Nucleosomes, octamers of histones wrapped in 147 bp of DNA, are the basic unit of chromatin. In eukaryotic cells, the placement of nucleosomes along the genome is highly organized, and modulation of this ordered arrangement contributes to regulation of gene expression. The SWI/SNF complex utilizes the energy of ATP hydrolysis to mobilize nucleosomes and remodel chromatin structure. Recently, the complex has also been implicated in oncogenesis as genes encoding multiple SWI/SNF subunits have been found mutated at high frequency across a wide spectrum of cancers. Given that epigenetic aberrations are now characterized as a hallmark of human cancer, hypotheses have been put forth that the SWI/SNF complex inhibits tumor formation by regulating key chromatin functions.

To understand how the SWI/SNF complex contributes to nucleosome organization in vivo we performed a genome-wide study in mammalian cells. We found that inactivation of SWI/SNF subunits leads to disruptions of specific nucleosome patterning and a loss of nucleosome occupancy at a large number of promoters. These findings define a direct relationship between the SWI/SNF complex, chromatin structure and transcriptional regulation. In this extra view, we discuss our findings, their relevance to gene regulation and possible links to the tumor suppression activities of the SWI/SNF complex.

Introduction

Eukaryotic cells compact their genetic material into chromatin ultimately achieving orderly, and dynamic, packaging of nearly two meters of DNA into a nucleus only a few microns in diameter.1 At the initial level of chromatin formation, while nucleosomes are found broadly throughout the genome, their placement is not random.2 Rather, stereotypical patterns occur, particularly at gene promoters and at other regulatory loci.3 Establishment of nucleosomal occupancy at these regions is in part contributed to by DNA sequence.2,3 However, ATP remodelers also serve an important role in this process. For example, the addition of crude yeast extract to purified histones and genomic DNA results in the reconstitution of nucleosome arrays in vitro.4 However, the addition of ATP results in generation of array positions that are a closer match for those found in vivo. Such results suggest that ATP-consuming chromatin remodelers are involved in the establishment, and also dynamic regulation, of nucleosome arrays and that they can override preferred nucleosome positions dictated by the underlying DNA sequence.

The transcriptional start sites of most active genes in metazoans are located in a nucleosome-depleted region (NDR) flanked by two specifically-positioned nucleosomes known as the -1 and +1 nucleosomes.5 Downstream, this structure is followed by a spaced array of nucleosomes leading into the gene body. In the promoter region, cis-regulatory elements are highly concentrated and can be targeted by various transcriptional regulators for transcriptional activation and initiation. However, the embedding of

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The SWI/SNF complex has also emerged as a critical tumor suppressor. The first clue to a role for the complex in tumor suppression came when the SNF5 (SMARCB1/INI1/BAF47) core subunit was found to be specifically inactivated in nearly all cases of rhabdoid tumors (RT), highly aggressive cancers that strike young children.1-4 Genetically engineered mouse models then revealed a bona fide tumor suppressor role and induced biallelic inactivation of Snf5 was shown to lead to the rapid onset of cancer in all mice.5-7 More recently, via cancer genome sequencing studies, mutations in genes that encode SWI/SNF subunits have been broadly linked to cancer.8 Recurrent, typically inactivating, mutations in at least eight SWI/SNF subunit genes have been identified across a wide spectrum of human cancers. These include mutations in ARID1A (Baf250A), SMARCA4 (Brg1), ARID1B (Baf250B), ARID2 (Baf200), PBRM1 (Baf170), and other SWI/SNF subunit genes. In total, SWI/SNF subunit genes are mutated in 20% of all human cancers, a high rate comparable to that of TP53 (27%).8-11

