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Research paper

Co-expression of antibody fab heavy and light chain genes from separate evolved compatible replicons in *E. coli*

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Abstract

Antibody molecules bind to antigen with six complementary determining region (CDR) loops, three of which are located on each variable heavy (V_H) and light (V_L) chains. Discovery and optimization of antibodies that bind antigen using *in vitro* techniques require diversification of one or more of these CDRs. Since antibodies are dimeric, simultaneous diversification of heavy and light chains on separate genetic elements would allow "chain shuffling" to occur simply and efficiently. Efficient expression of antibody V_H and V_L requires that the two separate replicons be compatible with one another, but also have similar properties, such as copy number in *E. coli*. Standard plasmids that are compatible with one another in *E. coli* exist at widely variable copy numbers. Recently we described the isolation of ColE1 mutants that have similar copy numbers but different incompatibility characteristics. Thus, new compatibility groups in the ColE1 family were established. Herein we describe the *E. coli* expression of V_H and V_L genes to form a functional Fab. The ability to express antibody heavy and light chains from separate but compatible high copy plasmids should allow new opportunities in antibody engineering, such as rapid chain shuffling and generation of more complex antibody libraries.

Keywords: Antibody engineering; Fab expression; Compatible plasmids; Origin of replication; ColE1

1. Introduction

There are now 18 therapeutic recombinant antibodies on the U.S. market (Reichert et al., 2005). Discovery of recombinant antibody therapeutics can involve traditional hybridoma technology (Kohler and Milstein, 1975), followed by PCR cloning of the heavy and light chain genes (Larrick et al., 1989), or can be done purely *in vitro* using a variety of display techniques (Hoogenboom, 2005). With regards to the latter, phage display (Smith and Petrenko, 1997), *E. coli* surface display (Daugherty et al., 1999), yeast display (Boder and Wittrup, 1997), ribosome display (Hanes and Pluckthun, 1997), and mRNA display (Wilson et al., 2001) have all been used as vehicles for expressing large antibody libraries and selecting antigen binders to various targets. Additionally, these techniques are also used to "affinity mature" antibody leads, in a process analogous to somatic hypermutation, which occurs naturally in the vertebrate immune response.

E. coli is a workhorse organism for the discovery, expression, and purification of antibody fragments. Indeed, both phage display and *E. coli* surface display

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utilize this bacterium. Furthermore biochemical characterization and structural biology are often done using Fab fragments produced in *E. coli* (Ulrich et al., 1995). The backbones of the plasmid and phagemid vectors used in these systems are based on those of some of the original recombinant DNA work (Bolivar et al., 1977). In general they use standard promoters like *lac* or *tac* to drive antibody expression, leader sequences like omp or pho to target the antibody into the non-reducing periplasm, and origins of replication like ColE1, which is found in several standard vectors like pBluescript, the pUC series, or the lower copy pBR322.

Antibody molecules are dimeric proteins composed of a heavy chain and light chain, both of which undergo somatic diversity generating processes (Smider and Chu, 1997). The diversity of antibodies arises both from the V(D)J recombination of each V-region gene, as well as the combinatorial association of different heavy and light chains (Lewis, 1994). In *E. coli*, Fab fragments consisting of the variable and first constant domain of each chain are often produced in the periplasm. Standard expression systems in *E. coli* use a single vector, often with one promoter driving a bicistronic mRNA (Ulrich et al., 1995). These vectors require independent cloning of the heavy and light chain genes, making chain shuffling or high throughput analysis of different heavy and light chain combinations difficult. The dimeric nature of antibodies suggests that their discovery and expression systems may be optimized by V_H and V_L co-expression from separate vectors, such that any heavy chain could be paired with any light chain in the cell, without independent cloning events (Collet et al., 1992). Recent analysis indicates this type of system would allow achievement of a far deeper complexity of antibody libraries with fewer cloning steps and less technical difficulty than standard libraries based on a bicistronic system (Ostermeier and Benkovic, 2000). This possibility could only be realized through the use of compatible plasmids.

Plasmid compatibility in *E. coli* is determined by the origin of replication (Novick, 1987). Most standard plasmid vectors use the ColE1 origin of replication. Plasmids compatible with some ColE1 origins, such as pMB1 and p15A, however, exist at a much lower copy number in cells. Thus, although attempts to co-express heavy and light chains from different plasmids have been made (Collet et al., 1992), these methods have not been widely adopted due to the disparity in copy number (and lack of equal expression control) between the divergent compatible plasmids. This difficulty is magnified by the fact that overexpression of a single antibody heavy or light chain is often toxic to the cell, and optimization of Fab production often necessitates balancing heavy and light chain synthesis (Humphreys et al., 2002).

We recently described the directed evolution of the ColE1 replication origin (Kim et al., 2005). Among the mutants discovered, several displayed compatibility with wild-type ColE1, and this property could be transferred by cloning the mutant origins into new plasmids. At least two new incompatibility groups of ColE1 were established. Since compatible ColE1 plasmids would be valuable to the co-expression of any dimeric protein, including antibodies, we evaluated the possibility of producing functional antibody Fab by co-expressing heavy and light chain genes from separate vectors in *E. coli* using the new mutant ColE1 origins. Herein we report that plasmid levels of cells co-cultured with two compatible ColE1 plasmids are at equivalent and high copy numbers, that heavy and light chains can be expressed separately at levels similar to expression from a single plasmid, and that the antibody is functional in binding antigen. This compatible plasmid system could offer significant benefits in antibody discovery, chain shuffling, and high throughput protein production.

2. Materials and methods

2.1. Strains and plasmids

E. coli XL1-Blue (Stratagene, La Jolla, CA) was used for cloning and Top 10F (Invitrogen, Carlsbad, CA) was used for antibody expression. The pV_HV_L-wt-Amp vector contains the RA7 heavy and light chain genes fused to the C_H1 and $C_\lambda2$ constant regions, respectively. Fab expression is driven by the *lac* promoter which produces a bicistronic mRNA. Vectors pV_HV_L-wt-Amp, pBS-3.3-Kan (Kim et al., 2005), and pBS-wt-Amp (pBluescript II SK-, Stratagene) were used in vector construction.

2.2. Construction of pV_L-wt-Amp

The pV_HV_L-wt-Amp vector is shown on the left in Fig. 1. The heavy chain gene was removed from pV_HV_L-wt-Amp using the restriction enzyme Nco I, which cleaves in the leader sequences of both V_H and V_L. The DNA (1 μg) was resolved on a 1% agarose gel and the ~5 kb band was isolated by gel purification using QIAEXII Agarose Gel Extraction (Qiagen, Chatsworth, CA). The vector (~100 ng) was then recircularized at 15 °C overnight using 40 units of T4 ligase (New England Biolabs) in 10 μl. Resulting recombinant plasmids were analyzed by Nco I restriction digest, and correct sequence confirmed by sequencing from the pCT-F primer (CCATGATTACGCCAAGCTT TGGAGCC).