The Year in Science and Medicine

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Mechanism of the year: epigenetics; it ain't new, but the progress it has made in the last year is astounding

Genome-wide high-resolution mapping and functional analysis of DNA methylation in *Arabidopsis*

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<http://epigenomics.mcdb.ucla.edu/DNAmeth/>

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Background: Cytosine methylation is important for transposon silencing and epigenetic regulation of endogenous genes although the extent to which this DNA modification functions to regulate the genome is still unknown.

Methods: Using a tiling microarray with 35 bp resolution, the authors carried out the first comprehensive mapping of DNA methylation in the entire genome of the flowering plant *Arabidopsis thaliana*.

Results: Pericentromeric heterochromatin, repetitive sequences and regions producing small interfering RNAs were heavily methylated. Unexpectedly, over one third of expressed genes contained methylation within transcribed regions, whereas only ~5% of genes contained methylation within promoter regions. Interestingly, genes methylated in transcribed regions were highly expressed and constitutively active, whereas promoter-methylated genes showed a greater degree of tissue-specific expression.

Conclusions: Whole genome tiling array transcriptional profiling of DNA methyltransferase null mutants identified hundreds of genes and intergenic non-coding RNAs with altered expression levels, many of which may be epigenetically controlled by DNA methylation.

The first genome-wide DNA methylation map has been published and provided important insights into how this DNA modification regulates gene expression. It turns out that almost 19% of the *Arabidopsis* genome is methylated. As expected, much of this methylation occurs in heterochromatin, including centromeres, which harbors transposons and repetitive elements, but a considerable amount of methylation is also found in euchromatin. Unsurprisingly, the highest levels are seen in pseudogenes and unexpressed genes, but around 5% of expressed genes have methylated promoters and 33% are methylated within a transcribed region (the body-methylated genes). A comparison of DNA methylation sites with microarray expression data from a number of tissues and conditions revealed that body-methylated genes are more highly expressed than unmethylated genes, with promoter-methylated genes showing the lowest levels of expression. Promoter-methylated genes tend to be expressed in a tissue-specific manner, whereas body-methylated genes tend to be constitutively expressed. The stage is set for other methylome analyses.

RNA-mediated non-Mendelian inheritance of an epigenetic change in the mouse

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Background: Paramutation is a heritable epigenetic modification induced in plants by cross-talk between allelic loci.

Results: The authors found a similar modification of the mouse *Kit* gene in the progeny of heterozygotes with the null mutant *Kit^{tm1Alf}* (a *lacZ* insertion). In spite of a homozygous wild-type genotype, their offspring maintain, to a variable extent, the white spots characteristic of *Kit* mutant animals. Efficiently inherited from either male or female parents, the modified phenotype results from a decrease in *Kit* messenger RNA levels with the accumulation of non-polyadenylated RNA molecules of abnormal sizes. Sustained transcriptional activity at the postmeiotic stages – at which time the gene is normally silent – leads to the accumulation of RNA in spermatozoa. Microinjection into fertilized eggs either of total RNA from *Kit^{tm1Alf/+}* heterozygotes or of *Kit*-specific microRNAs induced a heritable white tail phenotype.

Conclusions: This paper identifies an unexpected mode of epigenetic inheritance associated with the zygotic transfer of RNA molecules.

Paramutation, which was described 50 years ago, is inconsistent with Mendelian laws: an allele interacts with its homologue and somehow instigates a heritable change. In this era of epigenetics we aren't shocked any more [1]. But there is now evidence that one instance of paramutation in mice is mediated by RNA; a mutant RNA transcript seems to alter the expression of the wild-type allele and transmits the modified phenotype to future generations of offspring. The mouse proto-oncogene *Kit* encodes a transmembrane tyrosine kinase, with a strong structural homology to the colony-stimulating factor-1 receptor and the platelet-derived growth factor receptor. *Kit* mutations in the mouse produce spots of white fur on otherwise dark-colored mice, sterility, and anemia, attributable to failure of stem cell populations to migrate and proliferate effectively during development. The null allele of *Kit* that gives rise to this phenotype (*Kit^{tm1Alf}*) is dominant; however, most offspring of a *Kit* heterozygous mouse and a WT one had white spots, even when they carried two copies of the WT *Kit* allele. The explanation for this unusual inheritance was suggested by the fact that the WT *Kit* allele produces many oddly sized RNA transcripts. When RNA from *Kit* mutant cells was injected into wild-type fertilized eggs, mice developed white spots. The involvement of microRNAs in all this seems likely given their recognized association with paramutation and other epigenetic phenomena: the regulatory RNAs might degrade the normal *Kit* message, or modify the WT allele itself. The fitness benefits of an RNA-based inheritance system are easy to imagine: any advantage that a mutated allele conferred in one generation could be passed on to offspring, without the need to rely on mutation and selection to increase the representation of the fitter allele. If that sounds to you like Lamarckism inheritance of acquired traits, you may be right.

Fine-tune a flower power

Variation in the epigenetic silencing of FLC contributes to natural variation in *Arabidopsis* vernalization response

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Genes Dev 2006;20:3079–3083

Background: Vernalization, the cold-induced acceleration of flowering, involves the epigenetic silencing of the floral repressor gene *flowering locus c* (FLC).

Methods: This group investigated the molecular basis for variation in vernalization in *Arabidopsis* natural accessions adapted to different climates.

Results: A major variable was the degree to which different periods of cold caused stable FLC silencing. In accessions requiring long vernalization, FLC expression was reactivated following non-saturating vernalization, but this reactivation was progressively attenuated with increasing cold exposure. This response was correlated with the rate of accumulation of FLC histone H3 Lys 27 trimethylation (H3K27me3).

Conclusions: Variation in epigenetic silencing of FLC appears to have contributed to *Arabidopsis* adaptation.

