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Identification and initial characterization of POLIII-driven transcripts by msRNA-sequencing

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ABSTRACT

Non-coding RNAs (ncRNAs) are powerful regulators of gene expression but medium-sized (50–300 nts in length) ncRNAs (msRNAs) are barely picked-up precisely by RNA-sequencing. Here we describe msRNA-sequencing (msRNAseq), a modified protocol that associated with a computational analyses pipeline identified about ~1800 msRNA loci, including over 300 putatively novel msRNAs, in human and murine cells. We focused on the identification and initial characterization of three POLIII-derived transcripts. The validation of these uncharacterized msRNAs identified an ncRNA in antisense orientation from the *POLR3E* locus transcribed by POLIII. This msRNA, termed POLAR (POLR3E Antisense RNA), has a strikingly short half-life, localizes to paraspeckles (PSPs) and associates with PSP-associated proteins indicating that msRNAseq identifies functional msRNAs. Thus, our analyses will pave the way for analysing the roles of msRNAs in cells, development and diseases.

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Introduction

Current ncRNA sequencing strategies mainly focus on either long ncRNAs (e.g. lincRNA) with more than 200–300 nucleotides (nts) in length or short ncRNAs (e.g. miRNA) with a size below 30 nts. Due to library preparation protocols these analyses, however, barely cover medium-sized ncRNAs with a length between 50 and 300 nts. An essential limitation of small RNA library protocols is that 5′-end modifications in msRNAs, e.g. cap structures or triphosphates, are not removed routinely. The analysis of lncRNAs usually involves RNA fragmentation to achieve coverage of the complete RNA body. This essentially limits the coverage of msRNAs which are fragmented as well and thus fall below size limits of following library preparation steps or yield read peaks not reflecting the full-length transcript. Detailed protocols have been established for short ncRNAs as well as long ncRNAs highlighting the importance of, e.g., RNA fragmentation, size selection and 5′/3′-end modifications [1,2]. Finally, annotation analyses of msRNAs are complicated by the fact that these are frequently encoded by multiple gene or pseudogene loci. Despite these limitations, hundreds of msRNAs had been identified over recent decades. They are transcribed by all three RNA polymerases, but POLIII solely synthesizes msRNAs [3]. Like other RNAs, msRNAs are processed and/or modified, for instance the splicing and pseudouridylation of tRNAs [4]. Functional analyses revealed that msRNAs serve essential roles in splicing (e.g. snRNAs) [5], RNA processing (e.g. RNAse P) [6], transcription (e.g. 7SK) [7] and mRNA translation (e.g. tRNAs) [8]. In support of this, msRNAs have been implicated in human diseases like cancer (e.g. tRNAs) [9] or genetic disorders like the Prader-Willi-Syndrome (snoRNAs) [10]. Despite their multiple roles and potential pathophysiological relevance, a genome-wide experimental survey of msRNA-properties and -expression had not been performed systematically to the best of our knowledge.

Here we describe a protocol for the isolation, sequencing and computational analysis of msRNAs. In addition to the sole identification of msRNAs, we demonstrate that msRNAseq allows analysing the biogenesis, processing, editing and modification of msRNAs. Among the identified msRNAs we characterized POLAR, the first known msRNA recruited to paraspeckles.

Materials and methods

Cell culture and transfection

HEK293T/17 and B16-F10 cells were purchased from the ATCC and cultured in DMEM supplemented with GlutaMAX and 10% FBS (Life Technologies). For RNAi HEK293 cells were seeded at a density of 500.000 cells per well (6-well plate) and transfected with Lipofectamine RNAiMAX (Life Technologies) according to manufacturer's protocols. For La-directed siRNAs, cells were harvested after 72 hours post-transfection. Due to strong proliferation effects cells with BDP1-directed knockdowns were terminated after 36 hours. The POLAR gene was amplified from HEK293/T17

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Supplemental data for this article can be accessed here.