Cytoplasmic Genome

6

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Abstract

The chloroplasts and mitochondria are the organelles essential for normal growth and development of plants. They have their original genomes, and genes encoded in these organelles are expressed in concordance with factors originating from the nuclear genome. Researchers have analyzed cytoplasmic genomes to understand this complicated mechanism. The sizes of the chloroplast genome and the mitochondrial genome are relatively small, with the former being approximately 150 kbp and the latter being several hundred kbp to several Mbps. The widespread use of next-generation sequencing (NGS) in recent years has enabled sequencing of the entire genome of chloroplasts and mitochondria with relative ease and affordability. For example, several chloroplast and mitochondrial genomes have been sequenced for onions. Onions carry several different types of cytoplasms characterized by distinct cytoplasmic genomes. In particular, male sterility induced by certain types of cytoplasms is a trait that is crucial to F_1 hybrid breeding, and male sterility phenotypes are determined by their compatibility with the nuclear genome. This chapter explains the cytoplasmic form of these onion cultivars and lays out the characteristics of the cytoplasmic genome of each one of them.

6.1 Introduction

Cytoplasmic genomes are present in the cytoplasm and are distinct from nuclear genomes. In plants, these include chloroplast and mitochondrial genomes.

Mitochondria originated from an endosymbiosis between a host cell and an alpha-proteobacterium 1.45 billion years ago. Chloroplasts, on the other hand, originated from the endosymbiosis of a cyanobacterium 1.5 billion years ago. The host cell took control over the symbiotic fungi over the years, and eventually, mitochondria and chloroplasts came to play their own parts within the cell as an organ that synthesizes adenosine triphosphate (ATP) and an organelle responsible for carbon fixation, respectively. The genome of each bacteria disappeared as the host's nuclear genome acquired its genes. Although hydrogenosomes present in certain anaerobic protists (e.g., *Trichomonas*) have entirely lost their original genomes following an endosymbiotic process, mitochondria and chloroplasts still retain their original genomes with functional genes, which are crucial for

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proper functioning of the organelles and the successful passage of hereditary information to the next generation (Dyall et al. 2004; Keeling 2010; Dunning Hotopp 2011).

Particular attention should be drawn to the distinct characteristics of the chloroplast genome, the plant mitochondrial genome, and the animal mitochondrial genome. The chloroplast genomes of most plant species are composed of two inverted repeats that separate a large single-copy (LSC) region from a small single-copy (SSC) region. The plant mitochondrial genomes, on the other hand, are believed to take on the shape of a circle, but the size and sometimes even the structure of them vary with species. The mammalian mitochondrial genomes are well conserved, with almost identical structure and encoded genes.

The genes encoded in the nuclear genome are essential for these organelles to function normally. Of all genes that are associated with mitochondria, over 1000 are transferred to mitochondria. These proteins are indispensable for normal photosynthesis and respiration. Furthermore, genes originating from the nuclear genome are deeply associated with the expression of genes encoded in each organelle genome. A chloroplast contains genes that use a plastid-encoded RNA polymerase (PEP) and a nucleus-encoded RNA polymerase (NEP). Although PEP and NEP use different promoters, nearly all genes have both PEP and NEP promoters like in most cases of bacterial expression, and these promoters are transcribed as polycistronic RNAs. Although mitochondria are also expressed by the bacterial RNA polymerase, they do not carry their own polymerase and therefore are dependent on a nucleus-encoded RNA polymerase (Borner et al. 2015; Hammani and Giege 2014). Transcripts are subject to post-transcriptional modifications, such as splicing, RNA editing, and definition at their 3′ end and 5′ end.

The presence of signal transduction that enables interactive control and regulation of genes encoded in the nucleus and organelles has also been identified, which plays a crucial role in stress and development responses. Although not much is known about the metabolic pathways

and biosynthesis of these genes, several molecules have been identified to carry signals. These molecular components are referred to as anterograde (nuclear to organelle) and retrograde (organelle to nuclear) signals, which affect signal transduction pathways and switch gene expression on or off. Intermediates in the metabolic pathways of reactive oxygen species (ROS), calcium ion, and tricarboxylic acid (TCA) have been demonstrated to be involved in these mechanisms, and the alternative oxidase (AOX) pathway, in particular, has been studied extensively as a model for mitochondrial retrograde regulation (MRR) (Ng et al. 2014; Rhoads 2011).

In view of these facts, it is of extreme importance to focus attention on cytoplasms during the analysis of growth, development, and functionality of plants, and it is also essential to consider nucleus-cytoplasm compatibility. With agricultural crops, cytoplasmic male sterility/fertility restoration (CMS/Rf) systems are often used for F_1 hybrid breeding. The use of a line exhibiting CMS as the female parent and a line with restorer genes as pollen parent allows for F_1 hybrid seed production without the need for emasculation. These systems are especially useful for breeding agricultural crops not only because hybrids often exhibit hybrid vigor but also because they help protect information regarding genes carrying superior traits. With a typical CMS-Rf system, CMS-inducing genes are present in mitochondria, whereas the genes that restore male fertility are present in the nuclear genome (Hanson and Bentolila 2004; Bohra et al. 2016).

