Gastrulation as a self-organized symmetry breaking process

Alcides Castro-e-Silva[a,*], Américo T. Bernardes[b]

[a]*Departamento de Física, Instituto de Ciências Exatas, Universidade Federal de Minas Gerais, Caixa Postal 702, 30.123-970, Belo Horizonte/MG, Brazil*
[b]*Departamento de Física, Universidade Federal de Ouro Preto, Campus do Morro do Cruzeiro, 35.400-000, Ouro Preto/MG, Brazil*

Abstract

Among the stages a fertilized egg undergoes until reaching its final shape, gastrulation represents the first step in breaking its initial symmetry. This process is of enormous importance in development of the embryos sagittal symmetry plane or dorso-ventral axis. Gastrulation also results in the appearance of three regions, the germ layers, from which all of the organs and systems of the organism originate. In this paper, following the hypothesis which affirms that pattern formation at some stages of organism development are due to morphogens gradient, we introduce a model which mimics the early stages of gastrulation of many multicellular organisms. In this model, the cause of symmetry breaking is given by the intrinsic dynamics of the system.
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1. Introduction

From the latin *morphe*, shape, form, and *genesis*, to create, to generate; morphogenesis means the generation of new shapes and structures during the

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*Corresponding author.

development of organisms. Morphogenesis as a whole is an unsolved problem. It is remarkable that, after some weeks of incubation, an egg turns into an entire bird. In a short period of time, one single cell suffers transformations and reorganizations which lead to a final arrangement where many different cells, tissues and organs work together in a cooperative and orchestrated way. Morphogenesis, as described below, generally is constituted as a sequence of four basic stages, one of them, gastrulation, which is the earliest epithelial folding process. In this paper, we focus our attention on the basic mechanisms which cause the initial stages of rearrangement of embryo's cells. One of the first attempts to explain morphogenesis was the *homunculus*, which was supposed to be a completely formed organism, in reduced scale, stored in the spermatozoid. When the egg was fertilized, the *homunculus* was transferred to the egg, and with it, all information necessary to "build" a new organism. From this point of view, there was no increase in complexity.

Current knowledge extends far beyond the *homunculus* picture and many aspects of development are quite well understood. Unlike unicellular organisms, which reproduce by fission or conjugation, the majority of multicellular ones do not spring fully formed (of course there are exceptions like Hydra, an example of an animal which reproduce by budding). Generally, these organisms reach their final shape under a relatively slow process of change, with a progressive increase of complexity.

In most cases, the development of a multicellular being starts with a single cell, the fertilized egg called *zygote*. It is important to emphasize that there are many different pathways for the development of an organism. As pointed out by Keller et al. [1]: "this diversity of morphogenic mechanism is driven by the evolution of diverse reproductive strategies and life histories, which may entail the evolution of different egg sizes, amounts and proportions of yolk, and developmental rates." However, even after taking into account this diversity of mechanisms and processes, a general view of morphogenesis can be defined following four basic stages (most pattern of morphogenesis can be set using a combination and/or variation of these four cases. More than this, the stages overlapping is also common.) [2,3].

(1) Right after the formation of the *zygote, cleavage* takes place. At this stage, the egg undergoes an extremely rapid series of mitotic divisions, leading to a cluster of cells called the *morula*. At the end of *cleavage*, the cells (now called *blastomeres*) tend to form a hollow sphere known as the *blastula*. Its interior is called the *blastocoel*.

(2) Once the rate of mitotic divisions has slowed down, or even stopped, the blastomeres start to change their relative positions. This process of cell rearrangement is called *gastrulation*. These cellular migrations lead to an invagination of the blastula, forming the *gastrula*, a bilaterally symmetric three-layered structure. These *germ layers* are responsible for the origin of all organs, tissues and systems of the embryo. The outer layer—the ectoderm—produces the cells of epidermis and the nervous system; the inner layer—the endoderm—the lining of digestive tube and its associated organs (pancreas, liver) and the middle layer—the mesoderm—gives rise to several organs (heart, kidney, gonads), connective tissues (bone, muscles, tendons) and the blood cells (for a general and deep discussion of the mechanisms involved in gastrulation see Ref. [1]).

