Trans-acting small interfering RNA4: key to nutraceutical synthesis in grape development?∗

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The facility and versatility of microRNAs (miRNAs) to evolve and change likely underlie how they have become dominant constituents of eukaryotic genomes. In this opinion article I propose that trans-acting small interfering RNA gene 4 (TAS4) evolution may be important for biosynthesis of polyphenolics, arbuscular symbiosis, and bacterial pathogen etiologies. Expression-based and phylogenetic evidence shows that TAS4 targets two novel grape (Vitis vinifera L.) MYB transcription factors (VvMYBA6, VvMYBA7) that spawn phased small interfering RNAs (siRNAs) which probably function in nutraceutical bioflavonoid biosynthesis and fruit development. Characterization of the molecular mechanisms of TAS4 control of plant development and integration into biotic and abiotic stress- and nutrient-signaling regulatory networks has applicability to molecular breeding and the development of strategies for engineering healthier foods.

Functions of TAS loci
miRNAs and siRNAs are the specificity ‘guide’ RNAs for nucleases of the ARGONAUTE (AGO) class which cleave or otherwise repress mRNAs in a nucleotide sequence-specific manner [1,2]. TAS transcripts drive antisense transcription and feedback loops that amplify the production of siRNAs that in turn negatively regulate target gene expression in trans, an epigenetic process loosely described as ‘transitivity’ important for viral defense [3,4]. There are four known TAS genes – miR173 directs TAS1 and TAS2 siRNA biogenesis (unique to Arabidopsis) [5], whereas miR828 targets TAS4 transcripts and miR390 initiates TAS3 trans-acting small RNAs (tasiRNAs) that are deeply conserved in plants [3,6]. The functional significance of TAS3a–c loci in leaf polarity specification and morphogenesis has been established [7], and is suggested for TAS1a–c/2 as a means of dosage compensation by network repression of the large family of PENTATRICOPEPTIDE

REPEAT genes in Arabidopsis (Arabidopsis thaliana) [8]. The functions of novel TAS candidates in rice (Oryza sativa) [9–11], soybean (Glycine max) [12], grape [13], tobacco (Nicotiana tabacum) [14,15], and Norway spruce (Picea abies) [16] are not known. TAS loci, like MIRNA genes, have evolved independently at different times [17], and the derived tasiRNAs share some biosynthetic steps with miRNAs [18].

The fundamental difference between the miRNA and tasiRNA pathways is the origins of the respective small (~21–22 nt) RNAs: MIRnas are loci whose transcripts can fold back on themselves into imperfect hairpin structures (pre-miRNAs) that are ‘diced’ into duplexes with two nt 3’ overhangs by DICER-LIKE1 (DCL1), whereas TAS gene transcripts are copied after the action of a specific miRNA into long perfectly complementary double-stranded (ds)RNAs diced by DCL4 to produce tasiRNAs, which like miRNAs can target protein-coding genes for silencing. The activity of tasiRNAs as repressors of endogenous target miRNAs in Arabidopsis and maize (Zea mays) involves AGO1 as well as AGO7 (for TAS3a–c) [19,20], and is triggered by a two miRNA hit model [4,21,22] or through initial targeting by longer 22 nt miRNAs [23,24] or a bulged miRNA–miRNA* duplex [25]. In the TAS-specific pathway of RNA interference (RNAi), RNA DEPENDENT RNA POLYMERASE6 (RDR6) copies the miRNA-cleaved TAS product into dsRNA with the help of plant-specific SUPRESSOR OF GENE SILENCING3 (SGS3) [26]. There are distinct cellular pools of AGO1 loaded with miRNAs versus siRNAs, revealed by in planta studies of viral RNA silencing suppressor proteins [27] which underscore the evolutionary complexity of plant RNAi pathways and their perturbation by biotic stresses.

