Hypothesis

Dynactins p25 and p27 are predicted to adopt the LβH fold

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Abstract Dynactin is a multimeric protein essential for the minus-end-directed transport driven by microtubule-based motor dynein. The pointed-end subcomplex in dynactin contains p62, p27, p25, and Arp11 subunits, and is thought to participate in interactions with membranous cargoes. We used sequence and structure prediction analysis to study dynactins p25 and p27. Here we present evidence that strongly supports that dynactins p27 and p25 contain the isoleucine-patch motif and adopt the left-handed parallel β-helix fold. The structural models we obtained could contribute to the understanding of the complex interactions that dynactins are able to establish with cargo particles, microtubules or other dynactin subunits.

Key words: Dynactin; Structural model; Left-handed parallel β-helix

Membranous organelles of eukaryotic cells travel through the cytoplasm by the action of microtubule-based motor proteins. There are two groups of motor proteins which achieve opposite-polarity movements. Motors like kinesin and kinesin-like proteins account for the microtubule plus-end-directed movement [1]. The opposite movement towards the minus end of microtubules is driven by the complex dynein/dynactin molecular motors. The cargoes that these molecular motors could transport are as diverse as the Golgi apparatus, endoplasmic reticulum–Golgi vesicles, nuclei, mRNA particles, mitochondria, lipid droplets and viral capsids [2,3].

Dynactin was first described as an activator of the dynein-based vesicle movement in vitro [4,5] but now is believed to be required for the normal function of dynein [6]. Dynactin is a multimeric protein with a highly asymmetric structure [7,8]. This complexity could explain its multiplicity of functions. On the one hand, dynactin is the protein proposed to bind to the diverse membranous cargoes mentioned above. Also, it contains binding sites both for microtubules and for dynein which itself interacts with the microtubules [9–11].

One of the subcomplexes of dynactin is the pointed-end complex, which contains p62, p27, p25, and Arp11 proteins, and is thought to participate in interactions with membranous cargoes [8]. Here we present evidence that strongly supports that dynactins p27 and p25 contain the isoleucine-patch motif [12] and adopt the left-handed parallel β-helix fold (LβH) [13].

The isoleucine-patch or hexapeptide-repeat motif is described as [LIV]-[GAED]-X_2-[STAV]-X and is usually found as an imperfect tandem repetition along the protein sequence. This motif is found in a large and diverse superfamily [14], where most of the proteins are bacterial transferases. All proteins that display the hexapeptide-repeat motif whose structures have been determined adopt a LβH [13,15–19]. The six positions in the hexapeptide occupy different structural environments. If sites are identified with letters *i*, *i*+1,...,*i*+5, it is found that sites *i* and *i*+4 point towards the inside of the fold. The evolutionary divergence of these positions is structurally constrained, so that, when several LβH proteins are compared, the sites *i* and *i*+4 are the only truly conserved sites characterizing the hexapeptide motif [20].

To explore the distribution of LβH proteins we performed sequence similarity searches using well characterized LβH representatives (PDB ID, 1lxa (UDP *N*-acetylglucosamine acyltransferase), 1thj (γ-carbonic anhydrase), 1tdt (tetrahydrodipicolinate-*N*-succinyltransferase), 1hm9 (*N*-acetylglucosamine-1-phosphate-uridyltransferase and UDP *N*-acetylglucosamine pyrophosphorylase). We used PSI-BLAST [21] to search the non-redundant database of NCBI. Using an inclusion cutoff of 0.002, after convergence we found, on average over different runs, 1462 putative homologous sequences containing the hexapeptide-repeat motif. Among the retrieved proteins we found dynactin p25 and dynactin p27 with *E* values in the range of 10^{-7} to 10^{-4} which indicates a statistically significant sequence similarity.