Furthermore, comparing cytoplasmic genomes between different species or genera helps to identify a lineage relationship between those species or genera. The estimation of a lineage relationship by using the nuclear genome can be quite challenging with species that have large nuclear genomes or polyploidy genomes. Cytoplasmic genomes, on the other hand, are extremely small in size, and because they are transmitted from mother to offspring, investigating cytoplasmic genomes can reveal maternal-line ancestry.

The widespread use of NGS in recent years has enabled sequencing of the entire genome of chloroplasts and mitochondria with relative ease and affordability. As a result, organelle genome data has been steadily accumulated from diverse plant species, In fact, chloroplast and mitochondrial genomes have been completely sequenced for several onion species. This chapter lays out the characteristics of the cytoplasmic genome of these onions.

6.2 Cytoplasm Species and Their Origin in Onion

Onion, one of the most important crops in the world, is a diploid species ($2n = 2x = 16$) that has been cultivated for more than 5000 years (Khosa et al. 2016). Onions are cultivated in 147 countries in the world, with the world's onion production reaching 111 metric tons in 2014 (FAOSTAT 2014).

No wild form of onions has ever been found, and only cultivated varieties exist today. *Allium vavilovii*, which can be crossed with *Allium cepa*, grows naturally in the Tien-shan mountains and the Pamir–Altai mountains, where onions are believed to have originated (Kik 2002). *Allium* and its close relatives are classified into four grades, and it has been shown that *A. cepa* seems to originate from *A. vavilovii* or from hybridization between *Allium galanthum* and *Allium fistulosum* (Gurushidze et al. 2007).

The presence of CMS in onions has long been known, and onion was, in fact, the first crop to be used for F_1 hybrid breeding. Since the discovery of CMS in onion by Jones and Emsweller (1936), a considerable increase in crop yields has been achieved through heterosis breeding (Khosa et al. 2016). To date, two types of male-sterility-inducing cytoplasm, namely the CMS-S and CMS-T types, have been widely used for commercial breeding.

The CMS-S type shows normal development until the tetrad stage of pollen development, which is followed by degeneration of the protoplasm in the tetrads, resulting in empty pollen grain (Holford et al. 1991a). CMS-T, on the other

hand, has an abnormal pollen meiosis (Kik 2002).

S-type cytoplasmic male sterility is restored to fertility by a single nuclear locus (Jones and Clarke 1943; Jones and Davis 1944), whereas male sterility due to T cytoplasm is restored by three independent loci: *aa*, *bb*, and *cc* (Schweisguth 1973). The Normal-type cytoplasm, on the other hand, does not exhibit sterility. These three types of cytoplasms are identified by the restriction fragment patterns of chloroplast and mitochondrial DNAs.

Normal-type and CMS-T cytoplasms resemble one another and are, therefore, categorized into the M group, which is distinguished from the CMS-S-type cytoplasm (de Courcel et al. 1989; Holford et al. 1991b). In 2000, Havey showed that the CMS-S type originates from *Allium x proliferum* and that the CMS-T type and the Normal-type originate from *A. vavilovii* (Havey 2000).

Not much progress has been made in the improvement of *Allium* by use of wild-type subspecies in introgression breeding. This is attributable to the fact that onions are biennials that have a 2-year life cycle and that there are a limited number of onion species available for breeding. A previous study has demonstrated that while *A. vavilovii* can be crossed with *A. cepa*, crossing the triploid ovum *A. x proliferum* with *A. cepa* can be quite challenging (Kik 2002).

Introgression of *A. fistulosum* or *A. galanthum* with *A. cepa* produces sterility. Although CMS-induced by *A. galanthum* has been shown to produce empty pollen grains that are phenotypically similar to the pollens produced by the CMS-S type, CMS-S restorer genes do not restore fertility to *galanthum*-CMS (Havey 1999). A single locus has been found to restore male fertility in *A. fistulosum* (Japanese bunching onion) in which CMS has been induced by *A. galanthum* (Yamashita et al. 2005).

6.3 Chloroplast Genome

The chloroplast genomes are generally composed of two inverted repeats that separate an LSC from an SSC. This structure is common to all plants.

The same is true for the following five chloroplast genomes identified so far for onions: CMS-S type and Normal type identified by von Kohn et al. (2013) and CMS-S type, CMS-T type, and Normal-type identified by Kim et al. (2015).

