The Thesis focuses on eukaryotic transcription in individual cells, using human cell lines. It explores the phenomenon of transcriptional pulsing and its possible regulation by the level of chromatin condensation.

The Thesis consists of Review of the Literature, Aims, Materials and Methods, Results, and Discussion. The Review of the Literature section provides sufficient information about eukaryotic transcription with a special focus on what is known about pulsatile transcription. The text flows well, and I have only a minor comment about a statement on page 8: “…different protein levels among individual cells originate from transcription.” This is not entirely true as expression of many eukaryotic genes (most thoroughly studied in yeast and now in humans) is also regulated at the level of translation (for a recent review see e. g. Gunisova et al., 2017 doi: 10.1093/femsre/fux059). Materials and Methods then adequately describe the used techniques.

The Results section is divided into three parts, two publications and one manuscript that has already been submitted. In all three cases, Viola is the first author. The published studies are in peer-reviewed, impacted journals (IFs of 2.649 and 4.259, respectively).

The first project Transcriptional pulsing of a nucleolar transgene describes the behavior of a transgene under the control of an RNApol II promoter that localized to the cell nucleolus. The transcribed RNA was visualized via MS2 epitopes present in the transcript. Viola showed that, despite being in the nucleolus, this transgene was transcribed by RNApol II, its expression was pulsatile, and did not resume at the same time in daughter cells. Moreover, the transcripts were detected at the site of transcription and not elsewhere.

**Question 1:** The transgene encodes an antibiotic resistance for which the cell lines were selected. However, its transcripts were not detected elsewhere than in the
nucleolus. How do you explain this observation as the transcripts need to be translated in the cytoplasm to yield the protein required for the antibiotic resistance?

**Question 2:** The integration site of the transgene is unknown. Have you considered determining this site, e.g. by inverse PCR? Do you expect that it is integrated in/near rDNA clusters? Would you consider using a more specific approach (CRISPR) for creating targeted integrations of this transgene in rDNA and elsewhere to obtain more detailed insights into the role of RNApol II in the nucleolus?

**Question 3:** Could it be, that the structured transcript (the MS2 epitopes) may affect the localization of the transgene to a region (the nucleolus) where other structured RNAs (rRNA) are present?

The second project Transcriptional Spike upon Chromatin Decondensation uses physiological (P) and hyperosmotic conditions (HC) to test how condensation of chromatin (higher in HC) affects transcription. Interestingly, under HC, transcription was still observed, albeit somewhat decreased compared to P. After a HC to P shift, a short spike in transcription was observed. Viola proposed a plausible model for these observations, suggesting that under HC, only initiation is allowed and elongation blocked. Upon chromosome condensation relaxation, elongation is allowed, resulting in the observed increase in transcription.

**Question 4:** The localization of this transgene is outside of nucleolus. Have you tried these types of experiments (P, HC) with the cell lines from the previous project? What is known about chromosome condensation in the nucleolus?

The last project, A Peak of Transcriptional Activity at the Very Beginning of Cell Cycle, describes transcription of POLR2A and TRFC genes during interphase, metaphase, and telophase/early G1. A spike in transcription was observed in a narrow time window in telophase/early G1 and was possibly due to decondensation of mitotic chromosomes.

**Question 5:** Is there some defined organization of transcriptional spots in individual cell phases? In other words, have you observed some correlation between the relative positions of the POLR2A and TRFC transcription spots, suggesting a higher-order organization of DNA in the nucleus?
In summary, the Thesis brings new insights into our understanding of transcriptional pulsing and its regulation in human cell lines, and opens new questions for further research. It is well written and the presentation is at a good level; the only small blemish is the relatively tiny font (in the printed version the ink diffused, resulting in poorly legible text) used for description of graph axes (e. g. Fig. 4-12). Nevertheless, reading the Thesis was a pleasure and I recommend this Thesis to be classified as passed.

Finally, I wish Viola all the best in her further professional career.

Prague, 4 January 2018

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