HORMONE ELICITS STRUCTURAL REORGANIZATION OF TOPOLOGICAL DOMAINS IN A BREAST CANCER GENOME

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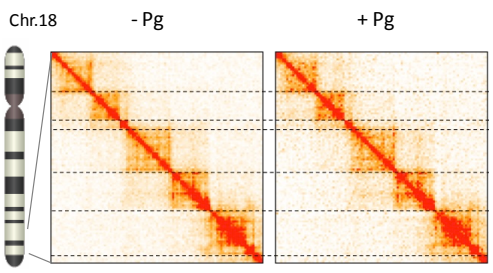
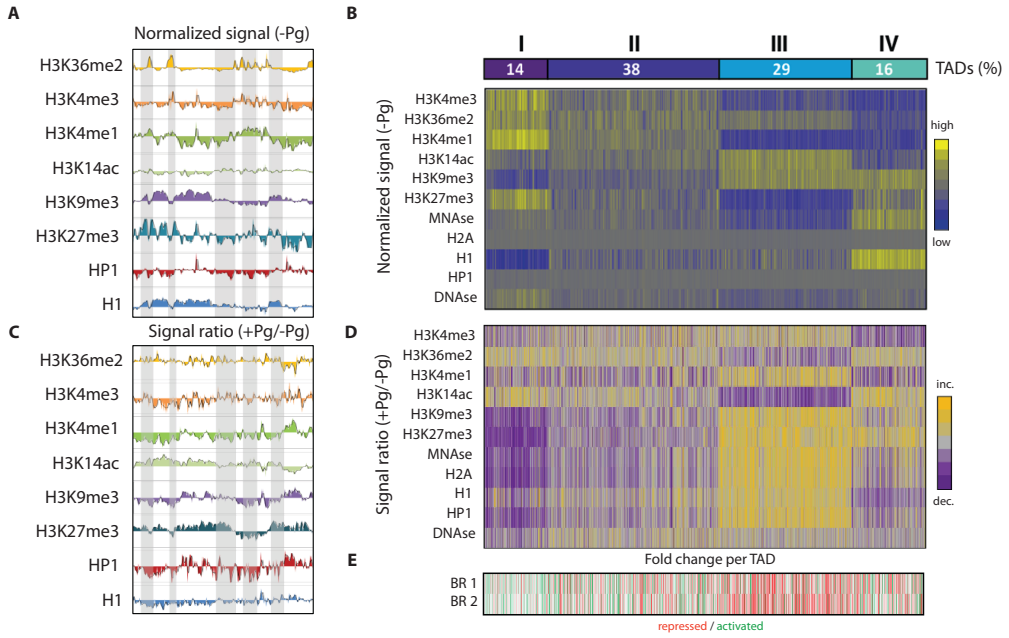


***Introduction**:* The genome is organized into Topologically Associating Domains (TADs), which appear conserved between cell types. To determine whether this scale of organization has a functional role during the dynamic changes of gene expression in terminally differentiated cells, we studied the relationship between the cell response to Progesterone (Pg) and the TAD structure in breast cancer cells. About 2,000 TADs were identified and found similarly demarcated before and after hormone treatment by applying Hi-C in T47D cells .

TADs are epigenetic domains that respond coordinately to external stimulation

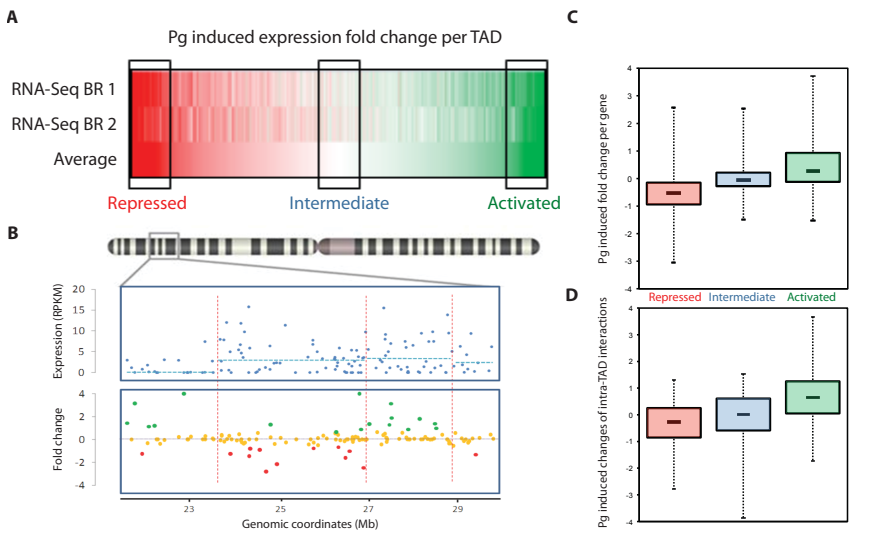
Histone marks frequently spread over entire TADs and individual TADs exhibit specific combination of epigenetic marks (A). Clustering TADs according to their overall enrichment/depletion of chromatin markers identified four main chromatin signatures (B). Pg-induced combinations of chromatin remodeling events were also topologically restrained within TADs and transition between responses preferentially occur at the TAD borders (C). Unexpectedly, the combinations of Pg induced changes were highly correlated with the basal chromatin type of TADs (D) and with the general changes in expression levels induced by Pg stimulation (E).



