Cancer Genetics 207 (2014) 352–357

Cancer Genetics

REVIEW ARTICLE

Chromatin remodeling: from transcription to cancer

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In this short review article, I have tried to trace the path that led my laboratory from the early studies of the structure of papova minichromosomes and transcription control to the investigation of chromatin remodeling complexes of the SWI/SNF family. I discuss briefly the genetic and biochemical studies that lead to the discovery of the SWI/SNF complex in yeast and drosophila and summarize some of the studies on the developmental role of the murine complex. The discovery of the tumor suppressor function of the *SNF5/INI1/SMARCB1* gene in humans and the identification of frequent mutations in other subunits of this complex in different human tumors opened a fascinating field of research on this epigenetic regulator. The hope is to better understand tumor development and to develop novel treatments.

Keywords SWI/SNF, tumor suppressor, development, differentiation, cell cycle

The genetic material of our cells, the DNA, is packaged in the nucleus in a structure called chromatin that remained biochemically ill defined for a long time. Breakthroughs in the 1970s, by both biochemistry and electron microscopy, followed later by X-ray crystallography, unraveled the structure of the nucleosome, the basic unit of chromatin. An octamer composed of two molecules of each one of the four core histones, H3, H4, H2A, and H2B, is surrounded by one and three quarters turns of double-stranded DNA comprising 147 bp (1). The nucleosomes are aligned like beads on a string along the long DNA chain of chromosomes. This chain of nucleosomes is further packaged in higher order structures that are not fully established yet. One possible structure is a 30-nm solenoid or a helix composed by packaging of six nucleosomes per turn. The linker histone H1 contributes to the formation of these higher order structures. The chromatin has to be further compacted to fit our more than 2 m of the genome in a nucleus with a diameter of roughly 10 μm. Furthermore, the degree of compaction can vary among the active chromatin, the euchromatin, and the inactive heterochromatin.

The nucleosome is a very stable structure with strong electrostatic interactions between the negatively charged DNA and the basic histones. These interactions can be disrupted only by sodium chloride concentrations above one molar and are thus very stable in the nucleus. The packaging of the DNA double helix around the histone core in the nucleosome restricts the access of specific DNA sequences to recognition by proteins involved in DNA repair or recognition of transcription control elements. The same difficulty is faced by RNA or DNA polymerases that have to locally separate both strands of DNA. Progression of these enzymes along the nucleosome fibers is hampered by the strong contacts between histones and DNA.

Early on, we studied the structures of the SV40 and polyomavirus minichromosomes, which contain roughly 5,200 bp of circular DNA packed in 24 nucleosomes. We investigated whether the histones could slide easily along the DNA chain, that is, whether the nucleosome is a static or dynamic structure. Testing for the accessibility of restriction sites in the viral genome to restriction enzymes such as EcoR1, we found that an important fraction of minichromosomes are protected from digestion after prolonged incubation, strongly arguing that the core histones do not move rapidly along the DNA chain (2). On the other hand, we and others could show that the template for DNA replication and transcription is the viral chromatin (3). It became clear that we were missing an activity or activities that would facilitate DNA-dependent transactions such as replication, transcription or repair.

The discovery of the SWI/SNF chromatin remodeling complex

Hints that biological systems can regulate nucleosome mobilization came from two distinct areas of research.

Received March 10, 2014; accepted March 13, 2014.

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2210-7762/$ - see front matter © 2014 Elsevier Inc. All rights reserved.
http://dx.doi.org/10.1016/j.cancergen.2014.03.006

Genetic studies in the yeast *Saccharomyces cerevisiae* on one hand and *Drosophila melanogaster* on the other led to the discovery of genetic complexes that control the expression of a group of genes. In yeast, these were mutations that block sucrose fermentation, the *SNF* genes (4). Mutations in what became apparent as the same group of genes also blocked mating type switch, the *SWI* genes (5). These mutations were suppressed by mutations that reduced the number of histone genes, indicating a link with chromatin structure and its capacity to block transcription (6).

For drosophila, these were mutations in the trithorax group of genes that were selected as suppressors of polycomb homeotic mutations, and the polycomb proteins were thought to function at the level of chromatin (7). Cloning of the yeast *SW2/SNF2* gene revealed that it encoded for a protein with motifs related to RNA or DNA helicases with an ATP-binding motif and DNA-dependent ATPase activity (8). A highly homologous protein was encoded by the drosophila *Brm* gene (7).

