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The potassium channel gene HK1 maps to human chromosome 11p14.1, close to the FSHB gene

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Abstract. Transiently activating (A-type) potassium (K) channels are important regulators of action potential and action potential firing frequencies. HK1 designates the first human cDNA that is highly homologous to the rat RCK4 cDNA that codes for an A-type K-channel. The HK1 channel is expressed in heart. By somatic cell hybrid analysis, the HK1 gene has been assigned to human chromosome 11p13-p14, the WAGR deletion region (Wilms tumor, aniridia, genito-urinary abnormalities and mental retardation). Subsequent pulsed field gel (PFG) analysis and comparison with the well-established PFG map of this region localized the gene to 11p14, 200–600 kb telomeric to the FSHB gene.

Introduction

Potassium (K) channels are highly diverse membrane proteins that seem to be present in nearly every eukaryotic cell (Hille 1984). The rat genome encodes a K-channel family (RCK) homologous to the *Drosophila* Shaker channels (Stuehmer et al. 1989). To date, only one member of this RCK family (RCK4) has been detected that expresses A-type (rapidly inactivating) K-channels.

Recently a full lenght human cDNA (HK1) has been cloned; it encodes a K-channel protein highly homologous to RCK4 (97% identity; Tamkun et al. 1991). HK1 mRNA is expressed in heart, in particular in the atrium and ventricle. Therefore, the K-channel formed by this protein might be important in the regulation of the fast repolarizing phase of action potentials in heart and thus might influence cardiac action potential duration (Josephson et al. 1984).

To further the genetic characterization of the human HK1 gene, we have determined the precise chromosomal localization of this gene. Using the polymerase chain reaction (PCR), we have produced a genomic HK1 DNA probe to map the HK1 gene on human chromosome 11p14 by somatic cell hybrid and pulsed field gel analysis (PFGE).

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Materials and methods

DNA probes

Two degenerated primers, backtranslated from the RCK4 protein (aa: 170–178, 406–414, Stuehmer et al. 1989) were used to amplify human RCK4 anologous DNA. The upstream and downstream primers were 5'-TCAGATCGATACAGNGA(C/T)TG(T/C)TG-(T/C)GAA-3' and 5'-AATGAATTC(G/T/A)AT(A/T/C)TCA-(A/G/A)TG(A/G/A)TG(T/A)TCAT-3'. The primers contain *Cla*I and *Eco*RI restriction sites for subcloning. Human HeLa cell DNA was used as a template for amplification (Grupe et al. 1990). The PCR reaction was performed in a Perkin Elmer/Cetus Thermocycler for 30 cycles: 60 s at 92°C, 30 s at 40°C and 90 s at 72°C. The amplified DNA-fragment was digested with *Cla*I/*Eco*RI and subcloned into Bluescript KS+ (Stratagene). The subcloned DNA-fragment, pHK4, was sequenced according to the method of Sanger et al. (1977).

Probe p202 is a 2.7-kb *Hin*dIII single copy segment from clone L202-1 that has been mapped to human chromosome 11p14 (Gessler et al. 1989a, b). The 500-bp *Hin*cII/*Sac*I fragment of pRS1.2 (Watkins et al. 1987) was used as a probe for FSHB.

Somatic cell hybrid analysis

The chromosomal complement of the somatic cell hybrid panel used in this study has been described elsewhere (Willecke et al. 1990). The J1 derived hybrids were obtained from Dr. C. Jones, ERCI, Denver (Glaser et al. 1990). The WAGR (Wilms tumor, amiridia, genito-urinary abnormalities and mental retardation) deletion hybrids cell lines were provided by Dr. G. Bruns, Children's Hospital, Boston (Gessler et al. 1989a). DNA preparation, restriction enzyme digestion, agarose gel electrophoresis and Southern blotting were performed according to standard protocols (Sambrook et al. 1989). Probes were labelled by oligonucleotide priming (Feinberg and Vogelstein 1984) and hybridized as described in Gessler et al. (1989a). Autoradiographic exposure times were 1–3 days at −70°C.

PFGE analysis

The lymphoblastoid cell line 6697, which formed the basis for the construction of the physical map of chromosome 11p13/p14, was