The mechanism by which the SWI/SNF complex serves as a tumor suppressor has become an active area of interest. Both in yeast and mammals, the complex has been implicated in DNA repair processes including double-strand break repair, non-homologous end-joining, nucleotide excision repair and DNA decatenation.12-14 This raises the possibility that mutation of the complex gives rise to cancer via disruption of DNA repair processes leading to genetic damage and/or genomic instability. However, an epigenetic mechanism underlying the tumor suppressor role has also been proposed. Interestingly, sequencing analysis of 35 human SNPS-mutant RT samples recently revealed that the genomes of these aggressive SWI/SNF-mutant cancers are remarkably simple: the average exome contained less than five mutations. SNF5 was the sole identified locus that contained repeated mutations and in two cases was the only mutant gene identified.15 This finding suggests that chromosomal instability is dispensable for the genesis of at least some SWI/SNF mutant cancers. Given that the SWI/SNF complex is capable of modulating chromatin structure, and that epigenetic aberration is now recognized as a hallmark of human cancers,16-18 these observations have led to speculation that the SWI/SNF complex may suppress tumor formation more by epigenetic transcriptional regulation than by modulation of DNA repair.19-21

The SWI/SNF Complex Regulates Promoter Nucleosomes

In order to investigate the contributions of the SWI/SNF complex to chromatin structure and transcriptional regulation in vivo, we utilized murine embryonic fibroblasts (MEFs) conditional for one of two core SWI/SNF subunits, both tumor suppressors, Snf5 (Smarcb1) and Brg1 (Smarc4).19 This enabled us to genetically inactivate these subunits in normal cells and compare the effects to wild type MEFs. We then performed genome-wide mapping of nucleosomes in these engineered cells with the Mnas-Seq technology. As others and we have found that the SWI/SNF complex is highly enriched at promoters, we analyzed the effects of subunit inactivation upon nucleosomes surrounding transcription start sites (TSS) genome wide. We found that Snf5 and Brg1 are essential for establishment or maintenance of the canonical nucleosome structure and transcriptional regulation than by modulation of DNA repair.20-21
The +1 nucleosome is unique in that it is the most tightly positioned nucleosome, and is highly modified, and incorporated with histone variants. Functionally, the +1 nucleosome is believed to be a gateway for transcription: for example, it is implicated in the random fluctuation of gene expression, maximal pausing and the recruitment of chromatin remodelers and/or transcription factors to gene promoters. The position of the +1 nucleosome may also influence the positions of the nucleosomes downstream. Perhaps consistent with these special roles, the occupancy and positioning of the +1 nucleosome always seem to be the last to be affected by mutations that disrupts nucleosome assembly in gene bodies.

The effects on the +1 nucleosome are also dramatically lower as compared with the other nucleosomes. But in our results, SWI/SNF perturbation caused a severe loss of the +1 nucleosome density and this was consistently observed in both the Snf5 and Brg1 deficient MEFs. The extent of nucleosome loss at the +1 position was also similar to that of the other positions. More strikingly, when we analyzed sequencing data derived from human CD36 erythroid precursor cells where BRG1 was knocked down, we made a nearly identical observation. These findings collectively argue that the SWI/SNF complex is involved in establishing +1 nucleosome density in mammalian cells.

The yeast SWI/SNF complex binds the +1 nucleosome and pulls it away from the NDR. In MEFs, consistent with findings in other mammalian cell types, we found that the SWI/SNF complex was substantially enriched at the +1 nucleosome as indicated by our Brg1 ChIP-Seq data. But unlike in yeast, Snf5 or Brg1 loss did not affect the positioning of the residual +1 nucleosomes in MEFs. This result raises the possibility that the mammalian SWI/SNF complex functions distinctively from its yeast counterpart in that we found no effect upon positioning of the +1 nucleosome. However, in order to directly evaluate this, it would be necessary to confirm that the +1 nucleosomes retained in the Snf5 or Brg1 deficient MEFs represented those at SWI/SNF target genes and not just nucleosomes from non-bound genes. Owing to our limited sequencing depth achieved when sequencing nucleosomes genome-wide, we were unable to perform this analysis and thus evaluation of this possibility will rely on future experiments. It is important to note that DNA associated with unstable remodelled nucleosomes, and in certain cases histone variants, can be more sensitive to Mønase digestion and can result in regions containing such nucleosomes appearing to have no or low nucleosome occupancy during Mønase-Seq analysis.

In this regard, our study cannot rule out the possibility that loss of Snf5 or Brg1 results in unstable nucleosomes, rather than complete nucleosome loss, at promoters. In addition, we did not evaluate placement of variant histones, which can also have effects upon Mønase sensitivity, and thus may be an interesting area of future study.

