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October 31, 2005

I am responding to your announcement for the position of Assistant Professor. Please find enclosed my curriculum vitae, a statement of past research accomplishments and future plans, and selected reprints. Four letters of reference are being sent separately by the individuals listed on my CV.

I plan to establish an independent research program aimed at understanding how specialized chromosome structures are assembled, and how cell physiology regulates the organization and segregation of chromosomes.

I am currently a postdoctoral fellow in the laboratory of Danesh Moazed in the Department of Cell Biology at Harvard Medical School. I have been studying how silent chromatin, or heterochromatin, is assembled and regulated in the budding yeast, *Saccharomyces cerevisiae*. Silent chromatin is a powerful model for how specialized chromatin structures assemble, are inherited and regulate developmental choices. The study of silent chromatin has uncovered exciting features of cell-fate determination, homologous recombination, epigenetic inheritance and critical insights into how histone modifications regulate chromatin structure and gene expression. These areas are ripe for further investigation using conventional biochemical analyses as well as novel biochemical and proteomic approaches that I plan to utilize in my own laboratory.

My postdoctoral work has focused on developing a mechanistic understanding of how structural components of heterochromatin interact with each other and with nucleosomes to build a stable and heritable chromatin structure. In addition to investigating the biochemistry of known components of silent chromatin, I have developed a new purification method that has allowed me to define the complete proteome of silent chromatin. These studies have revealed novel structural components and regulators of heterochromatin; my broad training in genetic and biochemical approaches allows me to determine their functions.

In the future, I will exploit this new purification method to study how chromosome structure and segregation change throughout the cell cycle and in response to cell physiology. Given the right institution, colleagues and departmental support, I hope to develop a research program that weds proteomic studies with classical genetic and cell biological tools to discover novel proteins and their functions.

I believe that my research interests, openness to collaborative research, attention to craftsmanship and commitment to training young scientists will complement and extend the strong research environment within your department. Thank you for considering my application.

Sincerely yours,

Adam Rudner

Research Interests **Adam Rudner**

My research interests focus on aspects of chromosome dynamics. How are chromosomal structures assembled and organized? How does this organization impact gene expression during the cell cycle and during development? How are replicated chromosomes faithfully segregated during mitosis? I address these questions using the budding yeast, *Saccharomyces cerevisiae.* As a graduate student I studied the cell cycle regulation of chromosome segregation; as a postdoctoral fellow I have investigated how specialized chromosomal structures are assembled and regulated. I am fascinated by these fundamental questions because the life of a dividing cell hinges on its ability to package its hereditary material so that it can be expressed, replicated, recombined, and then ultimately segregated.

In my work I take full advantage of the complement of genetic and biochemical techniques available to study yeast physiology. Moreover, during my postdoctoral training I developed a novel method for the purification of protein complexes. This method has allowed me to isolate specific chromatin regions and to define the complete proteome of these domains. I am now poised to dissect the structure of different chromosomal regions, and to analyze how chromatin domains change during the cell cycle and in response to environmental stimuli, stress and development. In the future I will continue my analysis of chromosome structure and exploit this novel purification method to address other aspects of chromosome biology. I plan to develop a research program that weds the broad information learned from proteomic studies with classical cell biological and genetic approaches to discover how novel proteins function in chromosome dynamics.

Assembly and regulation of the SIR complex and heterochromatin

Chromosomes in organisms from yeast to humans contain large tracts of transcriptionally inactive DNA called heterochromatin or silent chromatin. These specialized chromatin domains play a central role in the structure and transmission of chromosomes, and in the regulation of cell identity and proliferation. Heterochromatic regions of DNA have several hallmark features: they are transcriptionally "silent;" they are refractory to recombination; they are inaccessible to proteins that bind and modify the underlying DNA; and they are epigenetically inherited. My postdoctoral research has focused on dissecting how heterochromatin is assembled and how this assembly is regulated.

Heterochromatin contains nucleosomes that are hypoacetylated and in budding yeast these nucleosomes are bound by a complex of three proteins, Sir2, Sir3 and Sir4 (Silent information regulator), named the SIR complex. Sir2 is the founding member of a conserved family of NAD-dependent protein deacetylases and creates the hypoacetylated domains of nucleosomes within heterochromatin. Sir3 and Sir4 are both histone binding proteins that favor binding to deacetylated histone H4, the preferred substrate of Sir2.

