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Comprehensive analysis of tandem amino acid repeats from ten angiosperm genomes

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Abstract

Background: The presence of tandem amino acid repeats (AARs) is one of the signatures of eukaryotic proteins. AARs were thought to be frequently involved in bio-molecular interactions. Comprehensive studies that primarily focused on metazoan AARs have suggested that AARs are evolving rapidly and are highly variable among species. However, there is still controversy over causal factors of this inter-species variation. In this work, we attempted to investigate this topic mainly by comparing AARs in orthologous proteins from ten angiosperm genomes.

Results: Angiosperm AAR content is positively correlated with the GC content of the protein coding sequence. However, based on observations from fungal AARs and insect AARs, we argue that the applicability of this kind of correlation is limited by AAR residue composition and species' life history traits. Angiosperm AARs also tend to be fast evolving and structurally disordered, supporting the results of comprehensive analyses of metazoans. The functions of conserved long AARs are summarized. Finally, we propose that the rapid mRNA decay rate, alternative splicing and tissue specificity are regulatory processes that are associated with angiosperm proteins harboring AARs.

Conclusions: Our investigation suggests that GC content is a predictor of AAR content in the protein coding sequence under certain conditions. Although angiosperm AARs lack conservation and 3D structure, a fraction of the proteins that contain AARs may be functionally important and are under extensive regulation in plant cells.

Background

Tandem amino acid repeats (AARs), or homopeptides, are protein segments that comprise a continuous array of identical residues. As repetitive DNA is very abundant in eukaryotic genomes [1], AARs are frequently found in the proteomes of eukaryotes [2-4]. These simple peptides can be encoded by tandem repeats of the same codon, which are vulnerable to point mutations, or by a mixture of synonymous codons [5]. These repetitive codon tracts are primarily introduced by either replication slippage [6] or recombination [7].

AARs are often situated in disordered regions of proteins that lack regular 3D structures [8]. Nevertheless, over the past two decades, increasing attention has been paid on biological importance of AARs (see [9] for a recent review) which have long been regarded as junk sequences [10]. AARs have been shown to be associated

with several diseases. For example, the expansion of a glutamine repeat may induce Huntington's disease and other neuro-degenerative diseases [11]. Beneficial effects of AARs have also been uncovered. An example is the glutamine repeat that appears in a key component of the biological clock in the fungus *Neurospora crassa* White Collar-1. This AAR was suggested to control circadian period length [12]. Large-scale analyses indicate that AARs tend to participate in the regulation of transcription [8,13,14] and are frequently involved in protein-protein interactions [15].

AARs are highly polymorphic and fast-evolving sequences [9,16]. In line with the accelerated rate of evolution for protein segments that are in or near AARs, selective constraints are thought to be relaxed around AARs [8,17]. There are competing interpretations of the rapid evolution of AARs. Some believed that AARs evolve in a largely neutral fashion [18], partly as a consequence of the balance between replication slippage and point mutations [6]. Based on shifts of the frequency distribution of coding tri-nucleotide repeats compared to

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that of non-coding tri-nucleotide repeats, Mularoni *et al.* proposed that selection plays an important role in AAR evolution [19]. There is also evidence for positive selection on the AARs from case studies of a few mammalian genes [20,21].

The frequency and size of AARs show inter- and intra-species variation both in large-scale comparisons [17] and in studies focused on vertebrates [8,13,19] or fruit flies [22]. The causal factors underlying this variation are still a matter of dispute [13,17], and some have attributed them to GC content bias [16,18,23]. In plants, repetitive DNA is widely used as a genetic marker, and its variation among transcripts has been observed [24]. Nevertheless, in contrast to animal AARs, plant AARs have not been intensively investigated, except in a recent report based on two model plants, *Arabidopsis* (*Arabidopsis thaliana*) and rice (*Oryza sativa*) [14].

In this report, we revisit the questions surrounding AARs in plants in the light of current accumulation of plant whole genome sequences. A comparison of 1-to-1 orthologous proteins between ten sequenced angiosperm species revealed a positive correlation between AAR content and GC content, a finding that may be applicable to some other non-metazoan taxa. Other factors related to AAR content variation were also discussed. We attempted to summarize the functions of conserved long angiosperm AARs and their host genes in the context of the rapid evolution of AARs and their flanking regions. Our analysis also supports the idea that AARs are widely associated with protein structural disorder. Finally, we suggest that transcripts of repeat-containing proteins (RCPs) are under various levels of regulation in plant cells.

