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In reply — Cossee and colleagues dispute our observation that the *STM7* and *X25* sequences constitute a single transcriptional unit and hence, question that the protein product of this gene, a novel PIP4P5K, is defective in Friedreich's ataxia[1].

Their main argument focuses on the use of nested RT-PCR analysis for the connection of the STM7.I and *X25* sequences as a series of alternatively spliced variants and specifically, on the number of cycles used. Nested RT-PCR is a widely implemented technique for improving amplification specificity[7,8]. Nevertheless, the atrial variant reported in Carvajal *et al.*[1] came from a single round (35 cycles) of amplification, and 25 cycles can be sufficient to generate detectable products (Fig. 1*a*). Consequently, we would not expect these transcripts to be particularly rare.

To explain the generation of 'hybrid' transcripts derived from adjacent unrelated genes, the authors propose that this is due to non-recognition of polyadenylation signals, citing a single study that describes the splicing together of exons derived from adjacent sequences[4]. Contrary to the conclusion drawn by Cossee *et al.*, however, Ansari-Lari and colleagues acknowledge the possibility of a single transcriptional unit for these sequences, supported by the detection of a common transcript on northern analysis. This is directly analogous to our observation for *STM7*, where we have previously documented the detection of a common 1.3-kb transcript. Should non-recognition represent a genuine event, we would not expect to detect tissue-specific alternative splicing in these 'hybrid' transcripts. However, a single round of RT-PCR analysis results in the amplification of products linking the sequences which are i) not necessarily detected in all tissues and ii) can vary in size according to the origin of the mRNA template (Fig. 1*b*).

Identification of STM7.I-X25 products as natural cDNAs or indeed mutations within the STM7.I sequence would resolve this issue, but their absence so far is not definitive. The presence of the alternative polyadenylation site in exon 16, the average insert size of cDNA libraries and the relative abundance of each transcript, could mean that such clones would not be detected. Given the apparent importance of the *STM7* gene product in a variety of tissues including placenta, mutation within the kinase domain in particular, may not be compatible with life. In this context, however, it should be noted that all patients included in our original *STM7* (exons 1–16) mutation screen, subsequently proved to be homozygous for the expansion.

The differential incorporation of STM7.I exons can result in either the extension of the open reading frame to include the *X25* sequence or serve to relegate exons 18–22 to the 3'UTR; the latter being the case for the single variant containing *X25*/exon 1 described in our original communication[1]. However, we would expect to identify additional variants in which this exon contributes to the coding sequence. Consequently, the identification of a mutation in *X25*/exon 1 by Cossee *et al.* does not preclude the *STM7* concept, but simply indicates that this mutation will be of consequence only to those transcripts in which this exon is translated. In relation to the results of the RNase protection assay, we would argue that in the absence of information on the relative abundance of the respective splice variants in lymphoblasts compared to tissues more appropriate to the disease pathology, the statement that exons 1 and 2 of *X25* are always found spliced together has yet to be proven.

In our opinion therefore, Cossee *et al.* have failed to present either a plausible explanation for our original observations or a definitive argument to contradict our interpretation of the data. We therefore propose that the diversity of function and sites of activity postulated for phosphatidylinositol-4,5-biphosphate, a critical component of intracellular signaling pathways whose synthesis is regulated by PIP4P5K, necessitates multiple isoforms of the kinase, possibly resulting from alternative splicing. The constancy of the kinase domain in the splice variants suggests that the *X25* sequence may constitute the regulatory element of the gene, controlling functional/tissue specificity and level of expression. The presence of mutations in the *X25* component of this otherwise ubiquitously expressed gene, would therefore be compatible with the specific pattern of degeneration observed for this disorder.

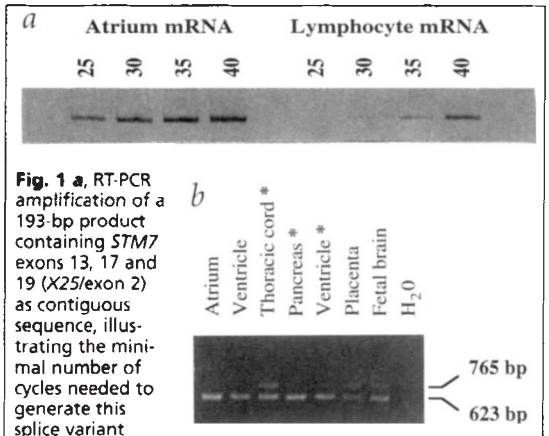

Fig. 1 ***a***, RT-PCR amplification of a 193-bp product containing *STM7* exons 13, 17 and 19 (*X25*/exon 2) as contiguous sequence, illustrating the minimal number of cycles needed to generate this splice variant from adult atrium (25) and lymphocyte (30) mRNA. PCR used [α-^{33}P]dATP (1000 Ci/mmol) with cycling conditions as follows: 94 °C for 30 s, 65 °C for 30 s and 72 °C for 45 s. Amplification reactions were stopped after 25, 30, 35 or 40 cycles and resolved by 10% polyacrylamide gel electrophoresis. ***b***, RT-PCR amplification (35 cycles) of mRNA isolated from a variety of human tissues including Friedreich's ataxia autopsy samples (*) used primers derived from *STM7* exons 13 and 22 (*X25*; exon 5a) and generated a 623-bp product, corresponding to exons 13, 17, 19–22 present as contiguous sequence, in all tissues analysed. This product constitutes part of a full-length transcript spanning exons 3–22; its encoded protein has kinase activity (unpublished data). A 765-bp product generated in thoracic cord, placenta and fetal brain provides evidence of tissue-specific alternative splicing. This product comprises exons 13–15, 17, 19, 21 and 22 as contiguous sequence. PCR conditions: 35 cycles of 94 °C for 30 s, 63 °C for 30 s and 72 °C for 3 min followed by a 10 min extension at 72 °C.

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