From mouse genetics to systems biology

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Introduction

It won´t be long before we have mutant alleles for every gene in the mouse genome. It also won´t be long before we can sequence an entire genome in a few hours and for less than 1000 $. But there is much more to come. And you will be surprised about the speed with which these developments will take place. Imagine you can follow the fate of every single cell during the development of a mouse from fertilization to birth and even beyond. Imagine you can watch the expression of a single molecule of any protein or the total expression of all proteins in a single cell continuously over time. Imagine you can titrate the expression of single genes in specific cell populations at will.

High throughput technologies have become the driving force in the analysis of biological systems. Biologists are increasingly taking advantage of automatization, miniaturization and computerization. In this sense biology follows the development of computer and information technology: smaller size, higher speed and capacity, lower cost. However we should remember that the age of computer and information technology was preceded by a pre-exponential phase during which important theoretical frameworks and concepts were developed: Alan Turing (1936)) and John von Neumann (1945) provided the mathematical basis for an automatic computing machine and a corresponding “computer architecture”. To Claude E. Shannon (Weaver, W. & Shannon, C.E. 1949) and Norbert Wiener (1948)we owe the mathematical theory of information and cybernetics. The convergence of electronic and mechanical engineering then triggered the development and application of systems control theory, a key requirement for the modelling and simulation of the dynamics of technical systems.

A major challenge in biology is to model, simulate and to eventually predict the behaviour of complex biological systems. The identification of the individual components that constitute a biological system, i.e. through the genome wide transcriptome-, proteome- and metabolome-analysis, will be required but will not be sufficient to achieve this goal. We will also need detailed information about the “network architecture” and the dynamics of

biological systems. This is where systems biology comes into place (Kitano 2002, Kirschner 2005, Palson 2006, Alon 2007).

From perturbation to model building: an iterative cycle in systems biology

Biological systems are emerging, adaptive systems, highly complex and often nonlinear. Their behaviour cannot be explained solely on the basis of their individual parts. Deep insight into the network structure, function and dynamics of biological systems can only be obtained through their systematic perturbation, followed by a detailed characterization of the molecular, cellular and phenotypic changes that follow these perturbations. Based on the perturbation consequences observed, a model can then be established or existing models modified or further developed, which grasp the important features of the underlying mechanisms (Sauro & Kholodenko 2004).

Mouse genetics has been an extremely powerful perturbation method for nearly a century. Loss of function and gain of function mouse mutants are able to reveal causal relationships between specific genes and specific phenotypes. An impressive mouse genetics toolbox is now available that allows us to perturb a wide range of biological systems. Methods such as the production of transgenic mice, gene targeting through homologous recombination in ES cells, phenotype or gene-driven mutagenesis strategies or RNAi-based knockdown are now used on a routine basis. Sequence diversity is also a form of natural perturbation. In combination with the analysis of gene expression and phenotype analysis a thorough comparison of the consequences of allelic variants can be very powerful. The main challenge will be the functional dissection of the combinatorial activity of small sequence changes, forming the core of “Complex Trait Analysis”.

Perturbing biological systems through genetic changes is only one possibility to obtain information to dissect the structure and function of genetic networks. Equally important and increasingly appreciated is the use of small molecules, which can act as agonists or antagonists of biological processes (Schreiber 2005). Whereas a while ago, combinatorial chemistry was largely a domain of pharmaceutical drug development, the power of small molecules as a means to study the function of specific proteins or pathways is increasingly appreciated. There are specific strengths and weaknesses to the use of small molecules. One of the most important aspects is specificity. Very rarely does a small molecule bind to one and only one target and in many cases the precise number and nature of targets is

unknown. Small molecules are more adaptable to the titration of dose-responses or pulse-chase studies. Similar to searching for modifiers in a genetic screen, chemical biologists now are starting to carry out combinatorial screens to unravel redundancies or pathway interactions that are not revealed by single small molecule screens. In fact, one may be able to stay below the "toxic window" of a specific small molecule by combining two or more of them each of which acting on different targets within the same pathway. Sooner or later we will see a convergence between the fields of small molecules and small animals, i.e. in the area of non-invasive imaging (Sako 2006). Molecular markers will become available that allow us to follow perturbations at the molecular and cellular level and in real time.

