CHEMICAL MORPHOGENESIS: TURING PATTERNS IN AN EXPERIMENTAL CHEMICAL SYSTEM

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ABSTRACT

Patterns resulting from the sole interplay between reaction and diffusion are probably involved in certain stages of morphogenesis in biological systems, as initially proposed by Alan Turing. Self-organization phenomena of this type can only develop in nonlinear systems (i.e. involving positive and negative feedback loops) maintained far from equilibrium. We present Turing patterns experimentally observed in a chemical system. An oscillating chemical reaction, the CIMA reaction, is operated in an open spatial reactor designed in order to obtain a pure reaction-diffusion system. The two types of Turing patterns observed, hexagonal arrays of spots and parallel stripes, are characterized by an intrinsic wavelength. We identify the origin of the necessary difference of diffusivity between activator and inhibitor. We also describe a pattern growth mechanism by spot splitting that recalls cell division.

KEY WORDS: pattern formation, morphogenesis, Turing patterns, reaction-diffusion, chemical reaction.

1. INTRODUCTION

How does organization spontaneously emerge in an initially uniform medium, be it chemical or biological? From egg to embryo then to adult organism, the development of any living thing goes through successive stages that involve complex mechanisms, a number of which are still poorly understood.

In spite of the crucial role of genes in biological systems, genetics cannot explain the onset mechanism of cell differentiation nor describe the mechanisms that drive pattern formation since each cell in a given organism has the same genetic information. Besides the genetic machinery, 'generic' physical mechanisms (Newman & Comper, 1990) have to be considered. The latter are organizing principles of nonliving as well as living systems. These physical processes include reaction-diffusion and mechano-chemical processes (adhesion, surface tension), gravitational effects, viscosity, phase separation and convection. In the following, we focus on reaction-diffusion patterning mechanism.

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Concerning reaction-diffusion, let us recall that the first clear cut analysis of spontaneous pattern formation through the sole coupling of reaction and diffusion processes is owed to the British mathematician Alan Turing. In his famous paper 'The chemical basis of morphogenesis' (Turing, 1952), he produces a model of reaction medium made of chemical substances called 'morphogens' which diffuse through an initially homogeneous tissue and react. In this paper, it is essentially shown that various types of patterns can emerge, among which stationary symmetry breaking patterns referred to as *'Turing patterns'*. The paper is concerned with the onset of instabilities which are supposed to be adequate to account for the basic phenomena of morphogenesis in biological systems. The beauty of Turing's idea lies in the counterintuitive organizing role of diffusion that usually smooths out any concentration inhomogeneity. Such a pattern formation mechanism calls for special but not too unusual kinetic features for the reaction, and for some differences in the diffusivity of reacting species.

In this report, we present experimental observations of Turing patterns in a chemical system. We first summarize the prerequisites for the onset of a Turing instability. Then, we describe the reactor and the reaction that made possible these observations, followed by a description of various types of observed patterns. Finally, an unusual and fascinating pattern growth dynamics is presented.

2. THE TURING INSTABILITY

Turing's idea instigated numerous theoretical studies among which the distinguished work of Prigogine and coworkers in Brussels (Prigogine & Nicolis, 1967). The expression *'diffusion-driven instability'* has been coined to characterize this mechanism that was thereafter shown by the Brussels school to be pertinent to the formation of spatial *dissipative structures* in many fields such as biology, materials science, plasma physics. Well documented reviews can be found both from the nonlinear physics (Nicolis & Prigogine, 1977; Haken, 1978; Field & Burger, 1985) and from the biological (Meinhardt, 1982; Babloyantz, 1987; Murray, 1989; Harrison, 1993) points of view. Concerning chemical reactions, it was shown that, in order to give rise to self-organization, a system i) has to be maintained *far from thermodynamic equilibrium* by a permanent supply of fresh reactants, that is, the *system has to be open*, ii) must involve *positive* (autocatalysis, substrate inhibition) and *negative feedback loops*. When so, the system can become unstable as one of the control (bifurcation) parameter is moved beyond a critical value. *Symmetry breaking instabilities* of the basic thermodynamic state can develop, leading to self-organized states. Temporal or spatial dissipative structures are obtained.

In these conditions, an isothermal chemical system kept homogeneous by vigourous mixing would exhibit uniform temporal changes, in particular periodic homogeneous oscillations. In the absence of stirring, reaction and transport processes can work together to produce spatial patterns. If convection or any type of global motion is excluded, the only active transport process is the molecular diffusion of species. We are left with a simple reaction-diffusion system. Such systems are described by a set of nonlinear partial differential equations, the *'reaction-diffusion equations'* of the following compact form:

$$\frac{\partial C_i}{\partial t} = f_i(...,C_j,...) + D_i \Delta_r C_i$$

where f_i accounts for the reaction rate (generally nonlinear), D_i and C_i are respectively the diffusion coefficient and concentration of the species i and Δ_r is the Laplacian operator.