To assess whether the dynactins found are actually LβH proteins we performed a series of sequence analyses. First, we performed reciprocal BLASTP searches using dynactin p25 and p27 sequences from diverse organisms as input. These searches detected LβH proteins with *E* values as low as 10^{-8}. Second, we aligned the dynactin sequences using CLUSTALW [22]. This showed that p25 and p27 have an average identity of 15% and average similarity of 30% (calculated using the Blosum 62 matrix). The presence of the hexapeptide motif is evident from inspection of Fig. 1, which shows the alignment of a group of p25 and p27 proteins. Based on the amino acid substitution patterns of different hexapeptide sites observed in LβH proteins [20,23], we selectively marked putative *i* and *i*+4 positions revealing the repetition pattern typically found in LβH proteins. Third, for a more quantitative test, we inspected the amino acid distributions of different hexapeptide positions. In Fig. 2 we show the amino acid distributions of hexapeptide sites *i* and *i*+4 of several well characterized LβH proteins, together with the distributions obtained for p25 and p27 dynactins. Clearly, these dynactins present the hexapeptide-repeat motif.

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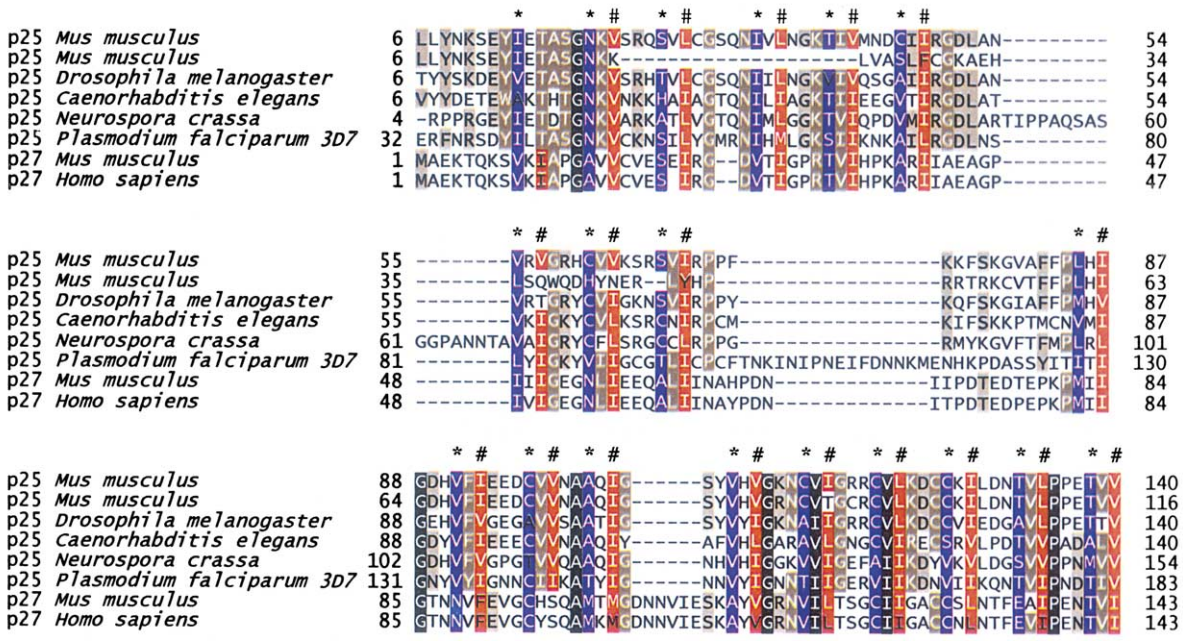

Fig. 1. Sequence alignment of representatives of dynactins p25 and p27. Putative *i* positions are marked with the symbol #, while * indicates *i*+4. Other conserved positions are shown with three levels of gray for 100%, 80%, and 60% of conservation. NCBI accession numbers of the sequences displayed in the alignment are: *Mus musculus* 11037798 and 25022593, *Drosophila melanogaster* 24584372, *Caenorhabditis elegans* 17510481, *Neurospora crassa* 28918512, *Plasmodium falciparum* 23612605, *Homo sapiens* 5730116, *Mus musculus* 6176552.