A need for integrative network analysis

Analyzing the individual components of a network is not sufficient. We need to understand how these components interact with each other and which are in direct or indirect contact. We must know how the components dynamically interact, what compensatory mechanisms are triggered, when components are defective or are inactivated. An understanding of a system therefore requires knowledge about the systems structure and architecture (Papin et al. 2005). Once we have sufficient information about the structure of a system we can begin to study systems dynamics. This will then help us to understand the control measures that are responsible for the overall behaviour of the system or its modules under external perturbations (Carpenter & Sabatini 2004, Barabasi & Zoltva 2004, Alon 2007). These cannot be automatically inferred from the parts list of a system. Biological information is passed through a number of highly integrated networks, including transcriptione, proteome or metabolome networks (Khammash & El-Samad 2004, Saez-Rodriguez et al. 2004). Methods to reconstruct or analyze biological networks have become an active field of research (Oda et al. 2005, Oda & Kitano 2006). Since Leonard Euler and Paul Erdöz the field of network analysis and graph theory has developed tremendously. Network analysis is also the basis of understanding disease pathogenesis and disease traits.

Modules: Making sense out of black boxes

A common theme in advanced technologies and engineering is to divide systems into modules that can be treated individually or in terms of connecting different modules as part of a higher order system. Since the discovery of the double helix structure of DNA, a reductionist approach to analyse biological systems has proved to be extremely successful. However we feel, that we are reaching a limit as to how much we can learn about complex

biological systems by looking with a still increasing resolution at individual components of a system. No doubt, at the end, we would like to understand biological phenomena on the basis of atomic resolution. On the other hand, the rise of systems biology reflects our increased appreciation and desire of looking at all the scales of biology, including the molecular, cellular, organismic and population based levels.

Partitioning biological systems into modules helps to achieve a more integrated picture. In order to understand causal relations among individual parts and modules, we need information about the directionality of flow of information or material between the edges within a network (Natarajan et al. 2006). We already know that systems behave differently dependent on whether we deal with single or a few molecules or millions of molecules. Stochastic and statistical approaches, i.e. Bayesian network reconstruction algorithms, need to be applied to deal with the uncertainties and probabilities of biological systems (Needham et al. 2006). The role of noise in biological systems is just being unravelled. Some of the most important contributions are currently made by physicists who are able to apply the repertoire of statistical physics to biological problems (Rao et al. 2002, Samoilov, M. et al. 2005, Sprinzak & Elowitz 2005, Kussell et al. 2005, Alon 2007).

Given their complexity a remarkable feature of biological systems is their robustness towards environmental perturbations (Kitano 2004, Kitano & Oda 2006, Kurata et al. 2006). How do biological systems preserve their function despite environmental conditions that can differ over magnitudes of scale leading to tremendous fluctuations in metabolic components or ligands? We do not yet understand the underlying mechanisms that are responsible for this robustness. Genetic redundancy, i.e. the presence of multigene families that can at least partially substitute for each other, are apparently one way to increase the robustness of a system. Similarly a redundancy of pathways could contribute to the potential of a cell to maintain the robustness of a biological system. On the other hand, there might be a price that has to be paid, i.e. under different environmental conditions, leading to a trade off of robustness vs. fragility dependent on the external factors that act on the system (Kurata et al. 2006). Robustness or fragility of biological systems can only be understood if we obtain insight into the structure and the dynamics of elements responsible for feedback control, an essential element in almost all complex systems (Schmidt & Jacobsen 2004).

Systems biology and drug development

Robustness and fragility are also of high interest to understand disease pathogenesis or the susceptibility or resistance towards the development of diseases (Butcher et al. 2004, Kitano 2004, Fishman & Porter 2005, Wagner 2005). What are the factors that drive a physiological system towards its disease state? How can we interfere with an unbalanced situation through preventive or therapeutic measures and maybe push back a disease state towards a more buffered state? What are the critical components which could be selected as a drug target? We are just at the beginning of identifying specific molecular components as indicators of the state of a system and more important as predictors for the future development of the system, i.e. as an early marker for disease development (Lage et al. 2007). These "biomarkers" do not necessarily need to be the same as those that qualify as drug targets. One of the frustrating issues in the drug development pipeline is the lack of sufficient preclinical predictability for safety and efficacy. Although many of the animal models are able to predict side effects of drug candidates, in many cases we miss adverse reactions and identify them at later stages of clinical development. By combining network analysis, statistic and high-throughput genetic and genomic approaches in order to identify new relevant biomarkers, systems biology bears great potential to improve the predictability of our preclinical *in vitro and vivo* models (Hood et al. 2004).

