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### Implications of STAT3 and STAT5 signaling on gene regulation and chromatin remodeling in hematopoietic cancer

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1 **Implications of STAT3 and STAT5 signaling on gene regulation and**  
2 **chromatin remodeling in hematopoietic cancer**

3

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34

**35 Abstract**

36 STAT3 and STAT5 proteins are oncogenic downstream mediators of the JAK-STAT  
37 pathway. Deregulated STAT3 and STAT5 signaling promotes cancer cell proliferation  
38 and survival in conjunction with other core cancer pathways. Nuclear phosphorylated  
39 STAT3 and STAT5 regulate cell-type specific transcription profiles via binding to  
40 promoter elements and exert more complex functions involving interaction with  
41 various transcriptional coactivators or corepressors and chromatin remodeling  
42 proteins. The JAK-STAT pathway can rapidly reshape the chromatin landscape upon  
43 cytokine, hormone or growth factor stimulation and unphosphorylated STAT proteins  
44 also appear to be functional with respect to regulating chromatin accessibility.  
45 Notably, cancer genome landscape studies have implicated mutations in various  
46 epigenetic modifiers as well as the JAK-STAT pathway as underlying causes of many  
47 cancers, particularly acute leukemia and lymphomas. However, it is incompletely  
48 understood how mutations within these pathways can interact and synergize to  
49 promote cancer. We summarize the current knowledge of oncogenic STAT3 and  
50 STAT5 functions downstream of cytokine signaling and provide details on  
51 prerequisites for DNA binding and gene transcription. We also discuss key  
52 interactions of STAT3 and STAT5 with chromatin remodeling factors such as DNA  
53 methyltransferases, histone modifiers, cofactors, corepressors, and other  
54 transcription factors.

55

**56 Keywords**

57 Signal transducer and activator of transcription, Janus kinases, epigenetics,  
58 transcriptional activation

59

**60 Conflict of interest**

61 The authors declare no conflict of interest.

62

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## 73 1. Introduction

74 Over the past decade, extensive Next Generation Sequencing (NGS) efforts and  
75 comparative data integration have provided insights into the mutational landscape of  
76 human cancer genome coding exons. These studies defined approximately 140  
77 different cancer driver genes within 12 core cancer pathways that, when mutated,  
78 can promote tumorigenesis<sup>1</sup>. These cancer driver genes regulate three main cellular  
79 processes: cell fate, cell survival, and genome maintenance<sup>1</sup>. Defining these core  
80 cancer pathways and acknowledging their multifaceted and interconnected nature  
81 has helped to stratify the complex genetics of cancer, which has significantly  
82 influenced both the intellectual approach of cancer biologists and the pharmaceutical  
83 development of specific inhibitors. However, recent research focuses not only on  
84 mutations that modify the specific key players of the core cancer pathways but also  
85 on mutations of chromatin regulatory sites within non-coding regions of DNA.  
86 Mutations in these regions very often result in epigenetic changes that influence gene  
87 expression in cancer cells. The interplay between mutations in the core cancer  
88 pathways and changed chromatin composition and its influence on transcription is  
89 considered as one of the most relevant concepts in current basic cancer research.  
90 Many of the genes that define core cancer pathways are directly involved in or  
91 converge in the Janus kinase/signal transducer and activator of transcription (JAK-  
92 STAT) pathway (**Figure 1**). Extensive studies utilizing STAT knock-out mice have  
93 revealed the mechanisms of canonical JAK–STAT signaling, that are influenced by  
94 several regulatory layers<sup>2</sup>. These include cell-type specific expression and cellular  
95 effector abundancies, differential affinity to receptors and their cognate tyrosine  
96 kinases (TKs), activity regulation by different post-translational modifications,  
97 nucleoplasmic shuttling, recycling by phosphatases, and the interactions with  
98 different co-regulators. Functional differences in the STAT proteins might be  
99 attributed to their ability to only recognize regulatory sequences in certain contexts,  
100 such as composite promoter elements or upon specific chromatin configurations. In  
101 fact, evidence is emerging that specific chromatin remodeling is required for STAT  
102 binding to a subset of loci<sup>3</sup>. It is suggested that other oncogenic transcription factors,  
103 such as steroid receptors, which bind to multiple sites nearby genes without having a  
104 transcriptional function, may play a role in the reconstruction of the genome

organization<sup>4</sup>. Similarly, STATs may be directly involved in the regulation of chromatin topology<sup>5</sup>, not only directly by binding to canonical binding sites in the active, tyrosine phosphorylated form, but also through their ability to form oligomers and to exert functions in the cytoplasm and nucleus as unphosphorylated dimers (uSTAT). However, the molecular mechanisms governing transcription factor-mediated structural rearrangements in the genome are still poorly understood.

Recent technological advances in molecular biology, resulting from the rise in 'next generation' techniques, have revealed new aspects of JAK-STAT signaling including recurrent somatic mutations of STATs, a plethora of novel DNA-binding sites, post-translational modifications (PTMs) and protein-protein interactions (PPIs), all of which have significant impact on the chromatin landscape. These findings have led to new mechanistic insights into the molecular processes of tumorigenesis that are not only induced by constitutively active STAT but also regulated by non-canonical STAT functions. STAT3 and STAT5 are of particular interest because they are not only activated by a wide variety of ligands that control proliferation, survival, cell-cell communication, adhesion, and angiogenesis<sup>6</sup>, but their dysregulation also facilitate tumor progression in various human cancers, particularly leukemia and lymphomas<sup>7</sup>,<sup>8</sup> (**Table 1**). STAT5 refers to two highly related genes, STAT5A and STAT5B, which are both found on human chromosome 17<sup>9</sup>. The STAT3 gene lies adjacent to the STAT5 locus and generates two isoforms STAT3 $\alpha$  and STAT3 $\beta$  by alternative splicing. Although we acknowledge the different biochemical and biological properties of the STAT3 and STAT5 isoforms<sup>2, 10</sup>, this is not in the focus of the following review. In most cells, STAT3 and STAT5 are activated by different mechanisms and bind to distinct loci to regulate specific target gene expression<sup>11, 12</sup>. However, STAT3 and STAT5 proteins can also bind to the same regulatory oncogenic loci resulting in compensatory or antagonistic signaling<sup>13, 14</sup>. Functional redundancy is particularly evident in definitive erythropoiesis where STAT3 compensates for a loss of STAT5<sup>14</sup>. Furthermore, competitive binding of STAT3 and STAT5 is best exemplified by the regulation of BCL6 expression, as discussed further below<sup>13</sup>. Additionally, STAT3 and STAT5 were both shown to contain gain-of-function mutations in hotspot residues in their SH2 domain or their extreme C-terminus<sup>15</sup>. As such, they were defined as driver genes predominantly in peripheral T-cell leukemia/lymphoma (PTCL) or T-cell prolymphocytic leukemia/lymphoma (T-PLL), rare but aggressive

138 forms of T-cell neoplasia. Interestingly, STAT3 and STAT5 mutations in  
139 hematopoietic cancers exist in a mutually-exclusive manner and often co-occur with  
140 mutations in DNA modifying enzymes such as DNA methyltransferase 1/3A  
141 (DNMT1/3A), Ten-eleven translocation methylcytosine dioxygenase 1/2 (TET1/2) or  
142 Isocitrate dehydrogenase 2 (IDH2), and corepressor molecules with histone  
143 deacetylase (HDAC) activity such as BCL6 Corepressor (BCoR)/Nuclear receptor co-  
144 repressor 1/2 (NCoR1/2)<sup>16</sup>.

145 Given that chromatin remodeling and the JAK-STAT pathway are both core cancer  
146 pathways (**Figure 1**) and are often co-mutated in various human cancers, it is of  
147 great interest to understand how these signaling nodes are interconnected. In the  
148 following, we will review different functions of STAT3 and STAT5 in controlling gene  
149 regulation and genome integrity during health or disease, specifically in the context of  
150 known protein-protein interaction partners, particularly those involved in chromatin  
151 remodeling.

