in vivo. We also cloned a *luc* gene (to express firefly luciferase) under the control of the *leu-500* promoter and a *lacZ* gene under the control of the T7 promoter. Using this unique system, we discovered that TDDS was able to significantly activate the *leu-500* promoter. In the second *in vivo* system, we studied how TDDS activated the *lue-500* promoter on the chromosome. Utilizing a transposon Tn7-based method, the divergently coupled $P_{leu-500}$ and $P_{T7A1/O4}$ promoters were inserted to the *attTn7* site of the chromosome of MG1655 and VS111. Using these two *E. coli* strains, we found that TDDS was able to activate $P_{leu-500}$ ~13- and 7-fold in VS111 and MG1655, respectively. Our studies suggest that TDDS has important biological functions in *vivo*.

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Nucleotide Structural Polymorphisms Formed by GGGGCC Repeats Cause C9orf72 Abortive Transcription and Nucleolar Stress

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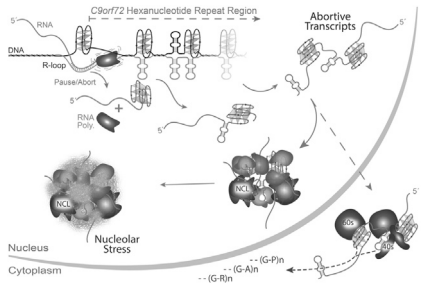
A hexanucleotide repeat expansion (HRE), GGGGCC, in the C9orf72 gene is the most common genetic cause of the neurodegenerative diseases amyotrophic lateral sclerosis (ALS) and frontotemporal dementia (FTD). The pathogenic mechanisms related to the repeat expansion are unknown. Here we identify a novel mechanism that leads to impaired transcription and ribonucleoprotein recognition in C9orf72 HRE carriers. The HRE impedes transcription and generates abortive RNA transcripts, which are aggravated by the formation of G-quadruplexes and RNA:DNA hybrids, R-loops. The accumulated RNA of the C9orf72 HRE binds to specific proteins in an HRE-conformation-dependent manner. One such protein nucleolin, which preferentially recognizes the G-quadruplexes on the RNA of the C9orf72 HRE, significantly mislocalizes in patient cells including induced pluripotent stem (iPS) cell-derived motor neurons, which suggests increased nucleolar stress. These findings provide a pathogenic mechanism for the C9orf72 HRE, including loss of transcriptional products and gain of RNA toxic properties. We propose that the unique nucleic acid structural motifs observed on the C9orf72 HRE are the fundamental cause of the age-dependent neurodegenerative diseases.



2468-Pos Board B160

Mechanical Strain Generated by RNA Polymerase during Transcription Initiation can Drive Structural Changes in DNA Topology that Relieve Repression

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DNA in cells is often topologically closed, and in some cases, tightly looped by proteins typically associated with transcriptional repression. During transcription initiation, RNA polymerase (RNAP) is challenged to open duplex DNA, wherein RNAP generates torque that consequently overtwists DNA flanking the melted promoter. As initiation proceeds, the transcription bubble expands as RNAP synthesizes downstream RNA while maintaining upstream DNA contacts. We hypothesized that the mechanical strain imparted to DNA during initiation would repress transcription from DNA templates that restrict the relief of torsional stress. To test our hypothesis, we constructed circular DNA templates that are 100 to 108 bp in size (i.e., each initially twisted to various degrees) and quantified transcription initiation by the bacteriophage T7 RNAP. We find that transcriptional repression during initiation is dependent on the sign and magnitude of initial twist within the DNA templates. Surprisingly, however, we observe that for the most overtwisted templates, repression is relieved at positions beyond the promoter that are dependent on the initial DNA twist. To interpret these results, we used elastic rod and molecular dynamics simulations to predict the structures of both the RNAP and the circular DNA template during initiation. Our modeling studies confirm that RNAP is capable of overtwisting the circular DNA template to the point of buckling from a planar into a supercoiled conformation. Further analysis reveals that initial DNA twist determines at what position along the template supercoiling will occur during initiation and that adopting a supercoiled structure substantially relieves the torque encountered by the RNAP. Our results demonstrate that repression of RNAP during initiation is determined not only by the initial mechanics of the DNA template, but also by the torque generated by RNAP itself.

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Sequence-Specific RNAP-DNA Interactions in Transcription Initiation and Elongation: Core Recognition Element (CRE)

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The crystal structure of RNA polymerase open complex (RPo) indicates that RNAP core interacts with the transcription-bubble nontemplate strand segment corresponding to positions −4 to +2 in which we designated it as core recognition element, CRE. In this study, we sought to investigate the sequence determinants, the recognition mechanism and the functional roles of CRE.

To determine whether RNAP-CRE interactions are sequence-specific, we constructed all possible nucleotide substitutions at each CRE position, and assessed effects on RNAP-DNA interaction in equilibrium binding experiments and high-salt-induced-dissociation off-rate experiments. We conclude that RNAP CRE interactions are specific and that the consensus CRE is t/g-n-n-n-T-G.

To identify individual RNAP amino acids that mediate specificity at CRE positions +2 and +1, we constructed single Ala substitutions of RNAP residues that contact CRE positions +2 and +1, and assessed effects on RNAP DNA interactions with promoter derivatives containing all possible nucleotide substitutions at CRE positions +2 and +1. We conclude that bR151, bD446, or bR451 mediate specificity at CRE position +2, and βW183 mediates specificity at CRE position +1.

To define the structural basis of specificity at CRE positions +1 and +2, we determined crystal structures of RPo derivatives containing all possible nucleotide substitutions at CRE positions +2 and +1. We conclude that specificity at CRE positions +2 and +1 manifests itself not only in quantitative differences in binding thermodynamics and kinetics, but also in qualitative differences in structure.

Our results establish that RNAP-CRE interactions are sequence specific. We propose that RNAP-CRE interactions contribute to the sequence specificity of promoter binding, promoter unwinding, promoter scrunching, and promoter escape during transcription initiation and also contribute to the sequence specificity of pausing during transcription elongation.

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Toward a General Mechanism for Transcription Initiation

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At the λP_R promoter, formation of initiation-competent open complexes involves a series of conformational changes in both RNA polymerase (RNAP) and promoter DNA after initial specific binding. The first set of these changes (described by equilibrium constant K_1) bend the downstream duplex DNA into the cleft of RNAP. These reversible steps are followed by the rate-determining step (rate constant k_2) in which the DNA is opened in the cleft using binding free energy. This initial open complex is unstable (lifetime ~1 s) but is greatly stabilized by irreversible conformational changes (quantified by equilibrium constant K_3) that reposition the nontemplate strand in the cleft and assemble downstream mobile elements (DME) of RNAP on the downstream duplex DNA, forming RP_o (lifetime ~1 day).

In all three phases of this mechanism, large conformational changes in RNAP and/or promoter DNA take place, and allosteric communication occurs over large distances. However, the details are not yet understood for λP_R or any other promoter. Are these mechanistic steps universal or promoter-specific? Which steps are the most important targets of regulation by promoter sequence, transcription factors, ligands, and solutes? What determines the efficiency of productive or abortive transcription?

To address these questions, we have determined the effects of sequence and length variants of the λP_R and T7A1 promoters and several RNAP variants on the kinetics of steps of the initiation mechanism. Taken together, our kinetic data suggest that the early and late steps of the mechanism are the most variable. We therefore propose that these steps are the targets of most cis- and trans-acting regulatory factors, while the opening/closing step is relatively unregulated. This is analogous to many enzyme mechanisms in which most regulation of catalytic velocity occurs in the early and late steps rather than the catalytic step itself.