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# Monoclonal Antibody Expression Enhancement by Insertion of Chromatin Modifying Insulator Elements in Mammalian Expression Vector

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***Abstract:*** **The isolation of stably transfected cell lines suitable for the manufacturing of biotherapeutic protein products such as monoclonal antibodies, is a challenging process and depends on the identification of high expressing clone. This phenomenon involves transgene amplification and maintenance of the expressing clones over numerous generations. Maintenance of cell line and stable protein expression is always depending on the nature of genomic environment at the site of transgene integration. Epigenetic mechanism leads to variable expression and silencing of the transgene. One of the major limitation of introduction of foreign gene into chromosome is, their expression appears to vary with chromosomal site of integration. There are certain elements present in the genomic environment called as chromatin function modifying elements. These elements have ability to negate chromatin insertion site position effect and to express and maintain the stable monoclonal antibody expression in mammalian host. Upon stable cell line generation, chromosomal integration site of the vector DNA has major impact on transgene expression. Here in the current article, different genome integration elements such as Ubiquitous chromatin opening elements (UCOE), stabilizing anti repressor elements (STAR), Chicken hypersensitive site 4 (cHS4), CCCTC binding factor (CCTF), Scaffold \Matrix Attachment Region (S\MAR) are considered.**

***Keywords:*** **Genome integrating elements, Expression vector, Monoclonal antibody.**

## 1. INTRODUCTION

Monoclonal antibodies are extensively used in the biopharmaceutical industry for the treatment of variety of human diseases [1] [2] . It is fastest growing and best-selling class of the biotherapeutics industry [3]. This type of drug is adventitious because of the comparative small size, longer half-life, higher binding affinity towards target molecule and ability to use host immune system effector function such as antibody dependent cell cytotoxicity (ADCC) and complement dependent cell cytotoxicity (CDCC)[4-7]. The monoclonal antibodies are mainly produced in the mammalian expression system containing CHO (Chinese hamster ovary) cell lines as they have well post translational modification properties such as glycosylation and disulphide bond formation [6, 8-10] . The successful expression of monoclonal antibody is depending on proper protein folding and expression vector assembly for the heavy and light chain genes [11-14]. A number of studies shown that the site of integration greatly influences its expression and a factor which determines the cell behavior with respect to prolonged stability [15, 16] . The generation of stably transfected cells usually begins with random integration of gene of interest (GOI) into target cell genome. However, the majority of genome consists of transcriptionally non-permissive heterochromatin in any given cell type, there is a high probability of the GOI integration into an area which is unfavorable for high level and stable expression. On the other hand, although the genome integration occurs in the transcriptionally active region, expression may silence due to DNA methylation within the integrated

transgene or its promoter region. These epigenic processes are resulted into, variegated transgene expression pattern which is also known as position effect variegation (PEV) or complete silencing over the time [16]. Linking certain genetic elements like ubiquitous chromatin opening elements (UCOE), Scaffolds matrix attachment region (S/MAR), Stabilizing anti repressor elements, and insulators to GOI can reduce epigenic process from negatively affecting transgene expression even if the gene has been integrated into an area of closed heterochromatin. The chromatin functioning modifying elements are as follows.

## 2. UCOE-UBIQUITOUS CHROMATIN OPENING ELEMENTS

UCOE is an insulator genetic element used against heterochromatin expansion [17]. These elements were initially identified in the study of TBP-PSMB1 and the HNRPA2B1-CBX3 housekeeping gene loci. The first genomic fragment found to possess a ubiquitous chromatin-opening function and protect against the epigenetic silencing of transgenes was derived from the TATA-binding protein (*TBP*) locus, which is a region encompassing the *TBP* and proteasomal subunit C5-encoding (*PSMB1)* housekeeping genes. *TBP* and *PSMB1* are closely linked (within 1 kb) and divergently transcribed. Typical of housekeeping genes, *TBP* and *PSMB1* express ubiquitously and contain promoters encompassed by methylation-free CpG islands (CGI) [18] [19].

