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Complete Sequence of a Gene Encoding KAR3-Related Kinesin-like Protein in *Candida albicans*

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In contrast to *Saccharomyces cerevisiae*, little is known about the kinesin-like protein (KLP) in *Candida albicans*. The motor domain of kinesin, or KLP, contains a subregion, which is well conserved from yeast to humans. A similarity search, with the murine ubiquitous kinesin heavy chain region as a query, revealed 6 contigs that contain putative KLPs in the genome of *C. albicans*. Of these, the length of an open reading (ORF) of 375 amino acids, temporarily designated *CaKAR3*, was noticeably short compared with the closely related *S. cerevisiae KAR3* (*ScKAR3*) of 729 amino acids. This finding prompted us to isolate a λ genomic clone containing the complete *CaKAR3* ORF, and here the complete sequence of *CaKAR3* is reported. *CaKAR3* is a C-terminus motor protein, of 687 amino acids, encoded by a non-disrupting gene. When compared with *ScKAR3*, the amino terminal region of 112 amino acids was unique, with the middle part of the 306 amino acids exhibiting 25% identity and 44% similarity, while the remaining C-terminal motor domain exhibited 64% identity and 78% similarity, and have been submitted to GeneBank under the accession number AY182242.

Key words: *Candida albicans, KAR3 gene, kinesin-like protein, Saccharomyces cerevisiae*

Microtubule-dependent movement is fundamental to many biological processes, including cell division and intracellular transport of membranous organelles in eukaryotic cells. The force for the movement derives from ATP hydrolysis, due to microtubule-based mechanochemical motor proteins, cytoplasmic dyneins and kinesin superfamily (KIF) proteins or kinesin-like proteins (KLPs) (Hirokawa *et al.*, 1998; Goldstein and Yang, 2000). Kinesin is composed of 4 domains; motor, neck, stalk and tail (Kosik *et al.*, 1990; Verhey and Rapoport, 2001). In general, the motor domain, possessing ATPase activity, has 5 regions (IFAYGQT, GKTY/HTM, VSYLEYINE, SSDSHAIF, and DLAGSE), which are highly conserved in various organisms. Fragments flanked by these sequences can therefore be amplified by PCR using degenerate oligonucleotide primers. Using this strategy, numerous KIF members have been identified (Nakagawa *et al.*, 1997).

When a similarity search was performed, using the evolutionarily conserved murine ubiquitous kinesin heavy chain region as a query against the database of *Candida albicans* (http://sequence-www.stanford.edu/group/candida), 6 putative KLP open reading frames (ORFs) were localized in the genome of the species. By comparison with 6 previously identified KLPs of *Saccharomyces cerevisiae*, the phylogenic relationship of the *C. albicans* KLPs was established (Fig. 1). Of these, the length of an ORF (375 amino acids) deduced from the contig6-2474, designated *CaKAR3*, was noticeably short compared with the closely related *S. cerevisiae KAR3* (*ScKAR3*) of 729 amino acids. Since *ScKAR3* is a kinesin, where the motor domain is

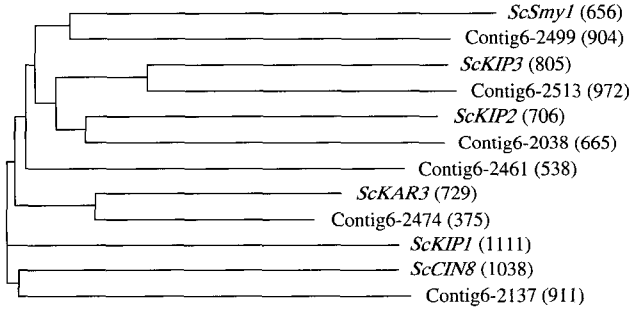

Fig. 1. Phylogenic relationship between kinesin-like proteins of *S. cerevisiae* and *C. albicans*. The six contigs shown were derived by a homology search using the murine ubiquitous kinesin heavy chain motor domain as a query in the database of *Candida albicans* (http://sequence-www.stanford.edu/group/candida). KLPs of *S. cerevisiae* were from http://www.proweb.org/kinesin//. A phylogenic relationship was established using the AlignX program within the Vector NTI Suite (InforMax, Bethesda, MD). The numbers of amino acids of each ORF are indicated in parentheses.