Plant scientists introduced epigenetics and still teach us a lesson: epigenetic silencing controls variation in flowering time – a key adaptive trait. Like many other plants, *Arabidopsis thaliana* (thale

cross, or mouse-ear cross, a small flowering plant related to cabbage and mustard) need to be exposed to a period of cold before they can flower – a response called vernalization. Growing in a wide range of environments, they show considerable differences in flowering times and vernalization responses. Initially thought to be genetic, this paper shows that there was no correlation between RNA levels of specific markers and the length of exposure to the cold. Rather, cold period quantitatively enhanced the stability of gene repression by differential accumulation of histone methylation. They propose that vernalization occurs in three phases: an insensitive phase is followed by a phase in which silencing occurs; when the levels of histone modification are sufficient, the third phase begins, in which the gene is fully silenced. The time taken to accumulate sufficient histone methylation levels determines the speed of the response to vernalization. Children respond to the environment in many ways, and for pediatric endocrinologists the secular trend of growth and puberty is an obvious analogue to vernalization. Epigenetics is here to stay and we will not be surprised when it eventually explains the secular trend.

Gene body-specific methylation on the active X chromosome

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Background: Differential DNA methylation is important for the epigenetic regulation of gene expression. Allele-specific methylation of the inactive X chromosome has been demonstrated at promoter CpG islands, but the overall pattern of methylation on the active X (Xa) and inactive X (Xi) chromosomes is unknown.

Methods: An allele-specific analysis of more than 1,000 informative loci along the human X chromosome was performed.

Results: The Xa displays more than two times as much allele-specific methylation as Xi. This methylation is concentrated at gene bodies, affecting multiple neighboring CpGs. Before X inactivation, all of these Xa gene body-methylated sites are biallelically methylated. Thus, a bipartite methylation-demethylation program results in Xa-specific hypomethylation at gene promoters and hypermethylation at gene bodies.

Conclusion: These results suggest a relationship between global methylation and expression potentiality.

If you thought that methylation silences genes, this paper used a genome-wide analysis of DNA methylation to show, surprisingly, that the transcribed regions of genes on the active human X chromosome (Xa) are hypermethylated, both in females and males. Equivalent regions on the Xi are hypomethylated. Prior to X inactivation, both X chromosomes are methylated, which suggests that methylation was lost. Paramutations were reported more than 50 years ago. They may be the oldest form of epigenetics, but they are probably not the most common; we are now almost friendly with imprinting. See the next article.

An RNA gene expressed during cortical development evolved rapidly in humans

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Nature 2006;443:167–172

Background: The developmental and evolutionary mechanisms behind the emergence of human-specific brain features remain largely unknown. However, the recent ability to compare our genome to that of our closest relative, the chimpanzee, provides new avenues to link genetic and phenotypic changes in the evolution of the human brain.

Methods: A ranking of regions in the human genome that show significant evolutionary acceleration was devised.

Results: The most dramatic of these ‘human accelerated regions’, HAR1, is part of a novel RNA gene (*HAR1F*) that is expressed specifically in Cajal-Retzius neurons in the developing human neocortex from 7 to 19 gestational weeks, a crucial period for cortical neuron specification and migration. *HAR1F* is co-expressed with reelin, a product of Cajal-Retzius neurons that is of fundamental importance in specifying the six-layer structure of the human cortex.

Conclusions: HAR1 and the other human accelerated regions provide new candidates in the search for uniquely human biology.

The brains of humans and chimpanzees are anatomically not so different, except in scale. About 2 million years ago, the hominin brain began to enlarge until, in modern times, it has become about three times larger than that of chimpanzees, contributing to the cognitive differences between the two species. This is followed now by a study of the speed at which various human genome regions have changed in recent times, and 49 Highly Accelerated Regions include the HAR1-HAR49. HAR1 is part of an RNA gene that is expressed in the embryonic neocortex, associated with higher cognitive functions, and influences the migration of neurons. It has accrued 18 changes in sequence in this time, when only one or no substitutions would be expected to occur by chance. Interestingly, all but two of the most-accelerated regions lie outside protein-coding sequences – in the enigmatic ‘dark matter’ of the human genome (*Yearbook 2006*). HAR1F is also expressed in the ovary and testis of adult humans; indeed, sexual selection of genes expressed in these tissues has often driven unusual sequence changes. Rapid human-specific evolution, and particularly the evolution of behavioral traits, may thus be associated more with epigenetic fine-tuning of gene expression than with altering the molecular functions of their encoded proteins.

Central role of p53 in the suntan response and pathologic hyperpigmentation

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Background: UV-induced pigmentation (suntanning) requires induction of α -melanocyte-stimulating hormone (α -MSH) secretion by keratinocytes. α -MSH and other bioactive peptides are cleavage products of pro-opiomelanocortin (POMC).

Results: The authors provide biochemical and genetic evidence demonstrating that UV induction of POMC/MSH in skin is directly controlled by p53. Whereas p53 potently stimulates the POMC promoter in response to UV, the absence of p53, as in knockout mice, is associated with absence of the UV-tanning response. The same pathway produces β -endorphin, another POMC derivative, which potentially contributes to sun-seeking behaviors. Furthermore, several instances of UV-independent pathologic pigmentation are shown to involve p53 ‘mimicking’ the tanning response. p53 thus functions as a sensor/effector for UV pigmentation, which is a nearly constant environmental exposure. Moreover, this pathway is activated in numerous conditions of pathologic pigmentation and thus mimics the tanning response.

Pigmentation of the skin results from the synthesis of melanin in the melanocyte, followed by distribution and transport of pigment granules to neighboring keratinocytes. Melanin is crucial for absorption of free radicals that have been generated within the cytoplasm by UV, and it acts as a direct shield protecting the DNA from UV and visible light radiation. It also prevents UV from its target vitamin D photosynthesis, and hence black-skin children are prone to develop rickets. This paper shows that the tumor suppressor gene p53, which ensures that DNA damage is detected early on and is mutated in 50% of human cancers, plays an important role in melanin generation, and that p53 absence ablates the tanning response, which endocrinologists call hyperpigmentation. UV light activates p53 in skin keratinocyte, which among others activates POMC, α -MSH and ACTH expression in the keratinocyte, which might involve primarily local paracrine, or perhaps autocrine, effects within the epidermis. The authors suggest that one of POMC end-products β -endorphin might contribute to sun-seeking behavior. We can at the time only speculate that multiple instances of clinical hyperpigmentation may arise due to such p53-mediated mimicking of the UV-pigmentation pathway. Previous dogma claimed that the entire spectrum of skin color is caused by polymorphism of the

MC4R to provide selective advantages at distinct latitudes based upon regulation of the UV-induced pigmentation response. It is possible that polymorphisms in p53 or p53-related pathways may also play a role.

