The Mouse Homologue of the Human Transcription Factor C1 (Host Cell Factor)

CONSERVATION OF FORMS AND FUNCTION*

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The assembly of the herpes simplex virus (HSV) α/IE gene enhancer complex is determined by the interactions of the Oct-1 POU domain protein, the viral αTIF (α-trans-induction factor, VP16, ICP25, VMW65), and the C1 factor (host cell factor, HCF). A unique transcription factor, C1 consists of a family of polypeptides derived from a common precursor by site-specific proteolytic processing. To analyze the role of this factor in the determination of HSV lytic-latent infection, cDNAs and genomic DNAs encoding the mouse homologue have been isolated. This factor is nearly identical to the human protein, contains multiple consensus proteolytic processing sites, and functions efficiently in the assembly of a specific HSV enhancer complex. Interestingly, the differential expression of the C1 factors in both human and mouse tissues may be important for the determination of HSV tissue tropism in these two organisms.

The genes of herpes simplex virus (HSV) form three major regulatory classes. The α/ immediate early (α/IE) genes are expressed immediately upon infection, and the products of these genes are required for progression to the later stages of viral gene expression (β and γ), resulting in viral DNA replication and capsid assembly (reviewed in Refs. 1 and 2). The expression of the α/IE genes is regulated by αTIF (VP16), a transactivator that is packaged in the tegument structure of the virus (3–6), released into the cell upon infection, and assembled into a specific transcription enhancer complex (7–16).

The assembly of the multiprotein α/IE enhancer complex is mediated by a complex set of protein-DNA and protein-protein interactions. The cellular POU domain protein, Oct-1, recognizes the octamer motif (ATGGTAAT) within the enhancer element via binding of both the POU-specific subdomain (ATGC) and POU-homeo subdomain (TAAATGA) (16–19). αTIF recognizes sequences in the 3′ domain of the enhancer element and cooperatively interacts with helix 1 of the Oct-1 POU-homeo subdomain (16, 20).

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The stable enhancer complex further requires the C1 factor (HCF) (9, 13, 16, 21, 22), an unusual cellular protein, which consists of a family of polypeptides (68, 100, 123–135, and 155–180 kDa) that are derived from a common 230-kDa precursor by site-specific proteolysis (23–25). This protein does not contain any apparent specific DNA binding activity but interacts, in a phosphorylation dependent manner, with αTIF (26, 27) and the POU domain of Oct-1. Since the C1 factor is a highly conserved protein (13, 16, 28) and has been implicated in the control of cell cycle (29), it is likely that this protein participates in a number of basic processes in addition to its role in the regulation of viral gene expression. Furthermore, the interaction of the C1 factor with a number of cellular proteins and transcription factors suggests that it is a significant component of the regulation complexes of cellular genes.

One of the key issues in the regulation of HSV gene expression concerns the biochemical mechanism(s) which determine the lytic-latent life cycle of the virus. Following a primary infection, an individual harbors the viral genome in the neurons of the sensory (trigeminal/sacral) ganglia where it remains in a latent state until complex stimuli induce reactivation and viral replication (reviewed in Ref. 2). Although little is known about the factors involved in determining the latent-lytic states of the virus, the expression of the α/IE genes may represent a critical regulatory point in this process. Specifically, C1-dependent modulation of the viral cycle is an attractive model as the factor consists of a set of proteolytic forms and modification states that would enable it to effectively regulate the latent-lytic switch, it has a critical role in the regulation of HSV IE gene expression, and it is involved in basic cellular processes.

As no tissue culture system faithfully represents a latent HSV infection, studies have focused upon the use of recombinant viruses and transgenes in the mouse model system (reviewed in Ref. 2). To develop a genetic system to analyze the role of the C1 factor in cellular processes and in the regulation of the latent-lytic cycle of HSV, the forms and function of the mouse C1 homologue have been characterized. cDNAs and genomic clones which encode this protein were isolated and demonstrate that the mouse homologue is nearly identical to the human factor. Interestingly, in contrast to the ubiquitous expression of the C1 factor in cultured cells (24), the protein is not expressed in all human tissues. Furthermore, the differential expression of the C1 polypeptide(s) in human and mouse tissues may account for the tissue tropism of HSV in these two organisms.