152

## 153 **2. Cell-type specific STAT5 target gene regulation**

154 STAT proteins act as transcriptional activators upon phosphorylation of a conserved  
155 tyrosine residue at the C-terminus followed by translocation into the nucleus, where  
156 they bind to DNA and activate target gene transcription<sup>2</sup>. STAT binding sites are  
157 usually found in enhancer and promoter regions as well as first introns of target  
158 genes and characterized by clusters of conserved motifs with an interferon gamma  
159 activated site (GAS)-like core sequence (TTCT/CNA/GGAA). The murine or human  
160 genomes comprise ~1 million GAS-like sequences, where ~10% are indeed bound  
161 by STAT molecules. Close proximity of multiple binding sites leads to binding of  
162 additional STAT molecules resulting in increased transcriptional activity<sup>17</sup>. STAT5  
163 expression is often upregulated in cancer, and this increased activity can promote  
164 additional STAT binding to less conserved GAS consensus elements. For example,  
165 growth hormone (GH)-induced STAT5 DNA binding was observed at 13,278 sites  
166 containing GAS motifs within the genome of mouse embryonic fibroblasts (MEFs),  
167 but enhanced STAT5 expression lead to a significant increase in genome binding  
168 sites, where up to 72,000 sites were mapped upon 20-fold overexpression of  
169 STAT5A<sup>18</sup>. Of the STAT5 binding peaks, 50% coincided with GAS motifs, confirming

170 that STAT5 binds to these specific sequence motifs. However, the nature of STAT5  
171 binding to sequences without a bona fide GAS motif is not clear.  
172 Overall, STAT5A dimers do not bind as efficiently to DNA as STAT5B dimers, which  
173 can also recognize 4 bp spaced motifs of TTCT/CN2A/GGAA. STAT5A preferentially  
174 forms tetramers even when two weak STAT5 affinity sites are in close proximity.  
175 Tetramerization has not been prominently reported for STAT5B, however upon  
176 heterodimerization with STAT5A, STAT5B can efficiently take part in the formation of  
177 DNA oligomers that are bound at enhancer or promoter regions. There are several  
178 amino acid differences in both the oligomerization and DNA binding domains of  
179 STAT5A/B and these could impact DNA binding efficiency as dimers or oligomers.  
180 However, to date there are no crystal structure analyses to provide a deeper  
181 understanding of STAT5A or STAT5B oligomer configuration.  
182 The occupation of STAT binding sites is cell-type specific. In fact, STAT binding is  
183 generally enriched in genes that are particularly important for the respective cell type.  
184 Bioinformatics analysis of murine and human ChIP-seq data estimated up to  
185 ~100,000 sequences occupied by STATs in cells that display high STAT activity (e.g.  
186 T-cells, macrophages, and hepatocytes), but binding sites were up to 20-fold lower in  
187 cell lines with less STAT abundance (e.g. MEFs and B-cells), where ~94% of such  
188 sites contained a GAS-like core sequence<sup>19</sup>. Interestingly, bioinformatics studies  
189 have also revealed that different transcription factors can bind to the same cis-  
190 regulatory elements as STATs<sup>20</sup>. Regulation of specific gene loci via association of  
191 STATs with tissue specific coactivators or corepressors comprises a mechanism by  
192 which activation of distinct STAT family members by different cytokines uniquely  
193 changes transcription (**Figure 2**). Moreover, the ability of STATs to access specific  
194 GAS sites could be pre-determined by the cell context via the chromatin status<sup>21</sup>.  
195 However, the overall mechanism, its association with PTMs and PPIs,  
196 interconnection with other core cancer pathways or connection to metabolism is  
197 poorly understood.  
198 The use of various transgenic mice and cell types for extensive analysis of genomic  
199 STAT5 binding patterns led to the identification of binding motifs for different  
200 transcription factors, which are enriched around the center of STAT5 binding sites  
201 (**Figure 2**). These include the C/EBP (CCAAT/enhancer-binding protein) family  
202 members, which were described to interact cooperatively with STAT5 in adipocytes<sup>22</sup>.

203 In particular, C/EBP $\alpha/\beta/\delta$  were found to highly occupy STAT5 binding sites in  
204 mesenchymal or epithelial cells<sup>19</sup>. In hepatocytes, binding motifs of C/EBP $\alpha$  and  
205 hepatocyte nuclear factor (HNF) family members, including HNF4/6, significantly  
206 coincide with STAT5 binding sites near liver sex-specific genes such as the major  
207 urinary protein downstream of GH signaling<sup>19</sup>. Also, binding sites of Forkhead box  
208 proteins FOXA1 and FOXA2, which are key regulators in initiating liver  
209 specification<sup>23</sup>, coincide with C/EBP $\alpha$  and STAT5 binding. FOXA proteins are  
210 particularly interesting since they have been described as ‘pioneer factors’ for cell-  
211 type specific transcriptional regulation. As such, they are involved in actively opening  
212 the local chromatin to allow other transcription factors to bind. Besides other factors  
213 like PBX-1, GREB1 or AP2- $\delta$ , FOXA1 has been linked to the modulation of nuclear  
214 hormone receptor signaling<sup>24</sup>. Interestingly, STAT5 is also able to interact with the  
215 glucocorticoid receptor via its N-terminal oligomerization domain, as well as other  
216 nuclear hormone receptors such as the estrogen, progesterone or androgen  
217 receptors. Thus, STAT5 might also act as a pioneer factor similarly to FOXA1, to  
218 enhance the binding of key regulators to chromatin in different cell types.  
219 Further interactions of STAT5 with chromatin binding proteins or other transcription  
220 factors such as nuclear factor kappa B (NF $\kappa$ B), the ubiquitously expressed octamer  
221 binding factor 1 (OCT1) and the more B-cell restricted OCT2 transcription factors  
222 were shown. Furthermore, centrosomal P4.1-associated protein (CPAP) was  
223 reported to act as a STAT5 coactivator to enhance transcription<sup>25</sup>. These protein-  
224 protein interactions antagonize chronic inflammation<sup>26</sup> in intestinal epithelial, are  
225 required for cell cycle progression<sup>27</sup>, and augment STAT-mediated transcriptional  
226 activity<sup>25</sup>. Overall, the cooperative activity of STATs with associated transcription  
227 factors appears to control cell type specific genes, while the accessibility of their  
228 target GAS sites seems to be pre-determined by chromatin configurations. Future  
229 studies will be required to elucidate which transcription factors act as pioneer factors  
230 that recruit co-transcription factors and/or influence chromatin modifications.

231

232 **3. Antagonistic regulation of STAT5 and BCL6 with consequences**  
233 **for target gene and STAT3/5 locus control**

234 B-cell lymphoma protein 6 (BCL6) is an evolutionarily conserved zinc finger  
235 transcription factor, which functions as a transcriptional repressor and has essential  
236 roles in germinal center B-cell differentiation, self-renewal of memory B-cells, as well  
237 as in the development of follicular helper T ( $T_{FH}$ ) cells<sup>28</sup>. BCL6 is found to be highly  
238 expressed in follicular lymphoma and Burkitt's lymphoma, and its locus is frequently  
239 translocated and hypermutated in diffuse large B-cell lymphoma (DLBCL) and  
240 nodular lymphocyte predominant Hodgkin lymphoma<sup>29</sup>. Intriguingly, STAT5B is also  
241 overexpressed in DLBCL<sup>30</sup>, and STAT5 is constitutively active in some DLBCL cell  
242 lines<sup>31</sup>, suggesting that both STAT5 and BCL6 may be important determinants in  
243 DLBCL pathogenesis.

244 The roles of STAT5A and STAT5B in the regulation of BCL6 expression are quite  
245 controversial. It has been demonstrated that STAT5B upregulates BCL6 in a subset  
246 of germinal center cells<sup>32</sup>. On the contrary, STAT5B represses BCL6 in liver epithelial  
247 cell lines by interaction with both p300 and HDAC3<sup>33</sup>. Similarly, STAT5A represses  
248 BCL6 expression at the transcriptional level in breast cancer cell lines<sup>34</sup>. STAT3 was  
249 found to increase the expression of BCL6 in breast cancer cells; however, the  
250 STAT5-mediated repression of BCL6 in these cells was dominant, because STAT5  
251 displaced STAT3 from the shared DNA binding site<sup>13</sup> (**Figure 3**). Repression of BCL6  
252 by increased binding of STAT5 to the BCL6 promoter has also been demonstrated in  
253 TH1 cells and natural killer (NK) cells after interleukin-2 (IL-2) stimulation<sup>35</sup>. Again,  
254 STAT3 binding was found to be reduced, suggesting competition between STAT5  
255 and STAT3<sup>35</sup>. Furthermore, it has been reported that STAT5 negatively regulates  $T_{FH}$   
256 cell generation due to upregulation of BLIMP-1 which results in repression of BCL6  
257 expression<sup>36</sup>. Since BCL6 and BLIMP-1 reciprocally repress each other, it is possible  
258 that STAT5 represses BCL6 expression, which allows BLIMP-1 to be upregulated,  
259 thereby preventing  $T_{FH}$  generation. A recent analysis of BCL6 binding sites in  $T_{FH}$   
260 cells revealed shared BCL6 and STAT5 binding sequences, as well as reduced IL-7  
261 receptor/STAT5 signaling by BCL6<sup>37</sup>. These reports suggest that STAT5 and STAT3  
262 modulation of BCL6 expression may affect helper T-cell lineages based upon BCL6  
263 regulation, and further support a role for STAT5 and STAT3 competing to regulate  
264 BCL6 expression.

