

head isoforms are encoded by distinct messenger RNA species differing in their 3' ends (Fig. 3). These experiments also revealed yet another transcript of 3.6 kilobases (kb) not detected in the head, and enriched in the adult body and embryo.

Results of genomic Southern blot and polytene chromosome hybridization were consistent with the existence of a single gene encoding the dynamin-related polypeptides, located within the 13F-14A region of the X chromosome (data not shown). Immunoblots of heads obtained from flies carrying duplications or deficiencies within the 13F-14A interval further localized the dynamin gene to the 14A region (data not shown) in which the *shibire* gene is known to be located^{12,13}. Temperature-sensitive *shibire* mutations cause reversible paralysis and defects in the endocytic pathway in neurons and other cell types^{9,10}. Dynamin is enriched in neuronal tissue (Fig. 1)¹⁷, and the dynamin-related yeast gene, *VPS1*, is itself involved in membrane sorting⁵, suggesting that dynamin could be the product of the *shibire* gene. The *shibire* locus has been cloned by chromosomal walking through the 14A region and the *shibire* gene identified within the cloned DNA segments by mapping the positions of chromosomal breakpoints known to disrupt *shibire* function (*In(1)shi*¹⁶, termed *In(1)shi*⁸⁻¹⁸ in reference 14, and *T(1;3)shi*¹⁹)^{13,14} (T. W. Austin, S. Park and C. A. Poodry, manuscript in preparation). A 2.3 kb partial embryonic cDNA clone (*shi-1*) was isolated by hybridization with the genomic DNA segment spanning the breakpoints noted above. Sequences within the *shi-1* cDNA were found to span the *In(1)shi*¹⁶ and *T(1;3)shi*¹⁹ breakpoints (Fig. 4a and data not shown). Results of two types of experiments indicate that the dynamin proteins are encoded by the *shibire* gene. First, we find that flies heterozygous for the *In(1)shi*¹⁶ mutation express about half of the wild-type amount of dynamin protein (Fig. 4b). Second, the deduced amino-acid sequence of the *shi-1* cDNA (Fig. 4c) exactly matches that of the Ddyn3 open reading frame from amino-acid position 461 to the terminator codon with two exceptions; the substitution of a lysine in the *shibire* sequence for Arg 598 in the Ddyn4 sequence, and the absence of six amino acids in the *shibire* sequence corresponding to amino acids 635-640 in the Ddyn4 sequence. The latter observation suggests that the *shi-1* embryonic cDNA represents yet another alternatively spliced form of mRNA from the dynamin/*shibire* gene.

Our results show that the *shibire* gene encodes the *Drosophila* equivalent of dynamin indicating a role for this protein in endocytosis. The paralytic phenotype is thought to result from a failure in synaptic vesicle reformation, reflected in a decrease in synaptic vesicles at the neuromuscular junction and an increase in coated or 'collared' pits^{9,10}. Aberrant membranous structures were also seen within the cytoplasm, but defects in later stages of the endocytic pathway were not specifically investigated. Microtubules have a role in translocation of endocytic vesicles from the periphery of the cell toward the centre^{15,16}, and some aspects of the *shibire* phenotype may reflect disruption of this process. As microtubules are not known to play a direct part in synaptic vesicle recycling, the effects at the synaptic terminal may reflect a backup of intermediates involved in the later stages of endocytosis. Alternatively, the few microtubules in the synaptic region may be more important for membrane budding than previously appreciated. A third possibility is that dynamin is multifunctional. In this regard, the dual membrane sorting and meiotic spindle pole separation defects observed in yeast *VPS1/SPO15* mutants are relevant. Hopefully, as a result of the findings of this study, the full range of dynamin functions will now be accessible to exploration *in vivo*. □

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Large differences in the helix propensities of alanine and glycine

