14. c_{ij} = 0.75 when the initial mice used to found an inbred strain were related as full sibs, (for example, C57BL and C57BR) because their gametes had 25% of their alleles identical by descent. c_{ij} = 0.375 for C3H and CBA because these strains arose from a single cross of the Bagg albino and DBA lines. DBA had been inbred for about 11 years so C3H and CBA should be identical by descent for all alleles from DBA and as full sibs for those alleles from Bagg albino. See (*5*) for more details and examples.
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16. The bootstrap procedure sampled with replacement the loci as many times as they were present in the original data set. The ANCESTOR program of Fitch (*15*) was used to find a most parsimonious tree and each clade (the group of taxa comprising all descendants of a given ancestral node) of that tree was given a unit of support. If there was more than one most parsimonious tree, say t of them, each clade of each most parsimonious tree received $1/t$ units of support. By repeating this procedure 100 times, the units of support could range from zero to 100 and hence can be treated as a measure of data's support of the tree. However, under circumstances where not all most parsimonious trees are guaranteed to be found (and that is the case here), or those trees found are not a random sample of all of them, the measure of support is biased in a way that would slightly inflate that estimate (W. M. Fitch, unpublished work). Nevertheless, because clades of trees with no measures of support give the appearance of being more certain than trees with them, we think that presenting those values is more likely to reduce rather than inflate confidence in the results. The boot strap values indicate the robustness of the data for a given result, not the representativeness of the data nor the probability that the correct tree has been found.
17. Weiss *et al.* (*19*) provide the following characterization of ecotropic and nonecotropic viruses: Ecotropic viruses will grow in cells of the species from which they were isolated—for example, mouse virus propagates best in mouse cells, to a much lesser extent in other rodent cells, and not at all in higher primates. Nonecotropic or xenotropic viruses, on the other hand, are endogenous to one species but cannot replicate in that species. Xenotropic viruses are not pathogenic in any animal. Endogenous ecotropic viruses, on the other hand may be pathogenic. Transmission may be from one host animal to another by contact; however, a frequent mode of transmission is from parent to offspring. Vertical transmission may be by contact infection or by genetic transmission. (Inbred mice have a single genetic locus, *Fv-1*, that controls susceptibility or resistance to different virus strains.)
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Activation of Early Gene Expression in T Lymphocytes by Oct-1 and an Inducible Protein, OAP^{40}

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After antigenic stimulation of T lymphocytes, genes essential for proliferation and immune function, such as the interleukin-2 (IL-2) gene, are transcriptionally activated. In both transient transfections and T lymphocyte–specific in vitro transcription, the homeodomain-containing protein Oct-1 participated in the inducible regulation of transcription of the IL-2 gene. Oct-1 functioned in this context with a 40-kilodalton protein called Oct-1–associated protein (OAP^{40}). In addition to interacting specifically with DNA, OAP^{40} reduced the rate of dissociation of Oct-1 from its cognate DNA-binding site, suggesting that a direct interaction exists between Oct-1 and OAP^{40}.

T LYMPHOCYTES CAPABLE OF RESPONDING to virtually any antigen are produced in the thymus as a result of intrathymic differentiation and selection. Mature T cells migrate to peripheral lymphoid organs and remain in a quiescent state until the presentation of a specific antigen. Antigen binding produces an orderly and sequential series of gene activations that result in proliferation and immunologic function. Once activated, T cells help to coordinate the immune response through the production of cytokines necessary for the function of B cells, macrophages, and other cell types (*1*). One of the first cytokines to be produced in this process is IL-2, which induces synthesis of its own receptor (*2*) and autoregulates T cell proliferation (*3*). The minimal IL-2 enhancer contains recognition sites for several DNA binding proteins, including two octamer motifs (Fig. 1A) (*4, 5*); however, it is not known which octamer protein or proteins function at these sites. We now demonstrate that Oct-1 (OTF-1, OBP100, NF-III) and an inducible auxiliary protein participate in activating transcription of the IL-2 gene. The association of Oct-1 with a second transcriptional regulatory protein is reminiscent of the VP16–Oct-1 interaction that reprograms gene expression after infection by herpes simplex virus (*6*).