Numerous modelling schemes which are able to exhibit Turing type instabilities have been proposed. For analytical and computational simplicity, most of the models consider only two variables. In such models, one can generally identify an *activator* and an *inhibitor*. The changes in the concentration of the activator tends to reinforce their own rates of change while the associated variations of the concentration of inhibitor opposes to this reinforcement. A pattern can only arise when the local balance between these antagonistic species is broken. This spontaneously occurs when the diffusion coefficient, D_h, of the inhibitor is sufficiently larger than that, D_a, of the activator. For realistic models, one typically finds that D_h and D_a must at least differ by an order of magnitude for a diffusive instability to occur over a significant domain of parameters.

The first known example of stationary reaction-diffusion patterns was not obtained in an isothermal chemical system. In 1944, Zeldovich experimentally observed a cellular arrangement in a flame front (Zeldovich, 1944).The pattern was shown to originate in a difference of diffusivity between the temperature and some reactive species of the gas mixture. Turing extended to isothermal reaction-diffusion systems the concept of coupling between a local activation and a long range inhibition. As mentioned above, many theoretical works make use of the Turing instability to account for self-organization phenomena in numerous biological systems (Newman & Frisch,1979; Hunding, 1981; Babloyantz, 1987; Harrison *et al.*, 1988; Lacalli *et al.*, 1988; Murray, 1989; Hunding *et al.*, 1990; Newman & Comper, 1990; Harrison, 1993). The expression of 'Turing pattern' and the concept of an instability induced by diffusion extends also to dissipative structures experimentally observed in fields as diverse as nonlinear optics, semi-conductors, gas discharge, heterogeneous catalysis.

However, the first experimental observation of Turing pattern in an isothermal single phase chemical system was made only in 1990 (Castets *et al.*, 1990). This long delay is due to practical impediments of two types: i) the difficulty of designing appropriate 'open spatial reactors', ii) the necessity of developing chemical reactions able to fulfil the required properties (nonlinear kinetics and differences in the diffusivity of species).

3. MATERIALS AND METHODS

3.1 The Open Spatial Reactor

As for temporal dynamics of nonlinear chemical systems, the extensive experimental study of which developed after the appropriate open stirred reactor was used, the prerequisite for obtaining sustained spatial organizations was the design of 'open spatial reactors'. Such reactors have to meet two apparently contradictory conditions: ensure a permanent feeding by chemicals and avoid any hydrodynamic motion. A convenient way consists in making the reagents to diffuse into chemically inert hydrogels, the polymeric matrix of which discards any parasitic fluid motion. The second impediment lies in assuming a system identically fed at every space point, an assumption usually made in most

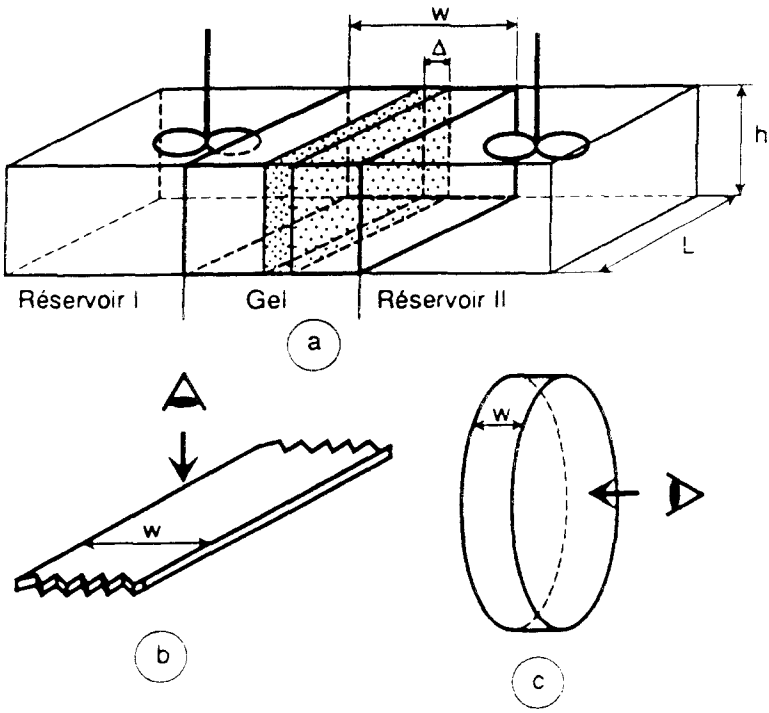

Fig. 1. Sketches of the open spatial reactors. a) Basic principles. Dimensions of the block of hydrogel: L × h × w; Δ is the width over which the chemical pattern of wavelength λ develops; b) thin strip reactor: h ≪ L. Dimensions used: L = 20mm, h = 0.5 or 0.14 mm, w = 3 mm; c) standard disc reactor (parallel faces): diameter = 21 mm, w = 3 mm.

of the theoretical studies but which is impossible to realize in practice. However, Turing patterns can also develop in systems only fed at boundaries (Herschkowitz-Kaufman, 1975; Dewel *et al.*, 1987; Boissonade, 1988).

