BIOGRAPHICAL SKETCH

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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)*	Start Date MM/YYYY	Completion Date MM/YYYY	FIELD OF STUDY
Santa Rosa Junior College, CA		09/1975	06/1977	Physics
University of California, Santa Barbara, CA	B.A.	09/1977	06/1979	Biochemistry
Brandeis University, MA	Ph.D.	09/1979	09/1984	Biochemistry
Massachusetts Institute of Technology, MA	Postdoc	09/1984	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. Some of my laboratory's high impact work over the past 32 years includes identifying genes encoding the RNA Polymerase (Pol) basal and regulatory factors TBP, TFIIA, Mot1, Brf1 (a TFIIB-like Pol III factor), the discovery that TBP functions in Pol I, II, and III transcription (with Ron Reeder's lab), structure determination of TBP-DNA and TFIIA-TBP-DNA (with Paul Sigler's lab), discovery of Rrn7 as a Pol I TFIIB-like factor, biochemical identification of Pol II preinitiation complex (PIC) assembly intermediates, determining Pol II PIC architecture, and uncovering important and conserved transcription initiation mechanisms. We've also made two breakthroughs in determining the mechanisms and features of transcription activation domains (ADs). Our NMR work (with Rachel Klevit) uncovered a dynamic fuzzy binding mechanism for AD-coactivator interactions and our computational work (with Johannes Söding and Bill Nobel) developed an accurate predictor for acidic ADs that uncovered important AD features. Together, our work revealed a molecular mechanism for AD function that had eluded the field for many years. Recently, our genomics studies have identified the genome-wide specificities of the coactivators TFIID, SAGA, Mediator and the BET factors and point to important conserved regulatory mechanisms.

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. Examples include: (i) X-ray structural analysis (with Paul Sigler); (ii) protein crosslinking-MS to decipher the architecture of large protein complexes (with Jeff Ranish); (iii) chemical biology to modulate the function of kinases important to transcriptional regulation (with Kevan Shokat); (iv) NMR to decipher AD function (with Rachel Klevit); (v) computational biology to characterize and predict AD function (with Johannes Söding and Bill Nobel); (vi) single molecule biophysics to probe transcription initiation mechanisms (with Eric Galburt); and (vii) recent genomics approaches, combining genome-wide protein-DNA mapping with rapid protein degradation and measurement of changes in nascent RNA production.

Lastly, I have a long history of service to the scientific community as well as successfully mentoring many graduate students, postdocs, and research associates, who have gone on to successful careers in academics, industry, medicine, and business.

Ongoing Research Support

NIGMS R35GM GM140823 5/2021 – 2/2026
Mechanisms of transcriptional regulation and transcription factor specificity
Role: PI
Regulation of gene expression by transcription factors and coactivators is a key mechanism for control of cell growth, differentiation, and development. Defects in transcriptional regulation directly contribute to many human illnesses. Our work identifies and characterizes conserved fundamental regulatory mechanisms and gene-specific functions of transcription factors and coactivator complexes that explain how diverse transcription factors and gene regulatory regions cooperate to regulate genome-wide transcription.

Completed Research Support – Past 3 Years

NIGMS R01GM053451 9/1995 – 4/2021
Molecular analysis of eukaryotic transcription
Role: PI
The overall goals of this project were to determine the mechanisms utilized by the transcription machinery to initiate transcription by RNA Pol II.

NIGMS R01GM075114 9/2005 – 6/2021
Mechanisms of Eukaryotic Transcription Activation
Role: PI
The overall goals of this project were to determine the mechanisms utilized by gene-specific activators to stimulate transcription by RNA Pol II

Citations

Warfield, L.*, Donczew, R*, Mahendrawada, L., and **S. Hahn**. Mediator is broadly recruited to gene promoters via a Tail-independent mechanism (2021). biorxiv doi: https://doi.org/10.1101/2021.12.21.473728

Tuttle, L., Pacheco, D., Warfield, L., Wilburn, D.B., **Hahn, S**., and R. Klevit (2021). Mediator subunit Med15 dictates the conserved "fuzzy" binding mechanism of yeast transcription activators Gal4 and Gcn4. Nat Commun. 2021 Apr 13;12(1):2220. doi: 10.1038/s41467-021-22441-4.

