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Octamer-Binding Proteins in Diverse Hemopoietic Cells

PETER N. COCKERILL* AND S. PETER KLINKEN†

The Walter and Eliza Hall Institute of Medical Research, Post Office Royal Melbourne Hospital, Victoria, 3050, Australia

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The immunoglobulin genes have B-cell-specific promoter and enhancer elements. The regulation of these elements is thought to be mediated to a large degree by the *trans*-activating factor oct-2, which binds the octamer element (ATTTGCAT). We have further examined the role of this octamer element in directing the lymphoid-specific expression of the immunoglobulin H enhancer. No direct relationship was found between the levels of expression of the Cμ gene and oct-2. Indeed, variable amounts of oct-2 were detected in all of the hemopoietic lineage cells tested in this study.

The immunoglobulin genes have been used extensively as a model for studying the regulation of tissue-specific gene expression (4). Their B-cell-specific expression is thought to be mediated via *trans*-acting factors which associate with both promoter and enhancer elements. One of these elements is the octamer (ATTTGCAT), which is found in the immunoglobulin H (IgH) enhancer and in all V-region promoters (4, 9, 26). This element appears to play a major role in immunoglobulin gene regulation, as genes introduced into B cells can be activated by the insertion of octamer elements (7, 12, 40). This activation element does, however, provide a paradox, as it contributes to the B-cell specificity of the immunoglobulin genes (7, 12, 24, 40) but is also found in many genes that show no tissue specificity, such as the histone 2B and U2 small nuclear RNA genes (8, 14, 23). Furthermore, the octamer can bind both ubiquitous and lymphoid-specific factors (oct-1 and oct-2 [12, 21, 36]). Its activity is apparently determined both by the complement of octamer-binding factors present in the cell and the nature and location of the other promoter elements within the gene (29, 31, 37). The ubiquitous factor oct-1 (also known as NF-A1, OTF-1, OBP100, and NFIII) binds the immunoglobulin octamer elements but only appears to activate other genes, such as the U2 small nuclear RNA gene (31, 37). The factor oct-2 (also known as NF-A2 and OTF-2) does, however, activate the immunoglobulin genes and is one of the only IgH enhancer-binding factors which is largely restricted to B cells. It has furthermore been observed that expression of oct-2 in HeLa cells is sufficient for activation of cotransfected B-cell-specific promoters (24). This reinforces the view that oct-2 is one of the major determinants of the B-cell specificity of the immunoglobulin genes. However, some IgH enhancer activity has been detected in cells outside of the B lineage. For example, nonproductive sterile transcripts of the IgH Cμ region have been detected in myeloid cells and T cells (2, 17, 18). If Cμ expression requires the presence of oct-2, then we may expect to detect oct-2 in those cell types in which limited Cμ expression has been observed.

With the above considerations in mind, we have investigated the role of oct-2 in immunoglobulin gene regulation. We have assayed for oct-2 in a variety of hemopoietic and fibroblast cell lines (1, 18–20, 25, 36). In this study, we have also made use of some unusual cell lines which have switched from B-lineage cells to macrophages [1-Bra/raf, Raf-1(M11), Ras 16(M)] (19). These cells were derived by introducing either the v-*raf* or the v-*ras* oncogene into B-lineage cells from Eμ-*myc* transgenic mice, in which c-*myc* is driven by the IgH enhancer (1). Upon switching hemopoietic lineage, these cells underwent gross morphological changes and, in the case of 1-Bra/raf and Ras 16(M), deactivated both the IgH gene and the Eμ-*myc* transgene (19). The myeloid cell line Raf-1(M11) has, however, maintained a level of Cμ expression similar to that found in its lymphoid parent (19). These cell lines provide a useful additional aid in determining whether a correlation exists between oct-2 and Cμ expression.

