Functional analysis of a gene-rich region of human chromosome 8 by comparative sequence analysis and targeted mutagenesis of the mouse

Thomas Schmitt-John [1] **and Matthias Platzer** [2]
[1] Entwicklungsbiologie & Molekulare Pathologie, Universität Bielefeld, 33501 Bielefeld [2] Abteilung Genom Analyse, IMB, Beutenbergstraße 11, 07745 Jena

The DNA sequences of most human and murine Genes have been determined in the course of the respective Genome Projects, but in many cases the function of these genes cannot directly be deduced from their DNA sequence. Thus mutagenesis of the mouse, the most important biomedical model organism, will become more important for the functional characterization of the sequenced genes.
In the course of this project we have started to utilize **cross-species comparative sequence analysis** and **targeted mutagenesis of the mouse** for functional analysis of a selected region of the human genome. The target region is located on chromosome 8p in man and corresponds to Chr 8 in mouse (see Fig. 1).

Cross-species comparative sequence analysis
For the structural analysis of the homologous genome regions known sequences of man, mouse and rat have been used. In addition to available sequences one Neuregulin 1 containing BAC has been sequenced in the laboratory of Matthias Platzer. For cross-species comparative sequence analysis, the PIPMaker program (Schwartz *et al*., 2000) and a newly developed program called Genalizer were utilized. The comparative sequence analysis revealed, besides the structures of the genes of the target region, an unexpectedly high number of "conserved non-coding sequences" (CNS). These will be investigated in more detail in collaboration with the group of Prof. R. Giegerich (Technical Faculty, University of Bielefeld). CNS elements might have important regulatory functions and thus might be potential targets for our planned phenotype driven target region specific random mutagenesis. Especially for the Neuregulin 1 locus, which is one of the most interesting target genes of the analyzed region, the comparative sequence analysis was carried out in detail.

Targeted deletion mutagenesis
The selected target area will be deleted by two different strategies in murine embryonic stem cells. As originally planned the radiation induced random deletion mutagensis followed by regionspecific selection as developed by You *et al*. (1997) has been started. For complementation a Cre recombinase mediated deletion strategy, modified according to Ramirez-Solis *et al*. (1995) will be utilized. The advantage of the latter strategy is the possibility to define deletion endpoints.

Progress in radiation induced mutagenesis
For the radiation induced strategy first targeting vectors were constructed (see Fig. 2) and first initial irradiations have been performend. In addition a set of polymorphic PCR markers (see Fig. 2) have been established, which allow the rapid screening of Chr 8 specific deletions. For radiation deletion mutagenesis a 129 x C57Bl/6J F1 hybrid ES-cell line is used and the loss of the polymorphic markers in Chr 8 of either of the parental strains can be scored for the estimation of the deletion sizes.

Progress on Cre mediated deletion mutagenesis
For Cre mediated mutagenesis targeting vectors were constructed for delivery of LoxP sites to the planned deletion end points (see Fig. 2). The vectors will be sequentially transfected into ES cells followed by selection. Twice targeted ES cell clones then will be transiently transfected with a Cre recombinase expression plasmid and screened for the deletion.

Phenotype-screening and DNA-chip mutation analysis
DNA microarray based expression analysis has successfully been tested in collaboration with Dr. J. Beckers (GSF-Institute for Experimental Genetics, München). Microarray technology is expected to facilitate the mutation analysis in future.

Schwartz S, Zhang Z, Frazer KA *et al.*: **PipMaker – a web server for aligning two genomic DNA sequences.** Genome research, 2000, 10: 577-586

You Y, Bergstrom, Klemm M *et al.*: **Chromosomal deletion complexes in mice by radiation of embryonic stem cells.** Nat. Genet., 1997, 15: 285-288.

Ramirez-Solis R, Liu P, Bradley A: **Chromosome engineering in mice.** Nature, 1995, 378: 720-724.

