6
Organisms and Levels of Autonomy

At the end of Chap. 4, we briefly mentioned that since the very beginning of life on Earth, organisms have established strong interactions (as opposed to weak ecological interactions) with each other, giving rise to several different types of stable associations. Unicellular organisms, which we took to be the prototypical example of autonomous systems, come together to form temporary bacterial aggregates, colonies, biofilms, and prokaryotic and eukaryotic multicellular ensembles. In turn, eukaryotic cells arise from symbiotic associations of prokaryotic cells and finally, colonial aggregates or more integrated societies establish groupings of multicellular systems with different degrees of cohesion. All these associations tend to occupy new niches and to increase the chances of survival of both the constituting units and the associations themselves as a whole. In certain cases, they even seem to behave as individual organisms.

One of the crucial issues discussed in the literature is to determine under what conditions these associations should be taken as fully-fledged organisms. The biological realm is full of examples of cellular ensembles or communities of cells, such as biofilms, slime moulds, lichens, sponges, mycelia fungi, clonal plants and colonial invertebrates, which may demonstrate some organism-like properties, but not all of them. In many cases, such composite multicellular systems dwell on the border between organismal and colonial behaviour, or between organismal and symbiotic relationships. It is therefore unclear in which cases they should be considered organisms, parts of organisms, or groups of organisms. As noted by Wilson (2000), assuming (as we do) that unicellular entities are organisms, the question would be: what sorts of multicellular systems meet equivalent requirements and can therefore be regarded as organisms? Actually, although we have pointed to unicellular entities as paradigmatic examples of organisms, it is more usual (or closer to our perspective as human beings) to think of highly evolved multicellular

This chapter relies on ideas previously formulated by Ruiz-Mirazo et al. (2000) and especially by Arnellos et al. (2014), from which several portions of the text are taken.

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systems as typical organisms (in particular metazoans, see Santelices 1999). Nevertheless, multicellular organisms represent a formidable challenge to any attempt to characterise or define them in precise terms, since cells have created many different kinds of collective entities over the course of evolution.

The contemporary literature shows that it is no easy task to determine which kind of organisation distinguishes "genuine" multicellular organisms from other forms of cohesive multicellular systems. Authors tend to offer a list of criteria (qualities and properties) that typify multicellular organisms, but often recognise that many exceptions exist. Sterelny and Griffiths' "spatial boundedness" (1999), Santelices' "unitary organism" (1999), Wilson's "paradigm organism" (1999), and the "functional integration" concept discussed by Wilson and Sober (1989) are examples of such criteria. Moreover, the criteria established in the literature are extremely heterogeneous; most are based on evolutionary considerations and even when they are conceived in organisational terms, they focus on very different aspects. So, although there is an intuitive grasp of the distinctive properties of organisms, there are always, as Clarke (2011, 2013) mentions, surprising cases of multicellular systems that force us to revise our criteria. Therefore, in order to make progress in this debate, what is required is a conceptual framework that, even if it does not completely succeed in clarifying the issue, at least provides us with the basic tools for interpreting most cases, including borderline ones, in a principled way.

In previous chapters, we have argued that individual biological organisms can be characterised as autonomous systems. When considering multicellular systems, the central question is whether the concept of autonomy developed so far also applies to such forms of multicellular organisation. What degree of integration and cohesion is required for multicellular systems to be taken as autonomous systems, and therefore as multicellular organisms? Supposing that we would agree that some multicellular systems indeed count as fully-fledged organisms, would it be in the same sense as for unicellular organisms? Furthermore, what is the status of the cells that constitute these different types of multicellular organisations? Are they still autonomous entities or just non-autonomous parts of an encompassing autonomous system[1]?

From the autonomous perspective, an organism is a regulated closed agential organisation that maintains itself while interacting with the environment. As we will see in Sect. 6.1.2, it seems reasonable to hypothesise that, in most cases, multicellular systems are self-maintaining closed organisations constituted by functionally differentiated parts (groups of cells) whose constituents (the individual

[1]The difficulty in applying the concept of autonomy to multicellular organisms was recognised by Maturana and Varela at the end of Chap. 4 of "The Tree of Knowledge" (1987), where they admit the problems involved in characterising multicellular organisms as "second-order autopoietic systems".

cells) are themselves closed systems. For example, a biofilm may contain many different types of microorganisms, e.g. bacteria, archaea, protozoa, fungi, and algae; each group performs specialised metabolic functions, and collectively they generate properties that emerge on free-floating bacteria of the same species. Accordingly, the biofilm constitutes a functionally integrated organisation that plays a causal role in the maintenance of the cells that actually constitute it. In our terms, biofilms realise a higher-level closure of constraints. Furthermore, multicellular systems are also integrated into ecological self-maintaining closed networks (see Chap. 4, Sect. 4.5 above), and often include deeply intertwined symbiotic associations.

In principle, as we argued in Chap. 1, closure constitutes a clear-cut criterion for marking the boundary between the system and its environment. In organisational terms, the set of constraints subject to closure constitutes the system, whereas all other constraints (and specifically those which have some causal interaction with the system) belong to the environment (as boundary conditions). When dealing with inherently intertwined multicellular biological systems, however, the question of the boundaries of closure may become much more complex, insofar as forms of strong (both intra- and inter-level) interactions between closed systems are considered. In spite of these difficulties, however, we do maintain that closure is a useful conceptual tool for identifying biological systems and, in particular, for distinguishing relevant levels of biological organisation. While we have previously discussed the realisation of different *orders* of closure (in relation to regulatory capacities), here we address the issue of *levels* of closure,[2] each level consisting of a set of closed constraints which is either made of constituents or included in an encompassing system, themselves realising closure.

At first approximation, the relations between levels of closure may consist in two different situations. In some cases, one can clearly distinguish between two or more distinct (and nested) levels of closure within the whole multicellular system. For example, in multicellular organisms, closure is realised by each individual cell on the one hand and by the organism on the other hand. Yet, it might be argued that there is no overlapping between the two levels of closure because individual cells do no exert functions that are subject to the higher-level closure. Only populations of cells (and, in ecosystems, populations of organisms) are subject to higher-level closure. In other cases, in turn, multicellular systems realise a kind of strong mutual dependence (symbiosis, for instance), which does not result in a sharp separation between the individual closures and the collective one. Within these systems, as argued by Ruiz-Mirazo and Moreno (2012), boundaries are not neat, though they can be established by "clusters" of mutual dependence. At some specific spatial

[2] As mentioned in Chap. 3, Sect. 3.2.2, each level of organisation can include one or more orders of closure, in particular if it possesses regulatory functions in addition to constitutive ones. Similarly, a given system can realise several levels of closure (and therefore of organisation), each of them including orders of closure. The conceptual distinction between orders and levels must be kept in mind to avoid confusion while reading the present chapter.

scale, in particular, many functions tend to be mutually dependent, such that one can identify discontinuities in order to establish the different levels of closure.[3]

Yet, as we already pointed out in Chap. 4, Sect. 4.5, when considering higher-levels of organisation, the realisation of closure does not involve as such the realisation of autonomy. As a consequence, the identification of higher-level closed organisations does not necessarily imply the identification of higher-level organisms. As a matter of fact, the encompassing multicellular organisation may perform a few functions (and simply maintain some relevant local environmental conditions for the different groups of autonomous agents that constitute it), while many others' functions are still subject to closure within the lower-level organisms. In the case of biofilms, for instance, different global properties such as density do play a role in the phenotypic shift of the bacteria. Moreover, because of the entanglement between the levels of organisation, it might be difficult to determine whether a specific function is performed by a given system or by an encompassing one, or by both of them. Thus, the main theoretical challenge consists in determining which of the hierarchically structured organisations that realise closure also meet the more demanding requirements for autonomy, and in precisely what sense.