(3) The next step is organogenesis. After the formation of the three layers, the cells interact with each other producing the organs. Many organs originate from cells of different layers and it is common that the outside of an organ is derived from one layer while the inside comes from another one. During organogenesis, the cells can perform long migrations from their original place to their final location.

(4) The final stage is growth and maturation, where most of the organs and systems, already formed, acquire their functional capabilities. At this point, another very important cell differentiation takes place, when a portion of egg cytoplasm are set apart to originate the *germ* cells, the precursors of the *gametes*. All the other cells are called *somatic* cells. The germ cells typically migrate to the *gonads*, where they differentiate into gametes in a process called gametogenesis. The production of the gametes is only completed when the organism reaches its sexual maturity, usually after its birth.

This paper is focused on the final steps of stage 1 and the beginning of stage 2 described above, that is: *how the blastula turns itself into a gastrula?*. The importance of gastrulation can be viewed in distinct ways. From the developmental biology point of view, gastrulation creates a spatial arrangement, originated by blastula folding, which makes possible the formation of the three layers as well as the contact between cells of different layers. At this stage, many biochemical processes lead to complex cells movements [1,4–6]. During gastrulation, embryo suffers a drastic reduction in symmetry. For many species, blastula can be viewed as a hollow sphere (some cases as a cylinder), which has an infinite number of symmetry axes; so, gastrulation reduces the number of symmetry axes to one. The question we are concerned about is: is it possible that this spatial symmetry breaking happens without any external agent and without even a privileged internal axis? In this paper, following the hypothesis which affirms that pattern formation at some stages of organisms development are due to morphogens gradient, we present a model where such type of breaking of symmetry can be achieved without any kind of external agent; all the information necessary to reduce the symmetry lies on the dynamics of the system, and only local rules are required. From this point of view, gastrulation occurs as a robust and self-organized process.

In the last decade, many different models have been proposed, dealing with the different stages of morphogenesis. The very first attempt to give a mathematical view to the biological patterns observed in Nature was done by D'Arcy Thompson, in his classic *On Growth and Form*, which connected mathematics, geometry and morphogenesis [7]. The problem of pattern formation was framed by Alan Turing, whose analysis serves as the conceptual basis of the present work. His work introduced the concept that morphological patterns can be regulated by biochemical agents, called morphogens, connected in a nonlinear system of reaction-diffusion equations [8,9]. In our case, we focused basically on models which mimic gastrulation. Changes in the structure of the blastula due to cell adhesion or chemotaxis may be modelled in many different ways [10–14], and some simulations described the entire life cycle of an organism [15]. It is interesting to observe that important aspects of the cell's movements are modelled by Potts like models (CPM) [16–18]. In this paper, instead of representing a cell by connected sites in a lattice, we

have a simplified version of those Potts models, where each site represents a cell type. As in other models inspired in magnetism, cell adhesion is simulated by "ferromagnetic interaction" between cells of the same type. Movements of the cells are governed by cell adhesion and diffusion of chemical substances, which is simulated in our model by a time/position varying "magnetic field". The balance of this paper is structured as follows: in the next section we introduce our model. After that, we present our results and discussions.

2. The model

In our model, the embryo as well as the medium where it grows are represented in a cubic lattice N^3. Each site of this lattice represents either a cell, part of the embryo, or a portion of that medium. Each site bears an integer valued variable S_i, the state of the ith site. $S_i = 0$ represents an empty exterior site, while $S_i = 1$ represents a cell (which will become a blastomere), and $S_i = 2$ site indicates the blastocoel.

