INVESTIGATION OF CONSERVATION OF BRD-1 IN *C. elegans*

by

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INVESTIGATION OF CONSERVATION OF BRD-1 IN *C. elegans*

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ABSTRACT

Our project focused on the conservation of activity of the protein brd-1 in *C. elegans*. *C. elegans* is a strong model organism for our study because Ce-brd-1 is the worm ortholog to BARD1 in humans. Specifically, our focus is on its function as an enzyme to attach ubiquitin to the H2A tail of nucleosomes. We studied a structural mutation of Ce-brd-1 that we predicted would interfere with its ability to bind its substrate, the nucleosome. We hypothesized that Ce-brd-1 is bound to the nucleosome at this mutation site based on prior research in the human protein. Therefore, we integrated mutations found in humans into the DNA that codes for *C. elegans* Ce-*brd*-1. A typical mutagenesis protocol was used to implement the mutations and then we expressed the proteins in *E. coli* cells. After that, nucleosomes were reconstituted by dialysis and enzyme activity was assessed using a ubiquitination assay. These assays showed that Ce-brd-1 in *C. elegans* does bind the nucleosome demonstrating conservation of the BARD1 function. Determining that function is conserved allowed us to determine that *C. elegans* is an appropriate organism to test mutations found in conserved areas between Ce-brd-1 and BARD1. This research has future clinical potential due to the ability to test mutations encountered in humans using a model organism and can aid with clinical treatment plans to help avoid the development of cancer.

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Introduction

There is a long list of variables that can contribute to the development of breast cancer. Somatic cells can mutate on their own and transform into lesions within the body. However, studies dating back to 1990 show genetic, inherited factors within a gene named *BRCA1*.[7] Risk begins to increase as diagnoses within familial relatives begin to increase; women whose mothers or sisters are diagnosed increases the risk of receiving their own diagnosis.[7] Mutations occur within the germline that predispose carriers to developing breast cancer.[1,2] Studies have shown mutation within the *BRCA1* gene can result in up to a 78% increased chance of developing breast cancer.[8]

While there are less concrete statistics concerning the partner gene of *BRCA1, BARD1,* it is still crucial in assisting in tumor suppression.[3] Together, these two genes create proteins (named the same as the genes) and are involved in many processes: DNA repair, DNA replication protection, transcription suppression, and most importantly for this study, tumor suppression.[3] These proteins both share a zinc-chelating RING domain that they utilize to associate with each other and thus create a heterodimer protein to perform their respective functions.[3] The BRCA1-BARD1 heterodimer is referred to as the BCBD complex and when the BCBD complex forms, it functions as an E3 ubiquitin ligase.[3] The enzyme complex, functioning as an E3 ligase, takes part in ubiquitination, a process that involves placing the molecule, ubiquitin, onto differing substrates. Ubiquitin can change the way the substrates interact with other molecules in the body which can cause visible changes within cellular function

Any mutations that occur within the RING domains that disrupts heterodimer formation results in degradation of the partner protein and loss of activity of the BCBD complex.[2-3] Studies have evaluated mutations that can take away ligase function and disrupt the heterodimer formation or solely affect the ligase function.[5] While much of the ligase function is attributed to the BRCA1 protein, it has been found that ligase activity is extremely limited when BRCA1 is isolated, thus emphasizing the importance of BARD1.[2] The BCBD complex ubiquitylates many substrates, the ones of most concern for this study are

the lysine residues that reside on the H2A portion of the octamer of the nucleosome.[1,3,12] Mutations that affect the E3 ligase activity and ubiquitylation are often identified in patients diagnosed with breast cancer.[3] For example, mutations in the RING domain of BARD1 in humans yield loss of ubiquitylation function that increases cancer risk.[12] The BCBD complex placing ubiquitin onto H2A is important for suppressing genes within the genome that have the potential to be hyperactivated and tumorigenic. Therefore, its ability to ubiquitylate is very important for tumor suppression. [3]

Just as the BCBD complex plays roles within somatic cells, its ability to place ubiquitin on to differing substrates is important for DNA double strand break (DSB) repair.[1,3,5] Specifically, BRCA1's function in recruitment of other proteins to assist in DSB repair as well as the ability of the BCBD complex to ubiquitylate play roles in DNA repair processes.[5] The BCBD complex recruits and associates itself with CtIP to begin the first step of DNA resection by relieving a blocking protein called 53BPI.[5] DNA resection is a process in DNA repair where nucleotides are removed to allow the broken DNA to line up correctly with its partner sequence and repair appropriately.[13] When WT BCBD complex is observed, there are high levels of ubiquitin on both H2A and 53BPI, but mutations in BARD1 cause a decrease in this ubiquitylation.[5] Therefore, mutations in the complex can lead to perpetual DNA damage due to the lack of ubiquitin placement on 53BPI. When lower levels of ubiquitination are observed on H2A there is increased gene expression and when this is paired with a reduced ability to repair DNA, it leads to an increased risk of developing cancer.

