Citation for published version (APA):
1. Introduction

The random integration of a transgene into a heterochromatic chromatin environment and the methylation of promoter DNA are major mechanisms that are antagonistic to gene expression, resulting in a variegated pattern of gene expression or silencing (Pikaart et al., 1998; Yang et al., 2010). Because stable and high level transgene expression are essential for the efficient and rapid production of clonal cell lines in biomanufacturing as well as for the lifelong expression of a transgene at a therapeutic level in gene therapy, there is a fundamental requirement for genetic regulatory elements, which can prevent gene silencing and maintain high levels of expression for long periods of time.

Genetic regulatory elements that confer a transcriptionally permissive state can be broadly dichotomised into those that actively function through dominant chromatin remodelling mechanisms and those that function as border or boundary elements to restrict the spread of heterochromatin marks into regions of euchromatin. The latter include insulators, scaffold/matrix attachment regions (S/MARs), and stabilising anti-repressor (STAR) elements, whilst the former comprise locus control regions (LCRs) and ubiquitous chromatin opening elements (UCOEs). LCRs and UCOEs are defined by their ability to consistently confer site of integration-independent stable transgene expression that is proportional to copy number, including from within regions of heterochromatin such as centromeres. UCOEs structurally consist of methylation-free CpG islands encompassing single or dual divergently-transcribed housekeeping genes. Since their discovery in 1999, UCOEs and their sub-fragments have found applications in areas of biotechnology requiring stable, reproducible, and high levels of gene expression. This review recounts the discovery of UCOEs and examines their current and future applications in protein therapeutic biomanufacturing and gene therapy.

2. Discovery of ubiquitous chromatin-opening elements

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, a tissue-specific regulatory elements that consist of multiple subcomponents characterised by DNase I hypersensitivity and a high density of transcription factor binding sites (Kim and Dean, 2012; Li et al., 2002; Tam et al., 2006). In contrast, UCOEs function ubiquitously and neither consist of multiple DNase I hypersensitive sites that are characteristic of LCRs, nor are they required to flank a transgene at both 5’ and 3’ ends in order to exert their function as in the case of insulators and S/MARs (Antoniou et al., 2003; Williams et al., 2005). Thus, structurally and functionally UCOEs represent a distinct class of genetic regulatory element.

UCOEs have found widespread usage in protein therapeutic biomanufacturing applications as a means to manage costs and resources as well as to reliably expedite the generation of highly expressing recombinant cell clones. Similarly, UCOEs show great promise in the field of gene therapy by providing stable ubiquitous or tissue-specific expression in somatic tissues as well as in adult, embryonic, and induced pluripotent stem cells and their differentiated progeny. This review recounts the discovery of UCOEs, and discusses their application in biomanufacturing and gene therapy.
region encompassing the TBP and proteosomal subunit C5-encoding (PSMB1) housekeeping genes (Fig. 1A) (Harland et al., 2002). 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). Functional expression analysis of a 44 kb TBP-PSMB1 fragment in stably transfected murine fibroblast L-cells after 60 days showed a level of TBP expression comparable to day zero, suggesting that this region protected the TBP promoter against silencing and enabled continuous and stable expression. In an effort to identify the location of regulatory elements of the TBP-PSMB1 locus, DNsase I hypersensitivity site mapping showed the presence of hypersensitive sites only at the TBP and PSMB1 promoters (Harland et al., 2002). The authors concluded that the combination of stable TBP expression within a transgene context, together with DNsase I hypersensitivity sites is structurally unlike that of previously identified LCRs (Li et al., 2002; Tam et al., 2006), indicating that a novel class of regulatory elements, which acts to negate epigenetic-mediated silencing and remodel chromatin structure, had been discovered.

