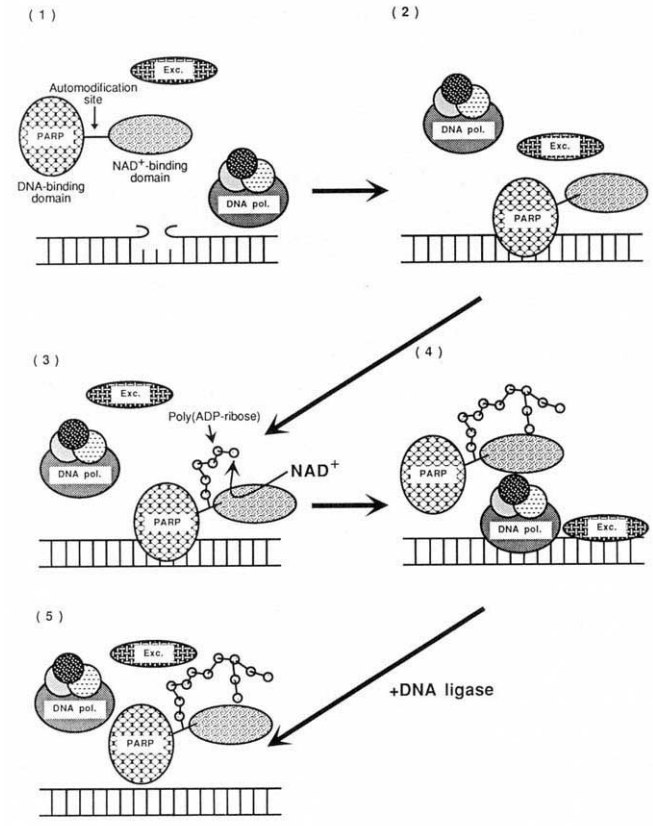

FIG. 4 Model for the involvement of poly(ADP-ribose) formation in DNA repair. After introduction of a DNA strand interruption with frayed termini by γ-irradiation, PARP, excision enzymes (Exc.) and DNA polymerases (DNA pol.) compete for binding to the damaged site (1); attachment of PARP interferes with access to the strand break for repair enzymes (2); poly(ADP-ribose) synthesis is activated by binding of PARP to damaged DNA (3); the automodified PARP has reduced affinity for DNA and is released, allowing availability of the lesion to DNA-repair enzymes (4); excision-repair seals the DNA strand break (5). Unmodified PARP is regenerated by degradation of poly(ADP-ribose) with a specific glycohydrolase.

DNA repair (Fig. 2*b*). One practical application could be to enhance the cytotoxic effects of DNA-damaging drugs and radiation by inhibiting poly(ADP-ribose) formation with nicotinamide analogues, given the paucity of specific inhibitors of DNA repair. The main function of the polymerase may not even be associated with DNA repair—the enzyme is abundant in chromatin and so could have a structural role in cell nuclei. Poly(ADP-ribose) is found in all higher and many lower eukaryotes but not in yeast or bacteria, although yeast nucleosomes are like those in higher organisms. On the other hand, the dinoflagellate *Crypthecodinium cohnii* lacks histones and has no nucleosome organization, but still has a polymerase for poly(ADP-ribose) synthesis and a poly(ADP-ribose) glycohydrolase[23]. These observations, together with our data, make it unlikely that histone modification is the main task of poly(ADP-ribose) polymerase, although chromatin decondensation may be achieved by modification of histone H1 in heterochromatin[24]. Poly(ADP-ribose) polymerase could help DNA strand breaks introduced temporarily for a purpose[11], for example in DNA associating with the nuclear scaffold, at origins of replication, or during cellular differentiation. Such a 'nick-protection' mechanism might prevent recombination leading to chromosome aberration[5] and spurious initiation of transcription at DNA strand breaks[25]. Alternatively, the binding of the poly(ADP-ribose) polymerase to strand breaks in DNA may constitute a signal that switches off DNA synthesis in γ-irradiated mammalian cells to ensure that lesions are not replicated before repair. □

Received 21 November 1991; accepted 15 January 1992.

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ACKNOWLEDGEMENTS. We thank G. Poirier, S. Sato, S. Shall and R. Wood for discussion, and L. Jones for help with γ-irradiation experiments. M.S.S. was a recipient of a Research Training Fellowship awarded by the International Agency for Research on Cancer. This work was supported by the Imperial Cancer Research Fund.

