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Cloning, Functional Characterization, and Mechanism of Action of the B-Cell-Specific Transcriptional Coactivator OCA-B

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Biochemical purification and cognate cDNA cloning studies have revealed that the previously described transcriptional coactivator OCA-B consists of a 34- or 35-kDa polypeptide with sequence relationships to known coactivators that function by protein-protein interactions. Studies with a recombinant protein have proved that a single OCA-B polypeptide is the main determinant for B-cell-specific activation of immunoglobulin (Ig) promoters and provided additional insights into its mechanism of action. Recombinant OCA-B can function equally well with Oct-1 or Oct-2 on an Ig promoter, but while corresponding POU domains are sufficient for OCA-B interaction, and for octamer-mediated transcription of a histone H2B promoter, an additional Oct-1 or Oct-2 activation domain(s) is necessary for functional synergy with OCA-B. Further studies show that Ig promoter activation by Oct-1 and OCA-B requires still other general (USA-derived) cofactors and also provide indirect evidence that distinct Oct-interacting cofactors regulate H2B transcription.

Highly restricted to cells of the B-cell lineage, the expression of immunoglobulin (Ig) genes is controlled both by developmentally regulated DNA rearrangements and by B-cell-specific DNA control elements (promoters and enhancers) and their cognate transcription factors (reviewed in reference 34). Most mammalian Ig promoters contain a conserved octamer element that is the major determinant for B-cell-specific promoter function, but this same element has also been implicated as a critical motif in a number of other genes that include ubiquitously expressed small nuclear RNA genes, the cell cycle-regulated histone H2B gene, and VP16-dependent herpes simplex virus immediate-early genes (reviewed in reference 20). Factors that can bind to the octamer element and act as activators belong to the POU domain family of regulators (reviewed in references 14 and 29) and include the ubiquitous Oct-1, tissue-specific Oct-2, and other developmentally regulated factors (reviewed in reference 31). An early hypothesis that Oct-2 was responsible for the B-cell-specific function of Ig promoters was based on the finding that Oct-2 is largely B cell restricted and further supported by the observation that ectopic expression of Oct-2 in non-B cells can stimulate transcription from artificial B-cell-specific promoters (reviewed in references 20 and 34). However, this early view was soon challenged by a number of studies which documented (i) the lack of a strict correlation between levels of Oct-2 and Ig transcription, (ii) the inability of ectopically expressed Oct-2 to specifically enhance authentic Ig promoters in transfected cells, (iii) the functional equivalence of Oct-1 and Oct-2 in binding to, and stimulating transcription from, Ig (and H2B) promoters in reconstituted cell-free systems, and (iv) the inability of purified Oct-2 to effect normal levels of Ig transcription in non-B-cell extracts (reviewed in reference 20).

Consistent with the implications of these studies, our previous biochemical studies provided direct evidence for a novel B-cell-specific coactivator designated OCA-B. OCA-B was first detected in affinity-purified Oct-1 or Oct-2 preparations (25) and later obtained in isolated form (20). It was shown to be a cell-, promoter- and activator-specific coactivator that enhances octamer-dependent Ig (but not H2B) transcription through direct (DNA-independent) interactions with Oct-1 or Oct-2. These observations indicated that OCA-B is a major determinant of B-cell-specific Ig promoter function and provided a clear concept of its mode of action via promoter-bound Oct-1 or Oct-2. Moreover, Oct-1 and Oct-2 immunodepletion studies indicated that OCA-B was associated almost exclusively with Oct-1 in B-cell extracts and that Oct-2 removal had no effect on Ig transcription. Consistent with these earlier biochemical knockout studies, the dispensability of Oct-2 for Ig transcription was confirmed by Oct-2 gene disruption in cultured B cells (8) and by an Oct-2 gene knockout in mice (6).

The discovery of a B-cell-specific coactivator that acts through protein-protein interactions with a ubiquitous promoter-bound activator provides a basis for further studies of key regulatory factors for Ig transcription and other B-cell differentiation events, as well as a general model that may be applicable to other genes whose presumed DNA-binding regulatory factors do not show the expected tissue restriction. Other possible examples of such coactivators include herpes simplex virus VP16 (38), adenovirus E1A (19, 32), mammalian CBP (16), and yeast Sug-1 (35) and GAL11 (12). Given that intrinsic activation domains have been localized, in some cases, both to DNA-bound activators and to cognate coactivators, an important question concerns their mechanism of action and possible synergism. Another relevant question is whether gene- and/or cell-specific mammalian coactivators such as OCA-B require any of the apparently general mammalian coactivators (e.g., USA-derived positive cofactors) that are required, along with TATA-binding protein-associated factors in TFIID, for the functions of various activators in vitro (reviewed in references 9 and 15).

To explore these questions, we have purified and cloned cDNAs encoding OCA-B proteins and here report novel aspects of the structure, function, and cell specificity of OCA-B.

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MATERIALS AND METHODS

In vitro transcription. Except where noted, transcription conditions described by Luo et al. (20) were used. Apart from the 5-μl transcription premix (200 mM

N-2-hydroxyethylpiperazine-*N*′-2-ethanesulfonic acid [HEPES; pH 8.4], 15 mM $MgCl_2$, 3 mM each ATP, GTP, CTP, and UTP, 20 mM dithiothreitol, 40 U of RNAsin), the 25-μl reaction mixtures contained 5 μl of a template solution and 15 μl of nuclear extract, factors, or cofactors (all in BC100-based buffers with compensating volumes of BC100–250 μg of bovine serum albumin [BSA] per ml when needed). Reaction mixtures were incubated at 30°C for 60 min, and transcripts were measured by primer extension.

Purification of factors and cofactors. Proteins were maintained in BC buffers described by Luo et al. (20). Numbers following BC indicate the millimolar KCl concentrations. Highly purified factors and cofactors (in BC100) were stabilized by 250 μg of BSA per ml. Oct-1 was purified from HeLa nuclear extracts by a method adapted from that of Pierani et al. (25). Oct-2 and its mutants (10) were expressed in recombinant vaccinia virus-infected HeLa cells and purified essentially as described elsewhere (20). For the purification of OCA-B, 600 ml (~5 g of total protein in BC100) of Namalwa nuclear extract was loaded onto a 50-ml immunoglobulin heavy-chain (IgH) octamer DNA affinity column equilibrated with BC100–250 μg of BSA per ml. After extensive washing with BC100, the column was developed with 5 column volumes of BC1000 to elute the bound proteins enriched for Oct-1, Oct-2, and OCA-B activity. Active fractions (75 ml, ~15 mg of protein) were pooled and passed through a 10-ml lectin agarose column equilibrated with BC1000–250 μg of BSA per ml. The flowthrough was dialyzed slowly against BC60 and loaded onto a 5-ml DE-52 column equilibrated with BC60. The column was washed with BC60 and developed with 5 column volumes of a BC60-BC400 linear gradient. OCA-B activity, essentially free of Oct factors, was eluted at ~180 to ~220 mM KCl. The pooled fractions (~4 ml, ~1 mg of protein) were dialyzed against BC100 and loaded onto a 1-ml heparin-agarose column equilibrated in BC100. OCA-B activity was recovered (2 ml, ~0.28 mg of protein) by elution with BC250. The fraction was mixed with 8 ml of BC0–250 μg of BSA per ml and loaded onto a 0.1-ml Oct-1 affinity column which had been saturated with BC500–250 μg of BSA per ml, washed with BC500, and then equilibrated with BC60. After washing of the loaded column with starting buffer, OCA-B activity was recovered (~0.2 ml) by step elution with BC500. Further purification involved dialysis against BC100–8% glycerol and fractionation on a 4-ml 10 to 40% glycerol gradient in BC100 by centrifugation in a Beckman SW60 rotor at 54,000 rpm and 4°C for 28 h. Sixteen fractions (~0.25 ml) were collected. Fractions 1 (bottom) through 15 (top) were analyzed both in a transcription assay (5 μl; Fig. 1B) and for peptide composition by sodium dodecyl sulfate (SDS)-polyacrylamide gel electrophoresis (PAGE) (8% gel) and silver staining (20 μl; Fig. 1C). Active fractions were pooled, trichloroacetic acid precipitated, and processed for microsequencing analysis.