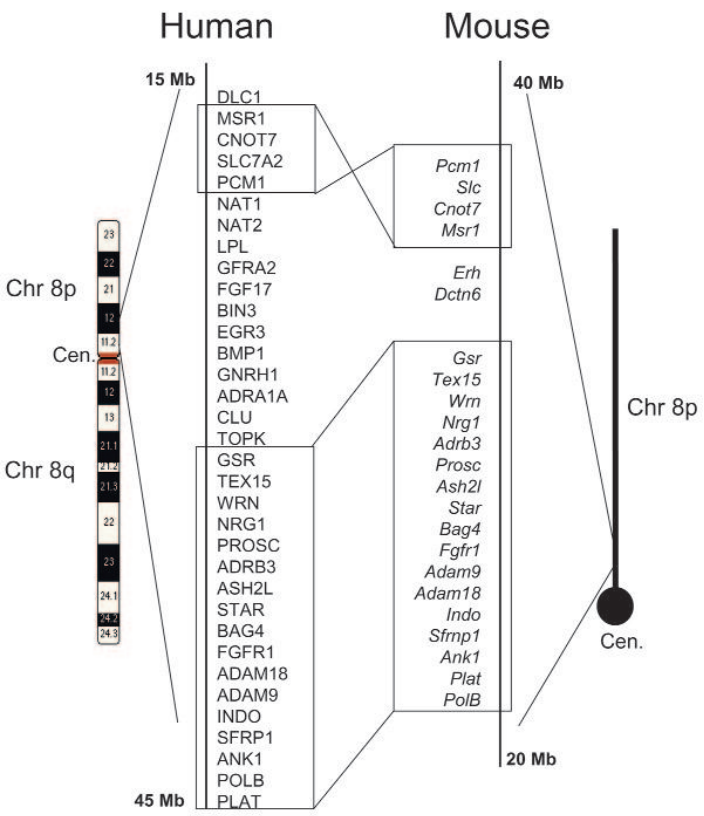

Fig 1: Target region on Chr 8 of man and mouse. Genes are listed according to the human and mouse genome draft versions; the homologous regions of conserved synteny are boxed.

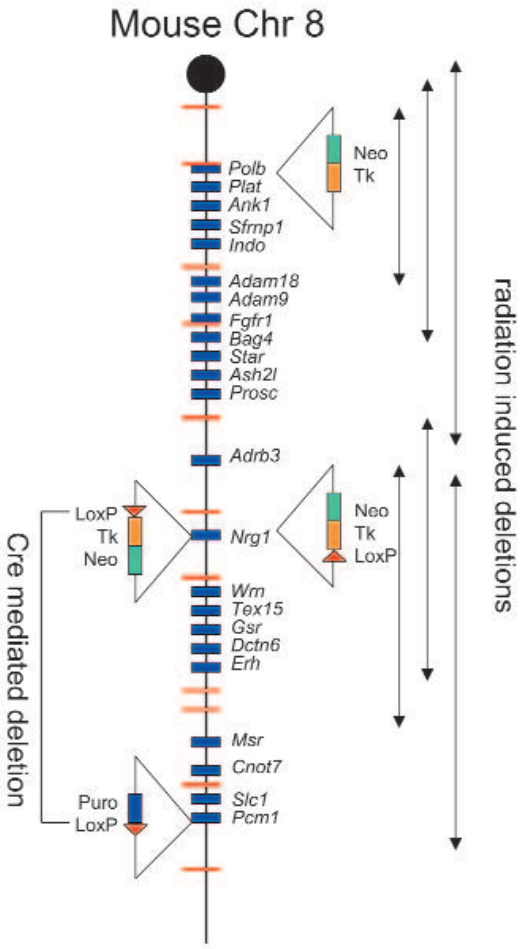

Fig 2: Deletion strategy and locations of planned deletions. Genes are shown as blue boxes, established polymorphic screening markers are indicated as red lines, targeting vectors are shown and the planned delectins are indicated.