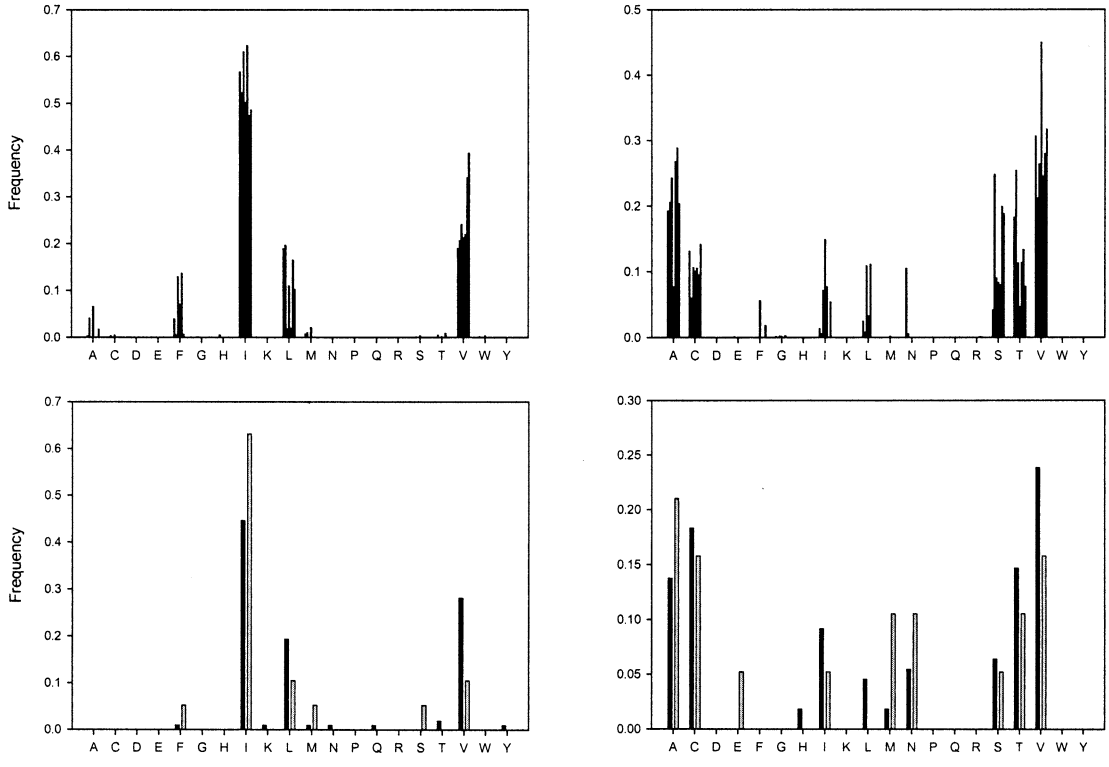

Fig. 2. Amino acid distributions of classes *i* and *i*+4 of well characterized LβH proteins (top) and p25 (black) and p27 (gray) dynactins (bottom). LβH proteins used in the calculations were (PDB ID): 1lxa (UDP *N*-acetylglucosamine acyltransferase), 1kk6 (streptogramin A acetyltransferase), 1kru (galactoside *O*-acetyltransferase), 1xat (xenobiotic acetyltransferase), 1tdt (tetrahydrodipicolinate *N*-succinyltransferase), 1qre (γ-carbonic anhydrase) and 1g97 (*N*-acetylglucosamine-1-phosphate-uridyltransferase and UDP-*N*-acetylglucosamine pyrophosphorylase). Capital letters indicate one-letter amino acid code.

