MEETING REVIEW

Transcription: from regulatory ncRNA to incongruent redundancy

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Transcription is such a fundamental process and has been studied by so many for so long that skeptics might ask what more there is to learn. Those who attended the meeting summarized here on the dynamics of eukaryotic transcription during development were not disappointed. Studying the transcription of genes in stem cells during early development and in model organisms has illuminated mechanisms for transcriptional control that would have been hard to accept even 5 years ago, and consistently challenges the textbook view of transcriptional regulation.

Rather than thinking of gene regulation as an on/off process, we now have to accept that our genomes are pervasively transcribed; that regulatory noncoding RNAs (ncRNAs) acting in cis complement transcription factors in gene regulation; that genes that are not expressed (which in the conventional sense are “off”) are often associated with engaged RNA polymerase II, producing short noncoding transcripts at their promoters; and that genes are differentially marked to respond to a particular developmental program long before they are actually expressed. Transcription factors are now associated with terms such as “rheostat” rather than “switch,” and, together with large coactivator protein complexes, often control the transition to the elongation stage of transcription. Not surprisingly, the chromatin environment in which genes reside has yielded some surprises, not least that there are new and uncharacterized chromatin domains, and challenges to the idea that histone modifications reflect the functionality of the complexes that deposit them. Many of the new views of our genomes and their transcription have been facilitated by technological developments, and prompt new ways of thinking using terms such as dynamic, stochastic, and complex systems. Although some of these new ideas have their critics, they were the subject of the meeting organized by Karen Adelman and Marc Timmers, sponsored by the Keystone Symposium Organization, and held in Big Sky, Montana, April 7–12, 2010.

Transcription in development and differentiation

A major theme of the meeting was the interaction of transcription factors with chromatin, and the mechanisms by which multipotent progenitor cells maintain themselves, choose certain cell fates, and permanently exclude others. Bivalent marks and the concept of many genes being poised for expression feature widely in the models presented. We know that a core set of transcription factors (Oct4, Sox2, and Nanog) maintains embryonic stem (ES) cells, while alternative combinations of factors (a dimer of Sox2 and Oct4, with either Klf4 and c-myc or Nanog and Lin28) reprogram somatic cells to pluripotency [iPS [induced pluripotent stem] cells] [Jaenisch and Young 2008]. At this meeting, screens to isolate other factors required to maintain stem cell lineages were presented. In the keynote address, Rick Young (Massachusetts Institute of Technology) described novel transcription factors and chromatin regulators required to maintain ES cells, discovered in a high-throughput genetic screen using shRNAs. Reassuringly, the screen identified many expected factors, including components of signaling pathways (Wnt, Lif, TGFβ, and Notch), transcription factors (Oct4, Sox2, Nanog, Esrrb, and Sal14), and chromatin regulators (PcG, setDB1, BAF, and ATPase). Other factors were more intriguing, and included 10 of the 30 subunits of the mediator complex. A mediator knockdown leads to reduced Oct4 expression and differentiation of ES cells. Evidence was presented that mediator function involves the formation of a loop between the core promoter and an upstream anchor point that has been detected at four differentially expressed genes so far using 3C [capturing chromosome conformation]. These loops are highly reminiscent of the organization of genes in bacteria, reminding us that we should not ignore the paradigms for gene regulation developed in these simpler organisms.

Barbara Panning (University of California at San Francisco) presented data showing that several subunits of the Tip60/p400 lysine acetyl transferase (KAT)/ATPase complex are required for ES cell self-renewal. In addition, Nanog and methylation of Lys 4 on histone H3 (H3K4me) are required for Tip60/p400 association with its target promoters. This is likely to be a conserved mechanism, as

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yeast NuA4, the Tip60 homolog, requires H3K4 methylation to associate with promoters to facilitate transcription elongation.

Ken Zaret (Philadelphia University) discussed chromatin signatures during development of pluripotent endoderm into pancreas and liver. He demonstrated that genes destined to be expressed in either liver or pancreas are differentially marked in undifferentiated cells. A cascade of Fox factors (D3 to A1 and A2) act as pioneer factors, marking poised liver-specific enhancers throughout development, and acting with repressors such as Groucho to compact chromatin. Groucho is displaced from chromatin by tissue-inductive signaling during early development, allowing FoxA to recruit additional transcription factors to enhancers. In undifferentiated progenitors, pancreas regulatory elements are marked by H3K9 acetylation and H3K27 trimethylation (H3K27me3) with the activating and repressing mark, respectively, creating a new kind of bivalent, poised state. Remarkably, the p300 KAT appears to be the major effector of the cell type choice for liver-specific genes.

