# The study of Interacting Partners of a Transcription Factor, YB-1

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MARCH 2005

# CERTIFICATE

This is certify that dissertation entitled 'The study of Interacting Partners of a Transcription Factor, YB-1' is the bonafide record of research work done by MOHAMAD HAFIZI BIN MANSOR during the period from Jun 2004 to March 2005 under our supervision

Signature of Supervisor : 

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Date 14 : March 2005

# ACKNOWLEDGEMENT

Assalamualaikum. Firstly would like to thank Allah S.W.T for giving me good health in finishing this project

I am indebted to Dr. Shaharum Shamsuddin, my supervisor for guiding, sharing him thoughts, encouragement and invaluable assistance in finishing this project successfully

I am also thankful to the Dean, PPSK, USM Health Campus, Kubang Kerian Kelantan, for permitting to do research.

I would also like to thank Pn. Norazizah Abdullah Scientific Officer of Molecular Biology Laboratory, PPSK for permitting and co-operation during my study.

I would also like to express my gratitude to Mr. Venugopal that very generous to pass their knowledge and given good guidance during my study. Without him, I may be having more problems to finishing this project.

My appreciation goes to all the Postgraduate Students that work in Molecular Biology Laboratory, PPSK for their help and co-operation during my study.

To my great family, thank you for your moral support for enable me to finish this project.

Last but not least, my appreciation goes to all my course mates, especially to Mr. Abdul Rashid Jusoh, Mr.Mohd Azim Patar, Mr Zaki Abdul Aziz, Mr Afif Mohd Nasir and Mr. Mohd Fikri Omar for all their supports

Finally gratitude goes to all those unmentioned who had shared their interest and given all the support needed.

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# ABSTRACT

The transcription factor, YB-1 was considered to be a transcription factor. YB-1 also involved in many genes regulation, such as genes involved in cell proliferation and cell growth, DNA repair, multi drug resistance, modification of chromatin, redox-state dependent-transcriptional enhancing, tissue cell specific processes, stress response, immune response (major histocompatibility class II genes), and many more.

Because of several important of YB-1, my supervisor proposed to study of YB-1's interacting partners. Base on interacting partner of YB-1, we hopefully can explore properties of YB-1.

In this study, we used culture of HeLa cells, and lysis the cells using Single-step lysis for extraction of total cellular protein, and nuclear and cytoplasmic proteinextraction methods to get YB-1 protein. Using co-immunoprecipitation analysis and several antibodies, we will detect interacting partners of YB-1.

Finally after co-immunoprecipitation analysis, we got YB-1 protein has specific interaction with P-glycoprotein *mdr1* protein which involved in multidrug-resistant cancer cells.

# Chapter 1

# INTRODUCTION

## 1.1 Cell Lines

In this study, we used HeLa cell derived from cervical cancer cells. He La cells are human carcinoma cells immortalized by transformation. It is monolayer monolayers for adherent cells. For growth, HeLa cell required Dulbecco's Modified Eagle's Medium (SIGMA, USA), supplemented with L-Glutamine, glucose, pyridoxine-HCl, $NaHCO_3$ 10% (v/v) penicillin-streptomycin (Bibco, BRL)and 10% (v/v) heat in activated Foetal Calf Serum (SIGMA,USA). HeLa cells require -20°C storage. Since HeLa cells are transformed, variations in the number of chromosomes will be observed between cells upon microscopic examination.

