Corrections

COMMENTARY

The authors note that, due to a printer’s error, on page 14715, middle column, first full paragraph, lines 1–6 “In murine and human T-LL cells, Zhao et al. (13) reported that RBPJ bind preferentially to promoter sites, but the majority of direct Notch target genes lack promoter-bound Notch1 (i.e., NICD1) or RBPJ site.”

Additionally, on page 14716, left column, first full paragraph, lines 1–6 “In proliferating lymphoblastoid cells (LCLs) expressing EBNA2, Wang et al. (14) found that EBNA2 and RBPJ bind predominantly at nonpromoter sites, with 72% of EBNA2 sites within 100 bp of an RBPJ site” should instead appear as “In murine and human T-LL cells, Wang et al. (14) reported that RBPJ binds preferentially to promoter sites, but the majority of direct Notch target genes lack promoter-bound Notch1 (i.e., NICD1) or RBPJ.”

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GEOLOGY

The authors note that, due to a printer’s error, the affiliation lines appear below. The online version has been corrected.

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IMMUNOLOGY

The authors note that, due to a printer’s error, the affiliation for Adam Round should instead appear as European Molecular Biology Laboratory, 38042 Grenoble, France. The corrected author and affiliation lines appear below. The online version has been corrected.

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EVOLUTION

The authors note that, on page 10238, left column, the third equation appears incorrectly. All three correct equations appear below. Additionally, the authors note the following statement should be added to the Acknowledgments: “A.K. was supported by the German Federal Ministry for Education and Research (BMBF) through the GerontoMitoSys project (FKZ 0315584).”

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Transcription factor RBPJ/CSL: A genome-wide look at transcriptional regulation

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The transcriptional regulator RBPJ, otherwise known as CSL ("CBF-1, Suppressor of Hairless, Lag-2," after its mammalian, Drosophila, and Caenorhabditis elegans orthologues) is a highly conserved DNA-binding protein that plays a central role in cell fate decisions in metazoa. RBPJ mediates canonical Notch signaling (1). In the absence of active Notch, RBPJ is thought to be primarily a transcriptional repressor that exists in complexes with corepressors (2). When bound to the active intracellular domain of Notch (NICD), RBPJ recruits a coactivator complex, including a Mastermind homologue (MAML1-3 in mammals), and drives a complex transcriptional program with pervasive phenotypic effects. This program is used during development and adult tissue homeostasis, and is frequently dysregulated in cancer (3, 4). In the hematopoietic system, Notch/RBPJ controls, among other things, the commitment of common lymphoid progenitors to the T-cell lineage and the expansion of marginal-zone B cells (5). Aberrant activation of the Notch–RBPJ transcriptional program in T-lineage cells leads to T-lymphoblastic leukemia (T-LL), and is the most frequent genetic alteration found in this malignancy (6). Epstein–Barr virus (EBV) oncprotein EBNA2, which is essential for the transforming activity of EBV, has been known for some time to mimic NICD and activate RBPJ (7, 8). EBV-induced immortalization is an important cause of B-cell malignancies such as Burkitt lymphoma and some Hodgkin lymphomas, as well as lymphoproliferative disorders in immune-suppressed patients (9, 10). The structure of DNA-bound RBPJ in complex with NICD and a fragment of MAML1 has been resolved (11, 12). However, the mechanism whereby RBPJ is capable of activating different transcriptional programs in different cells remains unclear. Understanding how RBPJ controls the gene expression programs of T and B cells will give us invaluable insights into physiological cell fate determination and neoplastic transformation. Two reports in PNAS (13, 14) used genome-wide ChIP/deep sequencing to identify extensively in these projects, used genome-wide ChIP/deep sequencing to identify genomic sites that bind RBPJ under different conditions and to seek other transcription factors that bind coactivatorially or competitively with RBPJ. Long-range interactions between chromatin sites were identified using chromatin conformation capture followed by deep sequencing.

