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Review

Dynamics of sequestering the limiting p300/CBP, viral cis-regulatory elements, and disease

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Many studies showed that the p300/CBP coactivator is limiting. Here we review three studies that showed how transcription complexes formed on viral cis-regulator elements compete with cellular transcription complexes by sequestrating the p300/CBP coactivator. According to the microcompetition model, this sequestering can cause disease. We use the microcompetition model to explain how a specific type of sequestering, caused by a latent virus that has an active cis-regulatory element in its promoter/enhancer that binds the transcription complex p300/CBP·GABP can cause diseases such as cancer, atherosclerosis, diabetes, and certain autoimmune diseases.

Keywords. cAMP; CBP; E1A protein; enhancer; HPV18; limiting; microcompetition; p300; sequester

1. The limiting p300/CBP

CBP and p300 are considered the most heavily connected coactivators in the mammalian protein–protein interaction network (Rual *et al.* 2005) with at least 315 different cellular and viral interacting partners (Kasper *et al.* 2006). CBP and p300 are histone acetyltransferases that control the transcription of numerous genes in humans, viruses, and other species.

p300/CBP is a 300 kDa protein that has a CH2 domain, which contains its acetyltransferase activity, and five binding domains (Kasper and Brindle 2005). Although two separate genes encode CBP and p300, they share a 61% sequence identity, and are often mentioned together as p300/CBP (Kasper and Brindle 2005). Countless research studies show that competition for limiting amounts of p300/CBP is an important mechanism used by the cell to regulate transcription and cellular behavior. This commentary discusses three of these studies (Bouallaga *et al.* 2003; Pelka *et al.* 2009; Banas *et al.* 2001), and connects the observations reported in these studies to the microcompetition model.

2. Competition for limiting p300/CBP

2.1 *Bouallaga et al. (2003)*

The heterodimer of JunB and Fos-related antigen 2 (Fra2) form the AP-1 transcription factor. JunB/Fra2 transactivates transcription of the E6 and E7 genes of the human papillomavirus type 18 (HPV18) by binding the enhancer on the early promoter.

The objective of the Bouallaga *et al.* study was to elucidate the relationship between the HPV18 enhancer, JunB/Fra2, and CBP.

First, Bouallaga *et al.* tested the effects of the JunB/Fra2 transcription factor on the HPV18 enhancer. They transfected HeLa cells with the HPV18 enhancer, or a collagenase promoter reporter plasmid, in the presence or absence of a plasmid expressing the JunB/Fra2 transcription factor. The results showed that overexpression of JunB/Fra2 caused a strong repression of the HPV18 enhancer, and activation of the collagenase promoter. These observations suggest that JunB/Fra2 is titrating an essential limiting factor needed for the activation of the HPV18 enhancer. This factor activates the collagenase promoter.

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Next, Bouallaga *et al.* examined the role of CBP in activating the HPV18 enhancer. The adenovirus E1A protein is known to bind the p300/CBP coactivators. Therefore, they transfected expression vectors of the adenovirus 12S E1A protein into HeLa cells, along with a HPV18 enhancer reporter plasmid, and a vector expressing CBP. They also transfected a set of cells with deletion mutants of the 12S E1A protein, which does not bind p300/CBP. Then, they measured the activity of the HPV18 enhancer. The results showed that the wild type E1A protein repressed the HPV18 enhancer. In contrast, the E1A mutant did not. Also, they observed that overexpression of CBP increased the HPV18 dependent transcription by about threefold, and this overexpression relieved the repression caused by the 12S E1A protein. Based on these results, Bouallaga *et al.* concluded that the E1A-mediated repression of the HPV18 enhancer was due to sequestering of the limiting p300/CBP.

To see if p300/CBP interacts with the multiple proteins bound to the HPV18 enhancer, Bouallaga *et al.* performed supershift experiments with an anti-p300 antibody. They observed that the anti-p300 antibodies supershifted the complex formed on the HPV18 probe. These results indicated that p300/CBP interacted with the HPV18 enhancer.

