[0005]

However, it has become clear that even when exogenous DNA is introduced into the genome, transformed cells with high expression levels cannot always be obtained. The expression level of exogenous DNA introduced into the genome generally differs depending on the position of introduction, and it has not always been possible to efficiently select desired transformed cells.

[0006]

Targeted integration (TI) has been proposed as one of the techniques capable of solving the problems in introducing exogenous DNA into the genome (PTL 1-3). In TI, a genomic region suitable for introduction and expression of exogenous DNA is identified in advance, and the exogenous DNA to be expressed is site-specifically integrated into the identified genomic region. TI can be said to be a new gene recombination technology created with the improvement of genomic site-specific recombination technologies. As a result of TI, the predictability of the expression level of exogenous DNA and such of the obtained transformed cells has increased, and efficient acquisition of transformed cells having the required traits can be expected. In TI, genomic regions suitable for introducing exogenous DNA are often referred to as hotspots. So far, hotspots have been found in wide-ranging regions on genomes in various cells used as host cells for transformation. Moreover, an attempt to apply TI to the production of monoclonal antibodies by animal cells to obtain transformed cells useful for the production has also been reported (PTL 4, NPL 1-2).

[Citation List]

[Patent Literature]

[0007]

[PTL 1] International Publication No. WO 2008/151219
[PTL 2] International Publication No. WO 2013/190032
[PTL 3] International Publication No. WO 2016/064999
[PTL 4] International Publication No. WO 2017/184831

[Non-Patent Literature]

[0008]

[NPL 1] Biotechnol. Prog., 2015, Vol. 31, No. 6, pp 1645-1656
[NPL 2] Methods 95 (2016) pp 3-12

[Summary]

[Technical Problem]

[0009]

An objective of the present disclosure is to provide a new hotspot that is useful for Targeted Integration (TI).

[Solution to Problem]

[0010]

The present inventors continued to search for regions that enable expression at a high level of exogenous DNA encoding a polypeptide such as an antibody in animal cells. In the production of transformed cells by TI reported so far, the initial expression level could not always be maintained for a long period of time, and there were times when the expression level would eventually decrease during culture (PLoS ONE, 2017 12(6): e0179902, Biotechnology Letters, 2018, Volume 40, Issue 8, pp 1209-1218). When such transformed cells are used for producing a polypeptide, the production amount of the expression product becomes unstable, and the production efficiency could decrease. Alternatively, if the transformed cells are unable to maintain industrial production levels, the production line would need to be restructured. The present inventors suspected that the decrease in the expression level of a polypeptide in culture may occur depending on the genomic integration site of the exogenous polynucleotide. Therefore, as a result of trying to search for hotspots that can be expected to maintain the expression level of exogenous DNA for a long period of time, the present inventors succeeded in finding a new hotspot, and achieved the present disclosure.

[0010a]

In one aspect, the present disclosure relates to a method for introducing an exogenous DNA encoding a polypeptide of interest into a CHO cell, wherein the method comprises introducing the exogenous DNA into the first intron of the CCDC91 gene in the CHO cell.

[0010b]

In another aspect, the present disclosure relates to a method for producing a polypeptide, wherein the method uses a CHO cell in which an exogenous DNA encoding a polypeptide of interest is introduced into the first intron of the CCDC91 gene.

[0010c]

In another aspect, the present disclosure relates to a method for producing a polypeptide comprising the following steps of: (1) introducing an exogenous DNA encoding a polypeptide of interest into a CHO cell, wherein the exogenous DNA is introduced into the first intron of the CCDC91 gene of the genome of the CHO cell; (2) culturing the CHO cell into which the exogenous DNA has been introduced; and (3) recovering the polypeptide of interest.

[0010d]

In another aspect, the present disclosure relates to an isolated CHO cell comprising a DNA cassette for introducing an exogenous DNA into the first intron of the CCDC91 gene by an exchange reaction.

[0011]

The present disclosure specifically encompasses the following examples:

[1] a method for introducing an exogenous DNA encoding a polypeptide of interest into a CHO cell, wherein the method comprises introducing the exogenous DNA into the genomic region specified by NCBI accession number NW_003614838.1 in the CHO cell;

[2] the method according to [1], wherein the integration site of the exogenous DNA is selected from a region comprising the coiled-coil domain-containing protein 91 (CCDC91) gene and its promoter region in the genomic region specified by NCBI accession number NW_003614838.1;

[3] the method according to [2], wherein the integration site of the exogenous DNA is selected from the CCDC91 gene region;

[4] the method according to [3], wherein the integration site of the exogenous DNA is in the first intron of the CCDC91 gene;

[5] the method according to any of [1] to [4], wherein the exogenous DNA is site-specifically introduced into the genomic region by any method selected from homologous recombination, recombinase-mediated cassette exchange (RMCE), and genome editing;

[6] the method according to any of [1] to [5], wherein the polypeptide of interest is an antigen-binding molecule;

[7] the method according to [6], wherein the antigen-binding molecule is an antibody;

[CLAIMS]

1. A method for introducing an exogenous DNA encoding a polypeptide of interest into a CHO cell, wherein the method comprises introducing the exogenous DNA into the first intron of the CCDC91 gene in the CHO cell.

2. A method for producing a polypeptide, wherein the method uses a CHO cell in which an exogenous DNA encoding a polypeptide of interest is introduced into the first intron of the CCDC91 gene.

3. A method for producing a polypeptide comprising the following steps of:
(1) introducing an exogenous DNA encoding a polypeptide of interest into a CHO cell, wherein the exogenous DNA is introduced into the first intron of the CCDC91 gene of the genome of the CHO cell;
(2) culturing the CHO cell into which the exogenous DNA has been introduced; and
(3) recovering the polypeptide of interest.

4. The method according to claim 3, wherein the step of introducing the exogenous DNA encoding the polypeptide of interest into the CHO cell includes the following steps (i)-(ii):
(i) introducing into the CHO cell a DNA cassette for introducing the exogenous DNA by an exchange reaction; and
(ii) introducing the exogenous DNA into the first intron of the CCDC91 gene by a recombinase which recognizes the DNA cassette of (i) as a target site.

5. The method according to any of claims 1 to 4, wherein the polypeptide of interest is an antigen-binding molecule.

6. The method according to claim 5, wherein the antigen-binding molecule is an antibody.

7. An isolated CHO cell comprising a DNA cassette for introducing an exogenous DNA into the first intron of the CCDC91 gene by an exchange reaction.

8. The CHO cell according to claim 7, wherein the exogenous DNA is an exogenous DNA encoding an antigen-binding molecule of interest.

9. The CHO cell according to claim 8, wherein the antigen-binding molecule is an antibody.