The growth process starts with the central site occupied by a cell ($S_i = 1$), representing the zygote; all the other sites are empty ($S_i = 0$). The zygote undergoes successive mitotic divisions, which is accomplished by replicating the cells, thus generating cells of the same type. At step one the central site splits in two, the original occupied site is kept and the new cell is placed at one of the 26 empty nearest or next-nearest neighbors, chosen at random. In the second step, the two cells split into four, with the new cells being placed at one of the available neighbors. The system grows under this dynamics until step k. When there is no empty neighbor available, the new cell division promotes a spatial rearrangement as follows: it pushes one of the 26 neighbors from its site and takes its place. The displaced cell does the same with its other neighbors, and the process goes on until it finds an empty site, in a three-dimensional random walk. After k steps we have a set of 2^k cells.

In order to simulate the blastula, we change the cell type under the following rules: a site i with $S_i = 1$ having at least one empty neighboring site remains as it is, otherwise it changes to state 2. After this *differentiation*, the blastula consists of a "skin" of sites in state 1, and an interior of type 2 sites, the blastocoel, as shown in Fig. 1. We caution that what we term "differentiation" is very different from true cellular differentiation. What we have is a set of simple rules which create a system with some characteristics in common with the most simple blastula found in Nature. Finally, a new skin of type 1 cells is created. In our model, like in real systems, the membrane of type 1 cells must avoid the contact of the inner part of the system with the exterior. Our system is defined on a lattice with rigid point-like cells, which cannot change in size and are not flexible. Due to this fact, the skin in our model can easily be broken. Thus, we have to create an extra layer of cells of type 1 to allow the movements of the embryo surface, avoiding rupture during the gastrulation process. At this stage we have an object with spherical symmetry, except for small surface fluctuations.
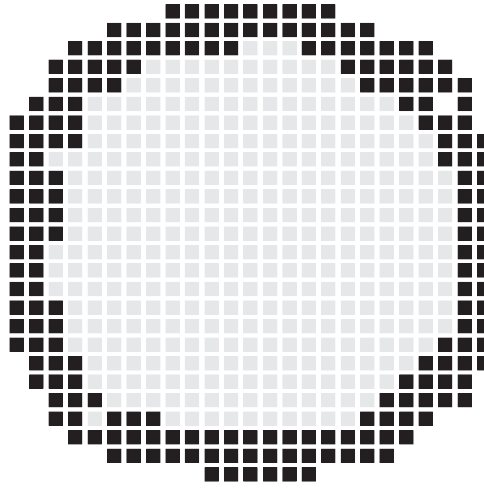
Fig. 1. Cross-section of a three-dimensional simulation of blastomeres migration under the minimization of H, without morphogenic field ($J_2 = 0$). $J_1 = 2.0$ and $T = 0.2$. Since there is no preferential region of movement, no spatial symmetry breaking occurs. In this picture, the blastomeres are represented by filled symbols and the blastocoel are represented by gray ones.

The embryo is now ready to undergo gastrulation via changes in the cellular arrangement. Like in other models, these movements are modelled via a Monte Carlo dynamics, minimizing a Potts-like energy given by

$$\mathcal{H} = -J_1 \sum_{i,j} \delta_{S_i,S_j} - J_2 \sum_{i,j} M_i \delta_{S_i,1} \,, \tag{1}$$

where J_1 and J_2 are parameters and M_i represents the concentration of morphogens at site i. We choose this Hamiltonian in order to describe the two ways in which embryonic cells obtain spatial information: (a) through direct intercellular interaction and (b) from a gradient of diffusible substances (*morphogens*), released by them.

The first term in this Hamiltonian is a local energy component representing the interactions between a cell and its neighbors. The sum is performed over the 26 nearest and next-nearest neighbors [19]. This interaction is supported by the differential adhesion hypothesis, which explains cell movements as a minimization of contact energy at cell interfaces. This hypothesis was successfully used to model cell sorting, where the cells migrate over distances much greater than one cell diameter, to restore disrupted patterns or create new ones [20,21].