Susan Mango (Harvard University) continued the discussion of the FoxA factor pha-4 in organogenesis in the worm Caenorhabditis elegans. Using artificial chromosomes, she showed that pha-4/FoxA is necessary and sufficient for large-scale decompaction of chromatin encompassing its target genes. In both worms and vertebrates, the FoxA proteins associate with different targets in different cell types, suggesting that binding is regulated. Mango described a role for emerin, a lamin-associated protein, in modulating differential pha-4 binding to chromatin. In the absence of emerin, pha-4 can bind its targets in cells where it normally would not bind. The altered binding profile of pha-4 is unlikely to be as a result of large-scale disruption of heterochromatin, which appears largely intact in emerin-deficient cells. Defects in lamins and lamin-interacting proteins such as emerin are associated with Emery-Dreifuss muscular dystrophy and syndromes such as Hutchinson Gilford Progeria in which there is a failure to repress pericentric heterochromatin. Clearly, there is much more to learn about the relationships between genes and the nuclear lamina.

Fiona Watt (Cambridge University) discussed epidermal stem cells in adult skin that are regulated by both intrinsic transcriptional mechanisms and environmental signals. She discussed how stem cells that are defined by expression of Lrig1, a negative regulator of EGFR signaling, exhibit different differentiation ability in different contexts. Potential environmental signals that regulate epidermal stem cell renewal and differentiation include extracellular matrix, cell-cell contacts, secreted factors, and physical factors such as substrate area and stiffness.

Hiroshi Handa (University of Tokyo) described his work on identification of the target of thalidomide teratogenicity, CRBN, using new bead technology to facilitate affinity chromatography of target proteins. CRBN forms an E3 ubiquitin ligase complex with DDB1, Cul4A, and Rock1 that is important for limb outgrowth and expression of the fibroblast growth factor Fgf8. Thalidomide initiates its teratogenic effects by binding to CRBN and inhibiting the associated ubiquitin ligase activity. This is confirmed by making the thalidomide-binding-deficient mutant (CRBNW/YA) with an E3 ubiquitin ligase activity. Fgf8 is often involved in reciprocal translocations associated with multiple myeloma, suggesting that thalidomide may have efficacy in its treatment by virtue of its ability to reduce Fgf8 function.

Poised RNA polymerase II at promoters

Part of the mechanism for maintaining the pluripotency of ES cells is to create bivalent domains that silence developmental genes while keeping them poised for activation with RNA polymerase phosphorylated at Ser5 of the C-terminal domain (CTD) and engaged at the promoter, demonstrated using a genome-wide run-on analysis coupled with deep sequencing (Gro-Seq) (Guenther et al. 2007; Core et al. 2008) to detect the nascent transcripts. One well-studied bivalent domain of overlapping repressive and activating histone modifications involves the polycomb group (PcG) [H3K27me3] and the trithorax group (Trx) [H3K4me3] proteins (Bernstein et al. 2006). On differentiation, many of these domains are resolved to yield active or repressed genes by removal of one of the marks. John Lis (Cornell University) reported that peaks of poised but transcriptionally engaged RNA polymerase can be found at >40% of genes in mouse ES cells. Poised polymerase is also referred to as paused polymerase, and promoter proximally paused RNA polymerase II molecules are prevalent across metazoan genomes due to the default process of negative elongation factors [NELFs] that include DRB sensitivity-inducing factor [DSIF; Spt4/5] and NELF. Although lacking a NELF activity, a transient pause is also evident at some yeast promoters during activation (Morillon et al. 2003, 2005). The positive transcription elongation factor P-TEFb phosphorylates NELF, DSIF, and the CTD of RNA polymerase II on Ser2, and facilitates the transition of these poised polymerases into productive elongation.

