Chapter 1
From Strict Determinism to Self-organization

Abstract We start from reviewing several ubiquitous approaches to morphogenesis and argue that for a more adequate presentation of morphogenesis, they should be replaced by explanatory constructions based upon the self-organization theory (SOT). The first step on this way will be in describing morphogenetic events in terms of the symmetry theory, to distinguish the processes driven either toward increase or toward decrease of the symmetry order and to use Curie principle as a clue. We will show that the only way to combine this principle with experimental data is to conclude that morphogenesis passes via a number of instabilities. The latter, in their turn, point to the domination of nonlinear regimes. Accordingly, we come to the realm of SOT and give a survey of the dynamic modes which it provides. By discussing the physical basis of embryonic self-organization, we focus ourselves on the role of mechanical stresses. We suggest that many (although no all) morphogenetic events can be regarded as retarded relaxations of previously accumulated elastic stresses toward a restricted number of metastable energy wells.

1.1 Deterministic Approaches to Development: Expectations and Impediments

1.1.1 Lessons from Embryonic Regulations

Please take a look at Fig. 1.1, displaying development of sea urchin embryo from a non-fertilized egg (Fig. 1.1a) up to a free-swimming larva (Fig. 1.1l, m). This is a textbook example of embryonic development, known for long ago in great details. Let us put a naïve question: Why just such a succession is taking place at all and why it is reproduced for innumerable set of generations? Obviously, our first suggestion will be that within any stage embryo, a certain set of "causes" is embedded providing its transition to the next stage. How large should be such a set? It is easy to see that as the development proceeds, the structure of embryo becomes ever more complicated: some structures not seen before are emerged. So-called

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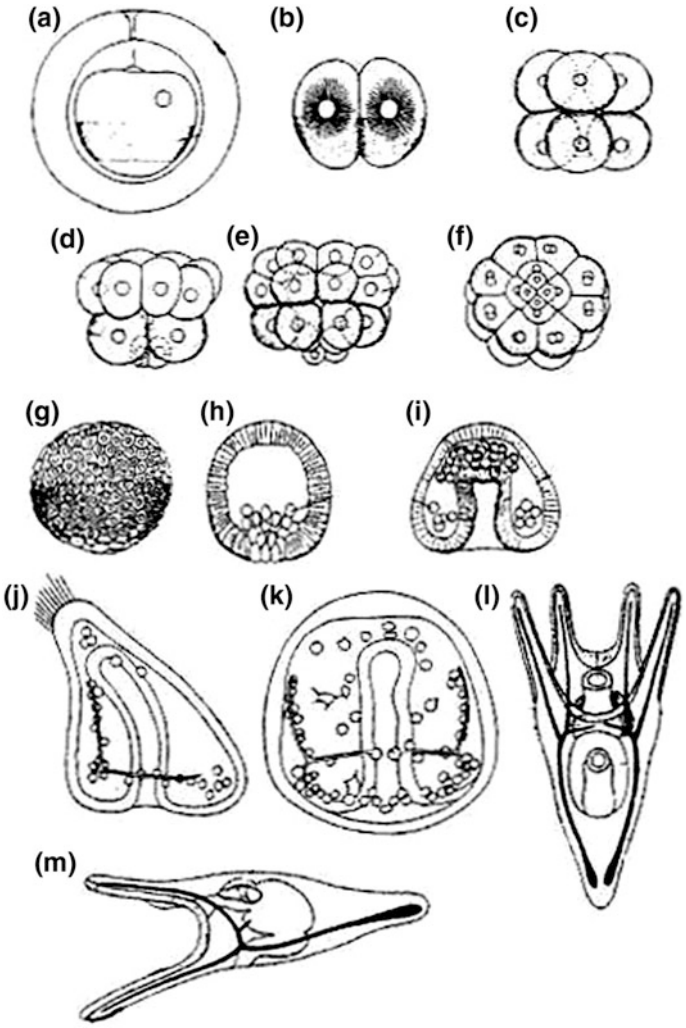

Fig. 1.1 **a**–**m** Successive stages of sea urchin development. **a** An egg within egg membranes; **b**–**f** cleavage; **g**, **h** blastula stage, surface view and sagittal section; **i**–**k** gastrulation in different projections; **l**, **m** pluteus larva in frontal and sagittal projections, correspondingly

arms of free-swimming larvae (Fig. 1.1l, m) are most obvious but not the sole examples of such a complication. Thus, if being consistent, we should suggest that any of the newly arisen structures had its own, individual "cause," settled within an egg in a definite position even before the start of development.

This is a brief exposition of influential ideology of a so-called preformism which dominated in embryology for several centuries and is keeping until now (although in a hidden form) rather strong positions in researchers minds. It is based upon the principles of a so-called Laplacian, or uniform determinism, ascribed (probably, not

at all justifiable) to the great French mathematician Pierre Simon Laplace (1749–1827). By this ideology, the only way for describing and exploring our world is to split it to such a set of cause–effect links that in each of them, a single cause cannot produce more than a single effect (the reverse is permitted: A single effect may require a combination of two or more causes). Until the rise of a quantum physics in the beginning of twentieth century, this ideology was regarded as only one compatible with natural sciences. It is worth mentioning, however, that in physics, it was always more or less shadowed by a law-centered approach (which puts the "causes" to a category of initial conditions and takes them usually as granted). However, in biology and the related sciences, the classical deterministic approach always dominated.

In embryology, it became a basis of one of the most important trends, the so-called Mechanics of Development ("Entwicklungsmechanik" in German) proclaimed by Wilhelm Roux about one and a half century ago (see Moček 1974). By this view, a developing embryo may be simulated by a clockwork which should be experimentally split into minor details in order to understand which one of them "determines" the next part activity; similarly, a task of a researcher would be in dissecting a developing embryo into single parts in order to see which one of them contains the "cause" enforcing another to develop further in a regular way.

By evaluating the role played by "Entwicklungsmechanik" in enlarging and improving our knowledge of development, we come to paradoxical conclusions. On one hand, by using the recommended analytical tools, we recognized a lot about interactions of embryo parts of quite different scales, from whole organs to single cells. But on the other hand—which is often neglected—the conceptual basis of Roux approach (the idea of a strict cause–effect determination) has been undermined already in few years after it was formulated.

This was done by another German embryologist, Hans Driesch, in his experiments on separating from each other two or four blastomeres of sea urchin eggs or on changing their mutual positions. Although Driesch's results have been described virtually in all embryological textbooks, almost never this description was accompanied by conceptual conclusions, forwarded already by Driesch himself and elaborated by recent authors.

As it is widely known, the main result of Driesch's experiments was that fairly normal (although proportionally diminished) larvae with all of their organs properly arranged could be obtained from a single embryonic cell (blastomere) containing no more than ½ (if two first blastomeres were separated) or even ¼ (in the case of four blastomeres separation) of the entire egg's material. Rather soon these effects (defined by Driesch as "embryonic regulations") were numerously confirmed and extended to the species belonging to almost all taxonomic groups of metazoans, from sponges to mammalians. The only noticed difference was the duration of a period of an egg/embryo capacity to regulations: In some groups, such as mollusks or ascidians, this period was rather brief (ending soon after egg's fertilization), while in others (flatworms), it extended over the entire living cycle (interesting, in ascidians, a regulatory capacity is lacking during larva development but restores in adult state). Importantly, after entire embryos lose their regulatory capacities, these

latter are still manifested by their parts: For example, whole limbs or eyes of Vertebrate embryos can be restored from small fragments of these rudiments, or even from dissociated cells. Embryonic regulations took place not only after removal, but also after experimental addition of some excessive amount of embryonic material.

Besides separating blastomeres, Driesch changed their mutual positions by compressing cleaving eggs for some time period. After being released, the eggs also developed in a normal way, although each of the blastomeres became surrounded by abnormal neighbors. Fairly normal embryos, although not in 100 % of cases have been obtained later from dissociated-reaggregated masses of sea urchin blastomeres (Spiegel and Spiegel 1975).

What can these experiments tell us about cause–effect relations? If continuing to apply deterministic approach to embryonic regulations, we have to conclude that complete sets of "causes" required for further development are contained not only within whole eggs/embryos but also in their halves, quarters, etc.; on the other hand, as a rule, the sets are not increased with the addition of embryonic material. Moreover, each time (depending upon the type of a disturbance performed) this hypothetical "set of causes" should change its arrangement for producing the normal pattern. Obviously, under these circumstances, a concept of an individual "cause" (precursor) for any embryonic structure becomes meaningless.

Driesch fully recognized this critical situation. So far as in his time scientific knowledge was in fact identified with strict cause–effect determinism, he concluded that embryonic regulations undermine the very basis of natural sciences. Such a position put this outstanding thinker outside the scientific mainstream, which hampered further study of embryonic regulations for several decades. Driesch formulated his final conclusion from his regulation studies as a law which in slightly simplified form sounds like this: "The fate of an embryo part is a function of its position within a whole" (Driesch 1921). Its idea is in the following. Suggest that both the normal and experimentally disturbed embryos possess a kind of a coordinate grid (including, for example, a polar axis and a set of latitudes) which is each time adjusted to embryo dimensions (being diminished in embryos having a part of their material removed and enlarged in those getting excessive additional amount of material). Each part of the embryo is endowed by a capacity to "read" its own coordinates and to develop accordingly, even if this does not coincide with the normal fate of this part.

Looking at the first glance as an adequate generalization of embryonic regulations and related phenomena, this statement contains nevertheless some hidden contradictions and leaves a number of questions unsolved. The first of them is about the reference points of the postulated coordinate grids. Do they correspond to certain small previously settled structural elements of otherwise homogeneous embryo, to entire embryo geometry and/or topology or to something else? How should the reference points be arranged for providing formation of similar adults out of differently disturbed eggs/embryos?

The second set of questions relates to the notion of "fate." So far as during embryo development any of its parts constantly changes, its position in any system

of coordinates and the notion of "fate" may include developmental periods of quite a different longevity—we are urged to define how long should be the developmental period determined by a given position. This question is closely connected with another, even more important one: What is the nature of the postulated connections between a position and a "fate" of embryonic element, whatever being the latter? Can we point to any universal dynamic component playing a leading role in all the position-fate dependencies or each of them has nothing in common with the others?

The most popular concept pretending to answer these questions is that of "positional information" (PI) (Wolpert 1969, 1996). Appearing after several decades of almost complete oblivion of Driesch's ideas, it aimed to modernize them because the very fact of positional dependencies in embryonic development could not be further ignored. By doing this, Wolpert started from postulating the existence of a few (as a rule two) structural elements of embryo acting as reference points for PI perceived by all the other elements (cells). In more concrete versions of PI concept, the reference points were identified as the source and the sink of a chemical substance (called morphogen) which creates concentration gradient between these points. It is the local morphogen concentration to be "read" and "interpreted" by any embryonic cell (independently of its neighbors) determining thus its fate.

If discussing the problem of reference points, the main trouble for PI concept is lack of robustness to mutual shifts of reference points which inevitably accompany any of experimental disturbances. Let us trace some examples, starting from the so-called French Flag (FF) model, a basic one for PI concept.

According to its name, FF model is dealing with 3-stripe axisymmetric pattern. If putting the "source" and the "sink" to the opposite poles of the main axis and making removals or additions of tissue pieces precisely axisymmetric, such a reference system will be formally suitable for preserving the initial pattern (Fig. 1.2a, b). However, if making tissue removals/additions even slightly asymmetric (which is almost usually the case), the reference points themselves will be shifted asymmetrically, thus distorting the resulting pattern (Fig. 1.2c). Even more important is to remind that axisymmetric eggs/embryos are rare exceptions among those capable of regulations: Rather, most of the eggs already soon after fertilization acquire irreversible differences [called dorso-ventral (DV)] between opposite sides. In these, any removals/additions of embryonic material will shift any pair of points into positions geometrically non-homologous to initial ones (a–a_1, b–b_1, Fig. 1.2d), thus inevitably distorting PI pattern. We can see that any formal way to save PI concept is to suggest that PI is "emanated" from *all* the material points of a given stage embryo, rather than from any previously selected ones. This brings us to the fundamental non-classical idea of *non-locality*, associated with *collective interactions* of a large number of equivalent elements. The both notions, central for a self-organization theory (SOT), will be discussed further in this and the next chapters. Meanwhile, if taken alone, the idea of the multiple PI bearers will be able to interpret embryonic regulations only if the initial shape of the embryo was not significantly changed after experimental perturbations. It will not work, for example, when pretty normal shapes

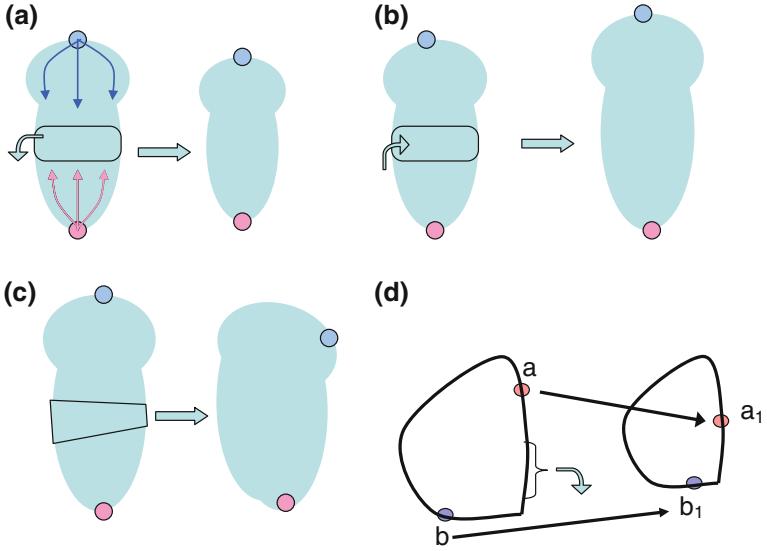

Fig. 1.2 Non-robustness of PI model. Small lilac and *blue circles* depict hypothetical sources of PI, the latter shown by *arrows* at the *upper left* frame. Following PI model embryonic regulations will become possible only if (as in frames **a**, **b**) embryonic body and PI sources are axisymmetric and removals (**a**) or additions (**b**) of embryonic material do not disturb axial symmetry. If, however, the pieces of removed or added material are asymmetric (**c**), or such is the initial shape of the intact embryo (**d**), no restoration of geometric similarity viewpoint is possible within the framework of the PI model

will emerge de novo out of completely chaotic cell arrangement, like in the above-mentioned Spiegel and Spiegel (1975) experiments. Such events belong to a "pure" self-organization and cannot be explained by any concepts demanding a more or less precise initial PI, whether it comes either from single elements or their collectives.