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.doi: 10.1016/j.molcel.2020.04.020.

Donczew R*, Warfield L*, Pacheco D, Erijman A, S. **Hahn S**. (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. doi: 10.7554/eLife.50109. PMID: 31913117

B. Positions, Scientific Appointments, and Honors

Current Appointments

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

Previous Appointments

1997-2005	Investigator, Howard Hughes Medical Institute
1996-2005	Affiliate Associate Professor, Department of Biochemistry, University of Washington.
1992-1995	Associate Professor, Division of Basic Sciences, Fred Hutchinson Cancer Research Center.
1988-1992	Assistant Professor, Division of Basic Sciences, Fred Hutchinson Cancer Research Center.

Honors:

2022	Fellow, American Academy of Microbiology/American Society for 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-Walter Winchell Cancer Fund.

Service:

2022	Site visit review team: EMBL Structural and Computational Biology Unit, Heidelberg
2013-2021	Editorial Board, Molecular and Cellular Biology
2017-2021	NIH MGB Review Panel (Member)
Feb 2015, Nov 2013, & Jun 2005	NIH MGB Review Panel Ad hoc.
2009-2014	Board of Reviewing Editors, Science Magazine.
2012	Site visit team, National Cancer Institute, Laboratory of Receptor Biology and Gene Expression.
2011, 2009, 2007	Co-organizer – Cold Spring Harbor meeting: Mechanisms of Eukaryotic Transcription.
2006	Co-Chair, FASEB meeting: Transcriptional Regulation During Cell growth, Differentiation, and Development.
Feb 2002	NIH CDF-1 Ad hoc
Feb 1996, Feb 1994	NIH MBY-1 Ad hoc.

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

1. Discovery of transcriptional regulatory mechanisms (1988-2014)

In pioneering work during the 1980's, the laboratories of Roeder, Sharp, and Chambon discovered the Pol II basal transcription factors, opening 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? As a postdoc, I teamed with Steve Buratowski on the breakthrough finding of TATA binding protein activity in yeast that complemented a mammalian transcription system. Subsequent purification of yeast TBP led to identifying the PIC assembly pathway (with Steve Buratowski) and the characterization and cloning of the TBP gene in my own laboratory. My laboratory identified and cloned many other Pol II basal and regulatory factors. Using biochemical approaches, we developed both crude and reconstituted transcription systems and used these to discover important mechanisms used by the basal factors in transcription initiation and reinitiation. We used both site-specific and lysine-specific crosslinking-MS to generate the first (correct) model for the architecture of the Pol II PIC that was subsequently validated and extended by CryoEM studies from the Cramer, Nogales, and Kornberg laboratories. From our PIC architecture studies, we developed a model for DNA unwinding during Pol II open complex formation that we later validated (see below). We also made great strides in identification of transcription activator targets and identified the dynamic fuzzy nature of activator-coactivator complexes (see below). Our combined work during this period, using a variety of experimental approaches, had high impact and revealed many fundamental principles and conserved mechanisms relevant to eukaryotic transcriptional regulation.

- Kim, Y., Geiger, J.H., **Hahn, S**. and Sigler, P.B. (1993). Crystal structure of a yeast TBP/TATA-box complex. Nature 365: 512-520. PMID: 8413604
- 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
- Brzovic, P.S., Heikaus, C.C., Kisselev, L., Vernon, R., Herbig, E., Pacheco, D., Warfield, L., Littlefield, P., Baker, D., Klevit, R. and **Hahn, S**. (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. PMID: 22195967
- 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, 19:788-796. PMID: 22751016