While this study aims to investigate the effects of specific mutations, it also aims to address the conservation of protein function between humans and the model organism, *C. elegans. C. elegans* is a great model organism to use to study the BCBD complex and how different mutations affect it. First, *C. elegans* share a considerable amount of gene orthologs with humans. [4, 9-10] Specifically, the orthologs of interest in this paper are Ce-brc-*1* and Ce-brd-*1.* These are the equivalent proteins of BRCA1 and BARD1 in humans, respectively. The *C. elegans* ortholog proteins, Ce-brd-1 and Ce-brc-1 are not essential to the development of the organism like they are in humans but still play special roles in DNA repair and

replication.[6, 11] While it has been shown that Ce-*brd-1* shares functions with the human *BARD1* gene, it is unclear where the complex binds the nucleosome and if mutations seen in humans will have similar effects in *C. elegans*. Due to this, the overall objective of this project is to determine if Ce-brd-1 has conserved enzymatic activity in *C. elegans* and to determine if *C. elegans* binds the nucleosome. It is expected that the BCBD complex in worms will interact with nucleosomes in similar fashion to that in humans and consequently mutations within the Ce-*brd-1* gene will create disruption equivalent to loss of function or degradation. To accomplish this, we created mutants, both with respect to zinc chelating residues in Ce-brd-1, and the binding interface of Ce-brd-1 and the nucleosome. These mutant proteins will be analyzed using *in vitro* ubiquitination assays to determine their enzymatic effects.

Results

The mutations were obtained using a typical mutagenesis protocol, confirmed with a genetic analyzer, and the proteins were isolated and purified from BL21DE3 E. *coli* cells. To obtain the results, a typical western blot protocol was implemented to visualize the proteins. The master mix that included all proteins and buffer was mixed with SDS-PAGE dye and run on an electrophoresis gel to separate proteins by size. The results were then transferred to a protein membrane and finally mixed with antibody solutions from Rockland Immunochemicals to see the non-ubiquitylated H2A protein (free H2A) and ubiquitylated H2A on the protein membrane. All three mutants underwent a statistical analysis including averages, standard deviation, variance testing and a student's t-test. All variance testing compared to the WT were found to be equal and the student's t-test showed that all mutant changes in ubiquitination activity were statistically significant with a p-value of <0.05.

Three mutant Ce-brd-1 proteins were created successfully. The specific amino acid residues that were changed in the nucleosome binding mutant were a lysine (K) and an arginine (R) to two glutamic acids (E) at amino acid 54 (KR54EE). The specific amino acid residues that were changed in the RING

domain mutants were a cysteine (C) to a tyrosine (Y) and a cysteine (C) to a tryptophan (W) at positions 40 and 21, respectively (C40Y, C21W). Both cysteine mutants are zinc coordinating residues and the KR54EE mutant is a structural mutant in a hypothesized nucleosome binding region.

Ce BCBD binds the nucleosome

The KR54EE mutant was slightly different than the others. The location of this mutant is hypothesized to assist in binding to the nucleosome and its effects can help better understand how and if Ce-brd-1interacts with the nucleosome. As observed in Figure 1, the amount of H2A protein that is not ubiquitinated (free H2A) at the ten and thirty-minute marks were increased compared to the wild-type protein at the same time. At the 10-minute and 30-minute period for the wild-type BCBD complex, there was an average of 74% and 29% of free H2A (not ubiquitinated) remaining compared to the sample at 0 minutes, respectively. Comparatively, the binding mutant at the 10-minute and 30-minute period had an average of 96% and 75% of free H2A remaining compared to the 0-minute sample, respectively. This indicates these residues are likely involved in binding to the nucleosome in Ce-brd-1.

RING domain function is conserved between Ce BCBD and Hs BCBD

The C40Y and C21W mutants generated are zinc chelating residues crucial in keeping the Ce-brd-1 protein together and in proper conformation. These specific mutants are named cancer-associated mutants because they have been witnessed in DNA sequences of humans with a genetic history of breast cancer. If these mutations share a similar effect in worms as in humans, then it will suggest *C. elegans* can

serve as a model organism for understanding BARD1 functions. We hypothesized that by changing the cysteine (C) residue, the amino acid would not interact with the zinc atom, therefore disrupting folding and ubiquitination. Like the nucleosome binding mutant, ubiquitination assays were performed using the wild-type BCBD complex as a comparison. As can be seen in Figure 1, the amount of free H2A at the ten-minute and thirty-minute period were markedly increased compared to the wild-type. Both the C21W and C40Y mutants at the 10-minute and 30-minute period had an average of 99% and 98% of free H2A remaining compared to the sample at 0 minutes, respectively.