Suppression of a myosin defect by a kinesin-related gene

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MOTOR proteins in cells include myosin[1], which is actin-based, and kinesin, dynein and dynamin[2], which are microtubule-based. Several proteins have recently been identified that have amino-acid sequences with similarity to the motor domains of either myosin[3] or kinesin[4–9], but are otherwise dissimilar. This has led to the suggestion that these may all be motor proteins, but that they are specialized for moving different cargos. Genetic analysis can address the question of the different functions of these new proteins. Studies of a temperature-sensitive mutation (*myo2-66*) in a gene of the myosin superfamily (*MYO2*) have implicated the Myo2 protein (Myo2p) in the process of polarized secretion in yeast (*Saccharomyces cerevisiae*)[10]. To understand more about the role of Myo2p, we have looked for 'multicopy suppressors' (heterologous genes that, when overexpressed, can correct the temperature sensitivity of the *myo2-66* mutant). Here we report the identification of such a suppressor (*SMY1*) that (surprisingly) encodes a predicted polypeptide sharing sequence similarity with the motor portion of proteins in the kinesin superfamily.

SMY1 (suppressor of myosin) was isolated by introducing a yeast genomic library, in a high-copy-number plasmid, into a *myo2-66* mutant strain and looking for yeast transformants that could grow at restrictive temperature (Fig. 1). Phenotypic analysis revealed that although *SMY1* is a relatively good multicopy suppressor, the suppression is not complete (Fig. 2). The lethality of the *myo2-66* mutation is suppressed at 30–33 °C but not at 35 °C. At 31 °C, the doubling time of the *myo2-66* strain suppressed by plasmid-borne *SMY1* is three to four times longer than that of the strain carrying plasmid-borne *MYO2*. The strain with vector alone undergoes less than one doubling before arresting as large unbudded cells at ≥30 °C, as expected[10].

The region of DNA sufficient for suppression was localized to a 2.3-kilobase (kb) fragment (Fig. 1). Sequencing of this region (Fig. 3) revealed a single long open reading frame. The

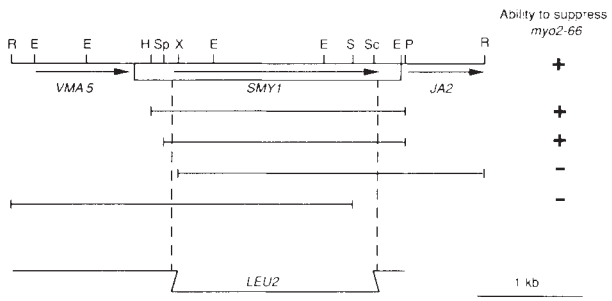


FIG. 1 Map of the *SMY1* locus. Top line, a subcloned segment of the *SMY1*-containing region obtained from the genomic library; other subcloned segments are aligned beneath it, and their ability to suppress the *myo2-66* mutation is indicated. Open box, the region whose sequence is shown in Fig. 3. Arrows, open reading frames identified by sequencing. *VMA5* (which encodes a 40K subunit of the vacuolar ATPase; Tom Stevens, personal communication) and *JA2* (ref. 23 and J. Arenas, personal communication) lie just 400 bp upstream, and 280 bp downstream of *SMY1*, respectively (the positions of the distal ends of these flanking genes shown in the figure are not exact). Bottom line, the construct used for *SMY1* deletion; the 1.9-kb *XbaI–ScaI* fragment of *SMY1* was replaced with a 2-kb fragment containing the *LEU2* gene. Vertical dashed lines, delineate the open reading frame. All restriction enzyme sites are shown for: E, *Eco*RI; H, *Hpa*I; P, *Pst*I; R, *Eco*RV; S, *Sst*I; Sc, *Sca*I; Sp, *Spe*I; X, *Xba*I.

METHODS. The *SMY1*-containing plasmid was obtained by transforming a *myo2-66 ura3-52* strain with the yeast library in YEp24 (a high-copy-number *URA3*-containing plasmid[24]). Transformation plates, selective for the *URA3* auxotrophic marker, were incubated at permissive temperature (~20 °C) for 2 days and then shifted to restrictive temperature (31 °C). The plasmid was recovered from a yeast transformant into *Escherichia coli*; restriction fragments were subcloned into the high-copy-number plasmid YEp352[25] and tested for ability to suppress a *myo2-66* mutant strain. This library screen only yielded two other temperature-sensitive$^+$ (ts$^-$) transformants out of the 16,000 Ura$^+$ transformants screened. Although this was not an exhaustive search, it does indicate that there are not a large number of genes capable of multicopy suppression of *myo2-66* (based both on a calculated expected frequency, and on observed frequencies in other multicopy suppressor screens with this library; S.H.L. *et al.*, unpublished results). *MYO2* was not recovered in this screen, presumably because its overexpression is deleterious (S.H.L. and S.S.B., unpublished results).

