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Chapter 3

SWI/SNF Chromatin Remodeling Complex Involved in RNA Polymerase II Elongation Process in *Drosophila melanogaster*

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Additional information is available at the end of the chapter

http://dx.doi.org/10.5772/55877

1. Introduction

After more than a decade of studying the chromatin remodeling, better view of the function mechanisms of the chromatin remodeling complexes has been developed. It was found that chromatin remodeling complexes facilitate transcription of genes by reducing the nucleosome density on specific genomic regions, such as enhancers and promoters, and increasing their affinity to activators and activator-binding complexes. Moreover, the importance of the chromatin remodeling complexes for transcriptional repression has been shown recently [1, 2]. Therefore chromatin remodeling complexes appear to be involved in nearly all aspects of transcription regulation [3].

At present, the SWI/SNF chromatin remodeling complex is considered to be a significant player in the process of RNA Polymerase II transcription initiation. Recruitment of the complex precedes other transcriptional events and is important for the binding of the general transcriptional machinery [4]. The interplay between chromatin remodeling and general transcriptional factors is so close, that these complexes may unite into physically stable formations termed supercomplexes [5]. An example of such cooperation has been demonstrated for the *Drosophila* SWI/SNF (dSWI/SNF) and TFIID complexes with the SAYP coactivator as a linchpin unit [6].

Recently, abundant evidence concerning SWI/SNF participation in the process of RNA Polymerase II elongation has been reported. It has been demonstrated that the SWI/SNF complex does not leave the promoter after general transcriptional factors recruitment but is involved in transcription elongation and co-transcriptional events. In addition SWI/SNF direct

influence on alternative splicing has been revealed in several studies. Using the human *CD44* gene as a model it has been shown that SWI/SNF decreases the elongation rate of RNA Polymerase II and facilitates the alternative exons incorporation. Moreover, biochemical interaction of human SWI/SNF (hSWI/SNF) complex with splicing machinery including both protein factor Sam68 and snRNAs of sliceosome has been demonstrated [7]. Later, a protein complex containing $p54^{nrb}$ and PSF factors of mRNA splicing and one of the hSWI/SNF ATPase subunit hBrg1 has been biochemically purified. The influence of the hBrg1 subunit knockdown on the alternative exons incorporation in the *TERT* gene transcripts has been demonstrated. It has been shown that hBrg1 knockdown leads to growth arrest and senescence of the human cells [8].

Further the several evidences concerning SWI/SNF complex role in mRNP processing in insects have been published. The knockdown of core *Drosophila melanogaster* SWI/SNF (dSWI/SNF) subunits has been shown to facilitate the alternative splicing of several *Drosophila* genes both in culturing cells and in the larvae [9]. Recently, physical association of the SWI/SNF complex with pre-mRNP of *Chironomus tentans* was demonstrated both biochemically and by immune-electron microscopy [10]. Thus participation of SWI/SNF complex in RNA Polymerase II elongation coupled events is not the distinctive feature of mammals but the evolutionary conserved phenomenon.

The participation in other important steps of the RNA Polymerase II elongation process has been described for the SWI/SNF complex in addition to its significance for the alternative pre-mRNP splicing. The accumulation of the SWI/SNF complex in the coding region of the genes during active transcription has been demonstrated for the yeast. Yeast genes, tested in that study, do not have introns. So, the presence of the SWI/SNF complex inside the coding region of yeast genes could not be explained by its interaction with splicing machinery. ySWI/SNF complex is rather important for the RNA Polymerase II elongation process. It has been shown, that the swi2Δ mutant (the ATPase subunit of the yeast SWI/SNF) possesses heightened sensitivity to the drugs that inhibit RNA Polymerase II elongation [11].

Similar data has been obtained for the human *hsp70* gene which, like the yeast genes, contains no introns at all. The SWI/SNF but not ISWI complex (another type of chromatin remodeling complex) binding to the coding region of the mouse *hsp70* during active transcription has been shown. Like the yeast counterpart, the homologous SWI/SNF complex of the human has demonstrated sensitivity to the drug (α-amanitin) which suppresses RNA Polymerase II elongation. Amanitin treatment led to a dramatic decrease in the level of SWI/SNF binding to the coding region of the *hsp70* gene during active transcription. It has been shown that point mutations of the HSF1 factor, which disrupts transcription elongation but not initiation of *hsp70* transcription, also causes a dramatic decrease in the SWI/SNF complex binding to the gene [12].

Participation of the SWI/SNF complex in RNA Polymerase II elongation process on the intronless genes like yeast genes and human *hsp70* indicates, that its function during transcription is not limited to splicing events. Moreover, importance of the SWI/SNF for the RNA Polymerase II elongation process itself has been demonstrated.

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Furthermore, the evidence concerning SWI/SNF complex participation in RNA Polymerase II transition from the initiation to the productive elongation state has been described in the last two years. *Drosophila melanogaster* developmental *ftz-f1* gene was used as a model gene to demonstrate the role of dSWI/SNF in RNA Polymerase II pausing process. It has been demonstrated that dSWI/SNF complex participates in the organization of the repressed gene state via the pausing of the RNA Polymerase II. Moreover dSWI/SNF has been revealed to be important for the transient pausing of RNA Polymerase II during active transcription. So, the significance of the dSWI/SNF for the proper elongation and Ser2 CTD phosphorylation marker loading has been demonstrated for the same gene during the active transcription state [13]. Furthermore, the influence of the SWI/SNF complex on the RNA Polymerase II transition to the elongation state has been reported for the human. It has been shown that human SWI/SNF stimulates the occasional transcriptional elongation of the HIV-1 provirus in the absence of the Tat activator thus disrupting the early termination of the short viral transcripts [14]. There are a number of studies that indicate association of SWI/SNF with the process of RNA Polymerase II elongation but the exact function of the complex during the process remains to be seen.

The first and simplest model of SWI/SNF function during elongation that comes to mind is that SWI/SNF assists RNA Polymerase II in overcoming the nucleosome barriers during elongation. This idea complies with the general view on SWI/SNF functions but is not in a good correlation with the results of the splicing studies. According to that studies the SWI/SNF complex slows down RNA Polymerase II elongation rate rather than stimulates it. This conclusion has been made by investigators on the base of the mutation and knockdown experiments where the incorporation rate of longer exons during transcription processing decreased upon SWI/SNF complex disruption. One more evidence could be concluded from these splicing studies: the RNA Polymerase II complex does not require the SWI/SNF complex for the productive elongation on the intron-containing genes. The SWI/SNF complex knock-down performed in the experiments impaired splicing of the genes transcripts but had no effect on the total transcription level [7]. On the other hand, it has been demonstrated recently that RNA Polymerase II complex alone could overcome the nucleosome barrier, suggesting that there is no urgent need for the special remodeling enzymes [15]. Thus, SWI/SNF functions during transcription elongation are not completely clear and still need to be investigated.

The *Drosophila melanogaster* (d) dSWI/SNF chromatin remodeling complex is comprised of the two types of the subcomplexes PBAP and BAP (in mammals, PBAF and BAF respectively). These subcomplexes share several common subunits and Brahma ATPase, but also contain several specific subunits: OSA in the BAP complex and Polybromo, Bap170 and SAYP in PBAP [16][17]. These subcomplexes control the expression of different, but partially overlapping gene patterns and are involved in different functions of the dSWI/SNF complex. For example, BAP but not the PBAP subcomplex is important for proper cell cycle progression [18]. However one question still remains uninvestigated: are there differences in the molecular mechanisms of the subcomplexes functioning, e.g., in the way they remodel histones or in the specific actions they perform.

