

Subunit Composition of the Human Mediator Complex in Neuronal Cell Lines

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Abstract

The human mediator complex is a 26 to 30 subunit complex that exists in various forms in cells. This multi subunit complex regulates all protein-coding genes and the expression of such genes. Though the mediator complex plays an important role in transcription, not much is known about it. In the quest to discover the structural composition, function and mechanism through which the mediator complex operates, most scientists and researchers have done extensive analysis of the mediator complex in He La cell lines and not so much in alternate cell lines. Analyzing two neuronal cell lines, LN-18, a malignant human glioma cell line, and SK-N-AS, a human neuroblastoma cell line, we hypothesize that the subunit composition of the mediator complex in these two neuronal cell lines might be different from that of HeLa cells due to the high specialization of the central nervous system. Employing the use of immunoprecipitation, gel electrophoresis, silver staining and ultimately mass spectrometry, we expect to observe “neuron specific” mediator complex. This research will open the door for further research on neuronal cell lines.

Introduction

The mediator complex plays an important role in transcription and regulation of synthesis of mRNA by interacting with RNA polymerase II enzyme [1]. The mediator complex was first discovered in *Saccharomyces cerevisiae* [2]. Studies have shown that the mediator complex in yeast comprises 20 subunits [2]. Further subunit composition analysis of *C. elegans*, *Drosophila* and mammalian complexes show that there has been limited evolutionary conservation of both structure and function of the mediator complex from yeast to man (Figure 3)[3, 4]. The mediator complex, in addition to other general transcription factors such as TFIIE and TFIIH are absent in microbial genomes and specific to eukaryotic organisms [1]. The functions of the mediator complex in transcription are numerous. Extensive data have shown that the mediator complex works cooperatively with most general transcription factors (GTF) to regulate the expression of protein-coding genes [1]. Although the mediator complex is unable to bind directly to specific DNA sequences like GTFs and RNA pol II, it is able to bind directly to RNA polymerase II and help in its recruitment [1,3]. The mediator complex is also a co-activator as it is a target for DNA-binding transcription factors [1,4]. Furthermore, studies have shown that this protein complex can stimulate basal transcription [1].

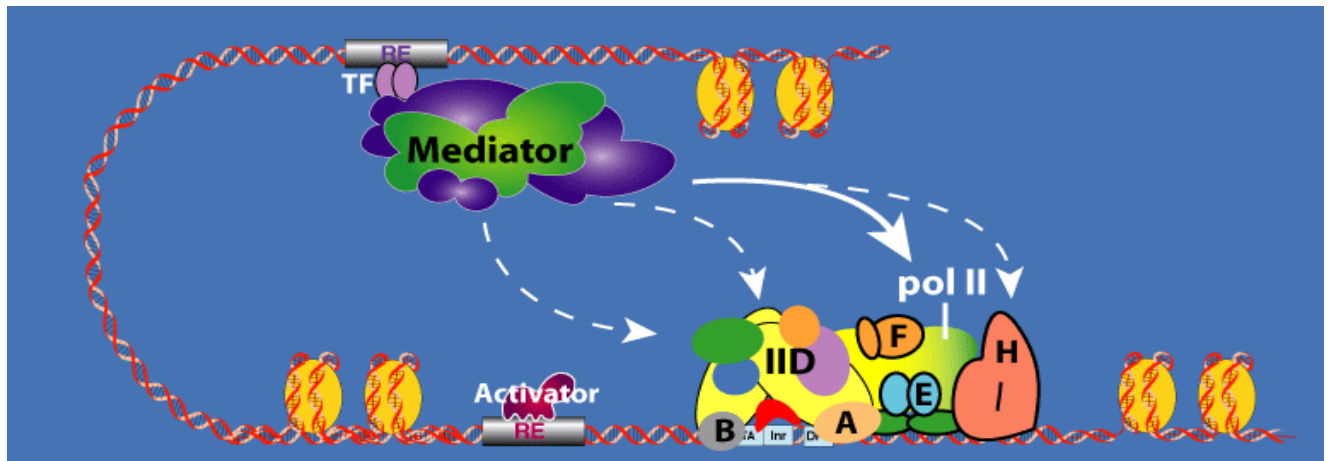


Fig 1: The interaction of the mediator complex with other transcription factors (TFIID, Pol II, TFIIF, co-activators of mediator complex) and the processes that take place to enable transcription [Image obtained from Dylan Taatjes' lab website].

