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Research paper

A fast and efficient procedure to produce scFvs specific for large macromolecular complexes

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Abstract

We have expanded the application of antibody phage display to a new type of antigen: ribonucleoprotein (RNP) complexes. We describe a simple and efficient method for screening antibodies specific for large intact RNPs and individual components. We also describe a fast and easy method to overcome the abundance of amber stop codons in the positive phage clones. The resulting antibodies have been used in ELISA and Western blot analysis.

Keywords: Antibody phage display; scFv; Macromolecular complexes; Ribonucleoprotein

1. Introduction

Antibody phage display is an attractive and powerful alternative to hybridoma technology. Beyond generating antibodies against a wide variety of proteins (Dong et al., 2003; Smith et al., 2003; Chang et al., 2006; Marcus et al., 2006), it has also been used to produce antibodies specific for unconventional antigens including small peptides (Rodriguez-Diaz et al., 2004), DNA photoproducts (Zavala et al., 2000), snRNAs (Teunissen et al., 1998) and lipids (Takkinen et al., 1996). However, this technology has not been efficient in producing antibodies that target subunits of macromolecular complexes (Rubinstein et al., 2003). Here we describe an optimized protocol to generate multiple monoclonal scFvs against a large ribonucleoprotein, the *E. coli* signal recognition particle–receptor complex.

Signal recognition particle (SRP) and its receptor (SR) are conserved in all organisms (Pool, 2005) as part of the molecular machinery that guides integral membrane and secretory proteins to the cellular translocation apparatus during translation (for details refer to Pool, 2005). *E. coli* SRP has two components: a protein subunit called Ffh (Bernstein et al., 1989; Romisch et al., 1989) and an 114-nucleotide RNA called 4.5S RNA (Poritz et al., 1990). *E. coli* SR is a single polypeptide molecule called FtsY (Luirink et al., 1994;

Abbreviations: scFv, single chain variable fragment; RNP, ribonucleoprotein; SRP, signal recognition particle; SR, signal recognition particle receptor; ELISA, enzyme linked immunosorbent assay; DTT, dithiothreitol; GMPPCP, guanylyl 5′-(beta, gamma-methylenediphosphonate); RT, room temperature; PBST, phosphate buffered saline with Tween-20; TBS, tris buffered saline; HRP, horse radish peroxidase; TMB, 3,3′5,5′-tetramethylbenzidine; AP, alkaline phosphatase; IPTG, isopropyl-1-thio-β-D-galactopyranoside.

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Miller et al., 1994). Ffh and FtsY each contain a homologous NG domain (Freymann et al., 1997; Montoya et al., 1997) which has GTPase activity and these domains associate to form a heterodimer upon GTP binding. Ffh contains an additional domain, the M domain, which binds to 4.5S RNA, whereas FtsY includes an additional domain – the A domain – which helps anchor FtsY to the membrane (de Leeuw et al., 1997). A three-dimensional model of SRP-SR predicts that the 4.5S RNA lies at the interface of Ffh and FtsY in the complex (Spanggord et al., 2005) (Fig. 1A). In this report we optimized the phage display protocol and generated scFv antibodies specific for the intact SRP–SR RNP complex or its components.

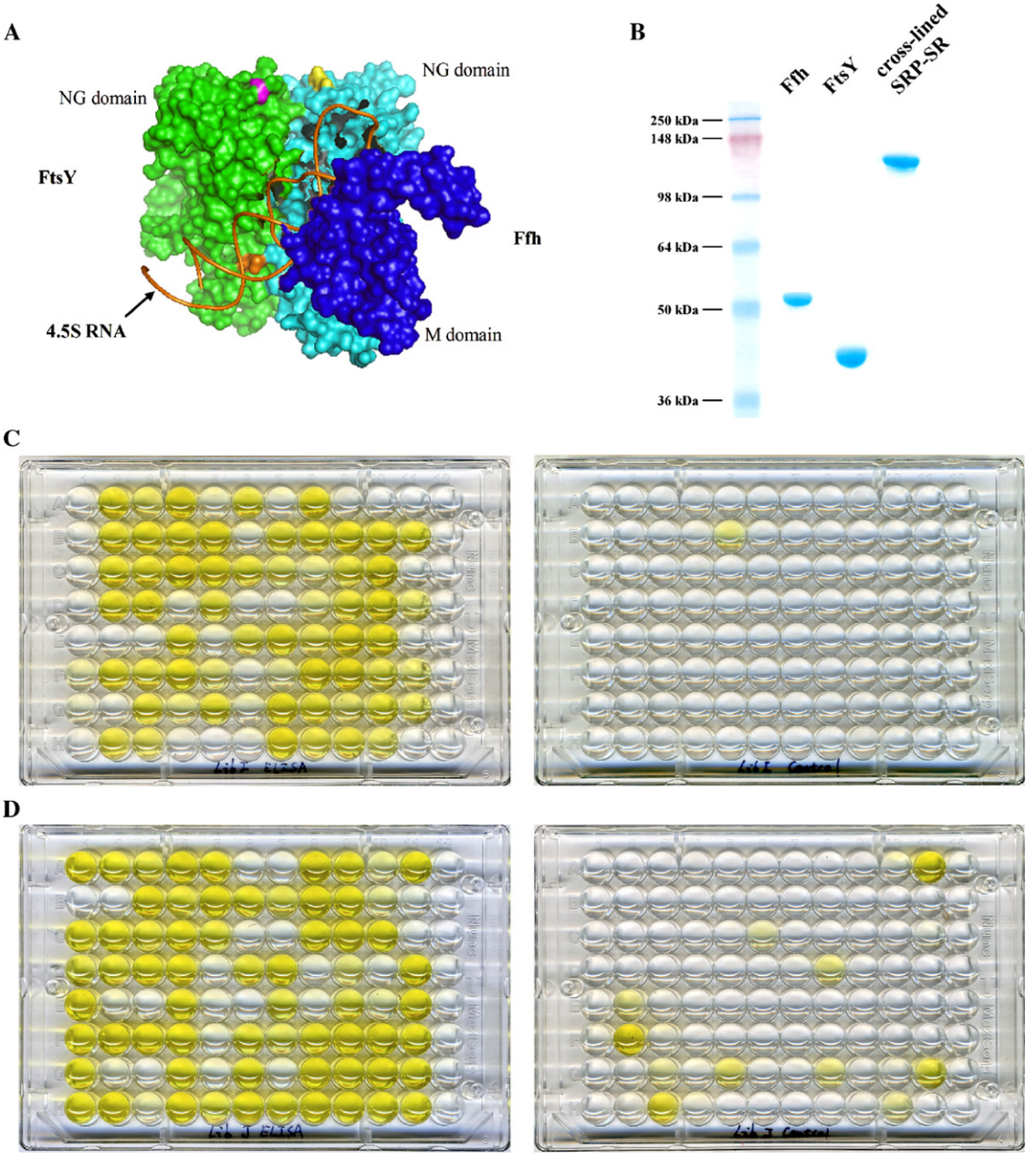

Fig. 1. Panning for scFvs specific for SRP–SR. (A) 3D model of SRP–SR complex showing the spatial relations of Ffh, FtsY and 4.5S RNA. (B) SDS-PAGE analysis of cross-linked SRP–SR. (C) Monoclonal ELISA plate of Tomlinson I library (left) and its control (right). (D) Monoclonal ELISA plate of Tomlinson J library (left) and its control (right).