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Function of YY1 in long-distance DNA interactions

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During B cell development, long-distance DNA interactions are needed for V(D)J somatic rearrangement of the immunoglobulin (Ig) loci to produce functional Ig genes, and for class switch recombination (CSR) needed for antibody maturation. The tissue-specificity and developmental timing of these mechanisms is a subject of active investigation. A small number of factors are implicated in controlling Ig locus long-distance interactions including Pax5, Yin Yang 1 (YY1), EZH2, IKAROS, CTCF, cohesin, and condensin proteins. Here we will focus on the role of YY1 in controlling these mechanisms. YY1 is a multifunctional transcription factor involved in transcriptional activation and repression, X chromosome inactivation, Polycomb Group (PcG) protein DNA recruitment, and recruitment of proteins required for epigenetic modifications (acetylation, deacetylation, methylation, ubiquitination, sumoylation, etc.). YY1 conditional knock-out indicated that YY1 is required for B cell development, at least in part, by controlling long-distance DNA interactions at the immunoglobulin heavy chain and Igκ loci. Our recent data show that YY1 is also required for CSR. The mechanisms implicated in YY1 control of long-distance DNA interactions include controlling non-coding antisense RNA transcripts, recruitment of PcG proteins to DNA, and interaction with complexes involved in long-distance DNA interactions including the cohesin and condensin complexes. Though common rearrangement mechanisms operate at all Ig loci, their distinct temporal activation along with the ubiquitous nature of YY1 poses challenges for determining the specific mechanisms of YY1 function in these processes, and their regulation at the tissue-specific and B cell stage-specific level. The large numbers of post-translational modifications that control YY1 functions are possible candidates for regulation.

Keywords: YY1, polycomb, condensin, cohesin, DNA loops, immunoglobulin loci

THE EARLY DAYS

Yin Yang 1 (YY1) was first identified in 1985 as a factor that yielded an *in vivo* B cell-specific DMS methylation interference pattern over the immunoglobulin heavy chain (IgH) intron enhancer (1, 2). The enhancer site that bound YY1 was defined as the μE1 site (3) and nuclear factors that bound to this sequence were identified by EMSA (4). Our laboratory isolated a cDNA clone expressing a protein that bound to the Igκ3′ enhancer as well as the IgH μE1 site and named the protein NF-E1 (5). Simultaneously the factor was cloned by Tom Shenk's laboratory and named YY1 (6) based on its ability to bind the adenoviral P1 promoter and both activate and repress transcription, by Robert Perry's laboratory and named delta (7) due to its binding to the delta motif in the promoters of ribosomal protein genes, and by Keiko Ozato's laboratory and named UCRBP based on its ability to bind to the upstream control region of retroviral LTRs (8). Ultimately, the name YY1 was adopted by all.

Yin Yang 1 contains four zinc fingers at its carboxyl terminus (amino acids 298–414) and a region rich in alanine and glycine between amino acids 154 and 201. The first 100 amino acids of YY1 encode several notable features. Sequences 43–53 contain 11 consecutive acidic residues while amino acids 70–80 consist of 11 consecutive histidine residues. These two segments are separated by a region rich in glycine (residues 54–69). In addition, sequences 16–29 have the potential to form an amphipathic negatively charged helix and sequences 80–100 are rich in proline and glutamine. Sequences near the carboxyl terminus (333–397), which overlap the YY1 zinc fingers, and sequences 170–200 have been reported to be involved in transcriptional repression (6, 9–15). These sequences are known to physically interact with a variety of transcriptionally important proteins including TBP, p300, c-myc, and HDAC2 (16). YY1 sequences important for transcriptional activation reside near the amino-terminus (9, 12, 13, 17). **Figure 1** shows various sequence features and functional domains of YY1.

DIVERSE AND COMPLEX ROLES OF YY1

Over the past 22 years, multiple diverse YY1 functions have been identified. YY1 is crucial for embryonic development because homozygous mutation of the *yy1* gene in mice results in peri-implantation lethality (18). YY1 is implicated in lineage differentiation of skeletal and cardiac muscle, and in cell growth control (13, 17, 19–24), as well as disease pathways such as dystrophic muscle disease (25–27). YY1 and its target genes are also believed to be central regulators of germinal center B cell development (28), and YY1 has been suggested to regulate genomic targeting of activation induced cytidine deaminase (AID) (29). YY1 is implicated in a number of cancers (30–32), and is overexpressed in B cell lymphomas that depend on AID function. YY1 is associated with B cell transformation and tumor progression in diffuse

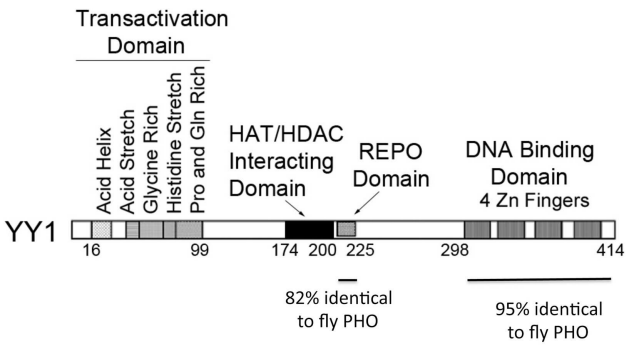

FIGURE 1 | Diagram of YY1 domains and functions. Domains of YY1 are indicated with specific functions listed. The regions of similarity to *Drosophila* Plieohomeotic are indicated below the diagram.