Of drivers and passengers in a cancer cell

Patterns of somatic mutation in human cancer genomes

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Context: Cancers arise owing to mutations in a subset of genes that confer growth advantage. The availability of the human genome sequence led the authors to propose that systematic resequencing of cancer genomes for mutations would lead to the discovery of many additional cancer genes.

Results: More than 1,000 somatic mutations found in 274Mb of DNA corresponding to the coding exons of 518 protein kinase genes in 210 diverse human cancers are reported. There was substantial variation in the number and pattern of mutations in individual cancers reflecting different exposures, DNA repair defects and cellular origins. Most somatic mutations are likely to be 'passengers' that do not contribute to oncogenesis. However, there was evidence for 'driver' mutations contributing to the development of the cancers studied in approximately 120 genes.

Conclusions: Systematic sequencing of cancer genomes therefore reveals the evolutionary diversity of cancers and implicates a larger repertoire of cancer genes than previously anticipated.

The Human Cancer Genome Project starts to provide us with important data. This paper reports mutations in the genes encoding all 518 protein kinases in 210 cancers, identifying 1,000 mutations, most of them novel. Mutations were relatively common in cancers of the lung, stomach, ovary, colon and kidney, and rare in cancers of the testis and breast, and in carcinoid tumors. Cancer provides an amazing evolutionary laboratory; by the time it is diagnosed, it comprises billions of cells carrying the DNA abnormalities that initiated malignant proliferation, but also many additional genetic lesions acquired along the way. Some of these secondary mutations emerge owing to selective pressure during tumorigenesis (drivers); others may be incidental (passengers), resulting from mutational exposures, genome instability or simply the large number of cell doublings that leads from a single transformed cell to a clinically detectable cancer. Using a statistical model, 158 predicted driver mutations were identified in 120 kinase genes. It is likely that the full range of somatic mutation patterns will not be apparent until thousands of cancer samples have been sequenced, each one yielding several dozen mutations each. For some cancers this may require sequencing of hundreds of megabases. This information will ultimately provide new opportunities for molecular diagnosis and therapeutics.

Sequencing and analysis of Neanderthal genomic DNA

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Science 2006;314:1113–1118

Background: Our knowledge of Neanderthals is based on a limited number of remains and artifacts from which we must make inferences about their biology, behavior, and relationship to ourselves.

Results: The authors describe the characterization of these extinct hominids from a new perspective, based on the development of a Neanderthal metagenomic library and its high-throughput sequencing and analysis. Several lines of evidence indicate that the 65,250 base pairs of hominid sequence so far identified in the library are of Neanderthal origin, the strongest being the ascertainment of sequence identities between Neanderthal and chimpanzee at sites where the human genomic sequence is different. These results enabled to calculate the human-Neanderthal divergence time based on multiple randomly distributed autosomal loci. These analyses suggest that on average the Neanderthal genomic sequence obtained and the reference human genome sequence share a most recent common ancestor approximately 706,000 years ago, and that the human and Neanderthal ancestral populations split approximately 370,000 years ago, before the emergence of anatomically modern humans. The finding that the Neanderthal and human genomes are at least 99.5% identical led to develop and successfully implement a targeted method for recovering specific ancient DNA sequences from metagenomic libraries.

Conclusion: This initial analysis of the Neanderthal genome advances our understanding of the evolutionary relationship of *Homo sapiens* and *Homo neanderthalensis* and signifies the dawn of Neanderthal genomics.

Modern humans and Neanderthals overlapped in Europe and West Asia for at least a few thousand years, and perhaps up to 10,000 years as of ~35,000 years ago; some researchers continue to argue that the two species interbred. We now get a genomic support for the view that Neanderthals are a separate branch of the hominid family tree that diverged from our own ancestors some 450,000 years ago or more. Ancient DNA is problematic, and the divergence between living people and Neanderthals is so small compared to the DNA damage and the sequencing error that it's still hard to be confident of any results. Comparing the new sequences to the modern human genome and to the chimpanzee genome and tallied the sequence differences between each pair of species, the two humans are signposts to changes that were key to their individual evolution. Eventually those changes could lead researchers to the genetic basis of *Homo sapiens* speciation. As expected, the Neanderthal and human genomes proved more than 99.5% identical, 27 bases varied between modern humans and Neanderthals, indicating sites where evolution occurred after the two species diverged. They conclude that the most recent common ancestor of the two human species lived 468,000 to 1 million years ago. As to the question of admixture, there were no sites where the Neanderthal possessed a rare SN found only in Europeans, which one would expect had interbreeding occurred. In parallel, a Leipzig group identified a 38,000-year-old Neanderthal fossil that was exceptionally free of contamination from modern human DNA [2]. Comparing with the human and chimpanzee genomes, they suggest that modern human and Neanderthal DNA sequences diverged on average about 500,000 years ago, even though evidence still suggests that modern human males might have invaded the Neanderthal gene pool by sometimes fathering children with Neanderthal females, but not necessarily vice versa. Because the extinct Neanderthals are our closest relatives, comparing their DNA to ours may one day reveal the mutations that set *Homo sapiens* apart from all other species, as well as the timing of key evolutionary changes.

Comparative genome sequencing of *Escherichia coli* allows observation of bacterial evolution on a laboratory timescale

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Methods: Whole-genome resequencing of *Escherichia coli* was used to monitor the acquisition and fixation of mutations that conveyed a selective growth advantage during adaptation to a glycerol-based growth medium.