Further functional expression analysis of the TBP-PSMB1 locus was undertaken, together with a second region of similar genomic architecture, the HNRPA2B1-CBX3 locus (Fig. 1B). The HNRPA2B1-CBX3 locus consists of a region of closely-linked, divergently-transcribed housekeeping genes: heterologous nuclear ribonucleoprotein A2/B1 (HNRPA2B1) and heterochromatin protein 1Hs-γ (CBX3). A 2.6 kb methylation-free CGI (defined as such because it has a GC content of 61.5% and an observed-to-expected CpG of ratio of 0.97, meaning that this region is GC rich and contains a far greater density of CpG dinucleotides than would be expected by chance, overlies the first alternate exons of CBX3 and the first exon of HNRPA2B1 (Antoniou et al., 2003; Williams et al., 2005). These early functional studies showed that the TBP-PSMB1 and HNRPA2B1-CBX3 loci, including their dual divergently-transcribed promoter regions, are able to dominantly open chromatin and confer stable gene expression as demonstrated by their ability to function even with transgene integration events into centromeric heterochromatin, with no observed position effect variegation (Antoniou et al., 2003), and thus represent the prototypical UCOEs.

The potential applications of UCOEs were first illustrated in 2005. Investigators linked fragments of the HNRPA2B1-CBX3 methylation-free CGI and associated promoters (A2UCOE) to the immediate-early promoter-enhancer region of the human cytomegalovirus (hCMV). Expression from the HNRPA2B1 and CBX3 promoters is relatively low and the hCMV promoter region represents a non-selective, strong promoter-enhancer combination commonly used in mammalian cells to achieve high level expression of linked genes. It was observed that the linkage of various sized fragments (1.5 kb, 4 kb, and 8 kb) of the A2UCOE to the hCMV promoter resulted in a marked elevation of transgene expression and a resistance to silencing for up to 107–199 generations within stably transfected Chinese Hamster Ovary (CHO) K1 cells (Williams et al., 2005). This ability of the A2UCOE to confer a site of integration-independent, dominant chromatin-opening function and to protect linked transgenes from silencing showed great promise for biomanufacturing and gene therapy.

Structural similarities between the TBP-PSMB1 UCOE and A2UCOE suggested potential functional mechanisms through common characteristics (Fig. 1). Both loci consisted of dual divergently-transcribed housekeeping genes with promoters encompassed by an extensive methylation-free CGI; it was therefore proposed that the chromatin remodelling capacity of UCOEs stemmed from the presence and combination of these features. Characterisation of the epigenetic signature of the native A2UCOE region in peripheral blood mononuclear cells revealed the coexistence of active histone H3 methylation and acetylation marks at the transcriptional start sites of HNRPA2B1 and CBX3. Euchromatic histone H4 acetylation was also observed to be present throughout the A2UCOE region. However, overall histone modifications within the proximity of the A2UCOE were sparse due to nucleosome depletion (Lindahl Allen and Antoniou, 2007; Majocchi et al., 2014). Reduction of the levels of repressive histone marks
Furthermore, the A2UCOE-based vector showed a higher number of expression of antibody relative to the standard driven expression system in CHO-S cells, it was observed that the use of a 4 kb A2UCOE-based vector against a standard promoter combination for a given cell line (Simpson et al., 2009). A comparison of the 3 kb Rps3 UCOE and 1.5 kb A2UCOE core subfragment linked to seven different heterologous promoters expressing a large B-domain deleted factor VIII (BDD-FVIII) blood clotting factor gene, was undertaken in the baby hamster kidney (BHK) fibroblast cell line (Nair et al., 2011). Linked to all heterologous promoters tested, the A2UCOE showed greater levels of BDD-FVIII production and activity in adherent and serum-free suspension cultures. However, in CHO cells, the Rps3 UCOE linked to hCMV was shown to outperform the A2UCOE, illustrating the importance of identifying the most effective UCOE-promoter combination for a given cell line (Simpson et al., 2009). A recent study systematically compared the effect of different factors on antibody production, including the use of UCOEs and promoters, in CHO-S cells (Rocha-Pizaña et al., 2017). The authors compared the use of double (Rps3 and 1.5 kb A2UCOE) and single (Rps3) UCOE-containing vectors, and found that the double UCOE-based vector gave a higher level of antibody production. In addition, three different heterologous promoters linked to the Rps3 UCOE were compared for their ability to drive expression of either the anti-TNFα or anti-CD20 + antibodies. The results varied, indicating that the most effective promoter is dependent on the protein being expressed. Furthermore, the order of heavy and light chain genes in the expression cassette was investigated and found to have no significant effect on antibody production (Rocha-Pizaña et al., 2017).