Assembly of heterochromatin in budding yeast initiates at telomeres or at specific DNA elements called silencers. These elements bind a group of DNA binding proteins that recruit the SIR complex which initiates the Sir2-dependent deacetylation of nucleosomes adjacent to silencers. The first SIR complex is thought to seed the recruitment and spreading of additional SIR complexes along chromatin, and this spreading is likely driven by the multimerization of Sir3, or the entire SIR complex.

Sir3 and Sir4 each can interact with deacetylated histones in vitro. An outstanding question in the field was whether these interactions are sufficient to assemble heterochromatin, or are Sir-Sir interactions also required. To answer this question I generated mutations in Sir4

that abrogate the binding of Sir4 to Sir3 in vivo. I discovered that when Sir3 cannot bind Sir4, heterochromatin does not assemble due to the inability of Sir3 to be recruited to silencers by Sir4. Moreover, my analysis revealed that the assembly of silent chromatin not only requires the deacetylation of nucleosomes adjacent to silencers, but also the recruitment of Sir3 by Sir4 (Rudner et al., MCB, 2005). In the future, I will define how the SIR complex transitions from recruitment to spreading. In particular, I will determine: 1) whether Sir3 binding to Sir4 is required for spreading, 2) how post-translational modifications (phosphorylation, ubiquitination, sumoylation, and ADP-ribosylation) of Sir proteins regulate the assembly and spreading of the SIR complex, and 3) whether localization of the SIR complex within heterochromatin is dynamic. A complete mechanistic understanding of the recruitment and spreading of the SIR complex will serve as a paradigm for how other chromatin domains assemble and spread.

Purification of the SIR complex

My work on the assembly of heterochromatin and studies by others have led to a model of how the Sir proteins assemble into the SIR complex and then multimerize and spread to form heterochromatin. It is not known, however, whether there are other core components of the SIR complex. In order to identify such components I developed strategies to purify the soluble SIR complex.

Previous purifications suggested that Sir3 binds to Sir4/Sir2 only on chromatin. However using different purification strategies I discovered that a large fraction of Sir4 and Sir2 are stably bound to Sir3 in solution. In addition, my analysis revealed that Asf2 (Anti silencing factor 2) is also a core component of the soluble SIR complex. Asf2 was identified previously as a gene that when overexpressed disrupted silencing, suggesting that it normally acts as an inhibitor of heterochromatin. My analysis is the first biochemical demonstration of a role for this protein in silencing. Consistent with the genetic observations, I have shown that overexpression of Asf2 disrupts the interaction between Sir3 and Sir4. Importantly, I demonstrated by chromatin immunoprecipitation that Asf2 also localizes to regions of heterochromatin, indicating that it may function to modulate the structure and function of silenced chromatin. Consistent with this idea, my recent characterization of Asf2 suggests that it regulates recombination of heterochromatic loci. This exciting finding suggests that repression of transcription and recombination by heterochromatin are separable activities. In the future I plan to further characterize this new core component of the SIR complex. I want to determine: 1) how Asf2 regulates recombination of these regions at heterochromatic loci, 2) how Asf2 alters the structure of the SIR complex, and 3) whether Asf2 has additional roles regulating heterochromatin.

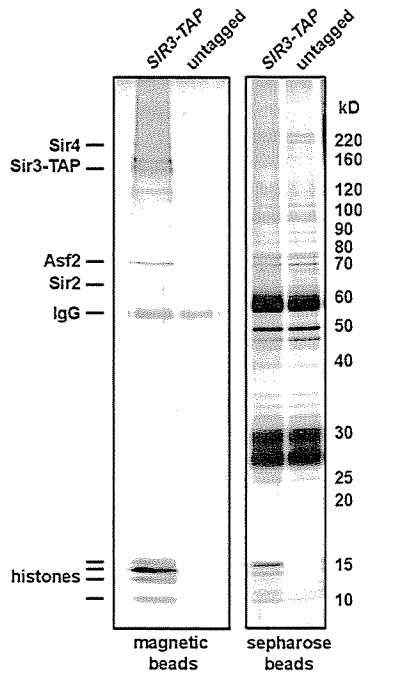
Purification of Heterochromatin

My identification of a new core component of the SIR complex suggested that other proteins involved in silencing may have been missed in previous analyses. Importantly, all previous affinity purifications focused on isolating soluble silencing complexes, rather than insoluble fragments of assembled heterochromatin. To address this, I developed a novel method for purifying chromatin that has allowed me to determine the complete proteome of heterochromatin. This method is a rapid one-step affinity purification using IgG-coupled magnetic beads. There are many advantages of this new protocol over previous methods: 1) The single step purification gives a 100-fold improvement in yield, 2) this improvement in yield allows less material to be used, and more conditions and strain backgrounds to be analyzed, 3) the speed of the single step preserves weak interactions that may be disrupted during longer

