

- 22 Boudonck, K. *et al.* (1999) The movement of Cajal bodies visualised in living plant cells by the green fluorescent protein. *Mol. Biol. Cell* 10, 2297–2307
- 23 Acevedo, R. *et al.* (2002) Coiled bodies in nuclei from plant cells evolving from dormancy to proliferation. *Chromosoma* 110, 559–569
- 24 Santos, A.P. *et al.* (2002) The architecture of interphase chromosomes and gene positioning are altered by changes in DNA methylation and histone acetylation. *J. Cell Sci.* 115, 4597–4605
- 25 Shaw, P.J. *et al.* (2002) The architecture of interphase chromosomes and nucleolar transcription sites in plants. *J. Struct. Biol.* 140, 31–38
- 26 Lam, E. *et al.* (2004) Visualising chromosome structure/organization. *Annu. Rev. Plant Biol.* 55, 537–554
- 27 Andersen, J.S. *et al.* (2002) Directed proteomic analysis of the human nucleolus. *Curr. Biol.* 12, 1–11
- 28 Saitoh, N. *et al.* (2004) Proteomic analysis of interchromatin granule clusters. *Mol. Biol. Cell* 15, 3876–3890
- 29 Rubbi, C.P. and Milner, J. (2003) Disruption of the nucleolus mediates stabilization of p53 in response to DNA damage and other stresses. *EMBO J.* 22, 6068–6077
- 30 Ideue, T. *et al.* (2004) The nucleolus is involved in mRNA export from the nucleus in fission yeast. *J. Cell Sci.* 117, 2887–2895
- 31 Misteli, T. *et al.* (1997) The dynamics of a pre-mRNA splicing factors in living cells. *Nature* 387, 523–527

1360-1385/\$ - see front matter © 2004 Elsevier Ltd. All rights reserved.
doi:10.1016/j.tplants.2004.10.003

Unveiling the molecular arms race between two conflicting genomes in cytoplasmic male sterility?

Pascal Touzet¹ and Françoise Budar²

¹Laboratoire de Génétique et Evolution des Populations Végétales, UMR-CNRS 8016, FR CNRS 1818, Université de Lille I, F-59655 Villeneuve d'Ascq cedex, France

²Station de Génétique et d'Amélioration des Plantes, Institut Jean-Pierre Bourgin, INRA route de Saint Cyr, 78026 Versailles, France

Cytoplasmic male sterility can be thought of as the product of a genetic conflict between two genomes that have different modes of inheritance. Male sterilizing factors, generally encoded by chimeric mitochondrial genes, can be down-regulated by specific nuclear restorer genes. The recent cloning of a restorer gene in rice and its comparison with restorer genes cloned in petunia and radish could be regarded as the beginning of a general molecular scenario in this peculiar arms race.

Gynodioecy is a mating system where both hermaphroditic and female individuals co-exist in a single population. This sexual polymorphism can be thought of as the outcome of a genetic conflict between two genomes that differ by their transmission: male sterilizing factors encoded by a cytoplasmic genome improve their transmission through resource allocation whereas the nuclear genome 'reacts' by re-establishing the male function through specific restorer alleles [1–4]. Frequently observed in wild and cultivated higher plant species, cytoplasmic male sterility (CMS) has been the object of extensive molecular investigation for the past decade. According to studies of numerous CMS systems in monocot and dicot species, the sterilizing factors are generally encoded by chimeric mitochondrial genes, probably as a result of intra-genomic recombination [5,6], a trait of the mitochondrial genome of plants [7]. Although extremely diverse, the CMS-related genes share common features such as containing parts of essential genes and/or being in the vicinity of genes enabling their 'opportunistic' transcription [8,9]. The restorer alleles generally act directly on the expression of the sterilizing

factor at the post-transcriptional level. Until recently, the nature of restorer genes was completely unknown, apart from the possibility that they might code for proteins targeted to the mitochondrion and specifically interacting with the sterilizing factor expression. The recent publication by Toshiyuki Komori and colleagues [10], who cloned a restorer locus in rice, completes the picture that had already emerged from earlier studies on petunia and radish of the current understanding of the recruitment of restorer loci.

