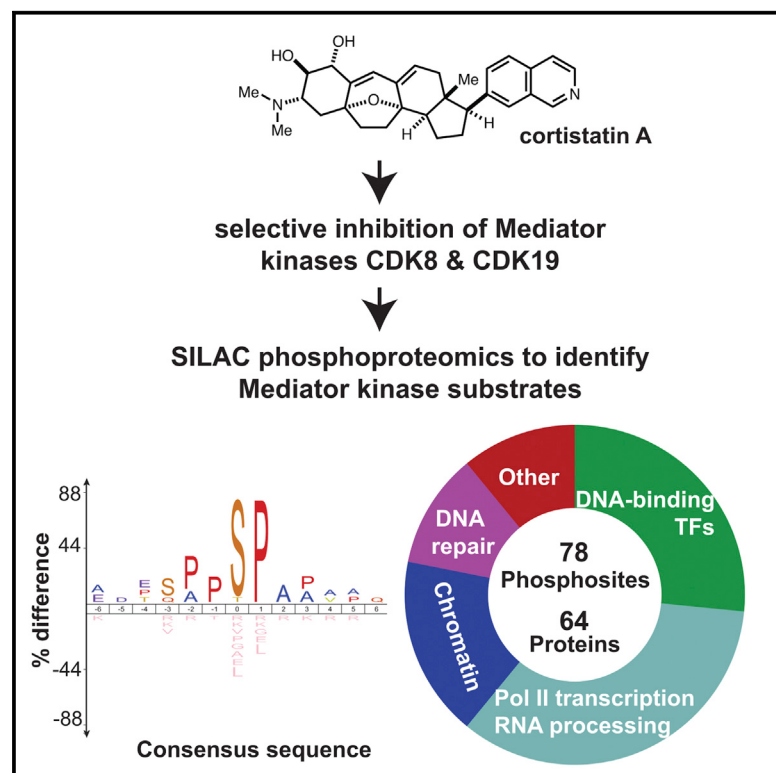


Cell Reports

Identification of Mediator Kinase Substrates in Human Cells using Cortistatin A and Quantitative Phosphoproteomics

Graphical Abstract



Authors

Zachary C. Poss, Christopher C. Ebmeier, Aaron T. Odell, ..., Robin D. Dowell, William M. Old, Dylan J. Taatjes

Correspondence

taatjes@colorado.edu

In Brief

Taking advantage of a recently characterized Mediator kinase (CDK8 and CDK19) inhibitor, Poss et al. use SILAC-based proteomics and phosphoproteomics, RNA-seq, and biochemical assays to provide an extensive analysis of human Mediator kinase function. The methods applied and targets identified provide a valuable resource for future studies.

Highlights

- Large-scale identification of CDK8 and CDK19 substrates in human cells
- Seventy-eight high-confidence targets identified within 64 different proteins
- Quantitative proteomics largely show no increased turnover of CDK8/19 targets
- Data support CDK8/19 roles in metabolism and DNA repair, as well as transcription

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Identification of Mediator Kinase Substrates in Human Cells using Cortistatin A and Quantitative Phosphoproteomics

Zachary C. Poss,¹ Christopher C. Ebmeier,² Aaron T. Odell,^{2,3} Anupong Tangpeerachaiikul,⁴ Thomas Lee,¹ Henry E. Pelish,⁴ Matthew D. Shair,⁴ Robin D. Dowell,^{2,3} William M. Old,² and Dylan J. Taatjes^{1,*}

¹Department of Chemistry and Biochemistry, University of Colorado, Boulder, CO 80303, USA

²Department of Molecular, Cell, and Developmental Biology, University of Colorado, Boulder, CO 80309, USA

³BioFrontiers Institute, University of Colorado, Boulder, CO 80309, USA

⁴Department of Chemistry and Chemical Biology, Harvard University, Cambridge, MA 02138, USA