Double-stranded DNA oligonucleotides (spanning positions −73 to −16 of the IgH [BCL_1] promoter) were coupled to CNBr-activated agarose (Pharmacia) to make DNA affinity resins. Purified Oct-1 (from HeLa nuclear extracts) was coupled to the activated agarose to make protein affinity resins (~20 μg/ml).

cDNA cloning and in vitro translation of OCA-B. Inosine-containing oligonucleotides were based on the sequences of peptides A and B (Fig. 2A). Two million phage from a B-JAB (a human B-lymphoma cell line) cDNA library (in λZAPII vector) were screened by using probe A. Two of the five resulting positive clones hybridized to probe B and were found to encode full-length OCA-B. In vitro translation of OCA-B cDNAs was performed as instructed by the manufacturer (Promega).

Immunodepletion and immunopurification of OCA-B. In addition to BC-based buffers, two more buffers were used in the procedure. HpH-BC500 was the same as BC500 except that 100 mM triethylamine (pH 12.0) replaced Tris (pH 8.0). LpH-BC500 was the same as BC500 except that 100 mM glycine (pH 3.0) replaced Tris (pH 8.0). For immunodepletion of OCA-B, 50 ml of Namalwa nuclear extract (in BC500) was passed through a 2-ml protein A-agarose column. The flowthrough was then loaded onto a 1.5-ml anti-OCA-B affinity column. Five flowthrough fractions (~10 ml each) were collected. The third fraction, with OCA-B almost completely depleted (see Fig. 6C), was used as OCA-B-depleted Namalwa nuclear extract after being dialyzed against BC100. (A small portion of the protein A column flowthrough was dialyzed and used as mock-depleted Namalwa nuclear extract.) OCA-B was purified as follows. The OCA-B-bound affinity column was extensively and successively washed with BC500, LpH-BC500, and BC500. The column-bound OCA-B survived the low-pH challenge but was efficiently eluted by HpH-BC500. The OCA-B fractions (~2.7 ml) were pooled, neutralized by 0.3 ml of 1 M Tris (pH 6.8), and dialyzed against BC100. The OCA-B so purified was highly active for transcription (e.g., Fig. 4). The in vitro-translated recombinant OCA-B (rOCA-B) was purified essentially as just described except that the column size was reduced accordingly.

Anti-OCA-B antibodies were raised against a protein containing the C-terminal portion of OCA-B (residues 171 to 256) fused to glutathione *S*-transferase (GST). Specific anti-OCA-B antibodies were purified from crude antisera by using an immunogen affinity column. The purified antibodies were then cross-linked to protein A agarose, yielding an anti-OCA-B affinity column.

Protein-protein interaction assays. For interaction of OCA-B with immobilized GST fusion proteins, GST and GST-POU domain fusion proteins were expressed in and purified from *Escherichia coli*. Aliquots of ^{35}S-labeled OCA-B (20 μl in reaction buffer) were mixed with 36 μg of GST and 18 μg each of GST–POU-1, GST–POU-2, and GST–POU-3 (all in 80 μl of BC100–250 μg of BSA per ml) and incubated at 30°C for 1 h. The mixtures were then loaded onto 45-μl glutathione-agarose minicolumns equilibrated with BC80–250 μg of BSA per ml. After extensive washing with BC80, the bound proteins were eluted with BC80–10 mM glutathione. A portion of each eluate, along with the in vitro-translated OCA-B, was resolved by SDS-PAGE and analyzed by fluorography. For electrophoretic mobility shift assay analysis of OCA-B–POU interactions on DNA, the conditions were the same as described by Luo et al. (20) except that PAGE on a 4% gel and purified OCA-B (instead of crude OCA-B activity) were used.

Elution and renaturation of OCA-B. Two milliliters of immunopurified OCA-B were trichloroacetic acid precipitated and resolved by SDS-PAGE, and the proteins were electroblotted onto polyvinylidene difluoride membranes. Membrane regions corresponding to p34 and p35 were cut and soaked in 200 μl of 2% SDS–2% Triton X-100–50 mM Tris (pH 8.8)–250 μg of BSA per ml for 15 min at room temperature. The eluted proteins were acetone precipitated three times and dissolved in 100 μl of BC100–6 M guanidine-HCl. After incubation at room temperature for 30 min, the samples were dialyzed against BC100–1 M guanidine-HCl for 1 h and against BC100 overnight. About 10% OCA-B activity was recovered.

RESULTS

Purification of OCA-B. Given that ectopic OCA-B can fully stimulate transcription of an IgH promoter in a HeLa nuclear extract (20), this simple complementation assay was used to monitor OCA-B activity during the combined ion-exchange and affinity chromatography procedures (Materials and Methods) that led to its successful purification. Figure 1B exemplifies in vitro transcription assays with fractions from the final glycerol gradient sedimentation step. When assayed with the template described in Fig. 1A, the input OCA-B activity stimulated transcription from the IgH promoter in a HeLa nuclear extract to a level comparable to that observed in Namalwa B-cell extract (Fig. 1B, leftmost three lanes). The slightly lower stimulation by the active glycerol gradient fractions (e.g., compare #12-14 with Inp in Fig. 1B) reflects dilution (~5-fold) of the OCA-B proteins. Nevertheless, the OCA-B activity was recovered as a single peak and correlated with two polypeptides with apparent sizes of 34 and 35 kDa (referred to p34 and p35, respectively) (Fig. 1C).

cDNA isolation and expression of OCA-B. Microsequencing analyses yielded identical partial internal sequences for p34 and p35 and a partial N-terminal sequence from p34, while the N terminus of p35 appeared to be blocked. Screening of a human B-cell (B-JAB) cDNA library with oligonucleotide probes based on these peptide sequences yielded two clones with full-length cDNAs that differ only in the length of the 3′ untranslated region. Inspection of the translated polypeptide sequences (Fig. 2A) indicated that OCA-B is a novel protein with noteworthy features. First, the protein is very rich in proline (~17%) and serine/threonine (15% in total). These features are characteristic of conventional proline-rich activation domains such as that in CTF (reviewed in reference 22) and reminiscent of Oct-2 activation domain II (10, 23, 37). This observation is consistent with the proposed role of OCA-B as a coactivator that provides an activation surface(s) after being tethered to target (Ig) promoters by Oct factors (reference 20 and this work). Second, some regions in OCA-B display significant similarities to other cellular and viral proteins implicated in mediating transcription (Fig. 2B to D). Of particular interest is the high degree of similarity (38% identity; 58% overall conservation) of an OCA-B domain (amino acids 203 to 226) to an E1A domain (amino acids 180 to 203) (Fig. 2B). The indicated E1A domain overlaps the C terminus of E1A conserved region 3 and is critical for tethering the viral coactivator to target promoters via protein-protein interaction (19). Thus, the OCA-B domain in question (residues 203 to 226) could be a motif for OCA-B binding to Oct factors. This view is supported by the demonstration that E1A conserved region 3 is required for binding to Oct-4 or Oct-3 (32). The similarities of OCA-B to other mammalian regulators (Fig. 2C) such as

