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**Regulation of *MYC* gene expression by aberrant Wnt/β-catenin signaling in colorectal cancer**

Rennoll S *et al*. Control of *MYC* expression in CRCs

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**Abstract**

The Wnt/β-catenin signaling pathway controls intestinal homeostasis and mutations in components of this pathway are prevalent in human colorectal cancers (CRCs). These mutations lead to inappropriate expression of genes controlled by Wnt responsive DNA elements (WREs). T-cell factor/Lymphoid enhancer factor (TCF/Lef) transcription factors bind WREs and recruit the β-catenin transcriptional co-activator to activate target gene expression. Deregulated expression of the *c-MYC* proto-oncogene (*MYC*) by aberrant Wnt/β-catenin signaling drives colorectal carcinogenesis. In this review, we discuss the current literature pertaining to the identification and characterization of WREs that control oncogenic *MYC* expression in CRCs. A common theme has emerged whereby these WREs often map distally to the *MYC* genomic locus and control *MYC* gene expression through long-range chromatin loops with the *MYC* proximal promoter. We propose that by determining which of these WREs is critical for CRC pathogenesis, novel strategies can be developed to treat individuals suffering from this disease.

**Key words:** Wnt; β-catenin; Wnt responsive DNA element; *MYC*; Chromatin looping

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**Core tip:** In colon cancer, mutations in components of the Wnt/β-catenin signaling pathway result in inappropriate *c-MYC* proto-oncogene (*MYC*)expression. To understand colorectal carcinogenesis requires the identification of Wnt responsive DNA elements (WREs) that control *MYC* expression in colorectal cancer (CRC). Through efforts to characterize *MYC* WREs, a model has emerged where several of these WREs appear largely dispensable for intestinal homeostasis, but are instead “hijacked” by oncogenic Wnt/β-catenin signaling to drive CRC. These findings raise the intriguing possibility that these WREs may be targeted therapeutically as an alternative approach to treat individuals afflicted by CRC. In this review, we summarize the literature describing the identification of *MYC* WREs and discuss how those involved in colorectal carcinogenesis may be targeted to limit progression of CRC.

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**INTRODUCTION**

The canonical Wnt/β-catenin signaling pathway regulates stem cell self-renewal, cell proliferation, and cell-fate decisions in the intestinal crypt microenvironment[1]. The β-catenin transcriptional co-activator governs the Wnt response, therefore, its distribution and levels within a cell are tightly regulated[2]. When cells, such as the differentiated cells in the apical regions of the intestinal crypts, are not exposed to Wnt, cytoplasmic β-catenin associates with a multi-protein “destruction complex”[3]. This complex contains adenomatous polyposis coli (APC), the axis inhibition proteins 1 and/or 2 (AXIN1/2), casein kinase 1 (CK1), and glycogen synthase kinase three beta (GSK3β). Here, APC and AXIN1/2 function as scaffolds to position β-catenin in proximity of CK1 and GSK3β. Phosphorylation of β-catenin by CK1 and GSK3β prime it for ubiquitination by the β-transducin-repeat-containing protein (β-TrCP) and subsequent degradation via the proteasome[4]. In the absence of Wnt, members of the T-cell factor/Lymphoid enhancer factor (TCF/Lef) family of sequence-specific transcription factors bound to Wnt responsive DNA elements (WREs) recruit transcriptional corepressor complexes. These complexes include transducin-like enhancer (TLE) and C-terminal binding protein (CtBP), which associate with histone deacetylases (HDACs) to repress target gene expression (Figure 1A)[5].

The basal regions of the intestinal crypts contain stem cells and transit-amplifying (TA) progenitor cells[6]. These cells are exposed to Wnt ligand, secreted by the surrounding mesenchyme and differentiated Paneth cells, which binds frizzled (FZD)/low-density liproprotein receptor-related protein 5 or 6 (LRP5/6) receptor complexes expressed on the cell surface[3]. This binding results in the subsequent recruitment of AXIN1/2 to the plasma membrane via interaction with dishevelled proteins (DVLs) and inactivation of the destruction complex. β-Catenin then escapes proteasomal degradation, accumulates in the cytoplasm, and translocates into the nucleus where it displaces TLE from TCF/Lef bound WREs[1]. β-Catenin/TCF/Lef complexes in turn recruit histone-modifying complexes, such as CBP/p300 protein acetyltransferases and MLL/Set methyltransferases, and chromatin remodeling complexes, including SWI/SNF, to induce expression of Wnt/β-catenin target genes and drive cellular proliferation (Figure 1B)[7].

Colorectal cancer (CRC) is the third leading cause of cancer related deaths in the United States. In 2015, 140000 individuals are predicted to be diagnosed with CRC and over 50000 patients are predicted to succumb to the disease[8]. Approximately 90% of sporadic CRCs contain mutations in components of the Wnt/β-catenin signaling pathway[9]. These mutations are found in the earliest neoplasms suggesting that this pathway serves as a critical gatekeeper to prevent colorectal carcinogenesis[10]. Most tumors contain a mutation in a single component of the pathway, although recent data from the cancer genome atlas (TCGA) consortium indicates that mutations in multiple components can co-occur[11]. The majority of mutations, some 85%, map to “hotspots” within *APC* and often lead to expression of a truncated APC protein from this allele[12]. This truncated protein is incapable of incorporating into a functional β-catenin destruction complex. Thus, when the wild-type *APC* is inactivated by mutation or lost through loss of heterozygosity (LOH), β-catenin levels inappropriately accumulate in the cell, leading to aberrant expression of Wnt/β-catenin target genes and the development of benign adenomas (Figure 1C)[1]. As these adenomas accumulate additional mutations in other signaling pathways, they transition into carcinomas[13]. Therefore, CRC is a disease of uncontrollable Wnt/β-catenin signaling where β-catenin/TCF complexes bound to WREs drive pathogenic expression of downstream target genes. To understand CRC initiation and progression requires identification of these genes and the WREs that control their expression.

The *c-MYC* proto-oncogene (*MYC*) was identified as a Wnt/β-catenin target gene using a differential RNA expression screen conducted in the human HT29 CRC cell line harboring mutant *APC* alleles[14]. MYC is a basic helix-loop-helix zipper (bHLHZ) transcription factor that heterodimerizes with bHLHZ factor MAX[15]. MYC:MAX heterodimers bind E-box sequence motifs to predominantly activate transcription of genes by recruiting histone modifying and chromatin remodeling complexes[16,17]. MYC:MAX regulates expression of thousands of target genes whose products control a wide-range of cellular processes including metabolism, ribosome biogenesis, and protein synthesis[18]. As such, MYC promotes cellular proliferation and cell growth[19].

*MYC* expression is deregulated in 50% of all cancers, including CRC[20]. In fact, *Myc* is required for tumorigenesis in mouse models of CRCs[21-25]. Given that deregulated *MYC* expression by oncogenic Wnt/β-catenin signaling is critical for colorectal carcinogenesis, several groups have sought to identify and characterize WREs that control its expression in human CRC cell lines over the years (Table 1). In this review, we summarize the literature pertaining to WREs that regulate *MYC* in CRC cells. We define a *MYC* WRE as a region of DNA that is: (1) bound by β-catenin/TCF complexes; (2) associated with histone modifications that demarcate enhancer elements, such as monomethylated lysine 4 on histone H3 (H3K4me1) and acetylated lysine 27 on histone H3 (H3K27Ac); (3) responsive to β-catenin/TCF complexes in luciferase reporter assays; and/or (4) juxtaposed to the *MYC* promoter region through chromatin loops, if it maps distal to the *MYC* gene. We then discuss future avenues of research aimed at determining which of these are the critical drivers for oncogenic *MYC* expression and how these results may be leveraged to develop new therapeutic options to manage individuals afflicted by CRC.

**PROMOTER PROXIMAL *MYC* WREs**

In the aforementioned study identifying *MYC* as a target of oncogenic Wnt/β-catenin signaling in human CRC cells, He *et al*[14] localized a β-catenin-responsive region within the *MYC* proximal promoter (Figure 2). This region contained two TCF binding elements, TBE1 and TBE2, which mediated β-catenin/TCF-responsiveness of this element in luciferase reporter assays. While expression of a dominant negative TCF4 protein lacking its amino-terminal β-catenin interaction domain decreased *MYC* expression in CRC cells, it was not evaluated at that time whether β-catenin/TCF complexes occupied this region at the endogenous *MYC* locus. However, this study identified the first *MYC* WRE and we have referred to it as the *MYC* 5’ WRE in our studies of WREs that control *MYC* expression in human CRC lines[26,27].

Sierra *et al*[28] characterized a region mapping approximately 1200-bp upstream from the *MYC* transcriptional start site (TSS), and slightly upstream from *MYC* 5’ WRE, that harbors a third TBE, TBE3. In an extensive set of chromatin immunoprecipitation (ChIP) analyses, they found that β-catenin/TCF complex binding to this region correlated with *MYC* transcription in CRC cells. They also demonstrated that when Wnt signaling was shut-off, APC removed β-catenin and transcriptional co-activators from this site and replaced them with transcriptional corepressors to repress *MYC* expression. Interestingly, unlike wild-type APC, truncated APC found in CRC cells was unable to mediate this corepressor exchange, providing one mechanism underlying how APC mutations lead to aberrant *MYC* expression.

