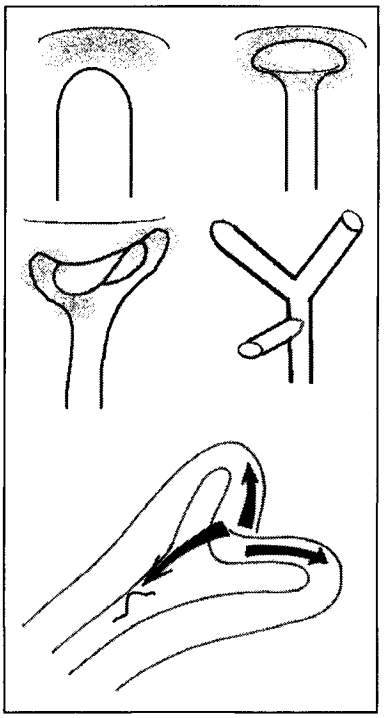

Figure 25. Saddle buckling of the epithelium and of the mesenchyme. Top left, a bud prior to be flattened; left. The T-shape. Middle left, buckling of the T, middle right: the final tube aspect. In presence of a pool of Fgf10 (shaded gray), one expects the growth promoter to be driven ahead in the regions of two siblings, but also downstream, towards "the back" of the T-shaped event, hence resuming growth at right angles, by budding. As growth resumes, the pool of Fgf10 is carried downstream by mesenchyme growth, and finds itself, eventually, somewhere down. Bottom, a tentative 3D sketch incorporating the deformation of the mesenchyme, at the moment of buckling, and just at the begining of budding. The arrows explain the physical displacements. The small tube is the internal epithelium, mesenchyme is around. Budding will resume down the trunk, with respect to the bifurcation point, in the region pointed by the arrow. This mechanism is still speculative (but see Chapter 8 for in vivo evidence).

The saddle buckling induces a breaking of the pool of Fgf10 into several pieces, which passively follow the deformation of the mesenchyme, in addition to possible inhibition-activation. As the mesenchyme is driven down the trunk in the direction of the small axis of the saddle, it will favor growth of buds in two regions, located at right angles of the main axis of the saddle, somewhere down the apical region and pointing outwards in the 3D dimension, with respect to the plane formed by the initial bud and the two axes defined by the tip-splitting event. This simple explanation of budding predicts that the well known structure of lungs with dichotomies "following each other at 90°" is in fact a misinterpretation of branching events produced somewhere in their past by dichotomous events. By this it is meant that as ducts elongate and tip-split, the dichotomy induces a new bud along the trunk from which the branches of the T have emerged. So, when an anatomist examines the branches, he or she may think that budding occured sequentially, first one branch and then another, because this is the spatial order which is seen on the final geometry, but the temporal order was actually the opposite, and so was the causality.

So much for the push in the apical region. The push of the mesenchyme along the trunk can also be implemented by finite element methods to show how a duct becomes a more ampulla-like or T-shaped pattern after strangling (Fig. 26).

This sequence of images is important, although the calculation is simplistic. First, it shows that recession of the epithelium has a stabilizing virtue. Indeed, for sport, we used a wavy edge of the growing duct, as may happen in vivo, spuriously. The mesenchyme push tends to smooth out the irregularity, and to reshape the tube to make it straighter. But there is more to this strangling: the ampulla adopts somewhat of a T-shape. If we have a more careful look at the T shape, we may notice that it has something like a smooth corner not at the top, but somewhere in the region where the trunk joins the ampulla. Such a "corner" or "parrot-beak" is actually seen in the experiments. The reader may identify such corners somewhat down the apex, in many images above, and in the more global image below (Fig. 27) in which the "parrot-beaks" are ubiquitous.

