Systems Biology

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Systems biology is a rapidly expanding novel research discipline aiming to describe interactions in complex biological systems. This is done by collection, integration and analysis of large quantities of data from multiple experimental sources employing powerful computational and informatics tools. The ‘omics’ techniques are typically used to collect quantitative data to construct and validate models. Starting with genomics, proteomics and transcriptomics, these techniques are constantly expanded by addition of new tools such as metabolomics, interactomics, cytomics, etc., that add to the increasing complexity of systems biology to be incorporated into biological networks. The following original and review papers published in 5/2006–3/2007 are a selection of works employing systems biology approaches to address important questions in endocrinology and general biology of relevance for human medicine.

New genes
Angry gene identified

Molecular analysis of flies selected for aggressive behavior

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Background: Aggressive behavior is common in most animals but little is known about its molecular background.

Methods: To address this problem, the authors developed a population-based selection procedure to increase aggression in Drosophila melanogaster. They measured changes in aggressive behavior in the selected subpopulations with a new two-male arena assay. In only 10 generations of selection, the aggressive lines became markedly more aggressive than the neutral lines.

Results: After 21 generations, the fighting index increased more than 30-fold. Using microarray analysis, the authors identified genes with differing expression levels in the aggressive and neutral lines as candidates for this strong behavioral selection response. A small set of these genes was tested through mutant analysis and found that one significantly increased fighting frequency.

Conclusion: These results suggest that selection for increases in aggression can be used to molecularly dissect this behavior.

Systems biology starts with a careful selection of the phenotypes. These investigators developed a population-based selection procedure to enhance aggression using two aggressive and two neutral fly populations derived from a single starting population and quantified the behavioral selection response with males that engaged in repeated fights over a female. After only 10 generations of selection, both aggressive selected lines were significantly more aggressive than both neutral lines, and these differences further increased under continued selection pressure. Differential expression of genes in the head (brain) of aggressive and neutral lines of flies was investigated by microarray. 28 genes showed higher expression (>25%) and 14 genes lower expression in aggressive flies. Five differentially expressed genes were mutated and aggressiveness of the offspring investigated. Two genes that produced a direct effect on aggression encode a cytochrome P450 (Cyp6a20) and an odor-binding protein. Translation of the present findings into humans is important but difficult. This study and forthcoming work using the same approach will reveal candidate genes to be explored in higher species. An appalling science fiction scenario fuelled by this work is the creation of the ‘Universal
Soldier’ (referring to the movie with the same name), in which selection for aggressiveness is an important tool. However, at the present stage, one must be happy that the insect species employed was small and harmless and that the studies were not conducted in wasps. Interestingly, aggressive animals were not more active than neutral ones.

**Sensitive ChIP-DSL technology reveals an extensive estrogen receptor α-binding program on human gene promoters**

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Proc Natl Acad Sci USA 2007;104:4852–4857

**Background:** Even though transcriptional initiation is a major research focus, little is known about how many genes are direct targets for a particular nuclear receptor. However, genome-wide chromatin immunoprecipitation (ChIP) coupled with microarray, known as ChIP-on-chip, offers a strategy to address these types of questions by determining promoters bound directly by transcription factors. Surprisingly, recent promoter and tiling array analyses suggest that ER-α frequently binds to intergenic regions and relatively rarely to gene promoters [http://www.pnas.org/cgi/content/full/104/12/4852-B12#B12](http://www.pnas.org/cgi/content/full/104/12/4852-B12#B12). In order to improve specificity and sensitivity, a new technology called ChIP-DSL, a modified ChIP-on-chip technology, was developed.

**Methods:** The authors developed a modified ChIP-on-chip technology using the DNA selection and ligation (DSL) strategy, thus permitting robust analysis with much reduced materials compared with standard procedures.

**Results:** Using the ChIP-DSL technology, general and sequence-specific DNA-binding transcription factors were profiled using a full human genome promoter, revealing approximately four times as many ER-α target promoters as were detected in previous genome-wide location studies. Gene expression profiling showed that only a fraction of these direct ER-α target genes were found to be highly responsive to estrogen. However, expression of those ER-α-bound, estrogen-inducible genes was associated with breast cancer progression in humans.

**Conclusions:** This study demonstrates the power of the ChIP-DSL technology in revealing regulatory gene expression programs that have been previously invisible in the human genome.