In this chapter, we will not develop a comprehensive analysis of higher-level autonomy. Accordingly, we will not discuss all the implications of how multicellular organisms realise biological autonomy, and whether or not they are endowed with the very same organisational properties than unicellular organisms (notably with respect to distinctive regulatory and agential capacities). More modestly, our aim will be to make a first step in this direction by discussing some of the necessary conditions required for a multicellular system to be a *relevant candidate* as a higher-level autonomous system, and hence as an organism. In particular, we will focus on the kind of functional integration that a multicellular organism must exhibit. Our central claim will be that the *functional integration of multicellular organisms requires, as a necessary condition, developmental functions and, therefore, developmental constraints*.

The reason why we focus on development is that, to be such, a multicellular organism should not only be capable of reproducing each of its own parts but also its own collective organisation, which in turn requires some kind of developmental process, understood in a broad sense. In this respect, the analysis undergone in the following pages will rely on two ideas.

The first idea has to do with the impossibility of realising higher-level autonomy without crossing a sufficient threshold of diversity in the constitutive functional parts of the multicellular system and their reciprocal interactions. In short, sufficiently broad higher-level functional diversity is a necessary condition for functional integration that is strong enough. If the number of cell types in a multicellular system or the number of ways in which cell types contribute to the maintenance

[3] In this chapter, we do not offer a detailed account of the relations that might exist between entities located at different levels of closure. For more (conceptual and formal) details, see Montévil and Mossio (2015).

of the whole is too limited, there are not enough resources for a cohesive form of collective autonomous organisation to emerge. In our terms, minimal closure of constraints in the higher-level system is not enough: the number and diversity of organised constraints in the system has to be high enough to realise autonomy. The role of developmental processes is precisely to enable the generation of such a higher-level functional diversity that in turn requires developmental functions be themselves complex and various enough.[4]

A second but no less important idea concerns the centrality of *control* and, closely linked with this, of dynamic decoupling as a requisite for the type of organisation that may support a multicellular organism. For the question of generating functional diversity goes intrinsically with the problem of controlling it. Without higher-level control in particular, the simultaneous generation of rich functional diversity and high integration would not be possible. That is why intercellular control mechanisms stand out in all complex forms of development. In fact, they are so essential and pervasive in these systems that they effectively modulate the behaviour of the underlying metabolic units, i.e., of each of the cells that become part of the developing whole (their growth, differentiation, division processes), in the interests of the more encompassing *modus operandi*. Indeed, a very delicate and subtle balance between *intra*cellular and *inter*cellular dynamics has to be managed in the system, and this is simply inconceivable without the control exerted by higher-level functions.

In Sect. 6.1 we first briefly review the two main existing views on the concept of multicellular organism; we then argue that multicellular organisms require a set of developmental mechanisms governing cell differentiation as a necessary condition, enabling the establishment of a higher-level functionally integrated organisation. In Sect. 6.2 we examine in detail the developmental mechanisms of three specific multicellular systems and in Sect. 6.3, we discuss those three examples by analysing how their respective mechanisms subtend different degrees of higher-level organised complexity. Section 6.4 concludes the analysis, by focusing on the reasons why some of these multicellular systems might be legitimately said to realise higher-level autonomy, and therefore be qualified as multicellular organisms. Lastly, we briefly address the issue of the relations between levels of autonomy, specifically in the case of multicellular organisms composed by cells being themselves – by hypothesis – autonomous.

6.1 The Concept of Multicellular Organism: Evolutionary and Organisational Views

During the history of life, various forms of multicellularity have arisen independently in each of the kingdoms. Prokaryotes have recurrently demonstrated their capacity to establish multicellular systems with relatively simple architectural and

[4]Determining the precise threshold above which those critical transitions are triggered should be a fundamental empirical target of scientific research, and goes beyond the objectives of the chapter.

morphological features, made of just a few different cell types (Bonner 1999). Similar levels of complexity are observed in many cases of eukaryotic multicellularity (Bell and Mooers 1997). It is true, however, that the macroscopic and more integrated multicellular forms found in animals, plants, and fungi show a much greater functional complexity, as well as a remarkable variety of morphologies and underlying organisations. Hence, it is important to remark, first, that not all multicellular organisations show the same degree or kind of integration and cohesion (Kaiser 2001; Rokas 2008) and second, that multicellularity must be taken as a multifarious phenomenon that has emerged independently in the evolution of many lineages.[5]

Given the variety of forms and degrees of integration of multicellularity, there is a wide debate about the conditions at which a multicellular organisation should be considered a true organism (see for instance Santelices 1999; Perlman 2000; Ruiz-Mirazo et al. 2000; Pepper and Herron 2008; Queller and Strassmann 2009; Folse 3rd and Roughgarden 2010; Clarke 2011). The aim of this debate is to provide a definition of organism that could be used to deal with various open biological questions, insofar as organisms seem to be the implicit or explicit point of reference for basic biological concepts such as fitness, adaptation, generation, trait, phenotype, metabolism, lineage, development, natural selection, and evolution. In what follows, we will review some existing characterisations of multicellular organisms, which can be grouped into two main views. The first view conceives the concept of multicellular organism from an evolutionary perspective, as a unit of selection; the second deals with this concept from an organisational standpoint.

6.1.1 The Evolutionary View

As mentioned at the beginning of Chap. 5, evolutionary thinking conceptualises organisms as biological units to the extent that, by exhibiting variation, differential fitness, and heredity, they are entities on which natural selection acts.

In this view, in which the units of selection are what matters most, fitness and its maximisation are usually taken as the fundamental criteria for defining organisms (Gardner 2009). For instance, drawing on an analogy with a pocket watch, Gardner suggests that biological adaptation does not imply perfection or optimality, but rather *contrivance* (the property according to which "all of the parts of the organism or of the watch appear contrived as if for a purpose") and *relation* ("all of the parts of the organism or watch appear contrived as if for the *same* purpose" ibid, p. 861). He then argues that fitness maximisation is the key design principle that explains

[5]Multicellularity has evolved independently in prokaryotes and eukaryotes (Grosberg and Strathmann 2007). Although certain requirements for multicellular organisation (as cell adhesion, cell-cell communication, and cell death) already evolved in prokaryotes, complex multicellular organisms evolved only in six eukaryotic groups: animals, fungi, brown algae, red algae, green algae, and plants.

how natural selection solves the problem of adaptation, i.e. the "packaging" of parts into units of common purpose (be they organisms or watches) (Gardner and Grafen 2009). Therefore, according to Gardner, an organism is a whole whose parts are all under selection to maximise its own fitness.

In the same vein, Queller and Strassmann (2009) argue that the distinctive feature of organisms is adaptation, through which they demonstrate "goal-directedness" (p. 3144). They focus on the fact that an organism exhibits adaptations as a whole, and that these adaptations are not disrupted (at least, not significantly) by adaptations of the parts. In agreement with Gardner and Grafen, they suggest that:

> the essence of organismality lies in this shared purpose; the parts work together for the integrated whole, with high cooperation and low conflict (p. 3144).

High cooperation and low conflict between the parts of a system are therefore the relevant criteria for considering a system as an organism, and inferring that this whole is the locus of natural selection and adaptation (Strassmann and Queller 2010). These authors claim that "organismality" is something that needs to be explained in biology, as natural selection seems to condensate into organisms. Their approach complements the fitness maximisation view of Gardner and Grafen because they focus on actual rather than potential cooperation and conflict: "organisms should be defined as what they actually do" (p. 3144). They view germline sequestration as a capacity that evolved for controlling selfish mutations (i.e. decreasing conflict), and argue that more serious conflict happens when the requirement of "unicellular bottleneck" is violated, i.e. the fact that all cells of the organism come from one single, fertilised cell. Accordingly, they view plants as organisms as well, but see them as having somewhat higher conflict rates than animals due to their growth from multicellular meristems, which sometimes leads to actual conflict.

