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14. ABSTRACT

For low-penetrance breast cancer risk alleles it is currently unknown how they lead to predisposition. Here, we study the Mcs5a locus that is associated with breast cancer risk in rats and humans. In our rat model we show that the presence of the resistant genotype of two components of the locus (Mcs5a1, Mcs5a2) down regulates the expression of the Fbxo10 gene in the T cells and that this reduced expression is associated with reduced mammary tumor multiplicity. We show that genetic elements in Mcs5a1 and Mcs5a2 are physically close to each other in the nuclear space. The spatial organization of the locus in primary T cells is conserved between rat and human. We present a model that begins to explain how the Fbxo10 gene could be regulated in T cells.

15. SUBJECT TERMS
mammary carcinogenesis, susceptibility, rat, human, breast cancer risk, Fbxo10, expression, T cells, chromatin organization, 3C, genetic elements

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INTRODUCTION

Breast cancer susceptibility is a complex, polygenic trait, in which the cumulative effects of low penetrance, high population frequency, risk-altering genetic variants (modifiers) determine the heritable fraction. To be able to construct genetic risk profiles and population-based intervention programs directed to those at highest risk, it is important to identify as many risk alleles as possible. Using whole-genome linkage studies in inbred rat models that vary in susceptibility to carcinogen (DMBA; 7,12-dimethylbenz(a)anthracene)-induced mammary cancer, we found mammary carcinogenesis susceptibility QTL *Mcs5* (Samuelson et al. 2003). Using congenic recombinant inbred lines that have small pieces of the resistant genome introgressed in the susceptible background, *Mcs5* was found to contain at least three distinct loci (*Mcs5a-c*) (Samuelson et al. 2005). *Mcs5a* has been mapped to ultra-fine resolution and was found to be a compound QTL, consisting of two loci (*Mcs5a1*, ~ 30 Kb; *Mcs5a2*, ~80 Kb) that synthetically interact only in *cis* (on the same chromosome) to confer resistance (Samuelson et al. 2007). Human *MCS5A* has essentially the same genetic features as rat *Mcs5a*. Interestingly, in two population-based case-control studies (~12,000 women), the minor alleles of a SNP (single nucleotide polymorphism) in human *MCS5A1* and a SNP in human *MCS5A2* associate significantly with an altered breast cancer risk (Samuelson et al. 2007). These SNPs could either be causative themselves, or be a marker for the causative SNP. This human association study clearly demonstrates the utility of rat models to identify unbiased potential human breast cancer candidates.

Since *Mcs5a* is entirely non-coding, the causative genetic elements will likely involve transcriptional regulation. All genes within 0.5 Mb flanking the QTL are expressed at similar levels in the mammary glands in susceptible and resistant congenic animals (Samuelson et al. 2007). However, *Fbxo10* and *Frmpd1*, the genes transcriptionally starting off in *Mcs5a*, are differentially expressed in thymus and spleen, respectively. However, only the expression level of *Fbxo10* in the thymus is correlated with mammary carcinogenesis susceptibility (unpublished). *Mcs5a1* and *Mcs5a2* also need to be both present to reduce the expression in the thymus. Flow cytometry experiments revealed that the *Fbxo10* differential expression is limited to T cells (unpublished). In addition, a mammary gland transplantation assay indicated that there is a host effect on mammary carcinogenesis, suggesting a mammary cell-non autonomous effect of the *Mcs5a* locus on mammary carcinogenesis susceptibility (Samuelson et al. 2007).

We hypothesize that in T cells, genetic elements in *Mcs5a1* and *Mcs5a2* are looping over to physically interact in order to regulate the expression of the *Fbxo10* gene, which thymic expression level is correlated with mammary carcinogenesis susceptibility.

BODY

Training

Lab Meetings and Seminars at the University of Wisconsin (SoW Task 1)
As part of my postdoctoral training, I participated by attending and presenting in the Gould lab meetings and in the student/postdoc seminar series organized by the McArdle Lab for Cancer Research. On a weekly basis, I attended seminars given by invited specialists on diverse cancer biology related topics, including transcriptional regulation, biostatistics, genetics, genomics, and more.

Visit Dr. Job Dekker's lab (SoW Task 2)
Completed (Annual Report 2008)

Scientific Meetings (SoW Task 3)
I took part in an international scientific meeting, Keystone Symposia 'Chromatin Dynamics and Higher-Order organization', Coeur D'Alene, ID, held February 25 – March 2, 2009. I was assigned a poster presentation. My abstract was awarded a Keystone Symposia Travel Scholarship.
I participated with a poster presentation in the BCRP LINKS meeting, Vienna, VA, held February 9-10, 2009.