The identification and characterization of *MYC* promoter proximal WREs was an important step forward in our understanding of how β-catenin/TCF complexes drive *MYC* expression. However, very low levels of *MYC* transcripts are produced from a transgene in mice containing the human *MYC* genomic locus, and regions 2.3-kb upstream and 0.4-kb downstream, suggesting that additional WREs may contribute to regulating *MYC* expression[29].

**THE DOWNSTREAM *MYC* PROXIMAL WRE**

To identify regulatory elements that control *MYC* expression, Mautner *et al*[30] mapped DNaseI hypersensitivity sites in proximity to the *MYC* gene in the Colo320 CRC cell line. This strategy mapped a strong hypersensitivity site 1.5-kb downstream from the *MYC* transcription stop site, although it was not determined at that time whether this region demarcated a WRE. In a subsequent study, some 13 years later, a strong β-catenin binding region that overlapped this hypersensitive site was identified in the HCT116 human CRC cell line[31]. Using a combination of ChIP assays, luciferase reporter assays, and *MYC* transcript analysis, it was determined that this β-catenin/TCF binding element demarcated a WRE termed the *MYC* 3’ WRE (Figure 2)[26]. Using chromosome conformation capture (3C), it was demonstrated that β-catenin/TCF4 complexes coordinated a chromatin loop that integrated *MYC* 5’ and 3’ WREs in CRC cells[27]. This chromatin conformation was absent in quiescent CRC cells when *MYC* expression is silenced, but was induced by serum mitogens as *MYC* levels increased, indicating that loop formation accompanied *MYC* transcriptional activation. Interestingly, in HEK293 cells that contain an intact Wnt/β-catenin signaling pathway, either treatment with Wnt3A ligand or activation of the pathway with the GSK3 inhibitor lithium chloride (LiCl), failed to induce the chromatin loop even though *MYC* expression was increased. However, over-expression of oncogenic β-catenin (S45F) in these cells induced the *MYC* 5’3’ loop. Thus, the *MYC* 5’3’ chromatin conformation required elevated levels of nuclear β-catenin that typifies CRC.

In the colonic crypts of mice harboring a germ-line deletion of the *Myc* 3’ WRE, there was a 2-fold increase in *Myc* transcript levels and a 2.5-fold increase in MYC protein levels relative to levels seen in the colons of wild-type littermates[32]. This increase in MYC correlated with an increase in the number of proliferative cells and a decrease in the number of differentiated cells comprising the colonic crypt epithelium. Two approaches were taken to ascertain the role of this WRE in colorectal carcinogenesis[33]. First, *Myc* 3’ WRE-/- mice were bred to *ApcMin/+* mice that harbor a mutation in one *Apc* allele and spontaneously form intestinal tumors as the mice age and the second wild-type *Apc* allele is lost[34,35]. In comparison to *ApcMin/+*, *ApcMin/+* *Myc* 3’ WRE-/- mice contained 4-fold more colonic tumors. Second, *Myc* 3’ WRE-/- mice and wild-type littermates were subjected to the azoxymethane/dextran sodium sulfate (AOM/DSS) model of colitis-associated colorectal cancer (CAC). *Myc* 3’ WRE-/- mice subjected to this protocol contained an elevated number of tumors along the entire colonic tract, most notably in the ceca, which presented 20-fold more tumors. Thus, the primary function of this WRE is to repress *Myc* in the colonic epithelium.

**DISTAL *MYC* WREs**

***The MYC -335 WRE***

The *MYC* locus maps within a gene-poor region on chromosome 8q24[36]. Upstream of *MYC*, results from genome-wide association studies (GWAS) identified regions that harbor single nucleotide polymorphisms that are associated with an increased risk for the development of colon, breast, and prostate cancers[37-44]. One SNP in particular, rs6983267, has received interest in the field, with the G allele associated with increased risk of CRC over the T allele[40,42,44]. Despite this relationship, it was unknown how this SNP contributed to CRC, as *MYC*, the nearest protein-coding region, resides 335-kb downstream[45]. Using the enhancer element locator (EEL) computational method, Tuupanen *et al*[46] found that a region encompassing rs6983267 was predicted to harbor a strong enhancer. Importantly, rs6983267 is adjacent to a TCF4 binding motif and was predicted to influence the binding of this factor. Indeed, the G allele conferred greater Wnt-responsiveness through this site that correlated with enhanced TCF4 binding. In an accompanying manuscript, Pomerantz *et al*[47] shed light on the mechanism for how the rs6983267 risk variant might influence *MYC* expression. They found that this *MYC* -335 WRE was physically juxtaposed to the *MYC* promoter region through a long-range chromatin loop. This result was confirmed by both Ahmadiyeh *et al*[48] and Jäger *et al*[49] in studies to identify regions of 8q24 that interact with the *MYC* -335 WREby 3C and capture Hi-C, respectively. Furthermore, Sotelo *et al*[50] also reported evidence for this long-range interaction and found that overexpression of β-catenin and TCF4 increased the frequency of the association. However, a subsequent study found that while the SNP had no effect on the efficiency of chromatin looping, the risk-associated allele increased expression of the linked *MYC* allele[51]. Although the precise mechanistic details remain to be fully defined, these findings offer a potential explanation for how transcription factors bound to a distal regulatory element can drive oncogenic *MYC* expression in CRC.

In 2012, Sur *et al*[52] reported findings from a mouse model containing a germ-line deletion in the *Myc* -335 WRE. As was the case in *Myc* 3’ WRE-/- intestines, deletion of the *Myc* -335 WRE did not cause gross phenotypic alterations of the intestinal architecture. Moreover, deletion of the *Myc* -335 WRE did not alter the cellular composition of the intestinal epithelium or influence expression of *Myc* in the duodenum. However, *Myc* expression in the colons of *Myc* -335 WRE-/- was decreased relative to levels seen in the colons of wild-type littermates. Importantly, when these mice were bred to *ApcMin/+*mice, deletion of the *Myc* -335 WRE led to a reduced number of tumors that formed in both the small intestines and colons. Thus, this element is a critical regulator of intestinal tumors that arise from pathogenic Wnt/β-catenin signaling.

As the *ApcMin/+* mouse is an important model for CRC, it is somewhat limited by the fact that tumors preferentially arise in the small intestine and not the colon[53]. A recent study suggests that this phenotype may be due to the fact that stem cells in the small intestine divide more rapidly than those in the colons and hence there is an increased chance of accumulating mutations in the small intestine[54]. In addition, tumors that arise in *ApcMin/+* mice are primarily adenomas, which do not progress to carcinomas because the mice become moribund[53]. Therefore, to determine whether the *MYC* -335 WRE is required in colorectal carcinomas requires deleting this regulatory element in a human CRC cell line. As part of an elegant study to functionally annotate genes whose expression is influenced by colon cancer risk SNPs, Yao *et al*[55] used clustered regulatory interspaced short palindromic repeats/Cas9 (CRISPR/Cas9) to delete the *MYC* -335 WRE in HCT116 human CRC cells. While *MYC* -335 WRE-/- cells expressed less *MYC* relative to parental cells, it was not reported whether this influenced the oncogenic properties of these cells.

In an intriguing study, Ling *et al*[56] offered an additional explanation for how the *MYC* -335 WRE influences *MYC*-driven colorectal carcinogenesis. They found that a long non-coding RNA, which they termed colon-cancer associated transcript two (*CCAT2*), is expressed from this region and contains the rs6983267 SNP. *CCAT2* expression is elevated in colon tumors relative to levels detected in adjacent and uninvolved colonic mucosa. Overexpression of *CCAT2* in HCT116 cells promoted tumorigenesis when these cells were implanted into immunocompromised mice, whereas *CCAT2* knockdown diminished the invasive capacity of the KM12SM CRC cell line. Moreover, *CCAT2* overexpression increased *MYC* expression in HCT116 cells, whereas *CCAT2* knockdown decreased *MYC* expression in these cells. *CCAT2* itself is a Wnt/β-catenin target gene, and it interacts directly with TCF4 to augment TCF4-dependent expression of Wnt/β-catenin target genes, including *MYC*. Further studies are necessary to fully understand how the rs6983267 SNP impinges upon *CCAT2* function.

In an effort to understand the function of rs6983267 in colorectal carcinogenesis, Kim *et al*[57] cloned seven lncRNAs derived from the 8q24.21 gene desert region. It was found that one of these lncRNAs, termed cancer-associated region long non-coding RNA number 5 (*CARLo5*), played an important role in driving CRC. *CARLo5* expression was elevated in CRC relative to expression in normal adjacent tissues, and knocking down *CARLo5* expression diminished CRC cell proliferation and growth of these cells as tumors in athymic nude mice. Interestingly, a region containing the *MYC*-335 WRE was juxtaposed to the *CARLo5* promoter region to drive its expression in CRC cells.

***The MYC distal super-enhancer***

In 2010, Bottomly *et al*[58] reported results from a ChIP-Seq screen to localize β-catenin binding sites throughout the genome in the HCT116 human CRC cell line. This screen confirmed that β-catenin bound the *MYC* 5’ WRE, 3’ WRE, and -335 WRE in these cells. In addition, they noted that a cluster of β-catenin binding sites mapped to a region approximately 400-500-kb upstream from the *MYC* TSS. In a follow-up study, it was found that these regions bound TCF4, β-catenin, and RNA Polymerase II (RNAP) and are demarcated by histones with modifications that typify enhancer regions including H3K4me1 and H3KAc[59,60]. Four of five of these distal β-catenin-bound regions formed long-range chromatin loops with the *MYC* proximal promoter region. Interestingly, these conformations were not only present in HCT116 cells, but also the non-CRC cell lines HEK293 and TIG-1, suggesting that β-catenin might use these pre-existing chromatin loops to activate *MYC* gene expression. Moreover, the interaction frequencies between two of these regions and the *MYC* proximal promoter were induced upon stimulation of the cells with serum mitogens.