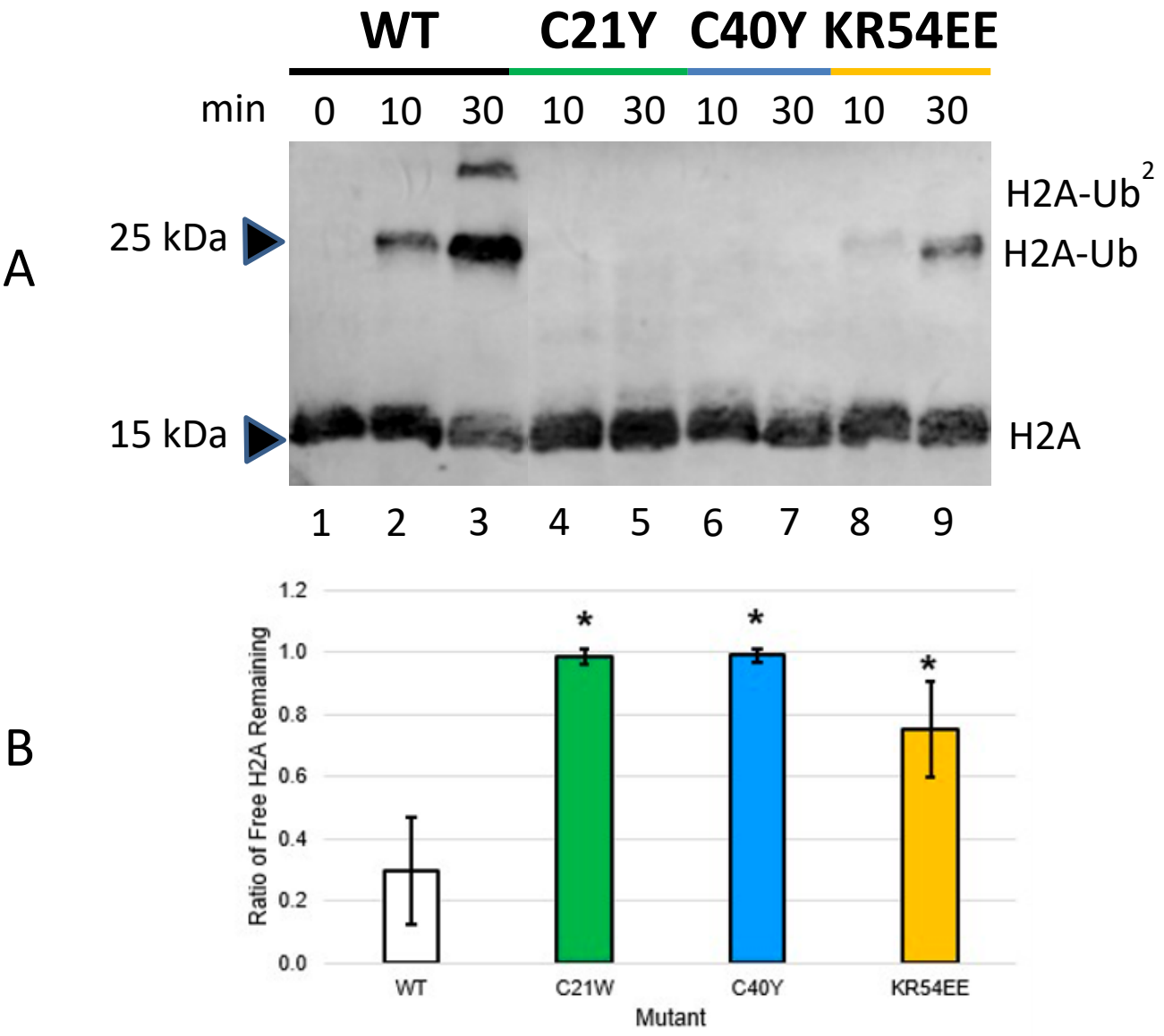

Figure 1. Ce-brd-1 is utilized to bind and ubiquitylate nucleosomes. Part A of the figure shows a western blot comparing the ubiquitylation activity of mutant Ce-brd-1 to wild-type Ce-brd-1. The substrate utilized is the H2A portion of the octamer that is incorporated in nucleosomes. Part B of the figure shows the averages and standard deviations for free H2A protein (non-ubiquitylated) for the wild-type (WT) and mutants at their 30-minute time point. All averages were calculated based off H2A present at the WT, 0-minute point. Student's T-test shows significant decreases in ubiquitylation by denoting an asterisk above each mutant using a p-value <0.05. The western blot was cropped between lanes 3 and 4 to remove additional mutant lanes that were not under study.