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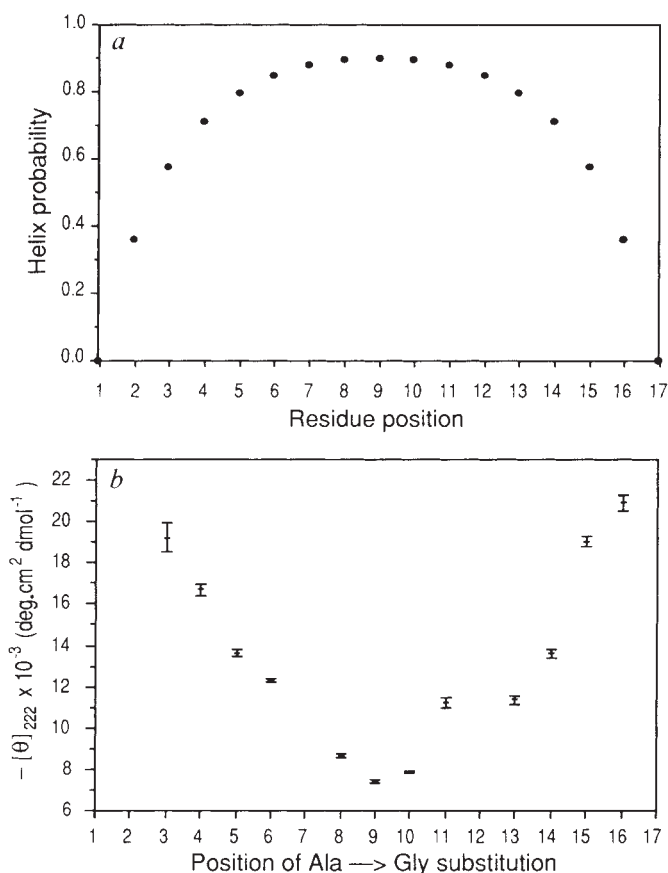
THE standard view of α helix formation in water, based on helix propensities determined by the host-guest method^{1,2}, is that differences in helix propensity among the amino acids are small, except for proline³, and that the average value of the helix propagation parameter s is near 1. A contradictory view of α helix formation in water is emerging from substitution experiments with short, unique-sequence peptides that contain only naturally occurring amino acids⁴⁻⁹. Short peptides that contain only alanine and lysine, or alanine and glutamate, form surprisingly stable monomeric helices in water⁹ and substitution of a single alanine residue by another amino acid in these or related peptides produces a wide range of changes in helix content, depending on which amino acid is substituted for alanine^{4-6,8}. We show here that the ratio of the helix propensities of alanine to glycine is large, about 100, in substitution experiments with a 17-residue reference peptide containing alanine and lysine. The helix propensity is identified with s , the helix propagation parameter of the statistical mechanics model for α helix formation, and the results are interpreted by the Lifson-Roig theory¹⁰. Single alanine \rightarrow glycine substitutions have been made at a series of positions in individual peptides. The helix-destabilizing effect of an Ala \rightarrow Gly substitution depends strongly on its position in the helix, as predicted by the Lifson-Roig theory if the ratio of s values for Ala:Gly is large.

Amino-acid substitution experiments, in which changes in α helix formation by short peptides are measured, yield nearly the same rank order of helix propensities for different types of reference peptides used^{4-6,8}, but a different rank order from that obtained by host-guest experiments^{1,2}. The change in helix content for a substitution (Ala \rightarrow X) nevertheless depends markedly on the choice of reference peptide in short-peptide experiments. Therefore, it is important to determine quantitative helix propensities by determining values of s , the helix propagation parameter of the Zimm-Bragg model¹¹, and to find out if the values of s vary with the choice of reference peptide. It has