Another problem associated with PI concept is that of relations between cell positions (in any reference system) and their "fates." Actually, PI concept is rather uncertain on the exact meaning of the "fate." Is it identical to the final cell differentiation (which is highly improbable if PI is assumed to be set at initial stages), or just to a next small step of development? In any case, the idea of transformation of cell position (local morphogen concentration) into its fate raises a number of problems. Some of them have been discussed by Furusawa and Kaneko (2006). The authors argue that even in most obvious examples of concentration-dependent action of certain agents (in their case, activin), "the pattern formation… is not predetermined from spatial information, but rather through intracellular dynamics and interaction. Spatial patterns and intracellular states mutually stabilize robust pattern formation…" They present model data showing that PI itself is not enough for establishing order in the population of heterogeneous cells, so that such notions

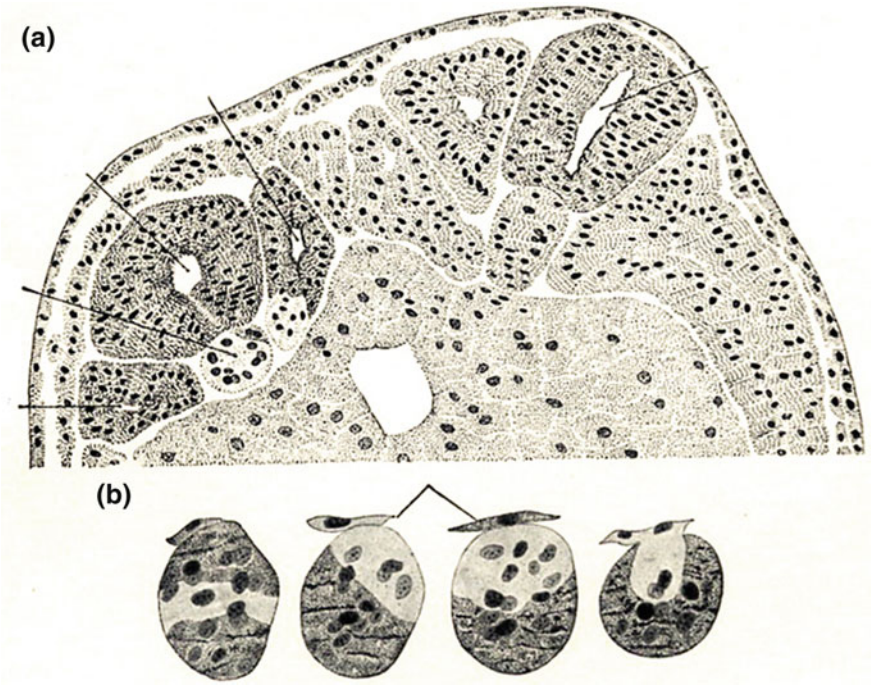

Fig. 1.3 Random mutual arrangement of transplanted inductor's tissue (*light*) and host tissue (*dark*) in the first Spemann and Mangold (1924) experiment on embryonic induction, abolishing the inductor's capacity to be a PI source. **a** Cross-sectional area of the host embryo with its normal axial organs to the right and induced organs to the left. **b** Several cross sections of the chimeric notochord. From Spemann (1936)

as nonlinear intracellular dynamics and attractors are required for getting realistic results. All of these belong to the SOT vocabulary.

By discussing these matters, we come to the most troublesome problem of development—actually going well beyond PI concept—which can be defined as that of *interpretation*.

If we have a certain signal (no matter being located inside of outside of embryonic body) which generates a definite response from the latter, our main interest is to know why such a relation between the signal and the response is taking place. As mentioned in Introduction, we have two epistemological models which can be used for solving this task: Either there is a reason to postulate each time a unique one-to-one cause–effect (signal–response) relation—in this case, our work will consist in compiling a comprehensive list of such relations; or we regard each relation as a particular manifestation of a general law. For example, if we relate velocity of a thrown stone to its position, we do not suggest that a new "specific" force is associated with each next position: Rather, we are searching for a common law embracing all the positions including those never occupied by the stone.

Considered in this context, PI concept resides a strange intermediate place: On the one hand, it ascribes that the leading role in development to a largely non-specific factor of position, which in physical sciences, is always used for constructing an embracing law (as a rule, describing certain field); but, on the other hand, each next position of embryonic elements is claimed to be connected with quite specific response, having no relations with another one.[1] This, by my view, makes the entire PI concept tautological, adding nothing to a mere descriptive approach which takes spatial patterns as given. Let us look now for the situation with interpretation problem in other branches of developmental biology.

1.1.2 Can Embryonic Inductions Be Regarded as Cause–Effect Relations?

The discovery and further exploration of embryonic inductions by Hans Spemann and his followers may look at first glance as a triumph of Wilhelm Roux causal approach: It was shown indeed that one part of embryo can be a crucial factor for the development of another. Does it mean, however, that the inductors can be regarded as a kind of blueprints, or as "PI sources" for induced tissues? It is enough to have a look to the picture from the famous first Spemann and Mangold paper (Fig. 1.3) for seeing how far this is from reality. We can see that the inductor's and host tissues (discerned by their pigmentation as being taken from two different Triton species) are mixed at random, both in the notochord and the neural tube. Nevertheless, the entire structure of the complex of axial organs is perfectly ordered. It means that the inductor tissue cannot serve not only as a spatial template, but even as a source of a hypothetical PI gradient for the reacting tissue: Formation of a proper set of axial organs under the influence of an inductor looks more as embryonic regulation in Driesch sense rather than a kind of a direct causation. Or, if speaking in terms of a SOT (to be later on accounted in this chapter), it was the long-range order, independent from the "micropatterns" of the inductors and host tissues, to be established in the first Spemann and Mangold experiments. At the intuitive level, this was perfectly apprehended by Spemann himself who considered the action of inductor as "abstract," that is, containing no "information" about spatial details (Spemann 1936). This conclusion was later on specified by Waddington as following: "Clearly, the problem [of induction] reduces to that of a complex response to a simple stimulus …somewhere along the line an increase in complexity occurs" (Waddington 1962).

Usually, the problem of complication during embryonic induction is resolved in terms of concentration gradients of inductive substances assumed to be set between

[1] My friend, American biologist Albert Harris, liked to compare PI with a price politics in non-marked economies: The prices (equivalent to local morphologies or cell types) are appointed ad hoc, without being regulated by any mutual feedbacks.

animal and vegetal embryo poles, or between its dorsal and ventral sides (e.g., De Robertis 2009). If accepting the presence of such a macroscopic gradient-like prepattern, the isolated small pieces of embryonic tissue cannot produce more than small parts of it. However, already in the old Holtfreter's (1938) experiment, a miniature copy of entire embryo was obtained from a piece of embryonic tissue extirpated from so-called marginal zone. As commented by Gerhardt (1998) "Holtfreter brought to light an individualistic and anti-authoritarian view of the embryo in which competent responsive cells interact in a self-organizing community, in place of conceptions of the embryo as a collection of naïve passive members dependent for their future on detailed directions from a central organizer."

A modern concept of so-called default induction, reducing the inductors' role to "inhibition of inhibitor" (Hemmati-Brivanloue and Melton 1997) may be regarded as a next step from the cause–effect ideology toward that of self-organization. Indeed, the inductors, instead of being the bearers of the positive "information," become a kind of releasers (triggers) of the potencies already preexisted in reacting tissues. As in the cases of embryonic regulations, this situation cannot be adequately described without using such notions belonging to SOT as a nonlinearity, potential relief (describing a state of embryonic cell), and others. A special question will be whether such a self-organization can be at least partly based upon morphomechanics. Later on, we hope to bring some evidences in favor of such a suggestion.

1.1.3 Genetic Program of Development: Does It Actually Exist?

In not so remote past, a claim that the course of development is "genetically programmed" was accepted as an absolute truth, even in spite of the lack of proper understanding what the "program of development" actually means. So stunned were the successes in deciphering the key roles of genes in "controlling" the development of embryonic rudiments that all the instructions for "making a fly" [a paraphrase of the title of famous Lawrence (1992) book] looked to be in our hands. Only closer to our days, it became realized that our believing to govern the development by switching on or off any genes or signaling pathways is the same as operating an electronic device by pushing its buttons without having even a slight idea on how it actually works.

For clarifying the situation, two main groups of evidences have to be mentioned: one of them related to classical biology and the other recently emerged as a result of unexpected discoveries in modern molecular genetics.

The first group of evidences claims that the factors determining space–time schedule of genes expression are non-genetic in their nature and topography. This statement, which creates the basis of biology for about a century and is supported by experiments on nuclei transplantations and many others, is on its own enough

for concluding that genes themselves should obey outside instructions which are called epigenetic. Meanwhile, recently it was complemented by numerous observations showing that relations between genes and signaling pathways on the one hand and their developmental targets on the other hand turned out to be quite far from being one to one: The products of activity of the same or closely homologous genes and/or of the same signaling pathways were found to be involved in quite different developmental events. Modern textbooks are full of such examples. Here are just a few of them:

- The interactions between msx-1 and msx-2 homeodomain proteins characterize the formation of teeth in the jaw field, the progress zone in the limb field, and the neural retina in the eye (Gilbert 2010).
- The transcription factor Pax-6 is expressed at different times and at different levels in the telencephalon, hindbrain, and spinal cord of the central nervous system; in the lens, cornea, neural and pigmented retina, lacrimal gland, and conjunctiva of the eye; and in the pancreas (Alberts et al. 2003).
- In *Drosophila* embryos, a gene Engrailed is involved in segmentation of a germ band, development of intestine, nervous system, and wings. In mouse, same gene participates in brain and somite development. In Echinodermata, it takes part in skeleton and nervous system development (Alberts et al. 2003).
- Delta–Notch signaling pathway regulates the following: neuro-epithelial differentiation in insects, feather formation in birds, fates of blastomeres in Nematodes, differentiation of T-lymphocytes, etc. (Alberts et al. 2003).
- Hunchback gene is involved at the early stage of Drosophila development as one of so-called gap genes and at the later stages participates in development of neural system.

For the similar conclusions, as related to signaling pathways, see Kupiec (2009). Shrewd remarks on this topic can be found in (Gordon 1999 V. 1, pp. 59–64).

Anyway, our present-day image on genetic regulation of development contains two great negations: (1) even complete knowledge of genome structure cannot tell us what gene will be expressed in a given space/time location; (2) even from exhaustive knowledge of space/temporal schedule of genes expression, one cannot predict what morphological structures will be formed in these definite locations.

Certainly, this is not to claim that the genes play no role in development at all. On the contrary, their role is crucial in permitting or abolishing development of the single structures and their ensembles; in particular, they may affect shapes of entire embryos or their parts. A proper conclusion from the above said is that their action should produce a definite morphological results only if being an integral part of quite extended and ramified regulatory contours, including the feedbacks coming from the upper-level events, such as cell shapes and mechanical forces. Actually, such a situation is in generally acknowledged, but the conclusion is in most cases expressed in an allegoric form, by claiming that genes action is "context-dependent." The urgent aim will be in transforming this vague formulation into a concrete research program.

