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The search for organizing principles as a cure against reductionism in systems medicine

Olaf Wolkenhauer[1,2] and Sara Green[3]

1 Department of Systems Biology and Bioinformatics, University of Rostock, Germany
2 Stellenbosch Institute for Advanced Study, Wallenberg Research Centre at Stellenbosch University, South Africa
3 Centre of Science Studies, Department of Physics and Astronomy, Aarhus University, Denmark

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Correspondence
O. Wolkenhauer, Department of Systems Biology and Bioinformatics, University of Rostock, Rostock, Germany
Fax: +49 381 498-7572
Tel: +49 381 498-7570
E-mail: olaf.wolkenhauer@uni-rostock.de

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Biological complexity has forced scientists to develop highly reductive approaches, with an ever-increasing degree of specialization. As a consequence, research projects have become fragmented, and their results strongly dependent on the experimental context. The general research question, that originally motivated these projects, appears to have been forgotten in many highly specialized research programmes. We here investigate the prospects for use of an old regulative ideal from systems theory to describe the organization of cellular systems 'in general' by identifying key concepts, challenges and strategies to pursue the search for organizing principles. We argue that there is no tension between the complexity of biological systems and the search for organizing principles. On the contrary, it is the complexity of organisms and the current level of techniques and knowledge that urge us to renew the search for organizing principles in order to meet the challenges that are arise from reductive approaches in systems medicine. Reductive approaches, as important and inevitable as they are, should be complemented by an integrative strategy that de-contextualizes through abstractions, and thereby generalizes results.

Introduction

Cell-biological systems are difficult to study because they are complex in several ways [1]. One aspect of biological complexity that is particularly important to systems medicine is multi-levelness: the structural and functional organization of the human body into organ systems and tissues composed of cells. From molecules to organs, levels are inter-related and inter-dependent, so that the organism is able to conserve and adapt the integrity of its structural and functional organization against a back-drop of continuous changes within the organism and its environment. This capacity, whether it is described as 'autoconservation' [2], 'functional stability' [3], 'evolvability' or 'robustness' [4–6], is a consequence of non-linear spatio-temporal intra- and inter-cellular interactions. To understand disease-relevant cellular processes, we therefore require methodologies that allow us to study non-linear spatio-temporal systems with multiple levels of structural and functional organization.

The most recent decades of research in the life sciences have been largely driven by development of new technologies, which have brought about unprecedented insights into the structural organization of cells [7,8]. Together with these technological developments, a form of reductionism, i.e. studying higher-level phenomena by analysing the lower levels, has been established [9]. While some aspects of this 'zooming in' are a pragmatic and indispensible response to biological complexity, we here demonstrate the negative side-effects of molecule-, pathway- and cell-centred approaches.

The emergence of systems biology is connected to the limitations of molecule-centred approaches [10]. Systems biology has shifted the focus from

identification and characterization of molecular components towards an understanding of networks and functional activity. As a consequence, dynamic systems theory has played an increasingly important role in understanding cellular processes [11,12]. We argue that, for the transition from systems biology to systems medicine, a further shift of perspective has to occur: re-focusing our attention away from pathway-centred approaches to an understanding of complex multi-level systems. Looking at the developments from biochemistry to systems biology, it becomes quite apparent that reductive approaches are rather limited when it comes to answering questions in systems medicine [13]. In systems medicine, our understanding of cellular functions must be integrated across multiple levels of structural and functional organization: from cells to tissues and organs to whole organisms, and from cell functions (growth, proliferation, differentiation and apoptosis) to the physiology of organs or the human body [14]. Multi-levelness is a hallmark of disease-relevant processes, which challenges conventional dynamic systems theory [15,16]. Here we provide an example from cancer research that demonstrates the limitations of pathway- and cell-centred approaches.

Our goal in this review is to evaluate, from a personal and necessarily biased perspective, reductive approaches and their limitations in answering questions at the tissue and organ level by conducting experiments at the molecular and cell level. We first consider how biological complexity challenges experimentalists and modellers alike, and then look at how the associated difficulties have led to specialization, fragmentation and the contextualization of knowledge. Following a discussion of reductive approaches and their negative consequences (in our view), we suggest possible future directions for research in systems medicine. In particular, we argue that the search for organizing principles may serve as a cure against the side-effects of reductive approaches in systems medicine.