The main part of the open spatial reactor is a piece of gel (fig.1a) of which two opposite faces are in contact with the solutions of reagents contained in two reservoirs I and II. Usually, reagents are distributed in the reservoirs in such a way that solutions I and II are not or little reactive on their own. The reagents diffuse from the boundaries into the gel where they meet and react. The reagents are permanently renewed by pumps and stirred in the reservoirs, ensuring constant and uniform boundary conditions. Due to the asymmetric feed compositions, steep ramps of chemicals establish between the feed surfaces. The reaction-diffusion instabilities will generally be confined to a region of width Δ between the two feed boundaries where appropriate concentrations of the major species are met. There only, concentration patterns can develop.

Polyacrylamide gel, previously used for studying sustained excitation waves (Noszticzius *at al.*, 1987; Dulos *et al.*, 1992) in the Belousov-Zhabotinskii medium was initially retained for the search of stationary patterns. Later, agarose gel was also used. During manufacturing, the gel is loaded with a colour indicator which is essentially immobile in the gel matrix. As we shall see later, this colour indicator can play a crucial role in the

pattern development mechanism.

Two geometries of reactors have been used but, in both cases, the distance *w* between the feed surfaces is the same so that the gradients of chemicals remain unchanged. i) In a first set of experiments, we used a *'gel strip reactor'* (fig.1b) a geometry that, in numerical simulations,was shown to be convenient to evidence Turing patterns (Boissonade, 1988). The piece of gel is a thin ribbon, 3 mm wide, fed by the two long edges. Observations are made from the top, allowing a view of the plane that extends between the feed surfaces. ii) We also used the *disc reactor* (fig.1c) as initially proposed by the group in Austin (Ouyang & Swinney, 1991a, 1991b). The piece of gel is now a flat disc, 3mm thick, fed by the two circular faces. Observations are made perpendicularly to the feed surfaces and give a view in a plane perpendicular to that in the gel strip reactor. In all cases, monitoring is provided by a CCD video camera fitted with a macrolens.

3.2 The CIMA Reaction

The reaction used is the 'CIMA' reaction, an oscillatory redox reaction involving chlorite (ClO_2^-), iodide (I^-) and malonic acid (MA) as initial reagents (De Kepper *et al.*, 1982).

The oscillatory mechanism of the reaction was elucidated by Lengyel *et al.* (1990). The oscillatory behaviour actually occurs when the initial chlorite and iodide ions are nearly completely consumed. Thereafter, besides malonic acid, the major species are chlorine dioxide (ClO_2) and iodine (I_2) while chlorite and iodide become the true variables and play the roles of the 'inhibitor' and of the 'activator', respectively.

The overall mechanism of the reaction is rather complex, but a simplified mechanism was proposed by Lengyel and Epstein (1992). It involves only three component processes for which the rate laws were experimentally determined:

$$MA + I_2 \rightarrow IMA + I^- + H^+ \quad (1)$$

$$ClO_2 + I^- \rightarrow ClO_2^- + 1/2I_2 \quad (2)$$

$$ClO_2^- + I^- + 4H^+ \rightarrow 2I_2 + Cl^- + 2H_2O \quad (3)$$

From a dynamical point of view, reaction (3) involves the crucial feedback process. It is shown that, starting with ClO_2, I_2 and MA, the reaction can immediately oscillate and that during each oscillation, the concentrations of iodide and chlorite change by several orders of magnitude. The rate of reaction (3) is inversely proportional to the iodide concentration, so that when iodide is consumed, this activates in own consumption. This *'substrate inhibition'* feature is the crucial nonlinear process in the reaction.

4. EXPERIMENTAL RESULTS

An appropriate colour indicator for the CIMA reaction is starch. It forms a dark blue complex with iodine and iodide.

Reagents are distributed as follows in the feed solutions: iodide is introduced symmetrically in both reservoirs, malonic acid in sulphuric acid solution only in reservoir I, and chlorite in basic solution only in reservoir II. Chlorite is the oxidizer. In the

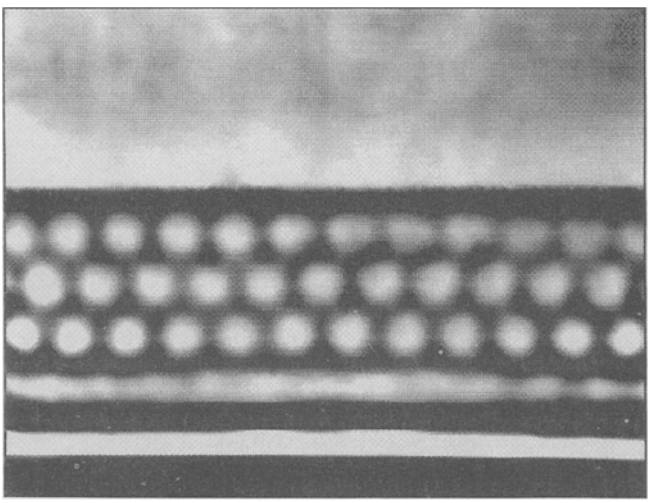

Fig. 2. A Turing pattern in the gel strip reactor. View size 2.1 mm × 1.7 mm. Experimental conditions: temperature = 6°C, residence time in reservoirs = 3 min, [starch] = 3 g/l of gel. Concentrations in reservoirs: $[I^-]_I = [I^-]_{II} = 2.5 \times 10^{-3}$ M, $[ClO_2^-]_{II} = 2.4 \times 10^{-2}$ M, $[MA]_I = 1.2 \times 10^{-2}$ M.

