Protein kinase CK2α is induced by serum as a delayed early gene and cooperates with Ha-ras in fibroblast transformation

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Protein kinase CK2 is an ubiquitous and pleiotropic Ser/Thr protein kinase composed of two catalytic (α and/or α') and two noncatalytic (β) subunits forming a heterotetrameric holoenzyme involved in cell growth and differentiation. Here we report the identification, cloning, and oncogenic activity of the murine CK2α subunit. Serum treatment of quiescent mouse fibroblasts induces CK2α mRNA expression, which peaks at 4 h. The kinetics of CK2α' expression correlate with increased kinase activity toward a specific CK2 holoenzyme peptide substrate. The ectopic expression of CK2α' or CK2α cooperates with Ha-ras in foci formation of rat primary embryo fibroblasts. Moreover, we observed that BALB/c 3T3 fibroblasts transformed with Ha-ras and CK2α show a faster growth rate than cells transformed with Ha-ras alone. In these cells the higher growth rate correlates with an increase in calmodulin phosphorylation, a protein substrate specifically affected by isolated CK2 catalytic subunits but not by CK2 holoenzyme, suggesting that unbalanced expression of a CK2 catalytic subunit synergizes with Ha-ras in cell transformation.

Protein kinase CK2 (previously known as casein kinase II) is an ubiquitous Ser/Thr kinase present in the cytoplasm and the nucleus of eukaryotic cells (for review, see Refs. 1–5). CK2 holoenzyme consists of two catalytic (α and/or α') and two regulatory (β) subunits assembled as stable heterotetramers, which in vitro do not dissociate unless under denaturing conditions. CK2 is unique among Ser/Thr protein kinases for its ability to use GTP, besides ATP, as phosphate donor and for its unusual site specificity, which is determined by multiple acidic and/or previously phosphorylated residues downstream (n + 3) from the phosphoacceptor amino acid, determining the minimum consensus (S/T-X-E/D/Y)p(S/p) (6).

More than 160 cellular proteins have been reported to be phosphorylated by CK2, and several are implicated in signal transduction, transcriptional activation, cell cycle progression, and cell differentiation. The nuclear proteins that are CK2 substrates includes: c-Myc (7), Max (7), c-Myb (8), serum response factor (SRF) (9), DNA ligase I (10), DNA topoisomerase 2 (11), p53 (12), and c-Fox (13). In mammalian cells phosphorylation of nuclear factors dependent on CK2 could be relevant for cell growth regulation and the progression into the cell cycle. A direct role of CK2 activity in cell cycle progression has been demonstrated by antibody-mediated CK2 depletion and by gene inactivation in Saccharomyces cerevisiae (14, 15). Although hundreds of papers have been published on the subject, it is still unknown how the enzyme is regulated in vivo (4, 16). CK2β undergoes stoichiometric autophosphorylation and both CK2β and CK2α (but not CK2α') are phosphorylated in vitro and in vivo by p34CDC2 kinase (17). However, these phosphorylations do not correlate with any regulation of activity. Moreover, it is not clear whether the holoenzyme represents an up- or a down-regulated form of the kinase, because some substrates are preferentially phosphorylated by the tetramer, but others, like calmodulin, are phosphorylated only by the free catalytic subunits (6).

In transgenic mice it was possible to demonstrate that in T cells the overexpression of the catalytic CK2α subunit enhanced the onset of lymphomas induced by either c-myc or tal-1 (18, 19). These results shed new light on the previous observations that cattle infected by the parasite Theileria parva developed T cell lymphomas, because parasite-infected cells show increased CK2 activity (20–22). Opposite results were obtained by the overexpression of CK2α in NIH 3T3 mouse fibroblasts. In these cells CK2α overexpression resulted in deactivation of the mitogen-activated protein kinase kinase and suppression of ras-dependent cell transformation (23).

To identify genes potentially involved in cell growth, we performed a differential screening for the isolation of transcripts induced by mitogenic stimuli within the G1 phase. Here we report the identification and cloning of the murine lower molecular weight catalytic subunit, CK2α'. We observed that in mouse fibroblasts CK2α' is induced by serum treatment as a slow-early gene. Together with CK2α' also CK2α and CK2β are induced. Cotransfection of an expression vector containing CK2α' together with a vector expressing Ha-ras induced foci formation in rat primary embryo fibroblasts. Moreover, ras-transformed BALB/c 3T3 fibroblasts overexpressing CK2α' showed a faster growth rate than cells transformed with Ha-ras alone. ras-Transformed fibroblasts overexpressing CK2α' also exhibited increased phosphorylating activity toward calmodulin, which is a specific substrate of CK2 catalytic subunits. These findings suggest that unbalanced expression of either CK2α' or CK2α plays a role in fibroblast cell transformation.

