12. F. J. Hasert *et al.*, *ibid.*, p. 121.
13. L. M. Barkov and M. S. Zoltorev, *ibid.* **85B**, 308 (1979).
14. G. Arnison *et al.* (UA-1 collaboration), *ibid.* **122B**, 103 (1983); G. Banner *et al.* (UA-2 collaboration), *ibid.*, p. 476.
15. For a review of electroweak symmetry breaking, see M. S. Chanowitz, *Annu. Rev. Nucl. Part. Sci.* **38**, 323 (1988).
16. B. Adeva *et al.* (L3 collaboration), *L3 Report #004* (CERN, Geneva, 1990).
17. D. Decamp *et al.* (ALEPH collaboration), *Phys. Lett.* **235B**, 399 (1990).
18. M. Z. Akrawy *et al.* (OPAL collaboration), *CERN-EP/90-27* (CERN, Geneva, 1990).
19. G. S. Abrams *et al.* (Mark II collaboration), *Phys. Rev. Lett.* **63** 1269 (1989).
20. Y. A. Golfand and E. P. Likhtman, *JETP Lett.* **13**, 323 (1971); D. V. Volkov and V. P. Akulov, *Phys. Lett.* **46B**, 109 (1973); J. Wess and B. Zumino, *Nucl. Phys.* **B70**, 39 (1974).
21. S. Weinberg, *Phys. Rev.* **D13**, 974 (1976); *ibid.* **D19**, 1227 (1979); L. Susskind, *ibid.* **D20**, 2619 (1979).
22. M. S. Chanowitz and M. K. Gaillard, *Nucl. Phys.* **B261**, 379 (1985); M. S. Chanowitz, M. Golden, H. Georgi, *Phys. Rev.* **D36**, 1490 (1987); *Phys. Rev. Lett.* **57**, 2344 (1986).
23. B. L. Ioffe and V. I. Khoze, *Leningrad Report 274* (Gatchina, Leningrad, 1976); J. D. Bjorken, in *Proceedings of the SLAC Summer Institute on Particle Physics, SLAC-198*, M. Zipf, Ed. (Stanford Linear Accelerator Center, Palo Alto, CA, 1976).
24. D. Decamp *et al.* (ALEPH collaboration), *Phys. Lett.* **236B**, 233 (1990).
25. D. Decamp *et al.* (ALEPH collaboration), *CERN-EP/90/16* (CERN, Geneva, 1990).
26. M. Akrawy *et al.* (OPAL collaboration), *Phys. Lett.* **236B**, 224 (1990).
27. D. Decamp *et al.* (ALEPH collaboration), *CERN-EP/89-168* (CERN, Geneva, 1990).
28. G. S. Abrams *et al.*, *Phys. Rev. Lett.* **63**, 2447 (1989).
29. M. Akrawy *et al.* (OPAL collaboration), *Phys. Lett.* **236B**, 364 (1990); D. Decamp *et al.* (ALEPH collaboration), *ibid.*, p. 511.
30. D. Decamp *et al.*, in (*29*); M. Akrawy *et al.* (OPAL collaboration), *CERN-EP/90-09* (CERN, Geneva, 1990).
31. B. Adeva *et al.* (L3 collaboration), *Phys. Lett.* **231B**, 509 (1989).
32. D. Decamp *et al.* (ALEPH collaboration), *ibid.* **236B**, 86 (1990).
33. M. Akrawy *et al.* (OPAL collaboration), *CERN-EP/89-176* (CERN, Geneva, 1989).
34. F. Abe *et al.* (CDF collaboration), *Phys. Rev. Lett.* **64**, 142 (1990).
35. M. Veltman, *Nucl. Phys.* **B123**, 89 (1977); M. S. Chanowitz, M. Furman, I. Hinchliffe, *Phys. Lett.* **78B**, 285 (1978); *Nucl. Phys.* **B153**, 402 (1979).
36. A. Sirlin, *Phys. Rev.* **D22**, 971 (1980).
37. For a recent review, see G. Altarelli, in *Proceedings of the 1989 International Symposium on Lepton and Photon Interactions at High Energies* (World Scientific, Singapore, 1990), p. 286.
38. J. Alitti *et al.* (UA-2 collaboration), *CERN-EP/90-22* (CERN, Geneva, 1990).
39. P. Schlabach *et al.* (CDF collaboration), presented at the meeting of the American Physical Society, Baltimore, MD, 16 to 20 April 1990.
40. D. Treille, *Polarization at LEP, CERN 88-06*, G. Alexander *et al.*, Eds. (CERN, Geneva, 1988), vol. 1, p. 265.
41. A. Blondel, *ibid.*, p. 1; E. Keil, *ibid.*, vol. 2, pp. 1 and 53.
42. D. Blockus *et al.*, *Proposal for Polarization at the SLC*, March 1986 (unpublished).
43. For a recent review, see G. Voss, *Proceedings of the 1989 International Symposium on Lepton and Photon Interactions at High Energies* (World Scientific, Singapore, 1990), p. 442.
44. P. Roudeau *et al.*, *Proceedings of the ECFA Workshop on LEP 200, CERN 87-08*, (CERN, Geneva, 1987), vol. 1, p. 49.
45. F. Borcherding *et al.*, in *Proceedings of the Conference on Fermilab in the 1990's* (World Scientific, Singapore, in press).
46. M. Chanowitz, in *Proceedings of the XXIII International Conference on High Energy Physics* (World Scientific, Singapore, 1987); in *Heavy Flavors and High Energy Collisions in the 1-100 TeV Range* (Plenum, New York, 1989), p. 443.
47. P. Langacker, R. Robinett, J. Rosner, *Phys. Rev.* **D30**, 1470 (1984).
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Research Article

Evidence That the Head of Kinesin Is Sufficient for Force Generation and Motility in Vitro

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Kinesin is a mechanochemical protein that converts the chemical energy in adenosine triphosphate into mechanical force for movement of cellular components along microtubules. The regions of the kinesin molecule responsible for generating movement were determined by studying the heavy chain of *Drosophila* kinesin, and its truncated forms, expressed in *Escherichia coli*. The results demonstrate that (i) kinesin heavy chain alone, without the light chains and other eukaryotic factors, is able to induce microtubule movement in vitro, and (ii) a fragment likely to contain only the kinesin head is also capable of inducing microtubule motility. Thus, the amino-terminal 450 amino acids of kinesin contain all the basic elements needed to convert chemical energy into mechanical force.