Several years later, Hnisz *et al*[61] found that this distal cluster of *MYC* WREs overlapped with a super-enhancer. According to Whyte *et al*[62], super-enhancers are “…large clusters of transcriptional enhancers – formed by binding of high levels of master TFs (transcription factors) and Mediator coactivator – that drive expression of genes that define cell identity”. These super-enhancers also bind high levels of the chromatin-associated protein bromodomain containing protein 4 (BRD4)[63]. Through its bromodomain, BRD4 associates with acetylated histones and recruits the positive transcription elongation factor (PTEF-b) to promote transcriptional elongation by RNAP[64,65]. BRD4 also interacts directly with the Mediator complex, and Mediator has been shown to play a role in chromatin looping[64-66]. JQ1 is a selective inhibitor of BRD4 activity that competes with BRD4 binding to acetylated substrates[67]. Interestingly, treatment of multiple myeloma cells with JQ1 led to a preferential loss of BRD4 occupancy at super-enhancers and repression of oncogene expression, including *MYC*[63,68]. A comparison of H3K27Ac patterns obtained from ChIP-Seq analysis in normal colonic mucosa and the HCT116 CRC cell line revealed that high levels of nucleosomes containing this modification map to the distal *MYC* super-enhancer in CRC cells[61]. By overlaying the ChIP-Seq profile for TCF4 binding in these cells, Hnisz *et al*[61] identified four putative WREs embedded within the distal super-enhancer that corresponded to four of the five *MYC* WREs identified previously in this region by Yochum[59]. Indeed, luciferase assays conducted with reporters containing these elements confirmed that they functioned as WREs[61].

As is the case for the *MYC* -335 WRE, a lncRNA termed colon cancer-associated transcript one (*CCAT1*), localizes within the *MYC* super-enhancer[69]. Two isoforms of *CCAT1* are expressed; a long form, *CCAT1-L*, which is 5200-bp in length, and a short form, *CCAT1-S*, which is 2600-bp in length. Both isoforms are expressed at higher levels in CRCs relative to levels in patient matched uninvolved colonic mucosa[69-71]. In contrast to *CCAT1-S*, which localizes to the cytoplasm, *CCAT1-L* localizes to the nucleus. *CCAT1-L* knockdown in CRC cells reduces *MYC* expression, while increased expression of *CCAT1-L* from its chromosomal locus enhances *MYC* expression. It was demonstrated that *CCAT1-L* is an important regulator of chromatin looping between *MYC* WREs and the *MYC* promoter. Interestingly, this lncRNA not only promoted chromatin looping between the *MYC* super-enhancer and the *MYC* promoter, but also between the *MYC* super-enhancer and the *MYC* -335 WRE. In addition, the CCCTC-binding factor (CTCF) was shown to play a role in mediating the interactions between distal *MYC* WREs and the *MYC* promoter. *CCAT1-L* interacted directly with CTCF and stabilized CTCF binding to distal WREs, providing a mechanism for *CCAT1-L*-mediated regulation of *MYC*.

**ACTIVATION OF *MYC* EXPRESSION BY ONCOGENIC WNT/β-CATENIN SIGNALING**

To summarize, mutations in components of the Wnt/β-catenin signaling pathway lead to aberrant *MYC* expression in CRCs through β-catenin/TCF transcription complexes bound to WREs. The *MYC* 3’ WRE, *MYC* -335 WRE, and distal super-enhancer are juxtaposed to the *MYC* 5’ WRE within theproximal promoter region through long-range chromatin loops (Figure 3)[27,47-51,59,69]. We therefore propose a model in which “hijacked” distal WREs align to the *MYC* proximal promoter and increase the local concentration of β-catenin/TCF transcription complexes to drive oncogenic *MYC* expression in CRC. Thus, the *MYC* proximal promoter serves as a “landing pad” to coordinate chromatin conformations at *MYC*. As the 3C technique represents an average interaction frequency across a population of cells, it is unknown whether these chromatin loops occur simultaneously at a single *MYC* allele[72]. It is also important to note that in several cases, these chromatin loops are not restricted to CRC cells and their formation does not depend on Wnt/β-catenin signaling[48,51,59]. Thus, the conformation itself, and not its formation, may poise the *MYC* locus to receive oncogenic signals. Finally, it is probable that additional WREs and other enhancer elements contribute to deregulated *MYC* expression in CRC cells[50,73].

**CONCLUSION**

Despite the identification of MYC over 30 years ago and numerous reports linking deregulated *MYC* gene expression to tumorigenesis, there is still no clinically available drug that targets MYC in cancer cells[74-76]. This is in part due to the fact that, unlike other proteins, *MYC* is very rarely mutated in cancers[76]. Instead, *MYC* is overexpressed due to aberrant activation of upstream signaling pathways or due to events that trigger amplification of the *MYC* gene locus or insertions of activating sequences[77]. It is, therefore, difficult to target MYC in cancer cells versus normal, healthy cells where MYC is required for cellular proliferation[76]. Despite these difficulties, recent approaches to inhibit the BRD4 chromatin reader have been demonstrated to specifically and profoundly diminish *MYC* gene expression in several cancer cells[63,68,78-80]. These studies indicate that targeting *MYC* at the transcriptional level is an effective therapeutic strategy for treating cancers with deregulated *MYC* gene expression. Therefore, further characterization of the *MYC* WREs discussed in this review will likely provide new avenues for targeting *MYC* gene expression in CRC cells.

Recent reports have indicated that active DNA enhancer elements express short, bidirectional RNAs termed enhancer RNAs (eRNAs)[81-84]. Expression level changes observed for eRNAs correspond to changes in mRNA levels of the target gene, but the mechanism through which eRNAs activate target gene expression appears to vary based on cellular context. eRNAs have been found to stabilize enhancer-promoter interactions and also to relieve transcriptional pausing by inactivating the negative elongation factor complex (NELF)[85,86]. It is unknown whether eRNAs are transcribed from the *MYC* WREs described in this report. It is feasible that eRNA transcripts derived from these *MYC* WREs facilitate *MYC* gene expression by stabilizing *MYC* WRE interactions with the *MYC* promoter. Additional factors could also be functioning to stabilize chromatin loops and activate *MYC* expression. β-Catenin was recently demonstrated to recruit cohesin and direct enhancer-promoter interactions in human embryonic stem cells[87]. If β-catenin also recruits cohesin at *MYC* WREs in CRC cells, it could explain how “hijacked” *MYC* WREs interact with the *MYC* promoter or suggest that β-catenin stabilizes pre-existing chromatin loops. CTCF could also be a critical factor for maintaining the genomic architecture at the *MYC* gene locus, as the interaction frequency of the *MYC* super-enhancer with the *MYC* promoter is diminished after CTCF knockdown[69]. To provide a better understanding of how β-catenin drives deregulated *MYC* gene expression in CRC cells, future work is needed to define the factors critical for *MYC* chromatin conformations and to determine the role of these conformations in activating *MYC* expression in CRCs.

Before candidate WREs can be considered as therapeutic targets, proof-of-principle experiments are required. Namely, whether these elements are dispensable in the normal colonic epithelium, but essential for colon carcinogenesis, which would suggest that a subset of WREs may be “hijacked” by oncogenic Wnt/β-catenin signaling. Indeed the mouse studies described by Sur *et al*[52] indicated that the *Myc* -335 WRE did not play a role in intestinal homeostasis, but was required for intestinal tumorigenesis caused by mutations in *Apc*. To determine whether this element was required in HCT116 human CRC cells, Yao *et al*[55] deleted it using CRISPR/Cas9 gene editing. While this deletion reduced *MYC* expression in these cells, it was not reported whether it altered the chromatin conformation at the *MYC* locus or reduced the oncogenic potential of these cells. Interestingly, the *MYC* distal super-enhancer is preferentially activated in CRC and not uninvolved colonic mucosa[61,88]. However, due to its size and complexity, it may be difficult to target using gene editing strategies. Although we reported that the *Myc* 3’ WRE suppressed colonic tumorigenesis in mice, we noted that deletion of this element decreased tumorigenesis in the small intestines of *ApcMin/+* mice[33]. Therefore, we used CRISPR/Cas9 to target the *MYC* 3’ WRE in HCT116 cells (SAR, GSY, unpublished). Using this approach, we generated clonal cell lines that harbored homozygous deletions in one of two TBEs within this element. These cells contain reduced *MYC* expression at the transcript and protein levels and also display reduced oncogenic properties.

Further investigation of the factors required for WRE-mediated transcriptional regulation of *MYC* will provide a more detailed model of how *MYC* gene expression becomes deregulated in CRCs. This model can then be applied to investigate deregulated *MYC* expression as a result of constitutive upstream signaling pathway activation in other cancers and also to identify potential therapeutic targets.