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Experimental Procedures

Cells and Cell Culture—NIH 3T3 and BALB/c 3T3 fibroblasts were grown at 37 °C in DMEM supplemented with 10% heat-inactivated FCS, penicillin-streptomycin, and glucose. The cells were expanded by trypsin-EDTA treatment and subcultured at a ratio of 1:3 every 2–3 days. Rat embryo fibroblasts were isolated as described previously (24). Briefly, 14-day P0–P3 F1/2 embryos were sacrificed, rinsed, and trypsinized at 37 °C. DMEM containing 10% FCS was added, and the cells were centrifuged, dispersed, counted, and plated on 100-mm tissue culture dishes at a density of 2 × 10^6 per dish. After 48 h, the cells were trypsinized, and aliquots were frozen in liquid nitrogen.

Differential Display and Cloning of the Murine CK2α' cDNA—To induce a relatively quiescent cell population, subconfluent NIH 3T3 fibroblasts were incubated for 48 h in DMEM plus 0.5% FCS. Cells were then treated for 2 and 4 h with DMEM supplemented with 10% FCS. Total cellular RNA was extracted using the guanidium thiocyanate method (25) from quiescent and serum-treated fibroblasts and subjected to the differential display technique as described previously (26, 27). The amplified cDNA fragments were compared in nondenaturing polyacrylamide gels. A serum-induced cDNA fragment, named L-0401, was excised, recovered by boiling, reamplified, and cloned into GEM-T vector (Promega). The L-0401 cDNA fragment (230 bp), whose corresponding mRNA was homologous to human CK2α', was labeled with [32P]dCTP by random primer labeling and used to screen a mouse cDNA library (27). The positive clones, inserted into the pBluescript SK vector, were sequenced on both strands either automatically using the Sequenase 2.0 kit (U. S. Biochemical Corp). A positive clone, named pBS38α' (FS304), contained the full-length mouse CK2α' cDNA.

Northern Blot Analysis—Total RNA (10 μg) was run on denaturing formaldehyde-agarose gels and stained with ethidium bromide to verify that each lane contained similar amounts of ungraded RNA. RNA was transferred onto nylon membranes and cross-linked by UV irradiation. Filters were hybridized with [32P]-labeled probes and washed as described (27). The mouse CK2α' probe was obtained from the full-length cDNA (pBS38α'). The cDNA fragments of murine CK2α' (base pairs 421–841) and CKβ' (base pairs 912–1321) were obtained by cDNA amplification of a mouse fibroblast cDNA library (27). The sequences of the primers used for amplification (5'-GCTTCGATATGAC-3' and 5'-GAG-GACTCAACTACTAAATCCG-3') were designed (27). The amplified cDNA fragments were sequenced, labeled with [32P]dCTP, and used in Northern blot analyses.

Construction of Expression Vectors—CK2α' open reading frame (from P-2 to R-350) was amplified by PCR from pBS38α' with a 5' primer containing a BamHI restriction site (5'-CGTCACG-3') and a 3' primer containing a KpnI restriction site (5'-GCGGGTAGTGATCTTTGCACG-3') and a 3' primer containing a BamHI and KpnI and cloned into the BamHI and KpnI site of the expression vector pcDNA3 (Invitrogen) under the control of the cytomegalovirus promoter, the 5' end of CK2α' was amplified by PCR from pT7–7CKII9 using a Sequenase 2.0 kit (U. S. Biochemical Corp). A positive clone, named pBS38α' (FS304), contained the full-length mouse CK2α' cDNA.