1.2 Main Notions and Principles of SOT, Applied to Developmental Events

Within one or two last decades, the word "self-organization" became among the most generally used ones, not only in science, but also in politics and every-day life. Meanwhile, for most of the users, it remains to be nothing more than a mere word, or a kind of vague metaphor; only few people knows that it is a designation of a strict theory, being in its essence mathematical but deeply rooted in physics, biology, economy, and even humanitarian sciences. SOT is treated in a number of perfect books ranging from very special to popular ones; among the latter, simplicity and strictness are adequately combined in the book by Capra (1996). For the readers who do not like math, a very qualified and perfectly illustrated account of the main SOT principles by Ball (2001) can be recommended. The aim of this section is more limited: It is in outlining only those notions and concepts of SOT which are necessary for interpreting adequately development of organisms. The first of them has been formulated and widely used well before the emergence of SOT: this is the symmetry theory. In certain sense, the term "symmetry" shares the destiny of a "self-organization": Both are widely used without apprehending their deep meanings. Meanwhile, not only for developmental biology but also for other branches of life sciences, the applications of the main notions of a symmetry theory are quite useful and adequate.

1.2.1 Translating Developmental Events into the Language of Symmetry Theory

A remarkable property of this theory is that it may be regarded as a compact model of any law-oriented science, aiming to search for invariable basis within a set of varying events. In other words, it is dealing with the so-called invariable transformations, keeping constant some properties of a body which in other relations is changing. The transformations used for testing the invariability are the movements in a broad sense, including so-called isometric transformations keeping the form and the dimensions of the object constant as well as the different kinds of deformations. The structural elements taken for testing the invariance may also be qualitatively different, being exemplified either by a macroscopic design (fitting in a particular case with the overall shape of a body), or by the positions of small (point-like) elements of a body. The symmetry evaluated by the first criteria is called geometric, while that using the second criteria is defined as colored (what means that the selected small elements are assumed to be distinguished by different colors). Although the distinctions between geometric and colored symmetry were introduced by persons non-familiar with biology, they luckily correspond to the differences between two main components of development: morphogenesis (overall shape changes) and cell differentiation (changes on the single-cell level).

1.2.1.1 Some Designations Related to Isometric Transformations

In elementary symmetry, three categories of isometric movements are considered: rotations, reflections, and translations (linear shifts). A number of movements of each category which brings a body into coincidence with itself are defined as a symmetry order, and the combination of all such movements for a given body is its symmetry group. Thus, rotational symmetry order of a square is 4 (this is the number of all the rotations—to 90°, 180°, 270° and 360°—matching a square with itself). Accordingly, rotation symmetry order for equidistant triangle is 3 (120°, 240° and 360°); any body (assuming that its shape is not changed during rotation) has at least rotation symmetry of the order 1. The presence of reflection symmetry is defined by letter *m* (the first letter of a French word "miroir" or English "mirror") (Fig. 1.4a, b). Numbers of reflection planes possessed by a given body are not included in the formulas of symmetry groups because it has been proved that if the reflection symmetry takes place at all, its order is equal to that of the rotation symmetry. Thus, symmetry group for a square which has four reflection planes (vertical, horizontal and two diagonal) is written as $4 \cdot m$, and symmetry group of an equidistant triangle as $3 \cdot m$ (reflection planes coincide with three bisectors).

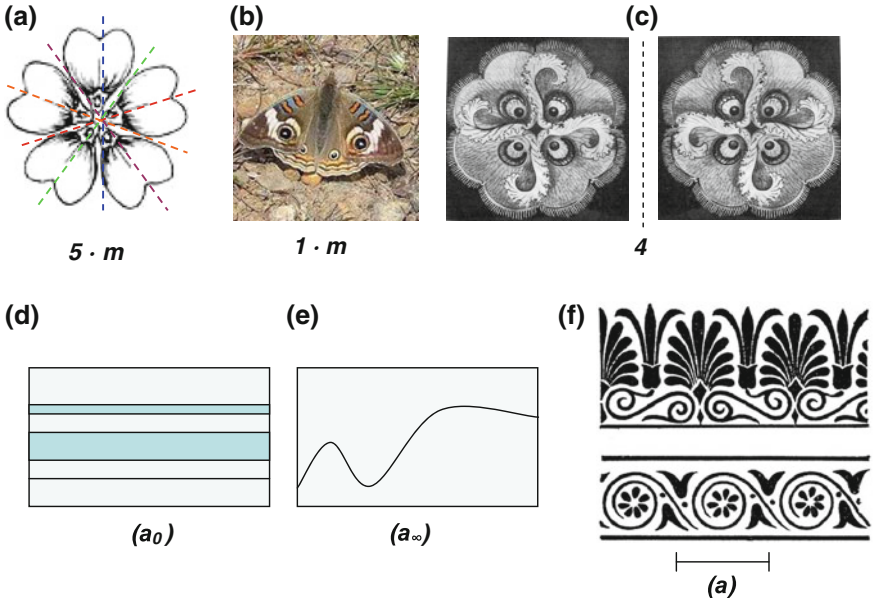

Fig. 1.4 Bodies belonging to different symmetry groups. **a** Rotation symmetry of order 5, combined with the same order mirror symmetry. **b** Mirror symmetry only. **c** A pair of enantiomorphic bodies with fourth-order rotation symmetry reflected to each other by a mirror plane (*vertical dashed line*). **d–f** Translational symmetries of zero, infinite, and finite orders correspondingly. Below each frame symmetry orders are shown. **a–c** and **f** from Shubnikov and Kopzik (1972) with the authors permission

On the other hand, a form depicted in Fig. 1.4c possesses only rotation, but not reflection symmetry. Accordingly, its symmetry group is 4. Any object lacking reflection symmetry has arbitrarily defined left and right configurations matching each other by a reflection. Such objects are called enantiomorphic.

For circles, disks, and cones, the order of rotational symmetry is infinite. So far as these bodies have also infinite number of reflection planes, their complete symmetry group is $\infty \cdot m$. A sphere possesses rotational symmetry around an infinite bundle of its central axes oriented at any angles to each other. Its symmetry group is defined as $\infty/\infty \cdot m$ (a slash means that the angles between rotation axes take arbitrary values). For comparing infinite symmetries, the notion of symmetry power is used. Accordingly, symmetry power of a sphere is greater than that of a disk.

For displaying a reflection plane perpendicular to the axis of rotational symmetry, a sign ":" is used. Thus, symmetry of a bi-cone as well as of a disk of finite thickness is $\infty : m$. Symmetry of a cylinder is $m \cdot \infty : m$.

Translational symmetry is that of linear shifts (transpositions) of a body in relation to its initial position. This kind of symmetry is evaluated by the length of a linear transposition matching a given structure with itself. It is defined as (a). Completely homogeneous bodies or those with a design arranged parallel to the shifts directions are self-coincided under any shifts and have symmetry order (a_0) (Fig. 1.4d). On the other hand, if the design is not at all repeated, the body has the infinite-order symmetry (a_∞) (Fig. 1.4e). An example of design having finite translational symmetry order is given in Fig. 1.4f.

In classical biology (both zoology and botany), the notions of symmetry are used in most cases for comparing static forms belonging to different taxonomic groups. Moreover, the compared symmetries are related as a rule to higher structural levels only. Playing an important role in morphology and taxonomic studies, this approach does not permit to penetrate deeply in developmental problems. Aiming to do just this, we shall compare now the symmetry orders of the different levels processes and the changes of symmetry orders at successive stages of development, using both descriptive and experimental criteria. We hope to show that such an enterprise will promote to clarify our views upon the driving forces of development.

1.2.1.2 Symmetry Orders on the Different Structural Levels

Whereas in ideal crystal bodies, the symmetry order of the crystal lattice is held on all macroscopic levels, in non-crystal bodies to which living beings belong, symmetry orders of different structural levels may be uncoupled. Even without using overtly the notions of symmetry, the researchers of a remote past knew this and believed in its biological importance. One of the most popular generalizations in the century back embryology was that "a whole is more precise than its parts" (e.g., Gurwitsch 1930). In the language of symmetry theory, this means that the symmetry order of a whole body or of its large enough areas is higher than that of its smaller parts (Fig. 1.5a); same are symmetry relations between the induced axial

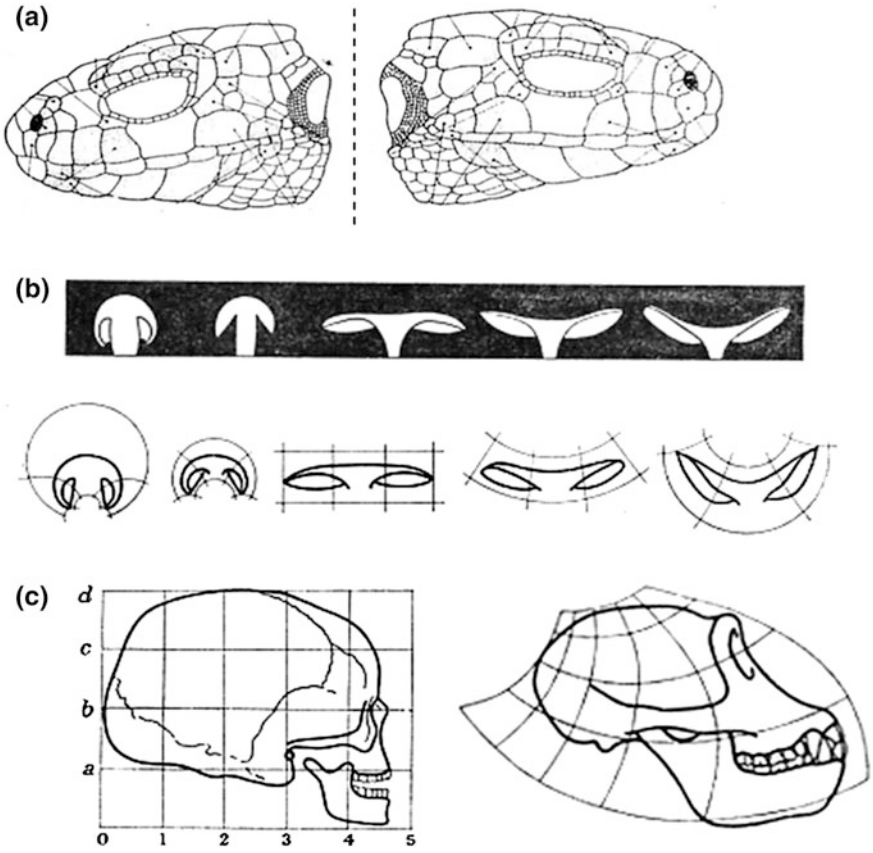

Fig. 1.5 Relations of symmetry orders between different-level structures. **a** A perfect mirror symmetry of *left* and *right* skull parts of the lizard, *Lacerta agilis*, taken as a whole (reflection plane shown by *vertical dotted line*) is combined with the lack of such symmetry at the level of bone plates. **b**, **c** Conformal symmetry transformations, preserving *rectangular shapes* of small parts (symmetry orders 4 m) under substantial deformations taking place at upper levels. **b** Growth of a mushroom fruit body. **c** Comparison of a human and chimpanzee skulls. **a** from Zacharov (1987); **b**, **c** from Petuchov (1981), with the author's permissions

organs taken as wholes and the areas occupied by inductor tissue (see Fig. 1.3). This was considered as a strong argument for holistic regulation of development. More recently, however, the reverse situations have been described (Petuchov 1981). In these cases, described by the so-called conformal symmetry, the shape and the symmetry order of small constituent parts remains invariable, while that of larger areas is extensively distorted (Fig. 1.5b, c).

At the present time, an interest to the symmetry of small body parts, including single cells, was essentially increased owing to discovery of so-called planar cell polarity, most overtly expressed in epithelial cells (Vladar et al. 2009; Eaton and Julicher 2011). This means that in addition to well-known apico-basal polarity

oriented perpendicularly to cell layer plane, a cell possesses also a polarity oriented in the layer's plane. Accordingly, symmetry group of a planarly cell should be reduced from $n \cdot m$ (taking n-edged cell with apico-basal polarity only) to $1 \cdot m$ (in case of the cells with mirror symmetry plane), and even to 1 (if a cell exhibits left–right dissymmetry, as shown by Xu et al. 2007). Although up to now our knowledge of 3-dimensional cell shapes is still rudimentary, there is no doubt that it affects cell differentiation (see Chap. 4 for more details).

What are relations between single-cell symmetries and those of higher structural levels? Does a "whole" dictate symmetry order to its parts? Is true the reverse or even the both levels' symmetries are established independently? The question is far from being solved, but some remarkable examples of a "symmetry orders exchange" between different levels can be traced and will be discussed in more details in Chap. 3. Here, it is worth to emphasize that the relations between the different-level symmetries are closely related to morphomechanics. As argued by Cademartiri et al. (2012), the direct transposition of the constituent parts symmetry to the upper structural levels corresponds to equilibrium state of solid bodies. On the contrary, the increase of a symmetry order at the upper level as compared to those of its constituent parts is typical for the equilibrium state of the liquids. Thus, by comparing the symmetry orders of different levels, we may conclude whether a living body becomes fluidized or instead solidified.

1.2.1.3 Curie Principle and Symmetry Breaks

One of the most important generalizations of symmetry theory is that formulated more than a century ago by a French physicist Pierre Curie (Curie 1894) for the crystal bodies and electromagnetic events. Only recently its applicability to a wider set of events including morphogenesis was acknowledged. Here is the initial formulation of Curie principle (op. cit):

> When certain causes produce certain effects, the elements of symmetry of the causes must be found in the produced effects.
> When certain effects show certain asymmetry, this asymmetry must be found in the causes that gave rise to them.
> The reverse of these propositions is not true, at least in practice that is to say that the produced effects can be more symmetric than the causes.

