ABSTRACTS / RÉSUMÉS

Functional analysis of the histone H3 variants of *Ustilago maydis*

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Two histone H3 variant proteins were purified from *Ustilago maydis* suspension cultures by guanidine extraction, BioRex-70 chromatography, and reversed phase hplc, and separated by acid-urea-Triton X-100 (AUT) gel electrophoresis. Histone variant H3.2, named based on the higher gel mobility, was shown by in vivo ^{35}S-methionine labeling to be the product of the methionine-containing H3 gene AACP01000090.1 (GenBank). It was predicted to be a cell-cycle regulated gene, based on the sequence of residues 87, 89, and 90: S-VM. Variant H3.1 from gene AACP01000135.1 with motif S-IG was predicted to be a constitutively expressed form, functioning to replace existing H3 protein displaced by chromatin transcription in so-called replication-independent (RI) nucleosome assembly. The predicted higher steady-state and dynamic acetylation of H3.1 relative to H3.2 was demonstrated in Coomassie-stained AUT gels by tritiated acetate and lysine fluorography. High levels of acetylation were observed on newly synthesized protein for both variants by tritiated lysine fluorography. Cultures synchronized by release from a hydroxyurea replication block demonstrated a strong S phase responsive synthesis pattern for H3.2, as predicted for replication-coupled (RC) chromatin assembly. Synthesis of H3.1 was stable, unresponsive to cell cycle phases. Contrary to prediction, substantial H3.2 synthesis occurred in non-S phase cells with concomitant RI incorporation into chromatin for both variants. The turnover half-life of both variant proteins in transcribing chromatin was 2–3 h. In contrast, RC assembled, nontranscribed chromatin was stable.

A new twist on GAGA factor

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The GAGA factor was originally identified as a transcription factor or an anti-repressor. It plays an important role in the heat-shock response of the hsp70 gene through recruitment to multiple binding sites over the promoter region. Its interaction with chromatin remodeling complexes has been identified, and a structural role for the GAGA factor has started to emerge. GAGA mutimers have been shown to form spherical structures of sizes similar to those of nucleosomes. We are investigating the role of GAGA factor in potentially redirecting the DNA to promote the formation of specific chromatin higher-order conformation, compatible with its role in transcription regulation of the hsp70 gene. Our results suggest that GAGA can facilitate chromatin long-distance interactions by bending the DNA upon recruitment. Interestingly, we have also shown that the GAGA factor can affect the supercoiling level of plasmid containing the hsp70 promoter region. The activity appears to have characteristics similar to that of eukaryotic topoisomerases I and to be endogenous to GAGA itself. Our current work focuses on further identifying the GAGA domain responsible for the topological relaxing activity, as well as understanding the details and biological significance of such an activity in the context of heat-shock response.