The proximal octamer motif is found in an antigen-receptor response element (ARRE-1) that confers transcriptional activation on a heterologous promoter in response to signals initiated at the antigen receptor (*4*). To assess the function of an octamer-binding protein in the inducible regulation of ARRE-1, we introduced site-directed mutations into the IL-2 enhancer (Fig. 1, A and B), which was ligated upstream of the chloramphenicol acetyltransferase (CAT) reporter gene. Transcriptional activity of these constructs was measured by transient transfection in Jurkat cells, a human T cell lymphoma line that mimics early events of T cell activation when stimulated through the antigen receptor (*7*). A 2-bp mutation that changed the proximal octamer sequence of the IL-2 enhancer to a related octamer motif found in the histone H2b promoter increased activity to 134% (mean) of the wild-type enhancer after stimulation with calcium ionophore and phorbol 12-myristate 13-acetate (PMA) (Fig. 1C). In contrast, when we mutated two additional base pairs to render the histone octamer nonfunctional (*8*), inducible activity dropped to 31% of wild-type (Fig. 1C). On the basis of methylation interference assays that identified the contact guanosines in the octamer motif (Fig. 1B), we mutated two noncontact guanosines immediately 5′ to the octamer site. These changes reduced activation to 28% of wild-type activity, indicating that the octamer motif is important for ARRE-1 induction but is not the sole sequence needed for inducible activity.

To further test the function of the sequences within ARRE-1, we developed an in vitro transcription system. The IL-2 enhancer is induced four- to fivefold by in vitro transcription, as compared to the 100-fold activation measured in transient transfections. Although not as sensitive as in vivo assays, in vitro transcription qualitatively reflects the complex requirements for activating the IL-2 gene (*9*). Using the internal

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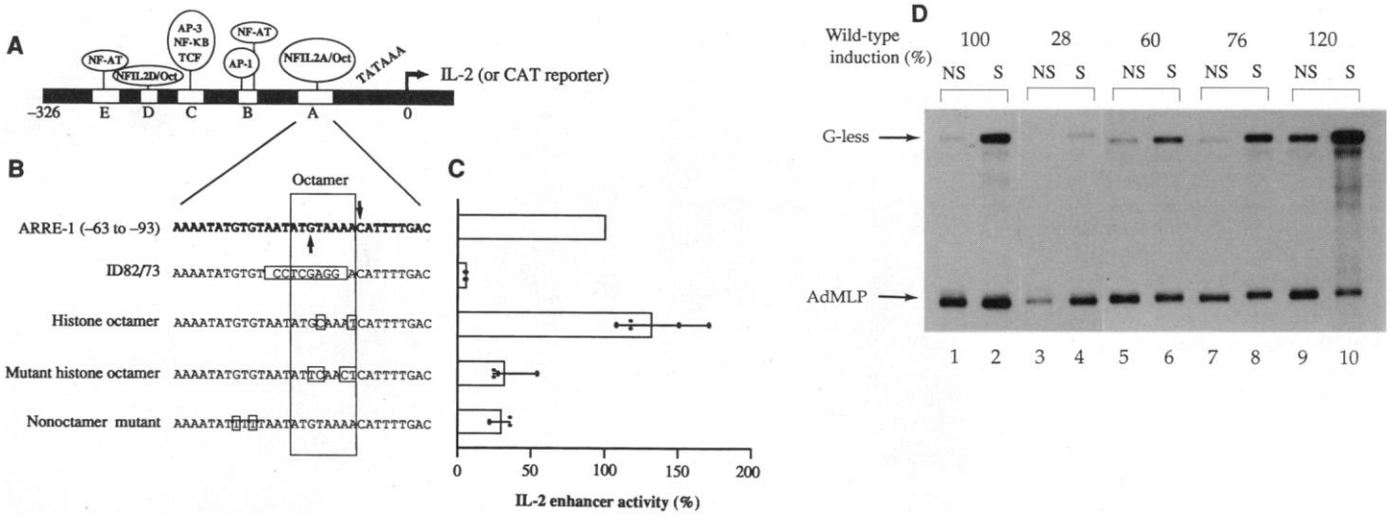

