CBI Student Sabbatical Proposal
03/14/2011

Chris Taylor, Chemistry Department
Advisor: Dr. Anna Mapp, Chemistry Department

Host: Dr. Colin Duckett, Department of Pathology
University of Michigan

Sabbatical duration: 8-10 weeks.

Sabbatical start date: April 1st

The overall goal of the sabbatical is to identify molecules that modulate the activity of the activator NF-kappaB. The host lab (Duckett) is a world leader in NF-kappaB function and mechanism and is thus an ideal environment to carry out this research. The specific sabbatical aims are as follows:
(1) To assess the potential of peptidomimetics that bnd to the coactivator and histone acetyltransferase CBP/p300 to inhibit the interaction between p65 and CBP/p300 and (2) to elucidate the effects of this inhibition on the transcriptional activity of p65. These aims will be addressed using cell-based assays that are familiar to the host lab. The results from these experiments will contribute to a larger investigation by the Mapp lab into the inhibition of CBP/p300-transcription factor interactions, and their functional consequences.

Peptidomimetic inhibitors of p65 mediated transcription.

The misregulation of transcription is associated with a wide variety of human diseases, such as cancer[1] and metabolic disorders.[2] In spite of this potential, generalizable methods to modulate transcription remain elusive.[3] An archetypal example of a transcription factor that drives pathological gene expression is the NF-κB subunit p65, which plays a key role in processes such as inflammation[4] and oncogenesis.[5] The aims of this sabbatical are (1) to assess the potential of peptidomimetics that bind to CBP/p300 (a known coactivator target of p65) to inhibit the interaction between p65 and CBP/p300 and (2) to elucidate the effects of this inhibition on the transcriptional activity of p65. This project will draw on the expertise of the host lab in a variety of molecular biology techniques, on their expertise with p65, and on the infrastructure that they have in place to examine p65 activity.

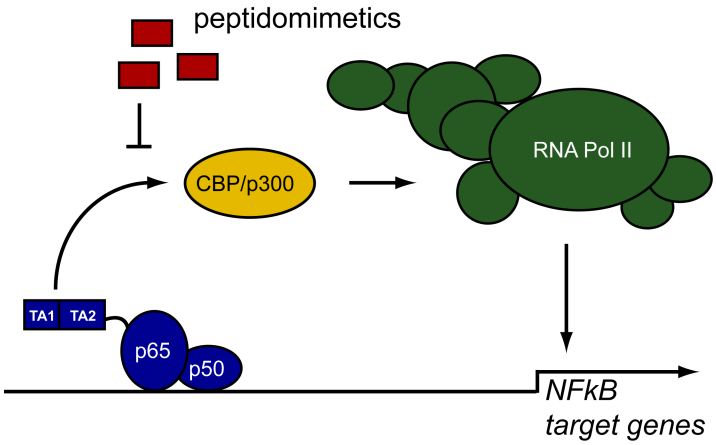


Figure 1: P65 forms a heterodimer with p50 to initiate transcription of NF-κB responsive genes. The coactivators CBP/p300 play key roles in this process, and inhibiting the interactions between p65 and CBP/p300 should curb NF-κB driven gene expression

Researcher Background

My research in the Mapp lab has focused on the design and evaluation of small molecule and peptidomimetics that modulate gene expression mediated by the transcriptional activator ESX. Our approach has been to design mimics of key regions of the activation domain of ESX– the region of a transcription factor that recruits the machinery necessary to initiate transcription. We have succeeded in designing a small molecule that curbs overexpression of the oncogene erbB2,(ref) and two additional manuscripts detailing the effects of this molecule as part of a combination treatment in breast and in head and neck cancer are in preparation. As part of an effort to extend these studies to additional oncogenes, we have identified the p50/p65 heterodimer of the NF-κB family as an ideal target because of its role in many human cancers, and because it has proven difficult to target NF-κB activity via traditional means. In contrast to ESX, there is less known about the detailed mechanism of how NF-kappaB up-regulates transcription. Thus the experience and expertise of the Duckett lab is essential to assessing our molecules.