The main idea of this work is to clarify the next issue: which of the two dSWI/SNF subcomplexes is involved in the new function of the dSWI/SNF complex and accompanies RNA Polymer-

ase II during transcription elongation. For that goal we have generated and characterized antibodies against the BAP170 and OSA subunits of the PBAP and BAP subcomplexes of the dSWI/SNF complex respectively. Using them we have investigated the changes in distribution of the subcomplexes along the *ftz-f1* and *hsp70* genes upon activation of transcription.

2. Results

2.1. Polyclonal antibodies against PBAP and BAP subcomplexes specific subunits generation and purification

Plasmids, containing two different fragments of BAP170 protein tagged with a 6xHis, for expression in prokaryotic system were generously provided by P. Verrijzer and Y. Moshkin (Erasmus University Medical Center, Rotterdam, The Netherlands)[16]. Expressed antigens were purified on Ni-NTA agarose. The quality of the purified antigens was examined by PAGE with subsequent Coomassie blue staining (Figure1A). Specific antibodies against BAP170 protein were raised in rabbits by series of immunization with both of the antigens. Antibodies were affinity purified from the obtained sera by the column with the antigens immobilized. The quality of the generated antibody was analyzed by Western blot (Figure1B). *Drosophila melanogaster* embryonic nuclear extract (from 0-12h embryo) were loaded on the Western blot for the analysis. Thus the affinity purified antibodies are effective against Bap170.

Antibodies against OSA specific subunit of BAP subcomplex were generated by the same scheme as BAP170 antibodies was. Sequence coding 108-330 aa of OSA were subcloned into pET system for the antigen expression in *E.coli*. Purified OSA antigen was used for the immunization (Figure1C). After series of immunization sera was affinity purified by OSA antigen immobilized on the sepharose column. The sera and purified antibodies were analyzed on Western blot with *Drosophila* embryonic nuclear extract loaded (Figure1D).

2.2. Antibodies against PBAP and BAP-specific subunits precipitate the common subunit but do not precipitate each other

The SWI/SNF chromatin remodeling complex of *Drosophila* is comprised of two different subclasses of remodeling complexes: PBAP and BAP. These subcomplexes reveal different targeting through the *Drosophila* genome and possess different functions. But the main divergence between the subcomplexes is their ability to form protein complexes, which could be separated biochemically. The specific subunits of the subcomplexes interact with the common subunits of dSWI/SNF (like BRM, MOR, BAP111, SNR1 etc.) but fail to precipitate the specific subunits of another subcomplex.

To confirm the specificity of antibodies generated against specific subunits of dSWI/SNF complex the immunoprecipitation experiment was performed (Figure2). The subcomplexes of the dSWI/SNF complex were precipitated from the crude lysate of the S2 Schneider cells by the generated antibodies against BAP170 and OSA subunits. S2 Schneider cells are the most widely used cells for the investigation of *Drosophila* proteins [19]. Both generated antibodies successfully

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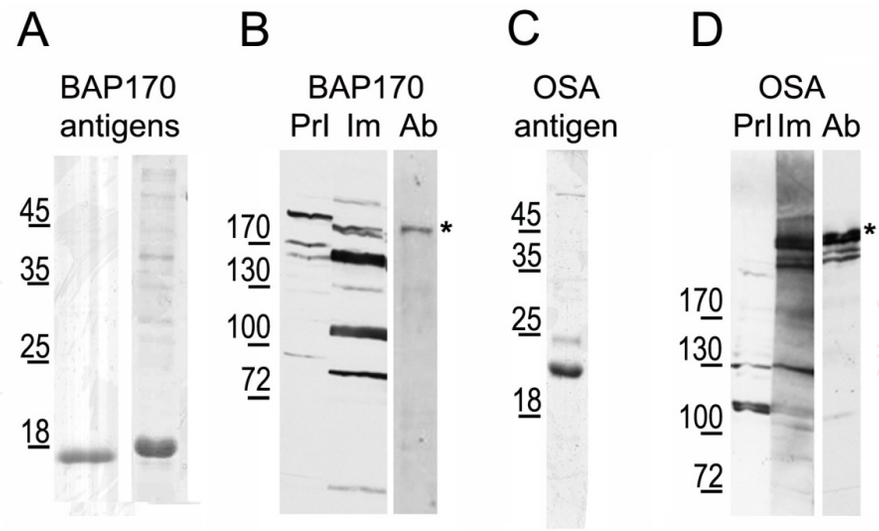

Figure 1. Polyclonal antibodies against PBAP and BAP subcomplexes specific subunits generation and purification A) The antigens, used for the antibodies against BAP170 subunit generation, were purified and loaded on the PAGE (Coomassie blue staining) B) The *Drosophila* embryonic nuclear extract was loaded on the Western blot and stained with the serum of rabbit immunized with BAP170 antigens (Im), the serum before immunization (PrI) and affinity purified antibodies against BAP170 protein. The BAP170 protein recognized with the antibodies is marked with asterisk. C) The antigen, used for the antibodies against OSA subunit generation, was purified and loaded on the PAGE (Coomassie blue staining) D) The Drosophila embryonic nuclear extract was loaded on the Western blot and stained with the serum of rabbit immunized with OSA antigen (Im), the serum before immunization (PrI) and affinity purified antibodies against OSA protein. The OSA protein recognized with the antibodies is marked with asterisk.

precipitated the corresponding proteins and completely depleted them from the cell lysate. Both specific subunits of the PBAP and BAP subcomplexes (BAP170 and OSA correspondingly) successfully co-precipitated the common MOR subunit of dSWI/SNF but failed to precipitate the specific subunits of other subcomplex. Therefore the generated antibodies against BAP170 and OSA could specifically precipitate the corresponding subcomplex.

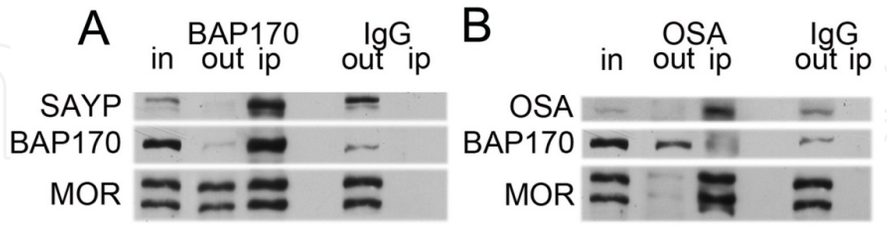

Figure 2. The immunoprecipitation of the dSWI/SNF protein complex from the S2 Schneider cells lysate by antibodies against BAP170 and OSA subunits (in – input; out – output; ip – immunoprecipitation) A) The immunoprecipitation of the dSWI/SNF complex with the anti-BAP170 antibodies. The Western blot was stained with the antibodies against SAYP, BAP170 and MOR subunits of the dSWI/SNF complex. As a negative control for the immunoprecipitation serum of non-immunized rabbits (IgG fraction) was taken. B) The immunoprecipitation of the dSWI/SNF complex with the anti-OSA antibodies. The Western blot was stained with the antibodies against OSA, BAP170 and MOR subunits of the dSWI/SNF complex. As a negative control for the immunoprecipitation serum of non-immunized rabbits (IgG fraction) was taken.