Mentoring Committee (SoW Task 4)
Although a formal meeting with the entire mentoring committee has not taken place yet, I had regular discussions with the members separately. I'm having discussions with my primary mentor, Dr. Michael Gould, at least once a week. I presented my work at the Transcriptional Mechanisms seminar series organized by Dr. Emery Bresnick, which was followed by a discussion. Discussions with Dr. William Dove and Dr. Sündüz Keles take place when needed.

Research

The 3C assay (SoW Task 1)

To identify a physical interaction between genetic elements in *Mcs5a1* and *Mcs5a2*, implementation of the chromatin conformation capture (3C) assay is essential. In collaboration with the lab of Dr. Job Dekker, who invented the 3C assay in 2002 and has a broad experience in using it (Dekker 2006), the 3C assay was established in the Gould lab (SoW Task 1a). To capture chromosomal interactions, cells are fixed using formaldehyde. The extracted fixed chromatin is digested with a restriction enzyme and religated in a strongly dilute fashion. In this procedure the ligation of genetic elements that were glued together by formaldehyde fixation is favored over ligation of random elements. Following reversal of the crosslinks, the ligation frequency of two elements of interest is determined quantitatively. The measurements will be relative to a fully digested and randomly ligated control template containing all restriction fragments of interest in equal molarity. To investigate the *Mcs5a1-Mcs5a2* interaction a fixed fragment in *Mcs5a1* was chosen and the relative interaction frequency to all restriction fragments in *Mcs5a2* was determined. This resulted in a regional profile in which fragments close to the fixed fragments give a high relative interaction frequency, due to random ligation

events. Such random events decrease with increasing genomic distance. Local peaks in the profile are indicative of a physical interaction.