EXPERIMENTAL PROCEDURES

Electrophoretic Mobility Shift Assay—Electrophoretic mobility shift assay reaction and electrophoresis conditions were as described previ...
ously (26) and included 15-fm HSVα0 DNA probe, 20 ng of affinity-purified Oct-1, 30 ng of purified α-TIF, and 50 ng of purified human C1 factor or 0.5 μl (3.5 μg of protein) of nuclear extract in a total of 10 μl. For antibody supershifts, the reactions were incubated at 30 °C for 10 min before the addition of 175 ng of affinity-purified anti-human C1 antibody (Ab2126) for an additional 10 min.

Production and Purification of Antiseros—pGST-FL29 and pGST-FL150.2 were produced by the insertion of cDNA sequences encoding an C1 ORF amino acids 3–454 and 382–1014, respectively, in the appropriate pGEX bacterial expression vector (Pharmacia). New Zealand White rabbits were inoculated with SDS-PAGE purified GST-FL29 (Ab2159) or GST-FL150.2 (Ab2159) protein according to standard procedures (30). The resulting immune sera were antigen affinity-purified as described previously (24).

Extracts and Western Blots—Nuclear extracts from HeLa, NA-AJ11, RMA, and P815 cells were prepared as described previously (31). 20 μg of each extract were resolved in a 7.5% denaturing SDS-polyacrylamide gel (acylamide:bisacylamide, 30:0.8), transferred to nitrocellulose, and probed with affinity-purified anti-human C1 antibody (Ab2126) as described elsewhere (24). Protein extracts from tissues of 7-week-old BALB/c mice were prepared by homogenization of 0.5 g of tissue in 2 ml of t-extract buffer (50 mM Heps (pH 7.4), 150 mM NaCl, 0.1% Nonidet P-40, 0.5 mM EDTA, 20 μg ml−1 RNase A, “complete” protease inhibitor (aprotinin, leupeptin, Pefabloc, EDTA; Boehringer Mannheim) in a tissue grinder using a Teflon pestle attached to a variable speed drill. The homogenates were incubated on ice for 15 min and subsequently cleared by centrifugation at 20,000 × g for 20 min. Human tissue extracts were purchased from CLONTECH. As various tissues contain the membrane with Coomassie Brilliant Blue prior to blotting as recommended (Pierce). Transfer efficiency was monitored by staining the membrane with Coomassie Brilliant Blue prior to blotting as described previously (32).

Northern Analysis—Human and mouse multiple tissue Northern blots were purchased from CLONTECH (human 7760-1: heart, brain, placenta, lung, liver, skeletal muscle, kidney, pancreas; human II 7759-1: spleen, thymus, prostate, testis, ovary, small intestine, colon, placenta, lung, liver, skeletal muscle, kidney, pancreas; human II 7761-1: brain, lung, liver, kidney; mouse 7762-1: heart brain, spleen, lung, liver, skeletal muscle, kidney, testis; mouse embryo 7763-1: 7-, 11-, 15-, and 17-day). DNA probes (human: cDNA clones FL29, FL150.2, H10; mouse: cDNA clones 53.11, 53.14, 5c.8; control: human β-actin DNA) were prepared by labeling of the appropriate cDNA fragments with [α-32P]dCTP (3000 Ci/μm) using random hexamer primers (Pharmacia). The probes were precipitated and hybridized with 2.0 × 106 cpm/ml of the appropriate DNA probe, and washed according to the manufacturer’s recommendations. C1 and β-actin hybridization signals were quantitated using a Molecular Dynamics PhosphorImager.

Screening λ Libraries for cDNAs Encoding the C1 Factor Polypeptide—Hybridization probes derived from the amino terminus (FL29), middle (FL53, FL150.2, and FL191.1), or carboxyl terminus (PB5c and H10) of the human C1 factor were prepared by isolation of the appropriate cDNA clone DNA fragments and labeling with [α-32P]dCTP (3000 Ci/μm) using random hexamer primers (Pharmacia). The probes were precipitated, hybridized with 2.0 × 106 cpm/ml of the appropriate DNA probe, and washed according to the manufacturer’s recommendations. C1 and β-actin hybridization signals were quantitated using a Molecular Dynamics PhosphorImager.

