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REVIEW

Post-transcriptional Regulation in Mitochondria of Rice and Wheat at Low Temperatures

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Abstract

Land plants respond to cold through complicated physiological, morphological, and metabolic processes, including abnormalities and changes in organelles, while plant mitochondrial gene expressions are regulated through several unique post-transcriptional events, such as *cis*-/*trans*-intron splicing and RNA editing. The author tries to clarify the relationship between these post-transcriptional events at low temperatures. Some intron-containing transcripts before splicing increase in rice and wheat after several days under cold conditions, while certain RNA editing events in IBS (Intron Binding Sites: which is present in exons and make complementary base pairing with Exon Binding Sites located in introns) have tight associations with splicing, some of which are sensitive to cold in intron-containing transcripts. However no correlation is observed between post-transcriptional events and the organization of introns: the primary sequence of introns, splicing manner (cis or trans), intron length, and splice-site sequence. These findings suggest that nucleus-encoded factors regulate mitochondrial gene expressions, some of which are cold-sensitive. In wheat, a cold acclimatable plant, accumulation of some intron-spliced transcripts also increase at low temperatures, as well as intron-containing transcripts, which may be one of the phenomena related to cold acclimation. The study about plant mitochondrial gene expression at low temperatures has the potential to become an alternative system to see how mitochondrial genes are regulated, which, in turn, has the potential to enhance plant breeding for cold tolerance.

Discipline: Plant breeding
Additional key words: group II intron, RNA editing, splicing

Introduction

Land plants respond to cold through complicated physiological, morphological, and metabolic processes[63]. These processes induce a number of changes, including gene expression, desiccation, solute accumulation, plant hormones, membrane alterations, abnormalities in anther, and so on in plant tissue[19,20,60,66,76,81]. Furthermore, changes and abnormalities are also observed in mitochondria and chloroplasts[4,36,48]. The response of mitochondrion to cold has become pronounced, as such changes are critical for the survival or adaptation of land plants. Cold-sensitive plants show increases in alternative oxidase (AOX) pathway activity and decreases in the cytochrome pathway activity of mitochondrial electron transport at low temperatures in mitochondria, resulting in decreases in cellular ATP, until they finally die (e.g. rice[26]; *Cornus stlonifera*[63]). In contrast, cold-tolerant plants show increases in cytochrome pathway activity and cellular ATP, and achieve respiration homeostasis by cold acclimation (e.g. wheat[60]; *A. thaliana*[3,79]). These changes are involved in both regulations and co-regulations by nucleus and/or mitochondria-encoded gene expressions at low temperatures. The expressions of organelles-encoded genes are regulated differently from nucleus-encoded genes: by polycistronic transcription, changes in the DNA copy number per cell, and the ampli-

Abbreviations: IBS, intron binding site; AOX, alternative oxidase; PPR, pentatricopeptide repeats; *cox2*, cytochrome c oxidase subunit 2; *ccmF*$_C$, cytochrome c maturation related protein; *rps3*, ribosomal protein S3; *nad7*, NADH dehydrogenase subunit 7

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fication of subgenomic molecules, as well as post-transcriptional processes which are the predominant regulation system. This review deals with the post-transcriptional regulation in mitochondria of rice and wheat at low temperatures; focusing on the relationship between splicing and RNA editing. Furthermore, the perspective of plant mitochondrial biological studies concerning crop breeding for cold stress tolerance is also described.

Post-transcriptional regulation in plant mitochondria-encoded genes

Organelle gene expressions in plants involve unique post-transcriptional steps, such as *cis*-/*trans*-intron splicing, RNA editing, maturation of transcript termini, and sometimes cleavage of polycistronic transcripts to monocistronic mRNAs[5,67].

Plant organelle-encoded genes contain a number of introns, which could be categorized as members of group I- or II families like introns of fungus and protist organelle genes, which are originally mobile genetic elements[7,51,75]. Group I and group II introns differ in terms of their higher order structure and splicing modes. In vascular plant organelles, group II introns predominate and group I introns are scarce. The only example of a group I intron in the plastid genome identified to date is found in *trn*L-UAA[38,80], in which intronic interruption is preserved in many angiosperms[73]. Mitochondrial group I intron has been identified in *cox1* of several angiosperm species[10]. The origin of the *cox1* intron may be fungi, while the ancestral intron is believed to have been horizontally transferred from the donor organism[10,78]. No other group I intron has been identified in the vascular plant mitochondrial genome, however, considering the horizontally transferred origin of the *cox1* intron, it is possible that another example will be found. Mitochondrial ge-