By defending a higher degree of cooperation than and a low degree of conflict between the interacting parts as the main criterion criteria for "organismality", Queller and Strassmann are not excluding the possibility that adaptations may take place above and below the level of the organism; rather, they argue that most adaptations will happen in discrete bundles, since the organism is, after all, the main focus of adaptation. In fact, these bundles of adaptations help identify organisms, because within each bundle almost all adaptations are directed towards a common end.

From a similar but more pluralistic perspective, Folse 3rd and Roughgarden (2010) emphasise that a definition based on the evolutionary concepts of fitness and adaptation would be preferable to one based on genetic and physiological characteristics. Following Maynard Smith and Szathmáry (1995), these authors claim that in an evolutionary approach to individuality, in which a new individual is considered as emerging from the interaction of previously independent ones, two main problems arise:

1. Selection operating at the lower level may be incongruent with selection operating at the higher level, and thus be fatal for the emergence of the new individuality;
2. Entities that were previously being reproduced independently, can now only reproduce *inter*dependently, as parts of a whole.

They then suggest what they call three "nested views of individuality", which should be jointly adopted to overcome these two difficulties. They call the first view "alignment of fitness", which stresses the importance of genetic relatedness and homogeneity, ensured by the unicellular bottleneck between generations in multicellular organisms. Basically, it is the idea that the organisation of cells avoids competition among themselves, so that the fitness appears as a collective property. The second view is called "export of fitness" and is based on the idea of germ-soma separation and the consequent division of labour between reproductive and non-reproductive tasks, which exports fitness from the lower to the higher level (see Buss 1987; Michod 1999, 2005).[6] The third view defines an individual organism as

> an integrated functional agent, whose components work together in coordinated action analogous to the pieces of a machine, thus demonstrating adaptation at the level of the whole organism (Folse 3rd and Roughgarden 2010: 449).

This third "functional concept" builds upon the "export of fitness", which transfers adaptations at the level of the whole organism, and therefore makes it the locus of fitness.

An important consequence of their tripartite and nested proposal is that "alignment of fitness" is not sufficient for individuality because, in this case, a multicellular organism would be equivalent, as Grosberg and Strathmann (2007) have suggested, to an ensemble whose parts (cells) stay connected after division.[7] Division of labour and functional organisation must be included to qualify a system as an individual from the "export of fitness"/"functional" point of view. As Folse 3rd and Roughgarden explain, the previous kind of multicellular ensemble (which just stays connected through generations without any cellular differentiation) would not demonstrate adaptation at the group level (the level of the whole), while the parts remain the locus of fitness. Therefore, the existence of the unicellular bottleneck is not sufficient for a transition to higher-level individuality: what is also required is an organisation of the constitutive cells that is complex enough to generate a functionally integrated multicellular unit. This is what we shall see next.

6.1.2 The Organisational View

The evolutionary view proposes a naturalised explanation for the design of organisms based on the mechanism of natural selection, analogous to the case of a watch. Kant had already used the same comparison in his *Critique of Judgment*, but in a rather different way. He noticed a fundamental *difference* between the

[6] More specifically, Michod (2005) has suggested that in a group of cells with complete germ-soma separation, the cell fitness of all cells will be zero, since none of the cells would be capable of both viability and reproduction (and the cell fitness is the product of them) although fitness at the group level could be considerably higher.

[7] As happens in all cases of multicellularity with an aquatic origin. See Bonner (1999) for details.

two: whereas the watch is formed by fixed components, fabricated beforehand and later assembled, the parts of an organism are formed for and from the others, some parts actually producing (and being in turn produced by) others. In our terms, organisms realise closure, while artefacts do not. Accordingly, while for the Darwinian tradition, the comparison between a watch and an organism – even regarding only contrivance and relation between parts – suggests an analogy, the organisational view requires an essential distinction.

As we explained in Chap. 1, an organism realises a closed organisation of constraints; its dynamic organisation plays a fundamental causal role in the generation of the constraints that actually make it possible. Closure, by definition, implies functional integration in the sense that the set of constitutive constraints exert mutually dependent functions that collectively maintain the whole organisation. Now, when dealing with associations of cells that not only become (temporary or relatively) cohesive systems, but may also turn into highly organised and functionally integrated entities, difficulties arise. Multicellular communities are made up of systems that are themselves functionally integrated, while at the same time they acquire some degree of functional integration and various degrees of interdependence at the collective level (Turroni et al. 2008). For instance, biofilms could be said to exhibit functional diversity in the sense that they bring together formerly differentiated groups of cells, performing several coordinated tasks (through the production of a common matrix, see Flemming and Wingender 2010; Ereshefsky and Pedroso 2013). In many biofilms, for example, there are groups of cells that belong to the multicellular entity only through the matrix provided by others. Just like biofilms, many other multicellular systems could also be considered, at least in a minimal sense, as organisationally closed systems.

Yet, the issue is that not all systems realising closure are eligible candidates for multicellular organisms. From our perspective, autonomy is the grounding of the concept of organism, be it unicellular or multicellular. Now, since we have argued that, in order to be considered autonomous, a system should realise a closed, regulated, agential organisation, the question is how and when these more demanding requirements are met in the multicellular domain. The organisational view should then clarify under what conditions multicellular closed organisations exhibit the relevant degree of functional integration for realising higher-level autonomy.

In this respect, the central remark is that, however different they might be, all highly integrated multicellular organisms are constituted by genetically homogeneous cells coming from one single fertilised cell ("germ cell"). In contrast to any artefact, or to weakly integrated multicellular systems, multicellular organisms result from a process of *differentiation* between their functional parts, and not from the *aggregation* of pre-existing entities. The main reason for this is that the forms of multicellularity constituted by genetically homogeneous cells, by enhancing integration, can considerably reduce intercellular conflicts. As Wolpert and Szathmary (2002: 745) have argued, only systems constituted by developmentally differentiated cell types are candidates as truly multicellular entities.

> It is advantageous for the unit of reproduction (the propagule) to be as small as possible (that is, a single cell), as the uniformity thus created will reduce the likelihood of conflict between cells. Mutation (. . .) will upset this uniformity, and selection against mutation may favour propagules of different sizes. Mutants that affect the organism but benefit the cell (such as those that lead to cancer) cannot be effectively selected out of large propagules, so their occurrence would favour a single-celled propagule. By contrast, uniformly deleterious mutants that affect the survival of both cell and organism can be successfully selected out of a multicellular organism, so their occurrence would favour propagules that are larger than a single cell (ibid.).

As these authors emphasise, only by meeting these requirements can multicellular systems evolve towards higher degrees of complexity:

> There are multicellular organisms, such as the cellular slime moulds, that develop by aggregation and not from an egg, but their patterns of cell behaviour have remained very simple for hundreds of millions of years. The evolution of more complex organisms increases the pressure to use an egg as a propagule (ibid.).

What is the link between differentiation and integration? Whenever multicellular organisms originate from germ cells, the generation of internal differentiation due to germ-soma separation entails some loss of freedom for single cells. More specifically, cells in a multicellular organism lose their totipotency through irreversible differentiation processes that make them apt to live only in a very specific environment, tightly surrounded by other cells, and therefore to contribute to the maintenance of the whole organism in a cooperative way. Therefore, the integration of functionally differentiated cells gradually emerges from early developmental stages onwards. For instance, inner cells depend on cells located at the physical boundary to obtain the material and energy resources required to carry out their own metabolism.