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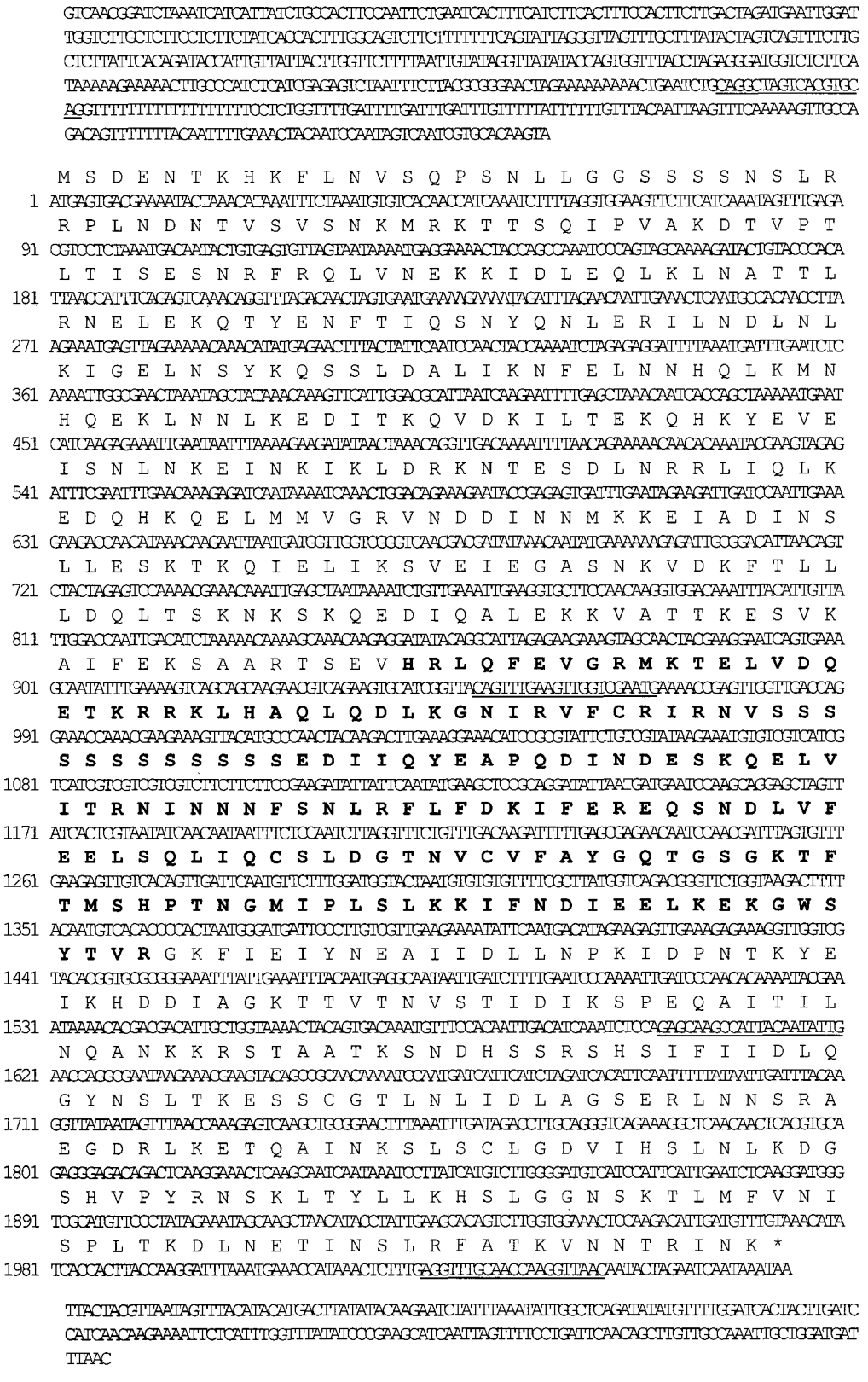

