BIOGRAPHICAL SKETCH

Provide the following information for the Senior/key personnel and other significant contributors.
Follow this format for each person. **DO NOT EXCEED FIVE PAGES.**

NAME: Steven M Hahn

eRA COMMONS USER NAME (credential, e.g., agency login): STEVEHAHN

POSITION TITLE: Professor

EDUCATION/TRAINING *(Begin with baccalaureate or other initial professional education, such as nursing, include postdoctoral training and residency training if applicable. Add/delete rows as necessary.)*

INSTITUTION AND LOCATION	DEGREE *(if applicable)*	Completion Date MM/YYYY	FIELD OF STUDY
Santa Rosa Junior College, CA		06/1977	physics
University of California, Santa Barbara, CA	B.A.	06/1979	biochemistry
Brandeis University, MA	Ph.D.	09/1984	biochemistry
Massachusetts Institute of Technology, MA	Postdoc	11/1988	biochem/mol genetics

A. Personal Statement

My laboratory uses an interdisciplinary approach to investigate mechanisms of transcriptional regulation. I have a long record of accomplishments in the eukaryotic transcription field, beginning with the identification of yeast TATA binding protein (TBP) (with Steve Buratowski) and the subsequent cloning of the TBP gene in my independent laboratory. Some of my laboratory's high impact work includes identifying genes encoding the RNA Polymerase (Pol) basal and regulatory factors (TFIIA, Mot1, Brf1), the discovery that TBP functions in Pol I, II, and III transcription (with Ron Reeder's lab), and structure determination of TBP-DNA and TFIIA-TBP-DNA (with Paul Sigler's lab). My laboratory subsequently used site-specific probes, genetics, and available structures to predict the architecture of the RNA Pol II Preinitiation Complex (PIC) and the function for many of the individual basal factors including how ATP is used to open DNA strands during transcription initiation. These predictions were later validated and extended through our biochemical studies and by x-ray and cryoEM studies by Cramer, Kornberg, Nogales and others. We also led a breakthrough in understanding the nature of transcription activators and how they bind their targets. In collaboration with Rachel Klevit, we found that acidic activators can recognize targets using a "fuzzy" protein interface rather than specific complimentary surfaces. In a parallel approach, in collaboration with Johannes Söding and Bill Nobel, we used random sequences and deep learning to devise a predictor for activators that also revealed sequence features that specify their dynamic molecular recognition properties. More recently, the lab transitioned toward genomics approaches to examine transcription factor (TF) and cofactor mechanisms. For example, we identified genome-wide specificities of the cofactors TFIID, SAGA and Mediator, and developed an approach to measure genome-wide transcription noise and gene activation at the single cell level. In our latest work, we mapped both the genomic binding sites and transcriptional regulatory targets for the near-complete set of yeast transcription factors and this led to many surprising conclusions. My laboratory has an outstanding record of applying cutting-edge approaches and technologies to answer important and timely biological questions in the gene regulation field. Lastly, I have a long history of service to the scientific community and have successfully mentored many graduate students, postdocs, and research associates, who have had successful careers in academics, industry, medicine, and business.

- Warfield, L.*, Donczew, R*, Mahendrawada, L., and **S. Hahn** (2022) Yeast Mediator facilitates transcription initiation at most promoters via a Tail-independent mechanism. Mol Cell, Nov 3;82(21):4033-4048.e7. PMCID: PMC9637718

- Erijman, A. Kozlowski, L., Sohrabi-Jahromi, J., Fishburn, J., Warfield, L., Schreiber, J., Noble, WS, Söding, J., and **S. Hahn** (2020). A high-throughput screen for transcription activation domains reveals their sequence features and permits prediction by deep learning Mol Cell, May 12;S1097-2765(20)30262-8. PMCID: PMC7275923

- Grünberg, S., Warfield, L., and **S. Hahn** (2012). Architecture of the RNA polymerase II preinitiation complex and mechanism of ATP-dependent promoter opening. Nature Struct. Mol. Biol., 19:788-795. PMCID: PMC3414687

- Brzovic, P.S., Heikaus, C.C., Kisselev, L., Vernon, R., Herbig, E., Pacheco, D., Warfield, L., Littlefield, P., Baker, D., Klevit, R. and **S. Hahn** (2011). The acidic transcription activator Gcn4 binds the Mediator subunit Gal11/Med15 using a simple protein interface forming a fuzzy complex. Mol Cell 44:942-953. PMCID: PMC3246216

