(Papavasiliou & Schatz, 2002), or by using other enzymes targeting somatic mutations to binding protein encoding sequences. For the generation of diverse collections of binding proteins, preferably antibodies or fragments thereof, by V(D)J recombination *in situ*, retroviral vector constructs and their utilization are disclosed, wherein said constructs contain variable (V), optionally diversity (D), and joining (J) gene segments arranged in "quasi-germline" configuration allowing assembly of coding regions for immunoglobulin or immunoglobulin-like binding proteins *via* recombination activating gene (RAG)-mediated rearrangement of the gene segments by the process known as V(D)J recombination (Grawunder *et al.*, 1998).

According to a further aspect, the present invention further illustrates how retrovirally transduced cells stably expressing diverse collections of recombinant binding proteins are subsequently labelled by binding to at least one ligand or antigen of interest, and how cells binding to the aforementioned ligand or antigen of interest are detected by appropriate secondary reagents. Methods for the specific labelling of ligand- or antigen-reactive cells and their enrichment or isolation, preferably by high-speed fluorescence-activated cell sorting (FACS), are described. Due to the stable expression phenotype of retrovirally transduced cells, it is described how antigen-reactive cells may optionally be isolated and again expanded in tissue culture, such that optionally iterative cycles of antigen labelling, antigen-directed enrichment, and expansion of ligand or antigen-reactive cells can be performed, until subcloning of the cells is performed allowing the identification of the nucleotide coding region for antigen-reactive antibodies by standard PCR cloning methods (Fig. 1).

comprise an antibody light chain sequence and multiple antibody heavy chain sequences.

According to another preferred embodiment, the antibody or fragment thereof comprises a variable binding domain encoded by the at least one retroviral expression construct enabling V(D)J recombination in order to generate a coding sequence for the variable binding domain upon retroviral transduction or

In a further preferred embodiment, step (b) of the above method is performed under mutagenizing conditions, preferably via the expression of activation induced cytidine deaminase (AID) which is either endogenously or ectopically expressed, wherein the ectopic expression of AID is performed under inducible conditions.

In one aspect of the above method, the at least one retroviral expression construct encoding said antibody or fragment thereof contains a combination of *cis*-regulatory promoter and enhancer elements allowing the targeting of AID mediated somatic mutation to a variable binding domain encoded by the expression construct, wherein the promoter and enhancer elements are selected from the group consisting of

(a) immunoglobulin heavy chain promoter, intron enhancer (EμH) and 3'α enhancer elements,

(b) immunoglobulin κ light chain promoter, κ intron enhancer (κiE) and 3'κ enhancer (3'κE) elements,

(c) immunoglobulin λ light chain promoter, λ2-4 and λ3-1 enhancer elements, and

(d) any functional combination thereof.

Description of the Figures

Fig.1: This figure illustrates the principle of 'Retrocyte Display' allowing the identification and isolation of a binding protein such as an antibody, specific for a desired antigen or ligand. In a first step, at least one retroviral

expression construct that can give rise to expression of a diverse collection of binding proteins is stably transduced into suitable vertebrate host cells ("selector cells"). This is accomplished by transfecting at least one retroviral vector encoding at least one binding protein into retroviral packaging cells (step 1), which may either constitutively or transiently express retroviral proteins Gag, Pol and Env. Packaging cells transfected with the at least one retroviral binding protein construct will then produce recombinant retroviral particles within 24-72 hours post transfection, containing the at least one retroviral expression construct. The resulting retroviral particles accumulate in the cell culture supernatant of the retroviral packaging cells, and can be used to transduce suitable vertebrate host cells ("selector cells") (step 2), which then express the binding protein. In the preferred method, the binding proteins such as antibodies or fragments thereof are expressed on the cell surface of the "selector cells" and the cells then are labelled with a desired antigen or ligand (step 3). Antigen- or ligand binding cells are then preferably analyzed by fluorescence-activated cell sorting (FACS) and cells that exhibit specific antigen binding, are separated from the non-binding cell population preferably by preparative, high-speed FACS (step 4). Antigen- or ligand reactive cells may optionally be expanded in tissue culture again, and due to the stable expression phenotype of retrovirally transduced cells, cycles of antigen-directed cell sorting and tissue culture expansion may be repeated, up to the point that a detectable antigen- or ligand reactive cell population is obtained. This antigen- or ligand reactive cell population may be subjected to a final, preferable, single-cell sorting step, or may directly be used for cloning of binding protein encoding genes on a population basis. In the next step (step 5), the coding regions of relevant binding domains are cloned from the antigen- or ligand-selected cell pools or cell clones, by RT-PCR or genomic PCR using primer