In his keynote address, Young described the role of c-myc in self-renewal and proliferation of ES cells through its ability to recruit P-TEFb (a positive transcription elongation factor composed of the Cdk9 kinase/Cyclin T) and promote efficient release of RNA polymerase II from poised promoters at one-third of the active genes. Knockdown of c-myc or a small molecule inhibitor directed at c-myc function does not change levels of Ser5-phosphorylated CTD on RNA polymerase II, associated with initiated RNA polymerase II at the c-myc-regulated promoters, but leads to a significant reduction in CTD phosphorylated at Ser2, deposited by P-TEFb and a marker of elongation.

Most of the P-TEFb is held in an inactive state by HEXIM1 in the 7SK snRNP. David Price (University of Iowa) provided results demonstrating that HIV Tat or human Brd4 can directly cause the release of P-TEFb from the 7SK snRNP. HEXIM1 was also released after P-TEFb, concomitant with a change in the conformation of 7SK RNA. These results suggest that regulation of HEXIM1 binding is the key to controlling the amount of P-TEFb in the 7SK snRNP. Importantly, it was hypothesized that
the release of P-TEFb from the 7SK snRNP directed by cellular and viral activators may allow gene-specific activation of expression at the level of elongation.

An important question is how P-TEFb is recruited to non-myc-regulated genes in ES cells to promote efficient elongation. P-TEFb is associated with a number of other factors and complexes. Kathy Jones (Salk Institute) discussed the interaction of P-TEFb with SKIP, a factor that links elongation, splicing, and H3K4me3 at inducible genes regulated by Tat, Notch, TGFβ, and/or stress responses. P-TEFb is part of the super elongation complex (SEC) with the elongation factors ELL1–3, EAFs, AFF1, AFF4, and several of the most common translocation partners of MLL1, including AF9 and ENL. SEC is required for transcription from poised promoters in metazoans and for the transcription of MLL1 chimera target genes that result from chromosomal translocations in leukemic cells, discussed by Ali Shilatifard (Stowers University). Joan Conaway (Stowers University) showed how the function of the SEC is linked to the mediator subunit Med26, which is required for the growth of cells, including ES cells, and expression of the immediate early genes such as c-myc. Med26 interacts with the SEC via its CTD and with TFIIH via its N-terminal domain (NTD), and, thereby, Med26 controls the switch from initiation to elongation.

Many promoters in mammalian and yeast genomes appear to be noisy and initiate transcription in both directions (Core et al. 2008; He et al. 2008; Preker et al. 2008; Seila et al. 2008). Lis showed very clearly that this is strongly correlated with the type of promoter. No divergent transcripts could be detected from 665 stringent TATA-box genes in mammalian cells or from any Drosophila gene in S2 cells. In contrast, CpG island promoters in mammalian cells produce divergent transcripts. Interestingly, the transcription start sites are broadly dispersed at these CpG island promoters, and there are two peaks of Ser5-phosphorylated RNA polymerase II and NELF, consistent with transcription in both directions.

Steve Buratowski (Harvard University) discussed the mechanisms in yeast by which these divergent transcripts are terminated, and proposed a role for the NuA3 KAT, antagonized by the Rpd3L histone deacetylase activity, in bypassing early termination close to promoters and controlling the amount of transcription over the main body of the gene. Buratowski also presented a provocative model suggesting a role for NELF in an early transcription termination pathway, using as an analogy the Nrd1/Sen1 pathway that terminates cryptic unstable transcripts [CUTs] shortly after initiation and relies on RNA polymerase II phosphorylated at Ser5. Thus, rather than simply blocking RNA polymerase, NELF would promote an abortive transcription cycle, which would result in multiple short RNA molecules per gene as opposed to a single RNA for a blocked polymerase. Whether an abortive cycle is consistent with the relatively uniform size of the short transcripts observed by Gro-seq remains to be seen, and, for remainder of this review, the peaks of initiated RNA polymerase at 5’ regions of genes are referred to as paused or poised. However, there is a real need to go back and examine the role of many more transcription-related factors at this step in the transcription cycle. In addition, the link between histone-modifying enzymes and early transcription termination/elongation needs to be explored. For example, a near universal mark of active genes in eukaryotes is promoter-associated H3K4me3. Perhaps this modification simply reflects the presence of RNA polymerase II in an abortive cycle or poised at promoters.