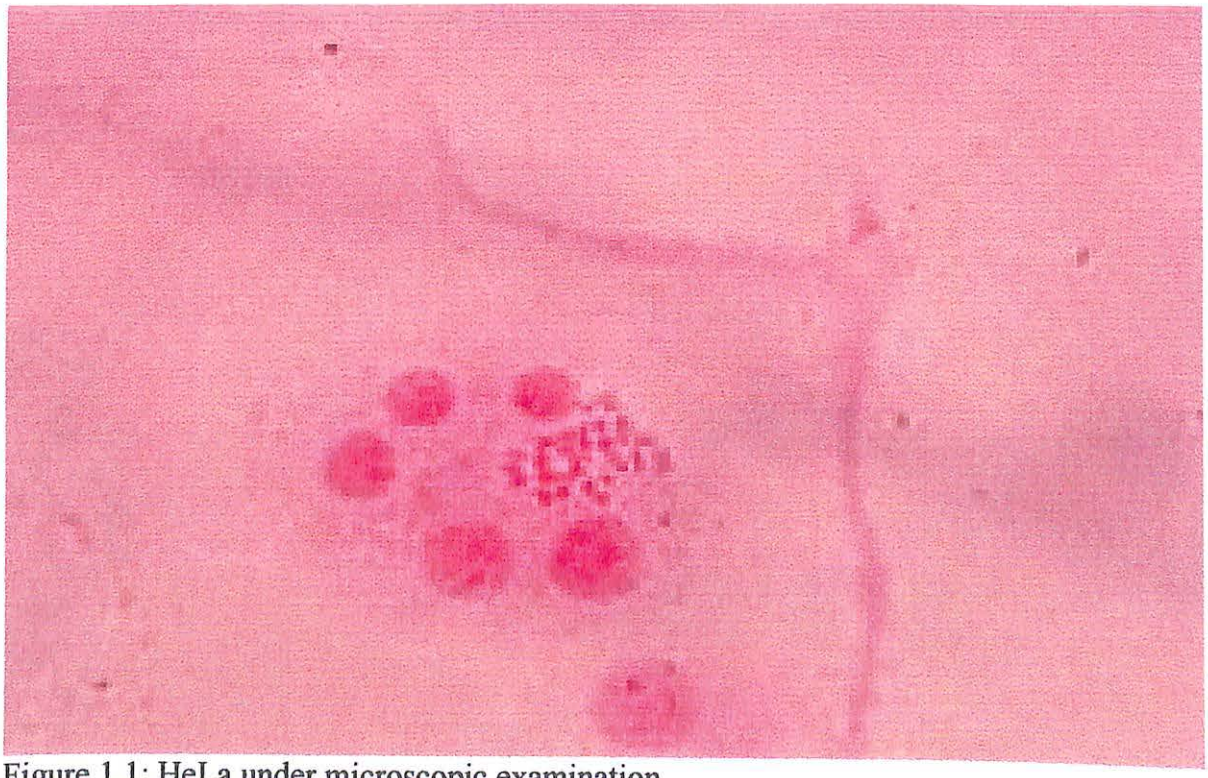

Figure 1.1: HeLa under microscopic examination

## 1.2 YB-1

The central role of DNA as the carrier of genetic information in all living species has become common knowledge in our society. How the hereditary information encoded in DNA (deoxyribonucleic acid) is brought to expression is, however, much less familiar. Many proteins and complexes play major role in regulation of the processes of the gene expression. One of the protein families involved in gene expression is the family of Y-box proteins. These proteins play a major role in the regulation of transcription and translation. Transcription is the process of copying DNA into messenger RNA (mRNA), while translation is the synthesis of proteins using mRNA as template. The Y-box protein are remarkable because, the most evolutionary conserved nucleic acid binding proteins yet defined in bacteria, plants and animal (Wolffe *et al.*,1994). This sequence, which consist of twelve nucleotides (given in figure), is found in all species mentioned above.

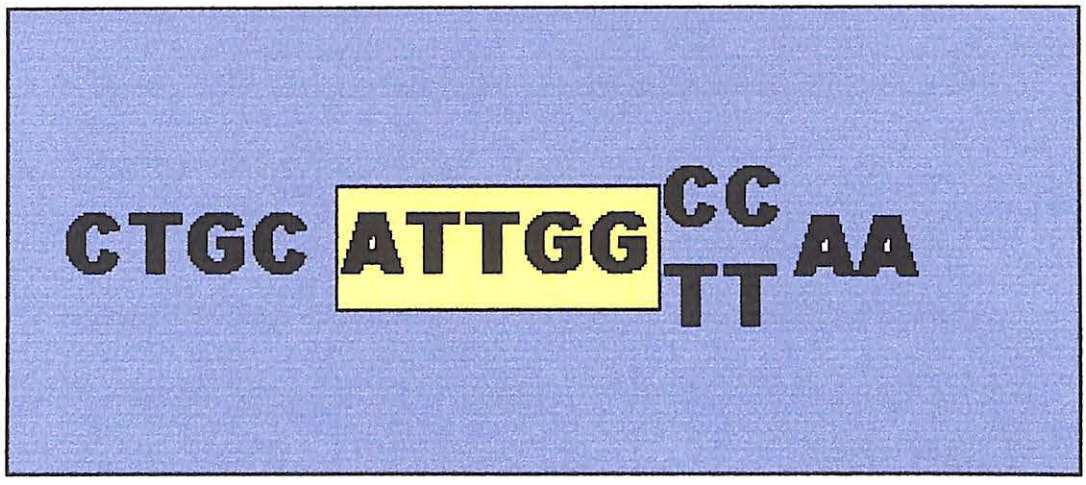


Figure1.2 : The consensus Y-Box sequence. The core of the Y-box sequence id boxed. The latters stand for the four different building blocks of DNA: the nucleotides adenosine (A), cytidine (C), guanosine (G), and thymidine of which adenosine and guanosine are purine and cytidine and thymidine are prymidines

YB-1 is one of the member of the family of Y-Box (an inverted CCAAT-box) binding factors (Wolffe, 1992) with each member containing a highly conserved 70 amino acid DNA domain, the so-called "cold shock domain" (Wolffe *et al.*,1994). The "cold shock domain" (CSD) region was identified as one of the most evolutionary conserved nucleic acid binding domains between bacteria, plants and animals. The name "Y box proteins" comes from the ability of the CSD to bind to the Y-box sequence [5' – CTGATTGG – 3'] of DNA, which is an inverted CCAAT box, in the promoter region of many genes (Wolffe, *et al.*, 1992). Among vertebrates, several Y Box protein genes have been cloned and characterized. The human YB-1 was originally cloned by Didier *et al.*, (1988) *via* screening of a human B cell expression library for a protein that binds to the Y-Box in the promoter region of Major Histocompatibility Complex (MHC) class II gene promoter. It can bind to double and

single stranded DNA in a sequence-specific manner (Wolffe, 1994), but shows preference for duplex DNA enriched with pyrimidines and purines on opposite strands (Ozer et al., 1990 and Sakura et al., 1988)

