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Rewriting the Genetic Text in Human Brain Development

How adaptive epigenetic changes that can rewrite genes contribute to human brain development and evolution Dr. Mae-Wan Ho

What makes brainy primates?

The brains of higher animals become increasingly complex in the course of evolution, reaching a pinnacle in primates and the human species. And among the most tantalizing discoveries since the sequencing of the human and other genomes are the genetic and epigenetic events associated with the evolution and development of the human brain.

Two classes of coincidental events stand out in the evolution of primates, the end result of which is to greatly expand the diversity of transcripts and proteins and to build the complex regulatory architecture required for human intellectual capacity.

The first is the dramatic increase in RNA editing, a process that systematically alters the genetic messages transcribed from the genome, creating new coding and non-coding RNAs, and hence new proteins as well as RNAi (interfering RNA) species that regulate networks of genes. The second is the expansion of primate-specific Alu retrotransposons, which multiply through RNA intermediates that are reverse-transcribed and inserted into the genome. It so happens that the increase in RNA editing in primates occurs almost entirely within primate-specific Alu elements.

John Mattick at University of Queensland St. Lucia in Australia and Mark Mehler at Albert Einstein College of Medicine, New York in the United States have recently speculated that this coincidental increase in RNA editing and Alu elements is indeed involved in the evolution and development of brainy primates and especially of human intelligence [1].

RNA editing in evolution

The most common form of RNA editing is to change adenosine to inosine (A to I), the I being recognized as G. This can cancel out a stop codon to create a read through message, alter the codon, or create new splice sites in introns (non-coding intervening sequences in interrupted genes) resulting in new exons (coding sequences for proteins in interrupted genes). The editing is catalyzed by members of the enzyme family adenosine

deaminase that act on RNA (ADARs), which specifically recognize double-stranded RNA longer than 30 bps (basepairs).

In reality, RNA editing encompasses a broad range of other RNA modifications, including insertion and deletion of nucleotides that change the entire reading frame of proteins.

RNA editing can act in concert with alternative splicing in interrupted genes to further enhance transcript diversity [2]. For example, in the *para* locus encoding a *Drosophila* voltage-gated Na^+ channel, two dozen processing sites for alternative splicing and RNA editing can potentially combine to generating more than two million 'isoforms' of the protein.

RNA editing occurs in all taxonomic groups of organisms, but increases dramatically in vertebrate, mammals and primates, with humans exhibiting the highest levels of edited and multiply-edited transcripts. RNA editing occurs in most, if not all tissues, but is particularly active in the nervous system, where transcripts encoding proteins involved in fast neural transmission, such as ion channels and ligand-gated receptors [1, 2]. These species-specific alterations have profound importance for normal nervous system function.

A to I editing is much more abundant in humans than in mice, and over 90 percent of this increased editing occurs in *Alu* elements in mainly noncoding regions of RNAs, i.e., in untranslated regions (UTRs) of mRNAs, in introns and intergenic transcripts.

ADARs have been shown to regulate neuronal gene expression through a variety of disparate processes including modulation of RNAi, creation of alternative splice sites, and abolition of stop codons. In addition ADARs have a novel role in primates in the widespread editing of Alu elements.

Robert Reenan and James Jepson at Brown University Providence Rhode Island in the United States share the view that the widespread editing of Alu elements [2] "may well have been fundamental to the evolution of complex behaviour."

RNA editing in embryonic and cognitive development

RNA editing has all the appearance of being crucial in development, especially of cognitive functions. For example, the loss of A to I editing in mice lacking the editing enzyme ADAR1 die at the embryonic stage from defects in the production of red blood cells and stress-induced programmed cell death, and degeneration of the liver. Mice lacking a second editing enzyme ADAR2 exhibit profound epileptic seizures and die shortly after birth.

In *Drosophila*, deletion of the single *adar* locus generates morphologically wild-type adult flies that display a range of behavioural abnormalities including severe non-coordination, temperature-sensitive paralysis, seizures and a complete lack of courtship displays and mating. Deletion of the RNA editing enzymes ADR1 and ADR2 in *C. elegans* similarly results in chemosensory defects.

RNA editing alters transcripts from genes encoding proteins involved in neural cell identity, maturation and function, as well as in DNA repair. This implies that RNA editing has a role not only in neural transmission and network plasticity but also in brain development.