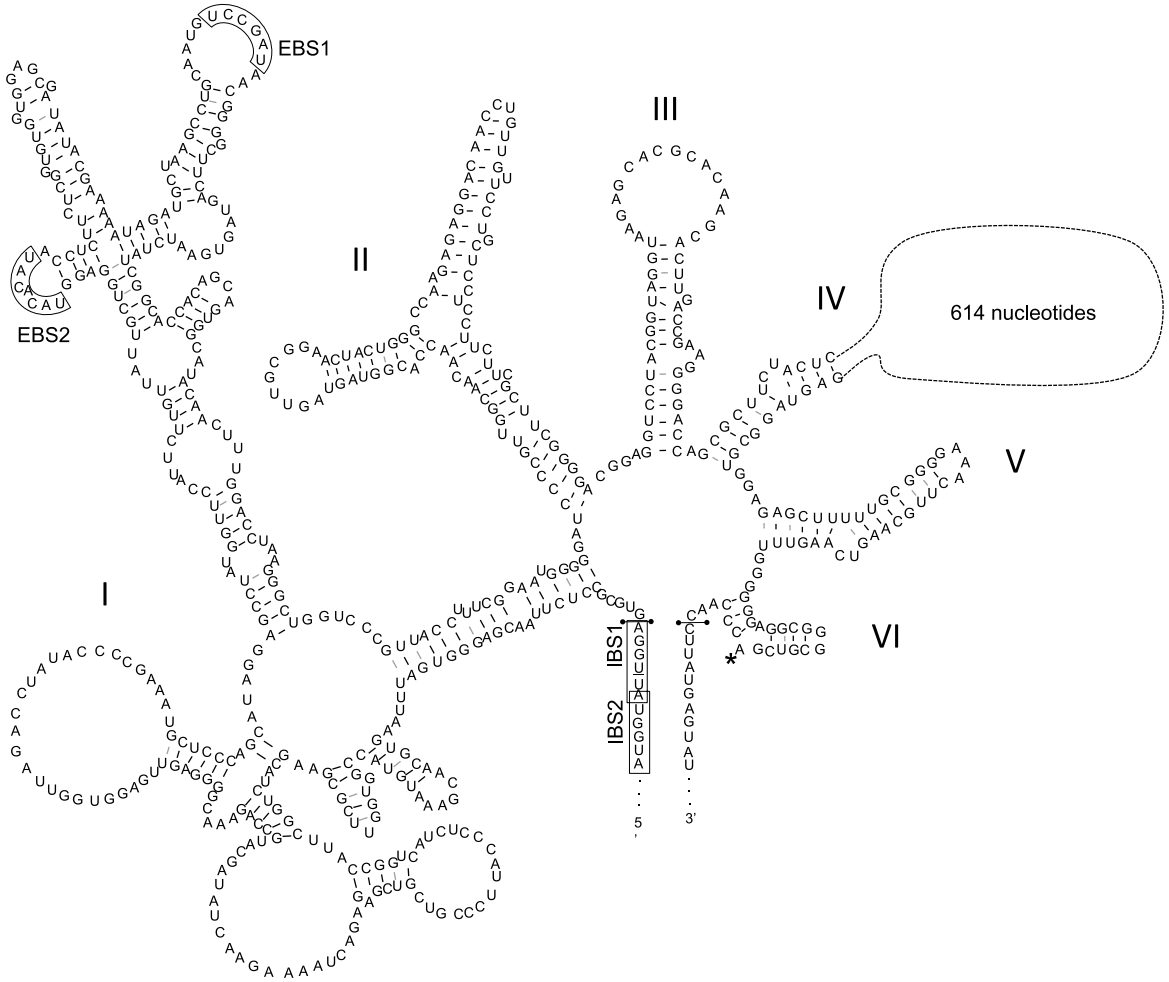

Fig. 1. Group II intron secondary structure model as illustrated by the wheat *cox2* intron, featuring a star-like secondary structure with a central hub and six protruding helical domains (I-VI)

EBS and IBS indicate exon- and intron binding sites, respectively. An asterisk at the bulged adenosine in domain VI shows the branch point forming the lariat structure for splicing. Note that *trans*-splicing introns in angiosperm mitochondria are disrupted with domain IV. Two lines indicate the boundaries of exon 1/intron and intron/exon 2.

nomes in angiosperm sequenced in their entirety to date contain 20 to 24 group II introns[11,21,37,57,59,72,77], while chloroplast genomes contain 17 to 20 group II introns[14]. Group II introns feature a star-like secondary structure with a central hub and six extending domains[8] (Fig. 1). The higher order structure of the intron, required for correct splicing, is formed by the interaction of tertiary structural elements in the intron and exons: Fig. 1 illustrates the secondary structure of the wheat *cox2* intron and examples of such tertiary structural elements: two exon binding sites (EBS1 and EBS2) and two intron binding sites (IBS1 and IBS2) present in domain I of the intron and at the 3' end of the preceding exon, respectively, which form complementary base pairings with each other. The splicing of group II introns proceeds through two trans-esterification steps: the 2' hydroxyl of a bulged adenosine in domain VI attacks the 5' splice site, followed by a nucleophilic attack on the 3' splice site by the 3' OH of the upstream exon[8]. The excised intron forms itself into a lariat with a 2'-5' linkage at the adenosine site having a 6-7 nucleotide tail. Group II introns are considered the ancestors of spliceosomal introns in nuclear genes because of their similar lariat forms. Group II introns in plant organelles have these general properties, albeit with some exceptions, such as a lack of self-splicing activity *in vitro*[8,9]. This indicates that an additional component, such as a helper protein, is required for splicing; however, none of the angiosperm organellar introns encodes an intronic ORF for splicing, except two (MAT-R encoded within *nad1* intron 4 in mitochondria and MatK encoded within *trnK* in chloroplasts), and no such ORF is encoded alone in the organellar genome[6]. Recently, a report suggested that MatK associates with some group II introns in tobacco chloroplasts[83]. Nevertheless, nucleus-encoded machinery must be imported into both mitochondria and chloroplasts to help splicing.

RNA editing in land plant organelles changes specific cytidine residues with uridine (C to U) by a deamination reaction, or specific uridine residues to cytidine (U to C), occurring at the post-transcriptional level[2,70,74]. In angiosperm organelles, it converses especially C to U[74]. The entire sets of editing sites in the protein-coding regions of all identified mRNAs in an angiosperm mitochondrial genome range from 357 to 491[18,21,54,57], while much lower numbers ranging within around 20 to 40 are predicted in chloroplasts[16]. A major role of this editing is to change the identity of genomically encoded amino acid residues, making the sequences more conserved and/or functional. In addition, RNA editing in plant mitochondria is required in several tRNAs to make them fold correctly by improving the intra-molecular base pairing and thus making them functional[49]. RNA editing in plant mitochondria is an essential step of RNA maturation, without which neither a working respiratory chain nor functional mitochondria can be assembled and maintained in the cell[74]. Factors involved in organellar RNA editing have been identified in the nuclear genome as PPR (*p*entatrico*p*eptide *r*epeats) proteins[16,35,82], supporting the view that RNA editing is governed by the nuclear genome, which will be described in the last chapter. Close associations between RNA editing and the other RNA processing steps are possible, for example, splicing; the editing status of IBS1 is negatively correlated with the abundance of unspliced precursor transcripts in germinating wheat *cox2*[44,45].

