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POINT-OF-VIEW

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Dynamic chromatin regulation at Notch target genes

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ABSTRACT
RBPJ is the central transcription factor that controls the Notch-dependent transcriptional response by coordinating repressing histone H3K27 deacetylation and activating histone H3K4 methylation. Here, we discuss the molecular mechanisms how RBPJ interacts with opposing NCoR/HDAC-corepressing or KMT2D/UTX-coactivating complexes and how this is controlled by phosphorylation of chromatin modifiers.

Signal transduction pathways and chromatin regulation determine gene expression programs during development and defects in chromatin regulation have been linked to disease occurrence, including carcinogenesis. The chromatin landscape of signal-dependent genes is controlled by activating and repressing chromatin modifiers that are recruited by transcription factors (TFs). How signaling to chromatin is conducted, remains largely unknown. RBPJ (recombination signal binding protein for immunoglobulin kappa J region) represents an excellent example to which extent a TF can act as a molecular switch, that either functions as an activator, in the presence of ligand, or as a repressor, in the absence of stimuli.

RBPJ, also known as CSL [CBF-1/RBPJ in vertebrates, Su(H) in *Drosophila* melanogaster, lag-1 in *Caenorhabditis elegans*], was originally identified by Honjo and colleagues as a protein able to bind the recombination signal sequence of the immunoglobulin Jκ segment[1] and, subsequently shown to regulate peripheral nervous system development in *Drosophila*.[2] RBPJ is a signal-sensitive TF that binds both to proximal and distal enhancer elements characterized by the CGTGGGAA motif.[3] RBPJ is the main and key TF

that governs the Notch signaling cascade. It actively represses transcription of target genes in absence of Notch signaling but, upon activation of the Notch pathway, it associates with the cleaved intracellular domain of Notch receptor (known as NICD or Notch-ICD; Fig. 1). The RBPJ/NICD-containing coactivator complex displaces the RBPJ-associated repressor complex and mediates the transcriptional activation of Notch target genes (Fig. 1).[4] The signal is terminated by proteasomal degradation of the NICD that occurs via post-translational modifications (PTMs) of the NICD itself[5,6] and, as consequence, the RBPJ-associated corepressor complex is reassembled at enhancer sites and repression of Notch target genes is reestablished (Fig. 1).
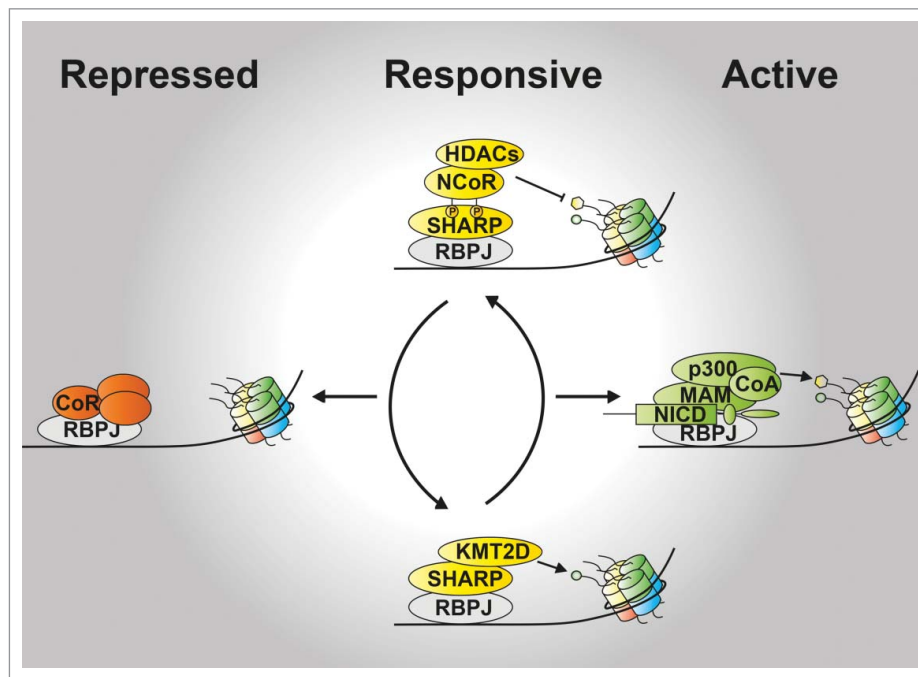
RBPJ consists of three main domains: the N-terminal domain (NTD) and the C-terminal domain (CTD) are similar to the Rel domain of Rel TFs; these domains, are separated by a β-trefoil domain (BTD) which is the characteristic of RBPJ and absent in Rel TFs.[7,8] While the NTD and BTD are both required for DNA binding, only the BTD interacts with the RAM domain of NICD. In addition, the CTD of RBPJ binds to the ankyrin repeats of NICD as well as to the

