High-resolution genome profiles of 8-oxodeoxyguanine, γH2AX and NBS1 reveal their co-association at transcribed long genes

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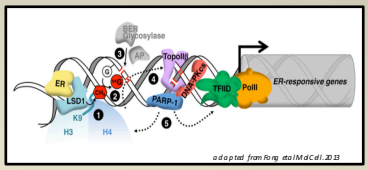
Background DNA is under persevering attack from both endogenous byproducts of cellular metabolism (e.g., reactive oxygen species) and exogenous sources of environmental stress (e.g., ultraviolet light). These genotoxic agents create DNA breaks and adducts that, if left unresolved, can be deleterious to both DNA replication and transcription and, ultimately, cell function and survival.

Accurate processing of genetic information by transcription is vital for development and survival of the organism. Execution of gene expression programs requires the coordinated assembly of the transcription apparatus at selected gene promoter and a highly choreographed cascade of events These events provide numerous points of regulation and fine-tuning but also make transcription particularly sensitive to perturbations in the genome including DNA damage.

On the other hand, transcription is per se a potential source of DNA damage, thus leading to mutagenic events, and for this reason it is constantly monitored by DNA repair factors in order to assure that DNA strands remains undamaged after gene has been transcribed and chromatin has been modified.

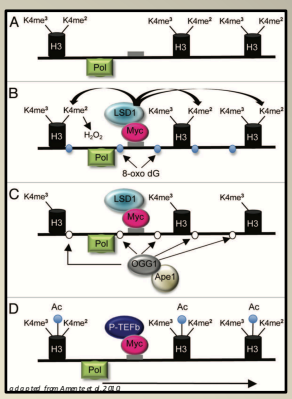
Transcription and DNA Repair are processes that involve intimate transactions with DNA that often overlap spatially and temporally and it is not surprising that a growing list of evidences has been revealing an unexpected tight connection between these two processes.

Generation of 8-oxodG (8-oxo-7.8-dihydro-2'-deoxyguanine) seems to be a crucial step for transcription activation mediated by ER (Estrogen Receptor) and c-Myc. Indeed, the repair of 8-oxodG contemplates the formation of DNA breaks to eliminate supercoiling generated by the progression of transcription fork. This allows the DNA double-helix to be relaxed, thus facilitating elongation of RNA polymerase throughout the gene body.



adopted from Kong et al Mol Cell. 2013

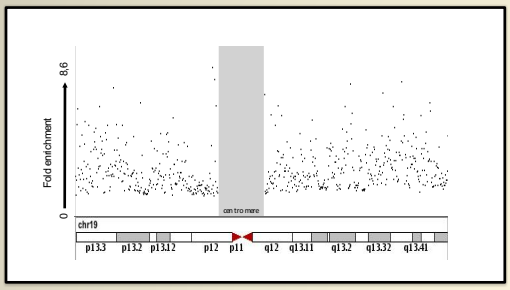
Upon ligand binding, estrogen receptor (ER) activates LSD1 at responsive genes (1). The demethylation reactions releases H_2O_2 that converts nearby guanines G into 8oxodG (•G) (2). •G removal by BER creates DNA nicks (3) that facilitate the entrance of Topoll β (4). Topoll recruits repair enzymes, e.g. PARP-1 and DNA-PKcs, which induce a permissive chromatin architecture for transcription initiation (5).



adapted from Amente et al. 2010

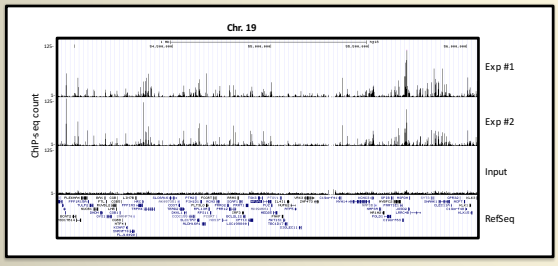
Schematic representation of Myc transcription activation model. (A) H3K4me/K4me2 is a signature for Myc activated targets with preloaded RNA PolII. (B) Myc binding induces chromatin relaxing: Myc-lsD1 complex is recruited on the e-box, transient de-methylation of H3K4me2. (C) BER requirement for repairing 8oxodG. (D) Histone acetylation and P-TEFb recruitment allow efficient transcription of Myc target genes.

