# Cdx 2 Homeodomain: A novel interaction with PP1 Gamma

Allyson Moore and David Lohnes

**Department of Cellular and Molecular Medicine, University of Ottawa**

## Abstract

Cdx 2 homeodomain is a transcription factor expressed early in embryonic development in the posterior embryo. Stable Isotope Labeling with Amino Acids in Cell Culture (SILAC) results indicate a list of potential protein-protein interactions with Cdx 2 gene in HEK 293 cells. In doing bioinformatics analysis of the data, a list of likely candidates was developed. Of the candidates, members of the PTW/PP1 Phosphatase complex stood out as this interaction was not expected. PP1 gamma, a member of this complex was chosen to investigate whether an interaction existed. Co-immunoprecipitation and Western Blot analysis suggests that the above is true, it is probable that there is an interaction between PP1 gamma and Cdx 2 within the cell.

## Introduction

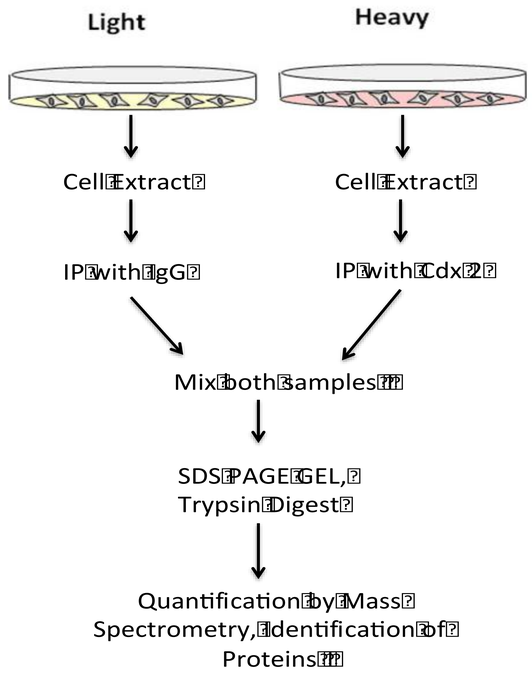
Cdx genes; Cdx 1, Cdx 2 and Cdx 4 encode homeodomain transcription factors. The Cdx2 gene in particular, is expressed early in embryonic development and there are significant phenotypic alterations when the gene is knocked out. For example, Cdx2 is highly expressed in the posterior embryo, and heterozygote mutants show a truncated tail. Homozygous mutants do not implant. This shows Cdx 2 is not only necessary for proper development of the posterior embryo but it is also vital for survival. What still is not clear however, is how Cdx 2 functions within the cell- does it work independently or (more likely) with other proteins or protein complexes?

Given that proteins within the cell rarely work alone, I predict that Cdx 2 interacts with one or more additional proteins to carry out its function as a transcription factor. Therefore, the goal of this project is to identify proteins that interact with Cdx 2 and subsequently to characterize the nature of the interaction.

To do this a stable isotope labeling with amino acids in cell culture (SILAC) experiment was performed followed by a series of Western Blots, which provide an analytical tool to determine whether or not a specific protein-protein interaction exists. HEK 293 cells were used throughout the project as they express Cdx 2 endogenously. The results of the study will provide a greater insight into the role of Cdx 2 and how it carries out its function during embryo development.

## Methodology

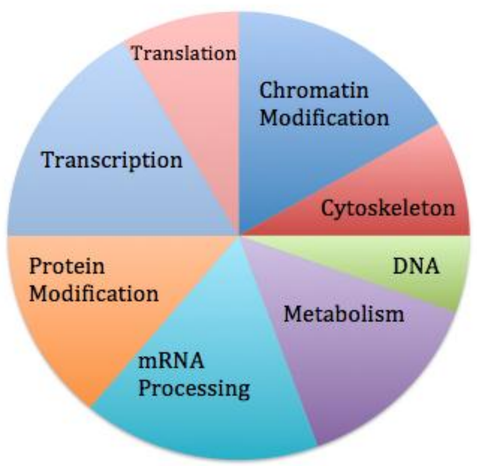
**Figure 1. Stable Isotope Labeling with Amino Acids in Cell Culture.** HEK293 cell cultures are grown in heavy amino acids and a control in light media. Proteins are purified using specific antibodies and the heavy and light cultures are mixed together. An SDS PAGE is run; followed by trypsin digestion. Fragments are quantified and analyzed using mass spectrometry. Proteins are identified and a log ratio is generated comparing the control to the heavy media cultures. A high log ratio indicates a likely candidate to interact with Cdx 2, as more protein was pulled down with Cdx 2 antibody beads.