In the analysis performed by von Kohn et al. (2013), 28 single nucleotide polymorphisms (SNPs), 2 restriction fragment length polymorphisms (RFLPs), and 1 insertion–deletion (InDel) were found in the genetic code between the CMS-S type and the Normal type. Furthermore, an InDel of 45 bp was present in the accD gene, with the CMS-S type showing the same sequence as tobacco, orchid, and rice. InDels of 99, 59, and 50 bp were also present in the trnL-trnT, psbM-petN, and rps16-trnQ intergenic regions, respectively, with CMS-S having the shorter sequence. An InDel of 90 bp was also found in the rps4-trnT intergenic region, with S having one repeat of 45 bp and N having three tandem repeats of 45 bp.

In the analysis by Kim et al. (2015), on the other hand, a SNP call for all the regions revealed 323 SNPs and 141 InDels between the CMS-S type and the Normal type. Furthermore, SNPs at four locations and InDels at two locations were found between the Normal type and the CMS-T type.

Between the two registered types of CMS-S, differences were found in SNPs at three locations and an InDel at one location (Table 6.1). Between the two Normal types and the CMS-T type, differences were found in SNPs at three locations and InDels at two locations (Table 6.2). None of the differences found in those SNPs and InDels were specific to the Normal type or the CMS-T type. Mutations seen in not only the S type but also between the Normal types or

between N and T appeared to be interspecies variation rather than mutations specific to Normal-type and CMS-T type. These findings suggest that while chloroplast genomes are useful for distinguishing between the CMS-S type and the Normal-type, they may be of little value when it comes to distinguishing between the Normal-type and the CMS-T type.

6.4 Mitochondrial Genome

As described in the previous section, it is important to distinguish between different types of onion cytoplasms because those that induce CMS are used with the Normal-type to develop F_1 hybrids. Like chloroplast genomes, mitochondrial DNAs are also widely used for classification of onion cytoplasms, and as demonstrated by Havey, these cytoplasms have been classified into the CMS-S type, the Normal-type, and the CMS-T type, the latter two of which belong to the M group (Havey 2000). These three types of cytoplasms (i.e., CMS-S type, CMS-T type and Normal type) were first identified through an RFLP analysis of mitochondrial DNAs, which revealed three types of mitochondrial genomes (de Courcel et al. 1989).

A specific gene called orf725 in mitochondria has been shown to induce CMS (Kim et al. 2009). This gene, which is a cox1 gene with an additional sequence of 576 bp attached to its 3′ end, is found in both the CMS-S type and the CMS-T type. This gene has been shown to be transcribed in both CMS lines, and due to a stop codon resulting from the editing at a position of 30 bases inward from the 3′ end, this gene in the CMS lines turns out to be 184aa longer than the normal-type cox1 gene. This additional sequence

Table 6.1 Positions of SNPs and InDels between two CMS-S chloroplast genomes

Position(KF728079; CMS-S type)	Type	KF728079; CMS-S type	KM088014; CMS-S type
32,557	Substitution	T	A
32,559	Substitution	A	T
141,791	Substitution	G	A
153,338–153,355	InDel	ACTCAAATTCGAGTGGAA	–

Table 6.2 Positions of SNPs and InDels among three chloroplast genomes classified as M group

Position(KF728080; N type)	Type	KF728080; N type	KM088013; N type	KM088015; CMS-T type
29,169–29,211	InDel	TTTCTATTCTATATGTACATC TACTATATACATATTGTAATTT	–	TTTCTATTCTATATGTACATC TACTATATACATATTGTAATTT
46,534–46,550	InDel	TAATATTAATAATATAT	–	–
50,776	Substitution	C	C	A
73,827	Substitution	A	G	A
119,803	Substitution	T	A	T
121,628	Substitution	A	G	G
122,659	Substitution	GAA	GAA	TTC

shows a high level of homology with *orfA501*, which is a gene specific to the CMS cytoplasm of chive (*A. schoenoprasum* L.) (Engelke and Tatlioglu 2002), and according to protein structure prediction, it contains transmembrane domains at two positions.

Although *orf725* is transcribed in both CMS lines, the normal-type *cox1* is not transcribed to the CMS-S type. However, in the CMS-T type, the normal-type *cox1* that is the same in size as that of the Normal type is transcribed (Kim et al. 2009). Furthermore, as mentioned earlier, the restorer genes in CMS-S and CMS-T are located at different loci, and the growth process of sterile pollen varies between these two cytoplasms. For this reason, this gene has long been considered a potential inducer of CMS; however, it has yet to be identified whether it is common to both the CMS-S type and the CMS-T type. On the other hand, a separation experiment carried out by using a line having a cytoplasm resembling CMS-T not only revealed an F_1 of 3:1 but also successfully identified the presence or absence of restorer genes by using the same locus markers as those of the restorer genes of the CMS-S type (Kim 2014). Thus, future research must reassess CMS-T. In addition, it is not yet known what systems are involved in the induction of male sterility and how it is restored to fertility, which would be an interesting topic to explore.

