Sequences of Sea Urchin Kinesin Light Chain Isoforms

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We have deduced the amino acid sequences of four sea urchin (Strongylocentrotus purpuratus; SP) kinesin light chain (KLC) isoforms (SPKLC 1-4) and compared them to rat brain light chain sequences. Examination of the SPKLC open reading frames (SPKLC1, 649; SPKLC2, 677; SPKLC3, 686; and SPKLC4, 451 amino acid residues) reveals that the first 500 or so residues of the KLCs are highly conserved but the C-terminal ends of rat and sea urchin light chains are divergent; SPKLCs 1, 2 and 3 share a highly basic, 86 residue C-terminal segment that is missing from the shorter rat light chains and SPKLC4. The insertion of 28 and 37 residue segments at residue 563 of SPKLCs 2 and 3, respectively, gives rise to sequence heterogeneity at the C-terminal ends of the sea urchin KLCs. C-terminal sequence differences between light chains may provide inter- and intraspecies differences in the functional properties of the presumptive cargo attachment elements of kinesin.

Keywords: kinesin; light chain sequences; organelle transport

Kinesin is a mechanochemical ATPase that transports its cargo towards the fast polymerizing ends of microtubules (Vale et al., 1985a,b; Brady, 1985; Scholey et al., 1985; Kuznetsov & Gelfand, 1986), and thus it is thought to drive the centrifugal intracellular transport of various types of membrane-bound organelles to which it binds (e.g. Pfister et al., 1989; Hollenbeck, 1989; Wright et al., 1991; Hirokawa et al., 1991; Leopold et al., 1992; Henson et al., 1992).

The purified kinesin molecule is reported to consist of a pair of heavy chains (KHC) together with equimolar amounts of copurifying light chains (KLCs; Kuznetsov et al., 1988; Bloom et al., 1988; Johnson et al., 1990). However, the enzyme can be purified in a light chain-depleted state (Hackney et al., 1991). Molecular analysis (Hirokawa et al., 1989; Yang et al., 1989; Scholey et al., 1989) reveals that the two KHCs are arranged in parallel with their N-terminal ends forming globular motor domains linked to a cargo-attachment element consisting of central alpha-helical regions that dimerize by coiled-coil formation (De Cuestas et al., 1992) and globular C-terminal regions that bind specifically to membranes in vitro (D. A. Skoufias & J. M. Scholey, unpublished results). The functions of the KLCs are unknown, although immunoelectron microscopy suggests that they are located at the C-terminal ends of the KHCs (Hirokawa et al., 1989) where they may regulate mechanochemical activity (Hackney et al., 1991) or attachment of kinesin to different membrane-bound organelles.

The amino acid sequences of KHCs have been deduced from cDNAs cloned from Drosophila (Yang et al., 1989), squid (Kosik et al., 1990), sea urchin (Wright et al., 1991) and man (Navone et al., 1992); sequence comparisons reveal a high level of conservation throughout the length of these KHCs. So far the only published KLC sequence is from rat brain (Cyr et al., 1991), where three isoforms differing at their C-terminal ends are thought to be produced by alternative splicing of a single gene. Here, we report the analysis of the sequences of four sea urchin KLC isoforms.

By using polymerase chain reaction and plaque hybridization to screen an unfertilized Strongylocentrotus purpuratus (SP) egg cDNA library, we obtained and sequenced cDNAs span-
Figure 1. Maps of the open reading frames (ORFs) that encode the 4 sea urchin kinesin light chains (SPKLCs). ORFs 1, 2 and 3 correspond to SPKLC1, 2 and 3, respectively. These isoforms share a 686 residue core sequence with predicted pl 6.3 (open boxes indicated by broken line below ORFs) but diverge at their basic C termini. The open box on the right of each ORF (e.g. residues 563 to 649 on ORF1) is an 86 residue, basic (pl 11.2) extension shared by SPKLC1, 2 and 3; the hatched box shows a 28 residue insert (pl 11.2) present in SPKLC2 (residues 563 to 591) and in SPKLC3, which also contains a 9 residue insert with predicted pl 11.7 (black box). The core sequence is presented in Fig. 2, whereas the sequences presented in this Figure (below ORF3 and above ORF 4) represent the sequences of the extra amino acid residues in the extension and insertions as indicated by the boxes. ORF4 is a 451 residue isoform that corresponds to residues 1 to 440 of the core sequence plus 11 residues that differ (stippled box plus corresponding sequence). The deduced number of residues and molecular mass (M,) for each isoform are also shown. DNA from a lambda ZAP S. purpuratus (SP) unfertilized egg cDNA library was used as template for polymerase chain reaction, with pBluescript universal sense primer and the degenerate oligonucleotide KLC antisense primer based on cow and rat sequences (SPKLC1 residues 251 to 259, HDHPDVATM). The amplified product was used to probe the cDNA library, yielding the short cDNA that encodes SPKLC4. Two 17mer sense primers (residues 254 to 259 and 338 to 363) were used with pBluescript universal antisense primers in nested polymerase chain reaction to amplify cDNAs corresponding to the C-terminal KLC variants. Subcloning, restriction mapping and sequencing were done using routine procedures (Wright et al., 1991).