EUKARYOTIC CELL FUNCTIONS AND GROWTH REQUIRE INTRACELLULAR motility, which is driven by molecular motors such as myosin, dynein, and kinesin (*1, 2*). The nature of the molecular mechanism by which these motors generate force is largely unknown. It is thought that these proteins generate motile force by cyclic cross-bridge interactions with actin filaments or microtubules (*1, 2*). These interactions may be coupled to conformational changes of the motors as a result of adenosine triphosphate (ATP) hydrolysis, converting the chemical energy stored in ATP into mechanical force. However, very little information is available about the way that energy produced by ATP hydrolysis leads to conformational changes of the molecule and what kinds of conformational change lead to motile force production.

Kinesin is a newly discovered cytoplasmic microtubule motor that may function in organelle transport (*3–5*), endoplasmic reticulum extension (*6*), and mitosis (*7*). In vitro, in the presence of ATP,

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kinesin attached to glass surfaces induces microtubule movement (*3*), thus giving rise to the simplest assay for kinesin motor activity. Kinesin is also a microtubule-activated adenosine triphosphatase (ATPase) composed of two heavy chains and two light chains (*8*). The kinesin heavy chain is composed of three structural domains (*9–11*): an amino-terminal head domain, which contains the sites for ATP and microtubule binding; a middle, or stalk domain, which may form an α-helical coiled coil that entwines two heavy chains to form a dimer; and a carboxyl-terminal domain, which probably forms a globular tail that interacts with the light chains and possibly with vesicles and organelles. The structural organization of kinesin is grossly similar to that of classical two-headed myosin, which is an actin filament–dependent motor (*2*). Although kinesin has a distinct tail structure, both proteins have two globular heads attached to an α-helical coiled-coil region. This similarity suggests that the two motors might generate movements by similar mechanisms.

Understanding the relation of the structure of kinesin to its ability to generate force requires the identification of the regions of kinesin that are responsible for inducing microtubule movements. For example, it is not known whether the heavy chain itself induces movement, and whether the kinesin "head" contains the elements necessary for generating motility. In the case of myosin, the head alone (the S1 subfragment), which consists of a segment of the heavy chain and the two light chains, is capable of moving actin filaments in vitro (*2, 12*). Since the kinesin head is likely to contain both the ATP and the microtubule binding sites (*9*), and since monoclonal antibodies that bind to the kinesin head inhibit in vitro kinesin-based motility (*11*), it seemed possible that the kinesin head alone might be able to function autonomously in generating the force for microtubule motility. However, a 45-kD proteolytic fragment of kinesin, which is likely to be the kinesin head, retains its microtubule-stimulated ATPase activity and its nucleotide-dependent microtubule-binding activity (*11, 13*), but, surprisingly, lacks microtubule motility activity (*13*). The loss of microtubule translocation activity could either result from the inability of the 45-kD fragment to adhere to a glass surface in the correct orientation or state in a motility assay, or from the absence in the 45-kD fragment of an important mechanochemical element.

To find the minimal component of kinesin needed for microtubule motility, we produced kinesin heavy chain and a number of truncated kinesin heavy chain molecules in *E. coli*. We found that the full-length kinesin heavy chain alone as well as several types of truncated kinesin heavy chain molecules are sufficient to generate in vitro microtubule motility. In addition, we constructed a chimeric protein containing the kinesin head and part of α-spectrin and found that it could generate microtubule motility. Thus, our in vitro studies suggest that the kinesin head contains the minimal elements necessary for generating force and motility.

Characterization of kinesin heavy chain made in *E. coli*. To determine whether kinesin heavy chain alone is sufficient to induce in vitro microtubule motility, we constructed three plasmids, pBS-KIN, pET-KIN, and pGEX-KIN (*14*). After induction with isopropyl-β-D-thiogalactoside (IPTG) (*15*), all three plasmids express full-length kinesin heavy chain in *E. coli*, but with slightly different NH_2-termini (Fig. 1). Although most of the expressed kinesin heavy chain formed insoluble aggregates when the cells were disrupted with lysozyme, approximately half of the expressed kinesin heavy chain was soluble when the cells were lysed by sonication or hydraulic pressure (*16*).

The soluble proteins were tested for activity in an in vitro microtubule-sedimentation assay (*17*). In this assay system, native kinesin co-sediments with microtubules in the presence of adenylyl-imido diphosphate (AMP-PNP), a nonhydrolyzable analog of ATP, and releases from microtubules in the presence of Mg^{2+}-ATP (*3*). Our initial preparations of kinesin heavy chain from bacteria grown at 37°C were not active in this assay system. Since the optimal growth temperature of *Drosophila* is 25°C, and higher temperature may affect the enzymatic activities of some proteins, we prepared kinesin heavy chain from *E. coli* grown at 22°C. A small amount of each protein prepared at 22°C was able to bind to microtubules in the presence of AMP-PNP and release when Mg^{2+}-ATP was added (Fig. 2).

After enrichment by microtubule affinity (*18*) or ammonium sulfate precipitation (*19*), the biochemically active, full-length kinesin heavy chain produced by each of the three constructs was tested in microtubule motility assays in vitro (*20*). These assays were