Restorer loci belong to the PPR gene family

Working on petunia, Stéphane Bentolila and colleagues [11] were the first to clone a restorer locus that is known to affect the expression of a CMS factor; earlier studies had identified *Rf2*, a 'metabolic' restorer locus in maize that might limit the effect of the CMS factor [12–14]. Bentolila *et al.* found that the petunia restorer belonged to a large gene family that codes for proteins characterized by tandem arrays of pentatricopeptide repeats (PPR) [15]. Subsequently, in radish [16–18], and recently in rice [10,19,20], restorer loci of unrelated CMS systems were shown to belong to the same family, which provided additional confirmation of the generality of the PPR feature, as judiciously foreseen in earlier studies [11,21]. Although the role of PPR genes was still hypothetical at the time, it appears now that the PPR genes are involved in organellar gene expression, probably by binding to specific transcripts, rather than being general unspecific RNA-binders, as suggested by the low redundancy of this huge family. Because obvious catalytic domains seem to be lacking in these proteins, they could be adaptors, directing the action of other factors [22]. Targeted to the mitochondrion through a transit peptide deduced in each restorer

Corresponding author: Pascal Touzet (pascal.touzet@univ-lille1.fr).

Available online 2 November 2004

allele that was sequenced, the restorer protein, probably an RNA-binding protein, might specifically be involved in the post-transcriptional regulation (RNA processing and translation) of the sterilizing factor. But as well as showing that they belong to the same gene family, the cloning of the genomic restorer loci revealed that they also share common features that could give some clue as to the process of their making.

Restorer loci have a complex genomic structure – the signature of a birth-and-death process?

The genomic structure of the restorer locus in rice comprises four PPR genes predicted to encode mitochondrial targeted proteins, one of which is the restorer gene. A similar structure has been observed in petunia (with potentially three PPR genes) and in radish (with three PPR genes). The complex structure of the restorer locus recalls the one observed in plant disease resistance genes, where the presence of leucine-rich repeats facilitates unequal crossing over, potentially leading to clusters of paralogues [23].

The comparison with the rice non-restorer locus completes the picture: the allelic homologue of the restorer PPR gene seems to have been partially deleted. Although two out of the three PPR genes share more homology with their allele than with their paralogue, the PPR gene in closest proximity to the non-restorer allele is lacking (Figure 1). These features recall the birth-and-death process that has been invoked to explain the dynamics of multigene families in the vertebrate immune system [24] and the plant disease resistance genes [23]: new genes are created by repeated gene duplication. Some duplicate genes are maintained for a long time whereas others are deleted or become non-functional through deleterious mutations. According to this process, alleles are expected to be more similar than are paralogues, as observed in the restorer locus in rice. The same constraints and common features (repeated motifs) of the genes of the immune system or resistance genes might shape the dynamics of plant nuclear restorer loci confronted with the lability of a 'virulent' mitochondrial genome. The recruitment of duplicated copies of mitochondrially targeted PPR genes, generated through unequal crossing over, might have facilitated the evolutionary arms race. In this context, a

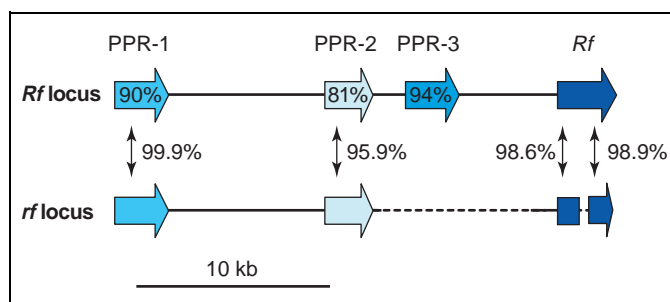


Figure 1. Scheme of the Rf-1 locus in rice. Comparison of the restorer (*Rf*) and non-restorer (*rf*) haplotypes (reproduced in print, with permission, from Ref. [10]). The arrows correspond to homologues of pentatricopeptide repeats (PPR). The percentage of nucleotide similarity between each PPR homologue and the *Rf* gene is indicated in each arrow. The percentage of nucleotide similarity between allelic homologues (same color) is indicated beside the vertical arrows. The broken line on the *rf* haplotype indicates regions of deletion.

large survey of the genomic diversity of restorer loci for a given CMS might reveal that even if some non-restorer alleles derive from restorer alleles, as is the case in the rice study (or in petunia, where the non-restorer allele seems to have arisen as a result of recombination between the restorer allele and an adjacent PPR gene), others might be ancestral non-restorer forms. Furthermore, one might expect the occurrence of complex restorer haplotypes composed of diverged PPR genes, each of them being specifically 'resistant' to a given CMS. This could be the case in Brassica: at a single locus two 'allelic' forms restore the nap or the pol CMSs [25].