RESULTS

C1 (HCF) is a unique transcription factor due both to its unusual specific proteolytic processing that gives rise to the family of polypeptides as well as its participation in the regulation of processes such as RNA polymerase II-directed transcription and cell cycle control. However, little is known concerning the biochemical role of this protein in these processes due to the ubiquitous expression of the factor in cultured cells and to the lack of an appropriate genetic system. To develop a genetic system to analyze the role of the C1 factor in cellular functions and in the lytic-latent cycle of herpes simplex virus infection, cDNAs and genomic DNA sequences that encode the mouse C1 factor have been isolated and characterized.

![FIG. 1](https://example.com/fig1.jpg)

**FIG. 1.** Conservation of forms and function of the mouse C1 factor. Left panel, nuclear extracts from human (lane 1) and mouse (lane 2–4) cells were resolved in an SDS-denaturing gel, transferred to nitrocellulose, and probed with purified anti-human C1 factor sera Ab2125. The molecular weights of the protein markers, in thousands, are indicated at the left of the panel. Right panel, protein-DNA binding reactions were done as described under “Experimental Procedures.” Reaction 1 contained only the Oct-1 protein, while reactions 2–4 contained Oct-1 and α-TIF in addition to the following protein or extracts: reaction 2, purified human C1 factor; reaction 3, nuclear extract of P815 cells; reaction 4, nuclear extract of P815 cells and purified anti-human C1 sera Ab2125.
The Forms and Function of the Mouse C1 Factor—The human C1 factor consists of a family of polypeptides ranging from 68 to 230 kDa that are derived from a common precursor protein (23–26). To determine if these polypeptides are conserved in the mouse, nuclear extracts of HeLa (human cervical carcinoma), NA-AJ11 (mouse neural), RMA (mouse lymphoid), and P815 (mouse lymphoid) cells were resolved by SDS-PAGE, transferred to nitrocellulose, and probed with affinity-purified anti-human C1 sera (Fig. 1, left panel). As shown, the sera specifically reacted with the family of C1 polypeptides in the HeLa (lane 1) and mouse cell extracts (lanes 2–4), illustrating the conservation of the processed C1 factor forms.

The function of the mouse C1 factor in the assembly of an αIE enhancer complex was determined by electrophoretic mobility shift assay in the presence of the HSV αTIF and the human Oct-1 proteins. As illustrated in Fig. 1 (right panel), the addition of the affinity-purified Oct-1 protein to a DNA binding reaction containing the HSV α0 DNA probe resulted in the

**Fig. 2.** cDNAs and amino acid sequence of the mouse C1 factor. *Top,* the assembled cDNA encoding the mouse C1 factor polypeptides is schematically illustrated with the positions of the 5′-untranslated sequence (nucleotides 1–54), ORF (nucleotides 55–6192), and 3′-untranslated domain (nucleotides 6193–7879). The cDNA clones that were isolated from a mouse fetal liver λ gtl0 library are shown above and below the complete gene. *Bottom,* the amino acid sequence of the C1 factor ORF is shown with the eight conserved proteolytic processing sites in *bold type.*
The inclusion of information of the M-C1 complex was strictly dependent upon the cells to a reaction containing the human Oct-1 and particularly, the addition of mouse nuclear extract derived from P815 cells to a reaction containing Oct-1, TIF, and the purified human C1 factor. The addition of mouse nuclear extract containing Oct-1, TIF, and the purified human C1 factor generated the characteristic C1 complex (M-C1) generated the characteristic C1 complex (M-C1).