Mitochondria-encoded gene expressions in rice (*Oryza sativa* L.), a cold-sensitive crop

It is indicated that the accumulation of intron-containing transcripts increase at low temperatures in rice mitochondria as shown in the profile of the *cox2* (cytochrome c oxidase subunit 2) transcript by Northern blots[41] (Fig. 2). The amount of intron-containing transcript before splicing increased over time at 12˚C, while the amount of transcripts harboring no intronic sequence after splicing remained unchanged. Similar results were also obtained for the transcript profiles of *rps3* (ribosomal protein S3) and $ccmF_C$, (cytochrome c maturation related protein)[41] (Fig. 2). It has been widely reported that cold-inducible genes also respond to a water deficit[27,81]. Thus, the effects of ABA, NaCl, and water deficit on the *cox2* transcriptional pattern were preliminarily examined; however, no accumulation change in the *cox2* intron-containing transcript was observed, suggesting no direct relationship between the increase in the *cox2* precursor transcript and osmotic stress[41].

The profiles of all other genes with introns were analyzed by semi-quantitative RT-PCR[41] and summarized in Table 1. The abundance of fourteen types of a total of twenty-three type intron-adjoining cDNAs increases and none decrease at 12˚C, while post-splicing transcripts remain mostly unchanged (Table 1). There seems to be no correlation between the cold-induced accumulation of intron-containing transcripts and organization of introns: the splicing manner (*cis* or *trans*), intron length, splice-site sequence, and length of domains V and VI[41]. Therefore, the association of nuclear genes with mitochondrial transcription and their cold responses should be investigated.

It was reported that some RNA editing sites in rice mitochondria are cold-sensitive[41] as shown in Figs. 3 and 5. In the intron-spliced *cox2* transcript, all 19 sites were almost fully edited irrespective of temperature. Con-

versely, a difference in editing in the intron-containing *cox2* transcript was observed between the two conditions (Fig. 3). At site 9, no editing was observed under 12˚C whereas about 40% of the transcripts were edited under 25˚C (Fig. 3). It should be noted that site 9 (four nucleotides upstream from the exon/intron boundary) is located in the IBS1 of *cox2*.

The effect of low temperature on the frequency of RNA editing in IBS was examined[41]. Among the 491 C-to-U editing sites in the protein-coding regions of rice mitochondria[57], six sites from four genes were selected as candidate RNA editing sites in IBS to be locally investigated.

All six sites were fully edited in the intron-spliced transcript; regardless of growth conditions. Conversely, of the six sites in intron-containing transcripts, two exhibited nearly complete editing, irrespective of growth conditions. One site exhibited incomplete editing and its