The plant mitochondrial genomes are far more complicated than the plant chloroplast genomes. Their sizes vary with species, ranging from several mega-bases to several dozen kilo-bases. Although the structure of plant mitochondrial genomes is often expressed as a single master circle, it has also been suggested that they may exist as multiple circles or linear molecules. Furthermore, mitochondrial genomes are characterized by the many repeats they have, through which numerous recombinant molecules exist.

Kim et al. were the first to identify the CMS-S type of onion mitochondrial genome in 2016 (Kim et al. 2016), followed by the identification of other species of the CMS-S type (Tsujimura et al. 2018) and then the Normal type by our group.

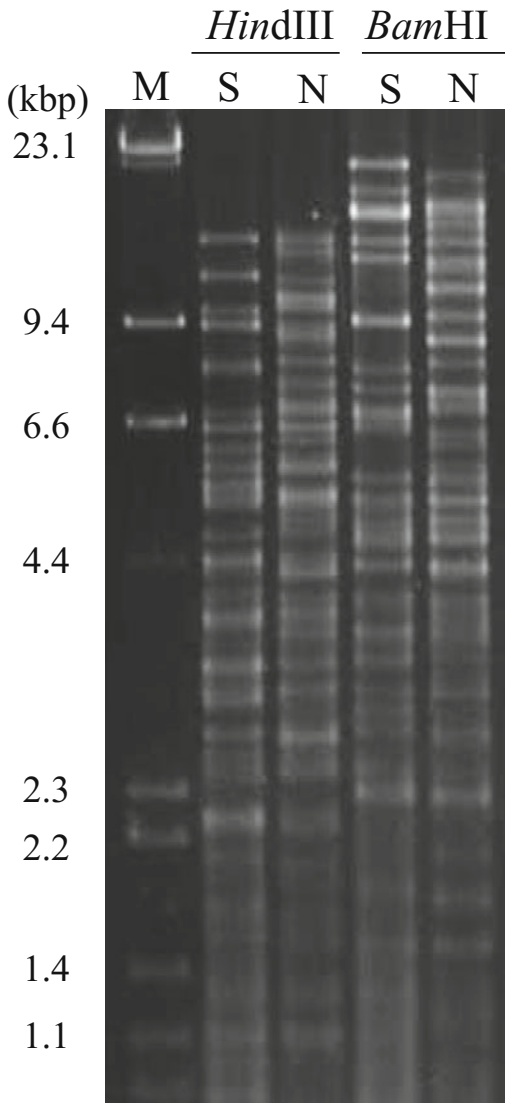Figure 6.1 shows the RFLPs of the mitochondrial genomes we identified. The

Fig. 6.1 Restriction fragment patterns of onion mitochondrial genome. M: molecular marker, S: CMS-S-type mtDNA, and N: Normal-type mtDNA

comparison of our RFLPs with the RFLP patterns presented by Courcel et al. shows that their RFLP patterns resemble those of our CMS-S type and Normal type (de Courcel et al. 1989). Furthermore, it is evident that the CMS-S type and the Normal-type are different species.

Interestingly, as it turned out, the CMS-S type we identified seemed to exist as three circles. Our initial NGS result found two circles, i.e., a

170-kbp (MC1) circle, and a 140-kbp circle. MC1 contains a direct repeat of 3.4 kbp, and when polymerase chain reaction (PCR) was carried out by using primers that had been designed to flank this repeat region, amplification and recombination were observed with all primer combinations. This PCR result suggests that the direct repeat yields two circles. Furthermore, another recombination occurred through a direct repeat of 3.4 kbp in MC1, possibly giving rise to two circles (Fig. 6.2). These findings suggest that the CMS-S type could be mapped as a total of three circles.

The Normal-type mitochondrial genomes, on the other hand, was illustrated as a single circle, according to the NGS result (Fig. 6.3). The total genome length was approximately 310 kbp for the CMS-S type and 537 kbp for the Normal-type, the latter of which turned out to be nearly 1.7 times longer than that of the "Momiji-3" onion cultivar. The size difference between these two species can be explained by the number of repeat regions. In fact, the percentage of the entire genome occupied by repeat regions was 1.5% for CMS-S-type and 30.7% for the Normal-type. Although it is possible that these repeat regions in the Normal-type induce recombination that could give rise to multiple circles like in the case of the CMS-S type, no band patterns were detected with pulsed-field gel electrophoresis (PFGE).

Table 6.3 summarizes the differences in the gene regions into which protein is coded by the CMS-S type and the Normal-type. Of the base substitutions and InDels found a total of 14 positions, 12 were accompanied by amino acid substitution, with a particularly significant difference, including a frameshift, found in *cox1*, *cox3*, and *nad6* (Fig. 6.4). Furthermore, we also obtained information on transcripts for each line by using RNA sequencing (RNAseq) and estimated the transcribed regions with respect to the coding regions.

