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From Genome to Gene: Causality, Synthesis, and Evolution

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Abstracts

(By Author)

Functional Characterization of Gene Regulatory Elements of Epilepsy-Associated Genes
Ramon Birnbaum (Ben-Gurion University of the Negev, Israel)

Epilepsy is a complex and heterogeneous disease making it difficult to precisely diagnose and provide effective treatments. Infantile spasms (IS) is an uncommon epilepsy syndrome that typically begins in infancy and is associated with ventral forebrain development and forebrain synapse function. Since a major cause of complex diseases, such as epilepsy, could be mutations in gene regulatory elements, we set out to identify these elements in the mouse forebrain at embryonic day 16.5 (E16.5) which could be associated with IS. Using chromatin immunoprecipitation followed by deep sequencing (ChIP-Seq) with enhancer marks (H3K27ac, p300, RNAPoll2), we identified active enhancer candidates in the mouse E16.5 forebrain. In addition, using chromatin interaction analysis followed by paired-end tag sequencing (ChIA-PET) on mouse E16.5 forebrain we are determining the physical interactions of these enhancer candidates and IS-associated genes. Several enhancer candidates are then tested for their enhancer activity using zebrafish transgenic enhancer assays. Our results provide a novel dataset of neuronal enhancers that could control the spatiotemporal expression of IS-associated genes. These sequences pose as great candidates for mutation analyses in IS patients. Combined, this work will shed light on neuronal gene regulation, in general, and identify novel genomic regions that could be involved in epilepsy pathogenesis and brain development.

Paleoepigenetics: Reconstructing the DNA Methylation Maps of Archaic Hominins
Liran Carmel (Hebrew University of Jerusalem, Israel)

Changes in gene activity patterns are important determinants of phenotypic differences between close lineages. However, the recent evolution of epigenetic regulation in humans, and its potential phenotypic effects, is largely unexplored. Advances in ancient DNA sequencing techniques have recently resulted in the sequencing of high-coverage archaic human genomes, providing an opportunity to study recent human evolution at the genomic level.

Here, we present a method to reconstruct genome-wide DNA methylation maps in high-coverage ancient genomes. To this end, we harness the asymmetry in the natural degradation processes of ancient DNA, where methylated cytosines are deaminated to thymines but unmethylated cytosines are deaminated to uracils.

We reconstruct the genome-wide DNA methylation maps of two archaic human groups: the Neanderthals and the Denisovans. Comparing these ancient methylation maps to those of present-day humans, we identified roughly 2000 differentially methylated regions (DMRs). Genes associated with DMRs have a tendency to be linked to diseases, and especially to neurological and psychiatric disorders. We identified substantial regulatory changes in the HOXD9 and HOXD10 genes that may explain some of the anatomical differences in limb morphology between archaic and present-day humans.

This work comprises the first DNA methylation map of an ancient genome, and provides the first insight to gene activity in archaic humans. Our method can be applied to any future high-coverage ancient genome, and thus opens a window to a new field – paleoepigenetics.

Long-range regulation during development and evolution: Back to preformation?
Denis Duboule (University of Geneva, Switzerland)

The emergence and evolution of digits was an essential step in the success of vertebrates. HoxD genes are amongst the key players in this developmental process and are coordinately regulated during digital development, following the principle of collinearity whereby the order of the genes along the chromosome reflects their place of transcription along the limb axis. We examined their long-range transcriptional regulation by using various biochemical and genetic approaches in vivo and showed that this enigmatic collinear relationship is, in fact, due to the asymmetric distribution of enhancer sequences flanking this gene cluster. We examined the similarities between this complex regulatory organization and that found during the development of the external genitals, another bud where HoxD genes play an essential function during fetal development and we show that an evolutionary hijacking of regulations occurred from one developing context to the other.

The Construction of Evolutionary Opportunity through Gene Regulation

Douglas Erwin (Smithsonian Institution, U.S.A.)

Ernst Mayr and GG Simpson, two of the primary theorists of the modern synthesis of evolution articulated a view of evolutionary innovation driven by ecological opportunity. They simply assumed that developmental innovations would be sufficiently frequent that their origin could be ignored. This view informed much of evolutionary biology until just the past decade. Recent developments have revealed far more about the complexity of reconfiguring the structure of developmental gene regulatory networks (dGRNs) for generating evolutionary novelty: the formation of novel individuated phenotypic structures. Comparative studies have revealed different patterns of genomic control of development and the evolutionary mechanisms responsible for these patterns, ranging from changes in the periphery of dGRNs associated with adaptive evolution between species to more fundamental regulatory changes responsible for differences in body plans. Such studies have also suggested that the distribution of GRN-based morphological novelty may be unevenly distributed with time. Integration of developmental and ecological approaches to understanding evolutionary novelty and innovation indicates the importance of developmental GRN evolution in constructing phenotypic spaces and evolutionary opportunity.

