TRANSLOCATION OF THE RIBOSOME IN TEMPERATURE-CONTROLLED MICROFLUIDIC CHANNELS

Bin Wang, Jingyi Fei, Ruben L. Gonzalez and Qiao Lin
Columbia University, USA

ABSTRACT

This paper reports on the application of temperature-controlled microfluidic channels for the single-molecule study of the temperature-dependent tRNA-mRNA translocation in the ribosome. Multiple parallel microchannels are formed between a quartz slide and a glass coverslip that is integrated with on-chip heaters and resistive temperature sensors. At precisely controlled temperatures, the fluorescent decay of single dyes is recorded, and the translocation time is analyzed to reveal the temperature-dependent behavior in tRNA-mRNA translocation.

Keywords: temperature-controlled microchannels, single-molecule studies, ribosome, translocation

1. INTRODUCTION

With sensitive kinetic assays enabled by single-molecule fluorescent spectroscopy and microscopy techniques, single-molecule studies of the translocation of the tRNA-mRNA complex within the ribosome, a fundamental step during protein biosynthesis, have drawn much attention lately [1]. In general, ribosomal translocation has a significant dependency on temperature. Such studies, therefore, require fast and accurate well-controlled environment within precise locations. However, conventional temperature control methods are cumbersome and inflexible, and are therefore not suited to single-molecule studies.

Due to its ease in integrating microscale thermal elements, MEMS technology has the potential to enable effective and accurate temperature control in a micro environment tailored to the needs of single-molecule studies. We have previously shown the detection of temperature-dependent photobleaching of single fluorophores in microchannels [2]. Here we present the application of microfluidics to single-molecule studies of the kinetics of mRNA-tRNA translocation within the ribosome at multiple, precisely controlled temperatures. Multiple parallel microchannels are integrated with heaters and temperatures sensors, and embedded within a total internal reflection fluorescence (TIRF) set-up. Analysis of measurement data at varying temperatures reveals a temperature-dependent change in the translocation rate, implying the existence of at least one temperature-dependent step in mRNA-tRNA translocation.

2. EXPERIMENTAL

The microfluidic device consists of parallel microchannels sandwiched between a quartz slide derivatized with biotin-polyethylene glycol and a microfabricated coverslip incorporating temperature-control elements (Fig. 1a). The device is outfitted onto the stage of a TIRF microscope (Fig. 1b). Temperature control is realized through the adjustment of heater load currents in response to a real-time feedback loop using temperature sensors

978-0-9798064-0-7/μTAS2007/$20©2007CBMS

underneath the microfluidic channels (Fig. 1c). Fluorescence is detected by a high numerical aperture objective and recorded by a CCD camera.

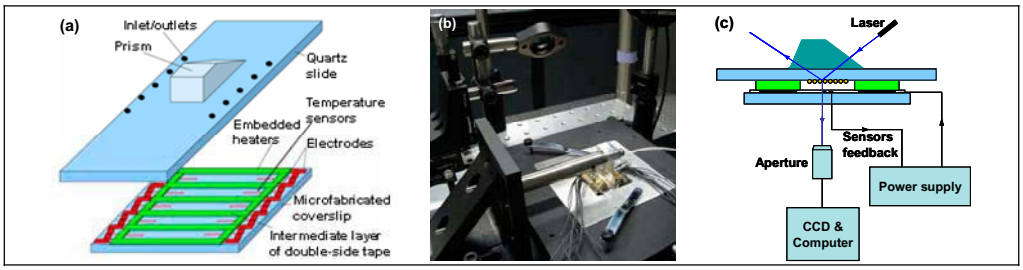

Fig. 1 (a) Device schematic. (b) Outfitted TIRF stage. (c) Temperature control scheme.

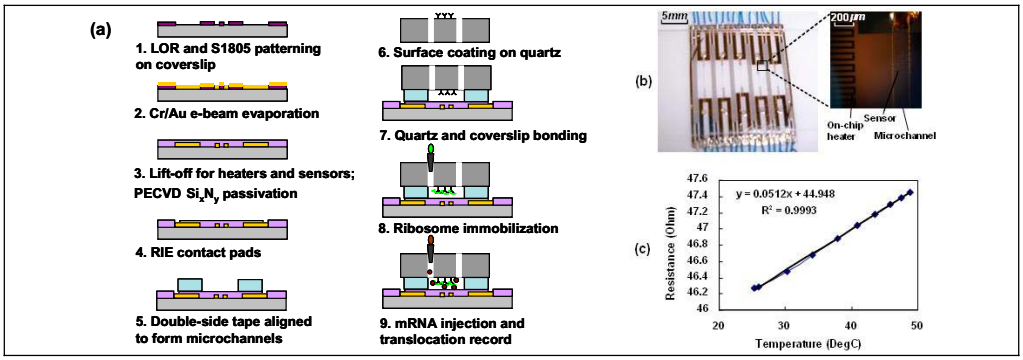

Fig. 2 (a) Fabrication and surface functionalization process. (b) Packaged device with a heater and temperature sensor near an inlet. (c) Characteristics of a typical temperature sensor.