Then, Bouallaga *et al.* tested the possibility that the HPV18 transcription repression observed with the JunB/Fra2 was due to the sequestering of p300/CBP, as in the case of the E1A-mediated repression. They transfected JunB/Fra2 and CBP expression plasmids in HeLa cells, and measured the HPV18 enhancer activity. The results showed that the excess CBP partially relieved the inhibition induced by JunB/Fra2. Since the CBP only partially relieved the transcriptional repression, Bouallaga *et al.* concluded that other limiting factors may be involved.

Next, Bouallaga *et al.* examined the effect of E1A transfection on the endogenous E6 expression in HeLa cells. The E6 transcription is controlled by the HPV18 enhancer. They co-transfected E1A with a GFP expression plasmids in HeLa cells, and measured the level of E6 transcription. The results showed that overexpression of E1A repressed E6 transcription.

Then, Bouallaga *et al.* examined the p53 levels in HeLa cells transfected with wild type E1A, or a mutant E1A that does not bind to p300/CBP. E6 induces the degradation of p53. The results showed that the wild type E1A prevented the degradation of the p53 levels, while the E1A mutant did not. This indicates that the induction of p53 degradation was dependent on p300/CBP.

To see if the inhibition of p53 degradation also occurred in the presence of excess JunB/Fra2, Bouallaga *et al.* transfected HeLa cells with JunB/Fra2 and GFP expression plasmids, and measured the p53 levels. The results showed that cells overexpressing JunB/Fra3 also prevented the degradation of p53. This indicates that JunB/Fra2 sequesters CBP *in vivo*.

The last experiment tested a possible interaction between JunB, p300, and HPV18 *in vivo*. Bouallaga *et al.* transfected HeLa cells with a p300 plasmid. Then, the cellular chromatin was immunoprecipitated with antibodies against JunB or p300, and the DNA of the HPV18 regulatory region was PCR amplified and analyzed. The results showed a more efficient amplification of the HPV18 DNA with the anti-JunB antibody and anti-p300 antibody compared with the controls. This indicates that both JunB and p300 bind to HPV-18 *in vivo*.

The results in the Bouallaga *et al.* study showed that the HPV18 enhancer recruited p300/CBP *in vitro* and *in vivo*. It also showed that Adenovirus E1A protein inhibited E6 transcription by sequestering the limiting p300/CBP, and thereby decreased the degradation of p53. Overexpression of JunB/Fra2 reduced the activity of the HPV18 enhancer, likely by titrating the limiting amounts of p300/CBP.

To conclude, the results of this study showed that p300/CBP is limiting, that p300/CBP is a coactivator that binds the complex on the HPV18 enhancers and increases the activity of the enhancer, and that the complex on the HPV18 enhancer competes with JunB/Fra2 by sequestering the p300/CBP coactivator.

2.2 *Pelka et al. (2009)*

The early region 1A (E1A) of the human adenovirus type 5 (HAdV-5) is an important viral gene for activating viral transcription. A region of the E1A protein, called CR3 (conserved region 3), interacts with various cellular transcription factors including p300/CBP. There are two isoforms of the E1A, the 13S E1A and the 12S E1A. Both isoforms bind to p300/CBP.

The objective of the Pelka *et al.* study was to better understand the relationship between E1A and p300/CBP.

First, Pelka *et al.* co-transfected U2OS cells with plasmids expressing a GAL4 DNA-binding domain-CR3 and E1A 12S, and measured the luciferase reporter activity. The results showed that increasing amounts of E1A 12S reduced CR3 activity. This

suggested that E1A 12S is a potent dose-dependent inhibitor of 13S and CR3-dependent transactivation. Pelka *et al.* speculated that E1A 12S may be sequestering or degrading a key transcription co-regulator required by CR3.