Fig. 1. Activity of an octamer site in the IL-2 enhancer. (**A**) Transcription factor–binding sites in the IL-2 enhancer (*1, 4, 5*). Different proteins have been shown to bind to site C; however, the functional regulation for this site has not been established. Two octamer sites are indicated by Oct. TATAAA, TATA element; 0 start site of transcription. (**B**) Sequence of ARRE-1 (in bold) and the octamer-binding site (large box). Site-directed mutations and internal deletions (small boxes) of this site are also listed. Contact guanosines detected by methylation-interference assays (*27*) are indicated with arrows. The internal deletion mutation ID82/73, in which 10 base pairs (AATATGTAAA) that overlap the putative octamer motif are replaced with a Xho I linker (CCTCGAGG), has been described (*4*). Site-directed mutations were created by polymerase chain reaction (PCR) as described (*28*). Histone octamer, octamer motif in histone H2b promoter; mutant histone octamer, nonfunctional histone octamer; and nonoctamer mutant, mutated guanosines 5′ to the octamer site. (**C**) Inducible activity of octamer mutations in transient transfections of mycoplasma-free Jurkat cells expressed relative to the wild-type enhancer activity. Each bar (mean) is aligned with the sequence tested. All transfections were internally controlled with either a Rous sarcoma virus enhancer–luciferase construct or an IL-2 enhancer–luciferase construct. After normalization to this control, the values for the mutant constructs were compared to the stimulated expression of the wild-type construct. With wild-type amounts in each of five independent transfections set to 100%, the individual results (expressed as percent of wild type) were as follows: H2b (107, 119/117, 172, 155); nonoctamer mutant (21, 32/32); and mutant histone octamer (53, 25, 23/24) (duplicate transfections with the same cells and DNA are separated by a solidus). We used electroporation to transiently transfect the Jurkat cells (250 V; 960 mF). The ID82/73 transfection data has been published (*4*). (**D**) In vitro transcription of IL-2 enhancer constructs. The percent induction after stimulation is calculated after normalization to AdMLP activity. Percent induction relative to wild type (lanes 1 and 2) measured in independent experiments was as follows: ID82/73 (lanes 3 and 4) (31, 28); nonoctamer mutant (lanes 5 and 6) (65, 60); mutant histone octamer (lanes 7 and 8) (75, 80, 76); and H2b (lanes 9 and 10) (150, 165, 120). For in vitro transcription, IL-2 enhancer mutants were cloned into a G-less cassette (*29*). The nuclear extracts and transcription reactions were carried out as described (*9*). For S extracts, Jurkat cells were treated with 2 μM ionomycin and PMA (20 ng/ml) for 2 hours before we made the nuclear extract. Transfection and transcription results were quantitated with a radioanalytic imaging system (AMBIS). The gel shown is one representative of three experiments.

deletion mutation ID82/73 (Fig. 1B), we found that inducible in vitro transcription is dependent on ARRE-1 (Fig. 1D, lanes 1 to 4). Substitution of the histone octamer site for the wild-type sequence gave a 1.2-fold increase in induction, as compared to wild-type, whereas nonfunctional histone octamer mutations had 76% of wild-type activity. The nonoctamer 5′ mutation decreased activity to 60% of the wild-type enhancer (Fig. 1D). These in vitro transcription results parallel the transient transfection data.

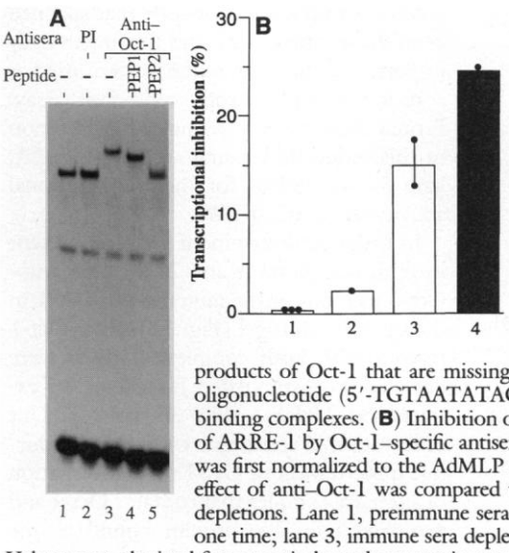

Fig. 2. Oct-1 binding to ARRE-1. (**A**) Anti–Oct-1 reacts with the ARRE-1–binding activity (*30*). Lane 1, ARRE-1–binding activity in nonstimulated Jurkat nuclear extracts as in Fig. 1; lane 2, preimmune sera (PI) was included in the binding reaction. Lanes 3 through 5, binding reactions in the presence of immune sera; lanes 4 and 5, peptide (2.8 μg) was included in the binding reactions. Other faster migrating complexes are also detected with this probe and extracts from S and NS Jurkat cells. These DNA-protein complexes do not react with anti–Oct-1, indicating that the proteins are either not Oct-1 or are partial degradation products of Oct-1 that are missing the NH_2-terminus. We used the ARRE-1B oligonucleotide (5′-TGTAATATAGTAAAACATTTTG-3′) to detect the DNA-binding complexes. (**B**) Inhibition of in vitro transcription directed by four copies of ARRE-1 by Oct-1–specific antisera (*30*). The ARRE-1–dependent transcription was first normalized to the AdMLP transcriptional activity, and then the inhibitory effect of anti–Oct-1 was compared to the values obtained with preimmune mock depletions. Lane 1, preimmune sera mock depleted; lane 2, immune sera depleted one time; lane 3, immune sera depleted twice; and lane 4, mutant histone octamer. Values were obtained from two independent experiments with the same nuclear extract.