Thus, Curie principle forbids "spontaneous" (causeless) *decrease* of symmetry order of a given system, but permits its spontaneous *increase*. It is directly related to the second law of thermodynamics. Indeed, the increase of the symmetry order is equivalent to homogenization of a body structure and to enhancement of the freedom degrees of its constituent particles—hence to the entropy increase. Accordingly, the decrease of the symmetry order means establishment of an ordered heterogeneity restricting the particles' degrees of freedom, which corresponds to the decrease of entropy.

Let us trace how the symmetry order of developing organism is changed from the very beginning of egg's development. In our analysis, we shall ignore left–right dissymmetry as being strictly determined by certain supramolecular structures (mictotubules and microfilaments). This issue will be discussed in Chap. 2.

Up to fertilization, the overall shape of oocyte is not changed at all or is changed in an irregular way. This does not permit to use geometric criteria of symmetry. On the contrary, the usage of colored symmetry (addressed to small regions of the body: polar bodies and markers of its future dorsal side) is much more adequate. Indeed, before the extrusion of polar bodies, the polar (future animal–vegetal) axis can take potentially any direction; hence, by these criteria, an egg has the highest possible (spherical) symmetry order ($\infty/\infty \cdot m$) (Fig. 1.6a). [Note that our evaluation is based upon considering an (imaginary) *set* of bodies, rather than a single one; same approach will be used in other cases as well]. When after the second polar body extrusion the position of the polar axis becomes strictly determined, the symmetry order is reduced to $\infty \cdot m$ (Fig. 1.6b). Next, after setting up location of the dorsal side (which is often associated with egg's fertilization) symmetry order becomes $1 \cdot m$ (Fig. 1.6c). Another routine example of the reduction of circular

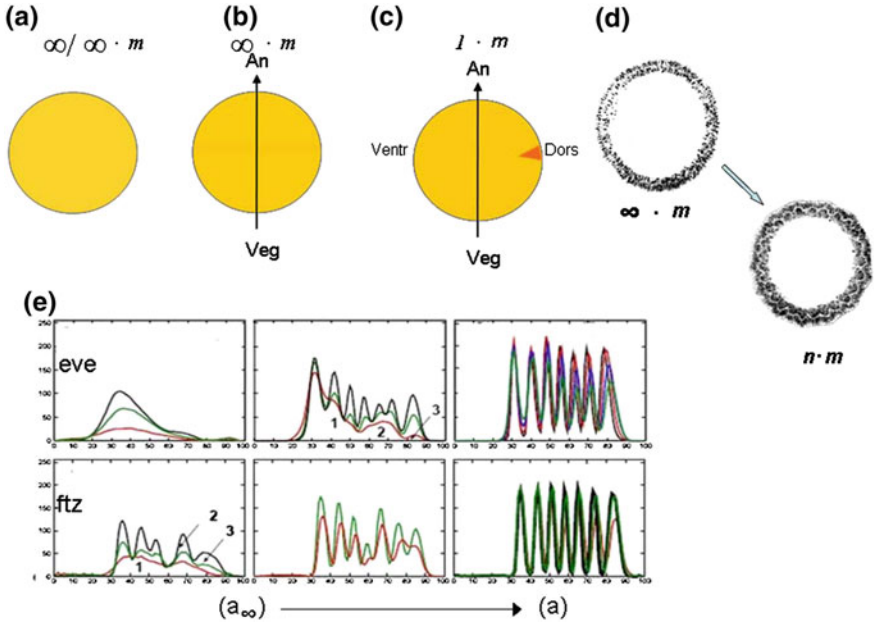

Fig. 1.6 Reductions of symmetry order (symmetry breaks) during development. **a** Non-germinated egg prior to establishment of animal–vegetal polarity. **b** Polarized egg. **c** Egg acquiring dorsoventrality. **d** Formation of tentacles ring in the oral region of hydroid polyp illustrating reduction of rotation symmetry order. **e** Transformation of infinite-to-finite translational symmetry in genes expression patterns of *Drosophila* embryos. *Upper line*: eve, *lower line*: fushi tarazu genes. Frames from left to right correspond to successive periods of development. **e** is from Surkova et al. (2013) with the author's permission, modified

symmetry order is provided by sectioning an initially homogeneous ring into a number of similar angular units, for example, tentacles (Fig. 1.6d).

More advanced stages of development are in many cases associated with acquiring a finite order of translational symmetry. The classical example of such transformation is segmentation of embryonic mesoderm (see Chap. 4 for more details). Recently to this one, the phenomena of the metameric genes expression in *Drosophila* embryos have been added (Alberts et al. 2003). For properly interpreting the finally established symmetries, it is necessary to know the developmental history of the arisen patterns. As shown by Surkova et al. (2013), instead of a previously expected homogeneity or smooth expression gradients, the regular segmented patterns have been emerged from quite variable ones (Fig. 1.6e, frames from the left to the right). This indicates the reduction of the translational symmetry order from infinite to finite and the corresponding entropy decrease.

So by a broadest survey, the general course of development looks as a succession of the reductions in symmetry order, defined also as symmetry breaks. (In no way, this excludes the existence of the periods of symmetry order increases, located between the breaks and/or on the other structural levels: see below for more details). Now the urgent question will be whether it would be possible (as demanded by Curie principle) to find for each symmetry break an external agent having a similarly broken symmetry which can be directly transmitted to a developing organism.

To the credit of experimenters almost surely unfamiliar with Curie principle be it said, they stubbornly looked for the agents which might serve as external "symmetry breakers." To a considerable part, their search was successful: In a number of species, the animal egg pole (the site of polar bodies extrusion) turned out to be determined by the position of the oocyte in relation to follicular cells; similarly, molecular determinants of a future dorsal side of an amphibian egg became located strictly opposite to sperm entrance point (see Chap. 3 for details). In brown algae, the egg pole giving rise to rhizoid became oriented oppositely to the source of light (Jaffe 1969). Thus, Curie principle seemed to be saved at a low price. However, further investigations of similar objects showed that the situation is not so straightforward. The rhizoid of algae eggs was growing in a polarized manner even in the case of isotropic illumination (op. cit); similarly, amphibian eggs underwent dorsalization in the absence of sperm (during parthenogenesis) or when the sperm was inserted exactly in the animal pole being thus unable to break symmetry (Nieuwkoop 1977); moreover, in mammalian eggs, the sperm entrance point is completely unrelated with embryo polarity. It is even more hopeless to find external dissymmetrizers for more advanced structures characterized by translational asymmetry: None of embryonic inductors are able to play this role.

In addition to the observations of intact embryos, the standard experimental procedures associated with changes of mutual positions of body parts can also be taken as adequate criteria of a symmetry order inherent for a given stage embryo. Indeed, if any possible replacements of embryo parts are compatible with its further normal development, we may say that by the criteria of a developmental fate, the given stage embryo has the highest possible symmetry order. Accordingly, if any

single replacement will disturb the entire developmental pathway, the symmetry order estimated by the same criteria should be reduced to 1. So by experimental criteria, similarly to morphological ones, the reduction of symmetry order coincides with the advancement of development.

In some cases, the intermediate steps of such reduction can be traced. One of the best examples is given by classical works of the American embryologist Ross Harrison performed almost a century ago (Harrison 1918). The researcher was interested in tracing the capacity of the limb rudiment to adjust its orientation to the entire body antero-posterior (AP) polarity at the successive stages of development. For this purpose, he turned a still flat limb rudiment (limb disk) of urodelean embryos in such a way that either both AP and DV axes, or only one of them was rotated to 180° in relation to the AP axis of entire body (To rotate just one axis was possible by transplanting a limb disk to the opposite side of embryo) (Fig. 1.7).

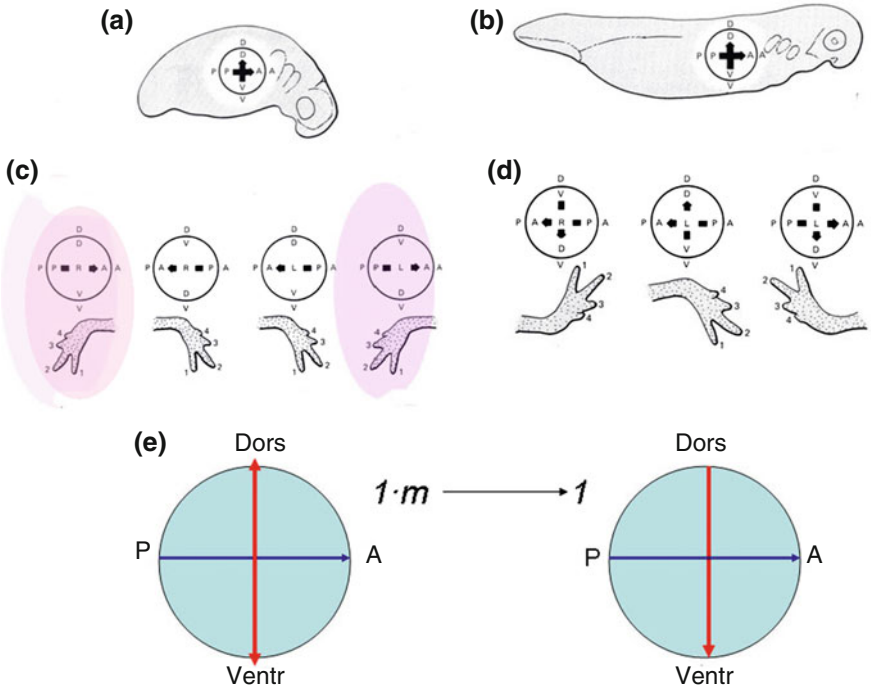

Fig. 1.7 Harrison's experiments on regulations of limb orientation according to body axes. **a**, **b** Ambystoma embryos at two successive stages of development. Shown are dorso-ventral (DV) and antero-posterior (AP) axes of entire bodies and limb buds. **c** A normal limb disk orientation (*left frame*) and results of its rotations at stage A. Only rotations of DV axis keeping AP axis intact (*right rose frame*) are compatible with normal orientation of a limb bud. **d** Results of limb bud rotations at stage **b**. None of these are compatible with normal limb orientation. **e** Harrison's results described in symmetry terms

The results crucially depended upon the stage when the operation has been performed. At the earliest stage, the normal limb orientation was restored after rotation of the both axes of a disk; at the intermediate stage, the restoration could be achieved after rotation of DV, but not AP disk axis (see frames marked by rose color), and at the most advanced stage, none of the axes' rotations were compatible with restoration of the normal orientation. In addition to demonstrate by experimental criteria the successive symmetry breaks, these results show that determination of limb polarity is in each stage essentially holistic: At the intermediate stage, it is AP axis as a whole, rather than any small material element which specifies its final fate. This property permits to describe the results of Harrison experiments in the symmetry terms: By a criteria of the developmental fate, at the initial stage, the limb disk has a rotational symmetry of infinite order ($\infty \cdot m$); at the intermediate stage, it is reduced to $1 \cdot m$ (symmetry axis coinciding with DV axis); and at the final stage the symmetry order becomes 1.

It is important (besides all, for satisfying Popper's falsification criteria) that it is possible not only to imagine but to reproduce experimentally some morphogenetic processes with exactly opposite symmetry dynamics, that is, tending to increase rather than decrease symmetry order. Among those, most important are the so-called cell sorting events described by Townes and Holtfreter (1955) and interpreted in terms of the differential adhesion hypothesis (Steinberg 1978). These experiments start from randomized arrangement of different types of strictly determined cells which become finally segregated into concentric layers (Fig. 1.8). Initial configurations, if evaluated by criteria of color symmetry, cannot be matched with themselves by any kind of movements due to cells heterogeneity: Hence, their symmetry order is 1. On the contrary, the final arrangement is much more symmetric, approaching roughly the symmetry order of a sphere ($\infty/\infty \cdot m$).

Another instructive example is given by Elsdale (1972) observations on the behavior of fibroblast monolayers in the presence of collagenase, destructing collagen fibers. Under these conditions, randomly oriented fibroblasts were grouping into vast domains of parallel oriented cells. Finally, all of the domains have been fused into a single giant one. To what kind of symmetry transformations should we attribute these processes?