As illustrated in Fig. 3, the predicted amino acid sequence of the mouse C1 factor is nearly identical (96% amino acid identity) to the human with the exception of a region of relatively high divergence within the central domain of the protein. Most significantly, the mouse ORF contains eight repetitions of the 20-amino acid sequence that represent the sites of specific proteolytic processing that generates the family of C1 factor polypeptides (PPS) (24). As shown, the consensus sequence for the mouse PPS is identical to that which was derived from six of the human C1 factor polypeptides (lanes 2 and 3) is due to the particular C1 factor cleavage products that are present in the two preparations. Whereas the mouse P815 nuclear extract contains the complete family of proteins (68–230 kDa), the purified human C1 factor consists primarily of the 68- and 100–123-kDa C1 polypeptides (24, 26).

The Mouse C1 Factor ORF—cDNAs encoding the mouse C1 factor were isolated from a λ gt10 mouse fetal liver library by hybridization with the human C1 factor cDNA (Fig. 2, top) (23, 24). Assembly of the complete 7879-nucleotide cDNA reveals an ORF of 2045 amino acids (Fig. 2, bottom). As illustrated in Fig. 3, the predicted amino acid sequence of the mouse C1 factor is nearly identical (96% amino acid identity) to the human with the exception of a region of relatively high divergence within the central domain of the protein. Most significantly, the mouse ORF contains eight repetitions of the 20-amino acid sequence that represent the sites of specific proteolytic processing that generates the family of C1 factor polypeptides (PPS) (24). As shown, the consensus sequence for the mouse PPS is identical to that which was derived from the six human processing sites (23, 24).

The genomic organization of the mouse C1 factor gene. Clones containing the mouse C1 factor genomic sequences were hybridized to immobilized metaphase mouse chromosomes. The arrow designates the location of the C1 (HCF) gene in band C1 of the X chromosome, a domain that is equivalent to the human C1 factor chromosomal locus (36).

The genomic organization of the mouse C1 factor gene. Clones containing the mouse C1 factor genomic sequences were hybridized to immobilized metaphase mouse chromosomes. The arrow designates the location of the C1 (HCF) gene in band C1 of the X chromosome, a domain that is equivalent to the human C1 factor chromosomal locus (36).

The genomic organization of the mouse C1 factor gene. The genomic organization of the 26 exon mouse C1 factor gene is schematically represented. The single exon that encodes the proteolytic processing domain is shown with the location of the consensus processing sites. The 5′ and 3′ untranslated regions (UTR) represent the untranslated mRNA sequence, while (GT)n and B2rep indicate the location of dinucleotide repeats and B2 repetitive sequence, respectively. NH2-20, B1–16, B1–32, and B2–19 represent the sequenced subclones of the original P1 phage.

Formation of Oct-1-DNA complex (lane 1), while a reaction containing Oct-1, TIF, and the purified human C1 factor generated the characteristic C1 complex (H-C1, lane 2). Similarly, the addition of mouse nuclear extract derived from P815 cells to a reaction containing the human Oct-1 and TIF proteins generated a stable C1 complex (M-C1, lane 3). The formation of the M-C1 complex was strictly dependent upon the inclusion of αTIF in the reaction and was not formed using HSVu0 mutant probes (data not shown). Furthermore, addition of Ab2125 to the M-C1 reaction specifically retarded the mobility of the C1 complex (lane 4), in contrast to the addition of control anti-GST antibodies (data not shown). It should be noted that the difference in mobility between the human and mouse C1 complexes (lanes 2 and 3) is due to the particular C1 factor cleavage products that are present in the two preparations. Whereas the mouse P815 nuclear extract contains the complete family of proteins (68–230 kDa), the purified human C1 factor consists primarily of the 68- and 100–123-kDa C1 polypeptides (24, 26).

The Mouse C1 Factor ORF—cDNAs encoding the mouse C1 factor were isolated from a λ gt10 mouse fetal liver library by hybridization with the human C1 factor cDNA (Fig. 2, top) (23,
Gene—As previously discussed, the development of a genetic system for the analysis of the C1 factor would significantly enhance the understanding of the role of this unique protein in cellular functions as well as the lytic/latent cycle of herpes simplex virus. Therefore, P1 phage containing the mouse genomic C1 gene were identified in an arranged library by polymerase chain reaction. Two recombinant phage (5341 and 5342) were subcloned and sequenced. The 25-kilobase pair deduced genomic structure of the C1 gene ORF (Fig. 4) is comprised of 26 exons with an unusually large single exon (501-amino acid/1503-nucleotide) encoding the entire C1 factor proteolytic processing domain.