Results and Discussion

Correlation between AAR content and GC content in plants

We predefined an AAR as an uninterrupted run of four or more identical amino acids. Our 1-to-1 ortholog dataset contains 4, 281 groups of proteins from six eudicots [25] [26] [27] [28] [29] [30] (*A. thaliana*, *Carica papaya*, *Glycine max*, *Malus × domestica*, *Populus trichocarpa* and *Vitis vinifera*) and four monocots [31], [32] [33] [34] (*Brachypodium distachyon*, *O. sativa*, *Sorghum bicolor* and *Zea mays*). The abbreviations used and general information about the genome sequence data of these ten species are given in Table 1. All of the following analyses within the spectrum of angiosperm were based on this dataset, unless stated otherwise.

Similar to other eukaryotes, angiosperm proteins are enriched in AARs (0.84 AAR per protein on average). Because short AARs may be derived from the interruption of a long AAR, we used repeated residues per 1000 amino acids (RRPK, Repeated Residues per Kilo Amino Acids, defined as the ratio of the total AAR length to the

protein length, multiplied by 1000) to represent the AAR content of a protein or protein segment. For example, the RRPK of peptide “QQQQSTWQQQAAE” is $9/15 \times 1000 = 600$. There is a nearly 3-fold variation in RRPK between these ten species, with values that range from 5.06 (grape) to 15.25 (rice). It is somehow striking that large genomes or proteomes do not necessarily have higher RRPK.

We noticed that orthologs in monocots have an elevated RRPK and that the GC content (of their coding sequences) is also higher. A strong linear correlation between RRPK and GC content can be observed (Pearson's correlation coefficient, $r = 0.87$, $p = 1.1 \times 10^{-3}$; Figure 1A), although no positive correlations observed within eudicots or monocots, partially due to a limited taxonomy coverage of the available genomes. To test whether this phenomenon is specific to angiosperms, we also tested a set of 1-to-1 orthologs between *Arabidopsis*, moss (*Physcomitrella patens* [35]) and green algae (*Chlamydomonas reinhardtii* [36] and *Volvox carteri* [37]) and also observed a strong positive correlation ($r = 0.97$, $p = 2.5 \times 10^{-2}$; Figure 1B). Moreover, within each angiosperm species, a weak but significant positive correlation between protein RRPK and GC content was observed ($r = 0.15\sim 0.43$, $p < 1.0 \times 10^{-10}$; Figure 1C and 1D). Finally, when protein sequences were equally divided into three parts, an accumulation of AARs in the N-terminus could be observed, which is in line with the elevated GC content of this region (Figure 1E). However, the C-terminus also had a higher RRPK in comparison with the middle segments for all of the species except rice (Figure 1E), similar to what has been observed in animals [16,22]. It is worth mentioning that AARs have been proposed to follow a negative gradient from the N-terminus to the C-terminus in plant proteins [14]. One explanation for this discrepancy is that there are differences in the definition of the protein terminus between the two studies. Zhang et al. measured the absolute position of the AARs [14] and thus may have counted all of the repeats that are present in short proteins as being in the N-terminus.

High GC content favors replication slippage and, thus, the generation of AARs, which has been proposed in a number of reports [13,16,38]. On the other hand, GC content has also been treated as an indicator of the local recombination rate [39]. Angiosperm species with high recombination rates (i.e., exactly, higher average centi-Morgan per megabase), such as *Arabidopsis* and rice [40], are relatively enriched for AARs (Figure 1A). We attempted to test the association of RCPs with recombination hotspots in *Arabidopsis* by exploiting publicly available extensive SNP data [41]. A total of 293 putative hotspot neighboring genes (see Materials and Methods) were identified. At the whole proteome level, the fraction of hotspot neighboring genes in genes encoding RCPs is higher than that in genes not encoding RCPs in our

Table 1 Summary of the ten angiosperm genomes included in this study

Organism	Abbreviation	Genome size (Mbp)	Number of proteins	Reference
Eudicot				
<i>Arabidopsis thaliana</i>	Arabidopsis	120	27, 1692	25
<i>Carica papaya</i>	papaya	372	27, 181	[26]
<i>Glycine max</i>	soybean	1, 100	46, 260	[27]
<i>Malus × domestica</i>	apple	742	62, 997	[28]
<i>Populus trichocarpa</i>	cottonwood	550	40, 664	[29]
<i>Vitis vinifera</i>	grape	500	26, 092	[30]
Monocot				
<i>Brachypodium distachyon</i>	false brome	272	25, 525	[32]
<i>Oryza sativa</i>	rice	382	56, 795	[31]
<i>Sorghum bicolor</i>	sorghum	735	27, 561	[33]
<i>Zea mays</i>	maize	2, 500	32, 606	[34]

