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Mapping of the human casein kinase II catalytic subunit genes: two loci carrying the homologous sequences for the α subunit

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ABSTRACT

The human serine/threonine protein casein kinase II (CK II) contains two distinct catalytic subunits, α and α′, which are encoded by different genes. A combination of segregation analysis of rodent-human hybrid cells and chromosomal *in situ* hybridization have localized the human CK II-α DNA sequence to two loci: 11p15.5-p15.4 and 20p13. In contrast, the CK II-α′ gene has been mapped to chromosome 16 by somatic cell hybrid analysis. Taken together with our previous assignment of the CK II regulatory β-subunit gene to 6p12-p21, these results indicate that although the products of these genes form a single biological complex, they are encoded on different human chromosomes. Further analysis should determine whether both loci of CK II-α are functional, or perhaps one of the two constitutes a pseudogene.

INTRODUCTION

Casein kinase II (CK II) is a ubiquitous messenger-independent protein serine/threonine kinase, localized in both the cytoplasm and the nucleus (1). The mammalian enzyme is isolated as an $\alpha_2\beta_2$, $\alpha'_2\beta_2$ or $\alpha\alpha'\beta_2$ tetramer, in which α and α′ are closely related, yet distinct catalytic subunits (2). The α and α′ subunits are encoded by different genes in yeast (3,4) as well as in humans (5,6). The carboxyl terminal regions of the human α and α′ subunits exhibit close sequence similarity to the human cell division cycle protein kinase CDC2Hs and its yeast homologs (7). The β subunit is the regulatory subunit (8,9,10), which is encoded in humans by a single gene (11,12,13) and shows no homology with any known protein. The occurrence of casein kinase II in all eukaryotic organisms, coupled with the extreme sequence conservation of both subunits during evolution, suggests that the enzyme has essential cellular function(s). Indeed, in *S. cerevisiae* casein kinase II is essential for viability (4). The enzyme has been shown *in vitro* to phosphorylate a broad range of endogenous substrates which include proteins that play key roles in regulating cellular growth and metabolism (14). Some of the evidence which links casein kinase II activity to regulation of cell growth is: (I) the activity of casein kinase II is transiently stimulated by polypeptide hormones, such as insulin (15,16), insulin-like growth factor I (16), and epidermal growth factor (15,17); (II) serum stimulation of deprived cells causes oscillations in casein kinase II activity, which seem to be associated with the cell cycle (18); (III) casein kinase II may have a role in the transition between prophase and metaphase during meiotic cell division of *Xenopus* oocytes (19). Mulner-Lorillon et. al. suggest that the meiotic function of CK II is initiated via phosphorylation of the regulatory β-subunit by the cdc2 protein kinase, leading to increased CK II activity (20); (IV) casein kinase II can phosphorylate *in vitro* several nuclear transcription factors at a subset of sites known to be phosphorylated *in vivo*. This has been shown for SV40 large T antigen (1,21), HPV E7 (22,23), adeno E1a (24), c-Myc (25), c-Myb (26), c-Fos (24), SRF (27) and c-ErbA (28). Moreover, the regions of the viral and the cellular oncoproteins listed above that are phosphorylated by CK II are important, in several cases essential, for transformation/immortalization of cultured cells. In the case of SV40 large T antigen, phosphorylation of its core transformation/immortalization region by casein kinase II may affect the efficiency of tranformation by determining the rate of large T antigen transport into the nucleus (29). A different mechanism may operate in the case of SRF (27) and the proto-oncogene Myb(26) whose DNA binding activities are increased and decreased, respectively, following their phosphorylation by casein kinase II.

The mechanism of mitogenic activation of casein kinase II is not known. Recently, it was observed that the intracellular distribution of CK II exhibited a striking shift toward an increased nuclear concentration during active proliferation of cells in culture (30). Further experiments should determine whether this nuclear translocation is linked to the hyperphosphorylation of the β-subunit which occurs upon mitogenic stimulation of CK II (31). On the basis of these observations and others (14), it seems that casein kinase II is an intermediate in a cascade of protein kinases which transduce signals from growth factor receptors to effector proteins within the cell nucleus.

