Development 133, 4393-4397 (2006) doi:10.1242/dev.02639

Transcription saga tells developmental stories

Barbara J. Graves[1] and John W. Tamkun[2]

The regulation of gene expression at the level of transcription is crucial for cell proliferation and differentiation, and thus, ultimately, for the development of the organism. A recent FASEB conference on 'Transcriptional Regulation during Cell Growth, Differentiation and Development' (Saxton's River, Vermont, USA) brought together molecular and developmental biologists with a common interest in the transcriptional control of gene expression. The program covered a wide range of topics, including transcriptional activators and repressors; the assembly of the pre-initiation complex; transcriptional initiation and elongation; and the role of chromatin in eukaryotic gene regulation. Recent advances in this long-standing field continue to reward biologists interested in molecular mechanisms.

Introduction

Held in August 2006, the conference was co-organized by Ken Zaret (Fox Chase Cancer Center, Philadelphia, PA, USA) and Steven Hahn (Fred Hutchinson Cancer Research Center, Seattle, WA, USA). A major goal of the meeting was to promote synergistic interactions between molecular and developmental biologists with a common interest in the field of transcriptional regulation. Here, we briefly review some of the highlights of the meeting, with an emphasis on mechanisms of transcriptional regulation that are essential for the development of multicellular organisms.

Getting started: transcriptional initiation and elongation

Recruitment of the TATA-binding protein (TBP) and other factors to promoters, followed by the assembly of the pre-initiation complex is a key step in transcriptional regulation. Several speakers described unconventional TBP-related factors and TBP-associated factors (TAFs) that function in tissue-specific developmental processes. Michael Green (University of Massachusetts Medical School, Worcester, MA, USA) presented evidence that the TBP-related factor TRF3 (Persengiev et al., 2003) plays an important role in vertebrate development. Using a combination of chromatin-immunoprecipitation (ChIP) and microarray studies, Green showed that TRF3 directly regulates the transcription of numerous genes, including transcription factors required for the commitment of mesoderm to hematopoietic lineages. Phenotypes associated with the loss of TRF3 are consistent with a role as a master regulatory switch that controls hematopoietic differentiation. Margaret Fuller (Stanford University, Stanford, CA, USA) discussed the role of tissue-specific TAFs in *Drosophila* spermatogenesis. Following several rounds of amplifying divisions and major changes in gene expression, germline stem cells differentiate into spermatocytes. In this cell type, testis-specific TAFs initiate the expression of genes required for the next step – spermatid differentiation – by counteracting transcriptional silencing by Polycomb group proteins (Chen et al., 2005). The two major complexes of Polycomb group proteins – PRC1 and PRC2 – are expressed in the male germline. Fuller presented recent data suggesting that PRC2 levels are downregulated soon after spermatocytes arise, while testis TAFs counteract silencing by sequestering PRC1 subunits in specific nuclear compartments. The above talks illustrated how variants of general transcription factors can be used to regulate specific pathways of differentiation.

Other speakers discussed more general aspects of transcriptional initiation. David Auble (University of Virginia, Charlottesville, VA, USA) presented recent work on how the association of TBP with promoters is regulated by MOT1, a SNF2/SWI2 ATPase that displaces TBP from the TATA box (Sprouse et al., 2006). Although MOT1 generally functions as a transcriptional repressor, it also activates transcription from a small number of promoters. Interestingly, a MOT1-activated promoter binds TBP in the incorrect orientation. Auble presented evidence that MOT1 stimulates transcription from this promoter by displacing TBP, allowing it to re-bind in a productive orientation. Owing to the large size and complexity of the pre-initiation complex, it has been difficult to conduct structural studies of the general transcription factors and RNA polymerase complex assembled at promoters. To circumvent this problem, Hahn has used a variety of photoreactive crosslinkers to analyze interactions between TBP, DNA and other factors within the context of native initiation complexes (Fig. 1). This approach has allowed him to map interactions with impressive resolution and to demonstrate that the TBP-mediated bending of promoter DNA is integral to the full interaction surface of the initiation complex. The approaches developed by Hahn to study the pre-initiation complex could be used to study numerous complexes of interest to developmental biologists.