Look at the similarities and treasure the differences

Maybe we have focussed so far too much on the similarities of model organisms instead of also trying to understand the differences. Maybe we should increase our efforts in comparative systems biology. We might have to take a much closer look at the differences between mice and humans in terms of their relevance for drug development and try to understand the mechanisms of species-specific absorption, metabolism, metabolism and excretion (ADME). Some of the species differences can be overcome by i.e. introducing human genes into the mouse genome or by xenografting human stem cells into mice (Schultz et al.2007). These efforts in "humanizing mice" are still at the stage of "trial and error". We urgently need a "Comparative Systems Analysis" that could guide us in selecting the most relevant genes or cell types that are the cause of differences in drug responses or disease pathogenesis and that should be prioritized in our efforts to improve the predictability of mice as a model system for human disease.

Biological systems are complex adaptive systems that emerge during the development from a fertilized egg to the development of an adult organism. During evolution changes in the environment leads to different constraints and fixation of certain degrees of freedom in genome structure and function. Components of genome networks can only be added or changed when the workability and functionality of the biological system is maintained, at least to a certain degree (Ottino 2004, Weitz et al. 2007).

Comparative systems analysis needs appropriate databases (Albeck et al. 2006, Kersey & Apweiler 2006). These are not yet sufficiently developed. The mouse comparative ontology database is useful but does not provide information about the components, interactions and dynamics of physiological systems.
(http://www.informatics.jax.org/menus/homology_menu.shtml).
We need all the information available, i.e. a user friendly easily retrievable information system on the level of transcripts in a given cell, the dynamic response of mouse vs. human cells to small molecules, the levels of redundancy in the two species, species-specific genes, splicing patterns, posttranslational modification etc.

A need for modelling and simulation

Networks of biological systems are so complex that they cannot be understood any more by intuition. Some systems properties are even counterintuitive! It is the iteration of experiment and simulation that will characterize future systems biology. We need to describe biological systems mathematically and treat them in an integrated and quantitative manner to come up with predictions about their behaviour (Gershenfeld 2006, Szallasi et al. 2006). So far biologists often formulate their conceptional picture of a biological system as a flowchart-type model. These are more or less static and do not encompass information about the behaviour of a system over time, i.e. after a specific environmental perturbation. Model building has often been done on an intuitive basis by biologists. Biologists are often not aware that there already exists a rich literature and toolbox in systems control theory (Csete & Doyle 2002, Tyson 2003, Brent 2004). We need to get used to applying systematic perturbations, observing the reaction of the system to these perturbations, developing a first approximation model, testing this model by further perturbation studies (Locke et al. 2005, Aldridge et al. 2006, Janes & Yaffe 2006). Biologists are fairly well trained in hypothesis testing, but not in hypothesis generation. This is where systems biology has its greatest potential. Description will converge with prediction.

Don´t be afraid of mathematics

Systems biology often tries to apply formal mathematical descriptions based on time series analysis of biological response. So far the sheer amount and the quality of data constituted significant roadblocks to tackle the dynamics of biological systems. Technological advances help us to overcome these problems. A more severe problem, at least for the current generation of biologists, is the limited training in mathematics. The first two years of an engineering training provides a mathematical toolbox necessary for a mathematical description of technical systems and is essential for modelling or simulating the behaviour of complex systems. It will neither be possible nor useful to turn every biologist into a mathematician. However we need to improve the capability for a dialogue between biologists and mathematicians, physicists and engineers. The basics of linear algebra, vector analysis and graph theory have to enter the curriculum of a biologists training (Wingreen & Botstein 2006).

Unfortunately formal tools for model production do not yet exist. In addition model building is not easy and requires a very good understanding of the biological system under study. A question often raised is where to start: bottom up, top down or a combination of both. An interesting suggestion is to start “middle out” where the modelling begins at that level at which there are rich biological data and then reach up and down to other levels. (Noble 2002). A major difficulty is also the transfer of a model from one application to another. We need to develop standardization frameworks, so that even novices in computational biology or systems biology are able to build, access and work with existing models (Wall et al. 2004).