The recent discovery of circular non-coding RNAs (ncRNAs) that function as ‘miRNA sponges’ in animals [28], and miRNA ‘target mimic’ ncRNAs in plants [29–31], raises the profile of TAS genes because these classes of ncRNAs are all examples of systemic innovations (e.g., signals, decoys, guides, scaffolds) that impact upon the circuit topology of miRNAs [32]. Unexpected positive correlations observed between expression of miRNAs and their predicted targets [33] support the notion that there are other processes (e.g., feedback loops and RNA decoys) that can impact upon miRNAs as canonical negative regulators. Intriguingly, predicted target mimics of miR858 [34], MYBL2 and MYB34, regulate the biosynthesis of
anthocyanin and aromatic amino acid-derived indole glucosinolates, respectively [35], that account for ~20% of the carbon fixed by plants to make secondary metabolite anthocyanins, defensive phytoalexins, and structural lignin. As elaborated below, particular MYB transcription factor genes are conserved key effectors of these pathways and are targeted directly by TAS4, which is also subject to a homeostatic autoregulatory feedback loop by the targeted MYBs [36,37].

TAS4, miRNAs, and MYBs

TAS4 was discovered in Arabidopsis [6] where it generates a ~1 kb ncRNA that spawns phased (21 nt registers) sense and antisense small interfering RNAs (tasiRNAs) triggered by miR828, a member of the class of 22 nt DCL1-dependent miRNAs that serve as specificity determinants for the TAS pathway of RNAi. In Arabidopsis, the tasiRNA TAS4-3′-D(−) (an antisense species four registers downstream from the miR828 trigger) targets a set of MYB transcription factors, PRODUCTION OF ANTHOCYANIN PIGMENT1 (PAP1/MYB75), PAP2/MYB90, and MYB113, that regulate the anthocyanin and lignin biosynthesis pathways [38,39] and are inducible by phosphate starvation, sugars, and the plant hormone abscisic acid (ABA) [36,37] important for seed development and stress responses including anthocyanin production [40,41]. The R2R3 MYB class of DNA-binding transcription factors is the most abundant superfamily in plants (126 members in Arabidopsis) [42], and several narrow MYB clades are targeted by miR828/TAS4 and miR858, both of which are involved in flavonoid and lignin biosynthesis [6,43–46]. The convergence of miR828/TAS4, miR858, and possibly miR855 endogenous target mimics on only this handful of MYBs involved in responses to and crosstalk between ABA, sugar, and phosphate signals leading to nutraceutical/lignin synthesis is remarkable from an evolutionary standpoint and warrants further study.

Because miRNAs and TASs act dominantly, they are a particularly good subject for studying the fitness landscape of interactions and genetic robustness in nature and at the bench. By their intrinsic nature as nodes in networks, siRNAs can lend themselves to biotechnological applications such as functional genomics and manipulating productivity and quality in specialty crops, forestry products, and horticultural species by RNAi. Accelerated breeding of crops and trees might be immediately achieved by selecting elite progeny with desirable target haplotypes (e.g., associated with pathogen resistance) regulated by TAS4 siRNAs and/or miR828/miR858, and eventual cogent engineering of traits such as fruit flavor and nutraceutical content, secondary metabolites for biomass feedstocks, beneficial microbe-plant interactions, and improved stress responses and productivity through better management of fertilizer and greater efficiency in phosphate uptake.

In this opinion article I present some direct and phylogenetic evidence that TAS4 may be a key effector controlling flavonoid synthesis, fruit development, and symptoms of a specific bacterial disease, at least in grape. The observations are drawn from genome-wide analyses across angiosperm clades and approach the key question in evolution – how high resiliency might come about through evolution that changes circuit topology gradually, one interaction at a time [47]. Bioflavonoid regulation through target MYBs by TAS4 siRNAs and miRNAs may be a corollary to sugar deposition in flowers and fruits – two prime examples of evolution through facultative symbiosis, whereby the abundance and nutritional compositions of plant organs aid insect pollination and animal seed dispersal, respectively. Because bioflavonoids have salutary effects on cardiovascular function, cancer prevention [48], and neurological processes [49], I speculate that TAS4 may have co-evolved with frugivores because of their derived fitness. Supporting this notion is that mammals generally, and birds to some degree, show a trend of brain expansion over evolutionary time that is absent in other vertebrates [50–53]. Much work remains to substantiate such an idea – for example, can nutraceuticals increase the reproductive longevity of species? What is the relationship between nutraceutical consumption and coloration in birds [54]?