Previously, we have assigned the human casein kinase II β-subunit gene to 6p12-p21 (32). We now report the chromosomal localization of the human genes which encode the catalytic subunits of casein kinase II.

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MATERIALS AND METHODS

Somatic Cell Hybrids

A mapping panel consisting of DNA from seventeen mouse-human (NA09925–09938, NA09940, NA10324, and NA10567) and two Chinese hamster-human (NA10611 and GM07298) hybrids was obtained from NIGMS cell repository.

Characterization and human chromosome content in these hybrids are described in detail in NIGMS catalog. One Chinese hamster-human hybrid cell line, GM07298, containing a part of chromosome 11 (11q) was used to confirm the regional assignment of the CK II-α locus on chromosome 11.

Hybridization probes

Two cDNA-derived probes, a 1260 bp coding DNA segment and an 822 bp fragment of 3'- nontranslated region (nucleotides 113–1372, and 1332–2153 in ref 5, respectively) were used to localize the CK II-α gene in humans. The CK II-α' locus was mapped by hybridization to a 414 bp fragment from the 3' nontranslated region of the human CK II-α' cDNA (nucleotides 1221–1634 in ref. 6).

Southern Blot Analysis

DNA samples were digested with restriction endonucleases, separated by electrophoresis on agarose gels, transferred to Hybond nylon filters (Amersham), and hybridized to the ^{32}P-labeled probes as described (33).

In Situ Hybridization

DNA probes were nick-translated with [^{3}H]dCTP and [^{3}H]TTP to a specific activity of $2-3\times10^7$ cpm/μg. Hybridization to human chromosome spreads, post hybridization wash, emulsion autoradiography, and silver grain analysis were carried out as previously described (33).

RESULTS

The Human CK II-α DNA Sequence Maps to Two Loci: 11p15.5-p15.4 and 20p13

Initially, for the chromosomal assignment of the human casein kinase II α-subunit gene, we have used a 1260 bp DNA fragment which contains all of the translated region of CK II-α cDNA (5,6). *In situ* hybridization of the cDNA probe to normal human metaphase spreads revealed two sites that were labeled above background (Fig. 1). Of 203 grains over 100 cells analyzed following hybridization, 27 (13.3%) and 15 (7.4%) were found to be at the distal short arms of chromosome 11, bands p15.5–15.4 and at chromosome 20, band p13, respectively. This result suggested that DNA sequences homologous to the CK II-α gene map to two different human chromosomes. Amino acid sequence comparison between the two casein kinase II catalytic subunits α and α' revealed 85% homology, which is reflected in 76% nucleotide identity between bp 168 to 1170 of the CK II-α cDNA (6). However, the 3' nontranslated regions of the two casein kinase II α and α' subunit cDNAs share little homology (6). To rule out the possibility of cross-hybridization with CK II-α' sequences, *in situ* hybridization to human metaphase chromosomes was performed with an 822 bp CK II-α cDNA 3' nontranslated region probe. In a total of 224 grains in 113 cells scored for the signals detected by the 3' nontranslated region probe, 30 (13.4%) were at 11p15.5-p15.4, and 13 (5.8%) were at 20p13 (data not shown). Further support for the existence in the human genome of an additional copy of CK II-α related DNA sequence was supplied by segregation analysis of rodent-human hybrid cells. Hybridization of the CK II-α cDNA 3' nontranslated region probe to DNA from 19 rodent-human somatic cell hybrids indicated that CK II-α gene maps to human chromosomes 11p and 20 (Fig.2 and Table 1). Three major human specific BglII fragments of 13.5, 6.7, and 1.6 kb and two faint bands of 4.7 and 3.0 kb were detected by this CK II-α