To determine the abundance of oct-2, nuclear extracts were prepared as described by Dignam et al. (6) and assayed for octamer-binding factors by gel mobility shift assays (10, 11), by the method of Staudt et al. (36). The DNA binding substrate was the sequence TAATTTGCAT inserted within the pUC19 polylinker. This substrate represents the slightly extended conserved octamer sequence (8) as it exists in the IgH enhancer. To demonstrate that we were visualizing authentic oct-2, we first examined 70Z/3 cell nuclear extracts, prepared before or after stimulation with lipopolysaccharide. Figure 1B confirms previous reports (36) that 70Z/3 cells produce two octamer-binding factors (oct-1 and oct-2) and that oct-2 production can be elevated by treatment with lipopolysaccharide. Each complex was readily inhibited by unlabeled octamer-containing DNA fragments (data not shown).

The three B-lineage cell lines, 70Z/3, 3-Mes, and 1-Bra, were found to produce comparable amounts of oct-2 (Fig. 1A). Significantly, the myeloid cell line 1-Bra/raf has maintained the same relatively high levels of oct-2 expression as its parental pre-B-cell line, 1-Bra (Fig. 1A), even though Cμ RNA production has ceased (19). Conversely, the myeloid cell line Raf-1(M11) produces only a small amount of oct-2 (relative to the B cells tested here) but maintains the same high level of Cμ RNA production seen in its lymphoid parent [Raf-1(L), (19)]. Of the three cell lines that have switched lineage from lymphoid to myeloid cells, only Ras-16(M) shows a low level of oct-2 (Fig. 1A) accompanying a large decrease in Cμ RNA production (Fig. 2). From this, we conclude that factors in addition to oct-2 are playing major roles in regulating the expression of Cμ after the lymphoid to myeloid lineage switch.

Having found oct-2 in all three myeloid derivatives, we investigated whether oct-2 was merely a legacy of their

* Corresponding author.
† Present address: Department of Biochemistry, University of Western Australia, Nedlands, WA, 6009, Australia.

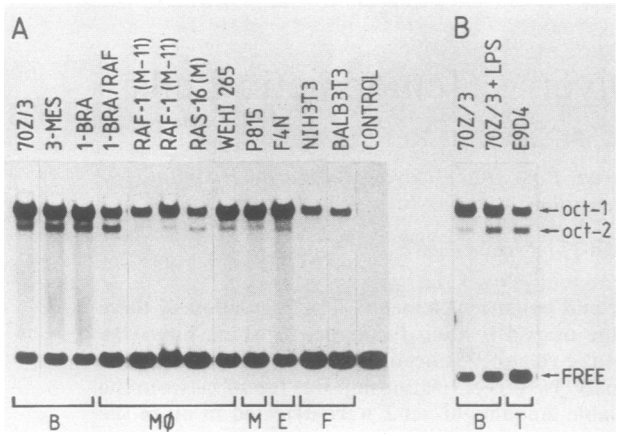

FIG. 1. Gel mobility shift assay of octamer-binding factors. Nuclear extracts were assayed with a 71-bp ^{32}P-labeled *Eco*RI-*Hin*dIII DNA fragment that includes the extended octamer sequence, TAATTTGCAT, inserted at the *Bam*HI site of the pUC19 polylinker. Each assay included 5 μg of nuclear protein, 5 μg of poly[dI-dC] · poly [dI-dC], and ~0.5 ng of probe DNA. For Raf-1(M11), an additional assay of 10 μg of nuclear protein was included to allow detection of oct-2 (righthand lane). The murine cell lines employed have been grouped as B-lineage (B), macrophage (MΦ), mast (M), erythroid (E), fibroblast (F), and T-lineage (T) cells. oct-1 and oct-2 represent complexes which are absent from the protein-free control. Panels A and B represent separate assays. LPS, Lipopolysaccharide.

