PERSPECTIVE

YY1 helps to bring loose ends together

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Ubiquitously expressed transcription factor Yin Yang 1 (YY1) has long been believed to play some role in immunoglobulin gene regulation, because it associates with multiple Ig enhancer elements including the heavy-chain intron and 3' enhancers as well as the Igk 3' enhancer (Park and Atchison 1991; Gordon et al. 2003). Early on, YY1 was noted to have bifunctional properties, in that it could either repress or activate transcription, depending on binding site context, protein interactions, or levels within the cell (Park and Atchison 1991; Seto et al. 1991; Shi et al. 1991; Lee et al. 1994, 1995; Bushmeyer et al. 1995). Numerous mechanisms of YY1 transcriptional control were observed and a number of activation/repression models were proposed (Galvin and Shi 1997). YY1 function in transcription at the Ig loci was enigmatic, however, because no evidence exists for differential expression of YY1 during B-cell development, and deletion of enhancer YY1-binding sites had a marginal impact on enhancer activity as measured by transient expression assays (Park and Atchison 1991). It was generally assumed that YY1 might control some aspect of chromatin structure at the Ig loci not completely recapitulated by transient expression assays.

Over the 16 years since its discovery, a very large number of genes regulated by YY1 were identified (Shrivastava and Calame 1994; Thomas and Seto 1999; Gordon et al. 2006). In addition, YY1 was observed to interact with a large number of proteins including coactivators, corepressors, and other transcription factors (Thomas and Seto 1999; Gordon et al. 2006). More recently, YY1 was implicated in cell cycle control, oncogenesis, imprinting control, X-chromosome inactivation, and Polycomb Group (PcG) function (Sui et al. 2004; Gordon et al. 2006; Kim et al. 2006, 2007; Donohoe et al. 2007). The huge numbers of processes believed to require YY1 function suggested that the protein would be crucial for development. Indeed, knockout of the gene results in peri-implantation lethality confirming its crucial function (Donohoe et al. 1999), but the role in B-cell development could only be revealed by the conditional knockout (CKO) approach that is used by Shi and colleagues (Liu et al. 2007) in this issue of *Genes & Development*.

Shi and colleagues (Liu et al. 2007) created CKO mice lacking the gene for YY1 in B lymphocyte progenitors and observed a block in pro-B to pre-B-cell development. They found that pro-B cells lacking YY1 have normal D_H-J_H recombination but reduced frequency of $V_H-D_HJ_H$ recombination, with the defect being most severe for more D_H -distal V_H genes. They further showed that pro-B cells lacking YY1 are defective in Ig heavy-chain (IgH) locus contraction, defined by close juxtaposition in nuclear chromatin of V and C gene segments separated from one another in the genome by large distances. Ig locus contraction has been observed previously by fluorescence in situ hybridization (FISH) in B cells undergoing V(D)J recombination (Kosak et al. 2002) and has been suggested to be mechanistically important for recombination of distal V regions. Importantly, introduction of a rearranged heavy-chain gene only partially complemented the YY1 CKO phenotype, suggesting additional roles for YY1 in early B-cell development. YY1 thus becomes the second transcription factor shown to be required for IgH locus contraction and $V_H-D_HJ_H$ rearrangement. Significantly, the results of Shi et al. (Liu et al. 2007) show that YY1 does not affect expression of Pax5, the other transcription factor known to be required for locus contraction, $V_H-D_HJ_H$ recombination, and B-cell commitment (Nutt et al. 1999; Fuxa et al. 2004). Furthermore, because faulty IgH locus contraction was the only defect related to $V_H-D_HJ_H$ recombination observed by Shi et al. (Liu et al. 2007) in the YY1 CKO pro-B cells, these data provide new support for the idea that locus contraction may, in fact, be required for $V_H-D_HJ_H$ recombination. This is particularly important because the role of locus contraction in $V_H-D_HJ_H$ recombination, as well as mechanisms regulating and mediating IgH locus contraction, remain poorly understood.