The genome size is the total length of chromosomal sequences. A gene with multiple protein products was counted as one protein in this table.

dataset (1.3% vs. 0.93%, Fisher's exact test, $p = 0.01$), indicating that the influence of recombination on the AAR frequency, although limited, cannot be excluded.

The relationship between AAR content and GC content is distorted by AAR composition and life history traits

There is controversy over the relationship between GC content and AAR content. A positive correlation between GC content and AAR content has been observed in some mammalian species [16], while in a wider spectrum of taxa, a negative correlation was proposed [18]. Thus, we examined the relationship between GC content and AAR content within two additional taxonomic groups from distinct eukaryotic clades, *Sordariomycetes* fungi and *Diptera* insects (summarized in Table S1 and Table S2 in Additional File 1, respectively). These 1-to-1 ortholog groups contain 4, 047 and 3, 680 proteins from fungi and insects, respectively.

A positive correlation was observed in fungi ($r = 0.78$, $p = 2.2 \times 10^{-2}$; Figure S1 in Additional File 1). Both plants and fungi harbor a large fraction of AARs that are encoded by GC-rich codons (39.3% and 36.9% on average, respectively; see also Figure 2), including alanine, glycine and proline repeats. Removal of these three types of AAR would diminish the positive correlation ($p = 0.19$ and 3.6×10^{-2} for plants and fungi, respectively). In contrast, fruit flies, whose RRPK shows a negative correlation with GC content ($r = -0.72$, $p = 8.9 \times 10^{-3}$, Figure S2 in Additional File 1), were relatively enriched for glutamine repeats (22.1% on average) but not for types of AAR that were encoded by GC-rich codons (31.7% on average), indicating the influence of AAR composition on the relationship between AAR content and GC content. Additionally, no significant correlation was identified in the insect group as a whole because mosquito proteins, which contain fewer glutamine repeats (15.9% on average) than their

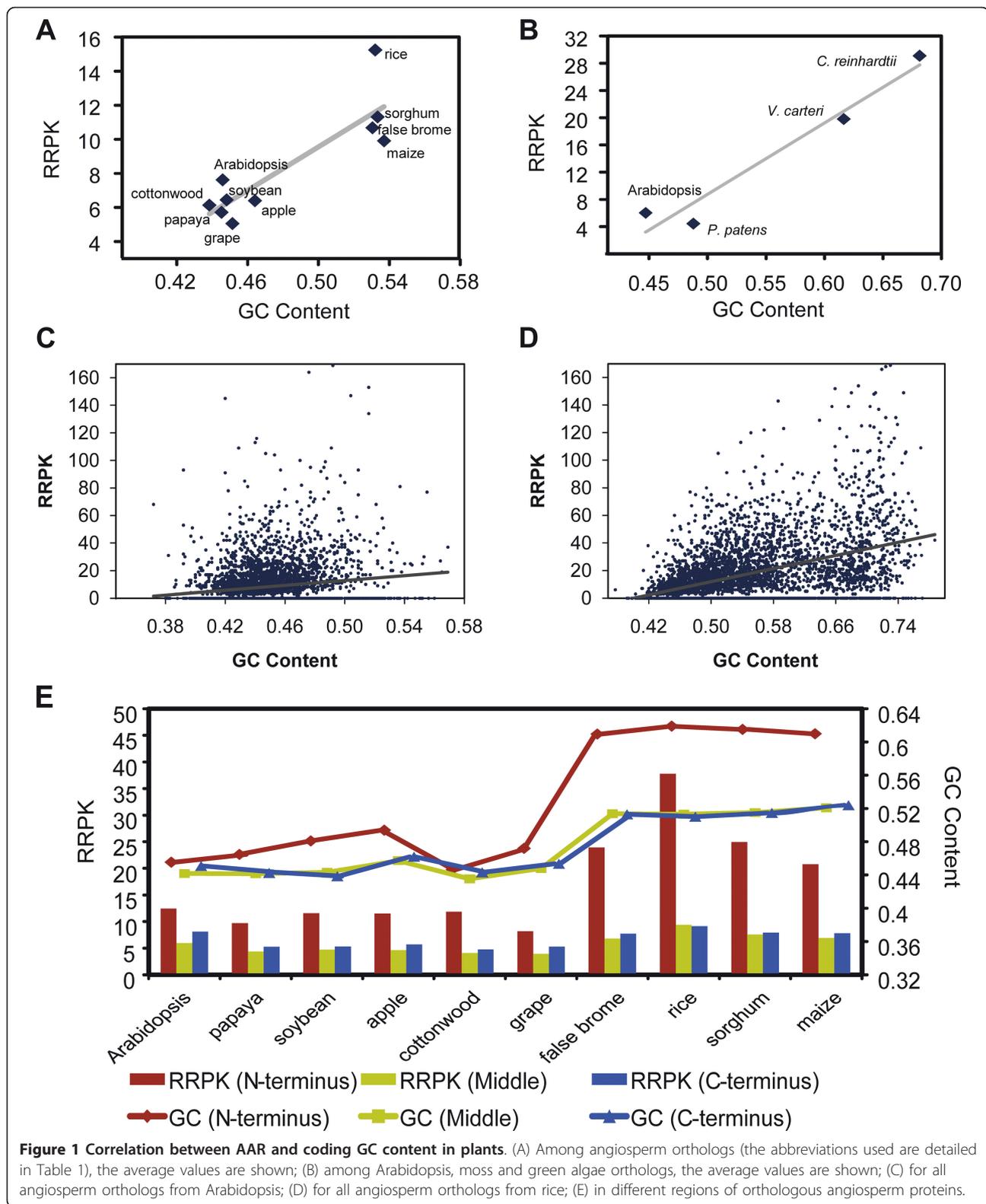
fruit fly orthologs, seem to accumulate AARs with elevated GC content (Figure S2).