**REFERENCES**

1 **Clevers H**. Wnt/beta-catenin signaling in development and disease. *Cell* 2006; **127**: 469-480 [PMID: 17081971 DOI: 10.1016/j.cell.2006.10.018]

2 **Willert K**, Nusse R. Beta-catenin: a key mediator of Wnt signaling. *Curr Opin Genet Dev* 1998; **8**: 95-102 [PMID: 9529612]

3 **Gregorieff A**, Clevers H. Wnt signaling in the intestinal epithelium: from endoderm to cancer. *Genes Dev* 2005; **19**: 877-890 [PMID: 15833914 DOI: 10.1101/gad.1295405]

4 **MacDonald BT**, Tamai K, He X. Wnt/beta-catenin signaling: components, mechanisms, and diseases. *Dev Cell* 2009; **17**: 9-26 [PMID: 19619488 DOI: 10.1016/j.devcel.2009.06.016]

5 **Cadigan KM**, Waterman ML. TCF/LEFs and Wnt signaling in the nucleus. *Cold Spring Harb Perspect Biol* 2012; **4**: [PMID: 23024173 DOI: 10.1101/cshperspect.a007906]

6 **van der Flier LG**, Clevers H. Stem cells, self-renewal, and differentiation in the intestinal epithelium. *Annu Rev Physiol* 2009; **71**: 241-260 [PMID: 18808327 DOI: 10.1146/annurev.physiol.010908.163145]

7 **Mosimann C**, Hausmann G, Basler K. Beta-catenin hits chromatin: regulation of Wnt target gene activation. *Nat Rev Mol Cell Biol* 2009; **10**: 276-286 [PMID: 19305417 DOI: 10.1038/nrm2654]

8 **Siegel RL**, Miller KD, Jemal A. Cancer statistics, 2015. *CA Cancer J Clin* 2015; **65**: 5-29 [PMID: 25559415 DOI: 10.3322/caac.21254]

9 **Giles RH**, van Es JH, Clevers H. Caught up in a Wnt storm: Wnt signaling in cancer. *Biochim Biophys Acta* 2003; **1653**: 1-24 [PMID: 12781368]

10 **Humphries A**, Wright NA. Colonic crypt organization and tumorigenesis. *Nat Rev Cancer* 2008; **8**: 415-424 [PMID: 18480839 DOI: 10.1038/nrc2392]

11 **Network CGA**. Comprehensive molecular characterization of human colon and rectal cancer. *Nature* 2012; **487**: 330-337 [PMID: 22810696 DOI: 10.1038/nature11252]

12 **Fearon ER**. Molecular genetics of colorectal cancer. *Annu Rev Pathol* 2011; **6**: 479-507 [PMID: 21090969 DOI: 10.1146/annurev-pathol-011110-130235]

13 **Davies RJ**, Miller R, Coleman N. Colorectal cancer screening: prospects for molecular stool analysis. *Nat Rev Cancer* 2005; **5**: 199-209 [PMID: 15738983 DOI: 10.1038/nrc1545]

14 **He TC**, Sparks AB, Rago C, Hermeking H, Zawel L, da Costa LT, Morin PJ, Vogelstein B, Kinzler KW. Identification of c-MYC as a target of the APC pathway. *Science* 1998; **281**: 1509-1512 [PMID: 9727977]

15 **Blackwood EM**, Eisenman RN. Max: a helix-loop-helix zipper protein that forms a sequence-specific DNA-binding complex with Myc. *Science* 1991; **251**: 1211-1217 [PMID: 2006410]

16 **Blackwell TK**, Kretzner L, Blackwood EM, Eisenman RN, Weintraub H. Sequence-specific DNA binding by the c-Myc protein. *Science* 1990; **250**: 1149-1151 [PMID: 2251503]

17 **Adhikary S**, Eilers M. Transcriptional regulation and transformation by Myc proteins. *Nat Rev Mol Cell Biol* 2005; **6**: 635-645 [PMID: 16064138 DOI: 10.1038/nrm1703]

18 **Dang CV**, O'Donnell KA, Zeller KI, Nguyen T, Osthus RC, Li F. The c-Myc target gene network. *Semin Cancer Biol* 2006; **16**: 253-264 [PMID: 16904903 DOI: 10.1016/j.semcancer.2006.07.014]

19 **Eilers M**, Eisenman RN. Myc's broad reach. *Genes Dev* 2008; **22**: 2755-2766 [PMID: 18923074 DOI: 10.1101/gad.1712408]

20 **Morton JP**, Sansom OJ. MYC-y mice: from tumour initiation to therapeutic targeting of endogenous MYC. *Mol Oncol* 2013; **7**: 248-258 [PMID: 23523308 DOI: 10.1016/j.molonc.2013.02.015]

21 **Yekkala K**, Baudino TA. Inhibition of intestinal polyposis with reduced angiogenesis in ApcMin/+ mice due to decreases in c-Myc expression. *Mol Cancer Res* 2007; **5**: 1296-1303 [PMID: 18171987 DOI: 10.1158/1541-7786.MCR-07-0232]

22 **Wilkins JA**, Sansom OJ. C-Myc is a critical mediator of the phenotypes of Apc loss in the intestine. *Cancer Res* 2008; **68**: 4963-4966 [PMID: 18593890 DOI: 10.1158/0008-5472.CAN-07-5558]

23 **Athineos D**, Sansom OJ. Myc heterozygosity attenuates the phenotypes of APC deficiency in the small intestine. *Oncogene* 2010; **29**: 2585-2590 [PMID: 20140021 DOI: 10.1038/onc.2010.5]

24 **Ignatenko NA**, Holubec H, Besselsen DG, Blohm-Mangone KA, Padilla-Torres JL, Nagle RB, de Alboránç IM, Guillen-R JM, Gerner EW. Role of c-Myc in intestinal tumorigenesis of the ApcMin/+ mouse. *Cancer Biol Ther* 2006; **5**: 1658-1664 [PMID: 17106247]

25 **Sansom OJ**, Meniel VS, Muncan V, Phesse TJ, Wilkins JA, Reed KR, Vass JK, Athineos D, Clevers H, Clarke AR. Myc deletion rescues Apc deficiency in the small intestine. *Nature* 2007; **446**: 676-679 [PMID: 17377531 DOI: 10.1038/nature05674]

26 **Yochum GS**, Cleland R, Goodman RH. A genome-wide screen for beta-catenin binding sites identifies a downstream enhancer element that controls c-Myc gene expression. *Mol Cell Biol* 2008; **28**: 7368-7379 [PMID: 18852287 DOI: 10.1128/MCB.00744-08]

27 **Yochum GS**, Sherrick CM, Macpartlin M, Goodman RH. A beta-catenin/TCF-coordinated chromatin loop at MYC integrates 5' and 3' Wnt responsive enhancers. *Proc Natl Acad Sci USA* 2010; **107**: 145-150 [PMID: 19966299 DOI: 10.1073/pnas.0912294107]

28 **Sierra J**, Yoshida T, Joazeiro CA, Jones KA. The APC tumor suppressor counteracts beta-catenin activation and H3K4 methylation at Wnt target genes. *Genes Dev* 2006; **20**: 586-600 [PMID: 16510874 DOI: 10.1101/gad.1385806]

29 **Lavenu A**, Pournin S, Babinet C, Morello D. The cis-acting elements known to regulate c-myc expression ex vivo are not sufficient for correct transcription in vivo. *Oncogene* 1994; **9**: 527-536 [PMID: 8290263]

30 **Mautner J**, Joos S, Werner T, Eick D, Bornkamm GW, Polack A. Identification of two enhancer elements downstream of the human c-myc gene. *Nucleic Acids Res* 1995; **23**: 72-80 [PMID: 7870592]

31 **Yochum GS**, McWeeney S, Rajaraman V, Cleland R, Peters S, Goodman RH. Serial analysis of chromatin occupancy identifies beta-catenin target genes in colorectal carcinoma cells. *Proc Natl Acad Sci USA* 2007; **104**: 3324-3329 [PMID: 17360646 DOI: 10.1073/pnas.0611576104]

32 **Konsavage WM**, Jin G, Yochum GS. The Myc 3' Wnt-responsive element regulates homeostasis and regeneration in the mouse intestinal tract. *Mol Cell Biol* 2012; **32**: 3891-3902 [PMID: 22826434 DOI: 10.1128/MCB.00548-12]

33 **Konsavage WM**, Yochum GS. The myc 3' wnt-responsive element suppresses colonic tumorigenesis. *Mol Cell Biol* 2014; **34**: 1659-1669 [PMID: 24567369 DOI: 10.1128/MCB.00969-13]

34 **Moser AR**, Pitot HC, Dove WF. A dominant mutation that predisposes to multiple intestinal neoplasia in the mouse. *Science* 1990; **247**: 322-324 [PMID: 2296722]

35 **Su LK**, Kinzler KW, Vogelstein B, Preisinger AC, Moser AR, Luongo C, Gould KA, Dove WF. Multiple intestinal neoplasia caused by a mutation in the murine homolog of the APC gene. *Science* 1992; **256**: 668-670 [PMID: 1350108]

36 **Grisanzio C**, Freedman ML. Chromosome 8q24-Associated Cancers and MYC. *Genes Cancer* 2010; **1**: 555-559 [PMID: 21779458 DOI: 10.1177/1947601910381380]

