Systems Biology

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Is systems biology just a trendy term for the attempt to integrate information of various hormonal networks into a more complete picture of human physiology, which has been a part of endocrine research ever since its beginnings? Or is systems biology a really new approach to the wealth of information on cellular mechanisms that is obtained with an increasing speed? Systems biology has developed as an academic field which studies the relationships and interactions between various parts of a biological system (e.g. gene and protein networks involved in cell signaling, metabolic pathways, organelles, cells, physiological systems, organisms, etc.), the goal being that eventually a comprehensible model of the whole organism can be developed. As with any new discipline, it is evolving rapidly. It has already created a forum with high visibility in the form of a Nature periodical: Nature Systems Biology. Nevertheless, many of the concepts of systems biology are not entirely new. Scientists have always anticipated that a detailed study of an individual protein or gene is only the initial step towards understanding the overall (integrated) life process. The initial step in systems biology is the collection of data about genes, proteins and metabolites in an organism, using high-throughput techniques attached to computerized data mining to quantify differences in the genome, proteome and metabolome in response to any stimulus from outside. Such high-throughput techniques involve microarrays to measure the changes in mRNAs of the genome and mostly mass spectrometry to study changes at the protein and metabolite level, which in different setups can be used to identify proteins, detect protein modifications, and quantify protein levels.

At odds with most other disciplines in molecular biology, systems biology per se does not study one part of a molecular process at a time, nor does it break down the cellular processes into all their parts. It rather tries to reassemble all the parts into a whole. If holism and vitalism are the slogans of the 'new age' spiritualist, systems biology is the holism of the scientist.

Skeptics criticize this approach as reduction and simplification for the sake of comprehensiveness that will most likely fail, mainly because of nature's redundancy and complexity. On the other hand, systems biology has already provided results for many specialists working in the field of molecular biology that were both helpful and unanticipated. There is no doubt that the amount of information that is generated with the new high-throughput technologies necessitates analytical techniques, which no longer are based on the tradition of postulating initial hypotheses to be approved or rejected. This does not mean that the goals of systems biology are vague or poorly defined. The system biologist uses the knowledge from molecular biology and mathematical models, which will attempt to explain the observed systems and thereby create new hypotheses, which in turn can be tested iteratively for their validity. A whole team of computational biologists, statisticians, mathematicians, computer scientists, engineers, and physicists are at the doors of our laboratories; let them in.

An introduction to the rationale of systems biology

Cell biology: a fishing buddy for hypothesis generators

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Background: In their editorial in *Science* introducing a series of articles dealing with systems biology, the authors give an introduction to the methodology and reasoning implemented by systems biology strategies. They emphasize that in the last half of the 20th century, the dominant experimental modality in biology was hypothesis-derived research. The article reflects on several examples where systems biology was able to delineate certain unexpected interrelationships between different biological systems. The

authors conclude that there is a proud tradition of important insights arising from undirected poking around and following hunches. However, until genomic biology made undirected fishing for information more respectable, the most common response to requests for money for such projects was dismissal with the term 'fishing expedition' or unfocused. They reflect on several examples where systems biology was able to delineate certain unexpected interrelationships between different biological systems. They conclude that 'fishing expeditions' and investigator reasoning might supplement one another, generating testable assertions about chains of causation and action in biological systems.

Methods: The article gives simple explanations to the techniques used, assigning gene products to pathways and learning the order of their actions. Additionally, it tries to introduce the concept and development of bayesian statistical methods, which are used to formalize common concepts of causation. Bayesian networks compute and display relationships of statistical dependency between system variables, the latter being represented by nodes in a network. Arrows between nodes represent some dependencies between corresponding variables. The form of such graphical models is familiar to geneticists, but the difference is that Bayesian network methods automatically generate numerous alternative models from experimental data, and assign probabilities to each different network structure and each different arrow.

Conclusion: Biologists are very good at making targeted perturbations. The authors postulate that for bayesian network methods to realize their promise, researchers will need to get much better at measuring relevant variables. For intracellular events, variables include numbers of regulatory molecules, specific molecular complexes, and the percent occupation of regulatory sites upstream of the genes. To be useful, measurement methods will need to operate on individual cells, or to allow large enough numbers of trials to yield causal assertions reliable enough to merit further experimental testing.