However, GC content and residue composition are not the only factors that influence the AAR content. For example, both maize and grape have relatively higher GC content (Figure 1A) and fewer glutamine repeats (< 4.5%), but their RRPK are the lowest among eudicots and monocots, respectively (Figure 1A). These two species share at least two life-history traits: (1) relatively "large body size" and (2) cross-pollination. We intentionally used quotes in this paragraph to emphasize that, owing to the high plasticity of plant development, caution should be used when linking body size to genomic signatures. Conversely, self-pollinating "small grasses", such as *Arabidopsis* and rice, have abundant AARs. Rice orthologs are so abundant in AARs that they appear as an outlier in the linear regression (Grubbs's test, $p = 0.015$; Figure 1A). A recent survey [24] showed that barley (*Hordeum vulgare*) has a higher fraction of RCPs than sugarcane (*Saccharum officinarum*). Interestingly, the former is a self-pollinating "small grass", whereas the latter is a cross-pollinating "large grass". In all, life-history traits are seeming cofactors of AAR content and deserve re-examination when more angiosperm genome sequences become available.

Taken together, the driving force shaping the presence and content of different types of AARs or low-complexity sequences appear to be complex, as was recently suggested for *Plasmodium falciparum* [42]. The interplay between GC content, AAR residue composition and life-history remains complicated and needs further investigation.

Rapid evolution of angiosperm AARs and their functions

Like their animal counterparts [8], many angiosperm AARs have not been conserved over a long period of evolution. This trend is indicated by the observation that approximately 75% of AARs fail to align to the corresponding



region in any of the other orthologs (i.e., the corresponding regions in the multiple alignment of other orthologs are filled with gaps). A faster rate of evolution, as estimated by

average dN/dS ratio of the AAR flanking regions in comparison to RCPs as a whole, was also observed (Mann-Whitney U test, $p < 1 \times 10^{-9}$ for all species; Table S3 in

