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Expanding the proteome: A-to-I RNA editing provides an adaptive advantage

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RNA editing refers to the post-transcriptional modification of RNA sequences. Diverse eukaryotes are capable of mRNA editing, most commonly through enzymatic deamination of adenosine-to-inosine (A-to-I) (1–3). The resulting 'I' is read by the translational machinery as guanosine ('G') (4). Thus, A-to-I editing within coding sequences can increase proteome complexity by generating new protein variants with amino acid substitutions or recoded stop codons. These new protein variants may be functionally distinct from their genome-encoded counterparts, but only a handful of protein variants generated by A-to-I editing have demonstrated functions. Widespread application of DNA- and RNA-sequencing has revealed that A-to-I editing is prevalent in mammals, cephalopods, insects, and other organisms including some fungi (5–9). The idea that A-to-I editing provides an adaptive advantage by recoding mRNAs is attractive, but evidence for this is limited. In PNAS, Xin et al. explore the role of A-to-I editing during sexual development of *Fusarium graminearum*, a filamentous ascomycete (10). The authors demonstrate that conserved A-to-I editing sites are functionally important during fungal development and provide evidence that A-to-I editing provides an adaptive advantage. In addition, these findings provide new mechanistic insights into multicellular development in the fungal kingdom and have major implications for understanding the complete coding potential of eukaryotic genomes.

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A-to-I editing was first identified through biochemical studies (11). In animals, members of the adenosine deaminase acting on RNA (ADAR) family target double-stranded RNAs for unwinding and adenosine deamination (12). ADAR enzymes can modify endogenous RNA duplexes to prevent aberrant antiviral responses (13). More recently, genomic approaches have uncovered thousands of A-to-I editing sites in a variety of metazoan organisms, including mammals, flies, and cephalopods (5–7, 9). The extent to which editing sites alter coding sequences varies between organisms. In primates, most editing sites occur in repetitive *Alu* elements, though a small fraction of A-to-I editing events impacts coding regions (5, 14). In flies and cephalopods, editing sites that generate nonsynonymous amino acid substitutions make up a significant fraction of total sites. The number of A-to-I editing sites that alter coding potential of mRNAs ranges from thousands (>1,000 in flies and >3,000 in humans) to over 70,000 in cephalopods (12). Genes with neuronal functions appear to be common targets of the A-to-I editing machinery in metazoans, but only a handful of observed A-to-I editing sites have demonstrated functions (6, 15, 16). For example, editing of the Q/R site in mammalian glutamate receptor 2 is essential for postnatal survival in mice (17). It is reasonable to assume that mRNA recoding via A-to-I editing provides an evolutionary advantage, especially given the widespread occurrence in metazoans, yet studies to address this question have provided contradictory results. A-to-I editing has been linked to temperature adaptation in flies and cephalopods, whereas researchers studying A-to-I editing in humans concluded that editing was nonadaptive (18–20).

It is experimentally challenging to demonstrate functional importance of individual A-to-I editing sites. Editing often occurs at low frequency, making it difficult to distinguish bona fide A-to-I editing sites from DNA sequencing errors. Labor-intensive genetic approaches are required to demonstrate that individual editing sites are functionally relevant, but results of genetic studies can be difficult to interpret because phenotypes are often subtle or tissue specific. The recent discovery that certain ascomycete fungi perform extensive A-to-I editing during sexual development is exciting because it creates an opportunity to apply facile fungal genetics to investigate A-to-I editing (8, 21).

In the present study, Xin et al. carried out comparative genomic analyses and genetic studies to investigate the role of A-to-I editing during fruiting body development of *F. graminearum.* Previous studies with *F. graminearum* identified over 26,000 putative A-to-I editing sites, including 429 sites that result in nonsynonymous substitutions and are shared across three species of sordariomycete fungi, *F. graminearum, Neurospora crassa,* and *Neurospora tetrasperma* (8, 21). These genes were classified as *conserved missense editing* (*CME*) genes. In this study, 21 *CME* genes that are edited at high frequency in both *F. graminearum* and *N. crassa* were investigated.

The authors first constructed deletion alleles of selected *CME* genes and examined their phenotypes. This revealed seven *CME* genes required for normal sexual development of *F. graminearum*. Next, *CME* deletion strains were transformed with an "nonedited" allele, which only produces the

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genome-encoded protein variant. In parallel, *CME* deletion strains were transformed with an allele that mimics the edited transcript (i.e., an 'A' to 'G' missense allele). The 'nonedited' allele was able to rescue the phenotype for most *CME* deletion strains, demonstrating that editing of these sites is not required for proper development. It remains possible that these editing-deficient alleles give rise to subtle phenotypes that were not investigated in this study. Significantly, the 'nonedited' allele of *CME5* failed to rescue developmental defects of a *CME5* deletion mutant, whereas an 'A' to 'G' missense allele restored normal development. These data demonstrate that A-to-I editing of *CME5* is essential for normal sexual development. *CME5* encodes a predicted kinesin-8 motor. Editing is predicted to result in an arginine to glycine substitution at position 986. Remarkably, phylogenetic analysis of *CME5* homologs across the fungal tree of life revealed that glycine is rarely hard-wired into the genome at this position, indicating the genome-encoded allele is conserved and likely ancestral. Together, these data indicate that the genome-encoded allele likely provides an important function during vegetative growth and that editing operates to produce a new protein variant with a critical function during sexual development.