While not essential to our arguments, here we understand systems biology as the science that studies how biological function emerges from interactions between the components of living systems, and how these emergent properties constrain the behaviour of these components. In practice, systems biology is an inter-disciplinary approach by which biological questions are addressed by integrating experiments in iterative cycles with mathematical and computational analysis. Systems medicine should be understood as application of the systems biology approach to disease-focused or clinically relevant research problems. A research challenge arising from systems medicine, that is discussed in detail here, is the fact that, for many diseases, it is necessary to study and model complex systems from the molecular to the organ level.

Reductionism and specialization

In studying networks rather than individual molecular components, some proponents of systems biology have considered systems biology a 'holistic approach' [17–19]. This unfortunate misconception ignores the fact that technological advances have continued to enforce reductive approaches, along with increasing levels of specialization. Ten years ago, the focus on pathways rather than single molecules may have been seen to be a more comprehensive approach, but even today we are still far down the reductive route, with the current dominance of pathway-centred approaches to understand disease phenomena. Reductive strategies are indeed an indispensible response to biological complexity, but, as we discuss here, they have negative side-effects. One such side-effect is over-specialization, which, in the current practice of systems biology, means that the choice of experimental and modelling strategies is more frequently guided and limited by personal and practical considerations than by the need to validate a general hypothesis that underlies the research project. The approaches chosen are frequently linked to decisions based on pragmatic considerations of the associated efforts in terms of time and costs required for experiments. For example, in research on metastasis, many projects are focused on single molecules or small pathways, frequently using specific cell lines. There is a mismatch between the research goal (understanding mechanisms underlying metastasis in humans) and the highly specialized projects, whose results are only valid in a narrowly defined context. There is an obvious need for integration of results from individual research projects and a need for generalization (de-contextualization) of results.

Below, we describe several reductive strategies used in biological and biomedical research. We first emphasize how the use of model organisms and the development of new experimental technologies provide key resources for biomedical research, but also require a high degree of specialization that may lead to fragmentation. Next, we indicate the difficulties arising from pathway-centred approaches and mechanistic modelling. Finally, we discuss the limitation of cell-centred approaches in cancer research.

The use of model organisms is one response to biological complexity, allowing us to study a complex organism by using another one that is either simpler or easier to handle in experiments. An example is yeast studies in cancer research, motivated by questions related to the

cell cycle and its consequences for carcinogenesis or tumor progression [20]. The experimental focus on a particular model organism, the decision to perform cell line *in vitro* experiments or the availability of a suitable *in vivo* model are our first examples of a common reductive approach, which also imply a disciplinary specialization with separate conferences and journals. However, research on model organisms also provides de-contextualized insights. A basic assumption in using model organisms or cell lines is that, while details may differ, there are some generalizable principles at work. We believe that the relationship between reductive choices, inevitable and successful as they are, and the generalization of results obtained, requires more attention from scientists, philosophers of science and funding bodies. For reductive approaches to succeed, they must be complemented by integrative strategies. We argue that these integrative strategies also require higher levels of abstraction than most biological and biomedical researchers currently feel comfortable with, and this requires further mathematical research.

What have been heralded as revolutionary advances in molecular and cell biology are largely due to technological developments, allowing us to study molecules and cells in greater detail and more comprehensively. The costs and the specialist expertise required to perform experiments with state-of-the-art measurement devices have meant that only one or a selection of technologies are used in any one study for most research projects. Whether the choice is microscopy, proteomics, transcriptomics or deep sequencing, their use requires a high degree of specialization. 'Omics' technologies are frequently tied to a focus on a particular class of subcellular processes, i.e. gene regulation (e.g. transcriptomics), signal transduction (e.g. proteomics) or metabolism (e.g. metabolomics). Again, a disciplinary fragmentation, with specialized conferences and journals, may be observed. Furthermore, another enforcement of scientific specialization is linked to the focus on a particular cell function, such as cell growth, proliferation, differentiation and apoptosis. It is quite obvious, albeit not generally appreciated, that, for application of systems biology approaches in biomedical research, there is not only a need for computational tools that enable integration of data from heterogeneous sources, but also a need for radically new methodologies that enable generalization of context-dependent experimental results.