(two step) purifications, 4) because I use magnetic beads, the resin is not collected by centrifugation, avoiding contamination of the purified complexes with other insoluble complexes (a major problem with previous methods); and finally, 5) the magnetic beads have low non-specific protein binding. These last two points virtually eliminate *all* non-specific background proteins (see Figure).

Taking advantage of this powerful purification protocol, I have purified the chromatin-bound fraction of the SIR complex. In collaboration with Scott Gerber, a postdoctoral fellow in the laboratory of Steve Gygi, we have identified all the proteins that co-purify with chromatin-bound Sir3 by mixture mass spectrometry. Scott Gerber and the Gygi lab are leading experts in mass spectrometry. Using their two dimensional ion-trap Fourier transform ion cyclotron resonance (FTICR) mass spectrometer, we have identified 300 proteins with coverage of at least two peptides in my heterochromatin preparation. This number of chromatin-associated proteins far exceeded all expectations.

Among the 300 proteins we identified is the SIR complex, histones, and at least six of the recruitment proteins that are required to initiate the assembly of heterochromatin. Importantly, these recruitment proteins have never been found physically associated with Sir proteins in purified preparations. This result demonstrates that the stable interaction between these proteins and the SIR complex occurs only on chromatin, and validates our purification of intact fragments of heterochromatin. In addition to the proteins described above we also identified many proteins that have been found genetically to regulate silencing. Not one of these proteins had previously been shown to physically interact with heterochromatin. Moreover, we have identified a large number of other chromatin proteins that co-purify with heterochromatin. I am now purifying centromeric, telomeric and euchromatic chromatin domains to determine which of these proteins are unique to heterochromatin, and which are more general. Needless to say, this collaboration has been extremely fruitful and I will continue collaborating with Scott Gerber as he builds his own group at Dartmouth Medical School.



Purification of Heterochromatin TAP (tandem affinity purification) tagged Sir3 was purified from yeast using IgG-magnetic or IgG-sepharose beads in one-step. Material was eluted with sample buffer, run on a protein gel and silver stained. The material eluted from magnetic beads is clearly more abundant and of greater purity than that eluted from sepharose beads. The identities of the proteins that co-purify with Sir3-TAP has been determined by mixture mass spectrometry. In addition to the components of the SIR complex and histones, hundreds of chromatin proteins are present in these purifications.

The TAP tag contains a CBP (calmodulin binding protein) tag and two protein-A tags separated by a protease cleavage site. Conventional Sir3-TAP purifications involve sequential binding of Sir3-TAP to IgG- and calmodulin-sepharose, each followed with gentle elution (not sample buffer elution, as in this figure). Although these preparations are quite pure, the yield is 100-fold lower, and interacting proteins and chromatin fragments are lost during the two steps of purification (Hoppe et al, MCB, 2002; Rudner et al., MCB, 2005).

A major effort in my future lab will be to determine the role of the heterochromatin-specific proteins in the assembly and regulation of silent chromatin. I anticipate there will be both proteins important for general chromatin metabolism as well as proteins that play a specific role in the assembly and regulation of heterochromatin. Using genetic and biochemical methodologies similar to the ones that I used in my analysis of the SIR complex and Asf2, I will focus on identifying the functions of these proteins, and integrate their activities into our current

model of the assembly, spreading and inheritance of heterochromatin. As mentioned above, the ability to perform this purification on such a small scale will also allow me to systematically compare the composition of heterochromatin purified from different stages of the cell cycle as well as during changes in environmental stimuli, stress, and development.

Regulation of chromosome segregation

My research for the past four years has focused on the structure of heterochromatin. However, my broader interests encompass all aspects of chromosome dynamics, including how cells regulate chromosome segregation and mitosis. Below I describe two examples of how the tools I have developed during my postdoctoral work will allow me investigate outstanding questions in this field.