Several speakers emphasized that transcription can be regulated at steps downstream of the recruitment of RNA polymerase to promoters. John Tamkun (University of California, Santa Cruz, CA, USA) presented evidence that the ATP-dependent chromatin-remodeling factor Kismet regulates an early step in transcriptional elongation in *Drosophila*, as well as the association of the Polycomb antagonists ASH1 and TRX with target promoters. Karen Adelman (NIEHS/NIH, Research Triangle Park, NC, USA) used ChIP and microarray studies to show that Negative Elongation Factor (NELF) plays both positive and negative roles in the transcription of many *Drosophila* genes. These and other examples suggest that the regulation of transcriptional elongation may be much more common than previously suspected.

Transcription factors bind DNA with surprising complexity

Sequence-specific DNA-binding transcription factors are essential players in dictating the correct execution of developmental programs and physiological responses. Although the biochemical principles of DNA recognition have been deciphered, many questions about the role of these factors in activation and repression during development remain unanswered. Zaret and Stephen Tapscott (Fred Hutchison Cancer Research Center, Seattle, WA, USA) addressed how initial developmental programs are set by early acting transcription factors. The pioneering role of FOXA in setting the

[1]Department of Oncological Sciences, Huntsman Cancer Institute, University of Utah, Salt Lake City, UT 84112, USA. [2]Department of Molecular, Cell and Developmental Biology, University of California, Santa Cruz, Santa Cruz, CA 95064, USA.

e-mail: barbara.graves@utah.edu; tamkun@biology.ucsc.edu

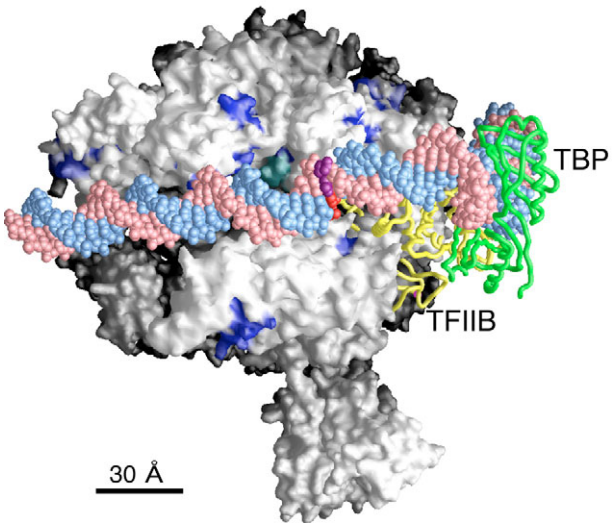

Fig. 1. A model for the structure of the RNA polymerase II transcription pre-initiation complex. The structure of RNA PolII (white), TFIIB (yellow), TBP (green) and promoter DNA, where the positions of the protein and DNA have been deduced from site-specific hydroxyl radical cleavage and photocrosslinking. The blue patches on the surface of RNA PolII are hydroxyl radical cleavage sites derived from Fe-EDTA probes inserted in the DNA backbone. The highlighted base pair (purple and red) is the presumed initiation site for DNA-strand separation. Modified, with permission, from Miller and Hahn (Miller and Hahn, 2006).

endodermal lineage involves the ability of FOXA to bind within the context of compacted chromatin (Cirillo et al., 2002). By analyzing selected loci using ChIP, Zaret detected the presence of FOXA at genes even prior to activation. Of equal importance, he observed that FOXA, but no other factor, is retained during mitosis as an epigenetic mark. Tapscott described the master role of MYOD in muscle lineage (Tapscott, 2005). Also using ChIP, he demonstrated the occupancy of promoters by MYOD at both early- and late-expressing genes. The highly related myogenin co-occupies targets with MYOD, perhaps for a distinct function. Finally, MYOD partners with PBX to provide specificity for late targets. It is likely that these scenarios will be recapitulated in other cell lineages.

