THE TOPOLOGICAL RELATIONSHIP BETWEEN RIBOGENESIS, mRNA TRANSCRIPTION/SPLICING AND THE TENSION OF ACTIN CYTOSKELETON

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Aims

To study the topological relationship between the nucleolar fibrillar centres, perinucleolar heterochromatin, nuclear speckles (splicesomes) and the lamin- associated heterochromatin after RNA transcription inhibition.

Methods

MCF7 breast cancer cells were treated in 3D preserving conditions by increasing concentration/time of Actinomycin D (AcD). Immunofluorescent staining, confocal microscopy, and image analysis were performed.

Results

In control cells with active rRNA and mRNA synthesis, the perinucleolar repressive heterochromatin labelled by H3K9Me3/cen forms extended structures bent around nucleoli, speckles located more externally are also extended, lamin B1 ideally outlines the nuclear envelope (NE), while actin filaments form fibrils both circular around NE and perpendicular or at angles to it, attached to the cellular membrane. When the nucleolar synthesis is initially suppressed by low AcD, the remnant Pol I cofactor RPA194 forms a few large granules at the nucleolar margin, H3K9Me3 heterochromatin condenses in round clumps between them, while speckles also condense as regular circular structures around the latter – all together revealing a radial-concentric order. The lamin B1-positive nuclear contour becomes irregular and lamin forms nuclear invaginations towards the nucleoli increasing ~3-fold frequency with suppression of both syntheses. Actin ring around the nucleus becomes thicker, while its extending cytoplasmic fibers less tense, further actin network is disappearing and it remains only under the cytolemma. Full suppression of both syntheses by high dosage/prolong AcD or a-amanitin brings to disorganization of the radial-concentric nuclear order.

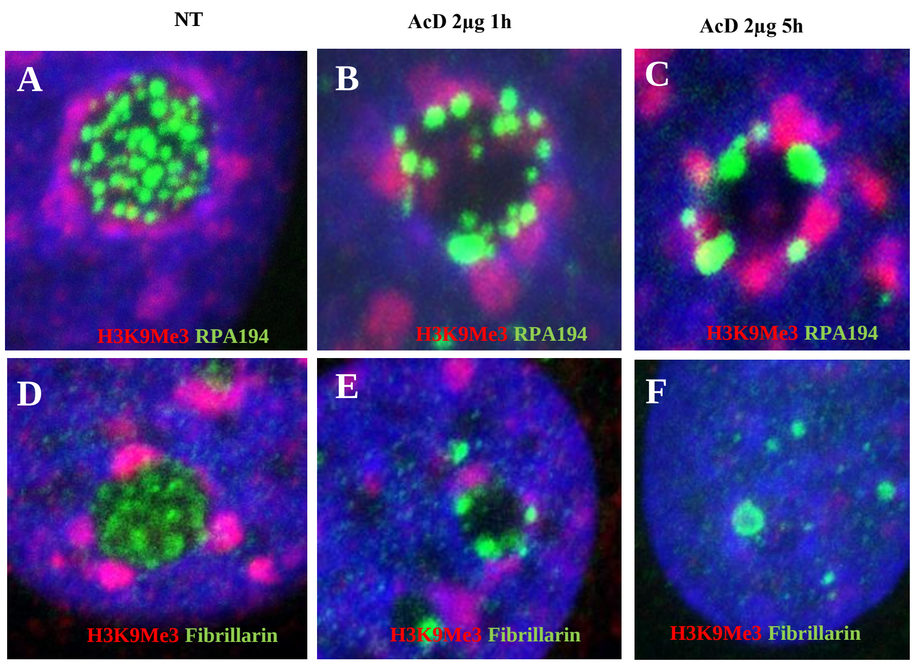

Fig.1. RNA transcription inhibition by Actinomycin D (AcD). (A, D) Control cells with active RNA synthesis show granular staining of nucleolar proteins Pol I cofactor RPA194 and Fibrillarin. (B, E) After short incubation time with AcD, RPA194 and Fibrillarin form a few large granules at the nucleolar margin showing RNA transcription suppression. (C, F) Prolonged AcD treatment causes even more clumping of nucleolar markers.

