MECHANISM OF TUMOR SUPPRESSION BY CHD5

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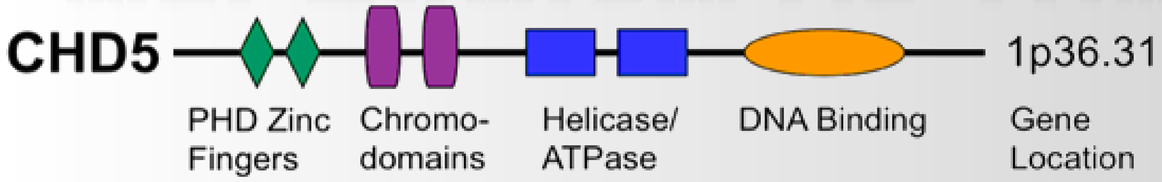


PARP-1. Photograph. *Atlas Genetics Oncology*. Jan. 2010. Web. 23 Feb. 2011. <http://atlasgeneticsoncology.org/Genes/Images/CHD5Fig1.png>.

❖Introduction

Malignant neoplasm, the uncontrolled growth of human cells commonly known as cancer, has become a focus of modern biological research. On a genetic level, cancer is thought to be caused by the activation of mutated oncogenes and the inactivation of tumor suppressor genes (TSGs) which act as “brakes” on cellular growth.

The “Knudson Hypothesis” explains cancer development. Mutation of an oncogene allele will not necessarily lead to cancer as normally functioning TSGs will act as counterbalances. Only a growth impetus from an activated oncogene *and* a damaged TSG will lead to unchecked cancer proliferation.

Cancer research diverged early on, one side of scientists searching for oncogenes genes and one side searching for TSGs. Cancer gene discovery has relied extensively on tumor analysis. One common technique, known as insertional mutagenesis, involves physically changing the genetic information of an organism, usually a transgenic mouse, to spawn cancer development for analysis.

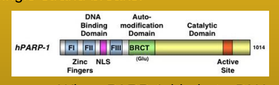
In genomic analyses of developed cancers, researchers consistently found deletions in 1p36, the outermost band of the short arm of chromosome 1 in 1977. The search for a 1p36 TSG had begun. In 2007, Dr. Anindya Bagchi and Dr. Alea A. Mills made a groundbreaking discovery by showing how CHD5, the mystery gene, functioned as a tumor suppressor *in vivo*, by controlling the proliferation, apoptosis, and senescence of cancer via the p19(Arf)/p53 pathway. However, CHD5’s mechanism of operation remained unknown.

Under Dr. Anindya Bagchi’s supervision, the Center for Genomic Engineering at the University of Minnesota began extensive research on CHD5. I have conducted a protein study of PARP-1 and CHD5 to discover more about CHD5’s mechanism of operation as a human TSG.

❖Background: PARP-1

We know chromatin remodelers such as CHD5 function as protein assemblies. This is where PARP-1, an enzyme associated with niacin, comes into the equation. PARP-1 has been shown to control processes including DNA repair and chromatin development.

PARP-1 is appealing to researchers working with CHD5 because of the enzyme’s structure. One study, observing PARP-1 interactions in Drosophila, observed the role played by the enzyme in inducing a “localized relaxation of chromatin structure, allowing DNA repair complexes to form and accomplish repair.” This is achieved through PARP-1’s two zinc fingers which allow the enzyme to bind to DNA at single-strand breaks.



PARP-1. Photograph. *Atlas Genetics Oncology*. Jan. 2010. Web. 23 Feb. 2011. <http://atlasgeneticsoncology.org/Genes/Images/CHD5Fig1.png>.

When PARP-1 binds to DNA, a collection of covalent and noncovalent interactions between the charged histones of chromatin and the enzyme unravel genetic information. As DNA damage in repaired, an enzyme named glycohydrolase will degrade the PARP-1, allowing the original coil to be restored.

Because of PARP-1’s role in DNA damage repair, PARP-1 inhibitors have become a major focus for cancer therapy. One clinical study explained the modern thesis of PARP-1 inhibition used in cancer patients. By targeting cells making use of the BRCA HR pathway, PARP-1 inhibitors can selectively impair DNA damage repair in cancer cells leading to cell lysis.

The study noted activity with BRCA1 and BRCA2 deficiencies, commonly found in breast and ovarian cancer patients. These deficiencies prevent double-strand break repair capacity while PARP-1 inhibitors impaired single-strand break repair in cancer cells.

However, animal models have shown that inhibition of PARP-1 function can lead to the development of leukemias. More must be discovered about PARP-1 activity to find an inhibitor which blocks PARP-1 synthesis while preventing NAD$^+$ depletion in human systems.