Results: Thirteen different de novo mutations in five different *E. coli* strains were identified and their fixation was monitored over a 44-day period of adaptation. The observed spontaneous mutations were responsible for improved fitness by creating single, double and triple site-directed mutants that had growth rates matching those of the evolved strains.

Conclusions: The success of this new genome-scale approach indicates that real-time evolution studies will now be practical in a wide variety of contexts.

Paleontologists look at the fossil record to study how evolution of dinosaurs and other animals occurred over millions of years, but in the case of the *E. coli*, new technology provides the ability to observe evolution as it is occurring over a matter of days. Using comparative genome sequencing technology, they grew *E. coli* in an environment that favored the emergence of mutants: the organism was fed glycerol, a poorly metabolized carbon and energy source. After 6 days of growth, mutations appeared in the glycerol kinase gene, which initiates the process of metabolizing glycerol. Cells with mutations grew 20–60% faster. Surprisingly, mutations also appeared in a second, unrelated gene for RNA polymerase, and these bacteria grew 150% faster than the starting strain. All the mutants arose in the experiments presumably as the result of naturally occurring errors in copying DNA into daughter cells during cell division. ‘Experimental evolution’ may refine the theory of evolution and the related process of natural selection of individuals with traits that convey a selective survival advantage. It may also be used as a tool for discovery and analysis, and could even be used to design bacteria to do useful jobs. A month later, and a second team developed a model for experimental evolution on a chip ‘fitness landscape’ [3]. They made a row of microscopic chambers on a silicon chip in which *E. coli* may grow. The bacteria can move between the chambers along narrow corridors. Adjusting the nutrient supply to each of these patches creates gradients in habitability, representing a fitness landscape that can be made arbitrarily ‘smooth’ or ‘rugged’.

Designed to kill

The second most prestigious scientific prize, the *Albert Lasker Award for Basic Medical Research*, was shared in 2006 by Elizabeth H. Blackburn, Carol W. Greider and Jack W. Szostak ‘for the prediction and discovery of telomerase, a remarkable RNA-containing enzyme that synthesizes the ends of chromosomes, protecting them and maintaining the integrity of the genome’.

Telomeres and telomerase are critical regulators of genomic stability and replicative lifespan in mammalian cells. Here is a shortlist of where to expect the utilization of telomeres and telomerase in pediatric endocrinology? Growth plate senescence and fusion: in vitro studies showed that chronic oxidative stress caused by repeated exposure to peroxide, or superphysiologic oxygen tension caused growth plate chondrocytes to senesce prematurely and before extensive telomere erosion occurred [4]. The presence of telomerase activity within primary endocrine tumors was shown to indicate a malignant tumor and might suggest the need for an attentive search for concomitant metastases. Quantification of human telomerase reverse transcriptase protein subunit mRNA could be used in clinical practice to exclude malignancy in most endocrine tumors [5]. Telomerase may soon become a drug and drug target for the treatment of thyroid cancer.

Transforming the architecture of compound eyes

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Background: Eyes differ markedly in the animal kingdom, and are an extreme example of the evolution of multiple anatomical solutions to light detection and image formation. A salient feature of all photoreceptor cells is the presence of a specialized compartment (disc outer segments in vertebrates, and microvillar rhabdomeres in insects), whose primary role is to accommodate the millions of light receptor molecules required for efficient photon collection. In insects, compound eyes can have very different inner architectures. Fruitflies and houseflies have an open rhabdom system, in which the seven rhabdomeres of each ommatidium are separated from each other and function as independent light guides. In contrast, bees and various mosquitoes and beetle species have a closed system, in which rhabdomeres within each ommatidium are fused to each other, thus sharing the same visual axis.

Methods: To understand the transition between open and closed rhabdom systems, the role of *Drosophila* genes involved in rhabdomere assembly were isolated and characterized.

Results: Spacemaker, a secreted protein expressed only in the eyes of insects with open rhabdom systems, acts together with prominin and the cell adhesion molecule chaoptin to choreograph the partitioning of rhabdomeres into an open system. Furthermore, the complete loss of spacemaker converts an open rhabdom system to a closed one, whereas its targeted expression to photoreceptors of a closed system markedly reorganizes the architecture of the compound eyes to resemble an open system.

Conclusions: These results provide a molecular atlas for the construction of microvillar assemblies and illustrate the critical effect of differences in a single structural protein in morphogenesis.

When evolution skeptics want to attack Darwin's theory, they often point to the human eye. How could something so complex, they argue, have developed through random mutations and natural selection, even over millions of years? If evolution occurs through gradations, how could it have created the separate parts of the eye – the lens, the retina, the pupil, and so forth – since none of these structures by themselves would make vision possible? Darwin acknowledged from the start that the eye would be a difficult case for his new theory to explain. Building on this enigma, Richard Dawkins' *The Blind Watchmaker* has been acclaimed as the most influential work on evolution written in the last hundred years, and is highly recommended and enjoyable reading. It is not often that we get such a clear glimpse of the blind watchmaker at work as in this article. We learn that contemporary flies see better than their ancestors. Those ancestral flies that did not see well contributed more to the evolution of frogs than to the fly lineage. Zelhof et al. reveal how flies improved the resolution of their eyes by evolving a specific modification to how their photoreceptor cells stick together. You may be interested to know that the mechanism utilized in insect vision has very little to do with the mechanism we operate.

Small is beautiful

Organization of interphase microtubules in fission yeast analyzed by electron tomography

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Dev Cell 2007;12:349–361

Background: Polarized cells, such as neuronal, epithelial, and fungal cells, all display a specialized organization of their microtubules. The interphase microtubule cytoskeleton of the rod-shaped fission yeast, *Schizosaccharomyces pombe*, has been extensively described by fluorescence microscopy.

Methods: A large-scale, electron tomography investigation of *S. pombe* is described, including a 3D reconstruction of a complete eukaryotic cell volume at sufficient resolution to show both how many microtubules there are in a bundle and their detailed architecture.