Experiments involving co-transfection of plasmid vectors harbouring separate light and heavy chain antibody cassettes under the control of the hCMV promoter with and without the A2UCOE, have been also reported (Nematpour et al., 2017). The results obtained indicate that including the A2UCOE as part of the heavy chain gene cassette is most crucial to obtaining increased and stable antibody output compared with constructs not containing the A2UCOE. (Nematpour et al., 2017). UCOEs are available commercially for biomanufacturing and research applications in the form of ready-to-use plasmid vectors contain-
ing expression and selection elements and as DNA sequences that can be cloned into a vector of choice. An illustration of using the commercial UCOE vector offering is shown in Fig. 2. Here expression of four different monoclonal antibodies were compared in the presence and absence of the 3 kb Rps3 UCOE in different CHO cell-based hosts. For this purpose, triplicate flasks of cells were stably transfected, placed under selection in bulk and then analyzed in batch cultures. Although expression of the individual monoclonal antibodies differed across the panel, the inclusion of the Rps3 UCOE in the vector improved bulk stable pool expression by > 10-fold for each antibody (Fig. 2).

The inclusion of the Rps3 UCOE confers benefits in the cell line development process beyond increased monoclonal antibody titres (Fig. 2). Bulk Rps3-UCOE and non-UCOE vector-derived cell pools (Fig.2) were separated into single cell clones by limiting dilution and expanded in static cultures for two weeks. At that time, > 500 clones for UCOE and non-UCOE control cultures were evaluated for cell number and monoclonal antibody titer. Titer was divided by cell number to provide a relative expression level and the most productive 100 clones for each pool are shown in descending order (Fig. 3). Strikingly, the specific productivity of UCOE-derived clones dwarfs the collective productivity of the control clones. Furthermore, each of the 100 UCOE-derived clones has higher relative productivity than 98% of the control clones. The most productive 20 clones were selected for batch culture titer evaluation and the results of the top five UCOE-derived and control cultures are shown in the inset of Fig. 3. UCOE-derived clones produced as much as 0.7 g/L in the small scale batch evaluation whereas the control cultures produced < 0.1 g/L. Since the incorporation of the Rps3 UCOE aids in promoting stable transgene expression, fewer clones need to be screened to isolate high producers of the desired therapeutic protein. Moreover, the increased productivity of UCOE-derived clones alleviates the need to amplify the integrated transgenes as in DHFR CHO based systems (Gu et al., 1992; Kaufman and Sharp, 1982). Therefore, UCOE-based vectors provide benefits in the cell line development workflow including reduced resources required as well as potential time savings to isolate highly productive clones.

In summary, UCOEs confer reproducible and stable high levels of linked gene expression when stably transfected into serum-free, suspension-adapted, and adherent cell lines widely used in biomanufacturing applications. Furthermore, UCOEs have shown utility in the rapid

Fig. 2. UCOE enhances bulk titer monoclonal antibody expression. Four different monoclonal antibodies were cloned into MilliporeSigma commercial offering of single expression or dual plasmid expression vectors with and without the Rps3 UCOE. DNA was stably transfected into suspension-adapted CHO host cells by electroporation and cells selected for transgene integration by addition of puromycin and then subjected to antibody titer evaluation secreted into the batch culture medium. Titers were obtained using HPLC.

Fig. 3. UCOE enhances the productivity of clonal cells. Bulk pools of stably transfected UCOE vector-derived and control cultures were sub-cloned using limiting dilution. Greater than 500 clones of each were analyzed for antibody titer secreted into the culture medium using an ELISA and analyzed for cell number by flow cytometry. Relative Productivity is represented as the ratio of monoclonal antibody titer divided by the cell number measurement. The inset shows the batch antibody titer of the five highest expressing UCOE-derived and non-UCOE control clones as determined by ELISA.
selection and isolation of highly expressing clones. The usage of UCOEs within biomanufacturing holds promise for expediting upstream biomanufacturing stages by facilitating the production and selection of a candidate clonal cell population in less than three months. However, there is still a requirement to select the most effective UCOE-heterologous (viral) promoter combination for maximum protein therapeutic biomanufacture depending on the nature of the protein to be produced and especially the cell line to be employed.