large B cell lymphoma (DLBCL) (33, 34), and high levels of YY1 expression are associated with reduced patient survival in DLBCL as well as follicular lymphoma. CTCF–YY1 elements are clustered in the imprinting domain of Tsix (35) and YY1 docks Xist particles on the X chromosome via DNA and RNA interactions during X chromosome inactivation (36). YY1 can also control imprinting at the Peg3 and Gnas domains (37). YY1 can control human immunodeficiency virus (HIV) gene expression and viral titers, and deletion of YY1 binding sites in regulatory regions of human papilloma viruses correlates with increased viral gene expression and the development of cervical cancer (38–46). Thus, YY1 function is related to transcriptional regulation, embryonic development, X-chromosome inactivation, imprinting, oncogenesis, viral gene expression, epigenetic function, and a growing list of diseases.

IDENTIFICATION OF THE PcG FUNCTION OF YY1

A significant new function of YY1 was suggested in 1998 when the Kassis laboratory cloned the *Drosophila* Pleiohomeotic (PHO) sequence and observed similarity to YY1 (47) (**Figure 1**). Girton and Jeon (48) demonstrated that PHO is a Polycomb Group (PcG) protein, a family of proteins involved in epigenetic chromosomal condensation, stable transcriptional repression, control of cell proliferation, hematopoietic development, as well as stem cell self-renewal. This raised the exciting possibility that YY1 is a vertebrate PcG protein. PHO is highly homologous to YY1 in two regions. These two regions include YY1 sequences 296–414 and 205–226 (the corresponding segments in PHO are residues 357–475 and 148–169, respectively). Sequences 298–414 constitute the four YY1 zinc fingers. The homology over this region is extraordinary for organisms as diverse as flies and humans (112 identities out of 118; 95%). Within this segment, zinc fingers 2 and 3 are 100% identical. The 205–226 segment is also highly homologous (18/22; 82% identity). Outside of these regions of high similarity, YY1 and PHO showed no discernible similarity. PHO does not contain an obvious transcriptional activation domain and lacks YY1 structural features such as acid and histidine stretches. However, the two regions of high similarity between YY1 and PHO, and their similar spatial locations within the proteins, suggested that they might carry out some of the same functions in vertebrates and flies, respectively.

Prompted by the possibility that YY1 functions as a PcG protein, we tested this hypothesis using a *Drosophila in vivo* transcription system, as well as a phenotypic correction assay. Our results showed that human YY1 does indeed function as a PcG protein *in vivo* (49–51). We found that YY1 can repress transcription in a PcG-dependent fashion, can phenotypically correct *pho* mutant flies, and can recruit PcG proteins to specific DNA sequences resulting in tri-methylation of H3 lysine 27 (49–51). The mechanisms responsible for targeting mammalian PcG proteins to specific DNA regions has long been proven enigmatic because none of the components of the PcG complexes bind to specific DNA sequences, yet the PcG complexes associate with specific DNA regions *in vivo*. Our demonstration that YY1 is a mammalian PcG protein with high affinity sequence-specific DNA binding activity suggested that YY1 is a crucial factor for targeting specific proteins to specific DNA sequences. The role of YY1 in PcG targeting has been confirmed in a number of studies (52–55) though clearly other factors are involved as YY1 (and PHO) does not co-localize with PcG proteins in all cell types (56–58). A particularly exciting aspect of YY1 PcG function is that PcG proteins are known to contribute to B cell development, and the PcG protein EZH2, like YY1, is required for Ig locus contraction (further explained below) (59). Nucleation of PcG proteins to specific target DNA sites by YY1 within the Ig loci thus opens up a new avenue for mechanistic evaluation of B cell development and Ig locus contraction, because PcG proteins are capable of mediating long-distance DNA interactions (60).

THE YY1 REPO DOMAIN

Using a fly transgenic approach, we set out to identify the YY1 sequences involved in PcG function (61). We found that the region of 82% YY1-PHO identity (the 25 amino acids between residues 201 and 226), when fused to a heterologous GAL4 DNA binding domain, was necessary and sufficient for PcG-dependent transcriptional repression. Amazingly, this small 25 amino acid segment was also necessary and sufficient for recruitment of PcG proteins to DNA resulting in tri-methylation of H3 lysine 27. Therefore, we named YY1 sequences 201–226 the REPO domain for their ability to REcruit Polycomb (61). A REPO domain YY1 mutant (Δ201–226) can mediate nearly all YY1 functions such as DNA binding, transcriptional activation, transient transcriptional repression, and interaction with HDAC proteins. However, this mutant fails to carry out YY1 PcG functions and fails to recruit PcG proteins to DNA (61). How the YY1 REPO domain recruits PcG proteins to DNA is now being elucidated. Two homologous proteins, YAF2 and RYBP, were previously identified as YY1 interacting proteins (62, 63). Functionally, RYBP associates with a subset of PcG complexes named PRC1L4 (64) and is involved in the repressive function of *hoxD11.12*, a mammalian "PRE-like" sequence (65). YAF2 was first identified by its ability to bind to YY1 (63) and we found YAF2 can interact with the REPO domain perhaps functioning as a bridge protein in PcG recruitment (52, 66). The importance of the YY1 REPO domain for B cell development is discussed below.