UCOE is methylation free CpG island that eliminates integrating position dependent effects and maintains the chromatin in "open" configuration to increase accessibility of the DNA region to transcription machinery. The recent research reported that Antibody production in CHO cells improved remarkably upon the incorporation of UCOE elements in the expression vector [20, 21]. It is claimed that UCOE enhanced the expression of antibody by six-fold in CHO stable transfection pools [22]. An alternative non-coding GC rich DNA fragment is proposed to be a novel UCOE as flanking the gene of interest with the GC rich fragment augments recombinant protein expression. It was subsequently proposed that rigidity of GC bond in a DNA double helix allows DNA secondary structure formation which can be affect methylation of the histones and finally resulted the configuration of chromatin. [23]

The UCOE gene used in the expression vector is 1.5 kb long or instead of using whole fragment 1.2 Kb sub -fragment can confer the stability of expression from linked promoters. In 2002, Benton and colleagues showed that A2UCOE-regulated transgenes integrated into CHO sub-clones adapted to grow in suspension and survive in serum-free media (CHO-S), are able to produce large quantities of protein rapidly, with the production up to 0.2 g/L of recombinant antibody obtained within five weeks of transfection. When UCOE contain vector was compared with non UCOE elements containing vector, it was observed that the presence of the A2UCOE increased recombinant protein expression per vector copy number at reproducible levels, with small variation in expression within and between cell lines transfected with UCOE-containing vectors [24]. Many biomanufacturing applications of UCOEs emphasis on the production of monoclonal antibodies. It was stated that the usage of A2UCOE-based vector system lead to increase a higher overall level of expression of antibody relative to the standard human Cytomegalovirus (*h*CMV) promoter when compared the efficiency of recombinant antibody production using a 4kb A2UCOE-based vector against a standard *h*CMV promoter-driven expression system in CHO-S cells [25]. Furthermore the A2UCOE-based vector exhibited a higher number of stably transfected clones, and outperformed constructs at medium-scale protein production levels however transgene expression was from the *h*CMV promoter alone, with antibody yields of 180 – 230mg obtained per 1L bioreactor flask [26] [27]. Similarly, when UCOE based vector system was compared against a commercially available immunoglobulin expression vector for stability and expression levels of heavy and light chains of the humanized anti-C2 monoclonal antibody, the UCOE-based vector, then it produced a greater number of stable clones with recombinant antibody production levels of up to 50 – 110mg/L [28].

## 3. STABILIZING ANTI REPRESSOR ELEMENTS (STAR)

The elements are composed of unique non-coding DNA sequences lacking CpG island. These elements were identified during the human genomic DNA fragment library screening [29]. The elements have ability to block chromatin associated repressors by allowing transfected cells to survive in the presence of antibiotic due to inactivation of silencing of linked resistance marker gene. Expression vector contain gene of interest is driven by different promoters and flanked by STAR elements results in to greater number of stably transfected cell colonies and increased transgene expression level proportion to transgene copy number [30].

## 4. CHICKEN HYPERSENSITIVE SITE 4 (CHS4)

It is Dnase I hypersensitive site, of the chicken beta- globin locus control region (cHS4). It functions as insulator element by shielding transgene from position effects. It is demonstrated that elements is having enhancer blocking as well as barrier activity. [31] [32]. Insulators are the elements which protect the transcribed region from distant unrelated regulatory sequences [33] [34]. 5' HS4 elements was first defined as insulator elements by the scientist Chung and Felsenfeld in the study of Drosophila. cHS4 is a 1.2 Kb DNA fragment derived by the 5' end of the chicken B globin locus [35]. Considerable activity of insulator was proved in the active 250 bp core region included in the cHS4 elements [36]. It is a GC rich region with the properties of CpG island and did not appear to function as promoter [37]. The recent study reveals that 250 bp core sequence of the cHS4 element can be used in the mammalian expression vector to have insulator activity [34].The function of the element found to be host cell dependent with effective insulator control [38].