Results: Most cytoplasmic microtubules are open at one end and capped at the other, providing evidence about their polarity. Electron-dense bridges between the microtubules themselves and between microtubules and the nuclear envelope were frequently observed. Finally, we have investigated structure/function relationships between microtubules and both mitochondria and vesicles.

Conclusions: Our analysis shows that electron tomography of well-preserved cells is ideally suited for describing fine ultrastructural details that were not visible with previous techniques.

In your future teaching, you will probably use these high-resolution, three-dimensional images of a cell developed this year by electron tomography. They scanned 250-nm-thick slices of frozen budding yeast, and assembled the scans into a computer-generated three-dimensional image. Save the spectacular photos. For more on far-field fluorescence microscopy with a focal-plane resolution of 15–20 nm in biological samples, see Donnert et al. [6].

Three boat lengths to the line

Increased App expression in a mouse model of Down's syndrome disrupts NGF transport and causes cholinergic neuron degeneration

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Background: Degeneration of basal forebrain cholinergic neurons (BFCNs) contributes to cognitive dysfunction in Alzheimer's disease and Down's syndrome.

Methods: This paper used Ts65Dn and Ts1Cje mouse models of Down's syndrome.

Results: The increased dose of the amyloid precursor protein gene, App, acts to markedly decrease NGF retrograde transport and cause degeneration of BFCNs. NGF transport was also decreased in mice expressing wild-type human APP or a familial Alzheimer-linked mutant APP; while significant, the decreases were less marked and there was no evident degeneration of BFCNs. Because of evidence suggesting that the NGF transport defect was intra-axonal, they explored within cholinergic axons the status of early endosomes. NGF-containing early endosomes were enlarged in Ts65Dn mice and their App content was increased.

Conclusion: This study provides evidence for a pathogenic mechanism for Down's syndrome in which increased expression of App, in the context of trisomy, causes abnormal transport of NGF and cholinergic neurodegeneration.

Intensive efforts over the last 3 years may finally lead to understanding of the enigma how a triple dose of chromosome 21 leads to the mental retardation of Down's syndrome. The neurotrophin, nerve growth factor (NGF), needs to be transported from the axon terminal to the neuronal cell body in order to promote cell survival. Following the development of an animal model of Down syndrome (Yearbook 2006), this paper shows that the retrograde transport of NGF malfunctions in a mouse model of Down syndrome results in neuronal death. The factor responsible for this deficit in NGF transport is amyloid precursor protein (APP), which is encoded by one of the genes triplicated in Down syndrome. A second article in the same journal [7] reports defects in the neurotrophin BDNF (brain-derived neurotrophic factor), which may prevent BDNF from driving neuronal survival in the cortex and hippocampus. Downregulation of the truncated BDNF receptor in the Down syndrome model restores BDNF signaling and reverses neuronal loss. Mechanisms provide promises for therapy; restoration of the neurotrophin signaling in the brain may be a promising therapeutic approach in

Down syndrome. From a pediatric endocrine angle, the abnormal thyroid functions seem to be an inherent feature of the trisomy rather than a problem in a subset of patients [8].

Fair trade-offs

Costs of encephalization: the energy trade-off hypothesis tested on birds

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Background: Costs and benefits of encephalization are a major topic of debate in the study of primate and human evolution. Comparative studies provide an opportunity to test the validity of a hypothesis as a general principle, rather than it being a special case in primate or hominid evolution. If a population evolves a larger brain, the metabolic costs of doing so must be paid for by either an increased energy turnover (direct metabolic constraint) or by a trade-off with other energetically expensive costs of body maintenance, locomotion, or reproduction, here referred to as the energy trade-off hypothesis, an extension of the influential *Expensive Tissue Hypothesis* of Aiello and Wheeler [Curr Anthropol 1995;36:199–221].

Methods: The authors tested these hypotheses on birds using raw species values, family means, and independent contrasts analysis to account for phylogenetic influences. First, we tested whether basal metabolic rates are correlated with brain mass or any other variable of interest.

Results: This not being the case, we examined various trade-offs between brain mass and the mass of other expensive tissues such as gut mass, which is approximated by gut length or diet quality. Only weak support was found for this original *Expensive Tissue Hypothesis* in birds. However, other energy allocations such as locomotor mode and reproductive strategy may also be reduced to shunt energy to an enlarged brain. There was a significantly negative correlation between brain mass and pectoral muscle mass, which averages 18% of body mass in birds and is indicative of their relative costs of flight. Reproductive costs, on the other hand, are positively correlated with brain mass in birds. An increase in brain mass may allow birds to devote more energy to reproduction, although not through an increase in their own energy budget as in mammals, but through direct provisioning of their offspring.

Conclusions: The trade-off between locomotor costs and brain mass in birds lets the authors conclude that an analogous effect could have played a role in the evolution of a larger brain in human evolution.

Ever since Darwin, anthropologists have been intrigued by the dramatic contrast in relative brain size between humans and our great ape relatives. As it goes for evolutionary theory, the costs of an enlarged brain, as ultimately only net benefit, i.e., benefits minus costs, will determine the selective advantage of a trait. The 1980s hypothesis that stressed the energetic costs of increased brain size rather than its adaptive benefits was the direct metabolic constraints hypothesis. A direct link between basal metabolic rate and brain size was postulated based on a similarity of scaling exponents of these parameters to body size. In the 1990s it was the dietary shift toward meat eating in hominins, which is likely to have occurred roughly at the same time as the major enlargement in brain size. As proposed by the *Expensive Tissue Hypothesis*, an animal is able to meet the high metabolic cost of a large brain without incurring a compensatory increase in basal metabolic rate by decreasing the amount of other metabolically expensive tissues (i.e., heart, lung, kidney, liver, and gastrointestinal tract). We now learn of a significantly negative correlation between brain mass and pectoral muscle mass, which averages 18% of body mass in birds and is indicative of their relative costs of flight. This is birds, but the trade-off between locomotor costs and brain mass suggests that an analogous effect could have played a role in the evolution of a larger brain in human evolution.