Proposal Background

It has been established that transcription via p65 is mediated by the homologous coactivators CBP and p300, although the direct interactions are poorly characterized. The C-terminus of p65 contains 2 activation domains (TA1 and TA2) either of which can activate transcription when fused to a

1 (a) Sotiriou, C.; Pusztai, P.; *N. Engl. J. Med.* **2009,** *360,* 790-800.
(b) Perou, C. M.; Sùrlie, T.; Eisen, M.B.; van de Rijn, M.; Jeffrey S.S.; Rees C.A. et al. *Nature*. **,** *406*, 747-752.
2 (a) Lehrke, M.; Lazar, M. A.; *Cell*, **2005**, *123,* 993-999.
(b) Tsuchida, A.; Yamauchi, T.; Kadowaki, T. *J Pharmacol Sci,* **2005**, *97*, 164-170.
3 (a) Berg, T. *Curr. Opin. Chem. Biol*. **2008,** *12*, 464-71.
(b) Majmudar, C.Y.; Mapp, A. K. *Curr. Opin. Chem. Biol.* **2005,** *9*, 467-74.
4 O'Shea, J. M.; Perkins, N. D. *Biochem. Soc. Trans.* **2008,** *36*, 603–608. and references therein.
5 (a) Singh, S.; Shi, Q.; Bailey, S. T.; Palczewski, M. J.; Pardee, A. B.; Iglehart, J. D.; Biswas, D. J. *Mol. Cancer. Ther.* **2007,** *6*, 1973-1982.
(b) Basse, D. B.; Baldwin, A. S. *Oncogene,* **2006,** *25*, 6817–6830.

DNA binding domain.[4] Early indications of the interaction between CBP/p300 and p65 came from the Nabel lab, where p300 was found to coprecipitate with p65-CDK complexes,[6] and to enhance the activation of p65 by the cell cycle regulatory protein p21. Work from Collins and co-workers[7] supports that both CBP and p300 can potentiate p65-driven transcription of a reporter gene. The results of these experiments also indicated interactions between p65 286-551 (which contains both the TA1 and TA2 domains) and the N-terminal regions of both CBP and p300. The results of a series of experiments by Zhong and coworkers(6c) indicate the presence of an interaction between the KIX domain of CBP and p65, which is dependent on p65 S276 phosphorylation, and a phosphorylation-independent interaction between the C terminal region (TA1 and TA2) of p65 and the N terminal region (1-450) of CBP, which contains the CH1 domain.[8] Taken together, these experiments indicate that CBP and/or p300, particularly the N terminal domains such as KIX and CH1, play a key role in mediating p65 dependent transcription. This makes sense, given that both the KIX and CH1 domains are well-validated targets for many other transcription factors.[9] However, the *functional* significance of these potential interactions is not clear. In particular, the existence of multiple interacting sites on both CBP/p300 and p65 indicate that the interaction between these two proteins is complex. By disrupting targeted parts of this interaction, we will develop a more detailed picture of the contacts neccesary for p65 driven gene expression and assess the potential effects of inhibiting specific interactions between these two proteins. Stapled peptides that bind to either the KIX or CH1 domains of CBP are known,[10] and these peptidomimetics will provide chemical tools to assess the role of these domains in p65 driven transcription. In order to accomplish this, these experiments will be carried out with Professor Colin Duckett, an international leader in NF-kappaB mechanism and function. Because of the complex potential effects of inhibiting p65 activity in living cells, Dr. Duckett's in-depth understanding of NF-kappaB activity will be an invaluable resources in the design and interpretation of experiments. In particular, his expertise will allow clearer identification of genuine effects, and more reliable identification of potential sources of systematic error from artifacts.

Specific Aim 1: *To assess the potential of peptidomimetics that bind to CBP/p300 to inhibit the interaction between p65 and CBP/p300*

Initial experiments will assess the ability of these peptidomimetics to inhibit the interaction between p65(286-551) and CBP(1-771), which encompasses the interacting regions of both proteins Define exactly what is known. These experiments will be carried out using a luciferase complementation assay such as that depicted in Figure 2, which can be easily assembled using material available in the host lab. This will enable us to determine the relative importance of both the KIX and CH1 domains for interaction with p65. Time permitting, additional experiments will examine

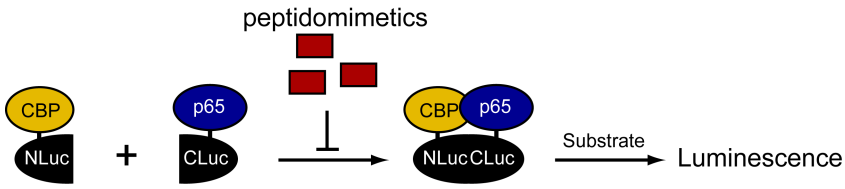


Figure 2: Luciferase complementation assay, in which regions of CBP and p65 are fused to the N anc C terminal fragments of firefly luciferase. The addition of inhibitor will block the CBP/p65 interaction, preventing luciferase activity.