2.3. Both PBAP and BAP subcomplexes of dSWI/SNF chromatin remodeling complex are detected on the promoter of the ftz-f1 gene

The generated antibodies were tested in the chromatin immunoprecipitation experiment on S2 Schneider cells. According to our previous studies, the promoter of the *ftz-f1* ecdysone cascade gene with a high affinity binds the PBAP subcomplex of the dSWI/SNF [13]. It was demonstrated for the PB- and SAYP-specific subunits of PBAP subcomplex. Therefore, BAP170 is expected to bind the promoter of the *ftz-f1* gene. There are no published results about the BAP subcomplex binding to the target genes. But there are some data which represents the PBAP and BAP subcomplexes overlapping targeting across the *Drosophila* genome. These data were obtained from the experiments with the polythene chromosome staining [18]. So, we expected both the BAP and PBAP subcomplexes binding to the *ftz-f1* gene promoter.

Two types of negative controls were used in chromatin immunoprecipitation experiment: PrA resin without any antibody bound (to demonstrate that there is no non-specific binding of *ftz-f1* promoter) and secondly, primers for the 28S rDNA region amplification for analysis the specificity of binding to *ftz-f1* promoter region but not throughout the genome. The locus of rDNA was chosen as a negative control because in our previous studies it did not bind the common subunits of the dSWI/SNF complex [13]. The results of chromatin immunoprecipitation are shown on Figure 3. As expected, both generated antibodies against the PBAP and BAP subcomplexes successfully precipitated the promoter region of *ftz-f1* gene ("0" primer pair in the description) while showed no affinity to the 28S rDNA locus.

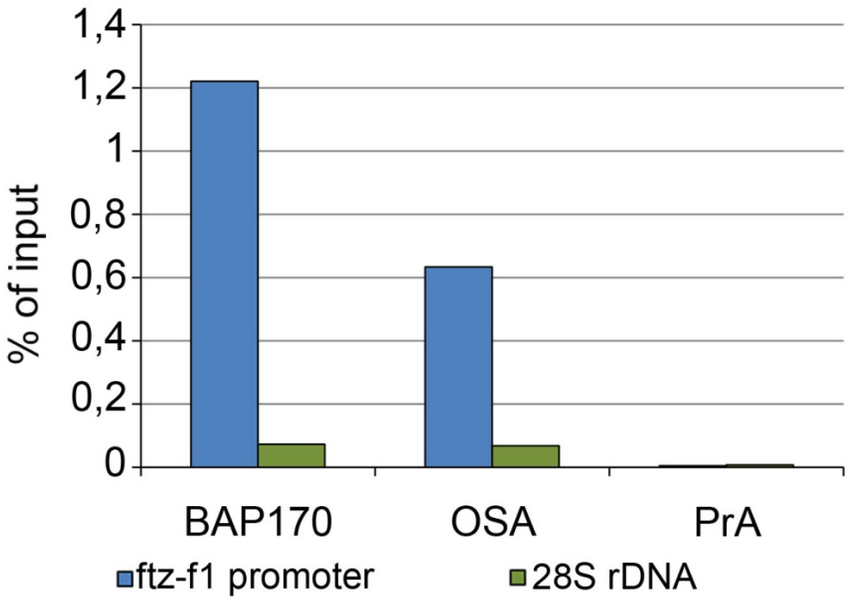

Figure 3. The immunoprecipitation of the chromatin (ChIP) from the S2 Schneider cells with antibodies against BAP170 and OSA subunits of dSWI/SNF complex. The blue bar represents the RT-PCR analysis of the ChIP experiment with the primers to the *ftz-f1* promoter region ("0" point in the description). The green bar represents the analysis with the primers to the 28S rDNA locus (negative control). The results of the chromatin immunoprecipitation experiment were calculated as a % of precipitated chromatin relative to input fraction.

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Therefore we have proved the fact of simultaneous PBAP and BAP recruitment on the promoter region of the same gene. The *ftz-f1* gene is an example of the target gene for both of the subcomplexes.

2.4. PBAP but not BAP subcomplex of the dSWI/SNF complex is accumulated at the coding region of the ftz-f1 gene after transcription activation

In our previous studies we have described in details the scheme which makes possible to activate the *Drosophila* developmental *ftz-f1* gene in S2 Schneider cells [13]. A simultaneous recruitment of the BAP170 and OSA subunits to the promoter of this gene in a non-activated stage (in normal S2 Schneider cells) was demonstrated above. To evaluate which one of the dSWI/SNF subcomplexes assists RNA Polymerase II in elongation process we have studied the re-distribution of the subunits on the *ftz-f1* gene upon transcription activation.

Earlier, we have described the multistep scheme of the *ftz-f1* gene activation by the addition and subsequent withdrawal of the 20 hydroxyecdysone (below, referred to simply as ecdysone) hormone into the S2 Schneider cells culturing medium [13]. The main steps of the induction system are schematically presented in the Figure 4. The ecdysone is the main regulator of the ecdysone cascade and its addition to the cell medium induces the expression of the DHR3 nuclear receptor, the activator protein for the *ftz-f1* gene. In spite of DHR3 activator recruitment on the *ftz-f1* promoter soon after DHR3 protein expression, the activation of the *ftz-f1* transcription does not start until the level of the ecdysone in the medium decreases close to basal level. Previously, we have shown that at the high ecdysone concentration, the DHR3 receptor settles on the promoter region of the *ftz-f1* gene and stimulates the formation of the PIC complex by increasing the level of TFIID and dSWI/SNF complexes and as a consequence RNA Polymerase II binding to the promoter. But transcription of the *ftz-f1* gene at this stage does not start because recruited RNA Polymerase II is not in fully active state. It bears the Ser5 phosphorylation marker on its CTD domain but lacks the Ser2 phosphorylation marker which is an illustrator of a RNA Polymerase II elongation-competent state. So, the *ftz-f1* gene in our scheme of activation has preliminary activation state with pre-recruited activator and with RNA Polymerase II poised for the transcription elongation. This phenomenon is called RNA Polymerase II pausing. The ecdysone withdrawal from the culturing medium causes removing of the repressive signal and disturbs the RNA Polymerase II complex pausing state. That leads to the productive transcription elongation and synthesis of the *ftz-f1* gene full-length transcripts.

Two steps, required to verify the *ftz-f1* activation scheme were performed: the 1 μM ecdysone addition (overnight) and subsequent triple washing with the fresh Schneider medium. The level of the *ftz-f1* gene transcription was measured in all activation stages: in the ecdysone-free medium (–), after overnight cultivation in the ecdysone-containing medium (+), and finally 4 hours after ecdysone withdrawal from the culturing medium (+;–). As expected, the *ftz-f1* was not transcribed during (–) and (+) stages and significantly activated 4 hours after the ecdysone removal in (+;–) stage (see Figure 5).