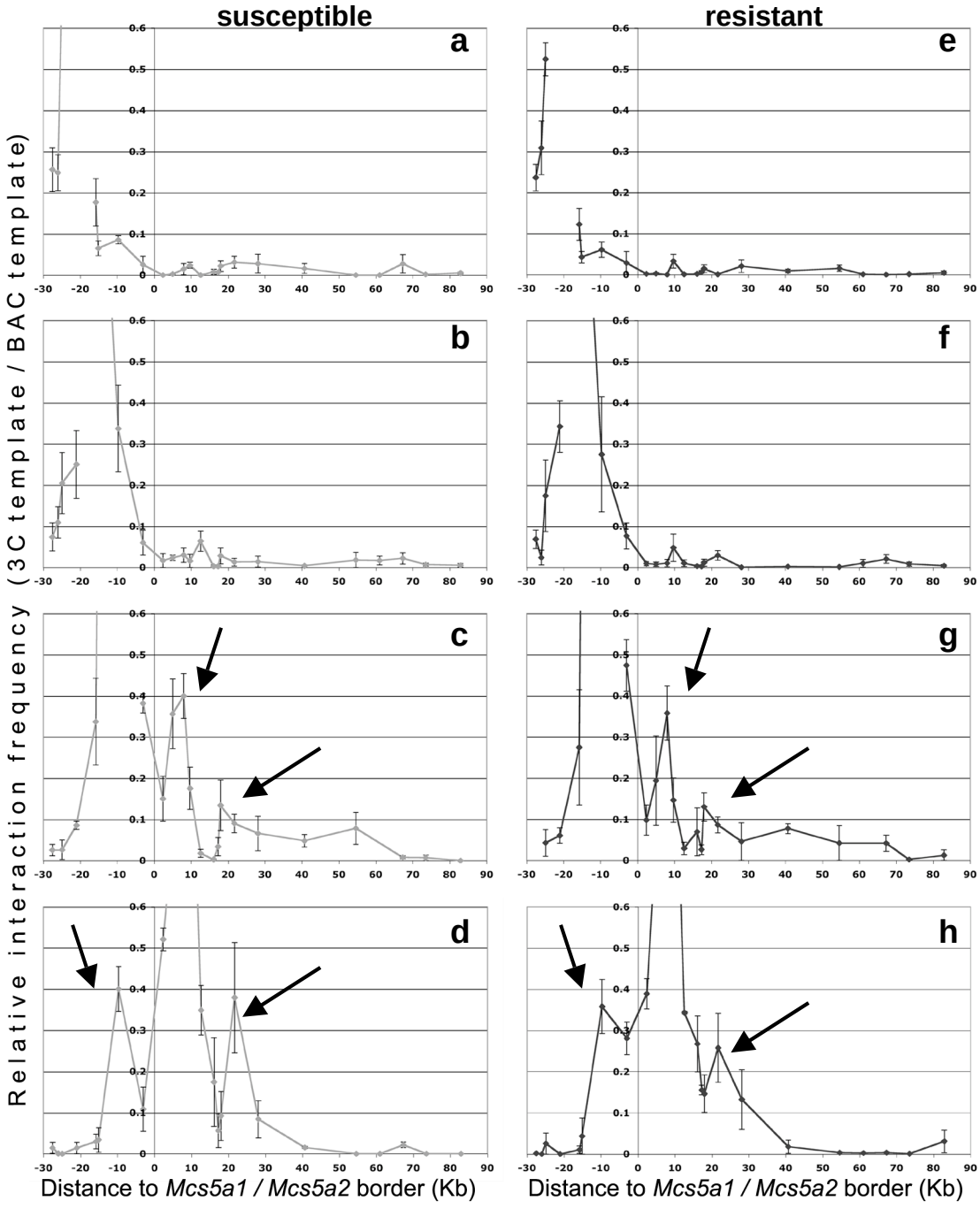

Figure 1: 3C profiles of the rat *Mcs5a* locus in splenic T cells of susceptible line WF.WKy (a-d) and resistant congenic animals WW (e-h). The fixed fragments used are within the gap of the profile. Each point is the average of at least five measurements on 3C template pools of six rats per genotype. Arrows indicate areas of potential looping.

Figure 1 shows the local 3C profiles of the susceptible (Fig. 1a-d), and the resistant (Fig. 1e-h) *Mcs5a* locus in rat splenic T cells, the tissue of interest (SoW Task 2). Initially, the fixed fragments in *Mcs5a1* were chosen to be close to the *Fbxo10* promoter and putative regulatory elements (Fig. 1a,b,e,f). Using these two fixed fragments no interactions with any elements in *Mcs5a2* were identified. Subsequently, the fixed fragment is slightly shifted towards putative upstream regulatory elements of the *Fbxo10* gene. Probing all *Mcs5a2* restriction fragments with this fixed fragment yielded locally enhanced interaction frequencies with at least two elements in *Mcs5a2*, close to the *Mcs5a1/Mcs5a2* border (Fig. 1c,g). Using one of the two *Mcs5a2* interacting elements as the fixed fragment and scanning both ways confirmed the 3-way interaction (Fig. 1d,h). The susceptible or resistant genotype had no effect on the local chromatin structure (Fig.1). Additionally, we discovered that the locus adopts a similar structure in splenic non-T cells, and in the mammary gland (shown in Annual Report 2008), suggesting that the locus higher-order structure does not directly determine the tissue-specific expression pattern of the *Fbxo10* gene (SoW Task 2). The higher-order chromatin structure is conserved between the rat and human *MCS5A* loci in primary T cells (shown in Annual Report 2008). The conservation of structure was not observed in HeLa, MCF7, and JURKAT cell lines, although the first peak after the *MCS5A1/MCS5A2* border was present.

Regulation of gene expression (SoW Task 2)

As much of the structural organization of the *Mcs5a* locus is currently known, the next step would be to understand how the locus would regulate gene expression that ultimately predisposes to breast cancer. Previous expression analysis of all genes within 1 Mb of the *Mcs5a* locus in the rat mammary gland of susceptible (WF.WKy) and resistant congenic (WW) animals yielded no expression differences (Samuelson et al., 2007). A co-worker in the Gould lab, Dr. David Samuelson, proceeded with expression analysis in other tissues of WF.WKy and WW animals and found the *Fbxo10* gene to be differentially expressed in the thymus and the *Frmpd1* gene in the spleen (SoW Task 2b). When these two genes were profiled in the thymus and spleen of the two susceptible congenic lines (*Mcs5a1, Mcs5a2*) that just have *Mcs5a1* or *Mcs5a*2 of the resistant genotype, only the expression level of *Fbxo10* in the thymus appeared to be correlated with the mammary carcinogenesis susceptibility phenotype. In other words, the *Mcs5a1-Mcs5a2* interaction is required for both down regulation of thymic *Fbxo10* expression and reduced tumor multiplicity in our carcinogenesis model.
The thymus consists mainly of T lymphocytes that could be expressing the CD4 receptor (CD4+), the CD8 receptor (CD8+), both receptors (double positive), or none of the receptors (double negative). Following flow cytometric separation of these cell types, *Fbxo10* was found to be differentially expressed in single positive CD4+, single positive CD8+, and double positive thymocytes. When isolated from the spleen, single positive CD4+ and CD8+ T lymphocytes persisted in their differential *Fbxo10* expression, whereas other cell types isolated from the spleen did not. At this point the T lymphocytes are considered the cell type of action (SoW Task 2b; Annual Report 2008).
In order to reveal where these regulatory elements that control the expression of *Fbxo10* in T lymphocytes could be located, it is essential to find its transcriptional start site (Annual Report 2008). An assay to identify transcriptional start sites of transcripts is the 5'RACE assay (RLM-RACE: RNA Ligase Mediated-Rapid Amplification of cDNA Ends; Ambion). Briefly, total RNA was isolated from rat spleen and thymus tissue. After removal of the 5'CAP structure an RNA-adapter was ligated to the 5'ends of mRNAs. Following an RT (reverse transcriptase) reaction to make cDNA, a PCR reaction with a