For any reader who would like to take a first step towards understanding systems biology and biological networks this would be a nice introduction. Brent and Lok give a plastic view on how systems biology is set up. The article is not skeptical about the approach per se, but it delineates the differences between our 'classical biology' thinking and the fundamental methods of the new tools. Though bayesian networks have been discussed in the introduction to Harrison's Textbook of Medicine for the last 15 years, they sound new to many of us endocrinologists or molecular biologists. Here you will find first access with a perspective on the relevant papers being published on this methodology so far.

The end of 'naive reductionism': rise of systems biology or renaissance of physiology?

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Background: In his review, the author emphasizes that physiology and systems biology share the goal of understanding the integrated function of complex, multicomponent biological systems, ranging from interacting proteins that carry out specific tasks to whole organisms. Despite this common ground, the author fears that physiology as an academic discipline runs the real risk of fading into the background and being superseded organizationally and administratively by systems biology. He discusses the cornerstones of modern systems biology, specifically functional genomics, non-mammalian model organisms and computational biology, and emphasizes the need to embrace them as essential components of 21st century physiology and research and teaching.

Methods: As the key instruments of systems biology, the author identifies the following molecular genetic tools: functional genomics develops and utilizes large-scale and high-throughput methodologies to define and analyze gene function at a global level. As the most evident modern tool, the value of DNA microarrays is beyond large-scale Northern blots. They provide unique insights into the function of uncharacterized genes, with the possibility to identify groups of interacting genes that give rise to a physiological process of interest. Reverse genetics is another tool, which can create valuable input data for systems biology. Whereas biologists utilized knockout and knockdown strategies to study the function of single genes, it is now possible to use double-stranded RNA (dsRNA)-mediated gene interference (RNAi) to carry out large-scale and whole genome reverse genetic analysis. Finally, protein-protein

interactions can now similarly be examined on a large scale. Large-scale protein interaction screening has been carried out in yeast, *C. elegans* and *Drosophila*, using high-throughput yeast two-hybrid analysis. Interaction screens have also been undertaken in yeast, using affinity purification and mass spectrometry to identify components of isolated protein complexes. This latter approach can identify complexes containing three or more proteins. As the key non-mammalian organisms for model studies in systems biology, the author identifies *Escherichia coli*, *Saccharomyces*, *C. elegans*, *Drosophila*, zebra fish, and the plant *Arabidopsis*.

Conclusions: The author concludes that the genome sequence must be deciphered into a set of instructions that allow researchers to understand when and where proteins are synthesized, when and how groups of proteins are assembled into functional complexes and pathways that accomplish specific tasks, and when and how such tasks are assembled to build and run organelles, cells, tissues, organs, and whole organisms. Enormous intellectual and technical challenges must be overcome to achieve this goal. The discipline of systems biology has arisen in the postgenomic era to undertake these challenges.

This is an interesting review article written from the viewpoint of a classical researcher, seeing the principles of his academic discipline, in this case physiology, at risk by the development of systems biology. However, he takes the challenge and summarizes all the aspects of his (and also our) research area which already has come into close contact with the new evolving research approach. Is there a 'risk' for traditional physiology or an opportunity? Researchers working in this area are welcome to fully embrace the cornerstones of systems biology research, including functional genomics, genetics, non-mammalian model organisms, computational biology, and interdisciplinary research efforts. We are all welcome to contemplate about our own areas of interest, and how they can advance with systems biology in the near future.

Now, where does systems biology already work today?

Causal protein-signaling networks derived from multiparameter single-cell data

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Background: The authors tested the validity of a new automated method of network modeling. They compared data obtained by a process of 'machine learning' to reconstruct the causal influences in a cellular signaling network with the data that already had been deduced in their cell system. The signaling network examined was the activation and inhibition signals to human T cells.