Subunits of the mediator complex

Approximately 30 subunits of the mediator complex have been identified through the analysis of mediator-like complexes in rats, human and mouse cell line [3]. Of the 30 subunits, only 8 subunits were found to be orthologs of the yeast mediator at first. More sophisticated bioinformatics analysis countered the notion that mediator-like complexes might only be distantly related in structure and function to the yeast [3,5]. These analyses have shown similarities between mammalian mediator-like complexes and all but three yeast mediator subunits (MED2, MED3, and MED5) [3]. Through purification and analysis of mammalian mediator-like complexes, subunits of the complexes have been found to be present in large complexes (1-2MDa) and smaller complexes (500-700kDa) [3]. The large complexes have been found to include some kinase module subunits MED12, MED13, cyclin C and cyclin-dependent kinase (CDK8). The smaller complexes on the other hand, have been shown to lack kinase module subunits [3]. Several experiments have been conducted

in determining the subunit composition of the mediator complex. The most recent and advance ones have been done using MudPIT (multidimensional protein identification technology). This mass spectrometry-based method has been used to analyze the subunit composition of mediator isolated from HeLa or HEK 293 cell line [3]. In addition to this, variant forms of kinase module subunits as described above were found during the analysis of the mediator complex. This has suggested to scientists that there might be cell-specific mediator complexes.

Mediator: a 1.2 MDa, 26-subunit complex

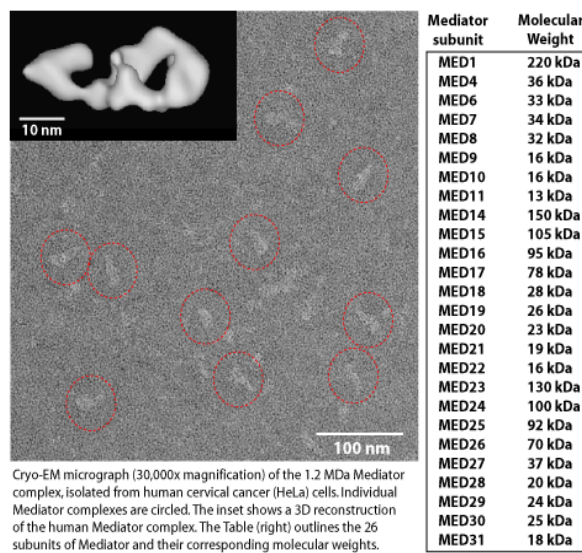


Figure 2: subunit composition of the Mediator complex isolated from human cervical cancer (HeLa cells).

Mediator has evolved most rapidly relative to other general transcription factors

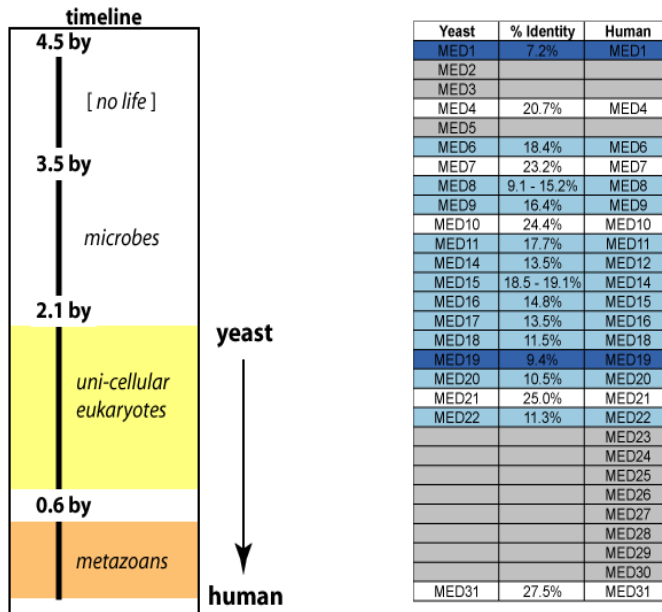


Figure 3: shows the evolution of subunits of mediator complex from yeast to human. Evolutionary conservation of the mediator complex is shown as a percentage of identity in structure in various subunits of the mediator complex.

Evidence of cell-specific mediator complexes

Evidence has shown that the mediator complex exists in various biochemically distinct forms [1]. Findings in several studies have suggested that there are changes to the mediator complex in terminally differentiated myotubes [6]. Also, recent experiments have found that mediator experience changes during mouse liver development [7]. In adult hepatocyte, there is a depletion of certain mediator subunits at both RNA and protein level. The changes observed in general transcription factors in specific cell types have provided a basis for the hypothesis for this study. Looking at two neuronal cell lines, LN-18 and SK-N-AS, we hypothesize that there might be a “cell-specific” mediator complex associated to these cell lines.

Results

1. Generation of neuronal cell nuclear extracts

LN-18 and SK-N-AS were grown at 37°C under 5% atmospheric CO₂. Cells were grown to confluency and then split into a 1:3 ratio. Since the human mediator complex is of low abundance in most cells, approximately 160 15cm² plates were harvested for each cell line. Harvesting involved the manual scraping of the cells off the culture dish. From the ~160 plates for each cell line, only 4.6ml of nuclei were isolated for each cell line.