Next, the distribution of the BAP170 and OSA subunits of the dSWI/SNF complex along the *ftz-f1* gene on different stages of activation was analyzed by the chromatin immunoprecipitation technique (see Figure 6A and B). Both of the proteins were readily bound to the promoter

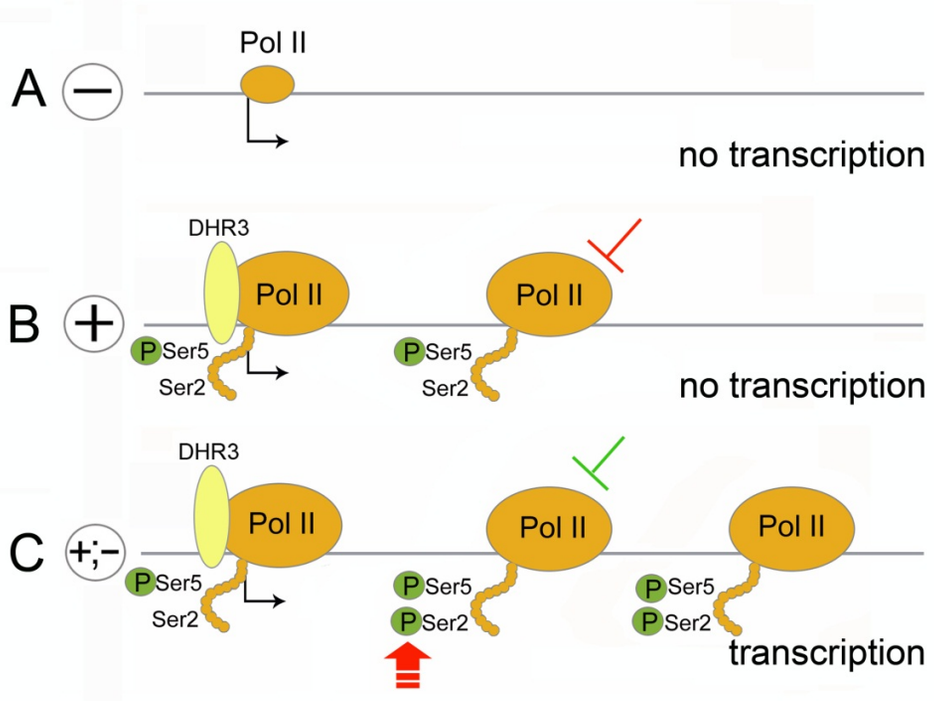

Figure 4. The scheme representation of the *Drosophila* developmental *ftz-f1* gene transcription induction by the ecdysone hormone in S2 Schneider cells. A) The *ftz-f1* gene is not expressed in the normal S2 Schneider cells. The promoter of the gene is not bound with the DHR3 activator and contains few RNA Polymerase II. The (-) mark on the scheme represents low ecdysone titer in the medium of the culturing cells. B) After ecdysone addition (represented with the (+) mark) the DHR3 activator is recruited on the *ftz-f1* promoter region. The DHR3 binding to the promoter stimulates RNA Polymerase II loading. Transcription of the *ftz-f1* gene is initiated but the RNA Polymerase II complex pauses close to the promoter. The RNA Polymerase II at (+) stage is not in the state competent for the elongation and is not phosphorylated on Ser2 of the CTD domain. The *ftz-f1* gene is not transcribed at (+) stage. C) After ecdysone withdrawal from the culturing medium the block on the RNA Polymerase II elongation is disposed. At the point of transient pausing (it completely coincides with the region of pausing at (+) stage) the RNA Polymerase II is phosphorylated on Ser2 of the CTD domain and continues the moving into the coding region of the gene. A few hours after ecdysone titer decreasing the *ftz-f1* gene is actively transcribed.

region of the studied gene with a high affinity in all stages of activation. The level of the promoter binding of the BAP170 protein increased twice in (+;-) stage after the *ftz-f1* transcription activation. The amount of OSA subunit bound to the *ftz-f1* promoter was increased in (+) stage during the stage of RNA Polymerase II recruitment and was not changed significantly after the withdrawal of the transcriptional block. Thus both of the dSWI/SNF subcomplexes bind the promoter region of the *ftz-f1* gene in all stages of activation.

The patterns of the PBAP and BAP subcomplexes distribution in the coding region of the *ftz-f1* gene during active transcription differ distinctly. The significant increase in the BAP170 subunit binding at the coding region of the *ftz-f1* gene during (+;-) stage of active transcription was detected. At that stage the binding level of the BAP170 subunit in the coding region exceeded by a factor of several times the negative control region of the 28S rDNA, while in all other stages was close to the background. At the same time, the binding level of the OSA subunit of the BAP subcomplex was close to the background throughout the coding region of the *ftz-f1* gene during all transcriptional stages.

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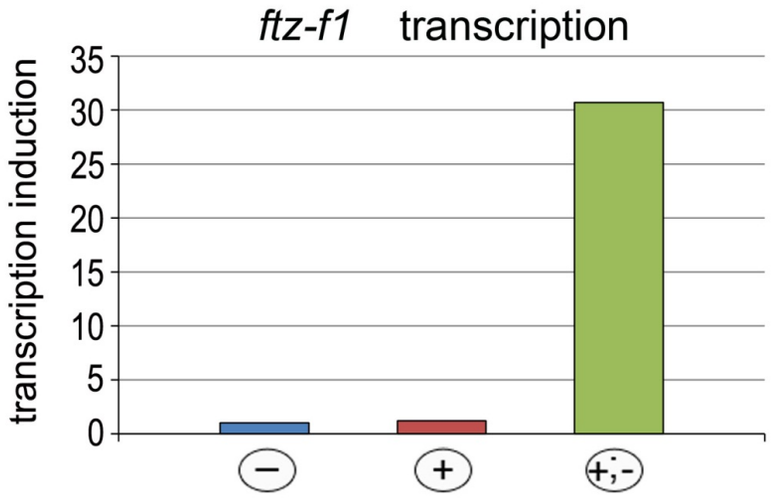

Figure 5. The *ftz-f1* gene transcription induction in the S2 Schneider cells with the ecdysone hormone addition ("+" state) and sequential removing ("+;-" state). The cells of the all states were collected for the analysis at the same time. The ecdysone was added overnight to the cells of (+) and (+;-) states and in (+;-) state it was removed from the cells medium 4 hours before analysis. The Y – axis represents the level of transcription induction relative to non-induced conditions.

Thus the accumulation of the BAP170-specific subunit of the PBAP subcomplex in the coding region of the *ftz-f1* gene during the active stage of the transcription was demonstrated. Thereby the participation of the PBAP but not the BAP subcomplex of the dSWI/SNF chromatin remodeling complex in the elongation process of the RNA Polymerase II was shown.

2.5. PBAP subcomplex of the dSWI/SNF complex is accumulated at the coding region of the hsp70 gene after transcription activation

To prove the wideness of the observation and non-specificity of the finding to the model of gene activation the recruitment of the PBAP subcomplex on the coding region of the active gene was studied in another system (on the model of *hsp70* gene).

The *hsp70* gene model system is widely used in transcriptional machinery studies. The model uses *Drosophila* cells treatment with heat shock conditions (37 °C for 20 min) for induction while the normal temperature for culturing is 25-28 °C [20]. In those stress conditions the transcriptional system of the *Drosophila* cell is drastically changed. Almost all of the genes stop being transcribed while several genes (called heat shock genes) exhibit very fast and high level of transcriptional activation. It should be taken into account that in such stress and non-physiological conditions the transcriptional machinery could not function by the same mechanisms as in the normal cell. But nevertheless the *hsp70* gene represents the system with the highest induction level which was ever being described for the *Drosophila*. Therefore these genes are a good model for the investigation of the subtle changes on the coding region of the gene during transcription activation.