Sequence-specific DNA-binding proteins are frequently encoded by gene families. Such proteins display highly conserved DNA-binding properties, yet are assumed to retain promoter selectivity. This dilemma was the theme of several talks. Barbara Graves (Huntsman Cancer Institute, Salt Lake City, UT, USA) addressed this issue for the ETS family of transcription factors (Hollenhorst et al., 2004). Using ChIP on full-genome promoter microarrays, she discovered both the redundant and the specific use of family members within a single cell type. Most interesting was the finding that multiple ETS proteins co-occupy proximal promoters of housekeeping genes that display strong consensus sites. In a complementary study, Arnold Berk (UCLA, Los Angeles, CA, USA) showed the alternative use of the TCF subclass of ETS proteins at the same promoter in different cell types. Other factor families could utilize these same diverse strategies. Another genomic approach to promoter specificity was undertaken by Stephen Small (New York University, New York, NY, USA), the focus of which is the promoter/enhancer architecture that dictates the spatial patterns of gene expression in early development. The development of segmental patterns in *Drosophila* requires the interface of a Bicoid gradient with Bicoid-binding sites in downstream targets (Ochoa-Espinosa et al., 2005). Unbiased genome-wide searching for Bicoid sites in clusters led to the identification of new Bicoid targets and to a hypothesis that relates the affinity of sites to the interpretation of the gradient. Keith Yamamoto (UCSF, San Francisco, CA, USA) addressed the factor-specificity issue with his update on the varied roles of the glucocorticoid receptor (GR). He presented evidence that two ligands, the small nuclear hormone and the larger DNA molecule, act to direct the activities of GR. Most fascinating were the data showing that different hormone ligands could affect promoter selectivity (Wang et al., 2006).

Next step: co-factor recruitment

Co-activators, co-repressors and chromatin-remodeling complexes must be recruited to genomic locations for accurate gene expression, and sequence-specific DNA-binding factors are important recruiters of this next tier of transcriptional machinery. Now that a large collection of co-factors have been identified, more mechanistic questions regarding the specificity and regulation of recruitment can be addressed. Joan Conaway (Stowers Institute for Medical Research, Kansas City, MO, USA) showed that a strong connection exists between the DNA-binding factor YYI and the INO80 complex, which is implicated in the sliding of nucleosomes that lie near promoter elements (Shen et al., 2000; Jin et al., 2005). YY1 can be purified as part of the human INO80 complex, and both are detected at promoter regions by ChIP. Finally, downregulation of YY1 blocks the recruitment of INO80 to promoter regions, and downregulation of an INO80 subunit reduces transcription of a YY1-activated target gene, suggesting that their activities are intimately related. Michael Carey (UCLA, Los Angeles, CA, USA) described the cooperative recruitment of the co-activator p300 and Mediator, a multi-protein co-activator complex, to both naked DNA and chromatin templates in vitro by viral co-activators. After recruitment, p300 dissociates via an autoacetylation pathway, thus allowing the subsequent recruitment of TFIID. Anders Näär (MGH Cancer Center, Harvard Medical School, Charlestown, MA, USA) reported the recruitment of a Mediator subunit, Med15, by the sterol-responsive-element-binding protein (SREBP) (Yang et al., 2006). The structural basis for the recruitment shows surprising similarity to the CBP-SREBP interface because of the commonality of a three-helix bundle domain in Med15, previously observed as the KIX domain in CBP/p300. Unlike CBP, which is recruited by many activators, Med15 appears to have a specific biological role in fatty acid metabolism. Richard Treisman (Cancer Research UK London Research Institute, London, UK) studies the regulation of serum response factor, which is regulated by RhoGTPases through the recruitment of the myocardin-related transcription factor (MRTF) family of co-activators. He showed that the nuclear accumulation and activity of the MRTF MAL is controlled by G-actin, and that both cytoplasmic and nuclear actin appear to play roles in its regulation. MAL contains a novel regulatory domain that binds multiple molecules of actin; the molecular mechanism underlying its activity is currently being characterized.