Methods: Intracellular multicolor flow cytometry was used as it allows more quantitative simultaneous observations of multiple signaling molecules in many thousands of individual cells. Hence, it was an especially appropriate source of data for bayesian network modeling of signaling pathways. Data were collected after a series of stimulatory cues and inhibitory interventions with cell reactions stopped at 15 min after stimulation by fixation of human primary CD4 T cells, downstream of CD3, CD28, and LFA-1 activation. Overall the authors made flow cytometry measurements of 11 phosphorylated proteins and phospholipids. The complete datasets were analyzed with a bayesian network structure inference algorithm. The resulting de novo causal network model was inferred with 17 high-confidence causal arcs between various components. To evaluate the validity of this model, the authors compared the model arcs (and absent potential arcs) with those described in the literature. Arcs were categorized as follows: (i) *expected*, for connections well established in the literature; (ii) *reported*, for connections that are not well known, but for which they were able to find at least one literature citation, and (iii) *missing*, which indicates an expected connection that the bayesian network analysis failed to find.

Results: In almost all cases, the direction of causal influence was correctly inferred (an exception was Plc-g to PIP3, in which case the arc was inferred in the reverse direction). All the influences were contained within one global model. Indirect connections were detected as well. Two influence connections in the

examined model that were found to be dependent were not well established in the literature: PKC on PKA and Erk on Akt. The authors used siRNA inhibition of these factors to confirm the so far unestablished connection between those factors. This result demonstrated that interventions were critical for effective inference, particularly to establish directionality of the connections.

Conclusions: In this work the combination of single-cell flow cytometry and the application of bayesian networks correctly reverse-engineered and rapidly inferred the basic structure of a classically understood signaling network that connects a number of key phosphorylated proteins in human T-cell signaling, using a map that was built by classical biochemistry and genetic analysis over the past two decades. The network was automatically constructed with no a priori knowledge of pathway connectivity.

Derived from a research field outside pediatric endocrinology, this paper is an example of a successful application of systems biology to a known biological question. The method was tested and validated against the wealth of published data on T-cell signaling. The authors are primarily engineers and specialists in the field of systems biology, and stress the importance of the right biological method to obtain data that are feasible for bayesian network analysis. The main advantage of their system was the reliance on a single cell type, and the simultaneous measuring of multiple phosphorylated proteins and phospholipids by multiparameter flow cytometry. Application of this approach to other sets of molecules, cell types and disease states such as childhood growth, obesity or diabetes and the possible interventions into these systems (e.g., pharmaceutical agents, siRNA and dominant negative screens) should be targets for such systematic approaches, and enhance our understanding of signaling networks, especially with respect to complex non-linear cross-talk between pathways.

A systems model of signaling identifies a molecular basis set for cytokine-induced apoptosis

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Background: Most extracellular inputs initiate complex signaling patterns that propagate through an intracellular network to change the response outputs that determine a cell's phenotype. Molecular signaling through this network is branched and dynamically interconnected to the molecular history of the previous inputs, signals, and outputs. The authors sought to develop a mathematical formalism to connect signals and outputs in such a way that cellular responses could be predicted from molecular signaling patterns alone.

Methods: To investigate how signaling networks coordinate cellular output responses, they used an established multi-input system, where HT-29 cells were stimulated with three biologically relevant cytokines: TNF, EGF, and insulin. The intracellular protein network downstream of these cytokine inputs is understood in reasonable detail. Within well-recognized network branches, 19 intracellular measurements of key receptors, kinases, caspase, and adaptor proteins were collected. Using nine distinct pairwise combinations of TNF, EGF, and insulin, the authors then sampled each molecular signal in triplicate at 13 time points between 0 and 24 h to compile 7,980 distinct molecular signals from the shared intracellular network. To test whether apoptosis could be connected to the measured signaling they selected four distinct apoptotic outputs (phosphatidylserine exposure, membrane permeability, nuclear fragmentation, and caspase substrate cleavage) and measured each output response by flow cytometry 12, 24, and 48 h after stimulation, thereby measuring an apoptotic signature, which characterizes early, middle and late responses of apoptosis. To analyze their data, the group had to model mathematically a 660-dimensional signaling metric space onto the 12-dimensional output response space, something that obviously was not feasible right away.