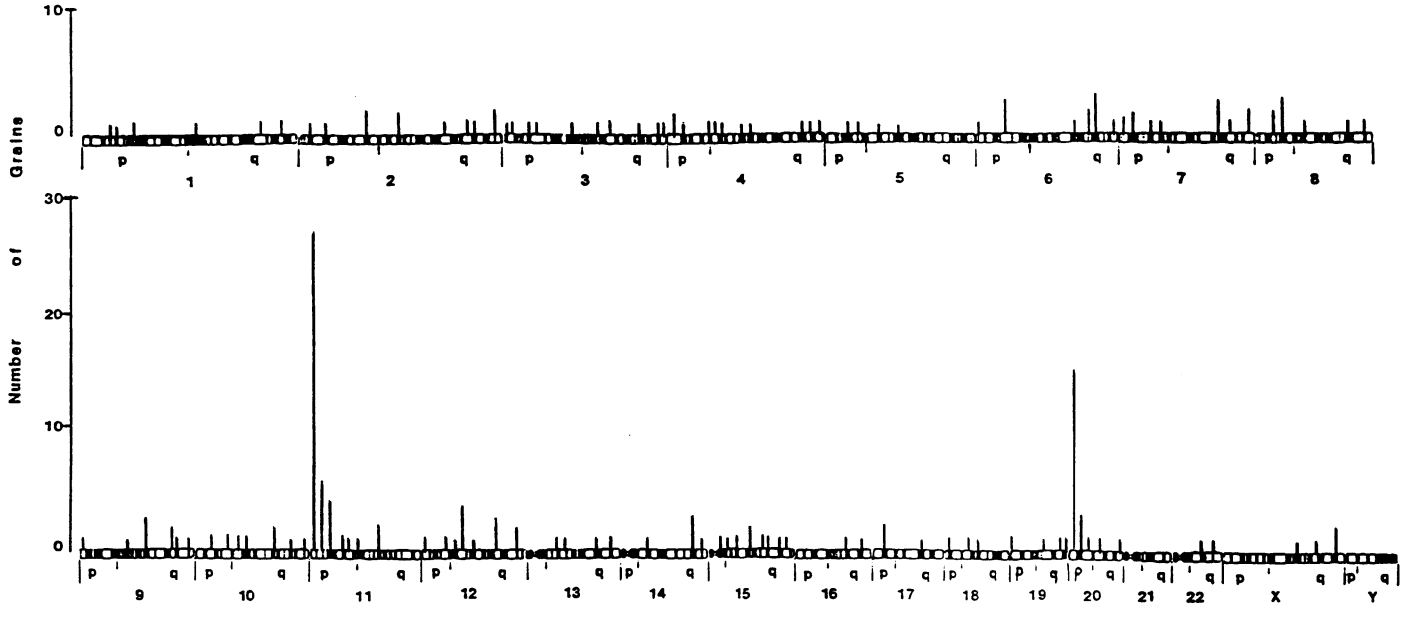

Fig. 1. Chromosomal *in situ* hybridization localizes the human CK II-α locus to 11p15.5-p15.4 and 20p13. The histogram shows the distribution of silver grains on human metaphase chromosomes after hybridization with the CK II-α cDNA coding region probe.

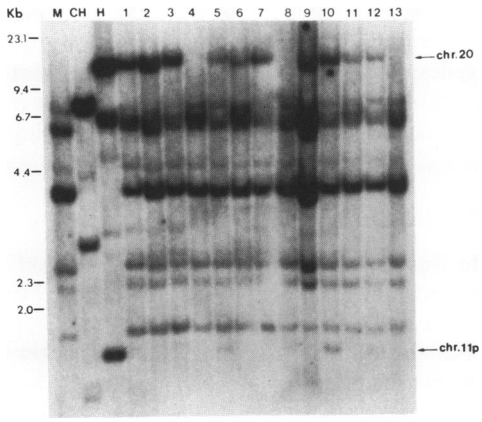

Fig. 2. Representative autoradiogram following hybridization of BglII-cleaved DNA from rodent-human hybrid cells with the CK II-α cDNA 3′ nontranslated region probe. Lanes are mouse (lane M); Chinese hamster (lane CH); human (lane H); hybrid NA09925 (lane 1); hybrid NA09926 (lane 2); hybrid NA09927 (lane 3); hybrid NA09928 (lane 4); hybrid NA09929 (lane 5); hybrid NA09930A (lane 6); hybrid NA09931 (lane 7); hybrid NA09932 (lane 8); hybrid NA09933 (lane 9); hybrid NA09934 (lane 10); hybrid NA09935A (lane 11); hybrid NA09936 (lane 12); and hybrid NA09937 (lane 13). As indicated at the right side, the 13.5 kb and the 1.6 kb bands segregate with human chromosomes 20 and 11p, respectively, although the data revealed by hybrid GM07298 containing only 11q is not shown in the autogradiogram.