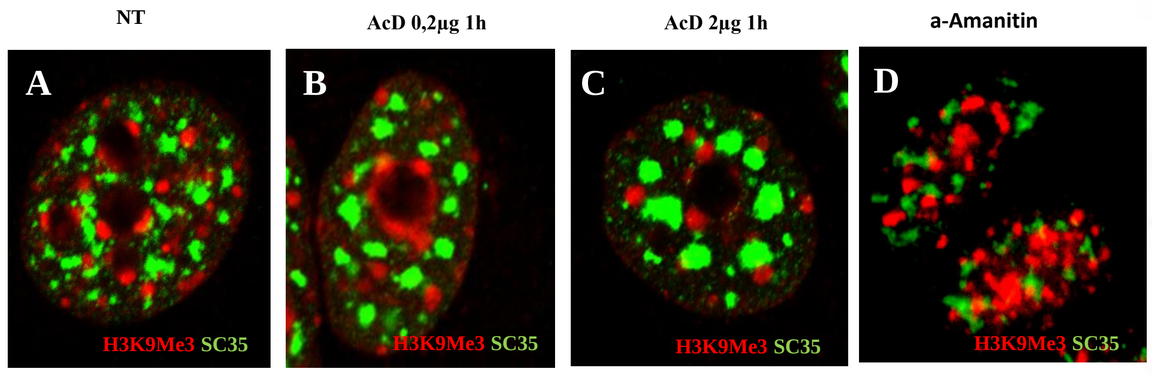

Fig.2. Topological relationship between perinucleolar heterochromatin and nuclear specles after transcription inhibition. (A) In control cells H3K9Me3 labelled heterochromatin clusters are mostly small, dispersed, and form extended structures around nucleoli. Nuclear specles SC35 are mostly elongated or round and "dashed", irregularly dispersed. (B) After mild AcD treatment the perinucleolar H3K9Me3 clumps appear and SC35 become concentrically clustered around nucleoli; (C) Higher AcD dosage shows radial pattern of SC35 around the nucleoli with condensed H3K9Me3; (D) Treatment with a-amanitin suppressing mRNA synthesis or prolonged treatment with AcD (not shown) impairs the radial-concentric nuclear order provoked by mild AcD. SC35 become decondensed and their decondensed material tend to surround H3K9Me3 heterochromatin clumps, which also become somewhat looser and move away from the nucleolus margin.

