

ods in *Enzymol.*, 101:228-245 (1983)). An integrating vector may be directed to a specific locus in yeast by selecting the appropriate homologous sequence for inclusion in the vector. See Orr-Weaver et al., *supra*. One or more expression constructs may integrate, possibly affecting levels of recombinant protein produced (Rine et al., *Proc. Natl. Acad. Sci. USA*, 80:6750 (1983)). The chromosomal sequences included in the vector can occur either as a single segment in the vector, which results in the integration of the entire vector, or as two segments homologous to adjacent segments in the chromosome and flanking the expression construct in the vector, which results in the stable integration of only the expression construct.

[0181] Expression and transformation vectors, either extra-chromosomal replicons or integrating vectors, have been developed for transformation into many yeasts. For example, expression vectors have been developed for, *inter alia*, the following yeasts: *Candida albicans* (Kurtz, et al., *Mol. Cell. Biol.*, 6:142 (1986)), *Candida maltosa* (Kunze et al., *J. Basic Microbiol.*, 25:141 (1985)); *Hansenula polymorpha* (Gleeson et al., *J. Gen. Microbiol.* 132:3459 (1986); Roggenkamp et al., *Mol. Gen. Genet.* 202:302 (1986)); *Kluyveromyces fragilis* (Das et al., *J. Bacteriol.* 158:1165 (1984)); *Kluyveromyces lactic* (De Louvencourt et al., *J. Bacteriol.* 154:737 (1983); Van den Berg et al., *Bio/Technology* 8:135 (1990)); *Pichia guilliermondii* (Kunze et al., *J. Basic Microbiol.* 25:141 (1985)); *Pichia pastoris* (Cregg et al., *Mol. Cell. Biol.* 5:3376 (1985); U.S. Pat. Nos. 4,837,148 and 4,929,555); *Saccharomyces cerevisiae* (Hinnen et al., *Proc. Natl. Acad. Sci. USA* 75:1929 (1978); Ito et al., *J. Bacteriol.* 153:163 (1983)); *Schizosaccharomyces pombe* (Beach and Nurse, *Nature* 300:706 (1981)); and *Yarrowia lipolytica* (Davidow, et al., *Curr. Genet.* 10:380471 (1985); and Gaillardin et al., *Curr. Genet.* 10:49 (1985)).

[0182] Methods of introducing exogenous nucleic acids into yeast hosts are well-known in the art, and typically include either the transformation of spheroplasts or of intact yeast cells treated with alkali cations. Transformation procedures usually vary with the yeast species to be transformed. See e.g., Kurtz et al., *Mol. Cell. Biol.* 6:142 (1986); Kunze et al., *J. Basic Microbiol.* 25:141 (1985) for *Candida*. See, e.g., Gleeson et al., *J. Gen. Microbiol.* 132:3459 (1986); Roggenkamp et al., *Mol. Gen. Genet.* 202:302 (1986) for *Hansenula*. See, e.g., Das et al., *J. Bacteriol.* 158:1165 (1984); De Louvencourt et al., *J. Bacteriol.* 154:1165 (1983); Van den Berg et al., *Bio/Technology* 8:135 (1990) for *Kluyveromyces*. See, e.g., Cregg et al., *Mol. Cell. Biol.* 5:3376 (1985); Kunze et al., *J. Basic Microbiol.* 25:141 (1985); U.S. Pat. Nos. 4,837,148 and 4,929,555 for *Pichia*. See, e.g., Hinnen et al., *Proc. Natl. Acad. Sci. USA* 75:1929 (1978); Ito et al., *J. Bacteriol.* 153:163 (1983) for *Saccharomyces*. See, e.g., Beach and Nurse, *Nature* 300:706 (1981) for *Schizosaccharomyces*. See, e.g., Davidow et al., *Curr. Genet.* 10:39 (1985); Gaillardin et al., *Curr. Genet.* 10:49 (1985) for *Yarrowia*.

[0183] In order to obtain expression polypeptides or proteins of interest, recombinant microbial host cells derived from the transformants are incubated under conditions which allow expression of the recombinant polypeptide-encoding sequence. These conditions will vary, dependent upon the host cell selected. However, the conditions are readily ascertainable to those of ordinary skill and knowledge in the art.

[0184] Detection of polypeptides expressed in the transformed host cell may be performed by several methods. For

example, a polypeptide or protein may be detected by its immunological reactivity with antibodies.

[0185] Polypeptides or proteins of the present invention may be isolated from the cell by lysis, if formed intracellularly, or isolated from the culture medium, if secreted, by conventional methods.

[0186] (g) Mammalian Constructs and Transformed Mammalian Cells

[0187] The present invention also relates to a mammalian recombinant expression vector comprising exogenous genetic material. The present invention also relates to a mammalian cell comprising a mammalian recombinant expression vector. The present invention also relates to methods for obtaining a recombinant mammalian host cell, comprising introducing into a mammalian cell exogenous genetic-material.

[0188] The mammalian recombinant expression vector may be any vector which can be conveniently subjected to recombinant DNA procedures. Many vectors are available for this purpose, and a suitable expression vector is one that is compatible with the desired function (e.g., transient expression, long term expression, integration, replication, amplification) and in which the control elements are compatible with the host cell. The control elements are those non-translated regions of the vector—promoters, enhancers, 5' and 3' untranslated regions—which interact with host cellular proteins to carry out transcription and translation.

[0189] Vectors suitable for replication in mammalian cells may include viral replicons, or sequences that ensure integration of the sequence encoding *D. v. virgifera* protein homologues or fragments thereof into the host genome. Suitable vectors may include, for example, those derived from simian virus SV40, retroviruses, bovine papilloma virus, vaccinia virus, and adenovirus. The components of the vectors, e.g. replicons, selection genes, enhancers, promoters, and the like, may be obtained from natural sources or synthesized by known procedures. (See, Kaufman et al, *J. Mol. Biol.*, 159: 511-521 (1982); and Kaufman, *Proc. Natl. Acad. Sci., USA*, 82:689-693 (1985)).

[0190] A suitable vector may be one derived from vaccinia viruses. In this case, a nucleic acid molecule encoding a *D. v. virgifera* protein homologue or fragment thereof is inserted into the vaccinia genome. Techniques for the insertion of foreign DNA into the vaccinia virus genome are known in the art, and utilize, for example, homologous recombination. The insertion of the foreign DNA is generally into a gene which is non-essential in nature, for example, the thymidine kinase gene (tk), which also provides a selectable marker. Plasmid shuttle vectors that greatly facilitate the construction of recombinant viruses have been described (see, for example, Mackett et al, *J. Virol.* 49: 857 (1984); Chakrabarti et al., *Mol. Cell. Biol.* 5: 3403 (1985); Moss, In: *Gene Transfer Vectors For Mammalian Cells* (Miller and Calos, eds., Cold Spring Harbor Laboratory, N.Y., p. 10, (1987)). Expression of the *D. v. virgifera* protein homologues or fragments thereof then occurs in cells or animals which are infected with the live recombinant vaccinia virus.

[0191] Suitable mammalian expression vectors usually contain one or more eukaryotic control elements that are capable of expression in mammalian cells. The control element is comprised of at least a promoter to mediate transcription of foreign DNA sequences. Suitable promoters for mammalian cells are known in the art and include viral promoters such as that from simian virus 40 (SV40), cytomegalovirus