How to deal with chromatin structure

The packaging of DNA into chromatin provides eukaryotic cells with a variety of other mechanisms for regulating transcription (Workman, 2006). The basic unit of chromatin structure – the nucleosome – can block the access of both gene-specific and general transcription factors to DNA. Nucleosomes also pose a significant physical barrier to elongating RNA polymerase.

Chromatin assembly and remodeling factors determine the density, composition and positioning of nucleosomes over promoters and other regions of the genome, thus regulating their accessibility to the transcription machinery. Histone-modifying enzymes covalently modify the surfaces of nucleosomes, thereby altering their interactions with a variety of structural and regulatory proteins. Alterations in higher-order chromatin structure can also have profound effects on DNA accessibility and transcription. A growing body of evidence suggests that global or gene-specific alterations in chromatin structure are crucial for a wide variety of development processes.

Several speakers discussed nucleosome assembly and turnover. Using high-density tiling arrays, Oliver Rando (Harvard University, Cambridge, MA, USA) mapped the global distribution of nucleosomes in budding yeast at an unprecedented level of resolution (Yuan et al., 2005). His characterization of more than 200 genes revealed that the vast majority of promoters are not masked by nucleosomes. Rando also showed that histone turnover varies widely in different regions: nucleosomes at promoters exchange rapidly, whereas nucleosomes within coding regions tend to be surprisingly stable. Rando proposes that rapid nucleosome turnover may be characteristic of DNA elements with specialized functions, including boundary elements. Jessica Tyler (University of Colorado Health Sciences Center, Aurora, CO, USA) discussed factors that regulate nucleosome disassembly in budding yeast, with a focus on ASF1 (antisilencing function 1) (Adkins et al., 2004). Tyler's work on *PHO5* regulation indicated that chromatin disassembly by ASF 1 is necessary to allow activation domains to recruit SAGA and SWI/SNF to promoters.

Exciting work on the molecular mechanisms that control nucleosome positioning was presented by several speakers. DNA sequence can have significant effects on the positioning of nucleosomes; for example, AT-rich sequences tend to be free of nucleosomes and may promote the formation of 'open' chromatin accessible to transcription factors. Toshio Tsukiyama (Fred Hutchinson Cancer Research Center, Seattle, WA, USA) presented evidence that ISW2 – a member of the ISWI subfamily of chromatin-remodeling factors – may repress transcription by sliding nucleosomes over AT-rich regions that would normally be nucleosome free (Whitehouse and Tsukiyama, 2006). Geeta Narlikar (UCSF, San Francisco, CA, USA) addressed the issue of how ACF – a chromatin-remodeling complex composed of ACF1 and the ISWI-related human SNF2H protein – senses the distance between nucleosomes. Using a powerful fluorescence resonance energy transfer (FRET) assay, Narlikar showed that ACF1 acts as a 'sensor' that regulates the nucleosome sliding activity of human SNF2H, based on the length of linker DNA adjacent to a nucleosome. These data help to explain the ability of ACF to create regularly spaced nucleosome arrays, which are generally considered to be repressive to transcription (Ito et al., 1997). The results presented by Tsukiyama and Narlikar suggest that DNA accessibility is determined by a complex interplay of DNA sequence, nucleosome spacing and ATP-dependent chromatin-remodeling factors.

Other talks focused on the role of histone modifications in eukaryotic transcription. For many years, methylation was considered to be an extremely stable, if not irreversible, epigenetic mark of nucleosomal histones. However, the identification of several histone demethylases raised the possibility that these marks may be reversible. Yang Shi (Harvard Medical School, Boston, MA, USA) showed structural data for the Jumanji domain (Chen et al., 2006) and reported that several Jumanji C-domain proteins have remarkably specific histone demethylase activities, suggesting that the methylation of nucleosomal histones may be far more dynamic and precisely regulated than previously suspected.