Further procedures were then performed on the isolated nuclei to generate nuclear extract. The isolated nuclei from each cell line were mixed with 0.9 volumes of Buffer C (1ml 1M HEPES, 25ml 50% glycerol, 4.2ml 5M NaCl, 75µL 1M MgCl₂, 20µL 0.5M EDTA, 19.7ml H₂O) and dounced 20 times. The douncing process serves to mechanically break the nuclear envelope and provide access to the mediator complex and other transcription factors in the nucleus. The sample was then dialyzed in Buffer D (40ml 1M HEPES, 67ml 3M KCl, 4ml 1M MgCl₂, 0.8ml 0.5M EDTA and 400ml 100% glycerol, 1482ml H₂O). About 4.5ml of nuclear extract were obtained from the 4.6ml of isolated nuclei for each cell line.

2. Isolation of the human mediator complex

The method of immunoprecipitation employed in this project aimed to purify the mediator complex from the nuclear extract. The process involves the exploitation of immunological function of antibodies to achieve purification of the human mediator complex in neuronal cell lines. Antibodies are known to bind specific antigens in the human body with a high affinity. Two antibodies—TRAP220/MED1 and CDK8—were used

in this project. These antibodies bind to two specific mediator subunits (MED1 and CDK8). Protein A and Protein G resins are beads that bind different antibodies with various affinities. In the process of immunoprecipitation, Protein A and Protein G mix were mixed with antibody TRAP220 and CDK8 and allowed to incubate for about 90 minutes to ensure the binding of the antibody to the resin. The nuclear extract of the cell lines was then added to the resin-antibody mixture and incubated for 3-4 hours. After the incubation period, 6 washes of HEGN Buffer were made and the resins were eluted. The eluent contained the purified mediator. The purified sample of the mediator complex was then analyzed through the utilization of gel electrophoresis and silver staining.

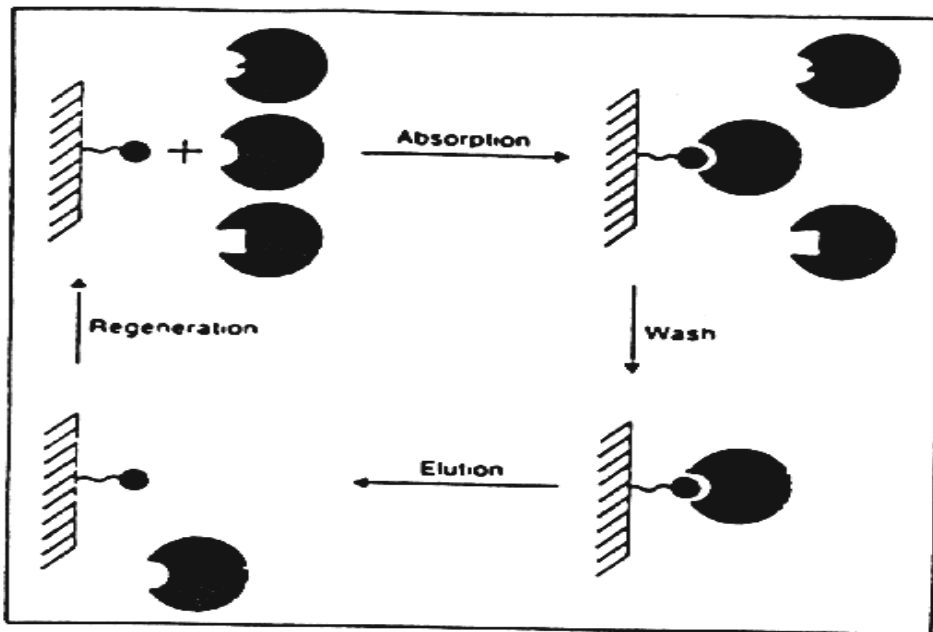


Figure 4: general schema of the isolation of the mediator complex using immunoprecipitation with antibodies. Antibody is bound to the mediator complex during incubation period. Antibody-resin mixture is washed in KCl buffer to remove proteins that are not bound to the antibody. An elution is performed to provide a purified mediator complex that is dissociated from the antibody.

SK-N-AS cells

In the human neuroblastoma cell line, a first attempt was made to purify the mediator complex with CDK8 antibody. The analysis of the silver stain of CDK8 immunoprecipitation suggests to us that this antibody wasn't effective in binding the subunit of the mediator complex well in this cell line. A second trial of immunoprecipitation was completed on SK-N-AS using TRAP220/MED1. The immunoprecipitation seemed to have worked well with this cell line as protein bands were present in eluent lane of the gel.