In order to map regulatory networks in mammalian cells the authors applied genome-wide chromatin immunoprecipitation (ChIP) coupled with microarray. This methodology has shown to provide a powerful tool for genome-wide detection of transcription factor binding. Aiming at improving the specificity and sensitivity of such analysis, the authors developed a new technology called ChIP-DSL using the DNA selection and ligation (DSL) strategy, permitting robust analysis with much reduced materials compared with standard procedures. The authors profiled general and sequence-specific DNA-binding transcription factors using a full human genome promoter array based on the ChIP-DSL technology, revealing an unprecedented number of the estrogen receptor-α (ER-α) target genes in MCF-7 cells. Only a small fraction of these genes are strongly regulated by estrogen. However, expression of genes that are under ER-α regulation is associated with breast cancer progression. In addition to developing a new technology and identifying novel transcriptional targets of ER-α, this study demonstrates how improvements of current technology may provide a much different answer to known biological questions. It also highlights the ever-increasing complexity of the regulatory networks induced by nuclear receptors in general and estrogen receptors in particular. Now when one knows what to look for it is easy to see translation of this work into the clinic in many disciplines including that of pediatric endocrinology.
Important observations for clinical practice
Regarding obesity, all mice are created unequal

Changes in gene expression foreshadow diet-induced obesity in genetically identical mice
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PLoS Genet 2006;2:e81

**Background:** High phenotypic variation in diet-induced obesity in male inbred mice suggests a molecular model to investigate non-genetic mechanisms of obesity.

**Methods and Results:** Feeding mice a high-fat diet beginning at 8 weeks of age resulted in a 4-fold difference in adiposity. The phenotypes of mice characteristic of high or low gainers were evident by 6 weeks of age, when mice were still on a low-fat diet; they were amplified after being switched to the high-fat diet and persisted even after the obesogenic protocol was interrupted with a calorically restricted, low-fat chow diet. Susceptibility to diet-induced obesity in genetically identical mice is a stable phenotype that can be detected in mice shortly after weaning. Chronologically, differences in adiposity preceded those of feeding efficiency and food intake, suggesting that observed difference in leptin secretion is a factor in determining phenotypes related to food intake. Gene expression analyses of adipose tissue and hypothalamus from mice with low and high weight gain, by microarray and qRT-PCR, showed major changes in the expression of genes of Wnt signaling and tissue re-modeling in adipose tissue. In particular, elevated expression of SFRP5, an inhibitor of Wnt signaling, the imprinted gene MEST and BMP3 may be causally linked to fat mass expansion, since differences in gene expression observed in biopsies of epididymal fat at 7 weeks of age (before the high-fat diet) correlated with adiposity after 8 weeks on a high-fat diet.

**Conclusions:** It is proposed that C57BL/6J mice have the phenotypic characteristics suitable for a model to investigate epigenetic mechanisms within adipose tissue that underlie diet-induced obesity.

Obesity is a multifactorial disease in which inherited allelic variation, together with environmental variation, determines the predisposition of an individual to developing the disease. Although the evidence in support of a genetic component to the development of obesity is overwhelming, and a number of promising candidate genes are being tested as underlying causes of obesity, it remains difficult to quantify the genetic contribution to the obesity epidemic during the past 25 years, a period too short for the accumulation of additional obesogenic alleles. The authors show that regarding fat accumulation, the phenotypes among C57BL/6J mice are highly variable by 6 weeks of age, even before they are fed a high-fat diet, indicating that some mice are destined to be high gainers, while others from the same litter are to become low gainers. The microarray analysis of gene expression in adipose tissue from high and low gainers suggests that the Wnt signaling pathway and genes associated with vascularization and tissue remodeling are major regulatory points controlling differences in adipose tissue expansion and that some of these genes are differentially expressed even before mice are fed a high-fat diet.

The findings strongly suggest that variation in energy balance in an inbred strain provides a model to explore epigenetic mechanisms that are powerful in causing obesity. Although an excess over the needs of calorie intake is the main cause of obesity, it has long been appreciated that the internal fate of absorbed calories may vary substantially between individuals. Numerous studies have tried to pinpoint the mechanisms and genetic factors involved in the regulation of these processes albeit without much success. The systems biology approach taken here gives promises for more rapid advances in the field which are highly warranted.
Whole genome microarray analysis of growth hormone-induced gene expression in bone: T-box 3, a novel transcription factor, regulates osteoblast proliferation

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Background: Growth hormone (GH) has an important role in bone metabolism, but the IGF-dependent and -independent molecular pathways involved are still largely unknown.