The device is fabricated (Fig. 2a) on a glass coverslip by lift-off to form Cr/Au (5/200 nm) heaters and resistive temperature sensors, which are passivated by PECVD silicon nitride (300 nm). The coverslip is then bonded to a derivatized quartz slide with a spacer layer defining the microchannels (Fig. 2b). From calibration, a typical temperature sensor (Fig. 2c) has a reference resistance of 46.30 Ω with a temperature coefficient of resistivity (TCR) of 1.14×10-3 /˚C and a linear similarity of 99.93%. Accordingly, a temperature measurement resolution of approximately 0.02 ˚C can be achieved.

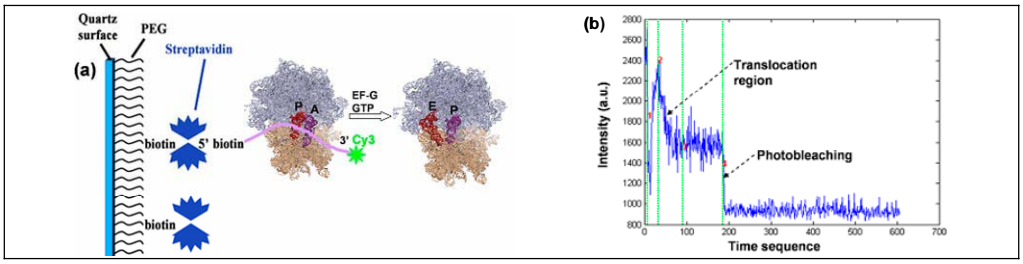

Fig. 3 (a) Surface functionalization scheme and ribosome translocation mechanism. (b) Typical fluorescent decay of a single dye.

Fluorescently-labeled mRNA undergoes a 10%-20% decrease of fluorescence emission intensity upon translocation, providing a direct measure of translocation rates for individual ribosome immobilized onto the microfluidic channel [3]. During experiments, ribosomal complexes containing a 5'-biotinylated/3'-Cy3-labeled mRNA and an initiator tRNA are in

vitro assembled, purified, and subsequently immobilized via biotin-streptavidin-biotin interaction on a derivatized quartz slide (Fig. 3a). Translocation is then initiated by aminoacyl-tRNA, in complex with elongation factor Tu and GTP, and elongation factor G, in complex with GTP [1]. Fig. 3b presents a typical single-molecule fluorescence intensity trace as a function of time.

3. RESULTS AND DISCUSSION

Translocations are measured at controlled temperatures of 22 (room temperature), 25, 31, 34, and 37 ˚C, respectively. The time required for translocation (i.e., translocation lifetime) on a single ribosome is defined as the time for the gradual decrease in fluorescence intensity to plateau. Fig. 4a shows the population histogram of the translocation as a function of time interval after initial exposure to the inducing laser light and its fitting to a sigmoidal distribution, through which the translocation lifetime can be obtained. The population results (Fig. 4b) at different temperature setpoints show a decrease in translocation lifetime (Fig. 4c), or an increase of translocation rate, with temperature. Such single-molecule results are consistent with bulk measurements [3].

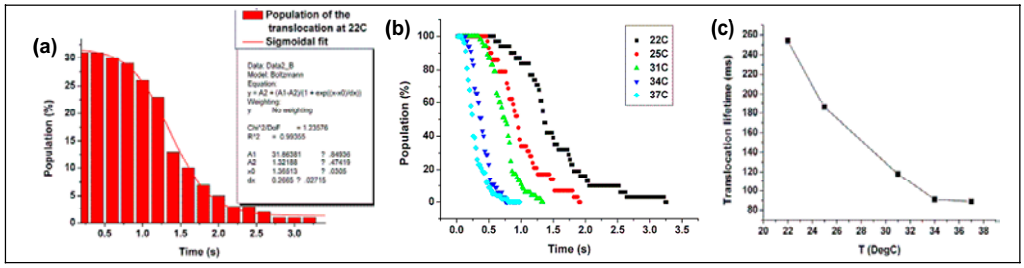

Fig. 4 (a) Population histogram of the translocation as a function of time at 22 ˚C. (b) Population of the translocation and (c) translocation lifetimes at different temperatures.

4. CONCLUSIONS

We have presented the application of microchannels with integrated temperature control to the study of tRNA-mRNA translocation in the ribosome. With on-chip heaters and temperature sensors, translocation at controlled temperatures has been measured, revealing significant temperature-dependent translocation rates. These results demonstrate the capability of this device for temperature-dependent kinetic studies at the single-molecule level with higher accuracy and sensitivity than afforded by conventional technology.

ACKNOWLEDGEMENTS

This work was supported by the National Science Foundation (Grant No. CBET-0639274).

REFERENCES

[1] S. C. Blanchard, et al. *tRNA dynamics on the ribosome during translation*, PNAS, 101, 12893-12898 (2004).

[2] B. Wang, et al. *Single-Molecule Detection in Temperature-controlled Microchannels*, IEEE-NEMS, Bangkok (2007).

[3] S. M. Studer, et al. *Rapid Kinetic Analysis of EF-G-dependent mRNA Translocation in the Ribosome*", J. Mol. Biol., 327, 369-381 (2003).