The data from Shi and colleagues (Liu et al. 2007) also help to bring together some concepts that have been at "loose ends" for understanding regulation of $V_H-D_HJ_H$ recombination. These include the roles of the core IgH intronic enhancer (iE μ), the transcription of nonrearranged IgH gene segments, and the alteration of chromatin structure in the IgH locus for locus contraction. Below, we review current understanding of the requirements for $V_H-D_HJ_H$ recombination in the context of the Shi paper (Liu et al. 2007) and discuss how YY1 may act in this process.

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V_H - D_HJ_H recombination

V(D)J recombination, a DNA rearrangement unique to immunoglobulin and T-cell receptor (TCR) genes, is highly regulated and is lineage-, developmental stage-, and allele-specific (Cobb et al. 2006; Jung et al. 2006). In developing B cells, the IgH locus rearranges before light-chain rearrangement, initiating B-lineage commitment and development (Cobb et al. 2006; Jung et al. 2006). D_HJ_H recombination occurs first, on both IgH alleles, but progenitors that have undergone D_HJ_H rearrangement are not yet committed to the B-cell lineage (Kurosawa et al. 1981). V_H - D_HJ_H recombination, which occurs after D_HJ_H recombination (Alt et al. 1984), is associated with commitment to the B-cell lineage (Nutt et al. 1999; Fuxa et al. 2004) and is also regulated to achieve heavy-chain allelic exclusion (discussed below). Given its critical role, regulation of V_H - D_HJ_H recombination has received extensive study and appears to involve *cis* DNA elements possibly including promoters, enhancers, and matrix attachment sites (MARs); *trans*-acting proteins; modifications in chromatin structure; changes in IgH chromosome subnuclear localization; and IgH locus contraction.

V_H - D_HJ_H recombination appears to occur sequentially on the two IgH alleles, allowing time to test the outcome of rearranging the first allele before the second allele is eligible for rearrangement. If rearrangement of the first allele encodes a functional μ heavy-chain protein, the transmembrane pre-B-cell receptor (pre-BCR), formed by association of μ with surrogate light chains ($\lambda 5$, Vpre-B), Ig α , and Ig β , signals the developing B cell to shut off V_H - D_HJ_H rearrangement of the second IgH allele. Only if rearrangement of the first allele is nonfunctional (i.e., out of translational reading frame) does the second allele rearrange. This sequence of events is necessary to achieve heavy-chain expression from a single allele (heavy-chain allelic exclusion) and the ultimate expression of antibody with a unique specificity on each B-cell clone. The mechanistic basis for sequential rearrangement is poorly understood. Based primarily on studies from the Ig κ locus, it may involve asynchronous replication of the two alleles (Mostoslavsky et al. 2004), monoallelic DNA looping (Sayegh et al. 2005), monoallelic activation (Liang et al. 2004), or monoallelic silencing (Liu et al. 2006). pre-BCR signaling to inhibit second allele rearrangement correlates with repressive chromatin modifications (Johnson et al. 2003; Chowdhury and Sen 2004), loss of locus contraction, and repositioning of IgH chromosomes within the nucleus (Roldan et al. 2005; Yang et al. 2005). However, it has not yet been possible to distinguish causal from correlative events in this context.

Since the V_H region covers >2.5 Mb, and the entire IgH locus is >3 Mb (Johnston et al. 2006), locus contraction, which has been observed by FISH analyses to be developmentally correlated with V_H - D_HJ_H recombination (Kosak et al. 2002), may be mechanistically important. More detailed analysis showed evidence for both locus contraction and DNA looping of the IgH locus in pre-B cells