The human YB-1 gene consists of 8 exons and 7 introns spanning 19 kb of genomic DNA (Figure). Exon 1 of YB-1 contains 166 bp of the coding sequence and 331 bp of the un-translated region. On the other hand, exon 8 consists only of the un-translated region. The C terminal domain of YB-1 is encoded by exons 5 - 7 (Toh *et al.*, 1998). The open reading frame of the full length YB-1 cDNA is 972 bp with a predicted molecular weight of YB-1 protein of 35.6 kDa. However, the protein migrates at 42 kDa in SDS-PAGE, probably due to particular composition of amino acids (Spitkovsky *et al.*, 1992). YB-1 protein consists of three domains; the N – terminal domain (rich in proline and alanine) is thought to play an important role in transcriptional regulation (Tafuri and Wolffe, 1992). This portion of YB-1 was previously reported to interact with p53 protein (Okamoto *et al.*, 2000), although the functional implication of this interaction is not known. The cold shock domain (CSD) is very important for binding to the Y-Box DNA sequence and is highly conserved in evolution from prokaryotes to eukaryotes (Ladomery and Sommerville, 1995). YB-1 can also bind RNA and single stranded DNA via the RNA binding motifs (RNP-1 and RNP-2), also much conserved between species (Kloks *et al.*, 2002).

High resolution NMR spectroscopy study of CSD showed that it adopts the five stranded antiparallel barrel structure (Kloks *et al.*, 2002). The hydrophilic C –

terminal domain (also termed as the charged zipper domain) is believed to facilitate both protein-protein interaction (Chen, *et al.*, 1995) and protein-nucleic acid interaction (Wolffe A.P., 1994). The C terminal domain of YB-1 consists of four alternating-regions of predominantly acidic (aspartate and glutamate) or basic amino acids (arginine, glutamine and proline), each of these is about 30 amino acids in length (Wolffe, 1994).

The YB-1 protein has been shown to affect gene expression at both transcriptional and translational levels. It can bind to a double and single stranded DNA in a sequence-specific manners (Wolffe, 1994), and also recognize and bind damaged DNA (MacDonald *et al.*, 1995) as well as the RNA (Hasegawa *et al.*, 1991). It has diverse regulatory targets which include the class II MHC genes (Montani *et al.*, 1998), the Multidrug resistance 1 gene (Bargou, *et al.*, 1997), the matrix metalloproteinase 2 gene (Mertens *et al.*, 1997), genes for the myosin light chain 2v (Zou and Chien 1995), the thyrotropin receptor (Ohmori *et al.*, 1996), chicked □-2 collagen (Bayarsaihan *et al.*, 1996), the HIV-1 (Sawaya *et al.*, 1998), HTLV-1 (Kashanchi *et al.*, 1994) and polyomavirus JVC (Chen *et al.*, 1995) promoters.

