Volume 55(1):197-212, 2011
Acta Biologica Szegediensis
http://www.sci.u-szeged.hu/ABS

Towards the understanding of genome-wide redistribution mechanisms of the RNA Polymerase II transcription machineries upon Ultraviolet B irradiation

Ákos Gyenis[1, 2]

[1]Institut de Génétique et de Biologie Moléculaire et Cellulaire (IGBMC), Illkirch, France, [2]Bay Z. Foundation for Applied Research, Institute for Plant Genomics, Human Biotechnology and Bioenergy (BAYGEN), Szeged, Hungary

One of the main reasons of skin cancer is the Ultraviolet (UV) irradiation coming from the Sun. Depending on the wavelengths we distinguish UV-A (400-320nm), UV-B (320-280nm) and UV-C (280–100nm) radiation. Although UV-C has the most genotoxic effect on cells, this wavelength is fully absorbed by the Earth's atmosphere, thus UV-B is the most common source of skin cancer by inducing pyrimidine dimers (CPD) and (6-4) photoproducts on the DNA. Intrinsic (block of transcriptional machinery) and extrinsic (cell membrane damage) signals will trigger the p53 pathway, which will lead to cell cycle arrest and DNA repair or apoptosis in a dose dependent manner. The given response to UV irradiation depends not only on the dose but also on the wavelength.

Transcription in eukaryotes is a tightly regulated, multistep process. Gene specific transcriptional activators, several different coactivators and general transcription factors are necessary to access specific loci to allow precise initiation of RNA polymerase II (Pol II) transcription. Upon different stress stimuli histone acetyltransferase (HAT) coactivator complexes play a crucial role in the maintenance of the eukaryotic chromatin architecture, in the regulation of locus specific transcription and in the establishment of consequent gene expression pathways (Toth et al. 2010).

Our goal is to gain insight in the alteration of the transcriptional machinery upon sub-lethal doses of UV-B irradiation in a human breast cancer model system (MCF7 cells). To this end first, we analyzed the global redistribution of the Pol II enzyme on the genome. We assume, based on several published results that the detectable presence of Pol II on the genome is mostly reflecting ongoing transcription. In addition, we would like to understand also how two of the major HAT complexes (ATAC and SAGA) participate in the maintenance of the genome and/or in gene regulation.

After 50 J/m^2 UV-B treatment we detected massive accumulation of CPD photoproducts by slot blot combined with western blot analysis, indicating that pyrimidine-dimers were still present in the genome 6 hours after irradiation. We examined the mRNA level of two well-characterized UV damage-responsive genes (*CDKN1A, GADD45A*) as a control using RT-QPCR. The results are concordant with previous observations, and show a slight gene induction between 2 and 6 hours after UV treatment. When we performed Chromatin Immunoprecipitation (ChIP) coupled Q-PCR and ChIP coupled to high throughput sequencing (ChIP-seq) using an antibody raised against the N-terminal end of the largest subunit of the RNA Polymerase II, we found a great decrease of Pol II occupancy at a large majority of promoters genome-wide 5 hours after UV irradiation when compared to the control (Gyenis et al. 2010). This overall promoter clearance can be due to a transcriptional block triggered by the known transcription coupled repair mechanism. By using several elaborate bioinformatics tools (*i.e.* seqMiner, DREM, etc.) capable of handling and compare genome-wide datasets, we were able to sort genes into several clusters based on Pol II alteration upon UV treatment at the different time points. We found that there is a slight increase of Pol II enrichment on stress response genes and we detected a dramatic and constant loss of Pol II enrichment on Histone gene clusters (Krishanpal et al. in preparation).

Our further objectives are:

To understand the molecular mechanisms by which Pol II presence at promoters is decreased genome-wide following UV-B treatment.

To investigate the role of HAT coactivator complexes in the regulation of UV-stress induced genes genome-wide. Moreover, we would also like to expand our above-described experiments to a human keratinocyte cell line to understand how skin cells respond to UV-B.

Gyenis A, Anamika K, Balint E, Tora L (2010) Examining the genome wide Pol II distribution and the transcriptional regulation of p53 target genes upon UV irradiation (9th EMBL Conference: Transcription and Chromatin, Heidelberg, Germany)

Tóth M, Gyenis Á, Bálint É (2010) A hiszton acetiltranszferáz inhibitor II (HATi II) kezelés hatása az egyes hiszton lizin oldalláncok acetiláltságára (Magyar Biokémiai Konferencia, Budapest, Hungary)

Anamika K, Gyenis A, Tora L (2011) Different Patterns of RNA Polymerase II Pause at the Transcription Termination Site Reveal Different Regulatory Mechanisms (Manuscript under preparation).

Supervisors: László Tora, Éva Bálint
E-mail: akosgyenis@gmail.com