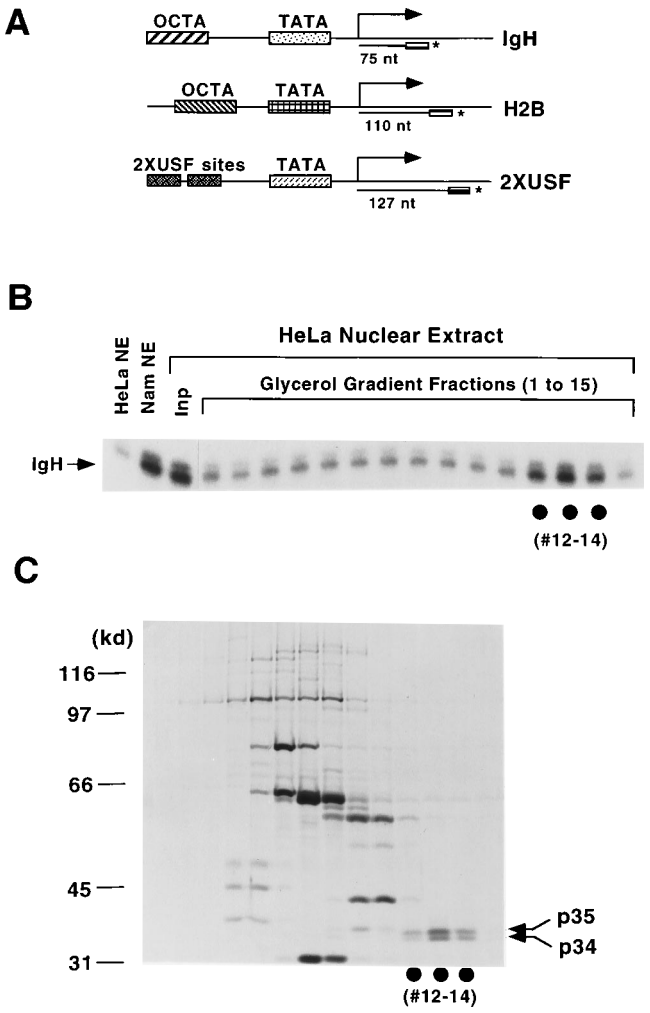

FIG. 1. Purification and peptide composition analyses of OCA-B. (A) Schematic diagrams of templates used for in vitro transcription assays. The construction of the templates is described in reference 20. Transcription levels were measured by primer extension using labeled template-specific primers, which created, as indicated for the respective templates, 75-, 110-, and 127-nucleotide (nt) signals from correctly initiated transcripts. Template dosages were 50 ng for 2XUSF, 100 ng for H2B, and 400 ng for IgH. (B) Purification of OCA-B activity by glycerol gradient sedimentation. OCA-B activity was assessed in a transcription assay by complementation of a HeLa nuclear extract (HeLa NE; 10 μl) with B-cell-derived glycerol gradient fractions (fractions 1 to 15; 5 μl of each) or input (Inp; 2 μl). Nam NE, Namalwa (B-cell) nuclear extract (8 μl, as a positive control). The active fractions (fractions 12 to 14) are marked by circles. (C) Silver stain analysis of glycerol gradient sedimentation fractions 1 to 15. The positions of size markers are indicated. Fractions corresponding to peak OCA-B activity (fractions 12 to 14) are marked by circles.

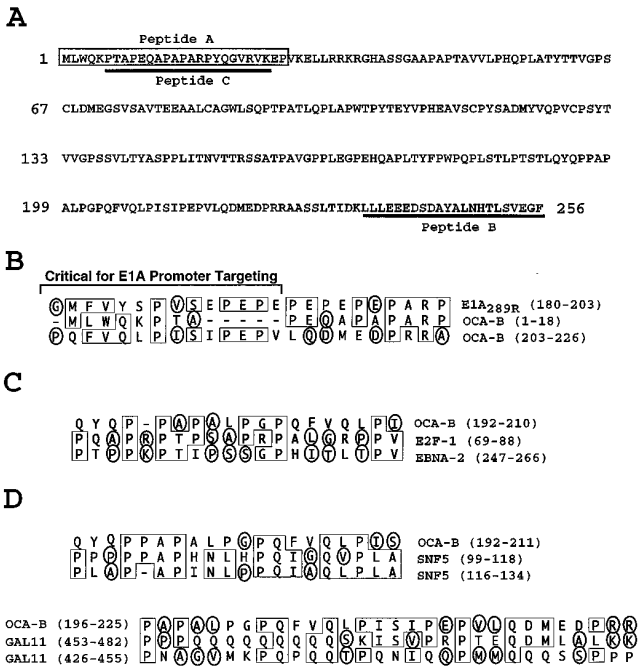

FIG. 2. (A) Predicted amino acid sequence of OCA-B. The sequences obtained from microsequencing of N-terminal (peptide A [boxed]) and internal (peptides B and C [underlined]) peptides are indicated. Oligonucleotides synthesized on the basis of the sequences of peptides A and B were used for cDNA cloning. (B) Similarity of OCA-B to E1A protein (289R). In this panel as well as in panels C and D, dashes denote gaps introduced to maintain the highest degree of conservation among proteins. In a particular position, identical residues are boxed and residues representing conservative exchanges are circled. Similarities were first identified by the BLAST program and then more precisely analyzed by LASERGENE NAVIGATOR. Conserved amino acids are as follows: A, G, P, S, and T; K and R; L, I, V, and M; and D, E, N, and Q. (C) Sequence similarities between OCA-B and E2F-1 and EBNA-2 proteins. (D) Sequence similarities between OCA-B and yeast transcription regulators SNF5 (top) and GAL11 (bottom).

E2F1 (13) and EBNA-2 (18), as well as to yeast coactivators (Fig. 2D) SNF5 (17) and GAL11 (7), may also have significant functional relevance.

In addition to the functional data presented below, several lines of evidence led us to conclude that the two cDNA isolates encode bona fide OCA-B. First, polyclonal antibodies raised against a segment of the putative OCA-B could efficiently inhibit IgH promoter transcription in (20a), and deplete the OCA-B activity from (see below), B-cell nuclear extracts. Second, coupled in vitro transcription and translation of either cDNA isolates in rabbit reticulocyte lysates yielded two polypeptides (Fig. 3), whose mobilities in SDS-PAGE (Fig. 3, lanes 5 and 7) were identical to those of two polypeptides in highly purified natural OCA-B (lane 3) or B-cell nuclear extracts (lane 2). Since no alternative initiation codon (ATG) was discovered either immediately upstream or downstream of the one encoding the first methionine indicated in Fig. 2A, it appears that p35 reflects a post- or cotranslational modification of p34 that takes place in reticulocyte lysates as well as in B cells.

Reconstitution of OCA-B activity from p34 and p35. Polyclonal antibodies were used to isolate large amounts of p34 and p35 from B-cell nuclear extracts by means of antibody affinity chromatography (Materials and Methods). This allowed us to determine, by elution and renaturation of SDS-PAGE-separated polypeptides, whether p34 and p35 could reconstitute OCA-B activity. As can be seen in Fig. 4A, p34 and p35 can independently stimulate the IgH promoter in a HeLa nuclear extract (compare lanes 4, 5, 7, and 8 with lane 2), to a level comparable to that observed with the highly purified OCA-B (lane 3) or with a complete B-cell extract (lane 1). OCA-B had no effect on a USF-dependent reference promoter (compare lane 2 with lane 3). Combination of p34 and p35 did not yield a synergistic activation (lane 6), suggesting that p34 and p35 are independently acting components rather than subunits of a common functional complex. Indeed, they were shown to arise from a single protein-coding sequence (see above). From these data, we conclude that the OCA-B activity is attributable to p34 and p35 polypeptides.