Compensatory growth impairs adult cognitive performance

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Background: Several studies have demonstrated that poor early nutrition, followed by growth compensation, can have negative consequences later in life. However, it remains unclear whether this is attributable to the nutritional deficit itself or a cost of compensatory growth. This distinction is important to understand both of the proximate and ultimate factors that shape growth trajectories and of how best to manage growth in humans and other species following low birth weight.

Methods: Sibling pairs of zebra finches were reared on different quality nutrition for the first 20 days of life only and their learning performance examined in adulthood.

Results: Final body size was not affected. However, the speed of learning a simple task in adulthood, which involved associating a screen color with the presence of a food reward, was negatively related to the amount of growth compensation that had occurred. Learning speed was not related to the early diet itself or the amount of early growth depression.

Conclusions: The results show that the level of compensatory growth that occurs following a period of poor nutrition is associated with long-term negative consequences for cognitive function and suggest that a growth-performance trade-off may determine optimal growth trajectories.

Although we are all familiar with the concept of catch-up growth, we have to realize that its mechanisms and regulators are poorly understood. The recognition that a growing organism can transiently grow much more rapidly to catch-up implies that the mean growth of a population is not maximized but selected by evolution as a trade-off between too fast and too slow. We knew of the negative role of early catch-up on subsequent cardiovascular risk. We also knew that poor development in early life affected subsequent intelligence. This paper addresses the influence of catch-up itself on cognitive performances in birds. Pairs of birds received either a normal diet or a deficient diet during the 20 days after hatching, resulting in a mean weight deficit of 14%. By day 70, all birds were fully grown and the deficient ones had caught up vs. their normally fed pairs. The influence of the marked individual variability of catch-down and catch-up growth on adult cognition was analyzed. There was no influence of the rate of catch-down, but a marked influence of catch-up growth with the animals growing more rapidly performing more poorly in adulthood. This suggests that the prioritization of central nervous system during development is variable between individuals. Some individuals might commit more energy into somatic growth, therefore compromising central nervous system development further. The mechanisms involved are unknown but could involve behavioral, endocrine or neurological changes. Although this is an experimental study in birds that does not apply to humans, it would be interesting to revisit the studies linking birth weight and cognition in the light of these findings. These data suggest that rapid catch-up might not be the best option in the long term.

Lipoprotein genotype and conserved pathway for exceptional longevity in humans

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Background: Alteration of single genes involved in nutrient and lipoprotein metabolism increases longevity in several animal models. Because exceptional longevity in humans is familial, it is likely that polymorphisms in genes favorably influence certain phenotypes and increase the likelihood of exceptional longevity.

Methods: A group of Ashkenazi Jewish centenarians ($n = 213$), their offspring ($n = 216$), and an age-matched Ashkenazi control group ($n = 258$) were genotyped for 66 polymorphisms in 36 candidate genes related to cardiovascular disease. These genes were tested for association with serum lipoprotein levels and particle sizes, apolipoprotein A1, B, and C-3 levels and with outcomes of hypertension, insulin resistance, and mortality.

Results: The prevalence of homozygosity for the 641C allele in the APOC3 promoter (rs2542052) was higher in centenarians (25%) and their offspring (20%) than in controls (10%) ($p = 0.0001$ and $p = 0.001$, respectively). This genotype was associated with significantly lower serum levels of APOC3 and a favorable pattern of lipoprotein levels and sizes. There was a lower prevalence of hypertension and greater insulin sensitivity in the 641C homozygotes, suggesting a protective effect against cardiovascular disease and the metabolic syndrome. Finally, in a prospectively studied cohort, a significant survival advantage was demonstrated in those with the favorable 641C homozygote ($p < 0.0001$).

Conclusion: Homozygosity for the APOC3 641C allele is associated with a favorable lipoprotein profile, cardiovascular health, insulin sensitivity, and longevity. Because modulation of lipoproteins is also seen in genetically altered longevity models, it may be a common pathway influencing lifespan from nematodes to humans.

Resveratrol improves health and survival of mice on a high-calorie diet

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Background: Resveratrol (3,5,49-trihydroxystilbene) extends the lifespan of diverse species including *Saccharomyces cerevisiae*, *Caenorhabditis elegans* and *Drosophila melanogaster*. In these organisms, lifespan extension is dependent on Sir2, a conserved deacetylase proposed to underlie the beneficial effects of caloric restriction.

Results: The authors show that resveratrol shifts the physiology of middle-aged mice on a high-calorie diet towards that of mice on a standard diet and significantly increases their survival. Resveratrol produces changes associated with longer lifespan, including increased insulin sensitivity, reduced insulin-like growth factor-1 levels, increased AMP-activated protein kinase and peroxisome proliferator-activated receptor- α coactivator 1 α activity, increased mitochondrial number, and improved motor function. Parametric analysis of gene set enrichment revealed that resveratrol opposed the effects of the high-calorie diet in 144 out of 153 significantly altered pathways.

Conclusion: These data show that improving general health in mammals using small molecules is an attainable goal, and point to new approaches for treating obesity-related disorders and diseases of aging.

