Characterization of Drosophila melanogaster histone deacetylases

A.L. Barlow, C.M. van Druenen, C.A. Johnson, B.M. Turner

We have started to identify and characterize the histone deacetylases of Drosophila melanogaster. Based on their homology with the mammalian enzymes three potential histone deacetylases have been identified. Two of these, dHDAC1 and dHDAC3 are class I deacetylases (related to yeast Rpd3), whilst the third, dHDAC6 is a class II deacetylase (related to yeast Hd1). In addition, we have shown that Drosophila dSir2 is a novel NAD-dependent histone deacetylase. To facilitate the characterization of these proteins we have produced stable Schneider cell lines expressing V5-tagged recombinant proteins.

dHDAC1 (520 a.a.) shows an exclusively nuclear distribution, it copurifies with the histone chaperone protein p55, as well as DMBP2/3, and is expressed throughout the whole Drosophila life cycle. We will show that dHDAC1 regulates its own expression, as the ectopic expression of the recombinant protein shuts off the expression of the endogenous gene. dHDAC3 (438 a.a.) has an apparently fluid cellular distribution, with some cells showing a nuclear distribution, some a cytosolic distribution, and others a combination of the two. Like dHDAC1 it is expressed throughout the Drosophila life cycle. dHDAC6 (883 a.a., with multiple splice variants) contains two active sites and has an exclusively cytosolic distribution. An antibody based site specificity assay shows all three enzymes are able to deacetylate all lysines of histone H4.

The deacetylases in Drosophila are part of distinct protein complexes. dHDAC1 is found in two complexes (800kDa and 200 kDa), whereas dHDAC3 is present in a single 700 kDa complex. Purification of these complexes is underway and preliminary data suggests that these deacetylases associate with several, as yet unidentified, protein partners.

Leishmania mexicana mexicana: Genetic Heterogeneity of Mexican Isolates using Restriction Lenght Polymorphism Analysis of Kinetoplast DNA

L. Becker, M. Berzunza-Cruz, R. Perez-Montfort
Facultad de Medicina, Instituto de FisioLogia Celular, UNAM, 04510 DF, Centro de Inversion en Enfermedades Tropicales, UAC, Mexico

Leishmania mexicana mexicana is endemic in Mexico where it causes 3 clinical forms of cutaneous leishmaniasis: localized cutaneous leishmaniasis (LCL), diffuse cutaneous leishmaniasis (DCL) and an atypical form of pseudodiffuse cutaneous leishmaniasis (PDCL). L. M. mexicana was isolated from 23 patients with the 3 clinical forms. RFLP analysis of kinetoplast DNA (kDNA) was done with 9 different endonucleases and the in vitro growth rate was analyzed. We found that the 23 L. M. mexicana isolates could be consistently classified into 6 groups. LCL isolates could have any of 5 patterns, DCL isolates only showed 2 patterns and PDCL consistently showed only 1 pattern. Additionally the L. m. mexicana isolates could also be differentiated into fast and slow growing groups. DCL isolates were fast growing, LCL fell into both categories and all PDCL isolates were slow growing. A clear correlation between digestion patterns, clinical disease and growth behavior was obtained for the PDCL group. This is the first study in which distinct and persistent genotypic characteristics of kDNA heterogeneity within the L. m. mexicana species could be directly correlated with clinical disease and growth behavior, suggesting that a distinctive restriction pattern could have important biological implications. It sheds new light on the biological significance of parasite kDNA.

DETECTION OF THE POLYMORPHISM LEVELS OF THE TURKISH BREAD WHEAT GENOTYPES BY SSR MARKERS

E. B. Büyüküna Bal and M. S. Akkaya
Middle East Technical University, 06531 Ankara-Turkey

Simple Sequence Repeats (SSRs) or microsatellites are one of the most powerful molecular markers for differentiation of genotypes. They are locus-specific PCR based markers and co-dominantly segregated. In addition, data obtained with SSRs are highly reproducible. In this study, we aimed to determine the polymorphism levels among the 11 Turkish bread wheat cultivars. Genomic DNA samples of total 65 randomly selected individuals from those 11 cultivars (5-6 genotypes from each cultivar) were amplified with wheat microsatellite markers, WMS 18, WMS 30, WMS 174 and WMS 194. Following the PCR reactions by labelling with [α-32P]-dATP, PCR products were separated on standard DNA sequencing gels in order to detect SSR length polymorphism. Results obtained with the studied markers indicated that they are highly polymorphic to detect the polymorphism among wheat cultivars. Moreover, 'Ikizce' and 'Kutluk-94' bread wheat cultivars have shown highest variation among their own genotypes, suggesting they are still segregating populations. Studies are in progress to test more wheat SSR markers on the samples to prepare a SSR data for construction of the genetic relationship trees.