clinicians will be most concerned about are the development of thrombosis and anti-drug antibodies (ADAs). Because this is nonsubstitutive therapy with a long half-life, thrombosis would be a potential issue if FXa generation was to continue in an uncontrolled manner. No such complication or rise in D-dimer or thrombin-antithrombin complex was observed in this small phase 1 study even though it was conducted on healthy volunteers with normal coagulation systems.

Two individuals were found to have anti-ACE910 antibodies following exposure to the bispecific antibody. One of these subjects also had antibodies before exposure to the drug but the antibody development was new in the second individual. The latter had a reduced ACE910 elimination half-life, and reduced APTT correction and thrombin generation following ex vivo FVIII neutralization, suggesting it was a functional ADA. A key issue in the long term will be the number of individuals who develop ADAs after repeated dosing with ACE910.

Although the initial aim of treatment with this antibody was as prophylaxis in patients with alloantibodies to FVIII, there is no physiological reason why this therapy would not be suitable for persons with hemophilia A and no alloantibodies, or for individuals with acquired hemophilia A and autoantibodies to FVIII. Furthermore, although ACE910 was administered once weekly in the nonhuman primate model[5] because its half-life is 28.3 to 34.4 days, it is likely that future trials will investigate drug injection rates that are less frequent than this.

We are currently at a very unusual time in hemophilia treatment with a large number of new therapies in development. The majority of these are standard or extended half-life substitutive products. ACE910, however, offers the option of a disruptive technology that could potentially alter the way hemophilia care is delivered in the future.

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● ● ● **HEMATOPOIESIS AND STEM CELLS**

Comment on Wilson et al, page e12

# Finding partners to play the music of regulation

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In this issue of *Blood*, Wilson et al generate and analyze a treasure trove of epigenetic data, such as transcription factor occupancy, histone modifications, and chromatin interaction frequencies, genome-wide (ie, epigenomic data), in a cell line model of hematopoietic stem/progenitor cells (HSPCs).[1] To appreciate the importance of these data, consider an analogy of gene expression being a song or symphony (transcripts) played by musicians (transcription factors and transcriptional machinery) reading the score encoded in the genome sequence. Previous studies[2] revealed the positions of a few transcription factors across the genome, so we only knew about, for example, the violinists and oboists. No wonder we did not understand how the music was being generated (how expression was regulated). By mapping the sites of occupancy of many more transcription factors (now a total of 29), as well as positions of 4 histone modifications and DNase hypersensitive sites, Wilson et al[1] reveal many more of the players and their partners. Furthermore, their data on 3-dimensional interaction frequencies of chromatin show how groups of musicians (protein complexes) come together in an orchestra to read the score and perform a symphony.

The breadth and diversity of epigenomic data on HPC-7 cells now are on a par with those for a small number cell lines studied intensively in multiple laboratories (such as embryonic stem cells) and major consortia (such as K562, HepG2, and GM12857 cells in the ENCODE Project Consortium[3]). Although data from transformed cells such as K562 and HepG2 are useful for deducing some general principles, data from primary cells are the most relevant to specific issues. Although a specialized approach has generated histone modification maps in HSPCs,[4] the scarcity of these cells precludes application of most genome-wide assays. This large collection of epigenomic data in HPC-7 cells is a great boon to hematology, as this multipotent cell line is capable of differentiating into several myeloid lineages,[5] and thus serves as a model for HSPCs.