Results: In the full model, 660 metrics derived from 7,980 signaling measurements were used to predict apoptosis. With the mathematical least square regression the model could be reduced to only 20 most informative metrics, as determined by the relative magnitude of their coefficients, which was nearly as predictive of apoptosis as one that used all 660 metrics.

Conclusion: By using a systems approach that combines quantitative experiments with data-driven modeling, the researchers identified two canonical axes – a stress-apoptosis axis and a survival axis – that together constitute a molecular basis set for the signaling network that controls apoptosis. These axes capture the dynamic intracellular signal processing of diverse stimuli, including autocrine feedback circuits.

This article is an example of the different strategies that systems biology follows. Using as little as 3 well-known biological cytokines alone or in combination, and measuring 19 well-established intracellular second messengers, left them with 7,980 different datasets that had to be weighed against the outcome, which was cell death in its different forms. Changes in the regulation of cell apoptosis for example most likely accompany many of the treatment modalities that pediatric endocrinology employs in stimulating growth in patients with short stature. The type of mathematical modeling that was used to finally achieve the goal of identifying the different relevant networks is impressive, though beyond my personal perception. This experience is most likely the cause of the skepticism that many of us have with this type of data manipulation. Therefore it is both reassuring and comforting to observe that some of the signal cascades characterized had been established before by the 'old fashion' methods by simply testing hypotheses and reasoning. This is an article which will be difficult to comprehend in its mathematical approaches for most of the readership, however is demonstrates how close the different disciplines involved in systems biology have to work together to produce biologically meaningful results.

An integrative genomics approach to infer causal associations between gene expression and disease

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Background: In their work the authors use a multistep procedure for identifying potential key drivers of a complex trait like obesity that integrates DNA variation and gene expression data with other complex trait data in segregating mouse populations. This is achieved by systematically testing whether variations in DNA that lead to variations in relative transcript abundances support an independent, causative or reactive function relative to the complex traits of obesity. The authors demonstrate how this approach can predict transcriptional responses to single gene perturbation, using single gene expression data in the context of a segregating mouse population. They also show that this approach can identify and experimentally validate the involvement of three unidentified genes of susceptibility to obesity.

Methods: Central to this method is a likelihood-based test for causality (LCMS) that takes into account genotypic, RNA and clinical data in segregating populations of mice to identify genes in the trait-specific transcriptional network that are under the control of multiple quantitative trait loci (QTLs) for the trait of interest. They used the LCMS procedure to evaluate the omental fat pad mass (OFPM) and liver gene expression data in order to identify the key drivers for the traits with enlarged OFPM. A whole set of perturbations had to be performed to account for artifacts that are encountered when two QTLs are connected to one another, for example by close linkage on a chromosome.

Results: Using this stepwise regression procedure they identified 113 transcripts as the most significant candidate causative genes with respect to OFPM. Among the ten most highly ranked genes, one of the strongest was 11 β HSD-1. The association of 11 β HSD-1 with the OFPM trait was previously established in a transgenic mouse overexpressing 11 β HSD-1 in adipose tissue. Next to this known gene, new susceptibility genes (Tgfb2, C3ar1 and Zfp90) were identified in the mouse population.

Conclusions: These results indicate that integrating genotypic and expression data may help the search for new targets for common human diseases like adiposity. They conclude that many statistical issues remain to be explored due to the high-dimensional nature of the problem. Nevertheless, the ability to partition genes into causal and reactive sets that are optimally placed in the gene network with respect to therapeutic intervention offers a promising approach to understanding the complex network of gene changes associated with common human diseases and also their future possible combat.

This work describes a new mathematical approach to data that are already on hand. For us endocrinologists it is mainly those three new genes that have evolved from this work as new candidates for causing obesity. However, it is rather the finding of the 'old' ones which makes this work reassuring that this new tool is for real. In the world of systems biology, the generation of a great body of unbiased data is more meaningful than the proof of a single well-developed hypothesis, a fact that lets the new discipline appear somewhat frightening for us. Yes – we have learned that fishing experiments can mean real science, but we also have to see that the first of our fishing cruises will just tell us how big the lake is and what equipment to take with us next time. The choice of the right experimental technique therefore seems crucial and is stressed by many systems biologists [1]. However, with all the high-throughput data we are able to obtain today, we have to admit that things have gotten out of our own hands. Despite the fact that we will not be able to develop tools ourselves for correctly exploiting their meaning, we still have the chance to decide which direction to go. Systems biology is generating a whole facet of new tools that we should at least be aware of. This paper is an honest attempt of system biologists to develop a useful strategy to analyze the wealth of data generated by the human genome project.