Our next example of a reductive strategy is the focus on selected pathways or networks. Pathways are frequently defined by practical considerations, meaning that only a relatively small number of molecules are considered in experiments. However, for most disease-relevant processes, these pathways are sub-systems of a larger whole. Rational criteria to identify modules or sub-systems are largely lacking. In practice, one is usually forced to define a boundary for the network as it is investigated experimentally. If this pathway is one of several that contribute to a particular cell function, for example, the notion of 'cross-talk' between pathways has been used. However, for most pathways that interact, this notion of cross-talk raises questions about the conceptual and experimental isolation of the two systems. In order to use the experimental results related to a specific pathway in a wider context (e.g. studying the Jak–Stat signalling pathway to investigate cell differentiation), we require new methodological and conceptual frameworks to de-contextualize and generalize. A similar situation occurs when studies at the cellular level (looking at single cells, cell cultures and single pathways) need to be related to tissue-level phenomena and the physiology of an organ. We believe that the problem of generalization through de-contextualization and the integration of experimental results requires more attention and research, as otherwise the currently favoured pathway-centred approaches will be of limited value.

Systems biology is largely defined as an inter-disciplinary approach that combines experiments with mathematical and computational modelling. Like experimentalists, who are often not free to choose any technology they want, most modellers are not really free to choose a conceptual framework for modelling. Despite the development of user-friendly tools that guide the modelling and simulation of biological systems, the construction of a model and its parameterization requires expert knowledge. Although the choice of an appropriate approach should in principle be guided by the question under consideration alone, more often, practical considerations and personal choices are decisive. Similar to the efforts required to perform experiments, the construction and analysis of a model may be challenging, requiring a high degree of specialization and experience. For example, non-linear ordinary differential equations are the most frequently used framework, but, for larger numbers of variables, parameterization and analysis of these models is difficult. Dynamic systems theory is particularly intuitive if systems can be reduced to a few variables. For systems with only two variables, and for systems that are linearized around a steady state, the theory is most powerful and well developed. It is therefore not surprising that some case studies are selected to fit the tools, rather than the other way round. In contrast to differential equation models, agent-based simulation models handle many variables and represent spatial

aspects more easily, but the 'model' is programmed, lacking the desirable simplicity of representation. Also, stochastic approaches, even if the most appropriate, are often avoided because they require a deeper understanding of the maths by the modeller. The choice of an appropriate modelling formalism, the construction of the model, the estimation of parameter values and subsequent exploration of the model through simulation and formal analysis are aspects of a craft that requires specialization. Tailoring a model around a particular question, making various assumptions and simplifications along the way, will unfortunately also make it context-dependent.

The creation of large collections of information from experiments using various experimental models and employing a wide range of technologies and methodologies requires integrative strategies through which fragmented information may be put together [13,21,22]. A pragmatic, computational way forward is to support integration of information through visualization of information in data management systems or data warehouses. However, this would only be a partial contribution to what is the actual scientific challenge: how can we, from large collections of information, extract principles, understood as robust generalizations, independent of the experimental context of any particular study? Take, for example, our understanding of cell functions, say apoptosis, for which numerous studies, using different technologies and experimental models (e.g. cell lines, genetic mouse models), have provided pieces of a puzzle that give us deeper insights into apoptosis in the context of carcinogenesis. Many experiments in molecular and cell biology are however valid only within a well and often narrowly defined experimental context, determined by the choice of technology and the biological model. Furthermore, most mathematical models are constructed to answer specific questions, and, while the assumptions made may be valid in this particular context, it is difficult if not impossible to merge models for complex multi-level systems. An important challenge for systems medicine is thus the integration and decontextualization of results, to put the pieces of a puzzle together.

A survey of review articles focusing on epithelial cell renewal in the context of colon cancer uncovers numerous speculations about the theories and (explanatory) models that may be formulated as organizing principles, including the 'unitarian hypothesis' of monoclonal conversion, the 'single stem cell hypothesis' or the 'stem cell niche hypothesis' in the context of niche succession, the 'hierarchical model' compared to the 'stochastic model' for niche homeostasis, the 'somatic mutation theory' versus 'tissue field organization theory' to explain carcinogenesis, or the 'top-down' versus 'bottom-up' hypothesis of clonal expansion linked to early growth of adenomas, or cancer progression being discussed in terms of the 'cancer stem cell model' versus the 'clonal evolution model' versus the 'interconversion model'. What this selection exemplifies is that the formulation of such principles and arguments for or against them are developed in exceptionally well-written review articles in biological journals: leading experts integrate knowledge by interpreting collections of fragmented pieces of information. Very often, the experimental studies are about cellular processes, but the results are interpreted with respect to consequences at the tissue level. What we propose is not simply to support this integrative process through data management and visualization tools. In addition, the search for organizing principles should be supported by systems theoretic approaches, specifically new forms of mathematical modelling to formalize cross-level relationships from molecules and cells to tissues and organs.