a

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24584372_PSS  CCCCCCCCCC CEEE..EECC CCEECCCCEE ....EECCCE EECC......
24584372_Seq  MEIPDTYYSK DEYV..ETAS GNKVSRHTVL ....CGSQNI ILNG......
  1lxa___Seq  ....MIDKSA FVHPTAIVEE GASIGANAHI GPFCIVGPHV EIGEGTVLKS
  1lxa___SS   ....CCCCCC EECCCCEECC CCEECCCCEE CCCCEECCCE EECCCCEECC

24584372_PSS  .......... ..CCEECCCC EE........ .ECCCCEEEE CCCCEECCCC
24584372_Seq  .......... ..KVIVQSGA II........ .RGDLANVRT GRYCVIGKNS
  1lxa___Seq  HVVVNGHTKI GRDNEIYQFA SIGEVNQDLK YAGEPTRVEI GDRNRIRESV
  1lxa___SS   CCEECCEEEE CCCCEECCCC EECCCCCCCC CCCCCCEEEE CCCCEECCCC

24584372_PSS  EECCCCCCCC CCCCCCCEEE CCCCEECCCC EE.CCEEECC CCEECCCCEE
24584372_Seq  VIRPPYKQFS KGIAFFPMHV GEHVFVGEGA VV.SAATIGS YVYIGKNAII
  1lxa___Seq  TIHRGTVQG. ....GGLTKV GSDNLLMINA HIAHDCTVGN RCILANNATL
  1lxa___SS   EECCCCCCC. ....CCEEEE CCCCEECCCC EECCCCEECC CCEECCCCEE

24584372_PSS  CCCCEECCCC EECCCCEECC CCEE
24584372_Seq  GRRCVLKDCC VIEDGAVLPP ETTV
  1lxa___Seq  AGHVSVDDFA IIGGMTAVHQ FCII
  1lxa___SS   CCCCEECCCC EECCCEEECC CCEE


 6176552_PSS  CCCCCCCCCE ECCCCEECCC CEECCCCEEC CCCEECCCCE EEECCCCEEE
 6176552_Seq  MAEKTQKSVK IAPGAVVCVE SEIRGDVTIG PRTVIHPKAR IIAEAGPIII
  1lxa___Seq  ...MIDKSAF VHPTAIVEEG ASIGANAHIG PFCIVGPH.. .VEIGEGTVL
  1lxa___SS   ...CCCCCCE ECCCCEECCC CEECCCCEEC CCCEECCC.. .EEECCCCEE

 6176552_PSS  CCCCEECCCC EEC..CCCCC CCCCCCCCCC ....CCE..E EECCCCEEEC
 6176552_Seq  GEGNLIEEQA LII..NAHPD NIIPDTEDTE ....PKP..M IIGTNNVFEV
  1lxa___Seq  KSHVVVNGHT KIGRDNEIYQ FASIGEVNQD LKYAGEPTRV EIGDRNRIRE
  1lxa___SS   CCCCEECCEE EECCCCEECC CCEECCCCCC CCCCCCCCEE EECCCCEECC

 6176552_PSS  CCEEC..... ..C.EEECCC CEECCCCEEC CCCEECCCCE EECCCEECCC
 6176552_Seq  GCHSQ..... ..A.MTMGDN NVIESKAYVG RNVILTSGCI IGACCSLNTF
  1lxa___Seq  SVTIHRGTVQ GGGLTKVGSD NLLMINAHIA HDCTVGNRCI LANNATLAGH
  1lxa___SS   CCEECCCCCC CCCEEEECCC CEECCCCEEC CCCEECCCCE ECCCCEECCC

 6176552_PSS  CEECCCCEE
 6176552_Seq  EAIPENTVI
  1lxa___Seq  VSVDDFAII
  1lxa___SS   CEECCCCEE
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b