Methods: Microarray analysis were used to evaluate GH signaling pathways in 4-week-old GH-deficient mice following a single injection of GH or PBS at 6 or 24 h after treatment.

Results: 6,160 genes were differentially expressed, and 17% of these genes were identified at both time points. More than half of the genes differentially expressed were previously unknown genes. Subsequent studies were focused on T-box 3 (Tbx3), a novel transcription factor, which increased more than 2-fold at both time points. Pretreatment with IGF-binding protein-4 did not block GH-induced Tbx3 expression, whereas pretreatment with TNF-α did block GH-induced Tbx3 expression. Tbx3 expression increased during osteoblast differentiation and following BMP-7 and Wnt3a treatment. Blocking Tbx3 expression by small interfering RNA decreased osteoblast proliferation and number.

Conclusions: GH caused changes in expression of several unknown genes, suggesting that several GH-induced signaling pathways and target genes remain to be discovered. Furthermore, Tbx3 expression is GH-regulated in osteoblasts and blockage of Tbx3 expression decreased cell number and DNA synthesis, thus suggesting that Tbx3 is a determinant of osteoblast cell number.

In this paper, the authors use microarray and a mouse model of GH deficiency (little mice) to explore the regulatory role of GH on bone metabolism. They identify the gene T-box 3, mutations of which causes ulnar-mammary syndrome characterized by skeletal defects, hypoplasia of mammary glands, micropenis, delayed puberty, and obesity, to be acutely regulated by GH in bone as well as an important regulator of osteoblast proliferation in vitro. The microarray analysis also identified several genes induced by GH of which little information is available, implicating novel direct actions of GH. Interestingly, several of these unknown genes include zinc finger motifs, thus indicating a potential role for these genes as transcription factors. This is good news for pediatric endocrinologists and other researchers working on GH research as the presented results open new gates to yet unexplored research fields that may be extended beyond bone.

Systems biology makes it possible to see the forest and the trees!

Cancer: a systems biology disease

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Biosystems 2006;83:81–90

Background: Cancer research has focused on the identification of molecular differences between cancerous and healthy cells. The emerging picture is overwhelmingly complex. Molecules out of many parallel signal transduction pathways are involved. Their activities appear to be controlled by multiple factors. The action of regulatory circuits, cross-talk between pathways and the non-linear reaction kinetics of biochemical processes complicate the understanding and prediction of the outcome of intracellular signaling. In addition, interactions between tumor and other cell types give rise to a complex supracellular communication network. If cancer is such a complex system, how can one ever predict the effect of a
mutation in a particular gene on a functionality of the entire system? And, how should one go about identifying drug targets?

Methods and Results: Review paper in which the authors argue that one aspect is to recognize where the essence resides, i.e. recognize cancer as a systems biology disease. Then, more cancer biologists could become systems biologists aiming to provide answers to some of the above systemic questions. To this aim, they should integrate the available knowledge stemming from quantitative experimental results through mathematical models.

Discussion and Conclusion: Models that have contributed to the understanding of complex biological systems are discussed. It is shown that the architecture of a signaling network is important for determining the site at which an oncologist should intervene. Finally, it is discussed the possibility of applying network-based drug design to cancer treatment and how rationalized therapies, such as the application of kinase inhibitors, may benefit from systems biology.

During recent years, there has been great progress in the knowledge in the field of molecular cell biology of cancer. At the same time, the emerging complexity of the entire ‘cancer system’ overwhelms us, leaving an enormous gap in our understanding. In this paper, different aspects of this complexity are discussed. Molecules out of many parallel signal transduction pathways are involved in carcinogenesis. Their activities appear to be controlled by multiple factors. The action of regulatory circuits, cross-talk between pathways and the non-linear reaction kinetics of biochemical processes complicate the understanding and prediction of the outcome of intracellular signalling. This is a systems biology view on cancer adding another useful dimension to our understanding of the biology of cancer disorders. It is easy to accept the view that cancer researchers should also be system biologists. The paper gives new concepts of clinical usefulness not only for oncologists.