The 3-dimensional chromatin interaction maps generated by Wilson et al[1] turn the static landscape inferred from the maps of nuclease accessibility, transcription factor occupancy, and histone modifications into a snapshot of regulatory regions working together (see figure). The complex interactions among regulatory regions first revealed in studies of hemoglobin genes also are found for many, if not most, genes regulated in a stage- and/or tissue-specific manner. Multiple candidate enhancers, as predicted by patterns of histone modifications and factor occupancy, can be identified for most genes, but the epigenomic maps do not reveal the target genes for the candidate enhancers. This is especially problematic in gene-dense regions. Although proximity and correlations of epigenomic signals can be used to infer targets, direct information about interactions between regulatory regions currently is the best guide. Generating maps of interaction frequency across an entire genome at high resolution[6] requires a staggering number of sequencing
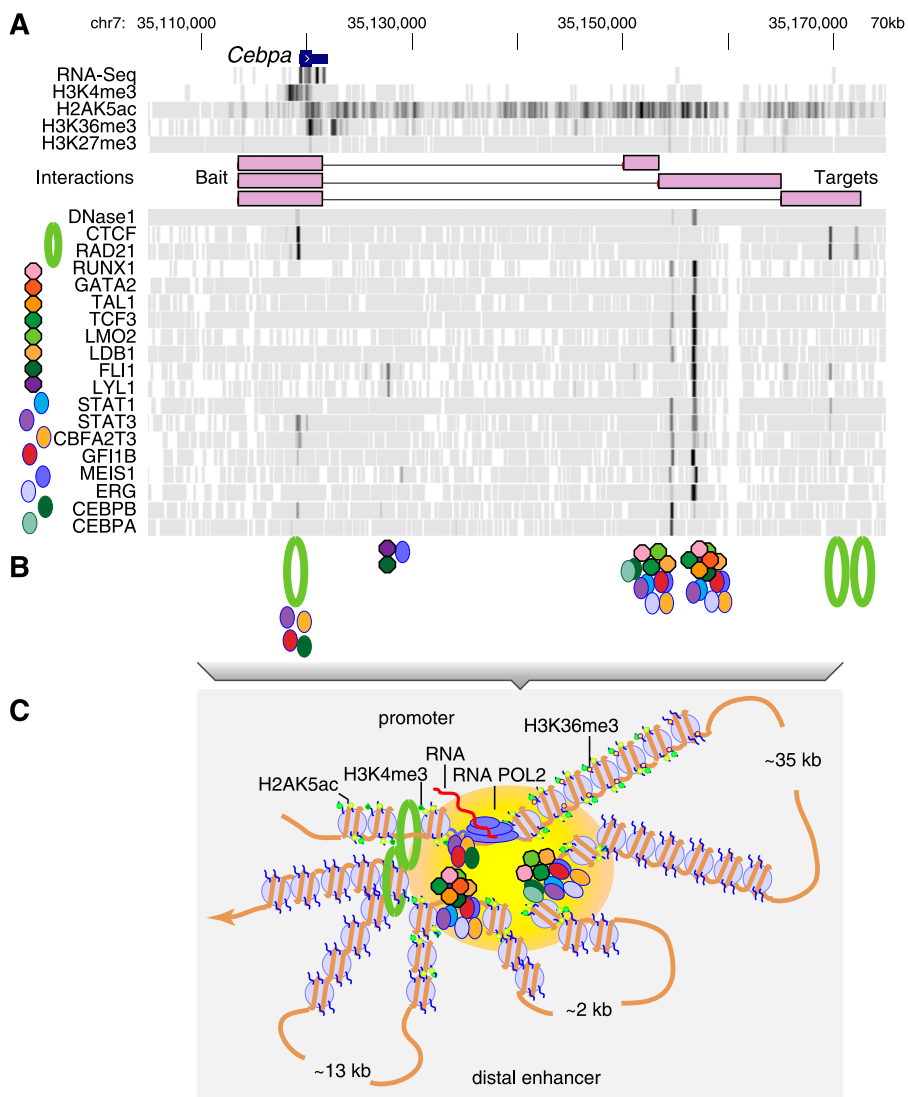
A landscape of diverse regulatory features coupled with chromatin interaction data leads to descriptive models of gene regulation. (A) As illustrated for the *Cebpa* locus (gene on top line), the strength of signals (proportional to the density of the gray along each track) from sequencing RNA, locations of cleavage by DNase, and chromatin immunoprecipitation of modified histones and transcription factors (named on the left and marked by distinctive icons) reveals where along the locus all these players in gene regulation are located. This panel was generated from data displayed at http://tinyurl.com/E-MTAB-3954. (B) Colocation of the transcription factors indicates the positions of at least 3 categories of protein complexes. Members of an octameric complex (a previously described heptamer[2] plus TCF3) are shown as octagons of distinctive colors, members of other complexes are shown as ovals, and the RAD21 component of cohesin plus CTCF are shown as a green circle. (C) Maps of the frequency of chromatin interactions (shown as connections between purple rectangles representing *Hind*III fragments in the promoter Hi-C experiment in A) show that at least 3 complexes of proteins bound downstream of *Cebpa* interact with complexes at the promoter. Thus, one can surmise a structure with the promoter and enhancers juxtaposed in a region with transcriptional activity (indicated by the yellow-orange oval), anchored on cohesin complexes, and with intervening DNA in 3 loops of widely differing sizes.