Conclusions and Discussion

After examining the ubiquitination assays of the KR54EE nucleosome binding mutant, it was concluded that the Ce-brd-1 protein does assist in binding the nucleosome. We hypothesized that the basic residues at position 54 and 55 were important for binding the nucleosome. This hypothesized binding region is in a loop of the protein, different from the human BARD1 nucleosome binding region. After interpreting the western blot, there is a decrease in free H2A over time at the 0-, 10- and 30-minute time points. However, the decline of free H2A is not to the same magnitude as the WT complex, indicating that the mutation disrupted binding to the interface and consequently, the ubiquitination activity.

After examining the ubiquitination assays, it is determined that Ce-brd-1 protein function in *C. elegans* is conserved. Specifically, by looking at the amount of free H2A on the western blots of the C40Y and C21W cancer-associated mutants, we see that activity of the E3 ubiquitin ligase was nearly eliminated. The amount of free H2A remained nearly constant for both mutants at their 0-, 10-, and 30-minute time periods, respectively. This suggests that the mutations seen in *C. elegans* disrupted the zinc coordination and disrupted the structure of the Ce-BCBD complex in some way. Because these similar effects are seen in human protein, we can conclude that the protein function of Ce-brd-1 is conserved between humans and worms.[12]

The cysteine, zinc chelating residue mutations were of specific interest due to their cancer association in humans. By mutating these cysteine amino acids, we hypothesized that ubiquitination rates would be decreased due to a disruption of the structure. No other amino acid is able to interact with the zinc atom in the same way that the cysteine does. Due to the loss of activity we observe in these mutants we suspect that as in humans, these mutants would result in the BCBD complex not being able to regulate gene expression on the nucleosome in *C. elegans*. The amino acid substitution most likely caused a change in conformation that shifted the nucleosome binding interface and made the complex unable to bind its substrate and utilize its E3 ligase activity. This helps confirm our hypothesis that Ce-brd-1

activity is conserved because disruption of zinc chelating residues within humans also causes a decrease in ubiquitination activity.[12]

The BCBD complex in *C. elegans* could be used as a proxy for visualizing effects of variants of unknown significance that are seen in humans. The mutations seen in human DNA sequences could be inserted into DNA sequences of live *C. elegans* organisms to see the effects on the BCBD complex and its cellular interactions in context of the development of the worm *in vivo*. This strategy only will work in areas of the complexes that are conserved between organisms. For example, mutations that are cancer associated and located in the zinc-coordinating region are an appropriate area of the protein to use for a comparison. However, the nucleosome binding interfaces, or mutations involved in substrate interactions are not appropriate to use for a comparison because they use different amino acid residues in different regions of the protein, making their binding mechanism different. While the function may be the same, the mechanism is different, and it is incorrect to assume the mechanism is similar strictly because the function is shared. When appropriate, the mutants and effects could be paired together in a catalog to assist in clinical decisions when considering mastectomies and other preventative actions. Additionally, now that we have confirmed the conservation of Ce-brd-1, *C. elegans* can now be confidently used as a model organism to study the protein interactions of the BCBD complex *in vivo.* This is not a reasonable possibility in humans primarily because of ethical dilemmas, and because mammals cannot progress past the embryonic stage and reproduce without at least one viable copy of both the BRCA1 and BARD1 proteins.[11] Therefore, mutations cannot be studied *in vivo* in humans because the organism will abort before development is complete.[11] Studying the development of *C. elegans* in combination with the BCBD complex *in vivo* can offer additional insight as to how the proteins work in cellular processes.

Methods

Creating Ce-brd-1 C21W, C40Y, KR54EE mutants

WT Ce-*brd*-1 plasmids were cloned at the University of Washington. The Ce-*brd*-1 construct (sequence in table 2) that was used was amino acids 1-107, which falls within the N-terminal domain of the Ce-brd-1 protein. Specific mutations in the Ce-*brd*-1 gene were created using the Agilent QuikChange Site-directed Mutagenesis protocol. In the creation of these mutants, mutated primers from Integrated DNA Technologies were generated with the specific mutations implemented. To begin, PCR reactions were started for forward and reverse primers for each mutation separately. All mutations were successful under different annealing conditions that are listed in Table 1. The products from these PCR reactions were combined with their respective complementary primer and annealed at 95 ˚C for 4 minutes, 90 ˚C for 1 minute, 80 ˚C for 1 minute, 70 ˚C for 30 seconds, 60 ˚C for 30 seconds, 50 ˚C for 30 seconds, 40 ˚C for 30 seconds, and then held at 25 ˚C indefinitely. Following the annealing reaction, samples were given 1 μL of the digestion enzyme, Dpn1, to digest the parent strands along with 4 μl of CutSmart buffer to increase the efficiency of the digestion. These samples sat at 37 ˚C for 1-3 hours. Finally, plasmids were co-transformed into DH5-α *E. coli* competent cells and plated on LB agar with kanamycin. The plates sat at 37 ˚C overnight to grow bacterial colonies and the DNA was recovered using the Qiagen Mini-Prep protocol. These mutations were then confirmed by DNA sequencing using a Hitachi Genetic Analyzer 3130XL.