TAS4 evolution

In addition to TAS4-3′-D(−) targeting of MYB113, miR828 also targets MYB113 directly, suggesting a close evolutionary relationship between MYB75/100/113, miR828, and TAS4 that function in a regulatory network or hierarchy that includes autoregulation [36,37]. miR828 is the trigger for production of TAS4 siRNAs, and was originally thought to be unique to Arabidopsis, but has subsequently been found in grape [55], poplar (Populus trichocarpa) [56], cotton (Gossypium hirsutum) [57], cabbage (Brassica rapa) [58], flax (Linum usitatissimum) [59], soybean (Glycine max) [21], common bean (Phaseolus vulgaris) [60], cary sage (Salvia sclarea) [61], melon (Cucumis melo) [62], rose (Rosa multiflora) [63], tomato (Solanum lycopersicum) [64], tobacco (Nicotiana tabacum and N. attenuata) [65,66], cocoa (Theobroma cacao) [67], Chinese apricot (Prunus mume) [68,69], peach (Prunus persica) [70,71], citrus (Citrus sinensis) [72], apple (Malus domestica) [73], hickory (Carya cathayensis) [74], cassava (Manihot esculenta) [75], Brazilian cherry (Eugenia uniflora) [76], Chinese tulp tree (Liriodendron chinense) [77], and Red Sea mangrove (Avicennia marina) [78]. Figure 1 shows a phylogram of the evolutionary relationships for eudicot species which have evidence of TAS4 in their genomes. It is intriguing that all of these species produce flavonoid nutraceuticals, framing the question of evolutionary significance of miR828 and TAS4. We previously found candidate MIR828 sequences in gymnosperms and Trillium camtschatcense, a basal monocot with broad, thin, soft leaves, net venation, and fleshy fruits, and suggested that miR828 or MYB pseudogenes may have evolved into TAS4 by neofunctionalization of an ancient MIR828 possibly important for the diversification of dicotyledonous flowering plants which occurred ~100 million years ago [79] concurrent with diversification of mammals and frugivory [80]. Three other groups also found miR828 and miR858 in Chinese fir (Cunninghamia lanceolata) [81] and Taxus mairei [82], or predicted miR828 and miR858 and MYB targets in Norway spruce (Picea abies) [83]. We predicted that grape MYBA6, an uncharacterized gene, was subject to regulation by VvTAS4, based on conservation at the protein level of
the putative TAS4-3’-D4(−) binding site in the mRNA [37]. Figure 2 shows evidence of selective sweep for the miR828 binding site of TAS4 and for the D4 species of tasiRNAs from numerous eudicot species, supporting the hypothesis that tasiRNA TAS4-3’-D4(−) plays an important functional role in fitness of eudicots, namely the targeting of MYBs involved in bioflavonoid and secondary metabolite biosynthesis.

Transitivity of MYB targets associated with miR828 and TAS4

Interestingly, clusters of ‘phased siRNAs’ (phasiRNAs) [21] map to MYB targets in grape [13,16], poplar [56], sweet potato (Ipomoea batatas) [84], apple [73], peach [71], soybean [21], and cotton (Guan, X. et al., Annu. Meeting Amer. Soc. Plant Biol., P16052; http://ycmcn.com/sites/my.aspb.org/resource/resmgr/Docs/Final_Program_Book.pdf) which have been attributed to miR828 activity, but the functional significance of this transitivity is unknown and evidence for miR828 causality is lacking. Recent reports documented miR828-directed cleavage of both TAS4 and numerous MYBs in apple [73] and peach [71], and invoked the ‘two-hit model’ [22] to explain observed transitivity of MYBs, based on weak predicted binding affinity of a different miR858 [73]. Below I provide evidence from publicly available datasets that the two-hit model does not explain transitivity for two novel MYBs in grape involved in polyphenolic nutraceutical synthesis, but instead is controlled by TAS4.