Assessment of genome-wide mapping of 8-oxodG



8-oxodG levels were plotted along the human chromosome 19 and shown as ideogram. Each dot represents an oxidized region and is expressed as fold enrichment respect a sample control. The plots clearly indicate a not uniform distribution of regions with high 8-oxodG levels and some regions conspicuously protected from DNA oxidation like centromere

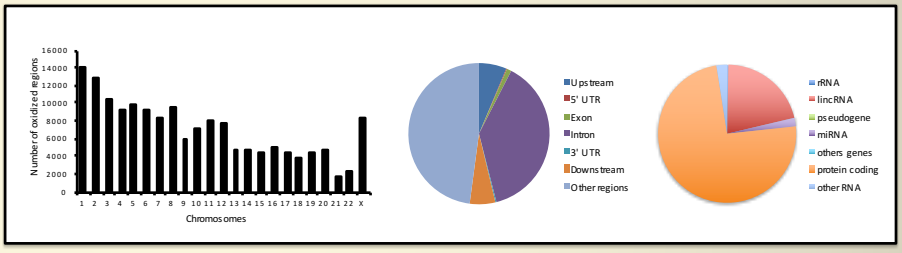
8-oxodG: signal watching



Data from two different biological experiments were analyzed to create a complete map of DNA oxidation for each chromosome of human genome

We have recently set-up a novel technique, we named OxiDIP-Seq, to profile the genome distribution of 8-oxodG at a single nucleotide level in human genome. We mapped 8-oxodG distribution in human non-tumorigenic epithelial breast cell line MFC10A. We found a non-stochastic distribution of DNA oxidation in the genome. Annotation of these peaks respect to different genomic features reveals that 8-oxodG peaks localize with a slight preference in the gene region (52%, including properly genes and regulative sequences) rather than in the intergenic region (48%), moreover a peculiar correlation between 8-oxodG residues and Polymerase II (Pol-II) coding genes is observed.

8-oxodG: distribution in genomic features



To confirm the hypothesis that oxidative DNA damage correlates with gene transcription, we decided to perform genome-wide analysis of γH2AX and NBS1 in MCF10a cells that together with 8-oxodG profile was useful to identify DNA damaged sites. Moreover we determined the transcriptional status of each gene by determining either occupancy of RNA Polymerase II phosphorylated on CTD-Ser5 and on CTD-Ser2, by ChIP-Seq, and mRNA levels by RNA-Seq.

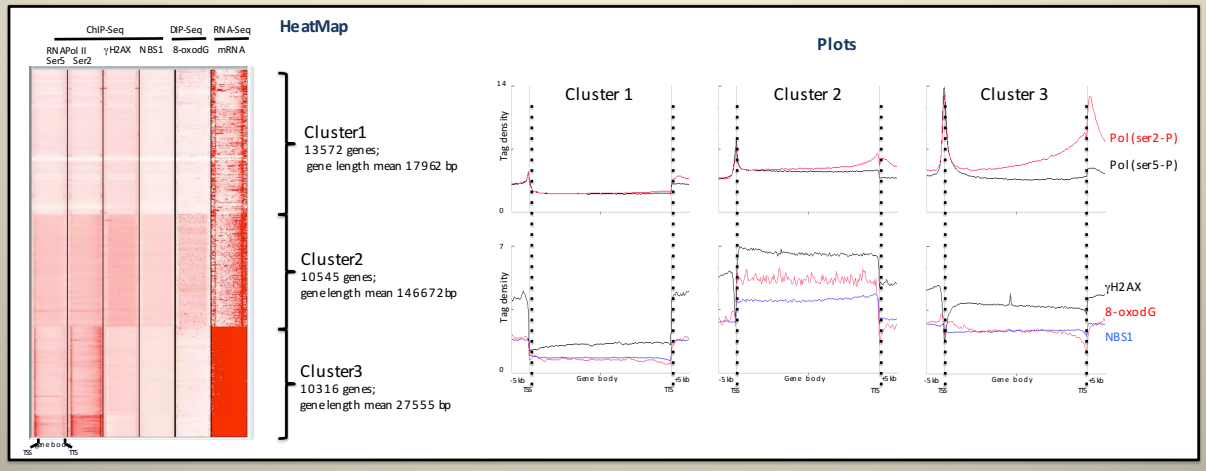
The relative occupancy of the 8-oxodG, γH2AX, NBS1 and PolII Ser2 and Ser5 along all annotated genes were analyzed using seqMINER Data are presented as:

1) Heatmaps where any single horizontal line show the read density mapped on the TSS, gene body, TTS and 5kb flanking regions of each unique gene, respectively. RNA-Seq data grouped genes in three clusters genes with low expression (or not expressed) are represented in Cluster 1; expressed genes characterized Cluster 2, and highest expressed genes are denoted in Cluster 3.

2) Plots that represent the average gene profiles of read density and is calculated averaging the read density mapped on total genes grouped in each cluster for each ChIP and DIP data set.

The analysis of the distribution of the DNA damage markers (8-oxodG, γH2AX and NBS1) with this bio informatics approach, revealed a peculiar correlation of DNA damage with the level of expression and with the length mean of the genes.

Correlation of DNA damage with transcription



Conclusions:

- **Set-up of a novel technique, we named OxiDIP-Seq;**
- **Mapping of the genomic distribution of 8-oxodG in human MFC10A cells;**
- **Correlation of DNA damage (8-oxodG, γH2AX and *NBS1*) with transcription;**
- **Identification of a set of long, transcribed and late-replicating genes showing high levels of 8-oxodG, γH2AX and *NBS1*.**