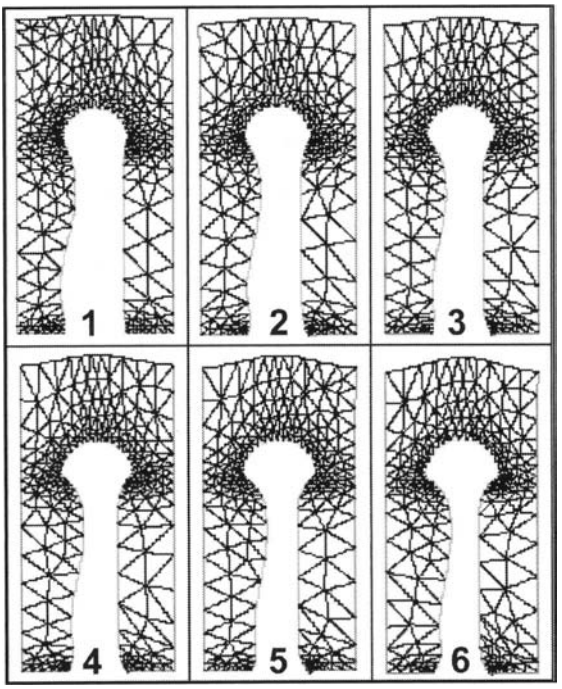

Figure 26. Ampulla and thin tube, obtained by a mechanical elasto-plastic push of the mesenchyme towards a duct, outside an apical region. The distance, outside the apical region, at which the mesenchyme push commences is chosen arbitrarily.

A very important feature of this "parrot-beak" is that it is **not** located at the junction between the region where the epithelium moves forward and the region where it recedes. Not at all. Because of the laws of elasticity, if there is a threshold below which the epithelium recedes (as is the case here) a corner bowing backwards appears, which finds itself in the region where the epithelium grows forward, neither at the tip, nor along the trunk, but on the side of the

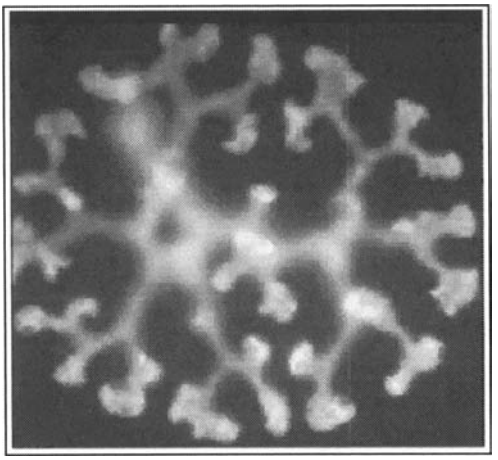

Figure 27. Typical kidney growth. The reader may identify many regions where the duct apex has a typical "parrot-beak" structure. The apex curves, or bows, with a sharp corner oriented backwards, with respect to the original growth direction. This backwards turn is due to the torque exerted on the epithelium apex.

region growing forward. As explained before, fingering is favored in sharp protruding regions. Therefore, we expect the growth to be accelerated in the region of the sharp corner, and hence to induce sibling ducts on either sides of the T. Moreover, if expression patterns are linked to curvature or stresses along the surface, the region where the contour is sharper will induce a ring of expression around the growing duct. This is indeed the pattern of expression of sprouty and of Fgfr2 during lung growth.[34]

However, in all these images, one needs to incorporate a threshold such that, outside the apical region, mesenchyme growth (hence recession of the epithelium) occurs. This threshold may well be given by some distance to the tip (decay of some diffusible molecule), by some time delay, or by some stress threshold which triggers mesenchyme development or maybe a conformatioal change of cell shape. This is an entirely open question, to our knowledge. Athough many features are known (e.g., change from cuboidal to squamous cells) it is not clear which feature is the cause and which features are mere consequences. However, it sounds awkward that one threshold should be needed to explain the existence of a soft apical region, and another threshold should be needed to introduce the growth and contraction of the mesenchyme around the growing ducts. It is more likely that there is a single explanation to these two facts. Above the threshold, the mesenchyme recedes and the duct moves forward, below the threshold, the mesenchyme moves forward and the epithelium recedes. There seems to be an imbalance rocking between epithelium and mesenchyme. The term rocking is in fact very apt: such a push-pull action around a threshold amounts to rotation around a fixed point (the boundary between the two regions). Then, the effect of the forces is to make the ducts rotate, in addition to growing forward. Movies of duct growth give this impression of systematic turns: the ducts swerve permanently. The result is the typical ampulla-like duct snaking between obstacles in the apical region, and straight and geometric tubes outside the apical region further down the tubes. The global result is an increase in size, but with a repeatedly branching structure, and isolated round balls of mesenchyme around each duct being pressed together in the capsule and forming pea-like aggregates, with septa separating different levels of the hierarchy.