37 **Amundadottir LT**, Sulem P, Gudmundsson J, Helgason A, Baker A, Agnarsson BA, Sigurdsson A, Benediktsdottir KR, Cazier JB, Sainz J, Jakobsdottir M, Kostic J, Magnusdottir DN, Ghosh S, Agnarsson K, Birgisdottir B, Le Roux L, Olafsdottir A, Blondal T, Andresdottir M, Gretarsdottir OS, Bergthorsson JT, Gudbjartsson D, Gylfason A, Thorleifsson G, Manolescu A, Kristjansson K, Geirsson G, Isaksson H, Douglas J, Johansson JE, Bälter K, Wiklund F, Montie JE, Yu X, Suarez BK, Ober C, Cooney KA, Gronberg H, Catalona WJ, Einarsson GV, Barkardottir RB, Gulcher JR, Kong A, Thorsteinsdottir U, Stefansson K. A common variant associated with prostate cancer in European and African populations. *Nat Genet* 2006; **38**: 652-658 [PMID: 16682969 DOI: 10.1038/ng1808]

38 **Gudmundsson J**, Sulem P, Manolescu A, Amundadottir LT, Gudbjartsson D, Helgason A, Rafnar T, Bergthorsson JT, Agnarsson BA, Baker A, Sigurdsson A, Benediktsdottir KR, Jakobsdottir M, Xu J, Blondal T, Kostic J, Sun J, Ghosh S, Stacey SN, Mouy M, Saemundsdottir J, Backman VM, Kristjansson K, Tres A, Partin AW, Albers-Akkers MT, Godino-Ivan Marcos J, Walsh PC, Swinkels DW, Navarrete S, Isaacs SD, Aben KK, Graif T, Cashy J, Ruiz-Echarri M, Wiley KE, Suarez BK, Witjes JA, Frigge M, Ober C, Jonsson E, Einarsson GV, Mayordomo JI, Kiemeney LA, Isaacs WB, Catalona WJ, Barkardottir RB, Gulcher JR, Thorsteinsdottir U, Kong A, Stefansson K. Genome-wide association study identifies a second prostate cancer susceptibility variant at 8q24. *Nat Genet* 2007; **39**: 631-637 [PMID: 17401366 DOI: 10.1038/ng1999]

39 **Easton DF**, Pooley KA, Dunning AM, Pharoah PD, Thompson D, Ballinger DG, Struewing JP, Morrison J, Field H, Luben R, Wareham N, Ahmed S, Healey CS, Bowman R, Meyer KB, Haiman CA, Kolonel LK, Henderson BE, Le Marchand L, Brennan P, Sangrajrang S, Gaborieau V, Odefrey F, Shen CY, Wu PE, Wang HC, Eccles D, Evans DG, Peto J, Fletcher O, Johnson N, Seal S, Stratton MR, Rahman N, Chenevix-Trench G, Bojesen SE, Nordestgaard BG, Axelsson CK, Garcia-Closas M, Brinton L, Chanock S, Lissowska J, Peplonska B, Nevanlinna H, Fagerholm R, Eerola H, Kang D, Yoo KY, Noh DY, Ahn SH, Hunter DJ, Hankinson SE, Cox DG, Hall P, Wedren S, Liu J, Low YL, Bogdanova N, Schürmann P, Dörk T, Tollenaar RA, Jacobi CE, Devilee P, Klijn JG, Sigurdson AJ, Doody MM, Alexander BH, Zhang J, Cox A, Brock IW, MacPherson G, Reed MW, Couch FJ, Goode EL, Olson JE, Meijers-Heijboer H, van den Ouweland A, Uitterlinden A, Rivadeneira F, Milne RL, Ribas G, Gonzalez-Neira A, Benitez J, Hopper JL, McCredie M, Southey M, Giles GG, Schroen C, Justenhoven C, Brauch H, Hamann U, Ko YD, Spurdle AB, Beesley J, Chen X, Mannermaa A, Kosma VM, Kataja V, Hartikainen J, Day NE, Cox DR, Ponder BA. Genome-wide association study identifies novel breast cancer susceptibility loci. *Nature* 2007; **447**: 1087-1093 [PMID: 17529967 DOI: 10.1038/nature05887]

40 **Haiman CA**, Le Marchand L, Yamamato J, Stram DO, Sheng X, Kolonel LN, Wu AH, Reich D, Henderson BE. A common genetic risk factor for colorectal and prostate cancer. *Nat Genet* 2007; **39**: 954-956 [PMID: 17618282 DOI: 10.1038/ng2098]

41 **Haiman CA**, Patterson N, Freedman ML, Myers SR, Pike MC, Waliszewska A, Neubauer J, Tandon A, Schirmer C, McDonald GJ, Greenway SC, Stram DO, Le Marchand L, Kolonel LN, Frasco M, Wong D, Pooler LC, Ardlie K, Oakley-Girvan I, Whittemore AS, Cooney KA, John EM, Ingles SA, Altshuler D, Henderson BE, Reich D. Multiple regions within 8q24 independently affect risk for prostate cancer. *Nat Genet* 2007; **39**: 638-644 [PMID: 17401364 DOI: 10.1038/ng2015]

42 **Tomlinson I**, Webb E, Carvajal-Carmona L, Broderick P, Kemp Z, Spain S, Penegar S, Chandler I, Gorman M, Wood W, Barclay E, Lubbe S, Martin L, Sellick G, Jaeger E, Hubner R, Wild R, Rowan A, Fielding S, Howarth K, Silver A, Atkin W, Muir K, Logan R, Kerr D, Johnstone E, Sieber O, Gray R, Thomas H, Peto J, Cazier JB, Houlston R. A genome-wide association scan of tag SNPs identifies a susceptibility variant for colorectal cancer at 8q24.21. *Nat Genet* 2007; **39**: 984-988 [PMID: 17618284 DOI: 10.1038/ng2085]

43 **Yeager M**, Orr N, Hayes RB, Jacobs KB, Kraft P, Wacholder S, Minichiello MJ, Fearnhead P, Yu K, Chatterjee N, Wang Z, Welch R, Staats BJ, Calle EE, Feigelson HS, Thun MJ, Rodriguez C, Albanes D, Virtamo J, Weinstein S, Schumacher FR, Giovannucci E, Willett WC, Cancel-Tassin G, Cussenot O, Valeri A, Andriole GL, Gelmann EP, Tucker M, Gerhard DS, Fraumeni JF, Hoover R, Hunter DJ, Chanock SJ, Thomas G. Genome-wide association study of prostate cancer identifies a second risk locus at 8q24. *Nat Genet* 2007; **39**: 645-649 [PMID: 17401363 DOI: 10.1038/ng2022]

44 **Zanke BW**, Greenwood CM, Rangrej J, Kustra R, Tenesa A, Farrington SM, Prendergast J, Olschwang S, Chiang T, Crowdy E, Ferretti V, Laflamme P, Sundararajan S, Roumy S, Olivier JF, Robidoux F, Sladek R, Montpetit A, Campbell P, Bezieau S, O'Shea AM, Zogopoulos G, Cotterchio M, Newcomb P, McLaughlin J, Younghusband B, Green R, Green J, Porteous ME, Campbell H, Blanche H, Sahbatou M, Tubacher E, Bonaiti-Pellié C, Buecher B, Riboli E, Kury S, Chanock SJ, Potter J, Thomas G, Gallinger S, Hudson TJ, Dunlop MG. Genome-wide association scan identifies a colorectal cancer susceptibility locus on chromosome 8q24. *Nat Genet* 2007; **39**: 989-994 [PMID: 17618283 DOI: 10.1038/ng2089]

45 **Yeager M**, Xiao N, Hayes RB, Bouffard P, Desany B, Burdett L, Orr N, Matthews C, Qi L, Crenshaw A, Markovic Z, Fredrikson KM, Jacobs KB, Amundadottir L, Jarvie TP, Hunter DJ, Hoover R, Thomas G, Harkins TT, Chanock SJ. Comprehensive resequence analysis of a 136 kb region of human chromosome 8q24 associated with prostate and colon cancers. *Hum Genet* 2008; **124**: 161-170 [PMID: 18704501 DOI: 10.1007/s00439-008-0535-3]

46 **Tuupanen S**, Turunen M, Lehtonen R, Hallikas O, Vanharanta S, Kivioja T, Björklund M, Wei G, Yan J, Niittymäki I, Mecklin JP, Järvinen H, Ristimäki A, Di-Bernardo M, East P, Carvajal-Carmona L, Houlston RS, Tomlinson I, Palin K, Ukkonen E, Karhu A, Taipale J, Aaltonen LA. The common colorectal cancer predisposition SNP rs6983267 at chromosome 8q24 confers potential to enhanced Wnt signaling. *Nat Genet* 2009; **41**: 885-890 [PMID: 19561604 DOI: 10.1038/ng.406]

47 **Pomerantz MM**, Ahmadiyeh N, Jia L, Herman P, Verzi MP, Doddapaneni H, Beckwith CA, Chan JA, Hills A, Davis M, Yao K, Kehoe SM, Lenz HJ, Haiman CA, Yan C, Henderson BE, Frenkel B, Barretina J, Bass A, Tabernero J, Baselga J, Regan MM, Manak JR, Shivdasani R, Coetzee GA, Freedman ML. The 8q24 cancer risk variant rs6983267 shows long-range interaction with MYC in colorectal cancer. *Nat Genet* 2009; **41**: 882-884 [PMID: 19561607 DOI: 10.1038/ng.403]

