Transgenic Mouse Technology in Skin Biology: Generation of Knockin Mice

Frederik Tellkamp[1], Farida Benhadou[2], Jeroen Bremer[3], Maria Gnarra[4], Jana Knüver[5], Sandra Schaffenrath[6] and Susanne Vorhagen[1]

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INTRODUCTION

Genetic modification of model organisms is a powerful tool to study human diseases *in vivo*. Mice have become the main subject of interest for basic researchers because they share a broad spectrum of physiological and pathological similarities with humans. These similarities, genetic accessibility, and a defined genetic background within the laboratory mouse strains provide the basis to analyze *in vivo* cellular and molecular pathways that are altered in human diseases. Knockout and knockin mice have become valuable tools to investigate the role of certain proteins and thereby have helped researchers to overcome cause and consequence problems arising from biochemical data (Hacking, 2008). For biomedical research, the knockin mouse opened the possibility of studying the *in vivo* pathological consequences of gene mutations associated with human disease and examining their similarity to human pathologies.

Whereas knockout technology aims to delete a gene or part of a gene, knockin technology introduces specific mutations or cDNAs into a known locus of the genome. The altered mice can then be used to study the molecular mechanisms by which specific mutations can contribute to human diseases. Examples of the potential of knockin technology are the introduction into mice of human orthologs—the human equivalent of the mouse gene—and investigation into how either single-point mutations or gene insertions/deletions/inversions observed in human disease affect gene function. In addition, knockin technology can be used to introduce reporter genes under the control of a cell type–specific promoter to follow their fate over time in a defined location within the genome (Vorhagen *et al.*, in press). An important advantage of knockin technology as compared with integration by chance (random integration) is its directed integration into a known locus in the genome. By contrast, random integration can result in multiple-copy integration and/or disturbance of genes where the transgene was inserted. For example, random integration may occur in the promoter of another gene, resulting in disturbed transcription of that gene.

BENEFITS OF KNOCKIN TECHNOLOGY

- Exact site of integration is known.
- Allows functional insight into proteins by studying single mutations or human orthologs *in vivo*.
- Constitutive, conditional, and inducible gene knockins are possible.
- Knockins can be used to generate mouse models of human diseases.

LIMITATIONS

- Combination of transgenes leads to growing complexity, which in turn can lead to more side effects.
- Only very few well-characterized genomic loci like the ROSA26 locus that can be used for conditional knockin of exogenous genes such as reporters or dominant mutations/truncations. This makes combinations of reporter and certain transgenes knocked in to the same locus difficult.
- Expensive.
- Time-intensive.

PROCEDURE

The generation of knockin mice utilizes a gene-targeting strategy similar to that used for constitutive and conditional knockout mice to modify genes in mouse embryonic stem cells. The general procedure of constitutive or tissue-specific gene targeting in mice was previously described

[1]*Department of Dermatology, Cologne Excellence Cluster on Stress Responses in Aging-Associated Diseases, Center for Molecular Medicine Cologne, University of Cologne, Cologne, Germany;* [2]*Department of Dermatology, Erasme Hospital, Université Libre de Bruxelles, Brussels, Belgium;* [3]*Department of Dermatology, Center for Blistering Diseases, University Medical Center Groningen and University of Groningen, Groningen, The Netherlands;* [4]*Dermatology Clinic, Catholic University of Sacred Heart, Rome, Italy;* [5]*Department of Dermatology, University of Cologne, Cologne, Germany and* [6]*Department of Dermatology and Venereology, Medical University of Innsbruck, Innsbruck, Austria*

Correspondence: Frederik Tellkamp, Department of Dermatology, Cologne Excellence Cluster on Stress Responses in Aging-Associated Diseases, Center for Molecular Medicine Cologne, University of Cologne, Cologne, Germany. E-mail: frederik.tellkamp@uk-koeln.de

