ARTICLE ADDENDUM

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Divergent regulatory mechanisms in the response of respiratory chain component genes to carbohydrates suggests a model for gene evolution after duplication

Raúl N. Comelli and Daniel H. Gonzalez*

Instituto de Agrobiotecnología del Litoral (CONICET-UNL); Cátedra de Biología Celular y Molecular; Facultad de Bioquímica y Ciencias Biológicas; Universidad Nacional del Litoral; Santa Fe, Argentina

The biogenesis of the plant mitochondrial respiratory chain needs the coordinated synthesis and assembly of the products of more than 100 genes located in the nucleus and within the organelle. One of the factors that regulate the expression of nuclear genes is the availability of carbohydrates. This regulation operates at the transcriptional level through elements present in the promoter regions of respiratory chain component genes. Recent studies of the promoters of two Arabidopsis genes that encode subunit 5b of cytochrome *c* oxidase suggest that these genes use different molecular mechanisms to respond to carbohydrates. A model is postulated in which one of the genes retained ancient expression characteristics while the other one incorporated novel response elements that allowed a progressive divergence of regulatory mechanisms.

Key words: cytochrome *c* oxidase, mitochondrial biogenesis, gene expression, response to carbohydrates, gene duplication

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*Correspondence to:
Daniel H. Gonzalez; Email: dhgonza@fbcb.unl.edu.ar

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and
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Plant energy metabolism depends mainly on processes located within two organelles acquired through endosymbiosis: choloroplasts and mitochondria. While the signalling pathways involved in chloroplast biogenesis have been studied in some detail,[1] much less is known about the processes that modulate mitochondrial biogenesis in plants. Particularly, the synthesis of the respiratory complexes, that are directly responsible for ATP production in mitochondria, requires the expression of more than 100 genes distributed among the nuclear and organellar compartments. The particular arrangement of respiratory compoments in a series of complexes and supercomplexes that operate sequentially to drive electrons from reduced coenzymes to oxygen immediately suggests the idea that there must be some sort of coordination in their biogenesis, operating at defined steps from transcription to subunit or cofactor assembly. Among the signals that influence the synthesis of respiratory complexes, tissue-specific and metabolic factors have been described.[2-4] One of the factors that produces a general increase in respiratory chain components in plant cells is the addition of sucrose to the culture medium.[5] This is due to activation of the synthesis of components encoded in the nucleus, that may be the limiting factors for complex assembly. Current evidence indicates that the regulation by sucrose and other carbohydrates operates mostly at the level of transcription of nuclear genes encoding respiratory chain components.[4] It is interesting that induction by carbohydrates is shared not only by genes encoding different components of the respiratory chain, but also by different genes encoding the same component, as is the case for subunit 5b of cytochrome *c* oxidase (COX) described below. A pertinent question is then which are the mechanisms that operate in the modulation of the response of different respiratory chain component genes to carbohydrate availability.

In two recent articles,[6,7] we describe the DNA elements that participate in induction by sucrose of the two nuclear genes that encode COX subunit 5b in Arabidopsis. These genes, *COX5b-1* and *COX5b-2*, have different expression