In the case of *cox1*, the sequence after the stop codon differed between the CMS-S type and the Normal-type as previously reported, and with the CMS-S type, *cox1* was encoded as *orf725*.
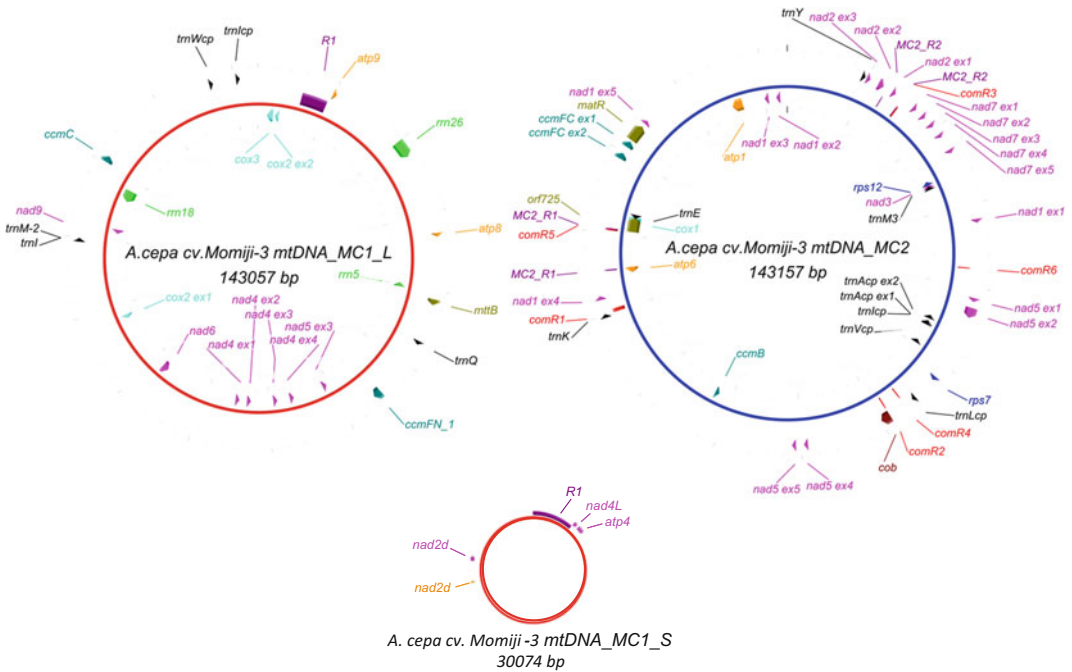
Fig. 6.2 Mitochondrial genome structure of *A. cepa* cv. "Momiji-3". Genes encoding proteins (red), rRNA (green), and tRNA (black) are shown outside (forward direction) and inside (reverse complementary direction)

direction) and inside (reverse complementary direction) the circle. A direct repeat sequence of 3.4 kbp (R1) is also indicated as a box (purple) outside the MC1 circles

Fig. 6.3 Mitochondrial genome structure of *A. cepa* "Normal type". Genes encoding proteins (red), rRNA (green), and tRNA (black) are shown outside (forward direction) and inside (reverse complementary direction) the circle
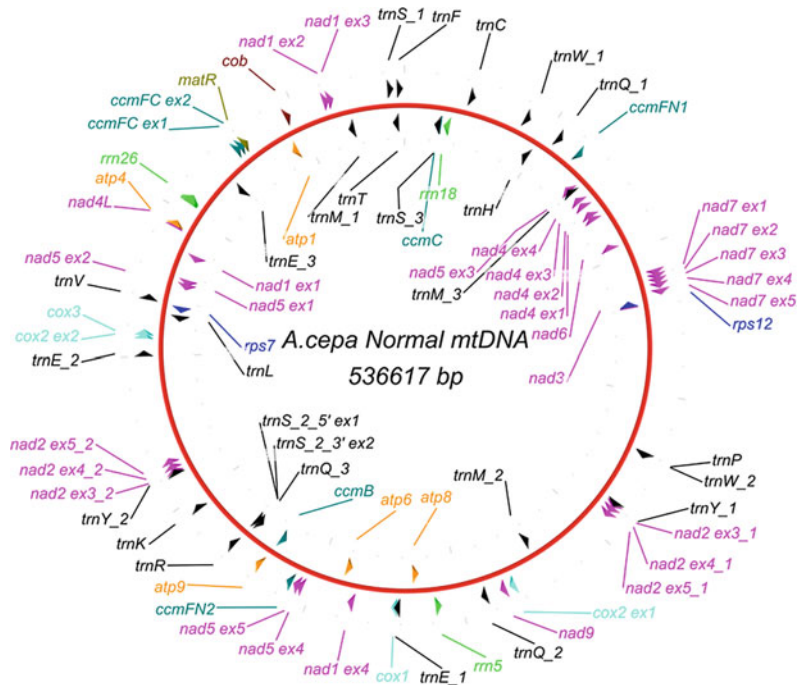
Table 6.3 Nucleotide differences in the coding region of protein-coding genes between "Momiji-3" and "Normal type"