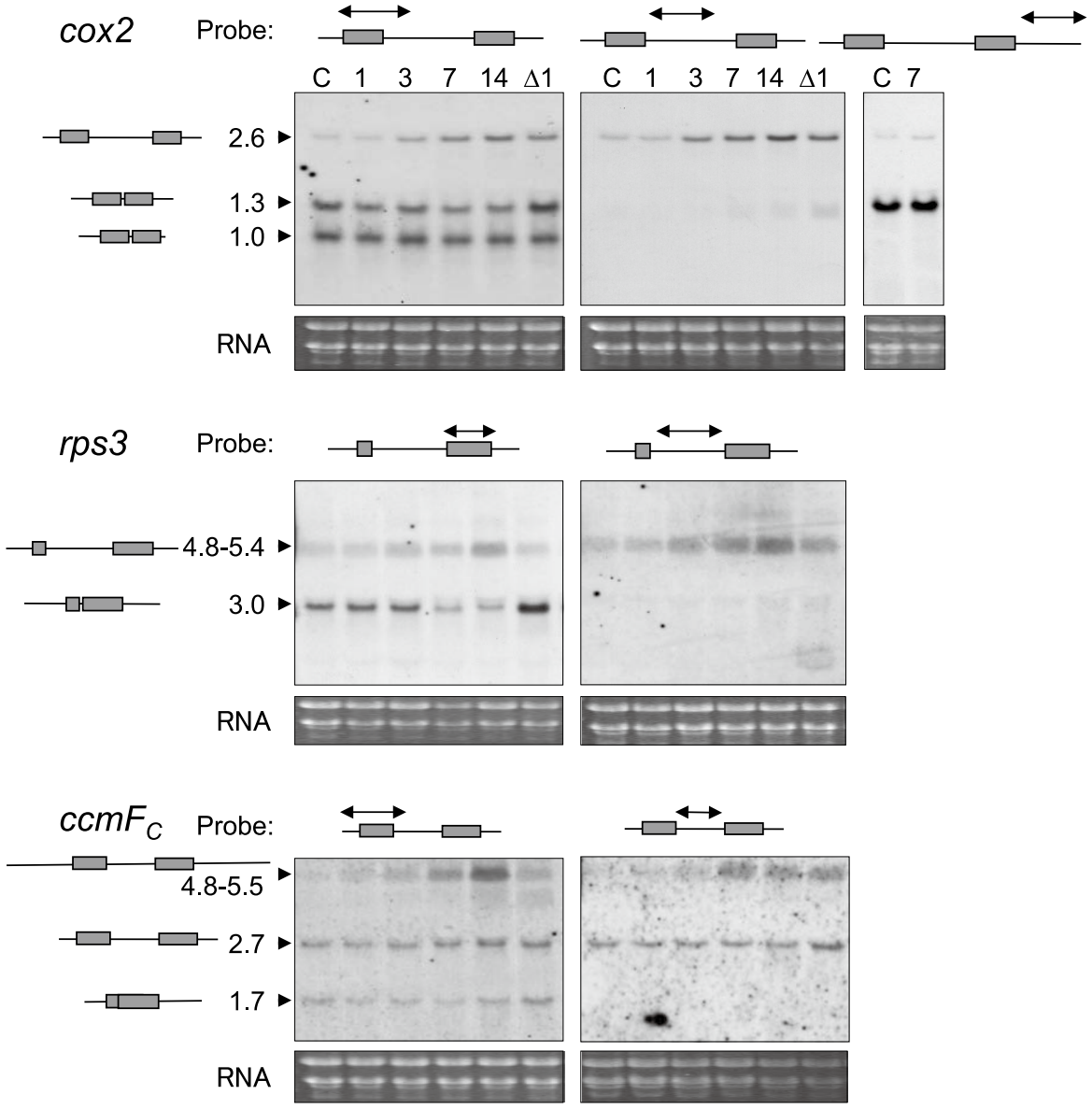

Fig. 2. Northern blot analyses of rice *cox2*, *rps3*, and *ccmF*$_C$

Total cellular RNA was isolated from plantlets grown at 25˚C (C), kept at 12˚C for 1, 3, 7, and 14 days (1, 3, 7 and 14, respectively), and 1 day after release from the 14-day treatment (Δ1). Probes corresponding to the indicated regions were used. The sizes of the signal bands are shown in kb. (Figures were referred from Kurihara-Yonemoto and Kubo[41].)

The amount of intron-containing transcript of rice *cox2* (2.6kb band) increased over time at 12˚C, while the amount of transcripts with no intronic sequences (1.3 and 1.0kb bands) remained unchanged. One day after removing plantlets from the 14-day cold treatment, the amount of intron-containing transcript declined slightly. In case of the transcript profiles of rice *rps3* and *ccmF*$_C$, similar results were obtained.

ratio of edited to unedited residues remained unchanged after 12˚C treatment. At another site, no editing at all was observed, irrespective of temperature (Fig. 4). At the remaining two sites, incomplete editing was observed in rice grown at 25˚C, while the frequency of editing gradually decreased at 12˚C (Fig. 5).

The six editing events stated in the former section can be classified into two types. The first type is the efficient editing of sites in the IBS of intron-containing transcripts before intron splicing, irrespective of temperature conditions. The second type of editing is tightly associated with splicing (Figs. 3, 4, and 5). The clear association between splicing and editing at site 20, the junction of exon 3/intron 3, of *nad7* (NADH dehydrogenase sub-

Table 1. Summary of transcript accumulation after low-temperature treatment of introns in 9 mitochondrial genes of rice and 8 mitochondrial genes of wheat

			Rice exposed to 12 ˚C for 14 days		Wheat exposed to 2/0.5 ˚C for 14 days	
	Intron		Accumulation after low-temperature treatment		Accumulation after low-temperature treatment	
Gene	No.	Mode of splicing	Intron-containing transcripts	Intron-spliced transcripts	Intron-containing transcripts	Intron-spliced transcripts
nad1	1	*trans*	Increased	Unchanged	Increased	Slightly increased
	2	*cis*	Unchanged	Unchanged	Slightly increased	Slightly increased
	3	*trans*	Unchanged	Unchanged	Increased	Slightly increased
	4	*trans*	Increased	Unchanged	Increased	Slightly increased
nad2	1	*cis*	Increased	Unchanged	Increased	Slightly increased
	2	*trans*	Unchanged	Unchanged	Increased	Unchanged
	3	*cis*	Increased	Unchanged	Slightly increased	Slightly increased
	4	*cis*	Increased	Unchanged	Slightly increased	Unchanged
nad4	1	*cis*	Slightly increased	Unchanged	Increased	Slightly increased
	2	*cis*	Slightly increased	Unchanged	Slightly increased	Slightly increased
	3	*cis*	Slightly increased	Unchanged	Slightly increased	Unchanged
nad5	1	*cis*	Unchanged	Unchanged	Increased	Slightly increased
	2	*trans*	Unchanged	Unchanged	Increased	Unchanged
	3	*trans*	Unchanged	Unchanged	Increased	Unchanged
	4	*cis*	Increased	Unchanged	Slightly increased	Unchanged
nad7	1	*cis*	Unchanged	Unchanged	Increased	Increased
	2	*cis*	Unchanged	Unchanged	Increased	Slightly increased
	3	*cis*	Unchanged	Unchanged	Increased	Slightly increased
	4	*cis*	Slightly increased	Unchanged	Increased	Unchanged
cox2	1	*cis*	Increased	Unchanged	Increased	Unchanged
rps3	1	*cis*	Increased	Slightly decreased	Increased	Slightly increased
rpl2	1	*cis*	Slightly increased	Unchanged	-	-
$ccmF_C$	1	*cis*	Increased	Unchanged	Increased	Slightly increased

Software Multi-Analyst (Bio-Rad laboratories, USA) was applied to an objective quantification of the bands to resolve some of the ambiguous results with the naked eye. 'Slightly increased' indicates 'about 10% increased'. Data of rice were extracted from Kurihara-Yonemoto and Kubo (2010) while those of wheat were referred to Kurihara-Yonemoto (2007).

