# Catalytic and non-catalytic functions of p300/CBP in zygotic genome activation

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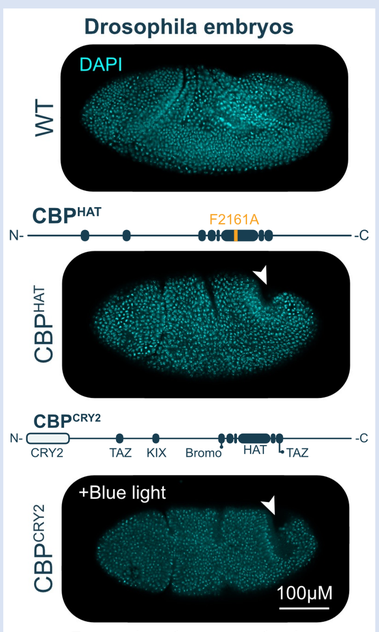
## Introduction

The p300/CBP acetyltransferase is a co-activator of gene expression that establishes the H3K27ac histone mark on active enhancers and promoters. The exact mechanism by which CBP-mediated acetylation promotes transcription remains elusive. Previous studies have shown that while substitution of H3K27 using histone replacement does not affect gene activation[1,2], chemical inhibition of CBP in cell culture affects transcription initiation and pause release of many genes[3,4]. Recently, it was shown that CBP is necessary for zygotic genome activation in zebrafish and fruit fly[5,6].

## Catalytic function of CBP

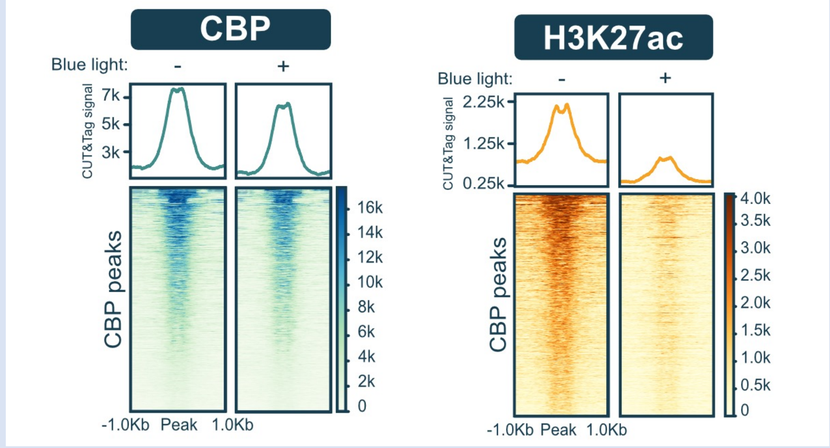
### Catalytic function of CBP is required for embryonic development and zygotic genome activation

To reveal catalytic function of CBP we inactivated endogenous CBP in the *Drosophila* zygote by two orthogonal approaches: loading of embryos with a catalytically dead CBP, and by optogenetic inactivation of CRY2-tagged CBP.
In all cases CBP has been edited at the endogenous locus.