(Sayegh et al. 2005), for locus contraction in the Ig κ locus (Roldan et al. 2005), and for decontraction of the IgH locus in pre-B cells when IgH rearrangements have ceased (Roldan et al. 2005). It has been suggested that contraction and/or looping facilitates juxtaposition of V_H gene segments with the D_HJ_H segments for V_H - D_HJ_H recombination, and that decontraction may inhibit recombination to ensure allelic exclusion. Locus contraction or looping might be especially important for recombination of the more distal V_H gene segments located >2 Mb 5' of the D_H region, bringing them into proximity with rearranged D_HJ_H gene segments so that V_H - D_HJ_H recombination can occur (Fig. 1; Roldan et al. 2005; Sayegh et al. 2005). A very recent report of reversible locus contraction in the TCR α and TCR β loci (Skok et al. 2007) provides further support for the idea that locus contraction is important for V(D)J recombination. The mechanism or protein(s) that mediate locus contraction were heretofore unknown except for the requirement for Pax5 (Fuxa et al. 2004) and Ezh2 (Su et al. 2003), underscoring the importance of the finding that YY1 is also required for this event.

Cis-acting elements in the IgH locus and the proteins that bind them

iE μ is a strong transcriptional enhancer located in the intervening sequence 3' of the J_H gene segments and 5' of C μ (Calame and Sen 2004). iE μ activates transcription of rearranged V_H promoters and other promoters in its vicinity, including $\mu 0$ (located 5' of DQ52) and I μ (within iE μ). In addition to activating transcription of rearranged heavy-chain genes, iE μ is important for V(D)J recombination of the heavy-chain locus. When recombination of a stably transfected recombination substrate was studied, iE μ was both required and sufficient for recombination (Oltz et al. 1993). More importantly, when endogenous iE μ was deleted in mice, a partial block in D_HJ_H recombination was observed and a more substantial, but still partial, block in V_H - D_HJ_H was observed, establishing

A. Binding of Pax5 and YY1 in the IgH Locus

B. Pax5 and YY1 Mediate IgH Locus Contraction

Figure 1. Models for direct action of YY1 and Pax5 in V_H - D_HJ_H recombination. See the text for details.

the importance of iEμ in heavy-chain recombination, especially V_H-D_HJ_H rearrangement (Perlot et al. 2005).

The mechanism(s) by which iEμ enhances V_H-D_HJ_H recombination is not established and analysis of mice harboring the germline deletion (Perlot et al. 2005) did not include an analysis of IgH chromosome condensation or looping. Many transcriptional regulators bind specifically to functional sites in iEμ (for review, see Calame and Sen 2004). Significantly, YY1 binds at the μE1 site (Park and Atchison 1991) and the Shi paper (Liu et al. 2007) shows binding of YY1 *in vivo* to iEμ. Pax5, the only other DNA-binding protein known to be required for V_H-D_HJ_H recombination, is not known to bind iEμ, although multiple Pax5-binding sites reside within the V_H genes themselves (discussed further below). Interestingly, although both YY1 and Pax5 bind in the 3' Cα enhancer region located at the far 3' end of the IgH locus (Gordon et al. 2003), a role for this enhancer in V_H-D_HJ_H recombination has not been demonstrated.

The action of iEμ and its associated proteins in V_H-D_HJ_H recombination could involve several mechanisms that are not mutually exclusive: (1) activation of transcription from V_H promoters or promoters near the DJ region, thereby opening chromatin structure; (2) recruitment of enzymes, such as histone acetyltransferases, histone demethylases, or chromatin remodeling complexes that are needed to form open chromatin structure; and/or (3) recruitment and/or stabilization of juxtaposed D_HJ_H and V_H gene segments during V_H-D_HJ_H recombination.

In B cells with iEμ deleted, there is a 10- to 20-fold decrease in μ0 and Iμ transcripts, but no change in unrearranged V_H transcripts (Perlot et al. 2005). If iEμ's primary mechanism in V(D)J recombination were to activate transcription of unrearranged genes, one would have expected its deletion to have a greater effect on D_H-J_H recombination than V_H-D_HJ_H recombination, because promoters in the vicinity of iEμ are activated by the enhancer, while unrearranged V_H promoters are not. In fact, the opposite is the case—deletion had a greater effect on V_H-D_HJ_H recombination (Perlot et al. 2005). It is also noteworthy that in the TCRβ locus, an enhancer has been shown to enhance D_β-J_β recombination by causing both general chromatin opening and facilitation of a complex between Dβ1 and Jβ (Oestreich et al. 2006).

