Use of the naturally occurring autoprotease N^{pro} of Pestivirus for production of heterologous polypeptides of interest may be limited though, as activation of autoproteolytic function of N^{pro} in vitro is susceptible only to specific renaturazing conditions. These conditions that allow for the cleavage activity of N^{pro} in vitro are inhibitory for certain other interactions which are necessary or desirable in some settings for production of heterologous polypeptides of interest. As an example of such interactions certain bio-specific affinities as e.g. selective peptide-protein affinity can be named. Also, due to other requirements of parameters, certain processes do not permit to create the favourable renaturazing conditions for N^{pro} and as a result N^{pro} can not be used in these processes. Therefore the naturally occurring N^{pro} of Pestivirus may be unsuitable for the production of certain polypeptides of interest and for use under certain conditions. Accordingly the need for an N^{pro} of Pestivirus with improved properties exists, in order to enhance cleavage efficiency, to obtain higher yields of polypeptide of interest, and in order to be able to use N^{pro} in a wider range of conditions, which allow for the application of new production processes.

Summary ~~of invention~~

Within the present ~~invention~~disclosure it has surprisingly been found, that certain derivatives of the naturally occurring autoprotease N^{pro} of Pestivirus, which have a lower pI and enhanced solubility are suitable for use in a wider range of conditions. In addition these derivatives were surprisingly found to have improved autoproteolytic activity in vitro.

Accordingly within the scope of the present ~~invention~~disclosure an improved process for the production of heterologous polypeptides of interest with homogenous N-termini is provided, which makes use of the derivatives of autoprotease N^{pro} of Pestivirus, also part of the present ~~invention~~disclosure.

In a first aspect, there is provided a process for the recombinant production of a heterologous polypeptide of interest, comprising,

(i) cultivation of a bacterial host cell which is transformed with an expression vector which comprises a nucleic acid molecule which codes for a fusion polypeptide, the fusion polypeptide comprising a derivative of an autoprotease N^{pro} of Pestivirus, wherein at least one cysteine residue of the naturally occuring autoprotease N^{pro} of Pestivirus is replaced by another amino acid residue, and a second polypeptide which is connected to the first polypeptide at the C-terminus of the first polypeptide in a manner such, that the second polypeptide is capable of being cleaved from the fusion polypeptide by the autoproteolytic activity of the first polypeptide, said second polypeptide being a heterologous polypeptide, wherein cultivation occurs under conditions which cause expression of the fusion polypeptide and formation of corresponding cytoplasmic inclusion bodies,
(ii) isolation of the inclusion bodies from the host cell,
(iii) solubilization of the isolated inclusion bodies,
(iv) induction of autoproteolytic cleavage of the heterologous polypeptide of interest from the fusion polypeptide, and

(v) isolation of the cleaved heterologous polypeptide of interest, wherein N^{pro} derivatives wherein at least one cysteine residue of the naturally occurring autoprotease N^{pro} of CSFV is selected from the group consisting of C112, C134 and C138 is replaced by a glutamic acid residue are excluded.

In a second aspect, there is provided a derivative of an autoprotease N^{pro} of CSFV, wherein at least one cysteine residue of the
naturally occuring autoprotease N^{pro} of CSFV is replaced by another amino acid residue, wherein N^{pro} derivatives wherein at least one cysteine residue of the naturally occurring autoprotease N^{pro} of CSFV is selected from the group consisting of C112, C134 and C138 is replaced by a glutamic acid residue are excluded.

In a third aspect, there is provided a derivative of an autoprotease N^{pro} of CSFV, wherein at least one cysteine residue of the
naturally occuring autoprotease N^{pro} of CSFV selected from the group consisting of C112, C134 and C138, is replaced by another amino acid residue, wherein N^{pro} derivatives wherein at least one cysteine residue of the naturally occurring autoprotease N^{pro} of CSFV is selected from the group consisting of C112, C134 and C138 is replaced by a glutamic acid residue are excluded.

In a fourth aspect, there is provided the use of a derivative of the autoprotease N^{pro} of CSFV as defined above in a process as defined above.

Detailed description of invention

The present invention relates to a process for the recombinant production of a heterologous polypeptide of interest, comprising

(i) cultivation of a bacterial host cell which is transformed with an expression vector which