The second term, and, in particular, the parameter J_2, plays a crucial role in the model, being the main factor that makes possible self-organization and spontaneous symmetry breaking, since it can force the movement of cells to a region where one has a high concentration of morphogens. In our model, the field M_i can be seen as a morphogenic "field" representing the concentrations of morphogenic substances dissolved in the medium. The morphogenic field is responsible for the transmission of the spatial information over the entire embryo, in a manner very different from

cell–cell interaction [22]. In embryos, the release of morphogens, and consequent creation of a morphogen gradient are due to many factors, related to the relative position of the cells [22]. So, this diffusion process (or change in morphogen concentration) is related to the diffusion or production of chemical substances when, for example and particularly significant for our model, a tension is produced in a membrane, changing its curvature. In our model, and representing the initial symmetry of the system, all sites have the same morphogen concentration in the beginning of the simulation. Movements of the skin cells may change the morphogen concentration, which will in turn influence the evolution of the cell configuration. Such approach is strongly supported by Cummings' model of gastrulation [23–26]. In his work, the Gaussian curvature of a cell layer is a function of a relative concentration of morphogens in the medium.

The Monte Carlo dynamics is performed using the following rules:

(1) Initially, all the M_i are set to zero.

(2) A $S_i = 1$ (blastomere) site and one of its neighbors S_j are randomly chosen.

(3) If this neighbor is $S_j = 0$ or 1 the choice is discarded, and we return to step (2).

(4) If $S_j = 2$ we try to move the cell $S_i = 1$ to position j. The difference in energy ΔE between the new and the old configuration is calculated and the movement is performed with probability 1 if $\Delta E \leqslant 0$, and $e^{-\Delta E/T}$ if $\Delta E > 0$. Temperature here represents the flexibility of the system. Like in other models, which show a qualitative picture of the dynamics, it can be adjusted in order to give us the possible results that can be obtained with this model.

(5) If the movement is accepted, the blastomere S_i occupies the site j. This movement leads to a rearrangement of the embryo's cells: site j pushes one of its neighbors, randomly chosen between its neighbors and avoiding the position i, producing a sequence of movements that reaches the embryo's surface. This happens when a site $S = 1$ moves to a site $S = 0$.

(6) After moving inwards, the blastomere (initially at i) pulls one of its neighbors of the same type 1, randomly chosen, which in turn pulls one neighbor and so on, as a stripe of $S = 1$ cells, avoiding rupture of the membrane. If during this rearrangement the skin breaks (the contact between $S = 0$ and 2 sites), the movement is not accepted.

(7) Once the choice is accepted and the movement is completed, we perform a change in the morphogenic field. Instead of solving reaction-diffusion equations [25], we use a linear function dependent on the distance of a given site to the site i. In order to simulate this feature, i.e., how the information of a cell movement can be transmitted to the environment, the concentration of morphogens at this new site (M_j) increases, and simultaneously decreases in other parts of the system, thus inhibiting cell movements far from that point. In this operation we may obtain a negative value for the field at a given point. It does not constitute a real problem in our model, since what matters is the relative difference in concentration—the gradient of morphogens— between neighboring sites. This operation represents a rapid diffusion of morphogens toward the migrating blastomere, which also increases the concentration of morphogens near the sites where the migration occurred. Thus, a linear gradient of morphogen concentration is established between

site j and a predefined distance (typically the radius of the embryo before gastrulation begins).

(8) Return to step (2).

Only sites with $S_i = 1$ are capable of movement, since they represent the blastomeres. Moreover, the blastomeres can only move to a site with $S_i = 2$, the blastocoel. This is done in order to allow the blastula to fold, and to prevent it from falling apart, since at this time, there is no further production of cells.