The region of iEμ deleted by Perlot et al. (2005) did not include the MARs, which flank iEμ on either side (Cockerill et al. 1987). Interestingly MARs are also found near V_H regions (Webb et al. 1991; Goebel et al. 2002), providing the possibility that they might help mediate juxtaposition of V_H and D_HJ_H gene segments to facilitate V_H-D_HJ_H rearrangement. Several proteins, including transcriptional activator Bright (Herrscher et al. 1995) and the repressive cut-like protein Cux/CDP compete for binding in iEμ and V_H MARs (Wang et al. 1999; Goebel et al. 2002). The atypical homeodomain protein SATB1, which packages active chromatin (Cai et al. 2006), also binds MARs (Dickinson et al. 1997). Interestingly, YY1 can bind to the nuclear matrix (Bushmeyer and Atchison 1998; McNeil et al. 1998), but the functional significance of this interaction is unclear. An ear-

lier study addressed the role of the MARs in V_H-D_HJ_H recombination by studying embryonic stem cells having mutant IgH alleles with or without the MARs flanking iEμ (Sakai et al. 1999). Using a RAG blastocyst complementation system, these authors showed a requirement for iEμ in V_H-D_HJ_H recombination, but no role for the flanking MARs. Subsequent work confirmed the importance of iEμ for V_H-D_HJ_H recombination using mice with germline deletion (Perlot et al. 2005). Mice with germline deletion of both MARs and iEμ have not been reported. Interestingly, however, germline deletion of iEμ and the 5' MAR was reported to have a strong effect on D_H-J_H recombination, although effects on V_H-D_HJ_H recombination were not reported for this mutation (Afshar et al. 2006). Thus, a role for iEμ MARs in V_H-D_HJ_H recombination is not yet clear.

There is evidence that sequences within the V_H gene region are also important for V_H-D_HJ_H recombination. In contrast to iEμ, V_H transcriptional promoters are relatively simple, depending primarily on octamer-binding proteins Oct-1 and Oct-2, and the coactivator OCA-B. Stat5 in association with octamer proteins is necessary for histone acetylation, transcription, and rearrangement of V_H gene segments, but STAT5 does not affect IgH locus contraction (Bertolino et al. 2005). However, a 1-Mb region in the far 5' end of the V_H region associates with the nuclear lamina and may be important for subnuclear localization or condensation of IgH alleles (Yang et al. 2005). In addition, many V_H gene segments have Pax-5-binding sites in the coding regions (Zhang et al. 2006). Pax-5 is required for V_H-D_HJ_H recombination of distal but not proximal V_H gene segments (Hesslein et al. 2003), although ectopic expression of Pax5 increases recombination of proximal, not distal V_H genes (Fuxa et al. 2004). Pax-5 is necessary for IgH locus contraction (Fuxa et al. 2004) and has several known activities in the context of V_H-D_HJ_H recombination. Pax5 can recruit RAG proteins (Zhang et al. 2006) to V_H recombination signal sequences (RSSs). Although this would be expected to facilitate RAG-dependent cleavage, it is not clear whether/how RAG recruitment would affect locus contraction. Pax-5 expression also causes loss of H3K9 methylation, a repressive chromatin modification, at V_H genes (Johnson et al. 2004), and this might affect locus contraction. Pax5 binding to multiple sites in the V_H region (Zhang et al. 2006) might facilitate V_H region juxtaposition/contraction, but the absence of known Pax5 sites in iEμ or the DJ region suggests a need for other proteins as well. Apparently, none of the Pax5 activities can substitute for YY1 in the context of V_H-D_HJ_H recombination, since Pax-5 levels are normal in the YY1 CKO pro-B cells that have defective V_H-D_HJ_H recombination.