6 Perkins, N.; Felzien, L.; Betts, J. C.; Leung, K.; Besch, D. H.; Nabel, G. T. Science 1997, 275, 523-527.

7 Gerritsen, M. E.; Williams, A. J.; Neish, A. S.; Moore, S.; Shi, Y.; Collins, T. Proceedings of the National Academy of Sciences of the United States of America 1997, 94, 2927.

8 Zhong, H.; Voll, R. E.; Ghosh, S. Molecular cell 1998, 1, 661–671.

9 (a) Freedman, S. J.; Sun, Z. J.; Poy, F.; Kung, A. L.; Livingston, D. M.; Wagner, G.; Eck, M. J. Proceedings of the National Academy of Sciences 2002, 99, 5367-5372.

(b) Vo, N.; Goodman, R. H. Journal of Biological Chemistry 2001, 276, 13505.

10 Henchey, L. K.; Kushal, S.; Dubey, R.; Chapman, R. N.; Olenyuk, B. Z.; Arora, P. S. J. Am. Chem. Soc. 2010, 132, 941-943.

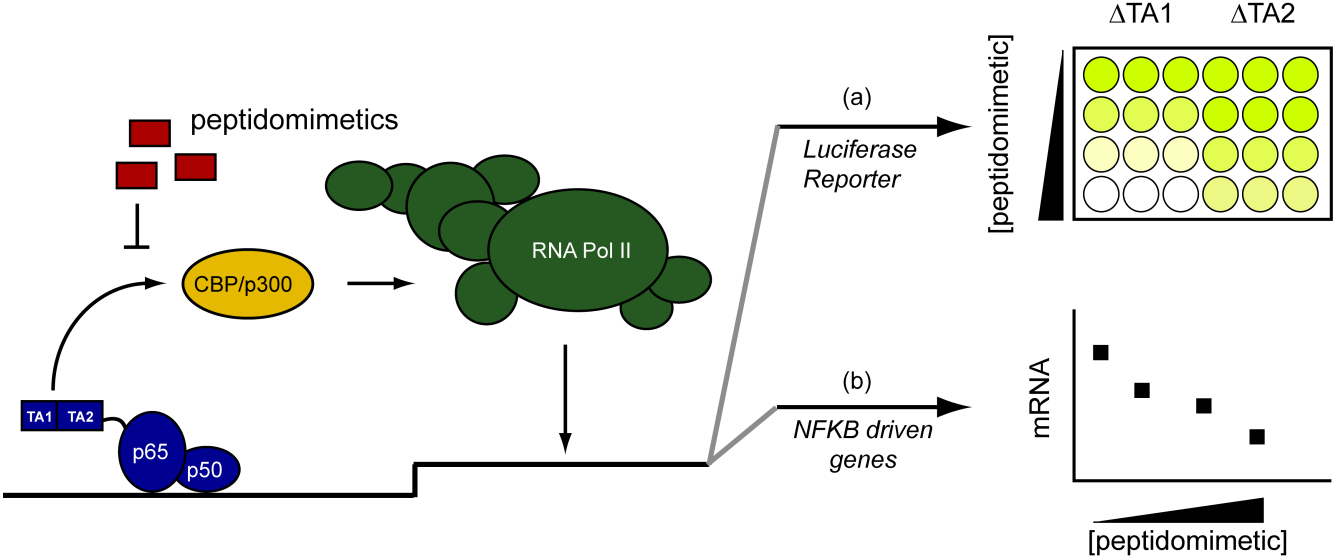


Figure 3: The effects of inhibition individual p65-CBP interactions will be assessed in a cellular context. (a) Initial experiments will use a p50/p65 driven luciferase reporter to measure the transcriptional activity of various p65 constructs, such as one with both the TA1 and TA2 domain, or one in which either the TA1 or TA2 domain has been rendered nonfunctional and only the other activation domain remains to drive transcription. (b) The effects of inhibition on a panel of p65 driven genes will also be measured. This will validate the biological relevence of inhibition, and may point to gene specific differences in coactivator requirements

interactions with individual transcriptional activation domains of p65 in order to elucidate the role of the individual domains in recruiting CBP.