One may wonder whether the threshold which is hypothesized in tip-growth (a diffusible molecule), could not itself be mechanical, in such a way that the process would, again, be entirely epigenetic instead of genetically-driven. Indeed, a mechanical stress setpoint might come into play in the following way. When a rigid medium is bent, the inner shells experience a contraction (compressive stress) and the outer shells experience a tensile stress. Although it is not very intuitive, a mechanical push inside a rigid elastic cylinder induces a compression close to the push, and a tension somewhat afar. The overall force, integrated over the entire body is tensile. However, in 3D, there exists 2 principle axis of curvature, for any surface. A cylinder has a finite radius of curvature R in one direction an infinite one in the other direction. The tissue is bent in one direction only. The internal pressure is sustained by a small deformation of the cylinder. At the tip of a duct, the surface is more spheroidal, and 2 radii of curvature, along two perpendicular axis come into play. The tensile or compressive stress is then less for a cell located at the tip of a duct, than along the cylinder. If there is a strangling of the duct, as described above, the situation is even less favorable for the tip: the tension is higher in the region of the parrot-beak. Then, if a mechanical set-point, either compressive or tensile triggers cell proliferation, it is more likely to trigger cell proliferation somewhat in the back of a duct, outside the apical region. Again, this comes from the 3D character of ducts, and it cannot be described by a 2D model. Whether the same threshold appplies to the recession in the region of the T-shape remains to be studied.

Orientational Order

So far, we have not taken into account the orientational order of the tissue. It is debated whether such an order already exists at the tip of a growing duct during growth.[22] It is at least not apparent in the epithelium, but it is very likely to exist in the mesenchyme.[20,45] Fibroblatsts are seldom represented without an orientational order (Fig. 28).

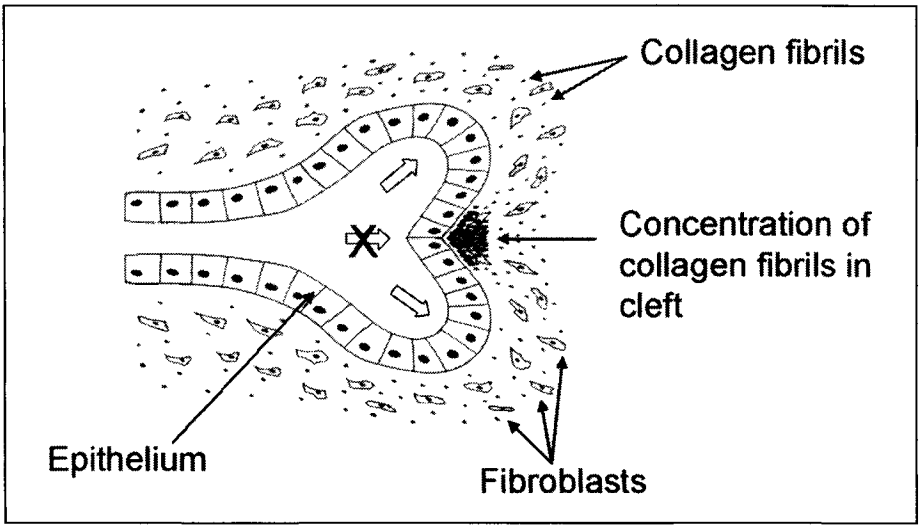

Figure 28. Typical representation of fibroblasts around a growing duct (after Nakanishi et al[46]).

This raises two questions: how does the shape and stress state of the surface influence the alignments of cells and even their own shape, and second, does the alignment of cells influence the pattern in return? This is the core of the **material** aspect of morphogenesis. If there is stereotypy, symmetry breaking, tubular structures, ampullae, push-pull actions, foldings etc., are all these features a consequence or a cause of the presence of morphogenic fields, be them concentration of chemicals or force fields?