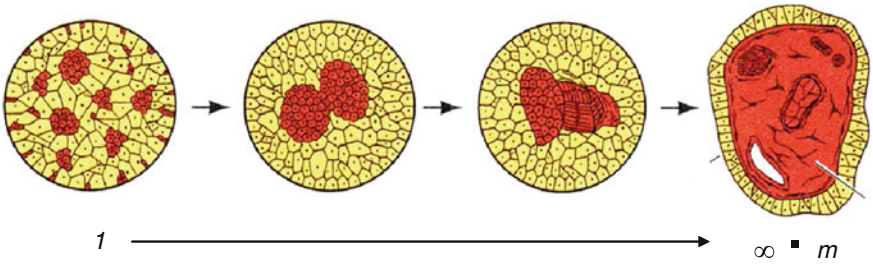

Fig. 1.8 Symmetry transformations under standard cell sorting experiments. For detailed description, see text

The key event here is the axiality ($2 \cdot m$ symmetry order) of each individual cell (lacking in the previous example). If we deliberately neglect it, we have to assume the initial symmetry order to be very high ($\infty \cdot m$ for the case of monolayer) so that the subsequent transformations should be estimated as the reduction of symmetry order down to $1 \cdot m$. This is not so, however, because the formation and fusion of the domains is based upon the inherent axiality of individual cells. Taking this into consideration, the initial symmetry will be of the order 1: a population of the randomly oriented axially symmetric cells would not coincide with itself under any shifts or rotations. Similarly to the previous example, the final stage is characterized by the increase of symmetry order up to $1 \cdot m$. Same is the evolution of the freedom degrees of the individual cells: Their mutual shifts are mostly hampered under random arrangement but facilitated under parallel one. In this respect, the behavior of aligned cells is quite similar to that of the liquid crystals (to be discussed below in this chapter).

> Noteworthy, such a type of behavior is taking place in the absence of long-range interactions, which may be introduced by seeding cells onto elastic substrates or by deposition of extracellular matrix. As we will show in Chap. 3, under the latter conditions, the initial symmetry order will be reduced.

Sometimes, the short-and long-range interactions coexist at different scales. By studying the formation of supramolecular structures (so-called ciliary units) in unicellular Ciliates, Frankel (1989) established a linear threshold (about 1 μm length) subdividing a smaller realm into which the symmetry order of the units' arrangement is "dependent only on the intrinsic properties of the building blocks" (op. cit) from the larger scale one where the units become oriented according to the entire body handedness, rather than their own chirality sign. Thus, the "Frankel's barrier" delimits the domination zones of the short- and long-range interactions.

It is of interest to note that in the most primitive metazoans, the short-range order and the corresponding tendencies to symmetry increase seem to dominate: Thus, in slime molds, the proper mutual positioning of their only two cell types, so-called prestalk and prespore cells, is achieved by a kind of cell sorting (Nicol et al. 1999) rather than according to their positions within a whole. On the other hand, in all the real metazoans developing from macroscopic eggs, the long-range types of order and the associated successions of symmetry reduction dominate from the earlier stages.

Now it will be important to address again to the Curie principle. How should we estimate it after recognizing that most of developmental symmetry breaks look as being proceeded spontaneously? Is this an argument for rejecting the principle? Before doing this, let us explore the situation in more details: This will permit us not only save Curie principle in relation to embryonic development at any price, but make this in a highly constructive way. The matter is that the Curie principle is not bound in any way to exact magnitudes of symmetry breaking agents: in fact, they may be indefinitely small. On the other hand, both whole organisms and their constituent parts are always exposed to some kinds of "noise," that is, to small perturbations of quite different nature coming from somewhere outside. Accordingly, instead of

rejecting Curie principle, one may suggest that for becoming able to symmetry breaks, the embryos should acquire a high sensitivity to some kinds of noise, perceiving them as dissymmetrizing agents; on the other hand during other periods, they should be indifferent to many external disturbances. This idea brings us closely to fundamental notions of SOT, and first of all to those of instability and stability. Let us look now how these notions and principles can help us in comprehending the main property of morphogenesis—regular complication of organic shapes.

1.2.2 Parametric and Dynamic Regulations: Several Basic Models

1.2.2.1 Stratification of Variables According to Characteristic Times

As mentioned in the Introduction, among the main properties of our world is its stratification to a number of more or less discrete levels distinguished from each other not only by characteristic dimensions (Lch) but also by "characteristic times," or Tch (reversed rates) of the events. Although in biology a scale of structural levels has been used for long ago, its real importance could be apprehended only within SOT framework. This is because there are differences in Tch which permit to distinguish two main categories of the variables: the *dynamic* ones characterized by small Tch and the *parameters*, whose Tch should be at least in an order greater.

In the following table, the dimensional and temporal ranges of Lch and Tch belonging to several levels most important for developmental processes are given:

Description of the level	Lch, meters	Tch, seconds
1. Macromolecules transducing chemical energy into mechanical	$\approx 10^{-8}$	$10^{-3} - 10^{0}$ (relaxation time)
2. Supramolecular non-covalently bound structures	$10^{-7} - 10^{-5}$	$\approx 10^{0}$ (assembly−disassembly time)
3. Single cells	10^{-5}	$\approx 10^{2}$ (average time for changing neighbors)
4. Embryonic territories capable of regulations (morphogenetic fields)	$10^{-4} - 10^{-3}$	$10^{2} - 10^{3}$ (time from formation to next step of segregation)
5. Whole organisms	$10^{-3} - 10^{1}$	$10^{5} - 10^{8}$ (duration of life cycle)

1.2.2.2 Linear and Nonlinear Feedbacks: Links to Embryology

Self-organization is impossible if the variable whose changes are a matter of our interest does not act back upon itself, whether positively or negatively. The simplest (but quite far from being the only one) way to explore feedbacks is to take as

examples autocatalytic or autoinhibitory chemical reactions. The first of them display positive and the second negative feedbacks between the amount of synthesized substance and the rate of its synthesis. The both can be described, in the first approximation, by linear differential equations: $dx/dt = kx - C$ for autocatalytic and $dx/dt = -kx + C$ for autoinhibitory reactions. Here, x is a dynamic variable while k and C are the parameters, their Tch being, ex definitio, at least an order greater than Tch for x. Even such simple feedbacks, as depending upon the sign of the parameter, can reproduce two main states of any self-organizing system: a dynamic (Lyapunov's) stability or instability. Indeed, at $k < 0$, a dynamic variable comes to a single stable state, while under $k > 0$, it becomes unstable and diverges toward $\pm\infty$; in practice, that means that it does not exist at all.

The feedback loops can be complicated in different ways: by including positive and negative ones in the same equation, by increasing the number of variables or by passing toward nonlinearity. Let us start from exploring the latter way.

While in the context of linear differential equations, instabilities lead to nothing except destructing the system, if introducing the second (and better a third)-order nonlinearity, they become to play a constructive role by increasing the system's complexity (reducing its symmetry order). In this way, nonlinearities cooperate with Curie principle. Let us also take into mind that nonlinearity (the existence of more than one solution for a given argument value) directly contradicts the classical determinism ("one cause–one effect" paradigm). Endowing the system with the elements of randomness (non-predictability), the nonlinearity provides at the same time its capacity to produce *novelties,* that is, something beforehand non-existed and even non-predictable. As claimed by Prigogine and Stengers (1984): "A novelty is a measure of a causal independence (indefiniteness) of successive states of a developing subject in relation to preceded ones." Neither individual, nor evolutionary development can take place without acquiring novelties, and hence without nonlinearity.

A simplest model illustrating the acquiring of novelties and reduction of symmetry order is described by the following third-order differential equation:

$$dx/dt = kx - k_1x^3 \ (k_1 > 0) \tag{1.1}$$

It combines a first-order positive feedback with a third-order negative one. As can be checked by simple algebraic calculations, at $k < 0$ the equation has only one solution ($x = 0$) which is stable, while at $k > 0$, it has three solutions ($x_1 = 0$; $x_{2,3} = \pm \sqrt{k/k_1}$) among which x_1 becomes unstable and new ones ($x_{2,3}$) are stable (Fig. 1.9a). While passing from $k < 0$ to $k > 0$, the variable x moves toward one of two new stable solutions from any point of the *phase space*,[2] leaving x_1 under any negligibly small perturbation along the x axis (this is so-called soft regime). At the same time, the indefinite-order translational symmetry characterizing $k < 0$ area is

[2] A phase space is a space in which all possible states of a system are represented, with each possible state of the system corresponding to one unique point in the phase space.

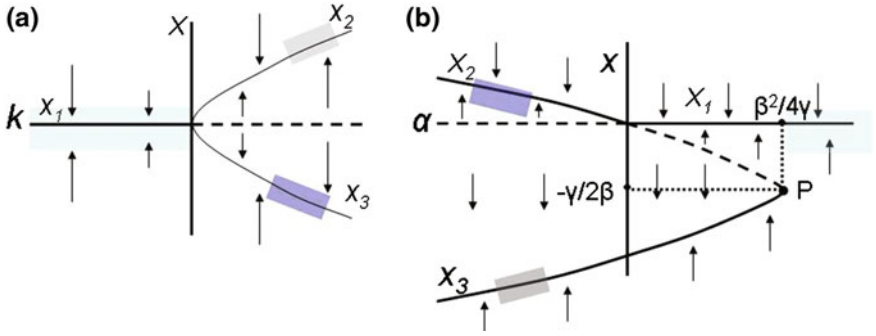

Fig. 1.9 **a**, **b** Soft and hard bifurcation regimes. In the both cases, parameters (k and α, respectively) are plotted along the horizontal and the dynamic variable (x) along the vertical axes. X1 is non-differentiated state (marked by *light blue*) split into alternative differentiation states x2 and x3 (*dark* and *light lilac*) either by infinitesimal perturbations (soft regime: **a**, $k > 0$, B, $\alpha < 0$) or by finite perturbations (hard regime: **b**, $0 < \alpha < \beta 2/4\gamma$)

completely lost. Let us note that variations of the parameter k values (with another parameter, k_1, being constant throughout) and those of the variable x play quite different roles in determining the system's behavior, namely a shift of the k parameter value from negative to positive endows a system by a *possibility to select* any one of the newly emerged stable states (x_2 or x_3), while *the result of selection* will depend upon whether the perturbation of a dynamic variable is shifted toward positive or negative x values. Hence, the first decision is regulated *parametrically*, that is by relatively slow and spatially smoothed evolution, while the second is regulated *dynamically*, due to faster and more local events. Such a strict separation between acquiring the ability to select one of the developmental pathways, and the selection itself is one of the most essential properties of organic development, being noticed by embryologists well before SOT was outlined. In embryological terms, the first (parametrically regulated) property is called *competence,* while the second (dynamically regulated) is defined as *determination* of a given part of embryonic tissue. A possibility to describe these properties in SOT language means that embryonic development obeys universal laws of nonlinear systems behavior.

Let us emphasize that at $k > 0$, initial values of the dynamic variable required for reaching a definite stable state do not need to be set up precisely: It is enough to limit their range either by a positive or by a negative semi-infinity. In mathematical terms, this means that dynamic regulation can be (and as a rule is) highly degenerative. This is in opposition to a widespread opinion that biological systems require very precise regulation. Actually, it is not so: in fact, their extreme reliability is based upon the capacity to produce precise responses to non-precise impulses. This again may take place only in nonlinear regimes.

Even more adequate for representing the universal properties of developing systems is a somewhat complicated version of Eq. (1.1) with an additional quadratic term, describing a new second-order feedback:

$$dx/dt = \alpha x - \beta x^2 - \gamma x^3 \tag{1.2}$$

This equation (Fig. 1.9b) always has one solution ($x = 0$) which is stable at positive and unstable at negative α values. Meanwhile, under $\alpha < \beta^2/4\gamma$ two other solutions appear in a peculiar and biologically relevant asymmetric manner, namely both of them are emerged in a single point P of a phase space as a kind of jump [rather being smoothly branched from a previously existed solution as it took place in Eq. (1.1)]. Among them, the middle one (x_2) is unstable under positive and stable under negative α values while x_1 solution behaves in a reverse manner. The solution x_3 is always stable.

These properties, associated with the appearance of a new feedback very much enrich the developmental potencies and regulatory properties of the imaged system. While that one described by Eq. (1.1) should pass toward new stable states under any infinitesimal perturbations (in a so called soft regime), the transition from x_1 to x_3 at $\alpha > 0$ will go now only under finite perturbations, because in this area of α values the two stable states are separated from each other with the instability barriers. The latter's existence opens new ways for the dynamic regulation of the system's behavior making it more reliable. At the same time, Eq. (1.2) learn us that the state of a competence (which depends upon the parameters values) may itself undergo a qualitative evolution from the area $\alpha > 0$ characterized by requirement of a finite perturbation for reaching x_3 to $\alpha < 0$ when the same transition can be reached in a soft regime.