Tools that may be useful for you in systems biology

Nuclear receptor signaling atlas (www.nursa.org): hyperlinking the nuclear receptor signaling community

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Background: The field of nuclear receptors has made rapid progress over the last several decades from the initial characterization of ligands and receptors to the cloning of receptor cDNAs for the discovery of an ever-increasing number of coregulatory proteins recruited by receptors to regulate target gene transcription. While the development of large public genomic and proteomic database collections has facilitated this process, there is a deficit in which data generated by the field are integrated and analyzed for the benefit of the research community.

Methods: NURSA laboratories are developing accelerated throughput initiatives in applications as diverse as microarray-based global transcriptomics, real-time quantitative PCR (Q-PCR)-based gene expression analyses, proteomic and kinomic profiling of cellular proteins and ligand screening. A central goal of the Bioinformatics Resource is to integrate these primary datasets to construct reciprocal models of the interplay between events at the molecular level (ligand, receptor and coregulator modulation of target gene expression) and functionality at the organism level (organ physiology, metabolic regulation and homeostasis).

Results: Molecular data for nuclear receptor ligands, coregulators and nuclear receptor orthologs from public repositories and literature databases is NURSA internally curated and cross-linked to a content management system built upon the unique identifiers for molecules, assays and tissue systems. For example, a page displaying a dataset for estrogen receptor contains links to all other NURSA datasets and journal articles relevant to that molecule.

Conclusion: Ultimately the goal of this website is to provide a platform for organizing and integrating the diverse data sources of this field into a framework to allow for better approaches to the biology of nuclear receptors.

This article describes a newly formed website constructed to compile data for those of us who investigate the biology of nuclear receptors, their ligands and coregulators. This may constitute a successful approach not only in compiling data but also make them directly accessible for analyses by systems biology approaches. Such an open access resource has to be moderated and carefully surveyed. As nuclear receptors play a pivotal role in many of the signaling pathways relevant for pediatric endocrinology, it may be helpful with one's own data. Click www.nursa.org

pSTIING: a 'systems' approach towards integrating signalling pathways, interaction and transcriptional regulatory networks in inflammation and cancer

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Background: The extraction of meaningful information in the context of physiological systems requires characterization of not only the function of individual genes or proteins but also the physical interactions and functional associations in which they participate. Recent genome-wide interactome projects involving two-hybrid, co-immunoprecipitation, protein chip analyses, affinity purification and mass spectrometry have produced a wealth of protein-protein interaction data. In order to understand these processes as interconnected and interdependent systems, there is a need to derive associations with corroborating evidence, which often means bringing together heterogeneous data.

Methods: The authors developed a Protein Signalling, Transcriptional Interactions and Inflammation Networks Gateway (pSTIING), a publicly accessible knowledgebase and web application to facilitate an integrative 'systems' approach towards understanding inflammation, cell migration and cancer. The knowledgebase integrates protein-protein, protein-lipid, protein-small molecule, ligand-receptor interactions, receptor-cell type associations, disease information and transcriptional associations with signal transduction modules. Experimental data from gene expression or proteomic analyses can also be directly cross-correlated with network information in pSTIING via CLADIST, a clustering and data analysis tool associated with pSTIING.

Results: The pSTIING web interface provides a number of starting points from which to navigate the database. Users may perform single or multiple searches using protein or gene name, synonyms or identifiers (such as SwissProt or TrEMBL accessions) which retrieve information about interacting entities, interactions, transcriptional associations and pathway modules, with supporting experimental evidence and cross-references to external databases. pSTIING dynamically generates graphical representations of interaction networks and displays maps in scalable vector graphics (SVG) formats.

Conclusions: Building on molecular interaction data provided by publicly available databases, pSTIING offers a resource in that it integrates transcriptional regulatory associations with molecular interaction data and signalling modules, allowing researchers and students to explore and extend regulatory networks using experimental data or by searching on protein domains/motifs, human diseases and orthologous proteins across species.