primer annealing to the translational start codon (ATG)-containing exon and a universal primer annealing to the 5'adapter was performed. This procedure potentially leads to the amplification of all transcriptional start sites of the *Fbxo10* transcripts. These start sites in rat thymus and spleen tissues were found to be located close to the only CpG island of the *Mcs5a1* locus (CpG1in Fig. 2). The same procedure on human thymus and spleen total RNA (Ambion) revealed the same transcriptional start site location for the human *FBXO10* gene. The start site of the *FBXO10* gene in immune tissue has never been found in the CpG island located close to the *MCS5A1/MCS5A2* border (CpG2 in Fig. 2), whereas in human breast total RNA this transcriptional start site location was identified for the *FBXO10* gene. These findings implicate that the active proximal promoter for the *FBXO10* gene in T lymphocytes is most likely located close to CpG1 in *MCS5A1*.

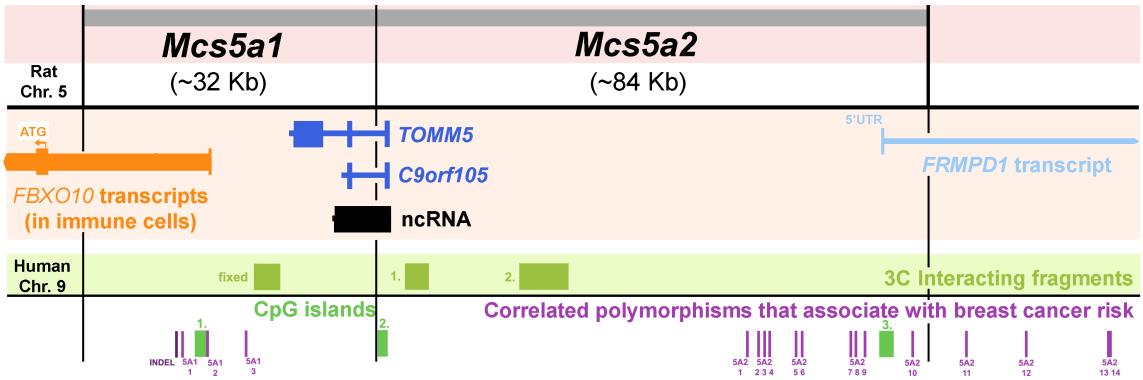

Figure 2: The *MCS5A* locus in a flat representation. Note that there are three major start site areas associated with CpG islands (in dark green). The predominant *FBXO10* transcript in immune cells is displayed in orange. The *FRMPD1* transcript is shown in light blue. The correlated polymorphisms that associate with risk are shown as purple bars. The SNPs are numbered according to their position in *MCS5A1* (INDEL, 5A1_1-3), or *MCS5A2* (5A2_1-14). The interacting elements in the 3C assay are shown in light green. Other small transcripts that start off from the promoter close to the *MCS5A1/ MCS5A2* border are shown in blue (*C9orf105; TOMM5*) and black (ncRNA).

Transcriptional activity of the breast cancer alleles (SoW Task 3 and 4)

The causative genetic variants of the breast cancer locus *MCS5A* are non-coding, suggesting a role in the regulation of gene transcription. Rat studies on gene expression regulation in various tissues identified the expression of the *Fbxo10* gene in T lymphocytes to be associated with the mammary carcinogenesis phenotype. Therefore, we hypothesize that the breast cancer alleles regulate the expression of the *Fbxo10* gene in T lymphocytes.
To mechanistically study how the correlated polymorphisms regulate transcription, I employed the pGL3-luciferase (LUC) expression vector system (Promega) in a human T lymphocyte cell culture model system (JURKAT). The system was successfully established by transiently transfecting JURKAT cells with control vectors having known luciferase activity (pGL3-basic, and pGL3-control) together with a vector resulting in renilla (REN) activity for internal normalization (SoW Task 3a). LUC and REN activities are read using a luminometer. Transcriptional activity is calculated as the ratio LUC/REN normalized against the activity of the control vector transfected in the same experiment. The higher-order chromatin interactions between *Mcs5a1* and *Mcs5a2* found by the 3C assay are not allele dependent. Therefore, they are not solely responsible for the expression regulation of the *Fbxo10* gene in the T cells and ultimately breast cancer