Fig. 2. Nucleotide and deduced amino acid sequences of *CaKAR3*. The nucleotide and amino acid residues are numbered from the initiation codon of an ORF. The four primer sequences used for the RT-PCR are underlined, and designated S1 (5'-CAGGCTAGTCACGTGCAG-3'), AS1 (5'-CATTCGACCAACTTCAAACTG-3'), S2 (5'-GAGCAAGCCATTACAATATTG-3') and AS2 (5'-GTTAACCTTGGTTGCAAACCT-3'), from the 5' end. Bold letters represent the overlapping region between contig6-2474 and cotig6-1523 (see text for detail). Boundaries of the *CaKAR3* transcript are unknown. These sequences have been submitted to Genbank under the accession number AY182242.

located at the carboxyl terminus, the amino terminal region of *CaKAR3* was suspected to be missing in the contig6-2474. In fact, when PCR was performed, using *C. albicans* genomic DNA as a template, with primers surrounding the initiation codon that appears in the contig6-2474 ORF, no product was generated. This finding prompted us to isolate a λ genomic clone containing the complete *CaKAR3* ORF, the sequence of which is reported here.

Materials and Methods

Preparation of probe

For preparation of a probe, DNA fragments covering the *CaKAR3* motor domain was amplified using *C. albicans* genomic DNA, with primers S2 and AS2, as shown in Fig. 2. Purified 440 bp PCR products were labeled and used for genomic library screening.

Genomic library screening

The basic procedure has been described elsewhere (Sambrook and Russel, 2001). Hybridization was carried out overnight, at 42°C, in 5 × SSC (1 × SSC was 150 mM NaCl and 15 mM Na-citrate), 0.1% BLOTTO and 50% formamide. Washing was performed in 2 x SSC at 42°C for 30 min, and then in 0.1 x SSC, 0.1% (w/v) SDS at 68°C for 30 min. Plaque hybridization was performed using replica nitrocellulose filters from plates containing 2×10^4 clones each from the *C. albicans* λ EMBL genomic library, using approximately 5×10^8 dpm of the probe, as prepared above. The genomic clones selected in the primary screening were subjected to secondary screening. Inserts from purified positive λ clones were subcloned into pBluescript, and their nucleotide sequence determined.

RT-PCR

Total RNA of *C. albicans* was prepared from a blastospore culture. First strand cDNAs were synthesized by transcribing 1 μg of RNA with 200 units of MMLV reverse transcriptase (Promega, USA), as recommended by the manufacturer. Using a combination of sense and antisense primers, PCR was conducted for 35 cycles at 94°C for 60 sec, 55°C for 60 sec and 72°C for 120 sec (Jin *et al.*, 2004; Kwon *et al.*, 2004). The PCR products were subjected to electrophoresis on a 0.8% agarose gel, with distinct bands purified by electroelution, followed by cloning into the pGEM T-easy vector (Promega, USA) and sequencing using the dideoxy termination method.

Sequence analyses

Database searches, with either DNA or protein sequences, were performed with the BLAST search program on the databases of the National Center for Biotechnology Institute NCBI) and Sanger Institute. Protein sequence alignment was performed using the AlignX program within the Vector NTI Suite (InforMax, USA).

Results and Discussion

Nucleotide and deduced amino acid sequences of CaKAR3

From the 10,000 *C. albicans* λ genomic clones screened, 3 were identified, using Southern blotting, as containing the putative *KAR3* gene. The longest insert was subcloned and completely sequenced (Fig. 2). Its deduced amino acid sequence showed that the putative *CaKAR3* gene encodes a polypeptide of 687 amino acids, with 313 residues extending from the N-terminal end of the contig6-2474 ORF. As expected, a motor protein with the motor domain located at the carboxyl terminus of the molecule was found to exist.