It has long been known that actively transcribed genes tend to be highly acetylated, but recent studies have shown that histone methylation is also important for transcription (Workman, 2006). For example, the methylation of lysine 36 of histone H3 (H3K36) in the body of genes is required for the deacetylation of nucleosomes following transcription, restoring the chromatin structure that is necessary to prevent transcription from cryptic promoters (Carrozza et al., 2005). Jerry Workman (Stowers Institute for Medical Research, Kansas City, MO, USA) presented data from Michael Carey suggesting that histone acetylation enhances the ability of the RSC chromatin-remodeling complex to promote transcriptional elongation through nucleosomal DNA. He also described work directed towards understanding how the methylation and deacetylation of nucleosomes is coupled in budding yeast. The chromodomain of the Eaf3 subunit of the Rpd3S histone deacetylase is essential for the recognition of methylated H3K36 and for the subsequent deacetylation of transcribed regions (Carrozza et al., 2005; Joshi and Sruhl, 2005). Eaf3 is also found in the NuA4 histone acetyltransferase complex, raising the interesting issue of how Eaf3 binds the H3K36 methyl mark in the context of Rpd3S, but not of NuA4. Workman presented evidence that other subunits of Rpd3S and NuA4 modulate the binding specificity of the Eaf3 chromodomain, a finding that is likely to be of general significance. Echoing this theme, Jane Mellor (University of Oxford, Oxford, UK) presented work demonstrating that the binding of the yeast 14-3-3 proteins, Bmh1 and Bmh2, to histone H3 phosphorylated on serine 10 augments the ability of histone acetyltransferases to acetylate histone H3 in vivo.

The incorporation of histone variants provides an additional mechanism for altering nucleosome structure. Kami Ahmad (Harvard Medical School, Boston, MA) presented work on a *Drosophila* histone variant, histone H3.3, that is deposited in transcriptionally active regions and may confer unique properties to chromatin that are permissive for transcription (Schwarz and Ahmad, 2005). Ahmad showed that individuals homozygous for H3.3 null alleles exhibit decreased viability; chromosomal instability; and behavioral, flight and nervous system defects that become increasingly severe with age. The further characterization of the phenotypes associated with the loss of variant histones should clarify their roles in transcription and in other processes.

Several talks highlighted direct connections between chromatin remodeling, modifying enzymes and developmental processes. Joseph Landry from Carl Wu's laboratory (National Cancer Institute, Bethesda, MD, USA) presented evidence that the NURF chromatin-remodeling complex is required for mouse embryogenesis, whereas Workman described recent studies that indicate that the SAGA histone acetyltransferase is required for axon targeting during *Drosophila* eye development. As illustrated by these two examples, factors that play relatively global roles in transcription can play surprisingly specific roles during development.

New directions: genomics and imaging

Exciting new approaches for studying transcriptional regulation are emerging because of technological advances. Two very different but equally powerful approaches made a strong appearance at this meeting. First, the ability of genome-wide approaches to obtain comprehensive answers to basic questions was demonstrated by speakers with varied interests. In addition to the genome-wide studies cited above, Michael Snyder (Yale University, New Haven, CT, USA)

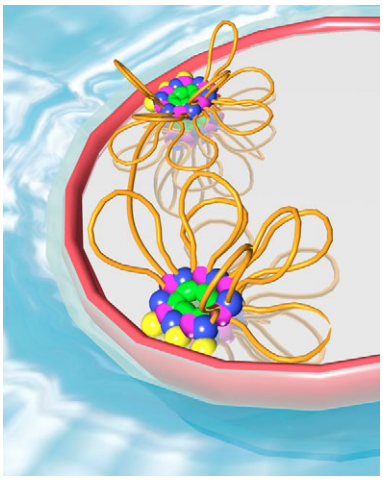

Fig. 2. Organization of the chromatin fiber by *gypsy* insulators. A model of insulator sites forming an insulator body in the nucleus of interphase cells. The interior of the nucleus is represented in gray, the nuclear lamina is depicted in red, and the nuclear membrane and cytoplasm are indicated in light blue. Dark blue, green, purple and yellow spheres represent various *gypsy* insulator proteins. The chromatin fiber is shown in gold. Image courtesy of Victor Corces.