A conserved TAS4 tasiRNA silences VvMYBA6 and VvMYBA7

By computational analysis of small RNA sequencing (sRNA-Seq) datasets from a wild species of grape, Vitis amurensis Rupe [85], Pinot noir variety of V. vinifera [86], and Merlot leaf, flower, and fruit libraries (GSE28755), compelling evidence of production of phasiRNAs from VvMYBA6 and MYBA7 triggered by TAS4b-3’-D4(−) has been obtained (see Figure S1 in the supplementary material online). Supporting evidence for production of perfect match tasiRNAs to VvMYBA7 phases 0 and 4 (not shown) was found in a Pinot noir degradoide library [86], and has been documented recently for VvMYBA6 and VvMYBA7 in a Merlot variety library (GSM803800) [16]. miR828 likely does not function on these target mRNAs owing to several mismatches at ‘seed’ position 7–11 in plants (see Figure S1B in the supplementary material online) which would preclude binding activity based on functional and structural analyses [87,88]. Almost all the perfect match 21 nt siRNAs mapping to the sense strand of VvMYBA6 and MYBA7 mRNAs are in perfect register downstream (3’) to the predicted cleavage site of TAS4-3’-D4(−) at ‘phase 0’ (see Figure S1A in the supplementary material online). Antisense reads for phases 3, 4, and 7 are found for VvMYBA6 in two libraries (Amur and tendril), whereas phases 4, 8, and 11 are found for VvMYBA7 in three (tendril, fruit, flower) libraries (data not shown). It is interesting that, for a majority of these antisense species, sense species are not observed (see Figure S1A in the supplementary material online), consistent with the notion that the antisense phasiRNAs are loaded preferentially into an AGO or otherwise stabilized at the expense of the sense phasiRNAs. An upstream sense siRNA at phase −8 (see Figure S1A in the supplementary material online) suggests that dsRNA can be generated and diced in both directions from the 3’D4(−) trigger, as has been shown in Arabidopsis [89]. Taken together, these observations show that TAS4 functions in grape to silence MYBA6 and MYBA7 in leaves, flowers, and fruits, and suggest a conserved molecular mechanism for the observed transitivity of MYB targets in numerous other dicots which have TAS4 (Figure 1).

Expression patterns of TAS4abc tasiRNAs elucidate gene activities

There are three TAS4 loci in grape (Figure 2) which generate isoforms of TAS4-3’-D4(−) with implications for differential MYB cleavage activities (see Figure S1B in the supplementary material online). TAS4a and TAS4b map to chromosome (Chr.) 14 and have expressed sequence tag (EST) evidence for expression, whereas Vvi-TAS4c was previously mis-annotated as Vvi-miR828b [55], a contention supported by its poor secondary hairpin structure (three gaps and three mismatches in the mature duplex;
www.mirbase.org) and similar extended homologies to those observed between Arabidopsis TAS4 and MIR828 [37]. All three isoforms are expressed, based on identification of perfect-match unique siRNAs in eight different RNA-Seq libraries, with TAS4c expression being lower than TAS4a or TAS4b by ~ three orders of magnitude and on a par with miR828 and miR828* abundance (see Figure S2C,D in the supplementary material online). The high reproducibility of abundances between libraries of the TAS4b species D1(+) and D4(−) (r = 0.78 and 0.77, respectively) compared to TAS4a D1(+) and D4(−) species (r = 0.67 and 0.20) supports the notion that TAS4b-3D4(−) is the trigger for the observed VvMYBA6 and MYBA7 phasiRNAs, consistent with a higher binding affinity of TAS4b-3D4(−) over that of TAS4a-3D4(−) (see Figure S1B in the supplementary material online).

VvMYBA6 and VvMYBA7 functions in bioflavonoid synthesis
The novel VvMYBA6, VvMYBA7, and other MYBs targeted by miR828/TAS4 comprise only ~7% of all R2R3 MYBs encoded in grape and ~2% of those in Arabidopsis. Those MYB targets of TAS4/miR828, remarkably, are most homologous [90] to those MYB transcription factors (F1, PA1/2, VvMYBA1–3) that have been shown to be effectors, respectively, of flavonol, proanthocyanidin, and anthocyanin pathways in apple [73], grape [91–95], and Arabidopsis (clades S5–S7) [96], the latter clade which is conspicuously absent (as are miR828 and TAS4) from monocots [97]. Figure 3 shows a phylogram of TAS4 – and miR828 [86] – validated MYB targets in grape, peach, and Arabidopsis [6], plus an outlier MYB from maize most homologous to the S6 clade [97]. Interestingly, the TAS4–validated or –predicted targets (in peach) are more diverged from their nearest miR828–targeted paralogs, suggesting they have evolved novel functions related to nutraceutical metabolism. Identification of MYB proteins involved in the control of secondary metabolism and development within subgroups across model species has shown that homologs participate in the same processes [98]. The central positioning of VvMYBA6 and VvMYBA7 between S6 and S5/S7/S3 clades for anthocyanin and proanthocyanidin/flavonol/lignin regulation [96], respectively, supports their potential function in each of these pathways.