Figure 1. Model for the regulation of Notch target genes. Activation of the Notch pathway via binding of ligands to Notch receptor, leads to the release of the NICD which interacts with the transcription factor RBPJ. The RBPJ/NICD complex activates gene expression via recruiting MAM, the histone acetyltransferase (HAT) p300 and additional coactivators (CoA; active state, right). Upon termination of the signal, SHARP is recruited to RBPJ-bound enhancer sites where it keeps genes in a responsive state (responsive state, middle) via interaction with KMT2D or with phospho-NCoR. Furthermore, repression of Notch target genes can be enforced via interaction of RBPJ with corepressors (CoR; repressed state, left).

coactivator Mastermind (MAM) the last of which, also interacts with the NTD of RBPJ.[7-10]

The DNA-binding affinity of RBPJ is surprisingly low, indicating that there is a rapid exchange between ON and OFF-rates of RBPJ-containing complexes. Therefore, the equilibrium between RBPJ-repressor and RBPJ/NICD-activator complexes is decisive to determine the transcriptional output. There is no biophysical evidence that binding of either the corepressor SHARP (SMRT- and HDAC-associated repressor protein) or NICD to RBPJ influences its DNA-binding affinity. It remains to be elucidated how the versatile DNA binding of RBPJ is regulated, possibly the underlying mechanism includes also the action of cofactors able to read the chromatin signature. A second scenario envisages that RBPJ cooperates with other DNA-binding proteins, which as consequence reduce its diffusion rate and prolong its chromatin association. RBPJ can also compete with other TFs for binding to the same enhancer site. One good example of this mechanism is the tumor suppressor and TF Ikaros. Given that Ikaros expression strongly increases during lymphopoiesis, Ikaros abundance leads to displacement of RBPJ.[11,12] A third scenario is given by

the observation that RBPJ/NICD-coactivator complexes dimerize at enhancer sites characterized by RBPJ-binding motifs which are oriented head-to-head.[13-15] The dimerization thus stabilizes the binding of each RBPJ monomer to its cognate motif on the DNA.

RBPJ either interacts with the SHARP-containing corepressor complex or the NICD-coactivator complex. The NICD-coactivator complex displaces the RBPJ-associated SHARP corepressor complex recruiting additional coactivators, among them MAM and the histone acetyltransferase (HAT) p300 play a key role for Notch target gene activation.[16] Several groups including our own have characterized the components of the RBPJ corepressor and coactivator complexes in recent years. Among the identified interactors, SHARP represents the key component of the corepressor complex in higher eukaryotes. SHARP directly interacts with RBPJ[17] and, via its highly conserved C-terminal SPOC domain (Spen paralog and ortholog C-terminal domain), it recruits the additional corepressors CtIP/CtBP[18] and ETO[19] as well as the NCoR/SMRT (nuclear receptor corepressor/silencing mediator for retinoid or thyroid-
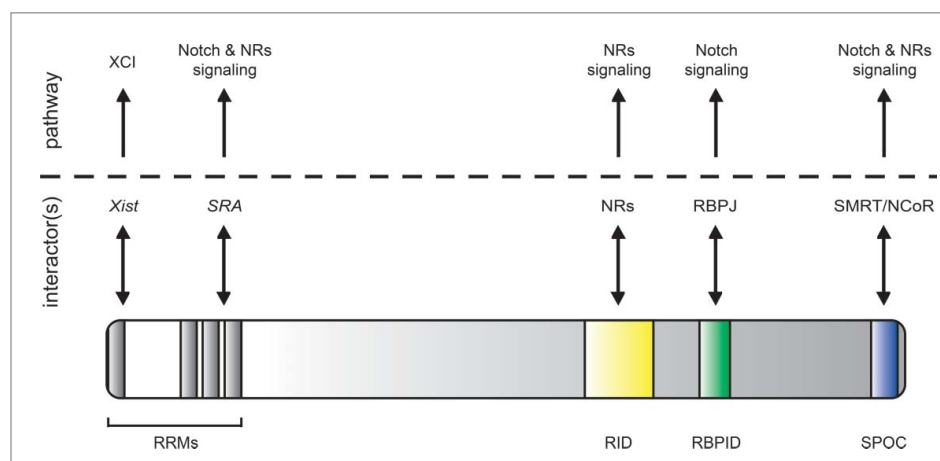
Figure 2. Schematic representation of SHARP and its functional network. SHARP is characterized by RNA recognition motifs (RRMs), the nuclear receptor interaction domain (RID) the RBPJ interaction domain (RBPID) and the SPOC domain. The RRMs of SHARP interact with the long non-coding RNAs *Xist* and *SRA* (steroid receptor coactivator). *Xist* links SHARP to X chromosome inactivation (XCI), whereas SRA involves SHARP in the regulation of the nuclear receptors (NRs)- and Notch signaling. The RID involves SHARP in NRs signaling, whereas the RBPID links SHARP to Notch signaling. The SPOC domain interacts with SMRT/NCoR linking SHARP to both NRs- and Notch-signaling. Additionally, the SPOC domain can interact with KMT2D, CtIP/CtBP and ETO (not shown).