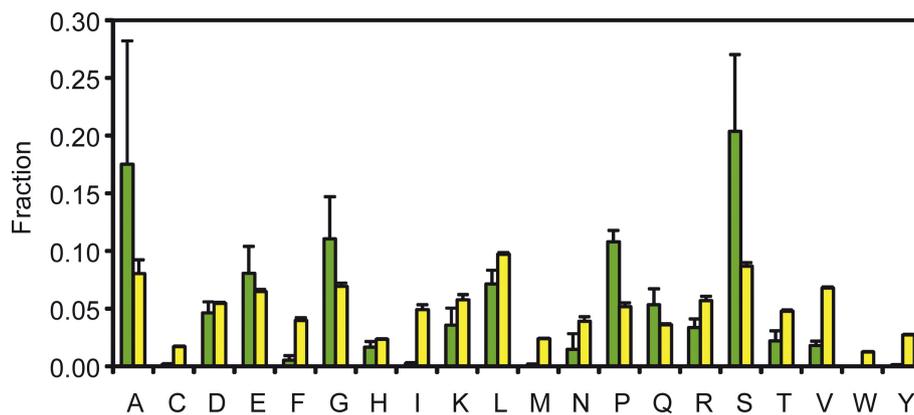


Figure 2 Residue composition in AARs and across the entire set of orthologs. The fractions of residues from AARs and from the entire set of orthologs are shown as green columns and yellow columns, respectively. Only positive error bars are shown.

Additional File 2), supporting previous work that was conducted in other species [17,43]. Although it has been suggested that purifying selection is relaxed in flanking regions of AARs, only about 3% of these flanking regions show signs of positive selection, i.e., a dN/dS greater than 1 (Table S4 in Additional File 2). Assuming that the fraction of regions under positive selection would be underestimated by the average dN/dS, we also calculated pairwise dN/dS for three pairs of species: (1) Arabidopsis and papaya, (2) rice and false brome and (3) maize and sorghum. The fractions were still limited (Welch's t-test, $p > 0.05$; Table S4), indicating that positive selection is not a ubiquitous evolutionary process in AAR flanking regions.

Some pathogenesis-related proteins from *P. falciparum* were enriched with long asparagines repeats, and this phenomenon has been proposed as a reflection of selection against human immune systems [44]. We tested the function enrichment of rice RCPs with higher (> 20) RRPK, but did not find specific terms other than the regulation of transcription (detailed data not shown). Even though some molecular functions are enriched for RCPs [14], a loss of conservation can make the detailed genetic or biochemistry assays of the function of the AARs difficult. Fortunately, a few conserved long AARs still exist and can serve as targets for further experimental analysis. We chose conserved long AARs based on two criteria: (1) longer than seven residues and (2) the corresponding regions from at least eight other orthologs could be aligned with a multiple sequence alignment identity that is not lower than 50%. We then mapped these 18 AARs onto the corresponding regions in the Arabidopsis orthologs (Table 2 and Table S5 in Additional File 2) and found a few with indirect evidence [45][46][47][48][49] of being functionally important in Arabidopsis (Table 2). For example, truncation of the N-terminal acid domains containing long serine repeats from ABI3 (ABA INSENSITIVE 3) largely abolishes its

activity [45]. Similarly, truncation of a domain with a glutamine rich region in SEU (SEUSS) can cause severe developmental defects [46]. Nonetheless, the functions of most of these 18 AARs and even the functions of their host genes remain to be surveyed (Table S5).

Angiosperm AARs tend to be structurally disordered and regulated at the transcript level

One explanation for the rapid evolution of AARs is that AARs tend to be disordered and thus lack structural constraints during evolution [50], an idea recently stressed by Simon and Hancock [8]. Indeed, disorder-promoting residues [51] such as serine, alanine, glutamine, glycine and proline are overrepresented in angiosperm AARs in comparison with the entire set of orthologs (Mann-Whitney U test, $p < 0.01$; Figure 2). To test whether angiosperm AARs tend to be fully disordered (i.e., embedded in disordered regions), we used PONDR[®] VSL2B [52] and IUPred [53] to predict disordered regions in our set of orthologs. These two software packages take advantage of distinct features and strategies to predict ordered/disordered status for each residue. PONDR[®] VSL2B and IUPRED disagree about the absolute fraction of fully disordered AARs (Table 3). In an independent benchmarking test with default cutoffs [54], IUPRED achieved 59.5% sensitivity with 95.6% specificity; the sensitivity of VSL2B reached 75.5%, but its specificity was 79.4% (the sensitivity of VSL2B was shown to be approximately 5% lower compared with VSL2 at a similar specificity [52]). Thus, this disagreement reflects the different trade-offs between false positives and false negatives that are inherent to different predictors. We tested whether the tendency of AARs to be fully disordered was significant by counting how many times, in 1000 trials, randomly selected equal length segments in RCPs showed a higher fraction of fully disordered segments. None of the 1000 random trials in