The P1 genomic clones subsequently were used to determine the chromosomal location of the C1 factor gene by fluorescent in situ hybridization analysis. As shown in Fig. 5, hybridization of mouse metaphase chromosomes with 5341/5342 resulted in specific labeling of the third largest chromosome (X chromosome). The identity of the chromosome was confirmed and the location of the C1 gene within band XC1 was determined by cohybridization of 5341/5342 with a probe that was specific for the centromeric region of the X chromosome.

Expression of the C1 Factor in Human and Mouse Tissues—
The C1 factor has been detected by immunofluorescence and Western blot in nuclei of many cultured or transformed human cell lines (24). However, it is not clear that the polypeptides are stably expressed in all primary tissue types (35). Therefore, to investigate the expression of the C1 factor in human tissues, equivalent amounts of protein from total extracts of representative human tissues (heart, brain, liver, kidney, skeletal muscle, lung, and testis) were resolved by SDS-PAGE, transferred to nitrocellulose, and probed with a mixture of affinity-purified anti-C1 antisera (Fig. 6, top right panel). Although the factor was detected in extracts of human heart, liver, kidney, and testis, the polypeptides were not found in extracts of brain, skeletal muscle, or lung, suggesting that the protein is not normally expressed ubiquitously. It should be noted that only a subset of the processed C1 factor polypeptides (100- and 125-135-kDa forms) are detected in these particular tissue extracts, likely due to the manner in which they were prepared. (4)

The mouse represents an important animal model system for human infection by herpes simplex virus (reviewed in Ref. 2). However, there are distinct differences in the infection tropism between these two organisms (see “Discussion”) (reviewed in Refs. 2 and 37). To determine if the expression of the C1 factor polypeptides correlates with this difference, replicate extracts from select mouse tissues (heart, brain, lung, spleen, and thymus; Fig. 6, left panels) were blotted with antisera directed against the amino terminus (NH2), the proteolytic processing domain (PPSD), or the carboxyl terminus (COOH) of the human C1 factor. In contrast to the human, the C1 factor polypeptides were detected in all of the tested mouse extracts.

To control for differences in the mouse and human extracts, duplicate titrations of both brain extracts were resolved by SDS-PAGE and probed with anti-C1 or with anti-neurofilament p200 sera. While the human extract clearly contains a

4 T. Kristie, unpublished observations.

FIG. 7. Expression of C1 mRNA in human and mouse tissues. Human and mouse multiple tissue Northern blots were hybridized with C1 factor and control β-actin cDNA probes. For each tissue set, the C1 signal was normalized according to the β-actin signal. The mouse and human adult tissues (bottom left) or the human adult and fetal tissues (bottom right) were further normalized to one another based upon the ratio of the β-actin control signal for a given tissue.

C.elegans C1 vs Human C1

Amino acid alignment of the human and C. elegans C1 factors. The amino acid sequence homology between the C. elegans C1 factor (top, 717 amino acids, GenBankTM accession no. U61948) and the human factor (bottom, 2035 amino acids) is shown with the amino acid numbers above or below the appropriate sequence. Colons (:) indicate gaps or divergence in the alignment while (+) are conservative amino acid changes. (∞∞) designates the domain of the human (amino acids 398–1811) and mouse C1 (amino acids 398–1822) factors that is not represented in the C. elegans homologue. KIEYSVY is a consensus site for tyrosine kinase phosphorylation (38).

