ADAR has roles independent of editing, during which it partners with proteins such as DROSHA and DICER1, targeting them to miRNAs and other noncoding RNAs, often in a tissue-specific manner [8]. Localization of different classes of catalytic machinery to active genes is also possible through their binding to flipons, usually by capturing them in the 'B'-conformation. Many of the resulting epigenetic nucleotide modifications (NMs) are known to impact flip energetics (Figure 1D and Table 1). By pushing flipons either left or right, NMs are able to lock in a particular chromatin state. Flip energetics also change when oxidative stresses and mutagens produce DNA and RNA adducts, especially on the C5 position of cytidine and the C8 position of guanosine. Here, flipons act as damage sensors, enabling a direct and rapid transcriptional response to cellular stress through changes in chromatin state. In all these cases, flipon conformation instructs on how to compile response-specific transcripts.

Generation of Diversity

The ease with which flipons form, along with their location, is subject to selection just like any other genetic variation. Flipons create phenotypic diversity by increasing transcriptome entropy. The genomes that emerge encode information by sequence and instructions by conformation (Table 1). Regions where flipon and codon sequences overlap have lower entropy (i.e., they have a fixed information content) and likely become hotspots for spawning species-specific phenotypic variability. Germline retrotransposition, recombination, and repair enhance transcriptome diversity by spreading flipon sequences to other parts of the genome. The noncoding IREs targeted by ADAR exemplify how this process works. During insertion into active genes, they bring flipons along for the ride [2]. While active transposons threaten genomic stability, the instruction sets they carry enhance the creation and capture of novel genetic programs.

Concluding Remarks: Entropy, Flipons, and Evolution

Codons enable the mapping of nucleotide sequence to protein sequence. Altering their usage is only one way to diversify phenotypes. Nature has discovered other strategies to create novelty through editing and splicing of RNA, resulting in multiple transcripts from the same reading frame. Flipons provide a novel innovation for changing the transcriptome by dynamically switching chromatin states to change how messages are compiled. The sequences encoding flipons often overlap those of codons and other regulatory elements. Each encodes a different set of information, is subject to natural selection, and causes Mendelian diseases in its own way. Flipons trade free energy for the extra possibilities that novel transcripts provide. The increased entropy enhances the reworking of existing adaptations and speeds the evolution of traits both new and unexpected.

Acknowledgment

Many thanks to the reviewer who highlighted the importance of Ilya Prigogine's work on dissipative structures.

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https://doi.org/10.1016/j.tig.2019.09.007

References

1. Shing, P. and Carter, M. (2011) DNA structure: alphabet soup for the cellular soul. In *DNA Replication: Current Advances* (Seligmann, H. ed), pp. 3–28, IntechOpen
2. Herbert, A. (2019) Z-DNA and Z-RNA in human disease. *Commun. Biol.* 2, 7
3. Herbert, A. (2019) Mendelian disease caused by variants affecting recognition of Z-DNA and Z-RNA by the Zα domain of the double-stranded RNA editing enzyme ADAR. *Eur. J. Hum. Genet.* Published online July 18, 2019. https://doi.org/10.1038/s41431-019-0458-6.
4. Shin, S.I. *et al.* (2016) Z-DNA-forming sites identified by ChIP-Seq are associated with actively transcribed regions in the human genome. *DNA Res* 23, 477–486
5. Zhang, F. *et al.* (2016) Histone acetylation induced transformation of B-DNA to Z-DNA in cells probed through FT-IR spectroscopy. *Anal. Chem.* 88, 4179–4182
6. Prigogine, I. (1978) Time, structure, and fluctuations. *Science* 201, 777–785
7. Athanasiadis, A. (2012) Zalpha-domains: at the intersection between RNA editing and innate immunity. *Semin. Cell Dev. Biol.* 23, 275–280
8. Nishikura, K. (2016) A-to-I editing of coding and non-coding RNAs by ADARs. *Nat. Rev. Mol. Cell Biol.* 17, 83–96
9. Xu, Y. *et al.* (2007) Photochemical determination of different DNA structures. *Nat. Protoc.* 2, 78–87
10. Herbert, A. (2019) ADAR and immune silencing in cancer. *Trends Cancer* 5, 272–282
11. Balasubramaniyam, T. *et al.* (2018) 2′-O-Methyl-8-methylguanosine as a Z-form RNA stabilizer for structural and functional study of Z-RNA. *Molecules* 23, 2572
12. Wang, S. *et al.* (2014) Systematic investigations of different cytosine modifications on CpG dinucleotide sequences: the effects on the B-Z transition. *J. Am. Chem. Soc.* 136, 56–59