asymptotic state, reduced forms of iodine (I_2 and I^-) are present in the gel along the malonic acid-fed side: there, the gel colours dark blue. Oxidized forms of iodine (e.g. IO_3^-) are present along the chlorite-fed side where the gel remains colourless. The temperature is maintained constant by a waterjacket.

4.1 Turing Patterns in the Gel Strip Reactor

The typical settling time for stationary concentration profiles across the gel strip 3 mm wide is about 3 hours (since D~ 10^{-5} $cm^2.sec^{-1}$). Thereafter, for feed concentrations conveniently chosen, a stationary pattern, as illustrated figure 2, can develop. Typically, from left to right, it is composed of:

- a broad dark band of reduced iodine state, parallel to the feed boundaries.
- another thinner dark band, parallel to the first one and separated from it by a thin clear stripe of oxidized iodine state.
- several rows of clear spots emerging from a slightly darker background. The rows of spots also develop parallel to the feed boundaries.
- moreover, a wide clear structureless region of oxidized iodine state occupies about two thirds of the width of the gel.

The pattern is stationary and can be sustained indefinitely. The arrangement in bands and rows preserves the symmetry of the boundaries, but the presence of clear spots in the rows breaks this symmetry. The spotted pattern is the result of a spontaneous symmetry breaking instability of the system. Spots are regularly spaced. They exhibit a regular hexagonal arrangement and the wavelength of the structure is about 0.17 mm. This wavelength remains unchanged in reactors with other geometries and dimensions(e.g. an

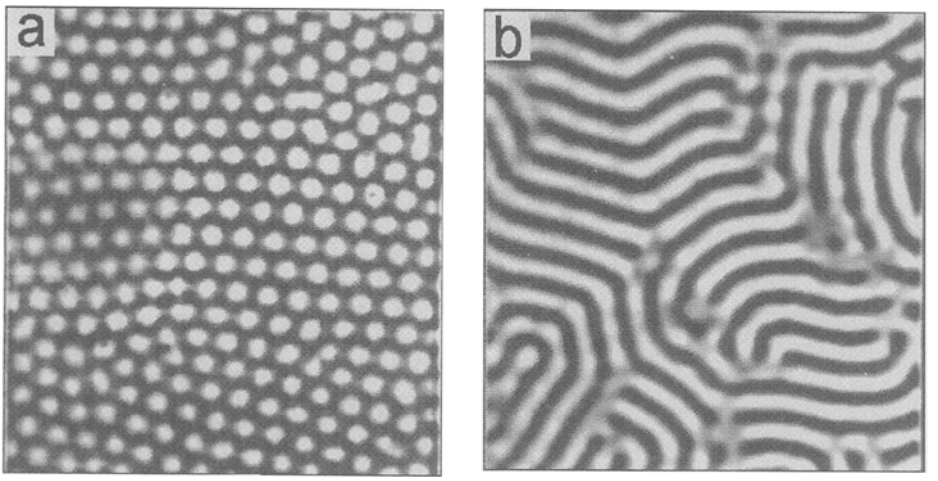

Fig. 3. Monolayer Turing patterns in the disc reactor. Contrast enhanced images of the central part of the reactor. View size 3.6 mm × 3.6 mm. Experimental conditions: temperature = 6°C, residence time in reservoirs = 10 min, [starch] = 1.75 g/l of gel. Concentrations in reservoirs: $[I^-]_I = [I^-]_{II} = 2.9 \times 10^{-3}$ M, $[ClO_2^-]_{II} = 2 \times 10^{-2}$ M. 3a) Hexagonal array of clear spots (oxidized iodine state, minimum of $[I^-]$): $[MA]_I = 3.5 \times 10^{-3}$ M. 3b) Striped pattern: $[MA]_I = 5 \times 10^{-3}$ M.

annular gel strip) (De Kepper *et al.*, 1991). The wavelength is intrinsic[1] to the system. Hence, the observed pattern meets all the characteristic properties of a Turing pattern, that is stationarity, spontaneous symmetry breaking, intrinsic wavelength.

The number of rows of spots strongly depends on the control parameters: as a function of the feed concentrations, patterns made of one to five rows of spots have been observed in the gel strip reactor (Castets *et al.*, 1990; De Kepper *et al.*, 1991; Boissonade *et al.*, 1994).

