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)*	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. Examples of our high impact work over the past 32 years include 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 and biochemical identification of Pol II preinitiation complex (PIC) assembly intermediates, PIC architecture, and 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) developed an accurate predictor for acidic ADs that uncovered important AD features. Together, our work identified a molecular mechanism for AD function that had eluded the field for many years. Finally, our genomics studies have revealed the genome-wide specificities of the coactivators TFIID, SAGA and Mediator and point to important conserved regulatory mechanisms. I also 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.

B. Positions and Honors

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

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

Honors:

Investigator, Howard Hughes Medical Institute. 1997-2005.
Scholar Award, the Leukemia and Lymphoma Society. 1993-1998.
Junior Faculty Award, American Cancer Society. 1990- 1993.
Postdoctoral Fellowship, Damon Runyon-Walter Winchell Cancer Fund. 1984-1987.

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

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, Roeder, Sharp, and Chambon's laboratories 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 yeast TATA binding protein that led to identifying the PIC assembly pathway and the subsequent characterization and cloning of the other basal factors. In my own laboratory, we identified and cloned many Pol II basal and regulatory factors from yeast. 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 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 determining 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 five 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: an initial 6-base-pair (bp) bubble that then 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. RNA polymerase II subsequently expands the bubble via transcription initiation and initial RNA synthesis. Our most recent work suggests that the expansion of the bubble from 6-13 bp occurs in steps during initial RNA synthesis of 2-5 nucleotides. Finally, recent genome-wide analysis showed that Ssl2 translocase is required for transcription from all 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 yeast genes are 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 MNase-based ChEC-seq method to map Mediator, TFIID and SAGA. 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 and the auxin degron system to determine in vivo functions and targets of all three coactivators. Rapid 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 nucleosome HAT activity. Our ChEC-seq mapping is in agreement with our findings, as SAGA and TFIID map to many yeast genes without regard to gene class. Using a similar approach we have also found gene-specific roles for Mediator subunits and mapped Mediator to a wide variety of genes. Our combined work has answered longstanding questions about TFIID and SAGA and opened up a new path for discovering gene-specific functions for all three 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 TFIID.

- 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.

- Warfield, L., Ramachandran, S., Baptista, T., Tora, L., Devys, D., and **S. Hahn** (2017). Transcription of nearly all yeast RNA Polymerase II-transcribed genes is dependent on transcription factor TFIID. Mol Cell 68:118-129. PMID: 28918900
- Baptista, T., Grünberg, S., Minoungou, N., Koster, M.J.E., Timmers, H.T.M., **Hahn, S**., Devys, D. and T., László (2017). SAGA is a general cofactor for RNA polymerase II transcription. Mol Cell, 68:130-143. PMID: 28918903
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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 how any of these facts translated into a molecular mechanism for AD function was unclear. Our breakthrough was the finding (with Rachel Klevit's laboratory) of the dynamic fuzzy binding mechanism used by acidic ADs binding the Mediator subunit Med15. In the past five 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 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 led 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., **Hahn, S**., and R. Klevit (2019). Mediator subunit Med15 dictates the conserved "fuzzy" binding mechanism of yeast transcription activators Gal4 and Gcn4. Bioxriv, doi: https://doi.org/10.1101/840348. *Currently under revision in response to reviewer comments at Nature Communications.*
- **Hahn S**. (2018). Phase Separation, Protein Disorder, and Enhancer Function (Preview). Cell 175:1723-1725. doi: 10.1016/j.cell.2018.11.034.
- 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]

Complete List of Published Works at ORCID: https://orcid.org/0000-0001-7240-2533

D. Additional Information: Research Support and/or Scholastic Performance

NIGMS 5RO1 GM053451
Molecular Analysis of Eukaryotic Transcription 9/30/1995 to 04/30/2021
The overall goals of this project are to determine the mechanisms utilized by the transcription machinery to initiate transcription by RNA Pol II.

NIGMS 2RO1 GM075114
Mechanisms of Eukaryotic Transcription Activation, 9/1/2005 to 6/30/2022
The overall goals of this project are to determine the mechanisms utilized by gene-specific activators to stimulate transcription by RNA Pol II.