265 Recently, it was shown that BCL6 serves as a male-specific transcription factor  
266 mediating GH-regulated sexual dimorphism of gene expression in the liver<sup>33</sup>.

267 Specifically, BCL6 binding was preferentially associated with repression of female-  
268 biased STAT5 targets in male liver. This suggests that STAT5 and BCL6 have  
269 opposing roles in the liver via reciprocal occupancy of the same DNA regulatory  
270 sequences<sup>38</sup>. This relationship is most likely a result of GH-activated STAT5B  
271 displacing BCL6 on a shared BCL6/STAT5 motif resulting in the expression of sex-  
272 specific genes. However, the mechanism of repression or activation by STAT5 is  
273 currently unknown. Most likely, the negative regulation by BCL6 and positive  
274 regulation by STAT5B involves interactions with epigenetic modifiers such as  
275 NCoR1/2, BCoR, p300/CBP, or NCoA-1<sup>39</sup> as described below.

276

#### 277 **4. Persistent STAT5 activation downstream of oncogenic proteins**

278 Cytokine-dependent STAT3 and STAT5 activation and effects on chromatin are  
279 physiologically transient. In contrast, cells that harbor STAT5 activating mutated or  
280 modified oncoproteins, like BCR-ABL, JAK2 V617F, mutant MPL (thrombopoietin  
281 receptor) and mutant calreticulin in myeloproliferative neoplasms will exhibit  
282 persistent activation of STAT5, with high constant levels in the nucleus<sup>40</sup>. Both BCR-  
283 ABL positive chronic myeloid leukemia and BCR-ABL negative MPNs are dependent  
284 on STAT5 signaling<sup>41</sup>. Chromatin immunoprecipitation (ChIP) and sequencing or  
285 ChIP on chip have shown that in cells with persistently activated STAT5 target genes  
286 are not identical to those in cytokine-activated cells, with a greater number of low  
287 affinity GAS sites occupied by persistently activated STAT5<sup>42</sup>. An interesting situation  
288 is represented by a group of genes pathologically overexpressed in MPN cells which  
289 are co-regulated by persistently activated STAT5 in JAK2 V617F cells together with  
290 p53 or mutated p53<sup>43</sup>. Among such targets is LPP (Lipoma-Preferred Partner) that  
291 hosts in an intron microRNA-28, which targets the MPL mRNA and inhibits pro-  
292 platelet formation<sup>44</sup>. In human erythroleukemia cells, which are homozygous for JAK2  
293 V617F and harbor p53 M133K mutation, down-modulation of p53 or inhibition of  
294 STAT5 activation prevents induction of LPP and similarly regulated genes;  
295 recruitment of p53 M133K is dependent on STAT5 chromatin binding, while down-  
296 modulation of p53 still allows STAT5 chromatin binding. This example illustrates a  
297 potential mechanism whereby presence of activated STAT5 on chromatin possibly at

298 low affinity sites might open up the chromatin for recruitment of different transcription  
299 factors or epigenetic regulators.  
300

301 **5. Gene transcription and chromatin remodeling by STAT3/5**

302 Since the STATs were discovered it has become increasingly evident that, in addition  
303 to their binding to GAS elements, epigenetic regulation is a crucial and dynamic part  
304 of their gene regulation activity. In response to DNA element binding of transcriptional  
305 activators or repressors, the modification of histones by methylation, acetylation, and  
306 phosphorylation results in important changes to chromatin structure regulating gene  
307 transcription<sup>45</sup>. Histone modifications such as histone H3 lysine 4 acetylation  
308 (H3K4ac) and histone H3 lysine 27 acetylation (H3K27ac) favor transcription factor  
309 binding and the formation of initiation and elongation complexes. Histone H3 lysine 4  
310 trimethylation (H3K4me3) and H3 lysine 27 trimethylation (H3K27me3) at gene  
311 promoters are associated with gene activation and repression, respectively.  
312 Moreover, dimethylated H3K4 as well as di- and trimethylated H3K36 have been  
313 detected at sites of transcriptionally active chromatin. Many key developmental genes  
314 have bivalent modifications where large domains of repressive H3K27me3 marks  
315 coexist with small domains of activating H3K4me3 modifications. Importantly, it is  
316 now evident that promoter bound transcription factors such as the STATs are also  
317 modified by histone modifying enzymes which has important consequences for target  
318 gene transcription (**Figure 4**).

319 STAT3 acetylation on Lys685 within the SH2 domain by the histone acetyltransferase  
320 (HAT) p300/CBP promotes STAT3 dimerization, DNA binding and transcriptional  
321 activation in human liver and prostate cancer cell lines<sup>46</sup> (**Figure 5a**). However, more  
322 recent data has suggested that this modification may play a more important role in  
323 unphosphorylated STAT3 (uSTAT3) regulated gene expression<sup>47</sup>. Additional  
324 p300/CBP-mediated acetylation sites have also been reported on STAT3 in  
325 hepatocytes, where again these modifications seem to promote STAT3 signaling and  
326 target gene expression<sup>48</sup>. Deacetylation by HDAC3, and to a lesser extent, HDAC1  
327 and HDAC2, inhibits transcription of STAT3 target genes<sup>49</sup>. Promoter-bound STAT3  
328 can also be methylated at Lys140 in an IL-6-dependent manner by the histone  
329 methylase SET9 in human colon cancer cells, and this modification appears to

reduce STAT3 binding to DNA, consequently reducing target gene transcription<sup>50</sup> (**Figure 5b**). This modification is removed by recruitment of the histone demethylase LSD1<sup>50</sup>. In regulatory T (T<sub>reg</sub>) cells, FOXP3 acts as a co-transcription factor that facilitates STAT3-mediated IL-10 expression by recruiting HAT1 to the *IL-10* locus<sup>51</sup> (**Figure 5c**). Recruitment of HAT1 results in epigenetic modifications to the *IL-10* promoter, creating space for subsequent docking of STAT3-FOXP3 complexes. Notably, STAT3 can also be methylated on Lys49 and Lys180 by EZH2, the lysine methyltransferase subunit of the Polycomb Repressive Complex 2 (PRC2) in glioblastoma, colon cancer, and breast cancer cell lines<sup>52, 53</sup> (**Figure 5d**). Methylation of STAT3 by EZH2 appears to lie downstream of AKT signaling and was shown to increase STAT3 transcriptional activity<sup>52, 53</sup>. Given that STAT3 is an important oncogenic transcription factor, and EZH2 is found to be mutated in many cancers, the interplay between these two proteins raises the possibility that targeting the STAT3-EZH2 axis might be beneficial to arrest or kill cancer cells. STAT3 has also been implicated in regulating epigenetic modifications and chromatin accessibility, adding another level of complexity to its role in transcriptional regulation. STAT3 was found to regulate H3K4 trimethylation, a mark of active transcription, at target gene loci in T-cells undergoing Th17 cell differentiation<sup>54</sup>. Furthermore, STAT3 can recruit the histone methyltransferase G9a to form a repressor complex that facilitates H3K9 dimethylation gene silencing marks at the promoter of *miR-200c*, in leptin-treated breast cancer cells<sup>55</sup> (**Figure 5e**). Interestingly, it was recently reported that STAT3 can bind to the promoter region of the *EZH2* gene in gastric cancer cells, implicating STAT3 as direct regulator of EZH2<sup>56</sup>. However, it remains to be determined whether this is a general or cell-type specific phenomenon, and further work will be required to examine potential STAT3-dependent global changes to histone methylation via regulation of EZH2.