STRUCTURE OF IMMUNOGLOBULIN LOCI DURING B CELL DEVELOPMENT

B cell development involves progression from Lin$^-$Sca-1$^+$c-kit$^+$ (LSK) progenitor cells through a number of intermediate B cell stages including pro-B, pre-B, immature B, mature B, and plasma cell stages. The early stages of B cell development can be delineated by the rearrangement status of the immunoglobulin heavy and light chain genes. Both heavy and light chain genes are produced during early, antigen-independent B cell development by a somatic rearrangement process that links together either V, D, and J segments (heavy chain), or V and J segments (light chain) to produce functional Ig genes (67–70). The Ig loci are huge (2.4–3.2 Mb) and for rearrangement of distal variable region genes to occur, the loci must go through a physical contraction process. Prior to the onset of rearrangement, Ig loci reside at the nuclear periphery in an "extended" configuration. However, at the pro-B cell stage, when the heavy chain genes undergo rearrangement, the loci take up an intranuclear localization with concomitant contraction of the loci (heavy chain first followed by light chain) (71–74). While IgH DJ and proximal V_H to D and Vκ to Jκ rearrangements can occur without contraction, the distal V genes require locus contraction and looping for rearrangement (71–73, 75–77).

Current data suggest that the Ig loci are organized as loops into rosette-like structures separated by spacer DNA (76, 78–80). A number of domains have been identified at the IgH locus, which adopt various conformations during development (76, 78–80). At the pre–pro-B cell stage, these rosette domains are in an extended conformation, but in pro-B cells the structure changes such that each V region domain is repositioned with all V_H regions approximately equidistant to the D_H and J_H regions, thus affording roughly equal access for recombination (79, 80) (**Figure 2**, left panel). Similar structures are believed to exist at the Ig kappa locus at pro-B and pre-B cell stages (**Figure 3**).

The mechanisms that control Ig locus contraction are unknown. A small number of transcription factors or protein complexes (YY1, Pax5, CTCF, IKAROS, cohesin, condensin, EZH2) are implicated in the DNA loops needed for V(D)J rearrangement (59, 78, 81–86), but the molecular details and regulatory processes that

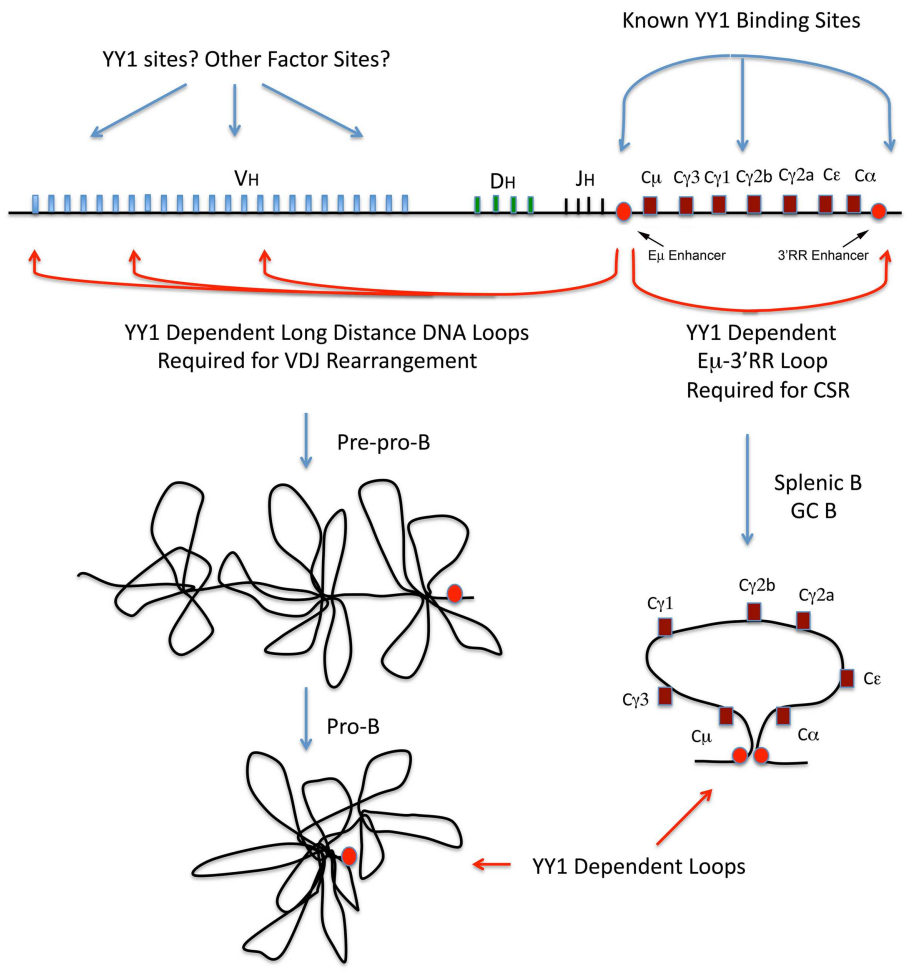

FIGURE 2 | Immunoglobulin heavy chain locus diagram with V, D, J, and C regions indicated and locations of known YY1 binding sites, and the approximate positions of long-distance DNA loops that are YY1-dependent. The red circles represent the IgH intron and 3′RR enhancers. The left panel models rosette-like loops encompassing the V_H regions at the pre–pro-B and pro-B cell stages. The right panel diagrams the Eμ-3′RR long-distance DNA loop required for CSR.