Longevity research is a hot topic these days and uses several approaches with two examples here, using classical genetic association studies and high throughput screening of chemicals affecting relevant pathways. In the paper by Atzmon et al., relevant gene variants were compared in a population of centenarians from the Ashkenazi Jewish community and in relevant controls. 25% of the centenarians

vs. 10% of the control were homozygous for the 641C allele in the APOC3 promoter, associated with lower APOC3 levels and more favorable lipoprotein profiles. The frequency of homozygosity for the 641C allele increased with age from 65 to 100 years and survival after the age of 75 was associated with the genotype. The APOC3 is a major component of very low density lipoproteins and chylomicron remnants and this study confirms the influence of lipoprotein metabolism on longevity in humans. Although the association does not prove causality and the data needs to be replicated in other ethnic groups, there is a strong case for the role of APOC3 in the factors affecting longevity. The second paper suggests a pharmacological approach to longevity and illustrates how new drugs are discovered, based on systematic targeting of relevant pathways. The sirtuin pathway has been identified as a mediator of diet-induced increased longevity in several species. Of the 7 sirtuin genes in mammals, SIRT1 regulates metabolism and cell survival and was the target of a systematic search of 20,000 molecules that enhance its activity. Resveratrol, a molecule produced by plants in response to stress, emerged as the most potent and was evaluated in simple organisms. The present paper reports its use in mice fed a high-fat diet. Resveratrol prevented body weight gain, increased survival and insulin sensitivity. Microarray analysis was used to identify the pathways targeted by the drug and identified Cidea, a regulator of energy balance in brown fat as a downregulated gene. Remarkably, resveratrol is a small molecule that can be administered orally that did not seem to prove toxicity during the limited studies represented here. Whether this is the next wonder drug capable of alleviating the negative effect of overfeeding in western societies will become apparent in the years to come.

New hopes. Prolactin, a heartbreaking hormone

A cathepsin D-cleaved 16 kDa form of prolactin mediates postpartum cardiomyopathy

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Background: Postpartum cardiomyopathy is a disease of unknown etiology and exposes women to a high risk of mortality after delivery.

Results: Female mice with a cardiomyocyte-specific deletion of STAT3 develop postpartum cardiomyopathy. In these mice, cardiac cathepsin D expression and activity is enhanced and associated with the generation of a cleaved antiangiogenic and proapoptotic 16 kDa form of the nursing hormone prolactin. Treatment with bromocriptine, an inhibitor of prolactin secretion, prevents the development of postpartum cardiomyopathy, whereas forced myocardial generation of 16 kDa prolactin impairs the cardiac capillary network and function, thereby recapitulating the cardiac phenotype of postpartum cardiomyopathy. Myocardial STAT3 protein levels are reduced and serum levels of activated cathepsin D and 16 kDa prolactin are elevated in postpartum cardiomyopathy patients.

Conclusion: A biologically active derivative of the pregnancy hormone prolactin mediates postpartum cardiomyopathy, implying that inhibition of prolactin release may represent a novel therapeutic strategy for postpartum cardiomyopathy.

This paper not only provides the mechanistic basis and the hope for an effective disease of a deadly disease, but is a great example of how careful hypothesis-driven observations can lead to important discoveries. The investigators made the hypothesis that oxidative stress and apoptosis were involved in postpartum cardiomyopathy and that STAT3 was involved in heart protection. This proved correct using a mouse model with targeted deletion of stat3 in the heart. The role of prolactin in pregnancy and postpartum as well as its angiogenic properties made this hormone an interesting candidate in the disease. Indeed, 23 kDa prolactin (the one we know as endocrinologists) is cleaved into a 16 kDa fragment which exerts antiangiogenic properties. Several experiments led to identify this 16 kDa

prolactin as a key player in postpartum cardiomyopathy. Moreover, a preliminary trial of bromocriptine to reduce prolactin levels in 6 women at high risk of postpartum cardiomyopathy improved heart function, as compared to 6 untreated women of whom 3 died within a few months after delivery. Whether prolactin is the only mediator and whether defective STAT3 function is associated with postpartum cardiomyopathy in humans remains to be determined. Large-scale trials of dopaminergic agents will represent hope for women who were so far counseled not to have additional pregnancies when they had survived postpartum cardiomyopathy [9].

New hopes. The origins of thymic epithelial cells

Clonal analysis reveals a common progenitor for thymic cortical and medullary epithelium

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Background: The thymus provides an essential environment for the development of T cells from hemopoietic progenitors. This environment is separated into cortical and medullary regions, each containing functionally distinct epithelial populations that are important at successive stages of T-cell development and selection. However, the developmental origin and lineage relationships between cortical and medullary epithelial cell types remain controversial.

Results: The authors describe a clonal assay to investigate the developmental potential of single, individually selected, thymic epithelial progenitors (marked with enhanced yellow fluorescent protein) developing within the normal architecture of the thymus. Using this approach, it is shown that cortical and medullary epithelial cells share a common origin in bipotent precursors, providing definitive evidence that they have a single rather than dual germ layer origin during embryogenesis.

Conclusions: These findings resolve a long-standing issue in thymus development, and are important in relation to the development of cell-based strategies for thymus disorders and the possibility of restoring function of the atrophied adult thymus.

Formation of a functional thymus initiated by a postnatal epithelial progenitor cell

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Background: The thymus is essential for the generation of self-tolerant effector and regulatory T cells. Intrathymic T-cell development requires an intact stromal microenvironment, of which thymic epithelial cells constitute a major part. For instance, cell-autonomous genetic defects of forkhead box N1 (Foxn1) and autoimmune regulator (Aire) in thymic epithelial cells cause primary immunodeficiency and autoimmunity, respectively. During development, the thymic epithelial rudiment gives rise to two major compartments, the cortex and medulla. Cortical thymic epithelial cells positively select T cells, whereas medullary thymic epithelial cells are involved in negative selection of potentially autoreactive T cells. It has long been unclear whether these two morphologically and functionally distinct types of epithelial cells arise from a common bipotent progenitor cell and whether such progenitors are still present in the postnatal period.

Results: Using in vivo cell lineage analysis in mice, the presence of a common progenitor of cortical and medullary thymic epithelial cells after birth is demonstrated. To probe the function of postnatal progenitors, a conditional mutant allele of Foxn1 was reverted to wild-type function in single epithelial cells in vivo. This led to the formation of small thymic lobules containing both cortical and medullary areas that supported normal thymopoiesis.

Conclusions: Thus, single epithelial progenitor cells can give rise to a complete and functional thymic microenvironment, suggesting that cell-based therapies could be developed for thymus disorders.