Major Scientific Contributions in the past seven years

2. Transcription initiation and open complex formation

Our prior mapping of the location for the Pol II basal factor TFIIH and its ATPase subunit Ssl2/XPB within the PIC led to a model for how ATP and Ssl2 are used to promote open complex formation: we predicted that Ssl2 is a DNA translocase that winds DNA into the PIC and the resulting topological force opens the DNA strands within the Pol II active site. This step is essential for Pol II initiation. In the past several years, we have validated this model using a variety of biochemical and biophysical assays. First, we demonstrated biochemically that Ssl2 is a DNA translocase and determined its important biochemical properties. Second, we collaborated with Eric Galburt's laboratory to develop a single molecule magnetic tweezers assay for DNA opening during transcription initiation. We showed that DNA opening unexpectedly occurs in two steps: (i) an initial 6-base-pair (bp) bubble that (ii) expands to 13 bp in the presence of NTPs. Our observations strongly support the following model: ATP-dependent Ssl2 translocation leads to a 6-bp open complex that is maintained during scanning for the transcription start site. Pol II subsequently expands the bubble via initial RNA synthesis. Our most recent work suggests that the expansion of the bubble from 6-13 bp occurs in steps during synthesis of the first 2-5 bases of the mRNA. Finally, recent genome-wide analysis showed that Ssl2 translocase is required for transcription from <u>all</u> yeast Pol II genes. Upon rapid degron depletion of Ssl2, transcription of all newly synthesized Pol II transcripts drops to essentially zero. Finally, we used molecular genetics and crosslinking-MS (with Jeff Ranish's laboratory) to characterize the molecular architecture of TFIIH and the function of its topological domains (including Ssl2). Our combined work revealed the answer to a longstanding problem in the field: how the Pol II system uses ATP to generate the open complex state.

- Fishburn, J., Tomko, E., Galburt, E. and S. **Hahn S**. (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. PMID: 25775526
- Warfield L, Luo J, Ranish J, and **Hahn S** (2016). Function of Conserved Topological Regions within the Saccharomyces cerevisiae Basal Transcription Factor TFIIH. Mol Cell Biol. 36:2464-75. doi: 10.1128/MCB.00182-16. Print 2016 Oct 1. PMID: 27381459.
- Tomko, E., Fishburn, J., **Hahn, S**., and E. Galburt (2017) TFIIH generates a six base-pair open complex during RNAP II transcription initiation and start-site scanning. Nat Struct Mol Biol, Nov 6. doi: 10.1038/nsmb.3500. [Epub ahead of print] PMID: 29106413

3. Specificity and mechanisms of transcription coactivators TFIID, SAGA, and Mediator

A longstanding and controversial topic has been the gene-specific requirement for the coactivators TFIID and SAGA. Both factors have the common function of TBP-DNA loading and both are targets of transcription activators, but each has unique functions; TFIID has DNA binding activity, binds acetylated nucleosomes, and recognizes short, specific sequence motifs in metazoan promoters while SAGA has HAT and deubiquitination activities. Early pioneering work suggested that transcription of individual yeast genes is dominated by either TFIID or SAGA, with many genes being largely TFIID-independent. Formaldehyde crosslinking assays suggested that TFIID was depleted from the TFIID-independent promoters. A second highly debated issue was the genome-wide binding of the coactivator Mediator, a large complex required for all Pol II transcription. ChIP assays had mapped yeast Mediator to a relatively small number of UAS elements but not at promoters–leaving the role of Mediator unclear. We initially partnered with Steve Henikoff's lab to use the formaldehyde-independent and MNase-based ChEC-seq method to map Mediator, TFIID and SAGA. Potential advantages of this approach include avoiding non-specific protein-DNA crosslinking in highly transcribed regions and efficient mapping of factors that do not directly bind DNA. My laboratory has subsequently improved the specificity of ChEC-seq so that it quantitatively maps coactivators and gene-specific TFs with high specificity. We've also used 4ThioU RNA-seq to label newly synthesized RNA and the auxin degron system to determine in vivo function and targets of all three coactivators. Sequencing labeled RNA avoids the known problem that mRNA stability is altered upon perturbation of genome-wide transcription and rapid protein depletion avoids potential indirect effects on transcription caused by long-term absence of a factor and incomplete depletion of function using a ts allele. Degron-depletion revealed two classes of yeast genes: TFIID-dependent and coactivator redundant (CR). Rather than being dominated by SAGA as earlier proposed, the CR genes can use either SAGA or TFIID and strong transcription defects at CR genes requires rapid depletion of both coactivators. We also showed that long-term depletion of SAGA decreases transcription from all Pol II genes, a function largely