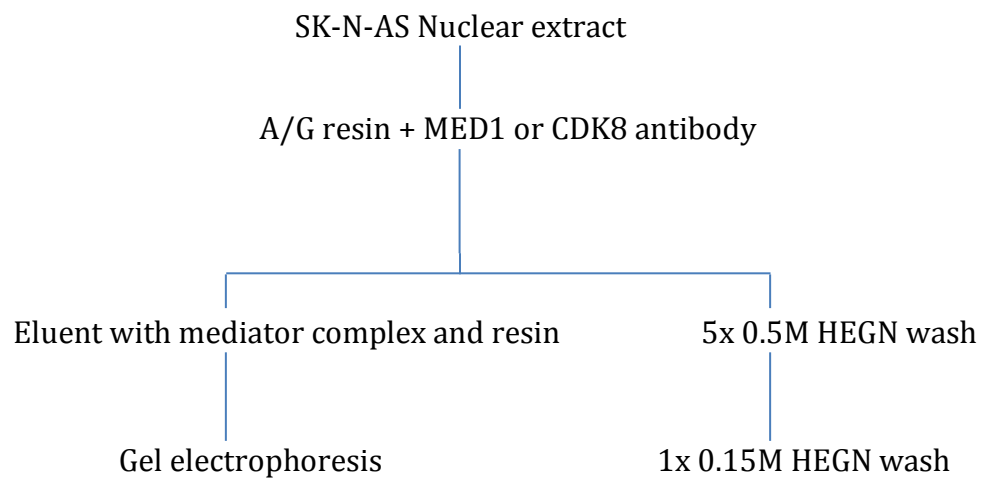


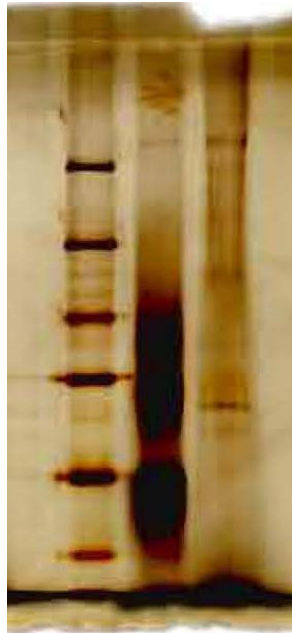
Fig. 5 General schematics of Immunoprecipitation in SK-N-AS using TRAP220/MED1 and CDK8 antibodies.

M 1 2

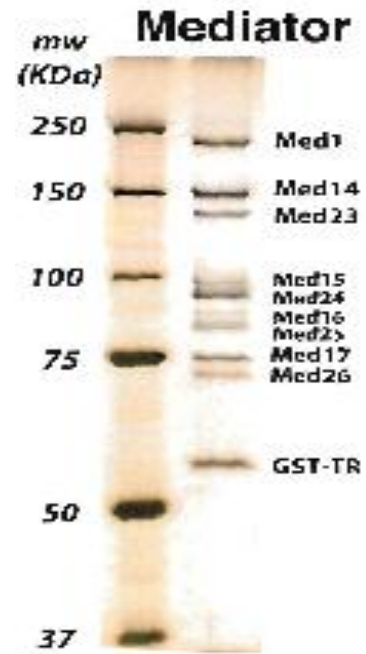


A

M 2 1



B



C

Fig. 6A& B: Silver-stained acrylamide gels of SK-N-AS. Immunoprecipitation of the mediator complex from SK-N-AS using TRAP220/MED1 antibody is shown in Figure 2A while that of CDK8 is shown in Figure 6B. The lanes M, 1, and 2 are unstained protein marker, IP eluent and A/G beads (negative control) respectively. Figure 6C shows the molecular weight of some of the subunit of the mediator complex in a gel electrophoresis. The first lane in this figure is the standard protein maker.

LN-18 cells

Using the same method as describe above, immunoprecipitation (IP) of mediator from LN-18 was completed. Unstained protein marker, A/G beads, eluent from the immunoprecipitation procedure was loaded into the wells of a gel. Afterwards, gel electrophoresis of the purified mediator complex then underwent a silver staining procedure. After the procedure, it was evident that mediator IP was not effective in the LN-18 nuclear extract (Figure 8).

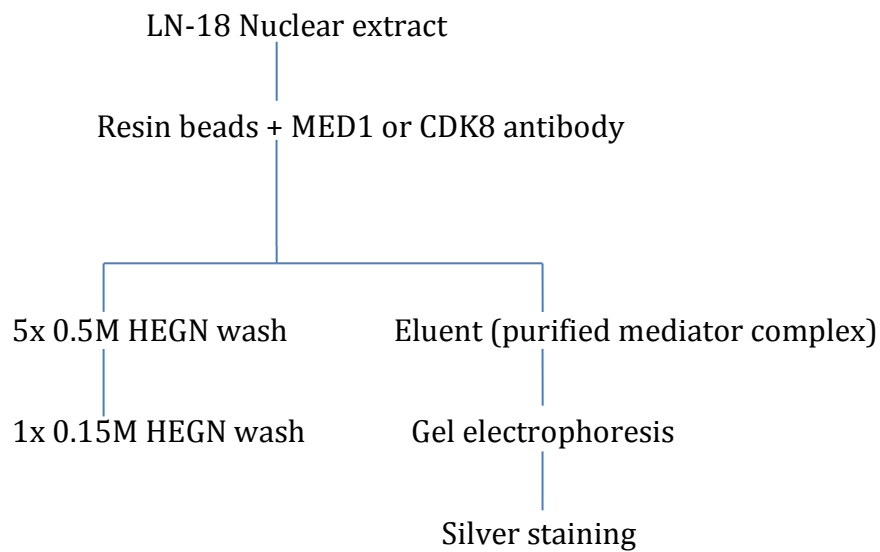


Figure 7: General schematics of immunoprecipitation procedure and silver staining procedure as completed on LN-18 using both MED1/ TRAP220 and CDK8 antibodies.