The connection between differentiation and integration has also been analysed by Buss (1987), who explains the origin of multicellular organisms from an evolutionary perspective as a unit of selection (from a similar perspective, it is also worth mentioning Michod 1999 and Bonner 2000). At the same time, he tries to integrate this evolutionary dimension into an organisational framework. Arguing that the germ-soma barrier is a derived evolutionary state, he shows how patterns in embryonic cleavage, gastrulation, mosaicism, induction, and competence arise as a consequence of the conflicting evolutionary interests of cells and the whole integrated multicellular entity. Buss explains that, in the evolution towards multicellular organisms, the germ line was initially not closed to genetic variations arising during the course of ontogeny. He studies the evolutionary emergence of homogeneous multicellular organisms as a competition between cell lineages to become germ cells, assuming that the unit of selection is the cell. In some organisms this evolution has produced homogeneity because germ cells are sequestered at very early stages of cell differentiation. Realising that there is a trade-off between the capacity for movement and the capacity for reproduction in single cells, Buss suggests that the appearance of gastrulation – where a hollow ball of cells is transformed into a multi-layered structure including diverse patterns of differentiated cells – was a crucial step in the origin of multicellular organisms. The idea was inspired by the

observation that the cells of a metazoan can be either ciliated or prone to divide, but not both. In other words, the gastrula would be the "solution" to this problem, with the cells on the surface remaining ciliated while those inside lose their cilia, so they can divide. Through gastrulation, cells begin to live in a more specific and spatially-organised environment, where migrated cells are surrounded by still-ciliated ones, which stay at the periphery of the group and provide the material and energy inflow required for the proliferation of the internal cells.

Buss' account, being consistent with natural selection (since cells find a way to maintain themselves and proliferate), can then be said to show that differentiation and integration processes go together. Moreover, it shows that this must happen at a very early stage of development, in accordance with constraints that have been internally generated and should continue until a fully integrated multicellular system is formed. Buss' perspective is, no doubt, interesting. His strategy of accounting for the evolution of developmental architectures in terms of trade-off solutions for the conflict between selective pressures acting on cells and multicellular individuals points to what, in our view, are the fundamental questions for understanding the nature of highly integrated multicellular systems. However, his focus is mainly on the evolutionary origin of multicellular organisms rather than on the question of the organisational requirements for achieving multicellular organismality.

In contrast, our aim in the following sections is to examine, in some detail, the network of relations, mechanisms, and couplings that these associations of cells have to establish in order to achieve a higher degree of functional variety and integration at the collective level, to the point at which they can be considered multicellular organisms. In turn, the emergence of multicellular organisms requires what is usually called a process of "development".

As Wolpert and Szathmary have argued (2002: 745):

> The development of a complex organism requires the establishment of a pattern of cells with different states that can differentiate along different pathways. One mechanism for pattern formation is based on positional information: cells acquire a positional identity that is then converted into one of a variety of cellular behaviours, such as differentiating into specific cell types or undergoing a change in shape and so exerting the forces required for the formation of different structures. This and other patterning processes require signalling between and within cells, leading ultimately to gene activation or inactivation. Such a process can lead to reliable patterns of cell activities only if all the cells have the same set of genes and obey the same rules.

Furthermore, every state/phase of this developmental process should be sufficiently robust and reliable to be compatible with the requirements of natural selection (i.e., always above a minimal threshold of overall fitness). On this basis, we agree with Pepper and Herron (2008) that there is a type of "*positive feedback loop between the process of natural selection and the pattern of functional integration*" (ibid.: 626). Thus, the primary goal will be to provide a feasible explanation of the developmental requirements and characteristics of the mechanisms and organisation that give rise to such a positive feedback mechanism.

From this perspective, a necessary condition for the realisation of highly integrated closure – and possibly, higher-level autonomy – is that the system must

include a specific class of functional constraints subject to higher-level closure, able to control the fate of the cells during the process of cellular differentiation. More specifically, this means that not only must the system possess constraints that are able to modulate *intracellular* epigenetic[8] mechanisms but also that they are also able to trigger off the generation of new developmental constraints during the process. Indeed, what matters for achieving multicellular organisms is the capacity to generate a high degree of phenotypic differentiation from genetically homogeneous cells. Under these conditions, not any form of higher-level control over development matters equally for the self-constitution and maintenance of the multicellular organism.

6.1.3 Multicellularity and Autonomy

Under what conditions may multicellular biological systems undergo the relevant complex collective process of ontogenetic development for getting higher-level autonomy? A sound answer to this question requires a characterisation of the endogenously generated cell-cell interactions resulting in the kind of functional integration of the systems under examination. One of the central challenges in this respect is to discern, as we will try to do below, what organisational level is ultimately "in charge" of the interactions (the individual cells or their collective organisation), paying attention to three specific, key features:

1. *Inter-cellular signalling mechanisms*, taken as one of the core aspects against which the size, diversity, and degree of sophistication of the interaction network can be assessed. This will be crucial for estimating the balance between *intra-* and *inter*-cellular constraints operating within the system as a whole.
2. *The plasticity, modularity, and robustness* of the network, trying to identify whether or not it includes higher-level functions. This in turn will provide an indication as to whether or not there is a set of interdependent constraints that functionally control the developmental process at the meta-cellular level.
3. *The degree of internal metabolic control over cell differentiation and cell division*. This will also provide an estimation of the extent to which the cell cycle is subordinate to the collective entity's global reproductive process.

[8]By the term "epigenetic" we mean processes and mechanisms by which a heritable phenotypic change is induced in the genetic system of a cell that does not involve a change in the nucleotide sequence of DNA (Berger et al. 2009). Epigenetic processes are basically the result of mechanisms allowing the selective activation of some genes and the inhibition of others. For example, DNA methylation or histone modification, which serve to regulate gene expression without altering the underlying DNA sequence. That is why epigenetic constraints affect the fate of the cells during development. Although there is no modification of the genome of the cell, epigenetic changes may remain through cell divisions for the remainder of the cell's life and may also last for multiple generations.

Taken together, these features provide a relevant measure of the degree and kind of control exerted by higher-level functions on the development and differentiation of individual cells. In turn, this gives an indication of the "taking over" of biological functions by the higher-level of organisation and, ultimately, of the degree of functional integration of the multicellular system as a whole. What matters from the autonomous perspective is that only those multicellular closed systems that have attained a sufficient threshold of collective functional integration are complex enough to realise higher-level autonomy. In particular, multicellular autonomous systems are those systems whose higher-level closed organisation includes the classes of functions required for autonomy, i.e. agential and regulatory. On the one hand, as described in Chap. 4, higher-level autonomy should include (in contrast with ecological organisations, see Sect. 4.5) agency, that is, the ability to deal with the environment *as an integrated (multicellular) unit*. On the other hand, regulatory higher-level functions are required, i.e. (Chap. 1, Sect. 1.8) *second-order* [9] constraints exerting their causal actions on changes of other constitutive constraints of the organisation.

As mentioned in the introduction, however, in this chapter we will not deal with the actual realisation of the interactive and regulatory capacities of multicellular organisms, nor even with the question of whether theoretical differences might exist concerning the way in which autonomy is realised at the different levels of organisation. Our focus here is on the control over development, as a general, necessary condition for attaining a sufficient degree of collective functional integration. In the next section, we will present and discuss three case studies in order to show how the appearance of increasingly integrated multicellular entities has required the appearance of increasingly complex strategies to manage the development of their internal functional variety and plasticity. In addition to illustrating several specific and empirically-grounded implications discussed in later sections, these cases help highlight the crucial importance of the kind of control exerted on cellular differentiation. In particular, we will examine the developmental processes of three multicellular systems: the cyanobacterium *Nostoc.punctiforme* as an example of a bacterial multicellular system; the green algae *Volvox.carteri* as an example of an early eukaryotic multicellular system; and the echinoderm *Strongylocentrotus.purpuratus* (a sea urchin) as an example of a metazoan multicellular system (Arnellos et al. 2014). As we will underscore, these systems exhibit substantial differences in the degree and complexity of the higher-level control exerted over development. These differences, in turn, underlie the differences in the functional complexity of the higher-level organisation. As a result, only one of these systems seems to be a candidate as a higher-level organism from the autonomous perspective.

It should be noted that, at both the prokaryotic and eukaryotic levels, there are many examples of multicellular systems (like biofilms) that are formed by

[9]The conceptual distinction between levels and orders of organisation is at work here. A given level of organisation, which is identified by the fact of realising closure, is a candidate as a level of autonomy if, among other things, it contains regulatory functions, subject to second-order closure.

aggregation, i.e. by the association of genetically inhomogeneous cells.[10] Yet, as we have argued, our underlying hypothesis is that only genetically homogeneous systems are relevant candidates as multicellular organisms.