4. Applications of UCOEs in gene therapy

Gene therapy is the treatment of a disease or medical disorder through the introduction of exogenous therapeutic genetic material into appropriate cellular targets in order to overcome the effects of specific genetic mutations and/or to normalise cellular function. Successful gene therapy therefore relies upon a means to introduce exogenous genetic material in an efficient, target-specific manner, and the stable incorporation and expression of the genetic material from within the target cell for the appropriate period of time.

Lentiviral vectors (LVs) represent one of the most promising gene delivery systems in the field of human gene therapy. LVs offer a reliable means by which exogenous genetic material can be integrated into dividing and non-dividing target cell genomes and their use has shown encouraging outcomes in a number of clinical trials for conditions such as primary immunodeficiencies (Sauer et al., 2014) and leukodystrophies (Aubourg, 2016). Once integrated into the target cell genome the therapeutic transgenes are inherited upon mitosis and in principle can be expressed over long periods. However, natural cellular defence mechanisms mediate the epigenetic silencing of proviral genetic material through DNA methylation, histone deacetylation, and chromatin condensation, acting to diminish or even reverse therapeutic effects (Antoniou et al., 2013). It is therefore important, if gene therapy using LVs is to be effective, that the epigenetic silencing of therapeutic transgenes be prevented by incorporating protective regulatory elements.

The first studies looking at UCOE function within LVs investigated the use of a 2.2 kb A2UCOE sub-fragment (Fig. 4A, fragment A) (Antoniou et al., 2003) linked to reporter genes expressing from the inherent HNRPA2B1 promoter (Fig. 4B, construct I). In this configuration the A2UCOE was shown to provide higher expression, greater stability, and more reproducible levels of expression per vector copy number than the spleen focus-forming virus (SFFV), hCMV, and elongation factor-1α (EF1α) promoters within ex vivo haematopoietic stem cell (HSC) transduction-transplantation experiments in mice (Zhang et al., 2007). The A2UCOE has also been shown to confer sustained expression of an EGFP reporter in vivo for 10 months, after the transplantation of transduced human fetal liver-derived HSCs into immunocompromised mice, whereas in the same period, expression from vectors driven by PGK and EF1α promoters showed a 5-fold and 22-fold reduction in expression, respectively (Dighe et al., 2014). Recently the utility of A2UCOE-based LVs has been further extended by the demonstration that they can confer stable, long-term expression following pre-natal delivery to the fetal liver and haematopoietic stem cells (Kao et al., 2016). Furthermore, post-natal haemophilia B curative levels of human FIX were produced following low level delivery of an A2UCOE-FIX LV to the fetal liver (Kao et al., 2016).

The capability of the A2UCOE to resist silencing has been found to be at least in part due to its ability to resist DNA methylation (Zhang et al., 2010). In summary, whereas in the short-term, there is no discernible difference in the levels of expression driven by promoters with and without a linked UCOE due to the time taken for transgene silencing (de Poorter et al., 2007), frequently used promoters from either viruses (SFFV, CMV) or native genes (EF1α, PGK) are invariably silenced over a period of weeks or months, resulting in a variegated expression pattern post-transduction and compromised transgene expression (Dighe et al., 2014; Zhang et al., 2010).

The potential utility of UCOEs within a gene therapy context was further extended when it was demonstrated that their linkage to tissue-specific heterologous promoters retains promoter tissue specificity, which frequently demands tissue-restricted therapeutic gene transcription. It has been shown that the 1.5 kb A2UCOE core sub-fragment (Fig. 4A, fragment B) linked to the muscle-specific desmin (DES) and granulocyte-specific myeloid-related protein-8 (MRP8) promoters (Fig. 4B, construct II) confers stable and appropriate tissue-specific expression (Brendel et al., 2012; Talbot et al., 2010). Furthermore, an A2UCOE-MRP8-gp91phox LV was shown to be able to completely rescue the X-linked chronic granulomatous disease phenotype in a mouse model system following ex vivo transduction-transplantation of HSCs at low average vector copy number per cell (Brendel et al., 2012).