4.2 Turing Patterns in the Disc Reactor

The rows of spots seen in the gel strip reactor correspond to parallel layers filled with patterns in the disc reactor. We will limit the discussion to the case where patterns develop in a monolayer. For appropriate feed concentrations, the pattern can be made of only one layer. Typically, depending on the control parameter value, two types of patterns can be observed. As illustrated on figures 3a and 3b, the pattern can be made either of spots arranged in hexagonal arrays or of parallel stripes.

More recently, we have designed bevelled disc reactors (Boissonade *et al.*, 1994) in order to obtain a continuous change of control parameter in the plane of observation. In these reactors, the circular feed surfaces are no longer parallel. The gradient of thickness

[1] Contrarely, in the Rayleigh-Benard cells, induced by convection in a thin layer of liquid with a vertical gradient of temperature, the wavelength of the structure strongly depends on the thickness of the fluid layer, a geometric factor of the system.

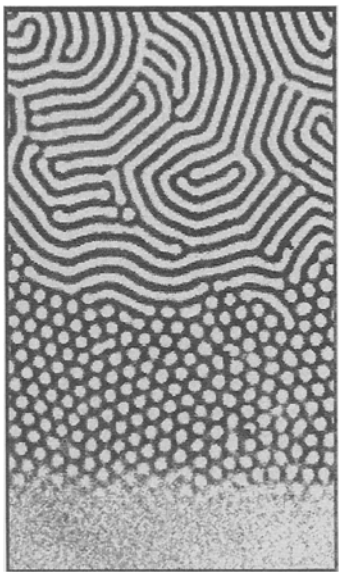

Fig. 4. Monolayer patterns in a bevelled disc. (Circular faces making an angle of 4 degrees, bottom width 1.9 mm, top width 3.4 mm). View of a bottom portion of the disc. From bottom to top: uniform state → hexagons of spots → stripes. Experimental conditions; temperature = 6°C, residence time in reservoirs = 6 min, [starch] = 2.1 g/l of gel, $[I^-]_I = [I^-]_{II} = 2 \times 10^{-3}$ M, $[ClO_2^-]_{II} = 5 \times 10^{-2}$ M, $[MA]_I = 1.3 \times 10^{-2}$ M.

produces a gradual change in the concentration ramps across the gel. As a result, for the same set of boundary feed concentrations, different types of patterns can be observed at different positions along the surface of the disc. This is examplified in figure 4 where various patterns can be seen extending over successive, almost parallel regions. They organize as follows with increasing thickness of the gel: i) a uniform region, ii) a band of clear spots exhibiting an hexagonal arrangement, iii) a domain of stripes. The same sequence *uniform-hexagons-stripes* is analytically predicted as a function of parameters in two-dimensional systems. It has also been produced in simulations close to onset, in three-dimensional systems where the patterns are confined to a monolayer, like in the experiments reported here (Dufiet & Boissonade, preprint 1996).

5. THE ORIGIN OF THE DIFFERENCE BETWEEN DIFFUSION COEFFICIENTS

As already mentioned, one of the main problems in obtaining Turing structures is linked to the requirement for a large enough difference between the diffusion coefficients of the activator and of the inhibitor. But, all the small molecules in aqueous solution such as those involved in the CIMA reaction have almost the same diffusion coefficient. It was theoretically shown (Hunding & Sorensen, 1988; Lengyel & Epstein, 1992; Pearson & Bruno, 1992) that the fast reversible binding of a species on immobile inert sites can lead

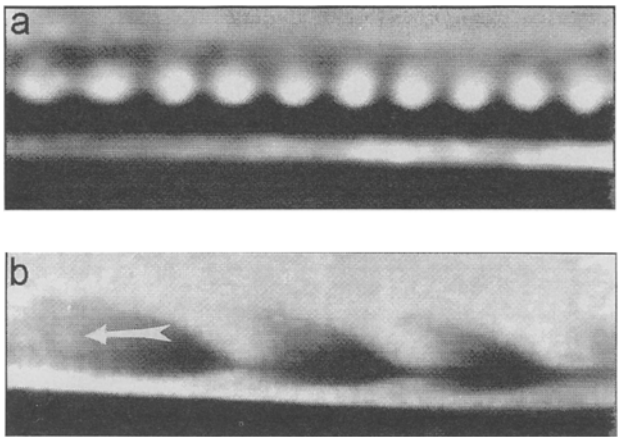

Fig. 5. Transition from stationary pattern to time-dependent pattern in the gel strip reactor. Agarose gel 2%, thickness of the strip: h = 0.14 mm. Experimental conditions: temperature = 2.5°C, residence time in reservoirs = 3 min, concentrations in reservoirs: $[I^-]_I = [I^-]_{II} = 2.5 \times 10^{-3}$ M, $[ClO_2^-]_{II} = 2.4 \times 10^{-2}$ M. a) One single row of stationary spots: [starch] = 1.26 g/l of gel, $[MA]_I = 5 \times 10^{-3}$ M. b) Travelling wave pattern: [starch] = 0.42 g/l, $[MA]_I = 8.5 \times 10^{-3}$ M. The whole pattern moves parallel to the horizontal feed surface.

to a decreased effective diffusivity of this species.