The spindle checkpoint halts mitotic progression if chromosomes are not properly attached via their kinetochores to the mitotic spindle. In the presence of unattached chromosomes, spindle checkpoint components, the Mad and Bub proteins, localize to kinetochores and generate a soluble signal that inhibits cell cycle progression. Although soluble complexes of spindle checkpoint proteins have been identified, the chromatin-associated checkpoint complex has not. Using the same methods I have used to purify heterochromatin, I plan to define the components of the chromatin-bound spindle checkpoint complex. Isolating this complex will provide insights into 1) how checkpoint proteins are recruited to the kinetochore, 2) how the complex changes during checkpoint activation, and 3) how the complex generates a soluble signal that halts mitotic progression.

The ultimate target of the spindle checkpoint is the anaphase promoting complex (APC), a multi-protein ubiquitin ligase that triggers the irreversible transition from metaphase to anaphase by catalyzing the degradation of the anaphase inhibitor, Pds1. The APC is a focal point for the regulation of chromosome segregation and I am interested in how its activity integrates information from the spindle checkpoint, the cell cycle, nutrient status and cell size in order to determine the timing of anaphase.

One way that the APC responds to cell physiology is by its phosphorylation. In my graduate work with Andrew Murray I showed that the protein kinase Cdk1 (Cdc28 in budding yeast) phosphorylates three subunits of the APC (Rudner and Murray, JCB, 2000). This phosphorylation is required for full mitotic APC activity and for timely initiation of anaphase onset. At the time I did this work it was unimaginable to map all the post-translational modifications on the APC (a complex composed of 13 proteins). As a test of the power of my new purification protocol, I purified the native APC and, in collaboration with Scott Gerber, identified 60 phosphorylation sites on 8 of its subunits! Importantly, we were able to carry out this analysis rapidly and from small quantities of material. Therefore, we are now poised to analyze a large collection of kinase and phosphatase mutants to identify all the regulators of the APC. In addition, using a heavy/light radioisotope labeling protocol, we will profile how the phosphorylation of these 60 sites changes during the cell cycle and in response to changing cell physiology.

In conclusion, I am excited to continue these proteomic studies and wed this approach to mechanistic studies using classical genetic and cell biological techniques. This two-pronged strategy will allow me to continue answering fundamental questions about chromosome dynamics.

Teaching Interests

One of the reasons I am drawn to academic research is the opportunity to work with students. I enjoy their energy and new perspective because it reminds me of how exciting it is to learn new things, and of the wonder of discovery.

My perspective on teaching has been shaped by experiences mentoring undergraduate and graduate students. During the course of graduate school and my postdoctoral training I have supervised four rotation student projects and mentored an undergraduate student during a summer internship. It was a pleasure to work with Phoebe Dann, the undergraduate student, and to teach her new skills so that by the end of the summer she had learned how to design and execute her own experiments. My experience mentoring her taught me that one of the greatest challenges as a teacher is deciding when to offer advice, and when to allow the student to learn on her own. Phoebe was a co-author on a paper published from our laboratory.

I look forward to teaching students in classes. My broad background will allow me to teach introductory courses in genetics, biochemistry and cell biology. My aim in any course would be to provide students with the skills that allow them to critically evaluate current research, design new experiments and rationally approach problem solving. Courses of this nature in college and graduate school shaped my interest in cell biology and my approach to problem solving. At the advanced level, I would like to teach seminars on how genetic and biochemical methods are used to understand chromosome structure, the regulation of mitosis, the cell cycle, and gene expression.

In my laboratory, or in laboratory courses, my principal goal will be to teach students the craftsmanship needed to perform experiments successfully, help them develop the critical thinking and creativity needed to design important and exciting experiments, and the problem solving skills to learn from failed experiments. I appreciate that these skills develop over many years, and although strong guidance may be needed for students early in their career, my eventual aim and delight will be for a student to design and execute their own independent experiments.

One of my greatest pleasures is sharing my wonder in the natural world with others. Whether I am explaining how specialized chromosome structures are assembled, my latest model that integrates various pieces of data, how to do a tetrad dissection, or how one of my beehives managed to survive the frigid New England winter, I strive to share my enthusiasm and excitement of discovery. I look forward to having teaching and mentoring as formal aspects of my job.