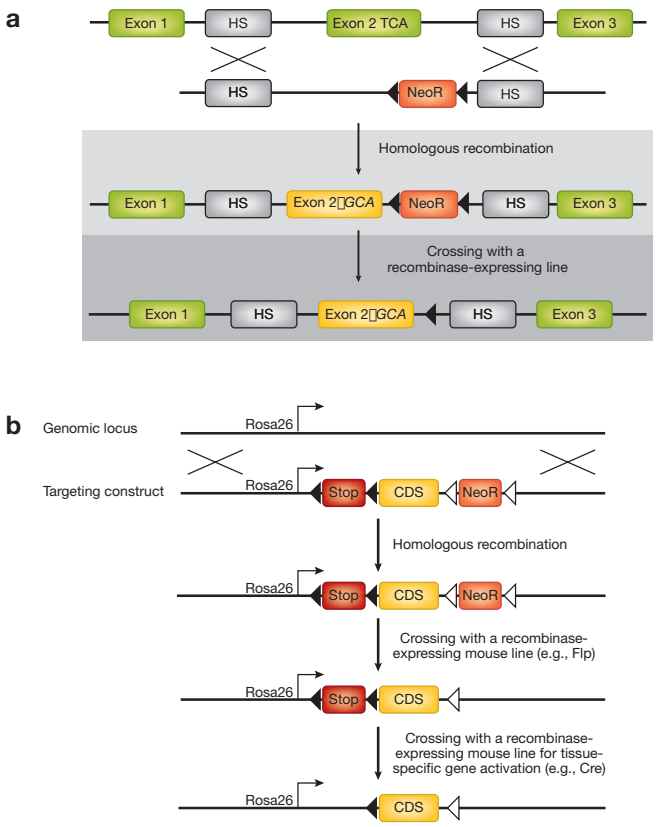

Figure 1. Site-specific integration of a transgene using homologous recombination. (**a**) Generation of a mouse model with a defined point mutation within one gene using knockin technology. The target vector contains the mutated sequence of the gene of interest (TCA → GCA), as well as a resistance cassette. Homologous sequences (HS) mediate the site-specific integration into the genomic locus. Crossing with a Cre line will delete the resistance cassette to ensure that the gene is expressed properly. (**b**) The Rosa26 locus is a widely used locus for transgenic expression. The target vector contains the coding sequence (CDS) of interest as well as a stop sequence and a resistance cassette flanked by sites of recombination, respectively. Removal of the resistance cassette occurs in a separate crossing with a Flp-expressing mouse line. Tissue-specific expression of the transgene is then achieved by crossing with tissue-specific Cre lines.

in two related Research Techniques Made Simple articles (Gunschmann *et al.*, 2014; Scharfenberger *et al.*, 2014). In brief, the central steps involve isolation and cultivation of embryonic stem cells from a blastocyst that are transfected with a targeting construct. This construct integrates into genomic DNA directed by homologous sequences flanking the construct. Cells are then selected for successful integration and injected into the cavity of an early embryo. After being implanted into a surrogate mother, the embryo develops into a chimeric mouse in which the transgenic cells may have inserted into the germ line. The progeny of these mice carry the transgene.

Whereas knockout mouse models target genes to delete parts of it (and sometimes the whole gene), knockin targeting enables the controlled insertion of genes into only one known locus of the genome. One example is the introduction of point mutations into an endogenous gene through homologous recombination (Figure 1a), which may, for example, mimic mutations observed in human conditions. Another example is the targeted insertion of a coding sequence of interest into the Rosa26 locus, a site that is frequently used for targeted transgene expression (Soriano, 1999). Insertion at this site ensures gene expression under the weak local Rosa26 promoter. To date, no disturbances of endogenous gene function have been reported on insertion into this locus. An exogenous promoter sequence can also be added, leading to enhanced expression of the introduced cDNA. Moreover, when a loxP-flanked stop cassette is placed between the promoter and cDNA, the Cre–loxP system can be employed for tissue-specific and induced expression. In general, the antibiotic resistance–selection cassette must be removed to allow the transgene to function properly (Figure 1b) (Gunschmann *et al.*, 2014).

Knockin technology can also be used to bring Cre under the control of an endogenous promoter (Figure 2a), which can then be used to follow the tissue-specific activity of this promoter (see below). One important feature of the knockin technology is its potential compatibility with inducible systems, such as the CreERT and TetO/TetR systems. This compatibility enables investigators to study the effects of mutations that occur in later stages of life, for example—effects that closely resemble the cause of many human diseases.