The relative abundance of intron-adjoining and -spliced cDNA between rice plantlets grown at 25˚C and those exposed to 12˚C for 14 days were compared by semi-quantitative RT-PCR, for each of the entire introns' set listed, which the rice mitochondrial genome has.

The relative abundance of intron-adjoining and -spliced cDNA between wheat plantlets grown at 20/15˚C (day/night) and those exposed to 2/0.5˚C (day/night) for 14 days were compared by semi-quantitative RT-PCR, for each of the entire introns' set listed, which the wheat mitochondrial genome has. Wheat *rpl2* is a truncated pseudogene, which has no intron.

unit 7) can be explained by the 'physical blocking' model[45], where the presence of intron could hinder the editing machinery accessing the site, but editing would immediately follow splicing (Fig. 4). Alternatively, given the cited importance of IBS for intron splicing[15], it cannot be ruled out that RNA editing at this site facilitates, or provides a cue for, immediate RNA splicing. As shown in Fig. 5, the frequency of editing at site 9 of *cox2* and site 3 of *rps3* began to decline 1 day after the onset of cold treatment, while intron-containing transcript levels remained unchanged as shown in the Northern blot, declined gradually at 12˚C until 14 days according to the increase in the intron-containing transcript; and the frequency was swiftly restored within a day of relief from cold treatment, while intron-containing transcript levels remained high. This observation indicates that RNA editing occurs before splicing in these cases. In any case, the residual editing activities after cold treatment seem tightly associated with splicing. Therefore, the type of RNA editing status of IBS sites could depend on the kinetics of the two phenomena (i.e. physically blocking RNA editing by the obstacle of an intron and facilitating RNA splicing by editing in the IBS).

Characterization of the machinery involved in RNA editing is yet to be clarified. However, it is thought that independent types of protein factors are involved in editing events, at least one of which is cold-sensitive. Interestingly, similar phenomena in the plastid are reported: some editing sites of tobacco *ndhB* are sensitive to a high temperature of 42˚C and antibiotics[28,29], which suggests that some editing factors in either organelle are temperature-sensitive.

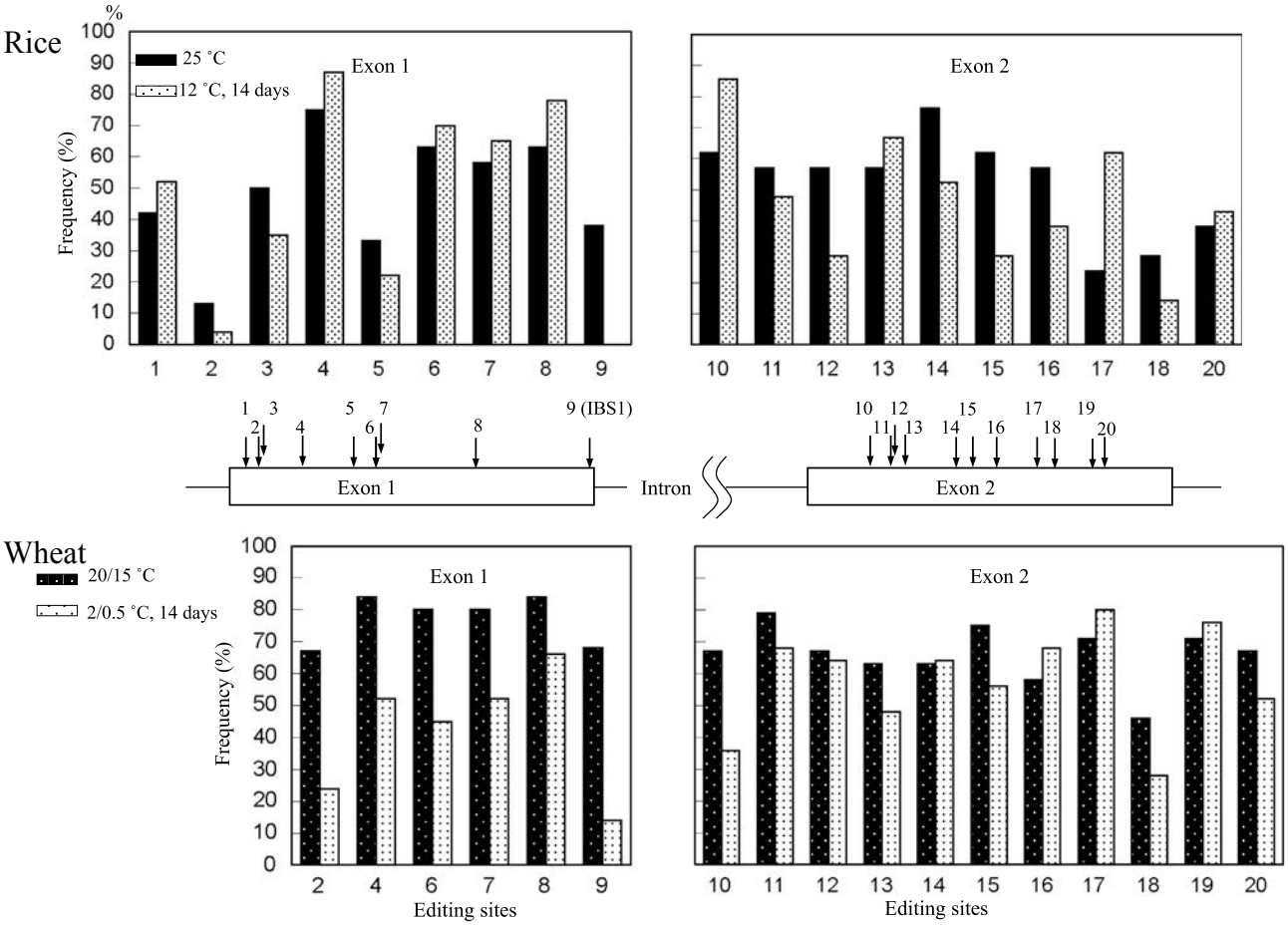

Fig. 3. RNA-editing frequency of intron-containing transcripts of rice *cox2* at the 19 sites at 25˚C (black) and after 12˚C treatment for 14 days (speckled), and of wheat *cox2* at the 17 sites at 20/15˚C (black with white speckles) and after 2/0.5˚C treatment for 14 days (speckled)