showed how transcription factor protein microarrays can be used to identify the proteins that bind to conserved motifs. Brenda Andrews (University of Toronto, Toronto, Canada) described a high-throughput approach to characterize systematically the phenotypes that result from the overexpression of genes in budding yeast (Sopko et al., 2006). By profiling changes in gene expression that result from the overexpression of transcription factors, Andrews deciphered cis-elements for sequence-specific factor binding that had a predictive value (Chua et al., 2006). John Rinn from Howard Chang's laboratory (Stanford University, Stanford, CA, USA) looked at global expression by isolating numerous fibroblast cultures from throughout the human body and detected a supra-anatomic organization of fibroblast differentiation (Rinn et al., 2006). Specifically, Hox gene expression in adult fibroblasts mirrored the previously described embryonic position-specific expression. These genomic approaches set the bar for future studies and caution us not to over-generalize from studies that focus on only a fraction of a biological problem.

Other striking views of transcription came from the imaging of genes and proteins in living cells in real time. Peter Fraser (Babraham Institute, Cambridge, UK) showed evidence for limited sites of transcriptional activity – so-called transcription factories – using both DNA and RNA fluorescence in situ hybridization (FISH) (Osborne et al., 2004). Most intriguing is the tendency for co-regulated genes to appear co-localized within the nucleus during active expression. Victor Corces (Johns Hopkins, Baltimore, MD) presented evidence that chromosomes in diploid *Drosophila* cells can be dramatically organized into loop structures bounded by insulators (Fig. 2), as well as data indicating that RNA is required for the formation of these structures (Lei and Corces, 2006). These views raise a variety of interesting questions about how genes – and the process of transcription itself – are organized within the nucleus. Other imaging techniques gave dynamic views of transcription. John Lis (Cornell University, Ithaca, NY) used a combination of two-photon fluorescence imaging combined with fluorescence recovery after photobleaching (FRAP) to study dynamic changes in *hsp70* transcription in live cells following induction (Yao et al., 2006) (Fig. 3). Heat-shock factor exchange at *hsp70* is surprisingly slow after induction, suggesting that its association with promoters is remarkably stable. By contrast, the initial recruitment of RNA polymerase II at *hsp70* following induction corresponds to the length of time required for the pioneering rounds of transcription, but then declines dramatically. These observations suggest that RNA polymerase II is efficiently recycled near the *hsp70* locus following such rounds of transcription. These images suggest that biological processes require dynamic switching between regulated states to enable appropriate responsiveness to environmental change and possibly also to progressive changes in development.

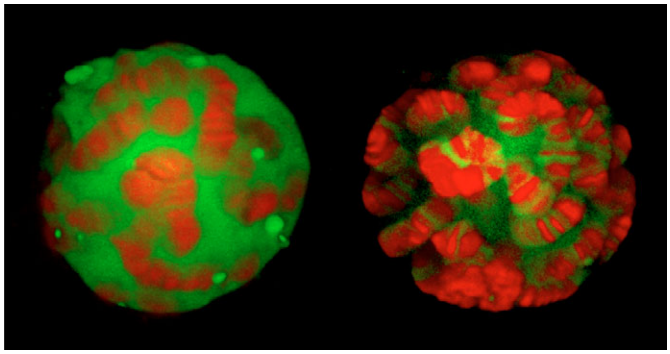

Fig. 3. Multiphoton microscopy imaging of a polytene nucleus. Multiphoton microscopy imaging of a polytene nucleus resolves individual, native, genetic loci and enables transcription factors involved in gene activation to be watched in real time. In the two 3D reconstruction images of a cell nucleus from *Drosophila* salivary gland tissues shown, heat-shock factor (green) resides in the nucleoplasm at room temperature (22°C, left). Upon heat shock (36.5°C, right), it rapidly associates with chromosomes (red) to activate the transcription of heat-shock genes. Image courtesy of John Lis.