3. Results and discussion

The results shown here were obtained with the parameter values $J_1 = 2.0$, $T = 0.2$, $N = 31$, $k = 13$ (2^{13} cells), using 1000 Monte Carlo steps. Larger systems, with $N = 101$, $k = 15$, were also simulated, with the same qualitative results.

Fig. 1 shows the result of a simulation with $J_2 = 0$, i.e., without the morphogenic field M. Here, the dynamics simply reduces the contact energy among the cells, a particular case of cell sorting [20]. In this picture, it can be seen that the blastomeres spread all over the surface, in a qualitatively homogeneous fashion. Thus, a Hamiltonian defined only by a contact energy is not capable of yielding a spherical symmetry breaking, since all sites with blastomeres experience similar fluctuations, and have the same probability to move.

The behavior of the system is drastically changed when M plays a role in the dynamics. Figs. 2a and b show an external 3d view of the system, before and after
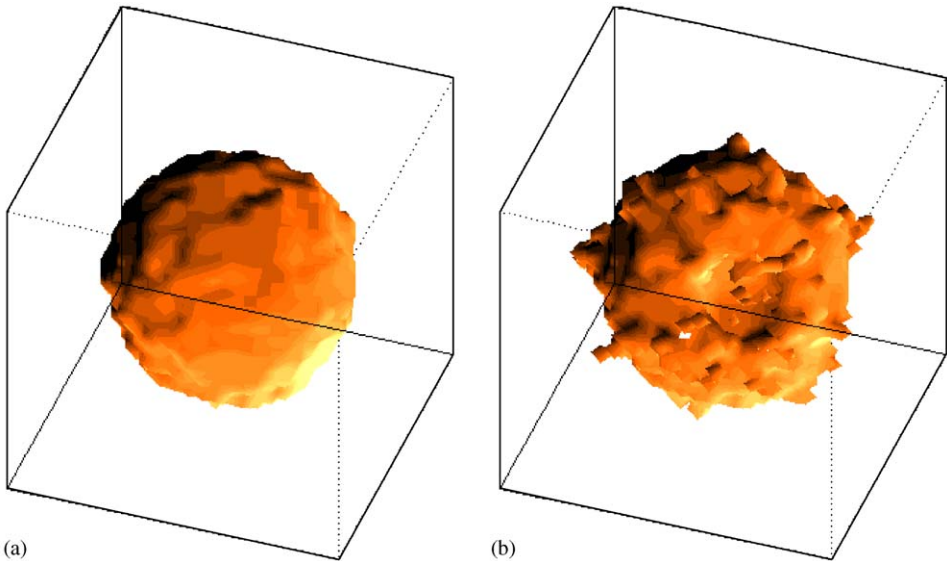

(a)   (b)

Fig. 2. Artistic view of the external region of the system before (a) and (b) after minimization of H. The parameters here are the same as in Fig. 1 except the presence of morphogenic field $J_2 = 1.0$. It can be seen the invagination as a hole in the center of the (b).

relaxation, now with the presence morphogens ($J_2 = 1.0$). Fig. 2a is a *spherical* cell aggregate, which, after the system reaches equilibrium, changes to the form shown in Fig. 2b. One can clearly see that an invagination has appeared on its surface. A better way to see this invagination is slicing the system, as shown in Fig. 3. Fig. 3a shows the border of the system; the concentration of blastomeres is high. Proceeding inwards, one sees in Fig. 3b the border of the invagination (right-bottom part of the figure). While the membrane is homogeneous in almost all regions of this figure, signs of different behavior are observed in the right-bottom region. Figs. 3c–e represent slices in the middle of the system; the invagination is evident. Finally, Fig. 3f shows the other border of the gastrula.