Transcriptional repression is mediated in part by non-DNA binding corepressors. The corepressors NCoR and silencing mediator for retinoid and thyroid receptors (SMRT) complex were originally identified to associate with nuclear hormone receptors thereby conferring transcriptional repression and subsequently, has been shown to be recruited to many classes of transcription factors and is also a component of multiple protein complexes containing HDAC proteins<sup>57</sup>. This association with HDAC activity provides an important mechanism that allows DNA-binding proteins for

363 interaction with NCoR/SMRT to repress transcription of specific target genes.  
364 Recruitment of SMRT associated with HDAC by STAT3 leads to the transcriptional  
365 inactivation of STAT3 and consequent down-regulation of IL-6-mediated multiple  
366 myeloma cell growth and gene expression<sup>58</sup>.  
367 STAT3 also mediates oncogenesis by recruiting DNA methyltransferase 1 (DNMT1)  
368 to gene promoters to silence tumor suppressor genes, such as *PTPN6*, *IL-2Rγ*,  
369 *CDKN2A*, *DLEC1* and *STAT1* by CpG methylation in malignant T lymphocytes and  
370 breast cancer cells<sup>59, 60</sup> (**Figure 5f**). Notably, acetylation of Lys685 on STAT3 has  
371 been shown to mediate this interaction with DNMT1<sup>60</sup>. STAT3 was also shown to  
372 occupy the *DNMT1* gene promoter in malignant T-cells inducing its expression<sup>61</sup>.  
373 This suggests a positive feedback mechanism, as inhibition of DNMT1 resulted in a  
374 loss of STAT3 activity, and therefore STAT3 may preserve its persistent activation by  
375 inducing DNMT1 which in turn acts to silence negative regulators of STAT3, such as  
376 the *PTPN6* gene product SHP-1<sup>61</sup>.  
377 Like STAT3, STAT5 also regulates the epigenetic landscape in a versatile manner,  
378 depending on its interaction partners and transcriptional targets. STAT5-dependent  
379 gene activation has been correlated with recruitment of HATs such as NCoA/SRC, or  
380 the TUDOR domain coactivators p100 and CBP/p300 to initiate prolactin (PRL)-  
381 dependent transcription<sup>62</sup> (**Figure 6a**). The PRL-activated receptor signals to  
382 STAT5B in mammary epithelial cells, which becomes acetylated at the lysine residue  
383 K694 by CBP/p300 and undergoes enhanced dimerization<sup>63</sup>. Furthermore, IL-7  
384 signaling leads to STAT5A acetylation at lysine K696, indicating that acetylation-  
385 dependent STAT5 dimerization is also observed in other cytokine signaling  
386 pathways<sup>64</sup>. Additionally, STAT5 binding and gene transcription is linked to  
387 acetylation of histones H3 and H4<sup>62, 65</sup>. It was also shown that STAT5 can recruit the  
388 DNA demethylases TET1/2 to the *FOXP3* promoter in T<sub>reg</sub> cells<sup>66</sup> (**Figure 6b**).  
389 Whether this is a more general epigenetic regulatory mechanism, especially in  
390 association with cancer-related constitutive JAK-STAT activity, remains to be shown.  
391 Multiple studies have shown that STAT5 can also function as a transcriptional  
392 repressor by recruiting demethylating or deacetylating epigenetic modifiers to specific  
393 gene loci<sup>67, 68</sup>. However, this negative transcriptional regulation by STAT5 is thought  
394 to be rare. The NCoR1/2 HDAC corepressor complex is probably the most important  
395 negative regulator directly interacting with STAT5<sup>69</sup>. NCoR associates with SHD1,

which in turn interacts with STAT5 and represses STAT5-mediated transcription in a cytokine-dependent manner in melanoma<sup>70</sup>. Interestingly, two hotspot mutations in the coiled-coil domain of STAT5A or STAT5B were found in human gastrointestinal cancers<sup>71</sup>, suggesting a perturbation of corepressor complex binding to STAT5<sup>69</sup>. Furthermore, it was reported that recruitment of the corepressor SMRT to STAT5 promoter regions can repress gene expression in response to IL-3 in murine 32D cells<sup>69</sup>. SMRT was found to interact with both STAT5A and STAT5B. Furthermore, due to the fact that the HDAC inhibitor TSA could re-activate target gene transcription, it was concluded that HDACs mediate this process of inhibiting STAT5-dependent transcription<sup>69</sup>.

Additionally, both lysine-specific histone demethylase 1A (LSD1) and HDAC3 exert transcriptional regulation of STAT5A targets and facilitate target gene repression by either deacetylation or histone demethylation, respectively (**Figure 6c**). Like STAT3, STAT5 is known to interact with SMRT in murine 32D cells<sup>69</sup>. LSD1 forms a complex with corepressor REST (CoREST)<sup>72</sup> and is reported to interact with HDAC3<sup>73</sup> or NCoR<sup>74</sup>. Considering these various reported PPIs, it is possible that STAT5 exists in complexes containing SMRT/NCoR-HDAC3 and/or LSD1. Furthermore, it was shown that STAT5 is able to recruit EZH2 via direct N-terminal binding in pre-B cells, thereby initiating the formation of H3K27me3 repressive chromatin<sup>68</sup> (**Figure 6d**). Although EZH2 was shown to directly methylate STAT3, it has not been found to methylate STAT5<sup>52</sup>.

Intestinal STAT5 has an important function in maintaining genome integrity, which was shown for gamma irradiation-mediated intestinal crypt damage. This damage was more severe upon complete STAT5 loss, and could be antagonized by inducible STAT5 expression<sup>75</sup>. pYSTAT5 could repress BMI1, an essential transcription factor within the polycomb repressor complex 1 (PRC1) that is involved in transcriptional regulation of intestinal epithelial stem and progenitor cells<sup>75</sup>. Notably, STAT5 is also able to directly regulate the expression of epigenetic regulators. It was shown that STAT5 directly binds to the promoter region of the DNA methyltransferase *DNMT3A* in human CD34<sup>+</sup> AML cells and thereby increases its transcriptional activity, which leads to methylation and thus transcriptional silencing of the tumor suppressor *PTEN*<sup>76</sup>. On the contrary, an association between STAT5 and DNMT3A in T<sub>reg</sub> cells could not be shown<sup>77</sup>.

429 These results suggest a mechanism that governs the switch between recruitment of  
430 coactivators versus recruitment of corepressors by STAT5. Attractive alternatives are  
431 either dimers versus tetramers or differential PTMs of STAT5 discriminating between  
432 coactivator or corepressor recruitment. Graded pYSTAT5 levels are likely key  
433 determinants in opening or closing gene loci. Upon cytokine-dependent activation,  
434 STAT5 not only binds to canonical DNA-binding sites as a dimer, but is also able to  
435 increase its transcriptional repertoire through binding to tandem repeats of such  
436 binding sites as a homo- or heterooligomer with STAT1/3/4<sup>17</sup>. Oligomer formation  
437 stabilizes the binding of STAT dimers to tandem low-affinity sites by decreasing the  
438 off-rate of the complex<sup>78</sup>. This can cause an amplification of ongoing transcription<sup>79</sup>.  
439 Additionally, tetramers offer a different protein surface accessibility, which allows  
440 selective recruitment of other transcription factors or coactivators<sup>80</sup>. It was  
441 demonstrated that the interaction of STAT5 with EZH2 is dependent on tetramer  
442 formation, while STAT5 dimers lack this repressive function<sup>68</sup>. It can be envisaged  
443 that the oligomer composition determines STAT-cofactor interactions at distinct target  
444 genes. Detailed mapping analyses of STAT5 coactivators or corepressors, as well as  
445 interaction studies in specific cancer types and their consequences for STAT5-  
446 regulated biology, remain enigmatic. Epigenetic modifiers such as HATs, EZH2,  
447 SMRT and TET1/2 are frequently mutated in human cancer, specifically in  
448 leukemia/lymphoma, carcinomas and sarcomas, all of which are associated with  
449 severely altered transcription. It will be essential to determine how these mutations  
450 influence their interaction with STAT dimers or oligomers to shape chromatin and the  
451 cancer genome.

452

453 **6. Impact of non-tyrosine phosphorylated STATs on chromatin  
454 formation**

455 Prior to activation, uSTAT is maintained as a pre-formed anti-parallel homo- or  
456 heterodimer in the cytoplasm, via interaction between the coiled-coil domains<sup>81</sup>.  
457 Notably, phosphorylated DNA-bound dimers and uSTAT dimers were shown to be  
458 quite distinctive in their structure, indicating different functionalities. Anti-parallel  
459 STAT dimers are bound to the cytoplasmic tails of cytokine receptors<sup>82</sup> via the N-  
460 terminal domain. STAT5A docking as anti-parallel dimers prevents its autoactivation,