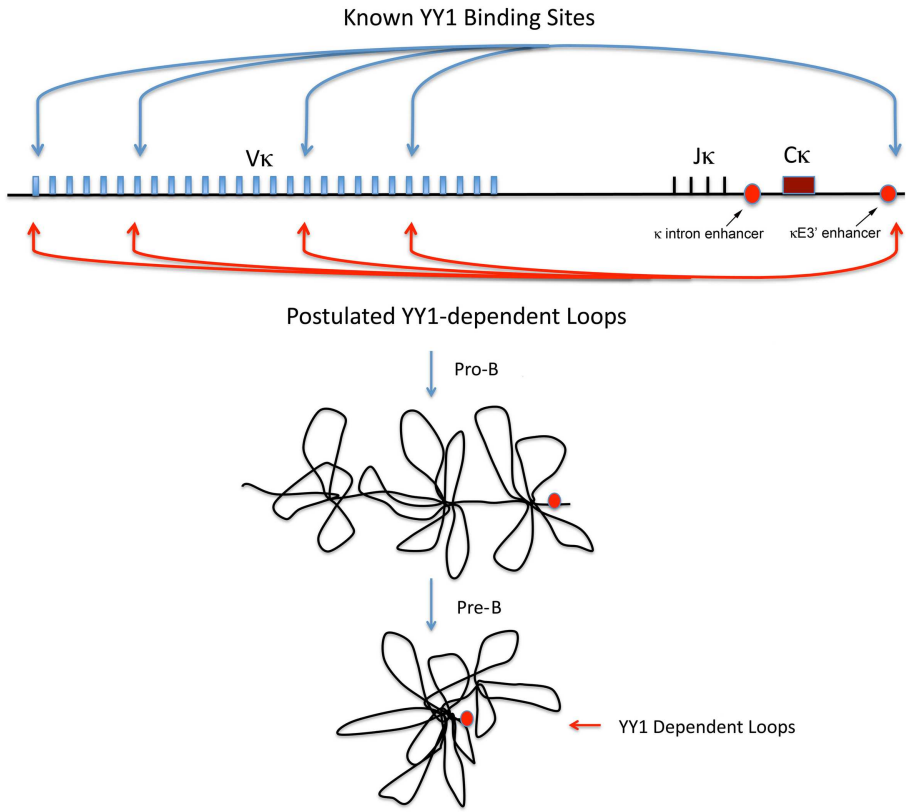

FIGURE 3 | Igκ locus diagram showing the location of known YY1 binding sites, and the postulated YY1-dependent loops required for Vκ–Jκ rearrangement. Locations of the Igκ intron and κE3′ enhancers and shown by red circles and arrows. Postulated locus structure in pro-B and pre-B cells is show below.

control this mechanism are not clear. Pax5 binds to multiple repeat sequences in the distal region of the IgH locus (PAIR sequences) and is believed to participate in rearrangement of distal V_H genes (83). Non-coding antisense transcripts expressed across the PAIR sequences correlate with VDJ rearrangement and are postulated to be involved with IgH locus contraction (83, 87, 88). Pax5 controls some of these transcripts (83), and recently YY1 was shown to regulate antisense transcripts across at least two PAIR sequences (87). Many Pax5 and YY1 potential binding sites exist in the IgH locus (89) and these transcription factors co-localize at some of these sites (87). Similar to the Pax5 and YY1 knock-out phenotypes (discussed below), PcG protein EZH2 knock-out results in arrest at the pro-B cell stage with impaired distal V_H to D_H–J_H rearrangement (59). CTCF and cohesin have been argued to regulate Ig locus structure and to control interactions of D_H and J_H regions with proximal V_H segments and Jκ regions with proximal Vκ segments (81, 82, 90–92). Ikaros knock-out also impacts IgH rearrangement as well as locus contraction (93).

THE ROLE OF YY1 AND THE REPO DOMAIN IN B CELL DEVELOPMENT

Yin Yang 1 has long been believed to play some role in immunoglobulin (Ig) gene regulation and B cell biology because it associates with multiple Ig enhancer elements including the heavy chain intron and 3′ enhancers, the Ig kappa 3′ enhancer, as well as to a site between the C_H γ1 and γ2b exons (1–5, 87, 94) (**Figures 2** and **3**). The Shi laboratory at Harvard provided insight into the role of YY1 in B cell development by demonstrating that conditional knock-out of YY1 in the B cell lineage (using mb1-CRE which is expressed early after B lineage commitment) resulted in arrest at the pro-B cell stage (84). Pro-B cells lacking YY1 have normal D_H–J_H recombination but reduced frequency of V_H–D_H–J_H recombination, with the defect being most severe for more distal V_H genes (84). These knock-out pro-B cells showed a defect in Ig locus contraction (84), and this phenotype has been confirmed by a number of studies (81, 88). Thus, conditional knock-out of YY1 using mb1-CRE results in arrest at the pro-B cell stage, lost Ig locus contraction, and reduced rearrangement of distal V genes. Importantly, despite the fact that proximal VDJ recombination does occur, very few mature B cells are generated in conditional knock-outs. Furthermore, introduction of a rearranged heavy chain gene only partially complements the YY1 conditional knock-out phenotype, suggesting additional roles for YY1 in early B cell development (84).