B. Positions, Scientific Appointments, and Honors

Current Appointments

2005-Present	Affiliate Professor, Department of Biochemistry, University of Washington
1995-Present	Professor, Division of Basic Sciences, Fred Hutchinson Cancer Center

Previous Appointments

1997-2005	Investigator, Howard Hughes Medical Institute
1992-1995	Associate Professor, Division of Basic Sciences, Fred Hutchinson Cancer Center.
1988-1992	Assistant Professor, Division of Basic Sciences, Fred Hutchinson Cancer Center.

Honors

2022	Fellow, American Academy of Microbiology
1997-2005	Investigator, Howard Hughes Medical Institute.
1993-1998	Scholar Award, the Leukemia and Lymphoma Society.
1990-1993	Junior Faculty Award, American Cancer Society.
1984-1987	Postdoctoral Fellowship, Damon Runyon Cancer Research Foundation.

Service (National and International)

2022	Site review panel: EMBL Structural and Computational Biology Unit, Heidelberg GE.
2017-2021	NIH MGB Review Panel (Member)
2013-2022	Editorial Board, Molecular and Cellular Biology
2012	Site review panel: NCI, Laboratory of Receptor Biology & Gene Expression
2011, 09, 07	Co-organizer – Cold Spring Harbor meeting: Mechanisms of Eukaryotic Transcription
2009-2014	Board of Reviewing Editors, Science Magazine
2006	Co-Chair, FASEB meeting: Transcriptional Regulation During Cell growth, Diff. and Dev.
1994-2015	Ad hoc CSR study sections: 2015, 2013, 2005, 2002, 1996, 1994

C. Contributions to Science (All work supported by NIGMS funding)

1. Transcriptional regulatory mechanisms (1988-2020)
Discovery of RNA Pol II basal transcription factors in the early 1980s opened a path for mechanistic studies on eukaryotic gene regulation. The next overarching questions were: what are the identities of these factors, how do they function, and what roles do they play in gene regulatory mechanisms? Work in my laboratory to address these questions using the model eukaryote *S. cerevisiae* began with the cloning of genes coding for TBP and many other Pol II (and III) basal and regulatory factors. We subsequently used biochemical approaches to investigate pathways for Pol II preinitiation complex (PIC) formation, transcription initiation, and reinitiation. We used both site-specific and lysine-specific crosslinking-MS, in combination with available structures, to generate the first correct model for the architecture of the Pol II PIC that was later validated and extended by CryoEM studies from other laboratories. From our PIC architecture studies, we developed a model for DNA unwinding during Pol II open complex formation that we later validated. As described above, parallel approaches of structural and molecular biology combined with deep learning analysis led to a breakthrough in discovering the nature of acidic transcription activation domains and how they dynamically interact with their targets. We subsequently transitioned to genomics approaches to examine the mechanisms of transcription cofactors (TFIID, SAGA, and Mediator), their genome-wide specificity, and identification of genes sets defined by cofactor usage. Our combined work during this period had high impact and revealed many fundamental principles and conserved mechanisms of eukaryotic transcriptional regulation.

- Donczew R*, Warfield L*, Pacheco D, Erijman A, S., and **S. Hahn** (2020). Two roles for the yeast transcription coactivator SAGA and a set of genes redundantly regulated by TFIID and SAGA. Elife. 2020 Jan 8;9. pii: e50109. PMCID: PMC6977968

- Tuttle LM, Pacheco D, Warfield L, Luo J, Ranish J, **Hahn S,** Klevit, R. (2018). Gcn4-Mediator specificity is mediated by a large and dynamic fuzzy protein-protein complex. Cell Reports, 22:3251-3264. PMCID: PMC5908246

- Fishburn, J., Tomko, E., Galburt, E. and **S. Hahn**. (2015). Double stranded DNA translocase activity of transcription factor TFIIH and the mechanism of RNA Polymerase II Open Complex formation. Proc Natl Acad Sci USA, 112:3961-3966. PMCID: PMC4386358

- Chen H-T. and **S. Hahn**. (2004) Mapping the location of TFIIB within the RNA Polymerase II transcription preinitiation complex: A model for the structure of the PIC. Cell 119:169-180. PMID: 15479635