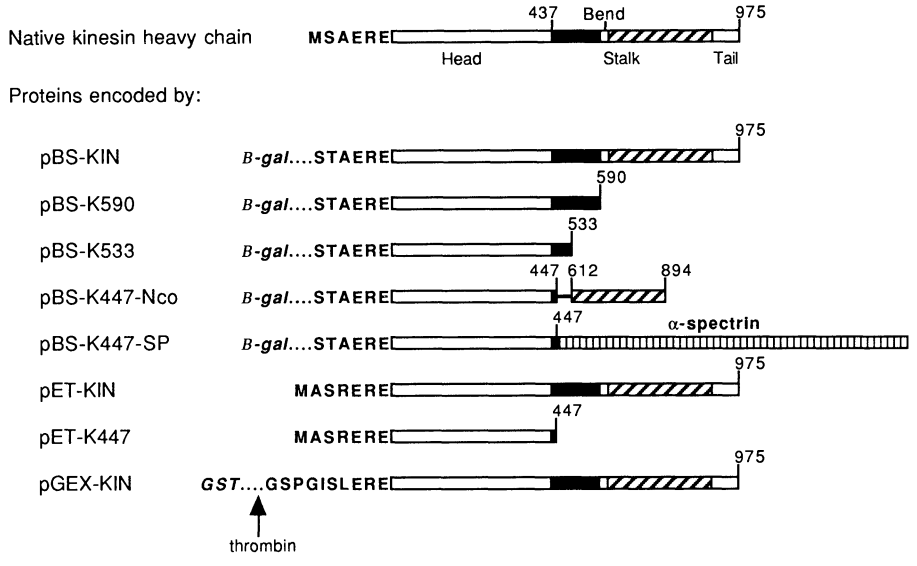

Fig. 1. Proteins encoded by the plasmids described in (*14*). Native kinesin heavy chain is shown at the top with the major structural landmarks indicated by different shadings. Full-length kinesin heavy chain was expressed from pBS-KIN, pET-KIN, and pGEX-KIN. Truncated kinesin heavy chain proteins ending at amino acid numbers 590, 533, and 447 (K590, K533, and K447, respectively), were expressed from pBS-K590, pBS-K533, and pET-K447, respectively. K590 contains the kinesin head, part of the stalk, and a portion of the bend. K533 lacks the bend but includes part of the stalk and the head. K447 includes the kinesin head and lacks all but 11 amino acids of the stalk. K447-Nco was expressed from pBS-K447-Nco and consists of the K447 protein fused to a near COOH-terminal segment of the stalk running from amino acids 612 to 894. K447-SP is expressed from pBS-K447-SP and consists of the K447 protein fused to a 1280 amino acid piece of *Drosophila* α-spectrin. The amino acid sequences in the region of the NH_2-termini of the kinesin heavy chain portions of each of the protein is also shown. Although each of the proteins produced in *E. coli* has a different NH_2-terminus than native kinesin heavy chain, this does not seem to affect their behavior. The protein expressed from pBS-KIN and the K590, K533, K447-Nco, and K447-SP proteins have 22 amino acids of β-galactosidase (B-*gal*) fused to amino acid 3 of kinesin heavy chain. The protein encoded by pGEX-KIN has 219 amino acids of glutathione S-transferase (GST) fused to amino acid 4 of kinesin heavy chain. Cleavage of this protein with thrombin occurs at the indicated site. Other novel sequences in the proteins K533, K447-Nco, K447-SP, and K447 are as described (*14*).

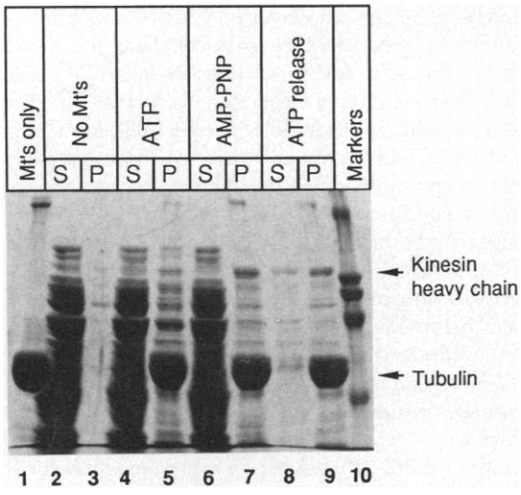

Fig. 2. A Coomassie blue–stained SDS-polyacrylamide gel showing a microtubule sedimentation assay (*17*) of the kinesin heavy chain produced by cells carrying pGEX-KIN. A fraction of the kinesin heavy chain expressed from pGEX-KIN binds microtubules in the presence of AMP-PNP and releases in the presence of ATP. The data presented do not permit a conclusion as to whether the material that remains bound to microtubules in ATP is active. (Lane 1) Microtubules (Mt's) only; (lanes 2 and 3) the supernatant (S) and the pellet (P) of the pGEX-KIN cell extract mixture with AMP-PNP but without microtubules; (lanes 4 and 5) the supernatant and the pellet of the cell extract–microtubule mixture with ATP instead of AMP-PNP; (lanes 6 and 7) the supernatant and the pellet of the cell extract–microtubule mixture with AMP-PNP; (lanes 8 and 9) the supernatant and the pellet of Mg^{2+}-ATP extraction; and (lane 10) molecular markers.

conducted with either purified microtubules or sea urchin sperm from which the membrane was removed (Fig. 3). All three proteins induced microtubule movement in both assays. In the presence of full-length kinesin heavy chain made from each of the three constructs and 10 mM Mg^{2+}-ATP, the sperm glided on glass in the direction of the sperm head. These results demonstrate that these proteins induce movement in the same direction as native kinesin relative to the intrinsic structural polarity of the microtubule, that is toward the plus end (*1*). The rate of movement induced by each of these proteins was assayed with purified microtubules and was similar to that of native kinesin from *Drosophila* (*21*). As a control, an ammonium sulfate–concentrated lysate of the same *E. coli* strain lacking any *Drosophila* kinesin heavy chain cDNA clones did not move microtubules, demonstrating that no *E. coli* proteins are capable of inducing microtubule motility under these conditions. The data show that the kinesin heavy chain itself, without added eukaryotic proteins other than tubulin, is sufficient to generate motile force and induce microtubule movement similar to that produced by native eukaryotic kinesin.

Characterization of the truncated kinesin heavy chain made in *E. coli*. To determine what regions of kinesin heavy chain are necessary for in vitro force generation and motility, we made in *E. coli* three truncated kinesin heavy chains, K590, K533, and K447 (*14*), that lack various structural elements of the protein (Fig. 1). Our previous analysis of the structural organization of kinesin heavy chain placed the NH_2-terminal end point of the α-helical coiled-coil stalk domain at approximately residue 437, where a heptapeptide repeat pattern begins (*9*). Moreover, a "bend" that was observed in the middle of the stalk by electron microscopy of porcine and chick kinesin (*10*) has been suggested to reside between residues 584 and 606, where the heptapeptide repeat pattern in the stalk is disrupted by a proline and several glycine residues (*9*). Both K590 and K533 contain the head and part of the stalk, but are missing the bend; K447 contains the head and 11 residues from the predicted stalk region (*9*). All of these proteins showed the nucleotide-dependent microtubule-binding activity of the full-length kinesin heavy chain.