Ce BCBD purification

Following successful implementation and confirmation of the mutations, another transformation took place using BL21(DE3) *E. coli* cells. This DNA was plated onto LB agar plates containing kanamycin for the Ce-*brd*-1 construct and chloramphenicol for the Ce-*brc-1* construct. The LB agar plates were allowed to grow overnight, and then small cultures were began using one bacterial colony suspended in the appropriate antibiotics and LB broth. These cultures were placed in a shaker shaking at 250 revolutions per minute (rpm) and at a temperature of 37 ˚C. After reaching an optical density of 0.5-0.6, the small cultures were transferred to 2 L culture flasks. These flasks contained 1 L of LB broth, 1 mL kanamycin, 1 mL chloramphenicol, and 100 µL of ZnCl. The ZnCl provided enough Zn^{+} atoms to assist the BCBD complex to fold. These large culture flasks grew at 37 ˚C until they reached an optical density of 0.5-0.6. Once the appropriate optical density was reached, 250 µL of the transcription factor, IPTG, was added to induce the expression of the protein. Next, the temperature was cooled to 16 ˚C and the cultures grew at this temperature in a shaker, shaking at 250 rpm overnight. The following day, harvesting began by spinning down the cells for 20 minutes at 4 ˚C at 3500 rpm. The cell pellet was then resuspended in nickel column binding buffer containing 500 mM NaCl, 20 mM TRIS, 5 mM Imidazole, pH 7.4 and placed in a -80 ˚C freezer for harvesting later. When it was time to harvest the proteins, the cells were thawed in a room temperature water bath. As the cells began to thaw, flakes of DNase, lysozyme, 500 µL of protein inhibitor complex without EDTA, and 30 µL of DTT were added to the nickel buffer solution. Next, the cells were lysed using sonication on ice at 60 MHz. The pulses were 10 seconds long with 30 second intervals between pulses for 10 minutes of total pulse time. The sonication product was spun down for 20 minutes at 4 ˚C at 25000 rpm to separate the protein contents from the cell pellet. The protein contents were then loaded on to the Nickel column and eluted slowly using 1x nickel buffer, 5x nickel buffer and water. During protein purification using column chromatography, samples were dispensed into test tubes containing 2 µL DTT to ensure the protein constructs remained in their reduced form. The proteins remaining in their reduced form is crucial because they are only active in their reduced conformation. After running the nickel affinity protein purification, the samples containing the

desired protein were concentrated using PALL 10k centrifugation tubes. After concentrating the protein samples, size exclusion chromatography was run to purify further using a buffer consisting of 25 mM HEPES, 150 mM NaCl, 1 mM TCEP-HCl, pH 7.0. Following the size-exclusion chromatography, protein samples were again concentrated in a 10k PALL centrifugation tube, aliquoted into 50 µL samples and frozen at -80 ˚C. Small samples were taken from the 50 µL aliquots and confirmed proper protein expression and purity by running a 15% SDS-PAGE gel. (See Figures 2 and 3).