Table 2 Putative functions for conserved long AARs in Arabidopsis

Protein	Type	Description (gene/AAR)	Clue
AT3G24650.1	S	<i>ABI3</i> is a key component of the ABA signal transduction pathway./See text for details of AAR function.	Biochemical [45]
AT1G43850.1	Q	<i>SEU</i> , together with <i>LUG</i> (<i>LEUNIG</i>), controls the development of several organs./See text for details of AAR function.	Phenotypic [46]
AT4G32551.1	Q	<i>LUG</i> , see above./The AAR is thought to be involved in the assembly of transcriptional co-repressors.	Speculation only [47]
AT5G67470.1	P	<i>FH6</i> (<i>FORMIN HOMOLOG 6</i>) binds profilin and is involved in actin-nucleating activity./The AAR may directly contribute to its binding activity.	Speculation only [48]
AT1G25540.1	Q	<i>PFT1</i> (<i>PHYTOCHROME AND FLOWERING TIME 1</i>) is a transcription factor that controls the flowering time./The AAR may be involved in transcriptional activation.	Speculation only [49]

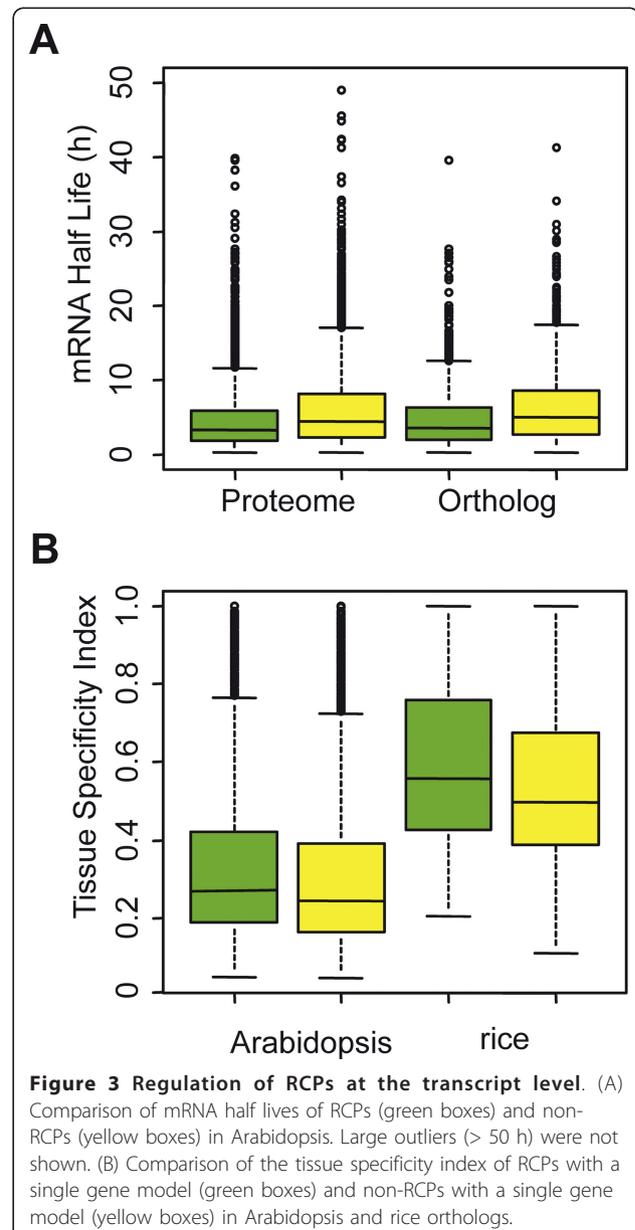
any of the 10 species resulted in a higher fraction of fully disordered segments, suggesting that the fraction of fully disordered AARs is indeed significantly high (i.e., $p < 0.001$).