   Figs. 4a and b show, respectively, one slice passing through the same position in the medium S and in the morphogenic field M. In this way, we can see how the morphogens are distributed in the medium. The contours increase from black (low concentration) to white (high concentration). Note that the difference in M between neighboring sites is of the order of unity. The white region of Fig. 4b coincides with the invagination. The figures help to understand the most important characteristics of the model: although there is no a priori a preferential region for gastrula formation, it is rapidly created due to the fluctuations in the concentration of morphogens. Once the process begins, it inhibits any other invagination on the surface of the system. Moreover, the initial process creates a flux of morphogens to
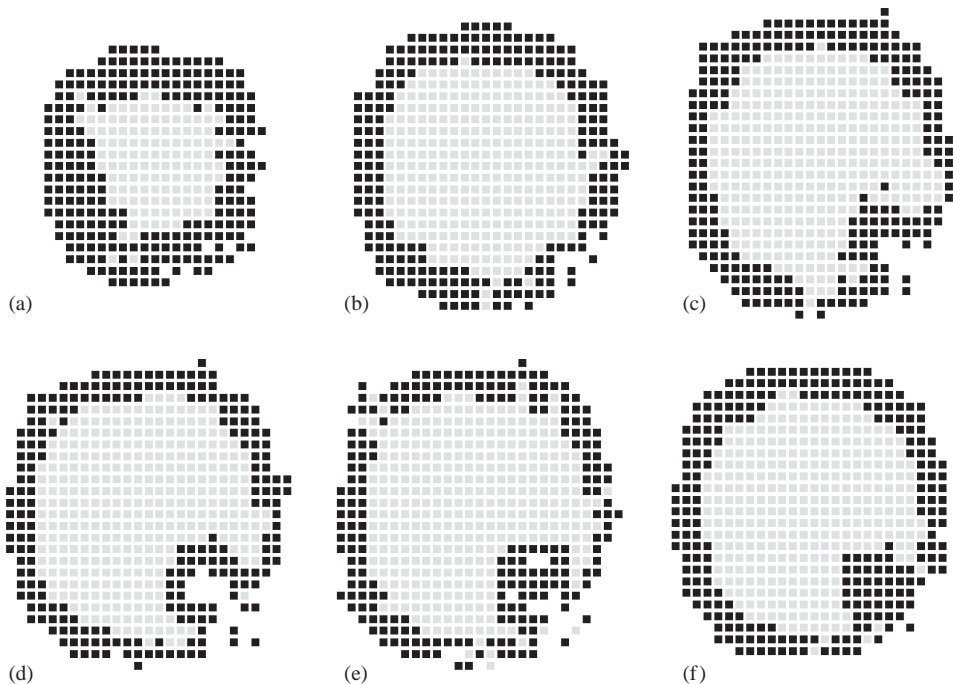

Fig. 3. Sequence of slices showing the inner region where the invagination occurred. The parameter are the same as used in Fig. 2. The meaning of the symbols are the same as in Fig. 1.
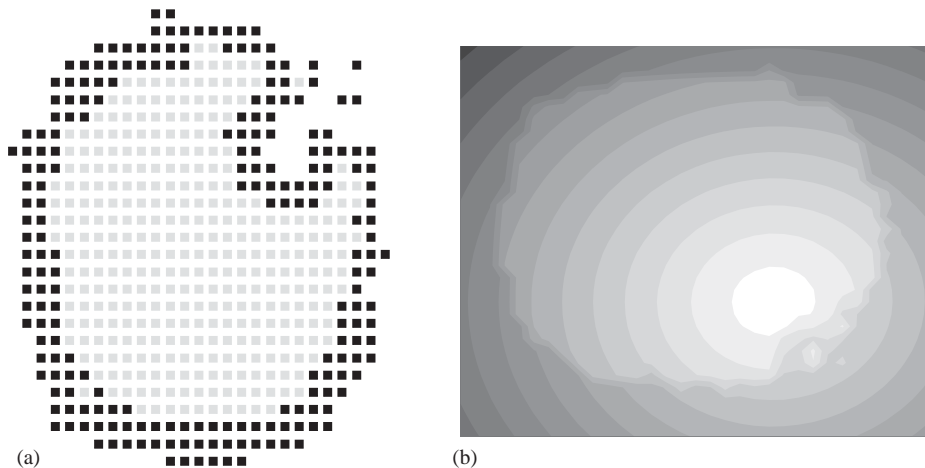
Fig. 4. Two slices of the system showing the medium S (a) and its related morphogenic field M (b). In (a), the meaning of the symbols are the same as in Fig. 1. In (b) higher concentrations are represented by lighter colors.