To find out which factor is involved in the repression of CR3, Pelka *et al.* created a series of E1A 12S deletion mutants. The *dl*1101, *dl*1103, *dl*1104 and *dl*1141 mutants, which were unable to bind to p300/CBP, lost the ability to repress CR3.

To see if E1A 12S was degrading or sequestering p300/CBP, Pelka *et al.* compared the levels of p300 in U2OS and HeLa cells transfected with either E1A 12S or E1A 13S. The results showed similar levels of p300 in both types of cells, indicating that E1A 12S is not degrading, but rather sequestering the coactivator.

Then, Pelka *et al.* tested the effect of adding p300. They co-transfected U2OS cells with GAL4-CR3 plasmids, acetyltransferase CBP, a GAL4-luciferase reporter plasmid, and E1A 12S, and measured the luciferase activity. The results showed that the higher levels of p300 (in the form of acetyltransferase CBP), increased CR3 transactivation in the presence of E1A 12S.

Pelka *et al.* also tested the effect of p300 depletion. They used siRNA specific to p300 mRNA to decrease the concentration of the p300 protein in human HeLa cervical cancer cells and U2OS osteosarcoma cells. They observed a decrease in transactivation of GAL4-CR3 in the presence of 13S.

Next, Pelka *et al.* tested a possible binding between CBP and CR3. They combined recombinant CBP and CR3 in a pulldown experiment, and detected a direct interaction between CR3 and CBP. They also observed that cellular factors, present in the HeLa cell lysate, reduced the interaction between CR3 and CBP. They concluded that some cellular factors present in the lysate may compete with E1A for CBP.

The final experiment determined the possible recruitment of p300 to the adenovirus E4 promoter during viral infection. Pelka *et al.* used chromatin immunoprecipitations with E1A and p300 antibodies on infected HeLa cells, and measured the binding of p300 to the E4 promoter. The results showed that p300 is recruited to the E4 promoter in the presence of E1A 13S, but not in the presence of E1A 12S.

To summarize, the Pelka *et al.* results showed that p300/CBP is limiting, that p300/CBP binds the CR3 region of E1A, and that p300/CBP is sequestered by being recruited to a complex on the adenovirus E4 promoter in the presence of E1A 13S.

2.3 *Banas et al. (2001)*

The transcription of human immunodeficiency virus HIV-1 is regulated by the NF-κB and cyclic AMP (cAMP) through the HIV long terminal repeat (LTR). p300/CBP binds the p65 subunit of NF-κB.

The objective of the Banas *et al.* study was to better understand the relationship between cAMP, NF-κB, p300/CBP, and the HIV-1 LTR.

First, Banas *et al.* treated Jurkat T cell clone infected with HIV-1 at three different concentrations of cAMP, and observed a decrease in viral replication. They repeated the experiment with HUT-78 cells and human peripheral blood lymphocytes (PBLs), and observed similar results. These observations indicate that cAMP reduces HIV-1 viral replication in various cell lines.

Next, Banas *et al.* tested the effect of cAMP on HIV-1 LTR transcription. They co-transfected human PBLs and Jurkat T cells with the full-length LTR HIV-1 sequence along with an expression plasmid encoding tat to achieve maximal LTR activity. The transfected cells were then treated with phorbol 12-myristate 13-acetate (PMA) alone, or PMA combined with 8-Br-cAMP. They observed that PMA alone induced the HIV-1 LTR, and co-incubation with cAMP repressed the LTR in both cell lines.

Then, Banas *et al.* concentrated on the role of CBP and p300 in the regulation of the HIV-1 enhancer region. They generated Jurkat T cell lines that over-expressed CBP or p300 by transfecting them with CBP or p300 encoding constructs. Then, they transfected wild-type Jurkat T cells with a luciferase construct containing both naturally occurring NF-κB binding sites (HIV-AB), and treated these cells with PMA and/or 8-Br-cAMP. For comparison, they transfected the cells overexpressing CBP or p300 with HIV-AB under the same conditions. They observed that PMA stimulation did not affect the luciferase activity in the CBP or p300 cell lines. Furthermore, the suppressive effect of cAMP, which was seen in the other cells, was eliminated. These results indicate that overexpression of CBP or p300 can overcome the inhibitory effect of cAMP.