Participation of an octamer-binding protein in regulation of the IL-2 gene is supported by an analysis of DNA-protein interactions. Electrophoretic mobility shift assays have shown that Oct-1 bound to the immunoglobulin octamer comigrates with a DNA-protein complex that is detected when Jurkat nuclear extracts are incubated with ARRE-1 (*5*). We used nuclear extracts from several cell types and also found that an octamer from the immunoglobulin promoter and ARRE-1 form DNA-protein complexes with identical patterns of migration and sequence specificity (*10*). We purified this common ARRE-1–binding activity (also called NFIL2A) from both liver and Jurkat cell nuclear extracts and found that the ARRE-1–binding activity is a protein that like Oct-1 (*11*) has an apparent molecular size of 90 to 100 kD (*12*).

To test whether the ARRE-1 binding activity was Oct-1, we made an antisera to a peptide from Oct-1 (anti–Oct-1) (*13*). The sequence of the immunogenic peptide (PEP2) is not found in Oct-3 (also called Oct-4 or Oct-5) (*14*) or Oct-6 (*15*), and the antisera does not cross-react with Oct-2 (*16, 17*). In an electrophoretic mobility shift

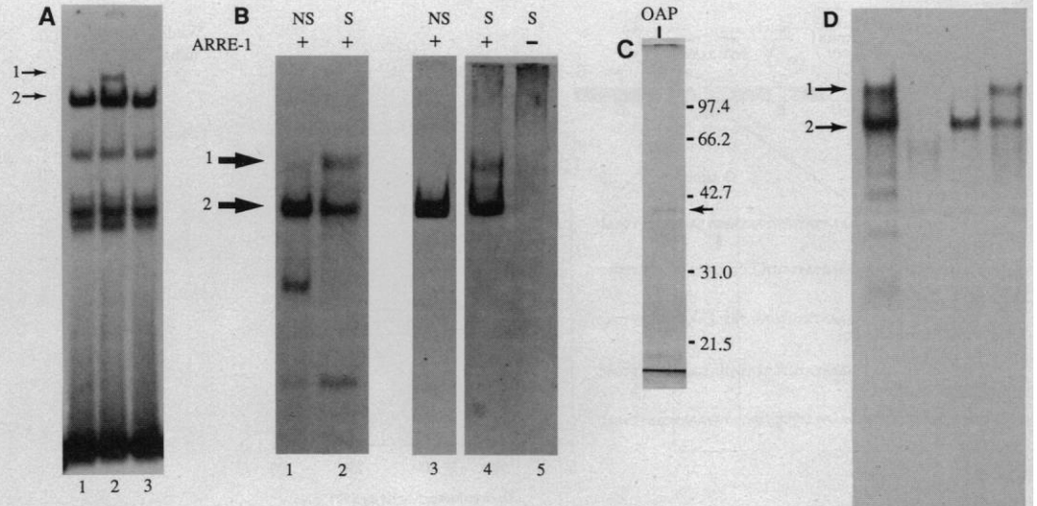

Fig. 3. Binding of a protein adjacent to Oct-1. (**A**) A binding activity was induced after stimulation and required protein synthesis for induction. ARRE-1–binding activity was assayed in nuclear extracts made from Jurkat cells that were NS (lane 1), S (75 min) (lane 2), or S (75 min) in the presence of 100 μM anisomycin, a protein synthesis inhibitor (lane 3). We used the ARRE-1 oligonucleotide (5′-TTTGAAAATATGTGTAATATGTAAAACATTTTG-3′) spanning both the octamer motif, as well as 5′ flanking sequences, in the binding assay. Complexes are indicated by arrows in the left margin. (**B**) Oct-1 is in complexes 1 and 2 (arrows). Electrophoretic mobility shift assays were performed with NS and S Jurkat nuclear extracts (as indicated) with either ^{32}P-labeled (lanes 1 and 2) or unlabeled (lanes 3 and 4) ARRE-1. No oligonucleotide was included in lane 5. Complexes visualized with anti–Oct-1 after transfer to nitrocellulose is shown in lanes 3 through 5 (*31*). No complexes were observed with preimmune sera. (**C**) OAP was purified on the basis of its interaction with ARRE-1 and has a molecular size of 40 kD (arrow) (*32*). Molecular size standards are shown at right in kilodaltons. (**D**) A renatured HPLC fraction in (C) forms complex 1 when Oct-1 is present. Lane 1, crude nuclear extract (for comparison); lane 2, HPLC fraction (OAP) alone; lane 3, Oct-1 alone; and lane 4, Oct-1 and OAP. Arrows indicate complex 1, complex 2, and ARRE-1.