the invagination region. The higher the concentration of morphogens, the faster is the invagination. In other words, when one cell moves, the concentration of morphogens around the region where the movement took place increases, also increasing the probability of a new movement in this region. At the same time, there is a decrease in such concentrations in regions far from the origin of the movement and then the probability of any cell belonging to those regions to move is reduced. This mechanism prevents the formation of more than one invagination region.

It is important to mention that the dynamics here reported is in accordance with the basic assumptions of previous works [23]. The creation of this invagination region occurs in a self-organized way, since it is not a result of any external agent, or even an internal privileged one. This means that it arises from the interaction of all the elements of the system, in non-linear dynamics. The main component responsible for self-organization is the morphogenic field M, which represents a memory of cellular movements.

From another point of view, one cell movement implies in an energy change, or, in a Hamiltonian's change, that, for its turn, reflects in the subsequent cell movements, creating a feedback loop between the cells and the morphogenic substances released by them. This loop is the key feature that makes possible the system self-organization. It resembles some other non-equilibrium processes, with self-catalytic loops. In those systems, the dynamics is governed by their own products, accelerating or slowing down themselves. Many works propose that self-organized processes driven by self-catalytic loops are responsible for the emergence of order and complexity in natural systems [27].

The model exhibits other remarkable features. The first one is its robustness, that is, the immunity to small changes in the parameters. This is a very desirable property for biological systems, once it introduces a kind of homeostasis, the capacity to

assimilate some perturbations introduced by environment or another source, without causing catastrophic damage. The second characteristic is that the system evolves to an equilibrium state. In the same way that the system "chooses" the region where the invagination occurs, it also defines this equilibrium state. After reaching this state, no further movements are accepted.

Such phenomenon can be seen in Figs. 5 and 6. Fig. 5 shows a cross section of the system at different times. Fig. 6 shows the evolution of the coordinates of the system's center of mass. Initially, the system experiences small changes, representing attempts to gastrulation. Once the region of the invagination is "chosen", a rapid rearrangement is produced, and changes occur in the CM coordinates in the interval of 100–200 MCS. Finally, the process stops. Only few movements are accepted. This occurs because the skin was stretched to its limit. More movements would break it.

Some care must be taken with this kind of assumption. The most relevant aspect is showing that a mechanical force is responsible for stopping the entire process and thus leading the system to reach an equilibrium state. This process resembles those which occur in living beings. In those cases, what is observed is a competition between curvature and an elastic tension, where cell adhesion plays a fundamental role [1]. In real systems, the skin is not stretched to its maximum, like in our
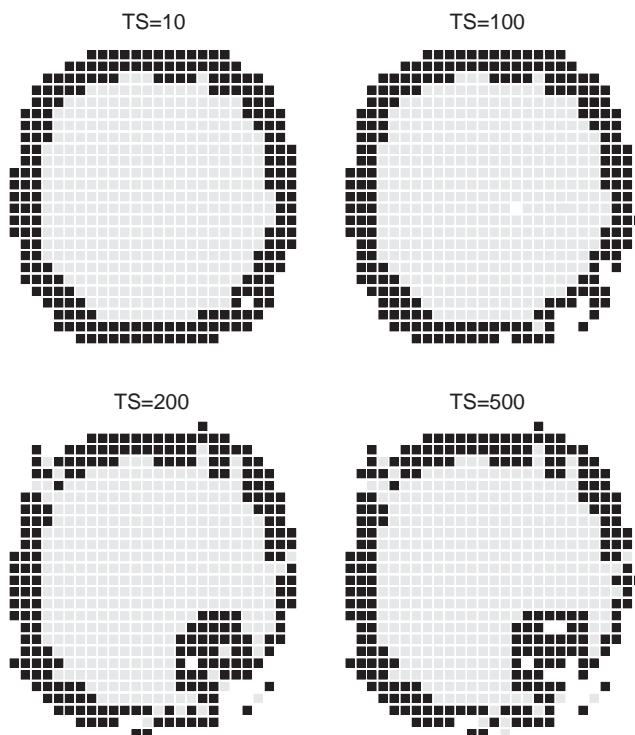