Horizontal axes indicate the editing sites, while vertical axes indicate the frequencies of editing (%). Editing sites 1 to 9 belong to exon 1 and sites 10 to 20 to exon 2[12,57]. (Figures were referred from Kurihara-Yonemoto and Kubo[41], and Kurihara-Yonemoto and Handa[40].)

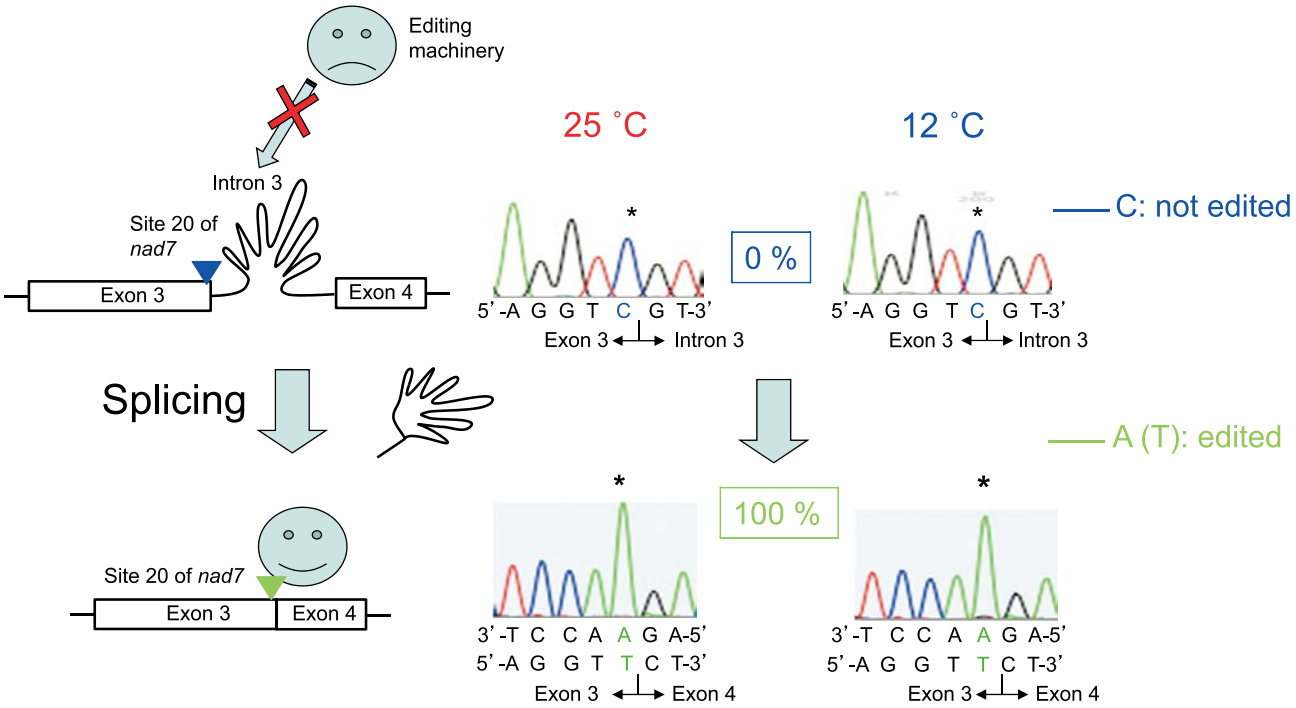

Fig. 4. The 'physical blocking' model: editing machinery could not access site 20 of rice *nad7* due to the presence of intron 3
No editing was observed at this site in intron-containing transcripts at either 12 or 25˚C, whereas intron-spliced transcripts were fully edited.

Mitochondria-encoded gene expressions in wheat (*Triticum aestivum* L.), a cold-acclimatable crop

The accumulation of intron-containing transcripts of *cox2* and other mitochondria-encoded genes has been shown to increase in wheat treated at low temperatures (2/0.5˚C (day/night))[39,40], as well as the case in rice (Table 1). The increase of accumulation reportedly started after some days of treatment in wheat, which resembles rice[39,40,41]. When some varieties were compared, the increase in winter wheat varieties tended to start later than that in spring ones, which suggests that this phenomenon is linked to cold tolerance[39]. No increases were observed in rice *cox2* after 4˚C treatment[39]. The difference in the temperature inducing the increases may be related to each species' intrinsic tolerance to low temperature, i.e. wheat acclimates to 2˚C but this is a lethal temperature for rice[26]. Interestingly, a shift up in the relative levels of precursors compared with the respective mRNAs has been reported in germinating wheat seeds[44], in which levels of mitochondrial activity exceed those in adult vegetative tissues[46]. A temporal difference exists between developmental- and cold-induced increases, in that the accumulation of intron-containing transcripts is maximized half to one day after imbibition, whereas 3 to 7 days are required after cold treatment. However, mitochondrial responses are shared by germinating seeds and cold-treated plants, such as increased O_2 uptake capacity, differential fluxes between cyanide-sensitive (mainly, cytochrome) and -insensitive respiratory (AOX) pathways, and the activation of some mitochondrial genes encoded in the nuclear genome[3,42,52,62]. It is possible that one or more of these responses is directly associated with the accumulation of intron-containing transcripts and will shed light on the physiological significance of the phenomenon. For wheat at 2/0.5˚C, the accumulation of the intron-spliced transcript has a tendency to increase[39], while in the case of rice at 12˚C, post-splicing transcripts remained almost unchanged[41] (Table 1). This does not contradict the increases in cytochrome pathway activity in cold acclimatable plants[3,79].