The number of cloned restorer loci that are involved in the repression of CMS gene expression is likely to increase in the near future. If the general picture that seems to be emerging is true, the PPR-targeted cloning approach holds the promise of results [9,21]. Analysis of the allelic diversity of restorer and non-restorer haplotypes should highlight the evolutionary process involved in this peculiar intra-genomic arms race and, more generally, the co-evolution of genes from two distinct genomes with different modes of inheritance.

Acknowledgements

We thank Jean-François Arnaud and Mathilde Dufayé for their comments, as well as the two anonymous referees who helped us to improve the clarity of the manuscript, and Licia Huffman for proof-reading this work.

References

- 1 Cosmides, L.M. and Tooby, J. (1981) Cytoplasmic inheritance and intragenomic conflict. *J. Theor. Biol.* 89, 83–129
- 2 Hurst, L.D. *et al.* (1996) Genetic conflicts. *Q. Rev. Biol.* 71, 317–364
- 3 Frank, S.A. (2000) Polymorphism of attack and defense. *Trends Ecol. Evol.* 15, 167–171
- 4 Budar, F. *et al.* (2003) The nucleo-mitochondrial conflict in cytoplasmic male sterilities revisited. *Genetica* 117, 3–16
- 5 Saumitou-Laprade, P. *et al.* (1994) Cytoplasmic male sterility in plants: molecular evidence and the nucleocytoplasmic conflict. *Trends Ecol. Evol.* 9, 431–435
- 6 Schnable, P.S. and Wise, R.P. (1998) The molecular basis of cytoplasmic male sterility and fertility restoration. *Trends Plant Sci.* 3, 175–180
- 7 Palmer, J.D. and Herbon, L.A. (1988) Plant mitochondrial DNA evolves rapidly in structure, but slowly in sequence. *J. Mol. Evol.* 28, 87–97
- 8 Budar, F. and Pelletier, G. (2001) Male sterility in plants: occurrence, determinism, significance and use. *C. R. Acad. Sci. III* 324, 543–550
- 9 Hanson, M.R. and Bentolila, S. (2004) Interactions of mitochondrial and nuclear genes that affect male gametophyte development. *Plant Cell* 16(Suppl. 1), S154–S169
- 10 Komori, T. *et al.* (2004) Map-based cloning of a fertility restorer gene, Rf-1, in rice (*Oryza sativa* L.). *Plant J.* 37, 315–325
- 11 Bentolila, S. *et al.* (2002) A pentatricopeptide repeat-containing gene restores fertility to cytoplasmic male-sterile plants. *Proc. Natl. Acad. Sci. U. S. A.* 99, 10887–10892
- 12 Cui, X. *et al.* (1996) The *rf2* nuclear restorer gene of male-sterile T-cytoplasm maize. *Science* 272, 1334–1336
- 13 Touzet, P. (2002) Is *rf2* a restorer gene of CMS-T in maize? *Trends Plant Sci.* 7, 434
- 14 Schnable, P.S. (2002) Is *rf2* a restorer gene of CMS-T in maize? *Trends Plant Sci.* 7, 434
- 15 Small, I.D. and Peeters, N. (2000) The PPR motif – a TPR-related motif prevalent in plant organellar proteins. *Trends Biochem. Sci.* 25, 46–47
- 16 Brown, G.G. *et al.* (2003) The radish Rfo restorer gene of Ogura cytoplasmic male sterility encodes a protein with multiple pentatricopeptide repeats. *Plant J.* 35, 262–272

- 17 Desloire, S. *et al.* (2003) Identification of the fertility restoration locus, Rfo, in radish, as a member of the pentatricopeptide-repeat protein family. *EMBO Rep.* 4, 588–594
- 18 Koizuka, N. *et al.* (2003) Genetic characterization of a pentatricopeptide repeat protein gene, orf687, that restores fertility in the cytoplasmic male-sterile Kosenia radish. *Plant J.* 34, 407–415
- 19 Kazama, T. and Toriyama, K. (2003) A pentatricopeptide repeat-containing gene that promotes the processing of aberrant atp6 RNA of cytoplasmic male-sterile rice. *FEBS Lett.* 544, 99–102
- 20 Akagi, H. *et al.* (2004) Positional cloning of the rice Rf-1 gene, a restorer of BT-type cytoplasmic male sterility that encodes a mitochondrial targeting PPR protein. *Theor. Appl. Genet.* 108, 1449–1457
- 21 Wise, R.P. and Pring, D.R. (2002) Nuclear-mediated mitochondrial gene regulation and male fertility in higher plants: light at the end of the tunnel? *Proc. Natl. Acad. Sci. U. S. A.* 99, 10240–10242
- 22 Lurin, C. *et al.* (2004) Genome-wide analysis of *Arabidopsis* pentatricopeptide repeat (PPR) proteins reveals their essential role in organelle biogenesis. *Plant Cell* 16, 2089–2103
- 23 Michelmore, R.W. and Meyers, B.C. (1998) Clusters of resistance genes in plants evolve by divergent selection and a birth-and-death process. *Genome Res.* 8, 1113–1130
- 24 Nei, M. *et al.* (1997) Evolution by the birth-and-death process in multigene families of the vertebrate immune systems. *Proc. Natl. Acad. Sci. U. S. A.* 94, 7799–7806
- 25 Li, X.-Q. *et al.* (1998) Restorer genes for different forms of *Brassica* cytoplasmic male sterility map to a single nuclear locus that modifies transcripts of several mitochondrial genes. *Proc. Natl. Acad. Sci. U. S. A.* 95, 10032–10037