## Results

| Protein Complex Name | Members pulled down with Cdx 2 |
|---|---|
| PTW/PP1 Phosphatase Complex | PP1CB, PP1A, UNQ9342 |
| SWI/SNF Complex | ARID1A, SMARCA4, ACTL6A |
| Splicesomal Complex: | EFTUD2, SNRNP40, PRPF6, GEMIN5, PAB1 |
| Translation Initiation Factor 1 Complex | EIF4H, EFTUD2, PPP1A |

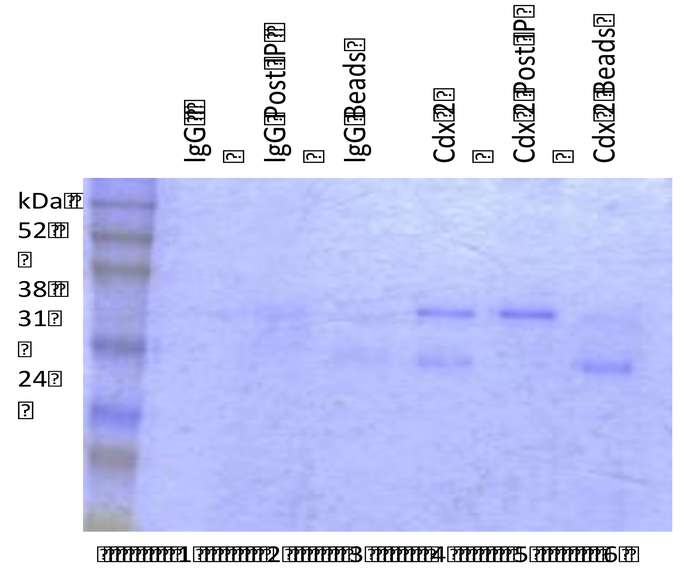
(A)




(B)

**Figure 2. Classification of Protein-Protein Interactions with Cdx 2.** (A) Specific examples of Protein complexes whose members were pulled down with Cdx 2 during SILAC, including PP1 gamma. (B) Categories are based on the prevalence of biological processes that specific proteins are involved in. These processes were expected, as Cdx 2 is a transcription factor. The interaction with PTW/PP1 Phosphatase complex was unexpected, and focus became to investigate into this novel interaction

**Figure 3. Verification of antibody-bound beads.** Both IgG and Cdx 2 antibodies were mixed in solution with PBS. An aliquot of this solution was taken as a negative control. Antibody-PBS solution, was added to Protein A/G Beads and were incubated overnight at 4°C. The solution was spun down and an aliquot of the supernatant was taken as a second control. Washed the beads/antibody mixture in PBS. Re-suspended beads in PBS- aliquot was taken.



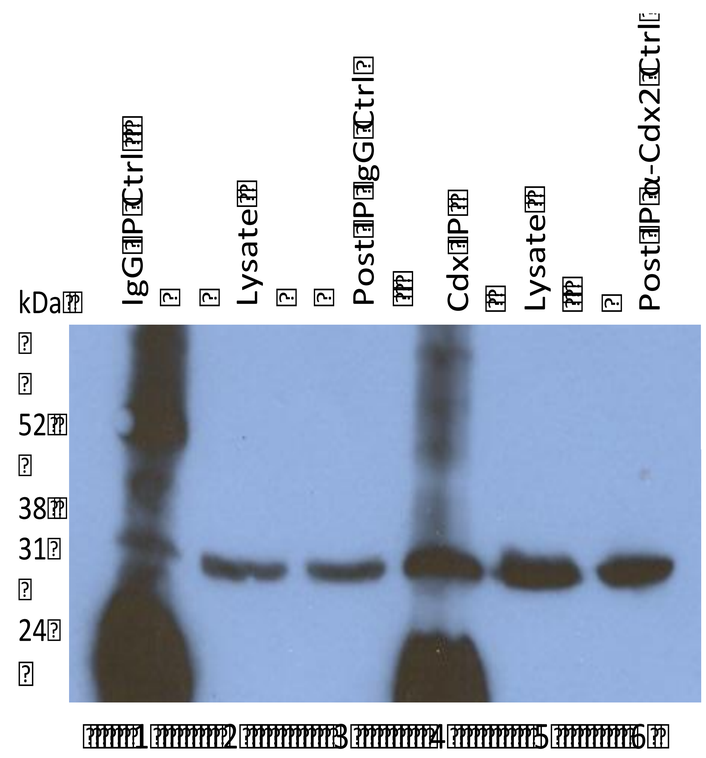




**Figure 4. Co-immunoprecipitation indicates a potential interaction between Cdx 2 and PP1 Gamma.** Protein A/G beads were coated with Cdx 2 antibody and non-specific immunoglobulin IgG. Proteins were immunoprecipitated from whole cell extracts of HEK293 cells and were resolved on a polyacrylamide gel. Subsequently, the proteins were transferred to a PVDF membrane, which was probed with an antibody that recognizes PP1 gamma. A primary antibody solution of PP1 gamma incubated overnight at 4°C. Secondary antibody of Donkey-anti-Goat incubated for 1 hour at 25°C. Secondary antibody was tagged and developed using ECL. Exposure time was 1 minute.

## Conclusions

PP1 gamma is one member of the much larger protein complex PTW/PP1 phosphatase complex. This complex has roles in phosphorylation and dephosphorylation within the cell. Western blot analysis further confirmed results generated from SILAC that PP1 gamma is likely to interact with Cdx 2. Cdx 2 antibody coated beads show a band in the position PP1 gamma is expected to migrate. Thus far, we can conclude there is a probable interaction between the two proteins. The next question to be answered is whether or not Cdx 2 interacts directly with PP1 gamma in the complex, or if the interaction is indirect, meaning there is a third protein component involved. Future work will be directed towards investigating the nature of these interactions.

## References

*http://www.molecularsciences.org/proteomics/protein_protein_interactions*
*http://silac.org/index_html*