❖Purpose/Applications

What is the biochemical interaction between PARP-1 and Chromodomain Helicase DNA Binding Protein 5 (CHD5) in DNA damage repair?

Operating Mechanism

CHD5 is responsible for two major functions of interest: repairing DNA damage and mediating pre-mRNA splicing. However, much is unknown about the mechanism by which CHD5 operates. Chromatin remodelers work in assemblies consisting of multiple proteins. By researching CHD5’s protein partners, we can develop a wealth of knowledge concerning CHD5’s biochemical mechanisms.

Cancer Therapy

One application of this knowledge is in cancer therapy. Cancers, such as neuroblastoma, which develop because of 1p deletions may be treated by a correction of defective tumor suppression due to a lack of CHD5 activity. My research will help with the development of specific therapies which can help activate CHD5 in mutated cancer cells to repair DNA damage or help direct synthetic replacement proteins.

❖Hypothesis

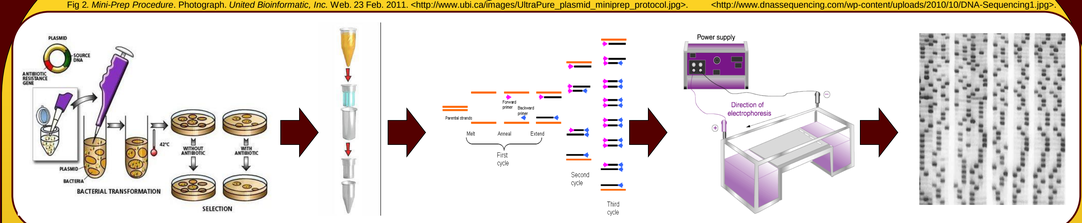
In humans, CHD5 indentifies and repairs DNA damage by a mechanism involving interaction with PARP-1. By monitoring this coiling and decoiling of chromatin, CHD5 acts as a tumor suppressor in human cells. PARP-1 will interact with CHD5 in the PHD domain because of the presence of zinc fingers which allow PARP-1 to uncoil chromatin.

❖Methodology

Fig 1, 3, 4. Photograph. *Austin Community College*. Web. 23 Feb. 2011. <http://www.austincc.edu/mlt/mdfund/pictures/Bacterial Transformation.jpg>
Fig 2. *Mini-Prep Procedure*. Photograph. *United Bioinformatic, Inc*. Web. 23 Feb. 2011. <http://www.ubi.ca/images/UltraPure_plasmid_miniprep_protocol.jpg>.

Constructing Plasmid DNA Vectors

Fig 5. *DNA Sequence*. Photograph. *DNA Sequencing*. Web. 23 Feb. 2011. <http://www.dnassequencing.com/wp-content/uploads/2010/10/DNA-Sequencing1.jpg>



I. Bacterial Transformation/ Selective Plating

DH5α *E. Coli* cells were made competent by heat-shock at 42˚ C and screened for antibiotic resistance (kanamycin).

II. Mini-Prep/ DNA Quantification

DH5α *E. Coli* cells were lysed and eluted by centrifugation to acquire pure DNA for analysis by digital spectrometer.

III. PCR Amplification

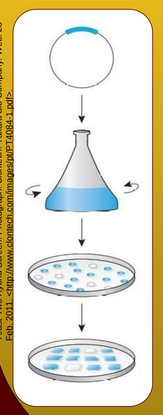
DNA vectors were annealed and amplified by polymerase chain reaction with reverse and forward primers employed to isolate my gene of interest.

IV. Agarose Gel Electrophoresis

PCR DNA fragments were segregated by gel electrophoresis to check cloned vectors for successful integration.

V. DNA Sequencing

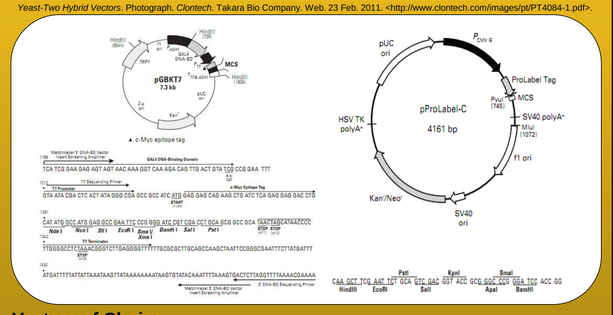
Samples were sent to the DNA Sequencing and Analysis Facility to check cloned vectors against cDNA protein library samples.



Yeast-Two Hybrid Screen. Photograph. *Clontech*. Takara Bio Company. Web. 23 Feb. 2011. <http://www.clontech.com/images/pt/PT4084-1.pdf>.

