IDENTIFICATION AND CHARACTERIZATION OF A UNIQUE RIBOSOMAL FRAMESHIFTING SIGNAL IN SARS-CoV ORF3A

Xiao X. Wang, Ying Liao, Sek M. Wong, and Ding X. Liu*

1. INTRODUCTION

Severe acute respiratory syndrome coronavirus (SARS-CoV) 3a gene encodes a protein of 274 amino acids. Recent studies showed that 3a is a minor structural protein. A heterogeneous population of sgRNA 3 transcripts, containing one, two, or three nucleotide insertion in a six T stretch located 18 nucleotides downstream of the 3a initiation codon, was identified in SARS-CoV-infected cells as well as in the sera of SARS patients. Here we report that a +1/-1 frameshifting event occurs in the insertion site. A mechanism of simultaneous slippage at both P and A sites may account for the frameshifting event.

2. MATERIALS AND METHODS

Construction of plasmids: Wild-type SARS 3a cDNA was amplified by PCR and was digested with EcoRV and EcoRl. The digested fragment was cloned into the two sites of pFlag vector, generating pF-3a/6T. Constructs with 7T and 8T were made by site-directed mutagenesis.

PCR fragment of EGFP from pEGFP-C1 (Clontech) was digested with BglII and EcoRV and cloned into these two sites on pSARS-3a/7T, generating pEGFP-3a. Deletions in EGFP or 3a regions were made by overlapping PCR.

In vitro transcription, translation, and transient expression of 3a and its mutants in Cos-7 cells: One microgram of plasmid DNA was transcribed and translated in a TnT coupled in vitro translation system (Promega) in rabbit reticulocyte lysate (RRL). The polypeptides were labeled with 35S-methionine.

* Xiao X. Wang, Sek M. Wong, Ding X. Liu, National University of Singapore, 117543. Ying Liao, Ding X. Liu, Nanyang Technological University, Singapore, 637551. Ding X. Liu, Institute of Molecular and Cell Biology, Singapore, 138673.
SARS-CoV 3a sequences placed under the control of a T7 promoter were transiently expressed in mammalian cells using the recombinant vaccinia virus (vTF7-3) system. In this study, the transfection reagent used was Effecten Transfection Kit (Qiagen).

**SDS-polyacrylamide gel electrophoresis (SDS-PAGE) and Western blot:** Electrophoresis of viral polypeptides was performed on SDS-12% polyacrylamide gels. After transferring to PVDF membrane by Semi-dry transfer (Bio-Rad), proteins were detected with 3a antibody raised in rabbits, anti-FLAG antibody (Sigma), and His-tag monoclonal antibody (Santa Cruz).

3. RESULTS

3.1. A Frameshifting Event Occurs in SARS-CoV ORF3a

Full-length cDNA covering the 3a and 3b region with six, seven and eight Ts were translated in vitro (Fig. 1a). A band equivalent to the full-length 3a protein was expressed from all three clones (Fig. 1a, lanes 1–3). To further confirm the 3a expression in intact cells, pF-3a with a Flag tag at the N terminal (Fig. 1b) was expressed in Cos-7 cells and detected by Western blot. The 3a expression was detected in all three clones except in control (Fig. 1b, lanes 1–3). These results suggested that a +1 frameshift in ORF3a with seven Ts and a –1 frameshift in ORF3a with eight Ts occurred during translational elongation in order to maintain the full length ORF3a expression.

3.2. Identification of the Slippage Site by Mutagenesis Studies

To characterize the slippage site, point mutations of the seven Ts (from T to C) were made, giving rise to pF-3a/7T(M1-M7). In addition, the nucleotide immediately downstream the seven T (A) was also mutated to C, giving rise to pF-3a/7T(M8). In vitro expression (Fig. 2a) showed that the full-length 3a was expressed in pF-3a/7T (lane 1). Mutation of any of the seven Ts significantly decreased the full-length 3a expression (lanes 2–8). However, the A to C mutation did not affect the full-length 3a production (lane 9).

Similar expression patterns were observed in Cos-7 cells transfected with these constructs (Fig. 2b). The full-length 3a was detected in pF-3a/7T (lane 1) and M8 (lane 9) but not in other mutants (lanes 2–8), confirming that mutation of any T to C could greatly reduce the full-length 3a expression.

**Figure 1a.** In vitro expression of 3a/6T (1), 7T (2), and 8T (3). 4: markers.

**Figure 1b.** Expression of 3a/6T (1), 7T (2) and 8T (3) in Cos-7 cells. C: mock transfection.