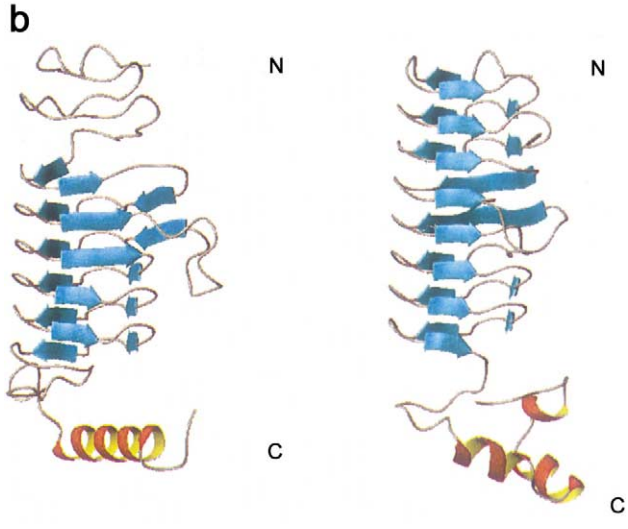

Fig. 3. a: Alignment of p27 from *M. musculus* (6176552) and p25 from *D. melanogaster* (24584372) with the structural template 1lxa (UDP *N*-acetylglucosamine acyltransferase). For each alignment the predicted secondary structure (PSS) using PSIPRED [25] and the secondary structure of the structural template (SS) is shown. Tandem occurrence of short predicted strands in p25 and p27 agrees with the presence of the LβH fold. In general these strands match with those in the template 1lxa. H means helix, E strand and C coil. b: Models for dynactins p25 of *D. melanogaster* (left) and p27 of *M. musculus* (right) obtained using Modeller [27] with 1lxa as template. Note the left-handed parallel β-helix, formed by several coils (five in p25 and eight in p27) wound to form a triangular prism. Generally, each coil contains three hexapeptide units. The tandem repetition of the hexapeptide motif is more conserved in the carboxy-terminus of the predicted LβH of p25 and p27 (C) than in the amino-terminus of the same domain (N). This feature is more evident in p25 than in p27.

To further the assessment, we performed a series of structural examinations. First, the results of secondary structure prediction performed on p25 and p27 with SOPM [24] and PSIPRED [25] show a tandem repetition of β-strands, interrupted only by one or two short α-helices, supporting the presence of the LβH fold in these proteins (Fig. 3a). Second, we submitted p25 and p27 sequences to the 3D-PSSM server for fold recognition by threading [26]. All the sequences submitted recognized an LβH representative fold with high statistical support. Third, we modeled the p25 of *Drosophila melanogaster* and p27 of *Mus musculus*. In the previous fold recognition test both of them selected the LβH fold of the enzyme UDP-*N*-acetylglucosamine acyltransferase (PDB ID: 1lxa) with significant *E* values (4.33×10^{-7} for p25 and 3.54×10^{-8} for p27). Thus, using 1lxa as a template and the alignment produced by the threading program, we built models for p25 and p27 using Modeller [27] in TITO server [28]. These models are shown in Fig. 3b. Using an evaluation criterion [29] based on ProsaII *Z* score [30], we found that both models are reliable.

To summarize, we have performed sequence and structural analyses that show that dynactins p25 and p27 contain the hexapeptide-repeat motif and strongly support that they adopt the LβH. Although all the LβH proteins crystallized so far show homotrimeric quaternary structures, it has been shown that p25 and p27 show a ratio of 1:1 in the dynactin complex and that they copurify [8]. This latter finding suggests a strong association between them to form a heterodimer. However, we have recently characterized three LβH proteins from *Arabidopsis thaliana* with putative carbonic anhydrase activity (Parisi et al., submitted) showing a stronger tendency to form heterodimers than homodimers (Perales et al., unpublished results). It is important to note that, as in the case of p25 and p27, *Arabidopsis* proteins are part of a multimeric protein complex, the mitochondrial complex I [31,32]. We can speculate that LβH proteins associated in multimeric assemblies could show slightly different structural and sequence patterns compared with homotrimeric LβH proteins.

We expect that the structural models of the two dynactin subunits reported here could contribute to the understanding of the complex interactions that dynactins are able to establish with cargo particles or other dynactin subunits.

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