In murine and human T-LL cells, Zhao et al. (13) reported that RBPJ binds preferentially to promoter sites, but the majority of direct Notch target genes lack promoter-bound Notch1 (i.e., NICD1) or RBPJ. Transcriptional regulation seems to result primarily from long-range interactions at enhancer sites. Putative enhancer sites were identified in the vicinity of such genes as MYC, DTX1, IGF1R, IL7R, and the GIMAP cluster. Interestingly, a significant fraction of genomic sites bound RBPJ only, in the presence of NICD1. This finding could have multiple explanations: (i) RBPJ may regulate transcription in Notch-independent fashions; (ii) RBPJ—“only” sites may not bind Notch1, but may be able to bind another one of the four mammalian Notch paralogues; or (iii) RBPJ—only sites identified in T-LL cells, which are predominately associated with repressive chromatin markers, could be inaccessible to Notch1 in these cells because of the presence of other competing proteins (e.g., stable corepressor complexes), but may be accessible to Notch1 in other cell types. Transcription factor binding motifs enriched within 250 bp of Notch1 sites included ZNF143 (which contains an embedded high-affinity RBPI sites. These sites are generally associated with markers of transcriptional activity. EBNA-LP is a coactivator protein recruited by the C-terminal acidic domain of EBNA2 (19).

Fig. 1. (A) Working model of ZNF143-Notch/RBPJ crosstalk. ZNF143 occupies DNA sites that overlap with RBPJ elements. When ZNF143 is bound, RBPJ is not. When ZNF143 dissociates, sites can be occupied by RBPJ-only complexes, which may contain corepressors SMRT and SKIP, required for RBPJ targeting to the nucleus (2). Both ZNF143 and RBPJ-only occupancy is predominantly associated with transcriptional repression. In the presence of active Notch, Notch/RBPJ/NICD complexes form. These recruit p300 and other coactivators and are predominantly associated with transcriptional activation. (B) Working model of EBF-EBNA2/RBPJ crosstalk. EBF consensus binding sites contain embedded high-affinity RBPI sites. The same sites are likely used by EBF dimers or RBPJ at different times. EBNA2/RBPJ complexes can bind DNA after EBF dimer dissociation, or EBF monomer could cobind with RBPJ/EBNA2 complexes. These sites are generally associated with markers of transcriptional activity. EBNA-LP is a coactivator protein recruited by the C-terminal acidic domain of EBNA2 (19).

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See companion articles on pages 14902 and 14908.

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CREB, ETS, and RUNX motifs were highly enriched within 250 bp of confirmed sites. CREB/RBPJ sites contained lower-affinity RBPJ motifs but had a stronger RBPJ signal. Taken together, these observations suggest that ETS and RUNX family factors cooperate with Notch/RBPJ in T-LL cells, consistent with their known roles in T-cell development and Notch signaling. RBPJ binding to low affinity sites may be modulated by CREB availability. A unique finding is the role of zinc finger protein ZNF143 in Notch/RBPJ signaling. ZNF143 was found with Notch1 and RBPJ at a significant fraction of sites. In vitro, DNA binding by ZNF143 and RBPJ was mutually exclusive. This suggests a model in which DNA-bound ZNF143 controls the accessibility of RBPJ sites to Notch/RBPJ complexes. ZNF143 sites were associated with a prevalence of repressive chromatin marks, as were RBPJ-only sites. Fig. 1A diagrammatically outlines a possible model for these interactions. A common question is the possible role of Notch transcriptional complex dimerization in long-range interactions controlling transcription. Notch transcriptional complex dimerization via Notch1 has been observed at the promoter level (15), and may conceivably occur over long DNA distances through chromatin looping.