Tissue specificity of OCA-B. To extend our previous demonstration (20) of the B-cell specificity of OCA-B activity rel-

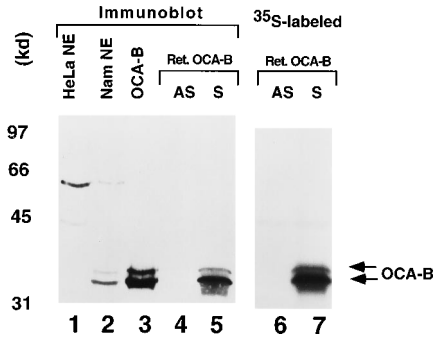

FIG. 3. Expression of OCA-B. OCA-B cDNA was used to produce in vitro-translated rOCA-B (Ret. OCA-B). S and AS indicate reactions programmed by sense and antisense RNAs, respectively. In lanes 6 and 7, the proteins were labeled by [^{35}S]methionine and detected by fluorography following SDS-PAGE (10 μl of each sample was used). This labeled OCA-B also was used in protein-protein interaction assays (Fig. 7B). In lanes 1 to 5, nonlabeled in vitro-translated proteins (10 μl) were resolved by SDS-PAGE along with HeLa nuclear extract (HeLa NE; 1 μl), Namalwa nuclear extract (Nam NE; 1 μl), and immunopurified OCA-B (1 μl). The OCA-B proteins were then detected by immunoblotting with anti-OCA-B antibodies. The in vitro-synthesized OCA-B was immunopurified (Materials and Methods) and used as a source for rOCA-B. The nature of the two cross-reacting bands observed in either extract is unknown. However, these bands were not enriched by immunopurification (lane 3).

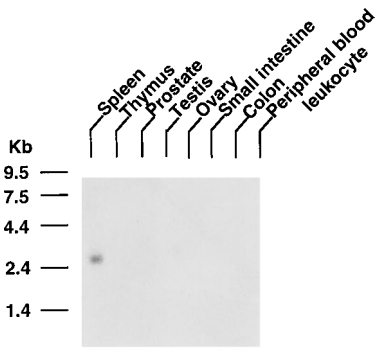

FIG. 5. Northern blot analysis of OCA-B. A blot (Clontech) containing poly(A)$^+$ RNAs from human tissues (as indicated) was hybridized to an internally labeled PCR probe (300 bp of OCA-B C-terminal coding region). All lanes contained comparable amounts of mRNA encoding the ubiquitously expressed general cofactor PC4 (15).

ative to HeLa cells, a Northern (RNA) blot analysis was carried out on poly(A)$^+$ RNAs prepared from a variety of human tissues (Fig. 5). OCA-B message is very restricted in expression, being detectable mainly in the B-lymphocyte-rich spleen. The failure to see OCA-B message in the peripheral blood leukocytes is consistent with the fact that these cells consist largely of non-B-lineage cells (1). In agreement with the Northern blot analysis, immunoblot analyses revealed the presence of the OCA-B proteins in nuclear extracts prepared from several cell lines representing the B-cell lineage (e.g., Namalwa; Fig. 3, lane 2) but failed to detect any OCA-B in extracts from HeLa cells (Fig. 3, lane 1), T cells, and muscle cells (33a). Interestingly, preliminary investigations (33a) have revealed a close correlation between OCA-B down-regulation and Ig gene extinction in B-cell × non-B-cell hybrids, arguing strongly that OCA-B is a B-cell factor critical for maintaining Ig gene expression.

Functional characterization of nOCA-B and rOCA-B. To fully establish that we had cloned the genuine OCA-B, the following analyses were conducted to show that rOCA-B behaves similarly, if not identically, to highly purified native OCA-B (nOCA-B). In each case, the octamer-dependent IgH template was used as the test promoter and the octamer-dependent H2B template was used as a control.

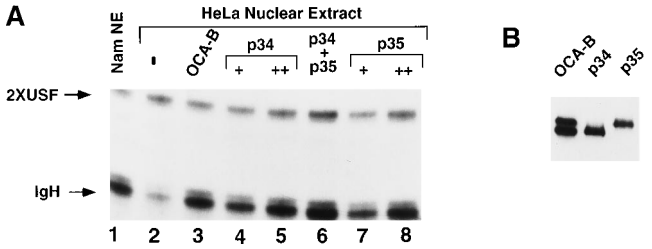

FIG. 4. Renaturation of OCA-B activity. (A) Transcription analysis. Compensating buffer (−; lane 2), immunopurified OCA-B (2 μl; lane 3), renatured p34 (lanes 4 and 5) or p35 (lanes 7 and 8), or a combination of renatured p34 and p35 (lane 6) was used to complement a HeLa nuclear extract (10 μl). + and ++ denote 2 and 4 μl of renatured proteins, respectively. The reaction in lane 6 used an aliquot (4 μl) from a mixture of equal volumes of p34 and p35. The same mixture (~20 ng of OCA-B protein per μl) was used as a source of most highly purified nOCA-B in later experiments. Nam NE, Namalwa nuclear extract. (B) Immunoblot analysis of immunopurified OCA-B (1 μl), renatured p34 (2 μl), and p35 (2 μl).

First, we compared the activation potential of highly purified nOCA-B with that of rOCA-B in a complete HeLa nuclear extract. Because of an inability to express full-length OCA-B protein in bacteria, rOCA-B was produced in a scaled-up in vitro transcription-translation reaction and purified via an anti-rOCA-B antibody affinity column (for details, see Materials and Methods). As can be seen in Fig. 6A, rOCA-B, like nOCA-B, stimulated transcription from the IgH promoter but not that from the control H2B promoter (compare lane 4 with lane 3). As a control, a corresponding fraction from the in vitro translation reaction programmed with antisense OCA-B RNA, otherwise identically prepared, had no effect (lane 5).

Second, we checked the functions of nOCA-B and rOCA-B with Oct-1 and Oct-2 in an Oct-depleted HeLa nuclear extract. As shown in Fig. 6A, removal of Oct-1 from the HeLa nuclear extract markedly reduced transcription from the H2B promoter without significantly affecting that from the IgH promoter (compare lane 8 with lane 7), presumably because the basal level of IgH transcription (even in the presence of Oct-1) was already quite low as a result of absence of OCA-B. In accordance with our earlier studies (20, 25), readdition of either purified Oct-1 or purified Oct-2 substantially and equivalently restored the H2B transcription (Fig. 6A; compare lanes 9 and 14 with lane 8); however, efficient IgH transcription (at a level comparable to that observed in B-cell extracts; lane 6) required a combination of both OCA-B and either Oct-1 or Oct-2 (compare lanes 12, 13, 15, and 16 with lane 8). Oct-1 or Oct-2 alone only marginally (~2-fold) stimulated the IgH transcription (lanes 9 and 14). Clearly, nOCA-B and rOCA-B act in indistinguishable manners (compare lanes 12 and 13 and lanes 15 and 16), no matter which octamer-binding factor is used (compare lanes 12 and 13 with lanes 15 and 16). As anticipated, OCA-B alone (lanes 10 and 11) had no significant effect on either promoter. Furthermore, OCA-B had no or only a marginal effect on the H2B promoter in the complete HeLa nuclear extract (compare lanes 3 and 4 with lane 2) and in the Oct-depleted HeLa nuclear extract complemented with either Oct-1 or Oct-2 (compare lanes 12, 13, 15, and 16 with lanes 9 and 14).