To further verify the authenticity of the gene, RT-PCR was performed using the primer located upstream of the N-terminal end (S1 of Fig. 2) and the internal primers, AS1, S2 and AS2, as shown in Fig. 2. Amplification with the 3 combinations of sense and antisense primers, S1/AS1, S1/AS2 and S2/AS2, gave rise to products with the expected sizes (Fig. 3), and their nucleotide sequences were in accordance with the genomic sequence (data not shown).

It was suspected that the nucleotide sequence of contig6-2474 in the *C. albicans* database might have resulted from an improper link between the fragments sequenced for the construction of the database, rather than from the existence of a truncated version of the *CaKAR3* gene. As mentioned above, no genomic PCR product was generated when a 21 bp primer preceding the initiation codon of the contig6-2474 (not included in Fig. 2, but located one residue ahead of amino acid number 314 of *CaKAR3* ORF) and AS2 were used for the PCR. Subse-

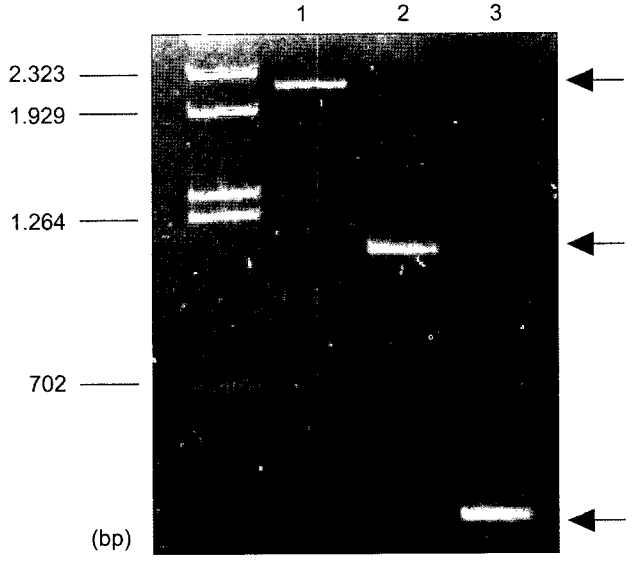

Fig. 3. RT-PCR of *CaKAR3* transcript. Amplification with 3 combinations of sense and antisense primers, S1/AS1 (lane 1), S1/AS2 (lane 2) and S2/AS2 (lane 3), gave rise to products of 2100 bp, 1100 bp and 440 bp, respectively. See Fig. 2 for their positions and expected sizes.

quently, a contig harboring the sequence encoding an N-terminal region, not included in the contig6-2474, was sought. The contig6-1523 was found to have an ORF, with 1-484 amino acid residues of Fig. 2, partially overlapping (171 residues) the *KAR3* ORF in the middle of the contig6-2474. Combining the two ORFs of contigs 6-2474 and 6-1523 resulted in the complete *KAR3* ORF of the genomic clone, as shown in Fig. 2. Accordingly, our genomic clone seemed to complement the C-terminal end of the contig6-1523 ORF. Thus, the putative *KAR3* gene of *C. albicans* was identified from genomic library screening and RT-PCR.

Homology to KAR3-related fungal kinesins

In contrast to higher mammals, the number of kinesin is rather smaller in single fungal species. The functions of

