two elements is usually on the 3´ side of an A residue. In vivo, the efficiency with which different polyadenylation sites are processed varies considerably. The assembly speed of the polyadenylation protein complex is a multistep process and correlates with the strength of the polyadenylation signal sequence. For example, due to faster assembly rate cleavage in the strong SV40 late polyadenylation signal occurs more rapidly than in the weaker SV40 early polyadenylation signal (Chao et al., Molecular and Cellular Biology, Vol.19 (8), 5588 – 5600, 1999).

There is the need to identify alternative strong or even very strong polyadenylation signals to accelerate the generation of high producer cell lines for the production of recombinant proteins. The use of strong or even very strong polyadenylation signals enhances transcriptional termination which in turn results in increased production, stability, nuclear export and/or translation of vector encoded mRNA. This should lead to higher mRNA levels and hence result in higher productivity of producer cells.

SUMMARY ~~OF THE INVENTION / SOLUTION~~

Here we describe a new polyadenylation signal isolated from the growth hormone of the Chinese hamster (*Cricetus griseus*). Surprisingly, it has been found that this newly identified polyadenylation signal, named HGH, outperforms the strong polyadenylation signals BGH and SV40 late. When using vectors comprising HGH as polyadenylation signal sequence protein titers in transient transfections of CHO-DG44 cells were increased up to 35 % compared to cells comprising BGH. In stable cells high specific productivities up to 45 pg/cell/day and titers in fed batch processes up to 6.3 g/L were obtained.

One embodiment of the present ~~invention~~disclosure is a polynucleotide sequence comprising at least one HGH polyadenylation signal and at least one heterologous nucleotide sequence encoding a product of interest. The HGH polyadenylation signal is downstream and operably linked to the heterologous nucleotide sequence(s).

Another embodiment of the present ~~invention~~disclosure is a novel vector comprising at least one heterologous nucleotide sequence encoding a product of interest and at least

one HGH polyadenylation signal. The HGH polyadenylation signal is downstream and operably linked to the heterologous nucleotide sequence(s). A further embodiment of the present ~~invention~~disclosure is a novel vector or polynucleotide sequence comprising at least one HGH polyadenylation signal operably linked to an upstream multiple cloning site which allows the cloning of the gene of interest via recognition sequences for restriction endonucleases. Yet another embodiment of the present ~~invention~~disclosure is a eukaryotic cell, preferably a mammalian cell, comprising the HGH polyadenylation signal. Yet a further embodiment of the present ~~invention~~disclosure is a method for producing a product of interest comprising culturing eukaryotic cells, preferably mammalian cells transfected with vectors or polynucleotide sequences comprising the HGH polyadenylation signal. In a preferred embodiment the product of interest is a polypeptide and the desired polypeptide is recovered from the culture medium.

The data of the present ~~invention~~disclosure show the impact of the HGH polyadenylation signal sequence on the transient expression of sICAM (Figure 6). Surprisingly, the highest sICAM expression is obtained with the polyadenylation signal sequence derived from the growth hormone gene of hamster. The titer is increased up to 21% (transfection series #1) compared to cells transfected with the vector pJR110 containing the BGH polyadenylation signal and increased up to 40 % (transfection series #1) compared to cells transfected with the vector pJR106 containing the SV40 late polyadenylation signal.

The data of the present ~~invention~~disclosure furthermore show the impact of HGH polyadenylation signal on the transient expression of an IgG4 antibody. Surprisingly, titers obtained with the HGH polyadenylation signal sequence are on average 35% higher than for the BGH polyadenylation signal (Figure 7).

The data of the present ~~invention~~disclosure additionally show a test of different HGH variants (Figure 8). The shortest HGH sequence of 113 bp contained in the expression vector pJR135 leads to a up to 78 % reduced sICAM expression in comparison to the HGH sequence of 324 bp contained in the expression vector pJR131. Whereas the HGH sequence of 189 bp contained in the expression vector pJR134

results in a very good sICAM expression comparable to the expression level achieved with BGH (Figure 7). The best expression result is achieved with the HGH sequence of 324 bp contained in the expression vector pJR131 (Figure 8), which is much better (35%) than the expression achieved with BGH polyadenylation signal (Figure 7).