Building a Minimal Bacterial Cell by Global Design and Synthesis

John Glass (for members of the Venter Institute Synthetic Biology Group, J. Craig Venter Institute)

The minimal cell is the hydrogen atom of cellular biology. Such a cell, because of its simplicity and absence of redundancy would be a platform for investigating just what biological components are required for life, and how those parts work together to make a living cell. Since the late 1990s, our team at the Venter Institute has been developing a suite of synthetic biology tools that will enable us to build what previously has only been imagined, a minimal cell. Specifically, it will be a bacterial cell with a genome that expresses only the minimum set of genes needed for the cell to divide every two hours that can be grown in pure culture. The minimal cell we are building has about half of the genes that are in the bacterium Mycoplasma mycoides JCVI syn1.0, the so called synthetic bacteria we reported on in 2010. We used transposon bombardment to identify non-essential genes, and genes needed to maintain rapid growth in M. mycoides. Based on those data, we designed and synthesized a reduced genome in eight overlapping segments. All segments were individually viable when combined with wild type versions of the seven other segments. Combinations of reduced segments that were not viable allowed us to identify synthetic lethal pairs of genes. These occur when two genes each encode an essential function. Those findings required re-design and re-synthesis of some reduced genome segments. Three cycles of design, synthesis, and testing, with retention of quasi-essential genes, produced JCVI-syn3.0 (531 kb, 477

genes), which has a genome smaller than that of any autonomously replicating cell found in nature. JCVI-syn3.0 retains almost all genes involved in synthesis and processing of macromolecules. Surprisingly, it also contains 150 genes with unknown biological functions, suggesting the presence of undiscovered functions essential for life. This minimal cell is a versatile platform for investigating the core functions of life, and for exploring whole-genome design.

This work was supported by Synthetic Genomics, Inc., DARPA Living Foundries contract HR0011-12-C-0063, and the J. Craig Venter Institute.

3D Genome Regulation in Cell Differentiation and Response to Hormones
Ofir Hakim (Bar Ilan University, Israel)

Gene expression networks that determine tissue-specific identity and responses are highly organized within the nuclear space. Yet the molecular basis of genome organization and the causal link between its structure and function are poorly understood. We uncovered that cell-type specific genome architecture of T lymphocytes is acquired by a profound shift from promiscuous to highly selective genome organization that accompanies cell differentiation. Importantly, the chromosomal contacts of the specialized cell are retained and strengthened precisely at DNA binding sites of specific lineage-determining transcription factors (TFs), suggesting a critical role for spatial aggregation of binding loci of these TFs in specification of higher-order nuclear architecture.

Intriguingly, the preferential co-localized functionally-relevant genes, show complex regulation profiles in response to glucocorticoid hormone in relatively static sub-nuclear environments. However, higher resolution analysis revealed that chromosomal domains within these environments harbor genes with similar transcriptional response. By compiling a catalogue of regulatory elements associated with glucocorticoid-responsive genes by chromosomal looping we uncovered novel potential co-regulators of the transcriptional response to glucocorticoids. Thus, once established, subnuclear environments may support a variety of orchestrated series of regulatory actions by spatial clustering of chromosomal domains which are central for the correct physiological response of the specialized cell.

A trade-off between constraints on embryo geometry and regulatory genome evolution
Patrick Lemaire (Centre de Recherche de Biochimie Macromoléculaire, France)

We present a quantitative digital representation of the stereotyped embryogenesis in the ascidian Phallusia mammillata, between the cleavage and initial tailbud stages. This dataset gives access to the position, shape, divisions and contacts of 1304 cells with a 2-minute temporal resolution and across 671 cell divisions. We first used this dataset to show that the mitotic history of cells is

diagnostic of their cell fate and derived a map of cell specification events until the end of gastrulation from the comparison of the cell lineage trees of sister cells. To understand the molecular basis of these decisions, we integrated measures of cell volumes, cell-cell contact areas and boolean spatio-temporal expression data for extracellular signalling molecules. Computational simulation reveals that remarkably simple cell induction rules, based on the precision of the measure of cell-cell contacts rather than on the concentration of extracellular ligand, explain most cell specification events up to the late gastrula stage. We thus propose the existence of a trade-off between constraints on embryo geometry and on quantitative signaling gene expression. This scenario may explain why organisms with an embryogenesis relying on invariant cell lineages combine very slow morphological evolution and rapid genomic divergence.