Correlative evidence for VvMYBA6 and VvMYBA7 functions
It is significant that non-small RNA targets VvMYBA1–3 isoforms clustered on Chr. 2 do not account for all observed
An anthocyanin phenotype [98] or flavonoid pathway [94], suggesting that VvMYBA6 and VvMYBA7 are effectors in a hierarchy of MYB proteins [96] controlling fruit pigment synthesis. Association mapping [99] demonstrated that only 62% of anthocyanin content variation could be attributed to the VvMYBA1–3 isogenes in a Syrah x Grenache F1 pseudotestcross. This finding is consistent with the hypothesis that other regulators (viz., VvMYBA6 and VvMYBA7 on Chr. 14 that are targets of Vvi-TAS4b) may control värison, the seed maturation phase in grape berry development that coincides with transition from acid to sugar accumulation, production of volatiles and antioxidant tannic pigments, and breakdown of chlorophyll and methoxypyrazines. Circumstantial evidence for MYB genes controlling fruit and leaf development are the pleiotropic phenotypes of Pinot Meunier sport ‘Samottot’, which has glabrous apices and leaves, a well-known phenotype caused by MYB mutations in many species [90], lower fruit set, lower yields, and higher sugar content than compared to Pinot Meunier or Pinot noir [100].

Nutraceutical properties and biosynthesis

Flavonoids (Latin: flauus, ‘yellow’) are secondary polyphenolic metabolites involved in many aspects of plant development, auxin transport [101], ABA biosynthesis [40], and structural reinforcement. They were called ‘vitamin P’ (not to be confused with the current euphemism for the antidepressants Prozac and Paxil) early in the last century owing to their effects on the ‘permeability’ of vascular capillaries. They contribute attractive colors to fruits and flowers that aid in seed and pollen dispersal, and adaptation to environmental conditions such as cold, phosphate starvation, UV stresses, pathogen attack, allelopathy, mediation of stigma–pollen interactions, and signaling to mutualist microbes for nitrogen fixation [102–104]. Regulation of flavonoid/polyphenolic biosynthetic genes is tightly organized in a spatial and temporal way orchestrated by ternary complexes involving transcription factors from the R2R3-MYB, basic helix–loop–helix (bHLH), and WD40 classes [104–107]. Flavonoids, stilbenes, and polyphenolics (tannins) are components of fruit that impart color, aromas, and flavor enhancers associated with purported nutraceutical benefits (e.g., antioxidant and anti-inflammatory properties) and in-mouth tactile sensations (astringency). They comprise four main classes of compounds (Figure 4) whose biosynthesis during fruit development and stress response is mediated by genes encoding enzymes for committed branch pathways from 4-coumarate [the product of phenylalanine (phe) catabolism by phenylalanine ammonia-lyase (PAL)]: flavonols by flavonol synthase (FLS) and UDP-glucuronic acid/galactose:flavonol-3-O-glucuronosyltransferases (GT5/6), anthocyanins...
by UDP-glucose:flavonoid 3-O-glucosyltransferases (UFGT), and proanthocyanidins by leucoanthocyanidin reductase (LAR) and anthocyanidin reductase (ANR), and anthocyanins (UDP-glucose:flavonoid 3-O-glucosyltransferase, UFGT). Arabidopsis, grape, and peach MYBs subject to miR828 [71,86], miR858 [6,43–46], or TAS4 regulation and inferred (from Figure 3) to regulate known [96,110,111] or predicted polyphenolic pathways are shown. eTM, predicted endogenous miR858 target mimics [34]. See supplementary material online for description of miR858 results. Figure modified from Cohen et al. [109]. Other abbreviations: C4H, cinnamate 4-hydroxylase; CHI, chalcone isomerase; CHS, chalcone synthase; DFR, dihydroflavonol-4-reductase; F3’/H, flavonoid 3’5’-hydroxylase; GST, glutathione S-transferase; HCT, leucoanthocyanidin dioxygenase; PAL, phenylalanine ammonia-lyase; Phe, phenylalanine; S5–S13, subgroups 5–13.