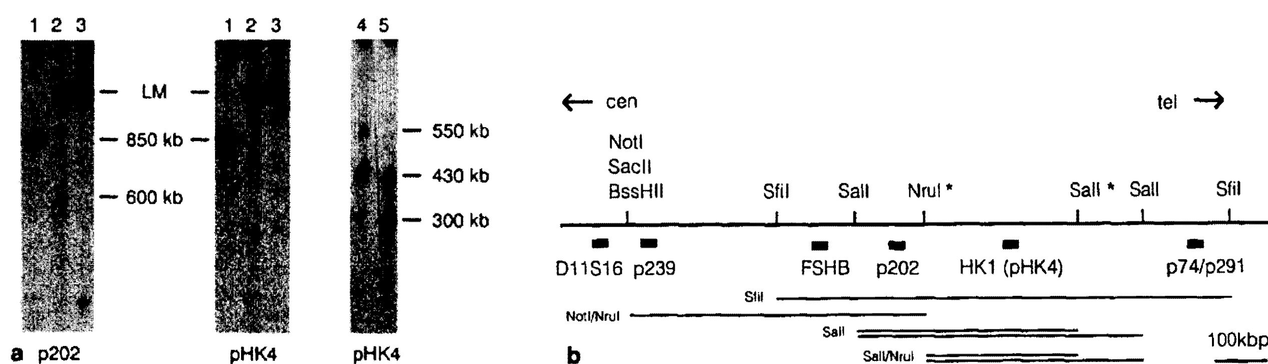

Fig. 1a. PFGE mapping of HK1. Probes pHK4 and p202 hybridize to a common 850-kb *Sfi*I fragment *(lane 1)*. Whereas p202 recognizes a 600-kb *NruI/NotI* fragment *(lane 2)*, pHK4 only shows hybridization in the limiting mobility region *(LM)* above 1 Mb. The *Nru*I site, which is only partially cut in 6697 DNA, thus separates p202 and pHK4. Neither *Nru*I alone *(lane 3) nor Not*I (not shown) produce fragments resolvable under the electrophoretic conditions used. The *Nru*I cutting site can be used to position the HK1 gene more precisely. *Sal*I restriction fragments of 430 kb and 550 kb *(lane 4)*, shared with p202, are shortened by double digestion with *Nru*I to 300 kb and 420 kb, respectively *(lane 5)*. Probe p202 on the other hand recognizes a much smaller *SalI/Nru*I fragment of approximately 130 kb (not shown). The double bands probably represent methylation differences. **b** Schematic drawing representing the mapping of HK1 within 200–600 kbp of FSHB. Sites that are incompletely cut are marked by *asterisks*. There are several additional *Sal*I sites on both sides of the p202/pHK4 region that are not shown. The hybridization fragments seen in **a** are drawn as *horizontal lines*

used for PFGE analysis. All procedures were carried out essentially as described in Gessler et al. (1989b). In brief, cells were embedded in LMP (low melting point) agarose blocks and used at a final concentration of 2.5 × 10 E5 cells per digest with 10–20 units of restriction enzyme. PFGE was carried out in a LKB Pulsaphor unit with the hexagonal electrode array using the following conditions: 170 V, 10–60 s switching time for 30 h at 12°C for lanes 1–3 in Fig. 1A and a switching time of 10–40 for lanes 4 and 5. Blots were prepared by alkaline transfer for 24 h. Size markers used were *Saccharomyces cerevisiae* chromosomes and phage lambda concatemeres.

Results and discussion

The sequence of the amplified human DNA fragment (pHK4) is identical to hPCN2 (Nt 527-1207; Philipson et al. 1990), a cDNA from a human skeletal muscle library, except for nucleotides 908/909, which are interchanged in pHK4, resulting in one amino acid replacement (D to S, data not shown). On the other hand, the deduced protein sequence of pHK4 is identical to that of HK1 protein (aa177–403; Tamkun et al. 1991), derived from a cDNA library from human ventricle.

To assess the chromosomal localization of the HK1 gene, the 681-bp DNA probe pHK4 was hybridized against a human-rodent somatic cell hybrid panel from 15 chromosomally characterized cell lines. The probe fragment used shows crossreaction with rodent DNA under high stringency hybridization conditions. The human signal, however, could be unambiguously identified based on its size in *Sac*I- and *Eco*RI-digested DNA, leading to an initial assignment to chromosome 11 (data not shown).

A subregional mapping was accomplished by using additional human-hamster somatic cell hybrids from the J1 hybrid series (Glaser et al. 1990) together with WAGR deletion hybrids (Gessler et al. 1989a) (Fig. 1a). The human HK1 gene could be detected in hybrid J1-11, retaining 11pter-11q11 as its only human material. No human-specific signal was detected in hybrids carrying deletions encompassing chromosome 11p13–11p14. The smallest region for HK1 was defined by the WAGR deletion in cell line G157A6 (prox11p14–11p13) (Fig. 1b).

The human chromosome 11 segment deleted in G157A6 has been extensively characterized because of the interest in the genes underlying the WAGR syndrome. The deletion breakpoints for that particular hybrid have been precisely positioned on a long range restriction map spanning the entire region. Thus, the position of any new probe mapping within this area can be directly inferred from is PFGE restriction map.

Initial experiments with probe pHK4 revealed restriction fragments in the megabasepair range for most rare cutting enzymes; this suggested a localization in the CpG poor 11p14 region (Gessler et al. 1989b), but provided little information about its precise position. However, the enzymes *Sfi*I revealed the presence of a unique 850-kb fragment that is also detected by a FSHB probe and several previously characterized DNA markers (Fig. 1). In addition, pHK4 shares *Sal*I fragments of 430 kb and 550 kb with probe p202, from which it is separated by a *Nru*I cutting site. These results unambiguously position HK1 telomeric to probe p202, approximately 200–600 kb distal to the FSHB gene. As the FSHB gene is located at 11p14, close to the 11p13/p15 boundary, the HK1 gene can thus be assigned to the proximal part of human chromosome 11p14, namely 11p14.1.

The genomic HK1 locus is included in a significant fraction of the known WAGR associated cytogenetic deletions, and affected individuals will be hemizygous for this gene. The loci for Wilms tumor/genito-urinary abnormalities and aniridia have been identified recently and both are located proximal to HK1 (Call et al. 1990; Gessler et al. 1989c, 1990; Ton et al. 1991). Only the mental retardation component of the syndrome has not

yet been unambiguously defined, but again a more centromeric localization appears likely. These data suggests that a hemizygous deletion of HK1 may have little phenotypic effect, perhaps because of less stringent requirements for the control of expression levels for this gene.

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