This shows that between the HGH region of bp 190 to 324 of SEQ ID NO:8 sequences are located which contribute to an efficient expression of a gene of interest.

The data of the present ~~invention~~disclosure furthermore show stable expression of proteins at high levels using the HGH polyadenylation signal. Cell pools and cell clones with specific productivities in the range of 10 - 45 pg/cell/day and titers in fed batch processes of up to 6.3 g/L are obtained (Figure 9).

The ~~invention~~disclosure relates to a polyadenylation signal comprising a nucleic acid comprising a sequence at least 75% identical to SEQ ID NO:9. The ~~inventiion~~disclosure further relates to a polyadenylation signal comprising a nucleic acid comprising a sequence having at least 75% identity to SEQ ID NO:9. The ~~invention~~disclosure furthermore relates to a polyadenylation signal comprising a nucleic acid comprising a sequence with at least 75% identity to SEQ ID NO:9. The ~~invention~~disclosure specifically relates to a polyadenylation signal comprising a nucleic acid consisting essentially of a sequence at least 75% identical to SEQ ID NO:9.

The ~~invention~~disclosure preferably relates to a polyadenylation signal comprising a nucleic acid consisting of a sequence at least 75% identical to SEQ ID NO:9. The ~~invention~~disclosure furthermore relates to a polyadenylation signal comprising a nucleic acid comprising SEQ ID NO:9.

In a specific embodiment the ~~invention~~disclosure relates to a polyadenylation signal comprising a nucleic acid comprising a sequence at least 75% identical to SEQ ID NO:8. The ~~invention~~disclosure specifically relates to a polyadenylation signal comprising a nucleic acid consisting essentially of a sequence at least 75% identical to SEQ ID NO:8. The ~~invention~~disclosure preferably relates to a polyadenylation signal comprising a nucleic acid consisting of a sequence at least 75% identical to SEQ ID NO:8. The

~~invention~~disclosure furthermore relates to a polyadenylation signal comprising a nucleic acid comprising SEQ ID NO:8.

In a further embodiment of the present ~~invention~~disclosure the polyadenylation signal comprises a sequence at least 80%, 85%, 90%, 95% or 98% identical to SEQ ID NO:9 or SEQ ID NO:8. In ~~a specific embodiment the invention relates to~~one aspect, there is provided a polyadenylation signal comprising a nucleic acid comprising a sequence at least 85% identical to SEQ ID NO: 9. In another specific embodiment the ~~invention~~disclosure relates to a polyadenylation signal comprising a nucleic acid comprising a sequence at least 95% identical to SEQ ID NO:9. In a specific embodiment the ~~invention~~disclosure relates to a polyadenylation signal comprising a nucleic acid comprising a sequence at least 85% identical to SEQ ID NO:8. In another specific embodiment the ~~invention~~disclosure relates to a polyadenylation signal comprising a nucleic acid comprising a sequence at least 95% identical to SEQ ID NO:8.

~~The invention relates to~~In another aspect, there is provided a nucleic acid the sequence of which comprises SEQ ID NO:9. Preferably, the ~~invention~~disclosure relates to a nucleic acid the sequence of which consists essentially of SEQ ID NO:9. More preferably, the ~~invention~~disclosure relates to a nucleic acid the sequence of which consists of SEQ ID NO:9.

The ~~invention~~disclosure relates to a nucleic acid the sequence of which comprises SEQ ID NO:8. Preferably, the ~~invention~~disclosure relates to a nucleic acid the sequence of which consists essentially of SEQ ID NO:8. More preferably, the ~~invention~~disclosure relates to a nucleic acid the sequence of which consists of SEQ ID NO:8.