To verify the induction scheme the S2 Schneider cells were exposed to the heat shock conditions (37°C) and the *hsp70* gene transcription was measured before and after the heat shock. The

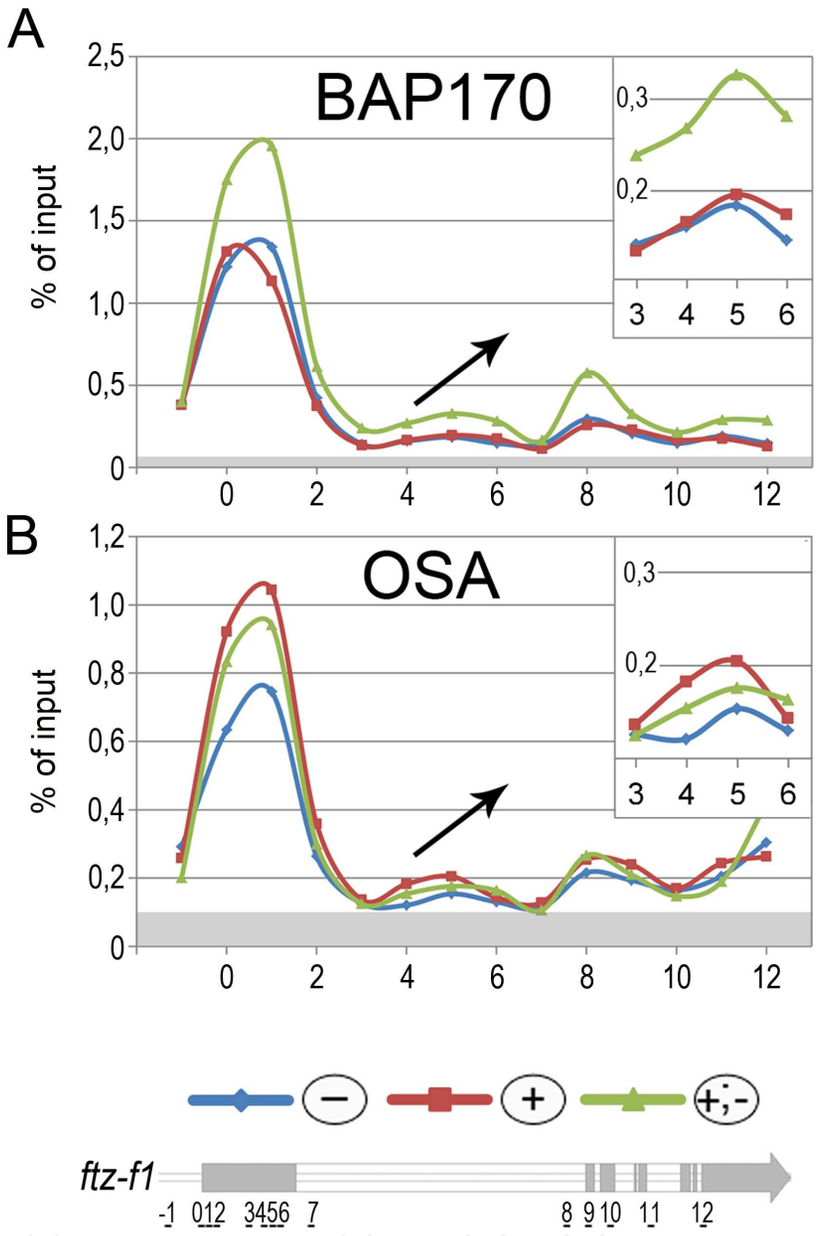

Figure 6. The BAP170 (A) and OSA (B) subunits distribution along the *ftz-f1* gene at different stages of transcription activation. The analysis was performed by chromatin immunoprecipitation technique on the S2 Schneider cells which was growing in the ecdysone-free medium (–), after overnight cultivation in the ecdysone-containing medium (+), and finally 4 hours after ecdysone withdrawal from the culturing medium (+;–). The positions of primer pairs which were used for the RT-PCR analysis of the *ftz-f1* gene are shown on the scheme of the gene. The precipitation level of the negative control (28S rDNA region) is shown on the graphs as a grey line. The results of the chromatin immunoprecipitation experiment were calculated as a % of precipitated chromatin relative to input fraction.

fifty fold increase in *hsp70* gene transcription was detected after the heat shock. The *hsp70* transcription induction was measured by Real-Time PCR using primer "7" from the description list.

To prove the involvement of the PBAP subcomplex in the process of RNA Polymerase II elongation the BAP170 subunit distribution was analyzed along the *hsp70* gene before and after

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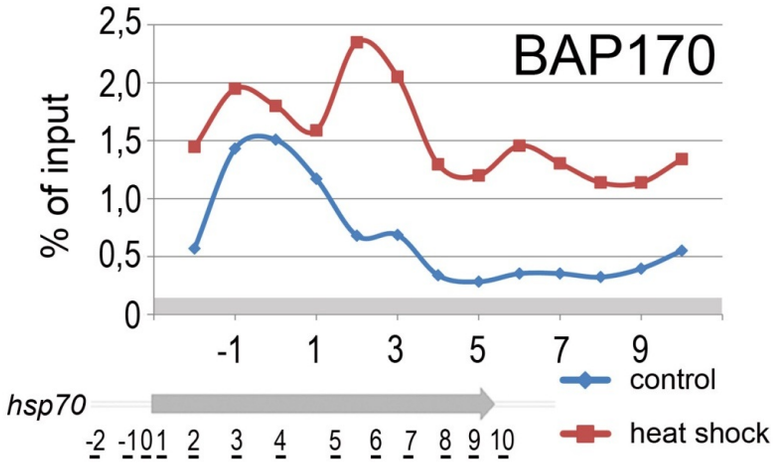

Figure 7. The BAP170 subunit distribution along the *hsp70* gene at heat shock (red) and non-heat shock (blue) conditions. The analysis was performed by chromatin immunoprecipitation technique on the S2 Schneider cells treated with the heat shock conditions (37°C) and harvested at room temperature (presented as "heat shock" and "control" line on the graph). The positions of primer pairs which were used for the RT-PCR analysis of the *hsp70* are shown on the scheme of the gene. The results of the chromatin immunoprecipitation experiment were calculated as a % of precipitated chromatin relative to input fraction.

heat shock (Figure 7). The BAP170 subunit of the PBAP subcomplex binding to the promoter region was detected both in the repressed and active state of the gene. But several times increase in the level of the BAP170 subunit binding inside the *hsp70* coding region was observed after transcription activation.

Thus the accumulation of the PBAP subcomplex inside the coding region upon transcription activation was demonstrated not only for the *ftz-f1,* but also for the *hsp70* gene.

3. Conclusions

Several pieces of evidence concerning SWI/SNF participation in the elongation process of RNA Polymerase II have emerged during the last few years [21]. These data describe SWI/SNF complex participation both in elongation process of RNA Polymerase II and in transcription elongation coupled events like pre-mRNP splicing [22]. There have been a few studies to date but there can be no doubt that the SWI/SNF complex travels with the RNA Polymerase II along the gene during active transcription. This research area is only starting to be investigated and attracts much attention because the participation in transcription elongation represents a novel function of the SWI/SNF complex.