related to its histone acetyltransferase (HAT) activity. Our ChEC-seq mapping was in agreement with these findings, as SAGA and TFIID map to many yeast genes without regard to gene class. Using a similar approach and, as described in this application, we have also found gene-specific roles for the activator-binding domain of Mediator and mapped Mediator recruitment to a wide variety of genes. Our combined work has answered longstanding questions about TFIID, SAGA and Mediator and opened a new path for discovering gene-specific functions for these coactivators. Finally, we played a supporting role (crosslinking-MS analysis of hTFIID) in breakthrough work from Eva Nogales' lab on the structure and mechanism of human TFIID.

- Warfield, L.*, Donczew, R*, Mahendrawada, L., and **S. Hahn**. Mediator is broadly recruited to gene promoters via a Tail-independent mechanism (2021). biorxiv doi: https://doi.org/10.1101/2021.12.21.473728
- 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. doi: 10.7554/eLife.69619. Online ahead of print.PMID: 34137374
- Donczew R, Warfield L, Pacheco D, Erijman A, **Hahn S** (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. doi: 10.7554/eLife.50109. PMID: 31913117
- Patel AB, Louder RK, Greber BJ, Grünberg S, Luo J, Fang J, Liu Y, Ranish J, **Hahn S**, E. Nogales (2018). Structure of human TFIID and mechanism of TBP loading onto promoter DNA. Science, 362. pii: eaau8872. doi: 10.1126/science.aau8872. Epub 2018 Nov 15.

4. Transcription activation mechanisms

Since the discovery of the first Pol II transcription activators Gcn4 and Gal4, it was recognized that understanding the mechanism of activators is a key problem in the gene regulation field. Pioneering work discovered unusual properties of activation domains (ADs) but left unanswered the important question of how these unusual properties lead to a molecular mechanism for AD function and specificity. Our breakthrough was the finding (with Rachel Klevit's laboratory) of the dynamic fuzzy binding mechanism used by acidic ADs to bind the Mediator Tail subunit Med15. In the past seven years, we have expanded our studies to examine larger and more diverse ADs including several natural tandem ADs and longer Med15 polypeptides containing up to four activator binding domains (ABDs). Our structural and biochemical studies have found that the fuzzy binding mechanism holds for these larger physiological complexes, that the protein-protein interface of the AD-ABD complexes resembles that of a hydrophobic cloud and, that biologically relevant affinity and specificity is generated by the combined interactions between polypeptides with multiple ADs and ABDs. Simultaneously, we pursued a computational approach (with Johannes Söding's laboratory) involving a high throughput screen for functional ADs. Our resulting data was used in two machine learning approaches to develop predictors for AD function. Our best predictor (*ADpred*; https://adpred.fredhutch.org) accurately predicts polypeptides with AD potential and successfully recognizes ADs in yeast, human and fly activators. Working backwards from the *ADpred* results, we identified important AD properties that lead to a molecular mechanism for acidic AD function. Our combined work is a breakthrough for this longstanding problem and opens a clear path to identify other AD classes.

- Erijman, E. 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, *in press.*
- Tuttle, L., Pacheco, D., Warfield, L., Wilburn, D.B., **Hahn, S**., and R. Klevit (2021). Mediator subunit Med15 dictates the conserved "fuzzy" binding mechanism of yeast transcription activators Gal4 and Gcn4. Nat Commun. 2021 Apr 13;12(1):2220. doi: 10.1038/s41467-021-22441-4.
- 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. https://doi.org/10.1016/j.celrep.2018.02.097
- Pacheco, D, Warfield L, Brajcich M, Robbins H, Luo J, Ranish J, **S. Hahn** (2018). Transcription activation domains of the yeast factors Met4 and Ino2: tandem activation domains with properties similar to the yeast Gcn4 activator. Mol Cell Biol, doi: 10.1128/MCB.00038-18. [Epub ahead of print]

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