Using the developing Drosophila embryo as a model, Mike Levine (University of California at Berkeley) very convincingly demonstrated a role for poised polymerase in ensuring coordinate and rapid responses to developmental signals. Much more variability was observed in the time of induction of genes lacking poised RNA polymerase in different cells in the embryo, and Levine proposed poised RNA polymerase II as a mechanism for reducing transcriptional noise and ensuring the fidelity of gene expression. A question that has not yet been addressed is whether the stochasticity at genes lacking poised polymerase is also an important component of the developmental process. Levine also demonstrated that the presence of poised polymerase acts as an effective insulator in enhancer-blocking assays, and suggested that repressors function at the post-initiation phase of transcription to block the release of poised polymerase.

### Nucleosomes and transcription

Work presented by Karen Adelman [University of North Carolina] and David Gilmour (Pennsylvania State University) gave further insights into the control of RNA polymerase at promoters, showing how transcription factors, elongation factors, nucleosomes, and the underlying DNA sequence act together to determine the kinetics of polymerase capture and release at each promoter. Rather than acting as an on/off switch, NELF appears to act more like a rheostat fine-tuning in response to signals. Moreover, contrary to predictions, loss of NELF often results in loss of expression and increased histone occupancy at many promoters. This suggests a new way of looking at NELF and paused RNA polymerase as a mechanism for clearing the promoter of nucleosomes to promote expression. In support of this, many NELF-dependent genes show a degree of nucleosome depletion in the promoter region, particularly those regulated by the GAGA factor in flies. In contrast, genes that are set up with a distinct nucleosome-depleted region [NDR] flanked with highly positioned nucleosomes are associated with the least paused RNA polymerase and show little dependence on NELF. Because they are nucleosome-depleted, they can be assumed to be transcriptionally noisy. Olivier Cuvier [University of Toulouse] described the insulator factor BEAF that binds to DNA and positions nucleosomes at promoters, particularly at the highly expressed NELF-regulated genes in flies. The insulating function of BEAF appears to regulate the expression of hundreds of genes involving discrete nucleosome-positioning conformations that are coupled with RNA polymerase II pausing. Although there is still debate in the field about the relative contribution of DNA and other factors in determining

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nucleosome positions, Jon Widom [Northwestern University] introduced the idea that the nucleosomal DNA sequence preference is sharply defined, and, moreover, that genomes explicitly encode both individual nucleosome locations and higher-order chromatin structure. To overcome breathing at the ends of nucleosomal fragments, and other problems of conventional nucleosome mapping methods that introduce significant errors in accurate determination of nucleosome positions, Widom went back to mapping the center of nucleosomes using cysteine-substituted histone H4 and hydroxyl radicals coupled with parallel DNA sequencing of nucleosome monomers. From this, he defined a rule for the most probable linker lengths between nucleosomes in yeast (10n + 5), meaning that nucleosomes in the yeast genome are, on average, offset and start on the opposite face of the dsDNA, leading to a zig-zag-shaped, flat, 30-nm fiber structure. While the most probable linker lengths in yeast obey the rule 10n + 5, other linker lengths are possible too, and could correspond, for example, to different “kinds” of chromatin, with different biological functions. The universal nature of the nucleosome-positioning code was illustrated quite dramatically by reconstituting eukaryotic nucleosomes with a 147-U length on the DNA from the Euryarchaeae Methanobacterium thermoautotrophicum, which normally contains nucleosomes associated with 60 base pairs [bp] of DNA.

Steve Henikoff [Seattle University] described a method, “catch-it,” to immunoprecipitate biotin-tagged histones to measure turnover of histones around origins of DNA replication and at Trx- and PcG-regulated genes, and proposed that silencing is reflected by a reduced rate of histone turnover. The rapid turnover of histones observed using catch-it would tend to rule out chromatin modifications as heritable epigenetic marks. Henikoff went on to discuss the effect of the variant histone H2A.Z (which is incorporated into chromatin reciprocally with CpG methylation) on the properties of nucleosomes, depending on whether there is one or two H2A.Z molecules in a nucleosomes. Two H2A.Z molecules were proposed to promote stable nucleosomes during transcription, and to be more easily assembled behind RNA polymerase II.

