

Title	Development of a new high-precision quantification methodology for gene expression analysis and its application in iPS cells
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Osaka University

Abstract of Thesis

Name (Panina Yulia)	
Title	Development of a new high-precision quantification methodology for gene expression analysis and its application in iPS cells (遺伝子発現解析における新しい高精度定量法の開発とiPS研究への応用)
<p>Abstract of Thesis</p> <p>Quantification of gene expression on mRNA level is one of the most important tasks of modern biology. High precision of such quantification is of utmost importance for drawing correct conclusions about cellular processes. Real-time quantitative polymerase chain reaction (RT-qPCR) is currently considered the most precise and most sensitive method of quantifying mRNA. However, the standard experimental procedure in RT-qPCR experiment requires the use of reference genes for normalization. The behavior of popular reference genes during long-term biological processes, such as iPS reprogramming, development or aging, has never been investigated. In the initial part of my work, I investigate the behavior of 12 commonly used housekeeping genes for their suitability in RT-qPCR experiments during a representative long-term process, iPS reprogramming, and find that these genes are unsuitable for normalization procedures due to their fluctuation, making standard RT-qPCR inapplicable to iPS reprogramming. Second, I proceed to develop a new methodology for RT-qPCR experimentation that does not require the use of reference genes. Importantly, my methodology increases the precision of obtained measurements while reducing experiment-associated labor and cost. Third, I go on to apply this new methodology to the investigation of the behavior of 70 housekeeping genes during the iPS reprogramming, demonstrating high potential of the new methodology for high-throughput use. The results obtained in the course of the analysis reveal previously unknown patterns of gene dynamics during iPS reprogramming. I found a collective pattern in the rise of most ribosomal genes' expression, with the exception of small ribosomal subunits Rps18 and Rps9, during the reprogramming process. Furthermore, I found that cell systems associated with growth inhibition, such as apoptosis-associated genes, ubiquitin system genes, or tumor suppressor genes, are collectively down-regulated. Moreover, the analysis showed that there exists a time-dependent pattern in gene expression dynamics of chosen genes, and hints at the existence of an unknown event early in the reprogramming process. These results showcase successful application of my newly developed methodology for gene expression analysis in long-term biological processes, and its notable precision in detection of gene expression changes.</p>	

論文審査の結果の要旨及び担当者

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論文審査の結果の要旨			
<p>Yulia Panina君は、人工多能性幹細胞研究における定量PCR(Polymerase Chain Reaction)計測について、(1)参照遺伝子（ハウスキーピング遺伝子）の発現の不安定さを示し、(2)その解決策として新しい解析手法を考案、さらに(3)開発した新手法を用いて人工多能性リプログラミング過程において解糖系関連遺伝子の発言が大きく変化していることを発見した。彼女の研究は、人工多能性幹細胞研究、特に、リプログラミング過程の解明に向けた研究において、重要な問題を提起したと共にその解決策を提供した。しかし残念なことに、新手法の論理的な検証、および、人工多能性リプログラミング過程における解糖系関連遺伝子の機能については調べ切れておらず、今後に期待される。</p> <p>総評として、Yulia Panina君は、生命科学に有用な方法を開発し、その方法は新たな知見を生み出す可能性を示している。課題は残されているものの、本研究は、博士学位論文として評価できるものであり、学位を授与するに値すると認める。</p>			
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