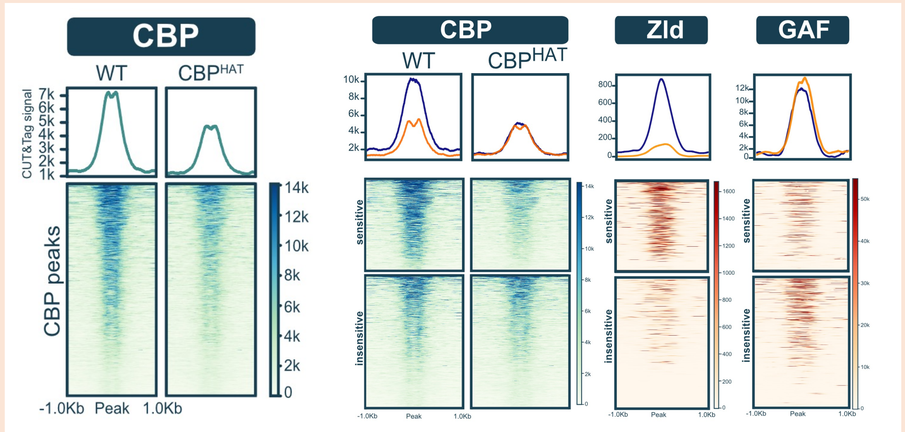



Impaired gastrulation

Blue light inactivates CRY2-CBP fusion protein, but it retains on chromatin.




### Mutant CBP$^{HAT}$ is partially lost from chromatin



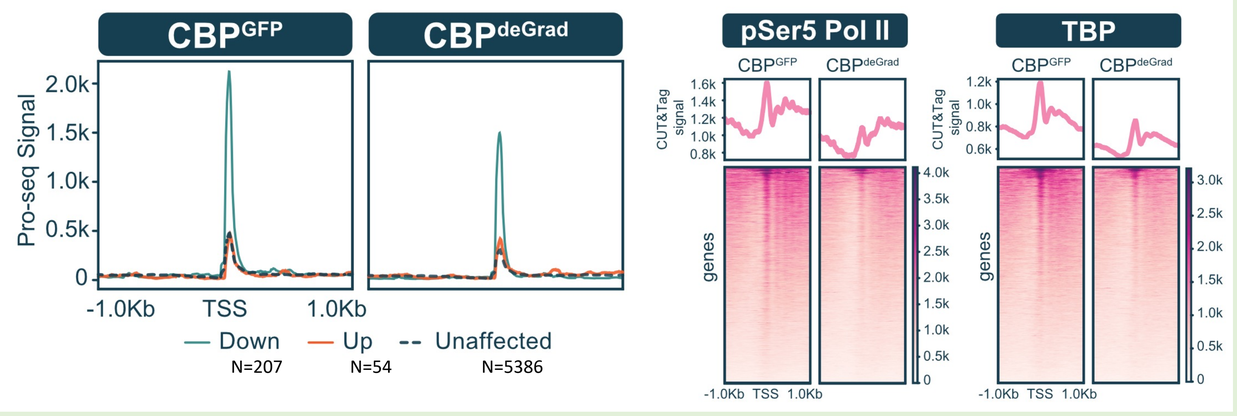

There are two groups of CBP peaks: sensitive and insensitive to catalytic activity. Catalytic sensitive and insensitive peaks are bound by different pioneer transcriptional factors: Zelda (Zld) and GAGA-binding factor (GAF).

## Non-catalytic function of CBP

### CBP targeted degradation leads to decreased transcription initiation

To find out possible non-catalytic function of CBP we made embryos where GFP-tagged CBP is degraded by maternally driven *deGradFP* transgene.