Transcription of unrearranged gene segments

Transcription of μ0, Iμ, and unrearranged V_H genes (initiating from V gene promoters) is developmentally correlated with V(D)J recombination (Yancopoulos and Alt 1985; Perlot et al. 2005; Jung et al. 2006). In addition,

antisense transcripts that are developmentally regulated and correlate with V_H - D_HJ_H recombination have been observed in the V_H region (Bolland et al. 2004). However, it remains unclear whether any of this transcription of unrearranged gene segments is required for V_H - D_HJ_H recombination or is a secondary consequence of the open chromatin structure that necessarily precedes RAG-dependent DNA cleavage. In A-MuLV-transformed pre-B lines, V_H - D_HJ_H recombination was demonstrated for V_H genes where transcription could not be detected (Angel-Duclos and Calame 1998). However, in the TCR α locus, transcription was shown to be required for V_α - J_α recombination (Abarrategui and Krangel 2006), suggesting the possibility of a similar requirement for transcription in V_H - D_HJ_H recombination.

Changes in chromatin structure of the IgH locus

In vitro, nucleosomes inhibit RAG-dependent cutting at RSSs (Kwon et al. 1998), and it is reasonable to imagine that higher order chromatin structure is important for determining whether RSS sequences are accessible to RAG proteins in vivo. Indeed, >20 years ago, Alt and colleagues (Yancopoulos and Alt 1985) suggested that chromatin accessibility of different Ig and TCR gene segments to recombination proteins was critical for lineage-, developmental stage-, and allele-specific regulation of V(D)J recombination. There is now much experimental data to support this idea. Numerous aspects of "open" chromatin have been shown to be correlated with V(D)J recombination, including nuclease sensitivity, transcription of unrearranged gene segments, histone acetylation, and lack of H3K9 methylation. However, most researchers agree that "open" chromatin appears to be necessary, but not sufficient, for V(D)J recombination (Jackson and Krangel 2006; Jung et al. 2006). Paradoxically, loss of Ezh2, a H3K27 methyltransferase typically associated with transcriptional repression, specifically decreases V(D)J recombination of distal V_H gene segments (Su et al. 2003). Nucleosome positioning over the RSSs in vivo has been reported (Baumann et al. 2003) and a requirement for nucleosome repositioning could be another determinant of accessibility. Finally, as discussed above, locus contraction is correlated with V_H - D_HJ_H recombination (Kosak et al. 2002).

Locus contraction and DNA looping observed by FISH are developmentally correlated with V_H - D_HJ_H recombination (Kosak et al. 2002; Sayegh et al. 2005). However, this raises interesting questions about the chromatin structure of the "contracted" region. Contracted or heterochromatic chromatin in other settings has characteristic repressive features including absence of histone acetylation, the presence of H3K9 methylation, and presence of HP1. However, during V_H - D_HJ_H recombination, the IgH locus is apparently both contracted and at the same time has an open and accessible chromatin structure characterized by histone acetylation, loss of H3K9 methylation, nuclease sensitivity, germline transcription, and accessibility to RAG-dependent cleavage. YY1 interacts with numerous chromatin-modifying proteins

including histone acetyltransferases, deacetylases, and methyltransferases (Thomas and Seto 1999; Rezai-Zadeh et al. 2003), and these interactions may contribute to IgH locus contraction and V_H - D_HJ_H rearrangement. Locus contraction may reflect looping back and tethering of the involved chromosomal segments rather than formation of compacted, inactive chromatin. In any case, the requirement for YY1 in locus contraction may provide entrée into characterizing the apparently unusual chromatin structure of the IgH locus during V_H - D_HJ_H rearrangement and may help us understand how the IgH region is simultaneously contracted and accessible to RAG-dependent recombination. It will also be important to determine whether YY1 is also required for recombination of the TCR α and TCR β loci, where locus contraction has been observed (Skok et al. 2007).