Conclusion

Owing to the broad range of topics, conference participants ranged from developmental to structural biologists. This diverse group showed enthusiasm for gaining mechanistic insight into biological problems. There was excitement about the complexity of the transcriptional control of gene expression, both at the molecular level and with an eye for the bigger picture provided by whole-organism and whole-genome experiments. Participants believe that deciphering these complexities using a broad range of techniques will lead us to finally understanding the process of development. This FASEB conference is held regularly and attendees look forward to the next conference in 2008.

We are grateful to Ken Zaret and Steven Hahn for organizing an outstanding conference. We also thank Joan Conaway, Grant Hartzog, Stuart Kim and Toshio Tsukiyama for providing feedback on this report; the many colleagues who granted permission to cite their unpublished findings; and John Lis, Steven Hahn and Victor Corces for providing figures. We apologize to the meeting participants whose exciting work could not be covered because of space limitations.

References

Adkins, M. W., Howar, S. R. and Tyler, J. K. (2004). Chromatin disassembly mediated by the histone chaperone Asf1 is essential for transcriptional activation of the yeast PHO5 and PHO8 genes. *Mol. Cell* **14**, 657-666.

Carrozza, M. J., Li, B., Florens, L., Suganuma, T., Swanson, S. K., Lee, K. K., Shia, W. J., Anderson, S., Yates, J., Washburn, M. P. et al. (2005). Histone H3 methylation by Set2 directs deacetylation of coding regions by Rpd3S to suppress spurious intragenic transcription. *Cell* **123**, 581-592.

Chen, X., Hiller, M., Sancak, Y. and Fuller, M. T. (2005). Tissue-specific TAFs counteract Polycomb to turn on terminal differentiation. *Science* **310**, 869-872.

