

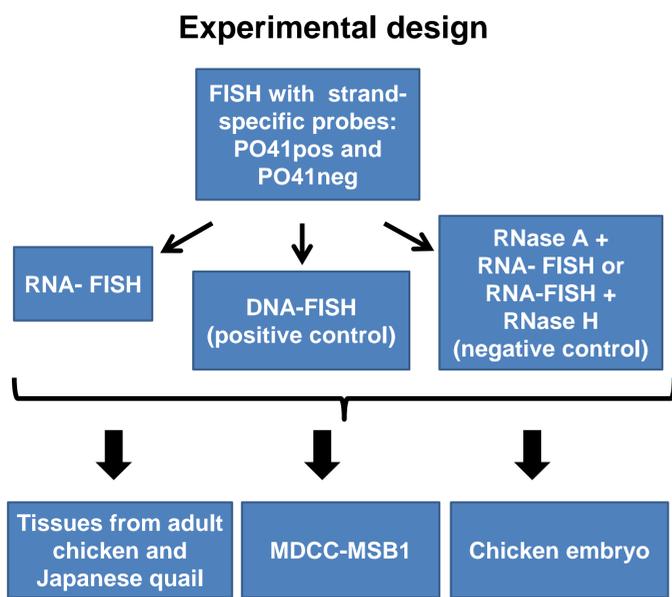


# Non-coding RNA derived from a conservative subtelomeric tandem repeat in embryos and adult tissues of Galliformes species revealed by FISH approach

Irina Trofimova, Darya Popova, Alla Krasikova

Cytology and Histology department, Saint-Petersburg State University, Saint-Petersburg, Russia

Transcription of tandemly organized DNA at terminal chromosomal regions is poorly investigated. In this study transcription of conservative subtelomeric tandem PO41 ("pattern of 41 bp") repeat in adult chicken (*Gallus gallus domesticus*) and Japanese quail (*Coturnix coturnix japonica*) somatic tissues and chicken embryos was examined.



### Subtelomeric PO41 repeat is transcribed in chicken lymphoblastoid MDCC-MSB1 cells, forming single-stranded non-coding RNAs with short hairpin structures

Detection of **C-rich** (upper row) and **G-rich** (bottom row) PO41 RNAs by RNA FISH on MDCC-MSB1 cells. Scale bars: 5 µm.

Scheme. Trofimova et al. *Molecular Cytogenetics* (2014) 7:102

PO41 RNAs from both strands of the repeat were detected in nuclei of chicken malignized cells. Focus enriched with PO41 RNAs was often localized adjacent to small-grained granules containing RNA polymerase II, that could suggest that in interphase nuclei the PO41 repeat is transcribed by RNA polymerase II. Pretreatments with RiboShredder RNase cocktail or RNase A (cleaves single-stranded RNA) before RNA FISH as well as with RNase H (cleaves RNA from RNA/DNA duplex) after RNA FISH effectively eliminated fluorescent signals. Treatments with RNase III (cleaves double-stranded RNA) or RNase H before FISH decreased the size and fluorescence intensity of PO41 RNAs foci and eliminated dispersed RNA-transcripts signals. Scheme illustrates major focus of PO41 RNA which associated with RNA polymerase II presumably represents nascent RNA (a) while dispersed PO41 repeat transcripts localized in euchromatin space presumably represent released RNA (b). PO41 RNA is predominantly single-stranded with short double-stranded regions (b).

### Subtelomeric PO41 repeat is transcribed in chicken embryogenesis and in chicken and Japanese quail adult somatic cells

Detection of **C-rich** and **G-rich** PO41 RNAs in chicken embryo by whole-mount FISH. Scale bars: 100 µm.

Detection of **C-rich** and **G-rich** PO41 RNAs in adult chicken somatic tissues (cryosections, upper row, whole-mount bottom row). Scale bars: 10 µm.

Detection of **C-rich** and **G-rich** PO41 RNAs in adult Japanese quail somatic tissues (whole-mount, upper row; cryosections, bottom row). Scale bars: 40 µm.

C- and G-rich RNase A sensitive PO41 RNAs were found in all tissues of chicken embryo and in adult chicken and Japanese quail somatic tissues: skeletal muscles, oviduct, cerebellum, telencephalon. In small and large intestine, only C-rich PO41 repeat transcripts were detected in both species.

### Redistribution of PO41 repeat transcripts during the cell cycle progression

Detection of **G-rich** PO41 RNAs in dividing MDCC-MSB1 cells. Scale bars: 3 µm.

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During prophase, PO41 RNAs form compact foci, which localize between condensed chromosomes (b). At metaphase stage transcripts distribute between condensed chromosomes (c, d). At early anaphase, transcripts localize predominantly in equatorial zone of the dividing cell (e), while at late anaphase and telophase, PO41 transcripts form clusters of granules, surrounding terminal regions of chromosomes (f, g, h). At a cytokinesis stage PO41 repeat transcripts form local foci in both daughter nuclei (i). In embryo dividing cells distribution of PO41 RNAs is similar to those obtained in MDCC-MSB1 cells.

### CONCLUSIONS:

- Subtelomeric tandem PO41 repeat is transcribed in chicken embryogenesis, in adult chicken and Japanese quail somatic tissues and chicken malignized cells.
- PO41 RNAs are single-stranded with partly shaped double stranded structures.
- Extremely uniform character of PO41 repeat transcription and transcripts distribution at different stages of development and adult tissues, in cell cycle, among species and cell types indicates on universal functions of PO41 RNAs.