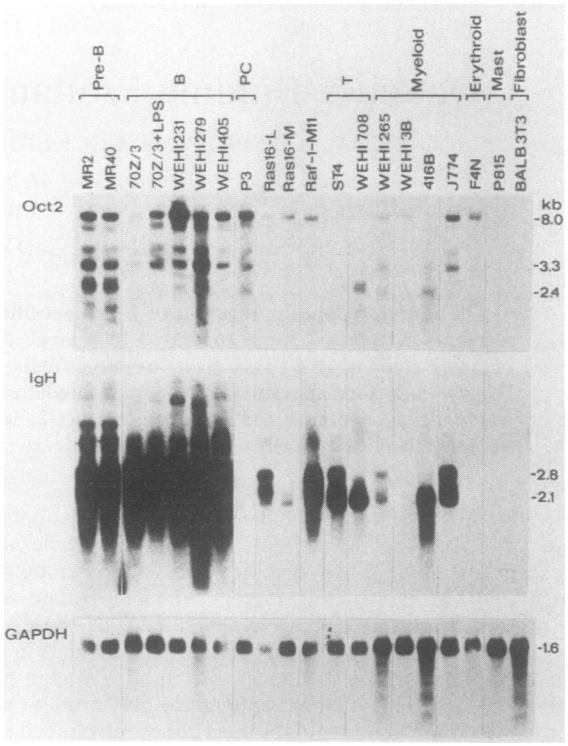

FIG. 2. Northern blot analysis of poly[A]$^+$ RNA. The same Hybond-N nylon membrane was probed sequentially with the human oct-2 gene (35), the Cμ region of the mouse IgH gene (19), and the housekeeping gene glyceraldehyde-3 phosphate dehydrogenase (GAPDH) (19), which was used as an internal standard. The major oct-2 RNA species appeared at 8 kb, and longer exposures revealed the presence of this band in all lanes except lane BALB3T3. Shorter exposures of the IgH-probed filter revealed that the B-lineage cells produced two dominant species, at 2.8 and 2.1 kb. The plasmacytoma cell line P3 has rearranged and deleted the Cμ region and so does not hybridize here with the IgH probe. Ras 16(M) is the macrophage derivative of the lymphoid line Ras 16(L). Note that Ras 16(L) was underloaded and gives a low GAPDH signal. LPS, Lipopolysaccharide; Pre-B, pre-B-cell line; B, B-lineage cells; PC, plasmacytoma cell line P3; T, T-lineage cells.

recent lymphoid history or a feature common to many hemopoietic lineage cells. Upon testing nuclear extracts prepared from cells of several different lineages, we found significant concentrations of oct-2 in all of the hemopoietic cell lines examined (Fig. 1). These included the myeloid line WEHI 265, the mastocytoma line P815, the Friend erythroleukemia line F4N, and the T-cell line E9D4. However, except for E9D4, these lines produced less oct-2 than was generally seen in B cells. No oct-2 was found in the fibroblast cell lines NIH 3T3 or BALB/3T3. These observations have been reproduced in assays with naturally occurring octamer-containing DNA fragments taken from either a V-region promoter or the IgH enhancer (data not shown). It therefore appears that oct-2 is not restricted to B cells and may in fact have a common usage in many types of hemopoietic cells. A similar finding has recently been made concerning the transcription factor NF-κB. This factor plays a key role in kappa immunoglobulin gene regulation and was also originally thought to be lymphoid specific. It too has recently been found to be constitutively expressed in macrophages (13).

The oct-2 gene has recently been cloned (24, 32, 35) and was used to determine whether the levels of oct-2 mRNA mirrored the levels of oct-2 protein found in nuclear extracts. Northern (RNA) blot analysis was performed on a selection of RNA samples (gift of J. M. Adams), which included most of the cell lines used in the above nuclear extract analysis and some additional hemopoietic cell lines. By probing the cell lines with the oct-2 gene, we found that the hemopoietic cell lines express a variable, but always demonstrable, amount of the 8.0-kilobase (kb) oct-2 RNA (Fig. 2; some lanes required longer exposures for this species to be seen). As expected, the highest levels of oct-2 were found in B-lineage cells, and oct-2 was markedly elevated after lipopolysaccharide induction of 70Z/3 cells. The cell lines tested cover all major stages of B-cell development, with MR2 expressing high levels of oct-2 even though no IgH gene rearrangement had occurred.