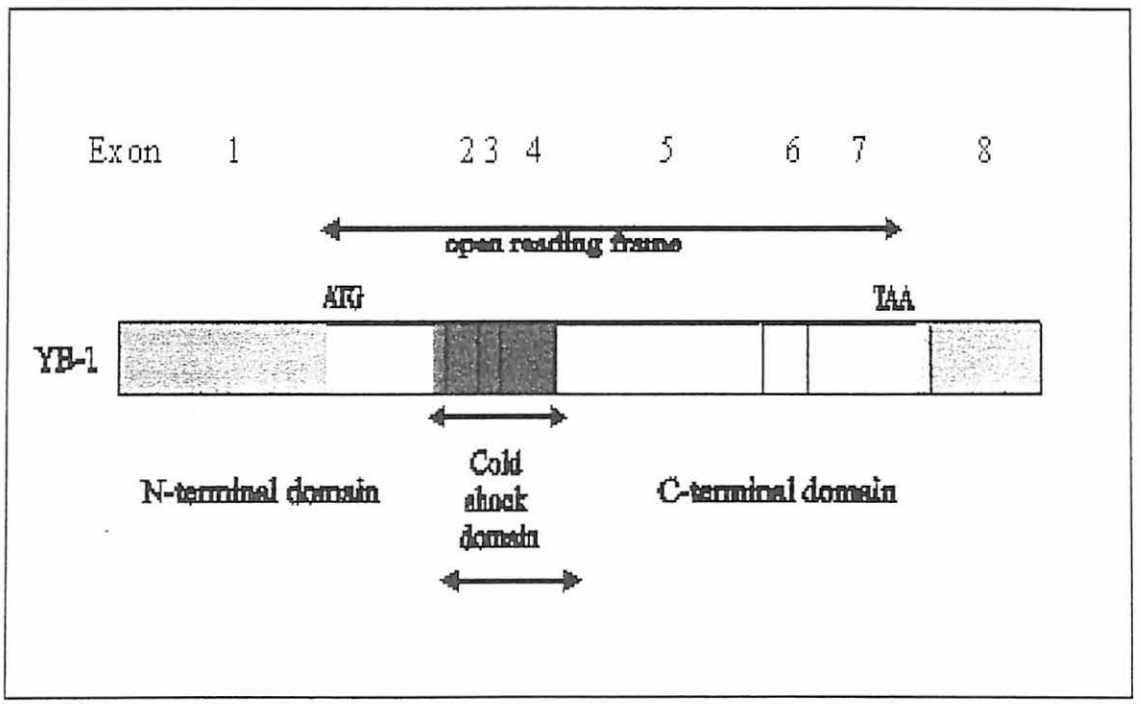


Figure1.3: Domain structure of human (YB-1). Light and dark shading represent non-coding sequences and the cold shock domains (CSD), respectively. Adapted from Toh *et al.*, (1998).

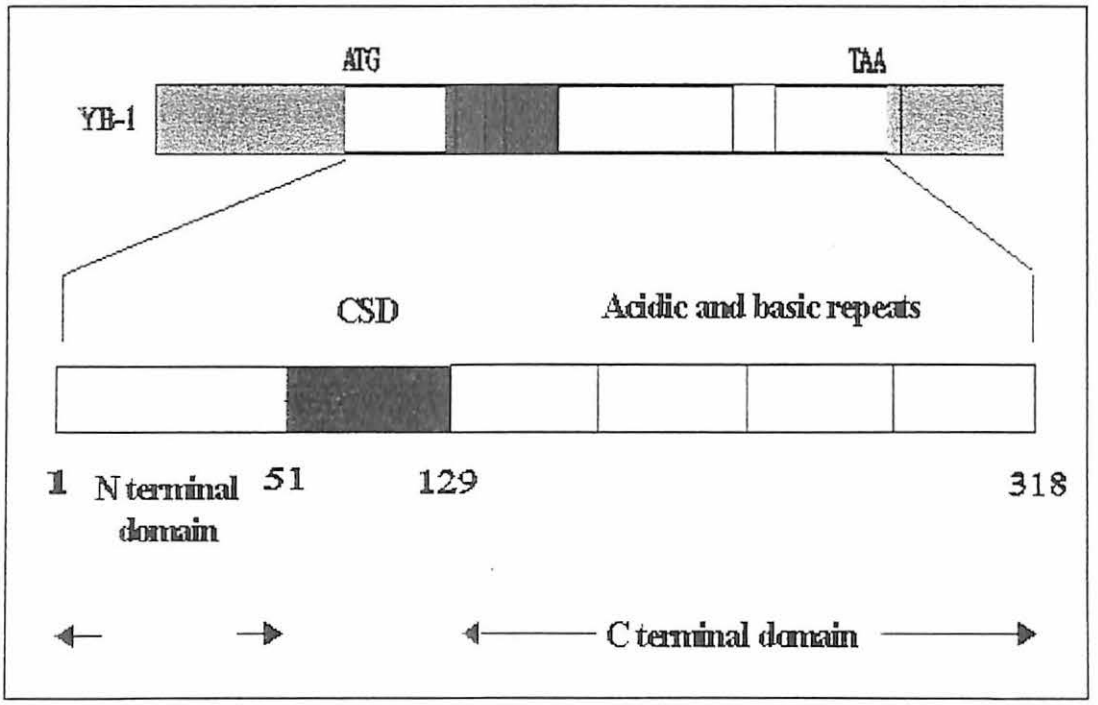


Figure1.4:Schematic diagram of the YB-1 whole molecule which contains a total of 318 amino acids (Safak *et al.*, 1999) and consists of N terminal domain, Cold Shock Domain (CSD) and the C terminal domain.

## 1.3 Immunoprecipitation

Immunoprecipitation is used to detect and quantitate target antigen in mixtures of proteins. The power of the technique lies in its selectivity. The specificity of the immunolglobulin for its ligand is so high that the resulting antigen-antibody complexes can be purified from contaminating proteins. Furthermore, immunoprecipitation is extremely sensitive and is capable with SDS-polyacylamide gel electrophoresis, the technique is ideal for analysis of the synthesis and processing of foreign antigen expressed in prokaryotic and eukaryotic hosts or in *in vitro* systems.

The target protein is usually immunoprecipitated from extracts of cells that have been radiolabeled. However, immunoprecipitation can also be used to analyze unlabeled protein as lo as sufficiently sensitive methods are available to detect the target protein after it has been dissociated from the antibody. Such methods include enzymatic activity, binding of radioactive ligands, and westerns blotting.