Similar to the article discussed before, this paper introduces a concept of handling data from numerous (65,228 at present) molecular associations comprising protein-protein, protein-lipid, protein-small molecule and transcriptional regulatory associations. The nice thing about it is that the web application allows graphical representations of networks which are comprehensive and comprehensible for myself, that it allows to compare molecular interactions across species and to explore networks in human diseases. Although this database has a major focus on chronic inflammation, cell migration and cancer, it contains more and is worth having a look at and see whether your molecule of interest is listed and tangled in one of the networks.

Systems biology at work

New members of the insulin family: regulators of metabolism, growth and now reproduction

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Background: A decade ago the insulin family was comprised of four members in mammals: insulin, IGF-I, IGF-II, and relaxin. In recent years, additional members of the family, termed INSL3, INSL4, INSL5,

INSL6, and INSL7, have been identified. The discovery of these new ligands initiated the search for their cognate receptors. These efforts resulted in the discovery that LGR8 is a member of the LGR (leucine-rich repeat-containing G-protein-coupled receptors) subfamily of GPCR (G-protein-coupled receptors). The authors review recent information about new members of the insulin family and the role of cellular, molecular and computational biology in the identification of new insulin-like ligands and their cognate receptors.

Methods: Whereas INSL3 was identified in the testis, INSL4 was found as a differentially expressed gene within the human placenta. Finally, INSL5 and INSL6 have been characterized by techniques of computational biology in three laboratories working independently [2, 3]. Similarly, the receptors were sought and identified as a group of G-protein-coupled receptors called LGR. So far, LGR 4, 5, 6, 7 and 8 have been identified [2].

Results: The overlapping of ligand specificity between the LGR receptors suggests that the tissue-specific expression profile of these receptors may enable these ligands to have differential biologic actions in various tissues. In this respect it is interesting that the LGR4 null mice exhibits intrauterine growth retardation and embryonic and perinatal lethality and the LGR5 null mice displays a phenotype similar to ankyloglossia, suggesting that the LGR4 and LGR5 systems may have biologic roles distinct from LGR7 and 8.

Conclusions: In relatively rapid succession, through the application of the techniques of molecular and computational biology, five new members of the insulin family and some of their cognate receptors have been identified. The two major themes that emerge from these discoveries are the expanding role of the insulin family of proteins in reproductive physiology and the identification of the GPC receptors as cognate receptors for some of these new ligands. This represents a paradigm shift because the canonical insulin/IGF-I receptors are tyrosine kinase receptors. The challenge now lies in identifying the biologic function of these ligands and receptor.

Systems biology after all is not that new. This review gives an account of approaches that entered our research already years ago and produced data that led to shifts of paradigms that have lasted for decades. However, with all the information collected about old insulin and IGFs and new ligands and their receptors, the research community seems to be at a point where the application of newer systems biology approaches may be helpful in developing an interconnection of the networks with the neighboring biological systems.

Minireview: the neuroendocrine regulation of puberty: is the time ripe for a systems biology approach?

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Background: The search for the neuronal networks most critically involved in controlling luteinizing hormone-releasing hormone (LHRH) release during sexual development has narrowed to those systems that utilize excitatory/inhibitory amino acids and the recently identified neuropeptide metastin/kisspeptin. A major conclusion derived from this work is that the pubertal activation of LHRH secretion can no longer be considered as an event exclusively driven by purely transsynaptic influences. Cell-cell signaling molecules produced in astroglial cells facilitate LHRH secretion. Genetic approaches are employed to define the physiological contribution of these molecules to the pubertal process.

Methods: High-throughput approaches have been devised to assess differences in protein expression. To identify proteins that might be affected by the disruption of astrocytic erbB-4 signaling, the authors compared protein expression profiles in the hypothalamus of wild-type mice and mutant DN-erbB4 animals which have a reduced LHRH secretion and delayed puberty, using the method of isotope-coded affinity tag microcapillary liquid chromatography tandem mass-spectrometry. Additionally, the authors used cDNA arrays to not only identify some of these gene networks, but also to define the existence of region-specific changes in gene expression that occur in the hypothalamus at critical windows of primate sexual development. By comparing gene expression profiles between different brain regions, they are identifying those changes that are specific to the neuroendocrine brain and those that are common to the hypothalamus and cerebral cortex.