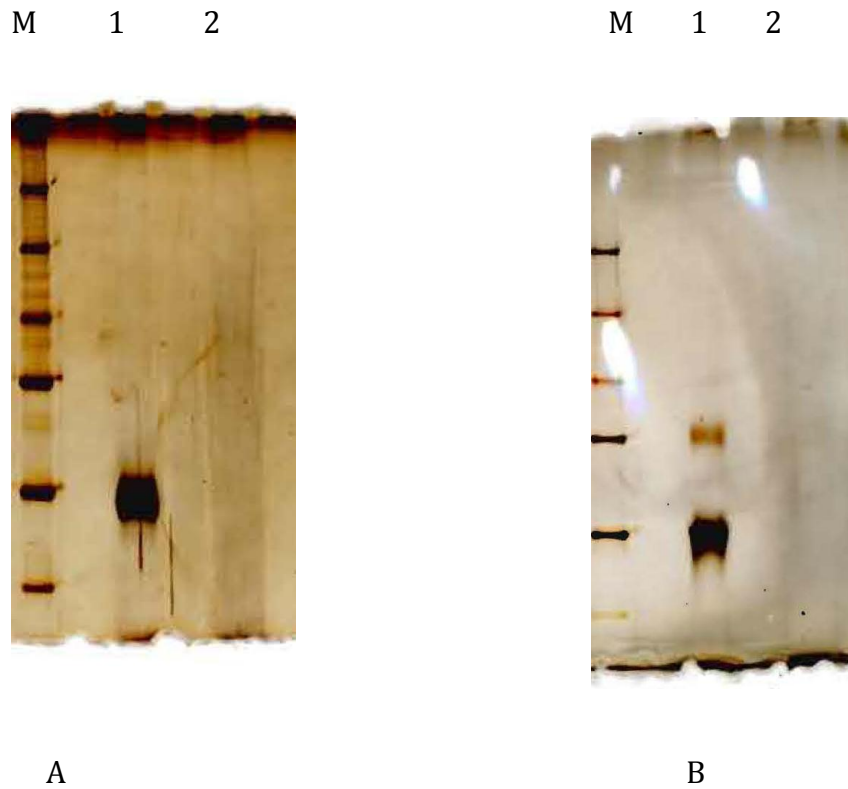


Fig. 8: Silver-stained gels of immunoprecipitation of human malignant glioma (LN-18) with CDK8 and MED1. Figure 8A shows the immunoprecipitation of LN-18 using CDK8. Lane M, 1, and 2 are unstained protein markers, A/G resin beads, and IP eluent respectively. Figure 8B shows the IP of the mediator complex from LN-18 with MED1 antibody. The protein marker is the first lane shown in the gel, then A/G beads and eluent of the IP being last.

Discussion:

For reasons unknown to us, the purification of mediator complex from LN-18 (human malignant glioma) using both MED1 and CDK8 did not seem to work. Looking at the gel in figure 8A and 8B above, one could see that the eluent well is blank while the bands of the standard protein marker is well defined on both gels. A possible explanation could be that a variant version of CDK8 and MED1 is present in LN-18, therefore making the antibodies incapable of binding to MED1 and CDK8 subunits with high affinity. If this is the case, then this will support our hypothesis of “cell-specific” mediator complexes in LN-18 and possibly other neuronal cell lines. But a definite conclusion cannot be made about this hypothesis yet. It is also possible that CDK8 or Med 1 might not be expressed in these cells. It would be interesting if future studies could complete a detailed subunit analysis of mediator present in this cell line. Future studies could utilize other antibodies for the immunoprecipitation procedure. Additionally, it is also possible that other subunits in the mediator complex of LN-18 have maintained the same composition as HeLa cells and the established antibodies for the mediator subunits would be able to bind to them. Utilizing a different antibody might lead to better analysis of LN-18.

The purification of the mediator complex in SK-N-AS has proven to be successful with the use of MED1 antibody. Although CDK8 was used in the process of immunoprecipitation, it is apparent that the CDK8 antibody did not bind the mediator complex in SK-N-AS with a good affinity as observed in Figure 6B. Looking at the silver stain of the MED1 immunoprecipitation purified SK-N-AS mediator complex, a typical mediator banding pattern is observed. Around the 250kDa molecular weight band, there