susceptibility. Subsequently, screening the interacting elements for transcriptional activity (as outlined in SoW Task 3b) would not explain how the *Fbxo10* gene is regulated. The 5'RACE assay indicated the start site and putative proximal promoter of the *FBXO10* gene in the human to be located amidst the correlated polymorphisms associated with breast cancer risk in *MCS5A1*. Hence, we decided to first screen all breast cancer-associated polymorphisms (both alleles, if available) for promoter activity (Fig. 3). Other (promoter) elements in the locus were also included to establish base line promoter activity levels (Fig. 3). This revealed that there are three main areas of promoter activity in the region, closely associated with the predicted transcripts. The activity of the *TOMM5* transcript promoter is strongest. Since the expression level of the *Fbxo10* is correlated with the mammary tumor multiplicity phenotype, I decided to focus on the *FBXO10* promoter and the 4 correlated breast cancer polymorphisms surrounding it. Only the fragment containing SNP 5A1_2 (Fig. 3) showed promoter activity, which is in accordance with the identified start sites of the *FBXO10* gene. The promoter activity is not different upon introduction of the susceptible allele (Fig. 3). This is not surprising as the rat expression level study suggested that the resistant allele of both *Mcs5a1* and *Mcs5a2* needs to be present to reduce *Fbxo10* expression.
It should also be mentioned that some elements containing breast cancer-associated SNPs in *MCS5A2* show promoter activity, of which one even shows differential promoter activity (SNP 5A2_11; Fig.3). These elements, however, are associated with the promoter of the *FRMPD1* gene, whose expression in the rat is not correlated with the mammary carcinogenesis susceptibility phenotype. Thus, these elements may not contribute to breast cancer susceptibility as promoter elements, but may have a distal effect on the expression level of the *FBXO10* gene.

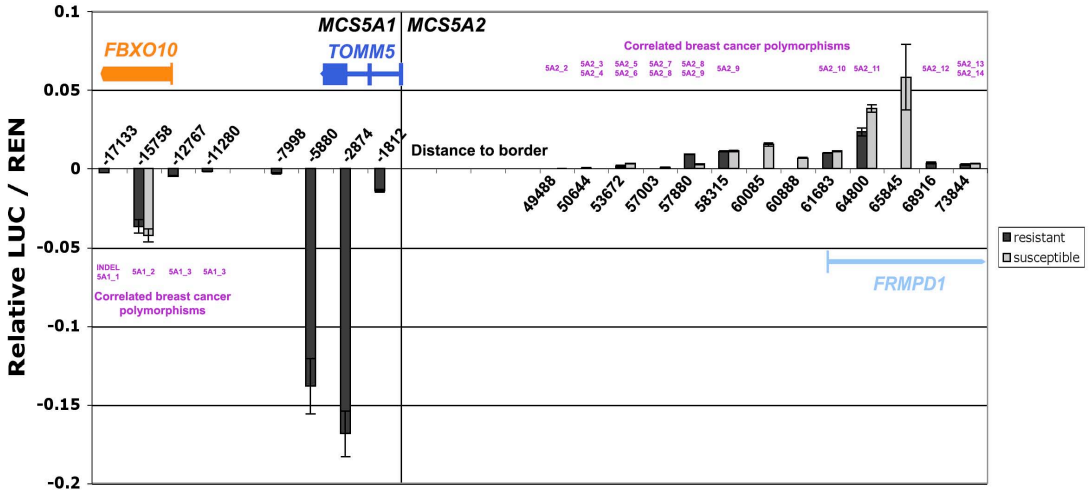

Figure 3: Promoter activity in the *MCS5A* locus (not to scale). Each element screened for promoter activity in the luciferase assay is between 800-1,400 bp in size. The number indicating each element represents the genomic distance of the middle of the element from the *MCS5A1-MCS5A2* border. The predominant *FBXO10* transcript in immune cells is displayed in orange. The *FRMPD1* transcript is shown in light blue. The *TOMM5* transcript is indicated in dark blue. The distribution over the screened fragments of the correlated polymorphisms that associate with breast cancer risk is shown in purple. The SNPs are numbered according to their position in *MCS5A1* (INDEL, 5A1_1-3), or *MCS5A2* (5A2_1-14).

To elucidate the mechanism through which *MCS5A1* and *MCS5A2* interact to regulate *FBXO10* expression, *MCS5A1-MCS5A2* combination LUC-expression vectors are made. These vectors have the active *FBXO10* promoter element in either the resistant (R) or susceptible (S) allele cloned in front of the LUC gene and elements containing the breast cancer polymorphisms of *MCS5A2* in both alleles cloned into the enhancer site. The elements from *MCS5A1* and *MCS5A2* are located >3,000bp away from each other in the vector to mimic the long-range nature of the *cis* interaction. The 14 polymorphisms of *MCS5A2* are distributed over 11 fragments to be inserted into the enhancer site. For each of the *MCS5A1* (SNP 5A1_2) – *MCS5A2* (SNP 5A2_1-11) combination there are four possibilities, namely susceptible 5A1-susceptible 5A2 (SS), susceptible 5A1-resistant 5A2 (SR), resistant 5A1-susceptible 5A2 (RS), and finally resistant 5A1-resistant 5A2. Thus, the complete set of combination vectors to be screened totals 44, of which 38 are currently ready. The remaining six vectors are anticipated to be ready within the next few weeks.