Experiments with a second gene provided additional evidence that A-to-I editing is adaptive in *F. graminearum. CME11* encodes a predicted chromatin remodeling enzyme. A-to-I editing of *CME11* results in the substitution of a putative phosphorylated residue, threonine-304 to an alanine. In contrast to *CME5,* developmental defects of *CME11* strains could not be rescued by the nonedited allele or a 'G' allele that mimics the editing product. A third experiment showed that introduction of both 'nonedited' and 'G' alleles into the *CME11* deletion strain rescued the developmental phenotype. Thus, both edited and unedited *CME11* transcripts are required for fruiting body development in *F. graminearum*. Together, these data show that nonsynonymous amino acid substitutions introduced by the A-to-I editing machinery are critical for fungal development and suggest that proteome diversification by A-to-I editing provides an adaptive advantage.

These findings have major implications for understanding regulatory mechanisms that control fungal physiology and development. In at least some Sordariomycetes, both genome-encoded mRNAs and novel mRNA isoforms generated by A-to-I editing are translated to produce functionally important proteins. This work provides strong motivation for future studies of A-to-I editing in other fungi. In addition to several Sordariomycetes, A-to-I editing operates during sexual development of *Pyronema confluens,* a member of the Pezizomycetes that last shared a common ancestor with the sordariomycete lineage over 400 Mya (22, 23). It seems likely that A-to-I editing is broadly important for fungal development. In both *F. graminearum* and *N. crassa*, A-to-I editing exhibits remarkable tissue specificity. Will studies of other fungi reveal A-to-I editing during additional growth conditions or cell types?

Identification of the A-to-I editing machinery and understanding how editing is regulated both spatially and temporally are important goals for future studies. Only a fraction of mRNA molecules is subject to A-to-I editing, enabling cells to express edited and unedited transcripts simultaneously, as observed here for *CME11* (Fig. 1). Editing levels at individual sites may be controlled by accessory

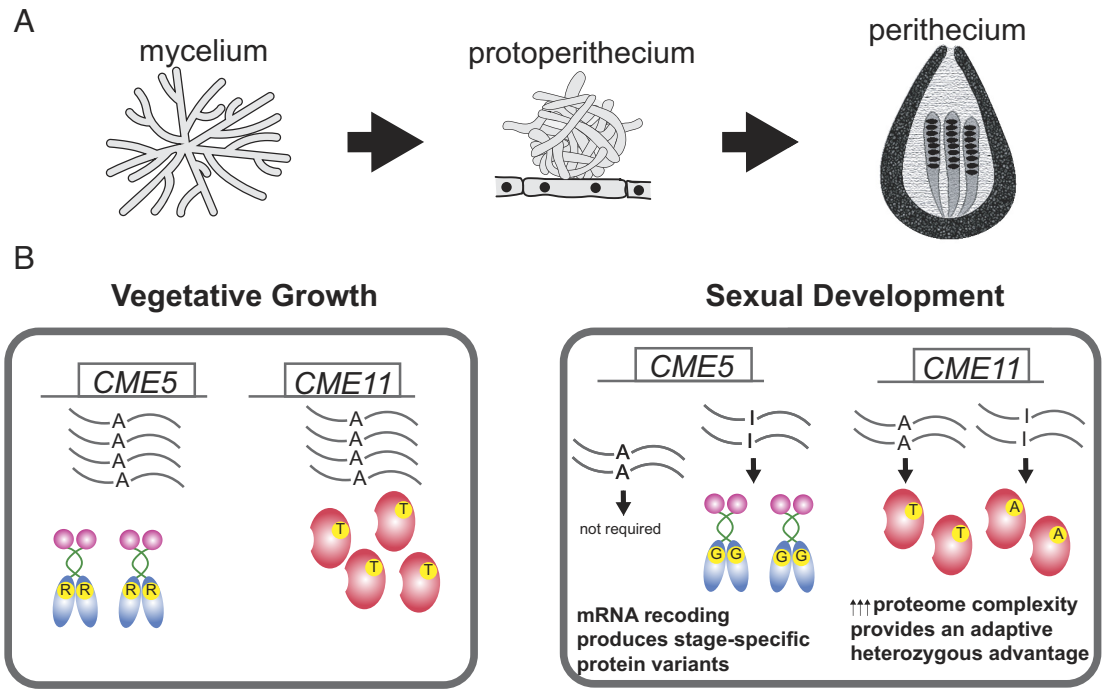

Fig. 1. A-to-I mRNA editing expands proteome complexity to drive fungal development and provide an adaptive advantage. (*A*) Sordariomycete fungi grow vegetatively as mycelia or differentiate to produce multicellular structures during sexual development (protoperithecia and perithecia). A-to-I mRNA editing operates exclusively during sexual development. (*B*) Genetic studies of two *conserved missense editing* (*CME*) genes in *F. graminearum* demonstrate that A-to-I editing provides an adaptive advantage. Recoding of *CME5* mRNA by A-to-I editing replaces the ancestral, genome-encoded arginine (R) with a glycine (G) to generate a stage-specific protein variant required for normal development of perithecia. In addition, expression of both edited and unedited *CME11* transcripts is required for normal perithecial development, suggesting that editing confers a heterozygous advantage.

factors, perhaps in response to specific stimuli. The fact that A-to-I editing is a dynamic process in fungi and metazoans supports the idea that unknown regulators of A-to-I editing are yet to be discovered. More broadly, it seems likely that proteome diversification by A-to-I editing is important for additional cell type-specific functions in metazoans. As mentioned above, editing is common in neuronal genes in insects, cephalopods, and mammals, suggesting that A-to-I editing may regulate the establishment or maintenance of neuronal cell identity or at least recode key neuronal functions.

A-to-I editing expands the coding potential of the genome, giving rise to new protein variants in the population that can be selected upon. The findings of Xin et al. provide evidence that A-to-I editing in fungi is both functionally important and adaptive. These results have far-reaching implications for understanding eukaryotic proteome complexity, developmental biology, and human disease states.

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