In humans, three ADARs (1-3) exist, all preferentially expressed in the nervous system, with ADAR3 being expressed exclusively in the brain. Within the brain, ADARs exhibit complex profiles of spatiotemporal regulation and dynamic changes in subcellular localization, and are themselves subject to alternative splicing. Moreover, the activities of ADARs are modulated by environmental cues, and modify signalling cues embedded within signal-transduction pathways containing edited targets. RNA editing is not only critical for cognitive behaviour; the deregulation of ADAR activity and associated hyper- or hypo-editing of RNA transcripts is associated with an increased risk of neurodegenerative disease and cancer, and neuro-developmental and psychiatric diseases in humans [1].

Mattick and Mehler suggest that "productive" epigenetic changes

resulting from RNA editing are communicated back to the genome of neurons, constituting the molecular basis of long-term memory and higher-order cognition.

There are at least 3 distinct ways that RNA editing can alter brain function in response to experience (learning) and contribute to the evolution of higher-order cognitive capacities. First by selectively editing codons and splicing signals in protein-coding sequences involved in modulating fast neurotransmission, the firing properties of neurons can be fine-tuned for appropriate neuronal output and neural network integration. Second, RNA editing can alter the processing properties and target specificities of microRNAs (miRNAs) and the RNA interference regulatory networks in which they participate. Third, RNA editing can modify the sequences and biophysical properties of a vast array of other gene products, notably pre-mRNAs and the large numbers of non-coding RNAs known to be specifically expressed in the brain and to play roles in many functional and regulatory pathways, including epigenetic phenomena associated with learning.

RNA editing and RNA interference

Numerous findings are suggesting that RNA editing is widespread in the brain, affecting not only the function of individual genes in the short-term, but its high-level long-term regulatory architecture that determines the epigenetic states of multiple networks of genes. This occurs through interactions between RNA editing and another ubiquitous epigenetic process, RNA interference [2].

RNA interference (RNAi) is present in all organisms that silence genes as well as viruses and transposons in the genome [3] (see Subverting the Genetic Text, *SiS* 24).. The triggers for RNAi are a range of small RNAs ranging from 21 to 29 nt (nucleotides) in length, mainly short interfering RNAs (siRNAs) and microRNAs (miRNAs). SiRNA and miRNA are generated from double stranded RNA (dsRNA) precursors that are bound and cleaved by members of the Dicer family of nucleases into small effector molecules. In the case of siRNAs, the dsRNAs are thought to originate from inversely orientated complementary viral or transposon sequences. SiRNA and miRNA function in similar ways by binding to mRNA that exhibit short stretches of complementary base sequences, resulting in cleavage of the mRNA or the inhibition of translation into protein.

MiRNA interference has recently been shown to control a wide variety of neurologically important processes in both vertebrates and invertebrates [2] including neuronal expression of chemoreceptor genes, neuron-specific splicing, circadian rhythms, morphogenesis of dendritic spine, learning and memory. In parallel, ADAR activity may be involved in negatively regulating the producing of miRNAs at multiple steps during maturation, and in redirectinig the actions of miRNAs by altering their targets.

Recent research has shown that ADARs may act to inhibit the actions of siRNAs through both their editing and dsRNA-binding activities. It is the unrestrained actions of siRNAs and/or miRNAs that result in the chemosensory defects of *C. elegans* lacking their RNA editing enzymes (see above).

Editing of miRNAs and other non-protein-coding RNAs that can redirect the miRNAs to different targets are involved in higher order cognitive functions dependent on entire suites of genes. The sites of RNA editing might also be sites of small nucleolar RNA-mediated RNA modification, again suggesting that editing is involved in genetic and epigenetic regulatory networks [1].

Some miRNAs subject to ADAR editing are derived from primate-specific *Alu* sequences, and recent results show that miRNA-mediated translational repression can be relieved by another class of editing enzymes, the APOBEC family members.

Given the abundance of miRNAs in the nervous system and their central roles in brain development, the fact that many miRNAs are derived from introns, and that many are primate specific, RNA editing for regulatory purposes might be widespread, and it is such high-level regulatory architecture that controls brain development and plasticity.

Primate-specific Alu elements are the most common

substrate for primate and human ADARs

RNA A to I editing is much more abundant in humans than in mice, and over 90 percent of this increase occurs in head-to-tail repeat Alu elements in mainly noncoding regions of RNAs, i.e., in untranslated regions of mRNAs and in introns and intergenic transcripts.

Alu elements represent a subclass of primate-specific short interspersed nuclear elements (SINEs) derived from 7S and tRNA sequences and spread around the genome by retrotransposition. Three successive waves of Alu expansion occurred during primate evolution resulting in more than 1.1 million copies in the human genome. The major waves consist of the old AluJ subfamily about 81 million years ago, the middle-aged AluS subfamily about 48-19mya, and the young, and still active AluY wave starting about 6 mya and continuing [4].