Work from Ana Pombo’s group [Medical Research Council-London] suggests that where genes are found [their chromosome territory [CT]] determines when and how they are transcribed, and may be critical for silencing by poising prior to induction. Her group also showed that, at PcG-associated poised genes in ES cells, RNA polymerase II lacking Ser2 phosphorylation is observed throughout coding regions, suggesting transcription without RNA processing and export. Moreover, this work describes two types of transcription factories (clusters of active RNA polymerase molecules) that can be distinguished by their RNA polymerase II CTD phosphorylation profile: one associated with active genes with Ser5 and Ser2 phosphorylated, and a second associated with poised genes in which only Ser5 is evident.

Bas van Steensel [University of Amsterdam] reminded us just how much still remains to be understood about how DNA is packaged into chromatin. The binding sites of 53 chromatin-associated proteins, representing 13 different functional classes, were mapped genome-wide in Drosophila Kc cells using DamID [DNA adenine methylation identification]. In this technique, the Escherichia coli Dam methylase is fused to the protein of interest, resulting in local GATC methylation in the vicinity of binding, allowing precise mapping of these sites. The data were subject to a principal component analysis to reveal five major lobes representing five distinct types of chromatin. Reassuringly, two of these chromatin types correspond to the classical PcG/H3K27me3 and HP1/H3K9me2 types of heterochromatin. The third type of chromatin represents about half of the fly genome [some 6000 genes] and is transcriptionally inactive, but contains no known repressive chromatin modifications. Six of the 53 proteins are found in this type of chromatin, including histone H1, a lamin, an insulator protein, a predicted histone H3 ubiquitin ligase, and a late DNA replication modulator. It is clear we still have a lot to learn about repressive chromatin. The remaining two types of chromatin are transcriptionally active, and are associated with the majority of the 53 proteins. One of these two classes is marked by a chromodomain protein named MRG15. In humans, MRG15 plays a role in embryonic development, cell proliferation, and cellular senescence. In this study, MRG15 binds to H3K36me2/3 found at the 3’ end of one of the active classes enriched with housekeeping genes. The second class comprises tissue-specific, intron-containing transcription units that lack both H3K36me2/3 and MRG15. This study also reveals that these five domains are found in a nonrandom organization in the nucleus, with active and repressed chromatin classes showing distinct preferences for their neighbors. This type of study provides a context for understanding the dramatic phenotypes associated with chromosome translocations and inversions, and epigenetic phenomena such as position effect variegation, which can be viewed as unstable situations resulting from positioning one type of chromatin into an incompatible second chromatin type.

Wendy Bickmore [University of Edinburgh] shook the foundations of our faith in histone modifications as effectors of events on chromatin with her provocative talk on the role of the PcG complexes [PRC1 and PRC2, associated with H2AK119 ubiquitylation and H3K27 trimethylation, respectively] in chromatin compaction and gene repression at the hoxb locus during differentiation in the early mouse embryo. Using a nicely controlled FISH technique in single cells, the physical distance between hoxb1 and hoxb9, shown to correlate strongly with activation or repression of the PcG target genes at the locus, was measured in cells lacking the various activities associated with PcG. This allowed a simple question to be addressed: Are the PcG complexes more than histone/protein-modifying enzymes? The answer seems to be that they are, and that the known modifying activities—particularly the H2A ubiquitylating activity of Ring1b in the PRC1 complex, which is generally envisaged as blocking transcription elongation by the poised RNA polymerase at the hoxb promoters—are not required for compaction or gene repression at the
hoxb locus. Although these observations rely on the mutation resulting in loss of Ring1b activity being recapitulated at the hoxb locus in vivo, they are highly provocative. Given the important roles for PcG proteins in regulating cellular senescence by repressing the INK4a–ARF locus, and recent observations that partial depression of PcG function actually leads to increased life span and stress resistance in flies, an answer to the question as to exactly how the PcG complexes repress gene expression is urgently needed.