assay (Fig. 2A), the Oct-1–specific antisera altered the migration of the complex formed with ARRE-1 and Jurkat nuclear extracts (lane 3). Preimmune sera had no effect on the complex (lane 2). The effect of the immune sera was blocked by PEP2 (amino acids 69 to 93 in Oct-1) but not by (PEP1) (amino acids 1 to 32 in Oct-1) (lanes 4 and 5). Peptide alone did not affect binding of Oct-1 to the ARRE-1. Thus, the ARRE-1–binding activity appears to be Oct-1.

To assess the function of Oct-1, we depleted nuclear extracts with anti–Oct-1 and tested them for transcriptional activity in vitro. Using the values obtained by mock depletions with preimmune sera as a base line, we found that two rounds of depletion with anti–Oct-1 decreased Oct-1–binding activity by 80% and specifically inhibited transcription driven by four copies of ARRE-1 by ~15% (Fig. 2B). Although this partial reduction may result from the fact that the concentration of Oct-1 exceeds that of active templates in the in vitro transcription reaction, it is also consistent with the finding that point mutations in the octamer site reduced in vitro transcription by only 24% (Figs. 1D and 2B). Therefore, although Oct-1 appears to participate in the induction of ARRE-1, there may be an additional protein functioning by means of this response element.

A binding activity induced by T cell activation (complex 1) was detected (Fig. 3A) when a 33-bp oligonucleotide that spanned both the octamer motif and the functionally important 5′ flanking sequences was used as a probe in an electrophoretic shift assay. Protein synthesis was required for detection of this inducible binding activity (Fig. 3A, lane 3), as well as for the transcriptional activity of ARRE-1 (*18*).

In order to determine if Oct-1 is present in both complexes 1 and 2, we used anti–Oct-1 to monitor the migration of Oct-1 in the mobility shift gel (Fig. 3B). Anti–Oct-1 reacted with both complexes, which were detected with stimulated Jurkat nuclear extracts and ARRE-1 (Fig. 3B, lane 4). This observation is consistent with either inducible dimerization of Oct-1 or the formation of a ternary complex that contains Oct-1 and a second inducible protein bound to the ARRE-1 site. To resolve this question, we