Third, we made use of polyclonal antibodies against rOCA-B to near quantitatively deplete the OCA-B proteins from a B-cell nuclear extract (Fig. 6C). OCA-B-mediated IgH

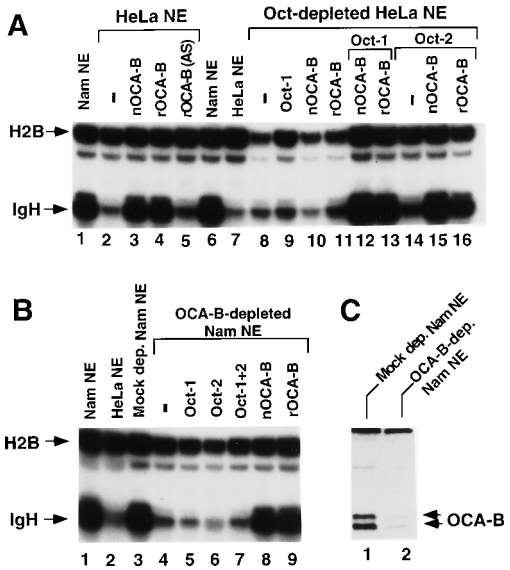

FIG. 6. Characterization of both nOCA-B and rOCA-B. (A) Function of OCA-B in a HeLa nuclear extract (HeLa NE; 10 μl; lanes 2 to 5) or an Oct-depleted HeLa nuclear extract (11.5 μl; lanes 8 to 16). In lanes 2 to 5, reaction mixtures contained compensating buffer (−) and 2 μl each of nOCA-B, rOCA-B, and rOCA-B(AS) (antisense), respectively. In lanes 8 to 16, compensating buffer (−), factors, or cofactors, as indicated, were added to the reaction mixtures. Ten nanograms of Oct-1 or Oct-2 was used when appropriate. (B) Function of OCA-B in an OCA-B-depleted Namalwa nuclear extract (Nam NE; 10 μl). Namalwa nuclear extract (8 μl; lane 1) and mock-depleted Namalwa nuclear extract (10 μl; lane 3) were included as positive controls. The depleted Namalwa nuclear extract was complemented by compensating buffer (−; lane 4) and factors or cofactors as indicated in lanes 5 to 9. The amount of each protein, when used, was the same as in panel A. (C) Analysis of OCA-B-depleted (lane 2) and mock-depleted (lane 1) Namalwa nuclear extracts by immunoblotting. One microliter of each nuclear extract was used, and the OCA-B was detected by anti-OCA-B antibodies.

promoter activation was severely impaired in this extract (Fig. 6B, lane 4) compared with a mock-depleted extract (lane 3). Addition of Oct-1 (lane 5), Oct-2 (lane 6), or Oct-1 and Oct-2 (lane 7) did not, as expected, rescue the transcription deficiency; however, addition of either highly purified nOCA-B (lane 8) or rOCA-B (lane 9) did so, to a level similar to that observed in either a complete B-cell nuclear extract (lane 1) or the mock-depleted B-cell nuclear extract (lane 3). As an internal control, the H2B template was shown to be almost equally transcribed under all circumstances, once again arguing strongly for the promoter specificity of OCA-B.

Taken together, the data clearly demonstrate that the cloned cDNA encodes a single polypeptide that accounts fully for the previously described (20) Ig promoter-specific coactivator (OCA-B) and that this single OCA-B polypeptide functions equally well with either Oct-1 or Oct-2.

Mechanism of action of OCA-B: interaction with the Oct POU domains and the role of Oct activation domains. Previous studies (20) indicated that OCA-B potentiates Oct factor or octamer-dependent IgH promoter activation through an interaction with Oct factors and further showed a direct physical interaction between Oct-1 and OCA-B in the presence or absence of DNA. Although in the earlier study a physical interaction of OCA-B (in a partially purified form) with Oct-2 was not evident in gel shift assays, the function of OCA-B in activating the IgH promoter with Oct-2 was nevertheless indistinguishable from that with Oct-1 (reference 20 and this work). Since Oct-1 and Oct-2 have virtually no homology outside their respective POU domains, it seems likely that Ig promoter activation by OCA-B results at least in part from the coactivator being tethered to the promoter by the conserved POU domain of either Oct-1 or Oct-2. The tethered OCA-B may in turn provide an activation surface(s) which could function synergistically with the previously described activation domains in Oct-1 or Oct-2 (10, 23, 37).

To test this hypothesis, we used a protein-protein interaction assay involving POU domains of Oct-1, Oct-2, and Oct-3 (termed POU-1, POU-2, and POU-3) fused to GST. Although Oct-1, Oct-2, and Oct-3 recognize the same DNA motif, Oct-3 contains a divergent POU domain and resides in a distinct subfamily of POU proteins (reviewed in reference 29). The respective GST-POU domain fusion proteins were incubated with radiolabeled OCA-B and passed through glutathione-Sepharose columns; after washing, the bound proteins were eluted with glutathione and analyzed by SDS-PAGE plus fluorography. The data in Fig. 7B indicate interactions of both OCA-B polypeptides with GST–POU-1 (lane 3), GST–POU-2 (lane 4), and, to a very small extent, GST–POU-3 (lane 5), but not the control GST (lane 2). Thus, OCA-B appears to be a POU domain-interacting coactivator which selectively binds to different POU proteins.

An electrophoresis mobility shift assay was also used to analyze interactions of OCA-B with Oct-1 and Oct-2 through corresponding POU domains (Fig. 7C). In this experiment, equimolar levels of intact Oct factors and derived POU domain proteins were incubated with a labeled IgH promoter fragment (see reference 20 for a description) in the presence or the absence of rOCA-B (Fig. 7C). Except for the POU-3 promoter complex (lanes 11 and 12), all of the Oct- or POU-IgH promoter complexes were quantitatively supershifted by OCA-B (compare lanes 3, 5, 7, 9 with lanes 4, 6, 8, and 10). An anti-OCA-B antibody could further retard the mobility of the supershifted complexes, confirming the presence of OCA-B in these complexes (20a). Our inability to see an Oct-2–OCA-B–DNA complex in the earlier study (20) probably reflected the use of a partially purified (and more dilute) OCA-B activity. In agreement with the proposed role of OCA-B as a non-DNA-binding coactivator (reference 20 and this work), OCA-B showed no independent DNA-binding activity (lane 2). Furthermore, titration studies over a wide range of Oct, POU, and OCA-B concentrations failed to show any differences between Oct-1/Oct-2 and POU-1/POU-2 with respect to OCA-B recruitment or any effect of OCA-B on the stability of Oct-1/Oct-2 or POU-1/POU-2 binding to the promoter (20a). Thus, the POU domains of Oct-1 and Oct-2 account quantitatively for OCA-B binding to the promoter, and conversely, OCA-B does not appear to stabilize the binding of either intact Oct factors or isolated POU domains.