In proliferating lymphoblastoid cells (LCLs) expressing EBNA2, Wang et al. (14) found that EBNA2 and RBPJ bind predominantly at nonpromoter sites, with 72% of EBNA2 sites within 100 bp of an RBPJ site. However, there were a significant number of EBNA2-only and RBPJ-only sites. Besides RBPJ, the 500 bp around EBNA2 sites were enriched in EBF, ETS, RUNX, PU.1, and NF-kB (RELA) sites. This correlated strongly with actual occupancy data for these transcription factors. ETS and RUNX sites, but not PU.1 and NF-kB sites, were also observed in T-LL cells in proximity to Notch/RBPJ sites. Cluster analysis of the EBNA2 sites based on associated factors classified EBNA2/RBPJ sites into six subgroups with different combinations of cofactors. All groups contained RBPJ in combinations with RELA-ETS, EBF-RUNX, EBF, ETS, no other factors, and RUNX. Chromatin marks (H3K4me1) and p300 occupancy suggested different transcriptional activity among these groups, with RELA-ETS being the most active and RUNX the least active. This suggests that the presence or absence of transcriptional cofactors modulates the transcriptional activity of EBNA2/RBPJ sites. Interestingly, when chromatin marks associated with EBNA2 sites in LCLs were compared with those in uninfected resting B lymphocytes (RLBs), there was remarkable overlap, with the same hierarchy of sites as in LCLs. This suggests that EBNA2 does not fundamentally alter the transcriptional program of RLBs, but takes advantage of their existing program by targeting RBPJ to sites of open chromatin that participate in B-cell fate determination. EBF, which binds to a DNA motif that contains an embedded high-affinity RBPJ site, may act as a “pioneer” factor, opening chromatin sites that are occupied by EBNA2/RBPJ complexes. How EBF facilitates EBNA2/RBPJ activity remains unclear. EBF dimers and RBPJ are likely to be mutually exclusive in binding these sites, but an EBF monomer and RBPJ could bind cooperatively. Whether such complexes exist remains to be tested. Fig. 1B illustrates two possible models for these interactions. Unlike ZNF143 sites, EBNA2/RBPJ/EBF sites were frequently associated with chromatin markers of transcriptional activity. As for Notch1 in T-LL cells, genes conditionally up-regulated by EBNA2 in LCLs appeared to depend largely on multiple long-range chromatin interactions with enhancers more than 2 kb from transcriptional start sites; 35% of these interactions were interchromosomal. The MYC gene is important in B-cell transformation and is a Notch target in T-LL. Zhao et al. (13) showed that the most likely candidate sites for EBNA2/RBPJ-mediated MYC regulation are EBNA2/RBPJ sites at −428 to −556 kb of the MYC transcriptional start sites. These sites had hallmarks of active enhancers, with EBF, RELA, H3K4me1, H3K9ac, PolII, and p300 signals. Remarkably, these sites were also active in RLBs, although less so, again suggesting that EBNA2 “hijacks” a preexisting transcriptional program that uses RBPJ in B-cell fate determination, and the difference between RLBs and LCLs is more quantitative (i.e., strength and/or duration of transcriptional activity) than qualitative. Interestingly, Notch1 and EBNA2 appear to activate MYC transcription through different putative enhancers in T-LL and LCL cells, respectively. Like Notch1 (15), EBNA2 is capable of self-association (16). An intriguing possibility is that differences in site use between NICD/RBPJ and EBNA2/RBPJ complexes may depend on physical interactions between Notch- or EBNA2-containing transcriptional complexes mediating chromatin looping.

“Context-dependence” is a term frequently used to describe poorly understood cellular conditions that modify the molecular and phenotypic effects of Notch/RBPJ signaling. There are likely many layers of “context” (e.g., activity of other pathways, coexistence of oncogenic mutations, microRNA expression). These two studies (13, 14) begin to give a mechanistic meaning to context at the chromatin level, showing how Notch and EBNA2 can drive RBPJ to enact different transcriptional programs depending on cooperation with other transcription factors and intra- and interchromosomal long-range interactions. The essential role of long-range chromatin interactions resembles what has been observed for the estrogen receptor-α (17, 18) and is likely a general phenomenon in transcriptional regulation.