REPLICATION PROFILE OF THE *FXN* LOCUS IN NORMAL HUMAN CELLS AND IN MUTATED CELLS CARRYING THE GAA/TCC-REPEAT EXPANSION

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Friedreich ataxia (FRDA), the most common inherited ataxia, is transmitted as an autosomal recessive trait and 98% of affected individuals are homozygous for an expanded GAA/TTC trinucleotide repeat in the first intron of the *FXN* gene (9q13), which encodes the mitochondrial protein frataxin. The mutation, resulting from the dynamic instability of the GAA/TCC repeat, causes the transcriptional inhibition of the *FXN* gene. Among several factors contributing to repeat dynamic instability, DNA replication is a key process. By molecular cytogenetic approaches we are currently evaluating the replication profile of the *FXN* locus, in normal cells, and in cell lines derived from FRDA patients and their heterozygous relatives. By interphase FISH we evaluated the proportions of non replicated (single fluorescent spot) and replicated (double fluorescent spot) alleles, both in asynchronous proliferating cells and in 4 (early-to-late) S-phase fractions obtained after FACS sorting; in parallel, for the same nuclei the BrdU staining pattern was recorded. The results indicate that replication of the *FXN* domain occurs during a wide temporal window corresponding to mid-late S-phase patterns. At present, we are considering if the *FXN* domain is replicated with similar or different timing in normal and mutated cells. In parallel, by molecular combing, we attempted to evaluate the position of activated origins, and the fork rates within the *FXN* locus; the global replication dynamics of normal and mutated cells has been also considered. Our preliminary data suggest the lack of activated origins within the *FXN* gene; therefore both the normal and the mutated allele are passively replicated by forks firing in the flanking regions and running at speed in the normal range for human cells. The analysis will be completed by the evaluation of possible altered replication patterns linked to the presence of the expanded repeat.