Immunoprecipitation of radiolabeled target proteins and their subsequent anlysis consist of the following steps, which can be completed in as little as one day or can becarried out over several successive days if desired:

- Radiolabeling of cells expressing the target protein
- Lysis of the cell
- Formation of specific immune complexes
- Collection and purification of the immune complex
- Analysis of radiolabeled proteins in immunoprecipitate

## 1.4 Lysis of Cells

This is perhaps the most crucial step in immunoprecipitation. The aim is to find a method that will solubilize all of the target antigen in a form that is immunoreactive, undergraded, and, for some purpose, biologically active. In view of the wide range of physical and biological properties of mammalian protein, it is not surprising thet no single method of lysis is sufficient for every purpose. Among the variables that have been found to influence the efficiency of solubillization and subsequent immunoprecipitattion of proteins are the ionic strength and pH of the lysis buffer; the concentration and type of detergent used; and the presence of divalent cation, cofactors, and stabilizing ligands.

Although there are exceptions, many soluble nuclear and cytoplasmic protein can be solubilized by lysis buffers that contain the nonionic detergent Nonidet P-40 (NP-40) and either no solt at all or relatively high concentration of salt (e.g., 0.5 M NaCl). However, the efficiency of extraction is often greatly affected by the pH of the buffer and the presence or absence of chelating agent such as EDTA and EGTA. Extraction of membrane-bound and hydrophobic protein is lesss affected by the ionic strengeh of the lysis buffer but often requires a mixture of ionic and nonionic detergents.

When attempting to solubilize a protein for the first time, there are two different strategies that can be employed. At one extreme, harsh conditions can be used in an effort to ensure that the protein is released quantitatively from the cell; however this may result in loss immunoreactivity. At the other extreme, gentle conditions can be used is determined in large part by the properties of the antiserum available for immunoprecipitation. For example, monospecific antisera raised against synthetic

peptides may react only denatured forms of the target protein, whereas monoclonal antibodies directed against native epitopes may be specific for the correctly folded form of the protein. To minimize problems, try to use polyclonal antisera or mixtures of monoclonal antibodies that react with all form of the protein. It is usually the possible to tailor the extraction condition to fit the characteristics of the target protein rather than the properties of the available antisera

Many method of solubilization, particularly those that involve mechanical disruption of cells, release intracellular proteases that can digest the target protein. The susceptibility of different protein to attack by proteases varies widely, with cell-surface and secreted proteins generally belong more resistant than intracellular proteins. Denatured proteins are much more likely to be degraded than native proteins. It is therefore advisable to take step to minimize proteolysis activity in cell extracts, especially when has conditions of extraction are used. It is important to keep the extracts cold (i.e., at 0°C or below, depending on the sensitivity of the target protein to freezing and thawing).

## 1.5 SDS-Polyacrylamide Gel Electrophoresis of Protein

Almost all analytical electrophoresis of protein is carried out in polyacrylamide gel under condition that ensure dissociation of the protein into their individual polypeptide subunits and that minimize aggregation. Most commonly, the strongly anionic detergent SDS is used in combination with a reducing agent and heat to dissociate the protein before they are loaded on the gel. The denatured polypeptides bind SDS and become negatively charged. Because the amount of SDS and is almost

always proportional to the molecular weight of the polypeptide and independent of its sequence, SDS-polypeptide complexes migrate through polyacrylamide gels in accordance with the size of the polypeptide. At saturation, approximately 1.4 g of detergent is bound per gram of polypeptide. By using marker of know molecular weight, it is therefore possible to estimate the molecular weight of the polypeptide chains. Modifications to the polypeptide backbone, such as N- or O-linked glycosylation, however, have a significant impact on the apparent molecular weight. Thus, the apparent molecular weight of glycosylated proteins is not a true reflection of the mass of the polypeptide chain.

In most cases, SDS-polyacrylamide gel electrophoresis is carried out with a discontinuous buffer system in which the buffer in the reservoirs is of a different pH and ionic strength from the buffer used to cast the gel. The SDS-polypeptide complex in the sample that is applied to gel are swept along by a moving boundary created when an electric current is passed between the electrodes. After migrating through a stacking gel of high porosity, the complexes are deposited in very thin zone (or stack) on yhe surface of the resolving gel. The ability of discontinuous buffer system to concentrate all of the complexes in the sam-le into a very small volume greatly increases the resolution of SDS-polyacrylamide gels.

The discontinuous buffer system that is most widely used was originally devised by Ornstein (1964) and Davis (1964). The most sample and the stacking gel contain Tris Cl (pH 6.8), the upper and lower buffer reservoir contain components of the system contain 0.1 % SDS (Laemmmli 1970). The chloride ions in the sample and staking gel contain Tris Cl (pH 8.8). All components of the system contain 0.1% SDS

(Laemmali 1970). The chloride ions in the sample and stacking gel form the leading edgr of the moving boundary, and the trailing edge is composed of gycine molecules. Between the leading and trailing edges of moving boundary is a zone of lower conductivity and steeper voltage gradient which sweeps the polypeptides from the sample and deposits them on the surface of the resolving gel. There the higher pH of the resolving gel favors the ionization of glycine, and the result glycine ions migrate through the stacked polypeptides and travel through the resolving gel immediately behind the chloride ions. Freed from the moving boundary, the SDS-polypeptide complexes move through thr resolving gel in zone of uniform voltage and pH and are separted according to size by sieving.