Figure 4 shows proof-of-principle of an allelic *cis* effect of an *MCS5A2* element on the transcriptional activity of the *FBXO10* promoter from *MCS5A1*. In Figure 4a, the susceptible allele of SNP 5A2_5 seem to selectively decrease the transcriptional activity of the resistant allele (5A1_2) of the *FBXO10* promoter element. Figure 4b shows an even more extreme case, where the resistant alleles of SNPs 5A2_5 and 5A2_6 together reduce the transcriptional activity of both alleles of the *FBXO10* promoter element. We anticipate constructing a complete landscape of the effect of all breast cancer alleles in *MCS5A2* on the transcriptional activity of the *FBXO10* promoter element, bearing the two alleles of SNP 5A1_2.

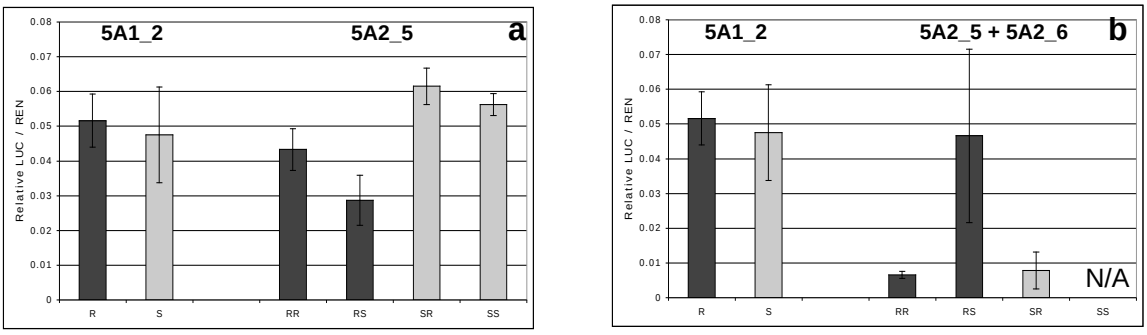

Figure 4: Effect of the *MCS5A2* breast cancer alleles on the *FBXO10* promoter activity. a) Effect of 5A2_5 on both alleles of *FBXO10* promoter element. b) Effect of 5A2_5 + 5A2_6 on both alleles of *FBXO10* promoter element. Transcriptional activity is measured by the LUC/REN activity assay in transiently transfected JURKAT cells. R=resistant allele; S=susceptible allele.

The approach to identify the interacting elements in *MCS5A1* and *MCS5A2* to regulate *FBXO10* expression as outlined above is more elaborate than initially anticipated in the SoW. As the majority of the fragments have a region of overlap with neighboring fragments, information on the minimal regulatory module around a SNP may be acquired (SoW Task 4). If needed, we will still follow the Erase-a-Base protocol to identify the sequence sufficient for the observed regulatory effect. However, considering the complexity of gene regulation, the effect of *MCS5A2* on the *FBXO10* promoter element in *MCS5A1* is likely not restricted to one element/polymorphism. Therefore, by screening the effect of all polymorphisms in *MCS5A2* on the transcriptional activity of the *FBXO10* promoter element, we will attempt to build a model to explain how the *FBXO10* gene could be regulated by the long-distance elements in *MCS5A2*. We anticipate finding multiple distal SNPs with a regulatory effect on the *FBXO10* promoter element that collectively explain the human version of the observed *Fbxo10* expression profile in the rat T cells.

Elucidating the mechanism of the breast cancer alleles: identification of the DNA-binding proteins involved (SoW Task 5)