The Contributions of the SWI/SNF Complex to Promoter Nucleosome Positioning

The yeast SWI/SNF complex binds the +1 nucleosome and pulls it away from the NDR. In MEFs, consistent with findings in other mammalian cell types, we found that the SWI/SNF complex was substantially enriched at the +1 nucleosome as indicated by our Brg1 ChIP-Seq data. But unlike in yeast, Snf5 or Brg1 loss did not affect the positioning of the residual +1 nucleosomes in MEFs. This result raises the possibility that the mammalian SWI/SNF complex functions distinctively from its yeast counterpart in that we speculate that several possible reasons may contribute to this phenomenon. First, nucleosome depletion in promoters is not strictly sufficient for transcription activation. For example, genes with very low levels of transcription frequency, e.g., with less than 1% of the maximum level can still have a well-configured NDR. Second, the loss of the nucleosomes, especially the +1 nucleosome, may represent the loss of modified histones, which serve important roles in marking transcriptional start sites and in recruiting transcriptional regulators to the promoter-proximal region. In this case, effects of removal of the +1 nucleosome barrier may be countervailed by impairment in recruitment of transcriptional activators and the transcription machinery. Third, the SWI/SNF complex may exert its effects on gene regulation by modulating gene enhancers, which are functionally as important as the promoters. For example, in embryonic stem cells, Brg1 binds to approximately 4% of the genome with its majority binding localized to distal regions that contain gene enhancers. Moreover, Brg1 binding correlates more with H3K4me3 than with H3K4me1, further indicating its enrichment at enhancers. Consequently, further analysis of the chromatin states at enhancers and their correlation with gene expression may provide additional insight in this regard.

Additional explanations are possible for the modest transcriptional changes identified. For example, optimal nucleosome occupancy and positioning conferred by chromatin remodelers have been shown to prohibit cryptic initiation and antisense transcription. Elevated cryptic initiation may lead to accumulation of dominant derivatives that can be detrimental to the cells, especially in the case of malignant transformation. In addition, the SWI/SNF ATPase subunit, BRM, has been shown to exert regulation on alternative splicing, which is again essential for normal cell function and impairment of splicing has been implicated in cancer development. Lastly, nucleosome occupancy in promoters controls transcriptional noise and plasticity. Here, transcriptional noise refers to the random fluctuation in the expression of a single gene allele in a cell population.
that is given under a constant condition. Transcriptional plasticity on the other hand refers to gene expression changes of the same gene under different conditions such as environmental stress, mutations or developmental transitions. As we evaluated cells in a uniform condition, our experiments would not detect such changes, although Hu and colleagues performed such an evaluation and identified changes in transcription factor binding at enhancers upon BRG1 mutation.48 Collectively, while our experiments implicated the SWI/SNF complex in establishment of nucleosome occupancy, much remains to be determined in how this chromatin remodeling and tumor suppressor complex regulates transcription.

Possible Links between SWI/SNF-Mediated Regulation on Promoter Nucleosomes and the Development of SWI/SNF Mutant Cancers

The disruption of the SWI/SNF complex by mutations is closely related to tumor formation. How would the regulation on promoter nucleosomes by the SWI/SNF complex fit into this process? Recent discoveries have indicated that mutations affecting epigenetic regulation may lead to a state of epigenetic instability, under which, cells would have more plasticity toward developing neoplastic heterogeneity.49 It is with this heterogeneity that cells could be selected for growth advantage and many other hallmarks of cancers, and then become tumorigenic.48 We thus postulate that the loss of nucleosome occupancy in gene promoters may promote epigenetic instability through mechanisms such as increased cryptic initiation, antisense transcription, aberrant RNA splicing and abnormal transcriptional noise and plasticity. In this model, the mammalian SWI/SNF complex resides in gene promoters and enhancers and modulates the deposition of nucleosomes. This regulation may facilitate transcription fidelity and protect the cells from undergoing epigenetic instability that can select for malignant transformation. Further evaluation of these ideas will have the potential to provide insights into the role of epigenetic dysregulation in oncogenesis.

Disclosure of Potential Conflicts of Interest
No potential conflict of interest was disclosed.

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