**Potential environmental forces driving the evolution of TAS4 and target MYBs**

Flowering plants presented Darwin with the most extreme exception to his strongly held notion *natura non facit saltum*, ‘nature does not make a leap’, resulting in his describing plant evolution as an ‘abominable mystery’ [116]. Defense against pathogens is one of the most potent drivers of evolutionary change in host organisms. Recent reports show that, similarly to viruses, various types of fungi and bacterial pathogens have evolved RNAi suppressor proteins important for disease etiologies [117–119]. The bacterium *Xylella fastidiosa* (XF) is the cause of Pierce’s disease in grapes and is a major threat to fruit, nut, and coffee groves. Obvious symptoms are anthocyanin accumulation in concentric zones of leaves and in xylem sap, and shriveling of undeveloped berries [120]. XF infection results in significant and reproducible decreases in leaf elemental phosphorus in several species including grape [121]. Phosphorus is the second most limiting macronutrient to plants affecting crop quality and production; the classic symptom of inorganic phosphorus (P$_i$) deficiency is anthocyanin accumulation. P$_i$ treatment induces systemic acquired resistance to pathogens and shares some common early steps (RNAi?) with tobacco necrosis virus acquired resistance [122]. P$_i$ deficiency causes delays in grape berry maturity and reduced berry size [123], but the molecular mechanisms are unknown.

In the context of XF disease symptoms of wilting and berry shrivel, it is interesting to note that vascular parenchyma cells at the boundary between xylem and phloem bundles are the site of drought-inducible ABA biosynthesis and transport [124] by the action of NINE-CIS-EPOXYCAROTENOID DIOXYGENASES1/4 (NCED1/4), the rate-limiting step of ABA biosynthesis, which is also upregulated during berry development [125]. Transport of ABA, TAS4 tasiRNAs, and miRNAs through the vasculature may be important for the same physiological processes driving evolution of plants and their ecological partners/pathogens. For example, phosphorus deficiency marked by miR399 induction and movement through the vasculature [126] has been identified as an etiological link to citrus Huanglongbing disease [127], caused by the Gram-negative bacterium Candidatus Liberibacter. Iron and phosphorus homeostasis are coupled [128], consistent with the notion that miRNAs regulating iron transport [119] and phosphate signaling could be targets of
pathogens. Another functional insight into a longstanding problem of why mutants affecting sRNA biogenesis and activity are ABA hypersensitive [129] is the recent finding that ALTERED MERISTEM PROGRAM 1, a negative regulator of ABA and drought response [130,131], mediates translational inhibition by miRNAs at the endoplasmic reticulum [132], which appears to be a site of action of AGOs [133–135].

Roles for ABA, Nb, and miR828/TAS4 in XF disease etiology have not been proposed. Because TAS4 expression is induced by phosphate starvation, sugars, and ABA in Arabidopsis [36,37], I hypothesize that TAS4 plays a role in disease etiology of Pierce’s disease and berry development by VvMYBA6 and VvMYBA7 regulation. Additional evidence is the mapping of a quantitative trait locus for Pierce’s disease resistance [136] very near to VvMYBA6/7 and a validated miR828 MYB target GSVVT1032467001 [86] on Chr. 14 (Figure 3). Taken together with the knowledge that microbes disrupt miRNA and RNAi pathways, the recent observations that grapevine leafroll-associated virus infection increases miR828 accumulation [137], blocks berry development [125], and phenocopies symptoms of Pierce’s disease [138] is strong correlative evidence of TAS4 and miR828 function in veraison and Pierce’s disease because viruses have evolved suppressor proteins that interfere with sRNA formation or activity [27].