Fig. 5. Time evolution of cellular movements. The parameters are $J_1 = 2.0$, $J_2 = 1.0$ and $T = 0.2$. Note that few changes occurred after 200 MCS.
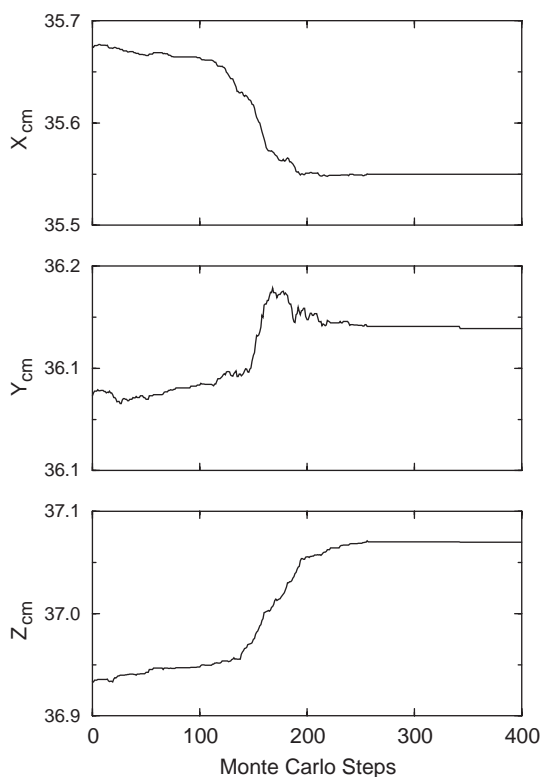
Fig. 6. Evolution of the system's center of mass, given by its spatial three-dimensional coordinates $X_c m$, $Y_c m$ and $Z_c m$, during the blastomeres migration. The system here shown is the same studied in Fig. 5.

simulations. Several models [16,17,21], where deformable cells are represented by many lattice sites, can represent this situation and could be an improvement of our model for future works.

Another important remark is the influence of temperature parameter T. As mentioned above, in this model T is not a real temperature, but gives some sort of softness to the system. Higher T makes the system easier to deform; lower T acts in the opposite way. However, the influence of T is very weak if compared with the other parameters. This can be explained by a spatial argument: since only cells $S = 1$ may move and since these cells are linked forming a skin of cells, it makes their mobility much more dependent on the geometry and the morphogenic field than on the temperature. In other words, mobility is not an advantage when there is no space to move.

The model introduced in this work does not have either the ability or the purpose of explaining the development process beyond the beginning of the gastrulation. It is an approximation even in this respect. It answers the question of how one region can behave differently from the rest, when all, initially, are under the same constraints. Of course, other models answer the same problem, by using mechanical properties or

the action of chemical substances. Our basic proposal was to emphasize the role of morphogens in this process. More than this, we would like also to emphasize that the final product, the final configuration of the system, is obtained by the cooperative action of many simple agents, our "cells", which obey very simple rules imposed by our Hamiltonian. From this point of view, each cell might be considered as an automaton programmed to execute simple set of movements. However, this dynamics becomes highly organized and coordinated when seen as a whole.

Acknowledgements

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