The final stage of the project focuses on elucidating the transcription factors that differentially bind to the breast cancer SNPs that are implicated in regulating the expression of the *FBXO10* gene. Before initiating this phase with computational transcription factor binding site predictions (SoW Task 5a), knowledge of the involved SNPs is required.
An important component of this task is the Chromatin ImmunoPrecipitation (ChIP) assay (SoW Task 5c). I established ChIP in our laboratory by examining binding of the CCCTC-binding factor CTCF to our locus. CTCF is a known insulator-binding factor, involved in mediating higher-order chromatin structure. I examined binding of CTCF to the fragments involved in the 3-way chromatin interaction across the *MCS5A* locus in both human and rat T cells. Briefly, T cells (human JURKAT, or rat splenic T cells) were formaldehyde fixed and sonicated to shear the chromatin. The sheared chromatin extract was incubated with a monoclonal antibody against CTCF. After collecting CTCF antibody-bound chromatin complexes, the DNA was recovered using phenol-chloroform extractions. Enrichment of certain DNA fragments in the CTCF antibody-collected sample versus a mock antibody (mIGG1)-collected sample was determined using a quantitative PCR method.
Figure 5 illustrates the position of the interacting fragments within the *MCS5A* locus. CTCF was found to be bound to two of the three interacting fragments. These two fragments are located at either side of the strong *TOMM5* promoter, likely providing insulation of that promoter within the nuclear space.
Ultimately, we anticipate employing this assay for those transcription factors predicted to bind to SNPs involved in regulating the transcriptional activity of the *FBXO10* promoter.

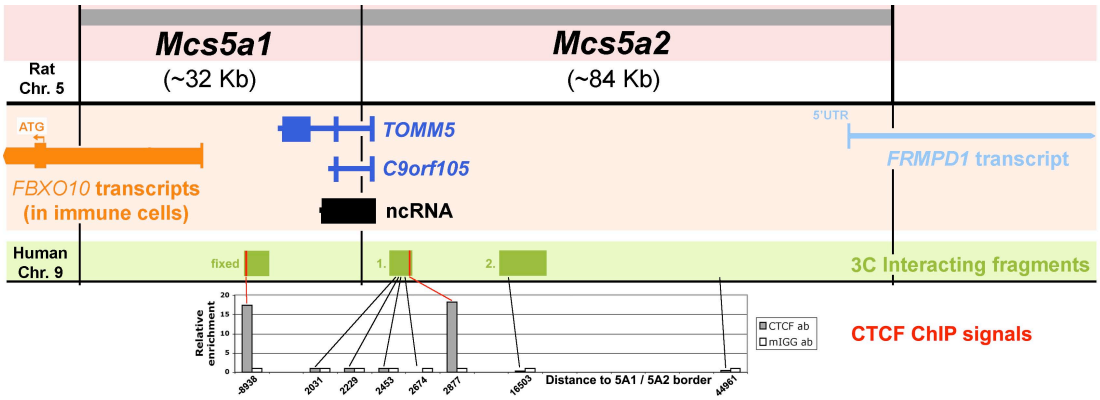

Figure 5: Chromatin ImmunoPrecipitation (ChIP) assay reveals binding of CTCF to two of the three interacting fragments in the *MCS5A* locus. The grey bars represent relative enrichment of a product of a linear PCR reaction on CTCF antibody-collected chromatin fragments versus mock antibody (mIGG1)-collected chromatin fragments (white bars; set to 1). Also see the legend of Figure 2 for explanation of the *MCS5A* locus.

Future work

The main focus of the near future experiments will be to understand how the distal elements in *MCS5A2* regulate the expression of the *FBXO10* gene. The plan is to finalize Task 3 (and 4) of the SoW, which involves screening of all alleles of the risk-associated polymorphisms in *MCS5A2* for influencing transcriptional activity of the *FBXO10* promoter element from *MCS5A1*. This data is anticipated to provide a model for the regulatory properties of the breast cancer SNPs on the expression of the *FBXO10* gene, whose expression in rat T cells is correlated with mammary carcinogenesis susceptibility. To ultimately understand how these alleles mediate breast cancer risk, the actual DNA binding factors will be identified that bind to the SNPs in an allele-specific fashion (Task 5).

KEY RESEARCH ACCOMPLISHMENTS

Training:

- Actively participated in the Gould lab meeting/journal club (SoW Task 1)
- Actively participated in McArdle Lab student/postdoc seminar series (SoW Task 1)
- Attended seminar series on a variety of cancer biology and related topics (SoW Task 1)
- Presented at an international scientific meeting: Keystone Symposia 'Chromatin Dynamics and Higher-Order Organization', Coeur D'Alene, ID (SoW Task 3)
- Presented a poster at the Era of Hope DoD BCRPM meeting, Baltimore, MD (SoW Task 3)
- Regular discussions with members of the mentoring committee (SoW Task 4)

Research:

- Completed the 3C experiments (SoW Task 1)
- Concluded 3C experiments, generated a model as a hypothesis for regulation of the *Fbxo10* gene by the *Mcs5a* locus in rats and humans (SoW Task 2)
- Established the luciferase assay (SoW Task 3a)
- Screened (nearly) all breast cancer polymorphisms in *MCS5A1* and *MCS5A2* for promoter activity (SoW Task 3b)
- Identified the promoter element of the *FBXO10* gene containing 1 breast cancer SNP (SoW Task 3b)
- Completed producing 38 of the 44 combination vectors to screen for distal transcriptional regulatory properties of the *MCS5A2* SNPs on the promoter element of the *FBXO10* gene (Task 3c).
- Showed proof-of-principle of allelic combinations specifically regulating gene expression (Task SoW 3c).
- Established ChIP assay showing CTCF binding to the interacting 3C elements (SoW Task 5c).