probe (Fig. 2). Since the 6.7 kb fragment was also detected in DNA from mouse and Chinese hamster, and the 4.7 and 3.0 kb human bands are too faint, only the major 13.5 and 1.6 kb DNA fragments could be scored for in hybrids. The 13.5 kb DNA fragment was concordant only with human chromosome 20 and the 1.6 kb fragment was present in hybrids retaining human chromosome 11 (Table 1). Moreover, the 1.6 kb human band was absent in one Chinese hamster-human hybrid, GM07298, containing only the long arm of human chromosome 11 (11q), indicating that chromosome 11q does not carry CK II-α sequences.

The human CK II-α′ gene maps to chromosome 16

Having assigned the CK II-α gene to human chromosomes 11 and 20, it was of interest to see whether the other catalytic gene (CK II-α′) is located proximal to any of these two sites. Southern blot hybridization of DNA from 19 rodent-human hybrid cell lines assigned the CK II-α′ gene to human chromosome 16 (Fig. 3 and Table 1). Hybridization of EcoRI-cleaved human DNA to the 414 bp CK II-α′ cDNA 3′ nontranslated region probe detected a single band of 4.6 kb (Fig. 3). Cross-hybridizing fragments of 16.5 kb and 8.5, 5.4 and 3.1 kb were detected by this probe in DNA from mouse and Chinese hamster, respectively. The 4.6 kb EcoRI human-specific band segregated with chromosome 16 (Table 1). One hybrid, CM/NA09925,

Table 1. Segregation of human sequences detected by CK-IIα and α′ probes with human chromosomes in human-rodent somatic hybrids

Presence of sequence/ presence of chromosome	Human Chromosomes 1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19	20	21	22	X	Y
α (BglII 1.6Kb)																								
Concordant																								
+/+	0	1	1	4	3	5	2	3	0	1	5	4	0	3	1	0	5	1	1	4	3	2	0	0
−/−	10	9	6	6	7	7	6	6	13	7	11	7	7	4	6	11	4	7	9	6	7	8	10	10
Discordant																								
+/−	5	4	3	1	2	0	1	0	5	3	0	1	4	1	1	5	0	4	1	1	2	2	4	4
−/+	4	5	8	5	6	7	8	7	1	3	0	6	5	10	8	2	10	7	5	7	6	5	1	3
Total Discordant Hybrids	9	9	11	7	8	7	9	7	6	6	0	7	9	11	9	7	10	11	6	8	8	7	5	7
Total Informative Hybrids	19	19	18	17	18	19	17	16	19	14	16	18	16	18	16	18	19	19	16	18	18	17	15	17
% Discordant	47	47	61	41	44	37	53	44	32	43	0	39	56	61	56	39	53	58	38	44	44	41	33	41
α (BglII 13.5Kb)																								
Concordant																								
+/+	4	5	6	8	7	9	8	6	0	4	4	8	5	11	6	1	11	7	5	11	7	6	0	2
−/−	7	6	4	5	5	4	5	3	6	4	6	5	6	5	4	6	3	6	5	7	5	6	6	6
Discordant																								
+/−	8	7	5	2	4	3	2	3	12	6	5	3	5	0	3	10	1	5	5	0	4	4	8	8
−/+	0	1	3	2	2	3	2	4	1	0	1	2	0	2	3	1	4	1	1	0	2	1	1	1
Total Discordant Hybrids	8	8	8	4	6	6	4	7	13	6	6	5	5	2	6	11	5	6	6	0	6	5	9	9
Total Informative Hybrids	19	19	18	17	18	19	17	16	19	14	16	18	16	18	16	18	19	19	16	18	18	17	15	17
% Discordant	42	42	44	23	33	32	23	44	68	43	38	28	31	11	38	61	26	32	38	0	33	29	60	53
α′ (EcoRI 4.6 Kb)																								
Concordant																								
+/+	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	1	0	0	0	0	0	0	0	0
−/−	14	12	8	6	8	6	6	5	17	9	10	7	10	4	6	16	3	10	9	6	8	9	13	13
Discordant																								
+/−	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	0	1	1	1	1	1	1	1	1
−/+	4	6	9	10	9	12	10	10	1	4	5	10	5	13	9	1	15	8	6	11	9	7	1	3
Total Discordant Hybrids	5	7	10	11	10	13	11	11	2	5	6	11	6	14	10	1	16	9	7	12	10	8	2	4
Total Informative Hybrids	19	19	18	17	18	19	17	16	19	14	16	18	16	18	16	18	19	19	16	18	18	17	15	17
% Discordant	26	37	56	65	56	68	65	69	11	36	38	61	38	78	63	6	84	47	44	67	56	47	13	24