6.2 Comparative Analysis

The three examples of multicellular systems examined in this section are relevant because they cover a wide range of configurations, while at the same time being highly integrated: they present a strong degree of contiguity (spatiotemporal neighbourhood) and, as we will explain in Sect. 6.3, they satisfy the criteria of alignment and export of fitness, exhibiting a high degree of collaboration. Accordingly, they might be taken as relevant candidates for multicellular organisms from an evolutionary perspective. Yet, as we will discuss, the analysis on how the developmental constraints operate in these three different multicellular systems may result in a different conclusion from the autonomous perspective.

6.2.1 Cyanobacterium Nostoc.Punctiforme

Nostoc.punctiforme is a multicellular genetically homogeneous system constituted by cyanobacteria, which form photosynthetic and diazotrophic filamentous organisations that obtain their energy from sunlight and their carbon from air and water, fixing molecular nitrogen as well. Initially, the cells are phenotypically homogeneous (all of them are vegetative cells), but then a developmental process takes place, leading to a phenotypic differentiation between two different types, photosynthetic vegetative cells and specialist nitrogen-fixing heterocysts.[11] Through this cellular differentiation, *Nostoc* can take energy from the sun and use it to make nitrogen compounds. The nitrogen products are then passed along to the photosynthesising cells.

The process of differentiation produces a semi-regular pattern of morphologically and metabolically different cell types. Several models have been proposed to explain this pattern of development (Kumar et al. 2010; Campbell et al. 2007). All models hypothesise that the pattern is ultimately determined by the action of a diffusible inhibitor produced by the differentiating heterocysts. The signal that

[10] An interesting case is *Physalia.physalis*, a highly integrated association of four specialised polyps and medusoids, whose constitutive parts can no longer disintegrate and continue living independently.

[11] Heterocysts are cells that specialise in nitrogen-fixing during nitrogen starvation. They fix nitrogen from dinitrogen (N_2) in the air in order to provide the cells in the filament with nitrogen for biosynthesis.

kick-starts development in all the vegetative cells is generated as a reaction to a nutrient limitation, namely, a lack of nitrogen. All cells in the filament detect the signal but only some of them respond to it, leading to a biased initiation process of differentiation. The cause of such biased initiation is not known, but it is assumed that it is associated with the physiological state of the cells (probably their position in the cell cycle at the moment the signal is detected). The nitrogen limitation triggers the activation of the global nitrogen regulator (*NtcA*) in all the cells that are in the appropriate cell stage. *NtcA,* in turn, at the same time, activates two different molecules, *HetR* (which induces the cell to differentiate into a heterocyst) and *PatS*. But whereas *HetR* operates intracellularly, *PatS* builds up as an intercellular gradient (as a molecular compound generated within the system), which diffuses rapidly among neighbouring cells. Diffusible *PatS* suppresses *HetR* and stops the differentiation of the neighbouring cell(s) as a heterocyst. If rapid diffusion drains *PatS* from the place of production (which is mostly the case), *HetR* synthesis is stabilised and the cell develops into a heterocyst. In neighbouring cells, the entry of *PatS* prevents the formation of *HetR*. In more distant areas, the diffusion of *PatS* may not be sufficient, so new centres of activation may be formed.

The crucial remark at this point is that no other cells have been found in the filament producing signals that act as intercellular constraints (i.e. inducing or suppressing cellular differentiation) on the cell that produced the diffusible *PatS*. Hence, it seems that the development of a differentiating cluster of cells into a single heterocyst (at any developmental site in the filament) operates, at a collective level, under the effect of a *single* constraint (*PatS* concentration). There seems to be no generation of other compounds/structures (i.e., no synthesis of any morphogen or some other kind of signal in the cells where *HetR* is suppressed by *PatS*) that act intercellularly on the phenotypic traits and organisation of the different cells that produced *PatS*, or indeed any other neighbouring cells.

Moreover, heterocysts undergo *terminal* differentiation, as they lose the ability to divide, because in this way they provide surrounding vegetative cells with combined nitrogen. In *Nostoc,* therefore, vegetative and reproductive functions are realised by the same type of cells. Furthermore, as explained by Christman et al. (2011), the transition from growth to nitrogen dependence (when heterocyst generation takes place) is not immediate. Given the limited number of developmental signals, cell division and cell differentiation cannot be modulated outside the core metabolic context. Consequently, the explicit dependence of the differentiation of a heterocyst on the vegetative cell life-cycle stage, and the terminal differentiation of heterocysts themselves, imply a mechanism of developmental modulation of differentiation that remains strongly coupled to the metabolic requirements of the vegetative cells.

6.2.2 Volvox.Carteri

Volvox.carteri is an eukaryotic multicellular system constituted by unicellular algae, which moves coherently towards the direction of light, and which has been

frequently studied in attempts to understand the transition to eukaryotic multicellularity exhibiting cellular differentiation and complete germ-soma separation. This multicellular system has a developmental process that results in asexual spheroid adults with two cell types: large reproductive cells (*gonidia*) and small motile somatic cells. The coexistence of these two cell types, however, generates a problem, known as the "flagellation constraint" (Koufopanou 1994), namely the incompatibility between cell division and motility in photosynthetic flagellates. This incompatibility is problematic because all swimming photosynthetic organisms need to be motile even when they divide in order to maintain a position that allows them to efficiently use light for growth and division. *Volvox* solves this problem by differentiating a subset of cells in the anterior end into somatic cells that do not divide but continue beating their flagella, thereby providing the system with the capacity to swim. The rest of the cells (the germ cells) divide and produce progeny. Since germ cells directly become reproductive *gonidia*, *Volvox* exhibits a complete germ-soma separation.

How is this differentiation achieved? *Volvox* embryos first cleave and then divide asymmetrically to produce one large gonidial "cell initial" and one small somatic "cell initial" each (Kirk 1998). The first gonidial cells produce additional somatic initials at each division. The gonidial initials then temporarily stop any cleavage activity, while the somatic initials continue to divide symmetrically about three more times. At the end of embryogenesis, the volume of gonidial initials is about 30-fold larger than that of somatic initials. However, at this stage, cells differ only in size (Kirk 2005). Subsequently, the size of each sister cell leads to either a somatic or germline developmental process (Kirk et al. 1993). Thus, small cells develop as biflagellate somatic cells for motility, biosynthesis of the extracellular matrix and phototaxis, and large cells develop as non-motile, germ cells specialised for growth and reproduction. Asymmetric division plays a crucial role in *V.carteri* development, as has been extensively discussed (see e.g. Kirk 1998, 2005; Hallmann 2011 for details). Specifically, *Volvox* cells that are below a certain size threshold at the end of cleavage will differentiate as somatic cells, while cells above that threshold will differentiate as gonidial. In the case of a *gls* gene mutation, all cells will keep on dividing symmetrically, becoming somatic cells since they are too small to undergo gonidial specification. There is also another gene, *RegA*, which plays a crucial role for complete, stable germ/soma separation. It operates by repressing chloroplast biogenesis, thus preventing somatic cells from growing enough to trigger cell division. *RegA* mutants will follow the path of their unicellular ancestor, beginning as small flagellated cells and then re-differentiating as gonidia. By contrast, *lag* genes act in gonidia to prevent the development of somatic features, such as flagella and eyespots. Now, considering that all three genes (*RegA*, *gls* and *lag*) act *intracellularly*, and that the initiation of the somatic or gonidial developmental process is explicitly dependent on the size of each cell, it seems that cellular differentiation is achieved only by intracellular cell fate specification. In other words, the development of cellular differentiation in *Volvox* takes place independently of any intercellular signal produced by other cells (Nedelcu and Michod 2004) (Fig. 6.1).