The possibility that Alu elements play a key role in the evolution of human cognitive capacity via RNA A to I editing is suggested by a number of observations [1]: RNA editing is most active in brain and important to brain function; humans show two orders of magnitude more editing than mice; most of the increased editing occurs in primate-specific Alu elements; and primates have experienced the highest evolution of cognitive capacities.

Several research groups used large scale analyses to identify novel human RNA editing sites among expressed sequences, and came up with 19 116 sites in 1 919 mRNAs, averaged over three estimates [1]. Almost all sites are within *Alu* elements inserted in inverse orientations. Editing sites are not evenly distributed along the Alu elements, but occur in hotspots, particularly the A residues at positions 27, 29, 136 and 162. In addition, editing is tissue-specific, with higher levels in the thymus and the brain.

Transcript of numerous genes involved in brain function subject to RNA editing

Analysis of the human RNA edited transcript databases reveal that transcripts from gene involved in fast neural transmission represent only a small subset of A to I RNA editing [1]. The thousands of targets include many transcripts from genes involved in nervous system development, encompassing proteins that modulate neural induction, and in three-dimensional patterning of the anterior portion of the evolving neural tube and the forebrain. Also edited are transcripts from genes involved in neural stem cell self-renewal, asymmetric cell division and modulation of proliferation, and early neuroblast development, including cell-cycle kinetics and migration. Other genes with edited transcripts are involved in neural maturation including differentiation, morphogenesis, polarity, axon guidance, dendrite formation, synapse formation, neural subtype specification and network connectivity. Also included are transcripts from genes encoding protocadherin a and protocadherin b subclasses of cell-surface molecules. Genes enoding protocadherins have been strongly implicated in the formation of neural circuitry by encoding an unusually large repertoire of isoforms that appear to provide the cellular address codes for directing appropriate cell-cell interactions during progressive stages of nervous system development.

Edited transcripts include protein-coding genes that play central roles in an extraordinary range of innovations in mature neural function: neurone survival, excitability, signal transduction, plasticity, axodentritic transport, energy metabolism, cell-cell and cell-environment interactions, organization of neuronal microdomains, signalling scaffolds and cooperative clustering of synaptic neural receptor subtypes. Many of these genes are associated with neurodegenerative diseases and brain tumours as well as neurodevelopment syndromes and psychiatric disorders.

These observations imply that not only synaptic strength but also brain development is influenced by environment and experience. Moreover if RNA editing is context dependent, this might explain the trafficking of non-coding RNAs (ncRNAs) and mRNA to the periphery of axons and dendrites where editing might be taking place in response to local cues, coincident with the activation of RNA regulatory networks, and before protein translation.

RNA editing of DNA surveillance and repair enzymes and rewriting DNA

Intriguingly, transcripts from genes encoding a broad range of DNA surveillance and repair enzymes are also subject to RNA editing [1].

Learning and memory in the brain is similar to the immune response in many ways. A key feature of the immune system is the alteration of DNA sequence in the genome to generate receptor diversity, in part catalyzed by the APOBEC family of cytidine deaminases that can catalyze cytosine to uracil (C to U) and cytosine to thymine (C to T) editing of RNA and DNA.

The possibility exists that DNA recoding – rewriting genome DNA - is a central feature of both the immune and nervous systems. DNA recoding may be involved at the level of establishing neuronal identity and neuronal connectivity during development, learning and brain regeneration. And it appears that the brain, like the immune system, also changes according to experience.

Mattick and Mehler suggest that the potential recoding of DNA in nerve cells (and similarly in immune cells) might be primarily a mechanism whereby productive or learned changes induced by RNA editing are *rewritten* back to DNA via RNA-directed DNA repair. (See the latest model of RNA-directed recoding of DNA proposed for the immune system [5] by Ted Steele at Australian National University Canberra). This effectively fixes the altered genetic message once a particular neural circuitry and epigenetic state has been established.

The suggestion that memory formation involves RNA-directed DNA modifications similar to those in the immune system is supported by a range of circumstantial observations over many years. For example, two enzymes involved in generating diversity in the immune system (Rag1 and Rag2) are expressed in the central nervous system and in olfactory sensory neurons that are actively involved in experience-mediated neural plasticity. Furthermore, recombination catalyzed by Rag1 and Rag2 and programmed genomic rearrangements in other organisms are RNA directed, although it remains uncertain whether such recombination occurs in the brain and is relevant to brain function.