In a preferred embodiment said polyadenylation signal is isolated. In a preferred embodiment the ~~invention~~disclosure relates to an isolated polyadenylation signal comprising a nucleic acid comprising a sequence at least 75% identical to SEQ ID NO:9. In another preferred embodiment the ~~invention~~disclosure relates to an isolated polyadenylation signal comprising a nucleic acid comprising a sequence at least 95% identical to SEQ ID NO:9. In still another preferred embodiment the ~~invention~~disclosure relates to an isolated polyadenylation signal comprising a nucleic acid comprising SEQ ID

NO:9. In a further preferred embodiment the ~~invention~~disclosure relates to an isolated polyadenylation signal comprising a nucleic acid comprising a sequence at least 75% identical to SEQ ID NO:8. In another preferred embodiment the ~~invention~~disclosure relates to an isolated polyadenylation signal comprising a nucleic acid comprising a sequence at least 95% identical to SEQ ID NO:8. In still another preferred embodiment the ~~invention~~disclosure relates to an isolated polyadenylation signal comprising a nucleic acid comprising SEQ ID NO:8.

Preferably, the ~~invention~~disclosure relates to an isolated nucleic acid the sequence of which comprises SEQ ID NO:9. More preferably, the ~~invention~~disclosure relates to an isolated nucleic acid the sequence of which comprises SEQ ID NO:8.

In a preferred embodiment said polyadenylation signal is operably linked to a heterologous coding sequence. In a specifically preferred embodiment said polyadenylation signal is characterized by that the titers / expression levels obtained with the said polyadenylation signal are at least 10%, preferably 20% and most preferably 30% higher than those obtained for the BGH polyadenylation signal. In a most preferred embodiment they are at least and/or on average 35% higher than those obtained for the BGH polyadenylation signal.

The ~~invention~~disclosure specifically relates to a nucleic acid the sequence of which comprises SEQ ID NO:9 operably linked to a heterologous coding sequence. Alternatively, the sequence of which consists essentially of SEQ ID NO:9 operably linked to a heterologous coding sequence. Preferably, the sequence of which consists of SEQ ID NO:9 operably linked to a heterologous coding sequence.

The ~~invention~~disclosure furthermore relates to a nucleic acid the sequence of which comprises SEQ ID NO:8 operably linked to a heterologous coding sequence. Alternatively, the sequence of which consists essentially of SEQ ID NO:8 operably linked to a heterologous coding sequence. Preferably, the sequence of which consists of SEQ ID NO:8 operably linked to a heterologous coding sequence.

A nucleic acid the sequence of which comprises SEQ ID NO:9 or 8 and has terminator function. Preferably said nucleic acid has terminator function and is operably linked to a heterologous coding sequence.

The ~~invention~~disclosure furthermore relates to a vector or polynucleotide sequence which comprises any one of the polyadenylation signals or nucleic acid sequences as described above. In another aspect, there is provided a vector or polynucleotide sequence comprising any one of said polyadenylation signals or said nucleic acids as defined herein. In a specific embodiment said polyadenylation signals or nucleic acid sequences are operably linked to an expression unit / expression cassette. In another embodiment of the ~~invention~~disclosure the vector comprises the selection and/or amplification marker dihydrofolate reductase (DHFR), glutamine synthetase or neomycin phosphatase (neo). In a preferred embodiment of the present ~~invention~~disclosure the vector or polynucleotide sequence comprises a heterologous gene of interest encoding for a heterologous product of interest. Preferably said product is a polypeptide. Preferably said polypeptide is an antibody, antibody fragment or fusion protein.

~~The invention, additionally relates to~~In another aspect, there is provided a cell comprising any one of the vectors or polynucleotide sequences as described above. Preferably, the cell comprises any one of the polyadenylation signals or nucleic acid sequences as described above operably linked to a transcription unit encoding a product of interest. Preferably said product of interest is a nucleotide / nucleic acid of interest. In another embodiment of the cell said product of interest is a polypeptide of interest encoded by a gene of interest. Preferably said polypeptide is an antibody, antibody fragment or fusion protein.