The generation of induced pluripotent stem cells (iPSCs) from adult human cells with well-defined reprogramming factors, followed by genetic modification, directed differentiation, and then transplantation into patients, holds great promise as a treatment in regenerative medicine (Karagiannis and Eto, 2016; Takahashi et al., 2007). One potential limitation of this technique is the epigenetic-mediated silencing of therapeutic transgenes introduced into iPSCs, especially upon their differentiation down the desired lineage. Because of this, several investigators have examined the function of UCOEs in this class of stem cells. The 1.5 kb A2UCOE linked to heterologous promoters has been found to consistently confer high and stable gene expression in human and murine iPSCs and in embryonic stem cells before and after differentiation into lineages representative of all three germ layers (Ackermann et al., 2014; Pfaff et al., 2013).

The inherent limited capacity of viral vectors requires that regulatory elements present within the incorporated therapeutic transcription unit must be minimised to allow for maximisation of transgene capacity. Hence, over the past decade various sized sub-fragments of UCOEs, particularly those derived from the A2UCOE, have been built and tested in order to maximise the size available space for linked transgenes (Fig. 4A). Initial studies showed that a 2.2 kb A2UCOE region encompassing both the CBX3 and HNRPA2B1 transcriptional start sites and the methylation-free CGI and with transgenes driven directly off the innate HNRPA2B1 promoter (Antoniou et al., 2003), maintains stable expression in a LV context both in vitro and in vivo (Zhang et al., 2007). The preferred A2UCOE sub-fragment that confers stability of expression when linked to heterologous ubiquitous and tissue-specific promoters, was the 1.5 kb core element (Fig. 4A, fragment B), which again extends over the alternative first exons of CBX3 and into the first intron of HNRPA2B1 (Fig. 4B, construct II) (Brendel et al., 2012; Zhang et al., 2010). A 1.2 kb sub-fragment of this 1.5 kb element, which lacks 300 bp from the HNRPA2B1 end, was first shown to possess an equal UCOE capability when linked to the highly silencing-prone SFFV promoter (Zhang et al., 2010). Uchiyama and colleagues investigated the function of a 0.6-kb A2UCOE sub-fragment that encompasses only the HNRPA2B1 promoter and 5′ flanking region, but no CBX3 sequences (Fig. 4A, fragment D). Linked to EGFP and the Wiskott-Aldrich syndrome protein gene (WAS) (Fig. 4B, construct IV), this element showed stable and long-term expression upon Foamy virus vector transduction into HSCs in vitro and in vivo comparable to the endogenous WAS promoter (Uchiyama et al., 2012). More recently a 0.7 kb A2UCOE-derived sub-fragment incorporating the alternate first exons and promoter of CBX3 (Fig. 4A, fragment C) was shown to possess anti-silencing functions comparable to that of the 1.5 kb A2UCOE in multipotent and pluripotent stem cells when linked to the SFFV and MRP8 promoters (Müller-Kuller et al., 2015). In addition, expression from the inherent CBX3 promoter of the 0.7 kb sub-fragment (Fig. 4B, construct III) was also shown to be stable, albeit at very low levels (Müller-Kuller et al., 2015).

Severe combined immunodeficiency (SCID) is a life-threatening group of primary immune deficiencies caused by a number of genetic mutations that result in the abnormal development of lymphocytes. The most common form of SCID, representing 50–60% of cases, is X-linked...
SCID (SCID-X1), which is caused by mutations in the interleukin-2 receptor gene (IL2RG) encoding the common \( \gamma \)-chain cytokine receptor subunit (Fischer, 2000). In 2007, Zhang and colleagues achieved efficient full immunogenic reconstitution within a SCID-X1 mouse model system using an LV-A2UCOE-IL2RG cassette based on expression directly off the HNRPA2B1 promoter (Fig. 4B, construct I) and an ex vivo HSC transduction-transplantation procedure at low vector copy number per cell, which was superior to that obtained with an SFFV-IL2RG vector (Zhang et al., 2007).