Gene	Position (Momiji-3)	Momiji-3	Normal	Description of differences
atp1	81	A	C	Synonymous substitution
atp6	92	A	C	Non-synonymous substitution
ccmFC	1168–1170	GGA	TCC	Non-synonymous substitution
ccmFN1	13–14	GA	TC	Non-synonymous substitution
	519–524	TGGAAG	–	InDels without causing frameshift
cox1 (orf725)	1595	C	T	Non-synonymous substitution
	1604–2151			Low homology within this region
cox3	52–58	GTATC	–	InDels causing frame shift
nad1	819–820	GA	TC	Non-synonymous substitution
nad4	297	T	G	Non-synonymous substitution
nad5	1314	A	C	Synonymous substitution
nad6	720–729	GACCT	–	InDels causing frameshift
rps7	268–269	GA	TC	Non-synonymous substitution
	360	A	C	Non-synonymous substitution

The C-terminus of the CMS-S type was longer than that of the Normal-type by 184aa, with each transcribed region covering the coding region. In the case of *cox3*, due to frameshift caused by an InDel of 5 bp at the 5′ side, the N-terminus of the Normal type was shorter than that of the CMS-S type by 43aa. The transcribed region started from the same position in both genomes. In the case of *nad6*, the C-terminus of the Normal type was shorter than that of the CMS-S type by 75aa due to an InDel. In both genomes, however, the transcribed region ended within the coding region. Even with *Arabidopsis*, transcription termination in *nad6* has been shown to occur upstream of the stop codon, suggesting the presence of a unique transcription termination system (Raczynska et al. 2006; Forner et al. 2007). Because the position of transcription termination is almost identical between the CMS-S type and the Normal-type, a similar transcription system is probably at work in onions.

Western blot analysis of *orf725*, a suspected inducer of CMS, using COX1 antibody demonstrated differences between the CMS-S type and the Normal-type (Fig. 6.5). No difference was found, however, between the CMS-S lines that have the *Ms* restorer gene and those that do not have the *Ms* restorer gene. This implies that the presence of a restorer gene does not affect the translated products of *orf725*. Two hypotheses can be inferred from this finding. First, genes other than *orf725* may actually be responsible for inducing CMS. Genes such as the above-mentioned *cox3* and *nad6* may show differences even at the translation level. Alternatively, there could be an unknown open reading frame (ORF) that may exhibit unexpected effects. Secondly, a restorer gene may be playing a part in fixing a defect caused during the *orf725* assembly process. *cox1* is a subunit comprising respiratory chain complex IV. It could be that, even when no functional protein is produced during the process of complex formation, the helping hand from a restorer gene ensures that a functional complex is produced. Further analysis is needed, which may include the investigation of the activity of respiratory chain complexes, to verify these hypotheses.
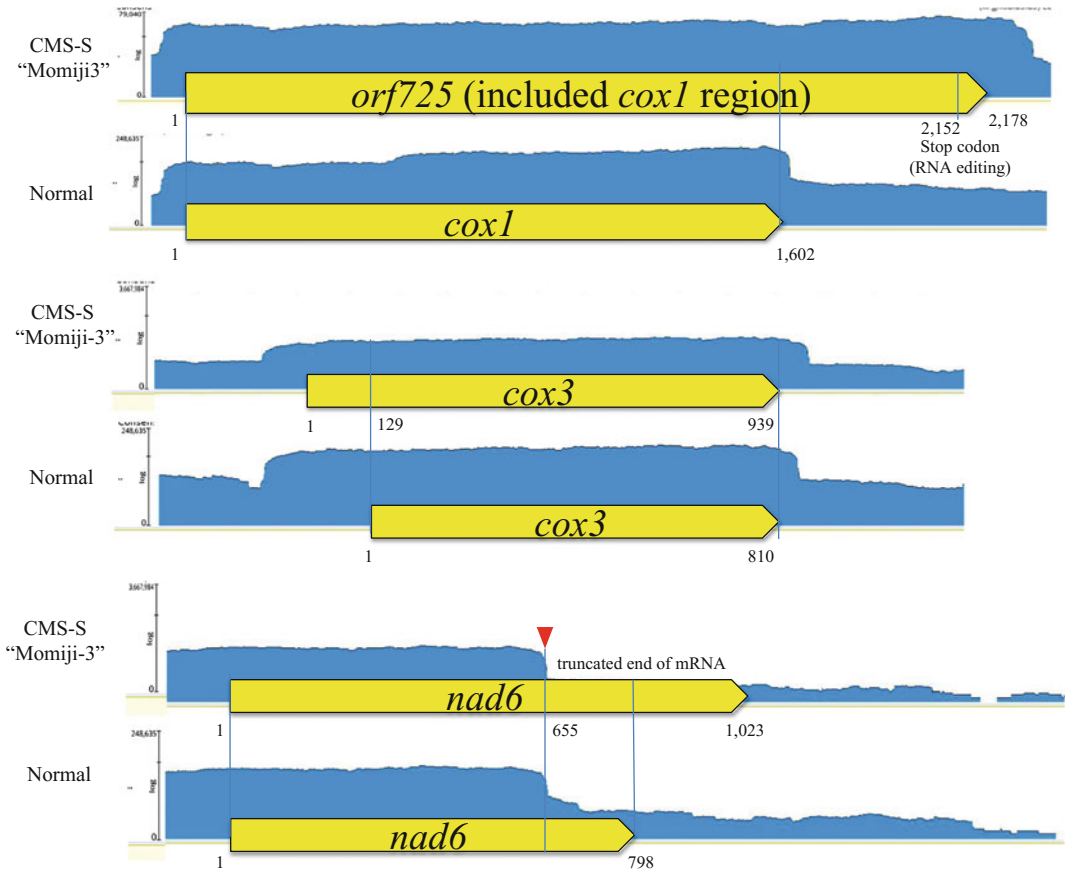
Fig. 6.4 Details of coding region differences in the genes *cox1*, *cox3*, and *nad6*. Block allows show protein-coding regions and graphs on the back of block allow indicate transcript abundance calculated by the coverage of sequence reads in RNA experiments
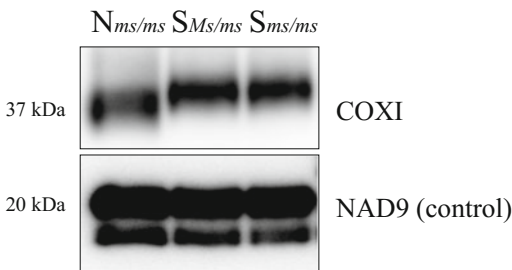