along genomic coordinates) coming together in an orchestra of regulatory molecules (see figure panel C). Wilson et al[1] show that this candidate enhancer activates reporter gene expression in a tissue-specific manner in transgenic mice embryos.

To facilitate access to and use of this information, Wilson et al[1] provide these data in the CODEX database[8] and at a stable URL for visualization in a genome browser. Thus, investigators can easily find levels of transcripts, maps of epigenomic features, and interaction frequencies in genes and loci of interest to them. These data should catalyze refinement and improve accuracy in identifying candidate enhancers and assigning them to target genes.

This improvement in the accuracy and completeness of our views of regulatory domains can also facilitate clinical research. More and more examples are being reported of the phenotypic effect of genetic variants and mutations in regulatory regions.[3,9,10] For phenotypes expressed in myeloid cells, the maps and resources provided by Wilson et al[1] will be particularly valuable.

These new data will serve as a strong resource for much future work, but they are not the final story. The promoter Hi-C data are valuable for the interactions they reveal, but the experimental design precludes the discovery of many interactions, and some chromatin interactions play key roles at other stages of differentiation. The binding patterns for some transcription factors are highly dynamic, and thus their occupancy needs to be mapped at multiple stages and in different lineages. The data in the article by Wilson et al[1] will help guide these additional studies and provide an important point of reference for comparison with new results.

These new data, coupled with the large amount of information from many laboratories, provide a rich description of the molecular players regulating expression of each locus. The next challenge is to build on this descriptive foundation to generate predictive models of expression, in which the role of each protein complex and each *cis*-regulatory module is defined quantitatively as an outcome on gene expression. Such models, after extensive experimental testing, could provide a basis for consolidating information about the many regulatory complexes at genetic loci into mechanistic rules for gene regulation that apply broadly across genomes and cell types. That would be a notable achievement in our understanding of gene regulation during hematopoiesis.

reads, well beyond the budget or capacity of most investigators. Thus, Wilson et al[1] adopted the promoter Hi-C approach[7] to reveal a highly informative subset of interactions: those between promoters and distal regions.

To illustrate the power of the new data, consider the gene *Cebpa* (see figure). The detailed maps (see figure panel A) identify the locations of transcription factors, which is analogous to knowing the locations of musicians resolved by instrument played (violin, oboe,

flute, percussion, etc). Aligning the maps reveals groups of colocated proteins (summarized in figure panel B) defining a complex of hematopoietic transcription factors (analogous to the string section in an orchestra), other complexes of transcription factors (analogous to the woodwinds), and components of cohesin (analogous to the percussion section). The 3-dimensional interaction maps show that all these components are close together physically, with the components (separated

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● ● ● **IMMUNOBIOLOGY** ▬

Comment on Das et al, page 1666

Alleviating the storm: ruxolitinib in HLH

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In this issue of *Blood*, Das and colleagues report their results on the use of the Janus kinase 1/2 (JAK1/2) inhibitor ruxolitinib in murine models of hemophagocytic lymphohistiocytosis (HLH), and the HLH-sibling macrophage activation syndrome (MAS).[1]