Our argument here is that a review of current practice leads us to the proposition that, if you want to understand a tissue, you need to study it as a whole! Interestingly, this argument mirrors an aspect in the transition from biochemistry to systems biology. In 1986, Kacser, commenting on whole–part relationships in metabolism, wrote 'to understand the whole, one must study the whole' [21]. Here, however, we reach an apparent contradiction because we also argue that reductive approaches, focusing on pathways and cells, are inevitable in the light of biological complexity and the experimental/technical challenges. How then may we escape the reductive cul-de-sac? One avenue is to 'up-scale' experiments and models, to incrementally increase the number of molecular components and pathways to be looked at. However, we have come to the conclusion that it is necessary to try to complement such reductive strategies by novel approaches that provide higher levels of abstraction, using systems theory. Abstraction in mathematical modelling allows us to link evidence and knowledge of the subcellular domain or cell level with the tissue and whole-organ level. A conceptual framework that provides a straightforward generalization of mechanistic models and that has been considered elsewhere is mathematical general systems theory [22,23]. An interesting problem that arises in this context is transition of a mechanistic model as an 'ontological' description of a biochemical and biophysical reality to a mathematical representation of what we know about the biological system – an 'epistemological' version of logical possi-

bilities that link evidence [24]. The move to higher levels of abstraction poses a number of challenges. For example, abstraction implies generalization, which in turn implies a lack of specificity – the more abstract the representation becomes, the less predictive the models are about a specific experimental context. In our view, this aspect is in fact showing the way forward: reductive approaches that 'zoom in' on cellular mechanisms in the context of human medicine ought to be complemented by a search for general organizing principles at higher levels of structural and functional organization in tissues and organs.

Below, we identify the challenges specific to systems medicine, leading up to a proposal for a way forward that addresses the complexity of disease-relevant processes. We argue that, despite its limitations, modelling is essential not only for systems biology and systems medicine, but for science in general. In our view, the response to biological complexity should not only be a reductive one. To go forward, there is also a need to strategically focus on the development of approaches that 'zoom out' to help us understand multi-level systems. Addressing experimentalists and modellers alike, we wish to proclaim that, to study disease-relevant processes in tissues, one should also study tissues through an active search for organizing principles.

Consequences for systems medicine

Many diseases represent problems of tissue organization: changes in the structure and function of a tissue may be the results of changes within cells (e.g. mutations), leading to cellular malfunction, but, simultaneously, tissue organization may also induce changes within cells (e.g. through epigenetic mechanisms). It therefore appears obvious that we require methodologies to investigate systems across multiple levels of functional and structural organization.

Cancer research is an example that illustrates the problems arising from reductive approaches, fragmentation and the dependency of results on a particular technological and/or experimental context. Hanahan and Weinberg's review 'The hallmarks of cancer' [25] may serve as a classification of research efforts. Most cancer projects focus on a particular cancer and on either carcinogenesis, tumour progression, or metastization and invasion. These high-level/tissue-level phenomena provide the motivation and background for the projects, but, in practice, the highly specialized research in most projects actually does not address such general questions directly. Instead, the current practice is rather 'pathway-centred', where most projects ask a very specific question, related to a specific pathway, say the Jak–Stat pathway or an MAPK pathway, or concentrate on the role of a particular molecule, say p53 or E2F1 [26]. The 'zooming in' on molecular components has been very important and has generated enormous amounts of valuable information. The work on a particular molecule, say p53, is argued to be justified on the basis of its role in a cellular process, like DNA damage response. This focus on a particular molecule leads to definition of a network of molecules linked to p53, small enough to be experimentally tractable. However, as the cancer biologist Lazebnik notes: 'the mystery of what the tumour suppressor p53 actually does seems only to deepen as the number of publications about this protein rises above 23 000 [27]. In this famous and provocative paper, Lazebnik asks whether biologists can meet two challenges described as analogous: fixing a radio and developing a general characterization of apoptosis. He comes to the conclusion that the strategy of biologists would fail in both cases, as this most likely would be to crush the radio down to all its components and analyse these, just as much of medical research has been a search for a miracle target whose malfunction is thought to explain the investigated disease. If no such master gene exists that can explain cancer, Lazebnik argues, the status of research is like the Chinese proverb alluding to the search for a cat in darkness that is not even there.