RESULTS

Isolation and Characterization of the Murine CK2α' cDNA—In cultured mouse fibroblasts growth factor depletion leads the cell to exit from the cell cycle and become quiescent. Serum treatment induces re-entry into the cell cycle, which is likely because of the induction of early genes. To identify new serum-induced genes, we used the mRNA differential display technique (26, 27). NIH 3T3 fibroblasts were serum-starved for 48 h and the RNAs were collected either from starved cells or from cells treated with serum at different time points. Several cDNAs, obtained only from serum-induced cells, were amplified and sequenced. Comparison of the cDNA fragments with the EBI Nucleotide Sequence Data Base revealed that a cDNA induced at 4 h after serum treatment was highly similar to the human protein kinase CK2α'. To clone the full-length cDNA coding for CK2α', a mouse fibroblast cDNA library (27) was screened. The few positive clones were sequenced using internal sequencing primers, and the nucleotide sequence of one clone of 1877 base pairs in length revealed a single open reading frame coding for a putative protein of 350 amino acid residues. The mouse and human predicted protein products shared 98.9% amino acid identity. The strong similarity of the murine CK2α' deduced protein sequence with its corresponding human homologue suggests a highly conserved function of CK2α'. The two proteins share 82.4% identity over 347 amino acids overlap (Fig. 1). The greatest difference is in the C-terminal domains, because the deduced CK2α' protein sequence is 41 amino acids shorter and thus lacks the p34Calc sites phosphorylated during the cell cycle (17). CK2α' also lacks the HEHKRL amino acid residues (166–171 of CK2α) that have been implicated in the interaction with protein phosphatase 2A (23).

To confirm that the cDNA encoded a biologically active CK2α' enzyme we expressed the cDNA in Escherichia coli.
Recombinant CK2α showed a molecular mass of about 41 kDa consistent with the predicted size of the protein, and the non-denatured soluble bacterial extract containing the immunoreactive CK2α was able to phosphorylate the CK2 peptide substrate RRRADDSDDDDD in vitro (not shown).

CK2 is an ubiquitously expressed protein kinase essential for cell growth. Northern blot analysis with a CK2α probe revealed two hybridizing transcripts of 2.2 and 4.2 kilobases, respectively. The expression of CK2α mRNA is relatively constant in all tissues with the exception of testis where a much stronger CK2α signal was detected (Fig. 2). These results contrast with CK2α expression, which is abundant in brain and barely expressed in testis (31, 32). Thus, CK2α and CK2α are differentially regulated in these tissues.

CK2α Is Induced by Serum Treatment in Cultured Fibroblasts—The identification of CK2α in a screening for mRNAs induced by serum treatment of quiescent fibroblasts suggested that this gene is induced by mitogenic stimuli. To measure the induction of the CK2α transcript, we performed Northern blot analysis on CK2α mRNA in fibroblasts before and after serum treatment. As can be observed in Fig. 3A the CK2α mRNA was low in quiescent fibroblasts and increased about 50% in serum-induced fibroblasts, with a peak induction at 4 h. The quantitative analysis of the transcripts normalized to the glyceraldehyde-3-phosphate dehydrogenase mRNA levels is reported in Fig. 3B. The serum-induced increase in CK2α transcripts was not blocked by the presence of the protein synthesis inhibitor, cycloheximide, as shown by the superinduction of CK2α mRNA (Fig. 3, A and B). Thus, in mouse fibroblasts CK2α is induced with slow kinetics by serum treatment, and this induction is independent of protein synthesis. Because protein kinase CK2 is a tetramer containing two catalytic and two regulatory subunits, we also tested whether the mRNA corresponding to CK2α and CK2β were induced by serum treatment of quiescent cells. Northern blot analysis of CK2α revealed three hybridizing transcripts of 1.6, 3.1, and 4.6 kilobases, respectively. The CK2α mRNA showed a less pronounced but detectable increase at 2 h after serum treatment. Similar to CK2α the CK2α mRNA increase was not...
A standard focus assay was used to assess the ability of various constructs to transform primary rat embryo fibroblasts. The number of foci were counted after crystal violet staining. The experiments were repeated four times, and the average foci number with their S.E. are listed.

| Transfected construct | Number of foci/100-mm dish |
|-----------------------|---------------------------|
| Ha-ras                | 0                         |
| CK2α’                 | 0                         |
| CK2α                  | 0                         |
| c-fos                 | 0                         |
| Ha-ras + CK2α’        | 68 ± 9                    |
| Ha-ras + CK2α         | 75 ± 10                   |
| Ha-ras + c-fos        | 95 ± 10                   |

CK2α’ Cooperates with Ha-ras in Rat Embryo Fibroblast Transformation—The above results showed that mouse fibroblasts respond to mitogenic stimuli with an increase of CK2 transcripts and kinase activity at the G1/S phase transition. Although the induction observed is not dramatic, we measured a reproducible increase of CK2 activity of about 80%. Previous experiments showed that as little as a 10% increase in CK2 expression in lymphoid organs of transgenic mice accelerated the onset of lymphomas induced by either c-myc or tal-1 oncogenes (18, 19). To test whether CK2α’ or CK2α play a direct role in tumor induction, we performed standard focus formation assay transfecting primary rat embryo fibroblasts with Ha-ras, CK2α’, or CK2α alone; the combination of Ha-ras with each catalytic subunit. Neither Ha-ras alone nor the CK2 catalytic subunits transfected independently induced foci formation in primary cells (Table I). However, transformed foci were visible within 10 days in the plates cotransfected with Ha-ras and either CK2α’ or CK2α. We therefore conclude that either CK2α’ or CK2α cooperate with oncogenic ras in primary cell transformation.