*Correspondence: taatjes@colorado.edu

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SUMMARY

Cortistatin A (CA) is a highly selective inhibitor of the Mediator kinases CDK8 and CDK19. Using CA, we now report a large-scale identification of Mediator kinase substrates in human cells (HCT116). We identified over 16,000 quantified phosphosites including 78 high-confidence Mediator kinase targets within 64 proteins, including DNA-binding transcription factors and proteins associated with chromatin, DNA repair, and RNA polymerase II. Although RNA-seq data correlated with Mediator kinase targets, the effects of CA on gene expression were limited and distinct from CDK8 or CDK19 knockdown. Quantitative proteome analyses, tracking around 7,000 proteins across six time points (0–24 hr), revealed that CA selectively affected pathways implicated in inflammation, growth, and metabolic regulation. Contrary to expectations, increased turnover of Mediator kinase targets was not generally observed. Collectively, these data support Mediator kinases as regulators of chromatin and RNA polymerase II activity and suggest their roles extend beyond transcription to metabolism and DNA repair.

INTRODUCTION

An important first step in understanding the cellular function of kinases is to identify the substrates that they modify. This first step has remained a persistent challenge, in part because of the difficulties in the development of highly potent and selective kinase inhibitors. The human CDK8 kinase exists in a 600-kDa complex known as the CDK8 module, which consists of four proteins (CDK8, CCNC, MED12, and MED13). The CDK8 module associates with regulatory loci on a genome-wide scale (Kagey et al., 2010; Pelish et al., 2015), and global targeting of the CDK8 module appears to reflect its association with Mediator (Allen and Taatjes, 2015). CDK19, a paralog of CDK8, emerged

in vertebrates and has high sequence similarity to CDK8, including nearly identical cyclin binding and kinase domains. Comparatively little is known about CDK19; however, it appears to assemble into an analogous CDK19 module in human cells (Daniels et al., 2013).

Based upon their association with Mediator—a global regulator of RNA polymerase II (pol II) transcription—CDK8 or CDK19 may broadly impact gene expression patterns; however, physical knockdown of CDK8 or CDK19 protein levels had relatively modest effects in HCT116 cells, with 2-fold or greater changes in expression of several hundred genes (Donner et al., 2010; Galbraith et al., 2013). Whereas knockdown studies do not address the role of the kinase activity per se, these data suggested limited roles for the Mediator kinases in transcriptional regulation. In agreement, gene expression analyses with the CDK8 ortholog in yeast, *Srb10* (Holstege et al., 1998), revealed that about 3% of genes were regulated by *Srb10* kinase activity. Similarly, limited effects on yeast transcription were observed in vitro and in vivo upon selective inhibition of *Srb10* (CDK8) kinase activity using an analog-sensitive mutant (Liu et al., 2004). Most genes affected by kinase-inactive mutant *Srb10* (CDK8) were involved in cellular response to nutrient stress (Holstege et al., 1998).

The biological roles of human CDK8 and CDK19 remain poorly understood, in part, because a more-comprehensive identification of their substrates or the genes specifically regulated by their activities has been lacking. Our recent studies with the natural product, cortistatin A (CA), showed that CA is a potent and highly selective inhibitor of the Mediator kinases CDK8 and CDK19 (Pelish et al., 2015). CA binds the CDK8-CCNC dimer with subnanomolar affinity ($K_d = 195$ pM) and two distinct kinome profiling assays, which collectively probed approximately 400 kinases, ultimately confirmed only CDK8 and CDK19 as targets of CA, even with analyses completed at 100 times the measured half-maximum inhibitory concentration (IC_{50}) for CDK8 (Pelish et al., 2015). Given these and other data showing the unusual selectivity of CA, we could begin to probe the cellular function and targets of CDK8 and CDK19.

Here, we report the large-scale identification of Mediator kinase (CDK8 and CDK19) substrates in human cells, using stable

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