The truncated kinesin heavy chains were enriched by microtubule affinity (*18*) or ammonium sulfate precipitation (*19*), and were then tested for their ability to induce microtubule gliding in the in vitro motility assay (*20*). Both K590 and K533 induced movement of microtubules at rates similar to that of native kinesin from *Drosophila* and the full-length kinesin heavy chain from *E. coli* (*21*). The direction of the sperm gliding induced by K590 and K533 was the same as that of the full-length kinesin heavy chain. However, K447 did not support microtubule motility and did not even cause microtubules to stick to the glass cover slip. The motile activities of K590 and K533 suggest that the COOH-terminal portion of kinesin heavy chain, from residue 533 to the COOH-terminus, including the bend, is dispensable for force generation in vitro.

Presence of the minimal elements essential for force generation in K447. Two general hypotheses can explain the failure of K447 to induce in vitro microtubule motility, even though K447 retains nucleotide-dependent microtubule-binding activity. Either this protein lacks an important mechanochemical region, or it fails to adhere to the glass surfaces in the in vitro motility assay in an appropriate orientation or state.

To distinguish between these two possibilities, we made two fusion proteins, K447-Nco and K447-SP (*14*), in *E. coli* (Fig. 1); both proteins contain the K447 fragment and a protein fragment attached to the COOH-terminal end of K447. The attached protein fragment for K447-Nco contains kinesin heavy chain residues 612 to 894, which include the COOH-terminal portion of the stalk but not the bend (*9*). As shown above, the truncated proteins K590 and K533, which lack this portion of the stalk, are capable of generating microtubule motility, indicating that this region is not essential for force generation. In contrast, K447-SP consists of K447 attached to 1280 residues of *Drosophila* α-spectrin. This portion of α-spectrin probably has an elongated α-helical conformation (*22*) and does not have any known motility activities. Thus, this protein is an ideal choice as an inert non-kinesin tail that might adhere to glass and

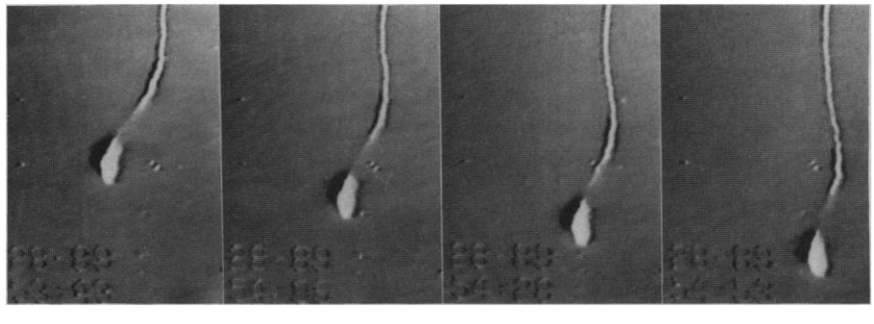

Fig. 3. An in vitro motility assay (*20*) of kinesin heavy chain produced by *E. coli* cells carrying pBS-KIN. The pictures are of moving demembranated sea urchin sperm, which were taken at 24-second intervals and correspond to a rate of movement of about 0.5 μm/s (*21*).

position the kinesin head portion of the chimeric protein appropriately for motility.

Both K447-Nco and K447-SP behave biochemically like other active kinesin heavy chain proteins made in *E. coli*. In an in vitro motility assay (*20*), both K447-Nco and K447-SP, enriched by microtubule affinity (*18*) or ammonium sulfate precipitation (*19*), induced microtubules to move on glass at a rate similar to that of the full-length kinesin heavy chain (*21*). The direction of the sperm gliding induced by K447-Nco and K447-SP was the same as that induced by the full-length kinesin heavy chain. As a control, we tested the spectrin piece produced on its own for microtubule motility activity and observed none.

The result from K447-Nco, in combination with the results for K590 and K533, suggest that the entire stalk, up to residue 447, is dispensable for force generation. It is not likely that the stalk has duplicated elements that can function for force generation because our data show that a non-kinesin protein fragment, which does not have any motility activity, can facilitate motility activity of K447. Thus, the NH_2-terminal portion of kinesin heavy chain, up to residue 447, contains all of the elements necessary to induce microtubule movement in vitro.

Basic elements for force generation in the kinesin head. To explore the nature of the minimal motile element of kinesin, we tested the in vitro motility activity of a series of truncated kinesin heavy chain molecules made in *E. coli*. Our results (Fig. 4) show that

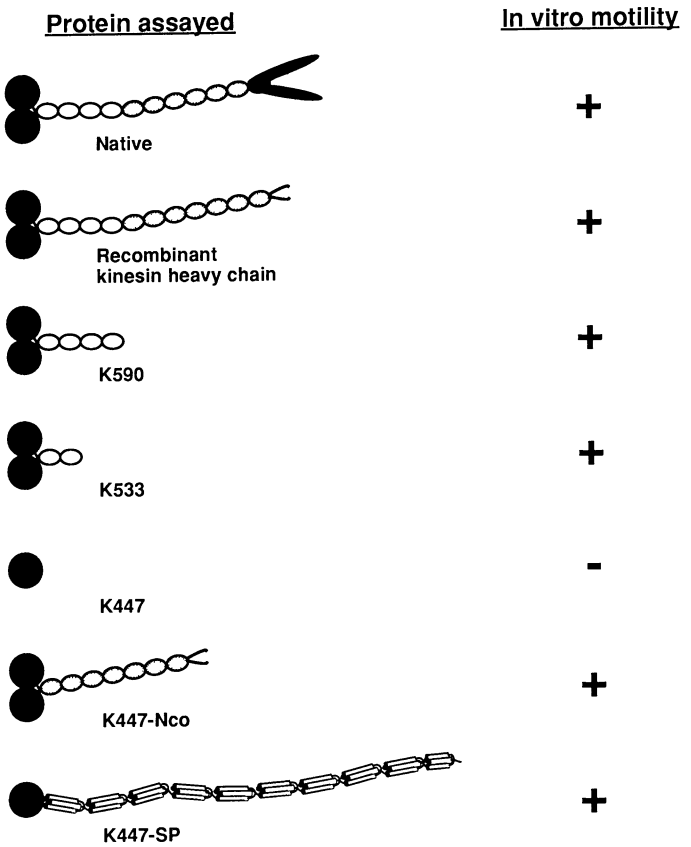

Fig. 4. Summary of the results from the in vitro motility assays of various kinesin proteins. The diagrams are based on the data from (*9, 10, 11*). The quaternary structures of the proteins are hypothetical at this time, hence, K447-SP, which is represented as a monomer, may in fact be a dimer (see text for discussion of this point). The solid circles at the left ends of each molecule represent the heads. The solid ellipses at the right end of the native molecules represent the light chains. The intervening ovals represent the stalk with an obvious bend in the middle; the two segments of the stalk flanking the bend are shaded differently. K447-SP is shown with a long spectrin tail attached to a single head.