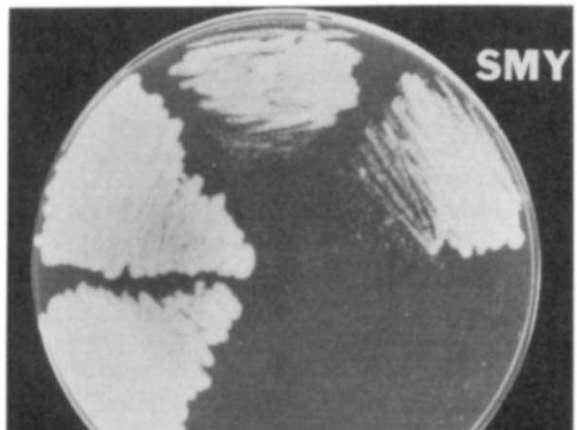


FIG. 2 *SMY1* suppression of the *myo2-66* mutation. Six independent transformants of a *myo2-66* mutant were spread on a plate and incubated at 31 °C for 36 h. Two contained the *SMY1* gene on a high-copy-number plasmid (SMY), two the high-copy-number vector alone (V), and two the *MYO2* gene on a low-copy-number plasmid (MYO). *MYO2* was introduced on a low-copy-number plasmid because the cells seem not to grow well with *MYO2* on a high-copy-number plasmid.

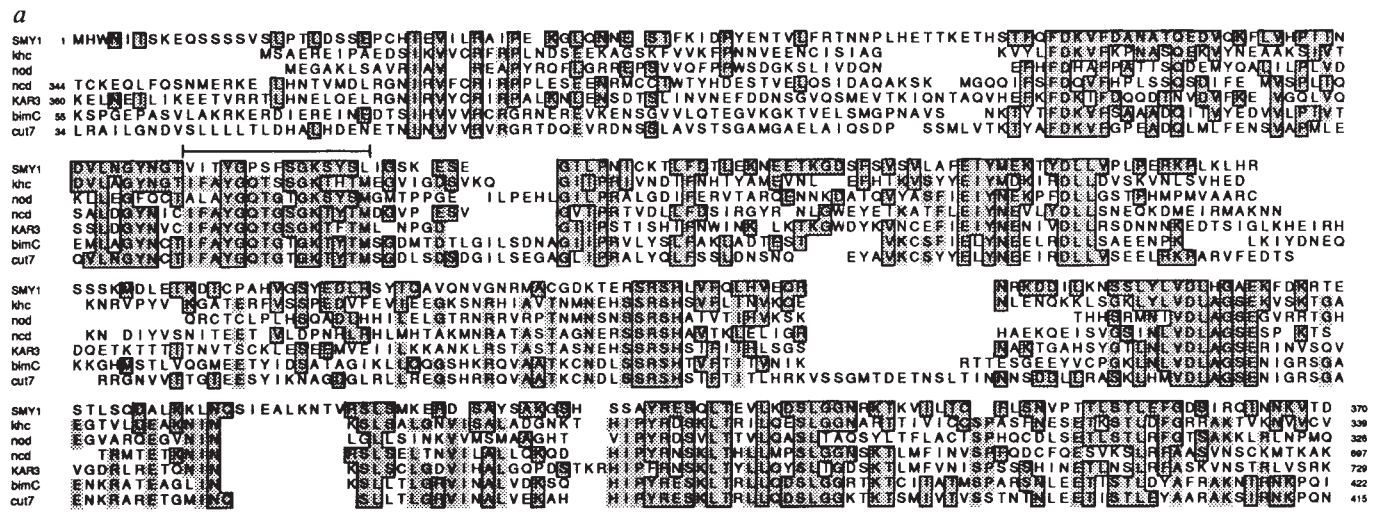
putative start codon is preceded by several stop codons in all frames within the region extending 150 base pairs (bp) upstream. The putative stop codon is followed by several in-frame stop codons. There is no recognition signal for splicing within or near the open reading frame, suggesting that this gene (like most yeast genes) does not contain introns. These considerations, together with the facts that the region sufficient for supppression starts 83 bp upstream of the putative start codon and ends 264 bp downstream of the putative stop codon, led us to conclude that the predicted protein product shown in Fig. 3 is the Smy1 protein (Smy1p). This protein has 656 amino-acid residues and a calculated relative molecular mass of 73,884.