1.2.2.3 Periodic Regimes and Creation of New Levels

Let us consider now a system of differential equations with two dynamic variables, x and y, having drastically different Tch: y evolving much faster than x. Accordingly, x is defined as a slow variable and y as a fast one. For equalizing the right parts rates, the term dy/dt is multiplied by so-called small parameter ε. A simplest system of this kind to be of interest is called Van der Pol equations. It looks like

$$dx/dt = y \tag{1.3a}$$

$$\varepsilon \cdot dy/dt = -\left(y^3 + ay + x\right) \tag{1.3b}$$

As one can see, the both variables are linked by "+, −" feedback loop: The variable y acts positively to the variable x, while x acts negatively to y. Another new property of the system is its three-level structure (if including the parameter a, assumed to be constant). The system's behavior is characterized by the presence of

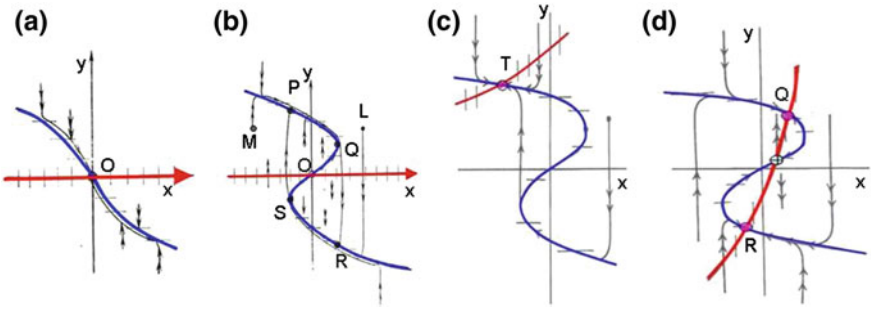

Fig. 1.10 **a**–**d** Phase portraits of auto-oscillations and related regimes. **a** Attraction toward a single stationary point under $a > 0$. In **b**–**d** $a < 0$. **b** Auto-oscillations. **c** Relay regime. **d** Trigger regime. x-zero isoclines are shown in *red* and y-zero isoclines in *blue*. In **a** and **b**, x-zero isoclines coincide with $y = 0$ axis, while in **c** and **d**, they deviate from this axis are inclined and slightly curved

a so-called attractor toward which the dynamic variables trajectories are rapidly (with dy/dt rate) approaching from all the points of a phase space. In its turn, the attractor's configuration crucially depends upon the sign of the parameter a. At $a > 0$, it is a cubic parabola after falling to which the variables are slowly (with dx/dt rate) moving toward a stationary point O which is called the stable nodule (Fig. 1.10a). Much more interesting is the system's behavior under $a < 0$: Now the attractor takes the shape of the so-called limit cycle consisting of two periods of slow movement (along the branches PQ and SR) and two fast "jumps" (QR and SP) (Fig. 1.10b). At the same time, point O becomes unstable. As a result, an entirely new temporal level is born, characterized by a non-damped oscillation period; remarkably, this temporal value depends upon those having no temporal dimensions at all: to these belong a constant parameter a and the entire structure of x, y feedbacks described by Eqs. (1.3a, 1.3b). This is a clear example of generation of a new quality.

The limit cycle is very robust in the sense that that the "phase point" (describing a system's state in the phase space) gets onto it without a possibility to escape from any point of a phase space (e.g., from points L or M, Fig. 1.10b). On the other hand, it principally differs from classical mechanical oscillations driven by external forces (that is, by perturbations alien to the oscillating body itself). Rather, in the case considered, the oscillations are supported by the kinetic properties of the oscillating system itself. Accordingly, such events are defined as *auto*-oscillations, taking place in the *active media*.

Auto-oscillations can be regulated and essentially transformed by including new parameters [mostly in Eq. (1.3a)], e.g., by adding a constant parameter b

$$dx/dt = y - b$$

The movement of the imaging point along the upper branch of the limit cycle is now slowed down compared to the lower branch; under large enough b values a new

stable point T is emerged (Fig. 1.10c) transforming non-damped auto-oscillations to the so-called waiting, or relay regime. Even more extensively, the system will be transformed by sloping x-zero isocline:

$$dx/dt = ky - b$$

If the slope is great enough, x-zero isocline intersects, in addition to the unstable branch QS, also PQ and RS sections of the stable branches (Fig. 1.10d). Under these conditions, two stable nodules, Q and R, are created. A switching from one to another is possible only under finite perturbations of x-variable, shifting it toward the verges of fast jumps. This model exemplifies the so-called trigger regime characterized by the existence of two alternative metastable states. As argued in Chap. 4, this regime plays a first-range role in regulating morphogenesis.

1.2.2.4 From Determinism to Stochasticity

The above-presented models are called deterministic in the sense that they generate predictable and as a rule uniform patterns of behavior even if starting from quite variable (noisy) initial conditions. Certainly, this designation does not mean that they obey the classical "one cause–one effect" determinism: Rather, the latter would always produce different results under different initial conditions which as we could see is not the case. The dynamic stability of the above models is provided by parametric regulation which is quite far from being precisely addressed but is instead essentially smoothed both in time and in space, bearing thus holistic properties.

A similar rise of precision in the course of development (which in a number of cases starts from rather variable initial stages and comes to more uniform results) was known to embryologists for a long time and was called equifinality. One of the greatest embryologists of a remote past, Carl Ernst von Baer, after tracing equifinality in a great set (more than 2,000 samples) of chicken embryos concluded: "it is not any stage by itself which, owing to its own properties, determines the next, but instead, more general and higher relations regulate all of this…" (Baer 1828). Today we may identify these relations with parametric regulation.

Remarkably, the parametric regulation can also bring to opposite results, namely to make a chaos out of order. One of the simplest scenarios of such transformation is based upon so-called logistic equation

$$dx/dt = \lambda x(1-x) \tag{1.4}$$

widely employed for describing the so-called restricted, or S-shaped growth, with its rate firstly increasing and then decreasing in a symmetric fashion. We shall use this expression as a discrete *reflection* written as

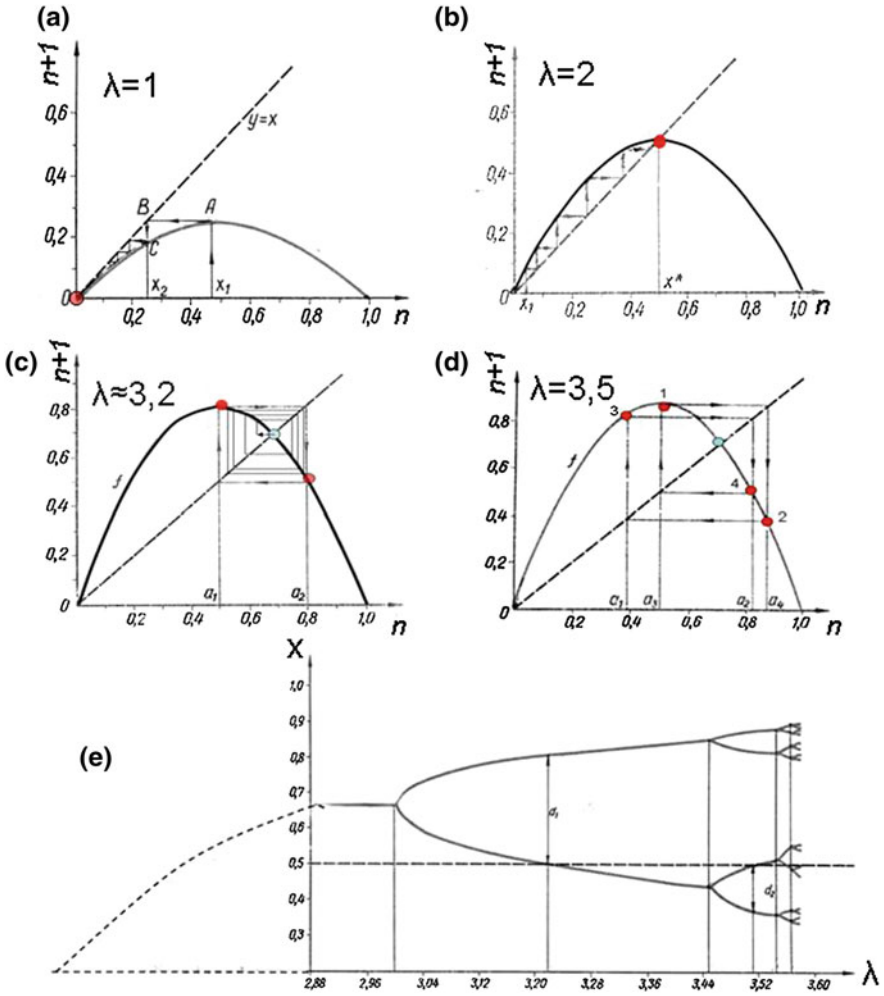

Fig. 1.11 From order to chaos. **a–d** Increase of parameter λ values (shown) transforms single stable point to an increased set of alternated states. **e** Splitting into increased number of alternated states as a function of λ values

$$x_{n+1} = \lambda x_n(1-x_n) \tag{1.4a}$$

where x_n and x_{n+1} are x values at discrete time points n and $n + 1$. The plot of (4a) is bell-shaped, its maximal height proportional to λ value (Fig. 1.11a–d). We have to explore what points at the reflection plot will be immobile and which ones among them will be stable under not too large variations of λ values. Obviously, a condition of immobility is $x_{n+1} = x_n$: The immobile points are always situated at the bisector of the coordinate angle (dashed lines in Fig. 1.11a–d). Also, it can be proved that the condition of stability is $[df(x_{immobile})/dx] < 1$. It is possible to derive

from these results that under the smallest λ values ($\lambda < 1$), there is only one stable point at $x = 0$ (Fig. 1.11a). With λ increase, this point at first shifts to non-zero x values still keeping its stability (Fig. 1.11b), but later on (when the bell-shaped graph becomes steep enough for intersecting the bisector by its descendant branch), the stability is lost. Most important is that in this case, contrary to the above-considered instabilities, the point is not moved toward infinity; rather, it starts to oscillate at first between two discrete points (Fig. 1.11c) and then (with further λ increase) between 4, 8, 16, 32… such points (Fig. 1.11d). In other words, a quasi-stable periodic solution will run through increased numbers of different x values until each next value will become practically unpredictable: a system will come to a state which we shall qualify as chaotic (Fig. 1.11e). Being plotted in the time coordinates, these regimes correspond to chaotic oscillations; if unfolded in space, they give rise to so-called fractal structures, remaining self-similar at quite different scales. A living matter is full of such examples.

The very existence of such structures is fatal for the classical determinism: It would be meaningless to search an individual cause for any single fractal structure—an entire set of these is generated at once by shifting just a single parameter λ. What might be its biological meaning? We can see that λ value determines the interval between two successive x values: If the interval is small, the system behaves in deterministic manner, while at larger intervals, it approaches a chaotic state. On the other hand, the interval between neighboring x values can be regarded as the gap (most probably temporal) between the action and the response or, in other words, as a measure of the feedback rate. Below (see Chap. 4), we shall see that modulations of the feedbacks rates may be among the main tools for regulating morphogenesis. In addition, as being non-spatial, such modulations are easily opened for being directly affected by genetic factors. All of this makes plausible that "playing" with λ values, a biological system employs one of the most effective ways to regulate development, sometimes approaching and sometimes leaving the verge of chaos.

1.2.2.5 Self-organized Criticality

As it was shown during the last few decades, first by theoretists and then by experimenters, the state maintained at the verge of chaos turned out to be, in some sense, rather stable. Consider a sand dune: being from time to time flattened by unpredicted avalanches of various sizes, it restores each time its typical inclination even if moving to some extent from one location to another. This peculiar state is defined as a "self-organized criticality" (see Ball 2001). Being non-equilibrial (a sand dune is created and supported by the energy of the wind), such a system exhibits from time to time large-scale perturbations (avalanches) for which only statistical probability rather than deterministic schedule can be estimated. In double-logarithmic coordinates, the frequency of different size avalanches versus their size obeys a linear slope: small avalanches appear more often than large ones. Events obeying this law are called the scale-free ones: being in a self-criticality state, a system looks as erasing the differences between characteristic times and/or spatial

scales typical for hierarchical systems in the "normal" conditions: Looking again on a sand dune, we can see that it possesses neither characteristic size nor characteristic frequency of the avalanches taking place from time to time. In a sense, this is an ideal case of a holistic, absolutely undivided system.

Many biological systems, from respiration of yeast cultures to electrical records of neural activity, reveal the state of self-criticality. In the next chapter, we shall discuss some similar events related to so-called glassy state of actin networks. However, quite few studies of this kind were performed on the developing organisms. One of the most relevant is that by Gamba et al. (2012) performed on fresh water hydra embryos. By studying the size distribution of gene *ks*1 expression spots, the authors found that it is close to scale-free patterns just at the time of determination of the main body axis. It would be of a great interest to know whether scale-free dynamics is a universal property of developing systems during their transition to a determined state.

1.2.2.6 Spatial Unfolding of Self-organized Regimes

Although nothing in principle forbids to unfold the parameters of the above models not only in time, but also in space, such a possibility is rarely used: To do this would be to consider the space a priori heterogeneous, which contradicts the main SOT demands. A usual way for providing a spatial unfolding by preserving the space homogeneity is to use a notion of diffusion in its broadest sense. It means that if within the active medium (endowed by a proper nonlinear dynamics) the concentration of a certain substance (or the amount of some measurable physical state) X is, due to a perturbation, locally increased, X will be propagated with the rate linearly proportional to the second derivative of X to space coordinate. The basic equation for the diffusion-mediated propagation along one-dimensional reactor is

$$\delta x/\delta t = f(x) + D_x\left(\delta^2 x/\delta r^2\right) \tag{1.5}$$

where *f(x)* describes the kinetics in any point of the active medium (the so-called point kinetics), D_x is the diffusion coefficient and r is the sole spatial coordinate of the reactor. If the point kinetics produces auto-oscillations, its spatial unfolding will look as the so-called autowave of a definite length, moving from the point of initial perturbation to the opposite edge of the reactor. Autowaves are the most remarkable examples of spatial structures created "out of nothing," that is, without any template (the above-described auto-oscillations exemplify temporal structures of similar origin). As claimed by Krinsky and Zhabotinsky (1981), "autowaves exemplify a new type of dynamical processes generating macroscopic linear scale due to local interactions, each of them possessing no linear scale at all." This definition captures the very essence of self-organization and is more precise than qualifying it as emergence of order out of fluctuations: the matter is that the notion of "order" itself requires further explication.