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FIG. 1 *a*, Fractional helicity at each residue in the reference peptide computed from Lifson-Roig theory¹⁰. The computation employed an average s value of 1.56 and a σ value of 0.0029. The average value of s was derived by fitting the experimental helix content of the reference peptide, using the value of σ determined by J. M. Scholtz *et al.* (manuscript in preparation). The ordinate gives the computed helicity at a specified residue position. The Lifson-Roig¹⁰ theory of the α helix-random coil transition gives the partition function directly as a matrix product using the formula $Z = v_r \cdot \Pi M_j \cdot v_i$, where ΠM_j is an ordered sequence of Lifson-Roig matrices M_j set up to correspond with the amino-acid sequence; v_i and v_r are the usual end vectors¹⁰. The matrices contain growth and nucleation parameters w and v^2 which correspond to the s and σ of the Zimm-Bragg theory¹¹; s and σ are computed from w and v^2 by the relations $s = w/(1 + v)$ and $\sigma = v^2/(1 + v)^4$ (H. Qian and J.A.S., manuscript in preparation). Individual values of w and v^2 may be assigned for each amino acid in the sequence. J. M. Scholtz *et al.* (manuscript in preparation) have tested the applicability of the theory by measuring thermal unfolding curves for peptides of varying chain length (6–51 residues) that contain repeating sequences (AAKAA or AEAAKA in one-letter amino-acid code). A tentative value for the helix nucleation parameter, $v^2 = 0.0037$ or $\sigma = 0.0029$, has been obtained in these experiments. Once the partition function has been set up, the helical probability for the j th amino acid in the sequence can be obtained by differentiating $\ln Z$ with respect to $\ln w_j$, where w_j is the growth parameter for the j th amino acid in the sequence. Summing over all residues gives the mean number of helical units per chain, from which the fractional helicity can be calculated. For discussion of the molecular nature of s and σ , see for example ref. 18, and for a discussion of helix-coil transition theory, see ref. 19. *b*, α -Helical contents of the glycine-substituted peptides. The data points show the mean and the standard deviation ($n = 5$). The experimental conditions are listed in Table 1.



been observed⁹ that the helix contents of alanine peptides, solubilized by insertion of a few lysine residues, are too large to be explained by the s values found using the host-guest method^{1,2}. A possible explanation has been given⁹, based on the special helix-forming properties¹² of the host residue (hydroxybutyl- or hydroxypropyl-L-glutamine), in host-guest experiments. The Lifson-Roig theory¹⁰ is used to interpret our results because it combines simplicity with an accurate representation of α helix cooperativity, and because it is well suited to the calculation of helicity at each residue position. The correct representation of cooperativity is particularly important in short helices because end effects are prominent. The results have, however, been converted to the more familiar s and σ parameters of the Zimm-Bragg theory. For details of the calculations by the Lifson-Roig theory, see the legend to Fig. 1.

The rationale of our experiments is as follows. Using $\sigma = 0.0029$, one finds that the end residues of short, partially helical

helices should be strongly frayed; see Fig. 1*a*. Consequently, the effect of substituting a helix-destabilizing residue will be larger at the centre of the helix than near either end. Consider the substitution Ala \rightarrow Gly, because substitution experiments⁴⁻⁸ show that Ala is one of the best helix-forming residues and Gly is one of the most helix-destabilizing residues. If the ratio $s(\text{Ala}) : s(\text{Gly})$ is large, the position-dependent effect of an Ala \rightarrow Gly substitution will also be large, according to prediction based on the Lifson-Roig theory. Thus, a series of single Ala \rightarrow Gly substitutions was made at different residue positions in individual peptides, using the same reference peptide (sequences are given in Table 1), and the change in helix content was measured for each substitution. The peptides contain tyrosine as the N-terminal residue in order to determine peptide concentration accurately from tyrosine absorbance⁹. The α -NH₂ and α -COOH groups are blocked to avoid unfavourable charge interactions with the helix dipole¹³.

FIG. 2 Experimental and computed fractional helix contents. See Table 1 for conditions. The data points and the curves represent experimental and computed values, respectively. Experimental fractional helix contents were determined from $[\theta]_{222}$ measurements using $-40,000(1 - 2.5/n)$ and $0 \text{ deg cm}^2 \text{ dmol}^{-1}$ as the values for 100 and 0% helix, respectively; n is the number of amino-acid residues in the peptide²⁰, which has an additional peptide group at each end because of the acetyl and amide blocking groups. The curves were computed from the Lifson-Roig equation using an average s value for Ala, Lys, and Tyr of 1.56, $s(\text{Gly}) = 0.015, 0.08, 0.20,$ and 0.50 , and $\sigma = 0.0029$.