Specific Aim 2: *To elucidate the effects of inhibiting the p65-CBP/p300 on the transcriptional activity of p65.*

Subsequent investigation will focus on the consequences of inhibiting this interaction. The host lab has assembled an assay in which NFKB binding sites allow p50/p65 dimer to drive expression of a driven luciferase reporter gene. This will provide direct evidence for inhibition of p65 mediated transcription in a cellular context. By using the native p50/p65 dimer rather than a p65 fragment linked to Gal4, this assay will preserve the many native protein-protein interactions of p65 and will provide more a more accurate picture of the effects of our peptidomimetics. Additional experiments will use expressed p65 constructs that lack functioning TA1 or TA2 domains, which will allow us to tease out any differences in how the TA1 and TA2 domains interact with CBP. The specific roles of these two domains in initiating transcription is poorly understood, and these experiments may provide valuable insight into this question. The host lab also has a panel of NF-κB genes that they routinely examine via qPCR, and which can be used to assess the downstream effects of inhibiting the p65-CBP/P300 interaction. There are indications that the coactivator requirements of p65 depend on the gene being activated.[11] If true, this may mean that the interaction between CBP/p300 and p65 is necessary for some p65 driven genes but not others. The selective inhibition of only a subset of an activator's target genes would be a novel and potentially useful phenomenon.

Dr. Duckett's research group is well versed in investigating protein-protein interactions that are part of complex regulatory mechanisms, especially those dealing with cell survival. They recently published a study in which the revealed a novel regulatory mechanism for NF-kB's transcriptional activity[12] and Dr. Duckett has a history of working with systems that either regulate or are regulated by NF-kB.[13] Carrying out the sabbatical in a lab that is intimately familiar with both the necessary

11 van Essen, D.; Engist, B.; Natoli, G.; Saccani, S. *PloS One*, **2009**, *7*, 549-562.
12 Wright, C. W.; Duckett, C. S. *Science*, **2009**, *323*, 251-255.
13 For examples, see:
(a) Wright, C. W.; Rumble, J. M.; Duckett, C. S. *J. Biol. Chem.* **2007,** *282*, 10252–10262.
(b) Burstein, E.; Duckett, C. S. *Current Opinion in Cell Biology*, **2003**, *15,* 732–737.

techniques and the signaling pathways under investigation will greatly facilitate rapid and accurate analysis of the resulting data.

Completion of this sabbatical would expand my current research on exogenous control of gene expression to a new system, and has the potential to answer key questions about the selectivity of this strategy. I would gain experience running several new assays that complement both my own research and ongoing work in the Mapp lab. Finally, this sabbatical would provide me with an opportunity to discuss the day to day operation of my project with a new set of researchers, and I will bring back a valuable set of new perspectives and insight when I finish.

(c) Duckett, C. S.; Gedrich, R. W.; Gilfillan, M. C.; Thompson, C. B. *Mol. Cell. Biol.* **1997,** *17*, 1535–1542.
(d) Duckett, C. S.; Perkins, N. D.; Leung, K.; Agranoff, A. B.; Nabel, G. J. *J. Biol. Chem.* **1995**, *270*, 18836-18840.

The University of Michigan
Medical School

DEPARTMENTS OF PATHOLOGY
& INTERNAL MEDICINE
Colin S. Duckett, Ph.D.
Associate Professor
Co-Director, Division of Cancer Cell Biology
University of Michigan Comprehensive Cancer Center

March 14, 2011

Chris Taylor,
Department of Chemistry
University of Michigan

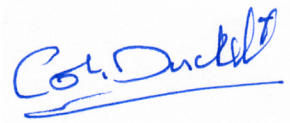
Chris,

I have enjoyed discussing your research proposal - 'Peptidomimetic inhibitors of p65 mediated transcription' and am pleased to be advising you as you work on it. There is sufficient space in my laboratory for your project and I am happy to welcome you to my research group for 8-10 weeks starting on or near April 1.

As you know, my research group investigates the regulation of cell survival and proliferation. My graduate and postdoctoral studies both focused on the regulation and role of NF-κB and I have considerable expertise in this area. I am particularly interested in the potential of peptidomimetics to selectively interfere with the activity of particular NF-κB family members, such as p65. These molecules will be a valuable tool for detailed biological investigations of NF-κB driven signaling. While in my lab, you will be working closely with students and postdocs who are experts in this area. We look forward to you participation in our weekly group meetings, and regular discussions of your work.

Thank you for bringing this proposal to me, I look forward to your work in our lab. If the committee for the Chemistry-Biology Interface Training Program has any questions, please let me know.

Sincerely,



Colin S. Duckett, Ph.D.

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