Mechanism of the year

Zac1 regulates an imprinted gene network critically involved in the control of embryonic growth

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Dev Cell 2006;11:711–722

Background: Genomic imprinting is an epigenetic mechanism of regulation that restrains the expression of a small subset of mammalian genes to one parental allele. The reason for the targeting of these approximately 80 genes by imprinting remains uncertain.

Methods and Results: It is shown that inactivation of the maternally repressed Zac1 transcription factor results in intrauterine growth restriction, altered bone formation, and neonatal lethality. A meta-analysis of microarray data reveals that Zac1 is a member of a network of coregulated genes comprising other imprinted genes involved in the control of embryonic growth. Zac1 alters the expression of several of these imprinted genes, including Igf2, H19, Cdkn1c, and Dlk1, and it directly regulates the Igf2/H19 locus through binding to a shared enhancer.

Conclusion: These data identify a network of imprinted genes, including Zac1, which controls embryonic growth and which may be the basis for the implementation of a common mechanism of gene regulation during mammalian evolution.

Imprinting is understood as a mechanism aimed at controlling the amount of maternal resources allocated to the offspring from conception to weaning. Imprinted genes are members of various gene families, but a recurrent theme in the biology of imprinted genes is the control of embryonic development. Analysis of gain- and loss-of-function mouse mutants indicates that a number of imprinted genes are critically involved in the control of embryonic growth, either directly or by modulating the transport of nutrients across the placenta. The number and identity of imprinted genes
involved in these processes, and the underlying gene networks, remain unclear. In order to understand the function of the maternally repressed transcription factor, Zac1, the authors inactivate the gene by homologous recombination. Next they identified the underlying network they did a meta-analysis of microarray data of genes that are co-expressed with Zac1 in different tissues and studied the regulatory effect of Zac1 on the members of the network. Zac1 was found to be critical for embryonic growth, and for gene expression of a large network of imprinted genes, including IGF2, Cdkn1c, and Dlk1.

Zac1 is identified as a central regulator of a large network of imprinted genes and may thus be part of a novel mechanism of gene regulation during mammalian development. Imprinted genes have often been observed to act in common developmental or physiological pathways. The present report exploring the function of the transcription factor Zac1 reveals just how extensive the transcriptional network of imprinted genes may be.

New paradigms

Circadian clocks are resounding in peripheral tissues
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Background: Circadian rhythms are prevalent in most organisms. Even the smallest disturbances in the orchestration of circadian gene expression patterns among different tissues can result in functional asynchrony, at the organism level, and may to contribute to a wide range of physiologic disorders. It has been reported that as many as 5–10% of transcribed genes in peripheral tissues follow a circadian expression pattern.

Methods: The authors have conducted a comprehensive study of circadian gene expression on a large dataset representing three different peripheral tissues. The data have been produced in a large-scale microarray experiment covering replicate daily cycles in murine white and brown adipose tissues as well as in liver. The authors applied three alternative algorithmic approaches to identify circadian oscillation in time series expression profiles.

Results: Analyses of our own data indicate that the expression of at least 7–21% of active genes in mouse liver, and in white and brown adipose tissues follow a daily oscillatory pattern. Indeed, analysis of data from other laboratories suggests that the percentage of genes with an oscillatory pattern may approach 50% in the liver. For the rest of the genes, oscillation appears to be obscured by stochastic noise. Phase classification and computer simulation studies based on multiple datasets indicate no detectable boundary between oscillating and non-oscillating fractions of genes.

Conclusion: Greater attention should be given to the potential influence of circadian mechanisms on any biological pathway related to metabolism and obesity.

The metabolism of living organisms changes over the 24-hour daily cycle in an oscillatory manner. This repeating pattern of ‘peak’ and ‘trough’ expression is known as a ‘circadian rhythm’. We now know that the body’s internal clock is controlled by a discrete group of genes. These important regulators are found in many different organs of the body, and they control expression of many other genes. In order to further understanding of the circadian rhythms of metabolism, the authors studied circadian gene expression using microarray analysis in liver, and in white and brown fat of mice using three different mathematical tests. They present data indicating that the majority of active genes fluctuate rhythmically over a 24-hour period. This work suggests that future studies should pay close attention to the influence of the circadian rhythm in obesity and in fat metabolism. Make sure in your clinical or animal studies to draw your samples at the same time each day.
Unraveling adaptive evolution: how a single point mutation affects the protein coregulation network

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Background: Understanding the mechanisms of evolution requires identification of the molecular basis of the multiple (pleiotropic) effects of specific adaptive mutations.