A search of the Genbank and EMBL databases revealed that the N-terminal portion of Smy1p shows significant sequence similarity to the motor domains of the kinesin superfamily, although it is the most divergent (Fig. 4). This superfamily includes kinesins[2], which display sequence similarity throughout their entire lengths[11–13], and several kinesin-related proteins that show sequence similarity only in the motor domains[4–9]. The motor domains have a number of highly conserved short stretches, some but not all of which are found in Smy1p (Fig. 4). For example, the putative ATP-binding region, indicated by the overline in Fig. 4, diverges from that typical of other kinesin-related proteins although all fit the consensus sequence of GX_4GKT[14]. In fact, Smy1p does not resemble kinesin-related proteins more than it does myosins[1,3] in the ATP-binding region. It would be interesting to know how much similarity Smy1p shows to the microtubule binding region of kinesin, but this region has not been sufficiently well localized[13] to permit a

```
-354  TTTTTTTTTTAGAATTTTCAACTTTTTTATTCATAGAGATAATACACTGTGATTGTGAATGTTTGAATAGTAATG
-279  GAACATAATCCGATAAAATATGCTTTTGATGAACGATTAGACATTTTTCAATTTTGTCGTTGAGTTCCAATCCTT
-204  CCTTATTTATGGTTAACAAGGAAAGAGGTGGAAGTTAAAAAAATGGAAATTAAAAAAAAAAAAAAAATCAAAAAAA
-129  GAAAAATAAAAATAGAAAAAATTGGCACGGAAATCTACAGGATTTAGCACTAGTAGGGGGTTTATTAAGCACTACA
 -54  GGTCGACTTTAAACCAGAAAAAATTATCATTCAGGGTAGGTGCAAACTTGTTAAAATGCACTGGAATATAATTTC
   1                                                  M  H  W  N  I  I  S
  21  GAAAGAGCAAAGTAGCTCTTCTGTATCGTTACCAACTCTAGACAGCAGTGAACCCTGTCACATCGAAGTTATCCT
   8  K  E  Q  S  S  S  S  V  S  L  P  T  L  D  S  S  E  P  C  H  I  E  V  I  L
  96  CAGGGCGATACCCGAAAAAGGATTACAAAACAATGAGTCGACCTTCAAAATAGACCCATATGAAAATACTGTGCT
  33  R  A  I  P  E  K  G  L  Q  N  N  E  S  T  F  K  I  D  P  Y  E  N  T  V  L
 171  ATTTCGCACAAACAATCCGTTACATGAGACAACCAAGGAGACCCATTCAACATTTCAATTCGATAAGGTATTCGA
  58  F  R  T  N  N  P  L  H  E  T  T  K  E  T  H  S  T  F  Q  F  D  K  V  F  D
 246  TGCTAACGCCACTCAAGAAGATGTTCAGAAATTTCTGGTGCATCCAATAATAAATGATGTTTTGAATGGTTATAA
  83  A  N  A  T  Q  E  D  V  Q  K  F  L  V  H  P  I  I  N  D  V  L  N  G  Y  N
 321  CGGTACTGTAATAACATATGGACCAAGTTTCAGCGGAAAGTCCTATTCCCTTATTGGATCAAAAGAAAGCGAAGG
 108  G  T  V  I  T  Y  G  P  S  F  S  G  K  S  Y  S  L  I  G  S  K  E  S  E  G
 396  AATTCTACCGAACATATGCAAGACCTTGTTTGATACGCTAGAAAAAAATGAAGAAACAAAGGGAGATAGTTTTAG
 133  I  L  P  N  I  C  K  T  L  F  D  T  L  E  K  N  E  E  T  K  G  D  S  F  S
 471  CGTAAGTGTTTTGGCATTCGAAATATATATGGAGAAAACGTATGACTTATTGGTACCTTTACCTGAAAGAAAACC
 158  V  S  V  L  A  F  E  I  Y  M  E  K  T  Y  D  L  L  V  P  L  P  E  R  K  P
 546  ATTAAAGCTTCACCGTTCTTCAAGCAAAATGGACTTAGAAATCAAAGATATTTGTCCGGCACATGTCGGATCGTA
 183  L  K  L  H  R  S  S  S  K  M  D  L  E  I  K  D  I  C  P  A  H  V  G  S  Y
 621  TGAAGACTTAAGAAGCTACATTCAGGCAGTCCAAAACGTCGGCAATAGGATGGCATGTGGCGACAAGACAGAGAG
 208  E  D  L  R  S  Y  I  Q  A  V  Q  N  V  G  N  R  M  A  C  G  D  K  T  E  R