The Expression Level of CK2α’ Correlates with Increased Growth Rate of Transformed Clones—To study further the effect of CK2α’ expression on the growth rate of ras-transformed cells, we compared the growth behavior of mouse fibroblasts, because these cells can be transformed with Ha-ras alone, and therefore it is possible to obtain transformed clones expressing or not ectopic CK2α’. For this experiment we chose immortalized mouse fibroblasts, BALB/c 3T3 cells were used for this set of experiments, because the efficiency of NIH 3T3 transformation with Ha-ras alone was too high, making it difficult to quantitate the effects of CK2α’. As shown in Fig. 4A in BALB/c 3T3 cells the cotransfection of CK2α’ and Ha-ras resulted in approximately a 3-fold higher number of foci compared with the number of foci induced by Ha-ras alone. Moreover, we observed that foci generated with Ha-ras alone were smaller than those from clones transformed with Ha-ras and CK2α’, suggesting that overexpression of CK2α’ contributed to the cell growth of transformed cells (Fig. 4B). In parallel sets of experiments we observed a similar effect following cotransfection of Ha-ras with the CK2α catalytic subunit. The transfection of CK2α’, or CK2α, alone did not induce foci formation (not shown).
vector plasmid (pcDNA3) or pcDNA a BALB/c 3T3 fibroblasts were cotransfected with Ha-ras subunits its phosphorylation is suppressed completely by subunits, because in reconstitution experiments with recombinant substrate for examining the contributions of the CK2 catalytic function when compared with ras. Eight other clones obtained from different transfections were analyzed, and those cotransfected with CK2a' and Ha-ras also exhibited increased growth rates (not shown).

To examine whether the growth differences correlated with the increased expression of CK2a' in the transformed clones, we tested the kinase activity mediated by CK2a' by measuring the phosphorylation of calmodulin. Calmodulin is an ideal substrate for examining the contributions of the CK2 catalytic subunits, because in reconstitution experiments with recombinant subunits its phosphorylation is suppressed completely by adding CK2β (5, 33–35). Clones, transformed with both Ha-ras and CK2a', showed higher levels of calmodulin phosphorylation when compared with ras-transformed clones (Fig. 5B). This growth-related phosphorylation of calmodulin was inhibited (>90%) by addition of either a molar excess (0.5 mM) of the specific peptide substrate, or 1 μg/ml heparin (not shown). These findings, in conjunction with the alkali-lability of the phosphate incorporated into calmodulin, which rules out the possibility of tyrosine phosphorylation, show that calmodulin phosphorylation is entirely because of CK2 rather than to any other protein kinase(s). Thus, although we observed clonal variability, the enhanced growth correlated with increased CK2a'-dependent calmodulin phosphorylation (Fig. 5C). Finally, in mice we tested the growth of Ha-ras versus Ha-ras plus CK2a'-transformed clones. Exponentially growing transformed clones were collected and injected subcutaneously into the scapular region of nude mice, and the resultant tumors were removed surgically after 2 weeks of growth and weighed. Consistent with the results from the cell growth in vitro, CK2a' was found to produce a significant enhancement of tumor growth (Fig. 5D).

**DISCUSSION**

Here we report the cloning of the murine CK2a' subunit and show that its mRNA and kinase activity are induced in response to serum stimulation of quiescent fibroblasts. Furthermore, we show that expression of CK2a' under the control of a constitutive promoter cooperates with Ha-ras in transformation of rat primary fibroblasts and increases cell growth of transformed cells both in vitro and in vivo.