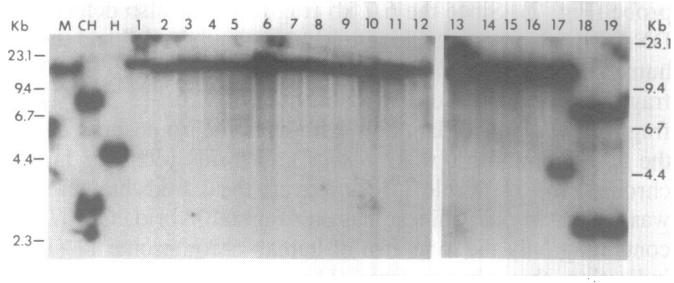

Fig 3. Hybridization of CK II-α′ cDNA 3′ nontranslated region fragment with EcoRI-cleaved DNA from mouse (lane M); Chinese hamster (lane CH); human (lane H); hybrids NA09925 to Na00937 (lanes 1–13) as shown in the legend to Fig. 2; hybrid NA09938 (lane 14); hybrid NA09940 (lane 15); hybrid NA10324 (lane 16); hybrid NA10567 (lane 17); hybrid NA10611 (lane 18) and hybrid GM07298 (lane 19).

reported to have chromosome 16 present at a frequency of 0.14 was negative for the 4.6 kb fragment. This discordancy is probably due to the limited hybridization sensitivity.

DISCUSSION

The human genes for the catalytic subunits of casein kinase II have been mapped by somatic cell hybrid analysis and *in situ* chromosomal hybridization. Whereas the CK II-α′ gene has been mapped to human chromosome 16, the CK II-α DNA sequence has been localized to two loci: 11p15.5-p15.4 and 20p13. Previously we have assigned the human casein kinase II β-subunit gene to chromosome 6, band p12-p21 (32). Taken together these results imply that although the products of these genes form a single biological complex, they are encoded on different human chromosomes. We do not know at this stage whether the two CK II-α homologous sequences are functional, or perhaps one of the two constitutes a pseudogene. Although isoelectric variants have been described for the α and α′ subunits of CK II from bovine testis (2) as well as from rat liver nuclei (34), they are thought to arise from post-translational modifications rather than by a change in primary sequence (2,6). The availability of somatic cell hybrids which contain only partial human chromosomal complements should help to resolve the issue of the coding potential of the two CK II-α genes.

The physiological roles and regulation of the two catalytic subunits of casein kinase II have not been clarified. The existence of distinct catalytic subunit genes, encoded on different chromosomes, raises the possibility of differential regulation and perhaps also specialized biological functions. In the case of *S. cerevisae* it has been shown that disruption of the gene encoding either the CK II-α (CKA1) or the CK II-α′ (CKA2) subunit gene by itself, had no gross effect on the wild type phenotype. However, simultaneous disruption of both genes was lethal (4). This suggested that for some essential processes the yeast CK II α and α′ subunits are functionally equivalent. Still, the possibility of uncovering, by employing more subtle tests, some specialized functions for each of the catalytic subunits, cannot be ruled out.

In situ hybridization with either the coding or the 3′ nontranslated region of CK II-α cDNA have shown preferred hybridization specificity at 11p15.5-p15.4. This region has a high frequency of loss of heterozygosity in several types of tumors (35), as well as paternal disomy in patients with the fetal overgrowth disease known as Beckwith-Wiedemann syndrome (36), suggesting the presence in this locus of a growth control gene or genes. It should be interesting to test whether casein kinase II, a protein kinase implicated in intracellular transduction of growth signals, is defective in any of these pathological states.

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