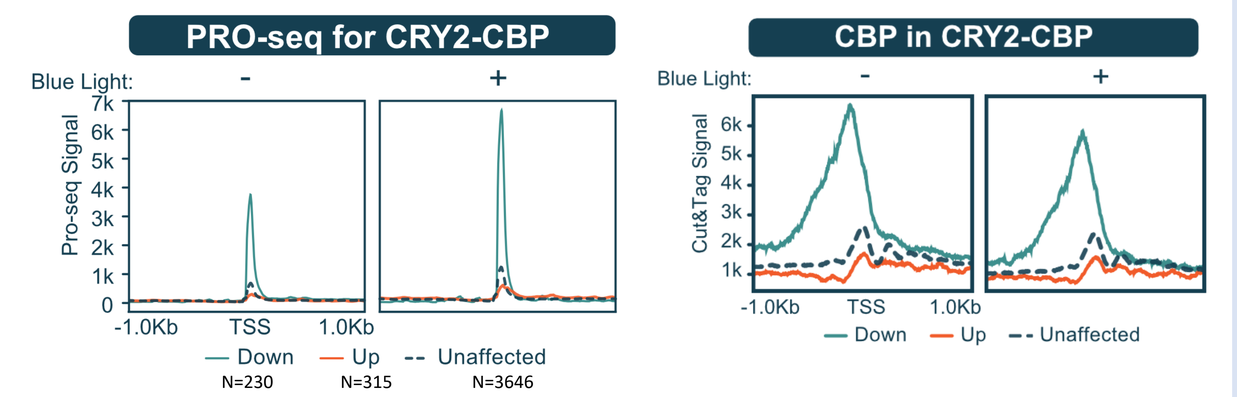



PRO-seq shows the ***reduction*** of promoter-proximal paused polymerases at downregulated zygotic genes after CBP ***degradation***.

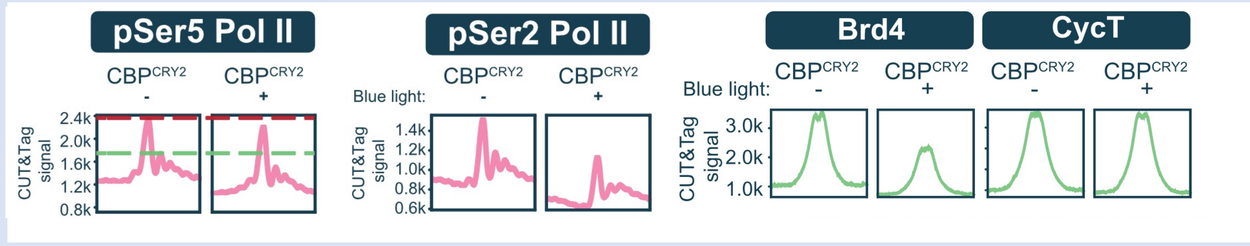
We observe the decrease in initiating Pol II (pSer5 CTD) and TATA-binding protein (TBP) detected by CUT&Tag.

### CBP catalytic activity releases Pol II from promoter-proximal pausing




PRO-seq shows the ***increase*** of promoter-proximal paused polymerases at downregulated zygotic genes after CBP ***inactivation***.

Downregulated genes are direct targets, because their promoters are bound by CBP.

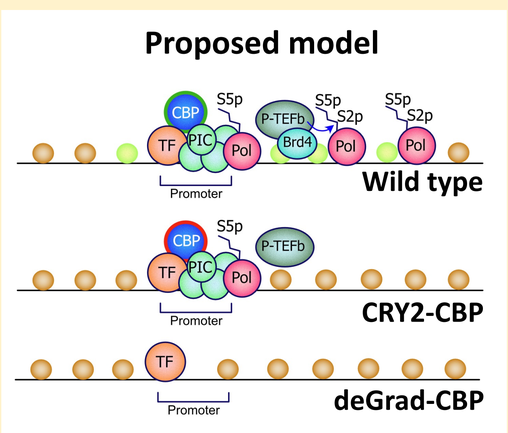



We observe no difference in initiating Pol II (pSer5 CTD), but the substantial loss of elongating form of Pol II (pSer2) by CUT&Tag.

CBP inactivation leads to BRD4 depleteion but not of P-TEFb complex (Cdk9 and CycT).

## Conclusions

1. CBP promotes transcription initiation and Pol II recruitment in non-catalytic manner.
2. Catalytic activity of CBP mediates pause-release into productive elongation, likely via Brd4 recruitment and P-TEFb complex activation.
3. CBP occupancy depends on catalytic activity at Zld-rich sites, while at GAF-rich sites CBP is recruited regardless of its activity.




[1] Pengelly, Copur et al, 2013
[2] Sankar et al, 2022
[3] Boija et al, 2017
[4] Narita, Ito, Higashijima et al, 2021
[5] Chan et al, 2019
[6] Ciabrelli et al, 2023

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