Our study originated from a screening for serum-induced messages in quiescent mouse fibroblasts, which allowed us to identify the CK2a' as an induced gene. We therefore cloned the murine full-length CK2a' cDNA, analyzed its expression pattern in vivo, in vitro, and activity in cultured cells. Northern blot analysis showed a CK2a' peak of induction at 4 h after serum treatment and that this induction does not require new protein synthesis. Therefore CK2a', like c-myc and MCP-1 (Refs. 36–38 and references therein), belongs to a subset of early genes induced with a slow kinetic. The analysis of induction revealed a lower but still measurable increase of both CK2a and CK2β at the same time points, suggesting that newly assembled CK2 tetramers can be formed following serum induction. In accordance with this prediction we observed an increased CK2-dependent phosphorylation activity in protein extracts from serum-induced cells. An active role of CK2 in cell cycle progression has already been suggested both in mammalian cells and yeast (14, 15, 39). Our data show, for the first time, that CK2 activity is indeed increased at the boundary between G0 and G1, suggesting that new CK2 synthesis is required at this stage of the cell cycle. Its specific induction could be necessary for several reasons. It is possible that the kinase already present in the cells is in a form which is not able to phosphorylate some critical substrates. Alternatively, because we observed a higher CK2a' induction compared with the other catalytic subunit, a higher proportion of αβ2 or α'β2 tetramers could be formed. The formation of these two types of tetramer may lead to different substrate specificity. Despite the fact that the catalytic properties of isolated recombinant CK2a and CK2a' are very similar (28, 34), which is consistent with their high sequence homology in their catalytic domains, significant structural differences suggest divergent functional commitments. Thus, the C-terminal segment of vertebrate CK2a', which lies outside the catalytic core and includes several phosphorylation sites affected both in vitro and in vivo by cyclin-dependent kinases, is absent in CK2a' (17). Likewise a motif (HEHRKL) responsible for association of CK2α with protein phosphatase 2A (23) is substantially altered in CK2a' (HQQKKL). This difference is especially remarkable as it occurs inside a region that is otherwise highly conserved between CK2α and CK2a'. Suggestive of specific function(s) of CK2α' in higher organisms is the observation that CK2α' could not be detected in *Drosophila*, *Xenopus*, and *Schizosaccharomyces pombe*. In *S. cerevisiae*, however, a somewhat atypical CK2α' subunit is found that exhibits functional differences from CK2α (39, 40).

CK2α' cooperates with oncogenic ras in the transformation of primary fibroblasts. Our data show that neither CK2 catalytic

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**Fig. 4. Analysis of foci formation in BALB/c 3T3 fibroblasts transformed either with Ha-ras or with Ha-ras and CK2a'**. BALB/c 3T3 fibroblasts were cotransfected with Ha-ras and either the vector plasmid (pcDNA3) or pcDNAa (a plasmid expressing CK2a' under the control of the cytomegalovirus promoter). Cells were transfected, and foci formation was monitored at day 10. A, number of foci measured in four different transfection experiments, with error bars representing the S.D. of the measurements. B, a representative experiment showing foci formation of BALB/c 3T3 fibroblasts transfected with Ha-ras or with Ha-ras and CK2a' as indicated. Cells were stained with crystal violet.

To analyze in more detail the growth rate of the transformed clones, four ras-transformed clones (R-1 to R-4) and four ras-CK2a'-transformed clones (Rα'1 to Rα'4) were chosen for further analysis. Fig. 5A shows the growth curves obtained by counting cells over a period of 5 days. The clones obtained by cotransfection of Ha-ras and CK2a' showed a marked increase in their growth rates compared with ras-transformed clones. Eight other clones obtained from different transfections were analyzed, and those cotransfected with CK2a' and Ha-ras also exhibited increased growth rates (not shown).

To examine whether the growth differences correlated with the increased expression of CK2a' in the transformed clones, we tested the kinase activity mediated by CK2a' by measuring the phosphorylation of calmodulin. Calmodulin is an ideal substrate for examining the contributions of the CK2 catalytic subunits, because in reconstitution experiments with recombinant subunits its phosphorylation is suppressed completely by adding CK2β (5, 33–35). Clones, transformed with both Ha-ras and CK2a', showed higher levels of calmodulin phosphorylation when compared with ras-transformed clones (Fig. 5B). This growth-related phosphorylation of calmodulin was inhibited (>90%) by addition of either a molar excess (0.5 mM) of the specific peptide substrate, or 1 μg/ml heparin (not shown). These findings, in conjunction with the alkali-lability of the phosphate incorporated into calmodulin, which rules out the possibility of tyrosine phosphorylation, show that calmodulin phosphorylation is entirely because of CK2 rather than to any other protein kinase(s). Thus, although we observed clonal
subunits nor Ha-ras alone induce foci formation when transfected in primary cells, whereas transformed foci become evident upon cotransfection with Ha-ras and either CK2α or CK2α. Therefore, we can conclude that although the structural differences between CK2α and CK2α may reflect distinct functional roles, at least with respect to the cooperation with ras in cell transformation, these differences are not critical.