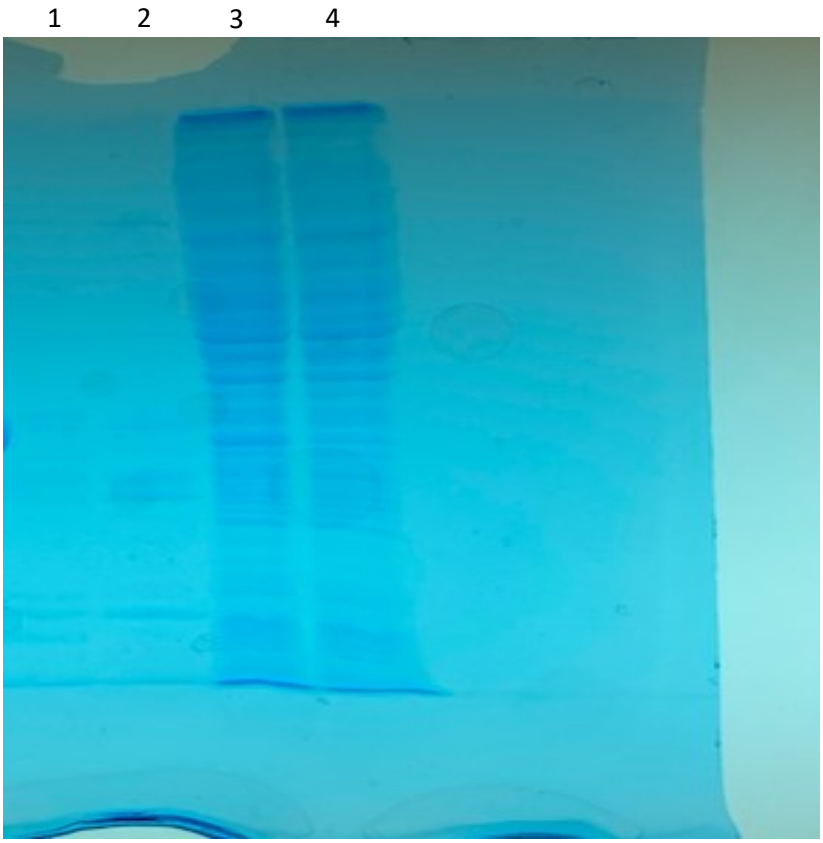

Figure 2. Ce-brc-1 and mutant Ce-brd-1 (C21W and C40Y) are both shown here, respectively in a 15% SDS-PAGE gel that denatured both proteins. The SDS-PAGE gel confirms proper expression of Ce-brc-1 and mutant Ce-brd-1 (C40Y) in lanes 1 and 3 with proper expression of Ce-BRC-1 and mutant Ce-*brd*-1 (C21W) in columns 2 and 4. Columns 3 and 4 are cell supernatant showing excess *E. coli* protein. BCBD complexes in columns 1 and 2 were determined to be approximately 50% pure, indicating that each volume should be doubled in future assays.

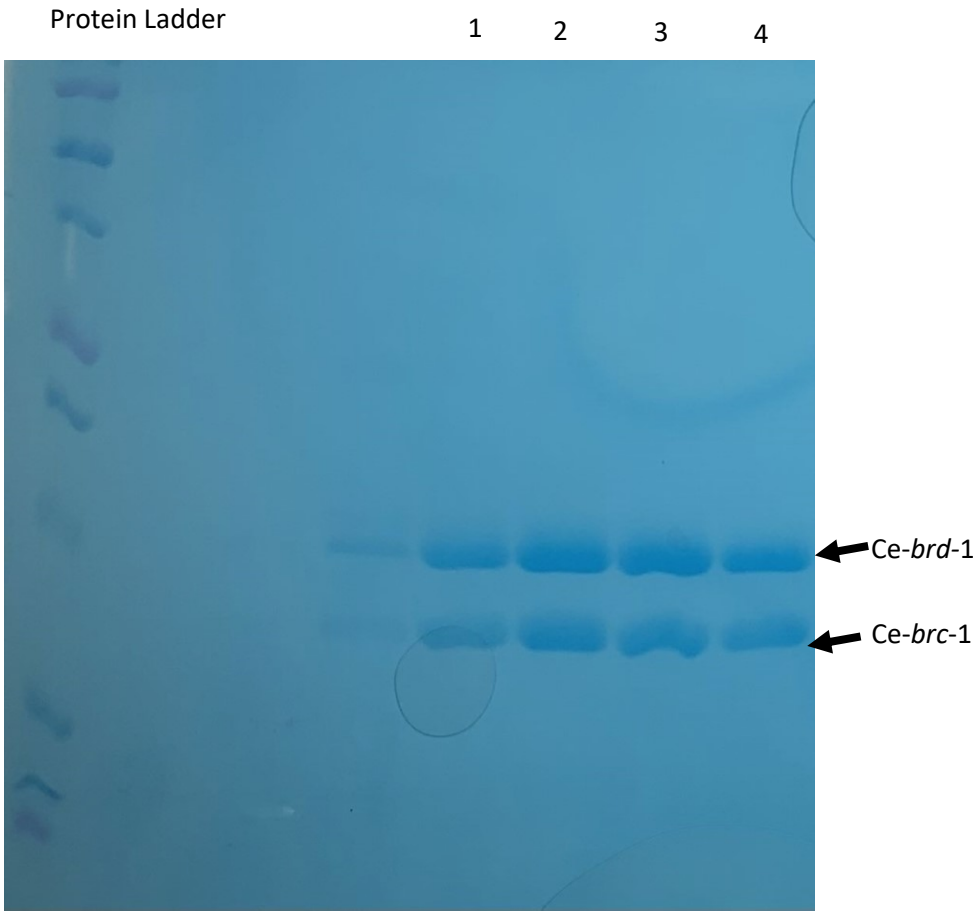

Figure 3. Ce-brc-1 and mutant Ce-brd-1 (KR54EE) are both shown here, respectively in a 15% SDS-PAGE gel that denatured both proteins. The SDS-PAGE gel confirms proper expression of Ce-brc-1 and mutant Ce-brd-1 (KR54EE) samples from gel filtration in lanes 1-4. Confirmation allows the ubiquitination assay to be run with confidence that the BCBD complex is present and pure.