SCID can also stem from deficiencies of RAG2, resulting in the total absence of T and B cells. In a murine Rag2 knockout model system, incomplete immunogenic reconstitution was achieved with a LV construct containing an SFFV promoter driving expression of RAG2. Although T cell proliferation and antibody responses, and plasma antibody and T cell receptor levels were restored, subnormal levels of B cells and double-positive T cells were observed. It was concluded that the silencing of the SFFV promoter through DNA methylation may have been responsible for the incomplete phenotype reversal. Replacement of the SFFV promoter with the 2.2 kb A2UCOE (Fig. 4A, fragment A) resulted in a greater correction of the phenotype, including B cell reconstitution to near normal levels (van Til et al., 2012).

Mutant O\(^6\)-methylguanine DNA methyltransferase (MGMT\(^{P140K}\)) is protective against certain chemotherapeutic agents and has shown clinical potential as a myeloprotective agent in glioblastoma patients treated with temozolomide (Adair et al., 2012). Transduction of myeloid and lymphoid cells with an LV containing an 1.5 kb A2UCOE-PGK construct provided high and stable levels of expression, which allowed for transgenic cell selection and conferred significant myeloprotection against a combination of O\(^6\)-benzylguanine and carmustine in a mouse model (Phaltane et al., 2014).

The risks of insertional mutagenesis are reduced through the usage of self-inactivating (SIN) viral vector systems. However, enhancer elements found in internal regulatory regions present within transgenes can still possess mutagenic potential. The A2UCOE is an enhancer-less element and as such vectors incorporating the A2UCOE are at reduced risk of insertional mutagenesis events compared to vectors containing enhancers (Zhang et al., 2007). A second safety consideration is the...
elimination of aberrant splicing. A number of studies have reported examples of vector-mediated aberrant splicing, a potential source of vector toxicity (Almarza et al., 2011; Cavazzana-Calvo et al., 2010; Moiani et al., 2012; Montini et al., 2009). Knight and colleagues reported that both native and activated cryptic splice donor sites within the A2UCOE act as sources of aberrant transcripts (Knight et al., 2012). However, targeted point mutations successfully inactivated splice donor sites within the A2UCOE, negating this source of potential insertional mutagenesis (Knight et al., 2012).

In addition to its use within LV systems, the A2UCOE has also been evaluated in a non-viral replicating episomal plasmid vector context. Upon incorporation into a plasmid, the 1.5 kb A2UCOE linked upstream of an hCMV promoter stably increased levels of transgene expression (Hagedorn et al., 2013).

The functional characteristics of UCOEs are unprecedented amongst genetic regulatory elements. Compared to other commonly used promoters, the A2UCOE, with expression from either the innate HNRPA2B1 promoter or when linked to a heterologous promoter, shows greater stability and levels of transgene expression. The A2UCOE also conserves tissue-specific expression and maintains stability of expression in adult, embryonic, and induced pluripotent stem cells before and after differentiation. Thus the A2UCOE is potentially a powerful tool in both LV and episomal plasmid vector gene therapy contexts. 5. Conclusion

The UCOE class of genetic regulatory elements shows broad and well-established utility in protein therapeutic biomanufacturing and encouraging applications in retroviral vector-based gene therapy. In cell lines commonly used for the production of recombinant monoclonal antibodies and other proteins, UCOEs based on the human HNRPA2B1-CBX3 and murine Rp3 loci have shown the ability to confer reproducible and high levels of linked gene expression and protein production. In many instances, expression is higher with the UCOE than with standard protocols and expression systems. The utility of UCOEs to markedly expedite the rapid selection of highly-expressing cell clones has also been shown. As such, the UCOE-based gene expression platform shows promise as a means to improve the time and cost efficiency of upstream biomanufacturing stages by increasing the proportion of stable highly-expressing clones and expediting clonal cell line isolation.

The A2UCOE has been the most widely investigated UCOE in the field of gene therapy. Alone or linked to heterologous ubiquitous or tissue-specific promoters, the A2UCOE has been shown to confer stable and long-term transgene expression not only in somatic cells, but also in adult, embryonic, and induced pluripotent stem cells, and their differentiated progeny. The A2UCOE has been shown to outperform other commonly used promoter elements over extended time periods, including the hCMV, SSFFV, EF1α, and PGK promoters. The A2UCOE functions in viral and plasmid vector contexts and functional subfragments of the A2UCOE have been identified, enabling the maximization of vector transgene capacity. Several studies have shown clinical promise for the A2UCOE as a ubiquitously-acting gene therapy regulatory element where long-term, high expression levels are required with minimal variation and insertional mutagenesis risk.