Yeast-Two Hybrid Gold Matchmaker

Y2H involves chimeric proteins. One is connected to the activating domain of GAL4 and one to the binding domain of GAL4. When interacting, GAL4 becomes active allowing for reporter gene expression coding for blue colony growth (AUR1-C, ADE2, HIS3, MEL1). Using a cDNA library, yeast strains containing CHD5 domain plasmids (CHD5, Chromo, SNF2, PHD) were used as bait proteins with Y187 yeast strains coding for the human universal protein used as the prey. Once mated and screened by selective plating, prey plasmids were recovered on blue colonies and sequenced to discover and identify CHD5’s protein partners.



Yeast-Two Hybrid Vectors. Photograph. *Clontech*. Takara Bio Company. Web. 23 Feb. 2011. <http://www.clontech.com/images/pt/PT3029-1.pdf>.

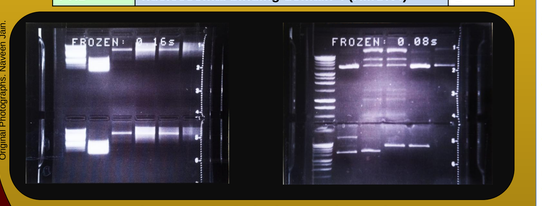
Vectors of Choice

pGBKT7 was used to construct my Y2H vectors while pProLabel-C was used to construct my CO-IP vectors. The multiple cloning site (MCS) is where mu gene of interest was integrated, flanked by SalI and BamHI sites for plasmid linearization. The Kan sequence of these vectors grants antibiotic resistance in *E. Coli* cells. The ProLabel Tag will be used in the next stages of experimentation.

❖Results

Bait Seq	Prey Seq	Hit #
CHD5	nibrin (NBN)	3
CHD5	ryanodine receptor 3	1
CHD5	phospholipase A2, group XIIA, PLA2G12A	1
CHD5	chromosome 20 open reading frame 132	1
chromo	THO complex 1	1
chromo	angiomotin like 1 AMOTL1	1
chromo	ribosomal protein L22 (LOC100129295)	1
SNF2	neuropilin 1 (NRP1), transcript variant 1	1
SNF2	hypothetical LOC157627 (LOC157627), non-coding RNA	1
SNF2	transmembrane protein 225 (TMEM225	1
SNF2	NIMA (never in mitosis gene a)-related kinase 3 (NEK3)	1
SNF2	mitochondrial poly(A) polymerase (MTPAP), nuclear	1
SNF2	THO complex 1	1
PHD	poly (ADP-ribose) polymerase 1 (PARP1)	2
PHD	epoxide hydrolase 2, cytoplasmic (EPHX2)	1
PHD	calponin 3, acidic (CNN3)	1
PHD	chloride channel 3 isoform e, chloride channel 3 isoform b	1
PHD	5-azacytidine induced 2 (AZI2)	1
PHD	family with sequence similarity 98, member A (FAM98A)	1
PHD	*Homo sapiens* high mobility group nucleosomal binding domain 4 (HMGN4)	1

Figure 1: Yeast-Two Hybrid Protein Screen

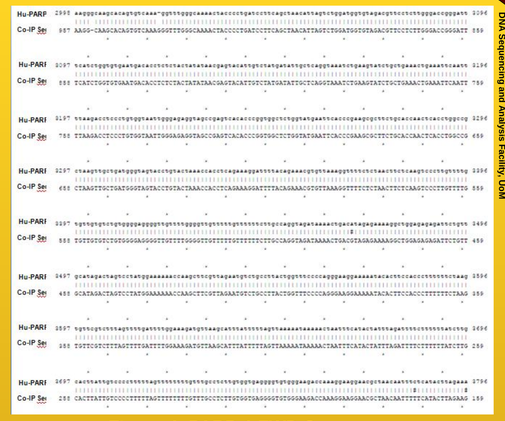


Original Photographs: Naveen Jain.

Figure 2, 3: Gel Electrophoresis

1kb DNA ladder, 100 kb DNA ladder, PHD/PAR-1 x4

1kb DNA ladder, CHD5 digested/CHD undigested x5



DNA Sequencing and Analysis Facility, UoM

Figure 4: PARP-1 FWD Sequence

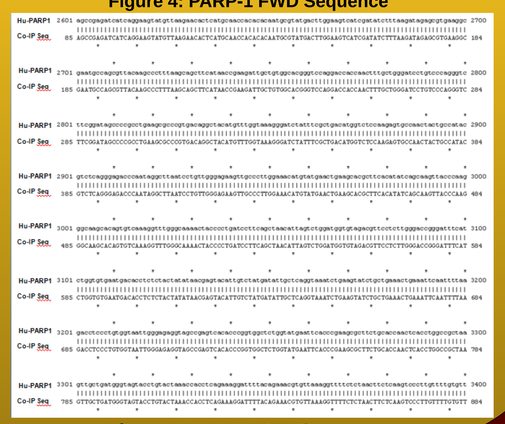
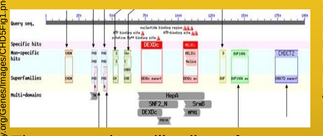


Figure 5: PARP-1 REV Sequence

❖Discussion

CHD5 Domain-Protein Screen

Using Y2H, plasmids were isolated and recovered from blue colonies. Figure 1 is a complete list of all the protein interactions observed using the Yeast-Two Hybrid system. While more domains exist, these 4 were the most active.