Methods: The authors have characterized the pleiotropic effects on protein levels of an adaptive single base pair substitution in the coding sequence of a signaling pathway gene in the bacterium Pseudomonas fluorescens SBW25.

Results: 52 proteomic changes were found, corresponding to 46 identified proteins. None of these proteins is required for the adaptive phenotype. Instead, many are found within specific metabolic pathways associated with fitness-reducing (that is, antagonistic) effects of the mutation. The affected proteins fall within a single coregulatory network.

Conclusions: The mutation ‘rewires’ this network by drawing particular proteins into tighter coregulating relationships. Although these changes are specific to the mutation studied, the quantitatively altered proteins are also affected in a coordinated way in other examples of evolution to the same niche.

This work demonstrates the power of a systems biology approach to dissect the pleiotropic molecular events accompanying evolution. The simple mutation introduced into the model bacteria allowed the occupation of an environmental niche that was not available to the ancestral strain. The single base pair mutation caused 52 proteomic changes involving 46 identified proteins, none of which were required for the adaptive phenotype of the host. The affected proteins all belonged to the same coregulatory network and were found to be associated with transport and metabolism of amino acids. The systems biology model used here will help to understand better the complex construction of integrated molecular systems and how they may be affected by evolution. The dramatic phenotypic change observed was the result of a simple mutation with profound effects on the proteome. The results demonstrate clearly that discrete mutational changes may result in dramatic effects on phenotypes, thus allowing major evolutionary steps. This challenges the view that evolution is driven by minute functional alterations of mutated proteins with negative or positive impact on their functions.

Mutations affecting gene expression may underlie common disorders

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Trends Genet 2006;22:456–461

Background: Changes in genetic regulation contribute to adaptations in natural populations and influence susceptibility to human diseases. Despite their potential phenotypic importance, the selective pressures acting on regulatory processes in general and gene expression levels in particular are largely unknown.

Methods and Results: Review article discussing the microarray-based observations that led to disparate interpretations from studies in model organisms suggesting that (1) expression levels of most genes evolve under stabilizing selection, although a few are consistent with adaptive evolution, and (2) gene expression levels in primates evolve largely in the absence of selective constraints.

Conclusion: It is concluded that in both primates and model organisms, stabilizing selection is likely to be the dominant mode of gene expression evolution. An important implication is that mutations affecting gene expression will often be deleterious and might underlie many human diseases.
This is an important review paper using recent systems biology data to advance the discussion on the evolution biology of species including man. Data from model systems in lower species and from pri-mates indicate that negative stabilizing selection is much more common whereas positive adaptive selection occurs more rarely. This suggests that changes in gene expression are deleterious and that they are involved in disease processes in humans. The specific sets of genes whose regulation are more commonly under adaptive pressures are yet to be defined. This paper is recommended as an up-to-date source of concepts and terminology in the field. The most interesting part is addressing novel views on the causes of common disorders in human.

**Reviews**

**How do women choose males? (Theory)**

Dissecting the complex genetic basis of mate choice

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*Nat Rev Genet* 2006;7:681–692

**Background and Methods:** Review paper discussing the topic of mate choice. The genetic analysis of mate choice is fraught with difficulties. Males produce complex signals and displays that can consist of a combination of acoustic, visual, chemical and behavioral phenotypes. Female preferences for these male traits are notoriously difficult to quantify.

**Results and Conclusions:** During mate choice, genes not only affect the phenotypes of the individual they are in, but can influence the expression of traits in other individuals. How can genetic analyses be conducted to encompass this complexity? Tighter integration of classical quantitative genetic approaches with modern genomic technologies promises to advance our understanding of the complex genetic basis of mate choice.