Recently, it was observed that exogenous expression of CK2α suppressed cell growth and inhibited foci formation induced by activated ras (23). Our results diverge from this observation. The reasons for such a discrepancy are presently unclear. It is possible that the inhibitory effect of CK2α previously observed (23) was dependent on the genetic background of the NIH 3T3 cells used in those experiments. Alternatively, the reduction of foci observed by Hériche and collaborators (23) may have resulted from a CK2α poisoning effect because of a too high expression of CK2α. In a standard focus formation assay we observed cooperation between CK2 catalytic subunits and oncogenic ras both in primary and immortalized fibroblasts, suggesting we are observing a general phenomenon. Moreover, our results are in agreement with experiments in transgenic mice where the constitutive expression of CK2α accelerated the formation of lymphomas induced by c-myc or tal-1 (18, 19). Comparison of the growth curves of fibroblasts transformed with Ha-ras alone versus fibroblasts transformed with Ha-ras and CK2α demonstrated that transformed clones, expressing constitutively CK2α, grew faster. The enhanced growth of Ha-ras and CK2α-transformed clones correlates with increased catalytic activity when monitored using calmodulin as a phosphoacceptor substrate, symptomatic of the presence of free catalytic subunit (33, 34). Thus, these data suggest that unbalanced expression of CK2α or CK2α leads to phosphorylation of some critical target(s) necessary to accelerate the cell cycle progression of ras-transformed cells. Therefore, it seems likely that the transforming potential of CK2 in each experimental model is because of a fraction of catalytic subunits not combined with CK2β to form the canonical holoenzyme. This hypothesis is supported by the phosphorylation of calmodulin, because this protein is unaffected by the CK2 holoenzyme.

In contrast the hypothesis that following serum treatment CK2α and CK2α not combined with CK2β could be transiently present in untransformed dividing cells is not consistent with available experimental data. Indeed, we observed that after serum treatment of fibroblast, CK2β was induced with the same kinetics of the catalytic subunits. In addition, by using the specific substrate calmodulin or by titrating in with recombinant CK2β, we did not detect free catalytic subunits in cell extracts (not shown). Therefore, it is likely that during the G1/S progression of the cell cycle the newly synthesized CK2α and CK2α are rapidly assembled into tetrameric CK2. Thus, the assembly of newly synthesized CK2 subunits into a tetrameric enzyme could represent a mechanism for the modulation of the too reactive free catalytic subunits necessary to reprogram the CK2 kinase activity during the progression of the cell cycle. Future studies aimed at the identification of specific target(s) of the CK2 catalytic subunits may unveil some target(s) critical for cell transformation.

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FIG. 5. CK2α overexpression accelerates proliferation of transformed cells. Individual foci from BALB/c 3T3 cells transformed with Ha-ras (R-1, R-2, R-3, R-4) or with Ha-ras plus CK2α (Rα-1, Rα-2, Rα-3, Rα-4) were picked and analyzed for their characteristics. A, growth curves of isolated foci. Cells were plated at a density of 10^5 cells/6-cm culture dish in medium containing 2% FCS and counted at daily intervals. The mean values of duplicate cultures are shown plotted against time. B, calmodulin phosphorylation by isolated foci. Confluent cells were lysed and total cellular extracts were used in phosphorylation assay using calmodulin as specific CK2 catalytic subunit substrate. The reaction products were run on SDS-polyacrylamide gel electrophoresis, and the blots were autoradiographed or directly scanned. All data are the mean of at least three separate determinations with a S.E. of less than 12%. C, correlation between calmodulin phosphorylation and growth curve rates. The number of cells corresponds to the number of cells described in A at day 5 of growth. The values of calmodulin phosphorylation are the same of the experiment described in B. Clones transformed with Ha-ras are represented with open circles, whereas clones transformed with Ha-ras plus CK2α are represented with black squares. D, tumor formation in nude mice. Mice were injected with 10^5 log-phase cells from the ras-transformed cells (R-1 to R-4) and from the Ha-ras with CK2α-transformed cells (Rα-1 to Rα-4). Tumors were harvested after 2 weeks of growth and weighed. The error bars represent the S.D. for three mice used for each clone.
Molecular Cloning and Oncogenic Activity of CK2α′