48 **Ahmadiyeh N**, Pomerantz MM, Grisanzio C, Herman P, Jia L, Almendro V, He HH, Brown M, Liu XS, Davis M, Caswell JL, Beckwith CA, Hills A, Macconaill L, Coetzee GA, Regan MM, Freedman ML. 8q24 prostate, breast, and colon cancer risk loci show tissue-specific long-range interaction with MYC. *Proc Natl Acad Sci USA* 2010; **107**: 9742-9746 [PMID: 20453196 DOI: 10.1073/pnas.0910668107]

49 **Jäger R**, Migliorini G, Henrion M, Kandaswamy R, Speedy HE, Heindl A, Whiffin N, Carnicer MJ, Broome L, Dryden N, Nagano T, Schoenfelder S, Enge M, Yuan Y, Taipale J, Fraser P, Fletcher O, Houlston RS. Capture Hi-C identifies the chromatin interactome of colorectal cancer risk loci. *Nat Commun* 2015; **6**: 6178 [PMID: 25695508 DOI: 10.1038/ncomms7178]

50 **Sotelo J**, Esposito D, Duhagon MA, Banfield K, Mehalko J, Liao H, Stephens RM, Harris TJ, Munroe DJ, Wu X. Long-range enhancers on 8q24 regulate c-Myc. *Proc Natl Acad Sci USA* 2010; **107**: 3001-3005 [PMID: 20133699 DOI: 10.1073/pnas.0906067107]

51 **Wright JB**, Brown SJ, Cole MD. Upregulation of c-MYC in cis through a large chromatin loop linked to a cancer risk-associated single-nucleotide polymorphism in colorectal cancer cells. *Mol Cell Biol* 2010; **30**: 1411-1420 [PMID: 20065031 DOI: 10.1128/MCB.01384-09]

52 **Sur IK**, Hallikas O, Vähärautio A, Yan J, Turunen M, Enge M, Taipale M, Karhu A, Aaltonen LA, Taipale J. Mice lacking a Myc enhancer that includes human SNP rs6983267 are resistant to intestinal tumors. *Science* 2012; **338**: 1360-1363 [PMID: 23118011 DOI: 10.1126/science.1228606]

53 **McCart AE**, Vickaryous NK, Silver A. Apc mice: models, modifiers and mutants. *Pathol Res Pract* 2008; **204**: 479-490 [PMID: 18538487 DOI: 10.1016/j.prp.2008.03.004]

54 **Tomasetti C**, Vogelstein B. Cancer etiology. Variation in cancer risk among tissues can be explained by the number of stem cell divisions. *Science* 2015; **347**: 78-81 [PMID: 25554788 DOI: 10.1126/science.1260825]

55 **Yao L**, Tak YG, Berman BP, Farnham PJ. Functional annotation of colon cancer risk SNPs. *Nat Commun* 2014; **5**: 5114 [PMID: 25268989 DOI: 10.1038/ncomms6114]

56 **Ling H**, Spizzo R, Atlasi Y, Nicoloso M, Shimizu M, Redis RS, Nishida N, Gafà R, Song J, Guo Z, Ivan C, Barbarotto E, De Vries I, Zhang X, Ferracin M, Churchman M, van Galen JF, Beverloo BH, Shariati M, Haderk F, Estecio MR, Garcia-Manero G, Patijn GA, Gotley DC, Bhardwaj V, Shureiqi I, Sen S, Multani AS, Welsh J, Yamamoto K, Taniguchi I, Song MA, Gallinger S, Casey G, Thibodeau SN, Le Marchand L, Tiirikainen M, Mani SA, Zhang W, Davuluri RV, Mimori K, Mori M, Sieuwerts AM, Martens JW, Tomlinson I, Negrini M, Berindan-Neagoe I, Foekens JA, Hamilton SR, Lanza G, Kopetz S, Fodde R, Calin GA. CCAT2, a novel noncoding RNA mapping to 8q24, underlies metastatic progression and chromosomal instability in colon cancer. *Genome Res* 2013; **23**: 1446-1461 [PMID: 23796952 DOI: 10.1101/gr.152942.112]

57 **Kim T**, Cui R, Jeon YJ, Lee JH, Lee JH, Sim H, Park JK, Fadda P, Tili E, Nakanishi H, Huh MI, Kim SH, Cho JH, Sung BH, Peng Y, Lee TJ, Luo Z, Sun HL, Wei H, Alder H, Oh JS, Shim KS, Ko SB, Croce CM. Long-range interaction and correlation between MYC enhancer and oncogenic long noncoding RNA CARLo-5. *Proc Natl Acad Sci USA* 2014; **111**: 4173-4178 [PMID: 24594601 DOI: 10.1073/pnas.1400350111]

58 **Bottomly D**, Kyler SL, McWeeney SK, Yochum GS. Identification of {beta}-catenin binding regions in colon cancer cells using ChIP-Seq. *Nucleic Acids Res* 2010; **38**: 5735-5745 [PMID: 20460455 DOI: 10.1093/nar/gkq363]

59 **Yochum GS**. Multiple Wnt/ß-catenin responsive enhancers align with the MYC promoter through long-range chromatin loops. *PLoS One* 2011; **6**: e18966 [PMID: 21533051 DOI: 10.1371/journal.pone.0018966]

60 **Heintzman ND**, Hon GC, Hawkins RD, Kheradpour P, Stark A, Harp LF, Ye Z, Lee LK, Stuart RK, Ching CW, Ching KA, Antosiewicz-Bourget JE, Liu H, Zhang X, Green RD, Lobanenkov VV, Stewart R, Thomson JA, Crawford GE, Kellis M, Ren B. Histone modifications at human enhancers reflect global cell-type-specific gene expression. *Nature* 2009; **459**: 108-112 [PMID: 19295514 DOI: 10.1038/nature07829]

61 **Hnisz D**, Schuijers J, Lin CY, Weintraub AS, Abraham BJ, Lee TI, Bradner JE, Young RA. Convergence of developmental and oncogenic signaling pathways at transcriptional super-enhancers. *Mol Cell* 2015; **58**: 362-370 [PMID: 25801169 DOI: 10.1016/j.molcel.2015.02.014]

62 **Whyte WA**, Orlando DA, Hnisz D, Abraham BJ, Lin CY, Kagey MH, Rahl PB, Lee TI, Young RA. Master transcription factors and mediator establish super-enhancers at key cell identity genes. *Cell* 2013; **153**: 307-319 [PMID: 23582322 DOI: 10.1016/j.cell.2013.03.035]

63 **Lovén J**, Hoke HA, Lin CY, Lau A, Orlando DA, Vakoc CR, Bradner JE, Lee TI, Young RA. Selective inhibition of tumor oncogenes by disruption of super-enhancers. *Cell* 2013; **153**: 320-334 [PMID: 23582323 DOI: 10.1016/j.cell.2013.03.036]

64 **Jang MK**, Mochizuki K, Zhou M, Jeong HS, Brady JN, Ozato K. The bromodomain protein Brd4 is a positive regulatory component of P-TEFb and stimulates RNA polymerase II-dependent transcription. *Mol Cell* 2005; **19**: 523-534 [PMID: 16109376 DOI: 10.1016/j.molcel.2005.06.027]

65 **Yang Z**, Yik JH, Chen R, He N, Jang MK, Ozato K, Zhou Q. Recruitment of P-TEFb for stimulation of transcriptional elongation by the bromodomain protein Brd4. *Mol Cell* 2005; **19**: 535-545 [PMID: 16109377 DOI: 10.1016/j.molcel.2005.06.029]

66 **Kagey MH**, Newman JJ, Bilodeau S, Zhan Y, Orlando DA, van Berkum NL, Ebmeier CC, Goossens J, Rahl PB, Levine SS, Taatjes DJ, Dekker J, Young RA. Mediator and cohesin connect gene expression and chromatin architecture. *Nature* 2010; **467**: 430-435 [PMID: 20720539 DOI: 10.1038/nature09380]

67 **Filippakopoulos P**, Qi J, Picaud S, Shen Y, Smith WB, Fedorov O, Morse EM, Keates T, Hickman TT, Felletar I, Philpott M, Munro S, McKeown MR, Wang Y, Christie AL, West N, Cameron MJ, Schwartz B, Heightman TD, La Thangue N, French CA, Wiest O, Kung AL, Knapp S, Bradner JE. Selective inhibition of BET bromodomains. *Nature* 2010; **468**: 1067-1073 [PMID: 20871596 DOI: 10.1038/nature09504]

68 **Delmore JE**, Issa GC, Lemieux ME, Rahl PB, Shi J, Jacobs HM, Kastritis E, Gilpatrick T, Paranal RM, Qi J, Chesi M, Schinzel AC, McKeown MR, Heffernan TP, Vakoc CR, Bergsagel PL, Ghobrial IM, Richardson PG, Young RA, Hahn WC, Anderson KC, Kung AL, Bradner JE, Mitsiades CS. BET bromodomain inhibition as a therapeutic strategy to target c-Myc. *Cell* 2011; **146**: 904-917 [PMID: 21889194 DOI: 10.1016/j.cell.2011.08.017]

69 **Xiang JF**, Yin QF, Chen T, Zhang Y, Zhang XO, Wu Z, Zhang S, Wang HB, Ge J, Lu X, Yang L, Chen LL. Human colorectal cancer-specific CCAT1-L lncRNA regulates long-range chromatin interactions at the MYC locus. *Cell Res* 2014; **24**: 513-531 [PMID: 24662484 DOI: 10.1038/cr.2014.35]

