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Identification of EhTIFIA: The putative *E. histolytica* orthologue of the human ribosomal RNA transcription initiation factor-IA

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Initiation of rDNA transcription requires the assembly of a specific multi-protein complex at rDNA promoter containing the RNA Polymerase-I (Pol-I) with many auxiliary factors. RNA Pol-I forms transcriptionally active enzyme, in association with a factor known as Rrn3P in yeast and Transcription Initiation Factor IA (TIFIA, Rrn3P homologue) in mammals. TIF-IA interacts with RPA43, a unique subunit of Pol I, and with two Pol I-specific TAF (TATA binding protein-associated factors), thereby serving as a bridge between Pol I and the pre-initiation complex at the rDNA promoter. TIFIA is phosphorylated at multiple sites, and signals in response to that affect cell proliferation and metabolism. In *E. histolytica*, the rRNA genes are present exclusively on 24.5 kb circular extra chromosomal plasmid named as EhR1. EhR1 contains two rDNA repeats (I & II), which are arranged palindromically, and separated by upstream and downstream spacers. The protein factors involved in regulation of rDNA transcription in *E. histolytica* are not known. We have identified and characterized the *E. histolytica* equivalent of the transcription initiation factor TIF-1A (EhTIFIA) which is known in other systems to control the initiation of rDNA transcription. EhTIFIA was identified by sequence analysis and have cloned and expressed the gene in E. coli. Immuno-localization studies showed, EhTIFIA co-localizes partially with Ehfibrillarin and the RNA Pol I specific subunit (EhRPA12) both of them are in the nucleolus (in eukaryotic cells), which in *E. histolytica* is located at the nuclear periphery. EhTIFIA was extensively phosphorylated in presence of *E. histolytica* nuclear extract and one phosphorylation site at S461 was identified through mass spectroscopy. EhTIFIA was shown to interact with EhRPA12 both in vivo and in vitro. Mass spectroscopy data showed the major interacting partners of EhTIFIA to be RNA Pol-I specific subunits as well as some nucleolar proteins Further, we are trying to characterize EhTIFIA in relation to RNA Polymerase-I transcription regulation. Our study demonstrates presence of similar transcriptional machinery in primitive *E. histolytica* as in higher eukaryotes with certain structural and functional reform.

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B16 Mouse melanosomes can serve as a chemoattractant for macrophages

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Previous research in the Rosenthal lab has shown that the inhibitor of differentiation-4 (Id4) stimulates pigment production, in vivo, resulting in histiocyte recruitment and tumor necrosis. Histiocytes are tissue macrophages derived from the monocyte lineage. However, the chemotactic factor that attracts macrophages to the melanoma has not been described. Two mouse macrophage cell lines (RAW264.7 and IC-21) were therefore incubated with different concentrations of conditioned medium (CM) containing melanosomes obtained from B16F0 mouse melanoma cells. HaCaT immortalized human keratinocytes were used as a negative control. After 3 hours of incubation with CM, macrophages were collected and melanosome uptake was measured by absorbance at 340 nm. Significant melanosome uptake was observed in both macrophage cell lines when compared to negative control, indicating that macrophages readily phagocytose melanosomes present in CM. A trans-well migration assay was performed to further examine the interaction between RAW264.7 and IC-21 macrophages and pigment-producing B16F0 melanoma cells. Our initial observation suggests a trend toward increased migration of macrophages toward pigment-producing melanoma cells and thus merits further investigation. Since Id4 expression was required for melanin synthesis in vivo, we investigated the effect of ectopic Id4 on the production of tyrosinase, the rate-limiting enzyme in controlling the production of melanin. However, no association was detected after 48 hours. Further time points, may be required to determine if Id4 alters tyrosinase production in B16 melanoma cells.

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