It appears that we have become so preoccupied with molecular details that we have forgotten to ask how all the research results relate to answering the big (higher-level) questions. We believe that, for some disease-related phenomena, we are failing to see the wood for the trees. It is paradoxical that most cancer research projects are motivated by a far more general research question that is largely ignored in the execution of these research programmes. The pragmatic reductionism that focuses on particular molecules and pathways creates a fundamental problem. The focus on a particular molecule or pathway may be justified by researchers on the basis of its relevance for an important cellular process (e.g. DNA repair), which in turn is associated to some cell function (e.g. apoptosis), that is then linked to some disease-relevant process (e.g. carcinogenesis). However, starting with a high-level phenomenon, say angiogenesis, one may easily identify a large number of molecules and pathways that are relevant. Therefore, how may any single project, motivated by a higher-level process but limited to a particular experimental context, provide any meaningful contribution? In our view, the current practice is not sustainable, and requires re-thinking of

how we go about answering bio-medically relevant questions in molecular and cell biology.

Systems biology emerged from a shift of focus, away from identification of cellular components and their molecular characterization towards an understanding of functional activity [28,29]. For systems medicine, it will be of utmost importance to move on from pathway-centred approaches. Rather than starting with subcellular mechanisms and models thereof, before generalizing these to the level of cell functions and their role in phenomena at the tissue level, we wish to promote an alternative route that starts with a hypothesized general principle about tissue organization, to then identify and investigate cellular functions and subcellular processes in an effort to validate the original hypothesis.

We believe that such a search for organizing principles is happening but is mostly hidden in a few review articles and left to the inspiration of a few scientists. Cancer research is an area in which review articles play a particularly important role due to the above-mentioned flood of information about individual molecular components. Exceptionally good review articles not only gather and list information in a summarized form, but the authors try to organize the information to speculate about the larger picture into which the pieces of the puzzle may fit. Take, for example, the highly cited review article 'The hallmarks of cancer' by Hanahan and Weinberg [25]. Looking at a quarter of a century of rapid advances in cancer research, the authors argue that rather than 'adding further layers of complexity to a scientific literature that is already complex beyond measure', the search for the origin and treatment of cancer will not only be driven by developments at the technical level 'but ultimately, the more fundamental challenge will be conceptual'. In 2000, Hanahan and Weinberg foresaw 'cancer research developing into a logical science, where the complexities of the disease, described in the laboratory and clinic, will become understandable in terms of a small number of underlying principles' [25]. In their seminal review article, Hanahan and Weinberg 'suggest that the vast catalog of cancer cell genotypes is a manifestation of six essential alterations in cell physiology that collectively dictate malignant growth' which 'are shared in common by most and perhaps all types of human tumors'. They refer to the functional capabilities that cancers acquire during their development as 'hallmarks of cancer'. A hallmark of cancer is here understood to be a generalization in the sense that it may be acquired by various cellular mechanisms. Hanahan and Weinberg's hallmarks therefore take us some way towards the search for organizing principles as an epistemological tool.

As discussed further below, organs and tissues are multi-level systems manifesting both 'regressive determination' and 'progressive determination': the whole (organ or tissue) is the product of the parts (tissue or cells, respectively), but the parts in turn depend upon the whole for their own functioning and existence. Karsenti's initial definition of self-organization implied that understanding of functions in living systems implied an understanding of (self) organization [30]. This also implies that we should focus on principles rather than on single molecules or pathways alone. In our view, the current practices in cancer systems biology require re-thinking. The technological advances that have enabled us to 'zoom in' should be complemented by methodologies that allow us to 'zoom out': the microscope of molecular and cell biology should be complemented by the 'macroscope' of systems theory.