TRANSLATIONAL DISEASE MODELS

Knockin technology allows the generation of mouse models that recapitulate human skin diseases (Chen and Roop, 2008). One prominent example is a mouse that expressed mutant keratin 10 (Fuchs *et al.*, 1992). Mice carrying this mutant K10 show epidermolytic hyperkeratosis, which closely reproduc-

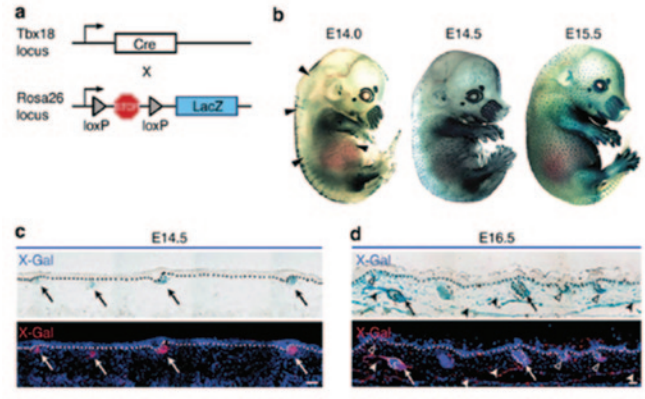

Figure 2. Example of a strategy that uses the knockin technology to investigate tissue-specific expression patterns. (**a**) With the knockin technology, a reporter gene (*LacZ*) is expressed under control of the Rosa26 promoter restricted by a stop sequence flanked by loxP sites. The Cre recombinase under the control of the *Tbx18* promoter deletes the stop sequence and thereby allows gene expression specifically in *Tbx18*-positive cells. (**b–d**) The reporter can be used to follow *Tbx18* expression in different tissues by different methods (X-Gal staining on embryos or tissue sections). Adapted from Grisanti *et al.* (2013).

es the phenotype observed in human patients carrying that mutation. This knockin mouse model identified suprabasal keratins as part of the molecular basis of epidermolytic hyperkeratosis and provided a basis for studying this human disease *in vivo* and developing approaches for therapy.

Knockin models also create the possibility of examining tissues and cells in which a specific promoter is active. For example, Grisanti *et al*. (2013) established a line in which Cre was knocked into the locus of the T-box transcription factor 18 (*Tbx18*), such that its expression was under the control of the endogenous *Tbx18* promoter. In combination with a β-galactosidase reporter knockin, *Tbx18* was shown to be active in dermal papilla precursor cells and thus was identified as a marker for these cells (Figure 2) (Grisanti *et al*., 2013).

KNOCKIN TECHNOLOGY: LIMITATIONS

Knockin models exhibit great potential for the analysis of biological processes and disease mechanisms, but several limitations still exist. Most are shared with the limitations of knockout models that are described elsewhere (Gunschmann *et al*., 2014; Scharfenberger *et al*., 2014). One potential limitation includes artifacts resulting from the forced overexpression of a gene, resulting in unspecific protein–protein interactions, for example. Perhaps the biggest disadvantage is that mouse physiology is not identical to that of humans. Using human orthologs of proteins to generate "humanized" mouse models for diseases therefore does not entirely reflect a human pathological situation.

CONFLICT OF INTEREST

The authors state no conflict of interest.

CME ACCREDITATION

This activity has been planned and implemented in accordance with the Essential Areas and Policies of the Accreditation Council for Continuing Medical Education through the joint sponsorship of the Duke University School of Medicine and Society for Investigative Dermatology. The Duke University School of Medicine is accredited by the ACCME to provide continuing medical education for physicians. To participate in the CME activity, follow the link provided. Physicians should only claim credit commensurate with the extent of their participation in the activity.

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SUPPLEMENTARY MATERIAL

A PowerPoint slide presentation appropriate for journal club or other teaching exercises is available at http://dx.doi.org/10.1038/jid.2014.434.

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QUESTIONS

This article has been approved for 1 hour of Category 1 CME credit. To take the quiz, with or without CME credit, follow the link under the "CME ACCREDITATION" heading.

1. The knockin of a transgene is _____ with _____

A. Site specific; one integration.

B. Random; one integration.

C. Random; multiple integrations.

2. The knockin can be combined with which of the following?

A. Only tissue-specific expression systems.

B. Only inducible expression systems.

C. Tissue-specific and inducible expression systems.

D. A constitutive expression system only.

3. The tissue-specific expression of a transgene relies on which of the following?

A. Promoter specificity.

B. Tissue-specific Cre expression.

C. Administration of tamoxifen.

4. Site-specific integration has what advantage?

A. Effects on other genes are minimized.

B. The transgene is always expressed.

C. The transgene cannot be lethal.

5. Site specific integration of a human gene allows investigators to do which of the following?

A. Study common mouse diseases.

B. Investigate mechanisms of human diseases.

C. Identify interaction partners on a protein level.