The Complexity of Mitochondrial DNA Regulation: Modulating the Once and Future Endosymbiont

Dan Mishmar (Ben-Gurion University of the Negev, Israel)

Two billion years have passed since the fusion event that gave rise to current eukaryotes and their energy producing mitochondria. The once free-living alpha proteo-bacterium, lost most of its genetic material during the course of evolution, and cellular-mitochondrial interdependence emerged. Only 37 genes, which are important for energy metabolism and mitochondrial protein translation, were retained in the current small circular mitochondrial genome (mtDNA). Hence, mtDNA replication, transcription and post transcriptional regulation are controlled by nuclear DNA-encoded proteins. It is believed that only a handful of dedicated proteins control mitochondrial function. It was further argued that mtDNA transcription regulation is fairly simple without any known enhancer elements, and no clear chromatin-like organization. These profound differences from the nuclear genome gave the impression that mitochondria are regulated separately from the rest of the cell. Our results imply a relatively different view: we show in vivo mtDNA binding and mitochondrial localization of known transcriptional regulators of nuclear DNA genes, a conserved pattern of DNase sensitivity sites across the entire mtDNA, and evidence for selective constraints on potentially novel mtDNA regulatory elements. Our findings support the hypothesis that mitochondrial transcriptional regulation and genome organization are more complex than once thought and have more common ground with the nuclear genome. Hence, the long co-existence of the ancient prokaryote within our cells was accompanied by regulatory adaptation.

Human germ line editing: A historical perspective
Michel Morange (École Normale Supérieure, France)

The development of the CRISPR-Cas9 system has given a new impetus to projects that are based on modification of the human genome. Whether this editing process must be extended to the germ line is presently at the core of a hot debate.

These projects are the achievement of a program of research initiated by the molecular revolution and supported by regular improvements of genetic engineering tools. However, the issue of modifying (and improving) human species has predated the rise of molecular biology.

There is a sharp contrast between the dramatic improvement in genetic engineering tools in recent years and the increasing doubts about the necessity of modifying the human genome and the human species.

Marking Developmental History on the Genome in Immune Cells
Ellen Rothenberg (California Institute of Technology, USA)

The DNA code in a fertilized egg lies open to be read by regulatory factors that can promote initial steps of differentiation for the embryo as a whole. The order in which genes involved in various developmental programs are turned on in the embryo can be understood as a hierarchy of complexity in gene activation requirements, where the first genes to be turned on can utilize maternally inherited regulators in the fertilized egg, but later genes are turned on only when the embryo has already begun to express genes coding for secondary, tertiary, or even later sets of regulatory factors. In these embryonic cases, the genome itself is assumed to be available to be engaged by all these regulatory factors in any cells that express them. However, what is the case in later development? Adult hematopoietic development depends on stem cells that have been set apart from differentiation during fetal life, and which are then activated one at a time in later life. Notably, as stem cells develop into particular types of blood cells, they use regulatory factors that have already established roles in the differentiation of completely different tissues as well, for example in development of the brain or bone or other structures. How do these factors maintain coherence in their roles, to guide differentiation of one program as opposed to another? This talk will focus on how parts of the genome are at least partially masked from regulatory factor action by default in blood progenitor cells, while others are flagged for preferential access. An interesting case will be examined where a highly regulated process unmasks a gene in a crucial developmental step, to measure the cost of the unmasking and thus reveal how this mechanism creates irreversibility in developmental transitions.

Genomic Spaces of the Possible and the Origins of Adaptations
Andreas Wagner (University of Zurich, Switzerland)

Genetic and biochemical studies combined with genome sequencing have accumulated a wealth of information about the complex chemical reaction networks we call metabolisms. Together with computational tools to predict metabolic phenotypes from genotypes, information about genome-scale metabolic networks allows us to answer long-standing and fundamental questions about the evolution of biological systems in new ways. One of these questions asks how metabolic adaptations and innovations originate. In general, some adaptations are known to originate non-adaptively as exaptations, or pre-adaptations, which are by-products of other adaptive traits. Examples include feathers, which originated before they were used in flight, and lens crystallins, which are light-refracting proteins that originated as enzymes. The question of how often adaptive traits have non-adaptive origins has profound implications for evolutionary biology, but it has been difficult to answer systematically. Here I discuss recent observations suggesting that any one adaptation in a complex metabolic reaction network entails multiple potential exaptations. Metabolic systems thus contain a latent potential for evolutionary innovations with non-adaptive origins. These observations suggest that many more metabolic traits may have non-adaptive origins than is appreciated at present. More generally, they challenge our ability to distinguish adaptive from non-adaptive traits.

Ethical and Political Findings of Whole-Genome Sequencing
Anna Zielinska (Ben-Gurion University of the Negev, Israel)

Many ethical questions arise regarding the recent development of whole genome sequencing. Several ambitious scientific projects streamed out of our capacity to grasp it, but also there are many philosophical worries that go them. There might be something very perilous in the unravelling of fundamental human mysteries, something disrespectful toward what one might see as human nature. What do those fears reveal about Western society? Are they justified, either as such, or as compared to other consequences of the developments of these technologies? In my presentation, I would like to suggest a shift from properly philosophical worries concerning whole genome sequencing toward more contextualized ethical and political qualms.

I will cover two distinct subjects that support this shift. First, I will offer a critical overview of various conceptions of human nature in contemporary literature (ranging from philosopher Jurgen Habermas to geneticist Francis Collins). Then, I will present a recent report of the German committee whose work has been devoted to the study of the ethical and legal aspects of whole genome sequencing (EURAT). I will subsequently show that the ethical focus on genetics mirrors the medical focus on it, and that they are both rather controversial, scientifically and politically.