The RNA editing status of wheat mitochondria at low temperatures also resembles that in rice[40]. The editing frequency of site 9 of wheat intron-containing *cox2* transcripts, which is located in IBS1 and corresponds to the cold-sensitive editing site in rice *cox2*, was very low under cold conditions, although about 70% were edited under 20/15˚C (day/night)[40] (Fig. 3). When the RNA editing status of *cox2* exon 1 in two species is compared, the frequency of all editing sites decreases in wheat after treatment at 2/0.5˚C, while that of only site 9 decreases markedly in rice after 12˚C treatment[40,41] (Fig. 3). This

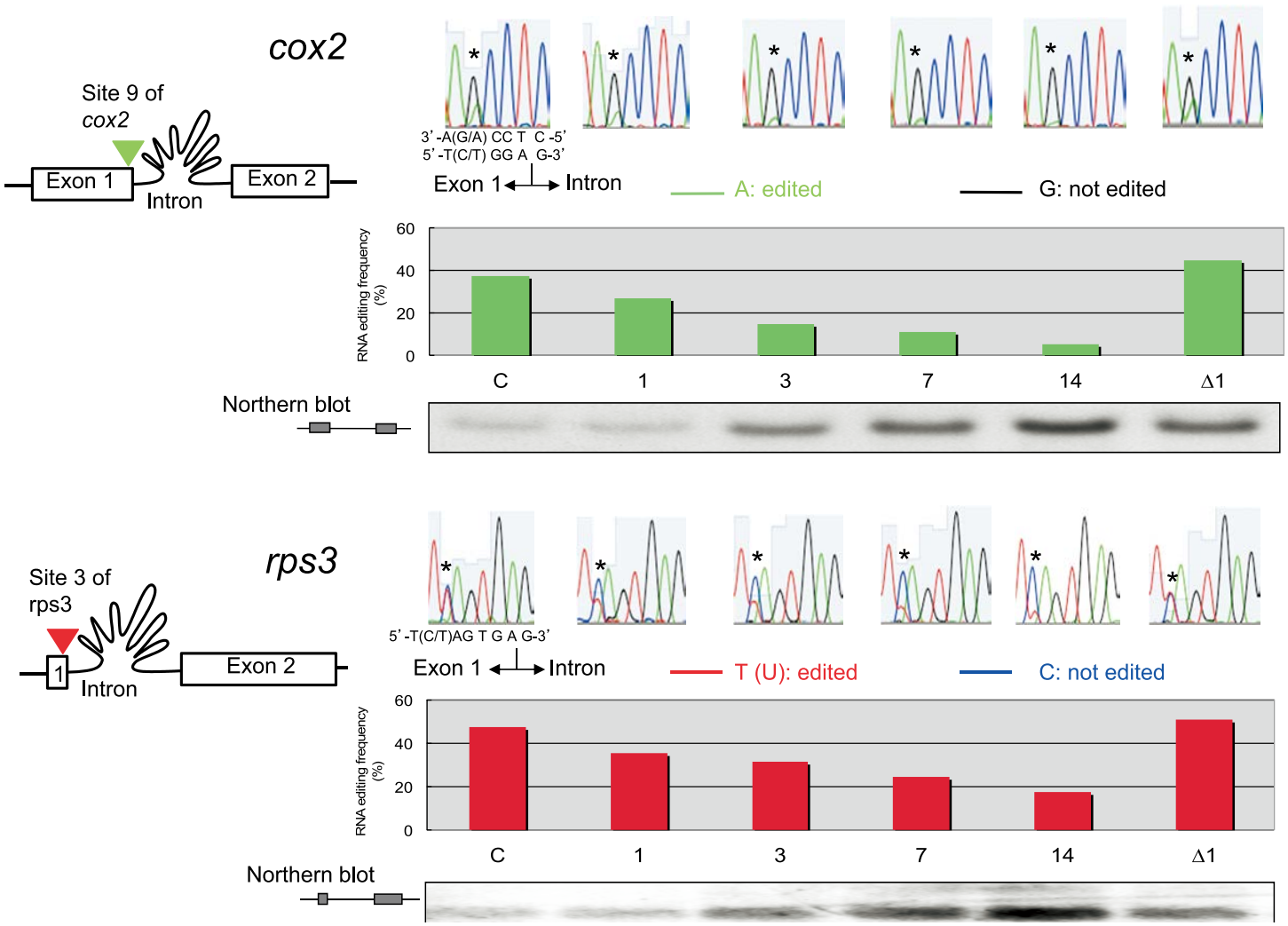

Fig. 5. Direct sequencing of RT-PCR products amplified from intron-containing rice *cox2* and *rps3* including IBSs