 696  ATCAAGATCACACCTAGTTTTTCAATTACACGTAGAACAAAGGAATAGAAAAGATGATATATTAAAAAATAGTTC
 233  S  R  S  H  L  V  F  Q  L  H  V  E  Q  R  N  R  K  D  D  I  L  K  N  S  S
 771  TTTATATCTGGTTGATTTACACGGGGCAGAGAAGTTCGATAAAAGAACTGAAAGTACGCTATCACAAGATGCGTT
 258  L  Y  L  V  D  L  H  G  A  E  K  F  D  K  R  T  E  S  T  L  S  Q  D  A  L
 846  AAAAAAATTAAACCAATCTATTGAGGCGTTGAAAAACACTGTGCGGTCCTTGTCAATGAAAGAGCGTGATTCAGC
 283  K  K  L  N  Q  S  I  E  A  L  K  N  T  V  R  S  L  S  M  K  E  R  D  S  A
 921  CTACAGCGCAAAAGGATCACATAGTTCTGCTTACCGTGAATCGCAATTAACTGAAGTGTTAAAAGATTCCCTTGG
 308  Y  S  A  K  G  S  H  S  S  A  Y  R  E  S  Q  L  T  E  V  L  K  D  S  L  G
 996  GGGAAATAGGAAAACAAAGGTGATATTGACATGTTTCTTAAGTAATGTTCCAACTACCCTATCAACACTAGAATT
 333  G  N  R  K  T  K  V  I  L  T  C  F  L  S  N  V  P  T  T  L  S  T  L  E  F
1071  TGGTGACAGTATTAGACAGATCAATAACAAGGTTACAGATAACACCACAGGTTTAAATCTGAAAAAAAAAATGGA
 358  G  D  S  I  R  Q  I  N  N  K  V  T  D  N  T  T  G  L  N  L  K  K  K  M  D
1146  TCTATTTATTCAGGACATGAAAATTAAGGATGATAATTATGTTGCCCAAATTAATATACTAAAGGCTGAAATAGA
 383  L  F  I  Q  D  M  K  I  K  D  D  N  Y  V  A  Q  I  N  I  L  K  A  E  I  D
1221  CTCTTTAAAAAGCCTTCACAATAAATCTCTTCCCGAAGACGACGAGAAAAAAATGTTAGAAAATACAAAGAAAGA
 408  S  L  K  S  L  H  N  K  S  L  P  E  D  D  E  K  K  M  L  E  N  T  K  K  E
1296  AAATATCAAACTAAAGCTTCAATTAGACAGTATTACCCAATTATTGAGTAGTTCTACCAATGAAGATCCTAACAA
 433  N  I  K  L  K  L  Q  L  D  S  I  T  Q  L  L  S  S  S  T  N  E  D  P  N  N
1371  TCGTATTGACCAAGAAGTTTCTGAAATATTAACGAAAAGATGCGAACAGATTGCTCAGCTTGAGTTATCTTTTGA
 458  R  I  D  E  E  V  S  E  I  L  T  K  R  C  E  Q  I  A  Q  L  E  L  S  F  D
1446  TCGACAGATGAATTCCAATTCTAAGTTGCAGCAAGAGTTAGAATACAAAAAGTCAAAAGAGGAAGCCTTAGAGTC
 483  R  Q  M  N  S  N  S  K  L  Q  Q  E  L  E  Y  K  K  S  K  E  E  A  L  E  S
1521  TATGAACGTTAGGCTACTAGAACAAATTCAGCTTCAGGAAAGAGAGATTCAAGAGCTTTTAACTACTAACGCCAT
 508  M  N  V  R  L  L  E  Q  I  Q  L  Q  E  R  E  I  Q  E  L  L  T  T  N  A  I
1596  CTTAAAGGGTGAACTAGAAACTCACACTAAACTTACTGAAACACGCAGTGAAAGGATAAAATCTTTGGAAAGTTC
 533  L  K  G  E  L  E  T  H  T  K  L  T  E  T  R  S  E  R  I  K  S  L  E  S  S
1671  TGTCAAAGAGCTTTCTCTTAATAAATCTGCAATCCCATCTCCTAGAAGAGGGTCTATGAGCTCGTCATCAGGAAA
 558  V  K  E  L  S  L  N  K  S  A  I  P  S  P  R  R  G  S  M  S  S  S  S  G  N
1746  TACTATGTTGCATATCGAGGAGGGTTCTGAAATATCAAATAGCCCTTGGTCAGCCAATACTTCCTCAAAACCTTT
 583  T  M  L  H  I  E  E  G  S  E  I  S  N  S  P  W  S  A  N  T  S  S  K  P  L
1821  AGTATGGGGCGCGAGAAAGGTTTCTTCTAGCAGTATAGCAACCACTGGATCACAGGAGTCTTTTGTGGCAAGACC
 608  V  W  G  A  R  K  V  S  S  S  S  I  A  T  T  G  S  Q  E  S  F  V  A  R  P
1896  GTTCAAGAAGGGATTAAACCTCCATTCGATAAAAGTCACTTCGAGTACTCCAAAAAGTCCCTCTTCTGGAAGGTG
 633  F  K  K  G  L  N  L  H  S  I  K  V  T  S  S  T  P  K  S  P  S  S  G  S  *
1971  ACTATAATTAAGTTCGATAATTGTACGATACAACTGTGTAGTATCTTTGTAATTATAATCATGATGATAACGCTA
2046  ATAATTTGTACGTATAATGCTGACTGAAAGATGTACTTTTCTTCTACCGTTTTTCTTCGGCGGCTATTCGTTTAC
2121  AATGATTTGGAAAAATGAGAAAAATTGAAAATAAAAAATTCTGCTCGATGAGATGAGATGAGGTTATGATATATA
2196  GTAAGAA
```