From thermodynamic point of view, autowaves belong to so-called dissipative structures, maintained only under a continuous flow of reagents and energy.

Meanwhile, dissipative structures can well be stationary. Such structures were firstly observed by a French physicist Benard in thin layers of a viscous liquid heated from below: These are so-called Benard cells separated from each other by coherent upward-directed convection flows of the liquid. The importance of these formations has been realized much later, when a British mathematician Alan Turing gave a model of formation of stationary waves of a given length out of a "noise," the latter containing the waves of any lengths (Turing 1952). Although Turing's model had no direct biological parallels, it became extremely influential as revealing some unique and most probably universal properties of spatial self-organization. They are the following.

The 1-dimensional reactor (either tubular or toroidal), if non-perturbed locally, should always contain an integer number of half-waves. Thus, if the reactor's length is gradually changed, it will always contain an integer number of half-waves: Accordingly, the half-wave numbers will be changed abruptly, after passing a certain threshold. Locations of the thresholds depend upon the direction of the length changing: If the length is increased, the thresholds are shifted toward greater values as compared to their positions under reverse movement. In other words, a system possesses a kind of primitive "memory" of its immediate past.

Under progressive shortening of the reactor, we reach the length threshold after crossing which only one half-wave (lacking mirror symmetry) can be formed from the random noise; formation of a complete (mirror symmetric) wave within the same length range is possible only by applying precisely located directed perturbations. The structures of the first class (those formed spontaneously, without requiring special perturbations) are called senior modes, while those of the second class (molded by perturbations) are called the junior modes. Under further shortening, the next threshold is reached marking a "homogeneity border": No structures can be now generated, whatever great would be the initial perturbations. These results belonging to pure mathematics have interesting parallels in the development of organisms. Spontaneous formation of asymmetric structures may explain the absolute domination of unipolar configurations over bipolar ones almost at any structural level of the living matter. The lack of differentiation in very small pieces of embryonic tissue is also a firmly established phenomenon. Thus, the Turing's model, being unrealistic in concrete details, captures several fundamental features of self-organizing systems.

Interestingly, in spite of calling his paper "On the chemical basis of morphogenesis," Turing in no way neglected a possible role of mechanical factors in providing self-organization (see Howard et al. 2011): The main reason for preferring chemokinetic models to mechanical ones was that the first ones were much easier to calculate. However, as we shall see later such a seeming easiness, when applied to morphogenesis has its own shortcomings.

During several decades following publication of a seminal Turing's paper, a great number of models based upon similar ideas appeared mostly known as chemokinetic models of morphogenesis (Meinhardt 1982). Common for all of them is the assumption of "chemical prepatterns," that is, local inequalities in the concentrations of certain substances (morphogenes) serving as precise templates for

morphological structures or/and certain differentiation pathways. Actually, this concept is quite similar to that of PI and is confronted with the same difficulties. Let us look in more details what this model means if being applied to the formation of serial evaginations, such as scales, appendages, and buds. For deriving specific shapes of these rudiments from chemical prepatterns, we have to assume a precise one-to-one dependence of the local curvatures upon the local concentrations of morphogenes. Same dependence should take place within an entire course of development. Thus, we have to accept that the postulated concentration gradients should evolve during development in a regular way, which will be another for any next rudiment: All of these demand separate explanations. Instead of simplifying the entire picture, we come to what is known as an increased multiplication of essences.

In certain cases, the chemical prepatterns, configured like more or less smooth gradients, can play a role of initial conditions, canalyzing somehow the course of morphogenesis, but to consider them as one-to-one morphogenetic templates seems unrealistic. Leaving for the future further elaboration of this important problem, let us ask ourselves, whether morphological structures can be created without any chemical prepatterns. In this chapter, we explore this possibility using in most cases the model examples.

1.2.3 Shaping Without Prepatterns

In this section, we come closer to the realm of mechanics. The reader unfamiliar with its main notions is asked to look for the Sect. 1.2.4, which includes, in addition, the list of recommended readings.

We start from considering a rod to which a compressing axisymmetric force is applied either from one side (the opposite one fixed) or from both sides equally. If the rod consists of a soft (easily deformable, plastic) matter, it will be shortened while remaining rectilinear. However, if it has some elastic resistance, when the compressing force exceeds a certain threshold, the rectilinear shape loses its stability and the rod will be bent to one of the sides. This is the case of the well-known Eulerian instability, described by a great eighteenth-century mathematician Leonard Euler. It obeys Eq. (1.1) and is the simplest case of mechanical instability, leading to the reduction of the symmetry order. On the other hand, it provides the basic model for a large group of morphogenetic processes, driven by increase of internal pressure in cell layers (see Chap. 3 for more details). By furnishing the rod with cross-beams which are also elastic, we may bring the model even closer to biological realities, imitating its cellular structure (Fig. 1.12a). Now, under even infinitesimal deviation of compressing force from the central axis of the rod, the cross-beams will be stretched in asymmetric manner (Fig. 1.12a, hatched lines), increasing elastic energy of transversal surfaces. Being driven toward the minimal elastic energy value, each "cell" tends to return toward rectangular shape which, under continuous action of the pressure force, is possible only by bending the entire

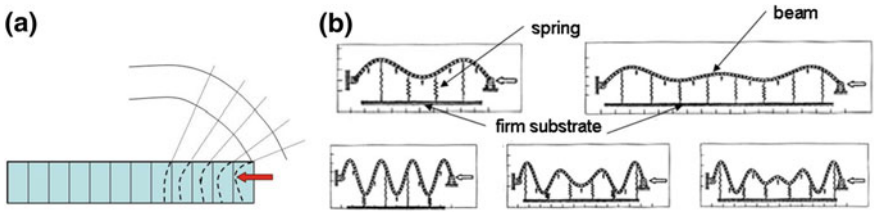

Fig. 1.12 Shape formation via Eulerian instabilities. **a** Bending of a rod split to "cells" with elastic walls (*hatched*) under the action of a slightly eccentric pressure force (*arrow*). The final deformation (*dotted*) is the result of the drift of the bent cell walls toward more relaxed symmetric state. **b** Some examples of bending patterns of laterally compressed elastic rods connected with a number of firmly fixed springs. In each case, the resulted pattern corresponds to the minimal elastic energy state of the entire two-components system and to the maximally homogeneous spread of its energy. Note that in no case, the bending wavelength fits the spring arrangement [From Green et al. (1996), with the authors permission, modified]

rod. It is easy to show that the bending will be directed to the side of the force deviation from the central axis. In this way, we get a two-leveled image of the rod's bending ("cells" first, the whole rod next) which closely imitates some basic epithelial morphogenesis (see Chap. 3 for more details).

As the next example, we take a deformable beam connected with a bundle of elastic springs firmly fixed from the opposite side (Fig. 1.12b). In this case, under the action of lateral pressure the beam will be bent as a rule in several points, always producing an integer number of half-waves. With the beam elongation, the number of half-waves is increased in a threshold manner, imitating thus Turing's behavior without any morphogens! Worth mentioning, in no way the wave pattern fits that of the springs' attachments. If the beam is circular, has finite thickness, and is compressed by its own internal forces (the most natural morphogenetic situation), the resulted half wavelength λ_n can be calculated by the formula

$$\lambda_n = 2\pi(D/k)\exp(1/4) \tag{1.6}$$

where D is the bending rigidity and k is the coefficient of the spring elasticity (Green et al. 1996). Interestingly, if the beam is thick enough, the "conflicts" may arise between its outer and inner perimeters, each one tending to arrange an integer number of half-waves; as a consequence, some irregularities of the resulted pattern will take place.

Now let us address to some examples of shape formation in balloon-like and vesicular bodies, covered with thin elastic shells. We start from considering a flattened puck-like balloon with radius a and height b, inflated through a central pore. As shown by Martynov (1982) if and only if $a > b/\sqrt{2}$, the inflation will stretch the lateral walls of ellipsoid in meridional (vertical) direction and compress it in the equatorial (horizontal) direction (Fig. 1.13a). If the shell is not too thick and rigid the inflation will produce N vertical folds which will be exchanged by the same amount of horizontal folds during deflation. In the both cases, $N \approx 4\sqrt{a}/S$ where S is the thickness of the shell. We can see that the uniformly applied forces within a

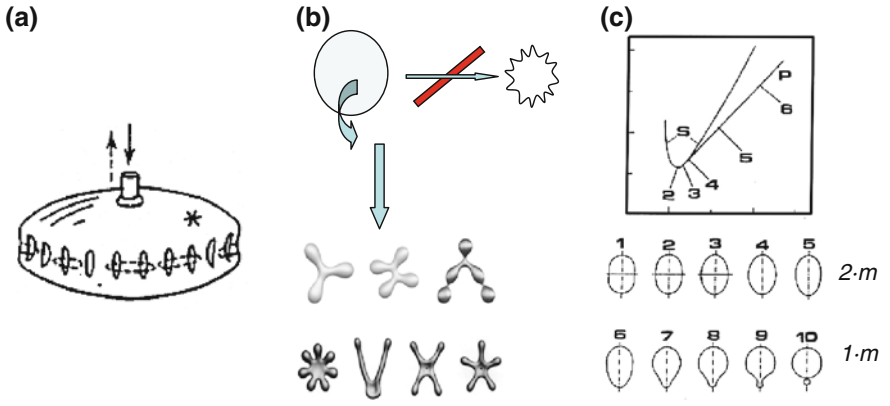

Fig. 1.13 Morphogenesis (symmetry breaks) without chemical prepatterns, driven by energy minimization. **a** Formation of folds under inflation/deflation of a flattened balloon. **b** Formation of "starfish vesicles" from a deflated baloon instead of its uniform shrinkage. **c** Svetina and Zeks (1991) model. Upper frame: a plot of the relative membrane bending energy (*vertical axis*) as a function of the average membrane curvature (*horizontal axis*). The curve denoted S is for shapes with 2 · m symmetry and the curve denoted P for shapes with 1 · m symmetry. Lower frame: numerically obtained axisymmetric shapes with minimum membrane bending energy. After exceeding a certain average curvature threshold, there are 1 · m rather than 2 · m symmetry figures which exhibit local energy minimae. **a** From Martynov (1982), with the author's permission; **b** from Wintz et al. (1996), with the publisher's permission. **c** from Svetina and Zeks (1991), with the authors' permission

restricted but not too small range of initial geometric conditions can produce extensive rotational asymmetry.

A set of the different deformations, each of them decreasing symmetry order, have been obtained in small lipid vesicles (Wintz et al. 1996). The osmotically driven shrinkage of initially spherical vesicles led to formation of peculiar figures with rotational ($n \cdot m$) and mirror-like ($1 \cdot m$) symmetry, some of them called by the authors "star-fish vesicles" (Fig. 1.13b).

So we can see that rather complicated deformations, associated with decrease of the symmetry order, and in some cases biomorphic, can be produced without any outside imposed patterning, including chemical gradients of any origin. What are the basic principles of these events?

The general answer will be quite simple: In full accordance to the second law of thermodynamics, all of the above-described transformations are driven by the tendency of a given body to reach the minimum of its free mechanical energy (which was pumped initially by an external force). Why, however, this tendency brings the bodies toward regular shapes with decreased symmetry order instead of increasing their randomness and homogenization? The response is: those are the geometrical and structural constraints which prevent the described bodies from reaching the "absolute" energy minimum, setting them instead for quite different (up to indefinitely long) time periods into the *metastable energy wells*.

In general terms, this can be achieved, if the system possesses a set of stable states to which it is relaxed with a greater rate than to the homogeneous state, and

which are far enough removed from the latter. Obviously, the first condition making this possible is nonlinearity, providing the multiplicity of stable states. Accordingly, the linear systems possessing only one stable state are incapable of morphogenesis. Next, for enough rapid relaxation to specific stable states, the system should possess a restricted number of *selected degrees of freedom*. The physical mechanisms associated with these tendencies will be discussed in the next chapter.

At the moment let us look how these conditions are fulfilled in the above-described shape-forming systems. First, all of them are nonlinear; the nonlinearity of the laterally compressed beams is expressed by Eq. (1.1). Our next question will be what are the pathways toward metastable states, permitting to avoid thermodynamic averaging?

In case of the compressed beam (Fig. 1.12a), the thermodynamic pathway is that directed toward its continuous rectilinear flattening, accompanied by heat emission. It is quite obvious, that this way is unstable, while just those directed away from it (toward bending which exemplifies elementary morphogenetic events) are stable.

In the second example (Fig. 1.12b), the system has the same fundamental properties although if aggravated by the presence of vertical elastic bonds. Morphogenetic stable states will be now determined by a compromise between the beam resistance to bending and the bonds resistance to stretching: in all the cases, the mechanical energy tends to be maximally equalized throughout the system's components. Under these conditions, the number of bending half-waves produced by the lateral compression will be more than one due to the tendency to equalize the elastic energy between all of the system components (including the beam itself and all the springs). Obviously, at the single half-wave bending, most of the energy will be concentrated in the beam itself and in central springs, contradicting the tendency to overall equalization of mechanical energy.