Fig. 6.5 Western blot analysis of COX1 and NAD9. N*ms/ms*; Normal-type with *ms/ms,* CMS-S type with *Ms/ms, and* CMS-S type with *ms/ms*

References

Bohra A, Jha UC, Adhimoolam P, Bisht D, Singh NP (2016) Cytoplasmic male sterility (CMS) in hybrid breeding in field crops. Plant Cell Rep 35:967–993

Borner T, Aleynikova AY, Zubo YO, Kusnetsov VV (2015) Chloroplast RNA polymerases: role in chloroplast biogenesis. Biochim Biophys Acta 1847:761–769

de Courcel AGL, Vedel F, Boussac JM (1989) DNA polymorphism in *Allium cepa* cytoplasms and its implications concerning the origin of onions. Theor Appl Genet 77:793–798

Dunning Hotopp JC (2011) Horizontal gene transfer between bacteria and animals. Trends Genet 27:157–163

Dyall SD, Brown MT, Johnson PJ (2004) Ancient invasions: from endosymbionts to organelles. Science 304:253–257

Engelke T, Tatlioglu T (2002) A PCR-marker for the CMS$_1$ inducing cytoplasm in chives derived from recombination events affecting the mitochondrial gene atp9. Theor Appl Genet 104:698–702

Forner J, Weber B, Thuss S, Wildum S, Binder S (2007) Mapping of mitochondrial mRNA termini in Arabidopsis thaliana: t-elements contribute to 5′ and 3′ end formation. Nucleic Acids Res 35:3676–3692

FAOSTAT, 2014. Agriculture Organization of the United Nations

Gurushidze M, Mashayekhi S, Blattner FR, Friesen N, Fritsch RM (2007) Phylogenetic relationships of wild and cultivated species of Allium section Cepa inferred by nuclear rDNA ITS sequence analysis. Plant Syst Evol 269(3):259–269

Hammani K, Giege P (2014) RNA metabolism in plant mitochondria. Trends Plant Sci 19:380–389

Hanson MR, Bentolila S (2004) Interactions of mitochondrial and nuclear genes that affect male gametophyte development. Plant Cell 16(Suppl):S154–S169. https://doi.org/10.1105/tpc.015966

Havey MJ (1999) Seed yield, floral morphology, and lack of male-fertile restoration of male sterile onion (Allium cepa) population possessing the cytoplasm of Allium galanthum. J Am Soc Hort Sci 124:626–629