Complex Trait analysis: The next frontier in systems biology

For more than 100 years mouse genetics has relied on the analysis of single monogenic mutants. The methods to identify or produce mutants has changed considerably over the years. Soon we will have in our catalogues and freezers mouse mutants for every gene in the genome (Collins et al. 2007). Extensive collections will also be available as a result of phenotype driven mutagenesis screens (Balling 2001). Whereas the analysis of these mutants might keep us busy for many years to come, the next frontier of mouse genetics is already on the horizon: systems genetics. We all know that the expressivity and penetrance of mouse mutant phenotypes can vary tremendously dependent on the genetic background. Modifier screens can be used to identify some of the genetic loci responsible for the strong

influence of genetic background on physiological and pathophysiological processes. Sequencing and as a cheaper substitute SNP-typing have provided us with a detailed picture of the genetic diversity of our main inbred mouse strains. Most of them are derived from a very limited pool of parental strains and strong selection was applied to obtain the handsome, highly adapted common lab strains of mice that we now use in our experiments.

Recombinant inbred strains and other reference panels of inbred strains are powerful tools to carry out a genome wide dissection of complex biological traits that are the result of multiple, quantitative and often highly interacting genes (Churchill et al. 2004, Flint et al. 2005, Hill et al. 2006, Peters eta l. 2007). The series of BXD strains has been a paradigm for the success of analyzing complex traits. Unfortunately the use of RI-strains does not fall under the category “quick and easy” experiments but requires a fair amount of logistics, infrastructure, an appreciation for the power of genetics and time. The major bottleneck however was the “power of mapping resolution” that the analysis of 30 to 80 strains provides. The “Complex Trait Consortium” has tackled precisely this problem (Churchill et al. 2004).

The goal is to produce approximately 1000 recombinant inbred strains (The Collaborative Cross) within the next 5 years and make them available as an open source to the scientific community. Importantly the parental strains chosen also include three strains that we would classify as “inbred wild mice”, i.e PWK/PhJ, WSB/EiJ and CAST/EiJ. The inclusion of these genetically highly diverse strains adds about 75 % additional sequence diversity. The availability of this large panel of diverse and well structured strains will allow experiments where mice with an identical genotype can be produced in large numbers and compared to an equally large number of mice with a wide range of different genetic and even environmental backgrounds. Sequencing of the parental strains and a community based complementary and additive phenotyping will eventually produce a resource that will help us to solve questions of gene function, epistatic genetic interactions, and genome-environment interactions that we can currently only dream about.

There are other approaches, i.e. the development of consomic mouse strains, that essentially target the same questions (Peters et al. 2007). It will be important to not look at these approaches as exclusive or competitive, but as a new toolbox of quantitative trait analysis where each one has specific pro´s and con´s. New phenotyping methods including

gene expression arrays or phenotyping based on non-invasive imaging will have to be integrated into the described complex trait studies. Microarrays are a new micro-phenotyping platform that allows us to look at the expression of thousands and hundreds of thousands of different genes (eQTLs). This shift to micro-phenotypes requires new statistical tools because of multiple testing issues but also a much higher computational capacity then ever before. To quote Denis Noble: *"Biology is set to become highly quantitative in the 21st century. It will become a computer-intensive discipline"* (Noble 2002).

For many years mouse genetics has been the driving force as a hypothesis generator for functional genomics. The production of mouse models, i.e. transgenic mice, knockout mice or mouse mutants identified from phenotype driven screens are great tools to identify candidates for human disease genes. The construction of mouse inbred strain panels derived from genetically diverse parental populations provides us with valuable model populations. At the same time, the power of human association studies has reached a point where some people even think that it heralds the end of mouse genetics. I think the opposite is true. The availability of mouse reference populations will allow us to ask question that complement those addressed by human association studies. More importantly, we can quickly validate hypothesis derived from human population studies by not only constructing equivalent mouse populations but also by probing the function of individual genes through the analysis of gene targeting or specific point mutation alleles. The argument that we can find such mutations also in human populations does not take into account that in mice we are not only able to study the effect of genetic variation, but also to "titrate the environment" much better than it will ever be possible for humans.

At this point of time, mouse geneticists and human geneticists have not connected well enough to exploit the power of their respective toolboxes. To quote Rob Williamson: *"There is still an impedance mismatch between human association and reductionist mouse studies"*. Maybe this special issue of *Mammalian Genome* can contribute to a more better cooperation between mouse and human geneticists. It will pay off for all of us.

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