To check whether such genes are conserved in higher eukaryotes, both the Crabtree laboratory and our group set up to search for the possible existence of human homologues of the yeast or drosophila helicase. Crabtree cloned a cDNA encoding *BRG1*, Brahma-related gene 1, also called *SMARCA4* (9), and we cloned a highly homologous protein, which we called human BRM or human Brahma, also called *SMARCA2* (10). *SMARC* stands for SWI/SNF-related, actin-containing regulators of chromatin. A second mammalian gene related to another member of the genetic *SNF* complex, a homologue of the yeast *SNF5* gene, was cloned as an HIV integrase−interacting protein and called *INI1* (11). The same cDNA was isolated by Claude Sardet in a two-hybrid screen and further characterized by us as the human homologue of the yeast *SNF5* gene, also called *SMARCB1* (12).

For BRM, it was shown that reexpression of the helicase in human cell lines lacking one or both of the helicases strongly increased transcriptional activation by the glucocorticoid hormone receptor (GR) (10). BRG1 could rescue SWI2/S*NF*2 mutants in yeast (9). These studies were reminiscent of the genetic data in yeast and drosophila, showing that the SWI/SNF complex is involved in transcription activation.

In parallel to these studies, several groups biochemically isolated protein complexes that facilitated ordered nucleosome assembly in vitro and nucleosome mobilization or disruption in the presence of ATP (13−16). Altogether, there are at least four families of chromatin remodeling complexes in yeast, drosophila, and mammalian cells defined by the nature of the helicase-related protein: SWI/SNF, ISWI, INO80/SWR1, and CHD. Each class contains several distinct complex subtypes with distinct biochemical activity and biological functions. Detailed description of these complexes is beyond the scope of this article and can be found in a recent review by Hargreaves and Crabtree (17).

As for the mammalian SWI/SNF complex, it is composed of 10 to 12 distinct subunits with composition partially different between different cell types (18) and a mass close to 2 MDa. The biological activity of the complex can be assayed with chromatin reconstituted in vitro. The complex can mobilize nucleosomes and facilitate the transcription of chromatinized templates. Similarly, by mobilizing the histones relative to the DNA sequences, the complex exposes restriction sites to cleavage by restriction endonucleases (19). How this is done is not yet fully understood. Electron microscopy images of a purified yeast RSC complex, which is highly homologous to the genuine SWI/SNF complex of the same organism, demonstrates that the complex is much bigger that the nucleosome itself, more than 1 Md versus 200 Kd, and that it has a cavity that can accommodate the nucleosome (20). It is believed that ATP hydrolysis is accompanied by transient disruption of DNA-histone contacts along 10 to 20 base pairs of the nucleosome, pulling out of such DNA segment and its translocation or repositioning relative to the histone octamer. Repeating such a process multiple times results in the sliding of the DNA sequences relative to the histone core (21−23).

The functions of the SWI/SNF complex in development and differentiation

Having isolated several of the genes encoding subunits of the SWI/SNF complex in the mouse, it was tempting to study in more detail their role in the development of higher organisms. Inactivation of the Brm helicase gave rise to viable mice that showed overgrowth and failure to arrest primary fibroblasts on confluency in culture (24). We observed increased compensatory expression of BRG1 in the absence of BRM. On the contrary, the BRG1 helicase was shown to be essential for early embryonic growth (25). S*NF*5/Ini1 is another subunit of what was defined as the core complex and is encoded by a single gene in mouse or man. Its inactivation resulted in very early embryonic lethality (26−28), identical to the phenotype observed with *Brg1-/-* embryos. In both cases, development was arrested around the time of implantation. Recovery of mutant blastocysts and their culture in vitro revealed that the cells in the inner cell mass do not multiply in the absence of one or the other of the core complex subunits and that they undergo apoptosis (25,26). The very early lethality of *Brg1-/-* mice was in agreement with the observations that Brg1 is expressed maternally and is the only SWI/SNF helicase present in very early development (29). SNF5/Ini1 is also expressed maternally, and its de novo transcription initiates very early, at the 2 to 4 cell stage of development (26). The fact that the phenotype of the *Brg1* and *SNF5/Ini1* knockout mice was identical strongly argues that both proteins function in the same biochemical complex, at least in early development. This difference in the behavior of the two alternative helicase subunits of the complex suggest that Brg1 is the basic helicase of the embryonic complex and that Brm takes over partially in a number of cell types during differentiation or growth arrest.

To investigate the function of the SWI/SNF complex at later stages of development, we and others used conditional inactivation of different genes encoding subunits of the complex in different cell types and at different stages of development. We have used a floxed *SNF5* allele and crossed these mice with a strain expressing the Cre recombinase in the hepatoblasts of the developing liver starting at day 10.5 to 11 of development. The mutant mice developed up to birth but died shortly after birth from acute liver failure.

The mice showed a major defect in maintaining normal plasma glucose concentrations caused by failure to accumulate glycogen and to synthesize glucose. Decreased