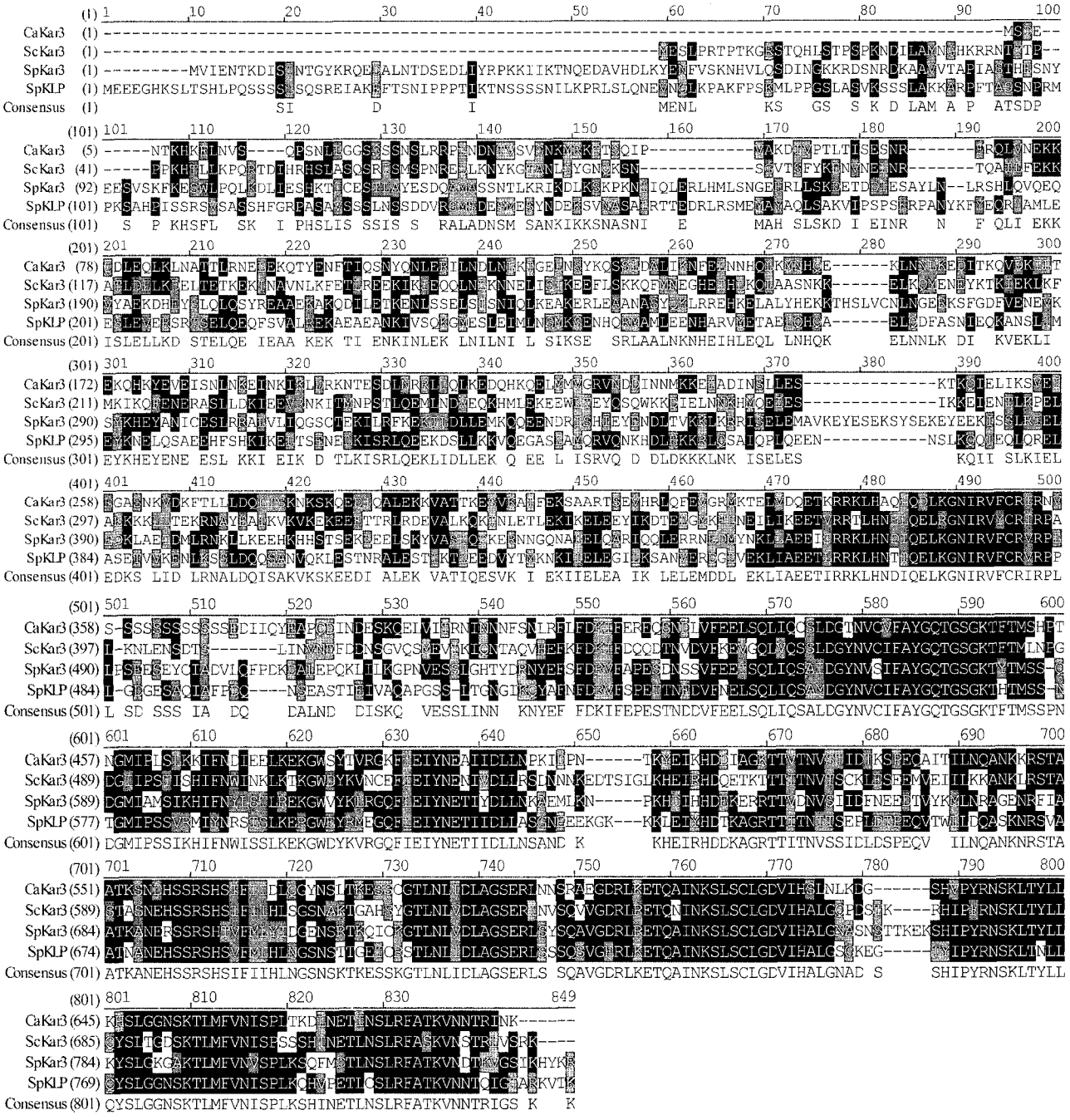

Fig. 4. Sequence comparison of *CaKAR3* with other fungal C-terminal motors. Sequence alignments of *Candida albicans KAR3* (*CaKAR3*; this study), *S. cerevisiae KAR3* (*ScKAR3*: GenBank ID; AY182242), *S. pombe KAR3* (recently renamed *SpoPkl1*; GenBank ID; U63916) and *S. pombe klp* (*Spklp*; recently renamed *SpoAC644.10;* Genbank ID; AL136235) were performed using the AlignX program within the Vector NTI suite (Infomax, Bethesda, MD). Identical amino acids are shaded in black, and similar amino acids are shaded in gray.