The properties of the SWI/SNF complex is under extensive study because subunits of the complex are indispensable for the living organism [23][24]. The ability to possess all types of nucleosome remodeling activities distinguishes it from other chromatin remodeling complexes [24]. The recent studies concerning the significance of SWI/SNF complex for the cell reprogramming and association of the SWI/SNF subunits mutations with cancer susceptibility have made this complex interesting for a wide circle of investigators [25][26].

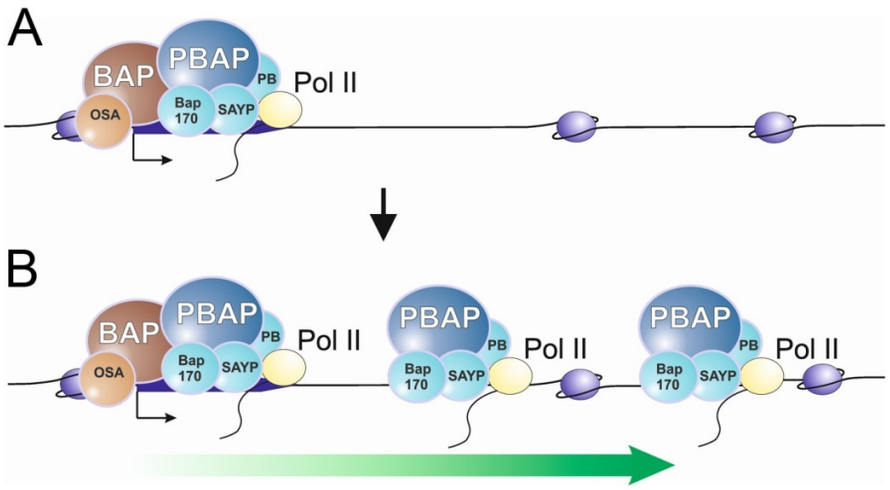

Figure 8. The descriptive model of the PBAP and BAP subcomplexes of the dSWI/SNF chromatin remodeling complex re-distribution along the gene before and after transcription activation. Both of the subcomplexes are bound to the promoter region before transcription induction (A). At the active transcription state the PBAP subcomplex of dSWI/SNF complex is detected on the coding region of the gene (B). The PBAP subcomplex of the dSWI/SNF assists the RNA Polymerase II in the process of transcription elongation.

It has been known for a few years that the SWI/SNF complex is comprised of PBAP and BAP types of subcomplexes (in mammals, PBAF and BAF respectively) [16]. The subcomplexes have partially overlapping but mostly distinct targeting throughout the genome [27]. These subcomplexes possess different functions. Thus, the BAP but not the PBAP complex is working in the cell cycle regulating pathway [18]. For the BAF250/ARID1-specific subunit of the BAP subcomplex an activity of the E3 ligase and ability to ubiquitinylate histone H2B has been demonstrated [28]. The capabilities of these subcomplexes to possess different functions obviously lie in their specific subunits.

The main idea of the current study was to investigate which one of the subcomplexes participates in the new functions of SWI/SNF during transcription elongation. The model describing results of this study is presented in Figure 8 A and B.

The drosophila developmental *ftz-f1* gene was chosen as a model for the investigation. The *ftz-f1* gene transcription induction was performed by developmental ecdysone hormone (so conditions were close to natural). This system of induction has advantages both in terms of the physiological non-stress conditions of gene activation and the simplicity of performing chromatin immunoprecipitation experiments on the culturing cells. The accumulation of the PBAP but not BAP subcomplex inside the coding region upon transcription activation was observed by chromatin immunoprecipitation with antibodies against specific subunits of the subcomplexes. Thus, for the *Drosophila melanogaster* it was demonstrated that the PBAP subcomplex of dSWI/SNF is not only important for the transcriptional initiation events but also assists the RNA Polymerase II in transcription elongation.

The discovered effect was confirmed in another inducible gene system. The *hsp70* gene is induced by subjecting the cell to the stress heat shock conditions and the transcription level after induction is characterized by the extremely high rate. In the inducible system of the *hsp70*

gene the significant accumulation of the PBAP subcomplex inside the coding region upon transcription activation was observed. Thus PBAP subcomplex participation in RNA Polymerase II elongation is not restricted to the solitary gene, but is realized at least on two inducible genes of *Drosophila melanogaster*.

The specification of the SWI/SNF subcomplex participation in the functions during the RNA Polymerase II elongation process will make easier further investigations of these functions.

The presence of the SWI/SNF complex inside the coding region of the intron-less genes testifies to the existence of some other functions during elongation in addition to the participation in the splicing process. The nature of these functions is not fully understood yet. But the functional link of the SWI/SNF complex with the process of RNA Polymerase II elongation definitely exists. It was shown for the yeast that mutants of the SWI/SNF subunit display heightened sensitivity to the drugs, inhibitors of transcription elongation [11]. The participation of the PBAP subcomplex in regulation of the RNA Polymerase II elongation rate has been described for the *Drosophila* using the *ftz-f1* gene as a model system. It was demonstrated that knockdown of the PBAP subcomplex subunit causes decrease in the level of the elongated RNA Polymerase II on the coding region, but at the same time it does not reduce the level of the promoter-bound RNA Polymerase II complex. Moreover, the PBAP subunit knockdown leads to a considerable decrease in level of the RNA Polymerase II CTD Ser2 phosphorylation state but does not change the Ser5 phosphorylation. The participation of the SWI/SNF complex in the process of CTD Ser2 phosphorylation could be one of the chromatin remodeling complex functions during elongation. This marker of active transcription is loaded close to the promoter area and increases towards the 3' end of the gene. The new kinase which is responsible for the Ser2 CTD phosphorylation RNA Polymerase II elongation through the coding region of the gene has been described recently [29]. The participation of SWI/SNF in this process could explain the observed accumulation of the complex inside the coding region of the gene upon transcription activation.

Experimental procedures

Drosophila embryonic nuclear extract purification

The Method of the *Drosophila* embryonic nuclear extract purification was described earlier in [30].

Experiments with S2 Schneider cell

The protocol of the *Drosophila* Schneider line 2 (S2) cells cultivation and *ftz-f1* gene induction was in the details described in [13]. For the *hsp70* gene transcription induction cells were incubated at the 37°C in water bath for the 20 min and briefly cooled to the RT. To extract proteins for IP, S2 Schneider cells were lysed in 10 mM Hepes (pH 7.9) buffer containing 5 mM MgCl2, 0.5% Nonidet P-40, 0.45 M NaCl, 1 mM DTT, and complete protease inhibitor mixture (Roche). IP was performed as described earlier [31].