As a result, in *Volvox* (even more explicitly than in *Nostoc*, since in *Volvox* there is a complete germ-soma separation) cell division remains either totally decoupled

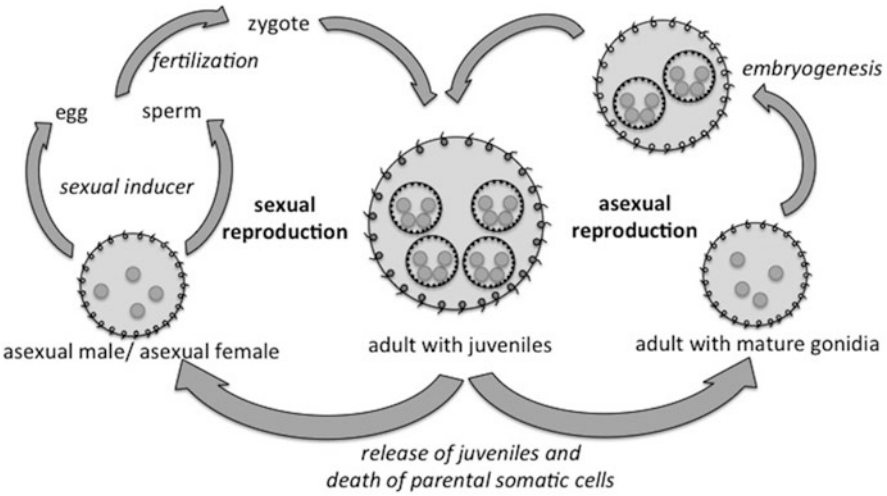

Fig. 6.1 Main steps in the development and reproduction of Volvox (*credits: Juli Peretó*)

(in somatic cells) or strongly coupled (in germ cells) to cell growth and global system reproduction. Consequently, *Volvox* does not present the flexibility required for further re-differentiation and growth in the somatic cells. The dissociation of cell division and cell growth does not occur through the asymmetric distribution of morphogens and germ-line factors during the asymmetric divisions in the cleavage phase, but rather by acting on the ancestral linkage of cell growth and cell division. While in *Nostoc* there is at least one intercellular signal, in *Volvox* developmental differentiation is entirely dependent on intracellular mechanisms and therefore it is strongly coupled to the core metabolic requirements of the processes of growth and division. Again, what is lacking is adequate higher-level control over development. As we shall see, this is precisely the difference with our next example.

6.2.3 Strongylocentrotus.Purpuratus

Strongylocentrotus.purpuratus, or the purple sea urchin, is a small invertebrate that belongs to the echinoderm phylum. Although it is a relatively simple metazoan, it has a very interesting developmental process leading to differentiated tissues and organs. Sea urchin embryos develop into free-swimming pluteus larvae.

At the beginning of development, pattern formation and cell differentiation in sea urchins employ two major mechanisms of cell fate specification (Peter and Davidson 2009, 2010, 2011):

1. The inheritance of maternal signals (structures playing the role of transcription factors) operating as intracellular determinants;
2. Intercellular signals (between cells of the same or of different lineages).

Initially the asymmetric distribution of the maternally provided signals along the major axes results in the establishment of domains of specific gene expression. This endows cells in different regions of the embryo with the capacity to send and receive intercellular signals. In this respect, the main difference with the two previous examples is that these signals lead to the variable expression of new sets of transcription factors, which by acting inter- and intracellularly, modulate the implementation and execution of *several different developmental processes*. According to these higher-level constraints, several developmental processes are initiated, stabilised or/and excluded, resulting in the spatiotemporal, timely production of specific proteins that characterise the state of different cell types, thus defining the overall organisational pattern in the developing embryo.

One of the most interesting aspects of sea urchin development is the mechanism of intercellular interactions that dynamically modulate key aspects of this development (Ben-Tabou de-Leon and Davidson 2007). These signals constrain the organisation of other cells, so that their developmental fate is appropriately specified and ensured. The results of the operation of intercellular signals are: (i) the initiation of the development of the endomesoderm; (ii) the timely separation between mesoderm and endoderm specification, and the initiation of mesoderm formation; and (iii) the timely separation between anterior and posterior endoderm specification, the initiation of their formations and the initiation of gastrulation. Let us briefly explain how all this happens.

Very early on, the intracellular operation of the maternally provided protein *β-catenin* creates a new signal, called *Wnt8*, whose intercellular operations result in a mutually constraining interaction between cells of the same lineage. In particular, *β-catenin* operates intracellularly, causing the creation of *Wnt8*, which in turn acts as a constraint on a neighbouring cell, in order that the nuclearisation of *β-catenin* in that second cell will be intensified, bringing about further production of *Wnt8*. This intercellular feedback mechanism ensures the continuous production of *Wnt8* across the lineage. This is essential for sea urchin development, since any disruption of that intercellular mechanism results in problematic specification of the skeletogenic and endomesodermal lineage (Oliveri et al. 2008). The intracellular operations of the increasingly nuclearised *β-catenin* will create another two intercellular signals: (i) an *early signal (ES)*, which is still undefined (Angerer and Angerer 2012), and (ii) a *Delta* signal, which will be used to drive mesoderm fate specification in the macromere lineage.

The indirect but mutually exclusive constraining actions between *Wnt8* and *Delta* operating intercellularly throughout the embryo's development are of particular interest here. The intercellular operation of *Wnt8* on the large micromeres induces *Delta*, whose intercellular operation results in: (i) the separation between mesoderm and endoderm developmental processes; and (ii) in the suppression of *Wnt8* in certain cells, permitting the creation of a new *Delta* signal in these cells. What happens in practice is that, as development proceeds, wherever *Wnt8* is generated in the endomesoderm, *Delta* is not, and vice versa (Peter and Davidson 2009). All these intercellular signals contribute to the precise activation of the mesodermal and endodermal developmental processes in space and time. Interestingly, this

constraining process is much more indirect, as it is the result of other intercellular signals that operated several developmental stages back. This intercellular signalling continues throughout development, allowing the formation of tissues critical to the survival of the embryo.

In sum, the developmental process of the sea urchin is characterised by intercellular signals that constrain intracellular processes, which further specify or directly initiate the developmental fate of the respective cell lineages, and/or affect (by inducing or suppressing) the production of other intercellular signals. In turn, these signals will constrain the intracellular processes of other cells in the embryo. Accordingly, the type of development coordination occurring in sea urchins differs from that which takes place in *Nostoc* and *Volvox* in three main aspects:

1. In sharp contrast to the single intercellular signal for the development of the differentiating filament operating in *Nostoc*, and the purely intracellularly-determined specification of the two cell types in *Volvox*, the development of the sea urchin depends on *several* intercellular signals (as we saw, it depends at least on *Wnt8*, *Delta*, and others like *Wnt16* and *V2*).
2. In sea urchins, different types of relations (combinations) exist between the intercellular signals, resulting in different types of intercellular mechanisms.[12] In all cases the result is the creation of intercellular mechanisms that modulate the developmental process.
3. As a consequence, sea urchins seem to have the capacity for much more elaborate cellular differentiation, which is decoupled from the ancestral mechanisms of cell growth and cell reproduction. Cells preserve a degree of differentiation potential for several developmental stages, and the sequence of their biochemical changes and the timing of their division and/or migration are largely modulated by the combinatory application of past and present intercellular mechanisms operating on them.

Sea urchins modulate development and cellular differentiation through the operation of intercellular mechanisms that coordinate the fate of different cell lineages, while allowing new possibilities for cell differentiation; this gives rise to a new form of collective multicellular organisation. In the next section, we shall discuss the nature of the coordination of the three multicellular organisations and shall argue that the type of developmental modulation exhibited by sea urchins consists in a much richer functional variety, leading to more complex (possibly autonomous) higher-level organisations.