## 5. CCCTC BINDING FACTOR (CTCF)

CCCTC is a 11-zinc finger protein and evolutionary conserved critical transcription factor involved in the various processes of gene regulation. It was originally identified as a repressor elements and later it was recognized as activator of a transcription. It was initially recognized for its ability to specifically bind regulatory sequences in the promoter proximal region of the MYC oncogene and in the silencer elements of the chicken lysozyme gene. CTCF is a long chain of 727 amino acids and contains 11 zinc finger central DNA binding domains. The full-length protein displaces 100% homology between mouse, chicken and human. CTCF is described as multivalent factor on the basis of its ability to bind to wide range of variant sequence and specific co regulatory proteins. This unique structural feature provided the first hint suggesting versatile role of CTCF in genome regulation distinct from most zinc finger proteins [39]. CTCF is reported to bind to a variety of DNA target sites those perform distinct function, including activation or repression of promoter, hormone responsive gene silencing, methylation dependent chromatin insulation and genomic imprinting. Out of these activities, insulator function of CTCF is attracted the greatest interest [40]. CTCF dependent insulators are characterized in the chicken B globin locus and in the imprinted igf2/H19 locus in mouse and human. B globin genes contain region and the erythrocyte specific enhancers are flanked on both sides by CTCF insulators in the 5' end , identified a 42 bp CTCF binding site both necessary and sufficient for enhancer blocking activity [41] [42].

The studies reveled that introduction of CTCF binding sites at either or both sides of the transgene cassette, led to remarkable reduction in the innate inflammatory response to vector and advances in the duration of transgene expression. On the other hand to typical definition of insulator, CTCF binding sites acted in position independent manner to inhibit innate immune response suggesting unexpected mechanism in play in the context of adenoviral vectors [43] [44]. Lloyd, Meller and colleagues studied the potential role of Drosophila CCCTC binding factor protein and proposed that drosophila CTCF has an evolutionary conserved role in the maintenance of imprinted states [45]. The versatile functions of CTCF are generally described according to a model in which CTCF confirmation is a function of differential zinc finger binding to divergent consensus sequences resulting in different binding partners , different post translational modification and finally multiple function role [46] [47].

## 6. SCAFFOLD \MATRIX ATTACHMENT REGION (S\MAR)

Matrix attachment region is AT rich genomic DNA sequence. It serves as attachment points within DNA [48]. MAR enables the anchoring of chromatin to the nuclear matrix during interphase [49].The most consistent features of the MARs include the prevalence of AT rich sequences, origin of replication, binding sites foe topoisomerase II, special AT rich binding protein 1 (SATB1) motifs, kinked DNA and curved DNA [50]. Recently use of MAR to produce recombinant protein has raised significant attention [49]. The studies demonstrated that these sequences enable increased production of recombinant proteins of biotechnological interest in mammalian cell lines [51]. Several studies also demonstrated that human MARs termed B globin MAR increase transgene expression and minimizes the probability of gene silencing in CHO cells [52]. Phenomenon of control of protein expression of transgene in CHO cells by MAR is not fully understood process. MARs were first discovered based on the principle of their association with relatively insoluble pretentious fraction of the cell nucleus called nuclear matrix or scaffold. It is proposed that MARs are structural components which anchor the chromosome to the matrix and partition chromosomes into loop structures [34]. It is hypothesized that MAR may form a genetic boundaries between chromosomal domain that independently organizes into structure permissive and non-permissive for gene expression referred as euchromatin and heterochromatin respectively [53]. A transgene flanked by S\MAR elements may therefore constitute an autonomous chromatin domain whose expression would remain

independent of the adjacent chromosomal environment. Consistent with this understanding, S\MARs are shown to increase the expression of adjacent transgene when co-inserted into a chromosomal environment. [54]. An alternative mechanism proposes that MARs directly recruit activities involved in a gene expression. For instance, S\MARs acts as entry site for regulatory proteins like histones acetyl transferases which modify the chromatin structure towards expression permissive states. Also, S\MARs were shown to mediate long range histone hyperacetylation and DNA hypomethylation effects in synergy with enhancers and may activate the expression nearby genes. The chicken lysozyme MAR was identified as one of the most active sequence in previous studies that compared the effect of several chromatin structure regulatory elements on transgene expression [55].

Research of monoclonal antibody expression in mammalian host over the two decades has demonstrated the ability of chromatin insulators and genome integrating elements to enhance both protein expression and ability of integrating vector system. There are many doubts about heterochromatin boundaries that remains yet to explored. Advantages and limitations of these boundary elements with respect to enhance protein expression can be solved by experimental analysis in all aspects with multiparameter study. From the current study of the insulators, it is suggested that, the study of chromatin modifying elements are very important in order to enhance protein production in mammalian host as the site of integration is key process.

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