Protein dialysis

At times during purification of the cysteine mutants, the amount of protein that was pulled from the nickel column was very low. Therefore, there was not enough protein to place into the size exclusion chromatography machine. When the small quantities of protein were placed in the size exclusion machine, there was not enough product to harvest, concentrate and run the ubiquitination assays at the proteins' proper concentration. Because the BCBD complex from the nickel column could not be purified further, the purity of the protein was determined to be approximately 50% as referenced in figure 2. As a

replacement, the protein was taken from the nickel column, concentrated to 2 mL, and poured into a bag made of dialysis membranes. This dialysis bag was allowed to float overnight in the same buffer made of 25 mM HEPES, 150 mM NaCl, 1 mM TCEP-HCl, pH 7.0. This slowly equilibrated the protein into the right buffer so that it would be ready for ubiquitination assays. Following the dialysis, protein expression was confirmed using a 15% SDS-PAGE gel as shown in Figure 2.

Nucleosome core particle (NCP) reconstitution

S. Witus at the University of Washington assembled the octamers as described in Witus et al. 2021 as well as provided the 185 base pair DNA. Dialysis buttons were first equilibrated in 200 mL of 20 mM Tris pH 7.5, 2 M NaCl. The reconstitution samples were then created obtaining a final DNA concentration of 7 μM, final octamer concentration of 7 μM, 20 mM Tris pH 7.5, and 20 mM Tris pH 7.5 with 5 M NaCl. These reconstitution reactions were then added to the dialysis buttons. Over a 36-hour period, 2 L of 20 mM Tris pH 7.5 was pumped into the bucket containing the dialysis buttons, gradually decreasing the salt concentration so the NCPs will form around 400 mM NaCl. This long period allows the DNA to wrap itself slowly and properly around the octamer to create the nucleosome before the salt concentration gets low enough to promote octamer aggregation. Next, a second dialysis took place using 20 mM Tris pH 7.5 and 50 mM NaCl. This dialysis was able to work as a storage buffer for the NCPs until they needed to be used. The NCPs begin to undergo degradation almost immediately following reconstitution, so it is best for them to be used for assays within 2 weeks. Finally, the reconstitution of the NCPs was verified by running a 5% polyacrylamide TBE gel as seen in figure 4.

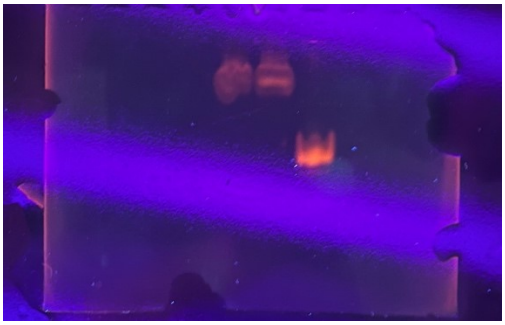

Figure 4: Confirmation of nucleosome core particle reconstitution. Figure 4 shows a 5% TBE polyacrylamide gel that confirms proper expression of reconstituted nucleosomes. Nucleosomes were reconstituted in two different concentrations relative to the DNA that was added which correlate to the two bands that are at the top of the gel. The band on the far right of the gel is a control that shows free DNA that was run to ensure that all DNA on the nucleosomes was bound.

H2A ubiquitination assays

The ubiquitination assays were performed to analyze the enzymatic effects that the specified mutations had on the E3 ligase activity. The assays all took place in a shaking incubator at 37 ˚C shaking at 500rpm. (These samples contained a final concentration of 25 mM HEPES, 150 mM NaCl, 20 µM Ubiquitin, 8 µM Ce BCBD, 4 µM Ce-LET70 (E2), 0.5 µM Human UBA1 (E1), 0.3 µM nucleosomes, 5 mM ATP, 5 mM $MgCl_2$, 0.1-0.2 mM TCEP-HCl at pH 7.0. The nucleosomes were added in the buffer solution from the nucleosome core particle reconstitution protocol listed above. At time 0, a 12 µL sample was added to a solution of SDS-PAGE load dye to denature the proteins and stop the reaction. Then, 1.4 µL of ATP was used to start the reaction and 12 µL reaction samples were taken from the shaking incubator at 10 and 30 minutes, respectively. Similarly, they were added to the SDS-PAGE load dye to denature the proteins and stop the reaction. These samples were run on a 15% SDS-PAGE gel and then protein activity was visualized using western blotting. The proteins from the electrophoresis gel were transferred onto a protein membrane and placed onto an orbital shaker with the primary antibody. The primary antibody from Rockland Immunochemicals was a rabbit antibody in a 1:10000 dilution. The primary antibody bound to the free H2A protein that was present on the protein membrane. After 30 minutes of shaking, the membrane was rotated 180 degrees to ensure full coverage and then shook for

another 30 minutes. The protein membrane was washed and placed back on the orbital shaker with the secondary antibody. The secondary antibody from Rockland Immunochemicals was a goat antibody in a 1:5000 dilution which bound to the primary antibody. After shaking for two, 30-minute time intervals, the protein membrane was washed and an alkaline phosphatase solution from Rockland Immunochemicals was poured over the membrane to help visualize the protein.