Multi-levelness and the search for organizing principles

Living systems, from organisms to organs, tissues and cells are phenomena of organized complexity [31] whose relationships and properties are largely determined by their function as a whole. The tissues of our human body are self-organizing systems: every cell owes its presence to the action of all its surrounding cells, and also exists for the sake of the others. The whole (tissue) and its parts (cells) reciprocally determine functioning of each other. For instance, the pacemaker rhythm of the heart is not only caused by the activity of the ion channels at the molecular level, but is also dependent on the functioning of the organ, and even the body, as a whole. The systems biologist Denis Noble elegantly demonstrated the importance of such downward determination in simulations of the heart rhythm, where feedback from cell voltage was removed and fluctuations in ion current ceased [32,33]. To understand such phenomena in multi-level systems, it is not only important to understand molecular mechanisms but also to understand the organizational maintenance of the system at higher levels.

The human body provides the prototypical example of a multi-level system, where molecules, cells, tissues and organs are sub-systems of physiological systems (e.g. the cardiovascular system, the digestive system, the immune system etc.) The human body is thus structurally organized into spatio-temporal scales and functionally organized into behavioural levels (Fig. 1). A characteristic of the system, as a whole, is its functional stability against a back-drop of continuously changing and perturbed sub-systems [3].

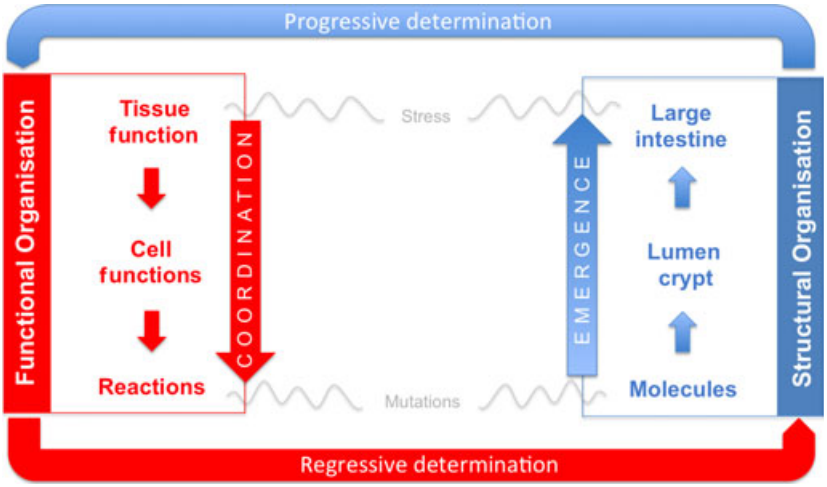

Fig. 1. Structural and functional (self) organization of tissues using the intestinal colon as an example.

Take, for example, the large intestine (colon) of the digestive system, which is also a common site for carcinogenesis. The inner lining of the colon is organized into millions of crypts [34,35]. The base of the crypts form a niche and micro-environment for a small number of stem cells that continuously renew the epithelial layer in order to maintain the physiological function of the colon (nutrient absorption) and to repair or avoid possibly detrimental effects from mechanical or chemo-toxic stress, which may lead to the formation of neoplasms and possibly carcinomas. The structural organization of the crypt emerges 'bottom-up', and its function is maintained through division and differentiation of stem cells. At the same time, the behaviour of these stem cells is coordinated by higher-level phenomena resulting from the need for tissue maintenance and repair. In the more general framework of multi-level systems with reciprocal and simultaneous cross-level determination, levels are inter-dependent but not necessarily causally linked [36]. Here, intra-level relationships may be conventional causal interactions, such as the mechanisms realized through subcellular biochemical networks, where causality is understood as a principle of explanation of change, not changes of things, but changes of states, represented with mechanistic models from dynamical systems theory. Inter-level relationships, on the other hand, constitute an interdependence in which levels are allowed a degree of autonomy [35,37]. The fact that levels are inter-dependent, but not necessarily causally linked, challenges the current practice of reductive approaches in experimentation and modelling. While systems approaches have been quite successful in describing mechanisms underlying intra-level relationships or 'causal interactions', we are in need of new ideas when it comes to understanding inter-level relationships. Below, we argue that mathematical general systems theory is one possible conceptual framework that abstracts conventional dynamical models and thus provides a basis for generalization from mechanistic models.