Basically, the question arises of how the presence of an orientational order modifies the behaviour of the material, and how it couples to all the fluidics and mechanics explained above. This is truly a fomidable task. However, simple features can be explained. The orientational order induces streams of aligned cells, and hence of deposited collagen. When a surface develops in 3D, an orientational order experiences a topological problem: certain surfaces cannot be dressed with a uniform orientation in a perfect manner, there always remain topological singularities. These singularities deform the surface. This is obvious on spheres, and at branching points. One may think also of fingerprints. In the simplest picture, an orientational order on a sphere, with cylindrical symmetry around a "singularity" induces a protrusion in the region of the "singularity". If one imagines the drawing of parallels and meridians on a sphere, the singularities are simply the north and south poles. But there exist more complex solutions, such as the pattern of stitching on a tennis ball.

In order to show that the existence of singularities has an impact on the shape, we calculate the deformation of a spherical "bubble" which would be fibered like a tennis ball (Fig. 29, left). This drawing is more complex than parallels or meridians, but it is commonly observed on fingers, and also on cartilage rings. A distribution of little rods which can flip statistically, but which interact with a tendency to align themselves with each other can be modelled by an energy which is called the Frank-Oseen elastic energy.[47] In such a model, the cost of making an element of surface depends on the local drawing of the lines. If the lines are bent, or twisted, or fanned out, instead of being gently parallel, there is an additional energy cost. Making a singularity is "very expensive" in terms of energy, and spontaneously, all the fibroblasts would prefer to be all parallel to one and the same direction. However, if little elongated elements (and we may think of fibroblasts as being such elongated elements) try to align themselves on a sphere, there is no way that they can all be parallel. Hence, they

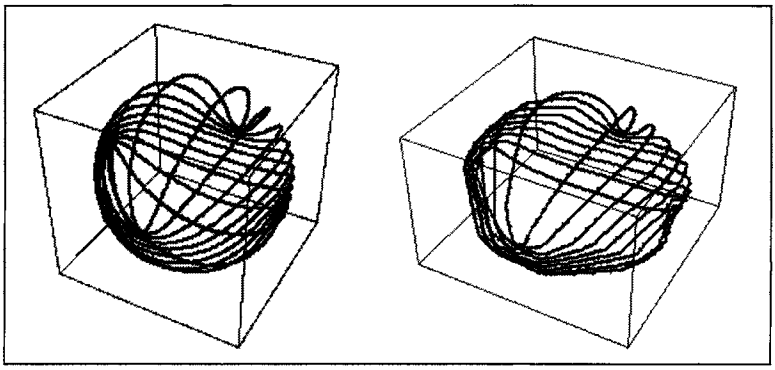

Figure 29. Calculation of the shape of a sphere dressed with fibers, in a simple Frank-elasticity model. Left the initial distribution of lines (e.g., fibroblasts), right the actual equilibrium shape. Hence, such a sphere... is not spherical. This shows that the laying down of collagen in an orderly fashion implies deformations of the surface.[49]

will make a pattern with singularities, and the surface energy associated to the drawing will locally depend on the specific drawing at each point. We have seen that there is a tension associated to the energy. If the tension is not uniform, then the "sphere" will not be spherical. One can find the surface which matches the equilibrium of the internal and external pressures and the non-uniform tension (Wulff construction).[43] This is how the equilibrium shape of crystals such as quartz or ice are mathematically constructed. In the case of the "tennis ball" it gives the following result (Fig. 29).

The region of the singularity (here the U-turn of the lines), induces, or favors, a bump in the region of the loop. However, the bump is not located right in front of the singularity, but shifted somewhat sideways. This appears in Figure 29 (right) in the fact that the region of the U-turn is not the foremost one pointing towards the reader. The part of the surface which protrudes most towards the reader is slightly below the U-turn (Fig. 29, right). The origin of this shift is in the symmetry breaking of the material properties, in the region of the singularity. This can be observed on anyone's fingers: if you find a loop on the drawing of your epidermal ridges, the center of the loop is not below the summit of the relief, but shifted more proximally, because a region of more curved lines is somewhat stiffer (think also of folding an edge of cloth and sewing the two half-edges: one finds a protrusion in the center, as for example on socks). Similar effects will occur with cartilage rings.

Therefore, in general terms, the presence of a singularity favors a forward growth in that direction. The mismatch between the maximum height of the relief and the position of the singularity is again associated to a torque (of surface tension), which bends the surface in this non-intuitive shape. The very center of the core is singular and must be accomodated by the tissue either with different cells, or with a hole, or with a uniform medium. Data about what happens in these regions are scarce.