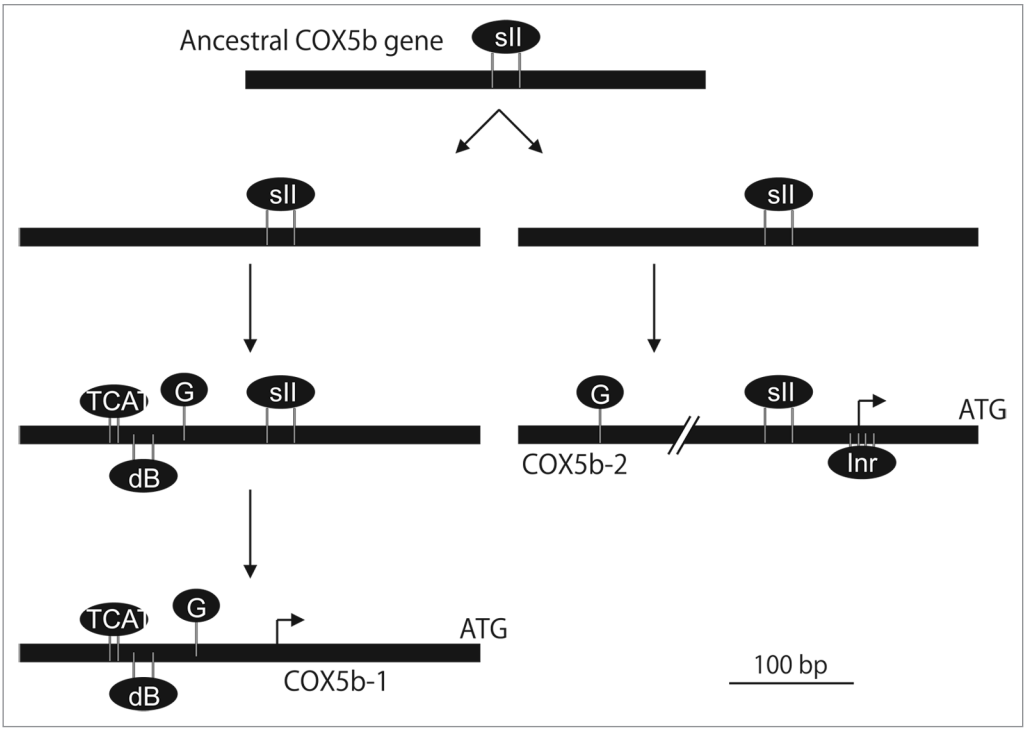

Figure 1. Proposed model for the evolution of COX5b genes. Arabidopsis *COX5b* genes probably derive from an ancestral gene that contained site II elements (sII) and that was duplicated during dicot evolution. After duplication, both genes diverged in expression patterns and responses through the incorporation of functional responsive elements. The *COX5b-1* ancestor incorporated elements with the core sequence TCAT involved in induction by carbohydrates and a G-box (G) involved in basal expression. This allowed the progressive elimination of site II elements. The *COX5b-2* gene retained site II elements and incorporated other elements, as the G-box that confers induction by UV-B light. Inr: initiator elements involved in basal expression of *COX5b-2*; dB: distal B motif, involved in the response of *COX5b-1* to abscisic acid. Broken arrows indicate the transcription start sites.

patterns and respond to several compounds, but share induction by sucrose and other carbohydrates.[8] Inspection of the respective promoter regions did not evidence the presence of common regulatory elements that may explain this behavior, apart from segments that contain a G-box[9] and a nearby ACGT motif, located in different regions respective to the transcription start sites in the two genes. Notably, the two G-boxes influence transcription of both genes but in rather different ways, none of them related with the response to carbohydrates: the *COX5b-1* G-box is essential for transcription while the *COX5b-2* G-box acts as a negative regulatory element involved in induction by UV light. Accordingly, yeast one-hybrid assays indicated that the two elements have different preferences for transcription factors from the ABRE-binding factor (ABF)[10] and G-box binding factor (GBF)[11] classes, respectively (reviewed in ref. 6). It is likely that the different specificities, probably brought about by subtle changes in DNA sequence around the G-box, are responsible for the different regulatory functions of these similar elements.

Detailed mutagenic analysis of the two promoters showed that regulation by carbohydrates is the opposite of the case described above: common regulation through different regulatory elements. For *COX5b-1*, a region located upstream of the G-box that contains elements with the consensus sequence TG(C/T) ATCATT(G/A)T is required for induction by sucrose. Preliminary results indicate that this region is recognized by transcription factors from the HD-Zip family. In the case of *COX5b-2*, motifs with the sequence TGGGYC located in the proximal promoter region are involved. Thus, both genes have acquired the same type of response through the incorporation of different regulatory elements.

Phylogenetic analysis indicated that the duplication that originated both Arabidopsis *COX5b* genes took place relatively recently within dicots.[8] Two posible scenarios arise then for the incorporation of elements involved in regulation by carbohydrates: either both of them were independently incorporated into the respective gene promoters or one of them was already present in the ancestral gene and was replaced by a different element in one of the genes after duplication. Current evidence suggests that the second option is probably the right one. This is based on the fact that elements similar to those present in the *COX5b-2* promoter, known as site II,[12] are also present in a majority of genes encoding respiratory chain components[13] and, at least for some of them, it has been shown that they are involved in the response to carbohydrates.[14,15] Thus, the evolutionary pathway that originated both Arabidopsis *COX5b* genes may be as schematized in **Figure 1**. This pathway is favored, in addition, by results obtained with the Arabidopsis *Cytc-1* and *Cytc-2* genes, that encode cytochrome *c*. *Cytc-2* contains site II elements and a G-box, as the putative ancestor of the *COX5b-1* gene depicted in **Figure 1**, and the effect of site II element loss on transcription is less

pronounced in *Cytc-2* than in other genes, including *Cytc-1* and *COX5b-2*.[15]

The conservation of the response to carbohydrates in both products of gene duplication, even if they have diverged in other aspects, suggests that this response is particularly important for their function. We speculate that site II element loss was only possible after the incorporation of additional elements that allowed induction by carbohydrates. The intracellular level of carbohydrates may be used by plant cells to establish the rate of respiratory complex biogenesis and, since carbohydrates are repressors of the synthesis of photosynthetic components,[16] to balance the processes of respiration and photosynthesis. Conservation of the response in both genes may be related with the necessity of induction in tissues where the genes are differentially expressed, or with the establishment of a more robust genetic system in face of possible changes that may produce alterations in the expression properties of one of the members of the gene family. Nevertheless, the results discussed here highlight the importance of regulation by carbohydrates for the function of respiratory chain component genes.

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