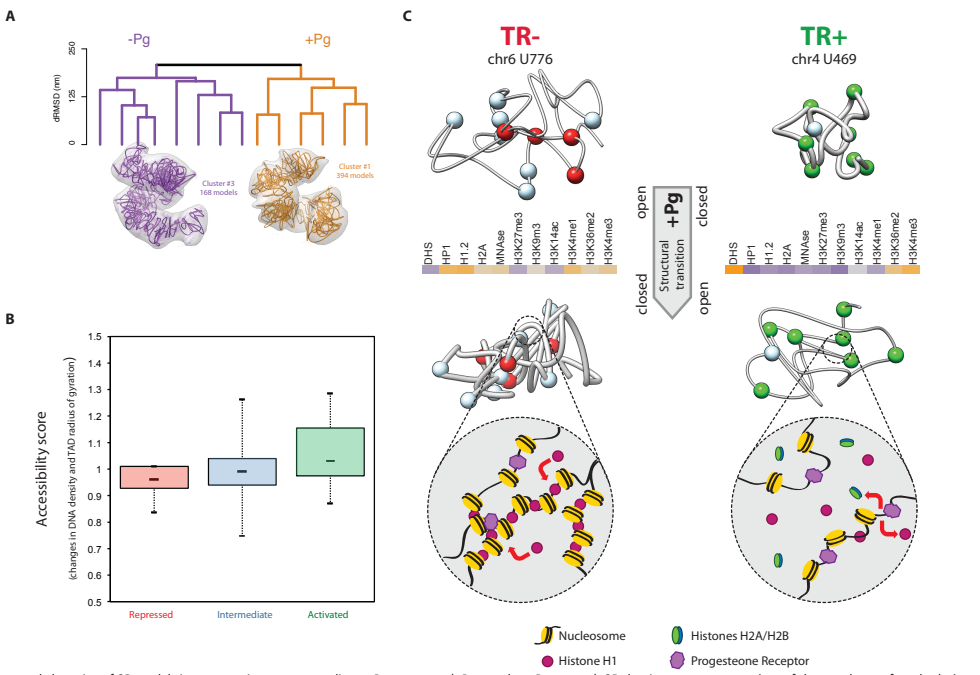
A. Log_2 normalised ChIP-Seq/Input ratio on windows of 100 kb showing different combinations of enrichment or depletion of epigenetic marks over TADs. **B.** The heatmap shows the 4 main TADs chromatin signatures (I to IV) identified after clustering according their relative enrichment of the histone marks and chromatin components listed (yellow: enrichment, blue: depletion). The fraction of TADs in each cluster is indicated at the top. **C.** Log_2 Ratio of normalized ChIP-Seq signals + Pg/- Pg treatment on windows of 100Kb on the region shown in A. **D.** Heatmap of the ratio of normalised ChiP-seq signals + Pg/- Pg treatment for whole TADs classified as in B (Pg induced decrease in purple and Pg induced increase in orange). **E.** Heatmap showing the global change of expression levels determined per TAD using two RNA-Seq replicates (BR1 and 2).

Interactions are differentially modified within Activated and Repressed TADs

TADs were classified as Activated and Repressed based on their global changes of expression (A). Genes within TADs were coordinately responding to hormone treatment (B) and an analysis of fold changes of individual genes confirmed that the majority of them followed the changes detected at the TAD scale (C). The changes in expression were accompanied by modifications of interactions within TADs (D). Intra-TAD interactions increase in activated TADs but decrease in repressed TADs suggesting a functional reorganization.



A. TADs deciles were classified in Activated and Repressed according the fold changes of their global expression after Pg. **B.** Consecutive TADs located on chromosome 1 with distinct ranges of expression level and biased enrichment of Pg activated and repressed genes. **C.** Distribution of fold changes determined for individual genes located within activated, intermediate and repressed TADs. **D.** Distribution of z-scores of Pg induced intra-TAD interactions changes per TAD.



A. Structural clustering of 3D models into two main groups according to Pg treatment (- Pg, purple; + Pg, orange). 3D density map representations of the top clusters from both time points are shown underneath of the dendrogram. **B**. Distribution of "accessibility ratio" as calculated from the 3D models for a subset of activated and repressed TADs. **C.** Model of Pg induced structural reorganization of responsive TADs. Prior to hormone treatment, repressed TADs are in a relatively opened conformation allowing basal transcription to occur. Upon Pg stimulation, inclusion of H1 within TR- chromatin leads to a more condensed chromatin fiber, which favours a global compaction of the TAD structure. Within activated TADs, Pg induced displacement of H1 and H2A increases chromatin fiber plasticity permitting the TADs structure to acquire a more open conformation.

TADs structure is functionally reorganized upon stimulation

We generated comprehensive 3D models of TADs based on our Hi-C datasets and observed that hormone treatment modified the overall structure of TADs, such that -Pg modelled TADs could not be structurally superimposed with the +Pg models (A). We calculated from our 3D models a "Pg induced accessibility score". The accessibility score was correlated with the basal chromatin state of the TAD as well as with the change of expression of their resident genes: activated TADs had higher accessibility score than the rest of the TADs whereas repressed TADs showed an opposite distribution B. (B). Thus, activated TADs were more prone to expansion and repressed TADs to compaction upon Pg stimulation compared to other TADs. Together these observations strongly suggest that the organization of the genome in TADs participate in the response to Pg by topologically restraining hormone induced large-scale chromatin remodeling events and consequently 3D structural transitions, which favor the transcriptional response towards stimulation or repression (C).

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