some fungal kinesins have been well defined by genetic analyses (Hildebradt and Hoyt, 2000; Schelmann and Fischer, 2000). A couple of fungal kinesins, which are C-terminal motors and, hence, apparently related to *CaKAR3,* have been registered in the database of the NCBI. In Fig. 4, their sequence alignment is depicted. *CaKAR3* shows a high degree of homology to *ScKAR3* and two *KAR3*-like kinesin-like proteins of the fission yeast, *Schizosaccharomyces pombe* (*Spkar3* and *Spklp* that are originally designated *pkl1*and *klp2*, respectively), mainly in the motor domain, but is somewhat divergent in the non-motor region. Compared with the most highly related *ScKAR3*, the amino terminal region of 112 amino acids was unique, with the middle part of 306 amino acids exhibiting 25% identity and 44% similarity, while the remaining C-terminal motor domain exhibited 64% identity and 78% similarity. Despite the primary structural similarity, *KAR3* or *KAR3*-like proteins of Saccharomyces species are different in their behavior and function (Bascom-Slack and Dawson, 1997; Troxelll *et al.*, 2001), which is probably attributable to the divergent amino terminal ends, and, for this reason, it is premature to predict how *CaKAR3* will perform.

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References

Bascom-Slack, C.A. and D.S. Dawson. 1997. The yeast motor protein, Kar3p, is essential for meiosis I. *J. Cell. Biol.* 139, 459-467.

Goldstein, L.S.B. and Z. Yang. 2000. Microtubule-based transport system in neurons: the roles of kinesin and dynein. *Annu. Rev. Neurosci.* 23, 39-71.

Hildebradt, E.R. and M.A. Hoyt. 2000. Mitotic motors in *Saccharomyces cerevisiae. Biochim. Biophys. Acta* 1496, 99-116.

Hirokawa, N., Y. Noda, and Y. Okada. 1998. Kinesin and dynein superfamily protein in organelle transport. *Curr. Opin. Cell Biol.* 10, 60-73.

Jin, U.H., S.H. Cho, M.G. Kim, S.D. Ha, K.S. Kim, K.H. Lee, K.Y. Kim, D.H. Chung, Y.C. Lee, and C.H. Kim. 2004. PCR method based on the ogdH gene for the detection of *Salmonella* spp. from chicken meat samples. *J. Microbiol.* 42, 216-222.

Kwon, T.W., D.K. Kim, J.S. Ye, W.J. Lee, M.S. Moon, C.H. Joo, H. Lee, and Y.K. Kim. 2004. Detection of Enterovirus, cytomegalovirus, and *Chlamydia pneumoniae* in Atheromas. *J. Microbiol.* 42, 299-304.

Kosik, K.S., L.D. Orecchio, B. Schnapp, H. Inouye, and R.L. Neve. 1990. The primary structure and analysis of the squid kinesin heavy chain. *J. Biol. Chem.* 265, 3278-3283.

Nakagawa, T., Y. Tanaka, E. Matsuoka, S. Kondo, Y. Okada, Y. Noda, Y. Kanai, and N. Hirokawa. 1997. Identification and classification of 16 new kinesin superfamily (KIF) proteins in mouse genome. *Proc. Natl. Acad. Sci. USA* 94, 9654-9659.

Sambrook, J. and D.W. Russel. 2001. *Moleclar Cloning: A Laborary Manual.* Cold Spring Harbor Laboratory Press, New York, New York.

Schelmann, R. and R. Fischer. 2000. Nuclear migration in fungi-different motors at work. *Res. Microbiol.* 151, 247-254.

Troxell, C.L., M.A. Sweezy, R.R. West, K.D. Reed, B.D. Carson, A.L. Pidoux, W.Z. Cande, and J.R. McIntosh. 2001. pkl1(+)and KLP2(+): Two kinesins of the Kar3 subfamily in fission yeast perform different functions in both mitosis and meiosis. *Mol. Biol. Cell* 11, 3476-3488.

Verhey, K.J. and T.A. Rapoport. 2001. Kinesin carries the signal. *Trends Biochem. Sci.* 26, 545-549.