I speculate that mycorrhizal arbuscular associations between plant roots and fungi, where the plant provides carbohydrates to the fungus in return for phosphate, may be evidence for the hypothesis of TAS4/miR828/MYB coevolution related to Nodulation factor (NodD) induction by flavonoids secreted by hosts in Rhizobium symbiosis [139,140]. Others have recently shown that several miRNA families function as master regulators of defense response by production of phasiRNAs [16,21,141] targeting the large family of NB-LRR genes, which together with MYBs are classified as PHAS loci based on the miR828-associated [13,21,56,73,84] and TAS4-triggered transitivity of VvMYBA6 and VvMYBA7 shown here. The diversity and conservation of PHAS loci across plant taxa, including soybean miR828 target MYBs [21,142], and their loss in virus- and bacteria-infected tissues [141,143] that results in pathogen susceptibility, demonstrates broad functions of miRNA PHAS effectors as master regulators [21]. This process amounts to exploitation by plants of pathogen-derived suppressors of RNAi to achieve inducible expression of defense- and/or development-related genes. It is remarkable that sRNA effectors DCL2, SGS3 (in legumes and soybean/grape, respectively), and AGO1 can spawn phasiRNAs themselves [16,21,144–146] and have acquired some miRNA target sites independently [21]. Furthermore, AGO2 and DCL1 are targeted by miR403 and miR162, respectively [3,147], and DCL1 encodes MIR838 in one of its introns [6], underscoring the evolutionary importance of recruitment of RNAi into homeostatic feedback circuits and ncRNAs.

Implications for healthier food and sustainable agriculture

Detailed, genome-enabled understanding of gene networks and the life cycles of RNAs can be translated into tools and resources for the development of crops that respond efficiently to local and changing conditions. Quality improvements depend on applying new genetic insights and new technologies to accelerate breeding through improved genotyping and phenotyping methods, and by increasing the available genetic diversity in germplasm [148,149]. It takes years of breeding and testing to obtain a new grape variety, and substantial space to grow out seedlings to a bearing year for phenotyping. Breeding is not widely practiced in viticulture because markets, traditions, and statutes dictate cultivar choice, thus varieties and clones lack recombination and the resulting opportunity to select for adaptability, for example for Pierce’s disease resistance. Molecular breeding can have significant impacts on specialty crops by: (i) applying deep knowledge from model species to reference genomes and expression datasets, (ii) facilitating optimal selection of parents and immediate selection of elite progeny with multiple desirable traits, (iii) circumventing biological and societal limits to genetic engineering, (iv) accessing abundant genetic variation, and (v) rapid linkage disequilibrium allowing single nucleotide polymorphisms of genes (e.g., VvMYBA6 and VvMYBA7) to be directly associated with phenotypes.

In the post-genomics era, the broad goals of translational science are to integrate growth and development processes to the environmental cues that shape an individual or species by evolution. Suppression and induction of the host sRNA silencing systems likely plays some fundamental role in plant–microbe interactions [21,119,141,143,150]. The availability of reference gymnosperm genomes such as P. abies [151], P. glauca [152], Pinus contorta, and Pinus resinosa [37] will facilitate future studies on the evolution of MIR828, TAS4, and MIR858 [16] and possible applications of the target genes to manipulate lignin biosynthesis. Interestingly, a recent report showed the possible convergence between P. abies, Amborella, Medicago, poplar, cotton, and grape for silencing of nucleotide-binding site–leucine-rich repeat (NBS–LRR) genes (and conservation of TAS4/miR828-targeted MYBs; my observations) by production of 21 nt phasiRNAs [16], demonstrating the deep evolutionary forces that have shaped the function of miRNAs and tasRNAs as master regulators of MYB and other large gene families in plants. Knowledge of the molecular mechanisms of TAS4 function will provide cogent means to manipulate productivity and quality in specialty crops, forestry products, and horticultural species by RNAi and transgenic technologies. The TAS4 mechanism may be important for other plant–pathogen interactions and may have significance for plant–microbe and frugivore–plant co-evolution. Detailed characterization of miR828 and TAS4 function in seed plants will reveal fundamental processes and mechanisms in plant biology which may change our understanding of healthy food. The recent publication of the National Institutes of Health (NIH) Microbiome Project [153] nicely frames the systems approach to transform understanding of the nature of human relationships (commensal, mutual, or parasitic) with our flora. I submit that ‘flora’ includes those plants that animals eat and that intestinal microorganisms process for immediate and generational fitness. For example, it is now a tractable question to address: how
did evolution adapt formation of flavonoid pathways that transform our preferred nutraceutical staples of cocoa and grape?

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Appendix A. Supplementary data

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