Recombination Mediates Genetic Instabilities of Triplet Repeat Sequences

A. Bacolla, P.D. Chastain, R. Dere, M. Filutowicz, J.D. Griffith, M. Hebert, J.P. Jakupciak, L. Mochmann, M. Napierala, P. Parniewski, A. Pluciennik, R.R. Iyer, A. Jaworski, J.E. Larson, N. Sakamoto, L. Spitz, A.A. Vetcher, J.H. Wilson, M. Wojciechowska, R. D. Wells*
Institute of Biosciences and Technology, Texas A&M University System Health Science Center, Center for Genome Research, 2121 W. Holcombe Blvd., Houston, TX 77030, USA

The expansion of triplet repeat sequences is an initial step in the disease etiology of a number of hereditary neurological disorders in humans. Diseases such as myotonic dystrophy, Huntington's, several spinocerebellar ataxias, fragile X syndrome, and Friedreich's ataxia are caused by the expansions of CTG•CAG, CGG•CCG or GAA•TTC repeats. The mechanisms of the expansion process have been investigated intensely in *E. coli*, yeast, transgenic mice, mammalian cell culture, and in human clinical cases. Whereas studies from 1994-1999 have implicated DNA replication and repair at the paused synthesis sites due to the unusual conformations of the triplet repeat sequences, recent work has shown that homologous recombination (gene conversion) is a powerful mechanism for generating massive expansions, in addition to or, in concert with replication and repair.

We have recently focused on the recombinational properties of long GAA•TTC repeating sequences analyzed *E. coli* to gain further insights into the molecular mechanisms of the genetic instability of this tract as possibly related to the etiology of Friedreich's ataxia. Intramolecular and intermolecular recombination studies showed that the frequency of recombination between the GAA•TTC tracts was as much as 15 times higher than the non-repeating control sequences. Homeologous, intramolecular recombination between GAA•TTC tracts and GAAGGA•TCCTTC repeats also occurred with a very high frequency (~0.8%). Biochemical analyses of the recombination products demonstrated the expansions and deletions of the GAA•TTC repeats. These results, together with our previous studies on the CTG•CAG sequences, suggest that the recombinational hotspot characteristics may be a common feature of all triplet repeat sequences (TRS). Unexpectedly, we found that the recombination properties of the GAA•TTC tracts were unique, compared to CTG•CAG repeats, since they depended on the DNA secondary structure polymorphism. Our results demonstrate that sticky DNA exists and functions in *E. coli*.