Intrigued by the similarity between the YY1 and PcG protein EZH2 B cell knock-out phenotypes (59, 84), we set out to determine the importance of YY1 PcG function for B cell development. Using YY1 wild-type and YY1ΔREPO retroviral constructs, we

transduced bone marrow from *yy1*$^{f/f}$ *mb1-CRE* mice and injected this transduced bone marrow into irradiated secondary recipients. Thus, within the B cell lineage of the transplanted mice, only the transduced YY1 constructs will provide YY1 function due to deletion of the endogenous *yy1* gene by mb1-CRE action. While wild-type YY1 largely restored B cell development, the YY1ΔREPO reconstituted cells arrested B cell development at the pro-B and pre-B cell stages (85). Interestingly, IgH VDJ rearrangement was largely normal, but Igκ rearrangement showed a dramatically skewed repertoire. Only a small number of Vκ genes underwent rearrangement with one third of rearrangements to the most distal 5′ V kappa gene. This dramatic result suggested that in the absence of YY1 PcG function, most of the DNA loops at the Igκ locus needed for Igκ rearrangement were abrogated, and a small number of loops that are independent of YY1 PcG function remained for Igκ Vκ–Jκ rearrangements. At least some of these loops may require E2A or Pax5 (85), although this is speculative.

MECHANISMS OF Ig LOCUS CONTRACTION

The dramatically skewed Vκ–Jκ rearrangement profiles in YY1ΔREPO compared to wild-type YY1 mice (85), suggested a possible direct effect of YY1 on Igκ locus structure, and loss of IgH locus contraction in a YY1 knock-out background suggested parallel effects at the heavy chain locus. Consistent with a direct effect on Igκ locus structure, RNAi knock-down of YY1 in bone marrow cultures reduced Igκ rearrangement at a subset of Vκ genes (85). Since the Shi lab showed YY1 is important for Ig locus contraction (81, 84, 88), we hypothesized that clusters of YY1 binding sites exist across the Ig loci, and that YY1 binding to these sites would result in recruitment of proteins needed for Ig locus contraction. As predicted, we identified clusters of YY1 binding sites across the Igκ locus that binds to YY1 (85). We found that PcG protein EZH2 co-localized with YY1 at these sites apparently as a result of recruitment by YY1 (85). We also identified several proteins that physically interact with the YY1 REPO domain providing potential insight into the mechanism of YY1 function in locus contraction. Intriguingly, we found that proteins from the condensin and cohesin complexes (SMC4 and SMC1) that are needed for contraction of chromosomes during mitosis (95–99), as well as lamin proteins, bind to the YY1 REPO domain. Lamin proteins are known to be involved in long-distance DNA interactions (100–103). Similarly, cohesin and condensin complexes, along with topoisomerase 2, are involved in mitotic chromosome contraction and higher order chromosome organization and dynamics (96, 104). During mitosis, condensin and cohesin proteins associate with the chromosomes and function in chromosomal contraction, cohesion, assembly, and segregation (96–98). A subpopulation of these proteins remains chromosome-associated at specific foci in the interphase nucleus (98). Importantly, cohesin and condensin proteins are involved in numerous long-distance DNA interactions (92, 105–114). Therefore, we hypothesized condensin and cohesin proteins associate with Ig loci in pro-B and pre-B cells by virtue of interaction with YY1, and thereby function to participate in Ig locus contraction. Consistent with this idea, we found that condensin proteins associate with the clusters of YY1 binding sites that we identified within the Igκ locus (85) in primary pro-B cells, but not in fibroblasts suggesting a B cell specific function of condensin and cohesin association with these sites (see **Figure 4**).

To test the functional consequences of YY1 and condensin binding at the Ig kappa locus, we performed RNAi knock-down and Ig kappa rearrangement assays. We found that knock-down of YY1 or condensin proteins resulted in reduced Igκ rearrangement at a subset of Vκ genes (85). Thus, YY1 binds to sites in the Ig loci, perhaps recruits PcG, condensin, cohesin, and lamin proteins to these sites, and results in specific Ig locus chromosomal contraction. The identification of condensin mutants that specifically affect T cell development supports the idea of condensin proteins (which are ubiquitously expressed) having lymphoid specific functions (115). These complexes can mediate long-distance chromosomal interactions (105, 107), and kleisin-β, a member of the condensin II complex is important for T cell development as is cohesin subunit Rad21 (92, 115). Cohesin subunit Rad 21 (a kleisin family protein) is recruited to CTCF binding sites throughout the Ig loci during B lymphocyte development (82). As condensin I is involved in the process of physically compacting DNA in the presence of hydrolyzable ATP (116), condensin complex proteins may also participate in bringing V genes in the Ig locus into close proximity with D and J gene segments.