(i) kinesin heavy chain by itself, without light chains and other eukaryotic factors, can induce microtubule movement in vitro; (ii) the NH_2-terminal portion of kinesin heavy chain up to residue 447 is sufficient to induce microtubule motility in vitro. Thus, a very small piece of kinesin (447 amino acids of kinesin heavy chain) is capable of in vitro force generation. This piece of kinesin is much smaller than the minimal element of myosin (900 amino acids of myosin heavy chain plus two myosin light chains) observed to be active as a molecular motor (*2, 12*).

Our previous studies suggested that the NH_2-terminal end point of the α-helical coiled-coil stalk domain is approximately at residue 437 (*9*). Therefore, the NH_2-terminal portion of kinesin heavy chain up to residue 447 is likely to contain the head domain and only about ten residues that may contribute to the stalk. This portion of the molecule is similar in size and properties to the previously reported 45-kD proteolytic fragment of intact kinesin. In addition, the NH_2-terminal 447 amino acid portion we describe is reactive with the SUK4 antibody, which also reacts with the 45-kD proteolytic fragment (*11, 13*). We thus suggest that the K447 protein is largely similar to the 45-kD proteolytic fragment. It is unlikely that the spectrin fragment in K447-SP contributes actively to the movements induced by the chimeric protein because spectrin does not have any known motility activities. Similarly, we think that it is unlikely that the ten residues of potential stalk that are included in K447 are required for force generation. We thus propose that the kinesin head contains all of the basic elements necessary for force generation. Interestingly, this portion of the kinesin heavy chain appears to be conserved within a group of recently discovered kinesin-like proteins (*23*).

An unresolved issue is whether a single-headed kinesin molecule can generate microtubule-dependent force or whether the native two-headed state is essential. In the case of myosin, single-headed molecules can generate movement (*2, 12*). However, as pointed out (*24*), the naturally occurring two-headed design must have some advantages, given the similarity of such a two-headed design for the motor functions of kinesin and myosin, which are not detectably related with respect to their primary amino acid sequences (*9*). Our current hypothesis is that the chimeric K447-SP protein is monomeric and that a single-headed kinesin is indeed functional. The K447 portion of this chimeric protein contains the kinesin head and 11 residues of the predicted stalk region. Since the kinesin head may not be able to dimerize (*13*), and the 11-residue peptide of the predicted stalk region is likely to be too short to form an α-helical coiled coil (*25*), the K447 portion of the chimeric protein is unlikely to be dimeric. The α-spectrin portion of this chimeric protein is also unlikely to be dimeric, based on electron microscopic observations (*26*). However, the quaternary structure of the kinesin head spectrin chimeric protein remains to be determined. Finally, to determine whether an individual single-headed molecule is capable of moving microtubules, it is necessary to test the kinesin head-spectrin chimeric protein in an assay system that can detect movements induced by a single protein molecule (*27*).

Mechanism of force generation. The overall similarities between the structure and behavior of kinesin and two-headed myosin (*2, 3, 9–11*, and our data) suggest that these two proteins might convert chemical energy into mechanical force by similar mechanisms. There are two major classes of mechanism that have been proposed to account for the force-generating properties of myosin.

The first model is the rotating-head model (*28*), which proposes that the myosin head, once bound to actin, undergoes a discrete change in orientation with respect to the axis of the actin filament, as a result of a conformational change in the head. This change leads to a tilting movement of cross bridges, thus pulling the motor forward along the actin filament. This model is supported by the observation

that the myosin head without a tail can induce movements of actin filaments in vitro (*2, 12*). However, with but one exception (*29*) changes of sufficient magnitude in the orientation of the myosin head with respect to the actin filament, or in the shape of the myosin head, upon actin-myosin interactions have not been detected (*30*). The second model is the helix-coil transition model (*31*), which suggests that a conformational change could occur in the hinge region within the myosin tail, leading to force generation. This model is based on the observation that the melting behavior of the α-helix in the hinge region correlates with ATP hydrolysis and muscle contraction (*31*). Although the observation that myosin S1 can induce movement on its own would seem to rule out this latter model, it has been argued that the in vitro movement induced by myosin head alone was under conditions of no or little load (*32*). Thus, the force generated by the myosin head may represent only a part of the overall force-generating process in muscle fiber contraction. This idea is supported by the observation that an antibody to the S2 subfragment of myosin suppressed the contraction of muscle fibers (*32*).

Our observation that the kinesin head is capable of inducing microtubule movement in vitro is most consistent with a rotating-head model. However, even though the stalk and the bend region of the stalk are dispensable for in vitro motility, they may behave differently in vivo. Under in vivo conditions, the stalk may provide part of the force, or play a regulatory role to control the rate of movement.

To conclude, our results provide substantial evidence that the kinesin head is capable of generating a force that is sufficient to induce in vitro microtubule movement. In addition, our development of an in vitro motility system with kinesin heavy chain produced in *E. coli*, and the availability of a small piece of protein that generates movement, should provide a basis for elucidating the general mechanism by which mechanochemical proteins generate force.