In a specific embodiment said cell is a eukaryotic cell, a mammalian cell, a hamster cell or a murine cell. Preferably said cell is a hamster cell. More preferably, said cell is Chinese hamster ovary (CHO) cell. Most preferably said cell is a CHO DG44, CHO-K1 or DUKX-B11 cell. In another preferred embodiment said cell is a NSO cell. In a preferred embodiment said cells as described are cultured cells. Preferably, said cells are cultured in serum-free medium. Preferably said cells are grown in suspension culture. In another preferred embodiment of the present invention the cell is characterized by that the titers / expression levels obtained with said polyadenylation signal or nucleic acid sequence are at least 10%, preferably 20% and most preferably 30% higher than

those obtained for the BGH polyadenylation signal. In a most preferred embodiment they are at least and/or on average 35% higher than those obtained for the BGH polyadenylation signal. Preferably, said cell has 35% higher expression levels.

~~The invention, additionally relates to~~In another aspect, there is provided a method of making a polypeptide of interest encoded by a gene of interest, the method comprising:

(a) Providing a host cell comprising a vector or polynucleotide sequence as described above or providing a cell as described above,

(b) Cultivating said cells, under conditions which allow the proliferation of the cells and the expression of the gene of interest,

(c) Harvesting the polypeptide of interest and

(d) Purifying the polypeptide of interest.

In a specific embodiment of said method the cell is a eukaryotic cell, a mammalian cell, a hamster cell or a murine cell. Preferably said cell is a CHO cell, most preferably a CHO DG44, CHO-K1 or DUKX-B11 cell. Furthermore preferred is a NSO cell.

In a preferred embodiment of said method the polypeptide of interest is a recombinant protein, preferably a secreted polypeptide, more preferably a therapeutic protein. Most preferably the polypeptide of interest is an antibody, such as a monoclonal, polyclonal, multispecific or single chain antibody, or a fragment thereof, e.g. Fab, Fab', F(ab')2, Fc and Fc'-fragments, heavy and light immunoglobulin chains and their constant, variable or hypervariable region as well as Fv- and Fd-fragments. In another preferred embodiment of said method the polypeptide of interest is a fusion protein or a scaffold protein.

The ~~invention~~disclosure further relates to a use of the cell as described above for the manufacturing of proteins.

~~The invention furthermore relates to a~~In another aspect, there is provided use of any one of the polyadenylation signals or nucleic acids as described above ~~for the use~~ as an insulator.

Additionally, the ~~invention~~disclosure relates to a use of any one of the polyadenylation signals or nucleic acids as described above for the generation of improved host cell lines.

The ~~invention~~disclosure specifically relates to a use of any one of the polyadenylation signals or nucleic acids as described above for the use in gene therapy.

~~The invention further relates to~~In another aspect, there is provided a kit comprising any one of the polyadenylation signals or nucleic acids as described above, a vector, a cell and a cell culture medium for cultivation of said cell.

DESCRIPTION OF THE FIGURES

FIGURE 1: BASIC EXPRESSION VECTORS

Figure 1 schematically shows the expression vector designs used for the transfection of CHO-DG44 cells. "P/E" means a composite unit that contains both CMV enhancer and promoter element, "P" a promoter element and "T" a termination signal for transcription, which is required for polyadenylation of transcribed messenger RNA. The polyadenylation signals "BGH", "SV40L" and "HGH" are termination signals for transcription derived from the 3' untranslated region of bovine growth hormone (SEQ ID NO:12), the SV40 late gene region (SEQ ID NO:11) and 3' untranslated region of Chinese hamster growth hormone (SEQ ID NO:8), respectively. These polyadenylation signals are flanked by restriction enzyme sites for "*Sfi*I" and "*Xba*I". The position and direction of transcription initiation within each transcription unit is indicated by an arrow. For cloning of the gene of interest a sequence region with multiple cutting sites for restriction endonucleases (multiple cloning sites – "mcs") is inserted after the promoter/enhancer element. The amplifiable selectable marker dihydrofolate reductase is abbreviated to "dhfr" and the selectable marker neomycin phosphotransferase is abbreviated to "npt".