The effect of an orientational order on a **branching region** has not, to our knowledge, ever been discussed. The problem of the branching of a uniform membrane has been adressed only recently.[50] However, a simple calculation (Fig. 30) of a cross-like membrane with vertical load and either parallel or transverse orientational order shows that, for identical pressure conditions, one has narrower necks in the region of the interconnection. Therefore, it is expected that the anisotropy of the fibroblasts and smooth muscle cells will contribute to build sharper connections between tubes (than with a uniform medium), if the cells run azimuthally, and to build rounder connections (than with a uniform medium) if the cells run longitudinally. Interestingly, this is seen in plants.

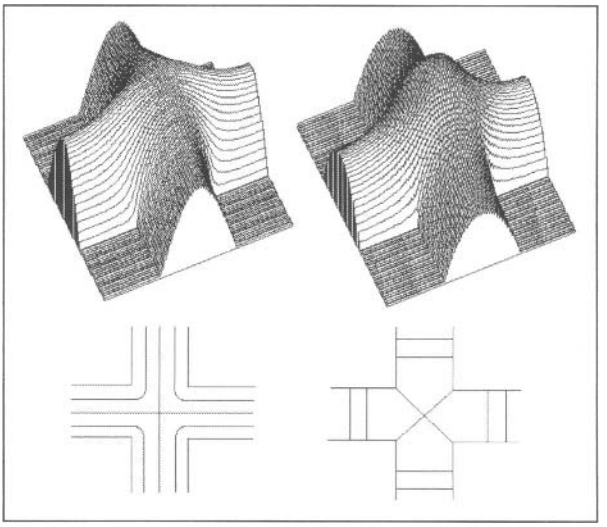

Figure 30. A membrane is stretched on a cross-like frame. The membrane is supposed to be constructed with fibers which have an anisotropic Young modulus, such that the membrane is more elastic in one direction (perpendicular to the lines) than along the lines. The membrane is flexed under a vertical load. The lines may be chosen longitudinally, or transversally to the arms of the cross. The deformation is not the same. If the stiff direction is transversal, the region of the interconnection is more curved (which amounts to sharper connections of tubes).

Conclusion

As a conclusion, a complete picture of branching morphogenesis, by no means quantitative, is probably as follows. In the presence of growth factors, especially FGFs, the tissue grows. The growth process is mediated by a mechanical push of osmotic origin. Under pressure, the mechanical (mostly tensile) setpoints of the pouch are overtaken; this tends to generate ducts with an active apex. The apex is curved and moves forward because there is an active region there, but the region is also active **because** the apex is curved. There is anyway an active region, even in the absence of any specific chemical activity, because of physical principles. This fact solves the chicken-and-egg problem raised by such a situation. The genetic actions, such as softening of membranes by degradation enzymes, "take over" and enhance physical cues.

The "pouch" is composed of two kinds of tissue, a more internal one (epithelium), and a more external one (mesenchyme). They do not experience the same mechanical fields, but still, they both grow. The ducts elongate more in the apical region by visco-elasto-plastic growth (fingering), while the mesenchyme pushes more outside the apical region (Dirichlet polygonal domains). In the apical region, a softer region, maybe even close to a fluid, forms a self-perpetuating finger-like structure, which swerves as it is contracted in its back, and collides repeatedly against the capsule, or against other fingers, finding its way in a self-organizing landscape of branches and pushing mesenchyme. When it collides, the duct is transformed into a disk, which undergoes a saddle-buckling which selects two siblings out of the disk, rarely more, and also induces buds down the trunk, depending on the context. Outside the apical region, the mesenchyme push straightens the tubes and makes them more polygonal.