Chromatin immunoprecipitation

For one ChIP experiment $3x10^6$ of S2 Schneider cells were taken. Crosslinking was made by 15 min incubation with 1,5% formaldehyde and was stopped by addition of 1/20 volume of the

2,5M glycine. Cells were triple washed with cold (4°C) PBS and resuspended in SDS-containing buffer (50 mM HEPES-KOH pH 7.9, 140 mM NaCl, 1 mM EDTA, 1% Triton X-100, 0,1% deoxycholate Na, 0,1% SDS, Protease inhibitors cocktail (Roche)). Chromatin was sheared to DNA size of appr. 700 b.p. and centrifuged (16 rcf, 20 min). For the one chromatin immunoprecipitation were taken: 10 μg of antibodies, 15 μl of PrA sepharose (Sigma), ssDNA and BSA up to 1 mg/ml. The precipitated chromatin was sequentially washed by buffers: SDS-containing buffer, SDS-containing buffer with 0,5M NaCl, LiCl-containing buffer (20mM Tris-HCl pH 8,0, 1mM EDTA, 250 mM LiCl, 0,5% NP40, 0,5% Deoxycholate Na) and TE buffer (20mM Tris-HCl pH 8,0, 1mM EDTA). Precipitated complexes were eluted by incubation in buffer (50mM Tris-HCl pH 8,0, 1mM EDTA, 1% SDS) at RT. Eluted chromatin was de-crosslinked for the 16 h at 65°C (16 μl of 5M NaCl was added) and treated with the 3 units of proteinase K for 4 h at 55°C (5 μl of 0,5 M EDTA was added to each sample). DNA was purified with the phenol/chloroform extraction and precipitated with the isopropanol. The precipitate was dissolved in TE buffer and subjected to the Real-Time PCR (RT PCR) analysis. The result of the chromatin immunoprecipitation experiment was calculated as a rate of precipitated fraction relative to the input chromatin fraction (presented as a percent). Each point was measured in at least five experiments and the mean value was calculated.

RNA purification and cDNA synthesis

The RNA purifications were performed as described in [13].

Primers for qPCR

ftz-f1 gene fragments:

Region	Forward primer	Reverse primer
–1	ACAAAAAACTGCTGAAGAAGAGACC	ACTGTGGGTATGGCATTATGAAAG
0	GAGGCAGAGGCAGCGACG	GCTTTGTCATCTATGTGTGTGTTGTTG
1	AGTCAATCGAGATACGTGGTTGATG	GTAACGCTTTGTCATCTATGTGTGT
2	GTTCTCTTGCTGCGTTGCG	GAAAGTGGGTCACGAATTTATTGC
3	ACCGCAACCTATTTTACTACC	TTAGAAGACCGAAGAGTTATCC
4	ACAACAACAATAACAACGACAATGATGC	CTGATTGCCGCTGCCACTCC
5	CAGCAGCAACAGCAACAGAATATC	GCGAGTGTGAGGAGGTGGTG
6	CTCCTCACACTCGCAACAGAGC	AGCAGCATGTAGCCACCGC
7	CTCCGTAAGAGTCAGCTTTAAC	CAGGGACATCACACATACG
8	CAACGCTTCACAGAAACAAACG	GTTGTACAAAGCGGCGTATGC
9	GTTCGAGCGGATAGAATGCGT	GATATGCTTGCTGGTAGCCCG
10	GAGGAGGAGGTGGCAATAATGC	GATCCTATTCCAGCCTCGTGG
11	TTCAATGCACATTCTGCCG	GCAGCAACATGGTTCAAAGC
12	AACATCTTACCGGAAATCCATGC	ATCTCCATGAGCAGCGTTTGG

Table 1. Primers for the amplification of the *ftz-f1* gene fragments.

hsp70 gene fragments:

Region	Forward primer	Reverse primer
-2	GCAACTAAATTCTAATACACTTCTC	TGCTGCGTTTCTAAAGATTAAAG
-1	GTGACAGAGTGAGAGAGCATTAGTG	ATTGTGGTAGGTCATTTGTTTGGC
0	TTGAATTGAATTGTCGCTCCGTAG	ACATACTGCTCTCGTTGGTTCG
1	GCAGTTGATTTACTTGGTTG	AACAAGCAAAGTGAACACG
2	ATGAGGCGTTCCGAGTCTGTG	CTACTCCTGCGTGGGTGTCTAC
3	CGCTGAGAGTCGTTGAAGTAAG	GTGCTGACCAAGATGAAGGAG
4	GCTGTTCTGAGGCGTCGTAGG	TTGGGCGGCGAGGACTTTG
5	CCTCCAGCGGTCTCAATTCCC	GACGAGGCAGTGGCATACGG
6	GGGTGTGCCCCAGATAGAAG	TGTCGTTCTTGATCGTGATGTTC
7	CTTCTCGGCGGTGGTGTTG	GTAAAGCAGTCCGTGGAGCAG
8	AGCTAAAATCAATTTGTTGCTAACTT	AGGTCGACTAAAGCCAAATAGA
9	GCTGTTTAATAGGGATGCCAAC	TATTGTCAGGGAGTGAGTTTGC
10	GTTGTTGAACTCCGTAACCATTCTG	GCCCCGCTAAGTGAGTCCTG

Table 2. Primers for the amplification of the *hsp70* gene fragments.

28S rDNA:

Forward primer	Reverse primer
AGAGCACTGGGCAGAAATCACATTG	AATTCAGAACTGGCACGGACTTGG

Table 3. Primers for the amplification of the 28S rDNA locus fragment.

Acknowledgements

We thanks S.G. Georgieva for her critical reading of the manuscript; P. Verrijzer and Y. Moshkin (Erasmus University Medical Center, Rotterdam) for the constructs with sequences coding BAP170 antigens and for the antibodies against MOR (described in [16]). This work was supported by the program "Molecular and Cell Biology" of the Russian Academy of Sciences, N.V. were supported by the RF Presidential Program in Support of Young Scientists, MK-5961.2012.4 and MD-4874.2011.4. N.V. acknowledges a fellowship from Dmitry Zimin Dynasty Foundation.

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References

[1] Rendina, R, Strangi, A, Avallone, B, & Giordano, E. Bap170, a subunit of the Drosophila PBAP chromatin remodeling complex, negatively regulates the EGFR signaling.," *Genetics*, Sep. (2010). , 186(1), 167-181.

[2] Baig, J, Chanut, F, Kornberg, T. B, & Klebes, A. The chromatin-remodeling protein Osa interacts with CyclinE in Drosophila eye imaginal discs.," *Genetics*, Mar. (2010). , 184(3), 731-744.

[3] Wu, J, Lessard, J, & Crabtree, G. Understanding the Words of Chromatin Regulation," *Cell*, (2009). , 136(2), 200-206.

[4] Métivier, R, Penot, G, Hübner, M. R, Reid, G, Brand, H, Kos, M, & Gannon, F. Estrogen receptor-alpha directs ordered, cyclical, and combinatorial recruitment of cofactors on a natural target promoter.," *Cell*, Dec. (2003). , 115(6), 751-763.

[5] Nakamura, T, Mori, T, Tada, S, Krajewski, W, Rozovskaia, T, Wassell, R, Dubois, G, Mazo, A, Croce, C. M, & Canaani, E. ALL-1 is a histone methyltransferase that assembles a supercomplex of proteins involved in transcriptional regulation.," *Molecular Cell*, Nov. (2002). , 10(5), 1119-1128.

[6] Vorobyeva, N. E, Soshnikova, N. V, Nikolenko, J. V, Kuzmina, J. L, Nabirochkina, E. N, Georgieva, S. G, & Shidlovskii, Y. V. Transcription coactivator SAYP combines chromatin remodeler Brahma and transcription initiation factor TFIID into a single supercomplex.," *Proceedings of the National Academy of Sciences of the United States of America*, Jul. (2009). , 106(27), 11049-11054.