FIG. 3 The nucleotide and predicted amino-acid sequences of *SMY1*. Numbers start at the first ATG of the open reading frame. DNA was sequenced by the dideoxy-chain termination method[26]; both strands were completely sequenced. In all cases we have sequenced across cloning junctions on at least one strand. The Genbank accession number is M69021.

a



b

	khc	nod	ncd	KAR3	bimC	cut7
SMY1	35	25	26	25	29	30
khc		37	41	37	45	40
nod			29	31	35	32
ncd				46	38	35
KAR3					36	38
bimC						58

FIG. 4 Comparison of the N-terminal amino-acid sequence of Smy1p to the motor domains of kinesin and kinesin-related proteins (khc, *Drosophila* kinesin heavy chain[13]; nod, *Drosophila nod* protein[4]; ncd, *Drosophila claret* nondisjunctional protein[5,6]; KAR3, *S. cerevisiae* Kar3 protein[7]; bimC, Aspergillus *bimC* protein[8]; cut7, *Schizosaccharomyces pombe cut7* protein[9]). The *ncd* and Kar3 proteins have their motor domains at the C- instead of N-terminus. *a*, Residues identical to Smy1p are boxed and shaded. Residues identical in at least four proteins excluding Smy1p are shaded but not boxed. Alignments were made by a combination of BESTFIT[27] and visual inspection; spaces represent gaps inserted to maximize matches. Overline, putative ATP-binding region. *b*, Per cent identity of the sequences in *a* is shown in tabular form. Pairwise comparisons were performed by BESTFIT analysis, using the default parameters.

comparison. The C-terminal portion of Smy1p (the 'tail') shows no sequence similarity to kinesin or other kinesin-related proteins. We looked carefully for similarity between the Myo2p and Smy1p tails, as our results indicate that they might carry the same cargo, but none was seen.

Secondary structure predictions[15] for Smy1p are similar to those for other kinesin-related proteins; amino acids 400–560 in Smy1p are predicted to be largely in an α-helical conformation, lacking turns. In conventional kinesin[13], this predicted domain has been shown to correspond to a coiled-coil region responsible for dimerization, preceded by the motor domain, and followed by a C-terminal domain that binds light chains and probably membrane vesicles (the cargo). A computer program[16] predicts that residues 272–306, 421–447 and 490–542 of Smy1p can form coiled-coils.

To learn more about the role of Smy1p , we deleted one copy of the *SMY1* gene in a diploid strain, using the construct shown in Fig. 1. This removed all but the N-terminal 16 and C-terminal 9 codons of *SMY1*. Southern blotting (data not shown) confirmed that the expected deletion had occurred in the diploid strain and the *SMY1* deletion mutant haploid (Leu^+) progeny. Phenotypic analysis of the haploid segregants showed no detectable differences between the deletion mutants and their wild-type sisters. The deletion mutants appeared to have a normal distribution of actin and tubulin, and appeared normal in their ability to mate, sporulate and grow in various media at various temperatures. Thus, the function of Smy1p must be either redundant or nonessential under the conditions tested. If redundant, the functional equivalent is not similar enough in sequence to *SMY1* to have been detected by Southern hybridization (data not shown).