Conflicts of interest

MNA holds inventor status on patents covering the biotechnological applications of UCOEs and receives income from out-licensing of the UCOE gene expression system and acts as a consultancy capacity to MilliporeSigma. JO and KM are employees of MilliporeSigma, who is the owner of the intellectual property rights to the UCOE gene expression platform. JJJN declares that he has no conflicts of interest.

Acknowledgements

Work that led to the discovery and subsequent biotechnological exploitation of UCOEs was funded by the Biotechnology and Biological Sciences Research Council UK, (grant numbers 18/GTH12532, iCASE Studentship 10497, iCASE Studentship BB/M016390/1) and Medical Research Council UK (iCASE Studentship G78/6209). ML Laboratories plc (UK) and EMD Millipore (USA) are industrial partners in the iCASE studentships.

References

Acknowledgements

Work that led to the discovery and subsequent biotechnological exploitation of UCOEs was funded by the Biotechnology and Biological Sciences Research Council UK, (grant numbers 18/GTH12532, iCASE Studentship 10497, iCASE Studentship BB/M016390/1) and Medical Research Council UK (iCASE Studentship G78/6209). ML Laboratories plc (UK) and EMD Millipore (USA) are industrial partners in the iCASE studentships.

References

A2UCOE also conserves tissue-specific expression and maintains stability of expression in adult, embryonic, and induced pluripotent stem cells before and after differentiation. Thus the A2UCOE is potentially a powerful tool in both LV and episomal plasmid vector gene therapy contexts. 5. Conclusion

The UCOE class of genetic regulatory elements shows broad and well-established utility in protein therapeutic biomanufacturing and encouraging applications in retroviral vector-based gene therapy. In cell lines commonly used for the production of recombinant monoclonal antibodies and other proteins, UCOEs based on the human HNRPA2B1-CBX3 and murine Rp3 loci have shown the ability to confer reproducible and high levels of linked gene expression and protein production. In many instances, expression is higher with the UCOE than with standard protocols and expression systems. The utility of UCOEs to markedly expedite the rapid selection of highly-expressing cell clones has also been shown. As such, the UCOE-based gene expression platform shows promise as a means to improve the time and cost efficiency of upstream biomanufacturing stages by increasing the proportion of stable highly-expressing clones and expediting clonal cell line isolation.

The A2UCOE has been the most widely investigated UCOE in the field of gene therapy. Alone or linked to heterologous ubiquitous or tissue-specific promoters, the A2UCOE has been shown to confer stable and long-term transgene expression not only in somatic cells, but also in adult, embryonic, and induced pluripotent stem cells, and their differentiated progeny. The A2UCOE has been shown to outperform other commonly used promoter elements over extended time periods, including the hCMV, SSFFV, EF1α, and PGK promoters. The A2UCOE functions in viral and plasmid vector contexts and functional subfragments of the A2UCOE have been identified, enabling the maximisation of vector transgene capacity. Several studies have shown clinical promise for the A2UCOE as a ubiquitously-acting gene therapy regulatory element where long-term, high expression levels are required with minimal variation and insertional mutagenesis risk.

Conflicts of interest

MNA holds inventor status on patents covering the biotechnological applications of UCOEs and receives income from out-licensing of the UCOE gene expression system and acts as a consultancy capacity to MilliporeSigma. JO and KM are employees of MilliporeSigma, who is the owner of the intellectual property rights to the UCOE gene expression platform. JJJN declares that he has no conflicts of interest.

Acknowledgements

Work that led to the discovery and subsequent biotechnological exploitation of UCOEs was funded by the Biotechnology and Biological Sciences Research Council UK, (grant numbers 18/GTH12532, iCASE Studentship 10497, iCASE Studentship BB/M016390/1) and Medical Research Council UK (iCASE Studentship G78/6209). ML Laboratories plc (UK) and EMD Millipore (USA) are industrial partners in the iCASE studentships.


