Non-coding RNA biogenesis

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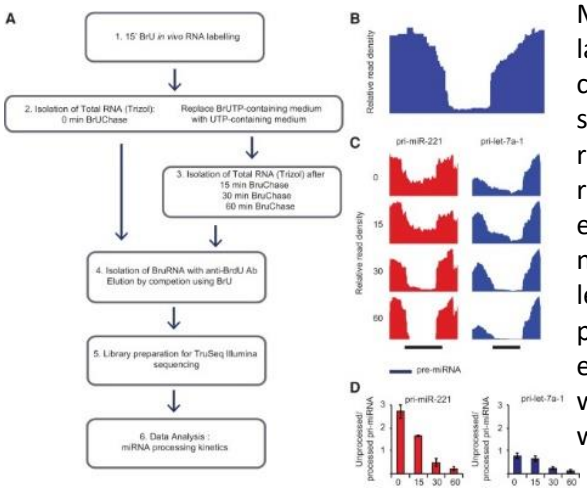
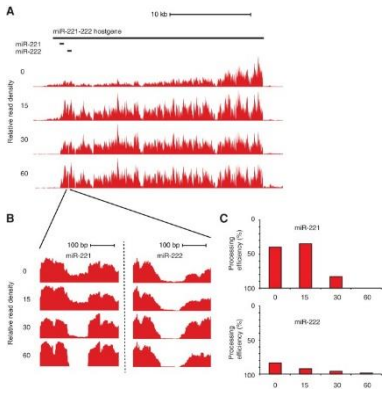
MicroRNAs (miRNA) are small RNAs that mediate post-transcriptional regulation of gene expression. miRNAs are transcribed as primary transcripts as long as 30 kb and processed by the Microprocessor complex in the nucleus. The Microprocessor complex is the minimal complex required for pri-miRNA processing in vitro, consisting of the two proteins, Drosha and DGCR8.
Sequencing of chromatin-associated RNA can reveal the steady-state processing efficiency of individual pri-miRNAs within the cell, demonstrating pri-miRNA processing as one of the most important factors for determining the level of mature miRNAs. To follow processing of pri-miRNAs endogenously over time without constraints of their cellular localization or differential transcription, we use pulse-labeled RNA and follow its processing over time.

Techniques



Measuring pri-miRNA processing kinetics with nascent RNA labeling. (*A*) Workflow for RNA pulse labeling with BrU and chase to follow nascent RNA. (*B*) Concept of processing signature in pri-miRNAs. Processing extent is calculated as the read density in the pre-miRNA region compared to the flanking regions. Processing efficiency is calculated as (1 − processing extent). (*C*) Processing signatures in RNA-sequencing data from nascent RNA in pulse-chase experiment for pri-miR-221 and pri-let-7a-1. (*D*) Quantification by PCR of unprocessed/processed pri-miRNA, for example, shown in *C* from two independent experiments. The *y*-axes show the relative read density as a window of minimum-to-maximum read counts within the window.



Differential processing within the pri-miR-221/222 polycistronic pri-miRNA transcript. (*A*) Overview of the genomic region and full pri-miRNA transcript. (*B*) Enlarged read densities around pre-miRNAs for miR-221 and miR-222. (*C*) Quantification of processing efficiency from *B*.

Projects

Processing co-factors and polyadenylation of pri-miRNA-222/221 using pulse-chase assays for nascent RNA processing, tissue-culture models, RNA interference, PCR, quantitative real-time PCR, western blot.

Links (f.eks. gruppens hjemmeside) og evt. publikationer af relevans for projekterne

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