CLAIMS

1. A method for the isolation and identification of at least one stably transduced vertebrate host cell that recombinantly expresses on its cell surface an antibody or fragment thereof specific for a desired antigen or ligand, comprising the steps of:
   (a) transducing at least one retroviral expression construct encoding an antibody or fragment thereof into vertebrate host cells by using replication incompetent retroviral particles, wherein the at least one construct stably integrates into the host cell genome such that the transduced host cells are capable of expressing and displaying said antibody or fragment thereof on their cell surface, and wherein the vertebrate host cells are precursor B lymphocytes that endogenously express Igα and Igβ molecules facilitating membrane deposition of said antibody or fragment thereof, and are unable to express endogenous antibody polypeptides and at least one surrogate light chain component;
   (b) stably expressing said antibody or fragment thereof in said vertebrate host cells and displaying the same on their cell surface;
   (c) enriching said vertebrate host cells expressing said antibody or fragment thereof on the basis of their ability to bind to said desired antigen or ligand by separating cells that exhibit specific antigen or ligand binding from a non-binding cell population, thus selectively isolating strong antibody binders having high affinity to said desired antigen or ligand; and
   (d) isolating and identifying said at least one stably transduced vertebrate host cell expressing said antibody or fragment thereof specific for the desired antigen or ligand on its cell surface from the retrovirally transduced and enriched vertebrate host cells.

2. The method according to claim 1, wherein step (d) is preceded by expanding said enriched vertebrate host cells in tissue culture.

3. The method according to claim 1, wherein step (c) is followed by expanding said enriched vertebrate host cells in tissue culture, after which step (c) as defined in claim 1 is repeated at least once.

4. The method according to any one of claims 1 to 3, in which the retroviral transduction is performed at a multiplicity of infection of equal to or less than 0.1.

5. The method according to any one of claims 1 to 4, in which the antibody is a full-length antibody.

6. The method according to any one of claims 1 to 4, in which the fragment of an antibody is selected from the group consisting of a heavy chain, a light chain, a single V_H domain, a single V_L domain, a scFv fragment, a Fab fragment, and a F(ab')2 fragment.

7. The method according to any one of claims 1 to 6, wherein the vertebrate host cells are derived from a group of species consisting of cartilaginous fish, bony fish, amphibians, reptilia, birds, mammals including pigs, sheep, cattle, horses and rodents including mice, rats, rabbits and guinea pigs.

8. The method according to claim 7, wherein the preferred vertebrate host cell species is mice.

9. The method according to claim 5, wherein the full-length antibody is selected from the group consisting of a fully human antibody, a humanized antibody, and a chimeric antibody.

10. The method according to any one of claims 1 to 9, wherein the antibody or fragment thereof comprises a variable binding domain encoded by said at least one retroviral expression construct enabling V(D)J recombination in order to generate a coding sequence for the variable binding domain upon retroviral transduction.

11. The method according to any one of claims 1 to 10, wherein step (b) is performed under mutagenizing conditions.

12. The method according to any one of claims 1 to 11, wherein the expression of said antibody or fragment thereof in the retrovirally transduced vertebrate host cells is operably linked to
    (a) at least one antibiotic selection marker,
    (b) at least one screening marker, and/or (c) a combination thereof,
    and wherein the expression of said antibody or fragment thereof is coupled using at least one internal ribosomal entry sequence (IRES).

13. The method according to claim 12, wherein the at least one screening marker is

(i) a fluorescent protein selected from the group consisting of green fluorescent protein (GFP), yellow fluorescent protein (YFP), red fluorescent protein (RFP), and blue fluorescent protein (BFP); or
(ii) a cell surface marker selected from the group consisting of CD7, CD34, and low affinity nerve growth factor receptor.

14. The method according to any one of claims 1 to 13, wherein the enrichment step (c) is performed by physical separation of cells from a non-binding cell population using:
(i) fluorescence-activated cell sorting (FACS);
(ii) micromanipulation; or
(iii) panning methods to immobilized desired antigen or ligand.