Regulatory noncoding transcripts

Gro-seq and strand-specific microarrays are being used widely to define the eukaryotic transcriptome. They are revealing that genomes from yeast to humans are pervasively transcribed [Core et al. 2008; Neil et al. 2009], and, at this meeting, we began to get a hint from several speakers about functions associated with these noncoding transcripts. W. Lee Kraus [Cornell University] redefined the estrogen response using Gro-seq, revealing many poised estrogen-responsive genes, a complex temporal response involving up-regulation and down-regulation of >3000 RefSeq genes, and many unannotated noncoding transcripts that are estrogen-responsive. Jen Kugel [University of Colorado] discussed ncRNAs that globally and directly inhibit RNA polymerase by binding in the active site and blocking the early steps of transcription initiation, including promoter melting and abortive initiation. The B2 ncRNA inhibits transcription during a heat shock, and, remarkably, is displaced from the active site by a purified factor only after extending the 3’ end of the B2 RNA using the RNA-dependent RNA polymerase activity of the enzyme [Lehmann et al. 2007].

The theme of ncRNA was continued in a talk by Ramin Shiekhattar [The Wistar Institute] describing a new class of long ncRNAs, known as enhancer-like ncRNAs (elRNAs), which function in cis and in an orientation-independent way to activate developmentally regulated genes. There may be as many as 10,000 similar transcripts in the human genome, suggesting that we still have a great deal to discover about how our genes are regulated. How these rare long transcripts differ in their regulation and function from the enhancer-associated bidirectional transcripts (eRNAs) mediated by p300/CBP that activate neighboring genes remains to be seen [Kim et al. 2010].

Jane Mellor [University of Oxford] continued the theme of regulatory RNA acting in cis. Her model focused on the general properties of classes of antisense transcripts at active yeast genes, and their role in the deposition of marks such as K36me2/3 in the vicinity of promoters to facilitate sense transcription through factors such as NuA4. The cis function of these transcripts could be observed only in the context of sense:antisense pairs (SAPs), with the antisense transcripts generated from the sites normally recognized as the termination sites for sense transcription. Careful analysis brings forward important regulatory properties of those termination sites, which act as de facto promoters for antisense transcripts. Debilitating production of antisense transcripts from those sites has direct effects on the activity of the promoters and the sense transcripts. Although much still remains to be understood in this mechanism, it clearly puts forward the idea of a transcriptional cycle that involves both sense- and antisense-regulated transcripts.

Predicting transcription factor-binding sites and recruiting them to chromatin

Predicting transcription factor binding at promoters is difficult, and, even using techniques such as chromatin immunoprecipitation (ChIP)-seq, there will always be a trade-off between false-positives and false-negatives. On the whole, conservative thresholds are set, resulting in a marked underestimate of genome-wide binding sites. Frank Pugh [Pennsylvania State University] described a new approach for more accurately predicting protein-binding sites by trimming cross-linked protein:DNA complexes using exonuclease and subjecting this to deep sequencing, yielding a characteristic set of peak pairs—one cluster on the Watson (W) strand, and the second on the Crick (C) strand, which, for Reb1, is 28 bp centered around the DNA-binding site. Using five parameters—number of reads, number of replicates (usually four), W–C distance, DNA-binding motif, and distance from the motif—allows much greater accuracy, and predicts 502 Reb1-binding sites that met all five criteria. The results suggest that Reb1 interacts with the −1 nucleosome.

Another method for predicting transcription factor in vivo occupancy was presented by X. Shirley Liu [Harvard University]. Using genome-wide nucleosome resolution ChIP-seq of H3K4me2 and bioinformatics analysis of transcription factor sequencing motifs, this approach can infer driving or pioneering transcription factors, their binding patterns, and the regulatory network during intestinal differentiation from progenitor crypt cells to villus cells. This is based on the observation that transcription factor binding is associated with an NDR flanked by two highly positioned nucleosomes enriched in H3K4me2, which is reversed when the transcription factor leaves the enhancer, resulting in deposition of a nucleosome at the NDR and an increased H3K4me2 signal. This allowed transcription factor-binding motifs in the dynamic regions to be examined, and the enriched transcription factors to be predicted. Liu and colleagues showed that the intestinal-specific transcription factor Cdx2 is found to colocalize with Gata6 in the progenitor cells and with Hnf4 in the differentiated cells. This will be a powerful technology for predicting stimulus-dependent transcription factor activity based on changes in chromatin structure.