REFERENCES AND NOTES

1. R. D. Vale, *Annu. Rev. Cell Biol.* **3**, 347 (1987).
2. H. M. Warrick and J. A. Spudich, *ibid.*, p. 379.
3. R. D. Vale, T. S. Reese, M. P. Sheetz, *Cell* **42**, 39 (1985).
4. T. A. Schroer, B. J. Schnapp, T. S. Reese, M. P. Sheets, *J. Cell. Biol.* **107**, 1785 (1988).
5. K. K. Pfister, M. C. Wagner, D. L. Stenoien, S. T. Brady, G. S. Bloom, *ibid.* **108**, 1453 (1989).
6. S. L. Dabora and M. P. Sheetz, *Cell* **54**, 27 (1988).
7. J. M. Scholey, M. E. Porter, P. M. Grissom, J. R. McIntosh, *Nature* **318**, 483 (1985).
8. S. A. Kuznetsov *et al.*, *EMBO J.* **7**, 353 (1988); G. S. Bloom, M. C. Wagner, K. K. Pfister, S. T. Brady, *Biochemistry* **27**, 3409 (1988).
9. J. T. Yang, R. A. Laymon, L. S. B. Goldstein, *Cell* **56**, 879 (1989).
10. L. A. Amos, *J. Cell Sci.* **87**, 105 (1987); N. Hirokawa *et al.*, *Cell* **56**, 867 (1989).
11. A. L. Ingold, S. A. Cohn, J. M. Scholey, *J. Cell Biol.* **107**, 2657 (1988); J. M. Scholey, J. Heuser, J. T. Yang, L. S. B. Goldstein, *Nature* **338**, 355 (1989).
12. Y. Y. Toyoshima *et al.*, *Nature* **328**, 536 (1987).
13. S. A. Kuznetsov, Y. A. Vaisberg, S. W. Rothwell, D. B. Murphy, V. I. Gelfand, *J. Biol. Chem.* **264**, 589 (1989).
14. The plasmids pBS-KIN and pBS-K590 were constructed in pBluescript (Stratagene) and were derived, respectively, from pBS1-2 and pBS2-10, which is one of the Exo III deletions of pBS1-2 (*9, 33*). Both pBS1-2 and pBS2-10 were digested and religated at the Sac II sites, so that the resulting plasmids would have the kinesin sequence in frame with the translational start site of the upstream β-galactosidase gene. The pBS-K533 plasmid is derived from pBS-KIN by digesting and religating at the Cla I sites in the plasmid, removing the sequence between the second Cla I site within the kinesin sequence and the Cla I site in the downstream polylinker region of the vector. K590 terminates immediately after the kinesin heavy chain portion of the sequence while K533 terminates downstream in the polylinker region and has the novel sequence TVDLEGGPGTQFAL appended to amino acid 533 of kinesin heavy chain. pBS-K447-SP was constructed by inserting the 1280 amino acids encoding the Eco RI fragment of *Drosophila* α-spectrin cDNA 9A (*22*), which was rendered blunt with the Klenow fragment of DNA polymerase I, into a Pvu II site within the kinesin sequence of pBS-K590. This insertion renders the kinesin sequence downstream from the Pvu II site out of frame so that the novel sequence FWTTRMRRSISRASTPSSSRSR is appended to the end of the α-spectrin portion of this protein. To construct pBS-K447-Nco, an oligonucleotide containing an Nco I site (AGCTTGGGCGACCTCACCATG-GAGCAAAAGCTCATTTCTGAAGAGGACTTGAATT) was inserted into the same Pvu II site of pBS-K590, followed by insertion of an Nco I fragment of the kinesin cDNA into this Nco I site. Insertion of the oligonucleotide led to the insertion of the novel sequence of SLGDL at the junction between residues 447 and 612 of kinesin heavy chain. It also puts the kinesin sequence downstream from the Pvu II site out of frame and resulted in the new sequence QKLISEEDLNSGR-QG being appended to amino acid 894 of kinesin heavy chain. The proteins produced by the plasmids above-described have 22 residues of β-galactosidase from pBluescript fused upstream of residue 3 at the NH_2-terminal end of kinesin heavy chain. The plasmids pET-KIN and pET-K447 were constructed by replacing an Nhe I–Bam HI fragment in the polylinker region of a pET-5 translation vector (*34*), respectively, with a Sac II–Bam HI fragment and a Sac II–Pvu II fragment of the kinesin heavy chain cDNA from pBS1-1 (*33*). The Sac II–Bam HI fragment contains the entire protein coding sequence of kinesin heavy chain, whereas the Sac II–Pvu II fragment contains the kinesin cDNA encoding the NH_2-terminal portion of kinesin heavy chain up to residue 447. For convenience, the Sac II site was converted into an Nhe I site before the Sac II–Bam HI and the Sac II–Pvu II fragments were taken out of the original vector. Proteins produced from these two plasmids have an alanine inserted after the first methionine residue of the kinesin heavy chain and an alteration of Ala^3 to Arg. As a result of terminating downstream from the kinesin heavy chain portion of the plasmid, K447 has the novel sequence DPNS appended to amino acid 447 of kinesin heavy chain. pGEX-KIN (*35*) was constructed as follows: The Hind III–Eco RI fragment of pBS-B3 (*36*) was inserted into the Eco RI site of pGEX-2T (*37*) by filling all the ends with the Klenow fragment of DNA polymerase I. The protein product of pGEX-KIN is a fusion protein with glutathione S-transferase (GST) attached to the NH_2-terminus of kinesin heavy chain. Removal of GST by thrombin cleavage leaves the remaining kinesin heavy chain intact, except that the protein has four additional residues (GSPG) at the NH_2-terminus and $Met^1 \rightarrow$ Ile; and $Ala^3 \rightarrow$ Leu. Except for pET-KIN and pET-K447, all the plasmids were transformed into *E. coli* strain 71/18. pET-KIN and pET-K447 were transformed into *E. coli* stain BL21 (DE3) (*34*).
15. For expression of kinesin proteins in *E. coli*, overnight cultures of transformed *E. coli* cells were diluted 1:100 into LB media supplemented with ampicillin and shaken at 37°C for 2 hours; The culture was made 1 mM with IPTG (0.1 mM for the cells carrying the pET plasmids). The culture was then shaken at 22°C for 10 hours.
16. Except for cells carrying pGEX-KIN and its derivatives, cells were lysed for protein preparation as follows. The cells were harvested by centrifugation (Sorvall GSA rotor; 8000 rpm, 5 minutes, 4°C). The pellet was resuspended in 1× AB buffer (0.1 M Pipes, pH 6.9, 1 mM $MgCl_2$, 1 mM EGTA, 2 mM dithiothreitol) and centrifuged in a weighed tube (5 minutes, 8000 rpm, Sorvall SS34 rotor, 4°C). The pellet was weighed and resuspended in 1× AB supplemented with protease inhibitors (leupeptin at 1 μg/ml, pepstatin A at 1 μg/ml, aprotinin at 2 μg/ml, tosyl-arginyl-methyl ester at 2 μg/ml, and phenylmethylsulfonyl fluoride at 1 mM). Each 1 gram of cells was resuspended in 4 ml of buffer. The resuspended cells were lysed in a French press cell that had been cooled at 4°C at 15,000 psi for two cycles. The lysed cells were released into a tube sitting on ice, and centrifuged (SS34 rotor, 10,000 rpm, 10 minutes, 4°C). The supernatant was cleared by centrifugation (150,000g_{max}, for 1 hour, at 4°C), the sediment was discarded, and the final supernatant is referred to as the cell extract. For cells carrying pGEX, the cells were harvested and washed as above, except that a phosphate buffer (16 mM Na_2HPO_4, 4 mM NaH_2PO_4, pH 7.3) was used. The final pGEX cell pellet was resuspended in the phosphate buffer supplemented with the protease inhibitors described above in 5 ml of buffer for each 1 gram of cell pellet. The cell suspension was placed in 1.5-ml Eppendorf tubes in 1-ml portions and sonicated on ice with a microprobe at a setting of 4 for 30 seconds. The sonicated samples were cleared in a microfuge at full speed for 10 minutes. For removal of GST from the fusion protein, a solution of 2.5 mM $CaCl_2$, 0.15 M NaCl, and thrombin (12.5 unit/ml) was added to the supernatant of the sonicated sample and incubated at room temperature for 1 hour. The digested sample was dialyzed against 200 ml of 1× AB for 4 hours with one change, and then centrifuged at 150,000g_{max} for 1 hour. This supernatant is referred to as the pGEX-KIN cell extract. In some preparations, the cell extracts were frozen in liquid nitrogen and stored at −70°C; this freezing step appeared to result in kinesin that moved microtubules at a slower rate than kinesin prepared from fresh extracts.
17. The microtubule sedimentation assay of the protein expressed from pGEX-KIN was conducted by mixing 20 μl of the pGEX-KIN cell extract (*16*) with microtubules (0.5 μg/ml) (assembled with 10 μM taxol and 2 mM GTP from tubulin purified on a phosphocellulose column), 20 μM taxol, 1 mM GTP, 2.5 mM AMP-PNP and 1× AB (0.1 M Pipes, pH 6.9, 1 mM $MgCl_2$, 1 mM EGTA, 2 mM dithiothreitol), and incubating at room temperature for 15 minutes. The mixture was centrifuged at 100,000g_{max} in an airfuge. The resulting pellet was washed in 20 μl of 1× AB supplemented with protease inhibitors (leupeptin at 1 μg/ml, pepstatin A at 1 μg/ml, aprotinin at 2 μg/ml, tosylarginyl methyl ester at 2 μg/ml, and phenylmethylsulfonyl fluoride at 1 mM), taxol, and GTP, and centrifuged at 100,000g_{max}. To extract the kinesin protein from microtubules, the pellet was resuspended in 20 μl of 1× AB containing 10 mM ATP and 10 mM $MgSO_4$, incubated at room temperature for 15 minutes, and centrifuged at 100,000g_{max} for 30 minutes at 22°C.
18. The kinesin heavy chain proteins were enriched by microtubule affinity as follows. The cell extract was mixed with microtubules (*17*), incubated at room temperature for 15 minutes, and centrifuged through a 2-ml sucrose cushion (15 percent sucrose, 20 μM taxol, 1 mM GTP in 1× AB with protease inhibitors) in a swinging bucket rotor (54,000g_{max}, 35 minutes, 22°C). The pellet was resuspended in 1× AB supplemented with protease inhibitors, taxol, and GTP, and centrifuged at 100,000g_{max}. The kinesin heavy chain protein was released from microtubules by resuspending the pellet (from 1 ml of cell extract) in 100 μl of 1× AB containing 10 mM ATP, 10 mM $MgSO_4$ and 0.1 M KCl, incubating at room temperature for 15 minutes, and centrifuging at 100,000g_{max} for 30 minutes at 22°C. The