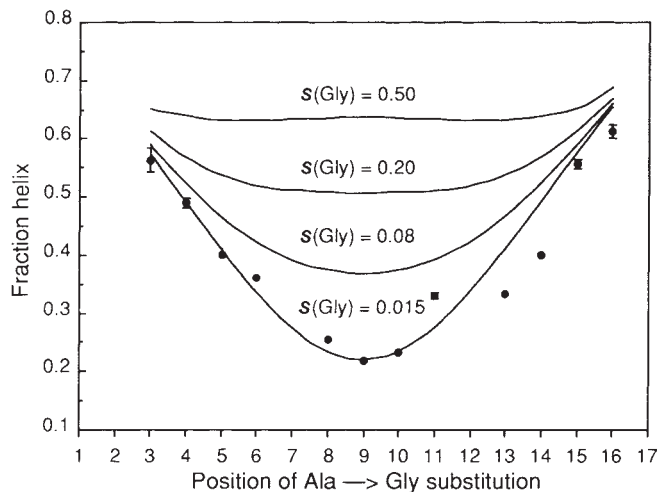


TABLE 1 Sequences and helix contents of the reference and glycine-substituted peptides

Name	Sequence	$[\theta]_{222}^*$ (deg cm ² dmol ⁻¹)
Reference	CH ₃ CONH-YKAAAAKAAAAKAAAAK-CONH ₂	-25,000 ± 300
Gly 3	CH ₃ CONH-YKGAAGAAAAKAAAAK-CONH ₂	-19,200 ± 700
Gly 4	CH ₃ CONH-YKAGAAKAAAAKAAAAK-CONH ₂	-16,700 ± 300
Gly 5	CH ₃ CONH-YKAAAGKAAAAKAAAAK-CONH ₂	-13,600 ± 200
Gly 6	CH ₃ CONH-YKAAAGKAAAAKAAAAK-CONH ₂	-12,300 ± 100
Gly 8	CH ₃ CONH-YKAAAAKGAAGAAAAK-CONH ₂	-8,700 ± 100
Gly 9	CH ₃ CONH-YKAAAAKAGAAKAAAAK-CONH ₂	-7,400 ± 100
Gly 10	CH ₃ CONH-YKAAAAKAGAAKAAAAK-CONH ₂	-7,800 ± 100
Gly 11	CH ₃ CONH-YKAAAAKAAAGKAAAAK-CONH ₂	-11,200 ± 200
Gly 13	CH ₃ CONH-YKAAAAKAAAAKGAAGK-CONH ₂	-11,400 ± 200
Gly 14	CH ₃ CONH-YKAAAAKAAAAKGAAGK-CONH ₂	-13,600 ± 200
Gly 15	CH ₃ CONH-YKAAAAKAAAAKAAAGK-CONH ₂	-19,000 ± 300
Gly 16	CH ₃ CONH-YKAAAAKAAAAKAAAGK-CONH ₂	-20,900 ± 400

Peptides were synthesized by the solid-phase method using the simultaneous multiple peptide synthesis procedure¹⁷. An active ester coupling procedure, employing pentafluorophenyl esters of 9-fluorenylmethoxycarbonyl amino acids, was used. The amino termini were acetylated with acetic anhydride and the peptides were cleaved from the PAL resin (Milligen) as peptide-amides with 95:5 trifluoroacetic acid/anisole mixture. The peptides were purified by C18 reverse-phase chromatography, and peptide identity was confirmed by FAB mass spectrometry. Peptide purity was assessed by capillary electrophoresis, and ranged between 94–100% purity (average purity, 98.6%).

* Mean residue ellipticity at 222 nm. Conditions: 0 °C, pH 7.00, 1.0 M NaCl, 1 mM each of sodium borate, sodium citrate, and sodium phosphate, and 10–30 μM peptide. Measurements were made on an Aviv 60DS spectro-polarimeter in a 1.0-cm path-length cuvette. The values represent the mean ± s.d. (n=5). There is an additional error of ±1% associated with each measurement which was due to the error of measuring peptide concentration by tyrosine absorbance.