hormone receptors) complex[20,21] which link RBPJ to histone deacetylases (HDACs) (Fig. 2). Recently, the role of SHARP in regulating RBPJ-dependent transcription has been better defined and surprisingly, more than a simple corepressor, SHARP represents a hub for signaling events that direct gene expression both in a positive and negative fashion.[22] Focusing on the SPOC domain of SHARP, we have defined its interactome by mass-spectrometry (defined as SPOCome). Surprisingly, we identified not only the NCoR complex but also the KMT2D/UTX-containing coactivator complex (hereafter referred to as KMT2D complex).[22] KMT2D is known to methylate lysine 4 of histone 3 (H3K4me1, me2 and me3), which is associated with active chromatin.[23] On the other hand, SHARP is a strong transcriptional repressor[17] and an RBPJ-SPOC fusion protein acts as a super-repressor of Notch target genes both *in vitro* and *in vivo*.[22] Thus, identifying the activating KMT2D complex within the SPOCome was unexpected. Importantly, NCoR and KMT2D compete for the binding to SHARP and the phosphorylation-state of NCoR plays a key role in regulating this competition. NCoR is phosphorylated on two highly conserved C-terminal serine residues located within a highly conserved LSDSD motif at its very C-terminus.[24] Phospho-NCoR outcompetes KMT2D for binding to SPOC[22] explaining how dynamic complex composition is achieved. Thus, a phospho-dependent mechanism regulates the occupancy of opposing

chromatin regulators (Fig. 1). These data suggest SHARP as a hub where signaling events converge and become integrated to determine time- and cell-specific transcriptional outputs. It remains to be established which kinase and phosphatase dynamically regulate the NCoR phosphorylation. So far, casein kinase-2β (CK2β) has been described to phosphorylate one serine residue within the LSDSD motif of NCoR[25] and we observed that casein kinase inhibition positively influences the expression of Notch target genes.[22] Since phosphorylation of both serine residues is required for tight binding of phospho-NCoR to SPOC, the identification of additional kinase(s) and unknown phosphatases represents an exciting challenge.

The KMT2D/NCoR competition has a significant impact on the chromatin environment at the enhancers of Notch target genes. While the NCoR complex contains HDAC activities that remove acetylation from the histone tails [for example acetylation of lysine 27 of the histone 3 (H3K27ac)] having a negative impact on transcription, the KMT2D complex, promoting H3K4 methylation states, has a positive function in transcription (Fig. 1). The bivalent function of the RBPJ/SHARP complex gives a significant contribution in defining the dynamics of the transcriptional RBPJ-dependent switch at Notch target genes. While the RBPJ/SHARP complex keeps Notch target genes responsive via KMT2D-dependent histone methylation, activation of Notch signaling leads