The orientational order of the fibroblasts contributes to the process in several respects. As the fibroblasts adopt an orientational order around tubes, they get more susceptible to circumferential stress (as receptors of tension, they are more sensitive), but, in addition, the pattern of force which they exert on the tube is more radial and centripetal. As a consequence, they tend to select or even create well defined, stable, directions of growth for the soft apex, and sharp

branching points. They probably contribute to selecting the direction of saddle-buckling of T-shaped ampullae, because the pattern of lines around the apex has a broken symmetry already (like fingerprints).[49]

It should be noted that the description given in this chapter implies that branching morphogenesis is a completely 3D process conceptually, and it cannot be described by any 2D scenario. This is because: (1) tubes are 3D entities which have 2 axis of principal curvature, a fact which disappears in 2D, (2) A T-shaped ampulla is a 3D entity which has a disk facing the mesenchyme, a fact which disappears in 2D, (3) the saddle-branching of a disk is a 3D phenomenon whose 2D equivalent produces only folds (like brain convolution), (4) an orientational order of fibroblasts is meaningless on a 2D drawing of a cross section of branches. (5)growth of a 3D organ requires symmetry breaking so that out-of-plane branching is favored.

It should also be insisted that a deterministic immutable branching rule cannot generate a confined tree. The construction of confined trees such as the organs is possible because small adjustments of the rules are always taking place at the scale of individual ducts.

It must also be said that the entire pattern implies both a push perpendicular to the surface and a push tangential to the surface. Whatever the molecular detail performing these pushes, a tangential push can only generate folds (as in brain), while a push perpendicular to the surface generates fingers, T's or pouches without branches. The perpendicular push is needed to move the ducts forward, the tangential push is needed to have them buckle. The perpendicular push is linked to osmotic balance, while the tangential push is linked to mitotic activity, with mitotic planes always perpendicular to the surface (cell anisotropy, or "polarity").[51] Hence, the mechanisms of these pushes are entirely different, and the balance between the two will generate different patterns, between super-folded structures (like *polymicrogyria* in brain), super-branched structures (like organs) or only "balloons" (like in polycystic kidney disease).[52,53]

What kind of genes are needed to perform these feats? First of all "geometrical" genes, i.e. genes which preserve the geometry of the system. These are essentially the polarity genes of the epithelium, and the genes regulating contact inhibition. Thanks to them, the epithelium remains a sheet, facing the lumen, with the basement membrane on the other side, facing the mesenchyme. Polarity genes also play a role in the shape of fibroblats and hence on the way collagen is deposited (and vice-versa). In the absence of polarity genes, a mass of epithelium could still penetrate into a mass of mesenchyme, but the filtering, breathing, etc. properties would be lost (no lumen). Such aberrant epithelia are observed frequently in squamous cell carcinoma.[51] These genes probably act also on the very shape of the epithelial cells, with an influence on growth. The original organ in the phyllogenetic order was probably already a bag or pouch, prior to evolving the branching. A second set of genes must rule the push of the epithelium and that of the mesenchyme. This set of genes acts on osmotic balances, in response to growth factors and to geometry, and on mitosis. Since one expects the epithelial push to be localized in the region of the apex, and the mesenchyme push outside that region, one is tempted to speculate that some non-linear autocatalytic reaction-diffusion system localizes the active regions. However, it is obvious from simple inspection of the growth that the system is very much influenced by mechanical cues, since it is able to turn, split or stop in the region of obstacles. Therefore it is possible that the dynamics of the growth is greatly influenced or even fully generated by a mechanical setpoint, probably a curvature or tension-related setpoint.[54,55] A third set of genes must monitor the material aspects, i.e. essentially density of fibroblasts and laying down of collagen in an orderly fashion, akin to a liquid crystal phase.[22]

Typical anatomical dimensions are certainly adjusted or fixed by material properties also, and not only by chemical gradients. It may be thought that a partial or even complete theoretical description of this branching morphogenesis, useful at a clinical level and even allowing one to design regeneration machines, is not too remote. At time this book is published, regeneration machines will probably be already under trial.

