Expression and function profiling of orphan nuclear receptors using bacterial artificial chromosome (BAC) transgenesis.

Eric Nemoz-Gaillard, Ming-Jer Tsai and Sophia Y. Tsai

Corresponding Author: stsai@bcm.tmc.edu

Department of Molecular and Cellular Biology, Baylor College of Medicine, One Baylor Plaza, Houston, Texas 77030

The long term goal of the Nuclear Receptor Signaling Atlas (NURSA) resides in unraveling the physiological and pathological functions of nuclear receptors (NRs) at the molecular, biochemical and cellular levels. This multi-oriented task requires complementary approaches in order to determine the specific function(s) and precise expression and receptor activity patterns for each individual conventional or orphan receptor. To attain this objective, we have chose to turn to technologies recently made available to engineer bacterial artificial chromosomes (BACs).

Introduction

The long term goal of the Nuclear Receptor Signaling Atlas (NURSA) resides in unraveling the physiological and pathological functions of nuclear receptors (NRs) at the molecular, biochemical and cellular levels. This multi-oriented task requires complementary approaches in order to determine the specific function(s) and precise expression and receptor activity patterns for each individual conventional or orphan receptor.

Figure 1. Modification of BAC plasmid by homologous recombination: linear recombination. The linear recombination construct is flanked by two homologous regions (A and B) for the targeted BAC clone and contains a prokaryotic selection marker (Sm) for the selection of recombinant-containing bacteria. Electropotent cells containing the BAC (BAC or PAC) are prepared and induced to express the recombination function (rec+). The recombinant construct is then introduced in these cells by electroporation and the recombinants are selected against the selection marker (Sm).

To attain this objective, we have chose to turn to technologies recently made available to engineer bacterial artificial chromosomes (BACs), E. coli F factor based vectors capable of maintaining cloned DNA fragments up to 300 kb. Due to their large size, BAC clones are more likely to contain the informative sequences necessary for directing the expression of transgenes in an appropriate spatio-temporal expression pattern, making them more amenable to study NRs.

BAC mediated transgenesis has indeed proven to be a highly reliable way to obtain accurate transgene targeting for in vivo studies of gene expression and function. BAC engineering technologies have been developed on the concept of homologous recombination in E. coli [Copeland et al., 2001; Lee et al., 2001; Muyrers et al., 2001; Yu et al., 2000; Zhang et al., 1998] and allow virtually any modification to be performed at any chosen position, including insertions, deletions, replacements and point mutations. Hence, NR gene-containing BAC clones can be isolated from a genomic BAC library and modified to introduce the features desired for the study of any particular aspect of the NR biology.

After presenting the BAC engineering system by homologous recombination, we will describe different transgene designs that we envision to address the questions of NR function, expression pattern and activity.

Modification of BACs by homologous recombination

Two main settings have been used to modify BACs by homologous recombination in E. coli, the first one involving RecA-mediated recombination with circular DNA [Gong et al., 2002; Yang et al., 1999], while in the second, linear DNA recombination is performed [Lee et al., 2001; Muyrers et al., 2000; Muyrers et al., 1999; Yu et al., 2000].
In both designs, homologous recombination occurs through freely-chosen homology regions, making possible the alteration of the target molecule at any position. In the first system, a circular plasmid DNA is designed to express the recombination function in the form of the RecA protein in addition to one or two regions homologous to the target, allowing the introduction of diverse modifications in the BAC (Figure 1).

For the second recombination system, RecBCD exonuclease activity is removed to allow for the use of linear DNA by expressing the gam gene or by using RecBCD deficient bacterial strains. The recombination is then performed by double strand break repair enzymes from bacterial (RecE/RecT, [Muyrers et al., 2000; Muyrers et al., 1999]) or prophage (Red-α/Red-β, [Lee et al., 2001; Muyrers et al., 2000]) origins (Figure 2).

Overall, the modification of NR gene-containing BAC clones allows the rapid generation of transgenic constructs to study the different aspects of NR biology. For defining the precise expression pattern of NRs, reporter transgenes such as fluorescent proteins or enzymatic reporters, such as the β-galactosidase, luciferase or the alkaline phosphatase genes, can be inserted into the NR gene locus. Each specific reporter presents different advantages and weaknesses in regard to their respective sensitivity, and spatial and temporal resolution (reporter gene design, Figure 3A).

For receptor activity studies, the DNA-binding domain (DBD) of the NR considered can be replaced by another DBD not active in mammalian cells, such as the well characterized Gal4 DBD, in such a way that the chimeric NR will then activate the expression of a reporter transgene (activity trap setting, Figure 3B). In a slightly different approach, ligand-activated receptor activity can also be addressed more specifically if the LBD of the NR...
– or putative domain, in the case of orphan receptors – is coupled to the Gal4 DBD to activate a reporter gene (ligand trap setting, Figure 4).

Finally, homologous recombination in E. Coli has been shown to be an efficient tool to generate gene-targeting vector constructions, whether complex or not, for the targeted disruption of genes in embryonic stem (ES) cells (Figure 2A). This method provides a simple way to produce sophisticated genomic manipulations such as conditional knock-outs. In addition, it has been demonstrated recently that intact BAC clones can be used to specifically target genes in ES cells with high targeting efficiencies.

References
Copeland, N. G., Jenkins, N. A. and Court, D. L. (2001) Recombineering: a powerful new tool for mouse functional genomics Nat Rev Genet 2, 769-79.

Gong, S., Yang, X. W., Li, C. and Heintz, N. (2002) Highly efficient modification of bacterial artificial chromosomes (BACs) using novel shuttle vectors containing the R6Kgamma origin of replication Genome Res 12, 1992-8.

Lee, E. C., Yu, D., Martinez de Velasco, J., Tessarollo, L., Swing, D. A., Court, D. L., Jenkins, N. A. and Copeland, N. G. (2001) A highly efficient Escherichia coli-based chromosome engineering system adapted for recombinogenic targeting and subcloning of BAC DNA Genomics 73, 56-65.

Muyrers, J. P., Zhang, Y., Buchholz, F. and Stewart, A. F. (2000) RecE/RecT and Redalpha/Redbeta initiate double-stranded break repair by specifically interacting with their respective partners Genes Dev 14, 1971-82.

Muyrers, J. P., Zhang, Y. and Stewart, A. F. (2001) Techniques: Recombinogenic engineering--new options for cloning and manipulating DNA Trends Biochem Sci 26, 325-31.

Muyrers, J. P., Zhang, Y., Testa, G. and Stewart, A. F. (1999) Rapid modification of bacterial artificial chromosomes by ET-recombination Nucleic Acids Res 27, 1555-7.

Yang, X. W., Wynder, C., Doughty, M. L. and Heintz, N. (1999) BAC-mediated gene-dosage analysis reveals a role for Zipro1 (Ru49/Zfp38) in progenitor cell proliferation in cerebellum and skin Nat Genet 22, 327-35.

Yu, D., Ellis, H. M., Lee, E. C., Jenkins, N. A., Copeland, N. G. and Court, D. L. (2000) An efficient recombination system for chromosome engineering in Escherichia coli Proc Natl Acad Sci U S A 97, 5978-83.

Zhang, Y., Buchholz, F., Muyrers, J. P. and Stewart, A. F. (1998) A new logic for DNA engineering using recombination in Escherichia coli Nat Genet 20, 123-8.