The tendency of AARs to be disordered indicates that RCPs may be under extensive regulation in plant cells, to prevent them from inducing dosage-sensitive phenotypes, as protein structural disorder was suggested to be associated with dosage-sensitive phenotypes in model metazoans [55]. The first line of evidence comes from the observation that transcripts encoding RCPs decay more quickly in Arabidopsis, similar to the transcripts of disordered proteins in human [56]. Both within orthologs and at the whole proteome scale, transcripts of RCPs have shorter half-lives than the rest (Welch's t-test, $p < 2.2 \times 10^{-16}$; Figure 3A). A similar result was obtained by comparing RCPs with non-RCPs encoded by GC-rich (GC content not smaller than 0.45) coding sequences (Welch's t-test, $p < 2.2 \times 10^{-16}$). Second, in the whole proteomes of Arabidopsis and rice, the fractions of alternatively spliced genes (i.e., genes with multiple gene models) were higher for RCPs than for non-RCPs (17.7% vs. 15.9% and 12.1% vs. 10.9%, respectively; Fisher exact test, $p = 0.021$ and 7.4×10^{-3} , respectively). Moreover, protein segments encoded by alternatively

Table 3 Fraction of fully disordered AARs

Abbreviation	VSL2B Fraction	IUPred Fraction
Arabidopsis	82.5%	48.0%
papaya	77.8%	41.6%
soybean	78.6%	46.3%
apple	67.9%	45.0%
cottonwood	79.6%	45.5%
grape	73.5%	39.7%
false brome	80.2%	44.6%
rice	81.5%	45.6%
sorghum	80.2%	44.9%
maize	78.4%	42.4%

All fractions of fully disordered AARs were significantly high in comparison with random samples ($p < 0.001$).



spliced exons showed significantly higher repeat content than those encoded by constitutively spliced exons (Welch's t-test, $p = 1.1 \times 10^{-28}$ and 5.1×10^{-47} for Arabidopsis and rice, respectively; Table S6 in Additional File 2), which further supports the association between AARs and alternative splicing that has been proposed by Haerty and Golding based on their observations in metazoans [57]. For Arabidopsis orthologous RCPs and rice orthologous RCPs, a higher average RRPK of protein segments encoded by alternatively spliced exons was also observed (Welch's t-test, $p = 7.6 \times 10^{-6}$ and 4.6×10^{-11} , respectively; Table S7 in Additional File 2), while we found no higher fraction of RCPs to be alternatively spliced genes in comparison with non-RCPs (23.2% vs. 24.3% and 31.3% vs. 37.7%, respectively; Fisher exact test, $p = 0.31$ and 7.7×10^{-3} , respectively). A third regulatory process proposed here is tissue-specific expression, as measured by the tissue specificity index (see Materials and Methods), may be another complementary regulatory process. Orthologous RCPs from both Arabidopsis and rice had a relatively higher tissue specificity index than non-RCPs (Welch's t-test, $p = 2.0 \times 10^{-4}$ and 1.4×10^{-15} , respectively). If only genes with a single model were considered, the difference seemed to be limited but could still be observed (Welch's t-test, $p = 1.5 \times 10^{-2}$ and 5.9×10^{-11} , respectively), indicating that tissue-specific expression may partially complement alternative splicing in the regulation of RCPs.

Conclusions

Angiosperm proteins are enriched in AARs whose content is positively correlated with the GC content in the coding sequences. It has also been suggested that the correlation between AAR content and GC content is influenced by residue composition of AARs as well as life-history traits. Similarly to AARs in many sequenced eukaryotic species, angiosperm AARs evolve rapidly and tend to be disordered. Although AARs are usually not well conserved, we identified 18 conserved long AARs for further detailed analysis. As potentially promiscuous molecules, RCPs are under at least three putative transcript-level regulatory controls in plant cells, including faster transcript decay, alternative splicing and tissue specificity of gene expression.

Methods

Collection of sequences

Sequences of *A. thaliana* (Version 9) and *O. sativa* (Version 6.1) were downloaded from TAIR [58] and RGAP [31], respectively. All of the other plant sequences were downloaded from the Phytozome 6.0 database [59]. The sources of the non-plant genome sequences are summarized in Tables S1 and S2 in Additional File 1. For genes with multiple protein products (gene models), only the

representative one (if available) or the longest one was retained.

To search for 1-to-1 orthologs between species within certain taxonomic groups, InParanoid 4.1, one of algorithms with the lowest false-positive rates [60], was initially employed to identify pair-wise orthologs between the reference proteomes (*A. thaliana* for plant species, *N. crassa* for fungus species and *Drosophila melanogaster* for insects, excluding proteins encoded by the mitochondrial/chloroplast genomes) and the proteomes of the other species, with a score cutoff of 40. Sets of 1-to-1 orthologs found in all of the species within each group were obtained by collecting the intersection of the ortholog pairs.