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Appendix 1

There exists to our knowledge one alternative model of epithelial morphogenesis. This model considers contraction waves propagating in the epithelium. It may contain part of the picture.[55] However, while it is easy to imagine a spontaneous contraction wave in a 1D circular epithelium, it is less so easy to imagine this scenario in 3D, because it amounts to proposing that tubes form by emission of self-organized rings of contraction waves emanating from the tip of the duct, a much less plausible structure. It is much more likely that the ring-like structures which are necessary to maintain the ducts have a material origin instead of a diffusible one. The rings which contract and maintain tubes, in this picture are true rings of cells, as classically observed in histological preparations. Although contraction waves may propagate, they need not carry themselves a morphological information. Also, it is a remarkable fact that ducts may collide against each other and form T-shaped dichotomies facing each other. If contraction waves in the epithelium were responsible for a T-shaped dichotomy event, then one would have to imagine a correlation between contraction waves in two tips facing each other, although their distance along the epithelium may be enormous, a very unlikely feature. Considering that the T-shaped dichotomy is almost the same (apart from the symmetry breaking, see text) whether tips collide against each other or against the capsule, it is tempting to state that the "information" needed to perform a T-shaped dichotomy is zero.

Glossary

Adaptation: the process by which cells, which sense mechanical forces, proliferate or change shape until they reach some mechanical setpoint which inhibits further mitotic activity.

Ampulla: the ducts are generally wider at the apex, thus having the shape of a lamp bulb (ampulla). Ampullae are easily obtained in falling or raising drops problems, they are characteristic of interfaces flowing or hanging in presence of a uniaxial force (like gravity, see nuclear mushrooms). The growth of a soft apex, which is fixed to a more polymerized trunk is somewhat analogous to the problem of hanging tethered drops.[56]

Anisotropy: a surface or physical process may admit a preferred direction of growth. For example, in crystal growth problems, the crystal lattice induces an anisotropy, which leads to beautiful six fold branching of snow-flakes, or conspicuous four-fold symmetry of pyrite. The nematoid order of cells, on a spheroidal surface, generates an anisotropy, in the direction of the center of the singularity. Anisotropy may cause (or be the result of) a symmetry breaking.

Apex, apical region: a narrow region, at the tip of a duct, where something different is going on, which is responsible for elongation and growth of the duct.

Buckling: mechanical "folding" by which a flat plate undergoing tangent stress breakes its symmetry. In 1D, buckling induces wavy folds. This type of folding may explain the folds of the hindbrain or of intestine villi. In 2D a flat sheet becomes folded in several directions, as a consequence of buckling. The simplest buckling mode of a circular or eliptical plate is the saddle.

Budding: a process by which a tip emerges on the side of a trunk. It is debated whether there is a specific molecular instruction which provokes budding, or whether it is epigenetic. The first branching events of a lung are more of a budding type than those of a kidney.

Dichotomoy: a process by which a tip splits into two (see budding). The first branches of a kidney are more dichotomous than those of a lung.
Duct: a tube of tissue (epithelium+basement membrane) with a roundish end which extends progressively, either in a culture medium, or in a supporting tissue.
Elastic deformation: reversible deformation of a body, with respect to a reference configuration when some external or internal force is applied. If the force is removed, the solid returns to the reference shape. The work of the force stores an energy into the solid which maintains the solid in the deformed state; when the force is removed, the body resumes its initial form, and gives the energy back.
Epigenetic: specified or controlled by an element of the context (temperature, pressure field, geometry, etc.) and not directly by genes. The epigenetic cue may of course be mediated by some genetic transduction. The number of fingers is genetically determined, the implantation of each hair, for example is epigenetic.
Field (biology): a domain containing cells which eventually will find themselves in the same organ or structure.
Field (physics): a non uniform quantity which is defined in space and whose value at each point is given by a mathematical function of the space coordinates, and possibly also of time. For example T(x,y,z), the temperature field, C(x,y,z) the field of concentration of some chemical, ε(xy,z) the deformation field, etc. Fields of different physical origins may sometimes satisfy analogous if not identical mathematical equations (because of "conservation laws"). For example, the diffusion equation is identical for temperature and for concentration fields, although they carry different names (Fick's law, Fourier's law). The fields may be more complex than just one scalar quantity, for example: fluid velocity is a vector, strain and deformation in a body are tensors.
Gradient: spatial variation of a quantity, such as pressure or growth factor concentration. High gradients correspond to big slope in the pressure or concentration landscape. Most growth processes tend to navigate up or down high gradients (fluxes and flows are greater in presence of higher gradients).
Minimal surface: a minimal surface is a surface which follows a thermodynamic equilibrium, but which, in addition, feels the same pressure on both sides. The result is a surface which has zero mean curvature (one concave and one convex). A typical minimal surface is a hyperboloïd, or a catenoid. A bubble is not a minimal surface (it has internal pressure). The name "minimal" comes from the fact that it is constructed automatically with the smallest possible area allowed by a given boundary condition.
Newtonian fluid: a fluid whose viscosity is constant and uniform, not dependent on velocity (e.g., water, oil). An intuitive characteristic of a Newtonian fluid is that objects sink into a Newtonian fluid, although sometimes very slowly (in honey, for example).
Nematoid order: order of oblate or elongated objects which interact with a tendency to align themselves. The simplest example is given by crystal liquids. Fibroblasts exhibit a nematoid order, epidermal ridges, cartilage rings, etc. exhibit a nematoid order.
Plastic Deformation: irreversible deformation of the body, with respect to a reference configuration. Plastic deformation requires energy dissipation in the body. In a solid, the dissipation is mostly by defects rearrangements inside the solid (up to rupture). In a living tissue, energy dissipation is both by physical polymer-polymer disentanglement (yield), and by cellular metabolism.
Prepattern: a distribution of chemicals on a biological surface, which is obtained by a Reaction-Diffusion mechanism à la Turing. The patterns are usually in the shape of spots, or lines. The spots usually adopt a square or hexagonal lattice, although more complex patterns exist. If one of the biochemicals is a growth factor, it is expected that growth will occur exactly in the region of one of the spots, and generate a bump, a duct, a needle, etc. However, mechanics must be incorporated at some stage to complete the growth model.