YY1 and V_H - D_HJ_H recombination

The phenotype of mice lacking YY1 in their B-cell progenitors is quite striking for several reasons. First, as described above, this is only the second transcription factor that when mutated yields a distal V gene rearrangement defect. Knockout of the Pax5 gene leads to arrest at the pro-B-cell stage with a similar defect in Ig locus contraction and distal V gene rearrangement (Fuxa et al. 2004). Second, the defect is similar to that observed by knockout of the PcG protein Ezh2, which also results in reduction of distal V gene rearrangement. Third, the above two points suggest a possible convergence of Pax5, YY1, and Ezh2 mechanisms in Ig locus contraction (described more fully below). Finally, the YY1 knockout defect is only partially reversed by provision of a functionally rearranged IgH gene, implying that functions in addition to IgH distal V gene rearrangement are disrupted by loss of YY1. A role in light-chain rearrangement could also be affected by YY1 knockout, or cell proliferation and expansion of various B-cell stages could be affected. These possibilities are not mutually exclusive.

A major unresolved question is whether the YY1 mutant phenotype affecting V_H - D_HJ_H recombination observed by Shi and colleagues (Liu et al. 2007) is due to a direct effect caused by YY1 DNA-binding activity within the Ig loci, or whether the phenotype is an indirect effect caused by YY1 gene regulation activities. Shi and colleagues (Liu et al. 2007) argue for a direct effect partly due to the observation that YY1 CKO does not affect expression of any of the proteins known to be involved in V(D)J rearrangement. According to the direct effect scenario, YY1 binds to the iE μ enhancer and perhaps to sites within the V_H regions (although such V_H -binding sites were not detected in initial chromatin immunoprecipitation [ChIP] studies). Pax5, along with YY1 or other proteins, binding to the IgH locus might stabilize rosette-like loops (see Fig. 1A), perhaps via self-association or interaction with additional proteins. Indeed, measurements of distances from the constant region gene segments to various V_H gene segments using high-resolution FISH are most consistent with a rosette or flower

structure for the V_H region (C. Murre, pers. comm.). Further contraction of V_H rosettes toward the constant region could be mediated by Pax5 and YY1 interacting with either a common structural component (such as the nuclear matrix) or by direct or indirect protein interactions (Fig. 1B). Possible direct effects by YY1 on Ig locus contraction are also implicated by its known associations with chromosomal structure mechanisms. YY1 can associate with the nuclear matrix (Bushmeyer and Atchison 1998; McNeil et al. 1998) and multiple YY1-binding sites lie within Imprinting Control Regions (Kim et al. 2006, 2007). In addition, YY1 serves as a cofactor with boundary factor Ctf and is implicated in X-chromosome inactivation (Donohoe et al. 2007). How all these functions relate to Ig locus contraction remain unclear, but the role of YY1 in these processes argues for YY1 involvement in global or large-scale regulatory mechanisms.

YY1 might also function in association with Pax5 in contraction and rearrangement. As mentioned above, the only other transcription factor with clear links to the Ig locus contraction mechanism is Pax5 (Fuxa et al. 2004; Zhang et al. 2006). Zhang et al. (2006) showed that Pax5 can stimulate V_H rearrangement as measured by *in vitro* assays and can even stimulate rearrangement of endogenous V_H genes in 293T cells when cotransfected with Rag1, Rag2, and E47. The similarity of YY1 and Pax5 knockout phenotypes suggests that they function in the same contraction and rearrangement processes. Since YY1 and Pax5 cannot compensate for one another in these mechanisms, it could be argued that they function either together or in cooperation with one another. Zhang et al. (2006) noted that many V_H regions contain Pax5-binding sites, and these sites can bind to Pax5 *in vivo* as assayed by ChIP assay.