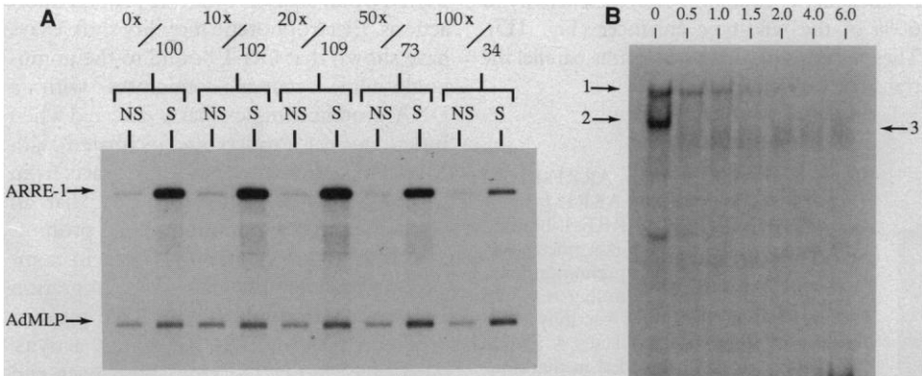

Fig. 4. Characterization of OAP. (**A**) OAP^{40}-binding activity is required for ARRE-1 transcriptional activity. Inducible transcription driven by four tandem copies of ARRE-1 was assayed in absence of ARRE-1A or in the presence of a 10-fold, 20-fold, 50-fold, or 100-fold molar excess of ARRE-1A (the top-most heading). The transcripts were quantitated and normalized to AdMLP activity. The inducibility of ARRE-1 was expressed relative to the induction of ARRE-1 without any oligonucleotide present (indicated under the ARRE-1A amounts). In vitro transcription reactions were carried out and quantitated as described in Fig. 1, as were the preparation of nuclear extracts. The ARRE-1A oligonucleotide (5′-GTCTTTGAAAATATGTGTAATAT-3′) was used as a competitor. (**B**) Measurement of Oct-1 dissociation kinetics. The dissociation of Oct-1 was monitored with the ARRE-1 probe over a period of 6 min at room temperature. Arrows indicate complexes 1, 2, and 3. Complex 1 is OAP^{40} and Oct-1; complex 2 is Oct-1 alone; and complex 3 is probably OAP^{40} binding to ARRE-1 without Oct-1. We measured the dissociation of Oct-1 from ARRE-1 by adding an excess of cold ARRE-1B and loading the reaction mixture onto a 4% native gel at the times indicated over each lane.

purified the activity that gave rise to the larger complex using ion-exchange chromatography, followed by DNA affinity chromatography and reversed-phase high-performance liquid chromatography (HPLC). A renatured HPLC fraction that contained a 40-kD protein (Fig. 3C) formed complex 1 when Oct-1 was included in the binding reaction (Fig. 3D). We refer to this protein as Oct-1–associated protein or OAP^{40}. We confirmed the molecular size of OAP^{40} by subjecting a sample of the HPLC fraction to electrophoresis on an SDS-polyacrylamide gel and eluting binding activity from the gel slices that was capable of forming complex 1 with Oct-1 and ARRE-1 (*19*). Amino acid sequence obtained from this purified protein has confirmed that it is not an Oct-1 degradation product (*20*).

Competition studies with oligonucleotides that spanned only the 5′ or 3′ part of ARRE-1 demonstrated that OAP-binding activity is dependent on the 5′ half of ARRE-1 (*21*). We used a 5′ half-site oligonucleotide (ARRE-1A) to titrate OAP^{40} DNA-binding activity and assess the transcriptional activity that remained in the nuclear extract. Depletion of OAP^{40}-binding activity led to a decrease in ARRE-1–dependent transcription, leaving 34% of the activity when a 100-fold molar excess of oligonucleotide was present (Fig. 4A). No effect on transcription driven by the adenovirus major late promoter (AdMLP) was seen. When nonspecific DNA (pUC polylinker) was used as a competitor, no inhibition was observed at the same concentrations (*22*).

Although Oct-1 and OAP^{40} can interact independently with ARRE-1, in vitro assays demonstrated that the complex was stabilized when Oct-1 and OAP^{40} were both included in the binding assay. This was detected by measuring the kinetics of dissociation of Oct-1. Using the long ARRE-1 probe to monitor both complexes, we found that dissociation of Oct-1 from its binding site was much faster when bound to DNA in the absence of OAP^{40} (Fig. 4B). Thus, OAP^{40} may form protein-protein interactions with Oct-1, as well as DNA-protein interactions.

In contrast to other homeodomain-containing proteins, Oct-1 has been described as a transcription factor that regulates commonly expressed genes such as those encoding histone H2b (*8*) and small nuclear RNAs (*23*). The demonstration that Oct-1 is required for the transcriptional activation of a gene required for T lymphocyte immune function indicates that the role of Oct-1 is not limited to the regulation of housekeeping genes. At least one other protein, OAP^{40}, participates directly with Oct-1 in the context of the IL-2 enhancer. OAP^{40} may account for the inducibility of ARRE-1 because the binding activity of OAP appears only after stimulation. Oct-1 has been shown to participate in the inducible transcription of herpes simplex virus (HSV)–1 immediate-early (IE) genes (*6*). During infection of the cell by HSV-1, the viral protein VP16 interacts directly with Oct-1 bound to an IE enhancer element (TAATGARAT; R is any pyrimidine) and may interact with general transcription factors (*24*). The importance of the context of octamer sequence for complex formation and activity indicates that Oct-1 and VP16 both contact DNA, as well as interact with each other (*25*). Thus, although we propose a different function for Oct-1, the mechanism of action follows the precedent that Oct-1 does not act independently (*26*).