The results in Banas *et al.*'s study show that p300/CBP is limiting, and that the increase in cAMP in the PMA-stimulated cells led to competition between the phosphorylated CREB and NF-κB bound to the HIV LTR for the limiting p300/CBP. In other words, the results show that the negative effect of cAMP on HIV-1 replication, and on the transcriptional activity of the HIV-LTR, may be due to the activation of CREB protein, which competed with phosphorylated NF-κB

and Ets-1 transcription factors that are bound to the HIV-1 LTR and sequester the p300/CBP coactivator.

To conclude, the results in in this studies showed that p300/CBP is limiting, and that transcription complexes bound to viral cis-regulator elements in the promoters, enhancers, or LTR compete with cellular transcription complexes by sequestrating the p300/CBP coactivator.

3. Disruption of competition by latent viruses

The results in these studies suggest that viral enhancers can interfere with cellular competition for the limiting p300/CBP, which is used by the cell to regulate transcription. According to the microcompetition model, such dysregulation can cause a disease. The microcompetition model was first described in the book *Microcompetition with Foreign DNA and the Origin of Chronic Disease*. (Polansky 2003; Polansky and Javaherian 2016) The model centers on one type of disruption of this regulation caused by viruses that include the strong cis-regulatory element found in their promoters/enhancers called the N-box. This element binds the cellular p300·GABP transcription complex during the latent phase. Some common viruses that include an N-box are the *Cytomegalovirus* (CMV), *Epstein-Barr Virus* (EBV), *Herpes Simplex Virus 1* (HSV-1), *Human T-Cell Lymphotropic Virus* (HTLV), and *Human Immunodeficiency Virus* (HIV). Since p300 is limiting, the p300·GABP complex is limiting. Therefore, the viral N-boxes decrease the availability of p300·GABP in the cell. The result is abnormal expression of the cellular genes that also bind the transcription complex. Those genes that are transactivated by the p300·GABP complex synthesize fewer proteins, while those that are transrepressed by the complex synthesize more proteins. The abnormal levels of these cellular proteins can cause diseases, including cancer, diabetes, atherosclerosis, and certain autoimmune diseases.

In the above-mentioned book, there is a list of many human genes that bind the p300·GABP complex. The list includes the β2 leukocyte integrin (CD18), interleukin 16 (IL-16), interleukin 2 (IL-2), interleukin 2 receptor β-chain (IL-2Rβ), IL-2 receptor γ-chain (IL-2 γc), human secretory interleukin-1 receptor antagonist (secretory IL-1ra), retinoblastoma (Rb), BRCA1, human thrombopoietin (TPO), aldose reductase, neutrophil elastase (NE), folate binding protein (FBP), cytochrome c oxidase subunit Vb (COXVb), cytochrome c oxidase subunit IV, mitochondrial transcription factor A (mtTFA), β subunit of the FoF1 ATP synthase (ATPsynβ), prolactin (prl), the oxytocin receptor (OTR), telomere repeat binding factor 2 (Terf2), 5α-reductase, androgen receptor (AR), and others. As predicted by the microcompetition model, these genes express abnormal levels of their proteins in various diseases, for instance, lower levels of BRCA1 in breast cancer (Polansky and Schwab 2019), lower levels of RB in many cancers (Jariwalla 2005), higher levels of CD18 in atherosclerosis and autoimmune diseases, lower levels of telomere repeat binding factor 2 (Terf2) in vascular disease (Polansky and Javaherian 2015), higher levels of 5α-reductase and androgen receptor (AR) in male-pattern baldness (Polansky and Kestenbaum 2018), etc.

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