to NICD-dependent displacement of SHARP with concomitant recruitment of MAM and, consequently, of the HAT p300[16,26] leading to full gene activation via histone acetylation (Fig. 1). Proteasomal-dependent NICD degradation terminates the signal allowing subsequently the recruitment of SHARP that drives histone deacetylation via the HDAC-containing phospho-NCoR complex (Fig. 1). Subsequently, two different scenarios can arise: Notch target genes can be maintained in a responsive state via SHARP-dependent recruitment of the KMT2D complex which function is to maintain H3K4 methylation; a recruitment which is dependent on the phospho-status of NCoR and eventually on PTMs of KMT2D (Fig. 1). Alternatively, responsiveness may be reduced via recruitment of histone demethylases (KDMs) leading to strong repression of Notch target genes. For example, KDM5A, the mammalian homolog of *Drosophila* Lid, is a direct interactor of RBPJ that decreases H3K4me3 at Notch-dependent enhancers.[27] Furthermore, repression of Notch target genes can be enforced by KDM1A, the homolog of *Drosophila* Lsd1, which is a negative regulator of Notch signaling.[28] Finally, additional mechanisms may be involved in modulating distinct Notch-dependent gene expression programs including, for example, Polycomb (PcG) complexes, histone variants and/or DNA methylation. Notably, the histone variant H2A.Z has been proposed to contribute to gene poising and activation but if and how it regulates Notch-dependent transcription has never been investigated.

The NCoR/KMT2D phospho-dependent switch is one of the first examples how changes of environmental signaling are integrated at the chromatin level. So far, mostly phosphorylation of transcription factors like NF-AT or FoxO have been described. Another example of a similar mechanism has been observed for the circadian gene expression program, which is regulated via acetylation of the histone methyltransferase KMT2A.[29] The existence of different intermediate RBPJ-associated complexes, namely the RBPJ/SHARP/KMT2D and RBPJ/SHARP/NCoR, suggests an additional layer of regulation of the Notch response that not only depends uniquely on the proteolytic release of the NICD but also on the specific time window when the release occurs. As a consequence, transcriptional responses may occur faster or slower based on the dynamic availability of RBPJ/SHARP-associated complex(es). Such regulation would be relevant during differentiation when not only gene activation is important but also gene repression as well as the timeframe required for turning ON and OFF a specific gene. Recycling RBPJ/SHARP-associated complexes might be relevant during somitogenesis when cycling expression of Notch target genes regulates the budding off of somites. Similar mechanisms might be used by other signaling pathways involved in somitogenesis like Wnt or FGF signaling pathways.

RBPJ can be seen as the prototype of such a group of TFs that are able to switch their function from repression to activation of transcription. Among those, TCF/LEF (T-cell factor/lymphoid enhancer factor), which is involved in the Wnt signaling pathway, switches from a negative to a positive transcriptional regulator upon interaction with β-catenin. As in the case of RBPJ, also the TCF/LEF switch involves a dynamic exchange of HDAC-containing corepressors with HAT-containing coactivators. Such dynamic exchange may be regulated by PTMs of chromatin modifiers. Differently to RBPJ, the switch of Gli TFs is regulated via proteolytic events. Gli proteins are converted into repressors via proteasome-dependent degradation but, upon activation of the Hedgehog signaling pathway, Gli proteins are stabilized and act as transcriptional activators. The switch of Gli proteins illustrates once more how PTMs, in this case ubiquitination, of transcriptional regulators convert a repressor into an activator. Together, in our view, RBPJ-associated factors actively shape the chromatin landscape at Notch target genes even in the absence of a Notch signal. This process is able to incorporate signaling inputs thereby modifying the outcome of the Notch response. So far we know that at the heart of this mechanism is the phosphorylation of the NCoR-corepressor.

Abbreviations

BTD	β-trefoil domain
CoA	coactivator
CoR	corepressor
CK2β	casein kinase 2β
CSLCBF-1/RBPJSu(H)	CBF-1/RBPJ/Su(H)/lag-1
CTD	C-terminal domain
CtIP	CtBP-interacting protein
CtBP	C-terminal binding protein
ETO	8–21
HAT	histone acetyltransferase

HDACs	histone deacetylases
KDM	lysine demethylase
KMT	lysine (K) methyltransferase
MAM	Mastermind
NCoR/SMRT	nuclear receptor corepressor/silencing mediator for retinoid or thyroid-hormone receptors
NICD	Notch intracellular domain
NR	nuclear receptor
NTD	N-terminal domain
PcG	Polycomb
PTM	post-translational modification
RBPID	RBPJ interaction domain
RBPJ	recombination signal binding protein for immunoglobulin kappa J region
RID	nuclear receptor interaction domain
RRM	RNA recognition motif
SHARP	SMRT- and HDAC-associated repressor protein
SPOC	Spen paralog and ortholog C-terminal domain
SPOCome	SPOC interactome
SRA	steroid receptor coactivator
Su(H)	suppressor of hairless
TCF/LEF	T-cell factor/lymphoid enhancer factor
TF	transcription factor
XCI	X-chromosome inactivation

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