Rocking the foundations of molecular genetics

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In PNAS, Nelson et al. present intriguing evidence that challenges the fundamental tenets of genetics (1). It has long been assumed that the inherited contribution to phenotype is embedded in DNA sequence variations in, and interactions between, the genes endogenous to the organism, i.e., alleles derived from parents with some degree of de novo variation. This assumption underlies most genetic analysis, including the fleet of genome-wide association studies launched in recent years to identify genomic loci that influence complex human traits and diseases. Not surprisingly, in contrast to mutations in protein-coding sequences, which underlie high penetrance monogenic disorders, the vast majority of the identified loci map to non–protein-coding intergenic and intronic regions, which comprise the vast majority of the genome. These regions contain the regulatory information that controls gene expression and underlies most phenotypic variation (2).

However, the perplexing and much debated surprise has been that most genome-wide association studies have superficially failed to locate more than a small percentage of the inherited component of complex traits. This may be a result of a number of possibilities that are not mutually exclusive (3, 4), including systematic underestimation of the fraction of the heritability and epistatic interactions measured by common SNPs used to monitor haplotype blocks, a larger than expected contribution of rare recent variants that lie under the SNP typing radar, and intergenerational epigenetic inheritance (5), which is not polled by DNA sequence. However, the latter has not thus far been paid much attention or given much credence as a major factor.

Evidence of Intergenerational Epigenetic Inheritance

Now Nelson et al. (1) provide data suggesting that epigenetic inheritance may be far more important and pervasive than expected. Although the genetics are complex, Nelson et al. (1) show in an elegant and comprehensive series of analyses that grand-maternal (but not grand-paternal) heterozygosis for a null allele of the *Apobec1* cytidine deaminase gene modulates testicular germ cell tumor susceptibility and embryonic viability in male (mouse) descendants that do not carry the null allele, an effect that persists for at least three generations. That is, female F0 mice carrying a null allele of Apobec1 had a transgenerational influence on the phenotype of male F1, F2, and F3 descendants, compared with WT and male ancestral controls, even though the allele was not present in F1, F2 or F3—an effect that can be reversed by backcrossing through the alternative germ lineage.

These findings add to a growing list of studies indicating that genetic influence of ancestral variants can commonly reach through multiple generations and rival conventional inheritance in strength. These include the demonstrations, with considerable molecular and genetic detail, of epigenetic inheritance (i.e., "paramutation") in plants, and, although still somewhat controversial, in animals (6).

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Mechanistically, epigenetic memory is embedded in DNA methylation and/or histone modifications, which are thought to be erased in germ cells, but may not be, at least completely, as some chromatin structure appears to be preserved (7). Some information may also be cotransmitted by RNA (7, 8).

RNA Regulation of Epigenetic State

Indeed, there is now good evidence that epigenetic inheritance is RNA-mediated (9), or, perhaps more precisely, RNA-directed, as it is becoming clear that a major function of the large numbers of noncoding RNAs that are differentially expressed from the genome (10) is to direct chromatin-modifying complexes to their sites of action (11, 12). This conclusion is consistent with the recent findings of the ENCODE project, suggesting that much if not most of the human genome may be functional (13), and explains the informational basis of the extraordinary precision and complexity of the epigenetic superstructure of the genome in different cells required to specify developmental architecture.

Interestingly, Apobec1 is an RNA-editing enzyme that is required for embryonic development, orthologues of which, including the "activation-induced" cytidine deaminase, modulate cytidine methylation following deamination to thymidine and presumptively mismatch repair (but which may involve more complex preprogrammed lineage- and/or context-specific mutation) (14–16). Moreover, activation-induced cytidine deaminase, which is involved in somatic hypermutation and rearrangement of Ig genes, is expressed in pluripotent cells (17), affects erasure of DNA methylation in mouse germ cells (18), and is required for reprogramming toward pluripotency (15).

Soma to Germline Inheritance

The available evidence not only suggests an intimate interplay between genetic and epigenetic inheritance, but also that this interplay may involve communication between the soma and the germline. This idea contravenes the so-called Weismann barrier, sometimes referred to as Biology's Second Law, which is based on flimsy evidence and a desire to distance Darwinian evolution from Lamarckian inheritance at the time of the Modern Evolutionary Synthesis. However, the belief that the soma and germline do not communicate is patently incorrect—as demonstrated by the multigenerational inheritance of RNAi-mediated phenotypes delivered to somatic cells in *Caenorhabditis elegans* (8).

Thus, if RNA editing can alter hard-wired genetic information in a context-dependent manner, and thereby alter epigenetic memory, it is feasible that not only allelic but also environmental history may shape phenotype, and provide a far more plastic and dynamic inheritance platform than envisaged by the genetic orthodoxy of the past century.

RNA at the Center

Moreover, the finding of extraordinarily dynamic noncoding transcription in complex organisms suggests that the long-held idea that gene expression is primarily controlled by combinatoric interactions between *cis*-acting transcription factors and their cognate binding sites is also incorrect, but rather that RNA may be

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the computational engine of the evolution and ontogeny of developmentally complex and cognitively advanced organisms (19).

It is time to reassess many assumptions in molecular biology and genetics.

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