Additional genetic evidence that Smy1p may interact with or substitute for Myo2p is provided by our finding of 'synthetic lethality'[17] between the *myo2-66* mutation and the *SMY1* deletion; from a cross of a *myo2-66* mutant with a *SMY1* deletion mutant no live double mutants were obtained. 36 tetrads were analysed at 20 °C (permissive temperature for the *myo2-66* mutant); these gave a frequency of 5:24:7 tetrads with 4, 3 and 1–2 live segregants, consistent with synthetic lethality. Of the 31 non-viable segregants for which the genotype could be inferred, 30 carried both mutations. The synthetic lethality indicates that Smy1p can compensate for the defect in *myo2-66* mutants not only under the abnormal conditions of overexpression (the multicopy suppression) but also when Smy1p is presumably present at normal levels.

Thus, Smy1p, a putative microtubule-based motor, can interact with or substitute for Myo2p, a putative actin-based motor. It is possible that Myo2p functions to organize or stabilize the actin cytoskeleton. Another possibility, proposed by Johnston *et al.*[10], is that Myo2p attaches to secretory vesicles and carries them along actin filaments to the bud. If a defect in vesicle transport is being corrected by *SMY1*, an obvious possibility is that Smy1p can carry the vesicles along microtubules, which are in the right location in yeast to direct vesicles to the bud. However, it has been demonstrated in yeast that microtubules are not required for delivery of secretory vesicles to the bud[17,18], nor do they substitute in actin mutants[19]. Nonetheless, microtubules could normally provide a minor pathway that can compensate for a partial defect in myosin at the nominally permissive temperature; this pathway may become more important under conditions of Smy1p overexpression, compensating for the more severe myosin defect at restrictive temperature. Such transport of vesicles along microtubules by Smy1p would represent the first clear-cut example of functional redundancy between actin and microtubules.

Another possibility is that Smy1p suppresses the myosin defect by carrying vesicles along actin filaments, although this seems unlikely, given the sequence similarity of Smy1p to kinesin. However, sequence similarity should be interpreted with caution, as shown by the recent finding that a kinesin-like protein has a directionality of movement *in vitro* opposite that of kinesin[20,21]. Furthermore, not enough is known about actin- or microtubule-binding sequences to look for them in Smy1p; studies of truncated kinesins[13] indicate only that the microtubule binding site(s) is somewhere in the C-terminal half of the motor domain. Thus, it will be important to test both Smy1p and Myo2p for motor activity, and to determine whether they move along actin or microtubules. As yeast actin and tubulins are similar in sequence to their mammalian counterparts and can copolymerize with them[22], it is unlikely that any unusual properties of these proteins are responsible for the intriguing relationship that we have found between Smy1p and Myo2p. □

Received 4 November 1991; accepted 28 January 1992.

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ACKNOWLEDGEMENTS. We thank G. Johnston and R. Singer for *myo2-66* mutant strains, *MYO2* DNA, and for communicating unpublished results, T. McKinney for sequencing assistance, and D. Gumucio, B. Haarer and J. Schiefelbein for critical reading of the manuscript. This work was supported by a grant from the American Cancer Society and in part by an NIH grant to the Kughn Clinical Research Center at the University of Michigan Hospital.

Diode-like behaviour of a mitochondrial electron-transport enzyme

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IN mitochondria, electrons derived from the oxidation of succinate by the tricarboxylic acid cycle enzyme succinate–ubiquinone oxidoreductase are transferred directly to the quinone pool. Here we provide evidence that the soluble form of this enzyme (succinate dehydrogenase) behaves as a diode that essentially allows electron flow in one direction only. The gating effect is observed when electrons are exchanged rapidly and directly between fully active succinate dehydrogenase and a graphite electrode. Turnover is therefore measured under conditions of continuously variable electrochemical potential. The otherwise rapid and efficient reduction of fumarate (the reverse reaction) is severely retarded as the driving force (overpotential) is increased. Such behaviour can arise if a rate-limiting chemical step like substrate binding or product release depends on the oxidation state of a redox group on the enzyme. The observation provides, for a biological electron-transport system, a simple demonstration of directionality that is enforced by kinetics as opposed to that which is assumed from thermodynamics.