461 whereas deletion of the N-terminal domain renders it persistently active<sup>83</sup>, which is  
462 not the case for the STAT5B N-domain upon deletion<sup>84</sup>.  
463 STAT proteins have three different functions in the non-tyrosine phosphorylated  
464 state<sup>85</sup>: (i) as transcription factors, and modifiers of transcription factors in the case of  
465 uSTAT1 and uSTAT3<sup>86</sup>, (ii) as mitochondrial effectors such as in the case of serine  
466 phosphorylated STAT3<sup>87</sup>, and (iii) as effectors of chromatin topology such as in the  
467 case of STAT5A<sup>88</sup>. As reviewed recently, stimulation of STAT1 and STAT3 through  
468 interferons (IFNs) and IL-6, respectively, induces increased expression and activity of  
469 each protein<sup>86</sup>. While the initial cellular response depending on STAT tyrosine  
470 phosphorylation is quite rapidly downregulated through dephosphorylation by SOCS  
471 proteins, increased amounts of uSTAT1/3 persist for several days. Notably, it has  
472 been shown that pYSTAT1/3 and uSTAT1/3 regulate a distinct set of genes. In  
473 breast cancer cells, uSTAT3 regulates genes with well-described roles in cancer,  
474 such as muscle RAS (MRAS) and MET, thus potentially contributing to  
475 oncogenesis<sup>89</sup>. uSTAT1 prolongs the expression of a subset of IFN-induced genes in  
476 combination with uSTAT2 and IRF9<sup>90</sup>. Additional transcription-related functions of  
477 uSTATs include an increased expression of uSTAT3 upon angiotensin II stimulation  
478 that leads to angiotensin II-induced cardiac hypertrophy<sup>91</sup>, and basal regulation of  
479 IFN-activated promoters by constitutive uSTAT2 binding prior to IFN stimulation<sup>92</sup>.  
480 Besides transcriptional regulation, uSTATs might have important roles in cellular  
481 compartments other than the nucleus, including mitochondria, Golgi apparatus and  
482 endoplasmic reticulum (ER). Recent work highlights important roles of serine  
483 phosphorylated STAT3 in the mitochondria, where it supports RAS-dependent  
484 oncogenic transformation in myeloproliferative neoplasms and participates in cellular  
485 respiration<sup>93, 94</sup>. Similarly, uSTAT5 was associated with the Golgi apparatus and the  
486 ER, as knockdown of STAT5 leads to a dramatic destabilization of these organelles.  
487 Additionally, an increasing number of reports demonstrate that T-cells employ  
488 uSTAT5 for diverse functions in the cytoplasm, mitochondria, and nucleus<sup>95-97</sup>.  
489 uSTAT5A was also shown to be involved in heterochromatin compaction through  
490 interaction with heterochromatin protein 1α (HP1α) in human cancer cell lines<sup>98</sup>  
491 (**Figure 7**). The authors proposed that uSTAT5A stabilizes heterochromatin, thereby  
492 suppressing tumor cell growth through epigenetic interaction of STAT5A through a  
493 PVVVI motif with HP1α bound to H3K9me. Interestingly, STAT5A genes from

494 different species contain an HP1-binding motif, PxVxl, in the DNA binding domain  
495 around amino acid position 467-472, which is conserved to *Drosophila* STAT92E<sup>99</sup>.  
496 Although there is evidence for these interactions for uSTAT5A, it is unclear whether  
497 this is true for uSTAT5B.

498 A recent study demonstrated that DNA-associated uSTAT5 and CTCF might  
499 influence each other's transcriptional activity<sup>97</sup> (**Figure 7**). CTCF is an architectural  
500 protein that binds to topologically associated chromatin domains in the vicinity of  
501 insulator sequences, which are hubs for open or closed chromatin structures.  
502 Together with cohesin subunits, CTCF has a diverse impact on gene transcription  
503 mainly through the establishment of long-range chromatin interactions. Interestingly,  
504 uSTAT5 co-localizes with CTCF resulting in repression of transcription. However,  
505 upon STAT5 activation by thrombopoietin, pYSTAT5 relocates its binding in the  
506 genome to STAT consensus sites, which triggers pYSTAT5-driven gene transcription  
507 controlling megakaryocyte proliferation, survival and differentiation<sup>97</sup>. It remains  
508 unclear whether uSTAT5 can also co-localize with cohesin subunits, such as STAG2,  
509 which are frequently mutated in cancer<sup>100</sup>. Indeed, STAT5 might also be involved in  
510 chromatin looping in a similar manner to cohesin, thereby providing mechanical  
511 stabilization of the ring-like structures and facilitating docking of other transcriptional  
512 regulators. Since both cohesin and STAT5 are additionally linked to DNA damage  
513 repair, a closer association of STAT5 with cohesin subunits might be likely. In line  
514 with this, a study examining FLT3-ITD-driven AML described enhanced pYSTAT5  
515 signaling upon haploinsufficient loss of *Smc3*, another subunit of multimeric  
516 cohesin<sup>101</sup>. Here, the authors demonstrated altered chromatin structure and  
517 increased expression of STAT5 target genes upon *Smc3* mutation, with enhanced  
518 STAT5 binding at its response elements due to more relaxed/accessible chromatin.  
519 Enhancer selection by STAT5 is cytokine-dependent, and the stability and function of  
520 STAT5 at enhancers and promoters is well characterized<sup>102</sup>. However, the ways in  
521 which STAT5 can affect surrounding chromatin upon binding to its target sites is less  
522 understood. Overall, STATs, when tyrosine phosphorylated, act as chromatin  
523 modifiers by providing a platform for chromatin remodeling enzymes, whereas  
524 uSTATs are associated with transcriptional repression and prolonged or alternative  
525 transcriptional subsets.

526 **7. Concluding remarks**

527 STAT3/5 proteins are the predominant oncogenic transcription factors of the JAK-  
528 STAT pathway and regulate gene expression in conjunction with other transcriptional  
529 regulators. Therefore, their expression levels and activity are crucial factors, in  
530 addition to their interaction with other transcription factors and various epigenetic  
531 modifiers such as EZH2, TET1/2, DNMT3A, the corepressor and histone deacetylase  
532 NCoR1/2 and the coactivator histone acetyl transferases p300/CBP. How these  
533 interactions are sustained in different cell types and how they change the chromatin  
534 landscape dependent on cytokine stimulation remains to be investigated. However,  
535 the concept of classic cytokine-mediated JAK-STAT signaling will need to be  
536 redefined in the era where we begin to understand chromatin dynamics and gene  
537 regulation.

538 Insights into cancer genome landscapes provide evidence that constitutive activation  
539 of STAT proteins and epigenetic gene reprogramming are important hallmarks of  
540 human cancer initiation, progression, and metastasis. Future studies will be required  
541 to elucidate which transcription factors act as pioneer factors that recruit other  
542 transcription factors or cofactors/corepressors to shape chromatin, and how they  
543 interplay with chromatin regulators. Detailed 3D chromatin architecture and  
544 transcription factor binding analyses combined with chromatin proteomic studies will  
545 increase our understanding of how the same key molecules participate in different  
546 cellular aspects, ranging from physiological processes like survival, differentiation  
547 and senescence, to transformation and cancer progression. Given the clear  
548 importance of the JAK-STAT pathway and their interplay with chromatin remodeling  
549 enzymes in the initiation and progression of cancer, targeting of STAT3 and/or  
550 STAT5 is of high therapeutic relevance. Furthermore, targeting these pathways in  
551 combination with inhibitors against epigenetic modifiers could provide novel treatment  
552 avenues.

553

554

### 555 **Author contribution**

556 BW, HN, and RM collected and analyzed literature information, designed the concept  
557 for the review article and created the figures. All authors further contributed to the  
558 content and editing, and have approved the final version of the manuscript.

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974 **Figure Legends**

975

976 **Figure 1: JAK-STAT signaling is interconnected with core cancer pathways.**

977 Core cancer pathways modulate or converge on the JAK-STAT pathway to control  
978 cell survival, differentiation, proliferation, and metabolism in response to extracellular  
979 stimuli. Arrows indicate either uni- or bidirectional interconnections of core cancer  
980 pathways. Dotted lines represent interactions independent of JAK-STAT signalling.

981

982 **Figure 2: STAT5 mediates common and cell-type specific gene regulation.** Upon  
983 activation of JAKs through cytokine stimulation, receptor associated and  
984 unphosphorylated STATs are phosphorylated (pYSTAT), which subsequently results  
985 in parallel dimerization and translocation to the nucleus to activate gene  
986 transcription. STATs regulate gene expression by binding to cognate GAS sites  
987 located in STAT-controlled regulatory sites. Common target genes (c-Myc, BCL-2, D-  
988 type cyclins) bind any STAT member in different cell types. STAT-controlled cell-  
989 specific binding sites coincide with cell-specific transcription factors such as C/EBP,  
990 HNF4/6, and FOX1/2 in liver tissue and adipocytes as exemplified.

991

992 **Figure 3: STAT5 and STAT3 compete for binding sites to regulate the**  
993 **expression of the oncogenic transcriptional modulator BCL6.** STAT3 increases  
994 expression of BCL6 and enhances recruitment of RNA polymerase II phosphorylated  
995 at a site associated with transcriptional initiation and elongation. STAT5, in contrast,  
996 represses BCL6 expression below basal levels and decreases the association of  
997 RNA polymerase II at the gene loci. Furthermore, BCL6 repression mediated by  
998 STAT5 is dominant over STAT3-mediated induction. STAT5 exerts this effect by  
999 displacing STAT3 from one of the two regulatory regions to which it binds.

1000

1001 **Figure 4: Binding sites of proteins that physically interact with STAT3 and**  
1002 **STAT5.** Various interacting proteins have been shown to activate or transcriptionally  
1003 modulate STAT3 and STAT5 (green and blue proteins, respectively) or act as  
1004 epigenetic modifiers to influence the chromatin landscape in the vicinity of STAT  
1005 binding sites.