Major Scientific Contributions in the past five years:

2. Cofactor specificity and mechanisms (Funded by 6R35 GM140823 and RO1GM053451/ RO1GM075114)

This project examined mechanisms and specificity of the cofactors Mediator (MED) and BDFs (double bromodomain-containing factors analogous to metazoan BET proteins). Yeast MED is a large, conserved complex that is essential for Pol II transcription and interacts with acidic activation domains via its Tail module. Before our work the consensus view was that, at most genes, MED transduces signals from transcription activators at the UAS to the transcription machinery. We used rapid depletion of MED subunits and monitored transcription changes using 4-thioU RNA-seq (measuring newly synthesized or “nascent” RNA). We surprisingly found that, counter to the prevailing model, MED Tail is important for expression of only ~ 6% of genes, while most genes are Tail-independent under standard growth conditions. However, complete rapid inactivation of MED function via depletion of the Head module or connector subunit (Med14) severely decreased transcription at all protein-coding genes. In agreement with this, we found that MED maps to promoters of most expressed genes, but only to a subset of UAS elements at mostly Tail-dependent genes. Thus, at most genes, MED seems to bypass the UAS and directly bind promoters where it has important functions in PIC formation and transcription initiation (e.g., stabilizing the PIC and positioning the Pol II CTD near TFIIH kinase). Our study has broad and important implications for the functions of TFs, coactivators, and

MED at different gene classes – e.g., at most genes, the critical functional targets of TFs must be factors other than MED.

Yeast BDFs were previously proposed to be loosely associated TFIID subunits, analogous to bromodomains in the human TFIID subunit Taf1. In contrast, we found BDFs have much greater similarity to mammalian BET proteins, cofactors that play roles in both enhancer function and transcription elongation. Using the rapid depletion and nascent transcription approaches described above, we found that BDFs are strongly required for transcription of the TFIID-class genes (~87% of protein coding genes) and that they also play a role in transcription elongation for many of these genes. Although the BDFs localize to the PIC, they are not essential for TFIID-promoter binding as predicted earlier. The striking functional similarities with mammalian BET factors suggest broad conservation of important BET functions in eukaryotes and opened an experimental system to study these conserved functions. Former postdoc Rafal Donczew has continued the BDF project in his independent laboratory.

- Warfield, L.*, Donczew, R*, Mahendrawada, L., and **S. Hahn** (2022) Yeast Mediator facilitates transcription initiation at most promoters via a Tail-independent mechanism. Mol Cell, Nov 3;82(21):4033-4048.e7. Epub 2022 Oct 7. PMCID: PMC9637718

- Donczew, R and **S. Hahn** (2021). BET family members Bdf1/2 modulate global transcription initiation and elongation in Saccharomyces cerevisiae. Elife, 2021 Jun 17;10:e69619. Online ahead of print. PMCID: PMC8266393

3. UAS-Promoter specificity, transcription noise, and gene activation (Funded by 6R35 GM140823)

Transcription cofactors function as intermediates between TFs that bind gene regulatory regions and the basal transcription machinery. In earlier studies, we used rapid cofactor depletion and nascent transcriptome analysis to identify three classes of yeast mRNA genes based on cofactor usage: (i) TFIID-dependent (87% genes), enriched for TATA-less and housekeeping genes, (ii) genes dependent on both SAGA and TFIID (cofactor redundant (CR)) (13% genes), enriched for highly regulated and TATA-containing genes, and (iii) the above MED Tail-dependent genes (~6% genes), a subset of the CR genes. Whether cofactor specificity is defined by the UAS, core promoter, or both, is an important question as it may explain important distinctions between constitutive and inducible genes and reveal distinct regulatory mechanisms at the three gene classes. In this project, we developed a large-scale reporter assay to measure transcription activity and cofactor specificity for >15,000 UAS-promoter combinations. We found that most UASs have broad specificity - activating promoters of different class. This is like the broad specificity of human enhancer-promoter compatibility but distinct from class-specific enhancers found in Drosophila. We next used rapid inactivation of TFIID, SAGA or MED Tail, in conjunction with our library, to show that TFIID-dependence is primarily determined by the core promoter while SAGA and MED Tail dependence is determined by <u>both</u> the UAS and core promoter. Interestingly, MED Tail dependence required both MED binding at the UAS as well as an appropriate core promoter. As no TFIID-specific promoter elements have yet been determined, our system will allow future detailed molecular investigation of what specifies a TFIID-dependent promoter and how this relates to TFIID binding. Our UAS studies defined the subset of UASs that recruit MED, allowing us to investigate which TFs have this function. Conversely, the Tail-independent UASs define another class of UAS that somehow activates independently of MED recruitment. In sum, we identified regulatory elements that define cofactor specificity and open a pathway for investigating the molecular basis of this response.