The results (Fig. 1b) show that the position effect is large for Ala → Gly substitutions. The largest drop in helix content is produced at residue 9, in the centre of the helix, and substitutions close to either end have comparatively small effects. The shape of the position-effect curve in Fig. 1b is inverse to that of the predicted curve of helix fraying (Fig. 1a). Figure 2 shows the data fitted to the Lifson–Roig theory for various values of $s(\text{Gly})$: a very low value (about $s = 0.015$) is required to fit the data. An average value of $s = 1.56$ fits the helix content of the reference peptide. As the reference peptide contains chiefly alanine, the ratio of $s(\text{Ala}) : s(\text{Gly})$ is about 100. A few data points deviate significantly from the theoretical curve. Possibly small but abrupt changes in the properties of the alanine helix arise at the position where each Lys⁺ residue is inserted. As measured by the host-guest method¹, the nucleation constant σ for glycine is reported to have the small value of 10^{-5} . We studied the effect of allowing $\sigma(\text{Gly})$ to be 10^{-5} on the predicted curve of peptide helicity versus position of an Ala → Gly substitution. The predicted effect is quite small and cannot account for the experimental results. Likewise, allowing $s(\text{Lys})$ to be 5-fold smaller than $s(\text{Ala})$ has only a small effect on the predicted curve of helicity versus position of an Ala → Gly substitution, when the average s for Ala, Lys and Tyr remains the same for the reference peptide.

Two effects have been noted that should contribute to the low value of $s(\text{Gly})$. The greater flexibility of the glycine peptide backbone (see ref. 14, and references therein) probably favours the random coil (but see ref. 15) and an enthalpic interaction between Cβ and the α helix backbone favours the helix¹⁶ and is missing in glycine.

To compare directly our results for Ala → Gly substitutions with other short peptide results, it will be necessary to determine the s values of Ala and Gly in the other reference peptides. Our peptide is similar to the 17-residue alanine-based peptide used by Merutka *et al.*⁵, although their peptide contains three possible helix-stabilizing ($i, i+4$) E⁻...K⁺ salt bridges, and the change we observe for an Ala → Gly substitution at a central position in the helix is similar to the change they observe. A considerably smaller change was found by Lyu *et al.*⁶: using a 21-residue peptide to make three central Ala → Gly substitutions, they found a change in helix content that is comparable to the change found

here for a single Ala → Gly substitution. Their reference peptide contains eight possible ($i, i+4$) E⁻...K⁺ salt bridges, whereas our reference peptide has no known helix-stabilizing side-chain interaction of importance. O'Neil and De Grado⁸ studied an Ala → Gly substitution on the solvent-exposed face of a dimeric coiled-coil helix, and interpreted the result by the two-state equation. More work is needed to relate their result to ours.

In summary, the large ratio found here for $s(\text{Ala}) : s(\text{Gly})$ indicates that the helix propensities of the amino acids are widely distributed, in contradiction to host-guest results. The existence of this contradiction suggests that context-dependent, or neighbouring residue, effects need to be analysed in future to understand fully the nature of substitution experiments. A strong dependence of the effect of a substitution on position in the helix has been demonstrated for the Ala → Gly substitution, and it has been explained by use of the Lifson–Roig equation. □

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Reduced binding of TFIIID to transcriptionally compromised mutants of VP16

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ACTIVATOR proteins that control transcription initiation by RNA polymerase II^{1,2} usually have two domains: one binds to DNA, and the other activates transcription^{3,4}. A particularly potent acidic^{5,6} activation domain at the C terminus of the herpes simplex virus protein VP16^{7–9} binds directly and selectively to the human and yeast TATA box-binding factor TFIIID¹⁰. We have now investigated the biological significance of this *in vitro* interaction by using mutant forms of VP16¹¹. For changes at the critical phenylalanine residue at position 442 of VP16 there was a good correlation between transactivation activity *in vivo* and the binding of VP16 to TFIIID *in vitro*. In contrast, mutants with reduced negative charge were more defective for binding than for activation.