Biochemical and biological plasticity and redundancy in gene regulation

Many coactivator complexes [for example, TFIID, SAGA, ATAC, and Mediator (MED)] that were originally isolated as discrete complexes are now being found as components of much larger complexes with discrete functions [for example, the SEC discussed earlier]. Laszlo Tora [University of Strasbourg] reported the isolation of a new meta
coactivator complex [MECO] composed of the Gen5 KAT-containing ATAC (G-ATAC) histone acetyl transferase complex and the active form Mediator complex [Med26-containing, PC2 form], along with some RNA polymerase II subunits. He showed that the leucine zipper factor, known as Luzp1, that is expressed in ES cells is a subunit of MECO, and that Luzp1 is required, but not sufficient, to bridge G-ATAC to MED. Interestingly, MECO controls the expression of many ncRNAs, including snRNAs. These findings establish that transcription coactivator complexes can form stable subcomplexes in order to facilitate their combined actions on specific target genes.

Bob Roeder [Rockefeller University] continued the theme of plasticity in biological systems, specifically related to the coactivator PGC-1α, which functions in the transition between p300-dependent and Mediator-dependent transcription through a Trα–RXRs heterodimer on the UCP-1 enhancer in brown fat adipocytes. In this model, PGC1α, recruited initially via its LXXLL domain to the thyroid hormone receptor [TR], functions in chromatin remodeling and potentiates mediator recruitment. MED1, via its LXXLL domain, then displaced PGC1α from TR to the CTD of MED1, where it enhances mediator-dependent transcription. Roeder also showed that the LXXLL domain of MED1 plays a role in mammary gland development, specifically mammary luminal epithelial cell differentiation and progenitor/stem cell determination through mediator–ERα interactions.

Marc Timmers [Utrecht University] described a role for the haspin kinase, which phosphorylates H3T3 and reduces gene expression in mitosis by interfering with the association of TFIID with promoters, specifically the TAF3 PHD-H3K4me3 interaction, found only in higher eukaryotes. He also discussed the dynamics of TBP association, and how this varies depending on the nature of the regulators and type of RNA polymerase in yeast. TBP is most dynamic at genes with a canonical TATA box that are also dependent on the Mot1 ATPase and SAGA for their expression. Genes regulated by TFIID generally show a lower TBP turnover rate. Pugh continued this theme by showing how the stress-induced SAGA-regulated genes are occupied by a large number of chromatin-modifying factors, while TFIID-regulated genes are occupied predominantly by a much smaller number of general transcription factors such as Rap1.

Frank Holstege [Utrecht University] dealt with the difficult question as to why two proteins are maintained in organisms when they apparently show functional redundancy, at least under the standardized laboratory growth conditions. Many of the genes encoding proteins that control transcription show some degree of functional redundancy; for example, strains containing an HTZ1 knockout encoding the variant histone Htz1 show virtually no phenotype, but show strong synthetic interactions with knockouts of virtually every other histone-modifying enzyme. Understanding the basis of these relationships will provide real insights into transcription and biological systems. Holstege used the functional redundancy between pairs of protein kinases, or kinases and phosphatases, to illustrate this. Using highly controlled genome-wide microarrays to profile 25 pairs of redundant kinases and/or phosphatases, he classified three types of redundancy. The first is complete redundancy, where each single knockout has no effect on genome-wide expression profiles, but the double has lots of changes. The second type is quantitative redundancy, where one knockout has an effect, the second has no effect, and the combination affects the same genes, but to a greater extent. The third, most common type is the most interesting, and is termed incongruent redundancy, in which different clusters of genes behave in different epistatic ways in the double knockout. This type of redundancy can be explained by a combination of partial overlap in function coupled to regulatory links between the redundant partners, and was modeled very convincingly for the kinases Fus3 and Kss1. Pairs of partially functionally redundant regulators linked in this way may allow regulation of distinct processes to be either coupled or uncoupled, depending on condition or context. This plausibly explains why genetically redundant pairs of regulators are maintained during evolution, and how different pathways are connected. Those at this meeting came away with no doubts that the transcription field is vibrant, with plenty of scope for creative scientists to make a real contribution in the near future. There are high expectations of the next transcription meeting 2 years from now.

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