Transcription

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Direct Observation of Single RNA Polymerase Processing through a Single Endogenous Gene in a Living Yeast Cell

*Barbara Treutlein and Jens Michaelis**

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The transcription of a gene into messenger RNA (mRNA) by the RNA polymerase II (RNAPII) is the first, fundamental step in gene expression and consists of the three major phases initiation, elongation, and termination. Each step of transcription is highly regulated by the binding and action of numerous transcription factors, and functional understanding of transcriptional networks and their dynamics is crucial for the understanding of cellular systems and their development. Classical biochemical studies of transcription are based on the determination of expression levels by the isolation of mRNA from cell populations. The applied bulk techniques, such as northern blotting and reverse-transcription quantitative PCR, however, comprehend the drawback of averaging over a large number of cells and hence obscure cell-to-cell variation and lack spatial information. Also, the dynamics and regulation of single transcription events cannot be resolved.

The implementation of in vitro single-molecule methods in the transcription field tremendously improved our mechanistic understanding of the transcription progress. Techniques such as single-molecule Förster resonance energy transfer (smFRET) and the use of magnetic and optical tweezers avoid ensemble averaging and enable the exploration of single RNAPIIs on a gene of interest in an extremely controlled environment.[1] Phenomena such as RNAPII pausing and backtracking and the influence of transcription factors could be revealed.[2–4] However, it remains to be seen how the simplistic mechanistic picture obtained from those in vitro studies has to be adapted to describe the complexity within organisms.

In recent years, as a result of fast advances in live-cell-imaging technologies, it has become possible to apply single-molecule fluorescence microscopy to cells and directly observe individual transcription events in single living cells.[5–8] Studies on the direct detection of nascent mRNA uncovered transcriptional bursting in mammalian cells[7] and bacteria,[5] however, single, uncorrelated transcription-initiation events in yeast;[8] further a high cell-to-cell variability of mRNA production and an inherently probabilistic nature of the transcription process (intrinsic noise).

Up to this point, single-molecule studies of transcription were performed by using exogenous reporter genes transfected into the investigated cell type. Larson et al. have now succeeded in directly observing the transcription of single nascent mRNA molecules from an endogenous, cell-cycle-regulated yeast gene in real time in living cells.[9] They also developed a novel, quantitative method of fluctuation analysis of fluorescently labeled mRNA to measure the kinetics of transcription initiation and the dynamics of elongation and termination. In this study, the nascent RNA was detected by genetically inserting a cassette that codes for 24 hairpin binding sites of the PP7 bacteriophage coat protein into the untranslated region (UTR) of the gene of interest (Figure 1 a,b). As the cassette is transcribed by RNAPII, RNA stem loops form and recruit the GFP–PP7 fusion protein that is constitutively coexpressed (GFP = green fluorescent protein). Thus, this fusion protein serves as a fluorescent tag.

Insertion of the cassette upstream of the coding region (5′UTR) enabled Larson et al. to obtain time-lapse data for whole transcription cycles (Figure 1 a): Shortly after initiation (t_1), the cassette is transcribed (t_2). Transcription is detectable by a stepwise increase in the fluorescence signal (Figure 1 c, green trace). The fluorescence signal of nascent RNA remains constant during elongation (t_2+t_3). When transcription terminates, it abruptly drops back to the background level as the transcript departs from the transcription site (TS) and freely diffuses away through the nucleus (t_4). Wide-field microscopy was used to monitor the active TS over time (Figure 1 c). Over a long period of time, many transcription events occurred. As a number of RNAPIIs were often transcribing simultaneously at different positions along the gene, multiple transcription events were often superimposed at a TS (Figure 1 a, t_3–t_5). Thus, the obvious question presented itself as to whether transcription by the different polymerases was cooperative, for example, owing to the continuous presence of important transcription factors. This information was obtained from quantitative analysis of the fluctuations in the mRNA fluorescence by computing the autocorrelation curve from long time traces of an active gene

[*] B. Treutlein, Prof. Dr. J. Michaelis
Ludwig-Maximilians-Universität München, Department Chemie
Butenandtstrasse 11, 81377 München (Germany)
E-mail: jens.michaelis@cup.uni-muenchen.de
Homepage: http://www.cup.uni-muenchen.de/pc/michaelis