are about 5 well defined protein bands. Using the mapped out proteins bands of the mediator complex shown in figure 6C, only Med1, Med12, and Med 13 could be accounted for as one of the proteins. Currently, the analysis from the gel suggests that there are more protein subunits besides mediator subunits in SK-N-AS Med1 immunoprecipitation. Med 14 and Med 23 protein bands in addition to two other protein bands were present around the 150kDa protein band. On analyzing the gel even further, the 100kDa protein band seem to have four protein bands which suggests the possibility of the presence of Med15, Med24, Med 16, and Med25. The three protein bands along the 75kDa suggest that 3 subunits in mediator complex of SK-N-AS weigh approximately 75kDa. Of the 3 protein bands, only 2 have been well defined as seen in figure 6B above. We do not fully know if the additional protein found around the 250kDa, 150kDa and 75kDa protein band in the gel are “cell specific” mediator complex or subunits associated with the Mediator complex found in every human cell lines. Additional analysis is needed before valid conclusions could be made about the specificity of the subunits in LN-18 and SK-N-AS. We do believe that with mass spectrometry of purified SK-N-AS nuclear extract, there might be subunits of the mediator complex that are specific to these neuronal cell lines.

Materials and Methods

Materials: SK-N-AS and LN-18 cell lines, growth medium as required by ATTC, gel electrophoresis materials and apparatus, silver staining materials, anti-CDK8, anti-TRAP220.

Methods: there are five methods that are crucial to this project. Of which are: cell subculture, harvesting and nuclei isolation, preparation of nuclear extract, immunoprecipitation and gel electrophoresis.

Cell Subculture

On obtaining the frozen vial of the two neuronal cell lines, the vial was thawed in a 37°C water bath as quickly as possible. The vial was removed from the water when thawed and sprayed with 70% ethanol to avoid contamination. The content in the vial was then transferred into a centrifuge tube with 9ml of growth medium. It was then spun at 125g for 5-7 minutes (ATTC, product information sheet). Then the cell pellet was resuspended and transferred to a 25cm² corning flask. When the cell has reached full confluency, the cell was transferred into a 150cm² plates. Since these two cell lines adhere to the surface of the plates, it is crucial to first aspirate off old media, then do a wash 5ml wash with PBSM and then add 1ml of trypsin. After adding trypsin, the plates were incubated for 3-5 minutes and then ~10ml of fresh media was added to resuspend the cells in the media. The subcultivation ratio as suggested by ATCC was 1:4 or 1:6 and cell medium was renewed every 3-4 days.

Isolation of cell nuclei

For this project, approximately 160 15cm² flasks for each cell lines were harvested. Harvesting was done by manually scrapping off the cells. The cells were then pipetted into 50ml conical flasks and spun at 4rpm in a centrifuge (4°C). The supernatant was pipetted off and the cell was resuspended in PBSM (containing protease inhibitors). To gauge the amount of PBSM (10ml 10x PBS (pH 7.0), 500 µL 1M MgCl₂, 989.5 ml 18MΩ H₂O) to add to the packed cell volume, a ratio of 3:5 was employed. After the cell was completely resuspended, they were spun again at approximately 4000rpm. The supernatant was then aspirated off and 5 times the volume of packed cell of Buffer A (10ml 1M HEPES pH 7.9, 1.5ml 1M MgCl₂, 3.33ml 3M KCl, 985ml 18MΩ H₂O) was added to the cells and resuspended. The cells were then spun at approximately 4000rpm and the supernatant was pipetted out. Again the cells were resuspended in 2 times cell volume of Buffer A (10ml 1M HEPES (pH 7.9), 1.5ml 1M MgCl₂, 3.33ml 3M KCl, 985ml 18MΩ H₂O) and was allowed to sit on ice for 20 minutes. Afterwards, they were dounced about 4 to 5 times and spun at about 2800rpm. After spinning, a clear demarcation could be seen between the supernatant and the pellet. The supernatant was carefully aspirated off and the pellet was frozen in liquid nitrogen and stored in a -70°C freezer for the next step in the project.

Generation of nuclear extract

The nuclei isolated in the step above were thawed in a water bath and placed on ice as soon as it was thawed. 0.9 volumes of cold Buffer C (1ml 1M HEPES, 25ml 50% glycerol, 4.2ml 5M NaCl, 75 μ L 1M MgCl₂, 20 μ L 0.5M EDTA, 19.7ml H₂O) was then added to the nuclei. Buffer C like all other buffers used in this project had DTT and protease inhibitors added to them. The resuspended mixture of the nuclei and Buffer C was then transferred to a dounce homogenizer and dounced 20 times. This step serves to mechanically break the nuclear envelope. The mixture was then transferred to a pre-cooled tube and stirred for 30 minutes at 4°C and then centrifuge for 30 minutes afterwards (15000rpm, 4°C). It is essential to make sure that the container in which the mixture is centrifuged in, is about 60% full but not more than 90% full to ensure efficient centrifuging and prevention of spilling. While centrifuging, the dialysis tubing was cut, rinsed with Milli Q water and equilibrated in Buffer D for about 20 minutes. The supernatant of the mixture is then obtained and carefully pipetted into the dialysis tube and dialyzed in 1L of buffer D (40ml 1M HEPES, 67ml 3M KCl, 4ml 1M MgCl₂, 0.8ml 0.5M EDTA and 400ml 100% glycerol, 1482ml H₂O). Protease inhibitors and DTT were added to Buffer D right before using in the dialysis step. The sample was allowed to dialyze for at least an hour and checked periodically thereafter. Dialysis was stopped once the conductivity \sim 0.1M. Once dialysis was completed, the sample was spun for 30 minutes at 15,000rpm, 4°C and the supernatant (nuclear extract) was put into smaller aliquots of 500 μ L and frozen in liquid nitrogen.