REPORTABLE OUTCOMES

- Abstract Keystone Symposia 'Chromatin Dynamics and Higher-Order Organization', Poster presentation

- Travel Scholarship Award Keystone Symposia 'Chromatin Dynamics and Higher-Order Organization'

- Abstract Era of Hope DoD BCRPM Meeting, Poster presentation

CONCLUSION

Integration of the structural organization data, the gene expression data, and the start site analysis resulted in a model that might explain how the *Mcs5a* locus could regulate the expression of *Fbxo10* in T lymphocytes (Fig. 6). The expression level of the *Fbxo10* gene in the rat thymus was found to associate with mammary carcinogenesis susceptibility in our model system. The *Fbxo10* expression difference between the susceptible and resistant animals was found to take place in the T cells. The 3C assay revealed three interacting components that explain how the locus is folded in the nucleus of human and rat T cells. It is thought to physically bring closer the regulatory genetic elements that are marked by the correlated polymorphisms that associate with breast cancer risk in our case-control study. Two of the three interacting elements are bound by CTCF, a known insulator-binding protein, also implicated in chromatin interactions. We believe that the CTCF-bound elements form an insulator loop to prevent interference of the strong *TOMM5* promoter with the downstream *FBXO10* promoter, located in *MCS5A1*.
Coincidently (or not), the human correlated polymorphisms in *MCS5A1* that associated with breast cancer risk in women, are located within and directly surrounding the CpG island in *MCS5A1* from which *FBXO10* is transcriptionally initiated in T lymphocytes (Fig. 6). Transcriptional activity assays using the luciferase reporter gene system elucidated an element containing one breast cancer SNP (5A1_2) to be the *FBXO10* promoter element. This SNP is now thought not to directly affect *FBXO10* transcriptional regulation in human T lymphocytes, but to interact with SNPs from the distal *MCS5A2* locus that may physically be brought closer to the *FBXO10* promoter by the formation of the insulator loop (Fig. 6). In order to explain how the correlated polymorphisms predispose to breast cancer it is important to understand how the SNP containing elements of *MCS5A1* and *MCS5A2* interact to regulate the expression of the *FBXO10* gene in T lymphocytes, in the context of their spatial organization.

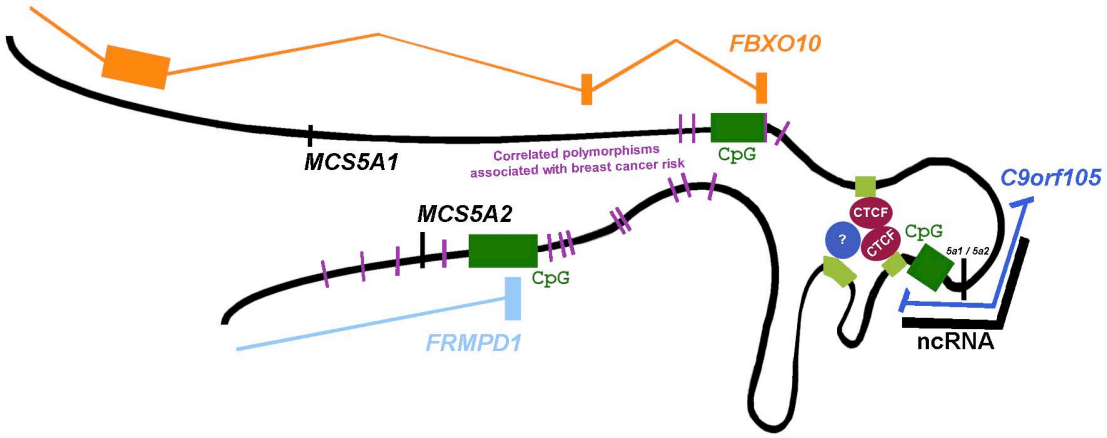

Figure 6: The *MCS5A* locus in a folded representation as determined by the 3C experiments. Note that there are three major promoter areas associated with CpG islands (in dark green). The predominant *FBXO10* transcript in immune cells is displayed in orange. The *FRMPD1* transcript is shown in light blue. The correlated polymorphisms that associate with risk are shown as purple bars. The interacting elements in the 3C assay are shown in light green. Two of those fragments are bound by CTCF (in pink). Other small transcripts that start off from the strong promoter close to the *MCS5A1/ MCS5A2* border are shown in blue (*C9orf105; TOMM5*) and black (ncRNA).

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APPENDICES

none