The coding regions of the four KLC isoforms are shown in Figures 1 to 3; the sequences of the SPKLCs are available from Genbank/EMBL under accession numbers L08258, L10233-35. SPKLCs 1, 2 and 3 share an identical “core” sequence (residues 1 to 563, predicted pl 6.3) that displays a strikingly high level of conservation with the rat brain KLCs (white blocks in Fig. 1; see best fit Fig. 2 and dot plots in Fig. 3); SPKLC4 is shorter than the other isoforms, but displays absolute identity to the first 440 residues of core sequence. As with rat KLCs (Cyr et al., 1991), the sea urchin sequences predict an N-terminal region rich in helix-forming residues and heptad repeats predicted to form coiled-coils (Fig. 4(a)). In both sequences, this is followed by a stretch of imperfect tandem repeats (Figs 3 and 4(b)), which are also predicted to form a helix containing a potential coiled-coil region (Fig. 4(a)).

Beyond the conserved sequences, the sea urchin KLCs have basic extensions not found in the rat KLCs (Fig. 1). SPKLC1, 2 and 3 share an 86 residue C-terminal segment that is separated from the core by 28 and 37 residue inserts to produce SPKLC2 and SPKLC3, respectively (Fig. 1). The additional nine residue insert in LC3 and the first five residues of the 28 residue insert are similar to inserts present in rat KLCs (Cyr et al., 1991) but otherwise the rat and sea urchin KLC C termini are divergent (Figs 1 and 3). The short SPKLC4 isoform has a unique 11 residue extension at position 441-451 (Fig. 1). The basic nature of the C-terminal domains of SPKLCs may be functionally important, for example in modulating the electrostatic binding of the basic KHC tail to negatively charged sites on membranes (D. A. Skoufias & J. M. Scholey, unpublished result).

Inter- and intraspecies differences in the primary structure of the C-terminal regions are thus a feature of KLCs (Cyr et al., 1991; this work), and additional heterogeneity in sea urchin KLCs is generated by phosphorylation (Buster et al., 1990). To further illuminate the nature of KLC heterogeneity, it will be important to learn exactly how sequence differences and post-translational modifi-
Figure 2. Best fit comparison of the sea urchin egg KLC core sequences (1 to 568; upper) with the rat brain KLC, LC-A (lower). Vertical lines show identities, conservative substitutions are indicated by dots, and gaps are represented by horizontal dotted lines. The sequences are 72% identical and 83% similar.

Figure 3. Dot matrix analyses of SPKLC isoforms (horizontal) versus rat KLCs (vertical). 1. SPKLC1 versus rat LC-A; 2. SPKLC2 versus rat LC-B; 3. SPKLC3 versus rat LC-C; 4. SPKLC4 versus rat LC-A. Window size 15, stringency 10. The sequences are conserved throughout most of their length. Four imperfect 42 residue tandem repeats (240 to 281, 282 to 323, 324 to 365 and 366 to 407) plus a 5th, shorter imperfect repeat (469 to 494) that is missing from the short isoform, SPKLC4, are revealed by the multiple off-axis diagonal lines.

Figure 4. (a) Predicted coiled-coil regions of SPKLC1. Residue numbers are shown on the horizontal axis; the vertical axis shows the probability of formation of an α-helical coiled-coil calculated by the method of Lupas et al. (1991). Two regions are predicted: an N-terminal region (residues 20 to 160), which may be involved in binding to the heavy chain, and a C-terminal region (342 to 395), which has not been described previously. The same regions of coiled-coil are predicted for all 4 isoforms. A very similar pattern is seen in the kinesin light chains from rat brain (not shown). Note that the C-terminal region falls in the 4th tandem repeat, but the algorithm does not predict coiled-coil in the other repeats. (b) Examination of an alignment of the tandem repeats shows that there is a conserved pattern of hydrophobic and charged residues between all the repeats, but suggests that the heptad pattern may be stronger in the 4th repeat than in the others. Letters a to g represent the 7 positions of the heptad repeat; the positions that are usually occupied by hydrophobic residues, a and d, are underscored. The 4th repeat contains 2 proline residues, one of which is conserved in all the other repeats. This would most likely result in a "kink" in the axis of the helix.

Lactations give rise to the multiple sea urchin KLC polypeptides (M, 75,000 to 85,000 and 50,000) identified by immunoadsorption and blot overlay methods (Johnson et al., 1990; D. Buster & J. M. Scholey, unpublished results; A. K. Gauger & L. S. B. Goldstein, personal communication) and also to determine if the KLC variants are expressed in a cell-type or temporally regulated fashion.

What is the functional significance of KLC heterogeneity? It is easy to imagine that KLCs that differ in their primary structures or state of phosphorylation may differentially modulate the process of mechanochemical coupling by KHCs. This might give rise to multiple kinesins with different motor activities (Hackney et al., 1991), each being adapted to perform specific intracellular transport functions. KLC epitopes are associated with the tails of KHCs, suggesting that light chain variants may play
important roles in the binding of kinesin to different types of membrane-bounded cargo. For example, KHC has been immunolocalized to membranes of the mitotic apparatus in dividing cells of the early sea urchin embryo (Wright et al., 1991) and to endoplasmic reticulum membranes and endosomes (but not mitochondria, Golgi membranes or lysosomes) in terminally differentiated cultured coelomocytes (Henson et al., 1992). Therefore, an appealing hypothesis is that SPKLCs that differ in primary structure and/or by post-translational modification, may play a role in targeting the sea urchin KHC to these different classes of membrane-bounded organelle.

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Note added in proof. Gauger, A. K. & Goldstein, L. S. B. (J. Biol. Chem. in the press) have obtained evidence that the heptad repeat region (SPKLC1 residues 20 to 160) of a Drosophila KLC, binds within the KHC "stalk-tail" region.