1006

1007 **Figure 5: Chromatin remodeling by the transcription factor STAT3.** In response  
1008 to binding of transcriptional activators or repressors to STAT3, modification of STAT3  
1009 itself or nearby histones by methylation or acetylation results in important changes of  
1010 the chromatin structure that regulate gene transcription.

1011

1012 **Figure 6: Chromatin remodeling by the transcription factor STAT5.** STAT5  
1013 regulates the epigenetic landscape by recruitment of activating or repressive proteins  
1014 conferring epigenetic protein modifications.

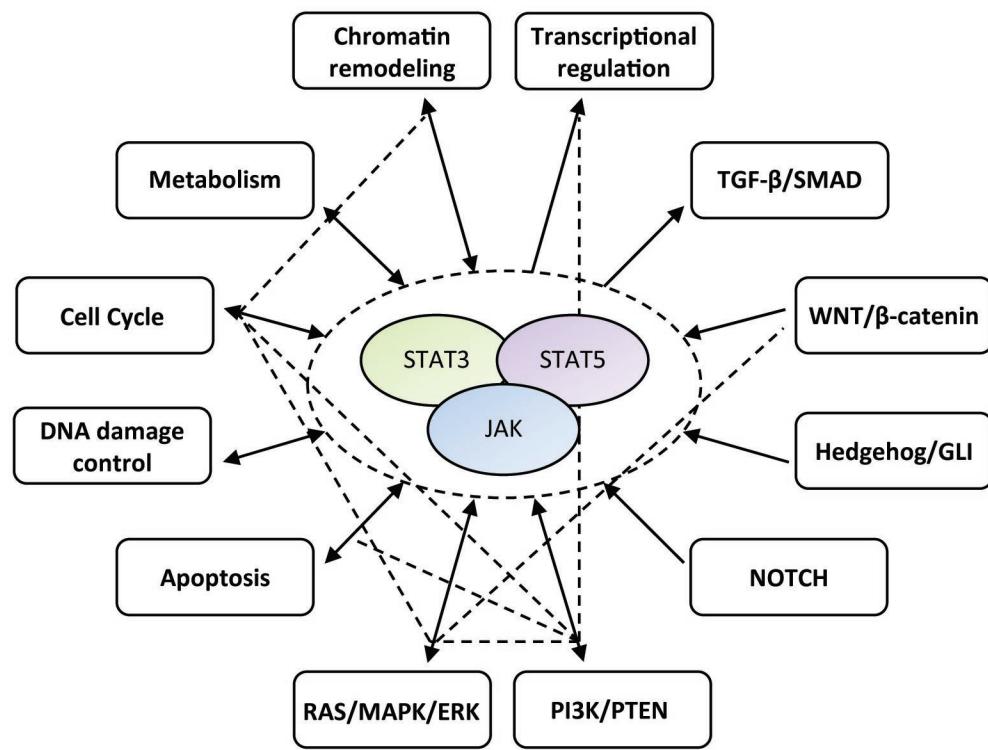
1015

1016 **Figure 7: uSTAT5 represses transcription and influences chromatin topology.**  
1017 Activation of canonical STAT5 signaling leads to transcription of common target  
1018 genes regulating immune cell function, survival, proliferation, and chromatin  
1019 regulation. uSTAT5 however is involved in chromatin compaction by interaction with  
1020 HP-1 and transcriptional silencing by indirect interaction with CTCF.

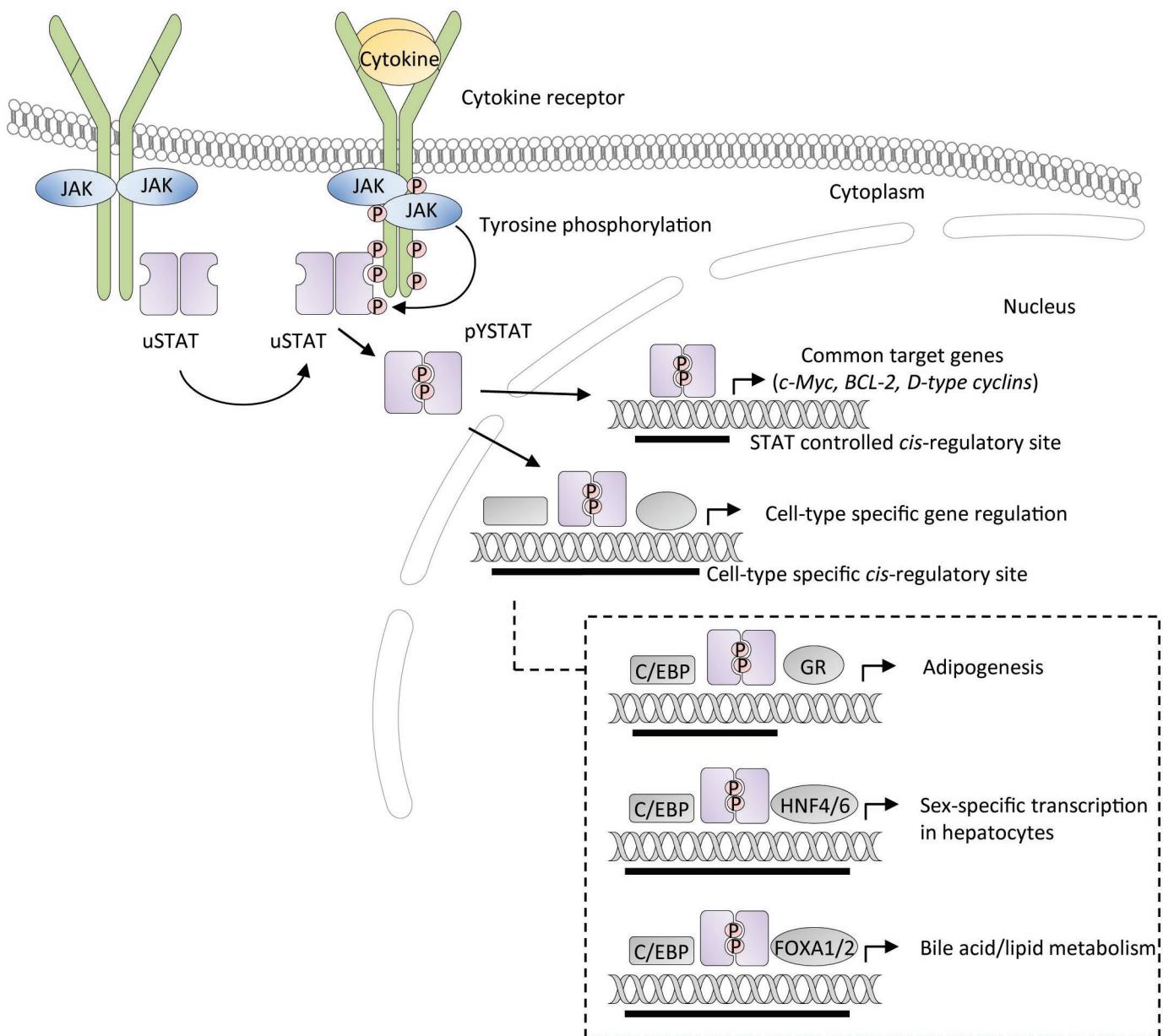
**Table 1: STAT3 and STAT5 activation in hematopoietic diseases**

Disease	Cell type	STAT activity
<b>Leukemia</b>		
ALL	B- or T-lymphocytes	STAT5, STAT3
CLL	B-lymphocytes	STAT3
Multiple myeloma	Plasma cells	STAT3
AML	Myeloid cells	STAT5, STAT3
APL	Promyelocytes	STAT5
Erythroleukemia	Erythroleukemia/blast cells	STAT5
AMKL	Megakaryocytes	STAT5
EMS/SCLL	Myeloid progenitor cells	STAT5
CML	Granulocytes	STAT5
PV	Erythrocytes	STAT5
ET	Megakaryocytes	STAT5
Idiopathic myelofibrosis	Megakaryocytes	STAT5
SCN	Promyelocyte/myelocyte	STAT5
CMM	Monocytes	STAT5
Mastocytosis	Mast cells/basophils	STAT5
CEL	Eosinophils	STAT5
<b>Lymphoma</b>		
B-cell lymphoma	B-cells	STAT5; STAT
EBV-related and Burkitts lymphoma	B-cells	STAT3
Hodgkin lymphoma	T-/B-cells	STAT5, STAT3
CTCL	T-cells	STAT5, STAT3
ALCL	T-cells	STAT5, STAT3
LGL leukemia	T-/NK-cells	STAT3
HTLV-1 infection	T-cells	STAT5, STAT3
HVS infection	T-cells	STAT3