Immunoprecipitation

To analyze the mediator complex in the nuclear extract of these two neuronal cell lines, mediator immunoprecipitation and purification using anti-MED1, anti-CDK8 affinity resin was employed. Protein A and G resins are beads with specific affinity for various antibodies. For the purpose of this experiment, two antibodies- CDK8 and TRAP220/MED1- were tested with each cell line to see which binds well to the mediator complex in that particular cell line. 50% of Protein A was mixed with 50% protein G of obtain a good mixture of resin beads. Resins are shipped and stored in 20% ethanol, so the ethanol needed to be removed by carrying out a 0.15M HEGN (2.5mL 3M KCl, 1mL 1M HEPES (pH 7.9), 10 μ L 0.5M EDTA, 0.1ml 10% NP-40, 10ml 50% glycerol, 36.40ml 18M Ω H₂O) wash before the antibody is added. After each wash, the resin was spun at low speed (3000rpm, 1 min, 4°C) and the supernatant was then removed. A fresh 35 μ L of 0.15M HEGN buffer (2.5mL 3M KCl, 1mL 1M HEPES (pH 7.9), 10 μ L 0.5M EDTA, 0.1ml 10% NP-40, 10ml 50% glycerol, 36.40ml 18M Ω H₂O) was then added to the washed A/G resin in addition to 35 μ L of MED-1/ CDK8 antibody. The tube was then inverted several times to make sure that the beads are resuspended in the antibody. The mix was allowed to incubate for 90 minutes, 4°C. When it was 60 minutes into the incubation period, the 500 μ L nuclear extracts of SK-N-AS and LN-18 were taken out and thawed. After thawing, they were immediately spun at 14,000rpm, 4°C, 20 minutes. After the high-spin, supernatant of the nuclear extract were pipetted out into new pre-cooled eppendorf and the pellet were discarded. The beads were then spun at low speed (3000rpm, 4°C). The supernatant was removed and 1mL of 0.5 HEGN buffer (8.33ml 3M KCl, 1ml 1M HEPES (pH 7.9), 10ml 50% glycerol, 10 μ L 0.5 EDTA, 0.5ml 10% NP-40) was added to the beads to resuspend it. The

beads were then spun at 3000rpm, 4°C. The supernatant was aspirated off each time a wash was completed. This process was repeated until a total of 3 washes of 0.5M HEGN were completed. Afterwards, the resin was washed twice with 1ml of 0.15M HEGN each time. After aspirating off as much buffer from the beads, the supernatant of nuclear extract that was obtained from the high-speed spin was added to the beads. The pellet of the nuclear extract was discarded at this point. The beads were mixed well and resuspended by inversion of the tube several times and placed on the rocker in the fridge to equilibrate for about 3 to 4 hours. After the incubation period the nuclear extract for both cell lines were then pelleted with a low speed spin (3,000rpm, 4°C, 1 min). Immediately after the 1 minute spin, the supernatant was removed and the beads were washed with 1ml 0.5M HEGN buffer. The tube was inverted several times and spun immediately at 3,000rpm, 4°C, 1 minute. Immediately after the 1-minute spin, the supernatant was taken off and another 0.5M HEGN wash was done again until 5 washes with 0.5M HEGN were completed. The supernatant was then removed and a final wash of 0.15M HEGN was done with a 3000rpm spin. After this final wash, all the supernatant were removed without taking any resin with the supernatant. A fine tip pipet was used to achieve this. After this process, elution was next. Two elusions of the beads were done using an elusion buffer (800µL 0.15M HEGN, 200 µL sarkosyl). To a 500 µL nuclear extract bead, 45 µL of elusion buffer was added and then left to mix well on the rocker at 4°C for 30 minutes. After the incubation period, the beads were then spun at 4,000rpm for 1 minute. The elusion buffer was then obtained from the beads and pipetted into a spin-filter eppendorf. A second elusion, using 45 µL of elusion buffer, was then done again using the same beads that were eluted first. The same set of beads were left to incubate for 30 minutes and spun at 4,00rpm for 1 minute. As much

supernatant that could be possibly pipetted out of the tube containing the beads was removed and transferred to the spin filter eppendorf. 10 μ L of loading buffer was then added to the remaining beads and stored in the fridge for gel electrophoresis. The spin-filter eppendorf was spun for 2 minutes at 4,000rpm at 4°C. 10 μ L of the eluent was collected and put in a new eppendorf while the rest was frozen in liquid nitrogen and stored. To the eppendorf with 10 μ L of eluent, 10 μ L of loading buffer was added and saved for gel electrophoresis.