[12]One type is the intercellular feedback mechanism of *Wnt8*. Another type is the intercellular mechanism established by the indirect and mutually exclusive operations of *Wnt8* and *Delta*. A case of a highly combinatorial type of mechanism is the one at work for the separation between anterior and posterior endoderm formation, a process which is eventually established by the intercellular operations of other signals – *Wnt16* and *V2* – but which needs other inputs from the operations of other intercellular mechanisms during prior developmental stages.

6.3 Developmental Conditions for Highly Integrated Multicellular Organisations

The examples described in the previous section constitute three different kinds of multicellular systems, each of which could be pertinently described (at least from a phenomenological perspective) as resulting in a high degree of collaboration – and low conflict – among the parts. Accordingly, this would lead to fitness maximisation for the multicellular systems, and the new higher-level organisation could be identified with the capacity of a group of cells to demonstrate adaptation at the level of the whole multicellular system. From an evolutionary perspective, hence, all these systems might be taken as multicellular organisms.

From the organisational perspective, in turn, relevant fundamental differences exist between these systems. In spite of their common features, indeed, what matters (again, as a *necessary* condition) for the realisation of higher-level autonomy is the following twofold issue:

1. Whether, in these systems, organisational closure includes developmental constraints; namely, intercellularly produced functional signals modulating the fate of the cells during differentiation;
2. Whether these signals can trigger the generation, during development, of new similar control signals, in order to guarantee the establishment of substantial high-level functional variety and, in particular, of regulatory capacities.

With respect to both of these aspects, the developmental mechanisms of the sea urchin are significantly and qualitatively different from those of *Nostoc* and *Volvox*. The developmental process occurring in two latter cases is strongly coupled to the reproductive and self-maintaining intracellular lower-level processes. As a result, their number and complexity is severely limited.

As described in the previous section, *Nostoc* possesses only *one* signal constraining the intercellular dynamics in development. Moreover, the underlying mechanism of differentiation remains strongly coupled to the metabolic requirements of the vegetative cells. As a consequence, there is no development of further cellular differentiation, resulting in a functionally diversified and integrated high-level organisation. Things remain essentially the same with respect to the capacity for cellular differentiation in *Volvox*. Although in this case there is a much more elaborate process of development and reproduction resulting in a complete germ-soma separation, it seems that *no* signals act intercellularly to further modulate the dynamics of development. Last, but not least, the way this multicellular system maintains its germ lineage precludes any possibility of further re-differentiation and growth of its cells. Here again, the lack of control over the intercellular collective dynamics prevents any further development of cellular differentiation and higher-level functional complexity.

It is worth emphasising that *Nostoc* does realise second-order closure; it therefore possesses collective interactions that are necessary for its operational coordination (functional division of labour) and global behaviour. For instance, in *Nostoc* there

is a rich exchange of metabolites between vegetative cells and heterocysts, which is necessary in order to meet the needs of the two cell types.[13] Yet – and this is the central point discussed in this chapter – this form of multicellular organisation does no generate a higher-level control subsystem operating on the developmental processes of the constitutive cells. In other words, in Nostoc, the multicellular system is unable to foster and support a process of development leading to the degree of functional differentiation required to get higher-level autonomy.

In the case of the sea urchin, things are substantially different. In sea urchins, the modulation of cellular development is based on several intercellular functional signals. These signals establish intercellular mechanisms that control the developmental process by triggering, activating, and suppressing intracellular processes responsible for the specification of the developmental fates of the respective cell lineages. As discussed earlier, different combinations exist of intercellular signals, which result in different types of intercellular mechanisms and, consequently, in qualitatively different kinds of developmental modulation. Subsequently, this allows for part of this set of intercellular signals to modulate intracellular processes that promote the production or suppression of other intercellular signals, which then constrain the intracellular processes of other cells, and so on and so forth (see Arnellos et al. 2014 for details).

These intercellular mechanisms constitute an endogenously created set of specific higher-level functions: through their constraining action, such a complex developmental process is effectively driven and the specification state of each cell lineage is spatiotemporally stabilised. As higher-level functions, these intercellular mechanisms are largely decoupled from the intracellular processes of the constituting units (because, among other things, their characteristic time scales are different; see Chap. 1), and can be varied without disrupting those more basic intracellular processes. At the same time, they act on the cellular epigenetic mechanisms, thus modulating their operations.

The specific organisation of the sea urchin can be usefully compared to different types of intercellular constraints that may also induce intracellular epigenetic changes, leading to some form of cellular differentiation. For example, some squids (*E.scolopes*) have a symbiotic relationship with certain bioluminescent bacteria (*Vibrio.fischeri*), which inhabit a special light organ in the squid's mantle. The bacteria are fed through a sugar and amino acid solution provided by the squid and, in return, "hide" the squid when viewed from below, by matching the amount of light hitting the top of the mantle. The light organ contains filters, which

[13]In the case of *Volvox*, the realisation of second-level closure is more debatable. Somatic cells achieve efficient swimming capacity that, thanks to their coordinated action, is beneficial to the whole system (and notably to reproductive cells). However, although there is coordination between reproduction (germ cells) and movement (flagellated cells), so that the network of cell-cell interactions results in a certain degree of functional differentiation, it remains unclear whether somatic cells could be said to depend on reproductive cells in the precise sense of "dependence" discussed in Chap. 1. Accordingly, the claim according to which *Volvox* is a multicellular organisation cannot be taken for granted, and would deserve further investigations.

may alter the wavelength of luminescence, making it closer to that of moonlight and starlight (McFall-Ngai 1999). In this symbiosis, the development of both the bacteria and the epithelial cells of the squid are modulated by each other. In particular, the bacterium *V.fisheri* induces several changes in the development of the squid's light organ, which lead to the loss of some superficial fields of the squid's epithelial cells; in turn, the squid induces two important developmental changes in *V.fisheri*, i.e. the loss of their flagella and the decrease in their cell volume. Yet considering that the bacterium has a prokaryotic genetic machinery[14] and that the type of cells responsible for the squid's intercellular developmental signalling network are completely different[15] from *V.fisheri*, the participation of *V.fisheri* in the squid's intercellular epigenetic network is severely limited, insofar as it does not have the capacity to significantly alter the network, in order to generate enough higher-level functional differentiation. From the organisational perspective, therefore, the symbiosis between the squids and *V.fischeri* does not set the conditions for higher-level autonomy. This suggests that the role played by symbiotic bacteria in the development of certain metazoans, although surely important (think of the human gut, for instance, as explored in Turroni et al. 2008), it does not succeed in reaching the degree of collective functional complexity of the multicellular systems constituted by genetically homogeneous eukaryotic cells.

In contrast, the genetically homogeneous (and epigenetically differentiated) eukaryotic cells in the sea urchin – and in the vast majority of metazoans – can participate in much more complex developmental functional interactions (e.g. leading to the formation of tissues and organs).[16] What the case of the sea urchin illustrates is the invention of a higher organisational level (an "intercellular epigenetic network") that enables the precise inter-level control of interactions

[14]For instance, *V.fisheri* has neither the ability to generate metabolically decoupled signals, such as *Delta* and *Wnt8*, nor the appropriate receptor mechanisms for their intercellular action.

[15]Eukaryotic epigenetic mechanisms are much more complex than prokaryotic ones because in the latter case, the processes of transcription and translation are operationally separated (see Chap. 5). Eukaryotic epigenetic control occurs even before transcription is initiated, and therefore in eukaryotic cells epigenetic mechanisms can control gene expression at many different levels. This means that intercellular signals modulating eukaryotic epigenetic cells can induce much more diversified effects.