To further substantiate the above-mentioned hypothesis, several Oct-2 mutants with small deletions in either of the two activation domains (Fig. 7A) were purified and used to complement an Oct-depleted HeLa nuclear extract in the absence or presence of OCA-B. Oct-2Δ10, Oct-2Δ5, and Oct-2Δ3 are mutants of Oct-2 that showed variably reduced (3- to 10-fold) abilities to stimulate an artificial octamer-dependent promoter in vivo (10). We reasoned that if the POU domains are sufficient to mediate the interaction of Oct factors with OCA-B (see above) and if OCA-B has intrinsic activation potential, then the activation domain mutants might lower the modest Oct-dependent basal-level transcription from the IgH promoter but still effect a potentiation by OCA-B. Data shown in Fig. 7D strongly support this idea. In the absence of OCA-B, Oct-1 and Oct-2 stimulated IgH transcription by ~3-fold (compare lanes 4 and 5 with lane 3), while the mutants, as expected, stimulated IgH transcription to somewhat lesser extents (com-

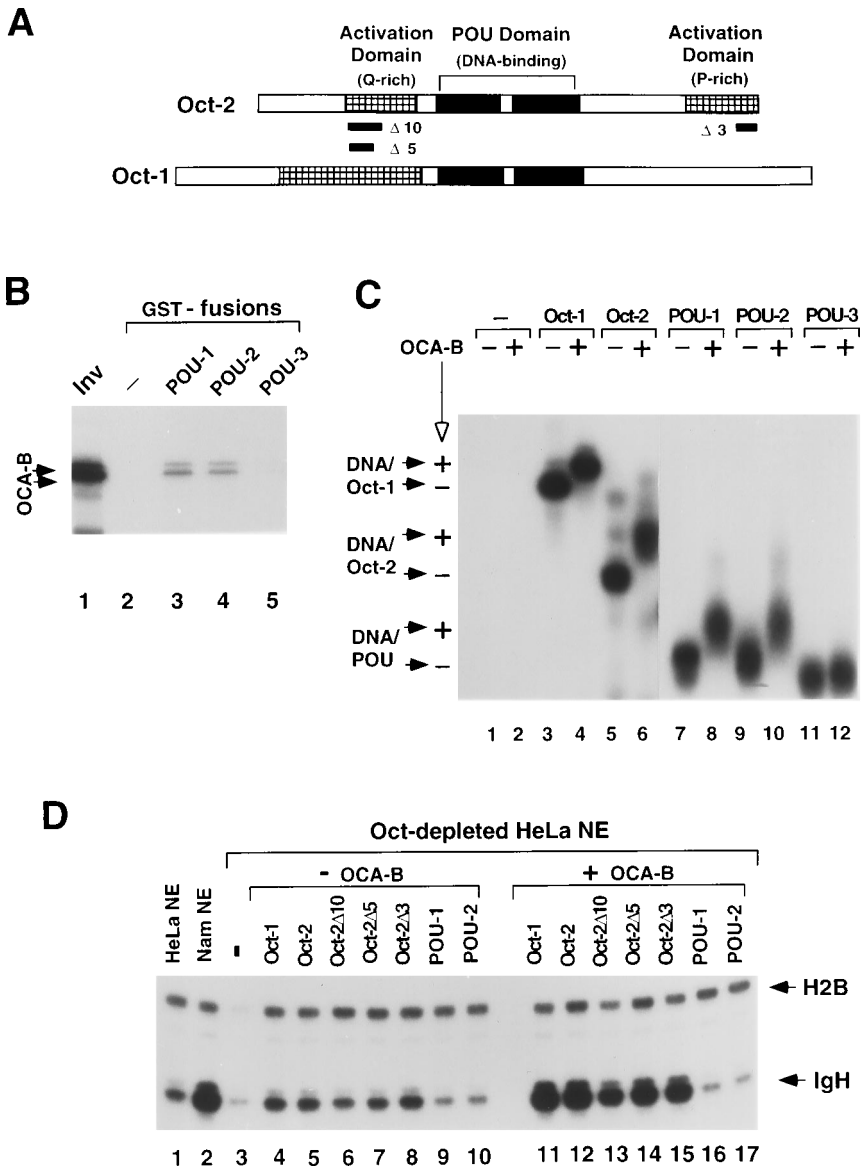

FIG. 7. (A) Schematic diagrams of Oct-1 and Oct-2. Deleted residues in the Oct-2 activation domain mutants are as follows: Oct-2Δ10, 102 to 160; Oct-2Δ5, 102 to 116; and Oct-2Δ3, 446 to 463. The numbering is according to Gerster et al. (10). POU-1 and POU-2, along with POU-3 (all made as GST fusion proteins), contain minimal POU domains indicated by the alignment (29). (B to D) Specific interaction of OCA-B with Oct-1 and Oct-2 POU domains (B and C) and analyses of OCA-B with Oct factors or Oct-derived mutants in a transcription assay (D). (B) Binding of OCA-B to immobilized POU domains. See text for explanations. Inv, in vitro-translated OCA-B. (C) Electrophoretic mobility shift assay performed with a labeled IgH promoter DNA fragment (~20 fmol, 0.5 ng) in the absence (−) or presence of ~100 fmol (5 to 10 ng) of octamer-binding factors (Oct-1, Oct-2, POU-1, POU-2, and POU-3) and, when present (+), ~500 fmol (15 ng) of purified OCA-B. Arrows on the left indicate the positions of the complexes; + and − following the arrows indicate whether or not a particular complex contains OCA-B. The data in lanes 1 to 6 and lanes 7 to 12 are from the same gel and autoradiographic exposure, and the bottom half of the gel showing the unbound probe is not shown because of space considerations. (D) Comparative analysis of Oct factors, Oct-2 activation domain mutants, POU-1, and POU-2 in an Oct-depleted HeLa nuclear extract (HeLa NE) without or with OCA-B (2 μl). Ten nanograms of Oct-1, Oct-2, or derivatives was used when present. Nam NE, Namalwa nuclear extract.

pare lanes 6 to 8 with lane 3). In the presence of OCA-B, however, both Oct factors and activation domain mutants of Oct-2 effected comparable levels of potentiation (5- 10-fold) by OCA-B on the IgH promoter (compare lanes 11 to 15 with lanes 4 to 8). This finding implies that the loss of function of either one of the two activation domains (Fig. 7A) in Oct-2 can be tolerated and raises the possibility (verified below) of a synergistic action of OCA-B and the remaining intact activation domain(s).

Should synergism between OCA-B and Oct activation domains be required for IgH promoter activation, the POU domain alone, although sufficient for tethering OCA-B to the promoter (see above), ought not to be sufficient to mediate the OCA-B action. To test this notion, isolated POU domains of Oct-1 and Oct-2 (POU-1 and POU-2) were used to complement an Oct-depleted HeLa cell extract in the absence or presence of OCA-B. As shown in Fig. 7D, POU-1 and POU-2 (compare lanes 16 and 17 with lanes 9 and 10), in sharp contrast to Oct-1, Oct-2, and the three above-mentioned Oct-2 mutants (compare lanes 11 to 15 with lanes 4 to 8), failed completely to stimulate the IgH promoter in conjunction with OCA-B. This differential was obviously not due to more effi-

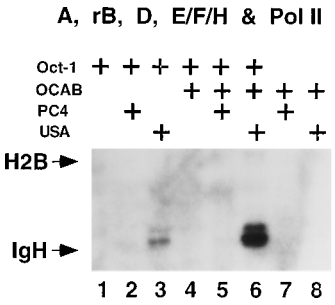

FIG. 8. Analyses of OCA-B in a reconstituted transcription system. OCA-B function requires general cofactor fraction USA. Reaction mixtures contained basal factors and RNA polymerase (Pol) II (listed at the top) and, when present (+), 100 ng of PC4, 2 μl (~1 μg) of USA fraction, 10 ng of Oct-1, and 2 μl of OCA-B. Virtually no activity was observed with basal factors alone (20a).

cient OCA-B binding to intact Oct factors than to isolated POU domains, because OCA-B was recruited to the IgH promoter equally well by either intact Oct factors or isolated POU domains (see discussion of Fig. 7C above). Thus, synergism between OCA-B, which displays an intrinsic activation function when linked to the GAL4 DNA-binding domain (34a), and an Oct activation domain(s) is essential for high levels of Ig promoter activation.