To investigate cofactor function and gene activation by an orthogonal approach, we developed a time-resolved nascent single cell RNA-seq method, allowing us to measure gene-specific transcriptional noise and the fraction of active genes in a population (Fon). We found that most genes are expressed with near constitutive behavior while a subset of genes (nearly all CR class genes) show high mRNA variance/cell suggestive of transcription bursting. To test the power of this approach in investigating transcription activation, we examined transcription of cell-cycle dependent genes. We found that histone genes are activated by modulating two transcription modes – transitioning from a low level, low-noise constitutive mode in M and M/G1, to an activated state in G1/S/G2 characterized by increased Fon and noisy/bursty transcription. Finally, we examined the roles of MED and SAGA using this assay. We found that, on average, both cofactors function primarily to

stimulate the Fon with only a modest effect on bursting. However, we found several gene-specific exceptions to this general rule where the cofactors have a greater effect on bursting compared with Fon. In sum, we developed a novel assay allowing measurement of genome-wide transcription parameters that reveals important insights into the mechanisms of transcriptional regulators and promoter elements.

- Schofield, JA and **S. Hahn** (2023) Broad compatibility between yeast UAS elements and core promoters and identification of promoter elements that determine cofactor specificity. Cell Reports Apr 12;42(4):112387. PMCID: PMC10567116

- Schofield, JA and **S. Hahn** (2024) Transcriptional noise, gene activation, and roles of SAGA and Mediator Tail measured using nucleotide recoding single cell RNA-seq. Cell Reports, Aug 27; 43:114593. PMCID: PMC11405135

4. Function and mechanisms of gene-specific transcription factors (TFs) (Funded by 6R35 GM140823)

The subset of DNA sequence-specific TFs expressed in a eukaryotic cell determines its fundamental properties including gene expression patterns, cell identity, and responses to signaling pathways. A central question in gene regulation is how TFs cooperate with other TFs, cofactors, and the basal transcription machinery to modulate transcription. For example, large-scale screens suggested that only a small number of TFs can activate transcription on their own, suggesting that most TFs must cooperate to regulate transcription. Further, it was found in several systems that TF-DNA binding in cells involves both the structured DNA binding domains and the disordered domains that are found in most TFs. To address these and other issues, we took a comprehensive approach to map the genome-wide roles for yeast TFs, mapping both binding sites and gene expression targets for 126 of the ~ 150 cellular TFs. For DNA binding, we mapped each factor using the MNase-based ChEC-seq method, as well as utilizing published ChIP-exo data. For identifying functional TF regulatory targets, we rapidly depleted individual TFs using the auxin degron system coupled with nascent RNA-seq. Our main conclusions include (i) for many TFs, DNA binding can be dependent on regions outside the DNA binding domain, raising the question of how TFs are targeted to many genomic sites (ii) for most TFs, there are many nonfunctional binding sites where TF depletion has no apparent effect on expression from the closely linked gene. This raises the question of why TFs work at some genes but not others. (iii) most TFs have dual function rather than being dedicated activators or repressors. While some dual function TFs have been previously identified, it was not realized that this is the major TF class. (iv) we found surprisingly low overlap between TF binding and regulatory target genes for many TFs. This raises the question of whether many yeast TFs are acting from either distant sites, from low occupancy sites, or by other unexpected mechanisms. In sum, our results from this project revealed unexpected and complex relationships between TF binding and function and form the basis for our future research program.

- Mahendrawada, L., Warfield, L., Donczew, R., and **S. Hahn** (2025). Low overlap of transcription factor DNA binding and regulatory targets. Nature. 2025 Apr 16. doi: 10.1038/s41586-025-08916-0. Online ahead of print. PMID: 40240607

Complete List of Published Works at My Bibliography:
https://www.ncbi.nlm.nih.gov/myncbi/steven.hahn.1/bibliography/public/