Havey MJ (2000) Diversity among male-sterility-inducing and male-fertile cytoplasms of onion. Theor Appl Genet 101:778–782

Holford P, Croft J, Newbury HJ (1991a) Structural studies of microsporogenesis in fertile and male-sterile onions (Allium cepa L.) containing the cms-S cytoplasm. Theor Appl Genet 82:745–755

Holford P, Croft JH, Newbury HJ (1991b) Differences between, and possible origins of, the cytoplasms found in fertile and male-sterile onions (Allium cepa L.). Theor Appl Genet 82:737–744

Jones HA, Clarke AE (1943) Inheritance of male sterility in the onion and the production of hybrid seed. Proc Am Soc Hortic Sci 43:189–194

Jones HA, Davis G (1944) Inbreeding and heterosis and their relation to the development of new varieties of onions. USDA Tech Bull 874:1–28

Jones HA, Emsweller SL (1936) A male-sterile onion. Proc Am Soc Hortic Sci 34:582–585

Keeling PJ (2010) The endosymbiotic origin, diversification and fate of plastids. Philos Trans R Soc Lond B Biol Sci 365:729–748. https://doi.org/10.1098/rstb.2009.0103

Khosa JS, McCallum J, Dhatt AS, Macknight RC (2016) Enhancing onion breeding using molecular tools. Plant Breed 135:9–20. https://doi.org/10.1111/pbr.12330

Kik C (2002) Exploitation of wild relatives for the breeding of cultivated Allium species. CABI Publishing, pp 81–100. https://doi.org/10.1079/9780851995106.0081

Kim B, Kim K, Yang TJ, Kim S (2016) Completion of the mitochondrial genome sequence of onion (Allium cepa L.) containing the CMS-S male-sterile cytoplasm and identification of an independent event of the ccmF$_N$ gene split. Curr Genet 62:873–885. https://doi.org/10.1007/s00294-016-0595-1

Kim S (2014) A codominant molecular marker in linkage disequilibrium with a restorer-of-fertility gene (Ms) and its application in reevaluation of inheritance of fertility restoration in onions. Mol Breed 34:769–778. https://doi.org/10.1007/s11032-014-0073-8

Kim S, Lee ET, Cho DY, Han T, Bang H, Patil BS, Ahn YK, Yoon M-K (2009) Identification of a novel chimeric gene, orf725, and its use in development of a molecular marker for distinguishing among three cytoplasm types in onion (Allium cepa L.). Theor Appl Genet 118:433–441. https://doi.org/10.1007/s00122-008-0909-x

Kim S, Park JY, Yang T (2015) Comparative analysis of the complete chloroplast genome sequences of a normal male-fertile cytoplasm and two different cytoplasms conferring cytoplasmic male sterility in onion (Allium cepa L.). J Hortic Sci Biotechnol 90:459–468. https://doi.org/10.1080/14620316.2015.11513210

Ng S, De Clercq I, Van Aken O, Law SR, Ivanova A, Willems P, Giraud E, Van Breusegem F, Whelan J (2014) Anterograde and retrograde regulation of nuclear genes encoding mitochondrial proteins during growth, development, and stress. Mol Plant 7:1075–1093. https://doi.org/10.1093/mp/ssu037

Raczynska KD, Le Ret M, Rurek M, Bonnard G, Augustyniak H, Gualberto JM (2006) Plant mitochondrial genes can be expressed from mRNAs lacking stop codons. FEBS Lett 580:5641–5646. https://doi.org/10.1016/j.febslet.2006.09.010

Rhoads DM (2011) Plant mitochondrial retrograde regulation. In: Kempken F (ed) Plant mitochondria. Springer, New York, pp 411–437. https://doi.org/10.1007/978-0-387-89781-3_16

Schweisguth B (1973) Étude d'un nouveau type de stérilité male chez l'oignon. Allium cepa L. Ann. Amélior Plant 23:221–233

Tsujimura M, Kaneko T, Sakamoto T, Kimura S, Shigyo M, Yamagishi H, Terachi T (2018) Multichromosomal structure of the onion mitochondrial genome and a transcript analysis. Mitochondrion (in press). https://doi.org/10.1016/j.mito.2018.05.001

von Kohn C, Kielkowska A, Havey MJ (2013) Sequencing and annotation of the chloroplast DNAs and identification of polymorphisms distinguishing normal male-fertile and male-sterile cytoplasms of onion. Genome 56:737–742. https://doi.org/10.1139/gen-2013-0182

Yamashita K-I, Takatori Y, Tashiro Y (2005) Chromosomal location of a pollen fertility-restoring gene, Rf, for CMS in Japanese bunching onion (Allium fistulosum L.) possessing the cytoplasm of A. galanthum Kar. et Kir. revealed by genomic in situ hybridization. Theor Appl Genet 111:15–22. https://doi.org/10.1007/s00122-005-1941-8