As a control for these transcription analyses, effects of Oct-1, Oct-2, Oct-2 activation domain mutants, POU-1, and POU-2 on Oct-dependent H2B transcription were analyzed. Significantly, the markedly reduced H2B transcription in the Oct-depleted extract (Fig. 7D; compare lane 3 with lane 1) was rescued fully and equivalently by Oct-1, Oct-2, POU-1, or POU-2 (compare lanes 4, 5, 9, and 10 with lane 3). Consistent with these results, the Oct-2 activation domain mutants were as effective as intact Oct-2 in restoring the reduced H2B transcription in Oct-depleted HeLa extracts, and their activities were unaffected by OCA-B (Fig. 7D, lanes 6 to 8 and 13 to 15).

These results indicate that the H2B and IgH promoters use distinct mechanisms with variable Oct activation domain and cofactor requirements and, relevant to Ig transcription, that full stimulation of IgH promoters requires synergism between activation functions of both Oct factors and OCA-B. It is interesting that the adenovirus E1A oncoprotein, tethered to target promoters by DNA-bound factors, also functions synergistically with certain activation domains (19).

Mechanism of action of OCA-B: requirement for USA. Our past (20) and present results have indicated that the specialized (cell-, gene-, and activator-specific) coactivator OCA-B functions in part through primary interactions with DNA-bound Oct-1 or Oct-2, while other studies have indicated a functionally analogous mechanism for the USA-derived (21) general coactivator PC4 acting in conjunction with DNA-bound GAL4-activation domain fusion proteins (9). In view of this parallel, and the general need for coactivators other than those in TFIID (reviewed in reference 9), one reasonable hypothesis was that the function of specialized coactivators like OCA-B might circumvent the need for generalized coactivators like the USA-derived PC4 component. To test this idea, the functions of OCA-B and Oct-1 were analyzed in a system reconstituted with highly purified HeLa-derived basal factors and RNA polymerase II, both in the absence and in the presence of USA (21) or recombinant PC4 (9, 15). As shown in Fig. 8, the marginal IgH transcription (see figure legend) observed with basal factors was not enhanced by Oct-1 (lane 1) or Oct-1 plus OCA-B (lane 4) in the absence of USA or PC4. Somewhat surprisingly, PC4 did not restore the modest Oct-1 effect or the potent Oct-1-plus-OCA-B effects seen in the crude reconstituted system (lanes 2 and 5), whereas the USA fraction did effect modest stimulation by Oct-1 (lane 3) and a large stimulation by Oct-1 plus OCA-B (lane 6). OCA-B had no effect in the absence of Oct-1, with either PC4 (lane 7) or USA (lane 8). Under identical assay conditions, the same preparations of PC4 and USA were both very active in stimulating transcription by GAL4-based activators (9). These results demonstrate that Oct-1 activator function in association with the specialized coactivator OCA-B still requires one or more ubiquitous coactivators in the USA fraction and that the very potent general coactivator PC4 does not suffice. Interestingly, the failure to see any Oct-1-mediated transcription from the H2B promoter above the marginal basal level (see the legend to Fig. 8) suggests, as predicted (20), that Oct-1-mediated H2B transcription still requires an as yet unidentified cofactor.

DISCUSSION

Our previous study (20) reported the biochemical identification of a specialized coactivator, OCA-B, whose tissue specificity and functional properties not only implicated this factor as the major determinant of B-cell-specific Ig promoter activation but also provided an initial insight into its mechanism of action through stable interactions with a ubiquitous factor (Oct-1) that directly recognizes the key promoter regulatory element (octamer). Our isolation and characterization of a cDNA clone that encodes OCA-B have allowed us to verify the key role of OCA-B, as a single polypeptide, in B-cell-specific Ig promoter transcription, to gain insights into the structure of OCA-B and possible relationships to other coactivators, and to investigate other aspects of its mechanism that may be of general importance.

Role of OCA-B in Ig transcription and B-cell development. B-cell differentiation is accompanied by a highly ordered program of gene expression comprising developmentally regulated DNA rearrangements of, and transcription from, Ig genes. Using a recombinant protein and corresponding antibodies, we show that the previously described Ig promoter-specific activation properties of nOCA-B, as well as function through either Oct-1 or Oct-2, can be attributed to a single polypeptide (or a modified form thereof). Remarkably, this single (recombinant) polypeptide has the ability to convert a non-B-cell extract to a B-cell extract with respect to Ig promoter transcription. Coupled with the B-cell-restricted expression of both OCA-B RNA and protein, these results demonstrate conclusively the preeminent role of OCA-B in the B-cell-specific transcription of active (rearranged) IgH and Ig light-chain promoters. Given that the ubiquitous Oct-1 appears to suffice for Oct-dependent B-cell-specific transcription of several other genes (B29, Cr2, and CD20) (5, 8), it is likely that OCA-B is also involved in their regulation.

Intriguingly, even prior to DNA rearrangements which result in functional Ig genes, transcription from both IgH and Ig light-chain genes can take place at several distinct sites in the germ line configuration to produce "sterile" (nontranslatable) transcripts (reviewed in reference 34). One hypothesis (e.g., reference 2) is that these novel (non-V_H or V_K) transcription events are prerequisite for DNA rearrangements by virtue of resulting chromatin structure disruptions that expose DNA to the recombination machinery. Careful searches have revealed that the octamer element (ATTTGCAT) found in most mammalian Ig promoters, and in both the IgH and Ig kappa enhancers, is also present in normal or variant forms (e.g., AT TTACAT and ATTTTCAT) at or near regions where the sterile transcripts are initiated (reviewed in reference 34). This

finding raises the interesting possibility that OCA-B is involved not only in Oct factor- or octamer-mediated promoter and enhancer functions but also in the induction of transcription from Ig germ line loci. This, if true, is of particular significance, for it suggests that OCA-B may function at the earliest stages of the B-cell differentiation program.

A related question concerns the role of Oct-2. In agreement with our in vitro Oct-2 knockout data (20), an analysis of Oct-2 knockout mice demonstrated that Oct-2 is dispensable for early B-cell differentiation and Ig transcription; however; it is still essential for the activation and maturation of B cells into Ig-secreting plasma cells (6). One explanation is that Ig secretion from activated B cells and plasma cells may require elevated levels of Ig transcription for which Oct-1 has become limiting, thus requiring compensation by Oct-2. A similar situation could occur during activated B-cell proliferation, when enhanced transcription of both ubiquitous and cell-specific Oct-dependent genes may render Oct-1 limiting. Indeed, very recent observations by Radomska et al. (28) and by Corcoran and Karvelas (5) are consistent with these explanations. In the first case, preventing the down-regulation of Oct-2 in plasmacytoma × T-cell hybrids was demonstrated to preserve the Ig gene transcription which was otherwise silenced, suggesting a role of Oct-2 in plasma cells. In the second case, Oct-2 was shown to be required during B-cell activation for G_1 progression and cell proliferation. These explanations do not exclude the possibility that, as suggested by Corcoran et al. (6) and Corcoran and Karvelas (5), Oct-2 possesses a unique function (e.g., in activating distinct target genes) not shared by Oct-1. Possibly related, other studies (3, 8) also have suggested that the functions of octamer elements from distal (enhancer) positions require Oct-2 as well as a B-cell cofactor for which OCA-B is a good candidate. Especially important in this regard is our direct demonstration of OCA-B function in conjunction with Oct-2.

Given the central role of OCA-B in activation of Ig promoters, and potentially a variety of other lymphoid cell-specific genes containing functional octamer elements, we envision a profound impact of OCA-B on B-cell development by virtue of its importance in maintaining an appropriate gene expression program. This idea can be readily tested once OCA-B knockout mutant mice become available.