70 **Nissan A**, Stojadinovic A, Mitrani-Rosenbaum S, Halle D, Grinbaum R, Roistacher M, Bochem A, Dayanc BE, Ritter G, Gomceli I, Bostanci EB, Akoglu M, Chen YT, Old LJ, Gure AO. Colon cancer associated transcript-1: a novel RNA expressed in malignant and pre-malignant human tissues. *Int J Cancer* 2012; **130**: 1598-1606 [PMID: 21547902 DOI: 10.1002/ijc.26170]

71 **Alaiyan B**, Ilyayev N, Stojadinovic A, Izadjoo M, Roistacher M, Pavlov V, Tzivin V, Halle D, Pan H, Trink B, Gure AO, Nissan A. Differential expression of colon cancer associated transcript1 (CCAT1) along the colonic adenoma-carcinoma sequence. *BMC Cancer* 2013; **13**: 196 [PMID: 23594791 DOI: 10.1186/1471-2407-13-196]

72 **de Wit E**, de Laat W. A decade of 3C technologies: insights into nuclear organization. *Genes Dev* 2012; **26**: 11-24 [PMID: 22215806 DOI: 10.1101/gad.179804.111]

73 **Jia L**, Landan G, Pomerantz M, Jaschek R, Herman P, Reich D, Yan C, Khalid O, Kantoff P, Oh W, Manak JR, Berman BP, Henderson BE, Frenkel B, Haiman CA, Freedman M, Tanay A, Coetzee GA. Functional enhancers at the gene-poor 8q24 cancer-linked locus. *PLoS Genet* 2009; **5**: e1000597 [PMID: 19680443 DOI: 10.1371/journal.pgen.1000597]

74 **Vennstrom B**, Sheiness D, Zabielski J, Bishop JM. Isolation and characterization of c-myc, a cellular homolog of the oncogene (v-myc) of avian myelocytomatosis virus strain 29. *J Virol* 1982; **42**: 773-779 [PMID: 6284994]

75 **Dang CV**. MYC on the path to cancer. *Cell* 2012; **149**: 22-35 [PMID: 22464321 DOI: 10.1016/j.cell.2012.03.003]

76 **Prochownik EV**, Vogt PK. Therapeutic Targeting of Myc. *Genes Cancer* 2010; **1**: 650-659 [PMID: 21132100 DOI: 10.1177/1947601910377494]

77 **Meyer N**, Penn LZ. Reflecting on 25 years with MYC. *Nat Rev Cancer* 2008; **8**: 976-990 [PMID: 19029958 DOI: 10.1038/nrc2231]

78 **Mertz JA**, Conery AR, Bryant BM, Sandy P, Balasubramanian S, Mele DA, Bergeron L, Sims RJ. Targeting MYC dependence in cancer by inhibiting BET bromodomains. *Proc Natl Acad Sci USA* 2011; **108**: 16669-16674 [PMID: 21949397 DOI: 10.1073/pnas.1108190108]

79 **Zuber J**, Shi J, Wang E, Rappaport AR, Herrmann H, Sison EA, Magoon D, Qi J, Blatt K, Wunderlich M, Taylor MJ, Johns C, Chicas A, Mulloy JC, Kogan SC, Brown P, Valent P, Bradner JE, Lowe SW, Vakoc CR. RNAi screen identifies Brd4 as a therapeutic target in acute myeloid leukaemia. *Nature* 2011; **478**: 524-528 [PMID: 21814200 DOI: 10.1038/nature10334]

80 **Shao Q**, Kannan A, Lin Z, Stack BC, Suen JY, Gao L. BET protein inhibitor JQ1 attenuates Myc-amplified MCC tumor growth in vivo. *Cancer Res* 2014; **74**: 7090-7102 [PMID: 25277525 DOI: 10.1158/0008-5472.CAN-14-0305]

81 **Kim TK**, Hemberg M, Gray JM, Costa AM, Bear DM, Wu J, Harmin DA, Laptewicz M, Barbara-Haley K, Kuersten S, Markenscoff-Papadimitriou E, Kuhl D, Bito H, Worley PF, Kreiman G, Greenberg ME. Widespread transcription at neuronal activity-regulated enhancers. *Nature* 2010; **465**: 182-187 [PMID: 20393465 DOI: 10.1038/nature09033]

82 **Lam MT**, Cho H, Lesch HP, Gosselin D, Heinz S, Tanaka-Oishi Y, Benner C, Kaikkonen MU, Kim AS, Kosaka M, Lee CY, Watt A, Grossman TR, Rosenfeld MG, Evans RM, Glass CK. Rev-Erbs repress macrophage gene expression by inhibiting enhancer-directed transcription. *Nature* 2013; **498**: 511-515 [PMID: 23728303 DOI: 10.1038/nature12209]

83 **Wang D**, Garcia-Bassets I, Benner C, Li W, Su X, Zhou Y, Qiu J, Liu W, Kaikkonen MU, Ohgi KA, Glass CK, Rosenfeld MG, Fu XD. Reprogramming transcription by distinct classes of enhancers functionally defined by eRNA. *Nature* 2011; **474**: 390-394 [PMID: 21572438 DOI: 10.1038/nature10006]

84 **Hah N**, Danko CG, Core L, Waterfall JJ, Siepel A, Lis JT, Kraus WL. A rapid, extensive, and transient transcriptional response to estrogen signaling in breast cancer cells. *Cell* 2011; **145**: 622-634 [PMID: 21549415 DOI: 10.1016/j.cell.2011.03.042]

85 **Li W**, Notani D, Ma Q, Tanasa B, Nunez E, Chen AY, Merkurjev D, Zhang J, Ohgi K, Song X, Oh S, Kim HS, Glass CK, Rosenfeld MG. Functional roles of enhancer RNAs for oestrogen-dependent transcriptional activation. *Nature* 2013; **498**: 516-520 [PMID: 23728302 DOI: 10.1038/nature12210]

86 **Schaukowitch K**, Joo JY, Liu X, Watts JK, Martinez C, Kim TK. Enhancer RNA facilitates NELF release from immediate early genes. *Mol Cell* 2014; **56**: 29-42 [PMID: 25263592 DOI: 10.1016/j.molcel.2014.08.023]

87 **Estarás C**, Benner C, Jones KA. SMADs and YAP compete to control elongation of β-catenin: LEF-1-recruited RNAPII during hESC differentiation. *Mol Cell* 2015; **58**: 780-793 [PMID: 25936800 DOI: 10.1016/j.molcel.2015.04.001]

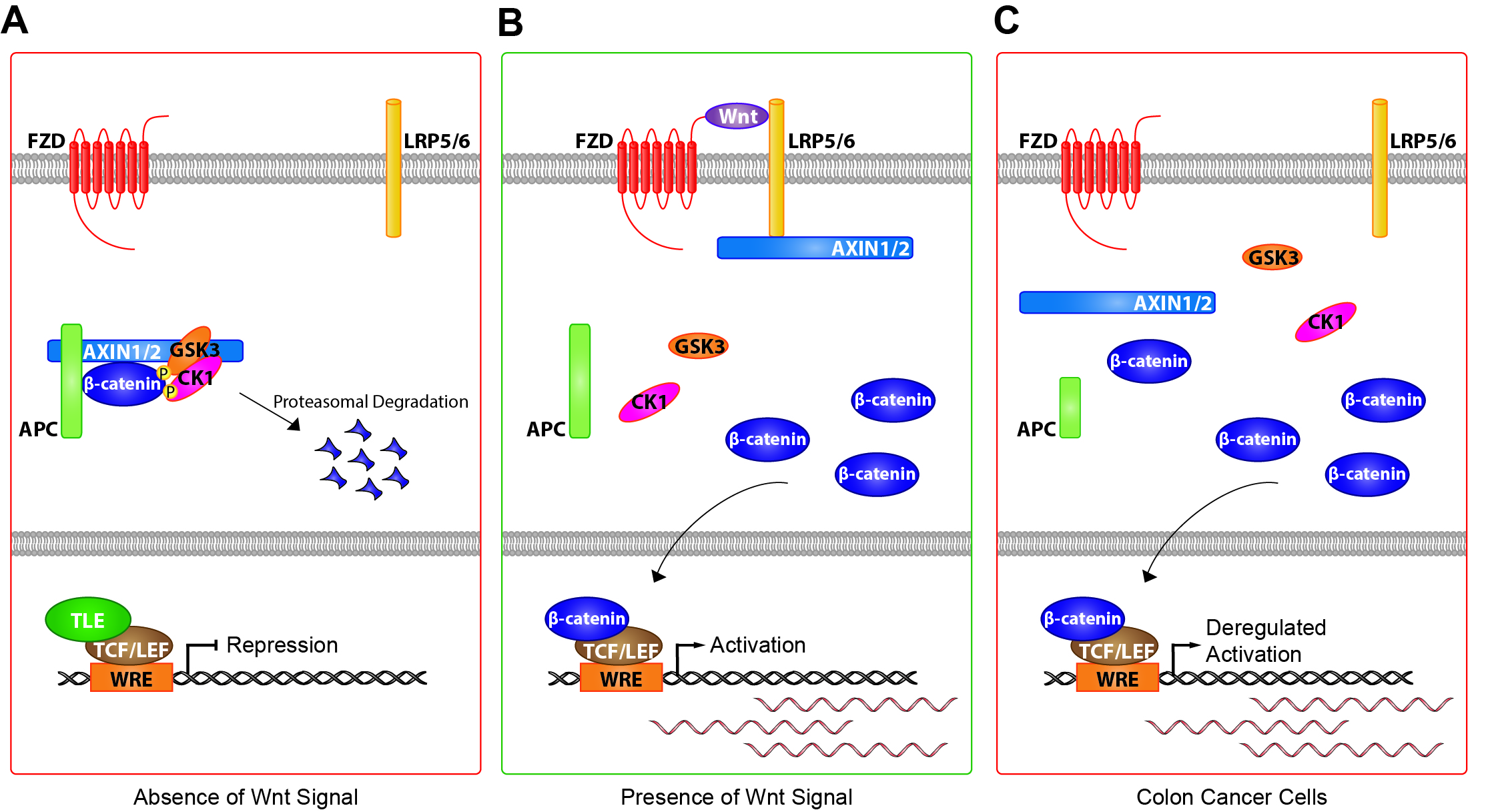
88 **Hnisz D**, Abraham BJ, Lee TI, Lau A, Saint-André V, Sigova AA, Hoke HA, Young RA. Super-enhancers in the control of cell identity and disease. *Cell* 2013; **155**: 934-947 [PMID: 24119843 DOI: 10.1016/j.cell.2013.09.053]