[16]Although plants share many of the requirements so far described for developmental modulation, we centre our analysis in metazoans, because plants are multicellular systems based on cells with walls. Now, as Gerhart and Kirschner (1997) have pointed out, the loss of the cell wall in some unicellular eukaryote ancestor was also a very important factor in the appearance of rich cell differentiation in multicellular systems. "One development of great importance for future metazoan multicellularity was the loss of the cell wall in some unicellular eukaryote ancestor. The lack of a cell wall (. . .) permitted the ancestors of animal cells to interact directly with each other through apposed plasma membranes, to adhere to each other, to crawl on surfaces, to differentiate into complex shapes, to engulf other cells by phagocytosis, and to engage in junctional communication with other cells. Cell adhesion and junctional communication are characteristics of the formation of epithelia and the segregation of an internal milieu, which are found in all metazoans" (p. 11). See also footnote 18.

between functionally differentiating cells. In turn, this control over development enables, as claimed, the emergence of a much richer functional diversity, combined to a high degree of integration.

The relevance of higher-level developmental functional mechanisms in the case of metazoans is found in the complex problems inherent in the generation, maintenance, and reliable reproduction of their organisation. Their constituents are cells that already have a genetically instructed metabolism, expressed in different phenotypes. Therefore, the multicellular organisation must modulate cell growth, cell differentiation, and cell division, so that its constitutive identity (or at least some key aspects of it) is specified and coordinated by a self-generated developmental process.[17]

Metazoans are tightly integrated systems that constitute modifications, or redefinitions, of their meta-cellular organisation. These increasingly complex forms of organisation are based on deep-rooted changes at the developmental, body-plan level. In turn, developmental plasticity is possible, among other things, because of the regulatory possibilities offered by many animal-specific genes (e.g. homeotic genes) which, combined with different levels of RNA editing processes, expand enormously at these stages (Mattick 2004), and give rise to the generation of elaborate intercellular communication and adhesion devices, complete germ-soma separation and its integration in further cellular differentiation, sexual reproduction, etc.

In sum, the specific case of the sea urchin's development exemplifies a kind of higher-level control over development (widespread in most multicellular animals[18]), which sets the conditions for the realisation of a rich domain of functionally integrated higher-level organisations.

[17]While many other functional constraints may also contribute to the constitutive processes and maintenance of the whole multicellular entity (i.e., symbionts, indirect action of other organisms, etc.), they do not belong to the same level as those we have studied (namely, those constraints which regulate epigenetic mechanisms of cellular differentiation and which are decoupled from metabolic-interactive processes).

[18]Although multicellular plants have their own developmental processes too, it is undeniable that metazoans' development has achieved a higher degree of complexity. There seems to be a number of different reasons for this. First, unlike animals, plant cells do not terminally differentiate, remaining totipotent, often with the ability to give rise to a new individual plant. While plants do utilise many of the same epigenetic mechanisms as animals, such as chromatin remodelling, it has been hypothesised that plant cells do not have a "memory" and reset their gene expression patterns at each cell division, using positional information from the environment and surrounding cells to determine their fate (Costa and Shaw 2007). Second, the loss of the cell wall, already mentioned in footnote 16, seems to be an additional condition contributing to the enabling of the unfolding of the functional potentialities of cellular differentiation when building a complex integrated multicellular organism. See also Caroll (2001).

6.4 Towards Higher-Level Autonomy

In Chap. 4, we developed the notion of minimal autonomy, which provides the conceptual framework for characterising unicellular organisms as prokaryotic and eukaryotic cells (Ruiz-Mirazo and Moreno 2004).

The background question of this chapter is to what extent can this concept of autonomy be applied to a multicellular system. According to our definition, any entity that achieves regulated agential closure should be considered an autonomous system, and therefore an organism. Yet, the situation changes here, because we are dealing with systems whose functional parts are themselves constituted by autonomous entities (living cells). Indeed, in some forms of collective associations, the constitutive autonomous units may be more integrated and cohesive than the multicellular system itself. In other cases, instead, the global multicellular organisation becomes more complex, functionally diversified, and cohesive than that of its constitutive units. In many cases, therefore, it might be difficult to determine what level of organisation is ultimately responsible for the production (and control) of the constraints that drive the behaviour and interactions between the constitutive cells, in such a way that the whole set becomes a cohesive, self-maintaining agent.

In this chapter we have focused our study on the "internal" dimension of the problem, leaving aside the question of agency. As we have explained, development plays a crucial role in the realisation of higher-level autonomy. In particular, it is our contention that higher-level autonomy requires a high-level closure, including a set of developmental constraints, that is complex enough to ensure the generation of the adequate degree of high-level functional diversity. In contrast to what happens in the cases of systems as *Nostoc* and *Volvox*, the global multicellular system must produce a set of second-level constraints that functionally harness the developmental processes of each of the parts. If the collective entity meets these additional requirements, then it constitutes a relevant candidate as an autonomous organisation.

As argued in the previous section, not all multicellular systems constituted by genetically homogeneous cells exhibit the same type of functional organisation. Multicellular systems like *Nostoc* and *Volvox*, for instance, do not possess the capacity to exert sufficient higher-level control over the epigenetic dynamics taking place at the lower level. In some cases (*Volvox*) there is a total absence of intercellular signals constraining the developmental proceses. In other cases (*Nostoc*) they do implement minimal intercellular mechanisms that have a constraining effect on intracellular dynamics, but these are not diverse enough to open up a new functional and hierarchical domain that could lead to collective autonomous organisations. This is why *Nostoc* and *Volvox* do not meet the requirements as second-order autonomous systems.

By contrast, sea urchins possess an operationally closed combination of different types of intercellular mechanisms that control the epigenetic intracellular processes, so that cellular differentiation is enhanced and immediately channelled. As a result, sea urchins possess the relevant degree of higher-level of functional variety and integration: in particular, they seem to possess higher-level regulatory functions

(Peter and Davidson 2009, 2010, 2011). According to our definition, hence, they do might comply with one of the requirements for higher-level autonomy. And if, moreover, sea urchins were shown to possess an integrated form of agency,[19] they should be considered as multicellular autonomous systems.

There is one final issue to address before concluding. The claim according to which multicellular organisms realise *higher-level* autonomy supposes that those systems are made of components which are themselves (lower-level) autonomous. As mentioned in the introduction, this might be questioned, insofar as the constitutive units (namely, their unicellular parts) are very heavily constrained by the encompassing organisation. As we discussed in the case of the sea urchin, for instance, cells in the different cell lineages need to adapt their characteristics (the initial pluripotency of all blastomeres) to serve the multicellular organisation. Consequently, they undergo irreversible differentiation processes that make them apt to live only in a very specific environment, tightly surrounded by other cells. Therefore, not only do they become heavily dependent on each other, but also undergo deep-rooted organisational changes that allow them to form tissues and organs (something which requires a qualitatively different degree of collaboration, beyond metabolic or associative/aggregative exchanges). Accordingly, it might be argued that when unicellular systems become a part of multicellular autonomous systems, they are no longer autonomous.

In our view, however, this conclusion is not compelling. The fulfilment of the highly specialised functions of the multicellular organism seems to require a type of unicellular entity that, from the point of view of its internal organisation, still meets the requirements for autonomy (in particular, it continues operating through its own intracellular regulatory mechanisms, retaining a certain degree of epigenetic plasticity and even maintaining interactive functions), even though it can maintain itself only within the boundary conditions generated by the higher-level intercellular mechanisms. In multicellular autonomy, each cell maintains its own identity, based on a closed network of chemical reactions that generates its constitutive and agential dimensions. The control exerted by the multicellular organisation on the individual cells is restricted by the need to preserve the metabolic coherence and minimal threshold of epigenetic plasticity of the unicellular units. In a word, it seems that multicellular autonomy does require unicellular autonomy.[20]

[19]Elsewhere (Arnellos and Moreno in press) one of us has argued that only eumetazoans *do* meet the criteria for being considered as multicellular agents; actually, such agential capacities are deeply related to the kind of development described in the case of sea urchins in the preceding pages.

[20]The actual characterisation of such nested levels of autonomy might not be an easy task. To mention again the issue of agency, it might be quite difficult, in some cases, to locate specific agential capacities at the relevant level of organisation. Deciding "who is the agent", so to speak, may therefore require fine-grained analyses when dealing with multicellular systems whose components are themselves autonomous.