Members of the DNA polymerase Y family involved in somatic hypermutation of genes encoding immunoglobulins have reverse transcriptase activity [6]. One of them, DNA polymerase-i is expressed in areas of the brain associated with learning and memory, as is DNA polymerase M, which is involved in rearrangement of genes encoding immunoglulins. The fact that transcripts from genes encoding enzymes putatively involved in DNA recoding are themselves edited suggests that the process is subject to contextual control, which might explain why some memories are more vivid and enduring than others. Moreover, A to G mutations correlate with nascent mRNA hairpin at somatic hypermutation hotspots, implying roles for both RNA editing and reverse transcription during somatic hypermutation [7], involving mismatch repair enzymes that are expressed in the hippocampus.

It has been shown recently that RNA-directed DNA repair can occur in eukaryotic cells. In addition, LINE1 elements that are active in the human genome encode several proteins, including a reverse transcriptase, and individual SINE elements including active Alu sequences can hijack and use the LINE1 reverse transcriptase.

The suggestion that there might be communication of RNA-encoded information back to the genome at the epigenetic and genetic levels would also potentially explain the surprising observation that diverse RNA species and associated regulatory signals are not only trafficked to the periphery of the nerve cell, but might also undergo retro-transport back to the nucleus. There is increasing evidence for retrograde transport of RNAs, including small RNAs, to the nucleus in a broad range of organisms, as well as for RNA informational exchange between cells through 'exosomes', specific RNA receptors and derivation of presynaptic RNA from surrounding glial cells.

There are clear evolutionary and functional parallels between

members of the immunoglobulin (Ig) superfamily and the protocadherins, as well as many other subclasses of nervous system-selective Ig superfamily domain-containing proteins involved in neuronal cell identity, connectivity, synaptic plasticity and developmental and adult brain homeostasis.

The pervasive presence of a broad array of functional subclasses of Ig-like CNS superfamily proteins might represent flexible modules for molecular recognition and also particularly amenable targets of APOBEC-mediated editing/mutation, as they are in the immune system.

APOBEC enzymes, as well as ADARs, also exhibit dynamic changes in nuclear-cytoplasmic translocation, intranuclear microdomain localization and editing functions in response to the environment. The APOBEC3 subfamily has been vastly expanded in primates, and complexes formed with APOBEC3 are recruited into RNA transport granules that contain both Staufen (RNA-binding protein) and Alu sequences. Staufen has been shown to be required for long-term memory formation in *Drosophila*, as has Armitage, a putative RNA helicase required for mRNA transport and translation at the synapse.

Numerous observations bear out the pervasive role of RNA editing and its potential coupling to DNA recoding via RNA trafficking between nucleus and synapse, and RNA-templated DNA repair enzymes in the evolution, development and function of the human brain.

Mattick and Mehler suggest that environmentally induced changes in neural development and brain architecture, cell identity and synaptic connectivity might become "hardwired in the genome", "potentially defining the complex and emergent properties of long-term memories and other structural and functional adaptations.

"If correct, this hypothesis predicts that individual neural cells will, in fact, have distinctive spatially and temporally defined genomic sequences and chromatin structure… It also predicts that memory consolidation, storage and retrieval and associated long-term adaptations of human brain form and function should be modifiable by the targeted and differential modulation of expression of genes encoding enzymes involved in RNA editing and DNA recoding."

Sperm-mediated gene transfer, the inheritance of acquired characters

Mattick and Mehler fall short of proposing that the RNA-templated recoding of the genome and the associated structural and functional adaptations could be transmitted to the next generation. This would appear to be crucial for brain evolution in primates leading up to humans, so that the gains made by each generation could be accumulated.

If the analogy with the immune system holds, then as suggested by Steele and colleagues, edited RNA messages or their reverse transcribed DNA counterparts could become inherited via the sperm [5, 7, 8] (see Epigenetic Inheritance Through Sperm Cells, *SiS* 41).

"Sperm-mediated gene transfer" has been well documented by Italian researcher Corrado Spadafora [9] as a process whereby new genetic traits are transmitted to the next generation by the uptake of DNA or RNA by spermatozoa and delivered to the oocytes at fertilization. The interaction of exogenous nucleic acids with sperm cells is mediated by specific factors, among which, a reverse transcriptase that generates "retro-genes" through reverse transcription of exogenous RNA or through sequential transcription, splicing and reverse transcription of exogenous DNA. The result is to transmit low copy transcriptionally active extrachromosomal structures capable of determining new traits. Retro-genes can be further transmitted through sexual reproduction from founders to their F1 progeny as new genetic and phenotypic features, unlinked to chromosomes, and thus be generated and inherited in a non-Mendelian manner. Rare instances of retro-gene integration into the chromosome could also occur, providing further potential for evolution.

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