In our system, two macromolecules of reduced mobility are in contact with the reacting species: these are polyacrylamide and starch. In order to evaluate the possible role of gel and starch in Turing pattern formation, experiments were performed in gel-free systems and in different types of gels (polyacrylamide and agarose gels) (Agladze *et al.*, 1992). The sequences observed in all cases are analog to that described in figure 2. This demonstrates that the gel plays no crucial role in the formation of the stationary Turing pattern. However, by decreasing the starch concentration in agarose and in gel-free media, a transition from stationary pattern to waves was found. Such a transition is illustrated figure 5 in a gel strip made of agarose. The row of stationary spots that develops at high starch concentration (fig.5a) is changed into a train of travelling waves at lower starch concentration (fig.5b). Waves travel, as indicated by the arrow, parallel to the feed boundaries. Thus, in agarose gel and in gel-free solutions, the presence of the colour indicator is fundamental to create the difference in diffusivity between activator and inhibitor required for Turing pattern formation. Starch reduces the effective diffusion coefficient of iodide (the activator) by forming a reversible complex with iodine and iodide.

6. GROWTH DYNAMICS OF THE PATTERN

Until now, we have studied the asymptotic states of the system, without considering the setting up dynamics of the stationary pattern. In most cases, starting from the uniform state, crossing the critical parameter value for the onset of Turing patterns induces the emergence of isolated spots at random locations.The pattern is often nucleated by heterogeneities in the gel (i.e. dust particles). Around these spots, the spatial pattern grows in concentric circles

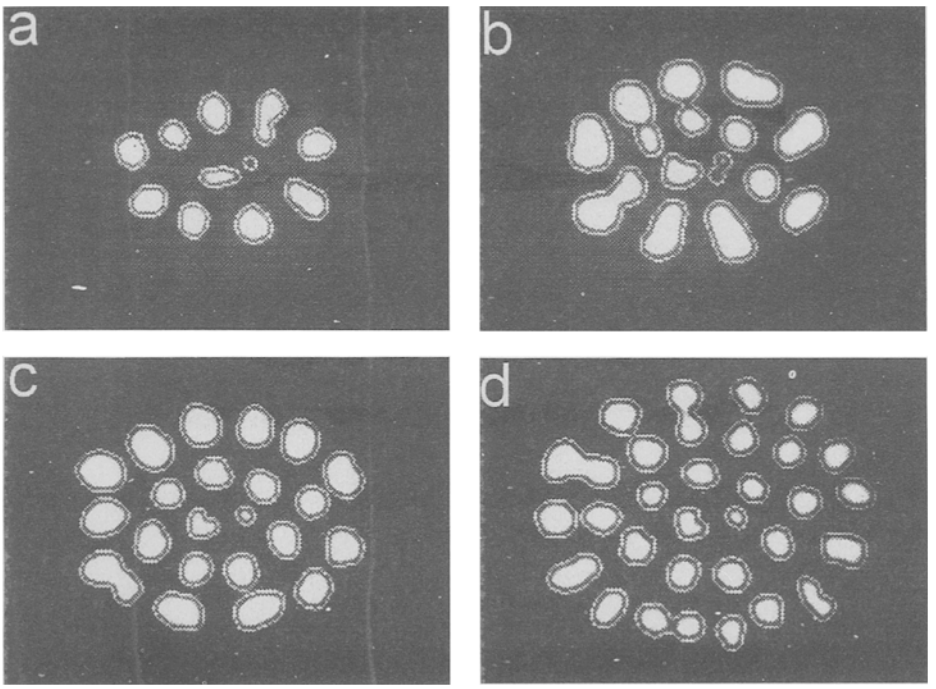

Fig. 6. Pattern growth dynamics by spot splitting. Experimental conditions: temperature = 5°C, residence time in reservoirs = 10 min. Concentrations in reservoirs: $[I^-]_I = [I^-]_{II} = 2 \times 10^{-2}$ M, $[MA]_I = 7 \times 10^{-3}$ M. a-d: series of snapshots taken at 4 min intervals.

that form at a distance from the already developed elements of the pattern. Then, the circles break into spots that ultimately organize in hexagonal arrays.

However, a more surprising pattern growth can be observed in an agarose gel loaded with polyvinylalcohol. Several hours after a small supercritical increase in the malonic acid concentration, growing patches of Turing spots appear in the previously uniform background. They are nucleated, as usual, by heterogeneities of the gel. The already developed spots elongate in the radial and azimuthal directions, and after having reached a critical size, they divide into two spots of quasi-identical sizes (fig.6). The separated daughter spots located at the border of the growing pattern repeat the splitting process. The divisions go on until the whole plane is filled. While pattern keeps growing at the border, spots in the inner part rearrange into regular hexagons. A similar growth mechanism has been found in numerical simulations (Pearson, 1993) and in another reaction (Lee *et al.*, 1994) exhibiting bistability between two uniform state branches. The spot splitting growth mechanism of the pattern is triggered by a spatial perturbation. This pattern formation strongly reminds us of cell division in living systems and could bring another perspective to replicating systems.