Complementary DNA was synthesized from rice plantlets grown at 25˚C (C), kept at 12˚C for 1, 3, 7, and 14 days (1, 3, 7 and 14, respectively), and 1 day after release from the 14-day treatment (Δ1). The nucleotide sequences are shown below the chart. Exon 1/intron boundaries are indicated below the sequences. Asterisks on the charts indicate the editing sites. The frequencies of RNA editing are shown in the bar chart in the middle, and accumulations of the intron-containing transcript are shown in the Northern blot below. (Figures were referred from Kurihara-Yonemoto and Kubo[41])

At site 9 of *cox2* and site 3 of *rps3*, incomplete editing was observed in rice grown at 25˚C, the frequency of editing began to decrease 1 day after cold treatment at 12˚C, while intron-containing transcript levels analyzed by Northern blot remained unchanged. They gradually decreased at 12˚C until 14 days and the RNA editing was restored quickly one day after relief from cold treatment, while intron-containing transcript levels analyzed by Northern blot remained high.

may be due to abundant increases in the substrate for RNA editing, the intron-containing transcript, in wheat, which would mask the edited transcript given limited editing activity. When the RNA editing status of *cox2* exon 2 in two species is compared, an increase in editing at some sites and a decrease at others are more often observed in rice than wheat[40,41] (Fig. 3). This may be involved in the absence of the correct tertiary structure of the group II intron in rice, which is essential for splicing.

Perspective of a plant organelle biological study, concerning crop breeding for cold-stress tolerance

Mitochondria and chloroplasts originate from bacteria symbionts in eukaryotic cells. Their host cells gained the respiration function from the ancestor of the mitochondria, α-proteobacteria, and the photosynthesis function from the ancestor of the chloroplast, cyanobacteria. Some nuclear genes encoding organellar proteins originate from an evolutionary gene transfer from the organelle to nucleus. An interesting question is why some genes have been retained in the organelle. It is possible simply because of the hydrophobicity-importability, which means that these proteins are too hydrophobic to import into mitochondria[13]. In contrast, a hypothesis termed 'CORR', for *co*-location for *r*edox *r*egulation, was proposed: mitochondria and chloroplasts contain genes whose expression must be under the direct regulatory control of the redox state of their gene products, or electron carriers with their gene products[1]. It is important to control redox under abiotic stresses, including chilling, heat shock, desiccation, and salt, which enhance the production of ROS: *r*eactive *o*xygen *s*pecies.

The fertility of rice, a cold-sensitive plant, decreases critically when it is exposed to low temperatures around 12˚C at the booting stage, due to the failure of microspore development[65]. Reproductive organs have high mitochondrial biogenesis activity[17,24,43,61,71]. Accordingly, if mitochondrial abnormalities can be ameliorated, rice fertility will improve in cold weather years.

Conversely, cold-tolerant plants such as wheat and *Arabidopsis* show cold acclimation: respiration capacity and mitochondrial gene expressions are augmented after some duration at low temperatures. It was reported that maintenance of growth rates at low temperatures in wheat cultivars with a high degree of respiratory homeostasis was associated with highly efficient respiratory ATP production[42]. It was also reported that *Arabidopsis* leaves developed at low temperatures showed increased COXII protein abundance and a drastically increased cytochrome pathway capacity, with a transient increase in alternative oxidase activity[3] that may only be significant in the early stages of cold treatment.

To recognize the large number of RNA editing sites in mitochondrial transcripts: 441 in *Arabidopsis thaliana* ORFs[18] and 491 in rice ones[57], a correspondingly large number of trans-factors will be required. This decade, the PPR protein family has been proposed as a candidate group of these specific factors[47]. In angiosperm, this family contains members of several hundreds: 450 and 477 PPR genes in *Arabidopsis thaliana* and rice, respectively[58]. The profiles of production programs *in silico* suggest that they are almost all targeted at either plastids or mitochondria, or both[47]. Moreover, considerable evidence of the involvement of PPR proteins in mitochondrial and chloroplast RNA editing was shown using PPR mutants with single amino acid substitutions[16,35,56,58,82]. It is becoming increasingly clear that PPR proteins, which are gene-specific RNA-binding proteins for organelles, are involved in all aspects of transcription and post-transcriptional processing, including not only RNA editing but also RNA cleavage, group II intron splicing and degradation[16,25,56,58]. Interestingly, *Rf* genes, which act to suppress mitochondrial cytoplasmic male sterility, often also encode PPR proteins[22,23,30,34]. To date, there has been little information regarding their changes to explain regulation under different environments because PPR genes are expressed at low levels[47]. Furthermore, group II intron splicing factors besides PPR proteins have been found: MRS2, a homologue of the yeast splicing factor[68,69], maturases, *Marchantia* or yeast group II intronic ORF homologs in higher plants[31,32,53,55], PMH2, a DEAD-box protein[33], and so on. Information on these genes will help develop environmental tolerances of crops.

The following examples suggest some correlation with chloroplast-encoded gene expressions and cold tolerances of plants. It was reported that low temperatures inhibit chlorophyll accumulations in actively growing leaves and that cold-sensitive rice lines accumulate less chlorophyll under cold conditions than cold-tolerant lines[64]. Albinism and chlorosis are phenomena decreasing chlorophyll accumulation, due to abnormalities in plastid biogenesis. Interestingly, it was reported that rice albinos in anther culture regenerants, which is one of the main problems linked with this breeding technique, arose from splicing abnormalities of a group II intron in chloroplast-encoded gene expression[50]. These reports suggest that group II intron splicing factors could be key for chlorophyll accumulation and cold tolerance. Further study of nucleus-encoded factors implicated in the post-transcriptional regulation of plant organelles is necessary, especially those subject to abiotic stresses.

The study of post-transcriptional regulation in mito-

chondria at low temperatures has just started. However, it has the potential to become an alternative system to determine how organellar genes are regulated, which enhances plant breeding for cold tolerances.

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