LONG-DISTANCE DNA INTERACTIONS AND CSR

Long-distance DNA loops are also required for class switch recombination (CSR), which recombines the rearranged VDJ segments that provide antibody specificity with various Ig heavy chain constant (C) regions with different effector functions (117, 118). CSR requires a large 220 kb long-distance DNA loop synapse between the IgH intron enhancer (Eμ) region, and the 3′RR enhancer downstream of the 3′-most Cα exon (119, 120) (the Eμ-3′RR synapse; see **Figure 2**, right panel). In addition, CSR to individual IgH C exons requires formation of inducible DNA loops from each switch region DNA sequence into the Eμ-3′RR synapse (119,

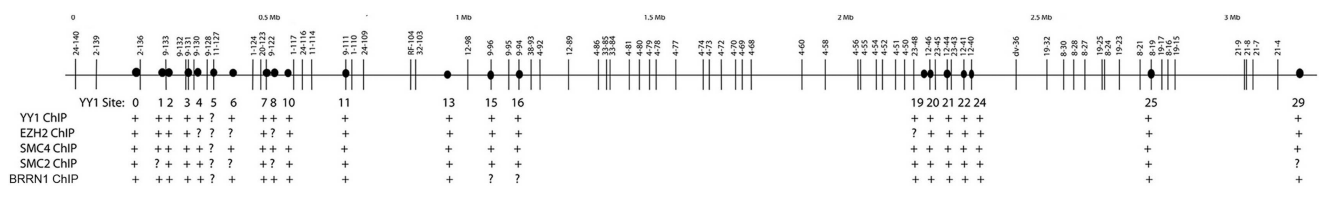

FIGURE 4 | Summary of protein co-localization data across the Igκ locus. The Igκ locus is shown in the top panel with Vκ genes represented by vertical lines. The identified YY1 binding sites are represented by black circles. Summary of ChIP data for YY1, EZH2, SMC4, SMC2, and BRRN1 are shown in the bottom panel. Positive ChIP signals are represented by a + symbol and question marks show inconclusive ChIP data.

120). Over 40 proteins are involved in the enzymology and mechanism of CSR and include DNA repair (base excision repair and mismatch repair) proteins, DNA damage sensors, factors that alter chromatin structure, factors that bind to AID, and transcriptional regulatory proteins [reviewed in Ref. (121)]. However, none of these factors are known to specifically impact the Eμ-3′RR DNA loop required for CSR.

Recent progress, however, has shed light on these long-distance DNA loops. CTCF and cohesin bind to the IgH 3′RR enhancer within the hs5–7 sites (81, 122, 123), and cohesin binding is induced at certain C_H switch regions in response to inducers of CSR implying a function for cohesin in CSR (123). Consistent with this, knock-down of cohesin subunits impairs CSR (123). In addition, knock-down of the cohesin loading protein NIPBL reduces CSR, reduces non-homologous end joining, and increases microhomology end joining (124). Interestingly, AID was shown to physically interact with condensin, cohesin, and INO80 complex proteins (123), precisely the same complexes that bind to YY1 (85, 125, 126).

Notably, we found that YY1 conditional knock-out in splenic B cells significantly reduces CSR (127). YY1 physically interacts with AID, leading to stabilization and increased AID nuclear accumulation, and this control of AID nuclear levels can regulate CSR. Control of nuclear levels of AID is crucial not only for regulating antibody maturation processes (CSR and somatic hypermutation), but also is important for maintaining integrity of the mammalian genome. Elevated levels of YY1 could cause aberrant accumulation of AID in germinal center B cells leading to increased mutagenesis and lymphomagenesis. Indeed, YY1 levels are elevated in germinal center-derived human DLBCL (34), suggesting that YY1 contributes to disease progression. However, we also found that YY1 has a second function important for CSR. In collaboration with Ranjan Sen (NIA), we found that YY1 is necessary for long-distance DNA loops formed between the Eμ and 3′RR enhancers (unpublished data). Recently, Kenter and colleagues identified a long-distance DNA loop between the Eμ and hs3b–hs4 sites of the 3′RR that is dramatically induced upon induction of CSR in splenic B cells (119). We found that this long-distance DNA loop is YY1-dependent (unpublished data). Thus, YY1 controls long-distance DNA loops in splenic B cells that are critical for CSR. Can the same be said of the long-distance DNA loops needed for IgH V(D)J rearrangement, and perhaps for other long-distance DNA loops? Recent evidence suggests this is the case.

YY1-DEPENDENT IgH LONG-DISTANCE DNA INTERACTIONS

The Sen Laboratory and colleagues indentified long-distance DNA loops in both the V_H distal and proximal regions, and at the 3′ end of the locus (78). They found YY1 bound to many of these segments and postulated either homotypic YY1 interactions to mediate these loops, or heterotypic interactions with other proteins (78). The essential nature of YY1 for these loops was subsequently demonstrated. In pro-B cells, YY1 conditional knock-out ablates long-distance DNA loops between the Eμ region and the distal and proximal V_H regions (87). In addition, YY1 knock-out in pro-B cells ablates loops between the Eμ region and the 3′RR enhancer, hs5–7 region (87). Thus, YY1 is essential for long-distance DNA loops within the IgH locus involved in either VDJ rearrangement, or CSR (**Figure 2**). Finally, YY1 is also involved in long-distance DNA interactions at the Th2 cytokine locus and controls IL4, IL5, and IL13 expression (128). These dramatic results indicate that YY1 is required for long-distance DNA loops that control IgH V(D)J rearrangement, CSR, and gene regulation. Our studies at the Igκ locus (85) also indicate a role for YY1 in long-distance DNA interactions needed for Igκ rearrangement (**Figure 3**).

REGULATORY MECHANISMS FOR YY1 FUNCTION

How might YY1 be functioning in these diverse long-distance DNA interactions? As described above, in pro-B cells, YY1 binds constitutively to the Eμ enhancer, to hs5–7 sites in the 3′RR enhancer, to a site between the Cγ1 and Cγ2b exons, and inducibly to the hs3b site in the 3′RR enhancer in splenic B cells (5, 78, 87, 94). The mechanism of regulation of developmental stage-specific function of YY1 in VDJ rearrangement at the IgH locus (pro-B cells), in Vκ–Jκ rearrangement at the Igκ locus (pre-B cells), and in CSR at the IgH locus (mature splenic B cells), is presently unknown. YY1 may participate in regulatory stage-specific functions to control locus accessibility (129), but other factors may control accessibility enabling subsequent YY1 DNA binding.