Polyacrylamide gels are composed of chains of polymerized acrylamide that are cross-linked by a bifunctional agent such as *N,N'*- metylenebisacrymide. The effective range of separation of SDS-polyacrylamide gels depends on the concertration of polyacrylamide used to cast the gel and on amount of cross-linking. Polyamerization of acrylamide in the absence of cross-linking agent generates viscous solutions that are of no practical use. Cross links formed from bisacrylamide add rigidity and tensile strength to the gel and form pores through which the SDS-polypeptide complexes must pass. The size of these pores decreases as the bisacrylamide- acrylamide ratio increases, reaching a minimum when the ratio is approximately 1:20, which has been shown on empirically to be capable of resolving polypeptides that differ in size by as little as 3%.

The sieving properties of the gel are determined by size of the pores, which is a function of the absolute concentrations of acrylamide and bisacrylamide used to cast the gel.

| Acrymide Concentration (%) | Liner range of separation (kD) |
|---|---|
| 15 | 12-43 |
| 10 | 16-68 |
| 7.5 | 36-96 |
| 5.0 | 57-212 |

Table 1.1: The liner range of separation obtained with gels cast with concentrations of acrylamide that range from 5% to 15%.

## 1.6 Staining SDS-Polyacrylamide Gels With Coomassie Brilliant Blue

Polypeptide separated by SDS-polyacrylamide gels can be simultaneously fixed with methanol: glacial acetic acid and stained with Coomassie Brillant Blue R250, triphenylmethane textile dyes also know as Acid Blue 83. The gel is immersed for several hours in a concentrated methanol/acetic acid solution of dye, and excess dye is then allowed to diffuse from the gel during a prolonged period of destaining.

## 1.7 Transfer Of Protein From SDS-Polyarylamide Gels To Solid Support: Immunological Detection Of Immolizer Proteins (Western Blotting)

Western blotting (Towbin et al. 1979; Burnette 1981) is to protein what Southern blotting is to DNA. In both technique, electrophoretically separated componentare

transferred from a gel to solid support and probed with reagents that are specific for particular sequences of amino acid (Western blotting) or nucleotides (Southern hybridization ). In the case of protein, the probes usually are antibodies that react specifically with antigenic epitopes displayed by the target protein attached to the solid support. Western blotting is therefore extremely useful for the identification and quantitation of specific protein in complex mixture of protein that is not radiolabeled. The technique is almost as sensitive as standard solid-phase radioimmunoassay and, unlike immunoprecipitation, does not require that the target protein be radiolabeled. Furthermore, because electrophoretic separation of protein is almost always carried out under denaturing conditions, any problems of solubilization, aggregation, and co-precipitation of the target protein with adventitious proteins are elimated.

The critical difference between southern and Western blotting lies in the nature of probes. Whereas nucleic acid probes hybridize with a specificity and rate that can be predicted by simple equations antibodies behave in a much more idiosyncratic manner. An individual immunoglobulin may preferentially recognize a particular conformation of its target epitope . consequently, not all monoclonal antibodies are suitable for use as probes in Western blots, on the other hand, are undefined mixtures of individual immunoglobulins, whose specificity, affinity, and concentration are often unknown, polyclonal antiserum will detect different antigenic epitopes of an immobilized, denatured target protein.

Although there is an obvious danger that comes from using from using undefined reagents to assay a target protein that may also be poorly characterized, most problems that arise with Western blotting in practice can be solved by designing adequate

control. These include the use of antibodies that should not react with the target protein and control preparations that either contain known amounts of target antigen or lack it altogether.

Often, there is little choice of immunological reagents from undefined reagent Western blotting it is simply necessary to work with whatever antibodies are at hand. However, if a choice is available, either a high-titer polyclonal antiserum or mixture of monoclonal antibodies raised against the denatured protein should be used. Reliance on a single monoclonal antibody is hazardous because of the high frequency of spurious cross-reactions with irrelevant protein. If, as is usually the case, monoclonal and polyclonal antibodies have been raised against native target protein, it will be necessary to verify that they react with epitopes that either resist denaturation with SDS and reducing agent or are created by such treatment, this can be done by using denatured target antigen in a solid-phase radioimmunoassay or in Western dot blots.

In Western blotting, the samples to be assayed are solubilized with detergent and reducing agent, separated by SDS-polyacrylamide gel electrophoresis, andtransferred to a solid support (usually a nirtcellulose filter) which may then be stained. The filter is subsequently exposed to unlabeled detected by one of several secondary immunological reagents (125 I-labeled protein A or anti-immunoglobulin or protein A coupled to horseradish peroxidase or alkaline phosphatase). As little as 1 – 5ng of an average –sized protein can be detected by Western blotting.