7. CONCLUDING REMARKS: REACTION-DIFFUSION AND MORPHOGENESIS IN BIOLOGICAL SYSTEMS

During the long time delay between Turing's prediction and the experimental realization of Turing patterns in a chemical system, reaction-diffusion processes received much attention from theoreticians, specially from biomathematicians which often use them to account for some aspects of morphogenesis. Since the individual steps of development are fairly independent, models can be written for such elementary developmental events. The reaction-diffusion theory has been now applied to quite a large number of biological situations. The basic principles on which models of this type rely are local self-enhancement and long range inhibition. Among the most extensive and famous works based on reaction-diffusion, we mention those by Meinhardt and by Murray.

Using its 'activator-inhibitor' or 'activator-substrate' systems or combinations of these with other features, Meinhardt (Meinhardt, 1982; Koch & Meinhardt, 1994) can account for events as diverse as pattern formation on mollusc shells, hydra regeneration, pattern formation on the coat of mammals, formation of reticulated structures like the fine veins on the wing of a dragonfly, the faceted eye of drosophila, etc...

Among the many works of Murray, we will select his fascinating, if not the most convincing studies of pattern formation on the coat of mammals (Murray, 1989). The author uses a reaction-diffusion model to account for the stripes of the zebra and the spots of the leopard. For him, stripes and spots result from a spatial distribution of the concentration of a morphogen due to a reaction-diffusion process. He envisions this inhomogeneous distribution of morphogen as a 'prepattern' and the final pattern on the coat of mammals would be a response to this reaction-diffusion prepattern. At a given stage of the development of the embryo, melanocytes would or would not produce melanin (the pigment which colours hairs)depending on their position in the morphogen concentration field.

A key problem in the application of the reaction-diffusion theory to morphogenesis lies in the necessity of identifying morphogens in developing tissues. It is only recently that genes and molecules controlling development can be isolated and that their specific function can be determined.

Several molecules which regulate cell adhesivity or extracellular matrix production are positively autoregulatory. In developing vertebrate limb,various such factors have been identified (Newman, 1990). These are the transforming growth factor β (TGFβ) which stimulates its own synthesis, and other differentiation factors which regulate the periodic deposition of the adhesive matrix macromolecule fibronectin. The latter periodic deposition is well simulated by a reaction-diffusion model mechanism elaborated for this particular case. This periodic deposition provides a prepattern for the skeleton. However, let us mention that a completely different model is able to account for the same prepattern formation: the mechanical model is proposed by Murray and Oster (Oster *et al.*, 1983) for generating the cell condensations which evolve in a developing limb and which eventually become cartilage.

In the case of hair formation in the green marine alga *Acetabularia*, calcium has been identified as a possible morphogen (Goodwin *et al.*, 1985). The distance between hairs that simultaneously develop on an annulus at the growing tip of the alga is constant and corresponds to the intrinsic wavelength of the pattern. A periodic distribution of calcium along this annulus has been experimentally demonstrated and simulated by a

reaction-diffusion model (Harrison *et al.*, 1988). It prefigures the hair pattern that subsequently develops. According to Murray (1988), "actual hair growth with its mechanical deformation of the plant is a subsequent process which uses and reflects the prepattern. It is possible that calcium is directly coupled to the mechanical properties of the cytoplasm. Such a coupling could be incorporated into the mechanochemical theory of morphogenesis."

The latter example together with that of vertebrate limb development underline the necessary interplay of several pattern forming mechanisms (reaction-diffusion, mechanochemistry, chemotaxis, surface tension, etc...) in morphogenesis.

REFERENCES

Agladze, K., E. Dulos and P. De Kepper (1992). Turing patterns in confined gel and gel-free media. J. Phys. Chem. 96: 2400-2403.

Babloyantz, A. (1987). Molecules, Dynamics and Life. New York, Wiley.

Boissonade, J. (1988). Stationary structure induced along a reaction-diffusion front by a Turing symmetry breaking instability. J. Phys. (France). 49: 541-546.

Boissonade, J., E. Dulos and P. De Kepper (1995). Turing patterns: From myth to reality. In: R. Kapral and K. Showalter, eds, Chemical Waves and Patterns, p. 222-268. Amsterdam, Kluwer.

Castets, V., E. Dulos, J. Boissonade and P. De Kepper (1990). Experimental evidence of a sustained standing turing-type nonequilibrium chemical pattern. Phys. Rev. Lett. 64: 2953-2956.

De Kepper, P., I.R. Epstein, K. Kustin and M. Orbán (1982). Batch oscillations and spatial wave patterns in chlorite oscillating systems. J. Phys. Chem. 86: 170-171.

De Kepper, P., V. Castets, E. Dulos and J. Boissonade (1991). Turing-type chemical patterns in the chlorite-iodide-malonic acid reaction. Physica D 46: 161-169.