Setpoint: a specific value of the surrounding epigenetic context at which a genetic switch modifies the behaviour of a cell.
Stereotypy: the laying down of branches tends to follow some regular pattern, which is somewhat of a constant. For example: dichotomies tend to repeat at constant interval, and turn each time by 90°. The stereotypy of kidney and of lung are different (there is more budding in lung than in kidney).
Surface tension (interfacial energy): if a surface is produced by a molecularly fluctuating material, and if the thermodynamical cost (interfacial energy) of an element of area is γ, then there exists a tension (force = energy per unit length) inside the surface equal to γ. The shape of the interface is the result of the equilibrium between the internal and external pressures, and the tension in the membrane. For a spherical object, this writes Pint - Pext = γ/R, where R is the radius of curvature of the sphere at equilibrium. Shapes which are not under thermodynamic fluctuations never reach equilibrium. They can have any shape, with or without tension (for example: a solid spherical shell, in the reference state, with or without residual tension, may be taylored with any diameter).
Shear-thinning fluid: a fluid which is less viscous, and hence flows more easily if it is sheared. Only big and heavy enough objects will sink in the fluid. Below some shear threshold, they behave like solids (typical of gels, wax, albumin, paints etc.)
Symmetry breaking: a mechanism by which, upon variation of some physical parameter, a structure becomes less symmetrical. For example, if elongated particles, deposited on a sphere, start interacting, then they tend to form filaments, but the orientational order of filaments on a sphere generates a symmetry-breaking, because the filaments organize themselves around singularities (for example a north and a south pole), which break the symmetry of the sphere.
Surface stiffness: the surface tension is generally supposed to be a constant. But in the true world, this is not so generally true. If the surface tension is not a constant, the relevant quantity, in order to find an equilibrium shape, is not the surface tension but the surface stiffness $\{\gamma + \partial^2\gamma/\partial^2\theta\}/R$ where $\partial^2\gamma/\partial^2\theta$ contains the effect of the spatial variation of γ, with respect to a polar direction θ. The equation to solve becomes Pint - Pext = $\{\gamma + \partial^2\gamma/\partial^2\theta\}/R$. This comes from the fact that the variation in surface tension acts as an additional torque which bends the surface. In many materials for which the elasticity of the surface interacts with the bulk, the concept of surface tension does not permit alone to find the shape.
T-shape dichotomy: when tips split they tend to have, transiently, a T shape which progressively transforms into a Y shape. This T-shape is easily obtained in moving drop problems, upon collision on a flat obstacle.
Viscous finger: elongated duct-like bubble which forms when one fluid penetrates into a more viscous one, especially in 2D. Viscous fingers are also observed in non-Newtonian fluids.

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