Results: The results of the profound work of this group shows that activation of LHRH secretion at puberty requires the coordinated activation of transsynaptic and astroglial regulatory systems. One level of coordination appears to be provided by a host of unrelated genes encoding proteins required for cell-cell communication. A second, but overlapping, level might be provided by a second set of genes engaged in specific cell functions required for productive cell-cell interaction. A third and higher level of control involves the transcriptional regulation of these subordinate genes by a handful of upper genes that, operating within the different neuronal and glial subsets required for the initiation of the pubertal process, sustain the functional integration of the network.

Conclusion: The existence of functionally connected genes controlling the pubertal process is consistent with the concept that puberty is under genetic control and that the genetic underpinnings of both normal and deranged puberty are polygenic rather than specified by a single gene.

We all knew that puberty is complex. The wealth of high-throughput expression data from the hypothalamus and its surrounding tissues, as produced mostly by the group of Ojeda, seems very well suited for a systems biology approach to identify the different components involved in the regulation of puberty onset, but also to test the model of hierarchies that exist between the different key players and their functional interactions. We can expect important discoveries from appropriate strategies as described above. Similar approaches have to be employed when examining the suspected interplay between already well-established hormonal axes as the somatotrophic and the gonadotropic axes [4].

Cell biology of the ghrelin receptor

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Background: Ghrelin, a gastric peptide involved in growth hormone release, is the ligand of the growth hormone secretagogue receptor type 1a (GHS-R1a), a G-protein-coupled receptor expressed in the pituitary and hypothalamus [5]. Next to the main ghrelin-stimulated endocrine actions, this hormone has some of the non-endocrine actions. Numerous non-endocrine actions associated with ghrelin appear to be mediated by various GHS-R1a-related receptor subtypes, which are widely distributed in the central and peripheral tissues.

Methods: The authors discuss the functional aspects of GHS-R1a by the examination of the genomic structure of the gene which gives hints to its potential regulation, they discuss published data about expression profiles in various parts of the central nervous system but also several peripheral tissues, they investigate the molecular structure of the receptor, which places it into a small family of receptors for peptides like motilin, neurotensin, GPR39 and neuromedin. They also discuss the kinetics of the receptor in the unbound and coupled state showing an internalization of this receptor as part of the modulation of receptor responsiveness. They further analyze the data obtained from transgenic animals with impaired GHS-R function and they follow the G-protein signaling pathways that are affected on ghrelin binding to GHS-R1a showing both changes in ionic currents and an activation of the MAP kinase cascade. Finally, they postulate the existence of specific uncharacterized ghrelin receptors due to the binding of ghrelin in various tissues that cannot be attributed to GHS-R1 expression.

Results: They identify the key role of Ghrelin in the regulation of GH secretion and food intake however the diversity of expression profiles and the differing responses of various tissues and signal cascades in response leave this picture fragmented into several pieces of a complex puzzle.

Conclusion: An unbiased systems biology approach to the data obtained so far seems promising in filling out some of the missing gaps of this fascinating regulatory network.

One may question what this paper has to do with systems biology. This work by Camina can be viewed as an initial step towards a systematic approach compiling and weighing all relevant data that have to do with ghrelin. This is the crucial step before this data undergoes the various steps of abstraction and calculations that may be beyond our horizons of reasoning. A systems biology approach is a demanding procedure and calls for a lot of insight and tolerance on both the biologist's

and the mathematician's side. As ghrelin was identified as a peptide that has a strong stimulatory effect on GH secretion, more potent than that of GHRH, the endocrinologist's focus was primarily on its role within the GH regulating network. The distribution of the GHS-R1 expression in various tissues and different modes of action can be expected in networks that control energy homeostasis, reproduction, sleep regulation, corticotroph secretion or gastroenteropancreatic functions [6]. With the data obtained so far, ghrelin seems to be a candidate for a systems biology approach.

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