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30. We generated the Oct-1–specific antisera by injecting BALB/c mice with PEP1 and PEP2 conjugated to keyhole limpet hemacyanin. Trehalose dimycolate emulsion (Ribi ImmunoChem Research) was used as an adjuvant. PEP1 is amino acids 1 to 32 in Oct-1 [MNNPSETSKPSMESGDGNTGTQTNGLDFQKQP(C)], and PEP2 is amino acids 69 to 93 [NVQSKSNEESGDSQQPSQPSQQPSV(C)]. We added C at end to cross-link proteins. Sera (1 μl) was included in a gel mobility shift reaction containing 5 μg of crude nuclear extract. Immunodepletion of Oct-1 from in vitro transcription extracts was carried out with fixed *Staphylococcus aureus* (Pansorbin, Calbiochem) that had been incubated with either preimmune sera or anti–Oct-1 immune sera before addition to the extracts. The treated Pansorbin was incubated with nonstimulated (NS) or stimulated (S) Jurkat in vitro extracts for 1 hour at room temperature. The samples were then cleared in a microcentrifuge at 4000 rpm for 4 min. In the case of the double depletions, this procedure was repeated one time. We used an aliquot of the depleted extracts to assess the amount of depletion by gel mobility shift, and the remainder was transferred to a transcription reaction containing the ARRE-1 DNA template. One round of depletion decreased Oct-1–binding activity by 36%; however, inhibition of transcription was not detectable until the majority of Oct-1–binding activity was removed. Abbreviations for the amino acid residues are as follows: A, Ala; C, Cys; D, Asp; E, Glu; F, Phe; G, Gly; H, His; I, Ile; K, Lys; L, Leu; M, Met; N, Asn; P, Pro; Q, Gln; R, Arg; S, Ser; T, Thr; V, Val; W, Trp; and Y, Tyr.
31. Crude extract (30 μg per binding reaction) was run on an electrophoretic mobility shift gel and then transferred to nitrocellulose following standard protocols. For the immunoblot, a 1:1000 dilution of the sera was used, followed by an incubation with horseradish peroxidase–conjugated rabbit antibody to mouse immunoglobulin (Zymed). The bound antibody was detected with chemiluminescence (Amersham).
32. We also used the Jurkat nuclear extracts described in (*12*) to purify OAP^{40} activity. Following the DEAE column, the active material was passed over carboxymethyl-Sepharose and heparin-Sepharose. We followed OAP-binding activity in gel mobility shift assays by mixing in Oct-1 and monitoring the

formation of complex 1. Fractions eluting from heparin-Sepharose (Pharmacia) at 400 to 500 mM KCl were pooled, and batch incubations were done with ARRE-1 DNA-affinity beads in the presence of nonspecific competitor. After approximately 12 hours, the beads were poured into a small column and washed extensively with 100 mM KCl and 200 mM KCl in wash buffer (50 mM Hepes, 0.1 mM EDTA, 0.03% lauryl dimethylamine oxide, and 1 mM dithiothreitol). Activity was eluted with 600 mM KCl in wash buffer, and 5% of the final material was denatured with guanidine-HCl and reduced with 50 mM dithiothreitol. After heating the sample 5 min at 65°C, we loaded it onto a reverse-phase HPLC C_8 column and eluted it with a gradient of 20 to 60% acetonitrile in 0.1% trifluoroacetic acid. Fractions were collected, and after adding 25 μl of 100 mM KCl in wash buffer we dried the fractions down to 25 μl. The pH of each fraction was adjusted to pH 7 to 8, and the samples were left on ice for 12 hours. Each fraction (10 μl) was run on a 10% SDS-polyacrylamide gel. The fraction corresponding to ~40% acetonitrile is shown in Fig. 3C. To detect OAP^{40}-binding activity, we incubated 0.5 μL of this HPLC fraction with and without a DEAE fraction containing Oct-1. Approximately 0.6 μg of protein from a heparin-Sepharose 200 mM KCl wash was also included in each binding reaction as a source of nonspecific protein.

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Establishment of Cell Type by Compartmentalized Activation of a Transcription Factor

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Early in the process of spore formation in *Bacillus subtilis* a septum is formed that partitions the sporangium into daughter cells called the forespore and the mother cell. The daughter cells each have their own chromosome but follow dissimilar programs of gene expression. Differential gene expression in the forespore is now shown to be established by the compartmentalized activity of the transcription factor σ^F. The σ^F factor is produced prior to septation, but is active only in the forespore compartment of the post-septation sporangium. The σ^F factor is controlled by the products of sporulation operons *spoIIA* and *spoIIE*, which may be responsible for confining its activity to one of the daughter cells.