It is again apparent that there is no direct relationship between oct-2 expression and Cμ RNA production. 70Z/3 cells expressed high levels of Cμ prior to oct-2 induction, while in contrast, the erythroid line F4N showed significant oct-2 but no Cμ RNA (Fig. 2). The T-cell line ST4 showed significant Cμ but little oct-2 expression, and the myeloid derivatives Ras-16(M) and Raf-1(M11) likewise showed no correlation between oct-2 and Cμ mRNA levels (Fig. 2). Only in the fibroblast cell line BALB/3T3 was there no detectable 8.0-kb oct-2 RNA, even after prolonged autoradiography (Fig. 2 and data not shown).

Other studies have also provided evidence for oct-2-like factors in cells outside of the B lineage. For example, Landolfi et al. (21) found that an octamer-binding factor (factor 3) resembling oct-2 was present at moderate-to-high levels in murine T-cell lines and at low levels in P815 mastocytoma cells but was absent from HeLa cells. Others have likewise observed expression of oct-2 RNA in T-cell lines and a glioma cell line (30, 35). Scholer et al. recently observed oct-2-like factors in the developing mouse embryo

and also in adult mouse brain, kidney, and sperm (33). Finally, we should add that octamer-binding factors may in fact represent a large family of related proteins that have different functions (5, 12, 21, 22, 30, 31, 33, 34). In some instances, octamer-binding factors may actually repress transcription (22). In the case of oct-2, it has been suggested that a minor variant form, OTF-2B, (which we have been unable to detect) is required for IgH enhancer activation (31, 34). Closer examination of Fig. 2 reveals that there are in fact several size classes of RNA that hybridize with the oct-2 probe. The major species, the 8.0-kb band, corresponds to the major species previously observed for both mouse and human B-cell oct-2 RNA (previously estimated to be 6.0 to 7.5 kb [24, 32, 35]). Two other prominent RNA species appear at 2.4 and 3.3 kb. It is interesting that the ratios among the different RNA species vary among cell lines, the 2.4-kb species being the most prominent band in some cases and absent in others. The significance of these smaller species is unclear, but they do not appear to arise from either nonspecific degradation (as Cμ and glyceraldehyde-3 phosphate dehydrogenase transcripts remained intact) or from cross-hybridization with other genes such as oct-1, as no signal was detected in fibroblast RNA. It remains possible that one or more of these specific products of the oct-2 gene account for the observed differences in Cμ expression in the cells examined in this study. Similarly, the oct-2 complexes identified in Fig. 1 do not necessarily represent forms of oct-2 capable of activating transcription.

As there is no simple relationship between oct-2 and Cμ expression, it is necessary to consider other factors which may influence the regulation of immunoglobulin genes. Araki et al. have recently purified a novel B-cell-specific 96-kilodalton protein (μB) which has the ability to activate the human IgH enhancer in the absence of oct-2 (3). If this factor, like NF-κB and oct-2, was also to be found in some hemopoietic cells outside of the B lineage, then it could perhaps account for the relatively high Cμ RNA expression seen in cell lines such as Raf-1(M11). Conversely, a lack of μB could account for the absence of Cμ expression in cells such as 1-Bra/raf, which do express high levels of oct-2.

It has also been demonstrated that the IgH enhancer is governed in part by negative regulators (15, 16, 38, 39). We have tested the extracts used in Fig. 1 for binding to three different negative regulatory regions of the IgH enhancer and have detected the previously described factors A, D, and E that bind at or close to these regions (27, 28). However, there is no clear evidence that these factors could account for the observed patterns of Cμ RNA expression (data not shown).

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