Mechanism of action of OCA-B. Using Oct-depleted nuclear extracts, we have shown that rOCA-B, as well as nOCA-B purified with antibodies to rOCA-B, can function equally well through either Oct-1 or Oct-2 to stimulate Ig promoter transcription and that OCA-B (natural or recombinant) is without effect on Oct-dependent H2B transcription. These results confirm and extend previous results obtained with partially purified OCA-B and, in addition, indicate that the electrophoretically distinct forms of OCA-B (p35 and p34) are functionally equivalent under the in vitro assay conditions and likely reflect different posttranslational modifications. Given the established identity of rOCA-B with nOCA-B, and the stable association of the latter with both Oct-1 and Oct-1–promoter complexes (20), it is clear that the proline-rich OCA-B polypeptide(s) described here must function through interactions with promoter-bound Oct-1. Given the further demonstration that OCA-B can interact directly with the POU domains of either Oct-1 or Oct-2, several simple mechanisms are possible. One possibility is that OCA-B contains potent activation domains which are merely tethered to the promoter by Oct-1 or Oct-2. A second possibility is that OCA-B contains activation domains which not only are tethered to the promoter by Oct-1 or Oct-2 but, necessarily, function in conjunction with activation domains within Oct-1 or Oct-2. Consistent with both possibilities is the observation that OCA-B contains regions characteristic of known activation domains (Fig. 2A; discussed in Results) and regions with sequence similarities to other proteins thought to effect transcription through protein-protein interactions. More consistent with the second possibility is the observation that although Oct-1 and Oct-2 POU domains suffice for OCA-B binding, they are unable to rescue transcription of the IgH promoter in an Oct-depleted cell extract, even in the presence of OCA-B. Moreover, mutants with deletions in either of the two known Oct-2 activation domains, while lowering the basal activity, still effect a stimulation by OCA-B. Therefore, it is likely that activation domains in OCA-B function cooperatively with activation domains in Oct-1 or Oct-2.

A similar mechanism may well apply to other situations in which DNA-binding activators and interacting cofactors have both been shown to require intrinsic activation domains. Examples include GAL4/GAL11 (12), ATF-2/E1A (19), and CREB/CBP (16). There are also several examples in which mammalian Oct factors and cofactors interact to exert full promoter activation. First, in differentiated embryonal carcinoma cells, the adenovirus E1A oncoprotein interacts with promoter-bound Oct-4 (also termed Oct-3) to effect distal octamer-dependent activation, while a cellular E1A-like protein may carry out the same function in undifferentiated embryonal carcinoma cells (32). Interestingly, OCA-B possesses a short segment with sequence similarity to an E1A region critical for promoter targeting (Results) and functions with Oct-1 and Oct-2 in a fashion similar to that indicated for Oct-4 and E1A. Hence, OCA-B may represent a class of cellular E1A-like proteins. Second, the viral regulatory protein VP16, with well-defined activation domains (36), is recruited to herpes simplex virus immediate-early promoters, in conjunction with another host cellular factor, by stable interaction with promoter-bound Oct-1, and the POU domain of Oct-1 is sufficient for this interaction (4, 26, 38). Third, mammalian 7SK gene activation requires the interaction of distal octamer-bound factors and a proximal sequence element-binding protein (PTF) whose promoter recruitment and function are potentiated solely by the POU domain of either Oct-1 or Oct-2 but further enhanced by the activation domains (24). Our present data (see Results) also argue indirectly for a distinct coactivator involved in H2B promoter activation and cell cycle regulation.

Taking the Oct coactivator promoter specificities into consideration, we propose related models to explain the differential activation of Ig and H2B promoters through common octamer elements and interacting Oct factors (Fig. 9). In each case, the POU domain of either Oct-1 or Oct-2 suffices to recruit the respective coactivator to the target promoter, and the combination of Oct-1 or Oct-2 and cofactors provides multiple activation surfaces. Significantly, and despite the involvement of a specialized coactivator, Ig promoter activation also involves one or more additional coactivators in the general USA cofactor fraction (discussed further in Results). Cofactors OCA-B and OCA-S (a presumptive Oct coactivator for S-phase-induced H2B promoter) differ in that the proposed activation domain(s) in OCA-S can function relatively independently (i.e., with the POU domain alone), whereas the activation domain(s) in OCA-B requires cooperation with at least one of the Oct activation domains. OCA-S thus resembles PTF, whose recruitment to promoters by the POU domain alone is sufficient for transcription activation (24), and potentially VP16, which contains intrinsic activation domains and is recruited to the promoter via the Oct-1 POU domain (4, 26). The models can explain many aspects of the different behaviors of H2B and the IgH promoters and may also be relevant to the ability of ectopically expressed POU domains to selectively

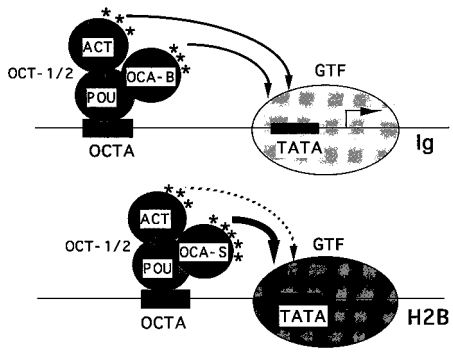

FIG. 9. Models of differential activation mechanisms for Ig and H2B promoters. Activation domains in Oct-1, Oct-2, or cognate cofactors are indicated by asterisks. According to the models, the coactivators (OCA-B and OCA-S) are recruited to the promoters via Oct-1 or Oct-2 POU domains, and while OCA-B function requires cooperation with one of the Oct activation domains, OCA-S can function relatively independently. Differential shadowing over the TATA box reflects the suggestion that the basal transcriptional machinery assembled on the Ig promoter may differ from that on the H2B promoter. GTF, general transcription factors; ACT, activation domain(s).

inhibit specific Oct-dependent promoters and to induce tumors (27). However, what determines the promoter specificities of OCA-B (for Ig) and OCA-S (for H2B) is not yet clear. It is conceivable that the downstream transcriptional machineries assembled on the respective core promoters have different compositions or configurations which respond differently to, and therefore distinguish between, distinct activation surfaces. Other sequences, such as those flanking the TATA and the octamer elements, or the spacing between the two elements, may also play a role.

Implication of OCA-B for other regulatory pathways. As previously argued (20), the function of promoter-bound Oct factors in higher eukaryotes may generally require stably interacting proteins (coactivators). Oct factors are members of both the POU domain (for reviews, see references 29 and 31) and the broader homeodomain (for a review, see reference 33) families of regulatory proteins. While a very few, such as Oct-1, are widely expressed, most show more restricted expression patterns, notably in developing embryos and in the neuronal system (reviewed in reference 29). However, quite often the target genes are more narrowly expressed than these upstream regulators (reviewed in references 11, 29, and 30). This observation suggests the existence of either activating (e.g., OCA-B) or inhibiting (e.g., I-POU; reviewed in reference 29) cofactors which serve to restrict the gene activation to more specialized territories, thus imposing sharper spatial or temporal boundaries for gene control. A relevant example from lower eukaryotes is provided by UNC-86, a POU domain protein essential for specifying neural identities in *Caenorhabditis elegans* (reviewed in reference 30). Whereas UNC-86 is expressed in 27 neuron types, the *mec-3* target gene is expressed in only 5 of the 27 neuron types. Genetic analyses (reviewed in reference 30) have identified candidates for an UNC-86 cofactor which, by virtue of its absence from the other 22 neuron types, may function in conjunction with UNC-86 to define the more restricted *mec-3* expression pattern. Conceivably, other classes of DNA-binding activators may also be modulated by interacting cofactors analogous to those discussed here.

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