Dewel, G., D. Walgraef and P. Borckmans (1987). Turing instability in anisotropic systems. J. Chim. Phys. Phys. Chim. Biol. 84: 1335.

Dufiet, V. and J. Boissonade (1996). Dynamics of Turing pattern monolayers close to onset. Phys. Rev. E., submitted.

Dulos, E., J. Boissonade and P. De Kepper (1992). Dynamics and morphology of sustained two-dimensional wavetrains. Physica A 188: 120-131.

Field, R.J. and M. Burger, eds. (1985). Oscillations and Travelling Waves in Chemical Systems. New York, Wiley.

Goodwin, B.C., J.D. Murray and D. Baldwin (1985). Calcium, the elusive morphogen in *Acetabularia*, In: S. Bonotto, F. Cinelli and R. Billiau, eds, Proc. 6th Intern. Symp. on *Acetabularia*. Pisa 1984. Belgian Nuclear Center, C.E.N.-S.C.K. Mol, Belgium, 101-108.

Haken, H. (1978). Synergetics, an Introduction. Berlin, Springer-Verlag.

Harrison, L.G., K.T. Graham and B.C. Lakowski (1988). Calcium localization during *Acetabularia* whorl formation: evidence supporting a two-stage hierarchical mechanism. Development 104: 255-262.

Harrison, L.G. (1993). Kinetic Theory of Living Pattern. Cambridge Univ.Press.

Herschkowitz-Kaufman, M. (1975). Bifurcation analysis of nonlinear reaction-diffusion equations II: steady state solutions and comparison with numerical simulations. Bull. Math. Soc. 37: 589-636.

Hunding, A. (1981). Possible prepatterns governing mitosis: the mechanism of spindle-free chromosome movement in *Aulacantha Scolymantha*. J. Theor. Biol. 89: 353-385.

Hunding, A. and P.G. Sorensen (1988). Size adaptation of Turing prepattern. J. Math. Biol. 26: 27-39.

Hunding, A., S.A. Kaufman and B.C. Goodwin (1990). *Drosophila* segmentation: supercomputer simulation of prepattern hierarchy. J.Theor. Biol. 145: 369-384.

Koch A.J. and H. Meinhardt (1994). Biological pattern formation: from basic mechanisms to complex structures. Reviews of modern Physics 66: 1481-1507.

Lacalli, T.C., D. Wilkinson and L.G. Harrison (1988). Theoretical aspects of stripe formation in

relation to *drosophila* segmentation. Development 104: 105-113.

Lee, K.J., W.D. McCormick, J.E. Pearson and H.L. Swinney (1994). Experimental observation of self-replicating spots in a reaction-diffusion system. Nature 369: 215.

Lengyel, L., G. Rabai and I.R. Epstein (1990). Experimental and modeling study of oscillations in the chlorine dioxide-iodinacid reaction. J. Am. Chem. Soc. 112: 9104-9110.

Lengyel, L. and I.R. Epstein (1992). A chemical approach to designing Turing patterns in reaction-diffusion systems. Proc. Nat. Acad. Sci. USA 89: 3977-3979.

Meinhardt, H. (1982). Models of Biological Pattern Formation. New York, Academic Press.

Murray, J.D. (1989). Mathematical Biology. Berlin, Springer-Verlag.

Newman S.A. and H.L. Firsch (1979). Dynamics of squeletal pattern formation in developing chick limb. Science 205: 662-668.

Newman, S.A. and W.A. Comper (1990). 'Generic' physical mechanisms of morphogenesis and pattern formation. Development 110: 1-18.

Nicolis, G. and I. Prigogine (1977). Self-Organization in Nonequilibrium Chemical Systems. New York, Wiley.

Noszticzius Z., W. Horsthemke, W.D. McCormick, H.L. Swinney and W.Y. Tam (1987). Sustained chemical waves in an annular gel reactor: a chemical pinwheel. Nature 329: 619-621.

Oster, G.F., J.D. Murray and A.K. Harris (1983). Mechanical aspects of mesenchymal morphogenesis. J. Embryol. exp. Morphol. 78: 83-125.

Ouyang, Q. and H.L. Swinney (1991). Transition from a uniform state to hexagonal and striped Turing patterns. Nature 352: 610-612.

Ouyang, Q. and H.L. Swinney (1991). Transition to chemical turbulence. Chaos 1: 411-420.

Pearson, J.E. and W.J. Bruno (1992). Pattern formation in an N+Q component reaction-diffusion system. Chaos 2: 513.

Pearson, J.E. (1993). Complex pattern in a simple system. Science 261: 189-191.

Prigogine, I. and G. Nicolis (1967). On symmetry-breaking instabilities in dissipative systems. J. Chem. Phys. 46: 3542-3550.

Turing A.M. (1952). The chemical basis of morphogenesis. Philos.Trans. R. Acad. Sci., London, B327: 37-72.

Zeldovich, Y.B. (1944). The Theory of Combustion and Detonation of Gases. Moscou, AN-SSSR.