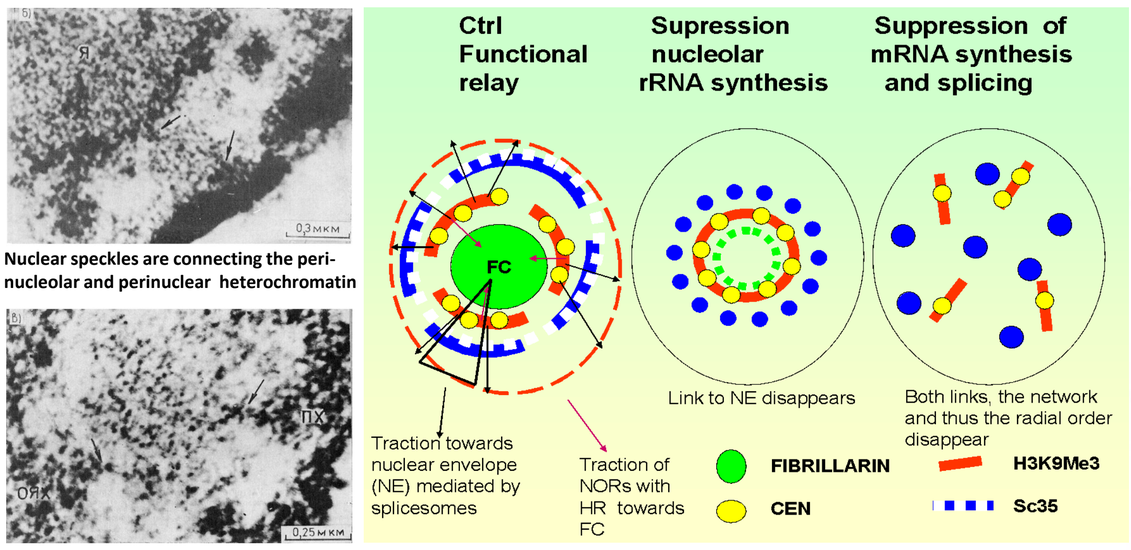

Fig.3. <u>Hypothesis</u> of the radial-concentric nuclear order. AcD treatment reveals connections between fibrillar centres (FC) and perinucleolar H3K9Me3-positive pericentric heterochromatin, which, in turn is linked via splicesome to the lamin-associated heterochromatin presuming topological coordination of rRNA and mRNA syntheses.

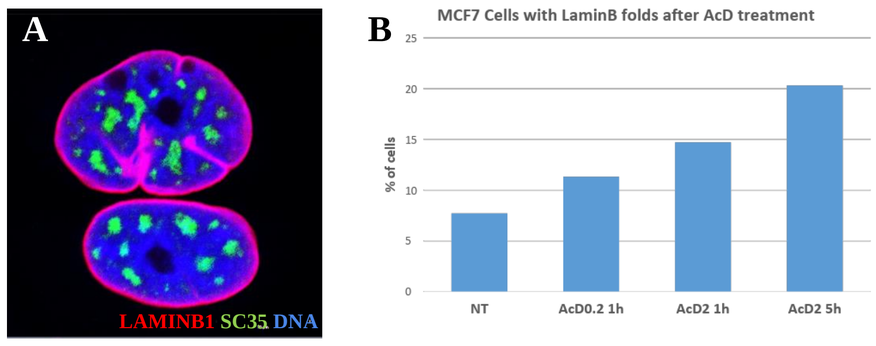

Fig.4. Increase of cell proportion with LaminB1 invaginations after AcD treatment. (A) Nucleus with LaminB1 invaginations; (B) Counts of nuclei with LaminB1 invaginations.

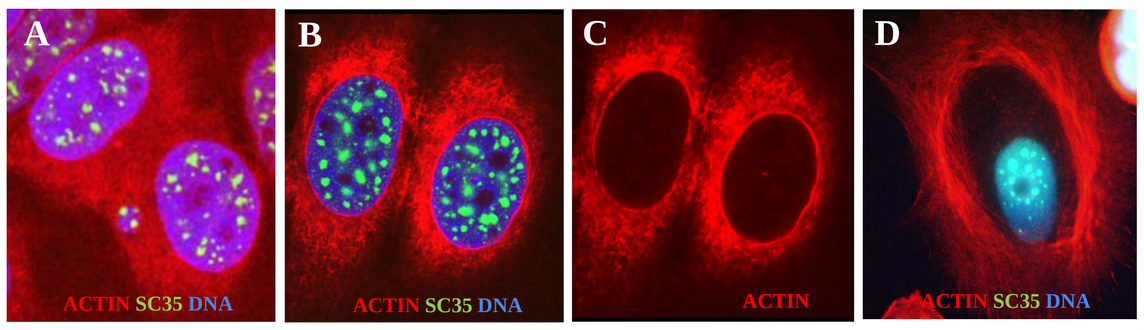

Fig.5. Actin ring after AcD treatment and further disappearance of actin network around the nucleus . (A) Control cells; (B-D) cells after 1h AcD treatment.

Conclusion: The links of the perinucleolar and lamin-associated heterochromatin with inner nuclear compartments are involved in topological coordination between the ribogenesis and mRNA maturation, where the radial tension of the actin cytoskeleton exerted via concentric elasticity of the nuclear lamin is part of this regulation.

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