## 1.8 Preparation and Electrophoresis of Samples

Two method are used to extract proteins for western blotting from cells, either the intact cells are dissolved directly in sample buffer or an extract is made as described

earlier for samples to be immunoprecipitated. Which of these methods is best in any individual case deepens on the type of cells and on the properties of the antigen.

- In general, bacterial expressing the target protein are lysed directly in SDS gel-loading buffer.
- Yeasts are first lysed by vortexing in the presence of glass beads or enzymatically, and the resulting extracts are then prepared.
- Mammalian tissue are usually dispersed mechanical and then dissolved directly in SDS gel-loading buffer.
- Mammalian cells in tissue cultured may be lysed with detergents, in which the cells are lysed directly in SDS gel-loading buffer, may be used if the target antigen is resistant to this type of extraction.

## 1.9Tranfer of Protein From SDS-Polyacrylmide Gels To Solid Support

A number of different solid supports have used for V Western blotting. These include diazophenylthio (DPT) paper ( Seed 1982a,b), diabenzyloxymethyl (DBM) paper (Renart et al. 1979), cyanogens-bromide-activated paper (Hitzeman et al.1980), cyanuric chloride paper ( Hunger et al. 1981), and activated nylon. In all of these cases, the proteins from the gel become covalently bound to the support. Although supports of this type may have the highest capacity and may retain the bound protein more securely, they are generally difficult to prepare and may require that gycine be soaked out of the gel before transfer. Furthermore, charge supports such as nylon membranes may not bind protein of the same charge with high efficiency.

Consequently, most Western blotting nowadays is carried out by direct electrophoretic transfer of proteins from gel to a nitrocellulose filter (Burnette 1981)

Two type of electrophoresis apparatuses are available for electroblotting. In the older type, one side of the gel apparatuses are available for electroblotting. In the older type, one side of the gel is placed in contact with a piece of nitrocellulose filter. The gel and its attached filter are then sandwiched between Whatman 3MM, two porous pads, and two plastic supports. The entire construction is then immersed in an electrophoresis tank, equipped with standard platinum electrode, which contains Tris-glycine eletrophoresis buffer at pH 8.3. The nitrocellulose filter is placed toward the anode. An electric current is then applied for about 12 hours, during this time, the protein migrate from the gel toward the anode and become attached to the nitrocellulose filter. To prevent overheating and the consequent formation of air bobbles in the sandwich, transfer is carried out in the cold.

In the newer type of apparatus, the gel and its attached nitrocellulose filter are sandwiched between pieces of Whatman 3MM paper that have been soaked in a transfer buffer containing Tris, glycine, SDS, and methanol. The sandwich is then placed between grahite plate electrodes, with the nitrocellulose filter on the anodic side. Transfer of the protein from the can be carried out at room temperature and is complete in 1.5-2 hours.

## 1.10Staining Protein Immobilized On Nitrocellulose Filters

Of the several procedures available to stain proteins immobilized on nitrocellulose filters, only one, staining with Ponceau S, is completely compatible with all methods of immunological probing because the stain is transient and is washed away during processing of the westrn blot. Staining with Ponceau S therefore does not interfere with the subsequent detection of antigen by chromogenic reactions catalyzed by antibody-linked enzymes such as alkaline phosphatase or lactoperoxidase. However, because the pink-purple color of Ponceau S is difficult to capture photographically, the stain does not provide a permanent record of the experiment. Instead, staining with Ponceau S is used to provide visual evidence that electrophoretic transfer of protein has taken place and to locate molecular-weight marker, whose position on the nitrocellulose filter are then marked with pencil or indelible ink.

If the western blot is to be probed with radiolabeled antibody or radiolabeled protein A, the protein immobilized on the nitrocellulose filter may be stained with India ink, which is cheaper and more sensitive than Ponceau S and provides a permanent record of the location on the nitrocellulose filter.

## 1.11 Blocking Binding Sites for Immunoglobulin on the Nitrocellulose Filter

Just as protein transferred from the SDS-polyacrylamide gel can bind to the nitrocellulose filter, so can protein in the immunoglogical reagent used for probing. The sensitivity western blotting depends on reducing this background of nonspecific binding solutions that have been devised, the best and least expensive is nonfat dried milk (Johnson et al.1984). it is easy to use and is compatible with all of the immunological detection system s in common use. There is only on circumstance under which nonfat dried milk should not be used, when western blots are probed for proteins that may be present in milk.

## 1.12 Binding Of the Primary Antibody to the Target Protein

Virtually all western blots are probed in two stages. An unlabeled antibody specific to the target protein is first incubated with the nitrocellulose filter in the presence of blocking solution. The filter is then washed and incubated with a secondary reagent anti-immunoglobulin or protein A that is either radiolabeled or coupled to an enzyme such as horse dish peroxidase or alkaline phosphatase. After further washing, the antigen-antibody-antibody or antigen-antigen-protein A complexes on the nitrocellulose filter are located by autoradiography or in situ enzymatic reaction.