Succinate dehydrogenase (SDH)[1] consists of two different hydrophilic subunits (F_p and I_p, relative molecular masses 70,000 and 27,000, respectively) containing one covalently bound flavin adenine dinucleotide and three Fe-S clusters (centres 1, 2 and 3). In complex II, succinate-ubiquinone oxidoreductase, these subunits are associated with two hydrophobic peptides that bind the enzyme to the mitochondrial inner membrane. In this physiological form, electrons are exchanged with the quinone pool:

$$\text{succinate} \rightleftharpoons \text{fumarate} + 2H^+ + 2e^- (\text{into ubiquinone pool})$$

For this study, we used reconstitutively-active SDH (ref. 2), which catalysed succinate oxidation by phenazine methosulphate or low concentrations of ferricyanide[3] with $k_{cat}(38\,^\circ\text{C}) = 10\text{–}12{,}000\ \text{min}^{-1}$. An edge-oriented pyrolytic graphite (PGE) electrode was used throughout[4]. We found that cyclic voltammetry of a solution of succinate (pH 8.3 at 35 °C) shows no faradaic activity over the range +200 to −500 mV against a standard hydrogen electrode. But introduction of SDH (0.6–1.2 μM) gives rise to an oxidation current (Fig. 1) which increases rapidly with potential to reach a steady-state value, i_{lim}.

The following observations (1) to (4) are based on first-cycle measurements and show that SDH can adsorb at the pyrolytic graphite electrode in a fully functional electroactive state: (1) i_{lim} is independent of scan rate over the range 2–500 mV s^{-1}. The half-height potential is constant (±10 mV) over the same range, and close to the thermodynamic reduction potential, $E^0_{F/S}$, of the fumarate (F)-succinate (S) couple. Experiments with a rotating-disc electrode[5] also showed that i_{lim} is independent of rotation rate (0–20 Hz). Thus, neither electron transfer (enzyme to electrode) nor mass transport limit the rate of turnover; (2) i_{lim} is independent of SDH concentration above 0.6 μM. This is consistent with catalysis by enzyme molecules that are saturatively bound at a small fraction of the electrode surface. The adsorbed nature of the catalyst is evident from two further observations: first, that activity increases initially with time at a rate dependent on SDH concentration (for example, below 0.6 μM, i_{lim} increases over the first few cycles at 10 mV s^{-1}); and, second, that electrodes incubated in SDH solution and rinsed with buffer show activity after transfer to a solution without enzyme; (3) i_{lim} varies with succinate

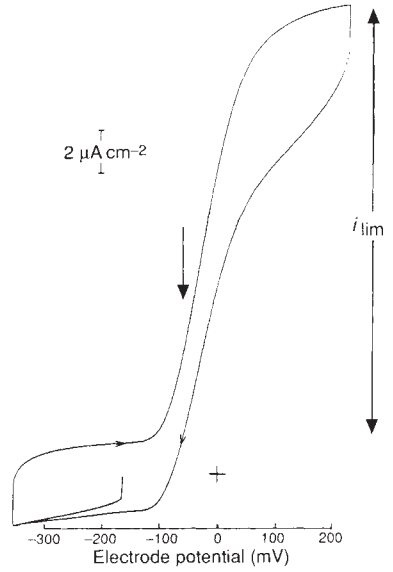

FIG. 1 Cyclic voltammogram at a freshly polished pyrolytic graphite edge electrode of a solution (0.1 M NaCl, 50 mM TAPS (*N*-tris(hydroxymethyl)-methyl-3-aminopropanesulphonic acid)) containing 6.2 mM succinate, 0.7 μM SDH at pH 8.3. Temperature was 35.5 °C, scan rate, 10 mV s^{-1}. The position of $E^0_{F/S}$, the formal reduction potential for the fumarate/succinate couple at pH 8.3, is indicated by the vertical arrow. The position of absolute zero current is marked with a cross. The limiting faradaic current i_{lim} is measured as indicated and expressed as current density. Repetitive cycles always showed progressive attenuation of i_{lim}, indicating inactivation of the enzyme. On the other hand, catalytic activity was largely retained if, instead of repetitive cycling, the electrode potential was held for an equivalent period at −200 mV. A likely explanation is degradation of centre 3 at the higher potentials required for succinate oxidation. It is known that loss of low-K_M ferricyanide reductase activity coincides with degradation of centre 3 as SDH is oxidized[3]. We also found that SDH samples with only 50% and 17% of the ferricyanide reductase activity of pristine enzyme gave respective degrees of diminished electroactivity.