Yin Yang 1 function can be regulated by a number of mechanisms. Stage-specific regulation could be at the level of YY1 DNA binding, such as the LPS inducible binding in the 3′RR enhancer in splenic B cells. YY1 binding to the Ig heavy chain 3′RR hypersensitive site 3b (hs3b) as well as to the Eμ enhancer is inducible by LPS (94). In this case, YY1 appears to be sequestered from DNA in resting B lymphocytes through interaction with hypophosphorylated retinoblastoma protein (Rb). However, after LPS induction, Rb becomes hyperphosphorylated and releases YY1 enabling it to bind to the hs3b and Eμ enhancers. Interestingly, hs3b and 4 hypersensitive sites are crucial for formation of Eμ: 3′RRl enhancer synapses with germline switch region promoters after cytokine treatment (119, 120). We hypothesize that LPS induction of CSR might partially result from induction of YY1 binding to the 3′RR and Eμ enhancers leading to induced DNA loop formation.

Alternatively, YY1 may be controlled by stage-specific post-translational modifications, or by stage-specific interaction with other proteins. A number of YY1 post-translational modifications can regulate YY1 DNA binding (phosphorylation of serines 180 and 184, and threonines 348 and 378) (130–132), and YY1 is sumoylated on lysine 288 (133), which can control protein–protein interactions. Phosphorylation of serines 180 and 184 is mediated by Aurora B kinase and expression of this kinase peaks in splenic germinal center B cells (www.immgen.org) when CSR is active. Several studies demonstrated that YY1 subcellular localization is regulated during cell cycle progression and development (132, 134–137) suggesting that YY1 might also regulate subcellular localization of interacting partner proteins. In addition, apoptotic stimuli promote rapid translocation of YY1 from the cytoplasm to the nucleus in asynchronous HeLa cells (138). Thus, YY1 might function to increase transport of proteins from the cytoplasm to the nucleus via the nuclear pore.

During B cell development, YY1 expression levels remain relatively constant, as defined by transcript levels (www.immgen.org). However, YY1 protein levels are regulated in some systems yielding biological responses. This is most well studied in skeletal muscle

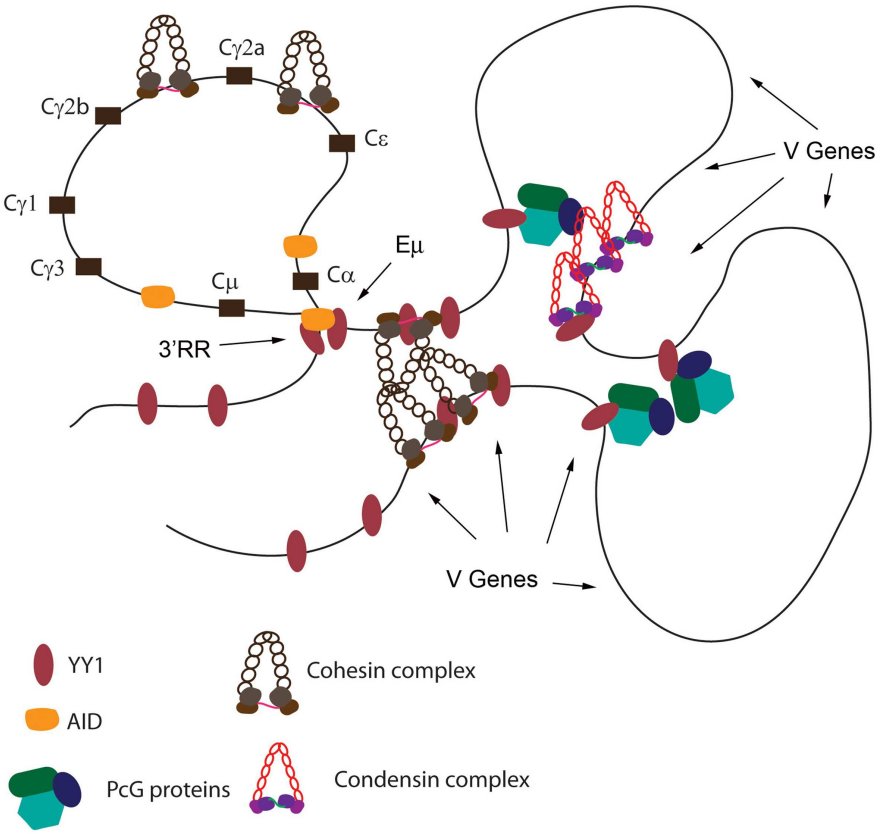

FIGURE 5 | Model of YY1 recruitment of proteins to DNA needed for long-distance DNA interactions. Using the IgH locus as a model, YY1 binding sites are indicated. Binding by YY1 then results in recruitment of condensin, cohesin, and PcG complex proteins. These proteins may form homotypic or heterotypic interactions to mediate long-distance DNA interactions. Positions of the Eμ enhancer, 3′RR enhancer, and various V genes are shown by arrows. Black rectangles represent various C_H constant regions. YY1 also physically interacts with AID, and AID is able to interact with condensin, and cohesin complexes, and thus may contribute to DNA loop formation in germinal center B cells.

differentiation systems where YY1 expression levels drop as a result of proteolysis (24), and in cardiac disease conditions (139, 140). Thus, regulation of YY1 protein stability may control DNA loop formation.