Let us consider an example from cancer research, where the need for identification and understanding of cross-level principles is of crucial importance. This example continues our discussion about the negative side-effects of reductive approaches. A widely accepted view on cancer is that it is a cell-based disease [38]. With cancer research following closely the developments in molecular and cell biology, pathway- and cell-centred (reductive) approaches have enforced the view that sporadic cancers are initiated and largely driven by accumulation of mutations in what may then be called a 'cancer cell' that loses control over its proliferation. Hanahan and Weinberg state that, 'By simplifying the nature of cancer – portraying it as a cell-autonomous process intrinsic to the cancer cell – these experimental models have turned their back on a central biological reality of tumor formation *in vivo*: cancer development depends upon changes in the heterotypic interactions between incipient tumor cells and their normal neighbors' [25]. Soto and Sonnenschein [39], who refer to the cell-centred view of carcinogenesis as the 'somatic mutation theory', have proposed an appealing alternative theory that considers cancer to be a problem of tissue organization. A key premise to their 'tissue field organization theory' is that 'carcinogenesis takes place at the tissue level of biological organization, as does normal morphogenesis'. Here cancer is not a cell-based phenomenon but a tissue-based phenomenon, comparable to organogenesis during early development. A startling conclusion is that

the genetic instability of tumours is likely to be a consequence, not a cause, of cancer. As new deep-sequencing technologies are pushing forward the reductionist agenda, we here call for a reflection about the original questions at tissue level, and ask whether the technology-driven reductionism should not be complemented by an equally well supported research programme into new, integrative and abstract methodologies. The purchase of technologies that dig deeper into the molecular details of a tumour sample is the seemingly more comfortable route. However, if cancer is a problem of tissue organization rather than of single cells, new experimental designs will be required. For modelling, the outlook is as challenging as it is exciting: if cancer is a problem of tissue organization, reciprocal interactions between cells and their environment come into focus, and ordinary differential equations are no longer sufficient to capture the spatial coupling of biochemical and biophysical/mechanical interactions. As discussed below, modelling complex systems across multiple scales of spatial and temporal organization may take two routes.

From multi-scale to multi-level systems analysis

How does one study multi-level systems, i.e. investigate, the functioning at higher levels of tissue organization? One possibility, proposed by several large-scale research projects such as the Virtual Physiological Human Project [14,40] or the Human Brain Project [41–43] is to simulate organs in physical and chemical detail, bottom-up, from molecules to organs. However, the attempt to meet biological complexity with a complexity of models that include ever increasing details seems somewhat to be analogous to Lewis Carroll's and Jorge Borge's fictions, where the art of cartography attains such perfection that the maps become as detailed and as big as the countries they represent. These maps are abandoned as useless, not because of the lack of precision, but because of their exact accuracy [44,45]. Similarly, it has been argued that the way forward in the biological and biomedical sciences is not to try to include all details and to add further levels of complexity to models and the scientific literature, but rather to develop approaches that zoom out and focus on key aspects of the phenomena studied [46–48].

An alternative response to the complexity of tissues and organs is to abstract away from the biophysical and biochemical details. The basis for such generalization of mechanistic models into more abstract representations is mathematical general systems theory [23]. While more abstract, and therefore less specific about a particular system, these approaches provide a framework to formulate and identify organizing principles [24,35,37]. An example of what such a theory should deliver is a formal framework to represent tissue organization, which may then be used to decide between the alternative theories of carcinogenesis discussed above.

The focus here on organizing principles is a re-introduction of an old regulative ideal in systems sciences dating back to Bertalanffy's ideals for a general systems theory [49], to Rashevsky and Rosen's notion of optimality principles [50–52], and to Savageau's so-called demand theory for gene expression, which exemplify design principles in biochemical systems theory [53,54]. The prospects of a more theoretically grounded biology searching for general and perhaps even law-like principles of living systems has been the issue of long debate in philosophy of biology [55–57]. However, the search for organizing principles need not rest on the widely criticized optimality approach [37,58,59], but is here understood as robust generalizations that account for the general behaviour of a class of (often different) systems. This strategy is not an attempt to reduce away biological complexity with abstract approaches. Our proposed focus on organizing principles is not an alternative to bottom-up approaches, or mechanistic modelling; it is a complementary approach. For that matter, it is also reductionist, but in a different sense. Every model or scientific theory is a reduction of something complex to something simpler [47]. The search for organizing principles is a matter of reducing the number of details and the amount of context-dependent information for the sake of the generality achieved through abstraction. This ideal is not in opposition to finding biological mechanisms but rather has a different aim, namely to find out how a class of systems works in principle.