[7] Batsché, E, Yaniv, M, & Muchardt, C. The human SWI/SNF subunit Brm is a regulator of alternative splicing.," *Nature Structural & Molecular Biology*, Jan. (2006). , 13(1), 22-29.

[8] Ito, T, Watanabe, H, Yamamichi, N, Kondo, S, Tando, T, Haraguchi, T, Mizutani, T, Sakurai, K, Fujita, S, Izumi, T, Isobe, T, & Iba, H. Brm transactivates the telomerase

reverse transcriptase (TERT) gene and modulates the splicing patterns of its transcripts in concert with p54(nrb).," *The Biochemical Journal*, Apr. (2008). , 411(1), 201-209.

[9] Waldholm, J, Wang, Z, Brodin, D, Tyagi, A, Yu, S, Theopold, U, Farrants, A. K. Ö, & Visa, N. SWI/SNF regulates the alternative processing of a specific subset of pre-mRNAs in Drosophila melanogaster.," *BMC Molecular Biology*, Jan. (2011). , 12(46), 1-12.

[10] Tyagi, A, Ryme, J, Brodin, D, & Ostlund, A. K. Farrants, and N. Visa, "SWI/SNF associates with nascent pre-mRNPs and regulates alternative pre-mRNA processing.," *PLoS Genetics*, May (2009). , 5(5), e1000470.

[11] Schwabish, M, & Struhl, K. The Swi/Snf complex is important for histone eviction during transcriptional activation and RNA polymerase II elongation in vivo.," *Molecular and Cellular Biology*, Oct. (2007). , 27(20), 6987-6995.

[12] Corey, L. L, Weirich, C. S, Benjamin, I. J, & Kingston, R. E. Localized recruitment of a chromatin-remodeling activity by an activator in vivo drives transcriptional elongation, *Genes and Development*, (2003). , 17, 1392-1401.

[13] Vorobyeva, N. E, Nikolenko, J. V, Nabirochkina, E. N, Krasnov, A. N, Shidlovskii, Y. V, & Georgieva, S. G. SAYP and Brahma are important for 'repressive' and 'transient' Pol II pausing.," *Nucleic Acids Research*, May (2012). , 40(15), 7319-7331.

[14] Mizutani, T, Ishizaka, A, Tomizawa, M, Okazaki, T, Yamamichi, N, Kawana-tachikawa, A, Iwamoto, A, & Iba, H. Loss of the Brm-type SWI/SNF chromatin remodeling complex is a strong barrier to the Tat-independent transcriptional elongation of human immunodeficiency virus type 1 transcripts.," *Journal of Virology*, Nov. (2009). , 83(22), 11569-11580.

[15] Kulaeva, O. I, Hsieh, F. -K, & Studitsky, V. M. RNA polymerase complexes cooperate to relieve the nucleosomal barrier and evict histones.," *Proceedings of the National Academy of Sciences of the United States of America*, Jun. (2010). , 107(25), 11325-11330.

[16] Mohrmann, L, Langenberg, K, Krijgsveld, J, Kal, A. J, Heck, A. J. R, & Verrijzer, C. P. Differential Targeting of Two Distinct SWI / SNF-Related Drosophila Chromatin-Remodeling Complexes," *Molecular and Cellular Biology*, (2004). , 24(8), 3077-3088.

[17] Vorobyeva, N. E, Soshnikova, N. V, Kuzmina, J. L, Kopantseva, M. R, Nikolenko, J. V, Nabirochkina, E. N, Georgieva, S. G, & Shidlovskii, Y. V. The novel regulator of metazoan development SAYP organizes a nuclear coactivator supercomplex.," *Cell Cycle*, Jul. (2009). , 8(14), 2152-2156.

[18] Moshkin, Y. M, Mohrmann, L, Van Ijcken, W. F. J, & Verrijzer, C. P. Functional differentiation of SWI/SNF remodelers in transcription and cell cycle control.," *Molecular and cellular biology*, Jan. (2007). , 27(2), 651-661.

[19] Schneider, I. Cell lines derived from late embryonic stages of Drosophila melanogaster," *J Embryol Exp Morphol*, Apr. (1972). , 27(2), 353-365.

[20] Brien, T, & Lis, J. T. RNA Polymerase II pauses at the 5′ end of the transcriptionally Induced Drosophila hsp70 Gene," *Molecular and Cellular Biology*, (1991). , 11(10), 5285-5290.

[21] Subtil-rodríguez, A, & Reyes, J. C. To cross or not to cross the nucleosome, that is the elongation question…," *RNA Biology*, May (2011). , 8(3), 389-393.

[22] Kornblihtt, A. R. Chromatin, transcript elongation and alternative splicing.," *Nature Structural & Molecular Biology*, Jan. (2006). , 13(1), 5-7.

[23] Shidlovskii, Y. V, Krasnov, A. N, Nikolenko, J. V, Lebedeva, L. a, Kopantseva, M, Ermolaeva, M. a, Ilyin, Y. V, Nabirochkina, E. N, Georgiev, P. G, & Georgieva, S. G. A novel multidomain transcription coactivator SAYP can also repress transcription in heterochromatin.," *The EMBO Journal*, Jan. (2005). , 24(1), 97-107.

[24] Hargreaves, D. C, & Crabtree, G. R. ATP-dependent chromatin remodeling: genetics, genomics and mechanisms.," *Cell Research*, Mar. (2011). , 21(3), 396-420.

[25] He, L, Liu, H, & Tang, L. SWI/SNF chromatin remodeling complex: a new cofactor in reprogramming.," *Stem Cell Reviews*, Mar. (2012). , 8(1), 128-136.

[26] Brownlee, P. M, Chambers, A. L, Oliver, A. W, & Downs, J. a. Cancer and the bromodomains of BAF180.," *Biochemical Society Transactions*, Apr. (2012). , 40(2), 364-369.

[27] Mohrmann, L, & Verrijzer, C. P. Composition and functional specificity of SWI2/SNF2 class chromatin remodeling complexes.," *Biochimica et Biophysica Acta*, Jan. (2005). , 1681(2-3), 59-73.

[28] Li, X. S, Trojer, P, Matsumura, T, Treisman, J. E, & Tanese, N. Mammalian SWI/SNF--a subunit BAF250/ARID1 is an E3 ubiquitin ligase that targets histone H2B.," *Molecular and Cellular Biology*, Apr. (2010). , 30(7), 1673-1688.

[29] Bartkowiak, B, Liu, P, Phatnani, H. P, Fuda, N. J, Cooper, J. J, Price, D. H, Adelman, K, Lis, J. T, & Greenleaf, A. L. CDK12 is a transcription elongation- associated CTD kinase, the metazoan ortholog of yeast Ctk1," *Genes and Development*, (2007). , 24, 2303-2316.

[30] Blank, T. A, Sandaltzopoulos, R, & Becker, P. B. Biochemical analysis of chromatin structure and function using Drosophila embryo extracts.," *Methods*, May (1997). , 12(1), 28-35.

[31] Georgieva, S, Kirschner, D. B, Jagla, T, Nabirochkina, E, Hanke, S, Schenkel, H, De Lorenzo, C, Sinha, P, Jagla, K, Mechler, B, & Tora, L. Two novel Drosophila TAF(II)s have homology with human TAF(II)30 and are differentially regulated during development.," *Molecular and Cellular Biology*, Mar. (2000). , 20(5), 1639-1648.