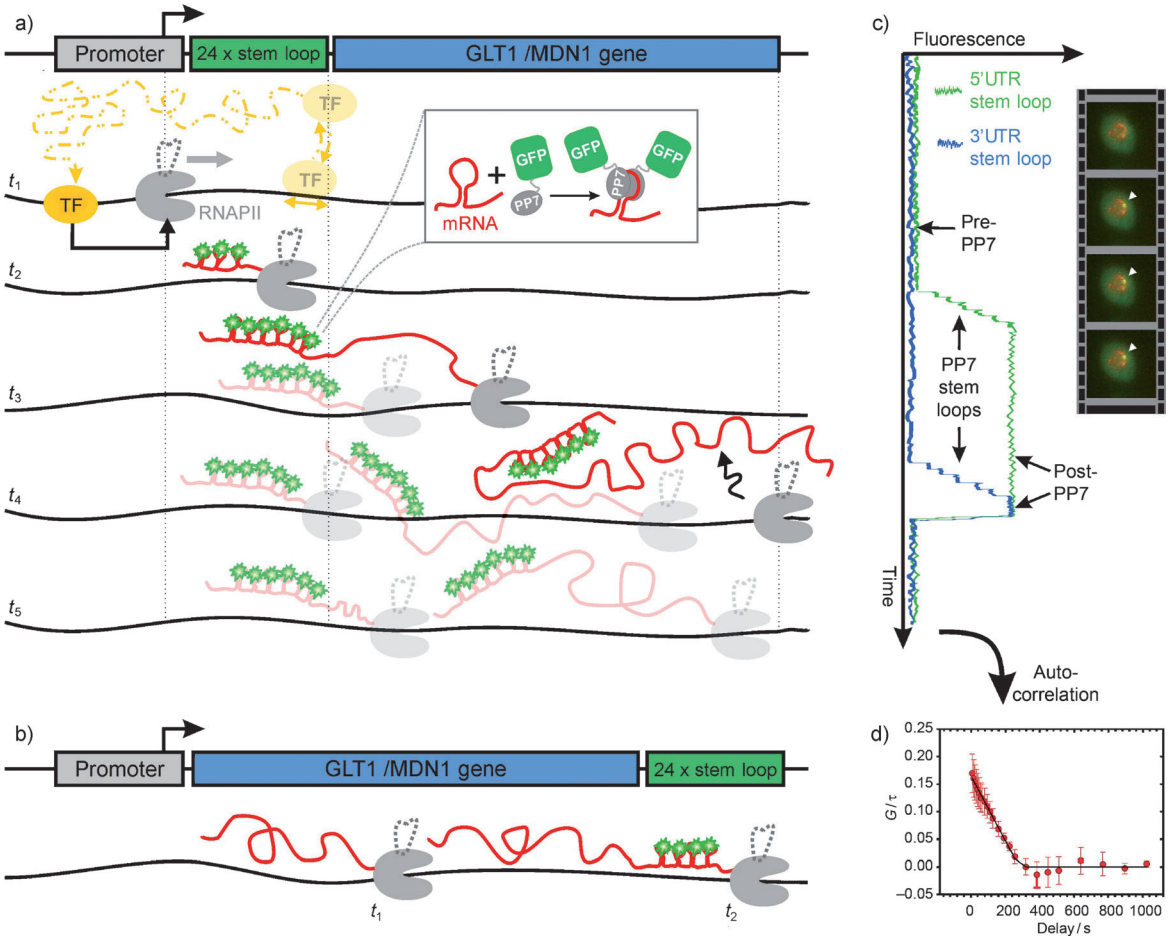

Figure 1. Real-time observation of the transcription initiation and elongation of an endogenous yeast gene and determination of transcription kinetics. a) Diagram of the engineered gene constructs used with 5′UTR insertion of the cassette of 24 PP7 binding sites. t_1–t_5: Schematic representation of the different steps in the transcription of the reporter gene (TF = transcription factor). b) Diagram of the alternative gene construct with the PP7 cassette inserted in the 3′UTR of the endogenous locus and schematic illustration of its transcription. c) Diagram of the fluorescence time traces of the TS of a gene with the stem-loop cassette in the 5′UTR (green) and in the 3′UTR (blue). An exemplary sequence of wide-field microscopy images is shown with a white arrow marking the TS. d) Autocorrelation curve for an active gene locus (GLT1), as computed from long time traces showing numerous transcription events. Red circles: data; black line: best fit; error bars: standard error of the mean.[9]

locus, and the data was best fit to a model that includes no correlation between initiation events (Figure 1d). The results showed that different RNAPIIs acted in an uncorrelated manner on the investigated gene, and that the initiation of transcription is a stochastic process.

Larson et al. then created a second reporter gene construct, in which the cassette with the PP7 binding sites was placed downstream of the endogenous locus into the 3′UTR and which was therefore sensitive only to late events in the lifetime of a nascent RNA molecule (Figure 1b and Figure 1c, blue trace). Nascent RNA could be detected fluorescently only after elongation of the regular gene, shortly before the termination of transcription. Measured fluorescence dwell times were dominated by termination, whereas dwell times for the 5′UTR construct were dominated by the elongation phase of the gene. By combining data from both constructs, it was possible to determine kinetic rates of initiation and elongation. The time between transcription of the stem-loop cassette and loss of the RNA signal was very consistent between different transcription events, which showed that elongation proceeded processively at a steady rate and was not interrupted by major pausing of RNAPII. Nevertheless, throughout the cell cycle, the elongation rate varied threefold, and initiation rates were also observed to be cell-cycle dependent.

Possibly the most interesting aspect of the study by Larsen et al. was the result of an additional experiment in which they investigated the abundance and intranuclear mobility of the transcription factor Mbp1p, which activates the gene through binding to its promoter. Mbp1p was fluorescently labeled by fusion to GFP, and the fluctuation of its fluorescence intensity within the nucleus was measured by two-photon excitation fluorescence correlation spectroscopy to reveal the diffusion behavior of the protein. Interestingly, the comparison of different models for the nuclear diffusion of Mbp1p with the measured time between transcription events yielded a good quantitative agreement. Hence, the initiation of transcription of the investigated gene is dependent only on the success of the transcription factor in its search for its particular promoter binding site. This situation in which a gene is controlled by

only one transcription factor in an environment that is not very dense is a very special case. More dense environments as well as the necessity of multiple factors are likely to be important for other genes and thus may form the basis for what are referred to as transcription factories. Data from genome-wide studies, such as chromatin immunoprecipitation (ChIP), on genes and their regulatory networks should now be used to select interesting candidate genes for further study by the quantitative, single-molecule, single-cell methodology developed by Larson et al. Such investigations will pave the way to a more mechanistic understanding of the dynamics of transcription regulation in the complex cell environment.

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