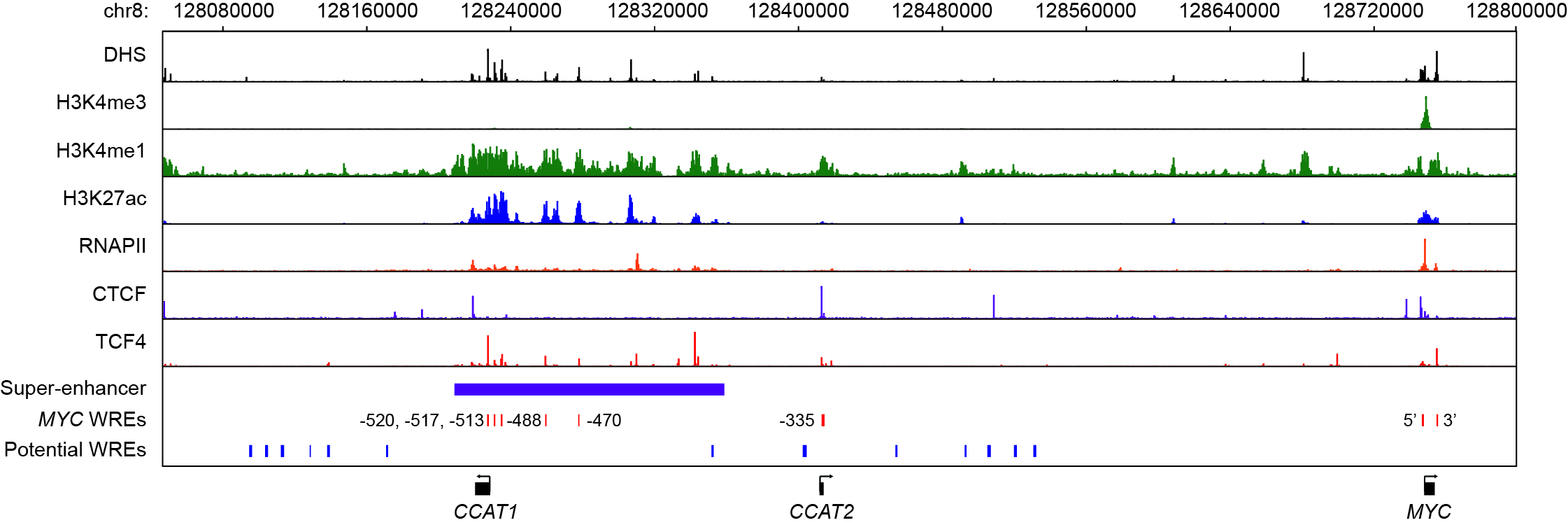
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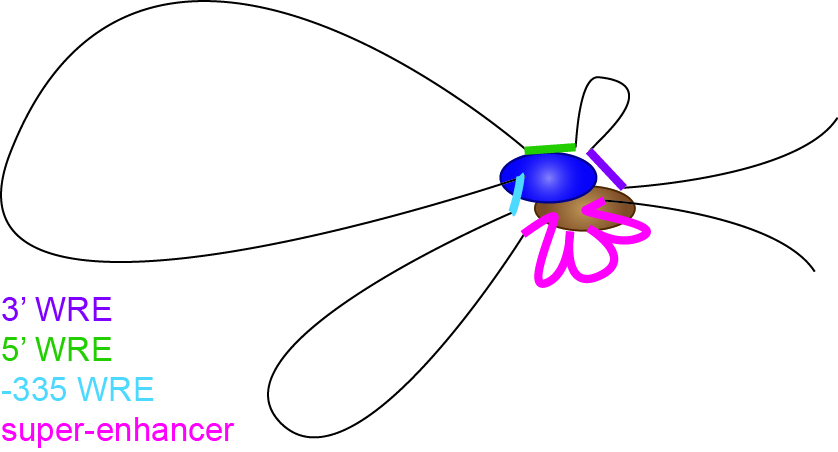
**Table 1 *MYC* Wnt responsive DNA element chromosomal locations**

|  |  |  |  |
| --- | --- | --- | --- |
| ***MYC* WRE** | **Chromosomal position** | **Distance to *MYC* TSS** | **Ref.** |
| *MYC* 5’ WREs | chr8: 128747425 | 89-bp upstream | [14,26] |
| *MYC* 3’ WRE | chr8: 128755419 | 7105-bp downstream | [29-31] |
| *Myc* 3’ WRE | chr15: 61992066 | 6725-bp downstream | [32,33] |
| *MYC* -335 WRE | chr8: 128413279 | 335036-bp upstream | [46-51,55,56] |
| *Myc* -335 | chr15: 61450712 | 534630-bp upstream | [52] |
| *MYC* -520 WRE | chr8: 128227619 | 520696-bp upstream | [59,61] |
| *MYC* -517 WRE | chr8: 128231269 | 517046-bp upstream | [59] |
| *MYC* -513 WRE | chr8: 128235209 | 513106-bp upstream | [59,61] |
| *MYC* -488 WRE | chr8: 128259779 | 488536-bp upstream | [59,61] |
| *MYC* -470 WRE | chr8: 128278129 | 470186-bp upstream | [59,61] |
| *MYC* super-enhancer | chr8: 128275000 | 473314-bp upstream | [61,69] |

TSS: Transcription start site; WRE: Wnt responsive DNA element.

**Figure 1 The Wnt/β-catenin signaling pathway**.A: In the absence of a Wnt ligand, cytoplasmic β-catenin is targeted for proteasomal degradation by a multi-protein “destruction complex”. Within the nucleus, TCF/Lef at WREs associates with the TLE co-repressor to repress Wnt/β-catenin target gene expression; B: Upon Wnt ligand binding to the FZD and LRP5/6 co-receptor complex, AXIN1/2 is recruited to the plasma membrane and the destruction complex is inactivated. β-Catenin subsequently translocates to the nucleus where it binds TCF/Lef assembled at WREs and recruits co-activator complexes to activate Wnt/β-catenin target gene expression; C: In CRCs, truncations of the APC protein prevent efficient targeting of β-catenin for proteasomal degradation. Therefore, nuclear β-catenin levels are elevated and aberrantly associate with TCF/Lef at WREs to drive deregulated expression of Wnt/β-catenin target genes. WRE: Wnt responsive DNA element; TCF/Lef: T-cell factor/Lymphoid enhancer factor; TLE: Transducin-like enhancer; FZD: Frizzled; LRP5/6: Liproprotein receptor-related protein 5 or 6; AXIN1/2: Axis inhibition proteins 1 and/or 2; CRC: Colorectal cancer; APC: Adenomatous polyposis coli; GSK3: Glycogen synthase kinase three; CK1: Casein kinase 1.

**Figure 2 The *MYC* genomic locus in colorectal cancer.** ChIP-Seq and DNase hypersensitivity (DHS) data in the HCT116 CRC cell line were downloaded from the WashU Epigenome Browser (http://epigenomegateway.wustl.edu/). The *MYC* distal super-enhancer is denoted as a purple rectangle, the *MYC* WREs discussed in this review are depicted as red lines, and potential WREs are denoted as blue lines[50,73]. WRE: Wnt responsive DNA element; CTCF: CCCTC-binding factor; H3K4me1: Monomethylated lysine 4 on histone H3; H3K27Ac: Acetylated lysine 27 on histone H3; RNAP: RNA Polymerase II; TCF: T-cell factor; *CCAT:* Colon-cancer associated transcript.



**Figure 3** **Model for the chromatin interaction network at the *MYC* gene locus in colorectal cancer cells with elevated nuclear β-catenin**. β-Catenin “hijacks” *MYC* WREs in CRC cells, therefore, driving or stabilizing a distinct promoter-enhancer interaction network that is required for deregulated *MYC* gene expression. TCF/Lef and β-catenin are depicted as brown and purple ovals, respectively. WRE: Wnt responsive DNA element; CRC: Colorectal cancer; TCF/Lef: T-cell factor/Lymphoid enhancer factor.