Indirect or two-stage probing has the major advantage of allowing a single secondary reagent to be used to detect a wide variety of primary antibodies, thereby eliminating the tedious task of purifying and labelling each individual primary antibody. Since secondary immunological reagents can be purchased quite

inexpensively from commercial sources, the resulting saving of time and money can be can be considerable.

## 1.13 Incubating the Nitrocellulose Filter with the Secondary Immunological Reagent

The secondary reagent (usually an anti-immunoglobulin or protein A) may be radiolabeled with $^{125}$ I or may be covalently coupled to an enzyme such as horsedish peroxidase or alkaline phosphates. Covalently coupled immunoglobulin and protein A are sold commercially.

Although both radiolabeled and enzyme-coupled secondary reagents can work very well, antibodies are sometime inactivated if radiolabeling process is carried out too enthusiastically.

# Chapter 2

# REVIEW OF LITERATURE

Survey of literature reveals that the YB-1 is implicated in regulation of multiple cellular functions. YB-1 can function as a regulator of transcription and of translation. It is also involved in the cellular responses to stress and DNA damage which a possible role in DNA repair and apoptosis has been suggested. Andreas G. *et al* (2003) suggested that an inhibitory effect of YB-1 on translation and protein synthesis mediates interference with oncogenic transformation. The resistance to transformation is correlated with the ability of YB-1 to bind to the cap structure of mRNA and with inhibition of translation and it also requires an intact RNA-binding motif in YB-1. Study in rabbit reticulocyte lysates, showed low concentrations of YB-1 promote translation, on the other hand high concentrations block it. YB-1 may prevent the assembly of the translational initiation complex 4F at the mRNA by competing with the 4E initiation factor for binding to the cap structure (33, 38). This activity would explain interference with cap-dependent but not with cap-independent translation.

Igor et al. (2000) suggested that YB-1and CTCF form specific complexes both *in vivo* and *in vitro*, and that interaction with YB-1 requires the zinc finger domain of CTCF. Furthermore, CTCF/YB-1 complexes were observed in various cell types possibly implying "universal" functions for the association of these two ubiquitously expressed factors. It is tempting to speculate that CTCF may, therefore, be involved in modulation of at least some of the multiple functions mediated by YB-1, including

those that are not mediated through direct DNA recognition. It also seems reasonable to suggest in a reciprocal manner that interaction with YB-1 may equip CTCF to perform functions beyond transcriptional regulation mediated by binding to promoters , hormone-responsive silencers, or enhancer-blocking elements in the globin genes loci or the differentially methylated imprinting control region upstream of the *H19* gene. Precedent for this comes from studies on the Wilms' tumor suppressor gene WT1 with a DNA-binding domain of four zinc fingers capable of mediating binding to at least two different sequences. WT1 is suggested to be "more then just a transcription factor" due to its apparent role in RNA processing and other functions not related to transcription

YB-1 shows aberrant activity in human cancers, for instance in carcinomas of the breast and of the lung. In both types of cancers, YB-1 levels are increased. Yet these cancers also commonly show a gain of function in the phosphatidylinositol 3-kinase signaling pathway. The apparent contradiction to the results reported here is resolved by the predominantly nuclear localization of YB-1 in these human tumors. YB-1 may therefore function as transcriptional regulator in these situations.

Among the transcriptional targets of YB-1 are the multidrug resistance gene 1 and matrix metalloproteinase 2, two genes important in cancer progression. On the other hand, adenocarcinomas of the lung in mice show transcriptional down-regulation of YB-1. Differential expression of YB-1 in cancers may therefore be positive or

negative, depending on the prevailing function of YB-1, transcriptional vs. translational, in a particular cell system. (Shibahara *et al.*, 2001)

The development of clinical drug resistance is a major limitation for effective cancer chemotherapy. The classical multidrug-resistant (MDR) phenotype is associated with increased transcription and translation of the *mdr1* gene, which encodes P-glycoprotein, a multifunctional drug transporters. Certain environmental stresses, for instance chemotherapy, UV light irradiation and hyperthermia, cause nuclear accumulation of Y-box protein (YB-1). Nuclear localization of the transcription factor YB-1 is associated with transcriptional activation of the human *mdr1* gene. Results from the literature suggest that YB-1 is involved in pleiotropic resistance to different classes of DNA-targeting drugs. YB-1 interacts with p53 and functions as transcriptional repressor of the cell death-associated *fas* gene, indicating that YB-1 is involved in certain processes that control cell survival. Y-box proteins are characterized by a highly conserved nucleic acid recognition domain, the so-called cold shock domain, and are acting as transcriptional, translational, and developmental regulators. Y-box proteins interact specifically with a sequence motif termed Y-box, which is characterized by the presence of an inverted 5'-CCAAT sequence.