FIG. 8. Amino acid alignment of the human and C. elegans C1 factors. The amino acid sequence homology between the C. elegans C1 factor (top, 717 amino acids, GenBankTM accession no. U61948) and the human factor (bottom, 2035 amino acids) is shown with the amino acid numbers above or below the appropriate sequence. Colons (:) indicate gaps or divergence in the alignment while (+) are conservative amino acid changes. (∞∞) designates the domain of the human (amino acids 398–1811) and mouse C1 (amino acids 398–1822) factors that is not represented in the C. elegans homologue. KIEYSVY is a consensus site for tyrosine kinase phosphorylation (38).
significantly higher level (>10 fold) of the neurofilament p200, the C1 factor polypeptides are detected only in the mouse extract (Fig. 6, bottom right panel). This expression difference is further emphasized by quantitative Northern blot analyses of mouse and human tissue mRNA where C1 mRNA was significantly more abundant in mouse but not human neural tissues (Fig. 7, left). Interestingly, quantitative comparison of fetal and adult C1 mRNA levels suggests that the C1 factor mRNA is more highly expressed in early developmental stages (Fig. 7, right).

**DISCUSSION**

The Human, Mouse, and Caenorhabditis elegans C1 Factors—C1 (HCF) is a unique transcription factor that consists of a family of polypeptides which are involved in the regulated assembly of the herpes simplex virus α/IE enhancer complex by direct interaction with αTIF and Oct-1. In addition, the protein has recently been implicated in the control of cell cycle suggesting that C1 is involved in a number of basic cellular processes. To develop a mouse genetic system for the analysis of the role of C1 (HCF) in cell and viral functions, cDNAs and genomic DNAs have been isolated which encode the mouse C1 factor homologue.

As suggested by the conservation of epitopes and functional assembly of a C1 complex, the mouse C1 factor is nearly identical to the human (94% amino acid identity/96.0% amino acid similarity). Most significantly, the mouse factor contains the identical 20 amino acid repeats which are the sites of specific proteolytic processing to generate the family of C1 polypeptides. Although the actual function of the processing of the C1 factor remains unknown, the series of amino- and carboxy-terminal products remain tightly associated, suggesting that the processing is a novel mechanism by which the function or activity of the protein is regulated. The high level of conservation of these sites in the mouse C1 factor suggests that this processing is critical to the function of the factor. In support of this, data from two-hybrid analyses have indicated that the PPS are not simply sites of specific proteolytic processing but are also sites for protein interactions with other cellular proteins. As illustrated in Figs. 3 and 8, C. elegans also contains a gene that is homologous to the human and mouse C1 factors. This homologue has a striking alignment to the amino-terminal and the carboxy-terminal portions of the mammalian factors. It, however, is a significantly smaller protein that lacks the central region and the processing domain of the human and mouse factors, suggesting that its functions may be more limited than the mammalian counterparts. Interestingly, the orthologous C1 factor which has been implicated in the control of cell cycle (29) but lacks the mammalian C1 factor domains which are involved in the interaction with several transcription factors, suggesting that these functions are not interdependent.

The Mouse C1 Factor and Herpes Simplex Virus—After a primary infection with herpes simplex virus, the virus remains in a latent state in the sensory ganglia (trigeminal, sacral) until stimulated to enter a reactivated replication mode (reviewed in Ref. 2). However, the biochemical mechanism(s) by which the latent/lytic cycle is regulated are largely unknown, primarily due to the fact that HSV does not establish latent infections in tissue culture systems. As the α/IE genes of herpes simplex virus encode factors that are required for the progression of the viral lytic cycle, the regulation of this gene class could represent a significant point in the lytic/latent decision.

The C1 factor provides a striking key target for regulation of the viral lytic/latent cycle due to its regulated interaction with the components of the HSV α/IE enhancer complex and its role in the control of cell cycle. Furthermore, the difference in the expression of the C1 factor in human and mouse brains correlates with the observed ability of the virus to productively replicate in this tissue type as evidenced by the significantly different frequency of viral encephalitis in these organisms following central nervous system infection (reviewed in Refs. 2 and 37). Most significantly, studies of the expression and localization of the C1 factor polypeptides in mouse trigeminal ganglia indicate that the protein is specifically activated by stimuli which induce the reactivation of herpes simplex virus from the latent state.

As studies of the events which determine latency or reactivation of HSV are restricted to animal model systems, the characterization of the mouse C1 factor allows for the development of a genetic system to investigate the role of this protein in viral as well as cellular processes.

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