The thymus is the central organ of the immune system and the source of mature T cells that fight infections. It is composed of developing thymocytes derived from the bone marrow and of a back-bone thymic epithelium. This epithelium is subdivided into the cortex and the medulla whose function are well identified and distinct. The cortex is responsible for the positive selection of T cells (i.e. the capacity to function in a major histocompatibility complex restricted fashion) and the medulla is in charge of purging the forming T cells from those that are autoreactive to self. The Aire gene is involved in the expression of peripheral self antigens in medullary thymic epithelial cells [10] and a defect in medullary thymic epithelial cells proinsulin expression plays a role in type 1 diabetes [11]. Although the functions and morphological characteristics of thymic epithelial cells are well known, their developmental pattern was unclear, with some believing that they had different (endodermal and ectodermal) origin and others that both types came from the endoderm. The two studies discussed here show that a single progenitor cell can give rise to both types of epithelial cells and that these cells exist in the adult thymus. The importance of these findings is not only theoretical since several human diseases result from thymic dysfunction. Rare immunodeficiencies can result from a loss of function of the FOXP1 gene, known in the mouse to result in the 'nude' phenotype (alopecia and deficient T cells). The data suggest that expressing FOXP1 in thymic precursors of affected patients will result in immune reconstitution. Beyond this rare disease, these findings open the way to manipulation of the thymus in autoimmune diseases, currently the only hope of re-establishing self tolerance.

Concerns. Drug safety monitoring

The FDA and drug safety: a proposal for sweeping changes

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Background: The current Food and Drug Administration (FDA) system of regulating drug safety has serious limitations and is in need of changes. The major problems include the following: the design of initial preapproval studies lets uncommon, serious adverse events go undetected; massive underreporting of adverse events to the FDA postmarketing surveillance system reduces the ability to quantify risk accurately; manufacturers do not fulfill the majority of their postmarketing safety study commitments; the FDA lacks authority to pursue sponsors who violate regulations and ignore postmarketing safety study commitments; the public increasingly perceives the FDA as having become too close to the regulated pharmaceutical industry; the FDA's safety oversight structure is suboptimal, and the FDA's expertise and resources in drug safety and public health are limited.

Conclusions: To address these problems, we urge Congress, which is ultimately responsible for the FDA's performance, to implement the following five recommendations: (1) give the FDA more direct legal authority to pursue violations, (2) authorize the adoption of a conditional drug approval policy, at least for selected drugs, (3) provide additional financial resources to support the safety operations, (4) mandate a reorganization of the agency with emphasis on strengthening the evaluation and proactive monitoring of drug safety, and (5) require broader representation of safety experts on the FDA's advisory committees.

Although this paper deals with US mechanisms of drug approval and safety surveillance, the issues discussed are valid worldwide and reflect the intrinsic difficulty of improving the health of the population with active drugs while avoiding adverse events due to these drugs. There is therefore a difficult balance between releasing a potentially lifesaving or useful drug and the data needed to have a full assessment of the risks of this drug. This is particularly true for rare events occurring late after the

drug is started. The paper lists six major problems with the current system of drug safety surveillance, including (1) that serious adverse events can be undetected during the approval process, (2) that only 10% of serious adverse events are reported, (3) that the threshold for action when an adverse event is detected is subjective and therefore variable, (4) that the FDA does not control the performance of postmarketing studies, (5) that the FDA (and other agencies) have a conflict of interest since it is both involved in drug approval and in safety actions if adverse events arise, and (6) that the FDA has a shortage of expertise in drug safety. The authors make several recommendations indicated above. This report reminds us that we have to balance our actions in the best interest of our patients and that we have to stay vigilant for adverse events in our daily practice.

Food for thought

A non-invasive test for prenatal diagnosis based on fetal DNA present in maternal blood: a preliminary study

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Background: Use of free fetal DNA to diagnose fetal chromosomal abnormalities has been hindered by the inability to distinguish fetal DNA from maternal DNA. The aim of this study was to establish whether single nucleotide polymorphisms (SNPs) can be used to distinguish fetal DNA from maternal DNA – and to determine the number of fetal chromosomes – in maternal blood samples.

Methods: Formaldehyde-treated blood samples from 60 pregnant women and the stated biological fathers were analyzed. Maternal plasma fractions were quantified at multiple SNPs, and the ratio of the unique fetal allele signal to the combined maternal and fetal allele signal calculated. The mean ratios of SNPs on chromosomes 13 and 21 were compared to test for potential fetal chromosomal abnormalities.

Results: The mean proportion of free fetal DNA was 34.0% (median 32.5%, range 17.0–93.8). Three samples with significant differences in the fetal DNA ratios for chromosome 13 and chromosome 21, indicative of trisomy 21, were identified; the remaining 57 samples were deemed to be normal. Amniocentesis or newborn reports from the clinical sites confirmed that the copy number of fetal chromosomes 13 and 21 was established correctly for 58 of the 60 samples, identifying 56 of the 57 normal samples, and two of the three trisomy-21 samples. Of the incorrectly identified samples, one was a false negative and one was a false positive. The sensitivity and positive predictive value were both 66.7% and the specificity and negative predictive values were both 98.2%.

Conclusions: The copy number of chromosomes of interest can be directly established from maternal plasma. Such a non-invasive prenatal test could provide a useful complement to currently used screening tests.

Non-invasive techniques are the Holy Grail of prenatal diagnosis and are already used for the diagnosis of sex in specialized laboratories and for specific indications (for instance for fetuses at risk of congenital adrenal hyperplasia). Non-invasive techniques for the diagnosis of fetal aneuploidies would alleviate the risk associated with amniocentesis and would pave the way for non-invasive techniques for the diagnosis of monogenic disorders. This study is based on the isolation of free fetal DNA in the maternal plasma and uses the ratio of the paternal fetal allele to the maternal alleles as an indicator of the number of chromosomes 21 and 13. As such the study is preliminary and only 2 of 3 fetuses with abnormalities were detected. In another study, based on the analysis of circulating placental RNA, fetal trisomy 21 was detected non-invasively in 90% of cases and excluded in 96.5% of controls [12]. Parents and health providers will be faced with more ethical problems as technology progresses in the future and will have to adapt to these new challenges.

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