They used genetically engineered perforin-deficient $Prf1^{-/-}$ mice as a model of familial HLH with impaired adaptive immunity due to deficiency of cytolysis. They also used a model for acquired HLH/MAS without perforin deficiency which induces inflammation via Toll-like receptor 9 activation. Due to the central role of cytokines in HLH with JAKs critical for cytokine signaling, Das and colleagues decided to test the pharmacologic JAK1/2 inhibitor treatment as a therapeutic strategy in HLH mouse models. HLH/MAS are hyperinflammatory syndromes that share many characteristics of other highly lethal cytokine storm-associated diseases like sepsis and systemic inflammatory response syndrome. All of these disorders have unresolved issues with respect to the underlying pathophysiology, precise diagnosis, and optimal treatment.[2,3] In familial HLH, a disease usually diagnosed in newborns and toddlers, immunosuppression with high-dose

corticosteroids, T-cell depletion by etoposide (VP-16), and cyclosporine A are used as a bridge to allogeneic stem cell transplantation. The HLH-1994 protocol is currently considered the therapeutic standard.[3] Despite establishing a new higher standard for HLH therapies, treatment according to the HLH-1994 protocol achieved a 5-year survival rate of 54% with a third of the patients not achieving a significant remission by induction treatment, and dying prior to hematopoietic stem cell transplantation. In adults, HLH has gained more attention, both through increased diagnostic vigilance and more patients treated with long-term immunosuppression or chemotherapy.[4] Treatment is more individualized due to a wider spectrum of underlying conditions and diseases that may trigger its onset, such as infections, cancer, and autoimmune disorders (see figure).[5] So far, a combined approach targeting both overt inflammation by immunosuppression and

the underlying trigger by disease-specific treatment is rapidly initiated at diagnosis. Unfortunately, there is no sound controlled data available on efficacy and toxicity of this adult HLH treatment approach.[3] Most recently, the first prospective clinical trial of adult patients with refractory HLH was published in this journal, demonstrating that intense immunosuppression combined with chemotherapy can rescue a significant fraction of patients not responding to the HLH-1994 protocol.[6] More targeted treatment to inhibit central inflammatory cytokine pathways like interleukin 1 receptor (IL1R), IL6, or IFNγ is also being used in HLH and reported as case series or phase 1 trials.[3,7]

Ruxolitinib has demonstrated remarkable activity in other hyperinflammatory, cytokine-governed diseases. It is approved for use in myelofibrosis (MF), where it reverses the hyperinflammatory state and thereby the conditional symptoms of MF.[8] More recently, corticosteroid-resistant acute graft-versus-host disease (aGVHD), another acute syndrome of inflammation, was reported to rapidly respond to ruxolitinib after failure of standard treatment.[9,10] Ruxolitinib suppresses proinflammatory cytokines, reduces T-cell proliferation, and reverses organ damage within days through interference with JAK–signal transducer and activator of transcription (STAT) signaling. The in vivo model provided by Das and colleagues shows similar immunologic effects: the master regulator IFNγ is significantly suppressed along with TNFα. Inflammatory liver foci and T-effector cells appeared reduced. Splenomegaly and weight loss were reversed. JAK-STAT–induced gene expression in HLH mice is significantly affected as shown by reduced expression of *IFNγ*, *STAT1*, and interferon-regulating factor 1 (*IRF1*) in ruxolitinib-treated HLH mice.

A plethora of cytokine receptors use JAKs as mediators of ligand binding and initiators of the STAT-regulated gene expression programs. Mechanistically, JAK inhibition seems to be a rather promiscuous business, that is, not "precision medicine." On the other hand, the cytokine storm in HLH is also quite promiscuous: IFNγ, IL1, IL6, IL18, TNFα, and other critical proinflammatory cytokines are responsible for inflammation-driven organ damage (see figure).[5] Conversely, IL10, an