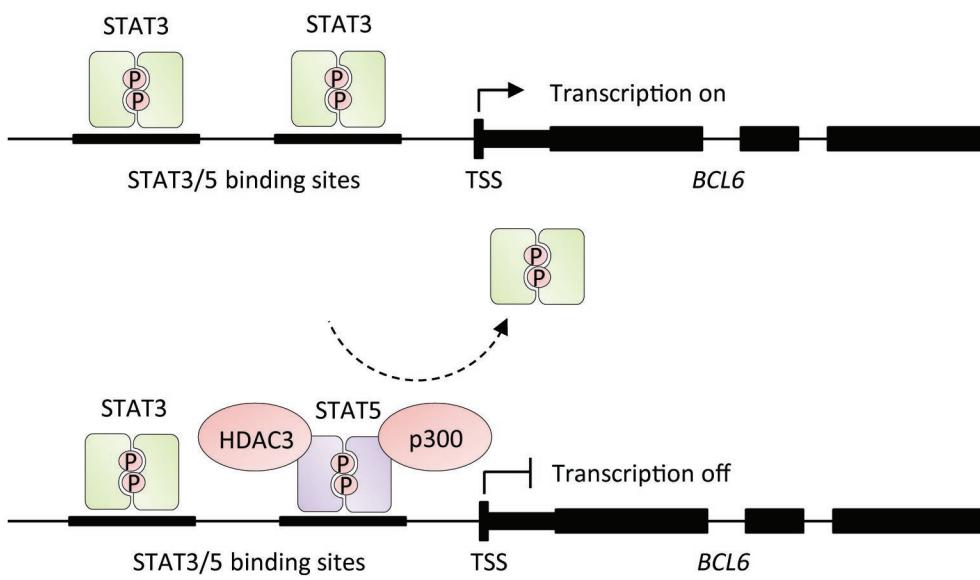
ALL Acute lymphocytic leukemia; CLL Chronic lymphocytic leukemia; AML Acute myeloid leukemia; APL Acute promyelocytic leukemia; AMKL Acute megakaryoblastic leukemia; EMS/SCLL 8p12 myeloproliferative syndrome/stem cell leukaemia-lymphoma syndrome; CML Chronic myeloid leukemia; PV Polycythemia vera; ET Essential thrombocythemia; SCN Severe congenital neutropenia CMML Chronic myelo-monocytic leukemia; CEL Chronic eosinophilic leukemia; EBV Epstein-Barr-Virus; CTCL Cutaneous T-cell lymphoma; ALCL Anaplastic large cell lymphoma; LGL Large granular lymphocyte; HTLV human T-cell lymphoma virus; HVS Herpesvirus Saimiri