A FUNDAMENTAL PROBLEM IN DEvelopmental biology is the process by which a cell of one type gives rise to one or more dissimilar cell types as a consequence of cell division. A primitive developmental process that has been cultivated as an experimental system for the establishment of cell type is the formation of endospores by the Gram-positive soil bacterium *Bacillus subtilis* (*1*). During sporulation an asymmetrically positioned septum forms, which partitions the developing cell (sporangium) into daughter cells called the mother cell and the forespore. These daughter cells differ in developmental fate from each other and from their parent cell; the forespore eventually becomes the spore, whereas the mother cell is discarded by lysis when maturation of the spore is complete. Each daughter cell type follows a distinct program of gene expression. At late developmental stages, differential gene expression is controlled by the compartment-specific transcription factors σ^G, which is present in the forespore, and σ^K, which is present in the mother cell. We now investigate the earliest regulatory events that establish distinct programs of gene expression in the two compartments.

σ^F is encoded by the promoter-distal member (*spoIIAC*) of the three-cistron sporulation operon *spoIIA* (*2, 3*) (Table 1). Expression of *spoIIA* commences prior to septum formation (*4*). Yet, σ^F directs the transcription of genes expressed in the forespore, such as *spoIIIG*, which encodes σ^G (*5–7*), and *gpr*, which encodes a forespore protease (*8*). Transcription of *spoIIIG* and *gpr* are also under the control of σ^G (*8–10*). Thus, it has not been possible to distinguish whether forespore-specific gene expression commences with the action of σ^F or, subsequently, with the action of σ^G.

To distinguish between these possibilities, we performed immunoelectron microscopy with antibodies to β-galactosidase (anti-βgal) to visualize the site of expression of *lacZ*, fused to a gene under σ^F control, in mutant sporangia that lacked σ^G. Because expression of *spoIIIG-lacZ* and *gpr-lacZ* in the mutant cells that lacked σ^G was too low to be detected by immunoelectron microscopy, we made use of a system for obtaining high-level synthesis of β-galactosidase from a σ^F-controlled reporter gene (*5, 11*). The system is based on an altered promoter recognition specificity mutant of σ^F that bears a valine-to-alanine substitution at residue 233 (VA233). The mutant σ^F directs transcription of the strongly expressed *sspB* gene, which is normally under the exclusive control of σ^G (*3*). Strain PM73 contains the VA233 mutant σ^F and a single chromosomally integrated copy of a plasmid (pPS490) (*12*) bearing an *sspB-lacZ* fusion and the gene encoding chloramphenicol acetyl transferase. To enhance σ^F-directed synthesis of β-galactosidase, PM73 was subjected to successive rounds of growth in the presence of increasing concentrations of chloramphenicol to select for amplification of pPS490 (*13*).

PM73 cells bearing approximately ten copies of pPS490 per chromosome were harvested during sporulation and subjected to immunoelectron microscopy (*13, 14*). As expected for *spoIIIG* mutants in which sporulation is blocked at engulfment, the sporangia were arrested at the stage (III) at which the forespore is pinched off as a free protoplast within the mother cell (Fig. 1). Although the level of decoration varied among sporangia, in all cases gold particles were more prevalent in the forespore than in the mother cell (Fig. 1, A to E). Cells bearing tandem chromosomal arrays of *lacZ* fused to mother cell–specific genes showed decoration of the mother cell and not the forespore (*15, 16*). Little decoration was observed in sporangia that lacked the *sspB-lacZ* fusion (Fig. 1F).

These observations are consistent with the view that σ^F-directed gene expression is largely restricted to the forespore chamber of the sporangium. However, because expression of *sspB-lacZ* is normally confined to the forespore (*12, 14*), we did not know whether the compartmentalization of σ^F-directed gene expression was controlled at the level of σ^F or *sspB* (for example, by the presence in the mother cell of a repressor of *sspB* transcription). To distinguish between these possibilities, we used an additional altered specificity mutant of σ^F bearing a valine-to-methionine substitution at residue 233 (VM233) (*11*). The VM233 mutant σ^F recognizes the promoter for *ctc*, a gene that is normally under the control of the nonsporulation transcription factor σ^B (*17*). *ctc* is normally

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