Mate choice is thought to be one of the fundamental means of Darwinistic evolution. Males produce signals and displays that can consist of complex combinations of phenotypic expressions including ornamental and behavioral traits. Females differ in their preferences for these male traits but studies in this field have been hampered by difficulties to quantify the female responses. This review article describes the present status and challenges of this important field of evolutionary and behavioral genetics. To be successful genetic analysis of mate choice requires integrative approaches taking onboard modern systems biology technologies. The authors are hopeful that tighter integration of classical quantitative genetic approaches with modern genomic technologies will advance our understanding of the complex genetic basis of mate choice. Although direct translation of this research into human medicine may seem far-sighted basic insights into this the field are of importance for better understanding of the physiology and pathophysiology of behavioral traits and disturbances that may occur during human adolescence and early adult life.

**New hope**

**How do women choose males II? (Practice)**

Testing the genetics underlying the co-evolution of mate choice and ornament in the wild

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**Background:** One of the most debated questions in evolutionary biology is whether female choice of males with exaggerated sexual displays can evolve as a correlated response to selection acting on genes coding
for male attractiveness or high overall viability. To date, empirical studies have provided support for parts of this scenario, but evidence for all key genetic components in a natural population is lacking.

**Methods:** Here the authors use animal-model quantitative genetic analysis on data from over 8,500 collared flycatchers (*Ficedula albicollis*) followed for 24 years to quantify all of the key genetic requirements of both fisherian and ‘good-genes’ models on sexual selection in the wild.

**Results:** It was found that significant additive genetic variances of all the main components: male ornament (forehead patch size), female mate choice for this ornament, male fitness and female fitness. However, when the necessary genetic correlations between these components were taken into account, the estimated strength of indirect sexual selection on female mate choice was negligible.

**Conclusion:** The results show that the combined effect of environmental influences on several components reduces the potential for indirect sexual selection in the wild. This study provides insight into the field of sexual selection by showing that genes coding for mate choice for an ornament probably evolve by their own pathways instead of ‘hitchhiking’ with genes coding for the ornament.

Current models of sexual selection (mate choice) suggest that males carry genes coding for sexual attractiveness (display of ornaments; fisherian model) and viability (good genes model) enhancing the competitiveness of their offspring. Females preferentially mate with highly ornamented males implicating a genetic correlation between the ornament and the mate choice with a linkage to good genes expressed in the offspring. The models have partial support from several studies but solid evidence covering all genetic components from a wild-life population perspective has been lacking. The authors test whether the preference of female collared flycatchers (*Ficedula albicollis*) for males with large forehead patches could have evolved as a by-product of selection acting on male patch size. They find that the crucial genetic correlation between female choice and male patch size is not significant, and conclude that preference for large patches must have been shaped directly by selection. This is good news! Lack of inherited good looks (strong ornaments) can be compensated for by hard work (environmental influence).

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**Mechanism of the year**

**Excitation by paracrine glutamate start you up**

Quantitative proteomics identifies a change in glial glutamate metabolism at the time of female puberty

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**Background:** Mammalian puberty requires activation of luteinizing hormone-releasing hormone (LHRH) neurons. In turn, these neurons are controlled by transsynaptic and glia-to-neuron communication pathways, which employ diverse cellular proteins for proper function.

**Methods:** The authors used a high throughput relative quantitative proteomics technique to identify proteins involved in pubertal activation. They selected the method of two-dimensional liquid chromatography tandem mass spectrometry (2DLC-MS/MS) and cleavable isotope-coded affinity tags (cICAT), to both identify and quantify individual proteins within a complex protein mixture. The proteins used derived from the hypothalamus of juvenile (25-day-old) and peripubertal (first proestrus, LP) female rats, and their identity was established by analyzing their mass spectra via database searching.

**Results:** Five proteins involved in glutamate metabolism were detected and two of them appeared to be differentially expressed. They were selected for further analysis, because of their importance in controlling glutamate synthesis and degradation, and their preferential expression in astroglial cells. One, glutamate dehydrogenase (GDH) catalyzes glutamate synthesis; its hypothalamic content detected by 2DLC-MS/MS increases at first proestrus. The other, glutamine synthetase (GS), catalyzes the metabolism of glutamate to glutamine; its content decreases in proestrus. Western blot analysis verified these results. Because these changes suggested an increased glutamate production at puberty, we measured...
glutamate release from hypothalamic fragments from juvenile 29-day-old rats, and from rats treated with PMSG to induce a premature proestrus surge of luteinizing hormone (LH). To determine the net output of glutamate in the absence of re-uptake, we used the excitatory amino acid transporter (EAAT) inhibitor L-trans-pyrrolidine-2,4-dicarboxylic acid (PDC). PDC elicited significantly more glutamate and LHRH release from the proestrus hypothalamus.