supernatant containing the enriched kinesin protein was divided into portions, frozen in liquid nitrogen, and stored at −70°C.
19. For enrichment, kinesin heavy chain proteins were precipitated in a saturated ammonium sulfate solution (supplemented with 10 mM EDTA, adjusted to pH 8.2 with NH_4OH, and stored at 4°C), which was added dropwise with constant stirring until the final concentration of ammonium sulfate was 35 percent. This concentration gave the best enrichment of kinesin heavy chain derivatives relative to other bacterial proteins. The mixture was stirred in the cold for 30 minutes, and centrifuged (SS34 rotor) at 10,000 rpm for 15 minutes. The pellet was resuspended in 1× AB with protease inhibitors (200 μl of buffer for 10 ml of cell extract), and dialyzed in 1 liter of 1× AB for 6 hours with one change. The dialyzed sample was clarified by centrifugation at $150{,}000g_{max}$ for 30 minutes at 4°C.
20. For the in vitro motility assays, the kinesin samples were clarified in an airfuge at $100{,}000g_{max}$ for 30 minutes at 4°C. Ammonium sulfate (*19*) or microtubule affinity enriched kinesin sample (14 μl) (*18*) was placed on a glass cover slip and kept in a humidified box for 10 minutes; the cover slip preparation was then supplemented with a solution containing 2 μl of 50 mM $MgSO_4$, 50 mM ATP in 1× EB buffer (0.1 M Pipes, pH 6.9, 0.9 M glycerol, 2.5 mM $MgSO_4$, 0.5 mM EDTA, 5 mM EGTA and protease inhibitors as described above), and 4 μl of purified microtubules (20 μg/ml) or demembranated whole sea urchin sperm (*38*). The cover slip was immediately placed on a slide, sealed with wax, and observed with video-enhanced differential interference contrast microscopy (*3*).
21. The velocities of microtubule movement produced by each of the proteins assayed (in micrometers per second) were as follows: native *Drosophila* kinesin: 0.3 to 0.7; protein encoded by PBS-KIN: 0.3 to 0.7; protein encoded by pET-KIN: 0.3 to 0.4; protein encoded by pGEX-KIN: 0.3 to 0.4; K590: 0.3 to 0.5; K533: 0.3 to 0.5: K447-Nco: 0.3 to 0.4; K447 to SP: 0.3 to 0.5. For the proteins produced in *E. coli*, velocities represent the range in the mean velocities of microtubule movement determined from at least two independent preparations of each protein. For native *Drosophila* kinesin, rates were determined from two independent assays of one preparation of protein. At least six microtubules were measured for each preparation. The methods have been described (*18–20*).
22. R. R. Dubreuil *et al.*, *J. Cell Biol.* **109**, 2197 (1989).
23. A. P. Enos and N. R. Morris, *Cell* **60**, 1019 (1990); P. B. Meluh and M. D. Rose, *ibid.*, p. 1029; R. D. Vale and L. S. B. Goldstein, *ibid.*, p. 883; S. A. Endow, S. Henikoff, L. Soler-Niedziela, *Nature* **345**, 81 (1990); H. B. McDonald and L. S. B. Goldstein, *Cell*, in press.
24. M. Schliwa, *Cell* **56**, 719 (1989).
25. S. Y. M. Lau, A. K. Taneja, R. S. Hodges, *J. Biol. Chem.* **259**, 13253 (1984).
26. R. Dubreuil, T. J. Byers, D. Branton, L. S. B. Goldstein, D. P. Kiehart, *J. Cell Biol.* **103**, 540 (1986).
27. J. Howard, A. J. Hudspeth, R. D. Vale, *Nature* **342**, 154 (1989).
28. H. E. Huxley, *Science* **164**, 1356 (1969).
29. C. L. Berger, E. C. Svensson, D. D. Thomas, *Proc. Natl. Acad. Sci. U.S.A.* **86**, 8753 (1989).
30. R. Cooke, *Cell Motil. Cytoskeleton* **14**, 183 (1989).
31. W. F. Harrington, *Proc. Natl. Acad. Sci. U.S.A.* **68**, 685 (1971).
32. S. Lovell, T. Karr, W. F. Harrington, *ibid.* **85**, 1849 (1988).
33. J. T. Yang, W. M. Saxton, L. S. B. Goldstein, *ibid.*, p. 1864.
34. A. H. Rosenberg *et al.*, *Gene* **56**, 125 (1987).
35. The plasmid pGEX-KIN was constructed in an attempt to purify kinesin heavy chain made in *E. coli* by glutathione affinity chromatography of the GST portion of the fusion protein and subsequent thrombin cleavage to remove GST from the fusion protein. However, the fusion protein GEX-KIN did not bind to glutathione beads, suggesting that the folding of the kinesin portion of GEX-KIN inhibited the glutathione binding activity of GST.
36. Bal 31 deletions at the 5′ end of the *Drosophila* kinesin heavy chain cDNA were generated by cutting at the Sph I site of pBS1-1 (*33*), digesting with Bal 31, stopping the reaction at regular intervals, and religating the plasmids in the presence of a Hind III linker. The resulting clones were sequenced to determine the 5′ end points. The 5′ end of the kinesin heavy chain cDNA in pBS-B3 is at nucleotide 329.
37. D. B. Smith and K. S. Johnson, *Gene* **67**, 31 (1988).
38. R. J. Lye, C. M. Pfarr, M. E. Porter, in *Cell Movement*, vol. 2, *Kinesin, Dyein, and Microtubule Dynamics*. F. D. Warner and J. R. McIntosh, Eds. (Liss, New York, 1989), p. 441.
39. We thank D. Kiehart for suggestions concerning temperatures of cell growth and lysis methods; D. Branton and R. Losick for suggestions on this manuscript, M. de Cuevas for making some of the plasmids, J. Scholey for purified tubulin, and R. Dubreuil for the α-spectrin cDNA fragment and a clone expressing it in *E. coli*. Supported by an American Cancer Society postdoctoral fellowship (W.M.S.), NIH grant HD16739 (E.C.R.), and NIH grant GM35252 (L.S.B.G.).