Wingelhofer et al, Figure 1

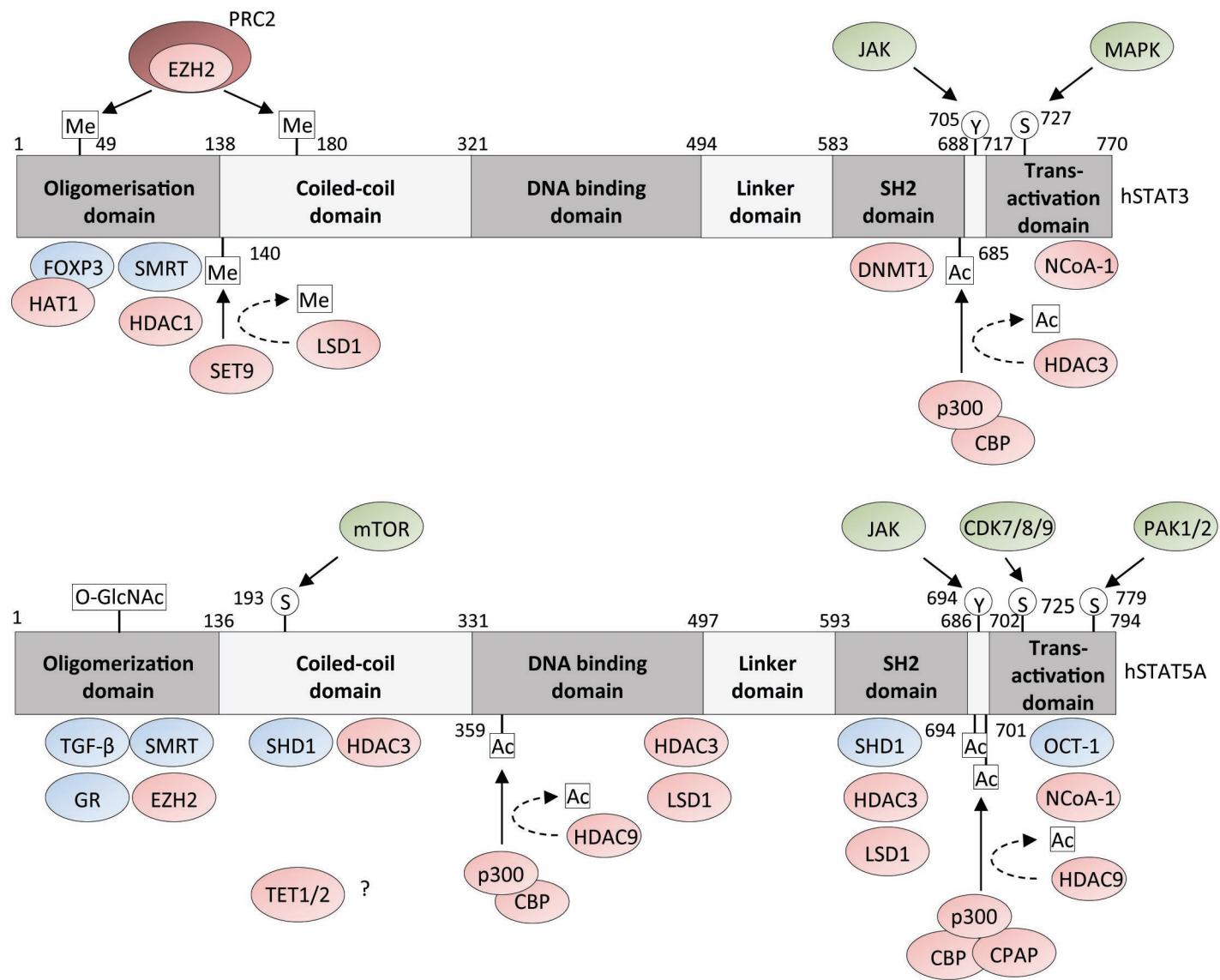


Wingelhofer et al, Figure 2

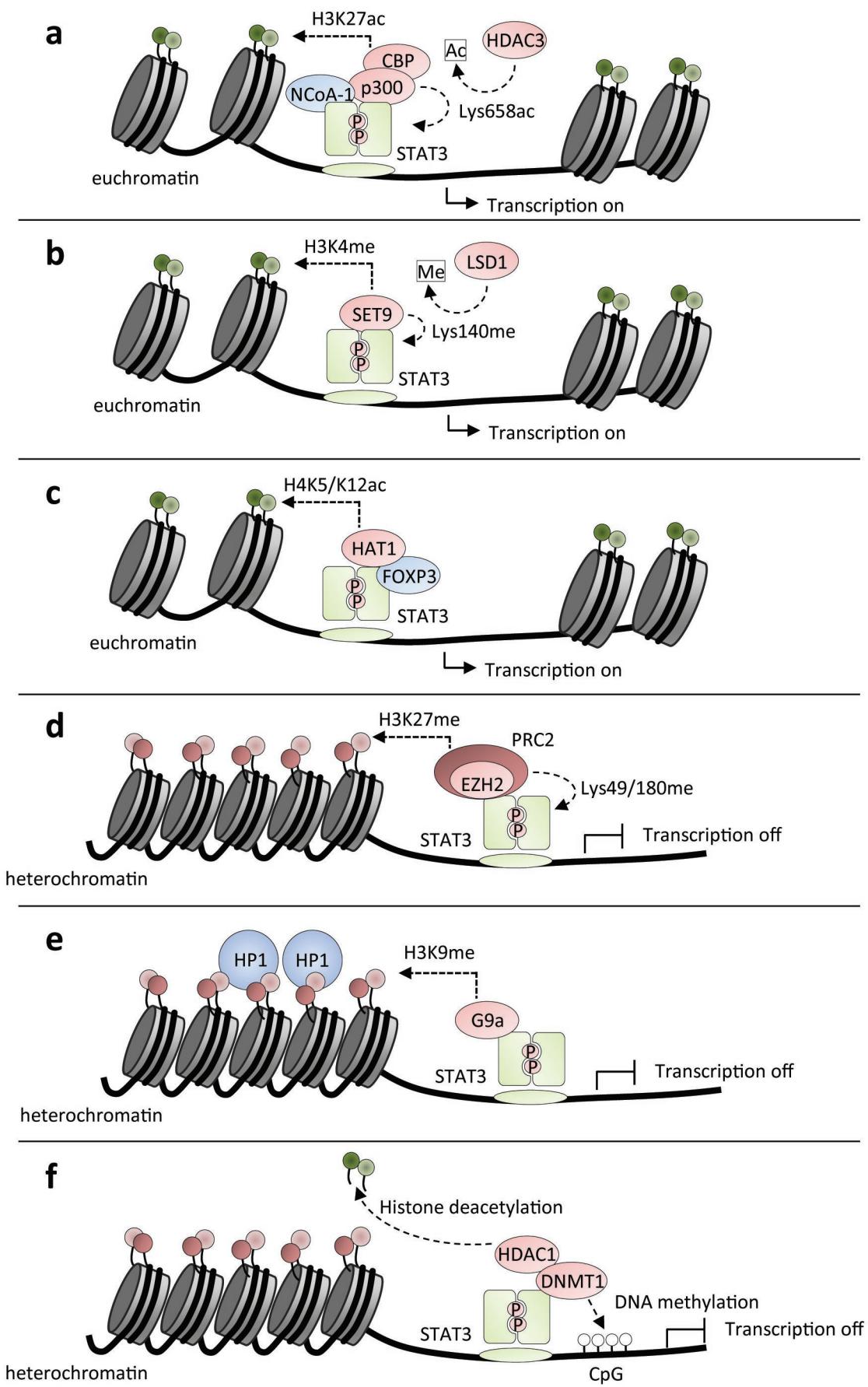


**Wingelhofer et al, Figure 3**

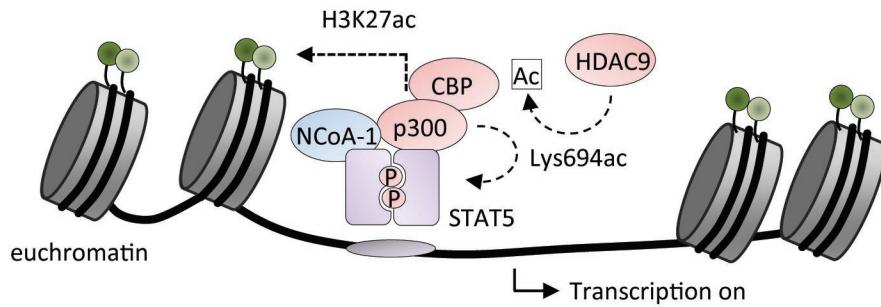
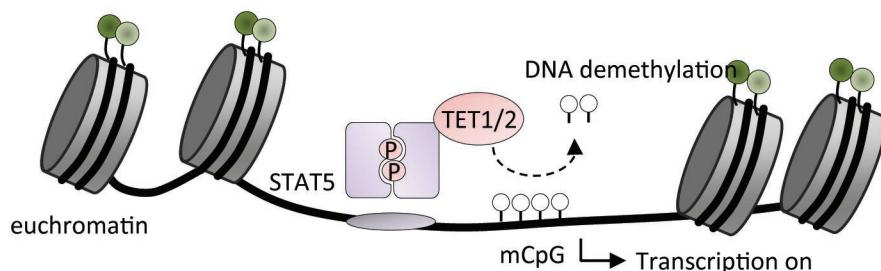
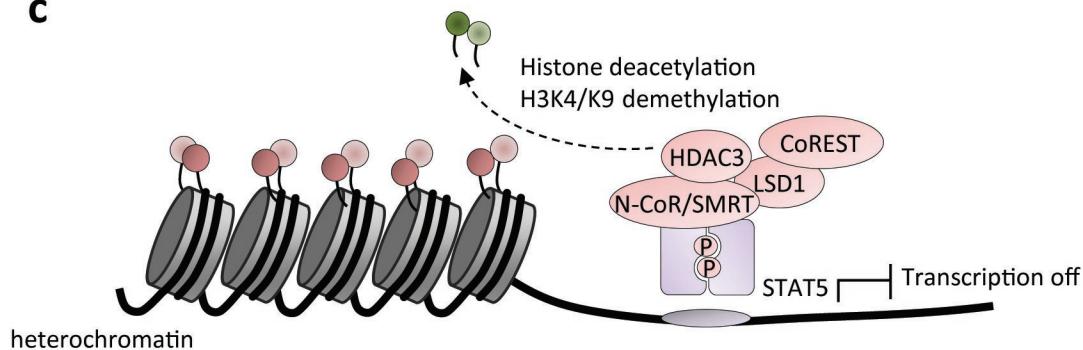
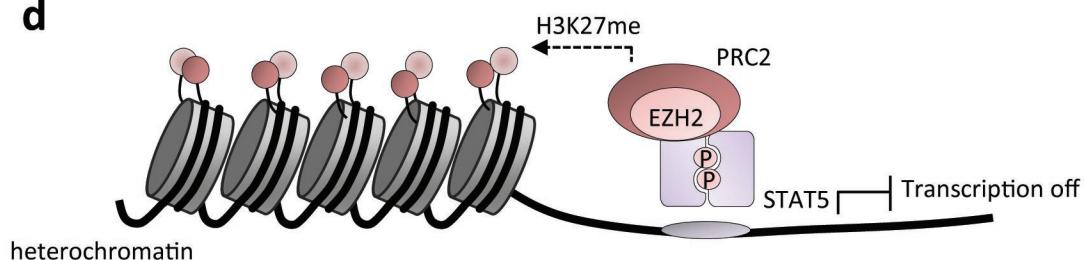
- Epigenetic modifiers
- STAT3/5 functional interactors
- STAT3/5 kinases



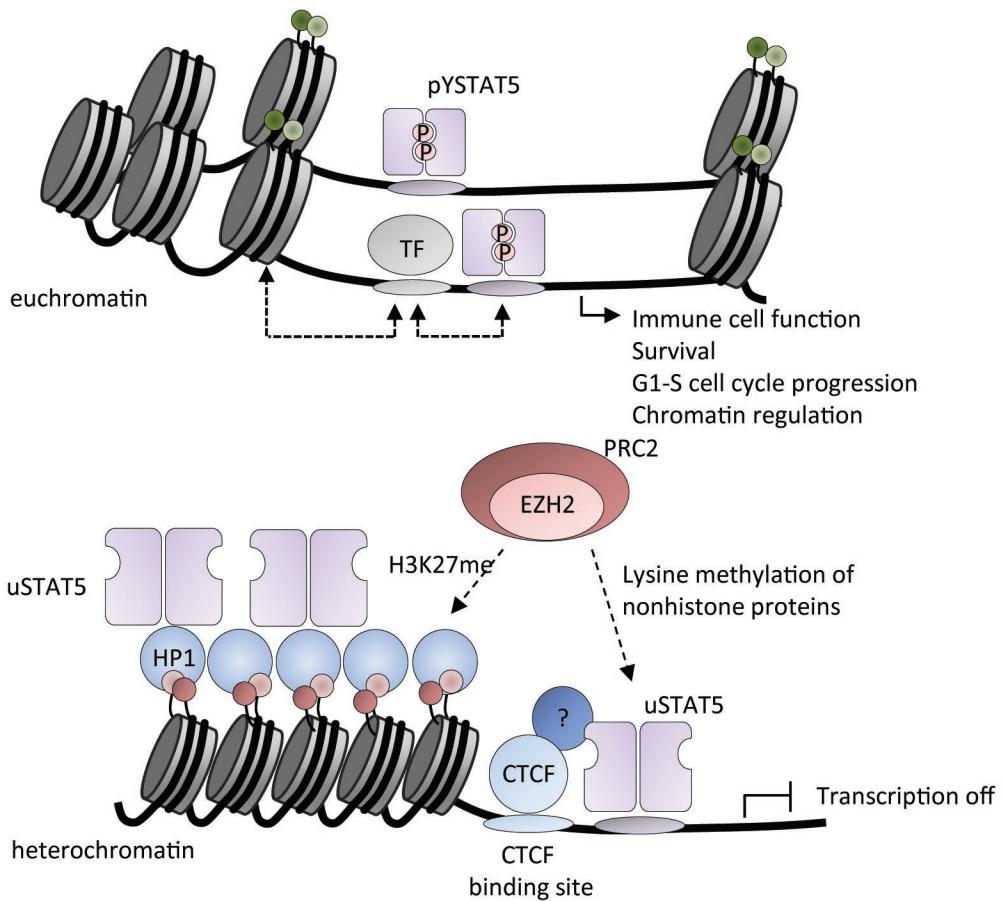
Wingelhofer et al, Figure 4



Wingelhofer et al, Figure 5

**a****b****c****d**

**Wingelhofer et al, Figure 6**



**Wingelhofer et al, Figure 7**