On the other hand, if the number of half-waves will be too great, the main beam would accumulate too much bending energy. By these ways, the system is working as integrated whole.

In the next two examples (Fig. 1.13a, b), there are not only mechanical, but also geometric constraints which canalize the drive toward free energy minimum along morphogenetic (rather than thermodynamic) pathways. In case of the flattened balloon, the constraints are exemplified by the radius/heights ratio, acting in a threshold-like fashion. Meanwhile, in case of the "star-fish vesicles," the situation is somehow more complicated. Here, the bending energy, which is proportional to the local curvature, plays the main role. Being unable, due to the shell's continuity, to reach the absolute minimum (that is, to flatten all the vesicle's surfaces), the maximal curvatures become concentrated in few small regions connected by tubular bridges (flattened in one dimension at least). Later (Chap. 4), we shall see that a similar tendency although if driven by other mechanisms takes place in real embryos. On the other hand, a great multiplicity of the shapes observed in Wintz et al. models indicates the existence of many metastable states which are not so numerous in biological samples. In any case, however, the thermodynamic minimum (which would correspond to a dense wrinkling of the shrunk shell) is avoided.

Svetina and Zeks (1991) modeled a similar situation imitating the increase of a vesicle's surface with its volume kept constant. They showed that after exceeding a certain threshold of the average curvature, there are $1 \cdot m$ symmetry structures (ovoid vesicle with a small bud) rather than $2 \cdot m$ ones (symmetric ellipsoid) which correspond to the minimal bending energy (Fig. 1.13c). This is another example of inherent drive toward the reduction of the symmetry order.

The second law of thermodynamics tells us that a drive toward the free energy minimum can be realized either by a decrease of enthalpy (which is a measure of the total energy of the system) and/or by an increase of entropy (which is defined as a measure of the number of specific ways in which a system may be arranged, often taken to be a measure of disorder). Which of these two members' contribution is the greatest for the systems we are interested in? According to experts' opinion (Cademarini et al. 2011), "free energy… for hard systems [to which the living matter belongs—LB] is dominated by entropic contributions. This leads to the somewhat surprising observation that ordered, close-packed structures are often more probable (i.e., they have higher entropy) than similar amorphous structures, in which "jamming" limits the mobility of the assembling components."

This suggestion can be adequately illustrated by the behavior of so-called liquid crystals, the highly ordered aggregations of rod-like particles. Usually, three main types of liquid crystals are distinguished: nematic, smectic, and cholesteric. While in cholesteric crystals, the particles, due to their intrinsic geometry, are arranged in spiral fashion, the first two types are characterized by parallel arrangement. Among them, in smectic crystals, the particles create parallel rows, while in nematic crystals, no such alignment is taking place although the parallel arrangement is kept. In the both cases, the particles have more freedom that under disordered arrangement because in nematic crystals, any parallel shifts of the particles are allowed, while in smectic crystals, the particles, although if unable to disturb the row, can incline cooperatively at any angle. Accordingly, the entropy of the liquid crystals in spite of their orderiness is higher than that of a dense population of disordered particles and after passing the density threshold the transition toward crystal state is spontaneous (entropy-driven). These arguments are of a direct interest for biologists because multicellular structures with parallel cells arrangement are widely presented in developing organisms (see Chap. 3).

According to the cited authors, from the thermodynamic view, all the structures arisen due to collective interactions of their components can be divided into three categories:

- equilibrial, that is those corresponding to the absolute (under given conditions) free energy minimum;
- non-equilibrial, trapped in long-living metastable states depending upon the history of the system and non-coinciding with the absolute energy minimum;
- dynamic (dissipative) ones which emerge and persist only under continuous presence of thermodynamic gradients and flows, preventing relaxation even toward metastable energy wells.

The above-described shape-forming systems best of all fit the second category. On the one hand, at the start of their formation, they should be pumped by mechanical energy, and on the other hand, they do not require its continuous flow for being maintained within prolonged time periods. At the first glance, this may be not true for compressed beams which seem to require permanent lateral forces for remaining bent; however, since their elementary structural units will be deformed, the mechanical energy becomes internalized within the system itself and what we qualify as the subsequent bending is an autonomous (independent from any outside forces) relaxation toward the mechanical energy minimum predetermined by the units deformations.

This is not to say that dynamic (dissipative) structures in sensu stricto play no role in morphogenesis. Indeed, their role may be very important, but in relation to morphological structures which we see under the microscope or even by a naked eye, it is mainly preparatory. Dissipative structures are visualized mostly as oscillations, flows, or vortices at supramolecular and sometimes cellular level (see next chapters) which are prerequisites of more stable higher level structures.

In general, the morphogenetic interest of the above-described models is in demonstrating that the relaxation of mechanical stresses established by a single force or by a manifold of symmetrically arranged force(s) can produce less symmetric and in many cases biomorphic macroscopic structures. Obviously, this can take place only if a substantial amount of mechanical energy which deforms a body is not immediately dissipated into heat but is instead stored in the form of elastic stresses to be later slowly relaxed to few metastable states. In the next chapters, we will demonstrate that the elastic stresses are taking place in quite different structural levels and are ultimately indispensable for coordinated morphogenesis.

On the other hand, it is to be emphasized that the relaxations are just single parts of the morphogenetic loops: another parts are associated with generation of new forces required for achieving the next mechanically stressed state. In each of the above-discussed models, the initial force was taken as given: this makes these models incomplete (non-closed). One of the main goals of our further account (see Chaps. 3 and 4) will be to search the ways for creating really closed morphogenetic models in which the both branches—generative and relaxatory—will be included in the common feedback contours.

1.2.4 Brief Biologically Oriented Exposure of Some Notions and Principles of Mechanics

1.2.4.1 Main Definitions

Mechanical stress (MS) **p** is defined as the average force per unit area S that some particle of a given body exerts on adjacent particle across an imaginary surface that separates them. **p** is a vector. More precisely, **p** is a limit of the ratio $\Delta\mathbf{p}/\Delta S$ under

$\Delta S \rightarrow 0$. By another definition, MS is a measure of internal forces arisen in the deformed body under the action of external forces. This expression fits our purposes due to emphasizing that the internal forces may be quite different by their values, directions, and spatial arrangement from the external ones.

When dealing with a single surface passing through a given material point of a body, one should distinguish MS oriented perpendicularly (normally) to the surface from those oriented in-plane. Normal MS can be either *tensile* (exemplified by pulling forces) or *compressive* (pushing forces). On MS plots, the first ones are taken as positive, while the second as negative. In-plane-oriented MS produce the so-called shear stresses. In our subsequent account, we shall be dealing almost always with normal MS.

Meanwhile, for getting a complete description of a *stressed state* of a material point belonging to 3-dimensional continuum (which is what we just want to obtain) to consider a single plane is not enough, in general, we have to introduce an indefinite number (a bundle) of such planes passing through the point and calculate MS within each of them. Usually, such a task is reduced toward evaluation of three mutually perpendicular MS components. In combination, they create a mathematical value called the *tensor*, which gives a full description of the stressed state of a material point. Its basic distinction from vector is a lack of unidirectionality: the simplest tensors are bidirectional. This property is of fundamental importance for biological morphogenesis.

Now we pass to deformations which in the case of linear ones are usually exchanged by the notion of *strain*—ratio of deformation over initial length of a sample. From the physical point of view, the deformations are divided into *elastic* and *inelastic* ones. Elastic deformations are those most closely linked with MS which they produce and vice versa: in the ideal case, the elastic strain/stress relation is linear (Hooke law): Real cases are more or less perfect approximations to Hookean ones. During elastic deformations, the mechanical energy is assumed to be preserved exactly in those inter-particles bonds to which it was directly applied by external force, rather than being dissipated over larger areas. Accordingly, elastic deformations are abolished "immediately" (in fact, with a sound wave speed) after cessation of the force action. Although the concept of elasticity in its strict sense is a kind of idealization (because any natural process is accompanied by energy dissipation), it is of an ultimate importance as a referent state.

Inelastic deformations are those characterized by the dissipation (transformation into a heat) of a considerable part of the pumped mechanical energy. The dissipation is accompanied by various and quite complicated rearrangements of the constituent body particles, driven toward thermodynamic equilibrium. Due to irreversibility of these transformations, at least a part of imposed deformation becomes preserved. In many cases, elastic deformations are transformed to inelastic ones under increase of the amount or of the duration of the force action.

For measuring a resistance of an elastic material to deformation (its stiffness), the so-called Young's modulus is used which is the ratio of the *stress* along *an axis* of deformation over the *strain* along that axis in the range of stress in which *Hooke's law* holds. Young's modulus is expressed in Pascals (Pa) or N/m^2.

Most of the living tissues, being stretched in one direction tend to contract in the directions, transverse to the direction of stretching. This is well-known *Poisson effect* which is measured by a Poisson's ratio υ: the fraction (or percent) of expansion divided by the fraction (or percent) of compression (for small values of these changes). The Poisson's ratio of a stable, *isotropic*, linear *elastic* material cannot be less than −1.0 or greater than 0.5.

A number of morphogenetically important deformations and MS are associated with the events phenomenologically quite similar (although never identical) to those taking place in the interphase borders and usually defined as a *surface tension*. So far as a free energy of the surface layer molecules are greater of those located inside a staff, the surface (interphase border) tends to contract up to a minimal (spherical) area enveloping a given volume. Correspondingly, to deviate a surface layer from a spherical shape (for extending the surface), a certain force should be applied (e.g., a pressure force within the surrounded volume). Similarly to inelastic deformations, those driven by surface tension are always directed toward minimum of free energy under the given initial/border conditions.

The main characteristic of a shape is a *curvature* that is deviation of a line from being straight or of a plane from being flat. A curvature k of an arch of a circle is inversely proportional to the circle's radius: $k = 1/R$. For most of our purposes, it will be enough to dissect in our images the contours of embryonic objects to a number of 1-dimensional circular arches (which in general case will have different radii) and to compare qualitatively their curvatures; the latter are called the local. However, for properly use the Laplace law (see below), a flat (1-dimensional) curvature of a line should be replaced by a 2-dimensional curvature of a surface

$$k = 1/R_x + 1/R_y$$

where R_x and R_y are the local curvature radii, oriented in mutually perpendicular planes. Their sum defines what is called the principal curvature in the intersection point of these two planes.

It is easy to see that the surface tension and local curvatures are inversely related to each other: Increase of surface tension tends to smooth the surface that is to diminish the local curvatures and vice versa. This is expressed by Laplace law describing the dependence of the hydrostatic pressure overfall $\Delta p = p_1 - p_2$ (where p_1 and p_2 are the pressures exerted to the surface from its concave and convex sides correspondingly) upon the interfacial tension σ and the local 2-dimensional curvature $\varepsilon = 1/R_1 + 1/R_2$:

$$\Delta p = \varepsilon\sigma$$

what means that the surface pressure is directly proportional and the surface tension is inversely proportional to the local 2-dimensional curvature.

1.2.4.2 Biological Reservations

For good or for bad when applied to biology, strict notions of mechanics to a great extent lose their preciseness, becoming to some extent vague and arbitrary. Probably the main reason for such a transformation is the appearance of the "activity–passivity" alternative almost unknown in classical mechanics but unavoidable in biological applications. To know whether the given MS (e.g., those demonstrating Poisson's effect) are born by external force or generated inside a given tissue piece is for a biologist in many times more important than to measure them accurately. Another principal difference between inorganic and biological samples is the latter's hierarchic structure leaving far behind that taking place, for example, in crystal bodies. In addition, a number of more particular uncertainties are taking place. For example, determinations of the absolute MS values and of the Young's moduli are largely aggravated by a lack of precise understanding what is the real square to which a given normal force is applied. Suggest that we make such estimations for a stretched bulk of biological tissue. As a first approximation, we can take the square of the entire transverse section through the bulk. Under more precise consideration, we have to conclude that the real square to which the force is applied is a total area of cell contact plaques oriented normally to the force. But this is also far from being the end of the story: Individual cell contact plaques also have complicated structure, and their areas are changed during force application, etc.

This is not to say that mechanics is incompatible with biological realities: our viewpoint is just the opposite. The main thing is to make clear the biological meaning of any mechanical measurement. In many cases, qualitative data will be of a greater importance than precise quantitative ones. Although in no way the latter should be rejected, they will make sense only if becoming the members of homologous sets of data permitting the direct comparison: It should be never forgotten that in biology, the relations are much more important than the absolute values.

1.3 Recommended Readings

For the full papers' titles, see reference list:

Schwarz and Gardel (2012)	Defining the main notions of mechanics
Blanchard and Adams (2011)	Describing techniques for measuring mechanical forces at the different structural levels
Diz-Muñoz et al. (2013)	Describes and explains techniques to measure and manipulate membrane tension
Ladoux and Nicolas (2012)	Gives a list of the cell-generated forces and the external forces used for studying the living cells' mechanics

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