Conclusion: An increase excitatory drive to the LHRH neuronal network provided by glutamatergic inputs of glial origin is an event contributing to the pubertal activation of LHRH secretion.

This study illustrates the power of a proteomics approach to study systemic time-restricted maturational events in biology, here verifying the important role of glutamate as an excitatory signal at the onset of puberty. The present study demonstrates that the onset of female puberty is accompanied by opposite changes in the hypothalamic content of glutamate dehydrogenase and glutamine synthetase, two enzymes involved in the homeostatic control of brain glutamate metabolism. The results also show that these changes in protein content are physiologically important, because they are accompanied by an increased capability of the hypothalamus to release glutamate. The relevance of these changes to the control of LHRH secretion is evidenced by the increased LHRH output that follows the peripubertal changes in glutamate release. The results thus verify that puberty starts after providing LHRH neurons an increased excitatory drive exerted by paracrine glutamate derived from local astroglial cells. The results are not exactly novel but the experimental context implicates a physiological significance. The next question is of course what comes before glutamate? Proteomics succeeded where genomics failed, suggesting that the onset of puberty is not necessarily in our gene DNA sequence but rather in gene expression, which may be regulated by the environment.

Food for thought
Males and females are more different than we think but not in the brain!

Tissue-specific expression and regulation of sexually dimorphic genes in mice
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Genome Res 2006;16:995–1004

Background: Sexual dimorphism in gene expression in various tissues is an important target of investigation but progress has been hampered by low-power and non-comprehensive approaches of hitherto published studies.

Methods and Results: The authors report a comprehensive analysis of gene expression differences between sexes in multiple somatic tissues of 334 mice derived from an intercross between inbred mouse strains C57BL/6J and C3H/HeJ. The analysis of a large number of individuals provided the power to detect relatively small differences in expression between sexes, and the use of an intercross allowed analysis of the genetic control of sexually dimorphic gene expression. Microarray analysis of 23,574 transcripts revealed that the extent of sexual dimorphism in gene expression was much greater than previously recognized. Thus, thousands of genes showed sexual dimorphism in liver, adipose, and muscle, and hundreds of genes were sexually dimorphic in brain. These genes exhibited highly tissue-specific patterns of expression and were enriched for distinct pathways represented in the Gene Ontology database. They also showed evidence of chromosomal enrichment, not only on the sex chromosomes, but also on several autosomes.

Conclusion: The analyses provided evidence of the global regulation of subsets of the sexually dimorphic genes, as the transcript levels of a large number of these genes were controlled by several expression quantitative trait loci (eQTL) hotspots that exhibited tissue-specific control. Many tissue-specific transcription factor binding sites were found to be enriched in the sexually dimorphic genes.

Common diseases often have a sex bias and systematic understanding of the physiological differences between sexes is therefore of great importance to advance the field. In this work the authors
present a comprehensive analysis of gene expression differences between sexes in multiple somatic tissues in mice. Thousands of genes in liver, fat and muscle, and hundreds of genes in brain were found to be sexually dimorphic, defined as >1.2-fold difference in expression between sexes. These genes exhibited a highly sex-specific tissue expression pattern and had a chromosomal enrichment not only restricted to the sex chromosomes. Liver showed the greatest degree of sex difference with more than 70% of expressed genes whereas the brain showed the lowest with less than 14% of expressed genes. Most differences were small and the percentages of genes with >2-fold difference in expression between sexes were 1.1% for liver, 0.8% for fat, 0.6% for muscle and 0.1% for brain. The sexually dimorphic genes were clustered into several functional categories such as immune response, lipid metabolism, steroid hormone metabolism and polyamine metabolism dependent on tissue (liver, fat, muscle). The brain was an exception showing RNA helicase activity as an enriched dimorphic category. The widespread sex differences in gene expression observed here are most likely due to differential effects of sex steroids, which contribute to sex differences both directly and indirectly (mediated by growth hormone profile). Despite the importance of hormones, an increasing amount of evidence supports a regulatory cascade concept of sexual dimorphism in gene expression; that is, the initiating events of sexual differentiation such as Sry expression trigger differential expression in many mediator genes that further regulate the sexually dimorphic expression of downstream genes. These results need extension to other tissues and organs and confirmation in humans, but the findings demonstrate clearly the potential impact of transcriptomics to advance our understanding of the pathophysiology behind the gender differences in common disorders.

