Review of: "The interaction between the Spt6-tSH2 domain and Rpb1 affects multiple functions of RNA Polymerase II"

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Context and Summary:

This paper presents a detailed functional analysis of one protein-protein interaction site in the RNA polymerase II elongation complex (RNAPII EC): that between the non-canonical, tandem SH2 domains (tSH2) in Spt6 and the phosphorylated linker region of Rpb1[1]. The discovery of this interaction by the Hill and Formosa labs in 2017 was unexpected, as previous work seemed to indicate that tSH2 bound the phosphorylated Rpb1 C-terminal domain (CTD) repeats[2,3]. This aligned with the general expectation in the field for two reasons: the general role for the CTD as a recruiting platform for the EC[4]; and the identification of phospho-tyrosine, the canonical interacting partner of SH2 domains, as a bona fide CTD modification[3,5]. Using budding yeast proteins, these labs made the surprising finding that tSH2 bound to Rpb1 by engaging a region just outside of the CTD termed the “linker.” Tight binding of tSH2 to the linker region required phosphorylation of Rpb1-S1493, bound in the C-terminal SH2 domain, and either Rpb1-T1471 or Y1473, bound in the N-terminal SH2 domain. Mutation of these residues or phospho-recognition residues in tSH2 caused phenotypes characteristic of *SPT6* loss-of-function in vivo[1]. In subsequent work, the relevant kinase responsible for Rpb1 linker phosphorylation was shown to be Bur1, functional analog of mammalian Cdk9[6]. This interaction is likely a highly conserved feature of the EC, as it was observed in a reconstituted mammalian EC that was phosphorylated by Cdk9[7].

Connell et al[8] use these findings as a starting point for in-depth investigation of the significance of the tSH2-linker interaction for transcription. They performed RNA-seq analysis on *spt6* and *rpb1* mutants (informed by previous structural work) that abolish the tSH2-linker interaction. The *SPT6* mutant in question ($spt6^{R,KK}$) eliminates the phospho-binding pockets in both SH2 domains, whereas the *RPB1* mutant ($rpb1^{TPY,FSP}$) eliminates all phospho-sites in the linker region. These mutants affected small but highly overlapping sets of genes, in support of the Rpb1 linker as the important binding site of tSH2 in cells. The remainder of the paper investigates the effects of these mutations on the expression of individual genes in more detail. The key insights obtained about the function of the tSH2:Rpb1 linker interface were the following: a role in regulating the stability of cryptic unstable transcripts (CUTs) transcribed from gene promoter regions, leading to altered transcription initiation site usage at a subset of genes; a role in regulating co-transcriptional splicing, particularly for transcripts with snoRNA-containing introns; a role in maintaining Spt6 occupancy genome-wide; a role in maintaining occupancy of the +1 nucleosome. None of these functions were shared with other histone chaperones, and were not shared with other *spt6* mutants that reduced Spt6 occupancy at transcribed genes, suggesting they are unique to the tSH2:Rpb1 interface. It was suggested that these functions reflect a role for this interface in organizing the Rpb1 CTD during transcription.

Strengths:

1. This was a beautifully executed study that managed to extract meaningful biological insights from genomics datasets that, overall, showed modest effects.
2. The nuance gleaned from the individual gene analyses was impressive. The role in CUT degradation originated from study of alternate transcription start site usage at *IMD2*. The splicing defect was evident in the RNA-seq data, but careful validation pointed to a specific effect at genes with a particular intron configuration.
3. The paper really drove home the point that the Rpb1 linker is THE physiological binding partner for tSH2, at least under the conditions examined here. This was mostly taken as a foregone conclusion by the authors, but recent studies, based on biochemical and structural data, continue to suggest that tSH2 binds the phosphorylated Rpb1 CTD[9]. The striking phenotypic concordance between the *spt6*^{R,KK} and *rpb1*^{TPY,FSP} mutants seen in this paper makes it hard to argue that the phospho-CTD interactions are meaningful, especially given that the *spt6*^{R,KK} mutant should eliminate all phospho-recognition by the tSH2[1]. One cannot rule out the possibility that there is some role for phospho-CTD binding under specialized growth conditions, although several of these have been tested in previous work and also showed close tracking of tSH2 and linker mutant phenotypes[1].

Alternate perspectives:

1. Phospho-regulation of the tSH2:Rpb1 interaction was not addressed in the paper. Previous work by the Hill/Formosa labs, as well as the cryo-EM study of Vos et al, strongly implicate Bur1/Cdk9 as the relevant kinase for the Rpb1 linker. It may be that the Rpb1 linker phospho-specific antibodies described previously[6] are not ChIP-grade, in which case detailed study of where and when these residues become phosphorylated during transcription is not currently possible. Nonetheless, it would be of great interest to use the *rpb1* linker mutants and the phenotypes described here to address potential functional overlap with other known Bur1/Cdk9 target sites, notably those in the C-terminal region (CTR) of Spt5. The Vos et al[7] paper provides a rationale for this idea. Their structure shows that the Spt5 CTR is positioned near the RNA exiting the RNAPII catalytic core, such that phosphorylation would be poised to recruit RNA processing factors. The proximity of Spt6 to the RNA exit channel, and the data in this paper, intimate a similar role for Spt6 tethering by the phosphorylated linker. Cdk9 orthologs are essential for RNAPII elongation (and viability) in diverse eukaryotes, but the underlying mechanisms remain unclear[10]. The availability of mutants specifically ablating different Bur1/Cdk9 targets affords a unique opportunity to ask questions about functional cooperation.

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