Forum

Compensatory Evolution of Gene Expression

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Determining the contribution of *cis* and *trans* components to differences in gene expression is a powerful approach for understanding gene regulatory evolution. Specifically, differences in gene expression that are due to linked polymorphisms (*cis*, allele-specific and local to the affected gene), or differences due to diffusible products that do not need to be linked with the affected gene (*trans*, affecting both alleles equally in diploids). Decomposing the evolution of gene expression into its

cis and *trans* components has contributed many important insights into evolution, including, for example, the frequency of *cis* and *trans* differences in interspecific versus intraspecific variation or the evolution of environment-specific phenotypes [1]. It has frequently been observed that *cis–trans* differences found in pairs are more frequently compensatory (+/–) rather than amplifying (–/–; +/+). A confounding factor associated with inferring compensatory *cis*- and *trans*-regulatory evolution from experiments comparing allele-specific expression to expression differences between parental strains has recently been identified [2–4], calling into question the role of compensatory changes in regulatory evolution reported by prior studies. However, as we describe later, evidence for compensatory changes being common in regulatory evolution does not come solely from this type of experiment, and the body of evidence remaining after taking this limitation of the allele-specific expression analysis into account still suggests that compensatory changes are common in regulatory evolution.

Evidence for compensatory evolution comes from a variety of study designs. Expression quantitative trait loci (eQTL) analysis correlates a molecular phenotype, such as gene expression, with genetic variation, and often finds an enrichment of antagonistic *cis* and *trans* effects which overall serve to normalize levels of gene expression (though eQTLs often have low power to detect *trans*-eQTL) [5]. Gene deletion experiments have found that the effects of a deletion are compensated for by mutations elsewhere in the genome [6]. Another approach identified *cis* differences and overall gene expression divergence but did not explicitly detect *trans* differences. This work found that while *cis*-regulatory divergence increased over time between species, the number of differences in total gene expression did not, which is consistent with compensatory evolution [7]. Leaving out situations in which the estimates are potentially correlated, a classic situation in which compensatory *cis–trans* pairs have been identified (and are due to stabilizing selection) is in F1 hybrid crosses. When both parents have the same level of expression of a given gene, but it shows both allele-specific expression and is misregulated in hybrids between the two species, this is likely due to compensatory *cis–trans* evolution [8]. Indeed, much of the literature on Bateson–Dobzhansky–Muller incompatibilities posits the accumulation of compensatory mutations to maintain gene expression output; as these require epistatic effects between loci, this is equivalent to *cis–trans* pairs.

Interestingly, compensatory evolution also need not occur exclusively between *cis–trans* pairs, and recent work on the *trans*-regulatory landscape suggests that *trans*-regulatory evolution may be compensatory; for example, Metzgar and Wittkopp (2019) found that hundreds of loci affect a single gene in *trans*, increasing and decreasing expression, and that the unique combination of *trans*-loci that any given individual inherits will lead overall to similar gene expression among strains [9]. While we cannot exhaustively compile examples here, the weight of the evidence from diverse sources suggests that this is a shared phenomenon rather than solely an artifact of a particular experimental approach.

All of this suggests that stabilizing selection is likely an important factor in the evolution of gene expression; from Bateson–Dobzhansky–Muller incompatibilities, to developmental systems drift, or antagonistic *cis–trans* mutations, a common theme is compensatory evolution to maintain a consistent output. Recent work by Albert *et al.* (2018) suggested that *trans*-eQTL arise preferentially from certain classes of genes, while local (*cis*) eQTL had little effect in *trans* [10]. It will be interesting in the future to understand how gene network context effects the evolution of genes, and how stabilizing selection is distributed throughout network nodes.

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https://doi.org/10.1016/j.tig.2019.09.008

References

1. Signor, S.A. and Nuzhdin, S.V. (2018) The evolution of gene expression in *cis* and *trans*. *Trends Genet* 34, 532–544
2. Emerson, J.J. *et al.* (2010) Natural selection on *cis* and *trans* regulation in yeasts. *Genome Res* 20, 826–836
3. Fraser, H.B. (2019) Improving estimates of compensatory *cis–trans* regulatory divergence. *Trends Genet* 35, 3–5
4. Zhang, X. and Emerson, J.J. (2019) Inferring compensatory evolution of *cis*- and *trans*-regulatory variation. *Trends Genet* 35, 1–3
5. Lovell, J.T. *et al.* (2018) The genomic landscape of molecular responses to natural drought stress in *Panicum hallii*. *Nat Comm* 9, 5213
6. Szamecz, B. *et al.* (2014) The genomic landscape of compensatory evolution. *PLoS Biol* 12, e1001935
7. Coolon, J.D. *et al.* (2014) Tempo and mode of regulatory evolution in *Drosophila*. *Genome Res* 24, 797–808
8. McGirr, J.A. and Martin, C.H. (2019) Hybrid gene misregulation in multiple developing tissues within a recent adaptive radiation of *Cyprinodon* pupfishes. *PLoS One* 14, e0218899
9. Metzger, B. and Wittkopp, P. (2019) Complex polymorphic genetic architecture underlies *trans*-regulatory variation among strains of *Saccharomyces cerevisiae*. *bioRxiv*. Published online March 4, 2019. https://doi.org/10.1101/566653.
10. Albert, F.W. *et al.* (2018) Genetics of *trans*-regulatory variation in gene expression. *eLife* 7, e35471