In recent years, interest in general principles underpinning the organization of biological systems has intensified, and we expect this to continue. Efforts in network modeling have led to the discovery of general topological aspects and shared functional constraints of various networks [54,60–63]. Evolutionary systems biology has initiated the search for evolutionary design principles that demonstrate general features of evolving networks [59]. Furthermore, attempts to develop abstract cell models and explore the potential of category theory and mathematical general systems theory have recently been initiated [35,37,64–68]. As these approaches address questions at a higher level of abstraction, the relationships between theoretical models and experimental practices will be an important

point of discussion in future biology and medicine [69]. Another example from our own work is the study of epithelial cell renewal in the context of colon cancer [35]. Using simple-order relationships to link the division of stem cells in their niche to the fate of the crypt, we formulated a theorem that shows how the fate of the tissue is determined by a single lineage. The approach does not use any numbers to characterize the system, but analyses what is logically possible 'in principle' [24]. In such approaches, the definition of (and assumptions about) variables and the subsequent formulation of the theorem create an argument about an organizing principle relating to a tissue. To identify or suggest a principle is to generalize a phenomenon from particular instances, to de-contextualize it, for example, generalizing it beyond a specific experimental context. We believe that, if the gap between systems theory and mainstream biology can be bridged through more research in this direction, theoretical models may be of high practical value because they address fundamental properties of the system under consideration.

In summary, we here considered the transition from systems biology to systems medicine by personal reflection upon the developments that took us from biochemistry and molecular biology to systems biology. We noted that advances in molecular and cell biology were largely technology-driven, leading to high degrees of specialization and a reduction of the validity of results to the specific experimental context. In the context of many diseases, which cross multiple levels of structural and functional organization, reductive approaches and conventional dynamic systems theory are limited in facilitating identification of general principles underlying these diseases. Another contribution of our analysis is the proposal for a strategy that promotes integrative approaches and the search for organizing principles. While new technologies are widely welcome and their development is well supported, we hope that our analysis contributes to a better appreciation of the development of new and abstract methodologies. We firmly believe that systems medicine not only requires new means of measuring things, but also new ways of thinking.

Conclusions

A review of the current practice of molecular and cell biology reveals negative side-effects of technology-driven reductive approaches. Although much has been learned about molecular components and subcellular processes, these sub-systems are part of a larger whole that is frequently ignored when it comes to understanding tissue- and organ-level questions. Many diseases are a problem of tissue organization, and require us to integrate our knowledge from the molecular level all the way up to the tissue and organ level. Multi-levelness is a hallmark of biological complexity, and, in our view, is the final frontier and the greatest hurdle in the success of systems medicine. In our analysis, pathway- and cell-centred approaches have severe limitations when it comes to understanding disease-relevant multi-level systems. As a consequence, we believe that the future of systems medicine will rely not only on technologies, but will also require a strategic focus on the development of new methodologies. Our analysis has revealed a need for generalization through abstraction, and we proposed the search for organizing principles as a cure against negative side-effects of reductive approaches. To this end, we suggest systems theory as systems medicine's next stethoscope.

The search for organizing principles is not only of theoretical value but of high relevance for solving practical problems. The ideal of general principles has a long history [49,50,70–72], but is still not fully appreciated [24,35,37,66]. The focus on general principles enables a shift away from molecule- and cell-centred studies and from what Robert Rosen called 'thinghood properties', towards an understanding of 'systemhood similarities' [57]. Organizing principles do not provide fine-grained causal explanations of biological mechanisms. Their epistemic value lies elsewhere; as higher-level abstractions, organizing principles may facilitate transfer of methods across disciplinary boundaries, and development of what Bertalanffy called 'in principle explanations' [49]. These are coarse-grained descriptions of the behaviour of a system that may be seen as templates for how such a system can be investigated. Organizing principles thus signify an epistemological framework for understanding complex phenomena. The formal framework of mathematical general systems theory forces us to be precise about our assumptions, and helps us to check the logical consistency of the argument made about a biology system [24,35]. Understood this way, they are not fruitful despite their abstract and often idealized nature, but because of it.

We believe that the limitations of reductive approaches will be particularly detrimental to progress in systems medicine. We provided an example from cancer research, demonstrating that many phenomena at the level of tissues and organs cannot be reduced to cellular events. Tissue organization, the tissue's structure and function are emergent properties that reciprocally determine the behaviour of the cells that make up the tissue. Cancer provides an example of a problem of tissue organization, and we argue that if one wants to

study tissues, one has to study tissues as a whole and not only focus on single pathways and single cells.

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