These V_H gene sequences have been evaluated for potential YY1-binding sites, though they are degenerate, and therefore difficult to identify. However, nearly all YY1-binding sites contain the core sequence CCAT (ATGG in the reverse complement) (Hyde-DeRuyscher et al. 1995). Remarkably, 73% of the 185 individual V_H1 , V_H3 , V_H4 , V_HJ558 , V_HJ183 , and V_HS107 genes contained one or more YY1 core sequences within 10 base pairs (bp) of a Pax5 site. While these sites are defined by only a 4-bp consensus, the close alignment of Pax5 and potential YY1 sites suggests that YY1 might cooperate with Pax5 either via DNA binding or by cooperating in protein interactions involved in the contraction or rearrangement processes. This possibility could be tested by assaying for cooperative Pax5–YY1 DNA binding by electrophoretic mobility shift assay and for increased VDJ rearrangement by transfection experiments.

Whether or not YY1 and Pax5 cooperate in Ig locus contraction, a major unresolved question is the identity of the proteins that interact with YY1 and Pax5 to mediate the mechanism. The PcG connection to distal V gene rearrangement is intriguing. Mutation of the PcG protein Ezh2 results in reduction in distal V_H gene rearrangement (Su et al. 2003). PcG function has been proposed to be involved in Ig locus contraction, but the

mechanism of targeting PcG proteins to the Ig locus is unknown. PcG proteins can assemble on DNA-binding elements called Polycomb Response Elements, but the individual PcG proteins do not bind to DNA with sequence specificity. Thus, the mechanism of their targeting to specific DNA sequences is unclear. Interestingly, YY1 is now known to function as a PcG protein and YY1 can recruit other PcG proteins to DNA, including Ezh2 (Atchison et al. 2003; Caretti et al. 2004; Srinivasan and Atchison 2004; Srinivasan et al. 2005; Wilkinson et al. 2006). Is YY1 PcG function needed for the Ig locus contraction mechanism? Fortunately, a functional test for the role of YY1 PcG function in Ig contraction, distal V_H rearrangement, and B-cell development is possible. A YY1 mutant (YY1 Δ 201–226) exists that selectively ablates YY1 PcG function, but which retains other known YY1 functions (Wilkinson et al. 2006). This mutant, expressed in YY1-null B cells, would enable one to determine the importance of YY1 PcG function for Ig locus contraction and for development past the pro-B-cell stage. If YY1 PcG function is needed for Ig locus contraction, YY1 might recruit PcG proteins to the Ig locus, which would then, in some presently unclear fashion, contribute to the locus contraction mechanism. YY1 might recruit PcG proteins either by binding adjacent to the Pax5 sites discussed above or by binding to the iE μ enhancer. Additional ChIP studies will be needed to explore these possibilities.

Additional YY1 functions in B-cell development

Even if YY1 PcG function is important for Ig locus contraction, additional mechanisms are affected by the YY1 CKO. Expression of a rearranged V_H gene only partially rescued the pro-B arrest defect caused by YY1 knockout. Two additional mechanisms are possibly affected. The first is simply an extension of the IgH locus contraction defect to include light-chain contraction. If light-chain loci cannot contract, B-cell development will be impaired. The Shi laboratory reports that Ig κ locus contraction is indeed affected by the YY1 CKO (Y. Shi pers. comm.). A second possible mechanism relates to the known effects of YY1 on cell proliferation. Knockout of YY1 can significantly impact cell growth, and thus, a stage-specific requirement for YY1 in controlling cell proliferation may exist (Sui et al. 2004; Affar et al. 2006). It should be noted that the PcG protein Bmi-1 can also control cell proliferation and is known to be necessary for hematopoietic stem cell self-renewal (Jacobs et al. 1999; Iwama et al. 2004). Thus, loss of YY1 may negatively impact both Ig locus contraction and B-cell proliferation and expansion.

The known links of YY1 with epigenetic, global chromosomal structural, and cell-proliferation mechanisms provide possible connection points between numerous control pathways. The mutant YY1 phenotype detected by Shi and colleagues (Liu et al. 2007) provides an important piece in the puzzle of early B-cell development and Ig locus contraction and rearrangement. Key proteins that are recruited to DNA by YY1 represent an

important next step in deciphering the Ig locus contraction and $V_H\text{-}D_HJ_H$ rearrangement processes.

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