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Hypothesis

Mutations at specific *atp6* codons which cause human mitochondrial diseases also lead to male sterility in a plant

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Abstract Defects in the human mitochondrial genetic system result in some diseases. These disorders are the result of rearrangements or point mutations in mitochondrial genes. In higher plants mutations and rearrangements in the mitochondrial DNA are believed to cause cytoplasmic male sterility (CMS), a mitochondrially inherited inability to produce viable pollen. In sorghum, formation of CMS is strongly correlated with anther-specific loss of mitochondrial *atp6* RNA editing. Here we show that this loss of *atp6* RNA editing mimics point mutations at codons that cause severe disorders in humans. We conclude that (i) loss of RNA editing in sorghum anthers probably causes CMS, (ii) similarities exist in the onset of mitochondrial dysfunction in plant and human tissues, and (iii) the evolutionary appearance of RNA editing provided a mechanism to compensate for otherwise lethal point mutations.

Key words: Cytoplasmic male sterility; Mitochondrial mutation; RNA editing; Mitochondrial dysfunction; Pollen fertility

1. Loss of RNA editing, human mutations and cytoplasmic male sterility

In 1988 the first experimental proof for a human mitochondrially inherited disease was established, when mitochondrial myopathies and Leber's hereditary optic neuropathy were shown to be caused by mutations in mitochondrial DNA [1,2]. In the meantime many different mitochondrial diseases have been characterised, which are due to large rearrangements or point mutations in specific mitochondrial genes. In addition nuclear gene defects may result in abnormalities in mtDNA. Although these defects cause a broad spectrum of multi-system disorders, neuromuscular involvement is prominent [3].

In a completely different area of genetics, plant breeding, cytoplasmic male sterility or CMS is known as a mitochondrially inherited inability to produce viable pollen, and has been observed in more than 150 plant species [4,5]. It is used in plant breeding to generate hybrid seed from a large number of different crops including beet, carrot, maize, rice, sorghum, sunflower and wheat [6]. CMS is often associated with the existence of chimeric ORFs, which are believed to result from mitochondrial rearrangements [7,8]. While human mitochondrial mutations exhibit a phenotype mainly in neuromuscular tissue, CMS exhibits a phenotype in certain anther cell-types only (tapetum and pollen).

We recently published data connecting CMS and mitochondrial RNA editing [9,10]. RNA editing in higher plant mitochondria is characterised by cytosine to uracil transitions due to an as yet unknown enzymatic mechanism [11]. In sorghum, a transcript-specific loss of RNA editing was observed in CMS lines. The effect was tissue-specific and occurs in anthers only. All 18 editing sites of the mitochondrial *atp6* transcript were affected, with editing frequency being about 20% of transcripts from fertile anthers [9,12].

Loss of *atp6* RNA editing is strongly correlated with pollen fertility, as demonstrated by comparison of F1 and F2 plants. However, the question remains whether loss of *atp6* RNA editing may cause CMS. Amazingly, work in the area of human mitochondrial genetics provides the pivotal clue to this question. Mutations at specific positions in the *atp6* gene are the cause of neurogenic muscle weakness, ataxia and retinitis pigmentosa (NARP or Leigh syndrome; [13,14]) and familial bilateral striatal necrosis (FBSN; [15]). Due to these mutations leucine residues at positions 156 and 217 in human *atp6* are replaced by other amino acid residues. A mutation at leucine 156 causes NARP, while a change at leucine 217 leads to FBSN. As shown in Fig. 1, in sorghum RNA editing is required to generate the codons that encode leucine residues at the equivalent positions. Loss of *atp6* RNA editing, as it occurs in sorghum CMS anthers, thus mimics mutations in human mitochondrial diseases. It should be noted, that in all ATP6 protein sequences extracted from databases, including protists, plants (edited sequence), fungi and animals, both amino acid positions are completely conserved.

Tatuch and Robinson [16] have shown that the leucine 156 exchange associated with the corresponding DNA mutation in human *atp6* results in a significant decrease of ATP synthesis rate. It is believed that this is due to a less functional proton channel in the F_0-subcomplex of ATP synthase. The leucine residue is part of one of the ATP6 transmembrane domains. In the case of sorghum, loss of editing would replace the hydrophobic leucine with a hydrophilic serine residue, which is likely to interfere with the integrity of the transmembrane domain. Site-directed mutagenesis of the equivalent leucine 207 in *Escherichia coli* ATPase6 (c subunit) was effective in decreasing the growth yield on 10 mM glucose minimal media [17], also showing that the identity of this residue is important. This is further emphasised by the fact that maize and wheat also edit these positions. In tobacco, at least at site 157 editing would be necessary; however, cDNA sequences are not available there (see Fig. 1). The human neuromuscular

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170       180       190                    230       240       250
FRALSSGIRLFANMMAGHSSVKILSGFA  //----//  ELGVAISQAHVSTISICIYLNDATNLH  sorghum, DNA
.....L.............L........  //----//  ......L..Y.F..L........I...  sorghum, edited RNA
.....................................  //----//  .....................................  maize, DNA
.....L.............L........  //----//  ......L..Y.F..L........I...  maize, edited RNA
.....L.............L........  //----//  .....................................  wheat, DNA
                                                .Y.F..L........I...  wheat, edited RNA*
.....................................  //----//  .....................L........I...  tobacco, DNA**
.....L.............L........  //----//  ......L..Y.F..L........I...  radish, DNA***
A..I.LGL..GS.IL...LLMV..A.LT  //----//  .FAIG.I.SY.W..LTAS..K.TLY..  yeast, DNA
IQPMALAV..T..IT...LLMHLIGSAT  //----//  .IA..LI..Y.F.LLVSL..H.N.     human, RNA
     156                                          217
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Fig. 1. Comparison of ATP6 amino acid sequences from plants, yeast and human. Deduced ATP6 amino acid (partial sequences) from genomic DNA and edited mRNA are shown. RNA editing is required in sorghum and maize to generate the leucine residues conserved in yeast and human. Mutations at these residues (156 and 217, bold face, underlined) in human mtDNA lead to diseases. In wheat (a) and tobacco (b) only at one site RNA editing is required, and radish ATP6 (c) requires no editing at all. *, Partial wheat cDNA sequences were obtained; a dot indicates identity to the upper sequence.

mitochondrial diseases such as Leigh syndrome are the consequence of a reduction in ATP production. Apparently neuromuscular tissues require much more energy than other tissues, which explains the tissue specificity of the phenotype observed [3]. The same holds true for plants. The anther cell types affected by CMS need more ATP than other tissues, which is reflected by the F_0F_1-ATP synthase activity in different plant tissues. In *Lilium longiflorum* F_0F_1-ATP synthase activity is highest in pollen [18] and tissue specificity apparently concerns the F_0 portion. Leaves exhibit an F_0F_1-ATP synthase activity only about half of that in the pollen in *Lilium* [18]. The high ATP demand during pollen development has been discussed earlier [19]; however, it remained an open question whether CMS is caused by a reduction in ATP production. The data presented here now strongly support this idea.

2. General implications

Aside from its significant implications for CMS in higher plants, for the first time it has been demonstrated that similarities exist in the onset of mitochondrial diseases in plants and humans. Two general implications are obvious. First, loss of RNA editing can have the same effect as point mutations in genomic DNA and thus represent a new source of mutations. Secondly, Covello and Gray [20] published a model to explain the introduction of RNA editing. According to their model, otherwise lethal point mutations could be corrected on the RNA level by RNA editing: a random process at first, which then would become fixed through evolution. The data and speculation provided here give direct support to this idea.

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