CHD5 Full-Length. Photograph. *Atlas Genetics Oncology*. Jan. 2010. Web. 23 Feb. 2011. <http://atlasgeneticsoncology.org/Genes/Images/CHD5Fig1.png>.

These results will allow for more directed research and experimentation with CHD5 and associated proteins. The proteins which the highest levels of CHD5 interaction were Nibrin and PARP-1. This makes sense because these two proteins are involved in DNA damage repair, especially in relation to double and single break strands.

PARP-1 Amplification Analysis

By electrophoresis (Figure 2), I confirmed that the PARP-1 CO-IP vector was correctly cloned because the base pair length was ~4161 base pairs. Upon sequencing (Figures 4 and 5), I confirmed that my gene of interest was a near-exact clone of human PARP-1. FWD and REV primers were used to capture the whole genetic sequence. With a successful clone I can move on to a functional analysis. However, my CHD5 clone was not successful (Figure 3). The electrophoresis gel confirmed the insert size was far larger than ~6000 base pairs meaning my bacterial transformation was unsuccessful. This explains why my sequences came out negative for CHD5.

❖Sources Of Error

Corrections

- Bacterial Transformation: The DNA to competent cell ratio was lowered from 10µl/100µl to 1µl/100µl for higher yield
- Samples PHD 3-1 1 2, PHD 3-1 2 2, and PHD 3-1 3 2 were accidentally plated on LB-ampicillin instead of LB-kanamycin
- PCR amplification was scrapped for a double digestion procedure to isolate and amplify my gene of interest

CHD5 Vector

My CHD5 CO-IP vector did not return sequencing results. Upon inspection, CHD5 was cloned incorrectly because the enzyme kit used in bacterial transformation had expired. Before I continue with the experiment, a new CHD5 CO-IP vector must be produced from full-length CHD5 cDNA.

❖Conclusions

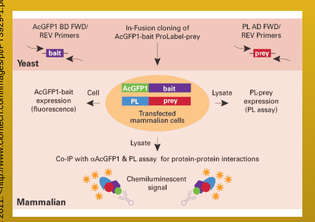
This experiment served as hard evidence of the interaction between CHD5 and PARP-1. Specifically, I know that the PHD domain of CHD5 is the region of interaction, proving my hypothesis. On top of this, I discovered from the DNA sequences (Figures 4 and 5) that PARP-1 interacts in the PHD domain through the C-terminal end of the polypeptide chain. These discoveries will allow for the development of more directed experimental study of PARP-1 and CHD5 protein interactions focused on the C-terminal end of the PHD domain.

❖Future Work

Protein Partner Identification (Yeast-Two Hybrid System):

I will continue to study other candidate proteins active in other CHD5 domains. Specifically, NIBRIN, HGMN4, and THOC-1 research will be undergone based on the results of my domain interaction experiment. Every protein I can successfully analyze will help me understand CHD5’s operating mechanism more comprehensively.

Protein Interaction Screen (Chemilluminescent CO-IP System):



CO-IP System. Photograph. *Clontech*. Takara Bio Company. Web. 23 Feb. 2011. <http://www.clontech.com/images/pt/PT3029-1.pdf>.

Using my co-immunoprecipitation (CO-IP) vectors, my PARP-1 and full-length CHD5 vectors will be placed in mammalian cells. A reporter gene will code for the expression of specific light colors. PARP-1 will code for green light and CHD5 for red. When PARP-1 and CHD5 interact in a mammalian cell, the light color will be distinct and identifiable. If successful, this will serve as hard data for the interaction between PARP-1 and CHD5 in humans.

Functional Protein Analysis (Gene Knockout Technique):

This evidence will open the way for a functional analysis of PARP-1 and CHD5. Testing and analysis will move from general to specific. First, using the same mammalian cell system, CO-IP vectors will be produced from modified versions of my Y2H vectors. Genes coding for either PARP-1 or CHD5 will be knocked out completely, allowing us to observe how the proteins impact one another. Eventually I will knockout individual portions of the genes for either PARP-1 or CHD5 allowing us to pinpoint the biochemical mechanism of interaction.