Identification of genetic networks involved in the cell injury accompanying endoplasmic reticulum stress induced by bisphenol A in testicular Sertoli cells

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Background: The molecular mechanisms mediating cell injury by xenobiotics are virtually unknown but are important to investigate in order to develop better diagnostic and preventive measures in reproductive toxicology.

Methods: To identify detailed mechanisms by which bisphenol A (BPA), an endocrine-disrupting chemical, induces cell injury in mouse testicular Sertoli TTE3 cells, the authors performed genome-wide microarray and computational gene network analyses.

Results: BPA (200 μM) significantly decreased cell viability and simultaneously induced an increase in mRNA levels of HSPA5 and DDIT3, endoplasmic reticulum (ER) stress marker genes. Of the 22,690 probe sets analyzed, BPA downregulated 661 probe sets and upregulated 604 probe sets by >2.0-fold. Hierarchical cluster analysis demonstrated nine gene clusters. In decreased gene clusters, two significant genetic networks were associated with cell growth and proliferation and the cell cycle. In increased gene clusters, two significant genetic networks including many basic-region leucine zipper transcription factors were associated with cell death and DNA replication, recombination, and repair.

Conclusion: The results will provide additional novel insights into the detailed molecular mechanisms of cell injury accompanying ER stress induced by BPA in Sertoli cells.
DNA replication, recombination and repair. Downregulated gene clusters included genes important for cell growth and cell cycle control. The principles applied in this laborious cellular toxicotranscriptomics study have a potential to become a model for toxicological screening programs. In Europe this has recently become highly relevant under the new EU legislation on registration, evaluation and authorization of new chemicals (REACH).

**Approaching the virtual cell**

**Establishing glucose- and ABA-regulated transcription networks in Arabidopsis by microarray analysis and promoter classification using a relevance vector machine**


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**Background:** Transcriptional regulatory networks are difficult but import to delineate. Novel techniques for analysis of gene expression data and promoter sequences show great promise in this respect.

**Methods:** The authors developed a novel promoter classification method using a relevance vector machine (RVM) and bayesian statistical principles to identify discriminatory features in the promoter sequences of genes that can correctly classify transcriptional responses. The method was applied to microarray data obtained from *Arabidopsis* seedlings treated with glucose or abscisic acid (ABA).

**Results:** Of genes showing >2.5-fold changes in expression level, approximately 70% were correctly predicted as being up- or downregulated (under 10-fold cross-validation), based on the presence or absence of a small set of discriminative promoter motifs. Many of these motifs have known regulatory functions in sugar- and ABA-mediated gene expression. One promoter motif that was not known to be involved in glucose-responsive gene expression was identified as the strongest classifier of glucose upregulated gene expression. It was shown it mediated glucose-responsive gene expression in conjunction with another promoter motif, thus validating the classification method.

**Conclusion:** The authors were able to establish a detailed model of glucose and ABA transcriptional regulatory networks and their interactions, which will help us to understand the mechanisms linking metabolism with growth in *Arabidopsis*. This study shows that machine-learning strategies coupled to bayesian statistical methods hold significant promise for identifying functionally significant promoter sequences.

Bioinformatic methods that define relationships between gene expression levels and putative regulatory sequences in upstream regions of genes are increasingly used to establish genome-scale transcriptional regulatory networks. The authors apply and validate such techniques. Microarray data obtained from cultures of *Arabidopsis* seedlings treated with glucose, ABA or both were analyzed using a RVM and bayesian statistics in order to establish genome-scale regulatory networks. A number of regulatory elements that are responsible for ABA- and glucose-regulated gene expression was identified. Based on the presence or absence of a small number of these regulatory elements, the authors were able to predict response to treatment. Some of these regulatory elements have not previously been associated with glucose-responsive gene expression. This is complicated methodology but the paper holds promises for integrated metabolomics and proteomics models that are important and necessary building blocks for creation of the ‘virtual cell’.

**References**

Readers who want a basic update and learn more about the methodologies employed in systems biology research are referred to the following review papers and books.