1360-1385/\$ - see front matter © 2004 Elsevier Ltd. All rights reserved.
doi:10.1016/j.tplants.2004.10.001

Letters

Controversy remains: regulation of pH gradient across the thylakoid membrane

Giles Johnson

University of Manchester, School of Biological Sciences, Oxford Road, Manchester, UK M13 9PT

In a recent review [1], David Kramer, Thomas Avenson and Gerald Edwards discussed a largely overlooked problem in photosynthesis – how the pH gradient (ΔpH) across the thylakoid membrane is regulated. ΔpH serves a dual role in chloroplasts, synthesizing ATP and regulating light harvesting, by inducing non-photochemical quenching (NPQ). As the authors point out, this results in a conflict of interest – conditions that require a slowing of linear electron transport (CO_2 fixation inhibited) also require the generation of a larger ΔpH to lower the efficiency of light capture.

The authors considered two mechanisms that might regulate ΔpH : (i) alternative routes of electron transport that generate ΔpH without ATP consumption, notably cyclic electron transport (CET) around the photosystem I reaction center; (ii) modulation of the conductivity of the chloroplast ATPase. The first model has been widely discussed for many years [2], although, as the authors point out, controversy remains. The second model is based on recent data from David Kramer's laboratory [3]. Kramer and colleagues seek to dismiss a major role for CET in regulating light harvesting. Given recent prominent publications by Yuri Munekage and colleagues [4,5], describing a mutant unable to perform NPQ and apparently deficient in CET, this argument might confuse non-specialist readers and requires further discussion.

Kramer and colleagues argue that to regulate ΔpH , CET would have to occur at high rates (up to 6 times linear electron flux) and that published evidence does not support the occurrence of such rates under steady-state conditions. The figures cited arise from an interpretation of the relationship between linear flow and NPQ [3]. At

low CO_2 levels, there is a low rate of linear electron transport owing to the inhibition of the Calvin–Benson cycle, but the extent of NPQ is greater than at high CO_2 levels. For a given level of NPQ, linear flow is about a sixth of that under high CO_2 levels. The argument then follows that for CET to compensate for this drop, it would have to be at a rate that is five to six times greater than the residual linear flow. However, this fails to take into account that linear flow to CO_2 is not a net generator of ΔpH – ATP consumption by CO_2 fixation is greater than the generation expected through linear flow alone. At low CO_2 levels, ATP consumption will drop in parallel with linear electron transport, so dissipation of ΔpH will be considerably reduced. CET generates ΔpH without being directly coupled to its consumption. Therefore, a modest increase in CET would be sufficient to generate a high ΔpH .

Analysis from my laboratory suggests that CET increases at low CO_2 levels, generating ΔpH to regulate light harvesting [6–8]. This conclusion is valid using both the decay of P700^+ as a measure of PSI turnover (the method questioned by David Kramer and colleagues [1]) and also using the 'active pool' of P700, as proposed by Christof Klughammer and Ulrich Schreiber [9]. It is consistent with a significant number of observations from other groups [10,11]. David Kramer and colleagues notably glossed over the *pgr5* mutant, identified for its inability to perform NPQ, which has been characterized as deficient in CET [5]. This phenotype remains to be tested under physiological conditions but is likely to provide a definitive test for the role of CET.

The model put forward for regulation of ATPase conductivity [1,3] is an interesting one, but the published evidence is open to alternative interpretations. The

Corresponding author: Giles Johnson (giles.johnson@man.ac.uk).