Tables

Table 1. Primer Sequences and Annealing Temperatures. Sequences and annealing temperatures of the forward and reverse primers for each mutation placed into DH5α cells. F refers to forward primer, R refers to reverse primer.

Primer Name and Mutation	Annealing Temperature	Sequence
F-Ce-BRD-1 C21W	**61ºC**	5’ CGAGTGTGTTAAATGGAAAAAACCGAGGGG 3’
R-Ce-BRD-1 C21W	**61ºC**	5’ CTCCCCTCGGTTTTTTCCATTTAACACACTCG 3’
F-Ce BRD-1 C40Y	**63ºC**	5’ GCAAACATGCTTATTACTGGGAATGTATCGCC 3’
R-Ce-BRD-1 C40Y	**63ºC**	5’ GGCGATACATTCCCAGTAATAAGCATGTTTGC 3’
F-Ce-BRD-1 KR54EE	**58ºC**	5’ CAACAGAAACCGTCTGGAGAAGAATCTTCAGTGGCTCGACACATG 3’
R-Ce-BRD-1 KR54EE	**58ºC**	5’ CATGTGTCGAGCCACTGAAGATTCTTCTCCAGACGGTTTCTGTTG 3’

F-T7	**50°C**	5’ TAATACGACTCACTATAGGG 3’
R-T7	**50°C**	5’ GCTAGTTATTGCTCAGCGG 3’

Table 2. Constructs and Sequences. This is a list of the sequences and constructs used throughout this study. Highlights in red indicate the insertion of the C-terminal tail from *C. elegans* to the human sequence.

Construct	Sequence
Chimera Histone H2A DNA	ATGTCGTACTACCATCACCATCACCATCACGATTACGATATCCCAA CGACCGAAAACCTGTATTTTCAGGGCGCCATGGGATACACAGACAT TGAGATGAACCGTCTTGGAAAGTCTGGTCGTGGTAAACAGGGTGGT AAAGCGCGTGCGAAAGCGAAATCTCGTTCTTCTCGTGCGGGTCTGC AGTTCCCGGTTGGTCGTGTTCACCGTCTGCTGCGTAAAGGTAACTA CGCGGAACGTGTTGGTGCGGGTGCGCCGGTTTACATGGCGGCGGTT CTGGAATACCTGACCGCGGAAATCCTGGAACTGGCGGGTAACGCG GCGCGTGACAACAAAAAAACCCGTATCATCCCGCGTCACCTGCAGC TGGCGATCCGTAACGACGAAGAACTGAACAAACTGCTGGGTAAAG TTACCATCGCGCAGGGTGGTGTTCTGCCGAACATCCAGGCGGTTCT GCTGCCGAAAAAAACCGGAGGAGACAAGGAATAG
Chimera Histone H2A Amino Acid	MSYYHHHHHHDYDIPTTENLYFQGAMGYTDIEMNRLGKSGRGKQGG KARAKAKSRSSRAGLQFPVGRVHRLLRKGNYAERVGAGAPVYMAAV LEYLTAEILELAGNAARDNKKTRIIPRHLQLAIRNDEELNKLLGKVTIA QGGVLPNIQAVLLPKKTGGDKE
Ce-*brd*-1	ATGGGATTTGAAAACACTAAAAAAGCATTGGAAACGTTTCGAACAGCTAT CGAGTGTGTTAAATGCAAAAAACCGAGGGGAGACTTGCAATATCTCGGAT CATCTTGCAAACATGCTTATTGCTGGGAATGTATCGCCACATTTCAACAGA AACCGTCTGGAAAACGCTCTTCAGTGGCTCGACACATGTGTCCGAGTTGT GCTTTCCCACTAGACACATCCAAAATCACAGAAGCTCATATGCTGAAAAC GTGCTTTGATACTTTGTCTGAACTAAACGACCTTTTACAGAAAGTCGGAAC AACATCTCTAACTCAAGCAGAGTTTAA
Ce-*brc*-1	ATGGCAGATGTTGCACTGAGGATCACAGAAACAGTGGCACGACTGCAAA AAGAACTGAAATGTGGAATTTGCTGTTCAACATACAAAGATCCAATTCTG TCCACATGTTTCCATATTTTCTGTCGTTCTTGCATTAACGCTTGCTTCGAAC GAAAACGAAAAGTTCAATGCCCAATTTGTAGAAGTGTACTGGATAAGCGA AGTTGTCGAGATACTTATCAAATTACAATGGCTGTGCAGAACTATTTAAA GCTATCAGAAGCATTTAAAAAAGATATTGAGAATATGAATACGTTCAATA A

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