Gel Electrophoresis

To analyze the subunit composition of the purified mediator complex that has been isolated in the eluent, 7% SDS polyacrylamide gel was used. Firstly, the protein gel apparatus was put together. The plates were washed well with soap and water and then rinsed with water and finally 95% ethanol. 1 gel of 7% resolving gel (0.9ml 30% acrylamide, 1.75ml 18M Ω water, 1.223ml Tris pH 8.8, 22 μ L of 20% SDS) was made in a disposable tube. Ammonium persulfate and TEMED were the last reagent to be added to the resolving gel. The mixture was mixed well and then pipetted between the gel plates. To ensure an even top in the resolving gel, 95% ethanol was carefully pipetted on the top of the resolving gel. The gel was let to polymerize for at least 20 minutes. While waiting for the gel to polymerize, 5% stacking gel (0.375ml 30% acrylamide, 1.75ml 18M Ω H₂O, 0.315 ml Tris pH 6.8 and 15 μ L 20% SDS) was made in a disposable tube with ammonium persulfate and TEMED being the last reagent added to the solution. After the 7% resolving gel has polymerized, the ethanol was then removed by turning the gel upside down. The remaining ethanol was blotted with paper towels. 5% stacking gel was then pipetted into

the plates and a 15-well comb was inserted in between the plates. The stacking gel was then let to polymerize for at least 12 minutes. The combs were removed after polymerization and then the plates were transferred to a gel electrophoresis unit. The unit was then filled with 1x SDS running buffer (100ml 10x glycine, 900ml 18MΩ water, 5ml 20% SDS). On the first lane of the well, 2 μL unstained protein marker was loaded. The bead sample and the eluent were loaded on the second and third lane respectively. The beads and the eluent were warmed for at least for 30 seconds before loading. The gel was then run at a constant voltage of 150V until the gel front is 2-5 mm from the end of the gel. This takes approximately 50-60 minutes. Using a razor blade, the plates were separated and then the stacking gel was separated from the resolving gel and discarded.

Silver Staining of Purified Sample

The gel was put in 250ml of 50% methanol for 10 minutes and placed on a rotating dock. The methanol was then poured off using a pipet tip to prevent the gel from falling from the plate. The gel was then incubated in 250ml of 5% methanol for 10 minutes. While the gel is incubating, 100ml of 35μM DTT sample was prepared. The 5% methanol was then poured off and then the gel was then incubated in 100ml DTT solution for 5 minutes while rotating the container on the rotating dock. While the gel was incubating in the DTT solution, the 250ml silver nitrate solution was prepared. When the incubation period was over, the DTT solution was poured out and the gel was then incubated for 10 minutes in 250ml silver nitrate solution (250ml 18MΩ H₂O, 250mg AgNO₃, 25 μL 37% CH₂O). During incubation, the developing solution (500ml cold 18MΩ H₂O, 15g NaCO₃, 250 μL 37% CH₂O) was prepared for the next step. The silver nitrate solution was then poured out and the gel

was rinsed twice with 50-100ml 18M Ω water for approximately 10 seconds during each wash. Then the gel is transferred into 40ml of developing solution and a 10 second wash is performed twice. The remaining developing solution was then poured onto the gel and placed on a rotating dock until mediator bands were visible. The developing of the gel was stopped by adding granular citric acid monohydrate. A pH of approximately 7 is an ideal pH to stop adding citric acid monohydrate. The citric acid was poured into the solution around the gel and not directly on gel. Afterwards, the gel was then washed with water and incubated in fresh water for 10 minutes. Finally, the gel was mounted and analyzed.

Conclusion

Utilizing both MED1 and CDK8 antibodies in the immunoprecipitation of the mediator complex from LN-18, we were unable to isolate the mediator complex from this cell line. It is possible that there is an isoform of MED1 and CDK8 subunits in LN-18. Alternatively, these subunits might not present in the LN-18 cell lines. This supports the idea that the composition of the mediator complex is altered in neuronal cell lines. Furthermore, the analysis of purified mediator complex obtained from SK-N-AS cell lines also supports the notion that there might be “cell specific” mediator complex in neuronal cell lines. The use of CDK8 antibody in the isolation of mediator complex from SK-N-AS was unsuccessful. This suggests a difference between HeLa and SK-N-AS mediator complex as CDK8 binds HeLa cells with a strong affinity in the immunoprecipitation procedure. Immunoprecipitation of the mediator complex of SK-N-AS with MED1 on the other hand was effective. Additional proteins were observed in the silver stained analysis of the purified mediator complex from SK-N-AS. It is unclear if these proteins are “cell specific” mediator complex subunits or subunits associated with the mediator complex found in every human cell lines. Further analysis must be completed before valid conclusions about the subunit composition of the mediator complex in SK-N-AS could be made. We believe with mass spectrometry, there might be subunits of the mediator complex that are specific to neuronal cell lines.

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