Chen, Z., Zang, J., Whetstine, J., Hong, X., Davrazou, F., Kutateladze, T. G., Simpson, M., Mao, Q., Pan, C. H., Dai, S. et al. (2006). Structural insights into histone demethylation by JMJD2 family members. *Cell* **125**, 691-702.
Chua, G., Morris, Q. D., Sopko, R., Robinson, M. D., Ryan, O., Chan, E. T., Frey, B. J., Andrews, B. J., Boone, C. and Hughes, T. R. (2006). Identifying transcription factor functions and targets by phenotypic activation. *Proc. Natl. Acad. Sci. USA* **103**, 12045-12050.
Cirillo, L. A., Lin, F. R., Cuesta, I., Friedman, D., Jarnik, M. and Zaret, K. S. (2002). Opening of compacted chromatin by early developmental transcription factors HNF3 (FoxA) and GATA-4. *Mol. Cell* **9**, 279-289.
Hollenhorst, P. C., Jones, D. A. and Graves, B. J. (2004). Expression profiles frame the promoter specificity dilemma of the ETS family of transcription factors. *Nucleic Acids Res.* **32**, 5693-5702.
Ito, T., Bulger, M., Pazin, M. J., Kobayashi, R. and Kadonaga, J. T. (1997). ACF, an ISWI-containing and ATP-utilizing chromatin assembly and remodeling factor. *Cell* **90**, 145-155.
Jin, J., Cai, Y., Yao, T., Gottschalk, A. J., Florens, L., Swanson, S. K., Gutierrez, J. L., Coleman, M. K., Workman, J. L., Mushegian, A. et al. (2005). A mammalian chromatin remodeling complex with similarities to the yeast INO80 complex. *J. Biol. Chem.* **280**, 41207-41212.
Joshi, A. A. and Struhl, K. (2005). Eaf3 chromodomain interaction with methylated H3-K36 links histone deacetylation to Pol II elongation. *Mol. Cell* **20**, 971-978.
Lei, E. P. and Corces, V. G. (2006). RNA interference machinery influences the nuclear organization of a chromatin insulator. *Nat. Genet.* **38**, 936-941.
Miller, G. and Hahn, S. (2006). A DNA-tethered cleavage probe reveals the path for promoter DNA in the yeast preinitiation complex. *Nat. Struct. Mol. Biol.* **13**, 603-610.
Ochoa-Espinosa, A., Yucel, G., Kaplan, L., Pare, A., Pura, N., Oberstein, A., Papatsenko, D. and Small, S. (2005). The role of binding site cluster strength in Bicoid-dependent patterning in Drosophila. *Proc. Natl. Acad. Sci. USA* **102**, 4960-4965.
Osborne, C. S., Chakalova, L., Brown, K. E., Carter, D., Horton, A., Debrand, E., Goyenechea, B., Mitchell, J. A., Lopes, S., Reik, W. et al. (2004). Active genes dynamically colocalize to shared sites of ongoing transcription. *Nat. Genet.* **36**, 1065-1071.
Persengiev, S. P., Zhu, X., Dixit, B. L., Maston, G. A., Kittler, E. L. and Green, M. R. (2003). TRF3, a TATA-box-binding protein-related factor, is vertebrate-specific and widely expressed. *Proc. Natl. Acad. Sci. USA* **100**, 14887-14891.
Ptacek, J., Devgan, G., Michaud, G., Zhu, H., Zhu, X., Fasolo, J., Guo, H., Jona, G., Breitkreutz, A., Sopko, R. et al. (2005). Global analysis of protein phosphorylation in yeast. *Nature* **438**, 679-684.
Rinn, J. L., Bondre, C., Gladstone, H. B., Brown, P. O. and Chang, H. Y. (2006). Anatomic demarcation by positional variation in fibroblast gene expression programs. *PLoS Genet.* **2**, e119.
Schwartz, B. E. and Ahmad, K. (2005). Transcriptional activation triggers deposition and removal of the histone variant H3.3. *Genes Dev.* **19**, 804-814.
Shen, X., Mizuguchi, G., Hamiche, A. and Wu, C. (2000). A chromatin remodelling complex involved in transcription and DNA processing. *Nature* **406**, 541-544.
Sopko, R., Huang, D., Preston, N., Chua, G., Papp, B., Kafadar, K., Snyder, M., Oliver, S. G., Cyert, M., Hughes, T. R. et al. (2006). Mapping pathways and phenotypes by systematic gene overexpression. *Mol. Cell* **21**, 319-330.
Sprouse, R. O., Brenowitz, M. and Auble, D. T. (2006). Snf2/Swi2-related ATPase Mot1 drives displacement of TATA-binding protein by gripping DNA. *EMBO J.* **25**, 1492-1504.
Tapscott, S. J. (2005). The circuitry of a master switch: Myod and the regulation of skeletal muscle gene transcription. *Development* **132**, 2685-2695.
Wang, J. C., Shah, N., Pantoja, C., Meijsing, S. H., Ho, J. D., Scanlan, T. S. and Yamamoto, K. R. (2006). Novel arylpyrazole compounds selectively modulate glucocorticoid receptor regulatory activity. *Genes Dev.* **20**, 689-699.
Whitehouse, I. and Tsukiyama, T. (2006). Antagonistic forces that position nucleosomes in vivo. *Nat. Struct. Mol. Biol.* **13**, 633-640.
Workman, J. (2006). Nucleosome displacement in transcription. *Genes Dev.* **200**, 2009-2017.
Yang, F., Vought, B. W., Satterlee, J. S., Walker, A. K., Jim Sun, Z. Y., Watts, J. L., DeBeaumont, R., Saito, R. M., Hyberts, S. G., Yang, S. et al. (2006). An ARC/Mediator subunit required for SREBP control of cholesterol and lipid homeostasis. *Nature* **442**, 700-704.
Yao, J., Munson, K. M., Webb, W. W. and Lis, J. T. (2006). Dynamics of heat shock factor association with native gene loci in living cells. *Nature* **442**, 1050-1053.
Yuan, G. C., Liu, Y. J., Dion, M. F., Slack, M. D., Wu, L. F., Altschuler, S. J. and Rando, O. J. (2005). Genome-scale identification of nucleosome positions in S. cerevisiae. *Science* **309**, 626-630.