It should be noted that RNA expression profiles of PcG proteins EZH2 and YAF2, as well as cohesin, and condensin subunit proteins SMC4, SMC2, SMC1, SMC3, CAP-G, CAP-H (BRRN1), and CAP-D2 all peak during B cell development at the pre-B cell stage (www.immgen.org). Expression levels are also high in pro-B cells, but peak in pre-B cells, then drop in immature B cell stages. This expression pattern is coincident with the timing of Ig rearrangement and is consistent with a role in Ig locus contraction and rearrangement. However, this timing is also coincident with high levels of proliferation in pre-B cells suggesting a possible effect of YY1 on the pre-B proliferative burst during development. All factors peak again in germinal center B cells (www.immgen.org) suggesting possible roles in proliferation, CSR, or somatic hypermutation.

Whatever the mode of locus accessibility or YY1 DNA binding, YY1 may then recruit proteins to DNA that are required for long-distance DNA interactions. As presented above, YY1 physically interacts with PcG, condensin, cohesin, and lamin proteins, all involved in long-distance DNA interactions, and we have noted co-localization of some of these proteins with YY1 at the Igκ locus (**Figure 4**). PcG proteins can mediate long-distance DNA interactions (60), and since YY1 recruits PcG proteins to DNA via the REPO domain (50, 61), we predict that this interaction will be important for long-distance interactions leading to DNA loop formation. Notably, condensin and cohesin complex proteins (105, 107), and lamin proteins (100–103) are all involved in long-distance DNA structures, suggesting that the DNA binding capacity of YY1 at IgH and Igκ sequences may nucleate protein–protein interactions that govern DNA looping mechanisms. In addition to co-localization of YY1 and condensin proteins at the Igκ locus, YY1 co-localizes with cohesin at the hs5–7 sites in the 3′RR enhancer (78, 81).

MODELS OF YY1-MEDIATED LONG-DISTANCE DNA INTERACTIONS

Based upon: (a) the crucial nature of the YY1 REPO domain for B cell development, (b) the ability of this domain to recruit PcG proteins to DNA, (c) the physical interaction of the REPO domain with PcG, condensin, cohesin, and lamin proteins, (d) the co-localization of YY1, EZH2, and condensin proteins across the Igκ

locus, (e) the co-localization of YY1 and cohesin proteins at the IgH 3′RR enhancer, (f) the effect of cohesin knock-down on CSR, (g) the effect of condensin subunit knock-down on Vκ–Jκ rearrangement, (h) the high levels of EZH2, YAF2, cohesin, and condensin proteins in pro-B, pre-B, and germinal center cells, (i) the critical role of YY1 in long-distance DNA loops in the IgH V region and 3′ region, and (j) the regulatory role of YY1 in CSR, we propose the following mechanism. We propose that YY1 binds to sites spanning the IgH and Igκ loci. Concomitant with YY1 DNA binding, increased EZH2, YAF2, cohesin, and condensin subunit expression results in these proteins binding to the same DNA regions, presumably due to interactions with YY1. The nucleated PcG, cohesin, and condensin proteins then mediate long-distance interactions between the YY1 binding sites resulting in contraction of the Ig loci in looped or rosette structures (**Figure 5**). These loops then control somatic rearrangement of IgH and Igκ genes as well as CSR. Immediately upon maturation to the immature B cell stage, or upon maturation to plasma cells, EZH2, YAF2, cohesin, and condensin protein expression drops dramatically (www.immgen.org), thus facilitating de-contraction of the Ig loci, perhaps assisting in regulation of the allelic exclusion process, and causing a decrease in the inducible loops needed for CSR. In the case of CSR, it is intriguing that AID binds to many of the same factors that bind to YY1 (condensin, cohesin, and INO80 complexes) (123). Thus, YY1–AID physical interaction may also contribute to DNA loop formation (**Figure 5**).

Finally, it has been proposed that YY1 function in long-distance DNA interactions relates to the regulation of non-coding antisense transcripts in the IgH V_H PAIR sequences (88). YY1 knock-out ablates some of these transcripts, and these transcripts have been proposed to play a role in IgH locus contraction (87, 88). Some RNA transcripts are known to regulate long-distance DNA interactions via interactions with the mediator complex (141). Whether YY1 functions in this mechanism is presently unclear.

FUTURE STUDIES AND REMAINING QUESTIONS

A number of outstanding questions remain. (1) Is recruitment to DNA of proteins involved in DNA loop formation dependent upon YY1 DNA binding? (2) What mechanisms enable YY1 to function at distinct loci at various developmental stages? (3) Is YY1 function controlled by post-translational modifications? (4) Is YY1 controlled by stage-specific protein interactions? (5) What functions and domains of YY1 are needed for DNA looping, V(D)J rearrangement, and CSR? (6) What are the biochemical mechanisms for Ig locus contraction and for DNA loop formation? These questions and others are important for immune function and control of gene expression. The ubiquitous nature of YY1 and its involvement in looping at multiple loci (78, 87, 128) suggests that paradigms learned in the Ig systems will be globally applicable to other long-distance DNA interactions.

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