AAR identification, GC content calculation and statistical tests

We used in-house PERL scripts to collect data on the length, composition and position of AARs in protein sequences and to calculate the GC content. All statistical tests were implemented in R 2.12.1 [61].

Recombination hotspots

We deduced the recombination hotspot at the Arabidopsis genome from SNP data described in [41]. Informative SNP markers in a chromosome were selected by the TAGGER application in HaploView 4.1 [62] with "-maxDistance 20 -aggressiveTagging -tagsqcutoff 0.8" options, excluding SNPs identified as "N" in more than three out of 20 Arabidopsis accessions. Mainly due to the greedy marker selection approach of TAGGER along the whole chromosomes, the total number of informative SNP markers selected here is 60,904. The hotspots were searched in 40-marker-long sliding windows by PHASE 2.1.1 [63], with "-MR1 1 -X10" options. These windows moved 20 markers per step. Windows that were longer than 100 kb were discarded. A hotspot was defined as a two-marker interval with a Bayes Factor that was higher than 10 in comparison with the background recombination rate [41]. Positions of the hotspot were compared with the position of genes in the Version 8 genome to collect genes that overlap with recombination hotspots. We called these genes putative hotspot neighboring genes (gene IDs were transferred to Version 9 for obsolete loci). We do not use Version 9 genomes here because approximately half of the markers fail to map to this version of genome [58].

dN/dS calculation

The alignment of orthologous coding sequences was guided by a multi-protein sequence alignment that was generated by MAFFT 6.849 [64]. A flanking region was defined as 33 amino acids on both sides of an AAR; this region was truncated if the end of a protein was reached

or if there was an adjacent AAR closer within 33 amino acids. PAML 4.3 yn00 tool [65] was used to calculate the dN/dS ratio with default parameters.

Disordered region prediction

PONDR[®] VSL2B [52] and IUPred [53] were used for predictions of disorder, with default parameters. We did not use PONDR[®] VSL2 because of limitations in computational capability. We used default thresholds (0.5) to predict disordered residues for both predictors.

Probing transcript level regulation features of RCPs

AtGenExpress [66] data (Accession: GSE5630, GSE5631, GSE5632, GSE5633 and GSE5634) were downloaded from the GEO database [67]. Data not derived from the Columbia-0 ecotype were discarded. A developmental time series of rice transcriptome data [68] (Accession: GSE13988, GSE14298, GSE14299 and GSE14300) was also downloaded from GEO [67]. Probes were mapped to loci in Arabidopsis and rice according to the mapping files that were provided by TAIR [58] and the Rice Array Database [69], respectively. All of the expression values that were presented above background (labeled as "Presence") were used and log-transformed. The expression values were normalized by subtracting the average expression value of a tissue and then adding the average expression value of the whole dataset. The tissue specificity index [70,71] was calculated as follows:

$$\text{Tissue Specificity Index} = \frac{\sum (1 - \frac{x_i}{x_{\max}})}{N - 1}$$

where x_i is the expression value in the i^{th} tissue, x_{\max} is the highest expression value among all of the tissues and N is the total number of tissues. For loci with multiple probes, the average tissue specificity index was used.

To calculate the RRPK of the protein segments that were encoded by different types of exons, the protein sequences of RCPs were mapped to their exon sequences using our in-house PERL scripts. Only exons that encode proteins were retained for calculation. The mRNA half-life for each probe was obtained from [72]. For loci with multiple probes, the average mRNA half-life was used.

Additional material

Additional file 1: The relationship between AAR content and GC content in fungi and insects. This file contains two tables (Tables S1 and S2) that list the sources of the fungi and insect genome sequences that were used in this study, as well as two figures (Figures S1 and S2) that show the corresponding relationship between AAR content and GC content.

Additional file 2: Fast evolving AAR flanking regions, conserved long AARs of unknown function and RRPK of alternatively spliced exons. This file contains Tables S3-S7.

Abbreviations

AAR: (Tandem) Amino Acid Repeat; RCP: Repeat Containing Protein; RRPK: Repeated Residues per One Kilo Amino Acids; SNP: Single Nucleotide Polymorphism.

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Authors' contributions

ZZ, JL and YZ conceived the study. JL and YZ performed most of the analyses. LH and ZGL helped perform the disorder prediction and the dN/dS calculation, respectively. YZ drafted the manuscript. ZZ supervised the study and revised the manuscript. All of the authors read and approved the final manuscript.

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