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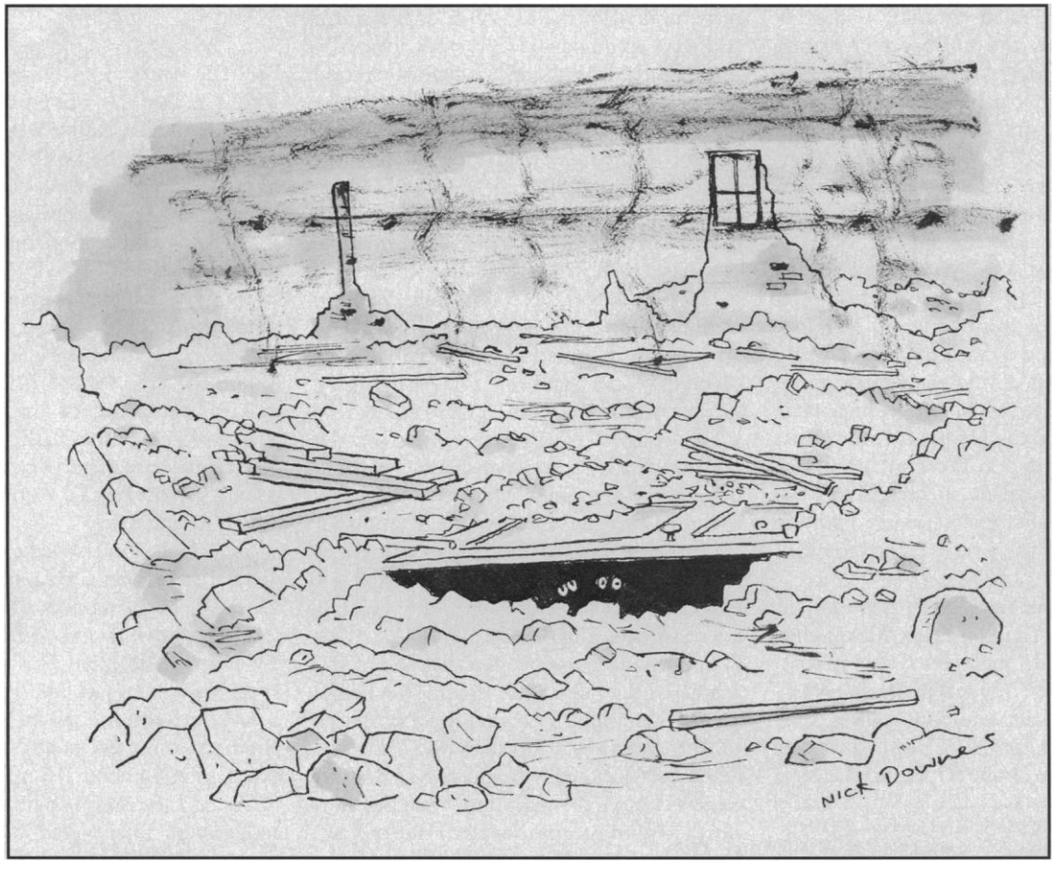

"I always get it confused. Was that an example of fusion or fission?"